Production of functional pharmaceutical nano/micro-particles by solvent displacement method using advanced micro-engineered dispersion devices

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Production of Functional Pharmaceutical Nano/Micro-Particles by Solvent Displacement Method Using Advanced Micro-Engineered Dispersion Devices

Rahimah Othman

A doctoral thesis submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy of Loughborough University

Department of Chemical Engineering
October 2016
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ABSTRACT

The rapid advancement of drug delivery systems (DDS) has raised the possibility of using functional engineered nano/micro-particles as drug carriers for the administration of active pharmaceutical ingredients (APIs) to the affected area. The major goals in designing these functional particles are to control the particle size, the surface properties and the pharmacologically active agents release in order to achieve the site-specification of the drug at the therapeutically optimal rate and dose regimen. Two different equipment (i.e. glass capillary microfluidic device and micro-engineered membrane dispersion cell) were utilised in this study for the formation of functional nano/micro-particles by antisolvent precipitation method. This method is based on micromixing/direct precipitation of two miscible liquids, which appear as a straightforward method, rapid and easy to perform, does not require high stirring rates, sonication, elevated temperatures, surfactants and Class 1 solvents can be avoided.

Theoretical selection of a “good solvent” and physicochemical interaction between solvent-water-polymer with the aid of Bagley’s two-dimensional graph were successfully elucidated the nature of anti-solvent precipitation method for the formation of desired properties of functional pharmaceutical nano/micro-engineered particles. For the glass capillary microfluidic experiment, the organic phase (a mixture of polymer and tetrahydrofuran/acetone) was injected through the inner glass capillary with a tapered cross section culminated in a narrow orifice. The size of nanoparticles was precisely controlled by controlling phase flow rates, orifice size and flow configuration (two-phase co-flow or counter-current flow focusing). The locations at which the nanoparticles would form were determined by using the solubility criteria of the polymer and the concentration profiles found by numerical modelling. This valuable results appeared as the first computational and experimental study dealing with the formation of polylactide (PLA) and poly(ε-caprolactone) (PCL) nanoparticles by nanoprecipitation in a co-flow glass capillary device.

The optimum formulations and parameters interactions involved in the preparation of paracetamol encapsulated nanoparticles (PCM-PCL NPs) using a co-flow
microfluidic device was successfully simulated using a $2^5$-full factorial design for five different parameters (i.e. PCL concentration, orifice size, flow rate ratios, surfactant concentration and paracetamol amount) with encapsulation efficiency and drug loading percentage as the responses. PCM-loaded composite NPs composed of a biodegradable poly($\delta$-L-lactide) (PLA) polymer matrix filled with organically modified montmorillonite (MMT) nanoparticles were also successfully formulated by antisolvent nanoprecipitation in a microfluidic co-flow glass capillary device. The incorporation of MMT in the polymer matrix improved the drug encapsulation efficiency and drug loading, and extended the rate of drug release in simulated intestinal fluid (pH 7.4). The encapsulation of MMT and PCM in the NPs were well verified using transmission electron microscopy (TEM), energy dispersive x-ray spectroscopy (EDS), x-ray diffraction (XRD), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR).

PCL drug-carrier nanoparticles were also produced by rapid membrane micromixing combined with nanoprecipitation in a stirred cell employing novel membrane dispersion. The size of the NPs was precisely controlled by changing the aqueous-to-organic volumetric ratio, stirring rate, transmembrane flux, the polymer content in the organic phase, membrane type and pore morphologies. The particle size decreased by increasing the stirring rate and the aqueous-to-organic volumetric ratio, and by decreasing the polymer concentration in the aqueous phase and the transmembrane flux. The existence of the shear stress peak within a transitional radius and a rapid decline of the shear stress away from the membrane surface were revealed by numerical modelling. Further investigation on the PCL nanoparticles loaded immunosuppressive rapamycin (RAPA) drug were successfully synthesised by antisolvent nanoprecipitation method using stainless steel (SS) ringed micro-engineered membrane. Less than 10 µm size of monohydrate piroxicam (PRX) micro-crystals also was successfully formed with the application of anti-solvent precipitation method combined with membrane dispersion cell that has been utilised in the formation of functional engineered nanoparticles. This study is believed to be a new insight into the development of integrated membrane crystallisation system.
ACKNOWLEDGEMENTS

I am very grateful to my supervisors, Dr. Goran T.Vladisljevic and Profesor Zoltan Nagy, for giving me the opportunity to undertake this research project. I am also greatly indebted to them for their expert guidance, considerate support and continual encouragement throughout the course of this project. Their enlightening suggestions and earnest comments were invaluable to the development of this report.

I would also like to acknowledge the following peoples:

- Dr. Keith Yendall from Department of Materials, Loughborough University for the operation training and the use of Field Emission Gun-Scanning Electron Microscopes (FEG-SEM) and X-ray diffraction (XRD) analyses.
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On a more personal level, I would like to thank my mum, Mrs Jamaliah Osman and my late father Mr Othman Awang, my siblings, Dr. Nor Fadzilah, Dr. Abdul Kadir, Omar, Fauziah, Dr. Abdul Rahim, Dr. Noor Badriah, Muhammad Firdaus, Dr. ‘Afifah, my nieces, nephews and friends, especially to Suria, Hidayah, Siti, Nani and Radhiah for their invaluable encouragement and continued support during the entire duration of my research works.

Rahimah Othman

2016
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Journal Papers:

**Conferences:**


University Research Conference 2015: Inspiring Research, Loughborough, 11\textsuperscript{th} November 2015.


\textbf{Achievements:}

1. 1\textsuperscript{st} poster winner in the British Association for Crystal Growth (BACG 2015) Conference, London, UK, 21\textsuperscript{st} – 23\textsuperscript{rd} June 2015.

2. Best presenter award in the Annual Postgraduate Seminar organised by Chemical Engineering Department, Loughborough University, 2016.
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<tbody>
<tr>
<td>Ace</td>
<td>Acetone</td>
</tr>
<tr>
<td>AFFD</td>
<td>Axisymmetric flow focusing device</td>
</tr>
<tr>
<td>API</td>
<td>Active pharmaceutical ingredients</td>
</tr>
<tr>
<td>ATR</td>
<td>Attenuated total reflection</td>
</tr>
<tr>
<td>BSE</td>
<td>Backscattered electron</td>
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<tr>
<td>CFD</td>
<td>Computational fluid dynamic</td>
</tr>
<tr>
<td>CLD</td>
<td>Chord length distribution</td>
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<tr>
<td>CryPRINS</td>
<td>Crystallisation process informatics system</td>
</tr>
<tr>
<td>CSA</td>
<td>Composite sensor array</td>
</tr>
<tr>
<td>CSD</td>
<td>Crystals size distribution</td>
</tr>
<tr>
<td>DDS</td>
<td>Drug delivery systems</td>
</tr>
<tr>
<td>DL</td>
<td>Drug loading</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic light scattering</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
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<tr>
<td>DNC</td>
<td>Direct nucleation control</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
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<td>EDS</td>
<td>Energy dispersive spectroscopy</td>
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<tr>
<td>EDX</td>
<td>Energy dispersive X-ray spectroscopy</td>
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<tr>
<td>EE</td>
<td>Entrapment efficiency</td>
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<tr>
<td>EELS</td>
<td>Electron energy loss spectroscopy</td>
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<tr>
<td>EL</td>
<td>Ethyl lactate</td>
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<tr>
<td>EPR</td>
<td>Enhanced retention and permeability</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
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<tr>
<td>FBRM</td>
<td>Focused beam reflectance measurement</td>
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<td>FDA</td>
<td>Food and drug administration</td>
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<tr>
<td>FEG-SEM</td>
<td>Field emission gun scanning electron microscopy</td>
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<tr>
<td>FFDG</td>
<td>Flow focusing droplet generators</td>
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<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
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<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<tr>
<td>HPMC</td>
<td>Hydroxypropylmethylcellulose</td>
</tr>
<tr>
<td>ICTAC</td>
<td>International confederation for thermal analysis and calorimetry</td>
</tr>
<tr>
<td>IPA</td>
<td>Isopropyl alcohol</td>
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<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
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<tr>
<td>MC</td>
<td>Microchannel</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>MFFD</td>
<td>Microfluidic flow focusing devices</td>
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<td>MMT</td>
<td>Montmorillonite</td>
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<tr>
<td>MPs</td>
<td>Microparticles</td>
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<td>MSZW</td>
<td>Metastable zone width</td>
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<td>Ni</td>
<td>Nickel</td>
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<td>NMR</td>
<td>Nuclear magnetic resonance</td>
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<tr>
<td>NPs</td>
<td>Nanoparticles</td>
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<td>NTA</td>
<td>Nanoparticle tracking analysis</td>
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<td>Pluronic-123</td>
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<td>PAT</td>
<td>Process analytical technology</td>
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<tr>
<td>PBM</td>
<td>Population balance modelling</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
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<tr>
<td>PCA</td>
<td>Poly-cyanoacrylate</td>
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<tr>
<td>PCL</td>
<td>Poly(ε-caprolactone)</td>
</tr>
<tr>
<td>PCM</td>
<td>Paracetamol</td>
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<tr>
<td>PDE</td>
<td>Permissible daily exposure</td>
</tr>
<tr>
<td>PDI</td>
<td>polydispersity index</td>
</tr>
<tr>
<td>PDLLA</td>
<td>Poly(δ,ʟ-lactide)</td>
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<tr>
<td>PEO-PLGA</td>
<td>Poly(δ,ʟ-lactic-co-glycolic acid) block copolymers</td>
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<td>PLA</td>
<td>Poly(lactide)</td>
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<td>PLG</td>
<td>Poly(δ,ʟ-glycolide)</td>
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<td>PLGA</td>
<td>Poly(lactide-co-glycolide)</td>
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<td>PRX</td>
<td>Piroxicam</td>
</tr>
<tr>
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<td>Teflon</td>
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<td>PVA</td>
<td>Polyvinyl alcohol</td>
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<td>Particle vision and measurement</td>
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<td>PVP</td>
<td>Polyvinylpyrrolidone</td>
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<td>RAPA</td>
<td>Rapamycin</td>
</tr>
<tr>
<td>SE</td>
<td>Secondary electron</td>
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<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
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<td>SS</td>
<td>Stainless steel</td>
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<td>SSC</td>
<td>Supersaturation control strategy</td>
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<td>TEM</td>
<td>Transmission electron microscopy</td>
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<td>Tetrahydrofuran</td>
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<td>$V_{aq}/V_{or}$</td>
<td>Aqueous-to-organic phase volume ratio</td>
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</tr>
<tr>
<td>$\delta_d$</td>
<td>Partial solubility parameters of dispersion forces</td>
<td>$(J \text{ cm}^{-3})^{1/2}$</td>
</tr>
<tr>
<td>$\delta_p$</td>
<td>Partial solubility parameters of polar contribution</td>
<td>$(J \text{ cm}^{-3})^{1/2}$</td>
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<tr>
<td>$\delta_h$</td>
<td>Partial solubility parameters of hydrogen contribution</td>
<td>$(J \text{ cm}^{-3})^{1/2}$</td>
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<tr>
<td>$\delta_r$</td>
<td>Partial solubility parameters of dispersion and polar contribution</td>
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</tr>
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<td>Combined solubility parameters of solvent-water</td>
<td>$(J \text{ cm}^{-3})^{1/2}$</td>
</tr>
<tr>
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<td>Combined solubility parameters of polymer-water</td>
<td>$(J \text{ cm}^{-3})^{1/2}$</td>
</tr>
<tr>
<td>$\chi_{\text{solvent-water}}$</td>
<td>Interaction parameter of solvent-water</td>
<td>-</td>
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<tr>
<td>$\chi_{\text{polymer-water}}$</td>
<td>Interaction parameters of polymer-water</td>
<td>-</td>
</tr>
<tr>
<td>$Q_{aq}$</td>
<td>Aqueous phase flow rate</td>
<td>mL h$^{-1}$</td>
</tr>
<tr>
<td>$Q_{or}$</td>
<td>Organic phase flow rate</td>
<td>mL h$^{-1}$</td>
</tr>
<tr>
<td>$C_i$</td>
<td>Polymer concentration</td>
<td>g L$^{-1}$</td>
</tr>
<tr>
<td>$C^*$</td>
<td>Saturation concentration</td>
<td>g L$^{-1}$</td>
</tr>
<tr>
<td>$K_g$</td>
<td>Particle growth rate</td>
<td>s$^{-1}$</td>
</tr>
<tr>
<td>$D_o$</td>
<td>Outer diameter of the nozzle</td>
<td>$\mu$m</td>
</tr>
<tr>
<td>$D$</td>
<td>Diffusion coefficient</td>
<td>cm$^2$ s$^{-1}$</td>
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<tr>
<td>$U_{aq}$</td>
<td>Aqueous phase velocity</td>
<td>m s$^{-1}$</td>
</tr>
<tr>
<td>$U_{or}$</td>
<td>Organic phase velocity</td>
<td>m s$^{-1}$</td>
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<tr>
<td>$\eta_{\text{water}}$</td>
<td>Water viscosity</td>
<td>mPa s</td>
</tr>
<tr>
<td>$\eta_{\text{THF}}$</td>
<td>Tetrahydrofuran viscosity</td>
<td>mPa s</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Dynamic viscosity</td>
<td>mPa</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Density of THF-water mixture</td>
<td>g cm$^{-3}$</td>
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</table>
\( \omega_i \) \hspace{1cm} \text{Mass fraction of species } i \text{ and } \text{ -}

\( j_i \) \hspace{1cm} \text{Relative mass flux vector} \hspace{1cm} \text{ -}

\( D_{ik} \) \hspace{1cm} \text{Multicomponent Maxwell-Stefan diffusivities} \hspace{1cm} \text{ -}

\( x_k \) \hspace{1cm} \text{Mole fraction} \hspace{1cm} \text{K}

\( M_i \) \hspace{1cm} \text{Molar mass of component } i \hspace{1cm} \text{cm}^3 \text{ mol}^{-1}

\( D_{i}^{T} \) \hspace{1cm} \text{Thermal diffusion coefficient of species } i \hspace{1cm} \text{cm}^2 \text{ s}^{-1}

\( D_{i}^{m} \) \hspace{1cm} \text{Final is the mixture-averaged diffusion coefficient} \hspace{1cm} \text{cm}^2 \text{ s}^{-1}

\( D_{N} \) \hspace{1cm} \text{Inner diameter of the nozzle} \hspace{1cm} \text{µm}

\( U_z \) \hspace{1cm} \text{Axial velocity} \hspace{1cm} \text{m s}^{-1}

\( U_x \) \hspace{1cm} \text{Radial velocity} \hspace{1cm} \text{m s}^{-1}

\( A_R \) \hspace{1cm} \text{Operating areas of the ringed membrane} \hspace{1cm} \text{m}^2

\( A_m \) \hspace{1cm} \text{Operating areas of the whole membrane} \hspace{1cm} \text{m}^2

\( T_c \) \hspace{1cm} \text{Crystallisation temperature} \hspace{1cm} \text{ºC}

\( T_g \) \hspace{1cm} \text{Glass transition temperature} \hspace{1cm} \text{ºC}

\( T_m \) \hspace{1cm} \text{Melting temperature} \hspace{1cm} \text{ºC}

\( M_w \) \hspace{1cm} \text{Molecular weight} \hspace{1cm} \text{g mol}^{-1}

\( r_1 \) \hspace{1cm} \text{Inner radius of ringed membrane} \hspace{1cm} \text{mm}

\( r_2 \) \hspace{1cm} \text{Outside radius of ringed membrane} \hspace{1cm} \text{mm}

\( T \) \hspace{1cm} \text{Absolute temperature} \hspace{1cm} \text{K}

\( V_P \) \hspace{1cm} \text{Molar volume of the polymer at normal boiling point} \hspace{1cm} \text{cm}^3 \text{ mol}^{-1}

\( W \) \hspace{1cm} \text{Width of the square capillary} \hspace{1cm} \text{µm}

\( Z_{ave} \) \hspace{1cm} \text{Average particle size} \hspace{1cm} \text{nm}
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CHAPTER 1

INTRODUCTION

1.1 Background

Engineered functional pharmaceutical particles have attracted intriguing attention of the scientific community due to their significant therapeutic potential for site specific delivery in their applications for drug targeting to particular organs/tissues, as carriers of active pharmaceutical ingredients (API), DNA in gene therapy, and in their ability to deliver proteins, peptides and genes through a per oral route of administration. Great attention has been given to this drug delivery strategy owing to its numerous advantages and rapid resolution:

“The late 1960s and early 1970s saw the advent of polymer microparticles (MPs) based on acrylamide micelle polymerization (Crommelin & Florence, 2013). Since then, along with different polymerization methods, preformed polymers also have been developed and studied. The majority of studies on nanoparticles (NPs) reported to date have dealt with microparticles created from poly(ᴅ,ʟ-lactide), polylactide (PLA), poly(ᴅ,ʟ-glycolide) (PLG), poly(lactide-co-glycolide) (PLGA), and poly-cyanoacrylate (PCA) (Barratt, 2000; Crommelin & Florence, 2013)”.

Various pharmaceutical carriers, including nano/micro-carriers, such as nano/micro-spheres, nano/micro-capsules, liposomes, micelles, cell ghosts, lipoproteins, nano/micro-crystals and many others, are widely used for the experimental and clinical delivery of therapeutic and diagnostic agents in order to enhance the in vivo efficiency of many drugs and drug administration protocols (Kreuter, 1996; Lee, 2003). Modifications of these carriers are often used to control their in vivo properties in a desirable fashion, for example: (i) stay (circulate) long in the body; (ii) specifically target the site of the disease; (iii) respond to local stimuli characteristic of the pathological site, such as intrinsically abnormal pH values or temperature, or externally applied or changing properties, (iv) provide an enhanced intracellular
delivery of drugs and genes as required, and (v) carry a reporter (contingrivast) component supplying real time information about the drug delivery systems (DDS) biodistribution and target accumulation (Torchilin, 2009).

Various methods for the manufacture of polymer nano/micro-particles have been described (De Jaeghere et al., 1999; Delair, 2004), such as solvent evaporation, salting-out, dialysis and supercritical fluid technology. They can be also directly synthesized by the polymerization of monomers using various polymerization techniques such as micro-emulsion, mini-emulsion, surfactant-free emulsion and interfacial polymerization (Legrand et al., 2007; Rao & Geckeler, 2011). The choice of production method is practically crucial depending on their physiological, anatomical, clinical behaviour and potential application (Otto et al., 2014). Among the different methods, precipitation, which is simple, fast and economic, has the advantage of using preformed polymers as starting materials rather than monomers, as well as employing non-toxic solvents (Fessi et al., 1989). Nanoprecipitation often enables the production of small nanoparticles (100–300 nm) with a narrow monomodal distribution and a wide range of preformed polymers can be used (Bilati et al., 2005; Nagavarma et al., 2012). A study by Legrand et al. (2007) showed promising results related to the influence of polymer behaviour in organic solution on the production of PLA nanoparticles by the nanoprecipitation process. Most of the previous studies have reported concerns about the mixing process enhancement with serious consideration given to polymer-solvent solubility, polymer-water and polymer-solvent interaction (Galindo-rodriguez et al., 2004; Legrand et al., 2007). All of these physico-chemical parameters are predominantly important for the precipitation process that is associated with nano/micro-particles formation.

In recent years, technologies for particle preparation using microfluidics have been developing very fast, because of their many advantages in material preparation, such as uniform flow and mixing, easy control, high efficiency, continuous operation and low cost. Requirements for particle monodispersity, chemistry, porosity, shape and size are becoming increasingly stringent (Dendukuri & Doyle, 2009), thus calling for controllable technologies and equipment for particle preparation. Novel morphologies and special properties particles can be synthesised with the use of controlled
microfluidics devices (Luo et al., 2011; Xu et al., 2005). Recently, a new micro-engineered nickel/stainless steel membrane has become available, consisting of an array of regularly spaced, rectilinear pores, which is analogous to an array of parallel microfluidic channels through with one fluid phase can be introduced into another fluid at an overall flow rate much higher than is possible in microfluidic devices (Laouini et al., 2013a; 2013b). Microporous membranes are increasingly used for the preparation of nano/micro-particles and emulsions, such as polymeric nanoparticles (Charcosset & Fessi, 2005a; Du et al., 2011), lipid nanoparticles (Charcosset et al., 2005b), nanocapsules (Charcosset & Fessi, 2005c), gel microbeads (Zhou et al., 2007), microcapsules (Wagdare et al., 2011) and liposomes (Jaafar-Maalej et al., 2011).

Membrane processes have recently been considered as being amongst the most promising of strategies to improvise on the existing crystallisation techniques with the main feature of a porous or a dense material acting as a physical semi-permeable barrier between the two phases (Chabanon et al., 2016). Thus, the integrated membrane crystallisation/precipitation process is seemingly possible; (i) to control and limit the maximum level of supersaturations due to the defined mass transfer across the membrane; (ii) to act as heterogeneous nucleation-inducing substrates; (iii) to control solid features such as crystals size distribution (CSD), polymorphic form, shape, and purity; and (iv) to reduce energy consumption compared to cooling or evaporative crystallisation. To achieve these goals, several membrane techniques have been reported: the membrane contactor, membrane templates, reverse osmosis and membrane distillation (Charcosset et al., 2010; Charpentier, 2002). In this study, a micro-engineered membrane dispersion cell was used to produce monodispersed micro-crystals as seeds for crystallisation process. Hence, it is often desirable to design a crystallisation process to avoid excessive fine generation, which help to minimise downstream processing problems, with its uniform and narrow CSD. The product quality and efficacy of the system are often contingent on adequate control of particle size and shape in the crystalliser, which may have a detrimental impact on downstream unit operations such as the filtration rate, drying and formulation operations (Aamir et al., 2010; Barrett et al., 2005; Nagy & Braatz, 2012).
Many researchers have tried to couple computational and experimental results in an effort to unveil the mechanism through which micro-confinement affects nanomaterial characteristics. Thus, computational fluid dynamic (CFD) models appeared as favourable computational tools to investigate how various system parameters (such as device geometry, flow rate ratio, total flow rate and etc.) affect the mixing dynamics with the ultimate goal of correlating device/system operational conditions to nano/micro-material output (Capretto et al., 2011). For example, Capretto et al. (2011) used a CFD model by considering the variation of the diffusion coefficient of the species according to the Stokes–Einstein equation, which pronounces the relation between the viscosity of the medium and the diffusion coefficient of the species; they investigated its effect on the microfluidic mixing process and the polymeric micelles size. To the best of our knowledge, this study proposed the first computational and experimental study dealing with the formation of nanoparticles by nanoprecipitation in a co-flow glass capillary device which indicated a good agreement with experimental results. This numerical simulation has also been applied in order to investigate velocity and shear profiles in the vicinity of the micro-engineered membrane surface associated with an effective inter-diffusion (between aqueous-to-organic phases) mixing area during the formation of nanoparticles. This then successfully revealed a better performance for the stainless steel ringed membrane compared to a whole nickel membrane.

1.2 Problem statements

The major goals in designing nano/micro-particles as a delivery system are to control the particle size, the surface properties and the release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. A few problems or pitfalls have been identified in this study, so that vital concerns should be focused on three major issues as follows: (i) the synthesis of nano/micro-particles by the precipitation method, (ii) micro-engineered dispersion devices applied in the formation of nano/micro-particles, and (iii) the development of a membrane crystallisation system by combining the nanoprecipitation-membrane technique for the formation of micro-crystals as seeds for the crystallisation process.
The original precipitation method suffers from some drawbacks. This technique is mostly suitable for compounds having a hydrophobic nature, such as indomethacin which is soluble in ethanol or acetone, but display very limited solubility in water. Thus, reduced or even zero drug leakage towards the outer medium leads to nanoparticles with entrapment efficiency values reaching 100% (Barichello et al., 1999; Fessi et al., 1989). When nano/micro-particles are produced by a method which involves the solvation of the polymer and the drug and, further, evaporation of the solvent leading to precipitation, the state of the drug and the polymer may vary from crystalline to amorphous. The drug can be present as a solid solution (dissolved) or it may form either a molecular or a crystalline dispersion among the polymeric matrix (Dubernet, 1995). Besides this, the state of the drug can be a combination of different possibilities, such that it can be partly solubilised in the polymer and partly deposited in the matrix as both amorphous and crystalline domains (Hung & Lee, 2007; Valencia et al., 2012). Correspondingly, if the drug remains dissolved in the polymer matrix, the polymer may interact with the drug (Izmailov et al., 1999). As a consequence, the state of the drug and the hosting polymer play an important role in determining the essential properties of the system, including the entrapment and release of the drug. In addition, the interactions of drugs and polymers in nano-particulate systems are not as widely studied as in micro-particle systems (Teychené & Biscans, 2008). Therefore, this study was aimed to formulate a new strategy to enhance the drug entrapment onto NPs polymer matrix by the use of nanofillers.

Microfluidic devices are designed to manipulate fluids in microchannels with a greatly reduced consumption of reagents, and they demonstrate intrinsically efficient heat and mass transfer due to high surface-area-to-volume ratios. Efficient mixing and rapid chemical reaction at the nanoliter to picoliter scales allow microfluidic devices to control the synthesis parameters better and thus the nanoparticle sizes and properties (Hung & Lee, 2007). However, this current method is not always applicable to all classes of nanoparticles, thus not all properties can be characterized, such as drug encapsulation and release, pharmacokinetics or biodistribution. This involves higher costs and complexities in fabrication and operation compared with the well plates, if they are not to be reusable, and if reusable, it would be difficult to keep them
sterile. For large-scale synthesis, it is difficult to build systems at low-cost that are comparable to a batch reactor which is able to prepare grams or kilograms of nanoparticles (Valencia et al., 2012). However, this problem can be solved by the application of a micro-engineered membrane that has been proposed in this study.

Crystallisation from solution is a core technology in pharmaceutical industries. Usually, this process is a part of a wide processing system, including solid–liquid separation, particle design, and formulation. In the pharmaceutical industry, chemical engineers must develop a robust crystallisation process that delivers the active pharmaceutical ingredient (API) with both high yield and the appropriate attributes that are conducive to drug product development (e.g., purity, polymorph and particle size distribution). To reach this goal, it is essential that fundamental data on nucleation kinetics, crystal growth and phase transitions should be determined precisely. A technique extensively applied in production crystallisation to help control the crystals size distribution (CSD) and the number of crystals produced, as well as the polymorphic form, is “seeding”. Seed crystals are added to a crystallizer before nucleation occurs; they provide the surface area for crystal growth and nucleation, hence offering the advantage of being able to control the onset of crystallisation. Unseeded batches tend to exhibit batch-to-batch point of nucleation consistency problems (this is process dependent), which can have a dramatic influence on the size and number of crystals produced. This latter technique enables the generation of crystallisation containers with perfectly controlled operating conditions (Laval et al., 2007; Selimović et al., 2009; Teychené et al., 2011; Zheng et al., 2004).

1.3 Objectives

The overall objective of this research is to develop outstanding methods/techniques for the formation of engineered functional pharmaceutical nano/micro-particles by taking into account the theoretical selection of a “good solvent” applied in the precipitation method. Two different devices (glass capillary microfluidics and the micro-engineered dispersion cell) were used, with the aim of investigating both synthesis/nucleation and the production stage involved during the formation of the
nanoparticles, respectively. Computational fluid dynamic (CFD) simulation was then examined to validate the experimental results attained from each of the micro-engineered dispersion device applications. Further study on the application of combined precipitation method and membrane dispersion cell was carried out by producing micro-crystals that could potentially be used as seeds in the batch crystallisation process with design efficient control strategies. This extension study is believed to be a new insight into the integrated membrane crystallisation system.

Six (6) important aims have been identified that lead to a logical progression through the research:

1. To measure the interaction between solvent-water-polymer for a better understanding on the selection of a “good solvent” and precipitation method.
2. To investigate the efficacy of using the glass capillary microfluidic device for the formation of NPs by evaluating the effects of various process parameters on effective mixing.
3. To examine the possibility of efficiently incorporating nanofillers and active ingredients (drug) into NPs by the microfluidic mixing/nanoprecipitation process.
4. To elaborate the interaction between each of the process parameters involved in drug-NPs encapsulation experiment using a microfluidic device by design of experiment (DOE) simulation approach.
5. To investigate the performance of membrane dispersion cell and the optimum process parameters involved in the formation of biodegradable NPs by nanoprecipitation method.
6. To apply solvent displacement method combined with membrane dispersion cell for the formation of monodispersed size-tuneable micro-crystals.

1.4 Research questions

Questions related to the proposed study are as follows:

1. What is the most suitable organic solvent and biodegradable polymer that can be used for the formation of nanoparticles by nanoprecipitation method?
CHAPTER 1

2. How to apply two different micro-engineered dispersion devices and what are the optimum parameters for the formation of nano/micro-particles?

3. What are the basic challenges in nano/micro-particle synthesis: firstly, to synthesise particles with the desired size/shape; and secondly, to reduce their polydispersity? The size and shape of the particles typically affects their physical and chemical properties.

4. How to extend the application of precipitation-membrane technique from the crystallisation perspective?

5. What is the appropriate numerical study that can be applied in order to verify the experimental observations? What are the significant parameters/model equations needed for this computational analysis?

1.5 Research novelties

The key contributions of this research are listed as follows:

1. Microfluidic devices that are designed to manipulate fluids in microchannels with greatly reduced consumption of reagents and that demonstrate intrinsically efficient mixing and rapid chemical reaction at the nanoliter to picoliter scales.

2. The miniaturization of synthesis systems provides new opportunities for advanced chemical synthesis, and also enables a broad range of biological and medical applications.

3. The encapsulation of nanofillers and drugs onto the NPs polymer matrix potentially appears as a novel finding for the engineered pharmaceutical particles, which results in a large specific surface area, and exhibits good adsorption ability, cation exchange capacity, and drug-carrying capability.

4. The use of micro-engineered membranes enables a better control over diffusive mixing at the liquid/membrane interface which may significantly provide fine control of particle size distribution and make it easier to extrapolate the results for industrial scale production.

5. A new exploration and a good start for the development of an integrated membrane-crystallisation system.
CHAPTER 1

1.6 Thesis structure

This thesis is divided into ten main chapters as discussed briefly below:

Chapter 1 gives a brief background to the work in this thesis as well as the objectives of this research.

Chapter 2 introduces the detailed background of nano/micro-particles formation by precipitation methods which includes a review of polymers, organic solvents, active ingredients (drug) and typical particle synthesis methods/techniques. A brief description of a glass capillary device and a micro-engineered membrane cell for the formation of nano/micro-particles are also presented. Further details on micro-crystals formation produced by the membrane dispersion cell with appropriate control strategies have been summarised in this chapter.

Chapter 3 explains the synthetic polymeric biodegradable nanoparticles produced by micro-mixing combined with nanoprecipitation in a co-flow glass capillary microfluidic device. The solubility criteria of the polymer and the concentration profiles found by numerical modelling indicated the locations at which the nanoparticles would form. The experimental results are in good agreement with the CFD simulation results showing a semi-spherical interface at a low organic phase flow rate and a widening jet at a high organic phase flow rate. The formation of spherically shaped nanoparticles and a narrow particle size distribution were shown significantly using dynamic light scattering, Nanoparticle Tracking Analysis (NTA) and microscopic observation.

Chapter 4 presents the interaction between water-solvent-polymer and measurement which reveals the good and poor solvents for the polymers applied in the nanoprecipitation method. The in situ mixing process which occurred in a glass capillary microfluidic device was monitored using a microscope video system with comprehensive investigation on the effect of operating parameters, system geometry and surfactants on the final particle size distributions.
Chapter 5 evaluates the various formulation variables involved in the preparation of paracetamol (PCM) loaded nanoparticles by nanoprecipitation method using a glass capillary microfluidic device with a two-level factorial design approach. The $2^5$-full factorial design was applied with five (5) different independent input variables included: (A) PCL concentration, (B) co-flow device orifice size, (C) flow rate ratios, (D) surfactant concentration and (E) acetaminophen percentage loading. These were investigated in order to obtain a mathematical model and the prediction of optimised formulations. The percentage encapsulation efficiency ($Y_1$), % drug loading ($Y_2$) and particle mean size, $Z_{ave}$ ($Y_3$) were used as the response variables. The produced PCM-loaded PCL NPs were then characterised according to their morphology, particle size, encapsulation efficiency, drug loading and in vitro drug release behaviour.

Chapter 6 investigates the possibility of efficiently incorporating nanofillers (nanoclay or also known as montmorillonite (MMT)) and paracetamol into polymeric NPs by microfluidic mixing/nanoprecipitation process. The effect of MMT on the physicochemical properties of the prepared NPs, the encapsulation efficiency of paracetamol inside the NPs and their in vitro drug release behaviour were examined.

Chapter 7 elaborates the effect of different aqueous-to-organic phase volumetric ratios, agitation speeds, transmembrane fluxes, membrane type and pore size, polymer and surfactant concentrations on the development and optimisation of novel micromixing/nanoprecipitation methods applied in a micro-engineered membrane dispersion cell for the fabrication of polymeric NPs. The velocity and shear profiles in the vicinity of the membrane surface were evaluated using a commercial CFD software package (ANSYS FLUENT 14.5) in dimensional form, which revealed the better performance of the stainless steel ringed membrane compared to the whole nickel membrane.

Chapter 8 presents the possibility of efficiently encapsulating immuno-suppressive agent of rapamycin (RAPA) onto poly($\varepsilon$-caprolactone) (PCL) NPs matrix using a stainless steel ringed membrane that fitted into the dispersion cell, combined with the solvent displacement method. The effect of RAPA on the physico-chemical properties of synthesised NPs were evaluated using transmission electron microscopy (TEM), x-
ray diffraction (XRD), differential scanning calorimetry (DSC) and attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR). The encapsulation efficiency of RAPA inside the NPs and the \textit{in vitro} drug release behavior were determined.

Chapter 9 explains the application of combined membrane-precipitation method for the formation of piroxicam, PRX monohydrate micro-crystals, than can be further used as seeds for a batch crystallisation process. The effect of PRX concentration, type and concentration of surfactant, different volume ratios and the effect of solvent removal are discussed in this chapter. A detailed comparison is drawn between bulk mixing, semi-batch, semi-batch membrane system and continuous membrane system applied for the formation of micro-crystals. The in-situ control strategy applied to the seeded-batch crystallisation system was evaluated using the PRX micro-crystals produced by the membrane dispersion cell.

Chapter 10 concludes this research as well as providing recommendations for future work.
CHAPTER 2

LITERATURE REVIEW

Chapter overview

Functional pharmaceutical nano/micro-particles have evolved into an exciting area of research due to their potential in drug delivery systems (DDS). To convey a sufficient dose of drug to the lesion, suitable carriers of drugs are needed. Nano/micro-particle carriers have important potential applications for the administration of therapeutic molecules. This chapter provides an overview of engineered functional pharmaceutical particles including polymeric nano/micro-particles, micro-crystals and the addition of nanofillers (nanoclay) into the particulate matrix. Screening results for the types of biodegradable polymers and the active ingredients (drugs) are well described in this part. The methods used for the formation of particles are briefly reviewed as well as the physicochemical parameters associated with nanoparticles (NPs) formation. Two different techniques are also reviewed: (i) the glass capillary microfluidic device and (ii) the micro-engineered membrane dispersion cell application for the formation of nano/micro-particles. Brief reviews of the different analytical instruments applied to characterize the physicochemical properties of nano/micro-particles are described. An overview of pharmaceutical crystallisation fundamentals for the formation of micro-crystals and their applications is given in this chapter. The techniques applied to characterise the crystal properties, including the main approaches to crystallisation operation and its control strategies, are described. The application of integrated membrane crystallisation is briefly discussed in this chapter.

2.1 Introduction

Rapid advancement in reducing colloidal drug carriers and Active Pharmaceutical Ingredients (API) has proved an efficient and reliable method of improving the bioavailability of relatively insoluble drugs that is often limited by poor dissolution rates.
Thus, by reducing the particles down to the micrometer or, in some cases, the nanometer (1/1000 of a micrometer) size, the particle surface area can be significantly improved, which corresponds to Noyes-Whitney equation, whereby the dissolution rate linearly depends on the surface area (Kohane, 2007; Lee, 2003). Functional pharmaceutical particles interacting with cells and the extracellular environment can trigger a sequence of biological effects. These effects largely depend on the dynamic physicochemical characteristics of nano/micro-particles, which determine the biocompatibility and efficacy of the intended outcomes (Barratt, 2000). The distinction is often made between micro- and nano- particles, which are particles with dimensions best described in micrometers and nanometers respectively (Naahidi et al., 2013). The major goals in designing these functional particles as a delivery system are to control the particle size, the surface properties and the release of the pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. The API substance/drug can be dissolved, entrapped, encapsulated or attached to a nano/micro-particle matrix.

The various advantages of nano/micro-particles are summarised as follows (Mohanraj & Chen, 2007; Madhav & Kala, 2011):

i. The ability to maintain unaltered physicochemical characteristics for long periods allowing long-term storage

ii. The possible administration through different routes (oral, intramuscular or subcutaneous) depending on their composition

iii. Effective delivery of agents which are insoluble or sparingly soluble in water

iv. Protect the drug from degradation

v. Produce a prolonged release of the drug

vi. Improve the bioavailability of the drug

vii. Provide the targeted delivery of the drug

viii. Decrease the toxic side effects of the drug

ix. Offer an appropriate form for all routes of administration

x. Allow rapid-formulation development
The development of effective drug delivery systems that can transport and deliver a drug precisely and safely to its site of action is becoming a highly important research area for pharmaceutical researchers. Thus, promising ways of delivering poorly soluble drugs, peptides and proteins have been devised. In addition, attractive drug delivery technologies, such as transdermal patches, nanodevices, bioadhesive systems, implants, micro-fabricated systems, cell encapsulation devices and novel nasal drug delivery systems are currently under intensive study (Labhasetwar, 2005; Parveen et al., 2012; Sahoo & Labhasetwar, 2003; Vasir & Labhasetwar, 2005). The aim of targeted drug delivery and controlled release is to improve the management of drug pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity and the biorecognition of systems in the quest for improved efficacy. However, advanced drug delivery systems are not restricted to the nanoworld (Jain, 2005; Labhasetwar, 2005; Vasir & Labhasetwar, 2005). Fig. 2.1 schematically illustrates the long march of controlled drug delivery systems from the mid-19th century enteric coated pills and other systems in the macroscopic size range, through to microcapsules and micro-particles, and on to the nano-domain (Yih & Al-Fandi, 2006). The late 1960s and early 1970s saw the advent of polymer microparticles based on acrylamide micelle polymerization (Crommelin & Florence, 2013).

The diameter of particles administered into blood vessels, airways or gastro-intestinal tract dictates their velocity, diffusion and adhesion to walls (Goldsmith & Turitto, 1986; Lamprecht, Schäfer, & Lehr, 2001; Patil et al., 2001). The movement of particles in tissues, whether arriving by migration or injection, is also limited by size due to steric hindrance in the extracellular matrix. The pathway of particle migration in the body directly impacts the final destination. When administrated intravenously, nanoparticles (NPs) should be sufficiently small (100–300 nm) to passively cross the tumour’s endothelial barrier and then be retained in the tumour bed for a prolonged time due to reduced lymphatic drainage, which is known as the enhanced permeability and retention effect (Kobayashi et al., 2014). Particles larger than 1 mm are not convenient for the intravascular delivery of drugs, since they can readily be opsonized with a possibility of
capillary occlusion, while NPs smaller than 5 nm can be cleared rapidly from the blood via extravasation or renal clearance (Elsabahy & Wooley, 2012). For pulmonary administration, 3 μm particles deposit deep in the alveolar region while larger particles accumulate in the upper airways and smaller particles are exhaled (Edwards et al., 1997). The particles having a size greater than 10 microns can be administered through nasal administration because they can get lodged in the nasal cavity, whereas particles that are too fine (having a size below 5 microns) should be warded off as they are inhaled directly into the lungs (Kushwaha et al., 2011).

**Fig. 2.1:** A schematic representation of the progress from macro- and micro- delivery systems to the nano-domain over the period from the 19th century to today, showing the indefinite boundary between the two domains, straddled, for example, by liposomes. The dates given represent early discovery and significant events after discovery when there are several dates (LDL = Low-Density Lipoprotein) (Yih & Al-Fandi, 2006).

### 2.2 Biodegradable functional pharmaceutical particles

#### 2.2.2 Polymeric nanoparticles

The polymeric nanoparticles (NPs) are prepared from biocompatible and biodegradable polymers where the drug is dissolved, entrapped, encapsulated or attached to a
nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed (Nagavarma et al., 2012). Fig. 2.2 shows the difference between nanospheres and nanocapsules. Polymer-based nanoparticles effectively carry drugs, proteins, and DNA to target cells and organs. Their nanometer size promotes effective permeation through cell membranes and also stability in the blood stream. Polymers are very convenient materials for the manufacture of countless and varied molecular designs that can be integrated into unique nanoparticle constructs with many potential medical applications (Peer et al., 2007). Abhilash (2010) and Peer et al. (2007) reviewed a few of the advantages of polymeric nanoparticles as follows:

- They increase the stability of any volatile pharmaceutical agents.
- They are easily and cheaply fabricated in large quantities by a multitude of methods.
- They offer a significant improvement over traditional oral and intravenous methods of administration in terms of efficiency and effectiveness.
- They deliver a higher concentration of pharmaceutical agent to a desired location.
- The choice of polymer and the ability to modify the drug release from polymeric nanoparticles have made them ideal candidates for cancer therapy, the delivery of vaccines, contraceptives and the delivery of targeted antibiotics.
- Polymeric nanoparticles can be easily incorporated into other activities related to drug delivery, such as tissue engineering.

![Fig. 2.2: Difference between the nanosphere and the nanocapsule (Courtesy of Nanomedicine (2010) Future Medicine Ltd).](image)
Fig. 2.3 depicts the increasing number of publications based on the polymeric NPs’ unique properties, demonstrating that they are quickly expanding and playing a pivotal role in a wide spectrum of areas ranging from electronics to photonics, conducting materials to sensors, medicine to biotechnology and pollution control to environmental technology during the past few decades (Rao & Geckeler, 2011). Table 2.1 shows the types of synthetic and natural polymers that can be used for the formation of polymeric NPs. The polymers should be compatible with the body in terms of adaptability (non-toxicity) and (non-antigenicity) and should be biodegradable and biocompatible (Rao & Geckeler, 2011).

**Fig. 2.3:** Graphical representation of the number of publications cited in the Scopus® database on polymer nanoparticles during the period 1996–2010 (Rao & Geckeler, 2011).

**Table 2.1:** Type of synthetic and natural polymers commonly used for the formation of NPs (Nagavarma et al., 2012).

<table>
<thead>
<tr>
<th>Synthetic Polymers</th>
<th>Natural Polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polylactides (PLA)</td>
<td>Poly(N-vinyl pyrrolidone)</td>
</tr>
<tr>
<td>Polyglycolides (PGA)</td>
<td>Poly(methyl methacrylate)</td>
</tr>
<tr>
<td>Poly(lactide co-glycolides) (PLGA)</td>
<td>Poly(vinyl alcohol)</td>
</tr>
<tr>
<td>Polyanhydrides</td>
<td>Chitosan</td>
</tr>
<tr>
<td>Polyanhydrides</td>
<td>Gelatin</td>
</tr>
<tr>
<td>Polyoxyesters</td>
<td>Sodium alginate</td>
</tr>
<tr>
<td>Polycyanoacrylates</td>
<td>Albumin</td>
</tr>
<tr>
<td>Polycaprolactone</td>
<td></td>
</tr>
<tr>
<td>Polylactic acid</td>
<td></td>
</tr>
<tr>
<td>Polymallic acid</td>
<td></td>
</tr>
<tr>
<td>Polymalic acid</td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>
2.2.3 Polymeric micro-particles

Drug delivery via biodegradable microparticles (mPs) benefits from both the protection of the encapsulated drug from hazardous conditions and the controlled release of the encapsulated drug, thereby reducing the administration frequency and improving patient compliance. “Microparticle” is defined as a spherical particle with a diameter in the micrometer range (typically from 1 µm to 1000 µm). With respect to the distribution of the active compound, there are two different categories of microparticles: “microspheres” and “microcapsules” (Fig. 2.4). “Microspheres” refers to microparticles composed of a homogeneous mixture of active compound and raw material, while “microcapsules” have a core (where the active compound is placed) which is delimited by a different material (usually the raw material). The core may be solid, liquid, gas and one or more discrete domains of active compound may be found in the microcapsule core (Coelho et al., 2010).

![Fig. 2.4: Different categories of microparticle (Coelho et al., 2010).](image)

Polymeric mPs used as a drug delivery strategy have advantages over other systems that make them particularly suitable for microencapsulation: (i) the controlled release of encapsulated materials, (ii) the protection of the encapsulated materials against degradative reactions (e.g., oxidation, dehydration, UV, heat acids and bases) in the external environment, which can also result in an improved shelf life, (iii) masking the organoleptic properties such as the colour, taste and odour of encapsulated materials, (iv) easy handling of the resulting powder-like materials, and (v) safe handling of toxic encapsulated materials (Sri et al., 2012). Various types of materials, including drugs (Wischke & Schwendeman, 2008), proteins (Morita et al., 2000; Xie & Wang, 2007),
food materials (Patel et al., 2012; Taylor et al., 2009), pesticides (Pérez-Martínez et al., 2001) and herbicides (Lobo et al., 2011), as well as cells (Mironov et al., 2009; Oliveira & Mano, 2011) can be encapsulated. Table 2.2 lists the types of natural and synthetic polymers commonly applied in the formation of microspheres.

Table 2.2: List of polymers used for microsphere formation (Lohani & Chaudhary, 2012; Ramteke et al., 2012; Vilos & Velasquez, 2012).

<table>
<thead>
<tr>
<th>Natural polymer</th>
<th>Synthetic</th>
<th>Biocompatible</th>
<th>Biodegradable</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Proteins</em>: albumin, gelatin, and collagen</td>
<td>Polyvinyl alcohol</td>
<td>Esters of hyaluronic acid</td>
<td>Poly (lactides)</td>
</tr>
<tr>
<td><em>Carbohydrates</em>: agarose, carrageenan, chitosan, starch</td>
<td>Polyamides polycarbonates, Polyalkylene glycols</td>
<td>Polyvinyl acetate</td>
<td>Poly (glycolides)</td>
</tr>
<tr>
<td><em>Chemically modified carbohydrates</em>: poly (acryl) dextran, poly (acryl) starch</td>
<td>Polyvinyl ethers, Polymethacrylic acid</td>
<td>Ethylene glycol</td>
<td>Poly (lactideco-glycolides)</td>
</tr>
<tr>
<td></td>
<td>Polymethyl methacrylic acid</td>
<td></td>
<td>Polycaprolactones</td>
</tr>
<tr>
<td></td>
<td>Methylcellulose</td>
<td></td>
<td>Polyalkyl cyanoacrylates</td>
</tr>
<tr>
<td></td>
<td>Ethylcellulose</td>
<td></td>
<td>Polyorthoesters</td>
</tr>
<tr>
<td></td>
<td>Hydroxypropyl cellulose</td>
<td></td>
<td>Polyphosphoesters</td>
</tr>
<tr>
<td></td>
<td>Hydroxypropyl</td>
<td></td>
<td>Polyhydrides</td>
</tr>
<tr>
<td></td>
<td>Methylcellulose</td>
<td></td>
<td>Polyphosphazenes</td>
</tr>
<tr>
<td></td>
<td>Sodium carboxymethyl cellulose</td>
<td></td>
<td>Chitosan</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Polyethylene oxide</td>
</tr>
</tbody>
</table>

The use of microparticles must initially take into account the chosen administration route. For oral administration, although there is no upper limit on microsphere size for administration, it has been found that decreasing the microsphere size from 7.2 µm to 2.1 µm doubled gastrointestinal adsorption (Gaumet et al., 2009; Lamprecht et al., 2001; Wei et al., 2008a; 2008b). For the pulmonary route, microspheres should be around 3 µm to achieve good results (Mohamed & Van Der Walle, 2008; Rawat et al., 2008). For subcutaneous, intramuscular or intravitreal administration routes, microspheres should be in the range of 10–250 µm in order to avoid particle uptake by macrophage phagocytosis and to minimise any inflammatory reaction (Gasparini et al., 2008; Herrero-Vanrell &
In the case of specific organs such as the brain, the microsphere size should not exceed 100 µm so as not to disturb the 3D structure of the brain (Tatard et al., 2005). Microspheres must not be too large as they will cause discomfort upon administration, but they should be sufficiently large to contain a reasonable amount of active ingredient (Mitragotri & Lahann, 2009; Wang et al., 1997). The global market for microspheres in 2010 was estimated at $2 billion (~£1.39 billion) and it was predicted to reach $3.5 billion (~£2.44 billion) by 2015 as reported by an extensive market research report entitled ‘Microspheres: Technologies and Global Markets’ (Lipovetskaya, 2010). According to the same report, the medical technology and life sciences industries were mentioned as emerging industries (Fig. 2.5).

**Fig. 2.5:** Global market for microparticles, by industry, in 2010 and estimates for 2015 (Lipovetskaya, 2010).

### 2.2.4 Active pharmaceutical ingredients (API) microcrystals

Drug powders containing micron-sized drug particles are used in several pharmaceutical dosage forms. Many drugs, especially newly developed substances, are poorly water soluble, which limits their oral bioavailability (Joshi, 2011). Many approaches have been attempted to reduce particle size, including mechanical micronization (Reis et al., 2014), the supercritical fluid technique (Tenorio et al., 2007) and controlled antisolvent precipitation (Chiou et al., 2007). Micronization is a term used to describe a size reduction technique where the resulting particle size distribution is less than 10 µm. Traditional
techniques for micronization focus on mechanical means, such as milling and grinding. The mechanical micronization methods need a high-energy input and shows some disadvantages in practice, such as electrostatic effects, broad particle size distributions, thermal degradation, agglomeration, reducing the available surface area, contamination and reproducibility problems among different batches (Varshosaz et al., 2013; Zhang et al., 2006). Thus, techniques that prepare the drug directly in the required particle size are of interest. Among them, antisolvent crystallisation has unique advantages because it can be carried out at ambient temperatures and in conventional crystallisation vessels. Antisolvent addition generates high supersaturation which favours rapid and abundant nucleation, and hence produces a large number of smaller crystals as compared with cooling and evaporation (Xie et al., 2010).

In the anti-solvent method, briefly, the drug is firstly dissolved in the solvent and the formed solution is quickly poured into the miscible solvent (anti-solvent). Precipitation happens instantaneously by a rapid desolvation of the drug. Currently, aqueous solutions containing some stabilizers are commonly used as the anti-solvent. Hydroxypropylmethylcellulose (HPMC) stabilizer is offered as a potential stabilizer to stabilize the small drug particles. HPMC, which consists of methoxyl and hydroxypropyl groups, can form hydrogen bonds between the drug molecule and the polymer; the stabilizer in the aqueous solution is absorbed on the surface of the formed hydrophobic drug particles to inhibit crystal growth (Raghavan et al., 2003; 2001). This technique is a rapid and direct process (Rasenack & Müller, 2002; Rasenack et al., 2003a; 2003b), which can be performed with ease. Table 2.3 summarises the type of APIs (drug) used by various researchers for the formation of microcrystals. In this research, the nonsteroidal anti-inflammatory drug, piroxicam (PRX), will be applied for the formation of microcrystals by the anti-solvent method.
**Table 2.3**: List of APIs used for microcrystal formation.

<table>
<thead>
<tr>
<th>Type of API</th>
<th>Type of surfactant</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Becomethasone-17,21-dipropionate (BDP), betamethasone-17-valerate (BV) Prednisolone (PDL)</td>
<td>hydroxypropyl methylcellulose (HPMC)</td>
<td>Raghavan et al. (2001)</td>
</tr>
<tr>
<td>Prednisolone (PDL)</td>
<td>hydroxypropyl methylcellulose (HPMC)</td>
<td>Li et al. (2007)</td>
</tr>
<tr>
<td>Paracetamol (PCM)</td>
<td>hydroxypropyl methylcellulose (HPMC)</td>
<td>Reis et al. (2014)</td>
</tr>
<tr>
<td>Piroxicam (PRX)</td>
<td>hydroxypropyl methylcellulose (HPMC), Brij35</td>
<td>Varshosaz et al. (2013)</td>
</tr>
<tr>
<td>Salbutamol sulfate (SS)</td>
<td>Lechitin, Span 85, Polyvinylpyrrolidone (PVP) and HPMC</td>
<td>Xie et al. (2010)</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>hydroxypropyl methylcellulose (HPMC)</td>
<td>Viçosa et al. (2012)</td>
</tr>
<tr>
<td>Budesonide</td>
<td>hydroxypropyl methylcellulose (HPMC)</td>
<td>Rasenack et al. (2003)</td>
</tr>
<tr>
<td>Siramesine hydrochloride</td>
<td>Sodium lauryl sulphate (SLS), hydroxypropyl methylcellulose (HPMC), and hydroxypropyl cellulose (HPC)</td>
<td>Zimmermann et al. (2009)</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>hydroxypropyl methylcellulose (HPMC)</td>
<td>Li et al. (2007)</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>hydroxypropyl methylcellulose (HPMC)</td>
<td>Zhang et al. (2009)</td>
</tr>
<tr>
<td>Griseofulvin and fenofibrate]</td>
<td>Methyl cellulose (MC), hydroxyethyl cellulose (HEC), HPMC, sodium dodecyl sulphate (SDS)</td>
<td>Meng et al. (2009)</td>
</tr>
</tbody>
</table>

**2.2.5 Addition of nanofillers (MMT) into the particulate matrix**

There is poor solubility of numerous drugs in aqueous media and biological fluids, and thus their effects are lessened due to insufficient bioavailability. To improve their solubility, drug molecules can be incorporated into the interlayer space of layered clays or nanofillers (San Román et al., 2013). Montmorillonite (MMT) is a natural clay mineral that belongs to the smectite group, in which a central alumina octahedral sheet is sandwiched between two silica tetrahedral sheets with the thickness of the individual layers being ≤1 nm. MMT has a large specific surface area, and exhibits a good adsorption
capability, cation exchange capacity, and drug-carrying capability. MMT is thought to be a good delivery carrier of hydrophilic drugs. MMT is a potent detoxifier with excellent adsorbent properties due to its high aspect ratio. It can adsorb excess water from faeces and thus act as an anti-diarrhoeic. MMT can also provide mucoadhesive capability for the nanoparticles to cross the gastrointestinal barrier (Dong & Feng, 2005; Feng et al., 2009). It has also been used as a controlled release system. MMT has been proved to be nontoxic by hematological, biochemical and histopathological analyses in rat models (Lee et al., 2005). MMT is utilised as a sustained release carrier for various therapeutic molecules, such as 5 Fluorouracil (Lin et al., 2002), sertraline (Nunes et al., 2007), vitamin B1 (Joshi et al., 2009a; 2009b) and buspiron hydrochloride (Joshi et al., 2010).

In addition to the therapeutic attributes of some unmodified MMT, their hybrids with ionic active pharmaceutical ingredients (APIs), that may be intercalated in the MMT interlayer space (Li et al., 2004) in a non-crystalline, amorphous state, can provide advantages such as: (i) the increased apparent solubility of APIs with poor aqueous solubility, (ii) controlled API release and, (iii) improved bioavailability. It has been hypothesized (Aguzzi et al., 2007; Dong & Feng, 2005; Li et al., 2004; Suzuki et al., 2001) that the addition of the MMT platelets (see Fig. 2.6) may improve the overall API stability by providing a tortuous path (see Fig. 2.7) that would slow down the API’s diffusion into the body fluids in the presence or absence of a polymeric excipient. The decrease of the API’s mobility inside the polymeric excipient would also help to prevent or slow down the aggregation of the API molecules. Thus, the crystallisation of the API molecules, which may lead to their delayed dissolution, could be minimised and the long term drug stability improved. It should be noted that hydrophilic polymers are, in general, chosen as excipients for the purpose of improving the APIs’ dissolution rate. It follows, then, that hydrophilic pharmaceutical nanoclays, such as montmorillonite and hydrotalcite, would tend to disperse better in hydrophilic polymers due to their improved affinity (Ha & Xanthos, 2011). Fig. 2.8 depicts the clay-drug complexation and in vivo drug release mechanisms.
(a) PLA nanocomposite with 3wt % MMT  (b) PLA nanocomposite with 5 wt % MMT

**Fig. 2.6:** Transmission electron micrograph of the PLA nanocomposite with MMT showing a platelet structure (Duan et al., 2013).

**Fig. 2.7:** Schematic diagram of Tortuous Path Model (Duan et al., 2013).
2.3 Anti-solvent precipitation method applied in nano/micro-particle formation

The properties of biodegradable nano/micro-particles have to be optimized depending on the particular application. Methods like solvent evaporation, salting-out, dialysis and supercritical fluid technology, involving the rapid expansion of a supercritical solution or rapid expansion of a supercritical solution into a liquid solvent, can be utilized for the preparation of functional particles from preformed polymers. These particles can be directly synthesized by the polymerization of monomers using various polymerization techniques such as micro-emulsion, mini-emulsion, surfactant-free emulsion and interfacial polymerization. Different preparation techniques for nano/micro-particles are given in Fig. 2.9, where the choice of preparation method is made on the basis of a number of factors such as the type of polymeric system, the area of application and the size requirement (Rao & Geckeler, 2011).
Fig. 2.9: Schematic representation of various techniques for the preparation of polymeric nano/micro-particles. SCF: supercritical fluid technology, C/LR: controlled/living radical (Rao & Geckeler, 2011).

Table 2.4 presents the most used polymers in functional particle formation according to the common practical techniques. Table 2.5 summarises the descriptions, advantages and disadvantages of different particle preparation techniques. Fig. 2.10 depicts the schematic diagrams of the different techniques for the preparation of nanoparticles and Table 2.6 summarises the screening results for the anti-solvent precipitation techniques applied by many researchers with different chemical formulations and strategies for the preparation of biodegradable polymeric NPs. This screening observation was used as a reference for further experimental investigation.
Table 2.4: List of commonly applied polymers based on particle preparation techniques (Mora-Huertas et al., 2010; Rao & Geckeler, 2011).

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Nanoparticle preparation technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic polymers</td>
<td></td>
</tr>
<tr>
<td>Poly(alkyl cyanoacrylate)</td>
<td></td>
</tr>
<tr>
<td>Poly(alkyl methacrylate)</td>
<td></td>
</tr>
<tr>
<td>Polystyrene</td>
<td></td>
</tr>
<tr>
<td>Poly(vinyl pyridine)</td>
<td></td>
</tr>
<tr>
<td>Poly(ε-caprolactone) (PCL)</td>
<td></td>
</tr>
<tr>
<td>Polylactide (PLA)</td>
<td></td>
</tr>
<tr>
<td>Poly(lactic-co-glycolic acid) (PLGA)</td>
<td></td>
</tr>
<tr>
<td>Poly(methacrylate) (PCL)</td>
<td></td>
</tr>
<tr>
<td>Poly(ε-caprolactone)</td>
<td></td>
</tr>
<tr>
<td>Polylactide (PLA)</td>
<td></td>
</tr>
<tr>
<td>Poly(lactic-co-glycolic acid)</td>
<td></td>
</tr>
<tr>
<td>Poly(β hydroxy butyrate)</td>
<td></td>
</tr>
<tr>
<td>Ethyl cellulose</td>
<td></td>
</tr>
<tr>
<td>Poly(alkyl methacrylate)</td>
<td></td>
</tr>
<tr>
<td>Ethyl cellulose</td>
<td></td>
</tr>
<tr>
<td>Polylactide (PLA)</td>
<td></td>
</tr>
<tr>
<td>Poly(lactic-co-glycolic acid) (PLGA)</td>
<td></td>
</tr>
<tr>
<td>Poly(lactic-co-glycolic acid) (PLGA)</td>
<td></td>
</tr>
<tr>
<td>Polylactide (PLA)</td>
<td></td>
</tr>
<tr>
<td>Natural polymers</td>
<td></td>
</tr>
<tr>
<td>Albumins</td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td></td>
</tr>
<tr>
<td>Gelatine</td>
<td></td>
</tr>
<tr>
<td>Alginate</td>
<td></td>
</tr>
<tr>
<td>Chitosan</td>
<td></td>
</tr>
<tr>
<td>Ethyl cellulose</td>
<td></td>
</tr>
<tr>
<td>Polylactide (PLA)</td>
<td>Supercritical fluids technology</td>
</tr>
<tr>
<td>Polylactide (PLA)</td>
<td>Salting out</td>
</tr>
<tr>
<td>Poly(β hydroxy butyrate)</td>
<td>Solvent evaporation</td>
</tr>
<tr>
<td>Poly(ε-caprolactone)</td>
<td>Nanoprecipitation</td>
</tr>
<tr>
<td>Poly(lactic-co-glycolic acid)</td>
<td>Polymerization</td>
</tr>
</tbody>
</table>

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Table 2.5: Descriptions, advantages and disadvantages of different functional particle preparation techniques (Nagavarma et al., 2012; Reis et al., 2006; Rao & Geckeler, 2011).

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Descriptions</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Solvent Evaporation</td>
<td>Emulsification-solvent evaporation involves two steps. The first step requires emulsification of the polymer solution into an aqueous phase (see Fig. 2.10 (i)). During the second step, the polymer solvent is evaporated, inducing polymer precipitation as nanospheres.</td>
<td>• Can be used for different types of emulsions; oil/water emulsions are of interest because they use water as the nonsolvent; this simplifies and thus improves process economics as it eliminates the need for recycling, facilitating the washing step and minimizing agglomeration.</td>
<td>• This method only can be applied to liposoluble drugs, and limitations are imposed by the scale-up of the high energy requirements in homogenization.</td>
</tr>
<tr>
<td>2. Nanoprecipitation</td>
<td>Nanoprecipitation is also called the solvent displacement method. It involves the precipitation of a preformed polymer from an organic solution and the diffusion of the organic solvent into the aqueous medium in the presence or absence of a surfactant (see Fig. 2.10 (ii)).</td>
<td>• Able to facilitate the formation of colloidal polymer particles during the first step of the procedure. • Allows the preparation of nanocapsules when a small volume of nontoxic oil is incorporated in the organic phase. • Applicable to lipophilic drugs because of the miscibility of the solvent with the aqueous phase.</td>
<td>• Not applicable to encapsulate water-soluble drugs. • Limited to water-miscible solvents, spontaneous emulsification cannot be observed if the coalescence rate of the formed droplets is sufficiently high.</td>
</tr>
<tr>
<td>Emulsification/Solvent Diffusion</td>
<td>This is a modified version of the solvent evaporation method. The encapsulating polymer is dissolved in a partially water soluble solvent such as propylene carbonate and saturated with water to ensure the initial thermodynamic equilibrium of both liquids (see Fig. 2.10 (iii)).</td>
<td>• High encapsulation efficiencies (generally &gt;70%), no need for homogenization, high batch-to-batch reproducibility, ease of scale-up, simplicity, and narrow size distribution. • One is efficient in encapsulating lipophilic drugs.</td>
<td>• The high volumes of water to be eliminated from the suspension and the leakage of water-soluble drug into the saturated-aqueous external phase during emulsification, reduce encapsulation efficiency.</td>
</tr>
<tr>
<td>Salting-out</td>
<td>Salting out is based on the separation of a water miscible solvent from aqueous solution via a salting out effect. Polymer and drug are initially</td>
<td>• It minimises the stress to protein encapsulants.</td>
<td>• The greatest disadvantages are exclusive application to lipophilic</td>
</tr>
</tbody>
</table>
dissolved in a solvent such as acetone, which is subsequently emulsified into an aqueous gel containing the salting-out agent (electrolytes, such as magnesium chloride, calcium chloride, and magnesium acetate, or non-electrolytes such as sucrose) and a colloidal stabilizer such as polyvinylpyrrolidone or hydroxyethylcellulose (see Fig. 2.10 (iv)).

### Dialysis

Dialysis offers a simple and effective method for the preparation of small, narrow-distributed PNs. The polymer is dissolved in an organic solvent and placed inside a dialysis tube with a proper molecular weight cut off. Dialysis is performed against a non-solvent miscible with the former miscible. The displacement of the solvent inside the membrane is followed by the progressive aggregation of polymer due to a loss of solubility and the formation of homogeneous suspensions of nanoparticles (see Fig. 2.10 (v)).

- **Salting out does not require an increase of temperature and, therefore, may be useful when heat sensitive substances have to be processed.**
- **The solvent used in the preparation of the polymer solution affects the morphology and particle size distribution of the nanoparticles.**
- **Allows the passive transport of solvents to slow down the mixing of the polymer solution with a non-solvent**
- **The mechanism of PNP formation by the dialysis method is not fully understood at present.**

### Supercritical Fluid Technology (SCF)

- **Supercritical fluid and dense gas technology are expected to offer an interesting and effective technique of particle production, avoiding most of the drawbacks of the traditional methods (see Fig. 2.10 (vi)).**
- **Two principles have been developed for the production of nanoparticles using supercritical fluids:**
  1. Rapid expansion of supercritical solution (RESS)
  2. Rapid expansion of supercritical solution into liquid solvent (RESSOLV).

A simple, but significant modification to RESS involves expansion of the supercritical solution into a liquid solvent instead of ambient air, termed as RESOLV.

- **Even though in the RESS technique no organic solvents are used for the formation of PNP s, the prime products obtained using this technique are microscaled rather than nanoscaled, which is the main drawback of RESS. In order to overcome this drawback, a new supercritical fluid technology known as RESOLV has been developed.**

---

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Fig. 2.10: Techniques of preparation for polymeric nanoparticles (Nagavarma et al., 2012; Reis et al., 2006).
Table 2.6: Screening for the anti-solvent precipitation method formulations.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Solvent</th>
<th>Non-Solvent</th>
<th>Stabilizing agent</th>
<th>Particle size (nm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA</td>
<td>Acetone</td>
<td>H₂O</td>
<td>PVA</td>
<td>95–560</td>
<td>Yallapu et al. (2010)</td>
</tr>
<tr>
<td>PBCA</td>
<td>Acetone</td>
<td>H₂O</td>
<td>Pluronic F 68 Polysorbate 80 Dextran</td>
<td>269±4 210±5 238±5</td>
<td>Yordanov et al. (2010)</td>
</tr>
<tr>
<td>Allylic starch</td>
<td>Acetone</td>
<td>H₂O</td>
<td>-</td>
<td>270</td>
<td>Tan et al. (2009)</td>
</tr>
<tr>
<td>PHB</td>
<td>Acetone</td>
<td>H₂O</td>
<td>Tween 80</td>
<td>100–125</td>
<td>Deepak et al. (2009)</td>
</tr>
<tr>
<td>Dextran ester</td>
<td>Acetone</td>
<td>H₂O</td>
<td>-</td>
<td>77</td>
<td>Hornig et al. (2009)</td>
</tr>
<tr>
<td>PLGA</td>
<td>Acetone/ethanol</td>
<td>H₂O</td>
<td>Tween 20</td>
<td>63–90</td>
<td>Chang et al. (2009)</td>
</tr>
<tr>
<td>PCL diol</td>
<td>Chloroform</td>
<td>H₂O</td>
<td>Pluronic F 127</td>
<td>17.4</td>
<td>Kim et al. (2009)</td>
</tr>
<tr>
<td>Eudragit L100-55</td>
<td>Acetone/absolute ethanol</td>
<td>H₂O</td>
<td>-</td>
<td>120</td>
<td>Nassar et al. (2009)</td>
</tr>
<tr>
<td>PLGA</td>
<td>Acetonitrile</td>
<td>H₂O</td>
<td>-</td>
<td>165±5 164±4</td>
<td>Nehilla et al. (2008)</td>
</tr>
<tr>
<td>PCL</td>
<td>Acetone</td>
<td>H₂O</td>
<td>PVA</td>
<td>365±5</td>
<td>Moinard-Chécot (2008)</td>
</tr>
<tr>
<td>PCA</td>
<td>Ethanol/water</td>
<td>H₂O</td>
<td>-</td>
<td>150</td>
<td>Stella et al. (2007)</td>
</tr>
<tr>
<td>PLA</td>
<td>THF</td>
<td>H₂O</td>
<td>-</td>
<td>100–300 741–924</td>
<td>Legrand et al. (2007)</td>
</tr>
<tr>
<td>PCL</td>
<td>Acetone</td>
<td>H₂O</td>
<td>Span 20</td>
<td>741–924</td>
<td>Limayem et al. (2006)</td>
</tr>
<tr>
<td>PCL</td>
<td>Acetone</td>
<td>H₂O</td>
<td>Polysorbate 80</td>
<td>266±11</td>
<td>Zili et al. (2005)</td>
</tr>
<tr>
<td>PLA</td>
<td>Acetone</td>
<td>H₂O</td>
<td>Poloxamer 188</td>
<td>250±50</td>
<td>Seyler et al. (1999)</td>
</tr>
<tr>
<td>PCL</td>
<td>Acetone</td>
<td>H₂O</td>
<td>PE/F68</td>
<td>308–352</td>
<td>Ferranti et al. (1999)</td>
</tr>
</tbody>
</table>

* Note: H₂O = water

2.4 Theoretical consideration for the choice of solvent and polymer

The selection of two solvents suitable for anti-solvent precipitation is based on the requirements of the method and the physicochemical characteristics of the polymer. The method dictates that the organic solvents must be able to dissolve the polymer, be miscible with water and have a low boiling point to facilitate their elimination by evaporation (Legrand et al., 2007). The main physicochemical parameters which may influence this ternary system, consisting of polymer, organic solvent and non-solvent
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(water) solvent and polymer selections, is the effects of the polymer–solvent interactions that can be defined by the polarity of the solvent. In general, by increasing the polarity of the polymer solvents and by decreasing the concentration of the polymer in the solvent, the yield of particle production can be increased and the size of the particles reduced (Galindo-rodriquez et al., 2004; Legrand et al., 2007; Murakami et al., 1999). This method can also be elucidated in terms of the interfacial turbulence and “diffusion-stranding” processes between the two unequilibrated liquid phases (Bagley et al., 1971; Bordes et al., 2010; Legrand et al., 2007; Van Krevelen & Hofyzer, 1976). The mechanism of particle formation can be described based on the water-solvent, water-polymer and solvent-polymer interactions (Galindo-rodriquez et al., 2004; Legrand et al., 2007). More detailed information related to this study will be given in Chapters 3 and 4 with a number of sequential equations and significant correlations. The following table (see Table 2.7) is a list of research references, which elucidate comprehensively the theoretical observations.

Table 2.7: References for physicochemical interaction studies.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Organic solvent</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(n, l-lactides) (PLA)</td>
<td>Acetone (Ace) and tetrahydrofuran (THF)</td>
<td>Legrand et al. (2007)</td>
</tr>
<tr>
<td>Monomethoxy poly(ethylene glycol)-poly(ε-caprolactone) block copolymer</td>
<td>THF, acetonitrile, Ace</td>
<td>Lee et al. (2009)</td>
</tr>
<tr>
<td>E L100-55</td>
<td>Ethanol, di-methyl sulfoxide, isopropyl alcohol, acetone, ethyl lactate</td>
<td>Galindo-rodriquez et al. (2004)</td>
</tr>
<tr>
<td>Poly(ε-caprolactone) (PCL)</td>
<td>Various of soluble, partially soluble, and non-soluble solvents</td>
<td>Bordes et al. (2010)</td>
</tr>
<tr>
<td>PCL</td>
<td>Acetone</td>
<td>Gomez et al. (2015)</td>
</tr>
<tr>
<td>PCL, PLA</td>
<td>THF</td>
<td>Othman et al. (2015a)</td>
</tr>
<tr>
<td>PCL, PLA</td>
<td>Ace, THF, ethanol, dimethyl sulfoxide, isopropyl alcohol, ethyl lactate</td>
<td>Othman et al. (2015b)</td>
</tr>
</tbody>
</table>

2.5 Micro-engineered dispersion devices

2.5.1 Glass capillary microfluidic device

Glass capillary microfluidic devices were recently developed to control the fine properties at the nanoscale. The use of microfluidic devices for functional pharmaceutical particle synthesis is significantly advantageous in many aspects,
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including (i) enhanced processing accuracy and efficiency, (ii) flexibility for multi-step platform design (iii) rapid turnaround results for fine tuning properties of synthesized nanoparticles, (iv) cost savings from reduced consumption of source materials and reagents, and (v) safer operation and environmental friendliness since the process consumes much reduced hazardous chemicals and reagents (Hung & Lee, 2007). To reach higher quality of chemical, physical, optical, and biological properties for various applications, researchers have resorted to miniaturization of reaction platforms (DeWitt, 1999). Nano/micro-particles are predominantly important to biomedical science since their critical size dimensions are close to cells, tissues, microorganisms, and biological molecules (Köhler & Henkel, 2005; Ratner & Bryant, 2004; Shchukin & Sukhorukov, 2004). Nanoparticles also have applications in advanced drug delivery systems, biomolecular sensing, targeted imaging, and thin film coatings. In nanoscale, the chemical, physical and biological properties are strongly affected by size dimensions and shape morphologies (Green & O’Brien, 1999; Song et al., 2006). Table 2.8 summarises the different type of microfluidic devices based on their geometries and structures. Fig. 2.1 depicts the schematic diagram for different type of microfluidic devices.

**Table 2.8**: Type of microfluidic/microchannel devices (Vladisavljević et al. 2012).

<table>
<thead>
<tr>
<th>Planar microfluidic devices</th>
<th>Microchannel array devices</th>
<th>Edge-based droplet generation</th>
<th>Three-dimensional axisymmetric microfluidic devices</th>
</tr>
</thead>
<tbody>
<tr>
<td>–T junction</td>
<td>–Grooved-type microchannel</td>
<td>–Edge-based droplet generation</td>
<td>–PDMS and acrylic axisymmetric devices</td>
</tr>
<tr>
<td>–Cross junction</td>
<td></td>
<td></td>
<td>–Glass capillary axisymmetric devices</td>
</tr>
<tr>
<td>–Y junction</td>
<td>–Straight-through microchannel</td>
<td>–Edge-based droplet generation (edge)</td>
<td>–Co-flow glass capillary device</td>
</tr>
<tr>
<td>–Microfluidic flow focusing devices (MFFD)</td>
<td>–Microchannel arrays</td>
<td></td>
<td>• Counter-current flow glass capillary device</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Three-phase glass capillary device</td>
</tr>
</tbody>
</table>

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Planar microfluidic devices: (a) droplet formation at a T junction, (b) droplet break up at a T junction, (c) merging of droplets with a target sample stream at a T, (d) mixing of two liquid streams within a droplet using two converging side channels, (e) mixing of three liquid streams within a droplet using three converging side channels, (f) droplet formation at a cross junction, (g) formation of droplets of alternating composition at a cross junction, (h) formation of droplets at a Y junction, (i) formation of two distinct fluid streams using a Y junction.

Planar microfluidic devices (Microfluidic flow focusing devices (MFFD)): (a) constriction placed downstream of three coaxial inlet streams. Liquid B wets channel walls, (b) modified design with three coaxial streams for generation of core/shell droplets. Liquid C wets channel walls, (c) two consecutive flow focusing droplet generators (FFDGs). FFDGs 1 and 2 are wetted by liquid B and C, respectively, (d) two parallel coupled FFDGs.

Microchannel array devices (Grooved-type MC plates): (a) dead-end plate, (b) cross-flow plate.

Microchannel array devices (Straight-through MC plates): (a) symmetric plate with microslots on both sides, (b) asymmetric plate with circular channels on upstream side and slots on downstream, (c) symmetric plate with micronozzles (MNs).

Fig. 2.11: Schematic diagrams of different type of microfluidic devices (Vladisavljević et al., 2012).
Three-dimensional axisymmetric microfluidic devices (Axisymmetric flow focusing device (AFFD) fabricated in PDMS): (a) fabrication process; (b) operation of AFFD oriented vertically to avoid accumulation of droplets on the wall of the outlet channel.

(vi) Three-dimensional axisymmetric microfluidic devices (Axisymmetric flow focusing device (AFFD) fabricated in PDMS): (a) co-flow of two immiscible fluids, (b) countercurrent flow of two immiscible fluids with flow focusing, (c) combination of co-flow and countercurrent flow of three immiscible fluids, (d) two sequential co-flow droplet generators, (e) injection of two distinct inner phases of double emulsions using a two-bore injection tube.

Fig. 2.11-cont’ Schematic diagrams of different type of microfluidic devices (Vladisavljević et al., 2012).

2.5.2 Micro-engineered membrane dispersion cell

Membrane dispersion technique has been previously applied for the preparation of emulsions (“membrane emulsification”) (Charcosset & Fessi, 2009; Charcosset et al. 2004). It has also been reported for the preparation of polymeric nanospheres and...
nanocapsules (Charcosset et al. 2005; Limayem Blouza et al., 2006), lipid nanoparticles (D’oria et al., 2009; Li et al., 2011), and liposomes (Jaafar-Maalej et al., 2011; Laouini et al., 2011). This technique has been employed in bi-phase emulsification (Joscelyne & Trägårdh, 2000; Nazir et al., 2010). By pushing the to-be-dispersed phase through a membrane into the cross-flowing continuous phase, an emulsion/particle is formed. The particle/droplet size can be controlled primarily by the choice of membrane, cross-flow velocity, and transmembrane pressure. Numerous advantages of this technique was highlighted such as low shear stresses, low energy requirement, uniform droplet size, less surfactant needed, ease of design and scale-up (Jia & Liu, 2013). Literature reviews about membrane dispersion are available (Joscelyne & Trägårdh, 2000; Vladisavljević & Williams, 2005) with an agreement to the several factors that influencing the final droplet/particle size, as follows; (i) shear on the membrane surface, (ii) dispersed phase flux, (iii) surfactant concentration, (iv) membrane wettability, (v) membrane pore size (droplet size is directly proportional to the membrane pore radius and the proportionality constant equals 3–20 (Vladisavljević & Williams, 2005; Williams et al., 1998), and (vi) viscosities of both continuous and dispersed phase.

Different values of shear stress significantly attributed to the higher drag force acting on the membrane surface area, which then help to detach droplets/particles from the membrane surface and allow better control over the droplet/particle size distribution. The surface shear can be generated by; (i) recirculating the continuous phase in cross-flow (Fig. 2.12 (a)) (Joscelyne & Trägårdh, 2000; Nakashima et al., 2000), (ii) vibrating (Kelder et al., 2007) or rotating the membrane (Fig. 2.12 (b) and (e)) (Aryanti et al., 2009; Schadler & Windhab, 2006; Vladisavljević & Williams, 2006), (iii) vibrating an element (e.g. a wire or plate) in the continuous phase at a short distance from the membrane (Fig. 2.12 (c)) (Hatate et al., 1997) or (iv) stirring the continuous phase using a stirring bar (Fig. 2.12 (f) and (g)) (Higashi & Setoguchi, 2000) or (v) a paddle stirrer (Fig. 2.12 (d)) (Kosvintsev et al., 2005; Stillwell et al., 2007), (vi) oscillating the membrane (Fig. 2.12 (h)) (Holdich et al., 2010) or (vii) combining the cross-flow with pulsation of the continuous phase (Fig. 2.12 (i)) (Holdich et al., 2013). Table 2.9 summarises the advantages and disadvantages of different techniques applied to create surface shear on the membrane surface area.
**Table 2.9:** A comparison of different techniques for shear stress generation.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Potential advantages</th>
<th>Potential disadvantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-flow, Tortuous membranes</td>
<td>Easy scale-up, constant shear stress at the membrane surface, modules widely available</td>
<td>Droplets can be damaged during recirculation in pipes and pumps, long operation times for concentrated emulsions</td>
<td>Joscelyne &amp; Trägårdh (2000), Nakashima et al. (2000)</td>
</tr>
<tr>
<td>Cross-flow + Membrane vibration</td>
<td>Additional control over droplet detachment, decrease in mean droplet size as compared with a simple cross-flow</td>
<td>Complicated design, no evidence that droplet size monodispersity is improved</td>
<td>Kelder et al. (2007)</td>
</tr>
<tr>
<td>Vibration in Continuous phase</td>
<td>Simple set-up</td>
<td>Poor control of shear stress, suitable only for small scale applications</td>
<td>Hatate et al., (1997)</td>
</tr>
<tr>
<td>Rotating microengineered membrane</td>
<td>Suitable for creation of fragile particles and viscous emulsions</td>
<td>Complicated and expensive design, high power consumption</td>
<td>Aryanti et al. (2009), Schadler &amp; Windhab (2006), Vladisavljević &amp; Williams (2006)</td>
</tr>
<tr>
<td>Stirring, tubular SPG membrane</td>
<td>Volume of continuous phase liquid can be as</td>
<td>Maximum transmembrane</td>
<td>Higashi &amp; Setoguchi (2000)</td>
</tr>
</tbody>
</table>

**Fig. 2.12:** Formation of surface shear in membrane dispersion cell.
In this study, the production of size-tuneable polymeric nanoparticles and microcrystals by anti-solvent precipitation method using membrane dispersion cell was discovered. Further discussion and experimental results attained from this study will be explained comprehensively in Chapter 5 and 6.

2.6 Analytical instruments

2.6.1 Particle size analyser

2.6.1.1 Delsa™ Nano HC

The Delsa™ Nano HC Particle Analyser (Beck-man Coulter, High Wycombe, UK) is the primary instrument used for both particle sizing and surface charge determination. Particle sizing is accomplished using dynamic light scattering (DLS), measuring the rate of fluctuations in a laser that is passed through the sample. As light is passed through the sample, the photons are scattered in all directions via Rayleigh scattering. The particles then diffused in the solution and the scattered photons will started to interfere constructively and destructively the particles surrounding. The intensity of this scattering undergoes a time-dependent fluctuation due to the motion of the...
particles in the sample. Fluctuations at a higher frequency are due to smaller particles while lower frequency fluctuations are due to larger particles, as illustrated in Fig. 2.13.

![Dynamic Light Scattering Diagram](image)

**Fig. 2.13:** Hypothetical dynamic light scattering of two samples: Larger particles on the top and smaller particles on the bottom (Submicron, 2011).

### 2.6.1.2 Nanoparticle tracking analysis (NTA)

Nanoparticle tracking analysis (NTA) is newly developed and was first commercialised in 2006. NTA is an optimal method for detection of spherical particles in a liquid based on laser-illuminated optical microscopy. The primary advantage of this method is its ability to detect, visualise in real-time, and directly analyse particles as they move in the liquid. All particles in liquid move by Brownian motion, and this principle is also applicable for NTA. As the particles moved by Brownian motion, a two-dimensional video is taken. This video is analysed by the NTA software on a frame-by-frame basis. The average speed and distance of movement of the particles is established and this information is used to calculate the diameter of each particle and thus a size distribution and concentration will also be evident. Since each particle is measured both simultaneously and individually, it is possible to measure both the particle size and the relative light scattering intensity, which provides a more precise result of liquid containing mixtures of different particle sizes, such as biological fluids.
Positioned on top of the microscope is an electron multiplying charged-couple device (EMCCD) camera. The EMCCD camera has a frame rate of 30 frames per second and typically this video is 60 seconds in duration. The NanoSight LM10 is equipped with 20 × magnification microscope objective just above the sample chamber, which is highlighted in Fig 2.14 (a). The sample chamber is equipped with an inlet where the sample is injected with a sterile syringe, and an outlet where the sample can be ejected through. The sample chamber is ~500 μm deep and can contain ~0.25 mL of sample. As Fig. 2.14 (b) illustrates, a laser beam passes through the sample chamber. The NanoSight LM10 system uses a finely focused 642 nm laser beam, which is introduced into the sample chamber through a glass prism-edged optical flat. The refractive index of the glass prism is as such that the laser beam is refracted just above the glass flat and into the liquid above it. This low angle refraction results in a thin laser beam that illuminates particles through the sample. The EMCCD camera collects the light scattered from each particles as it refracts the light in the field of view (see Fig. 2.14 (c)) (Carr et al., 2009; Dragovic et al., 2011).

**Fig. 2.14:** (a) Illustration of the NanoSight LM10-HS device with the (b) sample chamber highlighted to the right. (c) The computer shows the NTA 3.0 software as it is analysing a sample.
2.6.1.3 Malvern Mastersizer

The micron size of particles generated from membrane dispersion cell is measured by using Malvern Mastersizer, UK. It is based on light scattering: the particles passed through a laser beam and scatter light at an angle related to their size. Mastersizer 2000 software analyses the data to determine the size and size distribution of particles based on measuring the angular variability and intensity of the laser beam scattered by a series of photosensitive detectors. When laser beams pass through a dispersed particulate sample, the scattering of light depends on particles size in the sample; larger particles gives smaller angles of scattering while smaller particles give larger angles of scattering (see Fig. 2.15). The laser diffraction instrument in this study employed — Fraunhofer and Mie theory — to analyse the size of particles. This theory relies on the refractive indexes of dispersed particles and the dispersion medium (manufacturer personal communication). Deionized water and saturated monohydrate piroxicam solution were used as dispersion medium and their refractive index was ~1.34. The Malvern Mastersizer enables to measure particles in the size range of 0.1 to 10000 μm. The disadvantage of the Mastersizer is that it is an offline technique that needs sampling (Malvern Instrument Ltd., 2007).

Fig. 2.15: Schematic illustration of light scattering from small and large particles (Malvern Instrument Ltd., 2007).
2.6.2 Physicochemical properties analyser

2.6.2.1 Differential scanning calorimetry (DSC)

The TA Instruments Q100 Differential Scanning Calorimeter (DSC) is a powerful and versatile thermal analyzer that allows for property measurements on a broad variety of materials from -150 to 600°C. The DSC determines the temperature and heat flow associated with material transitions as a function of time and temperature. It also provides quantitative and qualitative data on endothermic (heat absorption) and exothermic (heat evolution) processes of materials during physical transitions that are caused by phase changes, melting, glass transitions, crystallisation, oxidation, and other heat related changes. It is used to characterize melting, crystallisation, resin curing, loss of solvents, and other processes involving an energy change. Differential scanning calorimetry may also be applied to the processes involving a change in heat capacity, such as the glass transition. In DSC analysis, the sample is placed in an aluminium pan, and the sample pan and an empty reference pan are placed on small platforms within the DSC chamber (TA Instruments Q100, 2007). Thermocouple sensors lie below the pans. DSC thermogram can be used to reveal four critical points; (i) the glass transition temperature ($T_g$), (ii) the crystallisation temperature ($T_c$), (iii) the melting temperature ($T_m$), and (iv) the curing temperature, as depicted in Fig. 2.16.

![DSC curve diagram](image)

**Fig. 2.16:** An idealized DSC curve showing the shapes associated with particular phase transitions (TA Instruments Q100, 2007).
2.6.2.2 Thermal gravimetric analysis (TGA)

Thermogravimetric analysis (TGA) or thermogravimetry is an analytical technique in which the changes of a substance mass are measured as a function of temperature whist the substance is subjected to temperature variation in a controlled atmosphere. The formal definition of thermogravimetry (TG is the preferred abbreviation, although TGA is also used) has been given by the Nomenclature Committee of the International Confederation for Thermal Analysis and Calorimetry (ICTAC) as “a technique in which the mass of a substance is measured as a function of temperature whilst the substance is subjected to a controlled temperature programme”. It is the most widely used experimental technique for determining the thermal change of solids. A high-precision scale is used to measure the mass loss of a very small sample.

In thermal decomposition, the mass of reactants disappears and forms gaseous products and possibly a residue of char. The record is the TG curve; the mass is normally plotted on the ordinate, decreasing down toward the origin, and temperature (T) or time (t) is on the abscissa, increasing from left to right according to the basic rules for plotting any kind of graph. The purpose is to determine the kinetic parameters of thermal decomposition of the material (TA Instruments, 2008). In this study, the thermal stability of the nanoparticles was assessed by TGA (Q5000IR Thermogravimetric Analyzer) at a heating rate of 10 °C min⁻¹ over the temperature range of 20–600 °C under dry nitrogen as the effluent gas. A typical TGA curve is shown in Fig. 2.17.

![Fig. 2.17: An example of typical TGA curve.](image-url)
2.6.2.3 X-ray diffractometry (XRD)

X-ray powder diffraction (XRD) is an alternative analysis method that derives its name from the fact the specimen is in the form of a crystalline powder. An X-ray diffractometer consist in an X-ray cathode tube, a sample holder, and a detector. X-rays are generated by the cathode ray tube, filtered to produce monochromatic radiation, collimated to be concentrated, and then directed towards the sample. X-ray diffraction is based on constructive interference of monochromatic light and the crystalline sample. Operates based on Bragg’s law: \( n \lambda = 2d \sin \theta \), where \( n \) is the order of the diffraction pattern; \( \lambda \) is the wavelength of the incident beam; \( d \) is the distance between the planes in the crystal; and \( \theta \) is the angle of beam diffraction. When a monochromatic X-ray beam strikes a plane of atoms at an angle \( \theta \), the rays are diffracted at the same angle \( \theta \) (see Fig. 2.18). These angles are corresponding to spaces between planes of molecules in the crystal lattice. Since no two compounds would form crystals in which their three-dimensional spacing of planes is the same in all directions, the technique is considered as the most conclusive by itself alone. If a single crystal is used, the technique is able to clearly differentiate between polymorphs, but high quality crystals of adequate size are hard to obtain. It allows observation of phase transitions such as polymorphic transformation and dehydration.

**Fig. 2.18:** X-Ray diffracted by a crystalline lattice following Bragg’s law.

2.6.2.4 ATR-FTIR spectroscopy

Attenuated Total Reflectance (ATR) is today the most widely used FTIR sampling tool. ATR generally allows qualitative or quantitative analysis of samples with little
or no sample preparation, which greatly speeds sample analysis. The main benefit of ATR sampling comes from the very thin sampling path length and depth of penetration of the IR beam into the sample (see Fig. 2.19). This is in contrast to traditional FTIR sampling by transmission where the sample must be diluted with IR transparent salt, pressed into a pellet or pressed to a thin film, prior to analysis to prevent totally absorbing bands in the infrared spectrum. The basic operation of the ATR-FTIR is similar to ATR-UV/Vis but it uses a radiation beam in the infrared region. The IR regions include the near-IR 12800–4000 cm\(^{-1}\), mid-IR 4000–200 cm\(^{-1}\) and far-IR 200–10 cm\(^{-1}\). Since most compounds absorb radiation in the mid-IR range, its applicability to different systems is widespread. It operates by imposing the radiation beam on a sample and measuring the amount of IR light absorbed at different frequencies. The IR energy corresponds to the energy related to bond vibrations e.g. bond stretching, bending etc. The frequencies at which IR energy is absorbed is a characteristic of a molecule, this fact can be used to identify a compound and hence the term fingerprint is used. IR absorptivities occur in CH, OH and NH groups, either of these exist in any organic molecule.

![Fig. 2.19: ATR measurement geometry (Lewiner et al., 2001).](image)

2.6.2.5 Raman spectroscopy

Raman spectroscopy is a characterization technique that is widely used in scientific field in recent years. This technique is also known as Raman radiation and was first observed by Raman & Krishnan in 1928 (Vankeirsbilck et al., 2002). It actually utilizes the unique Raman spectra for different components as a spectral “finger print”
to quantitatively determine polymorphic forms’ concentration in slurries or dry solid mixtures depends on the possibility of building a good calibration function using a correct experimental approach. This spectroscopic technique is, precisely, based on inelastic scattering of monochromatic light, usually from a laser source. The approximate spectral range of Raman spectroscopy is between 50−4000 cm$^{-1}$. When monochromatic light is incident on a material, the majority of the light or photons undergo Rayleigh scattering (elastic scattering) with no change in the energy or frequency of photons. A very small quantity of photons (0.0001%) however, undergoes Stokes (inelastic scattering) and anti-Stokes scattering. When the energy of the photon is absorbed by the molecule, it is termed as Stokes scattering resulting in red-shifted scattered light. An electron in the ground state is excited and lifted to a higher vibrational energy level. In case of anti-Stokes scattering, molecules give energy to the photon, resulting in blue shifted light, with a shorter wavelength and higher energy. An electron in the ground state is lifted to a higher level through a virtual state and ends up at a low vibration energy level. The possible energy transitions for vibrational spectroscopy are shown in Fig. 2.20.

![Energy transitions in Raman and IR spectroscopy](image)

**Fig. 2.20:** Schematic representation of energy transition in Raman and IR spectroscopy. Absorption of IR radiation, Rayleigh, Stokes and Anti-Stokes Raman scattering.

### 2.6.2.6 Focused beam reflectance measurement (FBRM)

FBRM is an extensively used *in situ* technique that gives information about nucleation, dissolution, metastable zone width, polymorphic transformation, crystals
growth and size distribution in particulate systems in real time (Bakar et al. 2009). The laser beam from FBRM is sent through fibre optics to an immersion probe tip where it is finely focused by a rotating lens, which causes the beam to scan in a circular path through a sapphire window at a fixed high speed. The beam then passes into the solution under study and when it hits a crystal suspended in the solution, light is scattered in many directions, but only light scattered back towards the probe is collected. The crystal continues to back-scatter the light until the beam reaches the opposite edge of the crystal (see Fig. 2.21 (a)). The variable measured is the chord length, which is related to the particle size and shape. The focused beam crosses the particles on a straight line between any two points on the edge of the particle as shown in Fig. 2.21 (b). Based on the rotating speed of the laser ($v_S$ and back scattering time ($\Delta t$), the chord length distribution (CLD) for the particles is obtained using equation; chord length = $v_S \Delta t$. The CLD obtained from FBRM is grouped in 90 channels from 0.8–1000 μm. The readings obtained from FBRM can be displayed in a variety of formats from simple total number of counts per second to square weighted or cubic weighted distributions.

**Fig. 2.21:** (a) Schematic of the mode of operation of an FBRM probe (b) chord length measurement of typical crystals.
2.6.2.7 Ultraviolet–visible spectroscopy (UV-VIS)

Ultraviolet and visible spectrometers have been in general applied for the last 35 years and over this period have become the most important analytical instrument in the modern day laboratory, due to its simplicity, versatility, speed, accuracy and cost-effectiveness. In this study, Lambda 35 UV/Vis spectrometer (Perkin Elmer, UK) is used with wavenumbers ranged 190–1100 nm and bandwidth between 0.5–4 nm (variable). The light generated from a Xenon flash lamp and is passed through the monochromator which splits the beam into different wavelengths out of the continuous spectrum, which can be explained by Beer-Lambert’s Law. The intensity ‘Io’ measured by the fraction of beam redirected using beam splitter. The transmitted intensity ‘I’ of the light beam is measured at photodetector and the absorbance is calculated by the following formula.

\[ A = A = \log \left( \frac{I_o}{I} \right) = \epsilon cl. \]  

(2.1)

where is A the absorbance, Io is the incident light intensity, I is intensity of light leaving, c is the molar concentration of the solute, l is the path length (cm) and \( \epsilon \) is the molar absorptivity. Based on this law absorbance is linearly dependent on concentration, provided that molar absorptivity and path length remain constant. However, absorbance also depends on the temperature. The presence of more than one absorbing species, interaction between solute and solvent can also cause deviations from this law.

2.6.2.8 High performance liquid chromatography (HPLC)

High performance liquid chromatography (HPLC) is a common technique to analyse liquid samples. It allows separation of analyte molecules is based on their differential partitioning between two non-miscible phases, i.e. the stationary phase and the mobile phase. Separation is governed by various interactions between the analyte and the stationary and mobile phases. These include: dispersion interactions (contributing to hydrophobic interactions); dipole-dipole interactions; hydrogen bonding; ionic (coulomb) interactions; and charged transfer or π–π interactions (Snyder et al. 2009). The stationary phase is either a solid, porous or surface-active material in small-particle form or, more commonly, a viscous liquid immobilised on these particles and
is fixed in the system. The mobile phase is a liquid which carries the mixture to be separated. Liquid chromatographic techniques that utilise elevated pressure to force the mobile phase through the small-particle packed bed are collectively termed high-performance liquid chromatography (HPLC) (Meyer, 2010). A simple schematic of an HPLC system is shown in Fig. 2.22.

![Fig. 2.22: Schematic of an HPLC system.](image)

A UV detector is placed at the end of the column for the quantification of each component. A selected UV wavelength is directed and absorbed by each component going out of the column. The non-absorbed light is then processed in order to quantify the amount of each component of the sample analysed using the Beer and Lambert law. The relative retention times are used to qualitatively identify each component of the sample.

### 2.6.3 Microscopic image analyser

#### 2.6.3.1 Field emission gun scanning electron microscopy (FEG-SEM)

A field emission gun scanning electron microscopy (FEG-SEM) is a type of electron microscope that produces images of a sample by scanning it with a focused beam of high energy electrons (see Fig. 2.23). The high-energy electrons interact with atoms in the sample, producing various signals which contain information about the sample’s surface topography, orientation and composition. FEG-SEM equipment mainly detects secondary electrons that result from interactions of the electron beam with atoms at or near the surface of the sample. In the standard secondary electron imaging mode, the FEG-SEM can produce very high-resolution images of a sample surface, revealing details less than 1 nm in size. Due to the very narrow electron beam, FEG-
SEM surface topographic images have a large depth of field yielding a characteristic three dimensional appearance useful for understanding the surface structure of a sample.

In this study, the microscopic structure of functional particles is analysed by Leo Elektronenmikroskopie GmbH model 1530 VP with an EDAX Pegasus (EBSD/EDXA) unit. Samples were mounted onto conventional aluminium sample holders with a surface of approximately 1 cm in diameter. The chamber was evacuated to \( \sim 5 \times 10^{-1} \) Pa, for imaging, Secondary Electron (SE) and Inlens detectors were used with accelerating voltages of 5–10 keV and a working distance of 5–10 mm, depending on type of sample. For general topographic images, SE imaging gave sufficient resolution. For higher resolution images, the Inlens detector was used.

**Fig. 2.23:** A schematic diagram of FEG-SEM machine.

### 2.6.3.2 Scanning electron microscopy (SEM)

The Hitachi TM3030 benchtop Scanning Electron Microscope (SEM) is an advanced scanning electron microscope; it uses electron technology to deliver excellent microscopic images for the samples. It is a small, lightweight machine, controlled via
software installed on a computer. The sample is placed in a vacuum chamber, and then a beam of electrons is applied to the sample. The Hitachi microscope offers many features and techniques to obtain much better images than those taken by conventional devices. This machine is also capable of providing compositional elements analysis of the sample via Energy Dispersive X-ray spectroscopy (EDX), which is essential for deeper analysis of the sample, as an X-ray sensor detects the emitted x-ray from the sample to produce the spectrum diagram of the elements. The main features of this tabletop microscope are; (i) magnification: $15 \times - 30.000 \times$ (digital zoom $\times 2$ or $\times 4$), (ii) resolution: 30 nm, (iii) maximum sample size is 70 mm in diameter, (iv) maximum sample height is 50 mm, and (v) signal detection is based on high-sensitivity semiconductor 4-segment backscattered electron (BSE) detector. Fig. 2.24 shows a schematic diagram of SEM analysis.

![Schematic diagram of SEM analysis](image)

**Fig. 2.24:** A schematic diagram of scanning electron microscope.

2.6.3.3 Transmission electron microscopy (TEM)

In transmission electron microscope (TEM) a very thin specimen (less than 100 nm thick) is irradiated by a beam of high-energy electrons (between few tens of kV up to 1 MeV). A schematic of a traditional TEM configuration is presented in Fig. 2.25. The TEM allows examination of specimens with an order of magnitude higher resolution than an SEM; the resolution of a non-aberration corrected TEM with field emission source is $\sim 1.2$ Å, whereas contemporary aberration corrected systems have been shown improve resolution down to 0.5 Å. Crystallographic information can be obtained in TEM using either selected area or convergent beam diffraction modes;
elemental characterization can be obtained by utilizing either energy dispersive spectroscopy (EDS) or electron energy loss spectroscopy (EELS). An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor such as a charge-coupled device (CCD) camera. X-ray emission consequent to the interaction of the primary electron beam with the sample, can also be detected by an energy-dispersive spectrometer (EDX) within the TEM. At smaller magnifications TEM image contrast is due to absorption of electrons in the material, due to the thickness and composition of the material. In this study, transmission electron microscope model JEOL, JEM-2000 FX operated at an accelerating voltage of 200 kV was used to characterize the morphology structure of functional particles.

Fig. 2.25: A typical setup of a TEM instrument.

2.7 Introduction to crystallisation process

Crystallisation from solution is a widely applied unit operation in both the pharmaceutical and bulk chemical industries for solid-liquid separations (Mangin et al., 2006; Olesberg et al., 2000; Shi et al., 2005). Researchers generally spend
considerable time and effort for the development of batch crystallisation processes for the production of crystalline compounds with consistent crystal properties, i.e. purity, shape, size, habit, morphology, and size distribution. The shape of the crystal size distribution (CSD) of the product obtained from the crystallisation process strongly affects the efficiency of downstream operations such as filtration, drying and washing (Chung et al., 1999; Mullin, 2001; Wibowo & Ng, 2001), but may also have considerable impact on the bioavailability of the active pharmaceutical ingredient (API). Most of the product properties (e.g. dissolution rate, bulk density, flow-ability, packing properties, etc.) are also directly related to the crystal size distribution (Chung et al., 1999).

2.7.1 Crystallisation mechanisms

The phase relationship of the solute-solvent system can be illustrated by a phase diagram; a typical example is shown in Fig. 2.26. In the figure, a solution whose composition lies on the solubility curve is said to be saturated, whereas those that lie in the regions below and above the curve are termed undersaturated and supersaturated, respectively. The terms indicate the relative amount of dissolved solid as compared to the saturated solution. The supersaturated solution is unstable because the dissolved solid and solvent are not in equilibrium. Like all other nonequilibrium systems, the supersaturated solution tends to reach equilibrium and in doing so it removes the solids in the form of nuclei, which then grow into crystals. The generation of supersaturation is therefore regarded as the first step in the crystallisation process (Davey & Garside, 2000; Mullin, 2001).

![Fig. 2.26: A typical phase diagram of a solute in a solvent (R. Davey & Garside, 2000; Mullin, 2001).]
2.7.2 Supersaturation

Theoretically, knowledge about the solubility curve in a given solvent (or solvent system) is a crucial step in performing a successful crystallisation process. It is worth noting that for a given solute in a given solvent, the solubility curve is fixed thermodynamically; however, impurities can have an impact on the solubility, and as impurities’ profiles and levels can change during process development or as process feedstock change from plant-to-plant, it is quite possible that the solubility could change. Hence, it is worth assessing the solubility of your material in the mother liquor from which it is crystallised (Barrett et al., 2005). Supersaturation of the solution may able to generate using five different methods, as listed and briefly described in Table 2.10. There are also combinational methods to generate supersaturation such as combined cooling–evaporation method, known as vacuum crystallisation and combined cooling anti-solvent addition method (Nagy et al., 2008). Generally, the choice of method for generation of supersaturation depends on the required product properties, as well as the economic aspects (Davey & Garside, 2000; Mullin, 2001).

Table 2.10: Methods of supersaturation generation (Davey & Garside, 2000; Mullin, 2001).

<table>
<thead>
<tr>
<th>Methods</th>
<th>Descriptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaporation</td>
<td>Removal of solvent from a solution by evaporation increases the concentration of solute in the solution, which results in supersaturation.</td>
</tr>
<tr>
<td>Cooling</td>
<td>If the solubility of a solid increases with increasing temperature, a saturated solution can be prepared at a higher temperature. The solution is then cooled and since the solubility decreases during cooling, the solution becomes supersaturated.</td>
</tr>
<tr>
<td>Anti-solvent addition</td>
<td>Addition of a second solvent that reduces the solubility of the solute in the resultant mixture.</td>
</tr>
<tr>
<td>Chemical reaction</td>
<td>When a new compound is formed by a chemical reaction, the concentration of this compound is higher than its solubility in a solution. Therefore, the solution becomes supersaturated with respect to the new compound. Variation of pH to produce a less soluble acid or base from a salt, or vice versa, can also generate supersaturation.</td>
</tr>
<tr>
<td>Freezing</td>
<td>Freezing of solvent from a solution increases the concentration of solute in the solution, which results in supersaturation. The frozen solvent can then be removed from a solution by sublimation under vacuum.</td>
</tr>
</tbody>
</table>
2.7.3 Nucleation

The formation of nuclei is an attempt of the system to reach equilibrium. It occurs spontaneously when the supersaturation was increased. The process of forming nuclei is called nucleation, whereas the point at which the nucleation occurs spontaneously is called the metastable limit. The metastable limit depends on kinetic variables, such as the rate at which supersaturation was created, the agitator speed, and the presence of impurities (Kim & Mersmann, 2001; Titiz-Sargut & Ulrich, 2002). The zones in the supersaturation region in which spontaneous nucleation would or would not occur are termed labile and metastable, respectively. Fig. 2.27 shows a possible location of labile zone, metastable zone, metastable limit and metastable width on a concentration against temperature diagram. Vital concerns should be focused on the metastable zone width (MSZW) in crystallisation because it provides information on nucleation kinetics, so that the nucleation behaviour of a system can be understood. The MSZW also can be considered as a characteristic property for each crystallising system (R. Davey & Garside, 2000; Mullin, 2001). In practice it is normally expressed in term of temperature rather than concentration for simplicity.

![Fig. 2.27: A typical phase diagram showing labile zone, metastable zone, metastable limit and MSZW (Kim & Mersmann, 2001; Titiz-Sargut & Ulrich, 2002).](image)

Nucleation is usually classified into either primary or secondary nucleation and can be further divided as shown in Fig. 2.28. It takes place spontaneously from a clear pure solution is called a homogeneous nucleation, whereas one induced by foreign
particles or surfaces is called a heterogeneous nucleation (Rawlings et al., 1993). Homogeneous nucleation, however, rarely occurs in practice because solutions usually contain impurities that act as substrates for nucleation (Giulietti et al., 2001). Since the presence of impurities in a supersaturated solution is known to reduce the energy required for nucleation, nucleation in a heterogeneous system generally takes place at a lower supersaturation than in a homogeneous system (Davey & Garside, 2000).

**Fig. 2.28:** Overview of the different types of nucleation (Davey & Garside, 2000).

Secondary nucleation occurs when a supersaturated solution is in contact with seed crystals of the solute. The seeds can either be deliberately added, or unintentionally present in the system. The latter crystalline resulted of initial breeding (the dislodgement of microcrystalline dust particles from the surface of the seed crystals), needle/dendrite breeding (the detachment of weak outgrowths from crystal surfaces), polycrystalline breeding (the fragmentation of a weak polycrystalline mass) or collision breeding, which is also known as contact nucleation (the collision of crystals with one another, with agitator blades, or with crystalliser’s wall) (Lounaci et al., 2010). There are other sources of secondary nucleation, where the seed crystals catalyse the nucleation and as a result, nucleation takes place at a lower supersaturation than that for the primary nucleation. For this reason, the secondary nucleation can be controlled more easily (Giulietti et al., 2001).

### 2.7.4 Crystallisation operation and control overview

Ideally, the mechanisms that govern crystallisation processes (nucleation, growth, polymorphic transformation) as well as on the modeling and control of crystallisation
systems is a very crucial things to be considered by most of the researchers. This significant knowledge might be enabled by the development and broader applications of process analytical technology (PAT) tools and increase in computing power. The key developments have occurred in four broad categories: (i) modelling, (ii) monitoring, (iii) control, and (iv) novel crystallisation concepts. In the modelling area major developments occurred in the use of multi-dimensional population balance models for morphological modelling of crystallisation processes, as well as in the better understanding of crystallisation in impure media (Nagy et al., 2013). Table 2.11 summarises the three main crystallisation approaches.

Table 2.11: Generic breakdown of crystallisation control approaches (Nagy et al., 2013).

<table>
<thead>
<tr>
<th>Modelling</th>
<th>Monitoring</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>–Population balance modelling (PBM) of crystallisation systems:</td>
<td>–Process analytical technology (PAT):</td>
<td>–Model-based control approaches:</td>
</tr>
<tr>
<td>• Modelling the dynamics of 2D and 3D particle sizes.</td>
<td>• It is defined by the U.S. Food and Drug Administration (FDA) as “a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e. during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality”.</td>
<td>• Model-free control.</td>
</tr>
<tr>
<td>• Modelling the many breakage and agglomeration phenomena resulting from multi-particle interactions prior to control crystals shape and CSD in real dispersed media and in particular, during crystallisation processes occurring under stirring.</td>
<td>• Imaging sensors.</td>
<td>• Simple linear cooling (constant antisolvent addition).</td>
</tr>
<tr>
<td>• Modelling the crystallisation and the separation of optically active molecules, notably for pharmaceutical R&amp;D purposes.</td>
<td>• Image analysis strategies.</td>
<td>• Supersaturation (concentration feedback) control (SSC/CFC).</td>
</tr>
<tr>
<td>• Modelling the inhibiting or promoting effects of impurities or additives on nucleation and crystal growth.</td>
<td></td>
<td>• Direct nucleation control (DNC).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Combined DNC and SSC approaches (simultaneous and sequential).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–Model-based control:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Programmed cooling/antisolvent.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Open-loop optimal control (nominal and robust).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Model predictive control (nominal and robust).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–Hybrid techniques:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Analytical CSD estimator based control of supersaturation controlled processes.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Application of SSC for intelligent experiments for parameter estimation.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Model-based optimization of optimal trajectories in the phase diagram.</td>
</tr>
</tbody>
</table>
2.8.4.1 Crystal nucleation in a particles based micro-channel crystalliser

In the field of pharmaceutical industry, chemical engineers must develop a robust crystallisation process that delivers the active pharmaceutical ingredient (API) with both high yield and appropriate attributes that are conducive to drug product development (e.g., purity, polymorph and particle size distribution). To reach this goal, it is essential that fundamental data on nucleation kinetics, crystal growth and phase transitions should be determined precisely. Recent advances in the state-of-the-art technology of micro-channel have led to the development of a glass made microfluidic chip to study nucleation and phase transition of organic materials confined in droplets of organic solvents. In fact, at the micrometric scale the two-phases flow is only controlled by capillary and viscous forces. Thus, monodispersed droplets, used as crystallisation containers, can be generated without a surfactant. This point can be important when studying nucleation and crystal growth rates, as surface-active molecules can have a significant effect due to molecular similarity (Davey et al., 1997) and can favour the appearance of an undesired polymorph or can alter the thermodynamic equilibrium (Yano et al., 2000). Table 2.12 summarises the examples of crystal nucleation based microfluidic devices for the formation of particles.

Table 2.12: Examples of crystal nucleation based microfluidic devices.

<table>
<thead>
<tr>
<th>Type of microfluidic devices</th>
<th>Crystal nucleation approached applied in microfluidic crystalliser</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microfluidic chip</td>
<td>Crystal nucleation in a droplet</td>
<td>Teychené &amp; Biscans (2012)</td>
</tr>
<tr>
<td>Microfluidic chip</td>
<td>An easy-to-use microfluidic with accurate method for metastable zone width measurement.</td>
<td>Ildefonso et al. (2012)</td>
</tr>
<tr>
<td>Microphotographs crystallisation in microfluidic devices with different channel heights</td>
<td>The channel height dependent nucleation and crystal growth were able to demonstrate, whereby the deep channels favorite the nucleation while shallow ones favorite the crystal growth.</td>
<td>Lounaci et al. (2010)</td>
</tr>
<tr>
<td>T-junction micromixer</td>
<td>Nanoparticles were synthesized as reactive crystallisation and anti-solvent recrystallisation examples, respectively, of using the microfluidic-based emulsion and mixing approach as a new avenue of</td>
<td>Su et al. (2007)</td>
</tr>
</tbody>
</table>
continuously producing inorganic and organic nanoparticles. A good control of humidity can be achieved and protein crystals could be conserved over a long period without dehydration.

Lounaci et al. (2007)

2.8 Application of PAT in crystallisation process monitoring

The application of process analytical technology (PAT) became extensively popular in 2004 when the Food and Drug Administration published the “Guidance for Industry”. PAT is defined as tools that enable process understanding for scientific, risk-managed pharmaceutical development, manufacture, and product quality (Guidance for Industry, 2004). These tools offer significantly valuable information to facilitate process understanding, continuous improvement, and development of risk-mitigation strategies. Spectroscopic techniques improved tremendously during the last decade. Ultraviolet (UV), visible, near-, mid- infrared, and Raman spectroscopy have been studied and implemented in manufacturing processes (Chew & Sharratt, 2010; Nagy et al., 2013). Many PAT tools have been used to monitor and control crystallisation processes (Giulietti et al., 2001; Helmdach et al., 2014), in particular, focused beam reflectance measurement (FBRM) (Yang & Nagy, 2014), particle vision and measurement (PVM), nuclear magnetic resonance (NMR), attenuated total reflectance (ATR)-UV/Vis, ATR-FTIR, and Raman spectroscopy.

Table 2.13 gives a brief summary of the most common PAT tools used in crystallisation processes of polymorphic compounds and in solid polymorphs screening. Raman, FTIR, and NIR are the most common techniques exploited to distinguish polymorphs, although FBRM, ATR-UV, and mid-IR can provide very useful information (Simone et al., 2014). Fig. 2.29 illustrates the first implementation of the crystallisation process informatics system (CryPRINS) concept with a composite sensor array (CSA) that consists of a focused beam reflectance measurement (FBRM), a particle vision and measurement (PVM), an ATR-UV/Vis and a Raman probe, developed at Loughborough University, UK. CSA or composite PAT array is used for simultaneously monitoring multiple process and quality properties at the same time, rather than one at a time by considering the combination of signals from various PAT measurements as a single bundle of complex information.
(Nagy & Braatz, 2012). The complementary and redundancy in the acquired information provided by the CSA allow the implementation of robust crystallisation control strategies.

**Table 2.13:** Summary of recent studies conducted using PAT tools for polymorph screening and transformation monitoring.

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Process studied</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raman</td>
<td>Determination of the polymorphic ratio of solid mixtures</td>
<td>Strachan et al. (2007)</td>
</tr>
<tr>
<td>FT-Raman, FTIR</td>
<td>Comparison between the two techniques in determining the polymorphic ratio of solid mixtures</td>
<td>Hennigan &amp; Ryder (2013)</td>
</tr>
<tr>
<td>Raman</td>
<td>Co-crystallisation of carbamazepine and nicotinamide monitoring</td>
<td>Rodríguez-Hornedo et al. (2006)</td>
</tr>
<tr>
<td>Raman</td>
<td>Solvent-mediated polymorphic transformation monitoring</td>
<td>Herman et al. (2012)</td>
</tr>
<tr>
<td>Raman, FBRM,</td>
<td>Study on the impact of operating parameters on polymorphic transformation of D-mannitol</td>
<td>Su et al. (2010)</td>
</tr>
<tr>
<td>PVM</td>
<td></td>
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<tr>
<td>Raman</td>
<td>Measurement of the total solid concentration in a polymorphic system</td>
<td>Caillet et al. (2008)</td>
</tr>
<tr>
<td>Raman</td>
<td>Measurement of solute concentration in a polymorphic system</td>
<td>Cornel et al. (2008)</td>
</tr>
<tr>
<td>FBRM</td>
<td>Solvent-mediated polymorphic transformation monitoring</td>
<td>Barthe et al. (2008)</td>
</tr>
<tr>
<td>Raman</td>
<td>Feedback strategies to control polymorphs of carvedilol</td>
<td>Pataki et al. (2013)</td>
</tr>
</tbody>
</table>

**Fig. 2.29:** An overview picture of a crystallisation system with a composite sensor array (CSA) and crystallisation process informatics systems (CryPRINS) (Nagy et al., 2013).
2.9 Summary of overall research study

Fig. 2.30 summarises all important techniques and methodologies applied in this study for the formation of biodegradable nanoparticles and microcrystals using different micro-engineered dispersion devices (i.e. glass capillary microfluidic device and membrane dispersion cell) by anti-solvent precipitation method.

Fig. 2.30: Summary of overall experimental works applied in this study.
CHAPTER 3

PRODUCTION OF POLYMERIC NANOPARTICLES BY MICROMIXING IN A CO-FLOW GLASS CAPILLARY DEVICE

This chapter was published in Chemical Engineering Journal

Chapter overview

Synthetic polymeric biodegradable nanoparticles were produced by micromixing combined with nanoprecipitation in a co-flow glass capillary device consisted of coaxial assembly of glass capillaries, fabricated by aligning a tapered-end round capillary inside a square capillary with 1 mm internal dimension. Micromixing of water and an organic phase (1 wt% polylactide or polycaprolactone dissolved in tetrahydrofuran) was modelled using a commercial software package Comsol Multiphysics™ and experimentally investigated using dynamic light scattering, Nanoparticle Tracking Analysis (NTA) and in situ microscopic observation. The organic phase was injected through a nozzle with a diameter of 60 µm at the organic-to-aqueous flow-rate ratios ranging from 1.5 to 10. The locations at which the nanoparticles would form were determined by using the solubility criteria of the polymer and the concentration profiles found by numerical modelling. The convective flux of the polymer in the radial direction was 2-3 orders of magnitude higher than the diffusive flux of the polymer; hence responsible for mixing the streams. The convective flux near the orifice was 3-4 orders of magnitudes higher than at the end of the computational domain. A maximum convective flux of 0.115 kg m⁻² s⁻¹ was found for polycaprolactone at the cloud point for the lowest flow rate ratio investigated. The numerical results were consistent with the experimental observations in terms of flow patterns and mean particle size. Narrower particle size distributions and smaller mean particle sizes were obtained at the higher organic-to-aqueous flow-rate ratios.

3.1 Introduction

Advances in microfluidic technology in recent years have provided alternative process strategies in diverse fields, such as materials science, chemical synthesis, biomedical
CHAPTER 3

diagnostics and drug screening (Stone et al., 2004; Mark et al., 2010; Song et al., 2006; Teh et al., 2008). Compared to conventional macro-scale reaction vessels, test tubes and microtiter plates, microfluidic technology offers many advantages: (i) possibility to use expensive or toxic chemicals due to picolitre fluid volumes; (ii) homogeneous reaction environments due to precise spatial control over process conditions; (iii) ability to continuously and systematically vary reaction conditions; (iv) fast reactions due to high heat and mass transfer rates as a result of high surface-to-volume ratios; and (v) ability to achieve high levels of parallelisation, integration, and automation of unit operations (DeMello, 2006; deMello & deMello, 2004; Karnik et al., 2008).

Although microfluidic techniques are experimentally well-established, optimisation of geometry and operating parameters in microfluidic devices is still challenging (Stone et al., 2004; Pan et al., 2008; Tonomura et al., 2004). Computational fluid dynamics (CFD) has appeared as an effective tool in providing visualized information on flow phenomena in complex geometries at both macroscopic and microscopic level (Ahuja & Patwardhan, 2008; Yamaguchi et al., 2004). Yamaguchi et al. (2004) applied CFD to simulate laminar co-flow in a microchannel with hairpin bends and the simulation results were consistent with experimental data. Bally et al. (2012) performed experiments and CFD simulations to investigate production of methacrylic nanoparticles in a multi-laminating micromixer under different operating conditions. Gradl et al. (2006) combined direct numerical simulation with Lagrangian particle tracking to simulate nanoprecipitation in a T-mixer and predict the size distribution of the produced nanoparticles. However, CFD simulations of flow phenomena arising from interaction between miscible liquid streams in microfluidic channels are still lacking. These simulations can provide valuable insight for optimising particle synthesis in two-phase microfluidic and millifluidic devices.

Synthesis of nanoparticles by bulk mixing (conventional method) typically leads to the lack of control over the mixing process, which may compromise the properties of the resulting nanoparticles (Pihl et al., 2005; Balda & Pohorecki, 1995). Bulk mixing is accomplished in two stages, macromixing (mixing at the scale of the whole system driven by convection and turbulent dispersion) and micromixing (mixing at molecular scale governed by Fick’s law) (Chan & Fuerstnau, 1967). A feature of
microfluidic mixers is that the macromixing stage, which is less controllable, can be avoided and mixing can be accomplished solely by molecular diffusion. Microfluidic mixing processes can be divided into active and passive strategies. Active mixing is based on providing an external source of energy to enhance mixing such as electric field or ultrasound, whereas passive mixing is any technique that requires no additional energy input, other than energy existing in the fluid flow. This study deals with passive microfluidic mixing that takes advantage of small lateral dimensions of microfluidic channels, which dramatically increase the effect of diffusion (Capretto et al., 2011; Locascio, 2004; Zhang et al., 2008).

Nanoprecipitation triggered by passive microfluidic mixing has been used to synthesise various nano-sized products, such as liposomes (Jahn et al., 2013; Jahn et al., 2008; Jahn et al., 2004; Jahn et al., 2010), solid lipid nanoparticles (Yun et al., 2009; Belliveau et al., 2012), micelles (Capretto et al., 2013), chitosan nanoparticles (Majedi et al., 2012), nanocrystals (Dev et al., 2013; Génot et al., 2010), and drug nanoparticles (Zhao et al., 2007; Ali et al., 2009; Dev et al., 2012). Nanoprecipitation requires two miscible solvents, but both the excipient and active ingredient (e.g. a drug) must be soluble in only one of them. The process is associated with a rapid self-assembly of macromolecules into nanoparticles occurring when a macromolecular excipient solution is added to a non-solvent phase, resulting in almost immediate drug entrapment within the nanoparticles (Fessi & Puisieux, 1989; Fessi et al., 1992). It is a single-step technique that allows production of nanoparticles from a wide range of preformed polymers (Bilati et al., 2005; Nagavarma et al., 2012).

Microfluidic devices that have been used in nanoprecipitation processes are flow-focusing devices and microfluidic Y- and T-junctions. Hydrodynamic flow focusing was used to synthesise PLGA-PEG nanoparticles by rapidly mixing polymer-acetonitrile solution and water (Karnik et al., 2008). Two lateral water streams were combined with a central organic phase stream and a narrow width of the focused organic stream enabled rapid mixing through diffusion. Y-junction has been used to produce nano-sized drug particles, thereby enhancing bioavailability of poorly water-soluble drugs (Zhao et al., 2007; Ali et al., 2009). The drug was dissolved in ethanol and then precipitated by mixing the organic phase with a non-solvent (water), which
resulted in amorphous spherical particles with a mean size of 500 nm (Zhao et al., 2007). T-junction has been used to prepare barium sulphate nanocrystals over a size range of 18–30 nm and boehmite nanocrystals (Ying et al., 2008).

To the best of our knowledge, this work is the first computational and experimental study dealing with the formation of polylactide (PLA) and polycaprolactone (PCL) nanoparticles by nanoprecipitation in a co-flow glass capillary device (Duncanson et al., 2012; Duncanson et al., 2012; Shah et al., 2008). Glass capillary devices have been mainly used for preparation of emulsions, emulsion-templated microparticles, and vesicles such as polymersomes, colloidosomes and liposomes (Duncanson et al., 2012; Duncanson et al., 2012; Shah et al., 2008; Vladisavljević et al., 2014; Vladisavljević et al., 2012). Recently, glass capillary devices have been used for fabrication of liposomes with a mean vesicle size in the range of 73–131 nm (Vladisavljević et al., 2014). Compared to planar flow focusing polydimethylsiloxane (PDMS) microfluidic devices more often used in nanoprecipitation, glass capillary devices offer several advantages: (i) fabrication is cheaper and does not require a master mould; (ii) many solvents commonly used in nanoprecipitation, such as tetrahydrofuran and chloroform, swell PDMS to a large extent, whereas glass has excellent chemical resistance against organic solvents; (iii) 3D geometry positions the organic phase at the centre of the collection channel in all directions.

When a 3D orifice is used (Fig. 3.1 (b)), the organic phase stream is completely surrounded by the aqueous phase and the walls of the collection channel are completely wetted by the aqueous phase. Since the particles are predominantly formed at liquid-liquid interface, which is fully displaced from the channel walls, 3D geometry minimises interaction between the particles and the walls. In a planar geometry (Fig. 3.1 (a)), the organic phase at the junction is focused in the substrate plane, but not in the vertical axis, which can lead to deposition of the particles on the walls of the collection channel and compromise control over the resultant particle size.
3.2 Solubility parameters

According to the “diffusion-stranding” mechanism, nanoprecipitation is caused by a rapid diffusion of the organic solvent to the aqueous phase, which is accompanied by the diffusion of the polymer in the same direction (Miller, 1988; Davies & Ridcal, 1963). The polymer becomes stranded at the organic/aqueous phase interface, due to its low solubility in the aqueous phase. The solubility of polymers in pure solvents and solvent mixtures can be predicted from the Hansen solubility parameters of components involved in the process.

Hansen divided the Hildebrand solubility parameter, $\delta$, into three components arising from different types of cohesive forces: a dispersion force component, $\delta_d$, arising from van der Waals forces, a polar component, $\delta_p$, reflecting permanent dipole-permanent dipole forces, and a hydrogen bonding component, $\delta_h$, arising from hydrogen bonding (Hansen, 2007). Hansen solubility parameters can be considered as coordinates in a 3D “solubility (Hansen) space” in which all liquid or solid substances may be localised. The more a solvent is close to the polymer in the “Hansen space”, the more likely the solvent will be a good solvent for the polymer (Hansen, 2007; Bordes et al., 2016).
Bagley et al. (Bagley et al., 1971) introduced the parameter $\delta_v = (\delta_p^2+\delta_d^2)^{1/2}$, which led to a 2D graph in which $\delta_h$ was plotted against $\delta_v$ (Van Krevelen & Hoftyzer, 1976). It was found that good solvents must be included in the circle of a radius of five $\delta$-units around the polymer (Sung & Lee, 2001; Choi et al., 2002; Van Krevelen & Hoftyzer, 1976; Hansen, 2007). Therefore, PLA or PCL are soluble in water-THF mixture if the following condition is satisfied:

$$5 > \sqrt{(\delta_{v,M} - \delta_{v,P})^2 + (\delta_{h,M} - \delta_{h,P})^2}$$

(3.1)

where indices $M$ and $P$ denote the solubility parameters of the water-THF mixture and polymer, respectively. The partial solubility parameters of the polymers (PLA and PCL), good solvent (THF) and pure solvent (water) used in this work are listed in Table 3.1.

The solubility parameters of THF-water mixture can be determined by averaging the solubility parameter values of the individual liquids by volume (Suh & Clarke, 1967):

$$\delta_{v,M} = \Phi_W \delta_{v,W} + \Phi_{THF} \delta_{v,THF}$$

(3.2)

$$\delta_{p,M} = \Phi_W \delta_{p,W} + \Phi_{THF} \delta_{p,THF}$$

(3.3)

where $\Phi_W$ and $\Phi_{THF}$ are the volume fractions of water and THF in the solvent mixture.

| Solubility parameters (J cm$^{-3}$)$^{1/2}$ | Water $^a$ | THF $^a$ | PLA $^b$ | PCL $^c$
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>$\delta_d$</td>
<td>12.28</td>
<td>16.77</td>
<td>17.62</td>
<td>17.00</td>
</tr>
<tr>
<td>$\delta_p$</td>
<td>31.30</td>
<td>5.71</td>
<td>9.70</td>
<td>4.80</td>
</tr>
<tr>
<td>$\delta_h$</td>
<td>34.17</td>
<td>7.96</td>
<td>11.77</td>
<td>8.30</td>
</tr>
<tr>
<td>$\delta_v$</td>
<td>33.62</td>
<td>17.72</td>
<td>20.11</td>
<td>17.66</td>
</tr>
</tbody>
</table>

$^a$ The partial solubility parameters of the solvents taken from Jain (2000).

$^b$ The partial solubility parameters of PLA calculated based on the classical method for Hansen solubility parameters (Lu & Chen, 2004).

$^c$ The partial solubility parameters of PCL calculated based on the classical method for Hansen solubility parameters (Davies & Rideal, 1963).

$\delta$ solubility parameter, subscripts $d$, contribution of the dispersion forces; $p$, polar contribution; $h$, hydrogen bonding contribution; $v$, dispersion and polar contribution.
3.3 Experimental

Co-flow microfluidic mixer used in this work consisted of two coaxial glass capillaries: (i) an inner tapered-end round capillary (1.15 mm outer diameter and 650 μm inner diameter) and (ii) an outer square capillary (1.15 mm inner dimension). A two-component epoxy glue (Five Minute Epoxy, ITW Devcon Ltd.) was used to fix the outer capillary onto a glass microscope slide that was used as a platform for the device. The inner capillary was pulled using a P-97 Flaming/Brown micropipette puller (Sutter Instrument Co.) to produce a sharp tip with a small orifice. The inner diameter of the orifice was enlarged to 60 μm by sliding the tip against abrasive paper. During this process, the tip was observed with a Narishige’s MF-830 microforge (Linton Instrument, Norfolk, UK). The inner capillary was flushed with water to remove any glass debris and then inserted in the square capillary. The capillary was then treated with 2-[methoxy(polyethyleneoxy)propyl]trimethoxysilane (FluoroChem, UK) to enhance the hydrophilicity of the orifice. After subsequent cleaning and treatment, the round capillary was positioned inside the square capillary such that the orifice coincides with the longitudinal axis of the square capillary. The device was placed on the stage of an inverted microscope (XDS-3, GX Microscopes) (Fig. 3.2 (a)), which was attached to a Phantom V9.0 high-speed camera (Vision Research, Ametek, US). Coaxial alignment of the two capillaries was ensured by measuring the distance from the edge of square capillary to the central axis of the round capillary. It was done by displaying live image from the camera on the computer’s screen. Two syringe needles with plastic hubs (B-D Precisionglide®, Sigma-Aldrich, 2.5 mm O.D. and 0.9 mm I.D.) were glued onto the slide such that the entrances to each capillary were situated inside the hubs. Two syringe needles with plastic hubs (B-D Precisionglide®, Sigma-Aldrich, 2.5 mm O.D. and 0.9 mm I.D.) were glued onto the slide such that the entrances to each capillary were situated inside the hubs.

Fig. 3.2 (b) is a schematic view of the device, showing the inner capillary partially inserted into the square capillary. Two syringe needles with plastic hubs were attached to the capillaries to serve as liquid inlets. The needle hub with a single groove was used to deliver organic phase to the opening of the inner capillary. The needle hub
with two grooves was used to deliver aqueous phase co-currently through the pockets between the two capillaries (Fig. 3.2 (c)). The organic phase was 1 mg ml\(^{-1}\) (1000 ppm) solution of PCL or PLA in THF and the aqueous phase was Milli-Q water.

The phase flow rates were controlled by two separate syringe pumps (Harvard Apparatus, model 11 Elite). The organic phase was delivered from a gas-tight syringe via a THF-resistant Teflon tubing (1.59 mm O.D. and 0.8 mm I.D.). Milli-Q water was delivered from another gas tight syringe via a polyethylene tubing (1.52 mm O.D. and 0.86 mm I.D.). The aqueous phase flow rate, \(Q_{aq}\), was constant at 5 ml h\(^{-1}\) and the organic phase flow rate, \(Q_{or}\), varied from 3.3 to 0.5 ml h\(^{-1}\). Therefore, the throughput of the NPs was 0.5–3.3 mg h\(^{-1}\). The mixing process was recorded by the camera at 25 frames per second and 576 \(\times\) 288 resolution. The particle size distribution was measured by dynamic light scattering using Delsa™ Nano HC particle analyzer (Beckman Coulter, Inc). Nanoparticle tracking analysis (NTA) was performed using a NanoSight LM20 (NanoSight, Amesbury, UK), equipped with a sample chamber with a 642-nm laser. The samples were injected in the sample chamber with sterile syringes (BD Discardit II, New Jersey, USA), and the measurement was performed at room temperature. The Transmission Electron Microscopy (TEM) measurements were carried out using a JEOL JEM-2000 FX transmission electron microscope operated at an accelerating voltage of 200 kV. The sample drop was deposited onto a carbon-coated copper mesh and left to dry before being observed. The mesh was coated by dipping it into a suspension of carbon particles in deionised water.
Fig. 3.2: Geometry of the glass capillary device modelled in this work: (a) A photograph of the experimental equipment; (b) A schematic top view showing coaxial assembly of glass capillaries glued onto a microscope slide. The magnified image is a tapered section of the inner capillary with 60 μm diameter orifice; (c) A schematic cross-sectional view showing round inner capillary and square outer capillary. All dimensions are in μm.

3.4 Computational modeling

The computational domain used for the simulations is shown in Fig. 3.3. The flow passage created by inserting a round capillary inside a square capillary is not entirely axisymmetric; hence a 3-D domain is required. By considering planes of symmetry, a quarter of the geometry along the axis was used in this numerical study. Sufficient channel lengths upstream and downstream of the nozzle have been selected to avoid inlet and outlet boundaries affecting the flow field and species distribution in the vicinity of the nozzle. The upstream and downstream channel lengths from the nozzle for the computational domain were selected to be 2885 μm and 3455 μm respectively. This selection was confirmed by a preliminary simulation with 50 % longer channels and we found no effect on the dependent variables solved.
The \( x \)-axis is perpendicular to the walls of the square capillary and equivalent to \( y \)-axis, due to coaxial alignment of the two capillaries. The \( x \) coordinate ranges from \( x = 0 \) at the capillary axes to \( x = r \) at the walls of the square capillary, where \( r = 576 \mu m \) according to Fig. 3.2. The \( z \)-axis is oriented in the direction of flow and coincides with the axes of the capillary. \( z = 0 \) corresponds to the orifice outlet position.

**Fig. 3.3:** The model geometry (3-D) of the co-flow microfluidic device.

### 3.4.1 Governing equations

The governing equations are the continuity and incompressible Navier-Stokes equations for laminar flow coupled with convection and diffusion equation for species transport. Here, we assume that the transient interfacial tension between the two miscible liquids used in this study is negligible due to similar viscosities and densities. The fluid flow equations were specified as follows:

\[
\frac{\partial \rho}{\partial t} + \rho \nabla \cdot \mathbf{u} = 0 \tag{3.4}
\]

\[
\frac{\partial \mathbf{u}}{\partial t} + \rho (\mathbf{u} \cdot \nabla) \mathbf{u} = \nabla \cdot \left[ -p \mathbf{I} + \eta (\nabla \mathbf{u} + (\nabla \mathbf{u})^T) \right] \tag{3.5}
\]

where \( \mathbf{u} \), \( \rho \), \( I \), \( \rho \) and \( \eta \) denotes the velocity field, pressure, identity matrix, density and dynamic viscosity respectively.
The density of THF-water mixture, expressed in g cm\(^{-3}\), was calculated using the following quadratic polynomial expression (Belandria et al., 2009):

\[ \rho = ay^2 + by + c \]  

(3.6)

where \( y \) is the mass fraction of water in the mixture and \( a = -0.1025 \), \( b = 0.2118 \), and \( c = 0.889 \) are constants.

The dynamic viscosity of THF-water mixture, in mPa s, was estimated based on the work of (Montgomery et al., 1949):

\[ \eta = \alpha y^3 + \beta y^2 + \gamma y + \delta \]  

(3.7)

where \( \alpha = -4.4983 \), \( \beta = 1.9205 \), \( \gamma = 3.0109 \), \( \delta = 0.5591 \). For pure THF (\( y = 0 \)), \( \eta_{THF} = 0.559 \) mPa s. For pure water (\( y = 1 \)) \( \eta_{water} = 0.99 \) mPa s.

The influence of mixture viscosity on the diffusion coefficient of the polymer was taken into account using Wilke-Chang equation (Wilke & Chang, 1955; Hayduk & Laudie, 1974):

\[ D = \frac{7.4 \times 10^{-8} (\zeta M_w)^{\frac{1}{2}} T}{\eta V_p^{0.6}} \]  

(3.8)

where \( D \) is the diffusion coefficient in cm\(^2\) s\(^{-1}\), \( M_w \) is the molecular weight of solvent, \( \zeta \) is the association parameter introduced to define the effective molecular weight of the solvent (for non-associated solvents \( \zeta = 1 \) and for water \( \zeta = 2.6 \)), \( T \) is the absolute temperature, \( \eta \) is the dynamic viscosity of the mixture in mPa s, calculated from Eq. (3.7), and \( V_p \) is the molar volume of the polymer at normal boiling point in cm\(^3\) mol\(^{-1}\). The diffusion coefficient was found to be equal to 4.88 and 13.5 \( \times \) 10\(^{-7}\) cm\(^2\) s\(^{-1}\) for PCL in pure water and pure THF, respectively and 2.24 and 6.20 \( \times \) 10\(^{-7}\) cm\(^2\) s\(^{-1}\) for PLA in pure water and THF, respectively.

The convection diffusion equation was solved using Transport of Concentrated Species interface available in Comsol Multiphysics\textsuperscript{TM}. A mixture averaged diffusion
model based on multicomponent Maxwell-Stefan diffusivities was selected along with convection to solve for species transport:

$$\frac{\partial (\rho \omega_i)}{\partial t} + \nabla \cdot (\rho \omega_i \mathbf{u}) = -\nabla \cdot \mathbf{j}_i \quad (3.9)$$

where $\omega_i$ denotes the mass fraction of species $i$ and $\mathbf{j}_i$ denotes the relative mass flux vector expressed as:

$$\mathbf{j}_i = -\left( \rho D^m_i \nabla \omega_i + \rho \omega_i D^{T}_i \frac{\nabla M_n}{M_m} + D^T_i \frac{\nabla T}{T} \right) \quad (3.10)$$

where $D^{T}_i$ is the thermal diffusion coefficient of species $i$ and $D^m_i$ is the mixture-averaged diffusion coefficient, given by:

$$D^{m}_i = \frac{1 - \omega_i}{\sum_{k=1}^{n} \frac{x_k}{D_{ik}}} \quad M_n = \left( \sum \frac{\omega_i}{M_i} \right)^{-1} \quad (3.11)$$

where $D_{ik}$ are the multicomponent Maxwell-Stefan diffusivities, $x_k$ is the mole fraction, and $M_i$ is the molar mass of component $i$.

### 3.4.2 Boundary conditions

The boundary conditions for the fluid flow were set as follows:

(i) no slip boundary conditions were applied on the walls of both capillaries; (ii) at the inlets, flow velocities and mass fractions for each liquid were set according to the experimental flow rates. The flow was assumed to be fully developed at the orifice and a parabolic profile for the axial velocity, $v_z$, was specified; (iii) at the outlet boundary, pressure was set to zero (the solution flows at the outlet of the co-flow device at atmospheric pressure); (iv) axial symmetry conditions were specified where surfaces were created in slicing the geometry.

### 3.4.3 Numerical method

The problem was solved using finite element method to predict the flow fields of the organic phase and aqueous phase within the device. A model was developed using laminar flow model (for fluid flow) and Transport of Concentrated Species (for convection and diffusion) available in Comsol Multiphysics™ 5.0. The computational
domain was discretised using 2,282,636 tetrahedral mesh elements with a finer mesh closer to the nozzle region. The number of degrees of freedom (DOFs) solved was 2,586,882. Mesh independency of the solution was confirmed by performing computations with a finer mesh of 3,178,218 elements. The variations between solutions for above two meshes were found to be less than 1% throughout the domain. All the flow rate ratios considered in this study were solved in one simulation using Parametric Sweep feature available under study Extensions. The total computation time was approximately 135 min on an Intel Core i7 64-bit 2.7 GHz processor.

Following the simulations, the results were analysed by plotting the nanoprecipitation lines for both PCL and PLA at five different aqueous to organic phase flow rate ratios \(Q_{aq}/Q_{or} = 1.5, 3.0, 4.5, 7.0, \text{ and } 10.0\). The flow rate ratio between the organic phase and aqueous phase was varied using parametric continuation feature available in the package.

3.5. Results and Discussion

3.5.1 Bagley’s two-dimensional graph for solubility of polymers in THF-water solution

Figs. 3.4 (a) and (b) are Bagley’s two-dimensional solubility graphs for PCL and PLA, respectively in interaction with pure water, pure THF and water-THF mixtures. As expected, water is located far outside the solubility circle of PCL or PLA, in agreement with the fact that water is a non-solvent for both polymers. The position of THF and PCL on the solubility graph in Fig. 3.4 (a) almost coincides (see also Table 3.1), indicating that THF and PCL have high affinity for each other and thus, THF is a good solvent for PLA. The THF-water mixtures are all situated on a straight line connecting two pure liquids, at a point corresponding in distance to the volume ratio of the liquids in the mixture. The higher the water content in the mixture, the greater the distance between the mixture and the polymer; hence lower the solubility of the polymer in that mixture.

As shown in Fig. 3.4 (a), a 50/50 THF/water mixture is a poor solvent for PCL since it is located outside the solubility circle, but a solution having 92 vol% of THF and 8
vol% of water is a good solvent for PCL. The mixture containing 84 vol% of THF lies on the solubility circle, which means that nanoprecipitation of PCL starts when the water content in THF-water mixture reaches 16 vol% (or 18 wt%). Fig. 3.4 (b) shows that nanoprecipitation of PLA starts when the amount of water in THF-water mixture reaches 31 vol% or 34 wt%. The different positions of PCL and PLA on the solubility graph reflect the fact that the polarity of PLA is higher than that of PCL, as can be seen from corresponding $\delta_p$ values in Table 3.1. The polarity of PLA and PCL is due to their polar ester groups, but PCL is more hydrophobic than PLA because it contains a longer hydrocarbon chain on each side of the ester group ($-(CH_2)_5-$ as compared to $-CH(CH_3)-$).
Fig. 3.4: Bagley’s two-dimensional graphs of partial solubility parameters for pure THF, water, and different water-THF mixtures with respect to: (a) PCL; (b) PLA. The solubility circle shown by the red dotted line (○) has a radius of 5 δ-units and the coordinates of its centre are $\delta_v = 17.66$ and $\delta_p = 4.80$ for PCL and $\delta_v = 20.11$ and $\delta_p = 9.70$ for PLA. Liquids outside the solubility circle are non-solvents.

3.5.2 Numerical simulation of two phase co-flow

The distribution of flow velocities and concentrations in a microfluidic mixer depends on geometry of the device, physical and thermodynamic properties of the ternary system (polymer-water-THF), such as polymer solubility, diffusion coefficients, viscosity and density of the mixture, interfacial tension, and operating conditions (flow rates of the aqueous and organic phase and the flow rate ratio). In this work, the Korteweg stress contribution at the mixing region separating the two fluids is not accounted for due to similar viscosities and densities of the fluids used. The contribution of Korteweg stress term is proportional to the viscosity contrast of the two miscible fluids; hence advection arising from this term is negligible for low viscosity ratios (Valentini & Moore, 2009).
3.5.2.1 Distribution of flow velocities in the co-flow microfluidic device

Fig. 3.5 shows velocity magnitude at a constant aqueous phase flow rate of $Q_{aq} = 5$ ml h$^{-1}$ and at five different organic phase flow rates, $Q_{or}$ (0.5, 3.0, 4.5, 7.0, and 10.0 ml h$^{-1}$). The colour bar on the right side of the figure represents normalised velocity magnitude within the computational domain. The precipitation lines for PLA and PCL calculated from Bagley’s solubility criteria are shown in the figure as solid red (PLA) and yellow (PCL) dashed lines, respectively. The polymers start to precipitate when they reach the respecting precipitation line during diffusion and convection in the radial direction.

A flow space downstream of the nozzle can be divided into two distinct regions separated by the precipitation lines: an unsaturated region located between the two precipitation (cloud point) lines, where a polymer is fully soluble in the liquid phase due to high concentration of THF and a supersaturated region situated between the precipitation lines and the wall of the collection capillary, where the polymer is insoluble and forms nanoparticles. As can be seen in Fig. 3.5, the unsaturated region is narrower for PCL than PLA, because PCL is less soluble in water and starts precipitating at the higher THF concentration compared to PLA.

The average velocity of the organic phase emerging from the nozzle is given by:

$$U_{N,or} = 4Q_{or} / (\pi D_N^2),$$

where $D_N$ is the inner diameter of the nozzle. For the conditions shown in Fig. 3.5, the average velocity of the organic phase in the nozzle ranged from 0.05 m s$^{-1}$ at $Q_{or} = 0.5$ ml h$^{-1}$ to 0.32 m s$^{-1}$ at $Q_{or} = 3.3$ ml h$^{-1}$. The average velocity of the aqueous phase at nozzle is given by:

$$U_{N,aq} = Q_{aq} / [a^2 - (D_o^2 \pi / 4)],$$

where $D_o$ is the outer diameter of the nozzle and $a$ is the inner dimension of the square capillary. For the conditions shown in Fig. 3.5, the average velocity of the aqueous phase at the injection point, $U_{N,aq} = 0.001$ m s$^{-1}$, is much lower than that of the organic phase, $U_{N,or} = 0.05$ m s$^{-1}$. As a result of the large difference in velocity between the two streams, momentum is exchanged between the fluid streams until a steady-state parabolic velocity profile is established downstream of the nozzle.
According to Fig. 3.5, the region where velocity exceeds 90% of the maximum velocity (represented by dark red colour) extends approximately 7 nozzle diameters downstream at \( Q_{or} = 3.3 \text{ ml h}^{-1} \), but is limited to approximately 2 nozzle diameters at \( Q_{or} = 0.5 \text{ ml h}^{-1} \). Also this high-velocity region changes its shape from an elongated ellipse at high \( Q_{or} \) values to a semi-sphere at low \( Q_{or} \) values. At the organic phase flow rates of 3.3 and 1.7 \text{ ml h}^{-1}, vortices are formed in the aqueous phase near the nozzle (Figs. 3.5 (a and b)), due to the large difference in velocity between the organic and aqueous phase. It is possible that nanoparticles formed near the nozzle in these cases be trapped within vortices and forced into circular motion instead of being immediately swept away, which may lead to much longer residence time of these particles compared to the ones formed further downstream. As a result of non-uniform residence time of particles in the device, a broader particle size distribution can be expected for higher organic flow rates (Fig. 3.5 (a) and (b)) compared to low organic flow rates shown in Fig. 3.5 (c-e), where vortices are absent.

At \( Q_{or} = 3.3 \text{ ml h}^{-1} \) (Fig. 3.5 (a)), the unsaturated (solvent-rich) region is narrow near the nozzle and reaches a maximum thickness of \( 28D_N \) downstream of the nozzle. This reflects the fact that the organic phase emerges from the nozzle at a relatively high velocity and a considerable distance is required for the velocities of the two phases to equilibrate. Once the maximum thickness is established, the unsaturated region remains unchanged until the end of the computational domain. At this point, mixing occurs mainly by diffusion in radial direction, therefore the effect of mixing is not visible. The mean residence time of fluid elements downstream the nozzle is inversely proportional to the total flow rate \( (Q_{or} + Q_{aq}) \). At \( Q_{or} =0.5 \text{ ml h}^{-1} \) (Fig. 3.5 (e)), a maximum thickness of the unsaturated region is \( 5D_N \) and the organic phase velocity is quickly reduced to the equilibrium value. Since the fluid elements have 50 % longer mean residence time in Fig. 3.5 (e) than in Fig. 3.5 (a), the effect of mixing on the shape of the precipitation lines is clear and the unsaturated region is narrower at the end of the computational domain. It can be explained by the progressive replacement of organic phase by water through the interfacial area, leading to shrinkage of the
unsaturated region. At a sufficiently long distance downstream of the nozzle, the two precipitation lines will join together at the central axis, which means that the supersaturated region will occupy the entire cross section of the collection capillary.

Fig. 3.6 shows velocity profiles at a cross-section one nozzle diameter downstream of the nozzle for the five different flow rate ratios. As shown in Fig. 3.6 (a), the axial velocity ($U_z$) for all flow rate ratios decreases from a maximum value at the central axis ($x = 0$) to zero at the wall ($x = 576$) of the capillary. A maximum axial velocity of 0.44 m s$^{-1}$ was found for the lowest flow rate ratio ($FR = 1.5$) while a minimum axial velocity of 0.02 m s$^{-1}$ is recorded for the highest flow rate ratio ($FR = 10$). These velocities are lower than the maximum axial velocities computed at the nozzle (0.64 m s$^{-1}$ for $FR = 1.5$ and 0.1 m s$^{-1}$ for $FR = 10$) due to viscous dissipation over a distance of $D_N$. For the given cross-section, precipitation of PLA or PCL starts 66 µm away from the central axis for $FR = 1.5$ and the corresponding axial velocity is 0.037 m s$^{-1}$. For $FR = 10$, the polymer precipitation starts at 160 µm from the central axis, where $U_z \approx 0$.

Fig. 3.6 (b) shows profiles of radial velocity ($U_x$) at a cross-section one nozzle diameter downstream of the nozzle. Negative values of $U_x$ represents velocity vectors directed away from the central axis (towards the wall). At $x = 0$, the radial velocity is zero for all the flow rate ratios, as no flow across the central axis is possible due to axisymmetric geometry. The radial velocity is negative near the central axis, reflecting the fact that the organic phase stream expands after injection from the nozzle. The highest negative radial velocity is observed at the highest organic phase flow rate. The radial velocity becomes positive farther away from the central axis, since the aqueous phase flows towards the interface. The maximum fluctuation of radial velocity is observed for $FR = 1.5$ due to the vortices formed on either sides of the jet emerging from the nozzle. The radial velocity becomes zero at a distance between 50 and 150 µm from the central axis, depending on the flow rate ratio, which roughly corresponds to the position of the interface.
Fig. 3.7 (a) shows axial velocity profiles $12D_N$ downstream of the nozzle. At $FR = 1.5$, the axial velocity at the central axis is $0.018 \text{ m s}^{-1}$, which is 5 times greater than the maximum velocity for fully developed laminar flow through a square duct given by: $U_{z,\text{max}} = 2.1(Q_{\text{air}} + Q_{\text{w})/a^2} = 0.0036 \text{ m s}^{-1}$. Thus, equilibrium velocity profile was not yet established $12D_N$ downstream of the nozzle. Furthermore, due to vortices formed within the aqueous phase, the axial velocity is negative at $x > 400 \mu\text{m}$. For $FR = 3, 4.5, 7$ and 10, the axial velocity profile is parabolic with a maximum velocity at the central axis ranging from $0.0023$ to $0.0035 \text{ m s}^{-1}$, which is close to $U_{z,\text{max}}$ values for fully developed steady-state laminar flows ($0.0024$ and $0.0029 \text{ m s}^{-1}$) calculated from the above equation. Fig. 3.7 (b) shows the radial velocity $12D_N$ downstream of the nozzle. Negative values indicate that the flow is diverted away from the central axis. Further down the capillary, fluid flow is fully developed and the radial velocity becomes zero. For $FR$ values from 3 to 10, the absolute value of the radial velocities are less than $0.0005 \text{ m s}^{-1}$, which indicates that fully developed velocity profiles are nearly established at 12 nozzle diameters downstream the nozzle. At $FR = 1.5$, the radial velocity exceeds $0.004 \text{ m s}^{-1}$ at certain locations, indicating that hydrodynamic equilibrium is not yet established.
Fig. 3.5: The distribution of velocity magnitudes at: (a) $Q_{aq}/Q_{or} = 1.5$, $Q_{aq} = 5.0 \text{ ml h}^{-1}$, $Q_{or} = 3.3 \text{ ml h}^{-1}$; (b) $Q_{aq}/Q_{or} = 3$, $Q_{aq} = 5.0 \text{ ml h}^{-1}$, $Q_{or} = 1.7 \text{ ml h}^{-1}$; (c) $Q_{aq}/Q_{or} = 4.5$, $Q_{aq} = 5.0 \text{ ml h}^{-1}$, $Q_{or} = 1.1 \text{ ml h}^{-1}$; (d) $Q_{aq}/Q_{or} = 7$, $Q_{aq} = 5.0 \text{ ml h}^{-1}$, $Q_{or} = 0.7 \text{ ml h}^{-1}$; (e) $Q_{aq}/Q_{or} = 10$, $Q_{aq} = 5.0 \text{ ml h}^{-1}$, $Q_{or} = 0.5 \text{ ml h}^{-1}$. Surface: normalised velocity magnitude. Black contours: stream lines. Solid red lines and yellow dashed lines are the cloud point lines for PLA and PCL, respectively. Vertical green lines in (a) are sections considered in Figs. 3.6 and 3.7. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
**Fig. 3.6:** (a) Axial velocity profiles and (b) radial (along x-axis) velocity profiles at one nozzle diameter downstream of the nozzle for five different flow rate ratios, $FR$, of the aqueous to organic phase.
Fig. 3.7: (a) Axial velocity profiles (along z-axis) and (b) radial velocity profiles (along x-axis) at 12 nozzle diameters downstream the nozzle for five different flow rate ratios ($FR$).

3.5.2.2 Distribution of THF and polymer in the co-flow microfluidic device

Fig. 3.8 shows distribution of mass fraction of THF at different flow rate ratios. The boundary between THF and water is very sharp near the nozzle, but becomes blurred...
farther downstream due to countercurrent mass transfer of THF and water across the interface. At $FR = 1.5$, the organic phase flow rate is the highest leading to the thickest organic phase stream and consequently, the longest mixing length. The mixing time is proportional to the square of the width of the organic phase stream and inversely proportional to the diffusivity of THF (Karnik et al., 2008). At $FR = 10$, the organic phase stream is so narrow that the mass fraction of THF on the central axis is below 0.9 at the end of the computational domain. At $FR = 10$, the organic phase stream is initially very thin, but widens further downstream as a result of its deceleration caused by the interaction with a slow-moving aqueous phase.

Fig. 3.9 shows profiles of mass fraction of THF at two different cross sections located at $z = D_N$ and $z = 5W$ ($W =$ width of the square capillary). At $z = D_N$ (Fig. 3.9 (a)), the mass fraction of THF in the liquid is 0.99 all the way from the central axis to the interface and then suddenly drops down to zero reflecting the fact that the mixing process has barely begun. However, for $FR$ of 1.5, the mass fraction of THF is not zero between the interface and the wall, due to retention of small amount of THF within the vortices. At $z = 5.5W$ (Fig. 3.9 (b)), the concentration profiles show a gradual variation due to progression of the mixing. When the mixing process is complete, THF will be uniformly distributed over the whole cross section and the concentration profiles will become ‘flat’ with a mass fraction of THF ranging from 0.37 at $FR = 1.5$ to 0.08 at $FR = 10$. According to Fig. 3.9 (b), the mass fraction of THF on the central axis is approximately 0.83, while the highest concentration was found at $FR = 3$. At $FR = 1.5$, mixing is enhanced by vortices formed and the concentration of THF is lower on the central axis than that at higher flow rate ratios.

Fig. 3.10 shows distribution of PCL within the device at different flow rate ratios. Clearly, PCL and THF show similar distribution patterns as mass transfer of both species is governed by similar factors. The total flux of PCL is the sum of diffusive and convective fluxes arising from concentration gradient and bulk fluid motion, respectively. As shown in Table 3.2, the mass transfer of PCL from the organic phase to the aqueous phase is convection-dominated, since the convective flux is 1–3 orders of magnitude higher than the diffusive flux. A maximum radial convective flux of
0.115 kg m\(^{-2}\) s\(^{-1}\) was found for PCL at the nozzle for \(FR = 1.5\). This is caused by vortices formed near the nozzle causing the radial velocity to reach a maximum (Figs. 3.5 and 3.6). It is also evident that the convective flux is \(3\)–\(4\) orders of magnitudes higher near the nozzle than that at the end of the computational domain, as the radial velocity is much greater near the nozzle compared to any downstream location. The diffusive flux of PCL follows the same trend with the maximum diffusion rate near the nozzle, due to the largest concentration gradient, as shown in Fig. 3.9.

3.5.2.3 Distribution of dynamic viscosities in the co-flow capillary device

Fig. 3.11 shows the variation of dynamic viscosity within the domain at 25 °C. Before mixing the organic phase has a viscosity of 0.559 mPa s (or 5.59 mP), while pure water has a viscosity of 1 mPa s. The viscosity of the mixture is higher than that of the feed streams and is given by equation (3.7). A maximum viscosity of 2.1 mPa s occurs at \(\sim42\) wt\% THF while the viscosity at the cloud point is 1.14 and 1.63 mPa s for PCL and PLA, respectively. At \(FR = 1.5\), radial mass flux near the nozzle is relatively high due to the vortices formed and thus, the viscosity of the mixture is higher in the vicinity of the nozzle than that observed for other flow rate ratios.
Fig. 3.8: Distribution of mass fraction of THF at: $Q_{aq}/Q_{or} = 1.5$, $Q_{aq} = 5.0$ ml h$^{-1}$, $Q_{or}$ = 3.3 ml h$^{-1}$; (b) $Q_{aq}/Q_{or} = 3$, $Q_{aq} = 5.0$ ml h$^{-1}$, $Q_{or} = 1.7$ ml h$^{-1}$; (c) $Q_{aq}/Q_{or} = 4.5$, $Q_{aq}$ = 5.0 ml h$^{-1}$, $Q_{or} = 1.1$ ml h$^{-1}$; (d) $Q_{aq}/Q_{or} = 7$, $Q_{aq} = 5.0$ ml h$^{-1}$, $Q_{or} = 0.7$ ml h$^{-1}$; (e) $Q_{aq}/Q_{or} = 10$, $Q_{aq} = 5.0$ ml h$^{-1}$, $Q_{or} = 0.5$ ml h$^{-1}$. Contours: Mass fractions of THF at 0.1 increment (0.1, 0.2, . . ., 0.9). Vertical green line is a section considered in Fig. 3.9(a).
Fig. 3.9: Profiles of mass fraction of THF for various flow rate ratios (FR) at a cross section located: (a) \( D_N \) downstream from the nozzle; (b) 5.5\( W \) downstream from the nozzle (\( D_N \) = internal diameter of the nozzle, \( W \) = width of the square capillary, and \( FR = \frac{Q_{aq}}{Q_{or}} \)).
Fig. 3.10: Distribution of mass percentage of PCL at: $Q_{aq}/Q_{or} = 1.5$, $Q_{aq}=5.0$ ml h$^{-1}$, $Q_{or} = 3.3$ ml h$^{-1}$; (b) $Q_{aq}/Q_{or} = 3$, $Q_{aq} = 5.0$ ml h$^{-1}$, $Q_{or} = 1.7$ ml h$^{-1}$; (c) $Q_{aq}/Q_{or} = 4.5$, $Q_{aq}=5.0$ ml h$^{-1}$, $Q_{or} = 1.7$ ml h$^{-1}$.
$Q_{aq} = 5.0 \text{ ml h}^{-1}, Q_{or} = 1.1 \text{ ml h}^{-1}$; (d) $Q_{aq}/Q_{or} = 7$, $Q_{aq} = 5.0 \text{ ml h}^{-1}, Q_{or} = 0.7 \text{ ml h}^{-1}$; (e) $Q_{aq}/Q_{or} = 10$, $Q_{aq} = 5.0 \text{ ml h}^{-1}, Q_{or} = 0.5 \text{ ml h}^{-1}$. **Black lines:** precipitation (cloud point) lines for PCL.

**Table 3.2:** The radial component of diffusive and convective fluxes of PCL on the cloud line just after the nozzle ($z \to 0$) and at the end of the computational domain ($z = z_{\text{max}}$) for two different flow rate ratios ($FR = 1.5$ and $10$).

<table>
<thead>
<tr>
<th>$FR$</th>
<th>$z = 0$ $\text{Diffusive mass flux of PCL/kg m}^{-2}\text{s}^{-1}$</th>
<th>$z = z_{\text{max}}$ $\text{Convective mass flux of PCL/kg m}^{-2}\text{s}^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>$37 \times 10^{-5}$</td>
<td>$0.27 \times 10^{-5}$</td>
</tr>
<tr>
<td>10</td>
<td>$25 \times 10^{-5}$</td>
<td>$0.047 \times 10^{-5}$</td>
</tr>
</tbody>
</table>
Fig. 3.11: Surface and contours of dynamic viscosity (in milipoise) at: $Q_{aq}/Q_{or} = 1.5$, $Q_{aq} = 5.0$ ml h$^{-1}$, $Q_{or} = 3.3$ ml h$^{-1}$; (b) $Q_{aq}/Q_{or} = 3$, $Q_{aq} = 5.0$ ml h$^{-1}$, $Q_{or} = 1.7$ ml h$^{-1}$; (c) $Q_{aq}/Q_{or} = 4.5$, $Q_{aq} = 5.0$ ml h$^{-1}$, $Q_{or} = 1.1$ ml h$^{-1}$; (d) $Q_{aq}/Q_{or} = 7$, $Q_{aq} = 5.0$ ml h$^{-1}$, $Q_{or} = 0.7$ ml h$^{-1}$; (e) $Q_{aq}/Q_{or} = 10$, $Q_{aq} = 5.0$ ml h$^{-1}$, $Q_{or} = 0.5$ ml h$^{-1}$.

3.6 Experimental validation

The video recordings of the process captured at $Q_{aq} = 5$ ml h$^{-1}$ and $Q_{or}$ of 0.7 and 3.3 ml h$^{-1}$ (Figs. 3.12 (a) and (b)) are in good agreement with the presented numerical simulations. At low $Q_{or}$ value, the interface is hemispherical and corresponds to the high velocity region shown in Fig. 3.5 (d). Although the equilibrium interfacial tension between water and THF is zero, a temporary interface is clearly visible, formed due to sharp differences in density and composition when the two liquids are suddenly brought into contact. The formed nanoparticles can be seen in the aqueous phase near the interface. As expected, nanoparticles are not visible between the two cloud point lines due to high THF concentration in this region. Self-assembly of PCL into nanoparticles is almost instantaneous near the nozzle due to high gradients of concentration and high fluxes of PCL, which results in high concentration of nanoparticles in that region. The formation of vortex flow at high $Q_{or}$ value can be seen in Fig. 3.12 (b), as predicted in Fig. 3.5 (a).
The interface has a widening shape due to decreasing velocity of the organic phase, as predicted in Fig. 3.8 (a). As a result of vortex flow, the nanoparticles formed near the nozzle and forced into circular motion, which leads to much longer and non-uniform residence time of these particles compared to the ones formed farther downstream. As a consequence, a broader particle size distribution and larger mean particle size was observed at the higher organic flow rate (Fig. 3.14). Fig. 3.12 (c) confirms the presence of monodisperse spherical nanoparticles with uniform size distribution at $Q_{aq}/Q_{or} = 10.0$ by tracking individual particles in liquid dispersions. The spherical shape of nanoparticles was clearly acquired from TEM analysis as presented in Fig. 3.13 for both synthesised PCL and PLA nanoparticles.

**Fig. 3.12:** The flow patterns observed in the micromixer at different aqueous to organic phase volume ratio, $Q_{aq}/Q_{or}$: (a) 7.0; (b) 1.5 and (c) size distribution from NTA measurement of mixtures of monodisperse PCL nanoparticles with the corresponding video frame at $Q_{aq}/Q_{or} = 10.0$. The internal diameter of the nozzle was 60 $\mu$m.

**Fig. 3.13:** Microscopic images of; (a) TEM image of individual PCL NPs; and (b) TEM image of individual PLA NPs.
Fig. 3.14: Particle size distributions of PCL particles prepared at various flow rate ratios by two different mixing strategies; (a) rapid mixing (co-flow device) and (b) bulk mixing. Each measurement was repeated for three times. The internal diameter of the nozzle was 60 μm.

3.7 Chapter summary

It was demonstrated that a 3-D co-flow microfluidic device constructed by inserting a round capillary inside a square capillary is suitable for fabrication of biodegradable polymeric nanoparticles through anti-solvent nanoprecipitation. Using a two-dimensional δ_h vs. δ_v graph, precipitation of PCL and PLA was found to start when the water content in the organic phase reaches 16 and 31 vol%, respectively. The
organic phase was injected at higher velocities than the aqueous phase to induce radial flow, which led to a significant increase in mass transfer but also in the formation of vortices near the nozzle at the flow rate ratio of 3 or lower.

In general, mass transfer in microfluidic devices is dominated by diffusion. However, for the investigated geometry and operating conditions, it was found that the convective flux of PCL at the cloud point was 1-3 orders of magnitude higher than the diffusive flux in the radial direction. In addition, convective flux of the polymer was 3-4 orders of magnitudes higher near the nozzle compared to the downstream end of the computational domain, which reflects the fact that mixing predominantly occurs in the vicinity of the nozzle. The diffusive flux of the polymer followed the same trend with the maximum rate of molecular diffusion observed near the nozzle due to high concentration gradients. The experimental results are in good agreement with the CFD simulation results showing semi-spherical interface at low organic phase flow rate and widening jet at high organic phase flow rate. A broader particle size distribution with larger mean particle size was found at higher organic phase flow rate due to longer residence time of nanoparticles as a result of vortex flow. The formation of spherical shape nanoparticles were significantly verified through the use of TEM analysis.
CHAPTER 4

PREPARATION OF BIODEGRADABLE POLYMERIC NANOPARTICLES FOR PHARMACEUTICAL APPLICATIONS USING GLASS CAPILLARY MICROFLUIDICS

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Chapter overview

The aim of this study was to develop a new microfluidic approach for the preparation of nanoparticles with tuneable sizes based on micromixing/direct nanoprecipitation in a coaxial assembly of tapered-end glass capillaries. The organic phase was 1 wt% poly(ε-caprolactone) (PCL) or poly(ᴅ,ʟ-lactide) (PLA) in tetrahydrofuran and the antisolvent was Milli-Q water. The size of nanoparticles was precisely controlled over a range of 190-650 nm by controlling phase flow rates, orifice size and flow configuration (two-phase co-flow or countercurrent flow focusing). Smaller particles were produced in a flow focusing device, because the organic phase stream was significantly narrower than the orifice and remained narrow for a longer distance downstream of the orifice. The mean size of PCL particles produced in a flow focusing device with an orifice size of 200 μm, an organic phase flow rate of 1.7 mL h⁻¹ and an aqueous-to-organic flow rate ratio of 10 was below 200 nm. The size of nanoparticles decreased with decreasing the orifice size and increasing the aqueous-to-organic phase flow rate ratio. Due to higher affinity for water and amorphous structure, PLA nanoparticles were smaller and exhibited a smoother surface and more rounded shape than PCL particles.

4.1 Introduction

Biodegradable polymeric nanoparticles (NPs) have attracted considerable attention of the scientific community in the last several decades due to their high potential for a site-
specific (targeted) drug delivery, especially for oral administration of proteins and peptides and gene therapy (Legrand et al., 2007; Douglas et al., 1987). Biodegradable polymeric NPs are solid carriers with a mean size of less than 1 mm, which are capable to dissolve, entrap, encapsulate or attach active ingredients to its nanoparticle matrix (Legrand et al., 2007). Depending upon the method of NPs preparation and formulation, nanospheres or nanocapsules can be obtained. Nanocapsules are carriers in which the drug is confined to a cavity surrounded by a polymeric shell, while nanospheres are matrix systems in which the drug is uniformly dispersed in a polymer matrix (Mohanraj & Chen, 2007; Soppimath et al., 2001).

Polymeric NPs can be prepared from preformed polymers by emulsification-solvent evaporation, salting-out, dialysis, nanoprecipitation, and supercritical fluid technology or directly synthesised by polymerisation of monomers using polymerisation techniques such as micro-emulsion, mini-emulsion, surfactant-free emulsion and interfacial polymerisation (Nagavarma et al., 2012; Rao & Geckeler, 2011; Galindo-Rodriguez et al., 2004). In nanoprecipitation, two mutually miscible liquids are required, a solvent and non-solvent of the polymer, typically a volatile organic solvent and water, respectively. The NPs are formed almost instantly when the polymer solution is mixed with an excess of non-solvent, after which the solvent can be evaporated off. The method does not require high stirring rates, sonication, elevated temperatures or surfactants, and Class 1 solvents can be avoided (Fessi et al., 1989, 1992; Jain, 2000).

Bilati et al. (2005) have investigated the effect of the type of solvent and non-solvent, solvent/non-solvent volume ratio and polymer concentration on the nanoprecipitation of poly(ᴅ,ʟ-lactide) (PLA) and poly(ᴅ,ʟ-lactic-co-glycolic acid) (PLGA). The size of NPs was dependent of the type of non-solvent and increased in the following order: methanol>ethanol>propanol. Lince et al. (2008) prepared poly-є-caprolactone (PCL) nanoparticles in a Confined Impinging Jets Reactor (CIJR) and found a significant effect of mixing on the final particle size. The mixing efficiency increased with increasing the
flow rate of the liquid phases entering the CIJR, which favoured nucleation and led to a marked reduction in the particle size.

In order to achieve a controlled drug release to the specific site of action at the therapeutically optimal rate, NPs should be prepared with a controlled size, adhesion properties and degradation rate (Mohanraj & Chen, 2007). The traditional bulk mixers lack precise control over the mixing process due to their relatively large volume, resulting in poor control over the particle size distribution. Microscale mixers/reactors handle very small fluid volumes, offering the possibility to achieve a homogeneous reaction environment, and have a larger surface-to-volume ratio than conventional bulk mixers, which can greatly reduce the mixing time that becomes comparable with the induction time for nucleation (Capretto et al., 2013).

Ali et al. (2009) prepared hydrocortisone NPs in a microfluidic Y junction. The size of the generated NPs was controlled by the flow rates of solvent and anti-solvent, with smaller particles being formed at higher flow rates. Su et al. (2007) prepared BaSO₄ and 2,2-dipyridylamine NPs using a microfluidic set-up composed of three T-junctions. Solvent and anti-solvent droplets were formed in two upstream T junctions and then merged together in a downstream T junction. Génot et al. (2010) positioned a glass capillary at the intersection of the two branches of a Y junction to construct a 3D microfluidic mixer that was used to prepare rubrene nanocrystals. Zhang et al. (2008) and Yun et al. (2009) produced solid lipid nanoparticles using flow focusing devices with cross junction geometry. The particle size was controlled by varying the flow rate ratio of the two phases and introducing gas bubbles downstream of the cross junction. Dev et al. (2013, 2012) used a microfluidic continuous flow rotating tube processor to produce NPs of meloxicam and curcumin by reactive crystallisation.

Membrane micromixing is an alternative strategy of controlled mixing at molecular scale that was combined with nanoprecipitation to produce inorganic nanoparticles (Jia & Liu, 2013), liposomes (Laouini et al., 2013a), micelles (Laouini et al., 2013c), and PCL
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nanoparticles (Khayata et al., 2012). In a membrane-dispersion reactor, one liquid phase is dispersed through a microporous membrane into another liquid under controlled shear conditions and injection rate.

In this work, a novel microfluidic strategy was developed for fabrication of PCL and PLA NPs based on bringing into contact two co-flowing or counter-current flowing streams in coaxial glass capillaries. Both polymers have been approved by FDA for drug delivery (Jain et al., 1998; Södergård & Stolt, 2002; Panyam & Labhasetwar, 2003) and widely used as excipients in nanoprecipitation processes (Jain, 2000; Lu & Chen, 2004). The main objectives of this study were: (i) to make appropriate choice of good and poor solvent of the polymers, (ii) to observe the mixing process in situ using a microscope video system, and (iii) to investigate the effect of operating parameters, system geometry, and surfactants on the final particle size distribution.

4.2 Materials and methods

4.2.1 Chemicals

Tetrahydrofuran (THF) (HPLC grade, purity ≥ 99.9%) and poly(ɛ-caprolactone) (PCL, $M_w = 14,000\ \text{g mol}^{-1}$ with a glass transition temperature of $60\ ^\circ\text{C}$) were purchased from Sigma-Aldrich (Dorset, UK). Poly(ᴅ,ʟ-lactide) (PLA, Ingeo™ 4060D, $M_w = 320,000\ \text{g mol}^{-1}$) was supplied by Natureworks LLC (Minetona, MN, USA). 4060D is an amorphous polymer with an average D-lactide content of 12 wt% and a glass transition temperature of 55-60 °C. Polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), Tween 20 and Tween 80 were obtained from Sigma-Aldrich (Dorset, UK) and used as water soluble surfactants. All chemicals other than THF were of analytical grade. The antisolvent phase was pure water produced by reverse osmosis (Milli-Q®, Millipore) or aqueous surfactant solutions. The role of surfactant was to prevent agglomeration, coalescence and imperfect surface formation, as well as to reduce the size of the NPs. The organic phase was a homogeneous solution containing 1 g L$^{-1}$ (1000 ppm) of the polymer (PCL or PLA) in THF.
4.2.2 Equipment

The experiments have been carried out using two different types of glass capillary devices shown in Figs. 4.1 (b) and (c). The main body of the device was made up of two coaxial glass capillaries: an inner capillary with a circular cross section (1 mm O.D. and 0.58 mm I.D.) and an outer capillary with a square cross section (1 mm I.D.). One end of the inner capillary was shaped into a tapered orifice with an I.D. of 60, 150, 200, 300 or 400 µm. Further fabrication steps have been described in Section 3.3.

4.2.3 Experimental set-up and preparation of polymeric NPs

In a co-flow device (Fig. 4.1 (b)), the organic phase was delivered to the inner capillary, the aqueous phase flowed co-currently through the space between the square and inner capillary and the product suspension exited through the square capillary. In a flow focusing device (Fig. 4.1 (c)), the organic phase was delivered to the outer capillary, the aqueous phase flowed counter-currently through the space between the two capillaries and the product suspension was collected from the inner capillary. These two phases were delivered to the device using a standard method described in Section 3.3.

NPs were formed downstream of the orifice when both streams were brought into contact. This was observed through an inverted microscope and recorded by a Phantom V9.0 high-speed camera (Vision Research, Ametek, US) at 25 frames per second with 576 × 288 resolution. The flow rates of the two phases and the orifice diameter were systematically varied in order to study their effects on the average size of the NPs and their particle size distribution. The fresh nanosuspension was collected in a vial via PTFE tubing (1.5 mm I.D.), after which the organic solvent was completely evaporated in a vacuum oven (Technico, Fistreem International Ltd, Loughborough, UK) under absolute pressures below 10 torr and the room temperature for about 30 min until the smell of THF had disappeared completely.
4.2.4 Characterisation of nanoparticles

4.2.4.1 Particle size analysis

The size distribution of NPs was determined by dynamic light scattering (DLS) using Delsa™ Nano HC Particle Analyser (Beckman Coulter, High Wycombe, UK), which measures the fluctuations of scattered light as a function of time. NPs were diluted 5-fold by Milli-Q water before being transferred into a 4 mL disposable cuvette which was then placed into the instrument. The measurement time was 120 s. Each samples were analysed by following the standard procedures described in Section 2.6.1.1.

4.2.4.2 Zeta potential determination

The zeta potential of NPs was measured by electrophoretic light scattering (Laser Doppler electrophoresis) using a Delsa™ Nano HC Particle Analyser (Beckman Coulter, High Wycombe, UK). The measurements were repeated three times after sample dilution with Milli-Q water. The zeta potential was calculated from the electrophoretic mobility using the Helmholtz-Smoluchowski equation (Submicron, 2011).

4.2.4.3 Microscopic observations (TEM and FEG-SEM)

The internal structure and surface morphology of the NPs was investigated using Transmission Electron Microscopy (TEM) and high resolution Field Emission Gun Scanning Electron Microscopy (FEG-SEM). For TEM analysis, a sample drop was deposited onto a carbon-coated copper mesh and left to dry before being observed by a JEOL JEM-2000 FX transmission electron microscope operated at an accelerating voltage of 200 kV. The mesh was coated by dipping it into a suspension of carbon particles in deionised water.

FEG-SEM images were obtained using a LEO 1530 VP (LEO Elektronenmikroskopie GmbH, Oberkochen, Germany) scanning electron microscope with an integrated EDAX TEAM™ Pegasus EBSD/EDXA (electron backscatter diffraction/energy dispersive x-ray analysis) system. FEG-SEM has the advantage over conventional SEM of providing
higher resolution images due to a smaller diameter of the electron beam, which gives a higher signal to noise ratio leading to improved spatial resolution. The samples were placed onto conventional aluminium sample holders with a diameter of ~1 cm. For NPs imaging, the chamber was evacuated to ~0.5 Pa and the images were taken using in-lens detector operating at an accelerating voltage of 5–10 keV and a working distance of 5–10 mm.

**Fig. 4.1:** (a) A schematic of the experimental set-up with a co-flow glass capillary device: (top) side view, (bottom) bird's-eye view; (b, c) Magnified views of a region near the orifice for: (b) co-flow; (c) flow focusing (c). $D_o =$ orifice diameter.
4.3 Results and Discussion

4.3.1 Prediction of solvent-water interactions

The choice of organic solvent is a crucial initial step that should be taken. The organic solvent must be able to dissolve polymer and must be miscible with water, which can be estimated using the combined solubility parameter (Van Krevelen & Hoftyzer, 1976):

\[
\Delta \delta_{\text{solvent-water}} = [(\delta_{d,S} - \delta_{d,W})^2 + (\delta_{p,S} - \delta_{p,W})^2 + (\delta_{h,S} - \delta_{h,W})^2]^{1/2} 
\]

(4.1)

where \( \delta_d \) is the dispersion solubility parameter due to London dispersion forces resulting from the existence of induced dipoles as two molecules approach each other, \( \delta_p \) is the polar solubility parameter due to Keesom forces occurring when two permanent dipoles are present, and \( \delta_h \) is the hydrogen bonding solubility parameter (Bordes et al., 2010; Hansen, 2007). The subscripts \( S \) and \( W \) refer to the organic solvent and water, respectively. Table 4.1 lists the combined solubility parameters of six potential volatile organic solvents: acetone (Ac), tetrahydrofuran (THF), ethanol (EtOH), dimethyl sulfoxide (DMSO), isopropyl alcohol (IPA) and ethyl lactate (EL), calculated from Eq. (4.1) using the partial solubility parameters from Table 4.1. The value of \( \Delta \delta_{\text{solvent-water}} \) increases in the following order: EtOH < DMSO < IPA < EL < Ac < THF. The smaller the \( \Delta \delta_{\text{solvent-water}} \) value, the higher the affinity of solvent for water and the higher its solubility into the aqueous phase, hence smaller NPs can be produced.

Solvent toxicity is another important aspect for pharmaceutical applications. All solvents in Table 4.1 except THF are categorised as class 3 by the U.S. Food and Drug Administration (FDA). The former permissible daily exposure (PDE) for THF was 121 mg/day and THF was categorised as class 3 solvent. Based on new toxicological data, the PDE for THF is 7.2 mg/day, and the new FDA's recommendation is to move THF from class 3 to class 2.
To completely explain the behaviour of solvent in nanoprecipitation process, the solvent-water interaction parameter must also be considered (Martin et al., 1993):

\[
\chi_{\text{solute-water}} = \frac{V_{\text{solute}}}{RT} (\delta_{\text{solute}} - \delta_{\text{water}})^2
\]

(4.2)

where \( V_{\text{solute}} \) is the molar volume of the solvent, \( R \) is the universal gas constant (8314 J kmol\(^{-1}\) K\(^{-1}\)), \( T \) is the absolute temperature, and \( \delta_{\text{solute}} \) and \( \delta_{\text{water}} \) are the total solubility parameters of the solvent and water, respectively, provided in Table 4.1. The values of \( \chi_{\text{solute-water}} \) calculated using Eq. (4.2) increase in the following order: EtOH < DMSO < IPA < Ac < THF < EL. Solvents that have a high affinity for water, which is evidenced by low \( \chi_{\text{solute-water}} \) values, tend to promote solvent diffusion and polymer partition into the aqueous phase, which leads to the formation of smaller NPs (Legrand et al., 2007; Galindo-Rodriguez et al., 2004). \( \Delta\delta_{\text{solute-water}} \) and \( \chi_{\text{solute-water}} \) in Table 4.1 are in good correlation with each other, indicating that EtOH, DMSO and IPA have the highest affinity for water. On the other hand, Ac, THF and EL show a relatively low affinity for water, either due to their low polarity (e.g. THF), or low hydrogen-bonding preference (Ac) or several combined factors (EL). In addition to solvent-water interactions, the polymer interactions with solvent and water must also be considered.

### 4.3.2 Prediction of polymer-solvent and polymer-water interactions

The extent of polymer-solvent interaction can be estimated from a 2-D graph (Bagley et al., 1971), in which a hydrogen bonding solubility parameter, \( \delta_h \), is plotted against Bagley's two-dimensional solubility parameter, \( \delta_v \), where \( \delta_v = (\delta_v^2 + \delta_d^2)^{1/2} \). The good solvents are those that are included within a circle of a radius of five \( \delta \)-units around the polymer (Van Krevelen & Hoftyzer, 1976; Choi et al., 2002; Su et al., 2007). Fig. 4.2 shows a Bagley’s two-dimensional solubility graph for two polymers (PLA and PCL), water and six organic solvents. The centre of the solubility circle of PLA corresponds to values of \( \delta_h \) and \( \delta_v \) in Table 4.1 listed under the heading PLA\(^a\), calculated based on the
classical method for Hansen solubility parameters (Van Krevelen & Hoftyzer, 1976). As expected, water appears far outside the solubility circle of PLA and PCL, in agreement with the fact that it is a nonsolvent of these polymers. IPA and EtOH are also outside the both solubility circles, due to high $\delta_h$ values as a result of extensive hydrogen bonding between their molecules. Therefore, both solvents are bad solvents for PLA and PCL, but with the highest affinity for water among all the solvents studied. The solubility graph also suggests that acetone is a bad solvent for PLA, whereas DMSO is a bad solvent for PCL. Thus, only THF and EL are good solvents for both polymers and suitable for the formation of PLA and PCL NPs. In a good solvent, polymer chains are more disentangled from one another and extensively solvated. Conversely, in a poor solvent, polymer chains are more shrunken and their solvation is limited (Galindo-Rodriguez et al., 2004).

The solubility of PLA and PCL in the investigated solvents can also be predicted using the Hansen sphere space theory. The distance between a solvent $(S)$ and the polymer $(P)$ in the “$2\delta_d - \delta_p - \delta_h$ solubility space” is given by:

$$D = [4(\delta_{d,S} - \delta_{d,P})^2 + (\delta_{p,S} - \delta_{p,P})^2 + (\delta_{h,S} - \delta_{h,P})^2]^{1/2}$$

(4.3)

The $D$ values for six selected organic solvents calculated from Eq. (4.3) are shown in Table 4.2. Good solvents for the given polymer lie within the solubility sphere of radius $R_0$, known as the interaction radius. The interaction radius for PCL with $M_w = 14,000$ g mol$^{-1}$ is 7.1 (Bordes et al., 2010). From Table 4.2, EtOH, IPA and DMSO are nonsolvents for PCL ($D > 7.1$), which agrees with the predictions from Fig. 4.2. The interaction radius for PLA at 25 $^\circ$C is 6.4 (Hansen, 2007), which means that EtOH, IPA, Ac, and DMSO can be regarded as nonsolvents for PLA.

Polymer-water compatibility can be predicted from the combined polymer-water solubility parameter, $\Delta\delta_{\text{polymer-water}}$: for a good compatibility, $\Delta\delta_{\text{polymer-water}}$ must have a small value (Van Krevelen & Hoftyzer, 1976). The values of $\Delta\delta_{\text{polymer-water}}$ in Table 4.1 increase in the following order: PLA$^a <$ PLA$^b <$ PCL. Clearly, PLA shows higher
compatibility with water, because PLA is more polar than PCL \( \delta_{p,PLA} = 9.7 \) and \( \delta_{p,PCL} = 4.8 \). The polarity of PLA and PCL originates from their ester bonds, but PCL has a longer nonpolar hydrocarbon chain between ester linkages, \([-\text{(CH}_2\text{s}\text{)}_-]\), as compared to PLA, \([-\text{CH(CH}_3\text{s}\text{)}_-]\).

The combined polymer-solvent solubility parameters are shown in Table 4.3. For PLA\(^a\), the \( \Delta \delta_{\text{polymer-solvent}} \) values increase as follows: EL < Ac < THF < IPA < DMSO < EtOH. Therefore, THF, Ac and EL show the highest compatibility with both polymers. The solvent-polymer Flory-Huggins interaction parameter, \( \chi_{\text{solvent-polymer}} \) is another measure of the interaction between polymer chains and solvent molecules and can be calculated as (Hansen, 2007):

\[
\chi_{\text{solvent-polymer}} = \frac{V_{\text{solvent}}}{RT} \left[ (\delta_{d,S} - \delta_{d,P})^2 + (\delta_{p,S} - \delta_{p,P})^2 + (\delta_{h,S} - \delta_{h,P})^2 \right]
\]  \hspace{1cm} (4.4)

The values of \( \chi_{\text{solvent-polymer}} \) for six different solvents are summarised in Table 4.3. For \( \chi_{\text{solvent-polymer}} < 0.5 \), the polymer is soluble in a solvent over entire concentration range (Bordes et al. (2010)) and if \( \chi_{\text{solvent-polymer}} > 0.5 \), the polymer is hardly soluble or insoluble. The results in Fig. 4.2 and Table 4.3 partially contradict each other, since Table 4.3 implies that only EL is a good solvent for PLA\(^a\), while Fig. 4.2 suggests that THF, EL, and DMSO are all good solvents for PLA\(^a\). It may be attributed to large variations in PLA solubility depending on the degree of crystallinity, which is determined by the ratio of \( d \) to \( l \) enantiomers. In this work, THF will be used as a solvent for PLA, since a 50:50 mixture of the \( d \) and \( l \) enantiomers is amorphous and soluble in THF, contrary to pure \( d \) or \( l \) forms. In addition, THF has a boiling point of 66 °C, which is much lower than the boiling point of EL of 151 °C and therefore, can be readily removed from the suspension through vacuum evaporation.
### Table 4.1

The partial solubility parameters, \( \delta_d \), \( \delta_p \), \( \delta_h \), and \( \delta_v \), and the total solubility parameters, \( \delta_t \), of different solvents and polymers, the combined solubility parameters, \( \Delta \delta_{\text{solvent-water}} \) and \( \Delta \delta_{\text{polymer-water}} \), and the interaction parameters, \( \chi_{\text{solvent-water}} \) and \( \chi_{\text{polymer-water}} \) (Ac = acetone, THF = tetrahydrofuran, EtOH = ethanol, DMSO = dimethyl sulfoxide, IPA = isopropyl alcohol, EL = ethyl lactate, PLA = poly(\( \delta, \lambda \)-lactic acid), PCL = poly-\( \varepsilon \)-caprolactone).

<table>
<thead>
<tr>
<th>Solubility parameters ((\text{J cm}^{-3})^{1/2})</th>
<th>Water</th>
<th>Ac</th>
<th>THF</th>
<th>EtOH</th>
<th>DMSO</th>
<th>IPA</th>
<th>EL</th>
<th>PLA(^a)</th>
<th>PLA(^b)</th>
<th>PCL(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \delta_d = \Sigma F_{di} / V )</td>
<td>12.28</td>
<td>15.46</td>
<td>16.77</td>
<td>15.77</td>
<td>18.36</td>
<td>15.80</td>
<td>15.95</td>
<td>17.62</td>
<td>18.50</td>
<td>17.00</td>
</tr>
<tr>
<td>( \delta_p = \left( \sum F_{pi}^2 \right)^{1/2} / V )</td>
<td>31.30</td>
<td>10.40</td>
<td>5.71</td>
<td>10.32</td>
<td>16.32</td>
<td>6.10</td>
<td>7.57</td>
<td>9.70</td>
<td>9.70</td>
<td>4.80</td>
</tr>
<tr>
<td>( \delta_h = \left( \sum E_{hi} / V \right)^{1/2} )</td>
<td>34.17</td>
<td>6.96</td>
<td>7.96</td>
<td>19.38</td>
<td>10.20</td>
<td>16.40</td>
<td>12.48</td>
<td>11.77</td>
<td>6.00</td>
<td>8.30</td>
</tr>
<tr>
<td>( \delta_v = \left( \delta_p^2 + \delta_d^2 \right)^{1/2} )</td>
<td>33.62</td>
<td>18.64</td>
<td>17.72</td>
<td>18.85</td>
<td>24.56</td>
<td>16.94</td>
<td>17.66</td>
<td>20.11</td>
<td>20.89</td>
<td>17.66</td>
</tr>
<tr>
<td>( \delta_t = \left( \delta_p^2 + \delta_d^2 + \delta_h^2 \right)^{1/2} )</td>
<td>48.08</td>
<td>19.90</td>
<td>19.42</td>
<td>27.03</td>
<td>26.60</td>
<td>23.58</td>
<td>21.62</td>
<td>23.31</td>
<td>21.73</td>
<td>19.52</td>
</tr>
<tr>
<td>( \Delta \delta_{\text{solvent-water}} ) or ( \Delta \delta_{\text{polymer-water}} )</td>
<td>0.00</td>
<td>34.45</td>
<td>36.91</td>
<td>25.91</td>
<td>28.91</td>
<td>31.04</td>
<td>32.36</td>
<td>31.57</td>
<td>36.04</td>
<td>37.33</td>
</tr>
<tr>
<td>( \chi_{\text{solvent-water}} ) or ( \chi_{\text{polymer-water}} )</td>
<td>0.00</td>
<td>23.93</td>
<td>27.33</td>
<td>10.61</td>
<td>13.45</td>
<td>18.84</td>
<td>32.94</td>
<td>6.85</td>
<td>6.46</td>
<td>7.13</td>
</tr>
</tbody>
</table>

The partial solubility parameters of the solvents were taken from Burrell (1975). \( \delta \), solubility parameter; subscripts: \( t \), total; \( d \), contribution of the dispersion forces; \( p \), polar contribution; \( h \), hydrogen bonding contribution; \( v \), dispersion and polar contribution. \( V \), molar volume of the compound; \( i \), structural groups within the molecule; \( F_{di} \) and \( F_{pi} \), molar attraction constants due to dispersion and polar interactions, respectively; \( E_{hi} \), energy of hydrogen bonding.

\(^a\) The partial solubility parameters of PLA calculated using the classical method of Van Krevelen & Hoftyzer (1976).

\(^b\) The partial solubility parameters of PLA calculated using the constrained nonlinear optimization method of Agrawal et al. (2004).

\(^c\) The partial solubility parameters of PCL calculated using the classical method of Van Krevelen & Hoftyzer (1976).
Fig. 4.2: Bagley’s two-dimensional graph of the partial solubility parameters of the solvents with respect to the partial solubility parameters determined for PLA and PCL. (line = solubility circle limit for PCL; line = solubility circle limit for PLA).

Table 4.2: The distance $D$ between a solvent ($S$) and the solute ($P$) in the “$2\delta_d - \delta_p - \delta_h$” space and the interaction radius, $R_0$. The points located outside of the solubility circle for the polymer are bolded. Abbreviations and superscripts have the same meaning as in Table 4.1.

<table>
<thead>
<tr>
<th>solvent</th>
<th>Ac</th>
<th>THF</th>
<th>EtOH</th>
<th>DMSO</th>
<th>IPA</th>
<th>EL</th>
<th>$R_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{PLA\text{-solvent}}$</td>
<td>6.50</td>
<td>5.77</td>
<td>8.49</td>
<td>6.96</td>
<td>6.90</td>
<td>4.02</td>
<td>6.4</td>
</tr>
<tr>
<td>$D_{PLA\text{-solvent}}$</td>
<td>6.19</td>
<td>5.63</td>
<td>14.47</td>
<td>7.84</td>
<td>12.26</td>
<td>8.52</td>
<td>10.5</td>
</tr>
<tr>
<td>$D_{PCL\text{-solvent}}$</td>
<td>6.53</td>
<td>1.08</td>
<td>12.62</td>
<td>11.99</td>
<td>8.55</td>
<td>5.44</td>
<td>7.1</td>
</tr>
</tbody>
</table>
Table 4.3: The combined polymer-solvent solubility parameters, $\delta_{\text{polymer-solvent}}$ and the polymer-solvent interaction parameters, $\chi_{\text{polymer-solvent}}$. Abbreviations and superscripts have the same meaning as in Table 4.1. The values of $\chi_{\text{solvent-polymer}} < 0.5$ are bolded indicating a good solvent for the polymer.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\Delta \delta_{\text{polymer-solvent}}$ (J cm$^{-3}$)$^{1/2}$</th>
<th>$\chi_{\text{solvent-polymer}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PLA$^a$</td>
<td>PLA$^b$</td>
</tr>
<tr>
<td>Ac</td>
<td>5.32</td>
<td>3.26</td>
</tr>
<tr>
<td>THF</td>
<td>5.58</td>
<td>4.77</td>
</tr>
<tr>
<td>EtOH</td>
<td>7.86</td>
<td>13.67</td>
</tr>
<tr>
<td>DMSO</td>
<td>6.84</td>
<td>7.84</td>
</tr>
<tr>
<td>IPA</td>
<td>6.14</td>
<td>11.33</td>
</tr>
<tr>
<td>EL</td>
<td>2.80</td>
<td>7.28</td>
</tr>
</tbody>
</table>

4.3.3 Effect of organic solvent removal

The average particle size, $Z_{\text{ave}}$ and the polydispersity index (PDI) were measured in fresh nanosuspensions and the samples stored in a vacuum evaporator (Table 4.4). Due to evaporation of residual THF from PCL particles, the particle diameter decreased 11−14 % of its original size, which is equivalent to the volumetric shrinkage of 28−37 %. After nanoprecipitation, THF is redistributed between the liquid and solid phase. Since $\Delta \delta_{\text{PCL-THF}} \propto \Delta \delta_{\text{THF-water}}$ ($\Delta \delta_{\text{PCL-THF}}$ =1.00 and $\Delta \delta_{\text{THF-water}}$ =36.91 J$^{1/2}$ cm$^{-3/2}$ from Tables 4.1 and 4.3), THF is much more compatible with PCL than water. As a result, the content of THF in the liquid phase immediately after PCL precipitation is 9.1 vol%, while its content in the swollen NPs is about 28–36 vol%. However, due to very small volume fraction of NPs of about 10$^{-4}$, more than 99.9 vol% of THF added to the system is present in the liquid phase, and less than 0.01 vol% is absorbed within the swollen NPs. As THF evaporates, its concentration in the aqueous phase decreases, which causes a decrease in the chemical potential of THF in the liquid phase and further diffusion of THF to the liquid phase until the equilibrium is reestablished. The process of THF dissolution continues until virtually all THF is removed from the NPs. The shrinkage percentage was independent on the initial particle size, which means that THF was completely removed from the particles in all
cases. In all subsequent experiments, THF was completely removed from the NPs before analysis. The PDI values for the fresh samples were in the range of 0.178–0.219 (Table 4.4). After solvent evaporation, the samples were significantly concentrated with a higher agglomera-tion tendency, which led to increased PDI values (0.219–0.294).

Table 4.4: The average size of NPs before and after solvent removal and the resultant linear and volumetric particle size reduction as a function of orifice diameter in a co-flow device at $Q_{aq} / Q_{or} = 10$ ($Q_{aq} = 5$ mL h$^{-1}$, $Q_{or} = 0.5$ mL h$^{-1}$). The organic phase was 1 g L$^{-1}$ PCL in THF and the aqueous phase was Mili-Q water.

<table>
<thead>
<tr>
<th>Orifice diameter (μm)</th>
<th>Without solvent removal</th>
<th>With solvent removal</th>
<th>Linear size reduction (%)</th>
<th>Volume reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$Z_{ave}$ (nm)</td>
<td>PDI</td>
<td>$Z_{ave}$ (nm)</td>
<td>PDI</td>
</tr>
<tr>
<td>60</td>
<td>359 ± 52</td>
<td>0.210 ± 0.016</td>
<td>309 ± 46</td>
<td>0.219 ± 0.010</td>
</tr>
<tr>
<td>150</td>
<td>471 ± 43</td>
<td>0.215 ± 0.008</td>
<td>418 ± 24</td>
<td>0.242 ± 0.014</td>
</tr>
<tr>
<td>200</td>
<td>319 ± 14</td>
<td>0.178 ± 0.057</td>
<td>279 ± 9</td>
<td>0.237 ± 0.060</td>
</tr>
<tr>
<td>300</td>
<td>396 ± 69</td>
<td>0.190 ± 0.008</td>
<td>355 ± 44</td>
<td>0.249 ± 0.025</td>
</tr>
<tr>
<td>400</td>
<td>594 ± 25</td>
<td>0.219 ± 0.008</td>
<td>509 ± 14</td>
<td>0.297 ± 0.050</td>
</tr>
</tbody>
</table>

4.3.4 Effects of aqueous-to-organic flow rate ratio, orifice size and polymer type

4.3.4.1 Constant aqueous phase flow rate and variable organic phase flow rate

In these experiments in a co-flow device, $Q_{aq}$ was kept constant at 5 mL h$^{-1}$ and $Q_{or}$ ranged from 3.3 to 0.5 mL h$^{-1}$ corresponding to $Q_{aq} / Q_{or}$ ratio from 1.5 to 10, respectively. The size of NPs was found to decrease with increasing $Q_{aq} / Q_{or}$, as shown in Fig. 4.3. At higher $Q_{aq} / Q_{or}$ ratio, the particle nuclei are more diluted after formation, which suppresses the rate of particle growth given by: $dl/dt = K_g (C_i - C^*)^b$, where $K_g$ is the particle growth rate constant and $C_i$ and $C^*$
are the polymer concentration on the particle surface and the saturation concentration, respectively. The value of the parameter b is usually between 1 and 3 (Zhao et al., 2007). The increased water flow rate decreases the polymer concentration on the particle surface, \( C_i \), leading to a decrease in \( C_i - C^* \) and the rate of particle growth, thereby resulting in smaller ultimate particle size. The increased water volume also decreases the tendency for particle aggregation due to lower frequency at which particles collide with and stick to each other. The particle aggregation is most likely near the orifice, where the local particle concentrations are high. In addition, a higher flow rate ratio provides a more rapid mixing in a microfluidic system (Génot et al., 2010). In a more rapid mixing process, the critical supersaturation needed for nucleation is reached faster, which allows for the generation of more nuclei, whose growth will be limited by the amount of available polymer in the liquid phase. Therefore, a larger number of nuclei will lead to smaller size of NPs. The smaller particle sizes at higher aqueous-to-organic volume ratios were also obtained by Laouini et al. (2013a, 2013b, 2013c) in the production of liposomes and polymeric micelles in membrane contactors and by Jahn et al. (2010) in the formation of liposomes in planar flow focusing microfluidic mixers.

At constant \( Q_{aq}/Q_{or} \) ratio, the particle size was found to significantly increase with increasing the orifice size, \( D_o \) over the range of 200-400 \( \mu m \) (Fig. 4.3). The mixing process is more efficient if the organic phase is injected through smaller orifice, due to greater shear stresses in the mixing zone and higher interfacial area per unit volume of the organic phase. At \( Q_{aq}/Q_{or} = 1.5 \) and \( D_o = 60 \mu m \), the velocity of organic stream in the orifice is 0.324 m s\(^{-1}\) and the velocity of the surrounding aqueous phase is 1.4 \( \times 10^{-3} \) m s\(^{-1}\). However, at \( Q_{aq}/Q_{or} = 1.5 \) and \( D_o = 400 \mu m \), the organic phase velocity in the orifice is only 7.3 \( \times 10^{-3} \) m s\(^{-1}\) and the aqueous phase velocity is 1.6 \( \times 10^{-3} \) m s\(^{-1}\). Due to small difference in velocity between the two streams, the mixing process is less efficient leading to higher particle size. At \( D_o = 60 \mu m \), the particle size was somewhat larger than that at 200 \( \mu m \), which may be due to susceptibility of 60-\( \mu m \) orifice to particle deposition and clogging, which may compromise the particle size.
Fig. 4.3: The average particle size, $Z_{ave}$ of PCL nanoparticles produced at different flow rate ratios in a co-flow device with different orifice diameters, $D_o$. The PCL concentration in the organic phase is 1 g L$^{-1}$ and $Q_{aq}$ = 5 ml h$^{-1}$.

The micrographs of mixing zone in the device with a 60-μm orifice size at various flow rate ratios are shown in Fig. 4.4. At $Q_{aq}/Q_{or}$ = 10 (Fig. 4.4 (a)), the interface is spherical and resembles a familiar shape which can be seen when one immiscible liquid is introduced into another in the dripping regime (Vladišavljević et al., 2012). It is hard to explain this shape without acknowledging some type of interfacial tension, although THF and water are miscible in all proportions and should have zero equilibrium interfacial tension. In fact, when two miscible fluids are suddenly put into contact, gradients of composition and density at the boundary can give rise to tension between the contacted fluids, which is known as the transient interfacial tension or Korteweg stress (Joseph & Venkatachalappa, 1999), given by:

$$\sigma = k\Delta C^2 / \delta$$  \hspace{1cm} (4.5)
where \( k \) is the proportionality constant, \( \Delta C \) is the change in concentration over the transition zone between two miscible fluids and \( \delta \) is the thickness of the transition zone. The transient tension decreases rapidly during the process of dissolution in proportion to \( \sqrt{D/t} \), where \( D \) is the diffusion coefficient and \( t \) is the interfacial age.

At each \( Q_{aq}/Q_{or} \) value, there is a certain equilibrium size of a droplet formed at the capillary tip. At equilibrium, the rate of diffusion of the organic phase from the interface, due to mutual mixing at the contact zone, is equal to the rate of convective flow from the orifice toward the interface. The produced NPs form dark concentric layers around the interface, due to capillary waves (Fig. 4.4 (a)). At \( Q_{aq}/Q_{or} = 4.5 \) (Fig. 4.4 (b)), the organic phase forms a widening jet due to increased inertial force that overcomes the transient interfacial tension and elongates the interface. The organic phase velocity at the orifice, \( U_{or} = 0.32 \text{ m s}^{-1} \), is much higher than the aqueous phase velocity, \( U_{aq} = 1.4 \times 10^{-3} \text{ m s}^{-1} \), leading to deceleration of the organic phase in the direction of flow and causing widening of the jet. With further increase in velocity of the organic phase, a flow instability phenomenon known as “viscous fingering” occurs (Fig. 4.4 (c)), which leads to distortion of the interface and formation of finger-like patterns. Such instability occurs typically when a less viscous fluid is injected into a more viscous one (it should be noted that the viscosity of THF at 293 K is 0.63 mPa s and the water viscosity is 0.99 mPa s). The penetration of the less viscous fluid is not uniform since part of the more viscous fluid forms fjords, named “viscous fingers” (Homsy, 1987). Viscous fingering was not observed when ethanolic solution of phospholipids was injected into water in the same type of capillary device (Vladisavljević et al., 2014), because the viscosity of ethanol of 1.25 mPa s was higher than the water viscosity. At \( Q_{aq}/Q_{or} = 1.5 \), two symmetrical vortices were formed at the lower and upper parts of the capillary tube (Fig. 4.4 (d)), due to high shear stress at the interface, caused by high difference in velocity between the organic and aqueous phase.
The particle size distribution curves at $D_o$=150 and 200 μm are in good agreement with the above observations featuring the minimum particle size at the maximum flow rate ratio (Fig. 4.5), due to shortest mixing time. The growth of nuclei is more limited if the mixing process is faster, which will lead to smaller NPs. At $Q_{aq}/Q_{or} = 10$, the mixing time is shortest due to the smallest amount of injected organic phase relative to aqueous phase. As a result, the interface disappears at the distance of just $4.4D_o$ downstream of the nozzle (Fig. 4.4 (a)) and the NPs have the minimum size. At $Q_{aq}/Q_{or} = 1.5$, the mixing time is long due to high amount of injected organic phase. In addition, as a result of vortex flow, the nuclei formed near the nozzle are forced into circular motion, which can lead to their much longer residence time compared to the nuclei formed more downstream. As a consequence, the particle size distribution is very broad, as shown in Fig. 4.5 (a).

The effect of polymer type on the size of NPs at the orifice size of 60 μm is shown in Fig. 4.6 (a). PLA formed smaller particles than PCL, because PLA is more compatible
with water, as can be seen by the lower $\Delta \delta_{\text{polymer-water}}$ value in Table 4.1. As a result, precipitation of PLA starts when the water content in THF reaches 31 vol%, while PCL starts precipitating out when the water content in THF is about 16 vol%. Therefore, PLA starts to precipitate from a more diluted polymer solution, which limits particle growth and leads to smaller particle size.

4.3.4.2 Constant organic phase flow rate and variable aqueous flow rate

In this set of experiments, the organic phase flow rate was kept constant at 1.7 mL h$^{-1}$ and the aqueous phase flow rate varied from 2.55 to 17 mL h$^{-1}$, corresponding to $Q_{\text{aq}}/Q_{\text{or}}$ value from 1.5 to 10 respectively (Fig. 4.6 (b-d)). At the same $Q_{\text{aq}}/Q_{\text{or}}$ value, the particle size was smaller when the organic phase flow rate was maintained at 1.7 mL h$^{-1}$ compared to the fixed aqueous phase flow rate of 5 mL h$^{-1}$. At $Q_{\text{or}} = 1.7$ mL h$^{-1}$, the total flow rate, $Q_{\text{aq}} + Q_{\text{or}}$ was in the range of 4.25-18.7 mL h$^{-1}$, whereas at $Q_{\text{aq}} = 5$ mL h$^{-1}$, the total flow rate was 5.5-8.3 mL h$^{-1}$. Probably, the mixing efficiency is higher at the higher flow rate in the collection capillary. Triple runs were carried out on each experiment to check reproducibility of the particle sizes and only small within-runs variations were observed, as indicated by small error bars. The opposite results were obtained by Jahn et al. (2010) in microfluidic preparation of liposomes, with smaller vesicle sizes obtained at smaller total flow rates. The minimum size of both PLA and PCL particles in a co-flow device was less than 250 nm and was achieved at $Q_{\text{aq}}/Q_{\text{or}} = 10$ and for a 60-μm orifice size.
Fig. 4.5: The size distribution of PCL NPs as a function of aqueous-to-organic flow rate ratio in a co-flow device at $Q_{aq} = 5$ ml h$^{-1}$. The orifice size, $D_o$: (a) 200 µm; (b) 150 µm. The organic phase was 1 g L$^{-1}$ PCL in THF and the aqueous phase was Milli-Q water.
Fig. 4.6: (a) The average particle size, $Z_{ave}$ as a function of flow rate ratio, $Q_{aq}/Q_{or}$ and orifice diameter, $D_o$ in a co-flow device. The flow rate of either organic or aqueous phase was kept constant in each series of experiments. The organic phase was 1 g L$^{-1}$ mm PCL or PLA in THF (Fig. a) or 1 g L$^{-1}$ PCL in THF (Fig. b–d) and the aqueous phase was Milli-Q water.
4.3.5 Co-current flow versus counter-current flow focusing

Micromixing in a glass capillary device has also been achieved by countercurrent flow focusing. The micrographs of the mixing zone of the device with an orifice size of 400 µm at \( Q_{or} = 1.7 \text{ mL h}^{-1} \) and variable aqueous phase flow rate are shown in Fig. 4.7. Due to high velocity of aqueous phase, the jetting regime occurs at all flow rate ratios with very long widening jets and no signs of interfacial instability. The phase boundary is sharp at \( Q_{aq}/Q_{or} = 10 \) and 7, becomes blurred at \( Q_{aq}/Q_{or} = 4.5 \) and almost completely disappears at \( Q_{aq}/Q_{or} = 3 \). A sharp interface occurs due to sharp concentration gradients at the contact zone resulting from high velocity of aqueous phase in the tapered section of the inner capillary. At \( Q_{aq}/Q_{or} = 3 \), \( \Delta C/\delta \approx 0 \) due to relatively long residence time of liquid elements and mutual mixing of THF and water upstream of the orifice and \( \sigma \approx 0 \); thus, the phase boundary is hardly visible. At \( Q_{aq}/Q_{or} = 1.5 \), the phase boundary is invisible (the image not shown here). The dark areas in Fig. 4.7 are the regions within the device where the NPs are formed at relatively high concentration. These regions are mainly contact zones between the two phases upstream of the orifice where the fluid velocities are relatively small due to large cross-sectional area available for flow.

Fig. 4.8 provides a comparison of the average particle size, \( Z_{ave} \) in a co-flow and flow focusing device for the same flow rates and device geometry. Triple runs were carried out on each sample and small error bars in the graph indicate high reproducibility. The mixing time in a microfluidic flow focusing device is given by (Karnik et al., 2008): \( \tau_{mix} \propto d_{or}^2 / D \), where \( d_{or} \) is the diameter of the organic phase stream and \( D \) is the diffusivity of the solvent. The organic phase stream is wider at smaller \( Q_{aq}/Q_{or} \) (Fig. 4.7), which results in longer mixing times and larger \( Z_{ave} \) value (Fig. 4.8). The smaller NPs were produced in a flow focusing device compared to co-flow device of the same orifice size, which was most pronounced at the orifice size of 400 µm. In a flow focusing device, the diameter of the organic phase stream in the mixing zone is significantly smaller than the orifice diameter (\( d_{or} \ll D \)), while in a co-flow device...
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the diameter of the organic phase stream corresponds to the orifice diameter \( d_{or} \approx D_o \). Therefore, under the same other conditions, \( \tau_{mis} \) in flow focusing device is much smaller than that in a co-flow device. At \( D_o = 60 \, \mu m \), there was no difference in performance between a co-flow and flow focusing microfluidic mixer. The orifice is prone to clogging in a flow focusing device by the particles formed upstream of the orifice, which can be deposited onto the inner walls of the collection capillary as they pass through the orifice. Therefore, the optimum diameter of the orifice in flow focusing device was about 200 \( \mu m \).

**Fig. 4.7:** The shape of liquid/liquid interface in a counter-counter flow device with a 400-\( \mu m \) orifice diameter at different flow rate ratios: (a) \( Q_{aq} / Q_{or} = 10.0 \); (b) \( Q_{aq} / Q_{or} = 7 \); (c) \( Q_{aq} / Q_{or} = 4.5 \); and (d) \( Q_{aq} / Q_{or} = 3.0 \). The organic phase was 1 g L\(^{-1}\) PCL in THF and the aqueous phase was Mili-Q water.
Fig. 4.8: (a) The comparison of average particle size, $Z_{ave}$ in a co-current and counter-current flow device at $Q_{or} = 1.7$ mL h$^{-1}$ for the orifice diameter, $D_o$ is: (a): 400 $\mu$m; (b) 200 $\mu$m; (c) 60 $\mu$m. The organic phase was 1 g L$^{-1}$ PCL in THF and the aqueous phase was Mili-Q water.

4.3.6 Effect of surfactant on NPs formation

In this section, PCL NPs were produced in a co-flow device at at $D_o = 200$ $\mu$m and $Q_{aq}/Q_{or} = 10$ in the presence of four different types of hydrophilic surfactant,
polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), Tween 20 and Tween 80. The concentration of each surfactant in the aqueous phase was fixed at 1 wt%, which is a typical value in nanoprecipitation process (Xie & Smith, 2010). The minimum particle size of 387 ± 7.0 nm was achieved using PVA, followed by PVP, Tween 20 and Tween 80. The $Z_{\text{ave}}$ value for the NPs produced under the same conditions without any surfactant was 279 ± 9 nm. The particle size was higher in the presence of surfactant in the aqueous phase which was due to the formation of surfactant layer on the particle surface. The presence of surfactant may also lead to an increase in the viscosity of aqueous phase which was reported to increase the particle size due to reduction in the rate of counter-diffusion of solvents (Tsukada et al., 2009).

**4.3.7 Stability of NPs**

The variation of the size of NPs with time is a good indicator of particle stability, since in most cases the particle size increases before macroscopic changes appear (Heurtault et al., 2003). In this study, the variations in mean particle size and zeta potential were measured over a storage period of 30 days at ambient temperature. Fig. 4.9 shows the average particle size, $Z_{\text{ave}}$ and the zeta potential as a function of time for PCL NPs produced in a counter-flow device at $D_o = 200 \mu m$ and $Q_{\text{aq}}/Q_{\text{or}} = 10$ in the absence and presence of surfactant (PVP) in the aqueous phase. In the absence of any surfactant, the size of NPs increased from 244 to 486 nm, indicating that the presence of PVP in the aqueous phase was vital in order to improve the storage stability of NPs and prevent particle aggregation. In the presence of 1 wt% PVP, the initial particle size was higher, since each polymer particle was surrounded by the surfactant corona. Nevertheless, the $Z_{\text{ave}}$ values increased only moderately over 30 days from 286 to 348 nm with negligible change in zeta potential from −3.72 to −3.37 mV. The same range of zeta potentials was reported by Filipović et al. (2013) for PCL-PVP NPs. The surfactant molecules are absorbed onto the surface of the newly formed PCL NPs with some chains extending away from the particle surface, which provides steric barrier and prevents particle coalescence (Lebouille et al., 2013).
Fig. 4.9: The average size, $Z_{ave}$, and zeta-potential of PCL NPs over a 30 day storage period at atmospheric pressure and ambient temperature. The particles were produced in a counter-current flow device at $Q_{aq}/Q_{or}=10$, $Q_{or}=1.7$ mL h$^{-1}$, and $D_o=200$ μm. The organic phase was 1 g L$^{-1}$ PCL in THF and the aqueous phase was Milli-Q water or 1 wt% PVP.

4.3.8 Microscopic images of NPs

Scanning electron micrographs of PCL and PLA NPs are shown in Fig. 4.10 (a) and (b). The PLA particle exhibits a nearly perfect spherical shape, as reported by Lai & Tsiang (2004). The PCL compared to the PLA NPs, due to crystallisation of PCL on the surface (Lin & Huang, 2001). The surface of PLA NPs was very smooth because a fully amorphous poly-(d,l-lactide) was used for particle formation. In addition, PCL NPs are larger than PLA NPs, which is in a good agreement with the dynamic light scattering data for the two polymers.

Fig. 4.10 (c) and (d) are TEM images of PCL and PLA NPs. The PLA particles in Fig. 4.10(d) have a very smooth surface and almost perfect spherical shape, as a result of
surface energy minimization during their formation. The size of both NPs was within a range of 200–320 nm. When administrated intravenously, NPs should be sufficiently small (100–300 nm) to passively cross the tumour endothelial barrier and then retain in the tumour bed for prolonged time due to reduced lymphatic drainage, which is known as the enhanced permeability and retention effect (Kobayashi et al., 2014). Particles larger than 1 mm are not convenient for intravascular delivery of drugs, since they can readily be opsonized with a possibility of capillary occlusion, while NPs smaller than 5 nm can be cleared rapidly from the blood via extravasation or renal clearance (Elsabahy & Wooley, 2012).

Fig. 4.10: (a) FEG-SEM micrograph of individual PCL particle; (b) FEG-SEM micrograph of individual PLA particle and; (c) TEM image of PCL NPs; (d) TEM image of PLA NPs.

4.4 Chapter summary

In this study, a new microfluidic method for the preparation of biodegradable nanoparticles was developed based on micromixing/nanoprecipitation in co-flow and flow focusing glass capillary devices. The particle size was precisely tuned by varying orifice size of the inner capillary, flow rate ratio and the total flow rate in the collection
capillary. The higher the aqueous-to-organic flow rate ratio, the higher the dilution factor of the polymer in the liquid phase and the lower the rate of particle growth after nucleation, resulting in smaller particle size. At the same liquid flow rates, the mixing process was faster when the organic phase was injected through smaller orifice, which led to the generation of more nuclei, whose growth was limited by the amount of available polymer in the liquid phase, thereby resulting in smaller ultimate particle size. At constant flow rate ratio and orifice size, PLA formed smaller particles than PCL, because PLA is more compatible with water than PCL and starts to precipitate from a more diluted organic solution, which limits particle growth. The PLA particles exhibited a smoother surface and more regular spherical shape than PCL particles, which can be related to fully amorphous structure of d-l type polylactide.

In a co-flow device, a decrease in the aqueous-to-organic flow rate ratio led to the following sequential changes in the shape of the phase boundary: spherical interface → widening jet → viscous fingering → vortex flow. A spherical interface suggests that a transient interfacial tension occurs between two miscible fluids (water and THF) immediately after injection as a result of high concentration gradients at the contact zone. In a flow focusing device, a widening-jet regime prevailed at all flow rates.

Smaller NPs were produced in a flow focusing device compared to a co-flow device of the same geometry, because in the former case the diameter of the organic stream was significantly smaller than the orifice diameter. PCL particles formed in flow focusing device with an orifice size of 200 μm at the organic stream flow rate of 1.7 mL h⁻¹ and a flow rate ratio of 10 were smaller than 200 nm. Such small NPs are capable of spontaneous accumulations in various pathological sites via the enhanced permeability and retention effect. The mean size of PCL NPs formed in a co-flow device of the same geometry under the same flow rates was 227 nm.

Further experimental work will be focused on encapsulation of hydrophobic drug (acetaminophen) within biodegradable polymer matrix and the optimisation of process parameters using design of experiments (DOE) software and methods. The nanoparticles will be embedded with nanoclays to modify their internal structure and drug release patterns.
CHAPTER 5

DEVELOPMENT OF ACETAMINOPHEN LOADED MONODISPERSED NANOPARTICLES FORMULATION USING FACTORIAL DESIGN APPROACH PRODUCED IN A GLASS CAPILLARY MICROFLUIDICS DEVICE

Chapter overview

Nanoencapsulation of acetaminophen (PCM) onto poly(ε-caprolactone) (PCL) polymer matrix by nanoprecipitation (“diffusion-stranding” process) was studied using glass capillary microfluidic device consisted of two different miscible liquids. PCL was chosen as a carrier for nanoparticle fabrication, because it is a biodegradable and bioresorbable polymer commonly used in pharmaceutical industry, while tetrahydrofuran (THF) was used as a water-miscible volatile solvent. Five (5) investigated independent input variables included PCL concentration (A), co-flow device orifice size (B), flow rate ratios (C), surfactant concentration (D) and acetaminophen percentage loading (E) were evaluated. Percentage of encapsulation efficiency (Y₁), % drug loading (Y₂) and particle mean size, Zₐᵥₑ (Y₃) were used as the response variables. A 2⁵-full factorial design was applied and allowed screening for the most influential variables. The optimum formulation with highest desirability percentage for each response was achieved by using the concentration of PCL (A) at 5.96 mg/ml, orifice size (B) at 60 μm, flow rate ratios, Qₐ𝑞/Qₒ𝑟 (C) at 10, PVP surfactant concentration (D) at 0.1 % (w/w) and acetaminophen loading (E) at 20 % (w/w). The size of PCM-loaded PCL NPs was 268 nm mean size with 49.25 % encapsulation efficiency and 6.96 % of drug loading which is well correlated to the predicted results given by simulation. The in vitro drug release profile showed a sustained acetaminophen up to 140 hours indicating the suitability of PCL nanoparticles in controlled acetaminophen release. Transmission electron microscopy (TEM), field emission gun scanning electron microscope (FEG-SEM), differential scanning calorimetry (DSC) and X-ray diffractometry (XRD) analyses were successfully confirmed the properties of functionised PCM-loaded PCL NPs.

5.1 Introduction

Paracetamol (C₈H₉NO₂), is used as an analgesic and antipyretic, in the treatment of a wide variety of arthritic and rheumatic conditions involving musculoskeletal pain and in other
painful disorders such as headache, dysmenorrhoea, myalgia and neuralgia (Billon et al., 2000; Kashyap et al., 2011). The conventional oral dose of paracetamol for adults must be in the range of 325–1000 mg (650 mg rectally) with total daily dose should not exceed than 4000 mg (Kashyap et al., 2011). Scientifically, it is known as acetaminophen and its toxicity is the foremost cause of acute liver failure in the Western world, and accounts for most drug overdoses in the United States, the United Kingdom, Australia and New Zealand (Bateman et al., 2006; Daly et al., 2008; Hawton et al., 2001; Hughes et al., 2003; Jefferies et al., 2011; Larson et al., 2005; Morgan & Majeed, 2005; Thomas 1993; Ward & Alexander-Williams, 1999). Since an overdose of acetaminophen can lead to a fatal centrilobular liver injury, a prolonged and controlled release rate of acetaminophen warrants a study of its nanoencapsulation in polymeric nanorospheres. In previous works, acetaminophen has been encapsulated within anionic acrylic resin microspheres by a W/O/O emulsion-solvent diffusion method (Lee et al., 2000). Sustained release systems using acetaminophen as the model drug were also investigated by Shimokawa et al. (2013). Lai & Tsiang (2004) studied the controlled release rate of poly(l-lactide) microspheres containing acetaminophen as the core material using the oil-in-water emulsification solvent-evaporation technique. Ward & Alexander-Williams (1999) also reported a new method to control acetaminophen and protein delivery at different stages from modified core–shell biodegradable microspheres produced by solvent evaporation method.

Biodegradable polymer nanoparticles are of great interest as drug delivery systems because of their ability to be reabsorbed by the body. Synthetic aliphatic polyesters, such as poly-ε-caprolactone (PCL), are often used in biomedical applications because they are biocompatible and non-toxic (Wu et al., 2000) behaviour. The advantages of PCL include its high permeability to small drug molecules, and its negligible tendency to generate an acidic environment during degradation as compared to polylactide (PLA) and poly(lactic-co-glycolic acid (PLGA). The degradation of PCL homopolymer is very slow as compared to other polyesters, making it more suitable for long-term delivery systems extending to a period of more than 1 year, and with appropriate blending the delivery can
be increased/decreased as desired (Abdolmohammadi et al., 2012; Woodruff & Hutmacher, 2010). PCL is a semi-crystalline polymer having a glass transition temperature ($T_g$) of $-60^\circ$C and melting point ranging between 59 and 64 $^\circ$C, dictated by the crystalline nature of PCL which enables easy formability at relatively low temperatures. Recently, Tshweu et al. (2013) discovered new formulation parameters for the encapsulation of lamivudine into poly(ε-caprolactone) (PCL) nanoparticles matrix using a double emulsion spray drying technique for improving HIV treatment. Spherical nanoparticles with an average size of 215 ± 3 nm and polydispersity index (PDI) of 0.227 ± 0.01 were obtained in their study. Bilensoy et al. (2009) designed a cationic nanoparticulate carrier system between 180–340 nm in size by encapsulating an intravesical chemotherapeutic agent Mitomycin C (MMC) onto PCL-chitosan (CS-PCL) and PCL-poly-l-lysine (PLL-PCL) matrix, respectively. Gupta et al. (2011) also developed a method to encapsulate influenza A virus (A/California/07/2009) H1N1 hemagglutinin (HA) recombinant protein onto the PCL-chitosan nanoparticulate. Behera et al. (2013) also focused on the development and enhancement of entrapment efficiency of isoniazid loaded poly-ε-caprolactone nanoparticles for the treatment of tuberculosis.

A central challenge in the development of drug-encapsulated polymeric nanoparticles is the inability to control the mixing processes required for their synthesis resulting in variable nanoparticle physicochemical properties (Karnik et al., 2008). The ability of microfluidics to rapidly mix reagents, provide homogeneous reaction environments, continuously vary reaction conditions, and add reagents at precise time intervals during reaction progression has made it an attractive technology for a myriad of applications (DeMello, 2006; deMello & deMello, 2004). Currently, Khan et al. (2013) succeeded to encapsulate high ketoprofen contents in acrylate-based copolymer microbeads by environment friendly UV induced free radical polymerization in off-the-shelf co-axial microfluidic device. Yeh et al. (2009) used a T-junction microfluidic chip for monodisperse calcium alginate microparticles and encapsulation of nanoparticles emulsification process. A microfluidic origami chip to synthesise monodisperse doxorubicin-loaded poly(lactic-co-glycolic acid) nanoparticles with optimum diameters.
of ~100 nm for cellular uptake and anticancer efficacy was successfully demonstrated by Sun et al. (2013). Capretto et al. (2013) also applied a hydrodynamic flow-focusing microfluidic device in the production of polymeric micelles for combined delivery of Dex and ascorbyl-palmitate (AP). Whilst, Jahn et al. (2007) produced liposome in a continuous-flow planar microfluidic network with precise control of size over the diameter range of 50–150 nm through the manipulation of liquid flow rates.

Solvent evaporation, monomer polymerization, nanoprecipitation and salting out procedure are some of the common methods applied for the preparation of nanoparticles from biodegradable polymers (Quintanar-Guerrero et al., 1998). However, nanoprecipitation method was developed by Fessi et al. (1989) represents an easy and reproducible technique and very often used to prepare colloidal carriers both matricidal (nanosphere) and vesicular type (nanocapsules). This method was based on the interfacial deposition of a polymer following diffusion of a semi-polar and miscible solvent in the aqueous medium in the presence of a surfactant (Barichello et al., 1999). Several important factors contribute to the effectiveness of this method in preparing particles with acceptable size range, shape and the percentage of the drug load, namely the amount of polymer, percentage of surfactant and volume of organic and aqueous phases. It is difficult to assess the effect of each variables or their combination, thus deriving a mathematical model suitable for establishing a quantitative relationship between the formulation variables is seemingly promising (Derakhshandeh et al., 2007; Yang & Zhu, 2002; Seth & Misra, 2002).

Limited number of studies has been reported on numerical analysis especially for the encapsulation of hydrophilic drug onto the polymeric nanoparticles matrix using flow-focusing approach. For instance, Thi et al. (2013) developed a new solution in optimizing the taste-masked formulation of acetaminophen using sodium caseinate and lecithin by $2^4$–full factorial experimental design by taking into account the inlet variables; temperature ($X_1$), the spray flow ($X_2$), the sodium caseinate amount ($X_3$) and the lecithin amount ($X_4$). Derakhshandeh et al. (2007) also demonstrated the effects of individual
input variables or in combination by deriving a suitable mathematical model for establishing a quantitative relationship between the formulation variables for 9-nitrocamptothecin (a novel anticancer drug) encapsulation in poly(d,l-lactide-co-glycolide) nanoparticles. The effects of dependent variables drug-polymer ratio ($X_1$) and surfactant concentration ($X_2$) on particle size and encapsulation efficiency of Glipizide loaded PCL nanoparticles were also studied by Lokhande & Mishra (2013) using a $3^2$–full factorial design. Kalani et al. (2011) optimised the process parameters resulted in production of nanoencapsulated paracetamol in l-polylactide including pressure, temperature, and polymer concentration, to produce fine small spherical particles with a narrow particle size distribution using a supercritical antisolvent method. While, Riddin et al. (2006) explained the analysis of inter- and extracellular formation of platinum nanoparticles by *Fusarium oxysporum* f. sp. *lycopersici* using experimental design for the response surface methodology. Zu et al. (2009) also tried to optimize the preparation process of vinblastine sulfate (VBLS)-loaded folate conjugated bovine serum albumin (BSA) nanoparticles for tumor-targeted drug delivery Design-Expert® Version 7.0.0 software.

In light of these above observations, this study is aimed to evaluate various formulation variables involved in the preparation of acetaminophen loaded nanoparticles by nanoprecipitation method using glass capillary microfluidic device. A two-level factorial design experiment was used for obtaining a mathematical model and prediction of optimized formulations. A $2^5$-full factorial design was applied with five (5) different independent input variables included; (A) PCL concentration, (B) co-flow device orifice size, (C) flow rate ratios, (D) surfactant concentration and (E) acetaminophen percentage loading were evaluated. Percentage of encapsulation efficiency ($Y_1$), % drug loading ($Y_2$) and particle mean size, $Z_{ave}$ ($Y_3$) were used as the response variables. The produced PCM-loaded PCL NPs (hereinafter called the PCM-PCL NPs) were characterised according to their morphology, particle size, encapsulation efficiency, drug loading and in vitro drug release behaviour.
5.2 Materials and methods

5.2.1 Materials

Tetrahydrofuran (THF) (for HPLC, purity ≥ 99.9 %) was supplied by Sigma-Aldrich Co., UK. Poly(ε-caprolactone) (PCL) (M_w=14,000 g mol⁻¹) in flakes form was purchased from Sigma-Aldrich Chemical Co. with a glass transition temperature (T_g) of 60 °C. Polyvinyl pyrrolidone (PVP) with M_w= 360 000 g mol⁻¹ in powder form was purchased from Sigma-Aldrich Co., UK applied as the surfactant in the aqueous phase. Acetaminophen (PCM) (purity ≥ 99.9 %) in powder was purchased from Fisher Scientific, UK. All chemicals and solvents used were of analytical grade. The aqueous phase used as an anti-solvent in microfluidic experiments was distilled water produced by reverse osmosis (MiliQ®, Millipore).

5.2.2 Preparation of acetaminophen loaded nanoparticles using co-flow device

The experimental set-up used for the experiments is shown in Fig. 5.1. Two 11 Elite syringe pumps (Harvard Apparatus, UK) were used to deliver the organic and aqueous phase from SGE syringes to their respective capillaries. The organic phase containing 0.3–0.6 % (w/w) polymer and 0.02–0.07 % (w/w) acetaminophen in THF was injected through the inner capillary tube with a tapered cross section culminated in a circular orifice via a Teflon (polytetrafluoroethylene, PTFE) tubing (1.59 mm O.D. and 0.8 mm I.D.), which was highly resistant to THF. While, the aqueous phase consisted of 0.2–0.5 % (w/w) PVP (surfactant) dissolved in Milli-Q water was delivered co-currently through the outer square capillary.

The formation of encapsulated nanoparticles occurred instantaneously in the outer capillary when both of the phases were brought into contact. These two phases were delivered to the device using a standard method explained in Section 3.3. This was observed through a Phantom V9.0 high-speed camera as described in Section 4.2.3. The size of orifice and the organic phase flow rate (Q_or) were varied in order to study their effects on the size of obtained nanoparticles. Initially, the aqueous phase flow rate (Q_auq)
was fixed at 5 mL h\(^{-1}\) and the organic phase flow rate ranged from 0.5 to 3.3 mL h\(^{-1}\) corresponding to the aqueous to organic phase volume ratio \(Q_{\text{aq}}/Q_{\text{or}}\) from 1.5 to 10.0.

**Fig. 5.1:** Experimental set-up for the preparation of acetaminophen encapsulated nanoparticles by nanoprecipitation method using flow focusing device.

### 5.2.3 Determination of the drug content

The nanosuspension was separated using ultracentrifugation (Beckman L-8 60M Ultracentrifuge) at 15,000 rpm for 1 h at 20 °C. The supernatant containing the dissolved free drug was discarded and transferred to UV–visible spectrophotometer (UV-2550, Shimadzu, Japan) for drug content analysis. The pellet was then freeze-dried (Edwards Modulyo Freeze-drier) for 48 h for further characterisation analyses. A stock solution of acetaminophen (10,000 mg/L) was used to prepare ten standard solutions in the range from 5–50 mg L\(^{-1}\) with milli-Q water. The following equations were applied in order to calculate the drug loading (DL) and the encapsulation efficiency (EE).

\[
\text{D.L.} \,(\%) = \left[ \frac{(W_i - W_2)}{W_i} \right] \times 100 \%	ag{5.1}
\]

where \(W_i\) is the initial weight of drug that was incorporated into the nanoparticles, \(W_2\) is the weight of free drug (unincorporated drug) and \(W_i\) is the weight of nanoparticles.

\[
\text{E.E.} \,(\%) = \left[ 1 - \left( \frac{W_f}{W_i} \right) \right] \times 100 \%	ag{5.2}
\]
where \( W_T \) is the total amount of drug in release medium and \( W_i \) is the total theoretical quantity of drug added initially during preparation (Calvo & Remunan-Lopez, 1997; Douglas & Tabrizian, 2005; Muthu et al., 2009; Zheng et al., 2009; Kumar et al., 2012).

### 5.2.4 Full factorial experimental design

Based on preliminary study of the effect of parameters on the drug loading of nanosphere, the experiments were performed by nanoprecipitation method using a two-level full factorial design. Design-Expert® Version 7.1.5 software was applied for designing the experiment. Five (5) parameters included the poly(\( \varepsilon \)-caprolactone) (PCL) concentration, mg/ml (\( A \)), orifice size, µm (\( B \)), flow rate ratios, \( Q_{aq}/Q_{or} \) (\( C \)), PVP surfactant concentration, (w/w) % (\( D \)), and the percentage (%) of paracetamol amount (\( E \)). Each factor is set at one of two levels i.e. low (−1) and high (+1) level as shown in Table 5.1.

**Table 5.1**: Values for the low (−1) and high (+1) levels of input variables investigated in the factorial design.

<table>
<thead>
<tr>
<th>Input variables</th>
<th>Coded units</th>
<th>Low level (−)</th>
<th>High level (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL concentration (g L(^{-1}))</td>
<td>( A )</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Orifice size (µm)</td>
<td>( B )</td>
<td>60</td>
<td>200</td>
</tr>
<tr>
<td>Flow rate ratios, ( Q_{aq}/Q_{or} )</td>
<td>( C )</td>
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<td>10</td>
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<tr>
<td>PVP surfactant concentration (w/w)(^ a )</td>
<td>( D )</td>
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<tr>
<td>Paracetamol amount (w/w)(^ b )</td>
<td>( E )</td>
<td>20</td>
<td>70</td>
</tr>
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</table>

\(^{a}\) Note: \(^{a}\) = Percentage values were calculated based on mass of aqueous phase (mili-Q water).

\(^{b}\) = Percentage values were measured based on amount of polymer (PCL).

In this screening step, a full factorial design involving five experimental variables was conducted to identify the processing and formulation parameters that have significant influence on the investigated responses. The full factorial required hence \( 2^5 \) or 32 experiments of which the experimental conditions are listed in Table 5.2, with five variables and three different responses; encapsulation efficiency (\( Y_1 \)), % drug loading (\( Y_2 \)) and particle mean size, \( Z_{ave} \) (\( Y_3 \)). Triple runs were carried out on each batch of experiments to check reproducibility.
Table 5.2: Experimental design and percentage of three different responses ($n = 3$).

<table>
<thead>
<tr>
<th>Run</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>Y1 (%)</th>
<th>Y2 (%)</th>
<th>Y3 (nm)</th>
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<td>70</td>
<td>28.20</td>
<td>11.61</td>
<td>425.9</td>
</tr>
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</table>

*Note: Each batch was prepared three times ($n = 3$) and the mean response values were determined.*
5.2.5 Characterisation of the encapsulated acetaminophen nanoparticles

5.2.5.1 Particle size analysis

The size of nanoparticles was determined by dynamic light scattering (DLS) using Delsa™ Nano HC Particle Analyzer (Beckman Coulter, Inc), which measures the fluctuations of scattered light as a function of time. Nanoparticles were diluted 5-fold by Mili-Q water before being transferred into a 4 mL disposable cuvette which was then placed into the instrument. The measurement was repeated three times for each of the measurement time was 120 s at 25 °C. Each samples were analysed by following the standard procedures described in Section 2.6.1.1.

5.2.5.2 Microscopic Observations (TEM and FEG-SEM)

The biodegradable polymeric NPs images and its microscopic structures were investigated using two different microscopic analysers; (i) Transmission Electron Microscopy (TEM) and (ii) Field Emission Gun Scanning Electron Microscope (FEG-SEM). Each samples were analysed by following the standard procedures described in Section 4.2.4.3.

5.2.5.3 Differential scanning calorimetry (DSC)

The thermal analyses were performed with a TA Instrument Q100 DSC. ~5−10 mg of pure PCL, pure acetaminophen, physical mixture of PCL and acetaminophen and encapsulated nanospheres. The encapsulated nanoparticles sediments obtained from ultracentrifugation process were then solidified using freeze-drier for 48 h before transferring to DSC analysis. The samples were individually put in aluminum pans and hermetically sealed. These sample pans were heated and compared against an empty pan from 30–220 °C at a heating rate of 10 °C min⁻¹, with dry nitrogen as the effluent gas (60 mL min⁻¹). Triple runs were carried out on each sample to check reproducibility.
5.2.5.4 X-ray diffractometry (XRD)

X-ray diffractometer, Bruker D8 diffractometer (Bruker, Germany), was used for diffraction studies. XRD studies were performed by exposing samples to CuKa radiation (40 kV, 20 mA) and scanned from 2° to 70°, 2θ at a step size of 0.02° and step time of 5 s. Samples used for XRD analysis were the same as those used for DSC analysis. The 2θ range was from 10−40° at a scanning speed of 0.5° min⁻¹. Samples for wide angle X-ray diffraction were injection molded under nitrogen at 100 °C and transferred at ambient temperature to a circular mold with a diameter of 20 mm and a thickness of 1 mm. Samples for this analysis can be either in the solid or liquid form.

5.2.5.5 In vitro drug release measurement

The in vitro release profile of acetaminophen-loaded nanospheres was determined as follows. An exact amount of nanospheres (after 30 minutes collection time) from microfluidic experiment was subjected to vacuum for 30 minutes in order to remove any excess organic solvents from the nanosuspension samples. The samples were then transferred to ultracentrifugation (Beckman L-8 60M Ultracentrifuge) at 15 000 rpm for 1 h at 20 °C. The supernatant water containing the dissolved free drug was removed from centrifugation tube and analysed for its acetaminophen content using an UV–visible spectrophotometer (UV-2550, Shimadzu, Japan) at absorbance of 243 nm (Wang et al., 2010) while the sediment (encapsulated nanoparticles) was remained in the centrifugation tube for further release experiment. Two different dissolution mediums (Mili-Q water and phosphate buffer (PBS) (0.01M, pH 7.4)) were applied in this study and the samples were incubated in a shaking bath at 50 rpm at 37 °C. The dissolution medium was withdrawn at precise intervals and replenished with fresh Mili-Q water or PBS to keep the releasing medium volume constant. UV–vis spectrophotometry was used to measure amount of drug released.
5.3 Results and discussion

5.3.1 Acetaminophen standard calibration by UV-Vis spectroscopy analysis

The acetaminophen (PCM) stock solutions in Mili-Q water were prepared for two different concentrations (1000 ppm and 10000 ppm) at various concentrations of standard samples from 5–50 ppm. In this analysis, the acetaminophen concentration detection limit and qualification was at 55 g mL\(^{-1}\). Two replications were determined and their mean values were calculated for each of the stock calibration solutions in order to ensure the accuracy of the standard calibration curve. The solutions were scanned in the wavelength range of 200–300 nm using UV–visible spectrophotometer. Fig. 5.2 depicts the standard calibration curve obtained for two different concentrations of pure PCM stock solutions at different investigated initial concentrations. Both standard curves were linear within investigated concentration range (\(y = 0.063x, r^2 > 0.9\)) and there was almost no difference was detected for both graphs.

![Diagram showing standard calibration curve with two sets of data points for 1000 ppm and 10000 ppm. The equations are \(y = 0.0633x, R^2 = 0.9747\) for 1000 ppm and \(y = 0.0636x, R^2 = 0.9964\) for 10000 ppm.]

**Fig. 5.2:** Pure acetaminophen (PCM) standard calibration curve.
Further analysis was then elucidated the interference of blank PCL nanoparticles existence on UV-Vis absorbance signal. Different concentrations of PCM were applied together with certain amount of suspended PCL nanoparticles was applied in this analysis. The PCM-W solution was added into PCL-W solution at various PCM concentrations before being transferred to UV-Vis spectroscopy for absorbance detection. Fig. 5.3 explicates the PCM-PCL NPs standard calibration at different concentration of PCM. Note that the PCL nanoparticles existence was found significantly interfered the UV-Vis signal with much higher detectable UV-Vis light was appeared compared to individually pure PCM component. The same observation was considered in Govender et al. (1999) experimental works for encapsulated PLGA nanoparticles. This finding significantly proved that slight amount of the drug was re-adsorbed or closes to the surface of the nanoparticles after the evaporation of organic solvents, a phenomenon recently by the other authors (Brigger et al., 2001) and not totally entrapped in the polymeric core. Although, centrifugation technique was applied to separate nanosuspension, it is entirely impossible to remove the most of the nanoparticles from the sample solution due to its tiny size.

![Graph](image)

**Fig. 5.3:** Pure paracetamol (PCM) and PCM-PCL NPs standard calibration curves.
5.3.2 Measurement of the marker concentration percentage reduction

Regan & Mulvihill (2009) evaluated the suitability of methylene blue and vitamin B\textsubscript{12} (Vit-B\textsubscript{12}) as water soluble inner aqueous phase (W\textsubscript{1}) markers for measuring the encapsulation efficiency and stability of water-in-oil-in-water (W\textsubscript{1}/O/W\textsubscript{2}) double emulsions stabilized by sodium caseinate (NaCN). The same technique was applied in this research in order to investigate the intrinsic PCM absorption effect onto the polymeric nanoparticles surface via blank experiment method by considering PCM as the marker. In this experiment, PCM was inversely dissolved in the aqueous phase with PVP as surfactant and in the organic phase, the polymer (PCL) was dissolved in organic solvent (tetrahydrofuran, THF). The blank experiment was done using co-flow microfluidic device at different flow rate ratios and concentration of PCL (3 g L\textsuperscript{-1} and 6 g L\textsuperscript{-1}). No intrinsic PCM absorption effect was detected on nanoparticles surface at 1 g L\textsuperscript{-1} concentration of PCL.

Different standard calibration graphs were performed at various PCM concentration values in aqueous phase at different flow rate ratios in order to measure the reduced concentration factors (%\textit{R}). The concentration of each marker (PCM concentration) in the recovered outer aqueous phase was measured from the absorbance values by referring to the standard curve for each marker. Fig. 5.4 (a–b) shows, the %\textit{R} values for 3 g L\textsuperscript{-1} at flow rate ratio of \(Q_{\text{aq}}/Q_{\text{or}} = 1.5\) and \(Q_{\text{aq}}/Q_{\text{or}} = 10.0\), respectively. While, Fig. 5.4c–d depicts the %\textit{R} values for 6 g L\textsuperscript{-1} at flow rate ratio of \(Q_{\text{aq}}/Q_{\text{or}} = 1.5\) and \(Q_{\text{aq}}/Q_{\text{or}} = 10.0\), respectively. The %\textit{R} values were then subtracted from the actual concentration measured from encapsulation experiment prior to get the real encapsulation efficiency. Equations; [%\textit{R} = 0.004\textbf{C}_{\text{PCM}} + 80.306] and [%\textit{R} = 0.002\textbf{C}_{\text{PCM}} + 81.252], with \textbf{C}_{\text{PCM}} as the marker concentration can be considered for both 3 g L\textsuperscript{-1} and 6 g L\textsuperscript{-1} organic phase concentration, respectively.

5.3.3 Statistical analysis by \(2^5\) factorial design

A technique of two-level factorial design offers the possibility of investigating five independent variables at two levels after performing only thirty two (32) batches of
different combinations were prepared by taking values of selected variables: A, B, C, D and E at two levels as shown in Table 5.1. The mathematical models of each response variables generated by the design are given in the following Eqs. 5.3–5.4.

% **Encapsulation efficiency** $(Y_1) = 38.09 + 2.20 \times A - 4.19 \times B - 0.73 \times C + 2.40 \times D + 2.37 \times E - 4.17 \times AB - 8.05 \times AE - 2.08 \times BC + 1.59 \times BD - 4.88 \times BE - 0.15 \times CE - 2.46 \times ABE + 2.97 \times BCE$

... (5.3)

% **Drug loading** $(Y_2) = 11.30 - 0.34 \times A - 1.80 \times B + 0.83 \times D + 5.34 \times E - 1.52 \times AB - 2.05 \times AE - 1.92 \times BE - 1.23 \times ABE$

... (5.4)

**Particle size, nm** $(Y_3) = 371.54 + 17.43 \times A + 19.48 \times B - 19.84 \times C + 31.22 \times D + 9.08 \times E - 8.89 \times AB + 7.19 \times AD + 1.44 \times BD - 25.25 \times BE - 19.51 \times ABD$

... (5.5)

The analysis of variance (ANOVA) was applied to determine the significance and the magnitude effects of the main variables and their interactions on the response variables (Zu et al., 2009). The summary of the ANOVA factorial design is presented in Table 5.3. Note that the lack of fit of each model was statistically significant if the values of "Prob $> F$" less than 0.0500. Values greater than 0.1000 indicate the model terms are not significant. For the first response $(Y_1)$, the model F-value of 32.71 implies the model is significant with individually input variables and combination variables; $A, B, D, E, AB, AE, BC, BD, BE, ABE, BCP$ are significant model terms. The "Pred R-Squared" of 0.7375 is in reasonable agreement with the "Adj R-Squared" of 0.8013. While, for second model $(Y_2)$, the model F-value of 68.11 implies the model is significant with influence variables; $B, D, E, AB, AE, BE, ABE$. The "Pred R-Squared" of 0.9216 is in reasonable agreement with the "Adj R-Squared" of 0.9454. Final response $(Y_3)$ showed 9.37 model F-value indicates as significant model with influenced parameters $A, B, C, D, BE, ABD$. The "Pred R-Squared" of 0.5747 is in reasonable agreement with the "Adj R-Squared" of 0.7296. Out of five different variables, parameter $A$ (PCL concentration), $B$ (orifice size), $D$ (PVP concentration) and $E$ (PCM concentration) were found significantly effects for each
responses and individual parameter $C$ (flow rate ratio) is the only relevant to response $Y_1$ (% encapsulation efficiency) and $Y_3$ (particle size, nm). Thus, any changes in flow rate ratio will not give pronounced effect to $Y_2$ (% drug loading) response. This is due to only minor differences were detected from response $Y_2$ corresponded to the parameter $C$. In fact, extremely small changes and indistinguishable values were detected in weight of nanoparticles over the percentage of drug loading ($Y_2$). Nevertheless, parameter $C$ also must be considered for response $Y_2$ as this response was intially defined by response $Y_1$.

The optimum formulations also offered by this software as shown in Table 5.4. The encapsulation efficiency ($Y_1$) response was set as the maximum and the other two responses ($Y_2$ and $Y_3$, nm) were set as the minimum target. This simulation strategy was applied based on the experimental results obtained by Govender et al. (1999). The calculated desirability factor for offered formulations was nearly 1.00 indicating suitability of the designed factorial model. Run 1 shows the selected optimum formulation for acetaminophen encapsulation nanoparticles. The concentration of PCL ($A$) is at 5.96 g L$^{-1}$, orifice size ($B$) at 60 μm, flow rate ratios, $Q_{aq}/Q_{or}$ ($C$) at 10, PVP surfactant concentration ($D$) at 0.1 % (w/w) and acetaminophen loading ($E$) at 20 % (w/w). The effect of the polymer concentration ($A$) increment values on the nanoparticles size appears mainly to be due to the higher resultant organic phase viscosity, which leads to larger nanoparticles’ formation. Meanwhile, the increasing values in drug content of the nanoparticles with increased theoretical drug loading ($E$) may have resulted in the increased particle sizes ($Y_3$) displayed. The low drug incorporation percentage values ($Y_2$) may be attributed to the water soluble nature of paracetamol. This led to its rapid partitioning into the aqueous phase and hence decreased entrapment into the nanoparticles during polymer deposition. The large surface area of the nanoparticle geometry may have also contributed to loss of drug into the aqueous phase during preparation. Low drug incorporation efficiency (drug loading) of another water soluble drug, 5-fluorouracil, into PLGA nanoparticles has also observed by Niwa et al. (1993).
The smaller orifice size ($B$), 60 μm was appeared as the optimum size in order to produce smaller particle size. The small value in flow rate ratio ($C$), 10 also profoundly induced the smaller size of particle size as requested in this study. Theoretically, the polymer in the organic phase will be more viscous and its tendency to form a much smaller particle is higher. In fact, the formation of more diluted nanoparticles suspension will hinder the fusion of small polymeric nanoparticles to larger particles. Mora-Huertas et al. (2010) also summarised a suggested composition of stabilizer agent within the range of 0.2–0.5 % of non-solvent in the preparation of nanocapsules by nanoprecipitation method. However in this study, 0.1 % (w/w) of surfactant ($C$) indicated as the optimum value in the formation of paracetamol encapsulated nanoparticles. This value is principally acceptable and helps to hinder the formation of particle agglomeration which potentially can be occurred simultaneously by the increasing amount of surfactant. The value of % $Y_1$ also increased with the increasing polymer concentration ($A$). Similar observation was reported in other research works (Mehta et al., 1994; Li et al., 1999; Rafati et al., 1997). High viscosity and fast solidification of the dispersed phase contributed to reduce porosity of the nanoparticles as well as delays the drug diffusion within the polymer particles (Bodmeier & McGinity, 1988). The concentrated polymer will also tend to precipitate faster on the surface of the dispersed phase and prevents drug diffusion across the phase boundary (Rafati et al., 1997).
Fig. 5.4: Calibration curves of reduced concentration factor (%) for 3 g L\(^{-1}\) at flow rate ratio of (a) \(Q_{aq}/Q_{or} = 1.5\) and (b) \(Q_{aq}/Q_{or} = 10.0\). Reduced concentration factor (%) for 6 g L\(^{-1}\) at flow rate ratio of (c) \(Q_{aq}/Q_{or} = 1.5\) and (d) \(Q_{aq}/Q_{or} = 10.0\).
Table 5.3: Analysis of variance for a $2^5$ Full-factorial design experiment for evaluation of some variables’ effect on acetaminophen encapsulated nanoparticles.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Response 1: % Encapsulation efficiency ($% Y_1$)</th>
<th>Response 2: % Drug loading ($% Y_2$)</th>
<th>Response 3: Particle size, nm ($Y_3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sum of squares</td>
<td>$df^*$</td>
<td>$F$ value</td>
</tr>
<tr>
<td>Model</td>
<td>5185.78</td>
<td>13</td>
<td>32.71</td>
</tr>
<tr>
<td>A</td>
<td>155.54</td>
<td>1</td>
<td>12.75</td>
</tr>
<tr>
<td>B</td>
<td>562.38</td>
<td>1</td>
<td>46.11</td>
</tr>
<tr>
<td>C</td>
<td>17.07</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>D</td>
<td>184.27</td>
<td>1</td>
<td>15.11</td>
</tr>
<tr>
<td>E</td>
<td>179.88</td>
<td>1</td>
<td>14.75</td>
</tr>
<tr>
<td>AB</td>
<td>555.19</td>
<td>1</td>
<td>45.52</td>
</tr>
<tr>
<td>AD</td>
<td>$-$</td>
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</tr>
<tr>
<td>AE</td>
<td>2072.88</td>
<td>1</td>
<td>169.96</td>
</tr>
<tr>
<td>BC</td>
<td>138.65</td>
<td>1</td>
<td>11.37</td>
</tr>
<tr>
<td>BD</td>
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<tr>
<td>BE</td>
<td>762.94</td>
<td>1</td>
<td>62.56</td>
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<tr>
<td>CE</td>
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<td>1</td>
<td>0.057</td>
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<tr>
<td>ABE</td>
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<td>1</td>
<td>15.89</td>
</tr>
<tr>
<td>ABD</td>
<td>$-$</td>
<td>$-$</td>
<td>$-$</td>
</tr>
<tr>
<td>BCE</td>
<td>281.62</td>
<td>1</td>
<td>23.09</td>
</tr>
</tbody>
</table>

$df^*$, degrees of freedom.

* Significance level based on 1 $df$; $p < 0.01$. 

Rahimah Othman 2016
Table 5.4: Factorial design optimised formulations.

<table>
<thead>
<tr>
<th>Run</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>( Y_1 ) (pre.)</th>
<th>( Y_1 ) (exp.)</th>
<th>( Y_2 ) (pre.)</th>
<th>( Y_2 ) (exp.)</th>
<th>( Y_3 ) (pre.)</th>
<th>( Y_3 ) (exp.)</th>
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</thead>
<tbody>
<tr>
<td>33</td>
<td>5.97</td>
<td>60.01</td>
<td>10.00</td>
<td>0.10</td>
<td>20.00</td>
<td>50.34</td>
<td>49.25 ± 2.17</td>
<td>6.98</td>
<td>6.87 ± 1.30</td>
<td>268.34</td>
<td>258.00 ± 2.17</td>
</tr>
<tr>
<td>34</td>
<td>5.81</td>
<td>60.06</td>
<td>10.00</td>
<td>0.10</td>
<td>20.02</td>
<td>49.58</td>
<td>47.30 ± 4.59</td>
<td>6.76</td>
<td>6.56 ± 3.88</td>
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<td>256.57 ± 4.19</td>
</tr>
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<td>35</td>
<td>5.96</td>
<td>60.00</td>
<td>9.99</td>
<td>0.10</td>
<td>20.47</td>
<td>50.55</td>
<td>48.24 ± 4.56</td>
<td>7.08</td>
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<td>268.43</td>
<td>259.14 ± 3.48</td>
</tr>
<tr>
<td>36</td>
<td>5.71</td>
<td>60.00</td>
<td>10.00</td>
<td>0.10</td>
<td>20.13</td>
<td>47.14</td>
<td>45.04 ± 4.47</td>
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<td>6.23 ± 2.94</td>
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<td>256.11 ± 4.38</td>
</tr>
<tr>
<td>37</td>
<td>6.00</td>
<td>69.00</td>
<td>10.00</td>
<td>0.10</td>
<td>20.00</td>
<td>46.65</td>
<td>44.71 ± 4.15</td>
<td>6.99</td>
<td>6.11 ± 3.77</td>
<td>275.04</td>
<td>252.62 ± 5.81</td>
</tr>
<tr>
<td>38</td>
<td>5.47</td>
<td>60.09</td>
<td>10.00</td>
<td>0.10</td>
<td>20.00</td>
<td>50.80</td>
<td>50.73 ± 0.14</td>
<td>6.30</td>
<td>7.12 ± 1.04</td>
<td>268.41</td>
<td>271.84 ± 3.09</td>
</tr>
<tr>
<td>39</td>
<td>5.29</td>
<td>60.00</td>
<td>9.90</td>
<td>0.10</td>
<td>20.01</td>
<td>48.56</td>
<td>48.48 ± 0.18</td>
<td>6.07</td>
<td>7.20 ± 0.88</td>
<td>268.41</td>
<td>255.65 ± 5.64</td>
</tr>
<tr>
<td>40</td>
<td>6.00</td>
<td>60.33</td>
<td>9.41</td>
<td>0.10</td>
<td>20.00</td>
<td>48.57</td>
<td>48.32 ± 0.51</td>
<td>7.00</td>
<td>6.93 ± 0.41</td>
<td>270.75</td>
<td>274.03 ± 2.94</td>
</tr>
<tr>
<td>41</td>
<td>6.00</td>
<td>77.54</td>
<td>10.00</td>
<td>0.10</td>
<td>20.02</td>
<td>50.84</td>
<td>48.91 ± 3.79</td>
<td>6.97</td>
<td>7.16 ± 5.97</td>
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</tr>
<tr>
<td>42</td>
<td>5.09</td>
<td>60.00</td>
<td>10.00</td>
<td>0.11</td>
<td>20.00</td>
<td>49.03</td>
<td>48.77 ± 0.53</td>
<td>5.84</td>
<td>6.96 ± 0.28</td>
<td>269.72</td>
<td>270.54 ± 2.78</td>
</tr>
</tbody>
</table>

*Note:* 1. Each batch was prepared three times. 2. Run 1 is the selected combined parameters for optimum formulation.

5.3.4 Nanoparticles characterisations

5.3.4.1 Differential scanning calorimetry (DSC) analysis

The DSC thermograms corresponding to acetaminophen, PCL, physical mixture of PCL and acetaminophen and acetaminophen encapsulated nanoparticles are shown in Fig. 5.5. This analysis is aimed to characterise the physical state of PCM encapsulated nanoparticles. The PCL thermogram displayed an endothermic peak at 60 °C, corresponding to the polymer melting point (\( T_m \)). The DSC curve of PCM (acetaminophen) showed a single melting peak at 169 °C and started to degrade as it melted. Very small PCM melting peak was visible in the case of drug loaded nanoparticles. This might be due to amorphous state of the drug dispersed in the nanoparticles. Since there was no shift in the \( T_m \) of the polymer, it can be concluded that there is no significant interaction occurring between the drug and the polymer. For physical mixture small peak was detectable at the melting point of PCM as PCM just mixed together with polymer. Since glass–liquid transition of PCL occurs at relatively lower temperature (− 60 °C), it is possible that at higher temperatures molecular dispersion of drug in polymer occurs during DSC process. The same observation was observed by Thi et al. (2013), Lai & Tsiang (2004) and Derakhshandeh et al. (2007).
Fig. 5.5: Differential scanning calorimetry (DSC) thermograms obtained for acetaminophen, polymer, acetaminophen-polymer physical mixture and encapsulated nanoparticles.

5.3.4.2 X-ray diffractometry (XRD) analysis

To confirm these results XRD was performed and results are presented in Fig. 5.6. As shown XRD patterns of PCM and physical mixture exhibit a sharp peak at about 2θ scattered angle 13.5 indicating crystalline nature of acetaminophen (PCM). This characteristic peak for drug was found nearly disappeared for the encapsulated nanoparticle XRD pattern which suggesting no crystal can be observed on the encapsulated particle surface. This finding indicates that the drug seems to be well entrapped in the polymeric nanoparticles core. This remarkable result was also reported by Zhang et al. (2006) and Derakhshandeh et al. (2007).
Fig. 5.6: XRD patterns of acetaminophen, polymer, acetaminophen-polymer physical mixture and encapsulated nanoparticles.

5.3.4.3 Microscopic image observation (FEG-SEM and TEM images)

The microscopic observations on blank PCL and acetaminophen encapsulated PCL nanoparticles presented in Fig. 5.7 (a-b) using FEG-SEM. Both nanoparticles showed a spherical shape of nanoparticles as well as individually distributed with homogeneous particle size distribution. Minor agglomerations were detected in these images probably due to the effect of the vacuum during free drying stage on the formed nanoparticles. The structure of blank PCL nanoparticles were nearly spherical compared to encapsulated nanoparticles, whereas the structure of encapsulated nanoparticles seems has been distorted by the encapsulated drug contents. The same observation was presented by Wang et al. (2010) and Lai & Tsiang (2004) for acetaminophen encapsulation microspheres using PLA and poly(lactic-co-glycolic acid (PLGA) polymer, respectively. The size of PCL nanoparticles was much larger compared to the encapsulated nanoparticles. These results showed a good results with
values obtained using Delsa™ Nano HC particle size analyzer with dynamic light scattering (DLS) approach of the measurement.

Similar observation was acquired for Transmission Electron Microscopy (TEM) images, as depicted in Fig. 5.8 (a-b) for blank PCL and acetaminophen encapsulated PCL nanoparticles, respectively. The spherical shape of nanoparticles can be seen clearly in Fig. 5.8 (a) for blank PCL and slight deformed structure was detected for the encapsulated nanoparticles due to the drug content. The size of PCL nanoparticles looks much bigger than PLA nanoparticles within the same magnification. The size of measured nanoparticles in this analysis was within the range of 200–280 nm for the encapsulated PCL nanoparticles which significantly similar with synthesised encapsulated nanoparticles (268 nm) at the optimum conditions.

5.3.4.4 In vitro release of encapsulated acetaminophen nanoparticles

The amount of acetaminophen released from nanoparticles was evaluated using two different dissolution mediums (Mili-Q water and phosphate buffer (PBS) (0.01M, pH 7.4)) and the samples were incubated in a shaking bath at 50 rpm at 37°C in order to mimic the normal blood stream conditions. The cumulative release profiles of acetaminophen from PCL nanoparticles are shown in Fig. 5.9. About 30 % of drug showed burst released over a period of 24 hours, followed by a distinct prolonged release up to more than 140 hours. The followed delayed release may be attributed to diffusion of the dissolved drug within the PCL core of the nanoparticle into the dissolution medium. The drug was dissolute and diffused from the polymer matrices to the aqueous phase due to the concentration gradient between dissolution medium, hence nanoparticles matrix was started to erode resulting from degradation of polymer. Minor different profiles were discovered for both dissolution mediums (water and buffer, pH 7.4). However, the release profile in buffer solution was much faster compared to the water solution because of its different concentration and charge dissimilarity. The drug profile exhibited nearly constant changes once it achieved 60 % cumulative drug release at 24 hours release period and kept persistent until 140 hours. This indicates that the drug was most likely amended onto the polymer core-matrix and a sustained drug release from PCL nanospheres could also be achieved due to the presence of the polymer layer.
Fig. 5.7: FEG-SEM images of; (a-b) blank poly(ε-caprolactone (PCL) and (c-d) acetaminophen encapsulated PCL nanoparticles at various magnifications.

Fig. 5.8: TEM images of; (a) blank PCL nanoparticles and (b) PCM encapsulated PCL nanoparticles at the same magnifications.
Fig. 5.9: In vitro release profile of acetaminophen from PCL NPs in two different release mediums (water and buffer solution in pH 7.4), data presents means ($n = 3$).

5.4 Conclusions

In the present study, the preparation and characterisation of nanoparticles of acetaminophen as an analgesic and antipyretic drug was carried out. For simultaneous analysis of the influence of different factors on the properties of the nanoparticles and to find optimum formulations, the formulation was optimized using a 32 factorial experimental design. The polymer amount, drug content and surfactant had a statically significant influence on the drug encapsulation efficiency and particle size. The optimum formulation with highest desirability percentage for each response was achieved by using the concentration of PCL ($A$) at 5.96 g L$^{-1}$, orifice size ($B$) at 60 μm, flow rate ratios, $Q_{aq}/Q_{or}$ ($C$) at 10, PVP surfactant concentration ($D$) at 0.1 % (w/w) and acetaminophen loading ($E$) at 20 % (w/w). In vitro release studies showed that PCL nanoparticles could successfully control the release of acetaminophen up to more than 140 h. In general the results show that the PCL nanoparticles may be considered as a promising carrier system for controlled release in clinical application.
CHAPTER 6

FABRICATION OF COMPOSITE POLY(\text{d,\text{L}-LACTIDE})/MONTMORILLONITE NANOPARTICLES FOR CONTROLLED DELIVERY OF ACETAMINOPHEN BY SOLVENT-DISPLACEMENT METHOD USING GLASS CAPILLARY MICROFLUIDICS

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Chapter overview

Paracetamol (PCM)-loaded composite nanoparticles (NPs) composed of a biodegradable poly(\text{d,\text{L}-lactide}) (PLA) polymer matrix filled with organically modified montmorillonite (MMT) nanoparticles were fabricated by antisolvent nanoprecipitation in a microfluidic co-flow glass capillary device. The incorporation of MMT in the polymer improved both the drug encapsulation efficiency and the drug loading, and extended the rate of drug release in simulated intestinal fluid (pH 7.4). The particle size increased on increasing both the drug loading and the concentration of MMT in the polymer matrix, and decreased on increasing the aqueous to organic flow rate ratio. The drug encapsulation efficiency in the NPs was higher at higher aqueous to organic flow rate ratio due to faster formation of the NPs. The PCM-loaded PLA NPs containing 2 wt\% MMT in PLA prepared at an aqueous to organic flow rate ratio of 10 with an orifice size of 200 \(\mu\)m exhibited a spherical shape with a mean size of 296 nm, a drug encapsulation efficiency of 38.5 \% and a drug loading of 5.4 \%. The encapsulation of MMT and PCM in the NPs was confirmed by transmission electron microscopy, energy dispersive x-ray spectroscopy, x-ray diffraction, differential scanning calorimetry, thermogravimetric analysis and attenuated total reflection-Fourier transform infrared spectroscopy.

6.1 Introduction

Many drugs are insoluble in aqueous media and biological fluids and, thus, their effects are diminished due to poor bioavailability. To enhance their solubility, drug
molecules can be incorporated in the interlayer space of layered clays (San Román et al., 2013). Montmorillonite (MMT) is a natural clay mineral that belongs to the smectite group, in which a central alumina octahedral sheet is sandwiched between two silica tetrahedral sheets with a thickness of individual layers of $\leq 1$ nm. The imperfection of the crystal lattice and the isomorphic substitution of $\text{Mg}^{2+}$ for $\text{Al}^{3+}$ induces a net negative charge that leads to the adsorption of alkali and alkaline-earth metal cations in the interlayer space (Joshi et al., 2009a). These cations can be exchanged with cationic forms of therapeutic molecules and drugs. The examples of such active species adsorbed onto MMT by cationic bonding are protonated forms of amino acids (Kollár et al., 2003), promethazine chloride (Seki & Yurdakoç, 2006), timolol maleate (Joshi et al., 2009b), buformin hydrochloride (Fejér et al., 2001), 5-fluorouracil (Lin et al., 2002), sertraline (Nunes et al., 2007), vitamin B1 (Joshi et al., 2009a; 2009b) and buspirone hydrochloride (Joshi et al., 2010). Anionic drugs can be intercalated into the interlayer space of MMT by hydrogen bonding between their functional groups and hydroxyl groups or oxygen atoms in MMT. For example, ibuprofen was adsorbed due to hydrogen bonding between its $-\text{COO}^-$ groups and hydroxyl groups of MMT (Zheng et al., 2007).

MMT exhibits high drug loading capacity due to high specific surface area, and provides mucoadhesive properties required for drug delivery across the gastrointestinal barrier (Carretero, 2002; Dong & Feng, 2005; Datta, 2013). MMT has been proved to be nontoxic by hematological, biochemical and histopathological analyses (Lee et al., 2005). MMT is a highly efficient detoxifier which has been used in the treatment of open wounds, hemorrhoids, stomach ulcers, intestinal problems and other diseases (Lee & Chen, 2004; Lee & Fu, 2003; Lin et al., 2002).

Paclitaxel- and docetaxel-loaded PLGA/MMT nanoparticles (NPs) have been prepared by an emulsion/solvent evaporation method using dichloromethane as organic solvent for PLGA (Dong & Feng, 2005; Feng et al., 2009). MMT was added to the aqueous phase and adsorbed onto the surface of the polymer NPs after solvent evaporation. Due to its hydrophilic nature, MMT is incompatible with most polymers and must be chemically modified to be incorporated within the polymer matrix. MMT can be rendered hydrophobic by replacing inorganic cations in the interlayer space.
with organic alkylammonium or alkylphosphonium cations (Datta, 2013; Suresh et al., 2010; Duan et al., 2013). Lee et al. (2000) encapsulated paracetamol within Eudragit® S100 microspheres using water-in-oil-in-oil double emulsion-solvent evaporation method. Shimokawa et al. (2013) prepared gelatin microcapsules loaded with the pain-relieving drug phenacetin by a simple coacervation technique. Lai & Tsiang (2004) produced PLA microcapsules containing paracetamol using O/W and W/O/W emulsion-solvent evaporation methods.

In this work, nanoprecipitation induced by microfluidic micromixing was used to produce novel composite paracetamol-loaded NPs consisting of organically modified MMT incorporated into poly(β,β-lactide) (PLA) NPs (hereinafter called the PLA/MMT NPs). PLA is a biodegradable and bioresorbable polymer (San Román et al., 2013) widely used as a drug carrier. The inclusion of organically modified MMT into the polymer matrix has proven to be a viable strategy to improve the thermal, mechanical, and barrier properties of PLA sheets (Duan et al., 2013; Pluta, 2004). In this work, MMT was incorporated into PLA NPs to increase drug bioavailability and modify its release. Paracetamol (acetaminophen, C₈H₉NO₂) was chosen as a model drug due to its significant therapeutic effects against a wide variety of arthritic and rheumatic conditions involving musculoskeletal pain and other painful disorders (Kashyap et al., 2011; Billon et al., 2000; Ward & Alexander-Williams, 1999).

Nanoprecipitation (solvent displacement) is a low-energy process for preparation of NPs driven by spontaneous counter-diffusion of polymer-laden amphiphilic organic solvent and water (Fessi et al., 1989; Galindo-Rodriguez et al., 2004; Chorny et al., 2002). Nanoprecipitation gives smaller polymeric NPs than emulsion-solvent evaporation and allows the use of non-halogenated organic solvents which are less toxic than halogenated solvents typically used in emulsion-solvent evaporation.

A crucial challenge in nanoprecipitation is to control the mixing conditions in order to produce uniformly sized particles (Karnik et al., 2008). The capability of microfluidic devices to rapidly mix fluids, continuously vary reaction conditions, and provide homogeneous reaction environments and small reactor volumes has made them attractive tools for a myriad of applications (DeMello, 2006; deMello & Rahimah Othman 2016).
Flow focusing polydimethylsiloxane (PDMS) devices are widely used for production of NPs (Karnik et al., 2008). However, PDMS swells in contact with organic solvents and the fabrication of PDMS devices by soft lithography requires the use of a master mould that must be manufactured using expensive photolithographic techniques. In this work, axisymmetric glass capillary devices were used (Utada et al., 2007) that are cheap to fabricate and have superior solvent resistance and optical properties for in-situ visual control of the process. These devices have been used by our group to prepare liposomes loaded with vitamin E (Vladisavljević et al., 2014), polymeric micelles (Laouini et al., 2013) and polycaprolactone NPs (Othman et al., 2015a; 2015b).

The aim of this study was: (i) to investigate the possibility of efficiently incorporating MMT and paracetamol into PLA NPs by microfluidic mixing/nanoprecipitation; (ii) to study the effect of MMT on the physical properties of the prepared NPs; and (iii) to investigate the encapsulation efficiency of paracetamol inside the NPs and their in vitro drug release behaviour.

6.2 Materials and methods

6.2.1 Materials

Poly(\\(d, l\)-lactide) (PLA, Ingeo\textsuperscript{TM} 4060D) was supplied by Natureworks LLC (Minetoka, MN, USA). 4060D is an amorphous polymer with a content of d- and l -lactide of 12 and 88 mol\%, respectively, a density of 1,240 kg m\(^{-3}\), a glass transition temperature of 55-60°C, and a weight-average molecular weight (\(M_w\)) of 89,000 g mol\(^{-1}\), as determined by gel permeation chromatography. Tetrahydrofuran (THF), HPLC grade (purity ≥ 99.9\%) was purchased from Sigma-Aldrich, UK. Polyvinyl pyrrolidone (PVP, \(M_w = 360,000\) g mol\(^{-1}\)) obtained from Sigma-Aldrich was used as a stabiliser to prevent agglomeration and coalescence of the NPs. Paracetamol (PCM) (purity ≥ 99.9\%) was purchased from Fisher Scientific, UK.

MMT used in this work was Cloisite® 30B with a density of 1,980 kg m\(^{-3}\) obtained from Southern Clay Products (Gonzales, TX, USA). It is MMT organically modified with N,N-Bis(2-hydroxyethyl)-N-methyl-N-tallow ammonium chloride to improve
its compatibility with the polymer matrix. PLA/MMT nanocomposite films were prepared by melt intercalation (Duan et al., 2013). Briefly, PLA granules were dried at 60 °C in a vacuum oven and then melt blended with 20 wt% of organo-MMT at 170 °C to make a masterbatch. The masterbatch was then mixed with dried PLA granules at 170 °C to form nanocomposites containing 2, 5, and 20 wt% of the clay. The organic phase was prepared by dissolving PCM and pure PLA or PLA/MMT composite film in THF and contained 6 g L\(^{-1}\) of the excipient (PLA or PLA/MMT) and 1.2–4.2 g L\(^{-1}\) of PCM. The aqueous phase was 0.2 wt% PVP dissolved in Milli-Q water.

**6.2.2 Preparation of PCM loaded PLA and PLA/MMT NPs**

The NPs were prepared using a co-flow glass capillary device consisting of a round capillary with a tapered tip inserted into a square capillary (Fig. 6.1 (a)). The organic phase was injected through the inner capillary (1 mm O.D. and 0.58 mm I.D.) with an orifice diameter of 200 μm via a Teflon tubing, which is highly resistant to THF. The aqueous phase was delivered co-currently through the outer square capillary (1.05 mm inner dimension). PLA precipitated almost instantaneously in the square capillary when the two fluids were brought into contact. This was observed through an inverted microscope as elucidated in Section 4.2.3. The aqueous phase flow rate \(Q_{aq}\) was 5 mL h\(^{-1}\) and the organic phase flow rate \(Q_{or}\) ranged from 0.5 to 3.3 mL h\(^{-1}\) to give \(Q_{aq}/Q_{or}\) between 10 and 1.5. These two phases were delivered to the device using a standard method described in Section 3.3. The duration of each experiment was 30 min.

A micrograph of the mixing zone within the glass capillary device at \(Q_{aq}/Q_{or} = 1.5\) captured by the high-speed camera is shown in Fig. 6.1 (a). The interface between the aqueous and organic phases is visible, although THF and water are miscible in all proportions and have zero equilibrium interfacial tension. When two miscible liquids are suddenly put into contact, concentration gradients at the boundary give rise to a transient tension between the liquids, \(\sigma\) given by (Joseph & Hu, 1999): 

\[
\sigma = kAC^2 / \delta
\]

, where \(k\) is the proportionality constant, \(AC\) is the change in concentration over the interfacial layer and \(\delta\) is the thickness of the boundary layer. The interface was found
to be blurred on the front side of the jet because of better mixing near the axis of the collection capillary due to high fluid velocities. The distortion of the interface known as “viscous fingering” is also noticeable. When a less viscous fluid is injected at high velocity into a more viscous one, a part of the more viscous fluid forms finger-like patterns due to non-uniform penetration. At 293 K, the viscosities of THF and water are 0.63 and 1 mPa·s respectively, supporting this assumption. Due to the 3D geometry of the micromixer, the continuous phase fully surrounds the organic phase and de-wets it from the walls (Fig. 6.1 (a)). Since the liquid/liquid interface is fully displaced from the walls and the NPs are formed at this interface, the deposition of the NPs onto the reactor walls is minimised. In a planar geometry, the organic phase wets the channel walls at the inlet, which can compromise the resultant particle size due to particle deposition onto the walls. A possible structure of encapsulated PCM-PLA/MMT NPs is depicted in Fig. 6.1 (b) with Na⁺ ions replaced by quaternary ammonium cations on the surface of the platelets. Paracetamol molecules and PLA chains are present between individual MMT platelets.

6.2.3 Determination of drug content

The organic solvent was completely evaporated from the prepared nanosuspension using a vacuum oven (Technico, Fisreem International Ltd, Loughborough, UK) at room temperature under a pressure of less than 10 Torr. The nanosuspension was then ultracentrifuged (Heraeus Labofuge 400R centrifuge, Thermo Scientific) at 15,000 rpm (23,000g) for 1 h. The supernatant containing the dissolved free drug was separated from the sediment, diluted with milli-Q water by a factor of 16 and analysed. The concentration of PCM in the supernatant was determined using a Shimadzu model UV-2550 UV-visible spectrophotometer at a wavelength of 243 nm (Wang et al., 2010). The drug encapsulation efficiency was calculated as:

\[
\text{%E.E.} = \left[1 - \left( \frac{M_R}{M_T} \right) \right] \times 100\%
\]

(6.1)

where \(M_R\) is the mass of drug in the supernatant and \(M_T\) is the total mass of drug in the sample (Zheng et al., 2009; Wang et al., 2010; Behera et al., 2013). The drug loading of the NPs was determined as:
where $M_NP$ is the total mass of NPs in the sample (Othman et al., 2015).

### 6.2.4 Characterisation of the prepared NPs

#### 6.2.4.1 Particle size measurement

The size distribution of the NPs was determined by dynamic light scattering using a Delsa™ Nano HC Particle Analyzer (Beckman Coulter, Inc), which measures the fluctuation in the intensity of scattered light as a function of time. Smaller particles cause the intensity to fluctuate more rapidly than large particles. The nanosuspension was diluted 5-fold by Mili-Q water before being transferred into a 4 mL disposable cuvette and placed into the instrument. The measurement time was 120 s. Each samples were analysed by following the standard procedures described in Section 2.6.1.1.

#### 6.2.4.2 Transmission electron microscopy (TEM) and energy-dispersive X-ray spectroscopy (EDS)

The internal structure of the NPs was investigated using TEM. Each samples were analysed by following the standard procedures described in Section 4.2.4.3.

#### 6.2.4.3 Differential scanning calorimetry (DSC)

Differential Scanning Calorimeter (DSC) was performed using a TA Instruments Model Q100. 5-10 mg of the sample (pure PLA, pure PCM, physical mixture of PLA and PCM, PCM-loaded PLA NPs and PCM-loaded PLA/MMT NPs) was used. PCM-loaded NPs were prepared by freeze-drying the sediment after centrifugation in a freeze dryer (Edwards, type EF4 Modulyo). Each samples were analysed by following the standard procedures described in Section 5.2.5.3.

#### 6.2.4.4 Thermogravimetric analysis (TGA)

The thermal stability of the NPs was assessed by TGA (Q5000IR Thermogravimetric Analyzer) at a heating rate of 10 °C min$^{-1}$ over the temperature range of 20–600 °C under dry nitrogen as the effluent gas.
Fig. 6.1: Synthesis of NPs in a co-flow glass capillary device: (a) experimental set-up consisting of two syringe pumps, plastic tubing and two coaxial capillaries glued onto a microscope slide. (b) Possible structure of encapsulated PCM-PLA/MMT NPs. Polycaprolactone (PCL) chains and paracetamol (PCM) are present between individual MMT platelets modified with a quaternary ammonium salt.

6.2.4.5 X-ray diffractometry (XRD)

Each samples were analysed by following the standard procedures described in Section 5.2.5.4.
6.2.4.6 Attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy

ATR-FTIR spectra in the range of 4000–400 cm\(^{-1}\) were recorded on a Thermo Scientific Nicolet iS50 ATR spectrometer with a monolithic diamond crystal. 2–3 mg of the powdered sample was placed onto the Universal diamond ATR top-plate and the spectrum was acquired within 32 s. Triple runs were carried out on each sample to check reproducibility.

6.2.4.7 In vitro drug release measurements

In-vitro drug release tests were carried out using PCM-loaded PLA and PLA/2 wt% MMT NPs in two different release media: Mili-Q water (the control fluid) and 0.01 M sodium phosphate-buffered saline (PBS) at pH 7.4 (the simulated intestinal fluid). Simulated intestinal fluid (pH=7.4) was chosen since paracetamol is a weak acid and should have a higher solubility in intestinal fluid than in simulated gastric fluid (pH=1.2). An aliquot of the prepared suspension was vacuum-evaporated in order to remove THF. The suspension was then transferred in a test tube and incubated in a shaking bath at 50 rpm and 37 °C to mimic the human blood stream conditions (Behera et al., 2013). At predetermined time intervals, the test tube was taken out of the shaker and centrifuged at 15,000 rpm for 1 h at room temperature. The supernatant was removed and used to measure the amount of drug released. The dissolution medium was replenished with fresh Mili-Q water or PBS to keep the total volume of the release medium constant.

6.3 Results and discussion

6.3.1 Control over the average size of the prepared NPs

Table 1 shows the effect of drug-to-excipient ratio in the organic phase and the type of excipient on the average particle size, drug encapsulation efficiency and drug loading of the prepared NPs at the flow rate ratio of 10. The average particle size increased from 285 to 398 nm for PLA and from 296 to 428 nm for PLA/2 wt% MMT with an increase in the drug-to-excipient ratio in the organic phase from 0.2 to 0.7. The average size of composite NPs was bigger than that of the pure PLA NPs, due to
the incorporation of MMT into the polymer matrix. A similar effect has been reported for docetaxel-loaded NPs when MMT was incorporated into the polymer matrix (Feng et al., 2009).

The drug loading in PLA NPs increased from 5.9 to 10.9 % as the drug-to-excipient ratio in the organic phase increased from 0.2 to 0.7 (Table 6.1). However, the drug encapsulation efficiency decreased from 35 to 27 % on increasing the drug loading in the NPs. Low drug encapsulation efficiencies can be attributed to the high solubility of PCM in water of 12.8 mg mL\(^{-1}\) at 20°C (Granberg & Rasmuson, 1999). Since the concentration of PCM in the organic phase was 1.2–4.2 g L\(^{-1}\) and \(Q_{aq}/Q_{or}\) was 10, the concentration of PCM in the suspension after THF evaporation was 0.12–0.42 mg mL\(^{-1}\), which is only 1–3% of the saturation point of PCM in water. Therefore, without entrapment within the NPs, all PCM added to the organic phase would dissolve completely in the aqueous phase. The specific surface area of the prepared NPs is another factor contributing to the significant loss of PCM into the aqueous phase. A decrease in drug entrapment with increased drug loading was probably due to the more porous polymer matrix formed as a result of the dissolution of PCM from the surface regions of the NPs. Owing to the increased amount of drug loaded, a more porous matrix may be formed through which the drug can easily escape to the aqueous phase, thereby decreasing the content of PCM encapsulated (Witschi & Doelker, 1998). Niwa et al. (1993) also observed decreased drug entrapment at high drug loadings due to enhanced drug leakage into the aqueous phase.

Table 6.2 shows the effect of \(Q_{aq}/Q_{or}\) and the type of carrier on the particle size and drug loading. Some initial research has been done to determine the particle size in THF of organo-modified non-intercalated MMT and dissolved PLA/MMT composite films. The size of intercalated/exfoliated MMT particles prepared by dissolving PLA/MMT films in THF at 1 g L\(^{-1}\) was (41 ± 6.1), (39 ± 4.0), and (78 ± 8.4) nm for the composite films containing respectively 2, 5, and 20 wt% of MMT. The particle size of pure MMT in THF was (426 ± 21.2) nm at 0.25 g L\(^{-1}\). After intercalation/exfoliation, MMT platelets are partially or fully separated from each other and therefore, the size of polymer-intercalated MMT particles is smaller than the size of non-intercalated/agglomerated particles. This observation proves that PLA...
was successfully intercalated into MMT and that PLA/MMT composite films were more suitable as drug carriers than a mechanical mixture of MMT and PLA powders. In all cases, the size of MMT NPs in the organic phase was significantly lower than the orifice diameter (200 μm).

The size of PLA/MMT NPs increased on increasing the content of MMT in the polymer matrix from 2 to 20 wt% and was larger than the size of pure PLA NPs (Table 6.2). The size of NPs decreased as the flow rate ratio increased, which was more pronounced for composite NPs. A higher flow rate ratio provides more rapid mixing due to higher flow rate in the collection capillary (British Standards Institution, 1997; Zheng et al., 2009). When fluids are mixed more rapidly, the critical degree of supersaturation needed for nucleation is reached faster, which leads to the generation of more nuclei per unit time. Since the growth of nuclei is limited by the amount of available polymer in the liquid phase, it results in smaller NPs. In addition, at higher $Q_{aq}/Q_{or}$ values, the NPs are more diluted after formation, which suppresses the rate of particle growth and agglomeration, due to the lower frequency at which the NPs collide with each other. Smaller NPs at higher $Q_{aq}/Q_{or}$ values have also been reported in membrane contactors (Laouini et al., 2013a; Laouini et al., 2013b; Laouini et al., 2013c) and flow focusing microfluidic devices (Jahn et al., 2010).

Table 6.2 also includes the drug encapsulation efficiency and drug loading at a PCM concentration in the organic phase of 20 wt% for various $Q_{aq}/Q_{or}$ values and two different drug-carriers, pure PLA and PLA/2 wt% MMT. The drug encapsulation efficiency and drug loading in the NPs increased with increasing $Q_{aq}/Q_{or}$, probably due to faster nucleation and faster inclusion of drug molecules within the polymer matrix. The incorporation of MMT into the polymer matrix improved both the drug entrapment efficiency and drug loading. This was due to reduced porosity of the composite polymer matrix compared with that of the pure polymer (Ekanem et al., 2015; Batra et al., 2011), adsorption of PCM onto the nanoclay particles and an increased diffusion path length of PCM molecules within the polymer.
Table 6.1: The effect of paracetamol-to-excipient mass ratio in the organic phase and the type of excipient on the average particle size, drug encapsulation efficiency and drug loading of the prepared NPs at $Q_{aq}/Q_{or} = 10$.

<table>
<thead>
<tr>
<th>PCM/excipient mass ratio in organic phase</th>
<th>Type of excipient</th>
<th>Average size of NPs, $Z_{ave}$ (nm)</th>
<th>E.E. (%)</th>
<th>D.L. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PLA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PLA/ 2% MMT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.20</td>
<td>285 ± 13.4</td>
<td>296 ±10.4</td>
<td>35.2</td>
<td>5.9</td>
</tr>
<tr>
<td>0.45</td>
<td>320 ± 19.4</td>
<td>357 ±11.0</td>
<td>32.8</td>
<td>10.2</td>
</tr>
<tr>
<td>0.70</td>
<td>398 ± 12.6</td>
<td>428 ±29.4</td>
<td>26.6</td>
<td>10.9</td>
</tr>
</tbody>
</table>

*Note: Each analysis was repeated three times ($n = 3$) and the average particle sizes were determined. The error bars are standard deviations.

Table 6.2: The effect of $Q_{aq}/Q_{or}$ and MMT content in the polymer matrix on the average particle size, drug encapsulation efficiency and drug loading.

<table>
<thead>
<tr>
<th>Excipient</th>
<th>$Q_{aq}/Q_{or}$</th>
<th>Average size of blank NPs, $Z_{ave}$ (nm)</th>
<th>E.E. (%)</th>
<th>D.L. (%)</th>
<th>E.E. (%)</th>
<th>D.L. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLA/ 2% MMT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLA/ 5% MMT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLA/ 20% MMT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLA/ 2% MMT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>270 ± 34</td>
<td>431 ±7</td>
<td>27.6</td>
<td>4.6</td>
<td>29.4</td>
<td>4.9</td>
</tr>
<tr>
<td>3.0</td>
<td>276 ± 21</td>
<td>383 ±11</td>
<td>27.2</td>
<td>4.5</td>
<td>31.1</td>
<td>5.2</td>
</tr>
<tr>
<td>4.5</td>
<td>237 ± 2</td>
<td>379 ± 5</td>
<td>32.5</td>
<td>5.4</td>
<td>34.3</td>
<td>5.7</td>
</tr>
<tr>
<td>7.0</td>
<td>316 ± 7</td>
<td>327 ± 4</td>
<td>30.7</td>
<td>5.1</td>
<td>34.7</td>
<td>5.8</td>
</tr>
<tr>
<td>10.0</td>
<td>265 ± 24</td>
<td>296 ±10</td>
<td>35.2</td>
<td>5.9</td>
<td>38.5</td>
<td>6.4</td>
</tr>
</tbody>
</table>

*Note: Each analysis was repeated three times ($n = 3$) and the average particle sizes were determined. The error bars are standard deviations.
6.3.2 Characterisation of the NPs

6.3.2.1 TEM and elemental analysis of the NPs

Fig. 6.2 shows the transmission electron micrographs of the NPs containing different concentrations of MMT in the polymer matrix. Only PLA and PLA/2wt% MMT NPs were found to have a spherical shape with a smooth surface. Clay platelets are visible in all NPs containing 2 wt% MMT, but the clay is incorporated entirely in the interior of the NP.

Due to the 2D structure of MMT platelets and their very low thickness compared with the diameter of a composite nanoparticle (individual platelet thicknesses are just 1 nm and the particle diameter was at least 300 nm), although 80% of the particle cross section was occupied by the MMT platelets, it was found that the volume of MMT was very low compared with the total particle volume. As a result of higher loading of MMT in the polymer matrix, the shape of NPs containing 5 and 20 wt% of MMT in the polymer matrix is distorted and significantly deviates from a spherical shape. The last two figures in Fig. 6.2 show TEM images of drug-loaded NPs prepared with the drug-to-excipient mass ratio in the organic phase of 1:5. Drug-loaded PLA NPs are spherical and exhibit a smooth surface and homogeneous interior indicating that the drug nanocrystals are finely and uniformly dispersed within the polymer matrix. Drug-loaded NPs containing 2 wt% MMT in the matrix are not perfectly spherical, due to inclusion of both MMT platelets and PCM nanocrystals in the polymer matrix. The polydispersity index of the NPs in all samples was less than 0.25.

The TEM images in Fig. 6.2 indicate that the nanoclay was successfully incorporated in the host polymer. Additional evidence came from the elemental analysis of the NPs (Table 6.3). The octahedral sheet of MMT has Al as the central atom (partly substituted by Mg and Fe) and the tetrahedral sheets have Si as the central atom. That is why Al, Mg, Fe and Si were all detected in MMT/PLA NPs but not in PLA NPs. A disproportionately high content of carbon and copper in all samples was due to carbon-coated copper grids used to hold the samples for EDS analysis. The significantly higher amount of carbon in drug-loaded NPs compared with blank PLA
and PLA/MMT NPs was due to the higher mass fraction of C in paracetamol (C₈H₉NO₂) compared with that in PLA, (C₃H₄O₂)n.

**Fig. 6.2:** TEM images of multi-functionalised NPs composed of different ratio of MMT and drug-loaded NPs prepared using the drug-to-excipient mass ratio in the organic phase of 0.2 at various magnifications.

**Table 6.3:** Elemental composition of different samples of the prepared NPs obtained using EDS.

<table>
<thead>
<tr>
<th>Chemical element (wt%)</th>
<th>PLA</th>
<th>PLA+2 w% MMT</th>
<th>PLA+5 w% MMT</th>
<th>PLA+20 w% MMT</th>
<th>PCM-loaded PLA</th>
<th>PCM-loaded PLA + 2 w% MMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>85.9</td>
<td>70.0</td>
<td>21.5</td>
<td>24.1</td>
<td>95.0</td>
<td>91.1</td>
</tr>
<tr>
<td>O</td>
<td>2.59</td>
<td>12.0</td>
<td>37.1</td>
<td>33.6</td>
<td>2.34</td>
<td>0.17</td>
</tr>
<tr>
<td>Mg</td>
<td>0.00</td>
<td>0.29</td>
<td>1.83</td>
<td>0.69</td>
<td>0.00</td>
<td>0.03</td>
</tr>
<tr>
<td>Al</td>
<td>0.00</td>
<td>1.62</td>
<td>9.72</td>
<td>12.1</td>
<td>0.00</td>
<td>0.81</td>
</tr>
<tr>
<td>Si</td>
<td>0.00</td>
<td>4.22</td>
<td>16.8</td>
<td>16.8</td>
<td>0.00</td>
<td>2.20</td>
</tr>
<tr>
<td>K</td>
<td>0.00</td>
<td>0.20</td>
<td>4.62</td>
<td>2.10</td>
<td>0.00</td>
<td>0.02</td>
</tr>
<tr>
<td>Fe</td>
<td>0.00</td>
<td>2.67</td>
<td>2.61</td>
<td>2.39</td>
<td>0.00</td>
<td>0.15</td>
</tr>
<tr>
<td>Cu</td>
<td>11.5</td>
<td>8.04</td>
<td>5.76</td>
<td>8.15</td>
<td>2.69</td>
<td>5.49</td>
</tr>
</tbody>
</table>

*Note: Each analysis was repeated three times (n = 3) and the average values were determined. The error bars are standard deviations.*
6.3.2.2 XRD analysis

The crystallinity of the fabricated NPs was investigated by XRD, as shown in Fig. 6.3a. The crystalline drug (PCM) shows sharp peaks at 13.6°, 16.4°, 23.2°, 23.8°, 26.2°, 26.9° and 30.8°, which are in good agreement with the reported values (Khanmohammadi, et al., 2010). The degree of crystallinity of PCM was very high (90.2 %), but less than 100% because inevitably some amorphous content is generated during the processes of milling, wet granulation, drying, re-crystallisation and compaction. The diffraction pattern of pure PLA powder includes only a broad ‘hump’ at 14.8°, due to the amorphous nature of poly(ᴅ,ʟ-lactide). MMT shows a characteristic diffraction peak at 20 of 6.4° corresponding to the 001 plane with a ‘d’ spacing of 1.36 nm (Datta, 2013). This peak cannot be seen because the 2θ axis starts at 10°, but two minor peaks at 19.5° and 36° are visible and they agree with previous results (Ekanem et al., 2015). The diffraction pattern of the PLA/MMT composite film shows a wide ‘hump’ between 20 values of 10° and 25° and the degree of crystallinity of 69.6 % is lower than that of pure MMT (76.4 %). As a result of intercalation of PLA in the interlayer spacing, there is an increase in ‘d’ spacing. The physical mixture of PCM and PLA exhibits two characteristic sharp peaks of PCM at 13.6° and 23.2°, but also a broad hump due to the contribution from PLA. However, PCM peaks disappear in the diffraction pattern of PCM-loaded PLA NPs, indicating that PCM was completely incorporated inside the polymer matrix. On the other hand, PCM-loaded PLA/MMT NPs produce a series of small peaks, which reveal the presence of crystalline MMT platelets protruding from the surface of the NPs.

6.3.2.3 DSC analysis

Fig. 6.3 (b) shows the thermograms of the prepared NPs, raw materials and physical mixtures of the drug and the polymer. The samples were heated above the melting points ($T_m$) of PCM. Pure PCM powder exhibited a large endothermic peak of melting with a maximum at 170°C and a shallow, broad endothermic peak at 60 °C corresponding to the glass transition temperature ($T_g$) of poly(ᴅ,ʟ-lactide). No peak was observed in the thermogram of pure MMT due to its high thermal stability. Two endothermic peaks corresponding to the $T_m$ of PCM and the $T_g$ of PLA were detected in the physical mixtures of PLA and PCM, and PLA/MMT and PCM, respectively.
The peaks of PCM-loaded PLA and PLA/MMT NPs were shifted to lower temperatures (~160 °C) indicating that PCM entrapped in the NPs was in an amorphous or disordered-crystalline phase. Moreover, the appearance of a single peak for the loaded NPs indicates that PCM was uniformly distributed in the NPs.

6.3.2.4 TGA

TGA was performed to determine the thermal stability of the prepared NPs. The TGA curve of MMT (Fig. 6.4 (a)) shows two inflection points, at ~270 °C due to free water evaporation and at 370 °C due to the release of structural (hydroxyl) water (Joshi et al., 2009; Joshi et al., 2009) with 22% weight loss recorded at 600 °C. For pure PLA powder, 10 % degradation occurred at 319 °C, which signifies the onset of degradation. The midpoint was at 346 °C and the powder was totally decomposed to CO₂ and H₂O at 370 °C. PLA/MMT composite film with 2 wt% MMT was more thermally stable than pure PLA powder and the amount of non-volatile residue at 600 °C was 3 %. The TGA curve for PCM showed a steep decrease between 230 and 300 °C with 99 % mass loss recorded at 600 °C. PLA NPs were less thermally stable than pure PLA powder due to their lower degree of crystallinity, as a result of fast polymer precipitation. It has been found that fast removal of polymer-dissolving organic solvent inhibits crystallisation of the polymer, because the polymer chains have less time to pack together in an organized manner (Izumikawa, et al., 1991). The PCM-loaded PLA NPs started to degrade at lower temperature than the blank PLA NPs due to the presence of PCM in the polymer matrix, which is another indicator of PCM encapsulation. PCM-loaded PLA/MMT NPs were fully degraded only at 370 °C and were more stable than the blank PLA and PCM-loaded PLA NPs, whose complete degradation occurred at 260 and 270 °C, respectively. The amount of non-volatile residue in PCM-loaded PLA/MMT NPs was 2 wt%, which corresponds to the amount of MMT in the composite film, indicating that MMT was efficiently entrapped in the NPs.

6.3.2.5 ATR-FTIR spectroscopy

The FTIR spectra of the prepared NPs and raw materials are shown in Fig. 6.4 (b). The IR peak of pure PLA at 1760 cm⁻¹ was due to the stretching vibration of the C=O
bonds along the PLA chain and this peak had the same position in all samples containing PLA. The FTIR spectrum of MMT showed an absorption band at 3600 cm\(^{-1}\) due to the stretching vibration of the O-H bond in Al-OH and Si-OH. The two bands that appear at 2930 and 2850 cm\(^{-1}\) can be attributed to the asymmetric and symmetric stretching of the methylene groups (-CH\(_2\)-) in the quaternary ammonium compound used to modify MMT. The band at 1465 cm\(^{-1}\) corresponds to the bending vibration of the methylene groups of the quaternary ammonium compound. The most intense band in the FTIR spectrum of MMT occurred near 1000 cm\(^{-1}\) can be attributed to the stretching vibrations of the Si-O groups in tetrahedral sheets. The bands at 915, 875 and 836 cm\(^{-1}\) were attributed to Al-Al-OH, Al-Fe-OH and Al-Mg-OH bending vibrations, respectively (Patel et al., 2007) and the band near 500 cm\(^{-1}\) may result from the stretching of the Al-O bonds.

No peak shifts or new peaks appeared in the spectra of the blank PLA NPs compared with PLA powder, which means that FTIR could not reveal any change in the molecular structure of PLA as a result of dissolution and nanoprecipitation, as opposed to XRD and TGA analysis. New peaks between 670 and 400 cm\(^{-1}\) detected in the spectra of PLA/MMT composite film and PCM-loaded PLA/MMT NPs, as compared with the spectrum of PLA powder, were caused by Al-O and Si-O bonds in MMT and verify the inclusion of MMT in PLA. The characteristic absorption peaks due to carbon–carbon stretching vibrations in the aromatic ring of PCM were visible in the region between 1265 and 1660 cm\(^{-1}\) for all PCM-loaded NPs. This indicates that PCM was encapsulated within the NPs, but has not strongly interacted with PLA and MMT since no peak shifts occurred (Zheng et al., 2007).
Fig. 6.3: (a) X-ray diffractograms and (b) DSC thermograms of: (1) pure PCM; (2) pure PLA; (3) physical mixture of PCM and PLA; (4) PCM-loaded PLA NPs; (5) physical mixture of PCM and PLA/MMT; (6) PLA/MMT composite film; (7) pure MMT and (8) PCM-loaded PLA/MMT NPs.
6.3.3 In vitro release of PCM from nanoparticles

The amount of PCM released from plain PLA and hybrid PLA/MMT NPs was measured over 190 h by recording the absorbance at 243 nm (Fig. 6.5). Zero-time
corresponds to the start of the incubation period, but the amount of PCM plotted in Fig. 6.5 excludes the amount of drug released during the nanoprecipitation process. For the conditions in Fig. 6.5, the total amount of drug released during the processes of fabrication and storage ranged between 84 and 90%. A fast drug release was observed over the first 9 h which can be attributed to relatively high solubility of PCM in water. It was followed by a very slow release over a long period of time due to diffusion of the drug from the PLA cores into the dissolution media. The rate of drug release from nanoclay-loaded NPs was reduced compared to plain PLA NPs due to the more compact polymer matrix (Batra et al., 2011), a physical adsorption of PCM onto MMT and a tortuous path of drug molecules due to obstacles imposed by non-permeable clay particles randomly distributed in the polymer matrix (Fig. 6.1 (b)). Slightly faster PCM release rates were found for Milli-Q water, probably due to higher solubility of PCM in pure water compared with PBS and different swelling properties of NPs in different solutions.

Fig. 6.5: In vitro release profile of paracetamol (PCM) from: (a) PLA NPs in Milli-Q water; (b) PLA NPs in phosphate-buffered saline (pH 7.4); (c) PLA/MMT NPs in Milli-Q water; (d) PLA/MMT NPs in phosphate-buffered saline (pH 7.4). The error bars represent the standard deviations of three repeated measurements ($n = 3$).
6.4 Conclusions

In this study, antisolvent nanoprecipitation in a co-flow 3D glass capillary device was used to produce paracetamol-loaded PLA/MMT NPs composed of a biodegradable polymer matrix filled with organically modified MMT clay. The size of the intercalated/exfoliated MMT particles in the organic phase was 41–78 nm. The incorporation of nanoclay in the polymer matrix improved both the drug encapsulation efficiency and drug loading in the final formulation, and extended the rate of drug release into simulated intestinal fluid. The particle size increased on increasing the drug loading and the content of MMT in the polymer matrix, and decreased with increasing aqueous to organic flow rate ratio in the glass capillary device. In addition, the drug encapsulation efficiency and drug loading in the NPs increased with increasing aqueous to organic flow rate ratio, due to more rapid mixing and faster formation of the NPs.

The 2 wt% PLA/MMT composite film was found to be the most suitable drug carrier due to the spherical shape of the fabricated NPs and almost complete inclusion of the nanoclay platelets inside the host polymer. The composite nanoclay-loaded NPs were significantly more thermally stable than the plain PLA NPs. The incorporation of MMT and PCM into PLA was confirmed by the new peaks detected in the FTIR spectra of the drug-loaded composite NPs in the regions between 670 and 400 cm\(^{-1}\) (MMT), and 1265 and 1660 cm\(^{-1}\) (PCM). In future work, the production rate of the NPs will be improved using microengineered membranes with regular pore spacing that will be used instead of glass capillaries.
CHAPTER 7

FORMATION OF SIZE-TUNEABLE BIODEGRADABLE POLYMERIC NANOPARTICLES BY SOLVENT DISPLACEMENT METHOD USING MICRO-ENGINEERED MEMBRANES FABRICATED BY LASER DRILLING AND ELECTROFORMING

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Chapter overview

Biodegradable poly(ε-caprolactone) (PCL) drug-carrier nanoparticles (NPs) were produced by rapid membrane micromixing combined with nanoprecipitation in a stirred cell employing novel membrane dispersion. The organic phase composed of 0.1–0.6 wt% PCL dissolved in tetrahydrofuran was injected into the aqueous phase (Mili-Q water or 0.2–1 wt% poly(vinyl alcohol) using two microfabricated membranes with different pore morphologies and spatial pore arrangements: ringed stainless steel membrane of reduced (annular) operating area with a square array of cylindrical laser-drilled pores and electroformed nickel membrane of full operating area with a hexagonal array of conical, funnel-shaped pores. The size of the NPs was precisely controlled over a range of 159–394 nm by changing the aqueous-to-organic volumetric ratio, stirring rate, transmembrane flux, the polymer content in the organic phase, membrane type and pore size. The smallest and most uniform particles with a Z-average of 159 nm and a polydispersity index of 0.107 ± 0.014 were obtained using a 10 lpore-sized stainless steel membrane at the transmembrane flux of 140 L m⁻² h⁻¹, a stirring rate of 1300 rpm, and an aqueous-to-organic phase volume ratio of 10 using 1 g L⁻¹ PCL in the organic phase. The existence of the peak shear stress within a transitional radius and a rapid decline of the shear stress away from the membrane surface were revealed by numerical modelling.

7.1 Introduction

Biocompatible functional nanoparticles (NPs) for drug delivery have attracted growing interest in the past several decades (Chaubal, 2004; Zhang et al., 2006; Barratt, 2000). These NPs should be able to preserve the drug from leakage while it is transported to a desired therapeutic site, so that the drug will not interact with non-
targeted cells or tissues and cause side effects, and they should be degraded or eliminated from the body after the drug has been released (Ghosh, 2000; De Jong & Borm, 2008). Polymeric NPs have been extensively studied as drug nanocarriers (Moinard-Checot et al., 2006; Quintanar-Guerrero et al., 1998; Legrand et al., 2007; Letchford & Burt, 2007). Synthetic aliphatic polyesters, such as poly-ε-caprolactone (PCL) and polylactide (PLA), are highly suitable polymers for these applications due to their good mechanical properties, biodegradability, nontoxicity (Sinha et al. 2004), and good compatibility with other polymers and inorganic nanofillers (Othman et al., 2016). PCL and PLA have been approved by the U.S. Food and Drug Administration (FDA) for use as drug carriers. The PCL biodegradation product, 6-hydroxycaproic acid, can be completely metabolised via the citric acid cycle, but the rate of degradation is very slow, which makes PCL ideally suited for long-term drug delivery.

Polymeric drug-loaded NPs can be fabricated using different methods (Nagavarma et al., 2012; Reis et al., 2006; Rao & Geckeler, 2011), including: (i) dispersion of preformed polymers (nanoprecipitation, emulsification-solvent evaporation/extraction/diffusion, salting-out, dialysis, spray drying, and supercritical fluid technology), (ii) polymerization of monomers, (iii) associative interactions among charged hydrophilic polymers (ionotropic gelation and complex coacervation), and (iv) particle replication in non-wetting templates (PRINT) (Rolland et al., 2005). Emulsification-solvent removal methods are associated with high energy consumption and the use of surfactants and toxic organic solvents. Nanoprecipitation is less energy demanding method that does not require surfactants and usually involves less toxic (Class 2 and Class 3) organic solvents. The process is based on the interfacial deposition due to the displacement of the polymer solvent by a non-solvent which is miscible with the polymer solvent (Fessi et al., 1989).

Membrane and microfluidic micromixing combined with nanoprecipitation have opened up new possibilities to prepare size-controlled NPs (Jia & Liu, 2013; Chen et al., 2004; Charcosset & Fessi, 2005; Pham et al., 2012; Jaafar-Maalej et al., 2011). Microfabricated membranes consisting of regular arrays of equally spaced pores manufactured by electroforming (Kosvintsev et al., 2005), percussion laser drilling (Vladisavljević & Williams, 2006) and etching (Kobayashi et al., 2005) have been
increasingly used for the preparation of emulsions (Nazir et al., 2011), microparticles (Imbrogno et al., 2015), and NPs (Laouini et al., 2013). They enable uniform liquid dispersion in micromixing processes, due to ordered pore arrays and uniform pore size, high flux through the membrane, and suppression of internal pore fouling due to thin and non-tortuous pores. Microfabricated pore arrays are similar to massively parallel T junctions, through which one fluid may be introduced into another at an overall much higher flow rate than is possible in individual channels (Vladisavljević et al., 2012). The mixing rate can be enhanced by providing a controlled shear at the membrane surface using cross flow (Laouini et al., 2011), stirring (Kosvintsev et al., 2005), flow pulsations (Holdich et al., 2013), radially or axially oscillating membrane tubes (Silva et al., 2015; Holdich et al., 2010), and rotating membrane (Vladisavljević & Williams, 2006). However, the role of different pore arrangements, pore shapes and fabrication methods on the performance of microfabricated membrane in micromixing process has not yet been systematically investigated.

In this study, membrane micromixing/nanoprecipitation process has been investigated using two microfabricated membranes (stainless steel membrane with laser drilled pores and electroformed nickel membrane) with different pore patterns (square and hexagonal), pore shapes (conical and cylindrical) and operating areas (reduced ringed area and full circular area).

The main objectives were to evaluate the effect of pore morphology and operating parameters on the particle size distribution of PCL nanoparticles and to investigate velocity and shear profiles in the vicinity of the membrane surface using computational fluid dynamics (CFD). The mathematical model was solved using a commercial software package CFD package ANSYS FLUENT 14.5 in dimensional form.

7.2 Materials and methods

7.2.1 Chemicals

Tetrahydrofuran (THF, HPLC grade, purity ≥ 99.9 %), poly(ε-caprolactone) (PCL, \( M_w = 14,000 \) g mol\(^{-1}\) with a glass transition temperature of 60 °C) and polyvinyl
alcohol (PVA, $M_w = 13,000 - 23,000 \text{ g mol}^{-1}$, 87 – 89 % hydrolysed) were purchased from Sigma-Aldrich (Dorset, UK). PLA was used as a water soluble stabiliser to prevent agglomeration and imperfect surface formation of the NPs and THF was used as a solvent for PCL. The anti-solvent phase was pure water produced by reverse osmosis (Mili-Q®, Millipore) or 0.2 – 1 wt% aqueous solution of PVA. All chemicals other than THF were of analytical grade.

### 7.2.2 Membrane dispersion cell

The NPs were prepared using a flat, disc-shaped membrane installed in a stirred cell shown in Fig. 7 ((a) – (b)). The cell and membranes were supplied by Micropore Technologies Ltd (Redcar, UK). The stirrer was driven by a 24 V DC motor (Instek model PR-3060) and its rotation speed was controlled between 200 and 1300 rpm by the applied voltage. A nickel (Ni) membrane with an effective diameter of 3.3 cm and an operating area, $A_m$, of 8.55 cm$^2$ had ~24,690 hexagonally arranged pores with a diameter of 10, 20, and 40 μm, spaced apart at a constant distance of 200 μm (Fig. 7 (d) and S2 (a–c)). A ringed stainless steel (SS) membrane had the same dimensions and a reduced operating (active) area of 2.76 cm$^2$ occupying space on the membrane surface between two concentric circles of radius $r_1 = 9 \text{ mm}$ and $r_2 = 13 \text{ mm}$ (Fig. S1 (b)). SS membrane contained ~6912 pores with a diameter of 10 lm arranged in a square array with a pitch of 200 μm (Fig. 7 (e)). The number of pores was estimated from Eqs. (S1) and (S2) given in the supplement.

### 7.2.3 Experimental set-up and preparation of polymeric NPs

The cell was filled with 30–60 mL of mili-Q water or aqueous PVA solution and the stirring rate was adjusted to achieve the peak shear stress at the membrane surface between 0.7 and 14 Pa. The organic phase was 0.1–0.6 wt% PCL in THF and was injected through the membrane using a Cole-Parmer model 230 VAC syringe pump. The organic phase flow rate ($Q$) through the nickel membrane was 2–5 mL min$^{-1}$ and thus, the transmembrane flux ($Q/A_m$) was 140–351 L m$^{-2}$ h$^{-1}$. To achieve the same flux through the ringed membrane, the organic phase flow rate was reduced to 0.64–
1.6 mL min\(^{-1}\), calculated from the equation: \( Q_R = \left( \frac{A_R}{A_m} \right) Q \), where \( A_R \) and \( A_m \) are the operating areas of the ringed and whole membrane, respectively.

The experiments were run until a predetermined aqueous-to-organic phase volumetric ratio was achieved. For the whole membrane, the operating time ranged from 2.6 min at the minimum flux to 6.5 min at the maximum transmembrane flux. For the ringed membrane, the operating times were 3.1 times longer than those for the whole membrane and ranged between 8.1 and 20.3 min. The aqueous phase turned cloudy as soon as the organic phase was brought in contact with the aqueous phase due to rapid exchange of two solvents at the interface, i.e. THF diffused from the organic to the aqueous phase and water diffused in the opposite direction (Fig. 7.1 (c)). THF was evaporated from the suspension in a vacuum oven at ambient temperature under a pressure of less than 10 Torr (Fistreem International Ltd, Loughborough, UK) until the smell of THF had disappeared (≈30 min). Each experiment was repeated at least three times.

After each experiment, the membrane was rinsed with Mili-Q water and then sonicated in THF for 30 min using a Fisher Scientific ultrasonic bath (model FB 15046) in order to remove any NPs leftovers from the membrane surface. The membrane was then rinsed again with Milli-Q water and finally washed with Milli-Q water in an ultrasonic bath for 5 min to restore its hydrophilic properties. The contact angle between water and the surface of the Ni membrane was measured after each cleaning step using a Krüss Model DSA 100 Advanced Drop Shape Analyser (Hamburg, Germany) and was found to be 86° ± 2.2° before cleaning, 68° ± 1.4° after treatment with THF and 59° ± 1.1° at the end of the cleaning process. Therefore, the membrane surface became progressively more hydrophilic as the cleaning progressed. The membrane should be hydrophilic during experiments to minimise wetting of the membrane surface by the organic phase and pore clogging by the deposited hydrophobic polymer.
Fig. 7.1: (a) A schematic diagram of the membrane dispersion cell with a paddle stirrer fitted above a micro-engineered membrane; (b) Formation of NPs by rapid solvent displacement above the membrane surface (yellow colour = solvent); (c) Optical micrograph of a 10 μm pore-sized nickel membrane; (d) Optical micrograph of a 10 μm pore-sized stainless steel membrane.

7.2.4 Characterisation of the prepared NPs

7.2.4.1 Particle size analysis

The particle size distribution was measured using a Delsa™ Nano HC Particle Analyser (Beckman Coulter, High Wycombe, UK) by dynamic light scattering (DLS) method, which measures the fluctuations in scattered light intensity as a function of time (Submicron, 2011). Smaller particles move faster than larger particles and therefore, the timescale of intensity fluctuations is shorter for smaller particles. A THF-free nanosuspension sample was transferred into a 4 mL disposable cuvette which was then placed into the instrument. The measurement time was 120 s. Each samples were analysed by following the standard procedures described in Section 2.6.1.1.
7.2.4.2 Zeta potential determination

The zeta potential of the NPs was measured using a Malvern Instruments Zetasizer 3000 HAS particle size analyser. The measurements were repeated at least three times after sample dilution in water. The zeta potential was calculated from the electrophoretic mobility using the Helmholtz-Smoluchowski equation (Hunter et al., 2001).

7.2.4.3 Microscopic observations (TEM, FEG-SEM and Benchtop SEM)

2-D micrographs of the prepared NPs were acquired using Transmission Electron Microscopy (TEM) and high resolution Field Emission Gun Scanning Electron Microscopy (FEG-SEM). Each samples were analysed by following the standard procedures described in Section 4.2.4.3.

3D micrographs of cross-section of the micro-engineered membranes were obtained using a Hitachi model TM3030 benchtop SEM fitted with an Oxford Instruments Swift ED3000 Silicon drift detector (SDD) operated at 5−15 kV voltage. A stage movement was controlled by a high resolution stepper motor with a 10 nm step size and a repositioning accuracy of 1 μm. The NPs were sputtered with gold to become electrically conductive and placed on a copper stub prior to the SEM imaging.

7.3 Computational modeling

The computational domain model geometry used to simulate fluid flow is shown in the supplementary material (Figure S2). A 3D simulation model is developed using the CFD package ANSYS FLUENT 14.5, constructed using ANSYS Design Modeller. Meshing pre-processor was used for the generation of the computational mesh. Considering the geometrical constraints, the fluid domain was meshed with tetrahedral cells while for the solid body (blades) hexahedral meshing scheme was used. The entire flow domain was then meshed using approximately one million cells. This was achieved by investigating the optimum number of cells that provide computational results which are independent of the number of cells and distribution of the computational grid. Hence, a grid independency test was performed. The
governing equations used for modelling are provided in the supplementary material (see S3.1).

7.4 Results and discussion

7.4.1 Effect of the aqueous-to-organic phase volumetric ratio

In this series of experiments, 6 mL of the organic solution composed of 1 g L⁻¹ of PCL in THF was injected at constant flux of 140 L m⁻² h⁻¹ through a 20-μm Ni membrane into 9, 18, 27, 42, and 60 mL of water to achieve an aqueous-to-organic phase volumetric ratio of 1.5, 3.0, 4.5, 7.0 and 10.0, respectively. The Z-average decreased with an increase in $V_{aq}/V_{or}$ (Fig. 7.2), which can be explained by the fact that the size of a NP formed during solvent displacement is a result of the relative rates of nucleation, particle growth, and agglomeration. The promotion of particle growth over nucleation leads to fewer and larger particles. The rate of particle growth is:

$$G = K_g (C_{PCL} - C_{PCL}^*)^g$$  \hspace{1cm} (7.1)

where $K_g$ is the growth constant, $C_{PCL}$ and $C_{PCL}^*$ are the local concentration and solubility of PCL in the solvent mixture, respectively, and $g$ is typically between 1 and 2 for organic systems (Zhao et al., 2007). The rate of nucleation is given by:

$$B = K_b (C_{PCL} - C_{PCL}^*)^b$$

where $K_b$ is the nucleation constant, and the index $b$ lies between 5 and 10. Since $b > g$, lower supersaturations, $C_{PCL} - C_{PCL}^*$, promote particle growth over nucleation, leading to fewer and larger particles, whereas higher supersaturations promote nucleation, resulting in a larger population of smaller particles. The organic phase was initially brought in contact with pure water and the rate of solvent exchange was very high. However, since THF gradually accumulates in the aqueous phase, the rate of solvent exchange decreases leading to lower supersaturations. The THF concentration in the aqueous phase at any time was higher for smaller $V_{aq}/V_{or}$ values, resulting in lower supersaturations and the formation of larger NPs under these conditions. In addition, at higher THF concentrations in the aqueous phase, the rate of Ostwald ripening was higher due to greater solubility of PCL, which may also play a role in the formation of larger NPs at smaller $V_{aq}/V_{or}$.
Agglomeration is another important factor in nanoprecipitation. Agglomerates form when growing NPs collide with each other and fuse together to form larger particles. Agglomeration is more pronounced at smaller $V_{aq}/V_{or}$ values, because the collision frequency of particles is proportional to the second power of their number density.

Fig. 7.3 shows particle size distribution of the samples prepared at different aqueous-to-organic ratios and rotation speeds. Smaller and more uniform NPs were formed at higher aqueous-to-organic volumetric ratios. The same behaviour in membrane micromixing was observed by Du et al. (2011) in the preparation of SiO2 NPs, Huang et al. (2013) in the preparation of ZnO NPs and Laouini et al. (2013a; 2013b; 2013c) in the production of liposomes and polymeric micelles. The same trend was obtained when NPs were fabricated in microfluidic devices (Othman et al., 2016; 2015a; 2015b).

### 7.4.2 Effect of agitation speed of aqueous phase

As shown in Fig. 7.2, an increase in the agitation speed from 200 to 1300 rpm caused a decrease in the Z-average, which can be attributed to the higher rate of solvent displacement, and hence higher supersaturation that can be achieved. Higher rotation speeds also helped to reduce agglomeration of freshly formed sticky particles near the membrane surface by providing higher mass transfer rates away from the membrane surface. The smallest Z-average (196 ± 5 nm) and PDI (0.128 ± 0.012) values were obtained at the highest rotation speed of 1,300 rpm and $V_{aq}/V_{or} = 10$. On the other hand, the broadest particle size distribution with a PDI of 0.269 ± 0.023 and the largest Z-average value were obtained at the lowest agitation speed of 200 rpm and $V_{aq}/V_{or} = 1.5$.

### 7.4.3 Effect of transmembrane flux

Fig. 7.4 shows the effect of flux on the particle size distribution, Z-average, and PDI at an agitation speed of 1300 rpm, an aqueous-to-organic phase volumetric ratio of 10, and a PCL concentration in the organic phase of 1 g L$^{-1}$. With an increase in flux
from 140 to 351 L m\(^{-2}\) h\(^{-1}\), a significant broadening of the particle size distribution was observed with PDI rising from 0.128 to 0.164. The increase in the organic phase flow rate leads to an increase in the rate of PCL mass transfer to the aqueous phase, which is given by: \(C_o Q_o\), where \(C_o\) is the PCL concentration in the organic phase and \(Q_o\) is the organic phase flow rate. The higher the PCL influx into the aqueous phase, the higher the concentration of particles near the membrane surface after polymer precipitation and hence, the higher the likelihood of the particle aggregation, which explains a broader particle size distribution at higher flux. On the other hand, the higher influx of PCL into the aqueous phase led to higher supersaturation, which is why the \(Z\)-average remained constant in the investigated range of transmembrane flux. Similar trends were observed for other NPs formed in membrane contactors (Laouini et al. 2011; Laouini et al., 2013a; Khayata et al., 2012; Sheibat-Othman et al., 2008).

**Fig. 7.2:** The average particle size, \(Z_{ave}\), of PCL NPs produced at different aqueous-to-organic phase volumetric ratios and different agitation speeds using a 20-\(\mu\)m Ni membrane. The PCL concentration in the organic phase is 1 g L\(^{-1}\) and the transmembrane flux is 140 L m\(^{-2}\) h\(^{-1}\).
7.4.4 Effect of polymer concentration

The effect of PCL concentration in the organic phase on the Zaverage and PDI at a rotation speed of 1300 rpm and a flux of 140 L m$^{-2}$ h$^{-1}$ is presented in Table 7.1. NPs with the largest Zaverage (347 ± 11 nm) and PDI (0.243 ± 0.023) values were obtained at 6 g L$^{-1}$. As discussed above, the size of the NPs is dependent on the rate at which organic solvent diffuses into the aqueous phase and the rate at which the particle nuclei collide and fuse together. At higher polymer concentration in the organic phase, more nuclei per unit volume were formed and hence, particle aggregation was more pronounced. In addition, at higher PCL concentration the viscosity of the organic phase was higher which resulted in reduced diffusion rate of THF into the aqueous phase and reduced supersaturation. At low supersaturation, polymer nuclei grow faster than they nucleate resulting in larger NPs. Similar behaviour was observed by Khayata et al. (2012) and Jaafar-Maalej et al. (2011) who prepared PCL NPs and liposomes using SPG membrane. Laouini et al. (2011) have prepared liposomes in a hollow fibre membrane contactor and found an increase in the mean vesicle size from 114 to 228 nm when the phospholipid concentration in the organic phase increased from 20 to 80 mg mL$^{-1}$.

7.4.5 Effect of membrane pore size

The effect of the pore size of nickel membrane on the particle size at an agitation speed of 1300 rpm, an aqueous-to-organic volumetric ratio of 10, a flux of 140 L m$^{-2}$ h$^{-1}$ and a polymer concentration in the organic phase of 1 g L$^{-1}$ is shown in Table 7.1. In membrane emulsification, the mean droplet diameter, $d_d$, in dripping regime is proportional to the mean pore diameter, $d_p$: $d_d = c d_p$, where $c$ is the proportionality constant that can vary between 2 and 10 (Charcosset et al., 2004). In nanoprecipitation, the fundamental role of membrane is to provide good mixing of solvent with antisolvent: since nucleation is much faster than mixing, the generation of nuclei is governed by the rate of mixing step. Poor mixing results in low nucleation rates and a small population of large NPs, whereas good mixing results in high nucleation rates and a large population of small NPs. Smaller pores provide better mixing and thus smaller NPs, probably because the organic phase is split into thinner jets after passing through the membrane and the mixing time increases with the square
of diffusion distance. On the other hand, smaller pore sizes can enhance membrane wetting, clogging and fouling, which can compromise the process. In this work, the Z-average decreased from 234 to 196 nm on reducing the pore size from 40 to 20 µm at $V_{aq}/V_{or} = 10$, revealing that the particle size in this pore size range was affected by the width of the jets formed at the pore outlets. However, the NPs formed using a 10 µm membrane were larger than those formed using a 20 µm membrane (Table 7.1), probably due to fouling that had occurred within the 10 µm pores. As can be seen from the SEM image of the membrane cross section in Fig. 7.7 (b), the pores of a nickel membrane have a conical shape and may be more prone to fouling.

![Graph showing the volume-weighted particle size distribution of PCL NPs as a function of aqueous-to-organic phase volumetric ratio.](image)

**Fig. 7.3**: The volume-weighted particle size distribution of PCL NPs as a function of aqueous-to-organic phase volumetric ratio. The pore size of Ni membrane is 20 µm, the transmembrane flux is 140 L m$^{-2}$ h$^{-1}$ and the PCL concentration in the organic phase is 1 g L$^{-1}$. 

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Fig. 7.4: The effect of transmembrane flux through a 20 μm nickel membrane on: (a) the volume-weighted particle size distribution, and (b) the Z-average and polydispersity index, PDI. The agitation speed was 1300 rpm, the aqueous-to-organic phase volume ratio was 10, the PCL concentration in the organic phase was 1 g L⁻¹.

Table 7.1: The effect of the PCL concentration, the membrane pore size and the aqueous-to-organic volumetric ratio on the Z-average and polydispersity index, PDI. The agitation speed was 1,300 rpm and the transmembrane flux through a nickel membrane was 140 L m⁻² h⁻¹.

<table>
<thead>
<tr>
<th>PCL concentration (mg mL⁻¹)</th>
<th>Membrane pore size (μm)</th>
<th>Volumetric ratio, V_{aq}/V_{or}</th>
<th>Mean size, Z_{ave} (nm)</th>
<th>Polydispersity index, PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>10</td>
<td>196 ± 5</td>
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</tr>
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<td>3</td>
<td>20</td>
<td>10</td>
<td>278 ± 7</td>
<td>0.221 ± 0.01</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>10</td>
<td>347 ± 11</td>
<td>0.242 ± 0.02</td>
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<tr>
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<td>10</td>
<td>218 ± 13</td>
<td>0.160 ± 0.04</td>
</tr>
<tr>
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</tr>
<tr>
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<td>0.198 ± 0.04</td>
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<tr>
<td>1</td>
<td>10</td>
<td>3</td>
<td>277 ± 5</td>
<td>0.219 ± 0.01</td>
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</tbody>
</table>
### Effect of Polyvinyl Alcohol (PVA)

NPs can be stabilised by adding an amphipathic compound to the non-solvent that adsorb at the interface and provide a steric barrier against particle growth and aggregation. In this work, amphipathic polymer PVA was added to the aqueous phase in the amount between 0.1 and 1.0 wt% and the NPs were prepared under optimum conditions specified in the caption to Fig. 7.5. The produced NPs were stored at ambient temperature over a period of 36 days to investigate the effect of PVA concentration on the long-term stability of the NPs.

Partially hydrolysed PVA is a copolymer of poly(vinyl acetate) and poly (vinyl alcohol) with considerable block copolymer character (Sahoo et al., 2002). The hydrophobic vinyl acetate part is preferentially attached to a hydrophobic surface of PCL, leaving the more hydrophilic vinyl alcohol segments dangling in the aqueous phase. At relatively low PVA concentrations ($C_{\text{PVA}} \approx 0.1$ wt%), when surface coverage is much below the saturation, NPs were highly unstable due to bridging flocculation as a result of the tendency of PVA chains to adsorb onto the surface of two or more NPs simultaneously (Seo et al. 2015). As $C_{\text{PVA}}$ increased to 0.2 wt% and the surface of the NPs became covered by PVA to more than 50% of the saturation coverage, steric stabilization dominated over bridging flocculation and the NPs were

<table>
<thead>
<tr>
<th>Concentration</th>
<th>PVA Addition</th>
<th>PVA wt%</th>
<th>NP Diameter</th>
<th>PVA Diameter</th>
</tr>
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<tbody>
<tr>
<td>1</td>
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<td>1.5</td>
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<td>0.245 ± 0.04</td>
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<td>10</td>
<td>234 ± 20</td>
<td>0.218 ± 0.04</td>
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<td>464 ± 11</td>
<td>0.389 ± 0.04</td>
</tr>
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</table>

$^a$: Each value is a mean of three repeated measurements. The error bars are standard deviations.

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stable throughout the investigated period (Fig. 7.5 (a)). The probability of forming flocs during bridging flocculation is proportional to $\theta(1-\theta)$ (Kim et al., 2011), where $\theta = \Gamma/\Gamma_{\text{max}}$ is the fraction of the NP surface coated with adsorbed flocculant. This function shows a maximum at $\theta = 50\%$, which implies that bridging flocculation is the most dominant for the surface coverage of $50\%$, but becomes increasingly less likely as the surface coverage approaches $100\%$.

However, with a further increase in $C_{\text{PVA}}$ above $0.2\, \text{wt}\%$, nonadsorbed PVA molecules play an increasingly dominant role by introducing depletion attraction, which gives rise to a depletion flocculation. The magnitude of depletion attraction is proportional to the osmotic pressure in the aqueous phase, leading to increased particle instability between $0.2$ and $1\, \text{wt}\%$ PVA (Fig. 7.5 (a)). Further increase in $C_{\text{PVA}}$ above $1\, \text{wt}\%$ would stabilize the particles again, which is termed as depletion stabilization (Kim et al., 2015). However, high PVA concentrations in the aqueous phase lead to large particle size, due to high viscosity of the aqueous phase so the PVA concentrations above $1\, \text{wt}\%$ were not investigated in this work. The presence of PVA in the aqueous phase contributed to an increase in the $Z_{\text{average}}$ size at day 0, from $196 \pm 5\, \text{nm}$ (without PVA) to $202 \pm 1\, \text{nm}$ for $0.2\, \text{wt}\%$ PVA, $219 \pm 8\, \text{nm}$ for $0.4\, \text{wt}\%$ PVA, $232 \pm 4\, \text{nm}$ for $0.6\, \text{wt}\%$ PVA, $252 \pm 13\, \text{nm}$ for $0.8\, \text{wt}\%$ PVA, and $313 \pm 14\, \text{nm}$ for $1.0\, \text{wt}\%$ PVA. There are several reasons that may contribute to this effect, such as depletion flocculation, coating of the NPs by a PVA layer and increase of the viscosity of the aqueous phase.

The effect of PVA concentration in the aqueous phase on the zeta-potential of PCL NPs during storage is shown in Fig. 7.5b. The negative charge of NPs was probably due to acidic nature of hydrogen atoms attached to alpha carbon atoms of residual acetate groups in the PVA chains, which remained after the manufacture of PVA by the hydrolysis of polyvinyl acetate (Wiśniewska, 2011). The zeta potential of the particles stabilized by $0.2\, \text{wt}\%$ PVA remained nearly unchanged within the storage period, revealing that the particle size was stable. The zeta potential has the highest negative value for $0.6–0.8\, \text{wt}\%$ PVA, but slightly decreased with time due to particle aggregation. It should be noted that PVA is a steric stabilizer, which means that low zeta potential values for $0.2\, \text{wt}\%$ PVA are not indication of poor particle stabilization.
Fig. 7.5: Effect of concentration of PVA in the aqueous phase on the storage stability of NPs at ambient temperature: (i) 0.2 wt% PVA, (ii) 0.4 wt% PVA, (iii) 0.6 wt% PVA, (iv) 0.8 wt% PVA and (v) 1.0 wt% PVA. The PCL NPs were prepared at a transmembrane flux of 140 L m\(^{-2}\) h\(^{-1}\), a stirring rate of 1300 rpm, and an aqueous-to-organic phase volumetric ratio of 10 using a 20 μm pore-sized nickel membrane. The PCL concentration in the organic phase was 1 g L\(^{-1}\).

7.4.7 Effect of pore shape and membrane fabrication process

The effect of membrane type on the average particle size, \(Z_{\text{ave}}\) and particle size distribution is shown in Fig. 7.6. The smallest Z-average (159 ± 8 nm) and most uniform particles (PDI = 0.107 ± 0.014) were obtained using a ringed 10 μm-SS
membrane, followed by 20 μm-Ni membrane ($Z_{ave} = 196 \pm 5$ nm, PDI = 0.128 ± 0.012) and 10 μm-Ni membrane ($Z_{ave} = 218 \pm 13$ nm, PDI = 0.160 ± 0.019). Khayata et al. (2012) produced vitamin E loaded PCL NPs stabilized with 12.5% (w/v) Tween® 80 using cross-flow SPG membrane with a pore size of 0.9 μm and obtained the smallest particle size of 165 nm and the smallest PDI of 0.18. Different particle sizes obtained using the same pore size but different membrane type can be attributed to different pore shapes arising from the different techniques used for membrane fabrication.

Laser drilling of pores occurs through rapid melting and vaporisation of stainless steel due to absorption of energy from a focused laser beam. The melt is expelled from the hole once the gas pressure in a cavity overcomes surface tension forces. A re-solidified material (dross) that cannot be fully ejected from the hole due to high viscosity of the molten material was formed at the pore exits (Fig. 7.7 (b) and Fig. S1 (d) in the appendix). Formation of dross can be minimised by using shorter wavelengths and pulse duration of laser beam. However, dross deposits did not have any adverse effect on the membrane performance, since the surface of the membrane on the laser exit side was in contact with incoming organic phase. Although the exit diameter of the pores was smaller than the inlet diameter, the pores were not significantly tapered, and thus the organic phase was injected from the pores at relatively high exit velocity contributing to the high mixing efficiency.

The main fabrication steps for Ni membrane are photolithography, nickel electroplating and membrane release. Photolithography starts with spin coating positive photoresist which is then irradiated with UV light through a mask that determines the geometry of the pores. The photoresist is then developed to remove the irradiated parts of the photoresist leaving cylindrical photoresist islands shown in Fig. S1 (d) in the supplement. The sieve is then electroformed in a nickel electroplating bath by depositing a Ni film in the voids left by the removed photoresist. The pores of nickel membrane had a conical shape with significant broadening toward the downstream side of the membrane (Fig. 7.7 (a)). The reason for that is that during electroforming not only upward growth between the photoresist islands, but also lateral overgrowth over the photoresist islands occurred. Shallow cylindrical cavities
on the upstream side of the membrane that can be seen in Fig. 7.7 (a) are the footprints of these photoresist islands. If the nickel growth takes place exclusively between the photoresist islands, the pore diameter would be equal to the diameter of these islands (~135 µm). A smaller pore size (10–40 µm) has been achieved by continuing to deposit Ni after the layer has reached the top of the resist pattern. The nickel then started to grow over the resist islands in the horizontal direction as well as in the vertical direction, as a result of which the pores became smaller and conical. Not all pores in Fig. 7.7 (a) are conical, since the membrane was not cut through the centre of each pore. If the cut edge is not perpendicular to the membrane surface and does not go through the centre of each pore, the cross section of some pores will be cylindrical rather than conical and the pores will not extend across the entire cross section of the membrane. Conical pores were more prone to fouling and less efficient in mixing than straight pores due to lower exit velocity of the organic phase.

Another reason for the formation of smaller NPs using SS membrane is that the pores of this membrane were strategically arranged over a reduced annular area on the membrane surface corresponding to the maximum shear stress.

![Graph](image)

**Fig. 7.6:** Effect of membrane type and pore size on: (a) volume-weighted particle size distribution, (b) average particle size, $Z_{ave}$ (nm). The NPs were prepared at a
transmembrane flux of 140 L m$^{-2}$ h$^{-1}$, a stirring rate of 1,300 rpm, and an aqueous-to-organic phase volumetric ratio of 10. The PCL concentration in the organic phase was 1 g L$^{-1}$ and no stabilizer was used.

Fig. 7.7: Scanning electron microscope (SEM) images of membrane cross sections at various magnifications: (a) whole nickel (Ni) membrane; (b) stainless steel (SS) ringed membrane.

7.4.8 Effect of membrane cleaning procedure

Membrane cleaning was performed using the procedure described in Section 7.2.3 in order to restore the original contact angle and remove all residual polymer particles from the membrane surface. By keeping a low contact angle, the membrane was preferentially wetted by the aqueous phase, which prevents the organic phase from spreading over the membrane surface and ensures that tiny jets of the organic phase emerging from the pores penetrate directly into the aqueous phase. Fig. S2 in the supplementary material shows optical micrographs of the downstream membrane surfaces before and after cleaning at different magnifications. The membranes were significantly fouled before cleaning with large particle aggregates deposited near the pore exits or inside the pores. After cleaning no particle could be seen on the
membrane surfaces and all pores were unclogged, indicating that the cleaning procedure was appropriate.

7.4.9 TEM and SEM images of produced nanoparticles

The FEG-SEM and TEM images of the NPs are shown in Fig. 7.8. As can be seen, PCL NPs have a spherical shape and relatively uniform size which is smaller than 200 nm. This particle size is in good correlation with the results obtained by static light scattering shown in Table 7.1 and Fig. 7.4 (a). FEG-SEM provided a 3-D image, while TEM produced a flat (2-D) image of the synthesised PCL NPs.

![Fig. 7.8: Micrographs of the formed PCL NPs: (a) FEG-SEM image; (b) TEM image.](image)

7.4.10 CFD simulation validation

In this section, the results of numerical modelling are reported in order to validate the experimental results and to better understanding flow pattern in the cell. Fig. 7.9 shows flow parameters in two different cross sections located above the membrane at the heights of 30 mm (vicinity of the blades) and 2 mm (vicinity of the membrane). Fig. 7.9 (a-i) shows the flow velocity vectors in the vicinity of the blades. The velocity is increasing by getting closer to the tips of the blades and decreases progressively by moving away from the blades. On the blade surface the velocity is roughly equal to the blade’s angular speed ($\omega_r$). The pressure contours around the blades are presented in Fig. 7.9 (a-ii). The highest pressure is on the leading faces near the tips of the blades because of the highest drag force. The pressure around trailing faces of the blades is low because of high velocity of the flow. Fig. 7.9 (a-iii) shows flow streamlines in the
vicinity of the blades. Two nearly symmetrical vortices were formed at the trailing edge of the blades near the tip because of high velocity. Cavitation occurs when the suction pressure on the back of the blades reaches the vaporisation pressure and the contours of cavitation-induced vapour volume fraction are shown in Fig. 7.9 (a-iv). Fig. 7.9 (b-i) illustrates velocity vectors of the flow in the plane near the membrane surface. The velocity vectors are parallel to the wall of the cell. The local velocity increases radially towards the wall until it reaches a maximum value and then suddenly drops to zero on the wall surface because of the no-slip boundary condition.

The pressure distribution in the plane positioned 2 mm above the membrane surface is shown in Fig. 7.9 (b-ii). The pressure is high near the wall of the cell due to generated centrifugal force and low in the centre. Therefore, the pressure distribution is axisymmetric, since the centrifugal acceleration, \( a_c \), is proportional to the distance from the axis of rotation (\( a_c = \omega^2 r_c \)). The pressure distribution in Fig. 7.9 (b-ii) is much more uniform than that in Fig. 7.9 (a-ii), because the flow near the membrane is more organised and less chaotic, Fig. 7.9 (b-i), indicating that the distance provided between the membrane surface and the impeller is adequate. Moreover, the pressure within the active region of the ringed membrane is very uniform, with local variations of less than 20 %, which ensures uniform flow distribution in the pores.

Fig. 7.10 is a plot of the shear stress on different planes parallel to the membrane surface as a function of the radial distance from the axis of rotation at 1300 rpm. The shear stress on the membrane surface (\( z = 0 \)) increases substantially towards the wall of the cell, reaches a peak value of 11.4 Pa at the transitional point, and then sharply declines to zero at the wall surface. The peak shear stress decreases exponentially with height and has a value of less than 2 Pa at the height of just 0.2 mm above the membrane surface. Therefore, the mixing rate is high on the membrane surface, where nucleation occurs and much smaller away from the membrane surface, which is useful because it limits the rate of particle aggregation.
**Fig. 7.9:** Flow in the cell at 1300 rpm rotation speed simulated at: (a) the cross section in the vicinity of the blades \((z = 30 \text{ mm})\), and (b) the cross section in the vicinity of the membrane \((z = 2 \text{ mm})\), with (i) velocity vectors, (ii) pressure contours, (iii) streamlines, (iv) vapour volume fraction contours (cavitation zones). All results are for counter-clockwise movement of blades and \(z\) is the vertical distance from the membrane surface.
Fig. 7.10: Local shear stress as a function of the radial distance from the axis of rotation at a stirrer speed of 1300 rpm for different distances from the membrane surface (CFD results).

Table 7.2 provides a comparison of numerical and analytical solutions for the transitional radius and peak shear stress on the membrane surface. The analytical solutions were derived using Eqs. (S18) – (S22) in the supplementary material. There is a good agreement between the numerical and analytical results with the peak shear stress on the membrane surface ranging between 11.4 and 14.0 Pa and the transitional radius between 10.7 and 13.3 mm. These values for the transitional radius are within or very close the operating region of the ringed (SS) membrane. Because the pores of SS membrane were located only in the high shear region near the rotational radius, the average shear stress was higher for that membrane than for the nickel membrane. Therefore, ringed membrane should provide better micromixing performance than the nickel membrane, as confirmed by the experimental results.
CHAPTER 7

<table>
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<th>Parameter</th>
<th>CFD results</th>
<th>Analytical results (Nagata, 1975)</th>
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<tbody>
<tr>
<td>Transitional radius, $r_{trans}$ (mm)</td>
<td>13.3</td>
<td>10.7</td>
</tr>
<tr>
<td>Maximum shear stress, $\tau_{max}$ (Pa)</td>
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<td>14.0</td>
</tr>
<tr>
<td>Average shear stress, $\tau$ (Pa)</td>
<td>-</td>
<td>7.03 (for whole membrane) and 13.1 (for ringed membrane)</td>
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7.5 Conclusions

New process for the preparation of biodegradable polymeric nanoparticles has been developed, which employed dispersion of a polymer-containing amphiphilic organic solvent into the aqueous phase through a micro-engineered membrane combined with nanoprecipitation. The particle size distribution has been precisely tuned by the membrane pore size and shape, the location of active region of the membrane, aqueous-to-organic volumetric ratio, agitation speed, transmembrane flux and polymer concentration in the organic phase. The higher the aqueous-to-organic volumetric ratio, the higher the nucleation rate and the lower the rate of particle growth and aggregation, resulting in a larger population of smaller particles. At the same aqueous-to-organic volumetric ratio, the interdiffusion rate of two phases was higher at the higher agitation speed, resulting in higher supersaturation and smaller particle size. Increase in the flux through the membrane and polymer concentration in the organic phase led to domination of particle agglomeration over nucleation and formation of fewer and larger particles. Steric stabilization of PCL particles has been achieved by adding 0.2 wt% PVA to the aqueous phase, with the lower and higher PVA concentrations causing fast bridging and depletion flocculation, respectively.

The ringed stainless steel membrane with laser drilled pores was found to provide better performance than whole nickel membrane due to straight pores localized at the
high shear stress region on the membrane surface. The numerical and analytical modelling revealed the existence of peak shear stress on the membrane surface at the transitional radius and rapid reduction in the shear stress by moving away from the membrane surface. Due to significant overgrowth of the resist islands during electroforming, nickel membrane had funnel-shaped pores, which led to less efficient mixing process probably due to lower exit velocity of the organic phase in the pores. The membrane cleaning procedure was highly efficient and enabled to restore fully the original membrane properties and remove all residual particles from the membrane surface.

The future work will be directed towards encapsulation of an immunosuppressive drug (rapamycin) within biodegradable polymer matrix using a ringed stainless steel membrane under the optimal operating conditions established in this study.
CHAPTER 8

PREPARATION OF FUNCTIONAL NANOPARTICLES LOADED WITH IMMUNOSUPPRESSIVE DRUG USING ENGINEERED MEMBRANE MICROMIXING COMBINED WITH SOLVENT DISPLACEMENT METHOD

Chapter overview

Immunosuppressive agent, rapamycin (RAPA) loaded polycaprolactone (PCL) nanoparticles (NPs) were successfully synthesised by antisolvent nanoprecipitation method combined with micro-engineered membrane dispersion cell. Stainless steel (SS) ringed micro-engineered membrane was applied due to well-defined maximum shear stress provided from its effective micro-mixing surface area, thus improving mixing process between aqueous-organic phase and leading to the production of smaller particle size. The incorporation of RAPA onto PCL NPs matrices under optimum operational parameters (rotation speed = 1300 rpm, aqueous-to-organic phase volumetric ratio = 10 and transmembrane flux = 140 L m⁻² h⁻¹) and process variables (PCL concentration = 6 g L⁻¹, RAPA concentration = 0.4 w/w over mass of PCL excipient and PVA surfactant concentration in the aqueous phase = 0.2 wt%) resulted in the average particle size of 204.0 ± 1.2 nm, encapsulation efficiency, %E.E. of 98.91 % and drug loading of %D.L. of 28.26 %, which are significantly suitable for intravascular delivery. The particle size increased on increasing both the drug loading in the polymer matrix at higher aqueous-to-organic volumetric ratio. 0.4 w/w of RAPA concentration was considered as the optimum drug loading for PCL NPs as it showed small differences in the percentage of drug loading, if up to this amount. The encapsulation of RAPA in the NPs was confirmed by transmission electron microscopy, X-ray diffraction, differential scanning calorimetry and attenuated total reflection-Fourier transform infrared spectroscopy.

8.1 Introduction

Rapid progress in the field of nanomedicine in recent years offers new promising approaches in systematic delivery of poor water solubility and poor bioavailability of
active ingredients, as well as in instability and dosing problems (Barratt, 2000). A wide selection of nanoscale materials such as polymeric nanoparticles (Soppimath et al., 2001), liposomes (Samad et al., 2007), polymeric micelles (Kevin et al., 2009) and dendrimers (Svenson & Tomalia, 2012) have been employed as drug carriers with a broad variety of useful properties, such as longevity in the body, specific targeting to certain disease sites, enhanced intracellular penetration, contrast properties allowing for direct carrier visualization in vivo, stimuli-sensitivity, and others (Torchilin, 2009). Biodegradable polymeric nanoparticles (NPs), such as poly-D,L-lactide-co-glycolide (PLGA), polylactide (PLA), poly-ε-caprolactone (PCL), poly-alkyl-cyanoacrylates, chitosan and gelatin are frequently used due to their controlled/sustained release property, subcellular size, biocompatibility with tissue and cells, stable in blood, non-toxic, nonimmunogenic, noninflammatory and applicable to various water insoluble drugs (Kumari et al., 2010). Polymeric NPs with size in the range of 10–1000 nm can be easily prepared by spontaneous solvent displacement or nanoprecipitation technique, which is less extensive, less energy consuming and more facile, as well as widely applicable technique in the presence/absence of a surfactant or any additives. The formation started by fast diffusion of solvent and non-solvent phases, leads to the instantaneous formation of a colloidal suspension (Fessi et al., 1992; Fessi et al., 1989; Jain, 2000).

Owing to the numerous advantages of applying polymeric NPs as a drug-carrier and an innovative therapeutic strategy discovered from rapamycin as a potent immunosuppressive agent to suppress organ transplant rejection and in treatment tumors (Zhang et al., 2013), rapamycin-loaded nanoparticles (NPs) with appropriate size and high loading capacity become more reliable to sustain drug delivery after surgical or transplantation procedures with smaller dose in an effective form and controlled drug administration within the body (Acharya et al., 2009). Rapamycin or also known as sirolimus is a triene macrolide antibiotic with immunosuppressive activity, was isolated from Streptomyces hygroscopicus in 1978 (Kim et al., 2011). Delivery is impeded by rapamycin’s poor solubility in water (2.6 μmL⁻¹ at 25 °C) (Simamora et al., 2001), and by its low bioavailability (Napoli et al., 1997) and dose-limiting toxicity, which eventually could be overcome by the use of polymeric NPs as its carrier. In addition, rapamycin is very unstable in phosphate-buffered saline and

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HEPES buffer under all conditions; their degradation effect on the drug is slower at 4 °C–8 °C and fastest at 37 °C with almost all drugs will be destroyed within 24 hours (Kim et al., 2011).

Currently, a number of studies demonstrated various types of biodegradable polymers with self-assembly capacity to solubilise rapamycin (RAPA) into hydrophobic polymer core and thus protect RAPA against inactivation in biological conditions and improve its bioavailability. Luderer et al. (2011) determine the applicability of RAPA-loaded biodegradable poly(D,L-lactide) (PDLLA) nanoparticles as drug carriers to prevent restenotic processes after stent implantation with average 250 nm sized 20% (w/w) RAPA-loaded nanoparticles were extensively characterised with regard to in vitro degradation, biocompatibility and in vitro drug release. RAPA-loaded polymeric poly(lactide-co-glycolide) (PLGA) nanoparticles were also prepared by Zou et al. (2011), Shi et al. (2014) and Acharya et al. (2009) for sustained inhibition in coronary surgical bypass and cancer treatment. Zweers et al. (2006) fabricated rapamycin-loaded nanoparticles from poly(ethylen oxide) and poly(D,L-lactic-co-glycolic acid) block copolymers (PEO-PLGA) without additional stabilizer by salting-out method and further in-vivo release study in PBS (pH 7.4) at 37 °C. RAPA-loaded poly(e-caprolactone)-poly(ethylene glycol)-poly(e-caprolactone) nanoparticles were also prepared and characterised by Zhang et al. (2013) purposely for corneal transplantation. Yuan et al. (2008) synthesised RAPA-loaded chitosan/PLA nanoparticles for immunosuppression in corneal transplantation with size of about 300 nm in diameter through nanoprecipitation method using cholesterol-modified chitosan as a stabilizer. Meanwhile, Macedo et al. (2012) developed tolerogenic dendritic cells (tolDC) to minimize the chronic administration of immunosuppression (IS) drugs (RAPA) in transplantation and autoimmune disease. Chen et al. (2013) also prepared rapamycin encapsulated in dual-responsive micelles for cancer therapy with extensively release profiles, uptake and in vitro/in vivo study.

With the emerging technologies applied for the formation of different pharmaceutical nanoparticles, such as polymeric nanospheres and nanocapsules (Charcosset & Fessi, 2005; Limayem et al., 2006), lipid nanoparticles (Charcosset et al. 2005; D’oria et al., 2009), liposomes (Laouini et al., 2011; Jaafar-Maalej et al., 2011), metal nanoparticles...
(Wagner et al., 2004), polymeric nanoparticles (Gasparini et al., 2008; Wei et al., 2008), barium sulfate nanoparticles (Chen et al., 2004) and silver nanoparticles (Kakazu et al., 2010), produced using different type of micro-porous membranes (i.e. shirasu porous glass (SPG), nickel (Ni), stainless steel (SS) membrane) (Vladisavljević & Williams, 2005) fitted in a membrane contactor combined with different physicochemical production process (i.e. solvent displacement, salting-out, emulsification-solvent evaporation) (Nagavarma et al., 2012), a narrower controllable particle size distribution and highly monodispersed particles formation is now seemingly feasible. In addition, the optimal sizes of a nano-sized agent, which can deliver an adequate dose of drugs distributed homogeneously and thus, induce the most efficient therapeutic, is reportedly should be between diameters of 100–300 nm or less for intravascular delivery which primarily relied on exploiting the well-known enhanced retention and permeability (EPR) effects (Kobayashi et al., 2014; Allen & Cullis, 2004; Decuzzi et al., 2009).

Recently, a new micro-engineered stainless steel, SS membrane, consisting of an array of regularly spaced, rectilinear pores and a perfect rectangular array of uniform pores fitted in a dispersion cell was introduced by the Micropore Technologies Ltd. This micro-engineered membrane is analogous to an array of parallel microfluidic channels which allow a much more uniform and controllable injection of organic phase (dissolved polymer and drug in organic solvent) into an aqueous phase at overall much higher flow rate than is possible in microfluidic devices (Laouini et al., 2013). A micro-engineered SS membrane is technically prepared by pulsed laser drilling drilled pores (Disperse Technologies Ltd, UK) (Geerken et al., 2008; Vladisavljević & Williams, 2005; Dowding et al., 2001) or end-milling (Kobayashi et al., 2008), which comprise of significantly straight-through pores.

The purposes of this study were: (i) to investigate the possibility of efficiently encapsulating rapamycin (RAPA) onto poly(ε-caprolactone) (PCL) NPs matrix (hereafter denoted as RAPA-PCL NPs) using SS ringed membrane that fitted in the dispersion cell combined with solvent displacement method; (ii) to study the effect of RAPA on the physical properties of the synthesised NPs using various physicochemical analytical methods, like transmission electron microscopy (TEM),

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x-ray diffraction (XRD), differential scanning calorimetry (DSC) and attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR); and (iii) to investigate the encapsulation efficiency of RAPA inside the NPs and their in vitro drug release behavior. Both RAPA and PCL are Food and Drug Administration (FDA) approved, so it is relatively easy to translate these methods to clinical application. PCL was chosen to serve as a most stable hydrophobic biodegradable polyester drug-carrier due to its suitability for the encapsulation of poorly water soluble drug, owing to its high permeability to small drug molecules and its negligible tendency to generate an acidic environment during the degradation process as compared to polylactids and polyglicolids, which led to the generation of inflammatory reactions (Lemoine et al., 1996; Woodruff & Hutmacher, 2010). Polyvinyl alcohol (PVA), one of the most popular stabilizers for the production of PCL nanoparticles was also selected for hindering aggregation and agglomeration between each newly synthesised particles (Woodruff & Hutmacher, 2010).

8.2 Materials and methods

8.2.1 Materials

Poly(ε-caprolactone) (PCL, $M_w = 14,000$ g mol$^{-1}$ with a glass transition temperature of 60 °C) and hydrophilic surfactant, tetrahydrofuran, THF (HPLC grade, purity ≥ 99.9%), polyvinyl alcohol (PVA, $M_w$ 13–23 kDa) were purchased from Sigma-Aldrich (Dorset, UK). Acetone (Ace) (HPLC grade, purity ≥ 99.8%), acetonitrile (HPLC grade, purity ≥ 99.9%), methanol HPLC grade, purity ≥ 99.8%), and acetic acid (HPLC grade, purity ≥ 99.7%) were purchased from Fisher Scientific Ltd. (Loughborough, UK). Rapamycin (RAPA) (sirolimus, purity ≥ 99 %) was provided by Chunghwa Chemical Synthesis & Biotech Co. Ltd. (New Taipei City, Taiwan). All chemicals were of analytical grade. The antisolvent phase was pure water produced by reverse osmosis (Mili-Q®, Millipore) or aqueous surfactant solutions. The hydrophilic surfactant was used to prevent agglomeration, coalescence and imperfect surface formation, as well as to reduce the size of the NPs. The organic phase was prepared by dissolving RAPA and pure PCL in Ace and contained 6 g L$^{-1}$ of the excipient (PCL) and 0.6–3.0 g L$^{-1}$ of RAPA. The aqueous phase was 0.2 wt% PVA dissolved in Milli-Q water.
8.2.2 Membrane and membrane module

The RAPA-PCL NPs were prepared using a membrane stirred dispersion cell with a flat disc SS ringed membrane fitted under the paddle blade stirrer as shown in Fig. 8.1 (a). Both stirred cell and membrane were supplied by Micropore Technologies Ltd. (Derbyshire, UK). The agitator was driven by a 24 V DC motor and power supply (INSTEK model PR 3060) with the paddle rotation speed could be controlled by the applied voltage at 1300 rpm, which corresponding to the 13 Pa average shear stress on the membrane surface. Stainless steel (SS) ringed micro-engineered membrane containing uniform cylindrical pores with a diameter of 10 μm arranged at a uniform spacing of 200 mm with effective diameter difference was 0.6 cm ($r_1 = 9$ mm, $r_2 = 13$ mm) with 2.76 cm$^2$ affective area were used for the optimum operating parameters investigation. A perfectly rectangular array of pores located circularly within the ringed area of the membrane surface as can be seen on the micrograph in Fig 8.1 (b).

8.2.3 Preparation of rapamycin-loaded PCL nanoparticles

Rapamycin-loaded PCL NPs were prepared by a solvent displacement technique (nanoprecipitation) as described in detail by Fessi et al. (1989). Briefly, 6 g L$^{-1}$ of organic phase containing a homogeneous solution of 0.6 % (w/w) PCL and 0.06–0.3 % (w/w) of RAPA was accurately weighted and dissolved in acetone. This organic solution was then injected through the membrane using a high pressure single-syringe infusion pump, 230 VAC syringe pump (Cole-Parmer Instrument Co. Ltd., UK) and the flow rate of 0.65 mL min$^{-1}$, corresponding to a dispersed phase flux of 38.8 m$^2$h$^{-1}$. The cell was filled with 60 mL of mili-Q water or dissolved surfactant in aqueous solution (PVA, 0.2 wt%) and the experiment was run until predetermined aqueous-to-organic phase volumetric ratio, $V_{aq}/V_{or}$ of 10 was achieved. The aqueous phase immediately turned cloudy as soon as the organic phase brought into contact with the aqueous phase, indicating the formation of nanoparticles suspension. Once the desired amount of organic phase had passed through the membrane, both pump and the agitator was switched off and the nanosuspensions were collected for further physicochemical analyses. Fig. 8.1 (b) showed a rapid solvent displacement mechanism occurred during the formation of NPs and a photomicrograph of 10 μm-SS ringed membrane. Blank PCL NPs also prepared by the same method without
adding rapamycin at any stage of the preparation. Every preparation was repeated at least three times.

After each experiment, the membrane was sonicated in acetone for 30 min, followed by treatment with Mili-Q water in an ultrasonic bath for 5 min (in order to improve the hydrophilicity of the membrane surface). While for storage purpose, the membrane was soaked in acetone purposely to remove all unwanted residuals.

### 8.2.4 Determination of encapsulation efficiency and drug loading

The newly formed nanosuspension must be evaporated in a vacuum oven (Technico, Fistreem International Ltd, Loughborough, UK) under absolute pressures below 10 Torr at room temperature for about 30 min until the smell of Ace had completely vanished. The nanosuspension was then ultracentrifuged (Heraeus Labofuge 400R centrifuge, Thermo Scientific) at 15,000 rpm (23,000 g) for 1 h. The supernatant containing the dissolved free drug was separated from the sediment and analysed by an established RP-HPLC method. Typically, 20 μL samples were injected on a Symmetry® C-8 column (4.6 × 50 mm; 3.5 μm; Waters, UK) eluted with methanol/1%(v/v) acetic acid in deionised water (75/25 v %) at a flow rate of 1.0 mL min⁻¹. A LC-10Avp Shimadzu UV-VIS detector was used to detect RAPA at 278 nm and ambient temperature. The amount of RAPA in the sample was calculated using a standard curve of RAPA in acetonitrile at various concentrations. The drug entrapment efficiency (E.E.) and drug loading (D.L.) were calculated from equations (8.1) and (8.2), respectively. The determinations were carried out in triplicate and the results were expressed as mean ± SD (n = 3).

\[
\% E.E. = \left[1 - \left(\frac{M_R}{M_T}\right)\right] \times 100\% \quad (8.1)
\]

where \(M_R\) is the mass of drug in the supernatant and \(M_T\) is the total mass of drug used in the sample (Zhang et al., 2013; Yuan et al., 2008; Chen et al., 2013). The drug loading of the NPs was determined as:

\[
\% D.L. = \left[\left(\frac{M_T - M_R}{M_{NP}}\right)\right] \times 100\% \quad (8.2)
\]

where \(M_{NP}\) is the total mass of NPs in the sample (Othman et al., 2016; Othman et al., 2015).
8.2.5 Characterisation of the prepared NPs

8.2.5.1 Particle size analysis

The polymeric NPs size was measured determined by dynamic light scattering (DLS) (Delsa™ Nano HC Particle Analyser (Beckman Coulter, High Wycombe, UK), by measuring the fluctuations of scattered light as a function of time (Submicron, 2011) with 120 s detecting time was allocated for each runs. Each samples were analysed by following the standard procedures described in Section 2.6.1.1. This method was developed by Provencher et al. (1978) describes bimodal and smooth distribution without the need of additional information, such as an initial estimate for the particle size.

8.2.5.2 Transmission electron microscopy (TEM)

The morphology structure of rapamycin-loaded PCL NPs was characterised by JEOL JEM-2000FX transmission electron microscopy (TEM), fitted with an Oxford Instruments Inca EDX system. Each samples were analysed by following the standard procedures described in Section 4.2.4.3.

8.2.5.3 Differential scanning calorimetry (DSC)

The physical state of ~ 5 mg pure RAPA, pure PCL, blank PLC NPs, the physical mixture of blank PCL NPs and RAPA as well as RAPA-PCL NPs was characterised using a differential scanning calorimetry (DSC) thermogram analysis (TA Instruments, Model Q100 DSC). RAPA-loaded PCL NPs and blank PLC NPs were lyophilised by freeze-drying the sediment after centrifugation in a freeze dryer (Edwards, type EF4 Modulyo). Each samples were analysed by following the standard procedures described in Section 5.2.5.3.
Fig. 8.1: Experimental set-up for the preparation of encapsulated polymeric biodegradable nanoparticles with; (a) membrane dispersion cell rig with a photomicrograph of 10 μm stainless steel, SS ringed micro-engineered membrane and (b) rapid solvent displacement mechanism during the formation of nanoparticles.

8.2.5.4 X-ray diffractometry (XRD)

X-ray diffraction (XRD) spectra of pure RAPA, pure PCL, blank PLC NPs, the physical mixture of blank PCL NPs and RAPA as well as RAPA-PCL NPs was detected using a Bruker D8 diffractometer by exposing samples to CuKa radiation (40 kV, 20 mA) over the 20 range from 5° to 50°. Each samples were analysed by following the standard procedures described in Section 5.2.5.4.
8.2.5.5 Attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy

2-3 mg of the individually powdered sample (pure RAPA, pure PCL, blank PLC NPs, the physical mixture of blank PCL NPs and RAPA, RAPA-PCL NPs) was analysed its ATR-FTIR spectra using Thermo Scientific Nicolet iS50 ATR spectrometer. Each samples were analysed by following the standard procedures described in Section 6.2.4.6.

8.2.5.6 In vitro drug release studies

In-vitro drug release studies were carried out using 0.3 % (w/w) of RAPA-loaded PCL NPs using two different concentrations of N, N-Diethylnicotinamide (DENA) in 3 M and 5.8 M were used as a release media. 0.3 % (w/w) RAPA-nanocrystals without PCL NPs host was also analysed as a control sample with dissolution medium of 3 M DENA. Briefly, DENA solution has been identified as an excellent hydrotropic agent for poorly soluble drugs, which also known as an amphiphilic agent that comprised of both hydrophilic and hydrophobic parts (Shukla et al., 2014). Recently, many studies have reported an increase use of DENA solution as a hydrotrope to accelerate the paclitaxel and rapamycin dissolution rate from drug-incorporated biodegradable stent matrices (Simamora et al., 2001; Alexis et al., 2004; Baek et al., 2004). Phosphate Buffer Saline (PBS) having pH 7.4 similar to pH of human blood previously has been applied as a release medium, but incomplete drug release due to lower solubility of drug in PBS was a major crisis for in vitro drug release study (Tailor et al., 2008). It may also be necessary to use hydro-alcoholic media, such as ethanol, ethanol and isopropyl alcohol to accelerate the dissolution rate (Alexis et al., 2004). In this study, a mixture of DENA solution, ethanol, hydrophilic surfactant (Tween-80), ethanol and PBS (pH = 7.4) was used as a dissolution/release medium in order to improve the RAPA solubility, hence to accelerate the RAPA dissolution rate. A known amount of lyophilised sample (5 mg) was dispersed in a centrifugation tube that consists of 5 mL prepared dissolution medium. The tube was then incubated in a shaking bath at 50 rpm and 37 °C to mimic the human blood stream conditions (Behera et al., 2013). At predetermined time intervals, the test tube was taken out of the shaker and centrifuged at 15,000 rpm for 30 min at −4 °C. The supernatant was
removed and the amount of drug released was quantified by RP-HPLC method as described above. The dissolution medium was replenished with fresh dissolution medium to keep the total volume of the release medium constant. All samples were in triplicate and the results were expressed as a mean value ± S.D.

8.3 Results and discussion

8.3.1 Optimum operational parameters validation

The selection of good organic solvent is a crucial initial step that should be taken for the formation of polymeric NPs by nanoprecipitation method. The good organic solvent must be able to dissolve polymer and must be miscible with water (Van Krevelen & Hoftyzer, 1976). Due to the higher solubility of RAPA in acetone and the incapability of THF to dissolve RAPA, this study was run to validate the optimum operational parameters (rotation speed = 1300 rpm and transmembrane flux = 140 L m\(^{-2}\) h\(^{-1}\), aqueous-to-organic volumetric ratio = 10) that have been obtained from the previous study. However, these optimum parameters were only obtained with the use of THF as the organic solvent. The variations on the average particle size of blank PCL NPs were investigated with the increasing of volumetric aqueous-to-organic phase ratio using two different organic solvents (Ace and THF) by applying 10-μm SS ringed membrane and the operational parameters, as can be seen in the Fig. 8.2. Indeed, at the higher aqueous-to-organic volumetric ratio, PCL from the organic phase become more diluted after mixing with the aqueous phase, which may result in the formation of smaller particles. Similar results were obtained were obtained by Seo et al. (2015) in itraconazole NPs formation using SPG membrane, Du et al. (2011) for SiO\(_2\) NPs production using a microfiltration membrane dispersion microreactor, Huang et al. (2013) in ZnO NPs formation and Laouini et al. (2013a, 2013b, 2013c) in the production of liposomes and polymeric micelles by membrane contactors.

Minor increasing in the average particle size was observed with nearly 3.4–7.5 % different in acetone compared to THF solvent, as can be seen in Fig. 8.2. This result was well correlated with the solubility distance, \(D_{PCL-solvent}\) calculated from Bagley's two-dimensional solubility circle as defined by Bordes et al.(2010) and Othman et al. 2015), where \(D_{PCL-Ace}\) for acetone was 6.53 and for THF, \(D_{PCL-THF}\) was 1.08. The
particle size was much smaller in THF as it was found to be a good solvent for PCL NPs formation by nanoprecipitation method. Nevertheless, acetone also can be concluded as a good solvent for PCL as well as for rapamycin. No further investigation has to be done for finalising the optimum operating parameters that should be applied in the rapamycin encapsulation experiment.

![Graph](image)

**Fig. 8.2:** The average particle size, $Z_{\text{ave}}$, of blank PCL nanoparticles produced using different organic solvents (THF and acetone) at different volume ratio and constant agitation speed, 1300 rpm using 20 μm size of SS ringed micro-engineered membrane. The PCL concentration in the organic phase is 1 g L$^{-1}$ and the transmembrane flux is at 140 L m$^{-2}$ h$^{-1}$.

### 8.3.2 Drug encapsulation efficiency and drug loading

Rapamycin was successfully encapsulated into PCL NPs matrices using SS ringed micro-engineered membrane dispersion cell combined with solvent displacement method under optimum operational parameters of; rotation speed = 1300 rpm, aqueous-to-organic phase volumetric ratio = 10, transmembrane flux = 140 L m$^{-2}$ h$^{-1}$ at different amount of rapamycin content dissolved in 6 g L$^{-1}$ of organic phase. As presented in Table 8.1, it was clearly observed that the average particle size of
Rapamycin-loaded PCL NPs increased from 189.4 ± 4.0 nm to 217.9 ± 5.0 nm and PDI ranged from 0.006 ± 0.025 to 0.073 ± 0.023 with an increase in the feed ratio of drug to polymer, which caused by more rapamycin molecules being attached to PCL NPs matrices. More hydrophobic aggregates were presumably formed at the higher concentration of RAPA, leading to the increment value of PDI. Meanwhile, RAPA was efficiently loaded in PCL NPs, reaching encapsulation efficiency, % E.E. in the ranged of 98.79 to 98.93 % with the increasing amount of drug loading from 0.1 to 0.5 w/w (see Table 8.1), due to the stronger hydrophobic interaction between RAPA and PCL. The drug loading, % D.L. was increased rapidly from 8.98 % (0.1 w/w RAPA content) to approximately 32.98 % (0.5 w/w RAPA content), proving that almost RAPA were successfully retained in the PCL NPs matrices. Small difference in % D.L. (nearly ~ 4.72 %) was observed from 0.4 w/w to 0.5 w/w RAPA content compared to the other loadings, thereby indicating 0.4 w/w as the optimum concentration of RAPA to be encapsulated. Zhang et al. (2013) showed similar trends with an increment in particle size (170 to 180 nm), encapsulation efficiency (84.1 to 99.7 %) and drug loading (5.03 to 14.91 %) by the increasing percentage of RAPA to polymer feed ratio from 5 to 15 %. Theoretically, the amount of drugs bound to NPs and the type of interaction between drugs and nanoparticles was reported significantly depend on the chemical structure of the drug, chemical structure of the polymer and the conditions of drug loading (Mahapatro & Singh, 2011).

Table 8.1: The effect of rapamycin-to-excipient mass ratio in the organic phase on the average particle size, drug encapsulation efficiency and drug loading of the prepared NPs at volume ratio, \( V_{aq/V_{or}} = 10 \). The PCL concentration in the organic phase is 6 g L\(^{-1}\) \((n = 3)\).

<table>
<thead>
<tr>
<th>RAPA/excipient mass ratio in organic phase</th>
<th>Volumetric ratio, ( V_{aq/V_{or}} = 10 )</th>
<th>E.E. (%)</th>
<th>D.L. (%)</th>
<th>Average size of NPs, ( Z_{ave} ) (nm)</th>
<th>Polydispersity index (PDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>E.E. (%)</td>
<td>98.79</td>
<td>8.98</td>
<td>189.4 ± 4.0</td>
<td>0.006 ± 0.025</td>
</tr>
<tr>
<td>0.2</td>
<td>D.L. (%)</td>
<td>98.80</td>
<td>16.47</td>
<td>194.8 ± 3.8</td>
<td>0.040 ± 0.031</td>
</tr>
<tr>
<td>0.3</td>
<td>Average size of NPs, ( Z_{ave} ) (nm)</td>
<td>98.92</td>
<td>22.83</td>
<td>198.5 ± 6.7</td>
<td>0.030 ± 0.024</td>
</tr>
<tr>
<td>0.4</td>
<td>Polydispersity index (PDI)</td>
<td>98.91</td>
<td>28.26</td>
<td>204.0 ± 1.2</td>
<td>0.066 ± 0.035</td>
</tr>
<tr>
<td>0.5</td>
<td>* Note: Each analysis was repeated three times ((n = 3)) and the average particle sizes were determined. The error bars are standard deviations.</td>
<td>98.93</td>
<td>32.98</td>
<td>217.9 ± 5.0</td>
<td>0.073 ± 0.023</td>
</tr>
</tbody>
</table>
8.3.3 Characterisation of the NPs

8.3.3.1 TEM analysis of the NPs

TEM micrographs exhibited discrete spherical shape of blank PCL NPs and RAPA-loaded PCL NPs, whilst disordered shape for RAPA nanocrystal without PCL host, as can be seen clearly in Fig. 8.3. The RAPA-loaded PCL NPs showed significantly larger particle size compared to the blank PCL NPs, proving that RAPA was successfully emended or solubilised onto PCL NPs matrices which are coherent with the values detected by light scattering particle size analyser. No free crystals were detectable. Some agglomerations and deteriorated shape of particles were detected in RAPA nanocrystal sample with bigger size of particle (≥ 500 nm), due to the absence of PCL host/excipient that leading to the aggregation of free RAPA. These TEM images were also well correlated with the intensity size distributions detected by dynamic light scattering particle analyser, as depicted in Fig. 8.4. Uniform and narrower intensity size distributions presenting how monodisperse was the particle were attained from both blank PCL NPs and RAPA-loaded PCL NPs. Conversely, a broader intensity size distribution was obtained from RAPA nanocrystal sample which attributable to its bigger size and uniformity.

8.3.3.2 XRD analysis

XRD analysis was used to check the changes in the rapamycin crystal morphology with the respect to a polymorphic transition and thereby to understand the solid state of nano-crystals of rapamycin drug (Chauhan & Cauchan, 2014). The crystallinity of blank PCL NPs, physical mixture of RAPA-PCL NPs and RAPA-loaded PCL NPs were inspected by XRD with pure PCL and RAPA samples as the references. As depicted in Fig. 8.5, the diffraction pattern of pure rapamycin showed characteristic high-intensity peaks at 7.2°, 9.9°, 10.3°, 11.1°, 12.5°, 14.5°, 15.3°, 15.5°, 16.2°, 20.0°, 20.4°, 21.8° and 23.5° were observed, while the specific peaks of pure PCL were attributed to 21.4° and 23.8°, accordingly. Similar results for both pure RAPA and PCL were attained by Kim et al. (2011) and Yeo et al. (2012), respectively. However, the characteristic peaks of blank PCL NPs were found slightly shifted from the pure PCL sample, with reducing crystalinity from 68.9 % to 67.1 %. This is due to the
polymer precipitation caused by rapid diffusion of organic phase to the outer phase and fast evaporation of polymer-dissolving organic solvent, which significantly reducing polymer network arrangement time and, thus inhibit the crystallisation process (Izumikawa et al., 1991).

In the spectrum of mixture of blank PCL NPs and RAPA, specific peaks of both blank PCL NPs and RAPA at 7.2°, 10.3°, 14.2°, 9.3°, 21.4° and 23.8° were observed, suggesting that rapamycin was in microcrystalline form in the mixture and that there was an absence of any possible interaction with PCL NPs. Nevertheless, in the spectrum of RAPA-loaded PCL NPs, only the specific peaks of PCL NPs nanoparticles at 21.4° and 23.8° were detected, while specific peaks of rapamycin disappeared, indicating that rapamycin was amorphously dispersed into the core of the PCL NPs and that the drug had a great affinity for PCL. This was also proved by the reducing crystallinity percentage obtained from pure RAPA (78.9 %) to RAPA-PCL mixture (71.3%) and RAPA-loaded PCL NPs (63.7%) sample.

8.3.3.3 DSC analysis

DSC is a thermal analysis instrument that widely used in the pharmacology and nanoscience to measure the material transitions and properties of materials/substances (Gill et al. 2010) as well as to gain more information about the crystallinity and possible interactions between PCL and the drug. The DSC profiles of pure RAPA, pure PCL, blank PCL NPs, mixture of RAPA-PCL NPs and RAPA-loaded PCL NPs are presented in Fig. 8.5. Pure rapamycin showed an endothermic peak in the region of 183−205 °C, corresponding to its melting point. The split peaks at 183 °C and 197 °C might be related to the two different crystal forms of rapamycin (Rouf et al., 2011).

As expected, pure PCL polymer exhibited an endothermic peak at 60 °C, corresponding to the melting point of PCL, which was in accordance with previous findings (Guilherme et al., 2014). Blank PCL NPs endothermic peak were significantly shifted to 52 °C which revealing the reduction in crystallinity percentage of PCL obtained from XRD analysis. Two-peaked melting exoterm of individual RAPA and blank PCL NPs were detected in the physical mixture of RAPA–PCL (Fig. 8.5 (b)) with a shallow, broad endothermic peak detected between 180 °C to 200 °C. The thermogram peaks of RAPA-loaded PCL NPs (Fig. 8.5 (b)) were shifted to lower
temperatures (\(\sim 50^\circ\text{C}\)) indicating that RAPA completely entrapped in the PCL NPs hydrophobic core was in an amorphous or disordered-crystalline phase. Moreover, the appearance of a single peak for the loaded NPs indicates that PCM was uniformly distributed in the NPs. Similar observations were also reported by Zhang et al. (2013 and Kim et al. (2011).

**Fig. 8.3:** TEM images of; (a) blank PCL NPs; (b) rapamycin (RAPA) encapsulated PCL NPs; and (c) RAPA nanocrystal without PCL host at various magnifications prepared using the drug-to-excipient mass ratio in the organic phase of 0.4.
Fig. 8.4: The intensity size distributions of blank PCL NPs, RAPA-PCL NPs and RAPA nanocrystals produced in a membrane dispersion using 10 μm size of stainless steel micro-engineered membrane at the transmembrane flux is at 140 L m$^{-2}$ h$^{-1}$, rotation speed 1300 rpm. The PCL concentration in the organic phase is 6 g L$^{-1}$. 
Fig. 8.5: (a) X-ray diffractograms; and (b) DSC thermograms of; 1- pure RAPA; 2- pure PCL; 3-blank PCL NPs; 4- physical mixture of RAPA; and 5- RAPA-loaded PLA NPs.
8.3.3.4 ATR-FTIR spectroscopy

FTIR analysis was used to characterise any chemical interactions that occurred in the polymer due to the addition of drug during the synthesis reaction (Acharya et al., 2009). The FTIR spectra of pure rapamycin, blank PCL NPs, mixture of RAPA-PCL NPs and RAPA-loaded PCL NPs are shown in Fig. 8.6. Characteristic bands due to different functional groups in pure rapamycin, appeared at 3418 cm\(^{-1}\), due to O–H stretching vibrations, and 2875 and 2932 cm\(^{-1}\), due to C–H stretching vibrations, while the peak 1718 cm\(^{-1}\) corresponded to C=O carbonyl stretching, whereas the peak at 1377 cm\(^{-1}\) was due to –CH bending/deformation, conjugated double bond at 1635 cm\(^{-1}\) and 995 cm\(^{-1}\), in which the peak of 1635 cm\(^{-1}\) is the stretching vibration of conjugated ethylene bond and a peak of 995 cm\(^{-1}\) is the bent vibration of C–H as a proof of trans-substitutive double bond existing. There are wide peaks of stretching and bent vibration of methylene, which show that there are many methylenes in the rapamycin structure (Acharya et al., 2009). Meanwhile, the peaks which appeared at 2942.37 cm\(^{-1}\) and 2867.18 cm\(^{-1}\) are due to the C–H stretching appeared in pure PCL spectrum. Beside, a strongest ester carbonyl absorption band, C=O stretching vibration located at 1719.54 cm\(^{-1}\) and 1722.17 cm\(^{-1}\) respectively. An asymmetrical C–H bending vibration in CH\(_3\) group showed an absorption band at 1453.33 cm\(^{-1}\) and 1466.56 cm\(^{-1}\), respectively. C–O–H bond shows a peak at 1378.95 cm\(^{-1}\) and 1365.30 cm\(^{-1}\) respectively. Additionally, FTIR spectra peaks present at 1181.48 cm\(^{-1}\) and 1167.31 cm\(^{-1}\) are attributed to C–O stretching, as reported by Liau et al. (2014).

No bifurcation of peaks or any bends of peaks detected in the spectra of the blank PCL NPs compared with pure PCL sample, which means that FTIR could not prove any changes in the internal crystal structure of PCL as a result of dissolution and nanoprecipitation, that proposed by XRD and TGA analyses. New small peak at 995 cm\(^{-1}\) was detected in the spectra of RAPA-PCL NPs mixture, which corresponding to the bent vibration of C–H inherited from pure RAPA sample. The characteristic absorption peaks due to conjugated ethylene bond stretching vibration at 1635 cm\(^{-1}\) in the RAPA constituent were seemingly visible in the region between 1600 and 1635 cm\(^{-1}\) for all RAPA-loaded PCL NPs. This indicates that RAPA was encapsulated.
within the PCL NPs matrices, but has not strongly interacted with PCL polymer since only small vibrations detected with no peak shifts occurred.

Fig. 8.6: ATR-FTIR spectrums of: 1- pure RAPA; 2- pure PCL; 3- blank PCL NPs; blank PCL NPs; 4- physical mixture of blank PCL NPs-RAPA; and 5- RAPA-loaded PCL NPs.

8.3.4 In vitro release of RAPA from nanoparticles

Efficient release of encapsulated drug from nanoparticles is an important parameter in developing successful formulations. In-vitro release of rapamycin from PCL NPs was measured over 18 h by recording the absorbance at 278 nm (Fig.8.7). Zero-time corresponds to the start of the incubation period by neglecting the amount of RAPA released during the nanoprecipitation process. The total amount of drug released during the processes of NPs and RAPA nanocrystals formation by nanoprecipitation method and incubation time was 100 % for RAPA nanocrystals without PCL NPs host, 91.34 % and 93.78 % for RAPA-loaded PCL NPs sample in 3 M and 5.8 M of DENA concentration in dissolution medium, respectively. As expected, with the
existence of higher DENA concentration in the release medium, RAPA tends to be soluble and more RAPA could be easily released (Baek et al., 2004). A burst drug release was observed over the first 2 h which can be attributed to relatively high solubility of RAPA in the dissolution mediums. It was followed by very slow drug diffusion over a long period of time from hydrophobic PCL cores into the dissolution media. The fastest released was observed from RAPA nanocrystals attributed by the absence of PCL core and RAPA dissolution. After 18 h of release, about 6.2 to 8.7 % rapamycin still remained in the nanoparticles, both for 5.8 M and 3 M of DENA concentration, which can be explained by the rapamycin still being embedded into the core of the nanoparticles with slow diffusion out from the PCL nanoparticles occurring in the release medium. Slow degradability of PCL polymer associated with the polymer matrix erosion mechanisms also contributed to this slower sustains release of rapamycin present inside the core of nanoparticles (Holland & Tighe, 1992; Musumeci et al., 2006; Yuan et al., 2008).

![Graph](image)

**Fig. 8.7:** In vitro release profile from; (a) rapamycin (RAPA) nanocrystals in 3 M DENA solution; (b) RAPA-PCL NPs in 3M DENA solution and; (c) RAPA-PCL NPs in 5.8 M DENA solution. The error bars represent the standard deviations of three repeated measurements ($n = 3$).
8.4 Conclusions

In this study, rapamycin-loaded PCL NPs was successfully synthesized by anti-solvent displacement method combined with micro-engineered membrane dispersion cell. A monodispersed (referring to the PDI and narrower particle size distributions) and spherical shape of nanoparticles within the size range of 153.4 ± 4.2 nm to 204.0 ± 1.2 nm, for blank PCL NPs and RAPA-loaded PCL NPs, respectively were produced under optimum operating parameters/conditions; rotation speed = 1300 rpm, aqueous-to-organic phase volumetric ratio = 10, transmembrane flux = 140 L m⁻² h⁻¹, PVA surfactant concentration in the aqueous phase = 0.2 wt%, PCL concentration = 6 g L⁻¹ and RAPA concentration = 0.4 w/w over mass of PCL excipient. The particle size increased on the increasing drug loading content in the polymer matrix, and decreased with increasing aqueous-to-organic volumetric ratio in the membrane dispersion cell. 0.4 w/w of RAPA concentration was considered as the optimum drug loading to be encapsulated onto PCL NPs matrices with encapsulation efficiency, %E.E. of 98.91 % and drug loading, %D.L. of 28.26 %. The encapsulation of RAPA in the PCL NPs was confirmed by transmission electron microscopy (TEM), X-ray diffraction (XRD), differential scanning calorimetry (DSC), and attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR). In-vitro release studies indicated that the rapamycin was released from PCL nanoparticle in a sustained manner over a period of 18 h. Rapid drug release was observed from the RAPA nanocrystal samples with 100 % release in 2 h, due to the absence of PCL NPs host.

Thus, we conceived that the rapamycin-loaded PCL NPs might be useful in clinical application primarily for intravascular delivery of anti-restenosis drugs. In future work, the potential of integrating micro-engineered membrane dispersion systems that consists of regular pore spacing membrane with the crystallisation system will be investigated in order to achieve smaller and more uniform size of active ingredients (drug) crystals growth.
CHAPTER 9

REVERSE ANTISOLVENT PRECIPITATION OF SIZE-TUNEABLE MICRO-CRYSTALS BY MEMBRANE DISPERSION MICROMIXING I: A NEW INSIGHT INTO INTEGRATED MEMBRANE CRYSTALLISATION SYSTEM

Chapter overview

Reverse antisolvent precipitation of nonsteroidal anti-inflammatory drug, monohydrate piroxicam (PRX) microcrystals were investigated using novel engineered membrane dispersion micromixing process. The organic phase comprising of 1.5–2.5 wt% PRX dissolved in acetone was injected into the aqueous phase (Mili-Q water or 0.1–0.5 wt% hydrophilic surfactant solution) using two micro-fabricated membranes with different pore morphologies and spatial pore arrangements of 10 μm pore-sized ringed stainless steel (SS) membrane fitted in a semi-batch membrane dispersion cell and 40 μm pore-sized whole nickel (Ni) membrane applied in a membrane continuous dispersion system. The size of monohydrate PRX microcrystals were precisely controlled over a range of 7–34 µm by controlling PRX concentrations, aqueous-to-organic phase volume ratios (V_{aq}/V_{or}), type and concentration of surfactants (hydroxypropyl methyl cellulose (HPMC), polyvinyl alcohol (PVA), and pluronic-123 (P-123)). Smaller crystals (< 10 µm) with uniform crystals size distribution (CSD) that are significantly suitable as inhaled drugs were produced using ringed SS membrane at the optimum process parameters of 25 g L^{-1} PRX concentration, 0.06 wt % HPMC and V_{aq}/V_{or} of 20. The monohydrate PRX microcrystals exhibited an elongated-rhombohedral shape at 0.06 wt % HPMC were confirmed by optical micrographs and scanning electron microscopic (SEM) images. Clear characterisation peaks of monohydrate PRX microcrystals were successfully verified by Raman spectroscopy and differential scanning calorimetry (DSC). A semi-batch membrane dispersion system indicated better performance in producing PRX crystals over the other designed systems (i.e. bulk mixing, semi-batch, and continuous membrane dispersion system. Further application of these PRX microcrystals also have been investigated in a controllable seeded crystallisation system.

9.1 Introduction

An increasing number of newly developed active pharmaceutical ingredients (APIs) suffer from poor water solubility with too low oral bioavailability and erratic
absorption (Douroumis & Fahr, 2007; Merisko-Liversidge et al., 2003; Müller & Peters, 1998). An estimated 40% of APIs fall under Biopharmaceutical Classification System (BCS) Class 2 or Class 4 for low solubility and low permeability (Löbenberg & Amidon, 2000; Varshosaz et al., 2013). These substances usually characterised by low bioavailability due to their low dissolution in gastrointestinal fluid (Teeranachaideekul et al., 2008). PRX is one of the most potent nonsteroidal anti-inflammatory drugs categorised in BCS Class 2 drug with high membrane permeability but low water solubility (Javadzadeh et al., 2007). The solubility and dissolution rate in water can be improved by producing salts, co-crystals or amorphous particles by increasing the surface area of the drug (Merisko-Liversidge et al., 2003; Müller et al., 2001). A very common way to increase drug substance surface area is via micronization, which produces particles in the size range of less than 10 microns (Joshi, 2011).

Many approaches have been tested to reduce particle size, including (i) mechanical micronization, such as milling or grinding (Krause & Müller, 2001), (ii) supercritical fluid technique (Tenorio et al., 2007) and (iii) controlled antisolvent precipitation (Chiou et al., 2007). However, these first two techniques show inevitable drawbacks, including the tendency of the produced particles to agglomerate, require a high-energy input, electrostatic effects, broad particle size distributions, thermal degradation, contamination and poor reproducibility among different batches, low yield and high equipment cost (Varshosaz et al., 2013; Viçosa et al., 2012). Nevertheless, with the use of antisolvent precipitation, the ultrafine drug particles formation for inhaled drug delivery is seemingly possible, thus appeared as the most promising technique (Dong et al., 2009; Zhang et al., 2006). The key motivation for producing these particles is to create conditions that favour very rapid particle formation and little or no particle growth. This technique is a straightforward method, rapid and easy to perform, does not require high stirring rates, sonication, elevated temperatures or surfactants, and Class 1 solvents can be avoided (Fessi et al., 1989; Othman et al., 2016a; 2016b; 2015a; 2015b). It has been successfully used to prepare several drugs, such as budesonide (Rasenack et al., 2003), danazol (Zhao et al., 2007), beclomethasone dipropionate (Wang et al., 2007), prednisolone (Li et al., 2007), atorvastatin (Zhang et al., 2009), griseofulvin and fenofibrate (Meng et al., 2009), salbutamol sulfate (Xie
et al., 2010), L-glutamic acid (Lindenberg et al., 2008), and paracetamol (Reis et al., 2014).

PRX((4-hydroxy-2-methyl-N-(2-pyridyl)-2H-1,2-benothiazine-3-carboxamide-1,1-dioxide) is one of the safest and most potent nonsteroidal anti-inflammatory drug that is most widely used in the treatment of rheumatic conditions like inflammation, pain due to injury, menstrual cramps, arthritis, and other musculoskeletal conditions and non-rheumatic conditions like biliary and ureteric colic, dysmenorrheal inflammation and fever (Kini et al., 2011; Silva et al., 2012). PRX inhibits two cyclooxygenase isozymes (COX-1 and COX-2) by accelerating the prostaglandins (PG) synthesis that helps to mediate many physiological functions of the body, including control of blood pressure, respiratory and gastrointestinal tract smooth muscles (Simmons et al., 2004). COX-1 is involved in maintaining the physiological function of the gastrointestinal and renal tracts and COX-2 participates in the pain and inflammation (Frölich, 1995). Anti-inflammatory actions of PRX are due to the inhibition of COX-2 only whereas the inhibition of COX-1 leads to the unwanted side-effects like dyspepsia, heartburn, nausea and vomiting (Brooks, 1998). PRX indeed can be formed into three anhydrous polymorphic forms (I, II and III) and one monohydrate form (Hansen & Qu, 2015; Liu et al., 2014). Among the four polymorphs, forms I and II can be produced by simple crystallisation from solution, whereas forms III and IV can be prepared by melt/quench cooling or by spray drying from amorphous piroxicam. However, form I is not stable in the used solvents, thus, transformation into the monohydrate form is significantly encouraging especially to store any suspension samples for extended periods (Hansen & Qu, 2015). Since polymorphism can change the pharmacokinetic and pharmacodynamics characteristics of PRX (Shet et al., 2004), it is important to develop a fast and effective technique/system for controlling the PRX polymorphism, as suggested in this study.

Despite of focussing on the antisolvent precipitation/crystallisation processes for the formation of monohydrate PRX microcrystals, the number of publications were also dedicated to the integrated membrane antisolvent precipitation/crystallisation processes in order to improve the performance of crystallisation operations (Charcosset et al., 2010; Curcio et al., 2003; Gugliuzza et al., 2009). The main features
of this integrated system are; (i) to control and limit the maximum level of supersaturation due to defined mass transfer across the membrane, so that crystal nucleation and growth is more uniform and predictable; (ii) to act as heterogeneous nucleation-inducing substrates; (iii) to control solid features such as size, structure (polymorphism), shape, and purity and level of agglomeration; and (iv) to reduce energy consumption compared to cooling or evaporative crystallisation (Charcosset et al., 2010; Ye et al., 2013). Several membrane techniques have been reported; reverse osmosis, membrane distillation, membrane contactor, and membrane templates (Charcosset et al., 2010; Drioli et al., 2012). Depending on the chemical–physical properties of the membrane and on the process parameters (e.g. temperature, concentration, flowrate, etc.), the solvent evaporation rate, and hence supersaturation degree, nucleation and growth rate can be controlled very precisely, by choosing a broad set of available kinetic trajectories in the thermodynamic phase diagram, that are not readily achievable in conventional crystallisation methods (Di Profio et al., 2007a; 2007b).

Integrated membrane crystallisation processes are frequently presented as a new alternative strategy to attain favourable crystals product. However, these studies are merely focused on the crystallisation or precipitation of a few model compounds (such as lysozyme, NaCl, carbonates, etc.) using a limited number of membrane materials (polypropylene (PP), polyvinylidene fluoride (PVDF), polyamide, and etc.) (Chabanon et al., 2016). To the best of our knowledge, this work is the first experimental study dealing with the formation of monohydrate PRX microcrystals by micro-engineered membrane dispersion cell, fitted with either ringed stainless steel (SS) or whole nickel (Ni) membrane. The same technique was successfully implemented for the formation of lipid nanoparticles (Charcosset et al., 2005), nanocapsules (Charcosset & Fessi, 2005), gel microbeads (Zhou et al., 2007), microcapsules (Wagdare et al., 2011), liposomes (Jaafar-Maalej et al., 2011; Laouini et al., 2013), polymeric nanoparticles (Othman et al., 2016) and barium sulfate nanoparticles (Chen et al., 2004).

In the reverse antisolvent crystallisation/precipitation procedure described in this work the membrane does not act as a sieving barrier for the selective transport of
specific components, but as a physical support that enable the generation and sustenance of a controlled supersaturated environment in which crystals can nucleate and grow. The effects of the composition of both the organic and aqueous phases and the operating conditions of the dispersion cell on the size distribution, agglomeration and morphology of the microcrystals produced were investigated in this study. Furthermore, both a semi-batch and a continuous membrane crystallisation/precipitation process were tested and compared. A novel membrane crystallisation technique that allows the production of micro-crystals with narrow size distribution and low degree of agglomeration is presented in this work. This approach is believed to be a very valuable alternative for the production of particles for inhalation because of its low cost, simplicity, the possibility to run in continuous and the high level of control on particle size and characteristics.

9.2 Materials and methods

9.2.1 Materials

Acetone (Ace, purity ≥ 99.98 %) was purchased from Fisher Scientific (Loughborough, UK), Piroxicam (PRX) (99% purity) for Form I and II were obtained from Hangzhou Hyper Chemicals Limited (Zhejiang, China). Polyvinyl alcohol (PVA, \( M_w = 13,000-23,000 \) g mol\(^{-1}\), 87-89 % hydrolysed), poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (Pluronic® P-123, \( M_n \sim 5,800 \)) and hydroxylpropyl methylcellulose (HPMC, typical \( M_w = 10,000 \)) were supplied by Sigma-Aldrich, Inc. (Dorset, UK) and used as water soluble surfactants. The antisolvent phase was deionized water obtained by reverse osmosis (Milli-Q®, Millipore) or aqueous surfactant solutions. The role of surfactant was to prevent agglomeration, coalescence and imperfect surface formation, as well as to reduce the size of the microcrystals. The organic phase was a homogeneous solution containing 15 – 25 g L\(^{-1}\) of PRX drug in Ace.

9.2.2 Membrane dispersion cell

Two different sets of experimental system (i.e. semi-batch and continuous membrane system) were performed in this study, as shown in Fig. 9.1.
In the first of experiment, the PRX microcrystals were initially prepared using a flat disc-shaped membrane fitted in a semi-batch stirred cell shown in Fig. 9.1 (a-(i-ii)). The cell and membranes were supplied by Micropore Technologies Ltd (Redcar, UK). The stirrer was driven by a 24 V DC motor (Instek model PR-3060) and its rotation speed was controlled at 1,500 rpm by the applied voltage. A ringed stainless steel (SS) membrane had the same dimensions and a reduced operating (active) area of 2.76 cm$^2$ containing ~6,912 pores with a perfectly rectangular array of 10 μm pores located circularly within the ringed area of the membrane surface with a pitch of 200 μm (Fig. 9.1 (a-iii)).

While, for the continuous membrane dispersion system (Fig. 9.1 (b-(i-ii))), a nickel (Ni) membrane with an effective diameter of 3.3 cm and an operating area, $A_m$, of 8.55 cm$^2$ with ~24,690 hexagonally arranged pores with a diameter of 40 μm, spaced apart at a constant distance of 200 μm was applied. Comprehensive observations on these two membranes have been clearly explained in our previous work (Othman et al., 2016a).

Fig. 9.1: Schematic diagram of; (a) Semi-batch membrane system containing paddle stirrer above a 10-μm of flat disc SS micro-engineered membrane with; (i) photomicrograph of 10-μm membrane, (ii) schematic diagram of the experimental set-up; (b) Continuous membrane system.
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up, (iii) rapid solvent displacement mechanism during the formation of PRX microcrystals; (b) Continuous membrane dispersion cell with a simple paddle stirrer above a 40-μm of flat disc Ni micro-engineered membrane with (i) photomicrograph of 40-μm membrane, (ii) schematic diagram of the experimental set-up for the formation of PRX microcrystals.

9.2.3 Experimental set-up and semi-batch preparation of monohydrate PRX microcrystals

The cell was filled with 30–180 mL of mili-Q water or aqueous surfactant solution and the experiment was run until a predetermined aqueous-to-organic phase volume ratio was achieved. The organic phase was 2.5–1.5 wt% PRX in Ace and was injected through the membrane using 11 Elite syringe pumps (Harvard Apparatus, Cambridge, UK). The organic phase flow rate, $Q$, through the ringed SS membrane was fixed at 18 mL min$^{-1}$ in order to achieve the same maximum transmembrane flux of 4000 L m$^{-2}$ h$^{-1}$ as acquired from the whole Ni membrane. To reach the same flux through the ringed membrane, the organic phase flow rate was reduced to 18 mL min$^{-1}$, calculated from the equation: $Q_R = (A_R / A_w)Q$, where $A_R$ and $A_w$ are the operating areas of the ringed and whole membrane, respectively. The stirring speed was fixed at the maximum value of 1500 rpm, which generated a shear stress on the membrane surface of 17.5 Pa. The aqueous phase simultaneously turned bright yellow and cloudy as soon as the organic phase was brought in contact with the aqueous phase due to rapid exchange of two solvents at the interface, i.e. Ace diffused from the organic to the aqueous phase and water diffused in the opposite direction (Fig. 9.1 (a–ii)). Each experiment was repeated at least three times. The maximum organic phase flow rate was applied in order to allow a rapid contact between the two phases and, thus, a faster generation of supersaturation that is associated to the precipitation of smaller crystals. The high shear stress at the membrane surface generated by the maximum stirring rate, instead, determines a quick removal of the newly formed crystals from the membrane surface, which is characterized by a high supersaturation, and their migration to the water-rich phase where crystal growth is not possible because of the low solute concentration. That also generates smaller crystals and a narrower size distribution.
After each experiment, the membrane was rinsed with Milli-Q water and then sonicated in Ace for 30 min using a Fisher Scientific ultrasonic bath (model FB 15046) in order to remove any microcrystals leftovers from the membrane surface. The membrane was rinsed again with Milli-Q water and then finally washed with Milli-Q water in an ultrasonic bath for 5 min to restore hydrophilic properties of the used membrane. The membrane surface was found progressively turn to hydrophilic throughout the cleaning procedures as has been proved in our previous study by measuring the membrane surface contact angle (Othman et al., 2016a). The membrane should be hydrophilic during experiments to minimize wetting of the membrane surface by the organic phase and pore clogging by the deposited hydrophobic drug.

**9.2.4 Experimental set-up for seeded batch crystallisation experiment**

The 350 g cooling crystallisations were carried out using a 400 mL jacketed glass vessel equipped with overhead stirring at 325 rpm (PTFE pitch blade turbine). Fig. 9.2 shows a schematic of the rig used for the experiments.

![Fig. 9.2: Schematic of the rig used for the experiments.](image)

The temperature was controlled using a PT-100 temperature probe connected to a Huber Ministat 125 thermoregulator. A PAT array was used, consisting of an RXN1 Raman analyser with immersion probe and 785 nm laser (Kaiser with iC Raman 4.1
software), G400 Mettler Toledo Focused Beam Reflectance Measurement (FBRM) probe (5-2000 microns equipped with iCFBRM software version 4.3) and a particle vision and measurement (PVM) V819 probe (Mettler Toledo) with an on-line image acquisition software (version 8.3). The pre- and post-processing of the data was done with Matlab R2013, iC Raman 4.1 and Excel 2010. The data from the FBRM and the Huber is transmitted in real-time to the CryPRINS software (Crystallisation Process Informatics System) which allows real time temperature control and simultaneous monitoring of signals from different probes (FBRM, ATR-UV/Vis, thermocouple, conductivity probes and pH-meter). The solvent for the seeded cooling crystallisation experiments was chosen based on solubility data as well as the available literature on piroxicam crystallisation (Hansen & Qu, 2015; Liu & Hansen, 2014).

A mixture of water and an organic solvent needs to be used in order to be able to grow crystals of the monohydrate form of PRX without excessively reducing its solubility (this compound is almost insoluble in pure water). Mixtures of 20 % water in acetone were used in this work since this solvent composition allows nucleation and growth of the monohydrate form (Hansen & Qu, 2015). The PRX solubility is still considerably increasing as the temperature rises, which is a required condition to grow crystals by cooling crystallisation. PRX solutions saturated at 40 °C (4.4 g of piroxicam in 350 g of solvent) were prepared and heated up to 50 °C. Temperature was kept constant for around 30 min to allow complete dissolution of solid piroxicam. After that, the solution was cooled down to 37 °C and seeded. The amount of seeds was 2 % of the total mass of PRX in solution in order to minimize crystal agglomeration right after seeding. Additional experiment using 6 % seeds was performed with the membrane crystallisation seeds for comparison.

9.2.5 Preparation of PRX microcrystals seed for crystallisation process

9.2.5.1 Membrane crystallisation

Monohydrate PRX microcrystals were prepared at the PRX concentration of 15 g L⁻¹, rotation speed of 1500 rpm (17.5 Pa), organic phase injection rate of 18 mL min⁻¹ (maximum transmembrane flux = 4000 L m⁻² h⁻¹) and aqueous-to-organic phase volume ratio, \( V_{aq}/V_{or} \) was 5 (i.e. \( V_{or} = 6 \) mL, \( V_{aq} = 30 \) mL) without surfactant addition.
by antisolvent precipitation method using a flat disc-shaped 40 μm of whole Ni membrane installed in a stirred cell. Similar experimental set-up, as shown in Fig. 1A was applied in this experiment. These operative conditions allow producing micro-crystals in the size range of 25–35 μm (volume mean diameter from Malvern Mastersizer). This slurry microcrystals were collected via pipette and inserted directly in the vessel during the seeded batch experiments.

9.2.5.2 Antisolvent crystallisation

Seeds from antisolvent crystallisation were produced pumping water in a solution of PRX in water (concentration of 35 mg g\(^{-1}\) acetone). The solution was prepared by dissolving solid PRX at 40 °C in the 400 mL vessel described in the Section 9.2.4. A peristaltic pump (Masterflex L/S 7544–06 drive, L/S 14 pump head, Cole-Parmer, IL, UK) was used to pump water in the PRX solution at a rate of 2.5 mL min\(^{-1}\). The rate was chosen after few preliminary experiments in order to obtain pure monohydrate PRX at the end of the batch. Both faster and slower flow rates determined nucleation of form II. FBRM was used during the experiments to monitor nucleation, agglomeration and shape of the particles. Crystals at the end of the experiment were filtered and dried. The CSD was measured using a Malvern Mastersizer.

9.2.5.3 Polymorphic transformation

Piroxicam form I (fine powder as purchased) was suspended in water and let transform to monohydrate. A total amount of 2.2 g of piroxicam was suspended in 500 g of water at 20 °C. The Raman immersion probe described in Section 9.2.4 was used to monitor the polymorphic transformation. At the end of the experiments the crystals were filtered and dried. Their CSD was measured by Malvern Mastersizer.

9.2.6 Characterisation of the prepared microcrystals

9.2.6.1 Particle size analysis

The crystals size distribution (CSD) was assessed using a Malvern Mastersizer 2000 (Malvern Instrument Ltd, Malvern, Worcesterhire, UK. This instrument uses laser diffraction and the full Mie theory to give an accurate CSD. The sample can be dispersed in a liquid and sent to the optical bench, which captures several scattering
patterns in order to average the results. The outcome of the analysis is the relative
distribution in volume percentage of the sample particles in the size classes, expressed
in μm. The results can be post-processed to calculate the surface, length or number
distribution or relevant statistics such as the derived diameters $D[m,n]$:

$$D[m,n] = \left[ \frac{\sum V_i d_i^{m-3}}{\sum V_i d_i^{n-3}} \right]^{1/m-n}$$  \hspace{1cm} (9.1)

where $V_i$ is the relative volume in class $i$ with mean class diameter of $d_i$. In particular,
$D[4,3]$ is defined as the Volume Weighted Mean and $D[3,2]$ as the Surface Weighted
Mean. Other relevant statistics are the standard deviation, the volume concentration
and the specific surface area (particle total area divided by total weight). The relative
span of a crystals size distribution was used to express the degree of crystals size
uniformity: (Malvern Instrument, 2007).

$$\text{span} = \frac{d(v,0.9) - d(v,0.1)}{d(v,0.5)}$$  \hspace{1cm} (9.2)

where $d(v,0.1), d(v,0.5)$ and $d(v,0.9)$ are the values of the particles diameter at
10 %, 50 % and 90 % of the cumulative distribution.

In this work, a saturated solution of piroxicam monohydrate in water at room
temperature was used as dispersant. The micro-crystals produced in the cell were
collected into a small Becker and then transferred to the wet dispersion unit using a
pipette. The final size distribution was the result of averaging two consecutive
measurements.

9.2.6.2 Raman microscopy

The polymorphic discrimination of the PRX crystalline structures was performed
using a Thermo Scientific DXR Raman Microscope (Thermo Scientific, UK) with
780 nm laser. The Raman spectrums were analysed with the accompanying version
8.3 of the Omnic software (Thermo Scientific, UK). Each spectrum was the results of
the averaging 10 measurement collected with 10 s scan time using a laser power of 15
mV. 10x Olympus of objective lens were applied in order to acquire higher samples
detection focus (Thermo Scientific, 2013).
9.2.6.3 Focused beam reflectance measurement (FBRM)

FBRM is an extensively used in situ technique that gives information about nucleation, dissolution, metastable zone width, polymorphic transformation, crystals growth and size distribution in particulate systems in real time. Based on the rotating speed of the laser ($v_s$ and back scattering time ($\Delta t$), the chord length distribution (CLD) for the particles is obtained using equation; chord length = $v_s \Delta t$. The CLD obtained from FBRM is grouped in 90 channels from 0.8–1000 μm. The readings obtained from FBRM can be displayed in a variety of formats from simple total number of counts per second to square weighted or cubic weighted distributions.

9.2.6.4 Differential scanning calorimetry (DSC)

Differential Scanning Calorimeter (DSC) analysis was performed using a TA Instruments Model Q100 to examine polymorphic purity and to validate the results obtained from Raman spectroscopy analysis. 5 – 10 mg of the sample (pure PRX and monohydrate PRX microcrystals) were accurately weighed into aluminium pans and hermetically sealed. Monohydrate PRX microcrystals produced by membrane dispersion cell were lyophilized by freeze-drying the sediment in a freeze dryer (Edwards, type EF4 Modulyo). The sample pan was heated from room temperature to 350 °C using a scanning rate of 10 °C min$^{-1}$ and a similar empty pan was used as the reference. Dry nitrogen at 60 mL min$^{-1}$ was used as the purge gas. Triple runs were carried out on each sample to check reproducibility.

9.2.6.4 Scanning electron microscopy (SEM)

Electron micrographs of PRX microcrystals samples precipitated using micro-engineered membrane dispersion cell without and in the present of surfactants (i.e. PVA, P-123 and HPMC) were obtained using a Hitachi model TM3030 benchtop SEM. Each samples were analysed by following the standard procedures described in Section 7.2.4.3.

9.3 Monohydrate PRX microcrystals formation system validation

In this study, a new insight into the integrate membrane crystallisation system can be fundamentally elucidated by comparing a few type of systems (System-1: bulk
mixing. System-2: semi-batch cell, System-3: semi batch membrane dispersion cell, and System-4: continuous membrane dispersion system). Two ultimate challenges of developing this integrated system have been addressed in several reports and reviews; (i) Product quality issues (quality by design) with the aims of achieving the desired polymorphic form, the CSD and the crystal shape factor to be mastered (Hermanto et al., 2007; Mangin et al., 2009; Variankaval et al., 2009; Roberts & Debenedetti, 2002; Tung, 2013), and (ii) Process issues include batch to continuous breakthrough approaches, scale up challenges, intensification and green engineering developments (Calabrese & Pissavini, 2011; Roberts & Debenedetti, 2002; Variankaval et al., 2009). Table 9.1 summarises the operating parameters/conditions utilised in each system (System-1, System-2, System-3 and System-4) with the average shear stress on the membrane surface was calculated based on the equations given in our previous study (Othman et al., 2016a). System-1 (bulk mixing) was conducted spontaneously without the aid of macro mixing effect (e.g. stirring speed) in order to represent the conventional technique. While, system-2 and -3 (as can be seen in Fig. 9.1 (a-i) were purposely investigated to validate the efficacy and impotency of using a micro-engineered membrane during the formation of microcrystals.

An extended observation/idea towards the development of an integrated membrane crystallisation system was further examined in System-4 (continuous membrane dispersion system), as depicted in Fig. 9.1 (b-i). This system was contributed by Micropore Technologies Ltd. (Redcar, UK), with the organic phase consisted of 15 g L\(^{-1}\) PRX in Ace and was injected through the membrane using a peristaltic pump (Masterflex L/S 7544–06 drive, L/S 14 pump head, Cole-Parmer, IL, UK) at a constant flow rate of 18 mL min\(^{-1}\) corresponding to the 1293 L m\(^{-2}\)h\(^{-1}\). The stirring speed was fixed at maximum value of 1500 rpm, which generated a shear stress on the membrane surface of 8.7 Pa. The aqueous phase was pumped into the cell at a constant flow rate of 90 mL min\(^{-1}\), which is equivalent to the aqueous-to-organic phase flow rate ratio, \(Q_{aq}/Q_{or}\) of 5. Spontaneous formation of PRX microcrystals started as soon as the organic phase was brought in contact with the aqueous phase. The PRX suspension was kept under stirring within the residence time of ~ 0.93 min (nearly 1 min) and finally the suspension was collected and its particle size was analysed using Malvern Mastersizer 2000 at different predetermined time intervals.
For clear observation and to prevent from having any agglomeration during the formation of PRX microcrystals, the organic phase was fixed at 15 g L\(^{-1}\) PRX in Ace, no surfactant was added in the aqueous phase and 40-µm of whole Ni was applied in System-3 and -4.

**Table 9.1:** The operating parameters and conditions utilised in each systems (System-1: bulk mixing, System-2: semi-batch cell, System-3: semi batch membrane dispersion cell, and System-4: continuous membrane dispersion system) and the volume weighted mean size (µm) of PRX microcrystals. The organic phase was 15 g L\(^{-1}\) PRX in Ace and the aqueous phase was Milli-Q water. No membrane was applied in System-1 and -2, whereas 40 µm whole Ni membrane for System-1 and -4.

<table>
<thead>
<tr>
<th>Systems</th>
<th>(V_{ow}/V_{or}) or (Q_{ow}/Q_{or})</th>
<th>Stirring speed (rpm)</th>
<th>Average shear stress, (\tau) (Pa)</th>
<th>Injection rate (mL min(^{-1}))</th>
<th>Flux (L m(^{-2}) h(^{-1}))</th>
<th>(D\ [4, 3]) (µm)</th>
<th>Span</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>86 ± 7.6</td>
<td>2.3 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>1500</td>
<td>-</td>
<td>18</td>
<td>33 ± 1.2</td>
<td>1.3 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>1500</td>
<td>8.7</td>
<td>18</td>
<td>1293</td>
<td>29 ± 0.7</td>
<td>1.1 ± 0.04</td>
</tr>
<tr>
<td>4(^a)</td>
<td>5</td>
<td>1500</td>
<td>8.7</td>
<td>18</td>
<td>1293</td>
<td>39 ± 1.4</td>
<td>1.4 ± 0.01</td>
</tr>
</tbody>
</table>

* Note: Each experiments were repeated at least thrice with the error bars measured as the standard deviations. \(D\ [4,3]\) = volume weighted mean size (µm) of PRX microcrystals. \(a\) = data collection at 1 minute microcrystals formation in a membrane continuous system.

### 9.4 Results and discussion

#### 9.4.1 Effect of the PRX concentration

The effect of different PRX concentration in the organic phase (ranged from 15 to 25 g L\(^{-1}\)) on the volume weighted mean size, \(D\ [4, 3]\) at a rotation speed of 1500 rpm and a transmembrane flux of 4000 L m\(^{-2}\) h\(^{-1}\) through a 10 µm ringed SS membrane at a constant aqueous-to-organic phase ratio, \(V_{ow}/V_{or} = 10\) is presented in Fig. 9.3(a). The microcrystals size, \(D\ [4, 3]\) decreased with an increase in PRX concentration with the highest size of 35 ± 1.0 µm was obtained at 15 g L\(^{-1}\) with span value of 1.5 ± 0.06 and...
the smallest crystals size of 24 ± 1.5 µm and span (1.1 ± 0.01) values were obtained at 25 g L$^{-1}$. The size of a microcrystals formed during antisolvent precipitation method is dependent on the rate of at which organic solvent diffuses into the aqueous phase and the relative rates of nucleation, crystals growth, and agglomeration. The promotion of crystals growth over nucleation leads to fewer and larger crystals. The rate of crystals growth is given by: 

$$G = K_g (C_{PRX} - C_{PRX}^*)^g$$

where $K_g$ is the growth constant, $C_{PRX}$ and $C_{PRX}^*$ are the local concentration and solubility of PRX in the solvent mixture, respectively, and $g$ is typically between 1 and 2 for organic systems.

The rate of nucleation is given by: 

$$B = K_b (C_{PRX} - C_{PRX}^*)^b$$

where $K_b$ is the nucleation constant, and $b$ lies between 0.5 and 2.5. Since $b \gg g$, lower supersaturations, $C_{PRX} - C_{PRX}^*$, should promote particle growth over nucleation, leading to fewer and larger particles, whereas higher supersaturations will promote nucleation, resulting in a larger population of smaller particles (Davey & Garside, 2000; Kurup & Raj, 2016; Mohameed et al., 2001; Zhao et al., 2007).

The volume crystals size distribution (CSD) depicted in Fig. 9.3 (b) are in a good agreement with the above observations featuring the minimum particle size at the maximum concentration of PRX, due to the higher creation of supersaturation level and a faster nucleation rate that resulting in smaller particle sizes. A uniform and narrow CSD was clearly shown at the higher concentration of PRX (25 g L$^{-1}$) with the smallest span value (1.1 ± 0.01). The same observations were also obtained by Park & Yeo (2010) in the observation of Roxithromycin crystal habit, Cho et al. (2010) in megestrol acetate microcrystals formation and Wang et al., (2007) in the preparation of ultrafine beclomethasone dipropionate by ant-solvent precipitation method.
Fig. 9.3: Effect of different PRX concentrations to; (a) the volume weighted mean size (µm), and (b) the volume size distribution at constant rotation speed = 1500 rpm, volume ratio and $V_{aq}/V_{or} = 10$, organic phase injection rate = 18 ml min$^{-1}$ using 10 µm size of SS micro-engineered membrane. No surfactant was added.

9.4.2 Effect of different type and concentration of surfactant

Monohydrate PRX microcrystals were prepared by reverse antisolvent precipitation using three type of surfactants such as PVA, P-123 and HPMC at constant PRX concentration (25 g L$^{-1}$), aqueous-to-organic phase volume ratio ($V_{aq}/V_{or} = 10$), organic phase injection rate (18 mL min$^{-1}$) and rotation speed of 1500 rpm (17.5 Pa). Three different concentrations (0.1 wt %, 0.25 wt % and 0.5 wt %) of each surfactants
were used and additional experiments were carried out with HPMC for further investigation on the optimum surfactant concentration. Fig 9.4 (a) shows the effect of different surfactant type on the volume weighted mean crystals size, $D [4, 3]$ at various concentrations. The main intention of this study was to find stabilizing agents which can stop the molecular association and the crystal growth by forming a protective layer around the nucleation germs in order to obtain micron-sized crystals, and indeed to hinder crystals agglomeration. This effect can be explained by the fact that the addition of surfactants may contribute to a reduction in mean particle size, from $24 \pm 1.5 \mu m$ (without surfactant) to $16 \pm 2.0 \mu m$ (at 0.1 wt % HPMC surfactant), due to the surface-active properties of the surfactants (Lim & Kim, 2002; Won et al., 2005).

By comparing experiments performed at the same concentration of different polymers (Fig. 9.4 (a)) it is clear that the most effective surfactant among those screened was HPMC with the smallest size of crystals reached at the end of the semi-batch experiments for a concentration of 0.1 wt% over the total PRX and a reduction of the $D [4, 3]$ of around 10μm compared to experiments conducted without the use of polymers. PVA and P-123 did not reduce significantly the size of the microcrystals produced compared to experiments carried out with similar operating conditions but in the absence of any kind of surfactant ($D [4, 3]$ around 25 μm). Furthermore, different concentrations of these two polymers generated very similar final CSD showing a very limited inhibition effect on growth and nucleation kinetics of PRX within the range of concentration used (see Fig. 9.5). The difference in action is probably due to the different strength and nature of interactions between each polymer and the surface of the microcrystals. Sahoo et al. (2002) explained that PVA is a partially hydrolyzed copolymer of poly(vinyl acetate) and poly (vinyl alcohol) with considerable block copolymer character. While, P-123 is a triblock polymer with the structure poly(ethylene glycol)-block- poly(propylene glycol)-block-poly(ethylene glycol) and is a nonionic surfactant (Zhang et al., 2009). Varshosaz et al. (2013) also revealed that when the $C_{P,123}$ increases, the concentration of the block copolymer increases and a great many particles come into being seriously agglomerated, P-123 molecules fragmentation reactions take over and the micelles are much longer, causing them to overlap and to form a transient network.
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The inhibiting effect of HPMC on the kinetic of nucleation of PRX monohydrate was already showed elsewhere (Hansen & Qu, 2015) but it is worth noticing how HPMC concentration higher than 0.1 generated an increase in the size of the microcrystals with a final CSD very similar to the one obtained in the absence of surfactants. Since this trend is not in agreement with most of the literature (Trasi & Taylor, 2012; Ilevbare et al., 2012a; 2012b; 2012c; Reis et al., 2014) a series of further experiments at lower concentration of HPMC was performed. The results are shown in Fig. 9.4 (b) where it can be observed that the addition of very small amounts of HPMC decreased the size of PRX microcrystals up to around 10 μm, obtained at a concentration of 0.06 wt% over the total PRX. This trend is in agreement with the previous literature. However, above this concentration the size of PRX microcrystals starts increasing again as the concentration of HPMC increases.

Optical images of the microcrystals obtained at those concentrations showed that the increase in size or the microcrystals is not due to increase of agglomeration. Therefore, it can be concluded that the inhibiting effect of HPMC decreases for concentrations above 0.06 wt % over the total PRX. A possible reason for this behaviour is an increase in the intermolecular interactions among polymer molecules (associated to higher HPMC concentrations) that reduces the contact with the surface of the microcrystals and, therefore, reduces the inhibition effect. However, further investigation is needed to elucidate this peculiar effect of HPMC concentration on the size distribution of PRX microcrystals.

9.4.3 Effect of different volume ratio

In this section, monohydrate PRX microcrystals were produced at the optimum operating parameters of; PRX concentration (25 g L⁻¹), HPMC surfactant concentration (0.06 wt %) and rotation speed of 1500 rpm (17.5 Pa) with 6 mL of PRX in Ace (organic phase) was injected at constant maximum flux of 4000 L m⁻² h⁻¹ though a 10 μm ringed SS membrane into 30, 60, 90, 120, and 180 mL of HPMC hydrophilic aqueous solution to achieve an aqueous-to-organic phase volumetric ratio of 5, 10, 15, 20, and 30 3.0, 4.5, 7.0. The D [4, 3] was drastically decreased with the increasing value of \(V_{aq}/V_{or}\) from 5 to 20 (see Fig. 9.6 (a)). When the drug solution is added to the anti-solvent, rapid reduction in the drug concentration occurs with an
increase in the amount of anti-solvent leading to rapid precipitation of the drug into microcrystals. Furthermore, a greater amount of anti-solvent lead to a greater nucleation rate and produces smaller nuclei and simultaneously the growth occurs. In the subsequent growth, the higher anti-solvent amount increases the diffusion distance for growing species and consequent diffusion becomes the limiting step for the growth nuclei (Kakran et al., 2012; Li et al., 2011). The nucleation rate is more dependent on supersaturation in comparison with the crystal growth rate and greatly affects the final particle size distribution. There is an inversely proportionality between the critical size and the logarithm of the supersaturation ratio. Therefore, high supersaturation condition results in small particles due to the formation of large number of nuclei (Paulino et al., 2013). However, a sudden increase in crystals size was detected as the volume ratio increases to 30, due to undistributed shear stress which predominantly hindered the effective rapid micromixing process, thus leading bigger size of crystals. These results can be also proved by the optical micrograph of monohydrate PRX microcrystals produced at different volume phase ratio, as depicted in Fig. 9.7, with highly uniform monodispersed crystals were detected at $V_{aq}/V_{or} = 20$.

### 9.4.4 Effect of organic solvent removal

Fig. 9.6 (b) revealed the effect of organic solvent removal during the preparation of monohydrate PRX microcrystals at constant optimum operating parameters; PRX concentration ($25 \text{ g L}^{-1}$), HPMC concentration (0.06 wt %), rotation speed of 1500 rpm (17.5 Pa), organic phase injection rate (18 mL min$^{-1}$) and $V_{aq}/V_{or} = 20$. As Ace (with boiling point 56 °C) evaporates using Rotavapor R-210 (Büchi Labortechnik, Oldham, UK), the tendency of Ace to diffuse into the liquid phase was decreased until the equilibrium crystals growth was achieved. No significant difference in the crystals size distribution (CSD) was observed in the microcrystals suspension before and after solvent removal, which means that the crystals formation process was mainly controlled and driven by the rate of inter diffusion of the two phases. Microcrystals were formed once the organic phase was brought into contact with the aqueous phase, irrespective of the acetone removal rate. Indeed, the critical concentration of PRX in the acetone–water mixture was reached without solvent evaporation, simply by dilution of the organic phase with water present in the cell. Therefore, as no difference was detected in CSD, it was not necessary to optimize experimental conditions in the...
rotary evaporator such as evaporation temperature, pressure, rotation speed of the flask, and etc.

9.4.5 Reproducibility test

Throughout the previous findings, the size-tuneable uniform monodispersed monohydrate PRX can be achieved at the operating parameters of; PRX concentration (25 g L\(^{-1}\)), HPMC concentration (0.06 wt %), rotation speed of 1500 rpm (17.5 Pa), organic phase injection rate (18 mL min\(^{-1}\)) and \(V_{aq}/V_{or} = 20\) and without organic solvent removal using ringed SS membrane. The experiment conducted under these optimum conditions was repeated thrice in order to test the reproducibility of the technique. The resulting data, presented in Fig. 9.6 (c), revealed very good reproducibility and repeatability, in terms of crystals size distribution (CSD) between different microcrystals batches produced at the optimum formulation parameters. Similar observations were also obtained by previous researchers (Laouini et al., 2013; Othman et al., 2015) with an agreement that no size alteration occurred during the organic solvent removal method.

9.4.6 Effect of membrane cleaning procedure

Membrane cleaning was performed using the procedure described in Section 9.2.3 in order to restore the original hydrophilicity of the membrane surface after being used in the formation of monohydrate PRX microcrystals. The reason for this treatment was to prevent the organic phase from being spread over the membrane surface and to ensure that tiny jets of the organic phase emerging from the membrane pores penetrate thoroughly into the aqueous phase without any obstruction. A clear scientific finding on the membrane cleaning procedures with intensive contact angle measurements were revealed in our previous study (Othman et al., 2016b) No difference in the monohydrate PRX microcrystals size distribution was observed between the two membranes, which means that the membrane properties were completely restored after cleaning, as depicted in Fig. 9.6 (c). The membrane was found significantly fouled before cleaning with large crystals aggregates deposited near the pore exits or inside the pores. After cleaning no particle could be seen on the
membrane surfaces and all pores were unclogged, indicating that the cleaning procedure was appropriate.

![Graph](image)

**Fig. 9.4:** Volume weighted mean size (µm) of PRX micro-crystals at different; (a) type of surfactant for 0.1, 0.25, 0.5 wt %, and (b) concentration of HPMC surfactant from 0.03–0.5 wt % at constant rotation speed = 1500 rpm, volume ratio, \( V_{aq}/V_{or} = 10 \), organic phase injection rate = 18 ml min\(^{-1}\) using 10 µm size of SS micro-engineered membrane. PRX concentration in organic phase was 25 g L\(^{-1}\).
Fig. 9.5: The volume distribution of monohydrate PRX micro-crystals as a function of particle size (µm) for different type of surfactants; (a) P-123, (b) PVA) and (c) HPMC at different concentration of surfactant, 0.1, 0.25 and 0.5 wt %, respectively. The rotation speed was fixed at 1500 rpm, volume ratio, $V_{aq}/V_{or} = 10$, organic phase injection rate = 18 ml min$^{-1}$ using 10 µm size of SS micro-engineered membrane. PRX concentration in organic phase was 25 g L$^{-1}$. 
Fig. 9.6: (a) Effect of different aqueous-to-organic phase volume ratio; (b) effect of organic solvent evaporation on monohydrate PRX microcrystals size (µm), and (c) experimental data reproducibility at constant rotation speed = 1500 rpm, organic phase injection rate = 18 ml min$^{-1}$ using 10 µm size of SS micro-engineered membrane. HPMC surfactant concentration was 0.06 wt % and PRX concentration in organic phase was 25 g L$^{-1}$. The volume ratio, $V_{aq}/V_{or} = 10$ for (b) and (c) was 10.
Fig. 9.7: Optical micrograph of monohydrate PRX microcrystals produced at different aqueous-to-organic phase volume ratio; (a) $Q_{aq}/Q_{or} = 5$, (b) $Q_{aq}/Q_{or} = 10$, (c) $Q_{aq}/Q_{or} = 20$, and (d) $Q_{aq}/Q_{or} = 30$. The rotation speed was fixed at 1500 rpm, organic phase injection rate = 18 ml min$^{-1}$ using 10 μm size of SS micro-engineered membrane. HPMC surfactant concentration was 0.06 wt % and PRX concentration in organic phase was 25 g L$^{-1}$.

**9.4.7 SEM images of produced monohydrate PRX microcrystals**

SEM micrographs of monohydrate PRX microcrystals synthesised at the optimum formulations of; PRX concentration (25 g L$^{-1}$), rotation speed of 1500 rpm (17.5 Pa), organic phase injection rate (18 mL min$^{-1}$) and $V_{aq}/V_{or} = 20$ are shown in Fig. 9.8 without and in the present of different surfactant type at the optimum concentration (0.06 wt %). As can be seen, for each samples, the monohydrate PRX microcrystals exhibited an elongated-rhombohedral shape. Without surfactant addition, the microcrystals tend to be agglomerated because of crystals instability and smaller size of crystals (see Fig. 9.8 (a)). However, a full steric stabilisation was detected at 0.06 wt % HPMC surfactant, whereas a bad saturated surface coverage was exhibited for...
the microcrystals produced in the present of P-123 and PVA, respectively. These observations confirmed that the microcrystals size uniformity can be controlled at low concentration of HPMC surfactant and also showed a good correlation with the crystals size results obtained in Fig. 9.4.

9.4.8 Monohydrate PRX microcrystals properties validation

As shown in Fig. 9.9 (a), the Raman spectra of the various forms showed enough differences to distinguish among form I, form II, and the monohydrate. The PRX polymorphs and monohydrate exhibited characteristic peaks in the Raman spectrum. The characteristic peaks of forms I and II are at 1335 and 1523cm\(^{-1}\) and at 1543 and 1568cm\(^{-1}\), respectively. The monohydrate PRX microcrystals showed characteristic peaks at 1007 and 1400cm\(^{-1}\). This result confirmed that a complete transformation of PRX can be easily done in a micro-engineered membrane dispersion cell without elevating the temperature or in supercritical conditions. Similar results were also obtained by (Hansen & Qu, 2015; Liu et al., 2014).

Fig. 9.9 (b) shows the DSC thermograms of pure PRX (Form I) and monohydrate PRX microcrystals synthesised at the optimum formulations. A specific and sharp endothermic peak of pure PRX (Form I) sample was identified at 203 °C due to its melting point. One single endothermic peak was also detected at 280 °C represents the degradation temperature of pure PRX. The DSC thermogram of the monohydrate showed a broad endothermic peak at 131 °C associated with the loss of the water molecule which is consistent with the literature (Lavric et al., 2010; Vrečer et al., 2003). A single endothermic peak represents the pure PRX composition also can be seen in the monohydrate PRX microcrystals thermogram.
Fig. 9.8: Scanning electron microscope (SEM) images of monohydrate PRX microcrystals synthesised by different formation methods; (a) without surfactant, (b) with HPMC surfactant, (c) with P-123 surfactant, and (d) with PVA surfactant addition inside an aqueous phase. The rotation speed was fixed at 1500 rpm, organic phase injection rate = 18 ml min$^{-1}$, PRX concentration in organic phase was 25 g L$^{-1}$ using 10 μm size of SS micro-engineered membrane with 0.06 wt % constant surfactant concentration.
Fig. 9.9: (a) Raman spectra of anhydrous form I, form II and monohydrate piroxicam microcrystals and (b) DSC thermograms of anhydrous form I and monohydrate piroxicam microcrystals.

9.4.9 Formation of monohydrate PRX microcrystals using different systems

Three different systems (i.e. System-1, System-2, System-3, and System-4) were studied for their efficacy in producing monohydrate PRX microcrystals. Table 9.1
summarises the operating parameters and conditions applied with volume weighted mean size, $D_{[4,3]}$ and span values obtained from each systems. The $D_{[4,3]}$ for System-1, -2, -3 and -4 were $86 \pm 7.6 \, \mu m$, $33 \pm 1.2 \, \mu m$, $29 \pm 0.7 \, \mu m$, $39 \pm 1.4 \, \mu m$, respectively with the span values for each system were $2.3 \pm 0.21$, $1.3 \pm 0.06$, $1.1 \pm 0.04$ and $1.4 \pm 0.01$. Indeed, these results elucidated that the crystals polydispersity (uniformity) and the crystals size distribution (CSD) increased in the bulk mixing and semi-batch system without the use of micro-engineered membrane, due to the lack of effective rapid micromixing intensity induced the supersaturation condition during the formation of monohydrate microcrystals (see Fig. 9.10). Although only minor differences in $D_{[4,3]}$ were detected from System-2 and -3 (without and with the membrane present), a clear dissimilarity can be seen in CSD results, whereby two peaks were appeared from System-3 instead of a narrow and uniform CSD obtained from System-2, represented highly monodispersed size of monohydrate PRX microcrystals.

The initial crystals size attained from the continuous system (System-4) showed slightly larger compared to the other semi-batch systems, due to highly contact and interaction between the organic and aqueous phases during the continuous system flow. As the continuous flow system approached 8 minutes of the operating time, the crystals size was getting constant and no crystals growth was detected. This system was implemented in order to have a new insight into the continuous crystals seeds production, which can be further connected to the crystallisation system. The optical images of monohydrate PRX microcrystals (Fig. 9.11) produced by each systems showed an agreement with these size observations, with a polymorphic mixture of Form II (needle like crystals) and monohydrate PRX microcrystals was found in the bulk mixing system (System-1). Uniform monodispersed monohydrate PRX microcrystals were obtained in the System-3, resulted by the effective rapid micromixing and intense shear stress on the membrane surface area.
Fig. 9.10: Different type of system validation; 1- Bulk mixing system, 2- Semi-batch membrane dispersion cell without membrane, 3- Semi-batch membrane dispersion cell with 40 µm pore size of Ni membrane, and 4- Continuous membrane dispersion system with 40 µm pore size of Ni membrane towards monohydrate PRX microcrystals volume weighted mean size (µm). The rotation speed was fixed at 1500 rpm, organic phase injection rate = 18 ml min⁻¹, PRX concentration in organic phase was 15 g L⁻¹ with aqueous-to-organic phase volume ratio, \( V_{aq}/V_{or} = 5 \) for System-1 until System-3 and flow rate ratio, \( Q_{aq}/Q_{or} = 5 \) for System-4. No surfactant was added.
Fig. 9.11: Optical images of monohydrate PRX microcrystals produced using different fabrication system; (a) System-1: bulk mixing system, (b) System-2: semi-batch membrane dispersion cell without membrane, (b) System-3: semi-batch membrane dispersion cell with 40 µm pore size of Ni membrane, and System-4: continuous membrane dispersion system with 40 µm pore size membrane at operating time of 1 min. The rotation speed was fixed at 1500 rpm, organic phase injection rate = 18 ml min⁻¹, PRX concentration in organic phase was 15 g L⁻¹ with aqueous-to-organic phase volume ratio, \( V_{aq}/V_{or} = 5 \) for System-1 until System-3 and flow rate ratio, \( Q_{aq}/Q_{or} = 5 \) for System-4. No surfactant was added.

9.4.10 Application of PRX microcrystals in seeded crystallisation process

The three different types of seeds prepared by different methods (i.e. membrane dispersion system, anti-solvent and polymorphic transformation) were used for batch temperature cycling crystallisation experiments. Fig. 9.12 (a-c) depicts the optical images of monohydrate PRX microcrystals produced by each methods with crystals size distributions (CSD) for each crystals. A uniform monodispersed elongated-rhombohedral shape of monohydrate PRX crystals (Fig. 9.12 (a)) were produced by
micro-engineered membrane dispersion cell over the other methods, with $D_{[4,3]}$ of 25 – 35 μm. The seeds obtained by antisolvent crystallisation and polymorphic transformation (Fig. 9.12 (b-c)) showed highly agglomerated with irregular shape of crystals. The transformation took almost two days and generated fine monohydrate crystals considerably agglomerated. Fig 9.12 (d) presented CSD results of each monohydrate PRX microcrystals after formation process, whereby a narrower CSD was generated by micro-engineered membrane compared to the other methods. Table 9.2 summarises the size of crystals produced by each methods, which confirmed the smallest and uniform CSD acquired from membrane method ($D_{[4,3]}= 34.81 \mu m$; span =1.03). The crystals size obtained by antisolvent and polymorphic transformation were $D_{[4,3]} = 54.98 \mu m$ with span = 1.16, $D_{[4,3]} = 133.83 \mu m$ with span = 1.28, respectively.

**Fig. 9.12:** Optical images of monohydrate PRX microcrystals produced by different methods; (a) Micro-engineered membrane dispersion cell, (b) antisolvent crystallisation of PRX with water flow rate of 2.5 mL min$^{-1}$, and (c) polymorphic transformation from form I PRX to monohydrate PRX. (d) Crystal size distribution (CSD) for each seeds.
### Table 9.2: Monohydrate PRX microcrystals diameter size at 10 %, 50 % and 90 % of the cumulative distribution obtained by Malvern Mastersizer. Volume and surface weighted mean diameters. All measurements are expressed in μm.

<table>
<thead>
<tr>
<th>Seeds production</th>
<th>(d(0.1))</th>
<th>(d(0.5))</th>
<th>(d(0.9))</th>
<th>Span</th>
<th>(D[4, 3])</th>
<th>(D[3, 2])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane</td>
<td>19.77</td>
<td>32.49</td>
<td>53.08</td>
<td>1.03</td>
<td>34.81</td>
<td>30.22</td>
</tr>
<tr>
<td>Transformation</td>
<td>28.75</td>
<td>50.61</td>
<td>87.52</td>
<td>1.16</td>
<td>54.98</td>
<td>46.03</td>
</tr>
<tr>
<td>Antisolvent</td>
<td>65.58</td>
<td>121.16</td>
<td>220.88</td>
<td>1.28</td>
<td>133.83</td>
<td>101.35</td>
</tr>
</tbody>
</table>

#### 9.4.10.1 Seeded growth experiments

The three different types of seeds prepared were used for batch cooling crystallisation experiments. Solutions of saturation temperature around 40 °C were prepared and heated up to 50 °C for the complete dissolution of the solids. Seeds were added after cooling down to 37 °C and then the solutions were further cooled down to 10 °C at a rate of -0.1 °C/min. The temperature was kept constant until complete consumption of the supersaturation. The solute concentration was monitored using Raman spectroscopy. In particular the peak intensity at 1443 – 1438 cm\(^{-1}\) (after calculation of second derivative and smoothing) was associated to the amount of PRX dissolved in solution. An inferential solubility curve was determined by measuring values of intensity of the solute Raman peak for several saturated solutions of monohydrate PRX at different temperatures. Data were interpolated using a polynomial function (shown in Fig. 9.13 (b)).

The complete consumption of supersaturation by growth, secondary nucleation and, partly, by agglomeration took around 8-10 hours, indicating slow kinetics of growth and nucleation. In order to remove fine crystals and induce de-agglomeration temperature cycling (nine cycles of 20 °C amplitude) was applied. Fig. 9.13 exhibits the evolution of the total counts/meas. during the experiment while the most significant FBRM statistics. A stable oscillatory trend for the total counts/meas. and mean of the squared weighted chord length distribution is reached after two cycles. The counts between 50 – 150 and 150 – 300 μm, mainly representing the size of
monohydrate agglomerates, also reached a stable trend after the same amount of cycles.

Fig. 9.13: Seeded crystallisation with monohydrate PRX microcrystals produced by micro-engineered membrane. (a) Total counts and temperature during the seeded experiments with 3% seeds, (b) intensity of the Raman peak corresponding to dissolved PRX (solute), (c) chord length distribution from FBRM during the experiment, and (d) statistical total counts grouped at different range of counts monitored by FBRM.

The trends for the total counts/meas. for the other three seeded cooling and cycling experiments are shown in Fig. 9.14. It is worth noting that a stable oscillating trend in the total counts/meas. was reached in a longer time compared to the seeded experiments with 2% membrane seeds in all cases. In fact, for the antisolvent addition (Fig. 9.14 (b)) and 6% membrane seeds (Fig. 9.14 (a)) nine cycles are not enough to reach a stable oscillating trend. With the polymorphic transformation seeds a stable trend is reached after around four temperature cycles (Fig. 9.14 (c)). It can be noticed...
that in this last case the maximum amount of total counts reached after 10 hrs at 10 °C is around 2100 #/meas., which is considerably lower than the maximum reached for all the other experiments (between 5000 and 7000 #/meas.). Fig. 9.15 presents the microscopic images of crystals obtained at the end of every cycling experiment for a better comparison, with the smallest and uniform crystals size detected for the seeds produced by membrane method (Fig. 9.15 (a)) compared to the others (Fig. 9.15 (b-d)). Although, the lowest degree of agglomeration is visible in the crystals obtained with seeds from antisolvent crystallisation (Fig 9.15 (d)) and polymorphic transformation (Fig 9.15 (d)), a larger size of crystals was still obtained at the end of experiment.

**Fig. 9.14:** (a) Total counts and temperature profile for the seeded experiment with 6% membrane seeds (b) 2% antisolvent addition seeds (c) polymorphic transformation seeds.
Fig. 9.15: Microscopic images of final monohydrate PRX microcrystals products manufactured by different methods; (a) micro-engineered membrane cell (3% of the total mass of PRX) (b) micro-engineered membrane cell (6% of the total mass of PRX) (c) antisolvent crystallisation, and (d) polymorphic transformation.

Fig. 9.16 shows the crystal size distributions (CSD) for all the samples obtained at the end of the cycling experiments. The crystals seeds produced by membrane and the polymorphic transformation methods generated narrow and unimodal distributions, whereas the antisolvent seeds led to a very broad CSD with higher amount of fines. The highest increment in $D$ [4, 3] were observed from membrane method (119.29 $\mu$m (for 2% seeding amunt) and 106.30 $\mu$m (for 6% seeding amount)) to antisolvent method (195.13 $\mu$m). A decrease in span values (from membrane to antisolvent method) were in agreement with the above observations, which indicates the narrowness of CSD obtained by each methods. Therefore, monohydrate PRX microcrystals produced by micro-engineered membrane dispersion cell appeared as a new novel method in order to obtain a uniform and smaller size of desired polymorphic crystals.
9.5 Conclusions

In this study, a new technique for the formation of monohydrate PRX microcrystals were developed by promoting a uniform rapid micromixing process due to ordered pore array and uniform pore size, high flux through the membrane, and suppression of internal pore fouling due to thin and non-tortuous pores. The size of monohydrate PRX microcrystals, a stable polymorphic nonsteroidal anti-inflammatory drug was precisely controlled over a range of 7–34 µm by controlling PRX concentrations, aqueous-to-organic phase volume ratios ($V_{aq}/V_{or} = 5$ to 30), type of surfactants (hydroxypropyl methyl cellulose (HPMC), polyvinyl alcohol (PVA), and pluronic-123 (P-123)) and concentrations of each surfactants. The higher the drug concentration, the higher supersaturations will be achieved, which leads to the promotion of nucleation and a larger population of smaller particles. At the higher aqueous-to-organic phase volume ratio increases the diffusion distance for growing
species and consequent diffusion becomes the limiting step for the growth nuclei. Smaller particles (< 10 µm) were obtained using SS ringed membrane at the optimum process parameters of 25 g L\(^{-1}\) PRX concentration, 0.0.6 wt % HPMC and aqueous-to-organic phase volume ratio of, \(V_{aq}/V_{or} = 20\) with significantly uniform narrow crystals size distribution (CSD), which can be practically applied as an inhalation drug. HPMC appeared as a good surfactant due to the stronger intermolecular hydrogen bonds between drug, surfactant and solvent that affecting the formation of different molecular clusters in the supersaturated solution. The monohydrate PRX microcrystals were confirmed by Raman spectroscopy, DSC, optical images and SEM. A semi-batch membrane dispersion system presented a better performance over the other designed systems (i.e. bulk mixing, semi-batch, and continuous membrane dispersion system) towards the formation of desired polymorphic monohydrate PRX microcrystals.

A novel procedure for seeds production was used in combination with temperature cycling to grow crystals of a highly agglomerating compound (monohydrate PRX). The seeds synthesised by membrane dispersion cell appeared as new, fast and efficient method over the other conventional, such as antisolvent addition and polymorphic transformation. A uniform and narrow crystal size distribution (CSD) was obtained at the end of the batch without having any agglomeration, for the seeds produced by micro-engineered membrane dispersion cell compared to the other crystal seed synthesise methods (i.e. antisolvent crystallisation and polymorphic transformation). The quality of final crystals produced by polymorphic transformation seeds also showed significantly improvement, but with much larger size of crystals.
CHAPTER 10

CONCLUSIONS AND FUTURE WORK

10.1 Conclusions

This PhD research aimed to create an outstanding method/technique for the formation of engineered functional pharmaceutical nano/micro particles using two different devices (glass capillary microfluidics and a micro-engineered dispersion cell). The main approach of this study was to investigate both synthesis/nucleation and the production stage involved during the formation of nano/micro particles, respectively. The theoretical selection of a “good solvent” and the physico-chemical interaction between the solvent-water-polymer successfully elucidated the nature of anti-solvent precipitation method for the formation of desired properties of functional pharmaceutical nano/micro-engineered particles. The experimental results obtained using both the glass capillary microfluidic device and the micro-engineered membrane dispersion cell were found to be significantly well correlated with the respective Computational Fluid Dynamic (CFD) simulation results applied to observe the formation of nanoparticles by taking into account the solubility parameter, diffusion and the convection interaction between two miscible liquids used in the anti-solvent precipitation method. The key conclusions from this research are as follows:

(1) This work is the first computational and experimental study dealing with the formation of polylactide (PLA) and poly(ε-caprolactone) (PCL) nanoparticles by nanoprecipitation in a co-flow glass capillary device. The locations at which the nanoparticles would form were determined by using the solubility criteria of the polymer and the concentration profiles found by numerical modelling.

(2) A new microfluidic method for the preparation of biodegradable nanoparticles was successfully developed based on micromixing/nanoprecipitation in co-flow and flow focusing glass capillary devices. The particle size was precisely tuned by varying the orifice size of the inner capillary, the flow rate ratio and the total
flow rate in the collection capillary which led to the generation of more nuclei and smaller sized nanoparticles.

(3) The optimum formulation parameters involved in the preparation of paracetamol encapsulated nanoparticles (PCM-PCL NPs) using the co-flow microfluidic device were simulated using a $2^5$-full factorial design for simultaneous statistical analysis of different experimental factors; (i) concentration of PCL, (ii) orifice size, (iii) flow rate ratios, $Q_{aq}/Q_{or}$, (iv) surfactant concentration and (v) paracetamol content with respect to encapsulation efficiency and drug loading percentage. In general, the results show that the PCL nanoparticles could be considered as a promising carrier system for controlled release in clinical application.

(4) Paracetamol (PCM)-loaded composite nanoparticles (NPs) composed of a biodegradable poly(d,l-lactide) (PLA) polymer matrix filled with organically modified montmorillonite (MMT) nanoparticles were also successfully formulated by anti-solvent nanoprecipitation in a microfluidic co-flow glass capillary device. The incorporation of MMT in the polymer was found to have significantly improved the drug encapsulation efficiency and drug loading, and to have extended the rate of drug release in simulated intestinal fluid (pH 7.4). The encapsulation of MMT and PCM in the NPs were well verified using transmission electron microscopy (TEM), energy dispersive x-ray spectroscopy (EDS), x-ray diffraction (XRD), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR).

(5) An extended approach for producing biodegradable polymeric drug-carrier nanoparticles (NPs) using a micro-engineered membrane dispersion cell was successfully defined by the rapid mixing process combined with the instantaneously fast diffusion process between two miscible liquids with respect to; (i) volume phase ratios, (ii) rotation speed, (iii) transmembrane flux, (iv) chemical composition of the two phases, (v) type of membrane and (vi) membrane pore size. A well-defined maximum shear stress within a transitional radius, associated with an effective inter-diffusion (between aqueous-to-organic phases) mixing area of the micro-engineered membrane was also successfully
proved by the Computational Fluid Dynamic (CFD) software and the model equations.

(6) An immunosuppressive agent of highly hydrophobic rapamycin (RAPA) drug loaded poly(ε-caprolactone) (PCL) nanoparticles (NPs) were successfully synthesised by the anti-solvent nanoprecipitation method combined with using a stainless steel (SS) ringed micro-engineered membrane. The incorporation of RAPA onto PCL NPs matrices at the optimum process/formulation parameters indicated an average particle size of 204.0 ± 1.2 nm, an encapsulation efficiency, %E.E., of 98.91 % and drug loading, %D.L., of 28.26 % which is significantly suitable for intravascular delivery. The RAPA-loaded PCL NPs capsulation PCM in the NPs was confirmed by TEM, XRD, DSC and ATR-FTIR analyses.

(7) Less than 10 µm sized monohydrate piroxicam (PRX) micro-crystals were successfully formed with the application of the anti-solvent precipitation method combined with a membrane dispersion cell, which has been utilised in the formation of functional engineered nanoparticles. This study is believed to be a new insight into the development of an integrated membrane-crystallisation system. A robust crystallisation process can be achieved with the presence of high purity, and the desirable polymorphic form, crystals size distribution (CSD) and number of produced crystals. To reach this goal, micro-crystal seeds could be added to a crystallizer before nucleation occurs, providing the surface area for crystal growth and nucleation, hence offering the advantage of being able to control the onset of crystallisation.

(8) The optimum experimental parameters (i.e. PRX concentration, type and concentration of surfactant and the different volume ratio), the effect of solvent removal and data reproducibility were well pronounced during the formation of the monohydrate PRX micro-crystals. A detailed comparison was given between bulk mixing, semi-batch, the semi-batch membrane system and the continuous membrane system applied for the formation of micro-crystals.

(9) The simulated results that were found remarkably answered the experimental results obtained by both type of micro-engineered dispersion devices (i.e. glass capillary microfluidic device and micro-engineered membrane dispersion cell)
for the formation of functional pharmaceutical engineered nano/micro-particles by anti-solvent precipitation method.

10.2 Future work

There are several suggestions that can be carried out in the future to develop the findings as outlined below:

(1) Functional pharmaceutical nano/micro-particles have progressed into an exciting area of research due to their potential in drug delivery systems (DDS). Thus, the efficacy of drug or nanofiller (i.e. nanoclay) incorporation into the polymer matrix is crucial and should be answered with a real simulated drug release mechanism model. In this study, the rate of drug release was investigated by mimicking the simulated intestinal fluid (pH 7.4). This idea may ideally complete the biochemical communication and is especially useful in developing a mathematical drug or model that can simulate real situations.

(2) The use of a synthetic biodegradable polymer is well pronounced in this study for the formation of functional pharmaceutical nanoparticles by nanoprecipitation. Natural biodegradable polymers, such as chitin and chitosan, are considerably versatile and promising biomaterials, which offer great possibilities for chemical modifications, and the formation of a large variety of useful derivatives that are commercially available or can be made available via graft reactions and ionic interactions.

(3) The clogging problem that usually occurs during the formation of nanoparticles when a smaller size of microfluidic orifice is used can be solved by controlling the operating experimental temperature. However, clear attention must be drawn to this suggestion as it may increase the solubility of the polymer/drug and result in the uncertainty of the functional particles properties (i.e. physico-chemical properties change/depletion).

(4) In this study, only two miscible solvents (i.e. water and a hydrophilic organic solvent) were applied in the anti-solvent precipitation method, which might cause some limitation on the allowable amount of drug or nanofillers that can be dissolved inside the organic solvent. Thus, the Hansen solubility interaction
between water and a mixture of organic solvents, which has been comprehensively discussed in this study, appears as a wise solution. In this way, the solubility of the drug or nanofillers in the organic solvent can be improved without elevating the temperature, hence enhancing the percentage of drug loading and the encapsulation efficiency.

(5) The effect of membrane fouling must be considered in future investigations, especially for the formation of micro-crystals by the anti-solvent precipitation method using both the semi-batch membrane dispersion cell and the continuous membrane dispersion cell.

(6) Statistical analysis using the $2^k$ factorial design simulation (i.e. Design of experiment (DOE), Design-Expert® Version 7.1.5 software) has been investigated only for the glass capillary microfluidic device experiment by taking into account two range factorial parameters (i.e. minimum and maximum range). Therefore, some extension study may be significantly useful if Central Composite Design (CCD) analysis can also be applied. This statistical engine is offered in the Design-Expert® Version 7.1.5 software package. Further analysis should also be implemented for the micro-engineered membrane dispersion cell.

(7) The application and formulation of the produced functional nanoparticle system can be improvised for antimicrobial drug delivery. Numerous antimicrobial drugs (e.g. penicillin, enzyme, etc.) have been prescribed to kill or inhibit the growth of microbes such as bacteria, fungi and viruses. These drugs are difficult to administer because of their low water-solubility, cytotoxicity to healthy tissues, and rapid degradation and clearance in the blood stream. Their antimicrobial activities against intracellular microbes are also severely limited by their poor membrane transport capability. Extensive studies have demonstrated that nanoparticles, such as liposomes, polymeric nanoparticles, solid lipid nanoparticles and dendrimers, are able to overcome these issues and facilitate antimicrobial delivery to microbial infection sites.

(8) Potential drug-release triggers by different release medium pH values should also be investigated for the functional pharmaceutical engineered particles produced by both the glass capillary microfluidic device and the micro-engineered membrane dispersion cell.
A different module of the continuous membrane dispersion system (e.g. a cross-flow membrane, an azimuthally oscillating membrane and etc.) can be applied to compare and validate the membrane continuous cell that has been applied in this study for the formation of microcrystals.

Extensive attention should be given to the allowable additives composition (e.g. surfactant, polymer, co-solvent) applied in the crystallisation process. These compositions might predominantly inhibit the crystallisation process, which would then result in major difficulties in the crystal control strategy (i.e. supersaturation control, slow cooling, direct nucleation control (DNC), temperature cycling strategy, etc.).

Future research will contribute to the further understanding of the drug delivery potential system and the possible strategy for enhancing the efficacy of functional pharmaceutical engineered nano/micro-particles. Progression should also be focused on the formation of micro/nano-crystals as monodispersed size-tunable seeds that can be applied in the crystallisation system.
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Figure S1. (a) A schematic view of the dispersion cell with a paddle stirrer used in this work ($a=24$ mm, $b=12.6$ mm, $T=36.7$ mm, $D_m=33$ mm, and $D=29.2$ mm); (b) A schematic view of the active region on the whole nickel and ringed stainless steel membrane ($r_2=13$ mm, $r_1=9$ mm). The grey areas denote active regions on the membrane surface through which the organic phase was injected, the dark area is an O-ring and the white regions are non-porous metal; (c) Pore morphology; (d) Laser drilling and electroforming fabrication method.

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S.2 Effect on membrane cleaning and number of pores

Figure S2. Optical micrographs of the membrane surface before and after cleaning: (a) 40-μm Ni membrane; (b) 20-μm Ni membrane; (c) 10-μm nickel membrane; (d) 10-μm SS membrane.

The total number of pores for a hexagonal pore array and whole Ni membrane is given by:

\[ N_p = \frac{2A_m}{L^2 \sqrt{3}} \]  

(S1)

where \( L \) is the pore spacing (200 μm), \( A_m = \pi D_m^2 / 4 \) is the effective cross-sectional area.
area of the membrane, and $D_m$ is the effective membrane diameter (total diameter excluding O-ring). For the nickel membrane used in this work, $N_p = 24,690$.

The total number of pores for a square pore array and ringed SS membrane is given by:

$$N_{p,R} = \frac{\pi (r_2^2 - r_1^2)}{L^2}$$  \hspace{1cm} (S2)

where $r_2$ and $r_1$ are the outer and inner radius of the active region. For the stainless steel membrane used in this work, $N_{p,R} = 6,912$.

S3. Numerical modelling

![Figure S3. A 3-D model geometry of the cell developed using the CFD package ANSYS FLUENT 14.5.]

S3.1. Governing equations

*RNG k-ε model*

The RNG model was developed using Re-Normalisation Group (RNG) methods to renormalise the Navier-Stokes equations, to account for the effects of smaller scales.
of motion (Yakhot et al., 1992). The turbulence kinetic energy, $k$ and its rate of dissipation, $\varepsilon$ were obtained from the following transport equations:

$$
\frac{\partial}{\partial t}(\rho k) + \frac{\partial}{\partial x_i}(\rho k u_i) = \frac{\partial}{\partial x_j}\left(\alpha_i \mu_{eff} \frac{\partial k}{\partial x_j}\right) + G_k - \rho \varepsilon + S_k
$$

(S3)

$$
\frac{\partial}{\partial t}(\rho \varepsilon) + \frac{\partial}{\partial x_i}(\rho \varepsilon u_i) = \frac{\partial}{\partial x_j}\left(\alpha_i \mu_{eff} \frac{\partial \varepsilon}{\partial x_j}\right) + C_{1\varepsilon} \frac{\varepsilon}{k} G_k - C_{2\varepsilon} \rho \frac{\varepsilon^2}{k} - R_{\varepsilon} + S_{\varepsilon}
$$

(S4)

In these equations, $G_k$ represents the generation of turbulence kinetic energy due to the mean velocity gradients, calculated as:

$$
G_k = -\rho \overline{u_i' u_j'} \frac{\partial u_j}{\partial x_i}
$$

(S5)

where $\alpha_k$ and $\alpha_{\varepsilon}$ are the inverse effective Prandtl numbers for $k$ and $\varepsilon$, respectively, $S_k$ and $S_{\varepsilon}$ are the source terms, and $C_{1\varepsilon} = 1.42$ and $C_{2\varepsilon} = 1.68$ are model constants.

The main difference between the RNG and standard $k$-$\varepsilon$ models lies in the additional term $R_{\varepsilon}$ in Eq. (S4), given by:

$$
R_{\varepsilon} = \frac{C_{\mu} \rho \eta^3 (1 - \eta / \eta_0) \varepsilon^2}{1 + \beta \eta^3} k
$$

(S6)

where $\eta = S_k / \varepsilon$, $\eta_0 = 4.38$, $\beta = 0.012$ and $C_{\mu} = 0.0845$.

**Mixture Multiphase model**

The mixture model is a simplified multi-phase model (Manninen et al., 1996). It can be used to model multi-phase flows where the phases move at different velocities, but assume local equilibrium over short spatial length scales. It can also be used to model multiphase flows with very strong coupling and phases moving at the same velocity.

The continuity equation for the mixture is:

$$
\frac{\partial}{\partial t}(\rho_m) + \nabla.(\rho_m \vec{V}_m) = 0
$$

(S7)

where $\vec{V}_m$ is the mass-averaged velocity:

$$
\vec{V}_m = \frac{\sum_{i=1}^{n} \alpha_i \rho_i \vec{V}_i}{\rho_m}
$$

(S8)

and $\rho_m$ is the mixture density:
\[ \rho_m = \sum_{i=1}^{n} \alpha_i \rho_i \]  
(S9)

where \( n \) is the number of phases and \( \alpha_i \) is the volume fraction of phase \( i \). By assuming the individual momentum equations for all the phases, the momentum equation for the mixture can be obtained as:

\[
\frac{\partial}{\partial t}(\rho_m \vec{V}_m) + \nabla \cdot (\rho_m \vec{V}_m \vec{V}_m) = -\nabla p + \nabla \left[ \mu_m \left( \nabla \vec{V}_m + \nabla \vec{V}_m^T \right) \right] + \nabla \left( \sum_{i=1}^{n} \alpha_i \rho_i \vec{V}_{dr,i} \vec{V}_{dr,i} \right) + \vec{F}
\]
(S10)

where \( \vec{F} \) is a body force, and \( \mu_m \) is the viscosity of the mixture:

\[
\mu_m = \sum_{i=1}^{n} \alpha_i \mu_i
\]
(S11)

\( \vec{V}_{dr,i} \) is the drift velocity for secondary phase \( i \):

\[
\vec{V}_{dr,i} = \vec{V}_i - \vec{V}_m
\]
(S12)

**Cavitation model**

The cavitation model proposed by Singhal et al. (2002) is used here, where the cavities are formed in the vicinity of the rotating blades by pressure drop. The transport equation for the vapour mass fraction is expressed as:

\[
\frac{\partial}{\partial t} (\rho f_v) + \nabla \cdot (\rho f_v \vec{V}_v) = \nabla \cdot (\Gamma \nabla f_v) + R_e - R_c
\]
(S13)

where \( \Gamma \) is the diffusion coefficient, \( \vec{V}_v \) is the velocity vector of the vapour phase, \( f_v \) is the vapour mass fraction, and \( R_e \) and \( R_c \) are the source terms which account for vapour generation and condensation rates, respectively. Singhal et al. [3] defined these phase change rates, based on the generalised Rayleigh-Plesset equation as:

\[
R_e = C_e \frac{V_{ch}}{\sigma} \rho_l \rho_v \sqrt{\frac{2(p_{sat} - p)}{3 \rho_l}} (1 - f_v), \quad \text{for } p \leq p_{sat}
\]
(S14)

\[
R_c = C_e \frac{V_{ch}}{\sigma} \rho_l \rho_v \sqrt{\frac{2(p - p_{sat})}{3 \rho_l}} f_v, \quad \text{for } p > p_{sat}
\]
(S15)

where \( \sigma \) is the surface tension of the lubricant and \( V_{ch} \) is the characteristic velocity associated with the local relative velocity between the liquid and vapour phases. In these models it is assumed that the bubble pressure, \( p \) equates the saturation (vapour) pressure, \( p_{sat} \) at a given temperature which is the case if one assumes that no dissolved

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gases are present. In addition, $C_e$ and $C_c$ are empirical constants which are considered to be 0.02 and 0.01 according to Singhal et al. (2002).

### S4. Analytical equations for shear stress at the membrane surface in stirred cell

The shear stress at the membrane surface is a function of the radial distance $r$ from the centre of the membrane (Nagata, 1975):

For $r < r_{\text{trans}}$  \[ \tau = 0.825\eta\omega \frac{1}{\delta} \]  \hspace{1cm} (S16)

For $r > r_{\text{trans}}$  \[ \tau = 0.825\eta\omega r_{\text{trans}} \left( \frac{r_{\text{trans}}}{r} \right)^{0.6} \frac{1}{\delta} \]  \hspace{1cm} (S17)

The transitional radius $r_{\text{trans}}$ is the radial distance from the centre of the membrane at which the shear stress has the maximum value:

\[ r_{\text{trans}} = 1.23 \frac{D}{2} \left( 0.57 + 0.35 \frac{D}{T} \right) \left( \frac{b}{T} \right)^{0.036} n_b^{0.116} \frac{Re}{1000 + 1.43 Re} \]  \hspace{1cm} (S18)

where $D$ is the stirrer diameter, $T$ is the cell diameter, $b$ is the blade height, $n_b$ is the number of blades, $Re = \omega \rho D^2 / 2 \eta$ is the rotating Reynolds number, $\rho$ and $\eta$ are the density and viscosity of the continuous phase, respectively, and $\omega$ is the angular velocity in rad s$^{-1}$.

The boundary layer thickness is given by:

\[ \delta = \sqrt{\eta / (\rho \omega)} \]  \hspace{1cm} (S19)

The average shear stress at the membrane surface for the whole membrane can be derived by integrating the local shear stress from $r = 0$ to $r = D_m / 2$ using Eqs. (S16) and (S17):

\[ \tau_{av} = \frac{6.6}{D_m^2} \eta \omega \frac{1}{\delta} \left\{ \frac{r_{\text{trans}}^3}{3} + \frac{r_{\text{trans}}^{1.6}}{1.4} \left[ \frac{D_m}{2} r_{\text{trans}}^{1.4} - r_{\text{trans}}^{1.4} \right] \right\} \]  \hspace{1cm} (S20)

The average shear stress for a ringed membrane with $r_1 < r_{\text{trans}} < r_2$ is:

\[ \tau_{av} = \frac{1.65}{r_2^2 - r_1^2} \frac{\eta \omega}{\delta} \left\{ \frac{r_{\text{trans}}^3 - r_1^3}{3} + \frac{r_{\text{trans}}^{1.6}}{1.4} \left[ r_2^{1.4} - r_{\text{trans}}^{1.4} \right] \right\} \]  \hspace{1cm} (S21)

The maximum shear stress $\tau_{max}$ can be calculated from Eq. (S16) or (S17):

\[ \tau_{max} = 0.825\eta\omega r_{\text{trans}} \frac{1}{\delta} \]  \hspace{1cm} (S22)