One-pot synthesis of micron-sized polybetaine particles; innovative use of supercritical carbon dioxide

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One-pot synthesis of micron-sized polybetaine particles; innovative use of supercritical carbon dioxide

Simon P. Bassett, Natasha A. Birkin, James Jennings, Emma Chapman, Rachel K. O’Reilly, Steven M. Howdle, Helen Willcock.

Polybetaines exhibit unique properties including anti-polyelectrolyte and low protein fouling behaviour, as well as biocompatibility. We recently presented the synthesis of ca. 20 nm polybetaine particles by aqueousRAFT polymerisation, but the synthesis of larger particles proved to be extremely challenging with standard emulsion and dispersion techniques being unsuccessful. Here we present the first reported synthesis of micron-sized, discrete cross-linked polybetaine particles, using polymerisation in scCO₂ with methanol as a co-solvent. Discrete particles are produced only when the methanol is efficiently removed in situ using scCO₂ extraction. A relatively high crosslinking agent initial concentration (10 wt%) was found to result in the most well defined particles, and particle integrity reduced as the crosslinking agent initial concentration was decreased. A monomer loading of between 3.0x10⁻³ mol/L and 1.8x10⁻² mol/L resulted in discrete micron sized particles, with significant agglomoration occurring as the monomer loading was increased further. A spherical morphology and extremely low size dispersity is observed by SEM analysis for the optimised particles. The particles are readily re-dispersed in aqueous solution and light scattering measurements confirm their low size dispersity.

Introduction

Polybetaines have found a wide range of commercial uses in recent years, from viscosifying agents in the formulation of cosmetics, to anti-fouling agents for biosensors. There are several detailed reviews covering the breadth of techniques used for their synthesis as well as their applications. The McCormick group in particular have reported extensively on the synthesis and applications of polybetaines, mainly from acrylamido based monomers and have published numerous high quality publications in this area.

There are several examples of the use of polybetaines for both non-fouling coatings and filtration membranes. In non-fouling surface coatings the amphiphility of the coatings was tuned by incorporating side groups of varying hydrophobicities, with a combination of fluorinated side groups and zwitterionic moieties resulting in efficient non-fouling surfaces, whereas hydrophobic groups alone caused significant protein adsorption. When used in membranes, incorporation of the polybetaines reduces the protein adsorption of these materials as well as increasing their water permeability. Since the first reported synthetic polybetaines in the 1950s, which were made using conventional free radical techniques, there have been various reports on their synthesis using polymerisation techniques from conventional free radical polymerisation to single electron transfer living radical polymerisation (SET LRP) and more recently reversible addition fragmentation chain transfer (RAFT) polymerisation. These advances in synthetic techniques have allowed for the development of block co- and ter-polymers, with ever expanding complexity of architecture. However, the synthesis of discrete particles of polybetaines has been somewhat limited by their complex solubility characteristics. Polybetaines are in general only soluble in very polar solvents such as water and fluorinated alcohols, though the monomers can also be solubilised in methanol and acetone. The polymers display antipolyelectrolyte behaviour in aqueous solution, becoming more soluble upon the addition of salts. Moreover, the highly charged nature of these polymers results in increased interactions between the polymer chains, and this phenomenon leads them to have an upper critical solution temperature (UCST) in water, becoming more soluble with increasing temperature. It also results in an increased likelihood of aggregation of polymers with increasing molecular weight, limiting the possibility of incorporating high molecular weight polybetaines into discrete particles. There have been very few reports detailing the incorporation of betaine monomers into large particles (~100nm). In 2008 Das et al reported the synthesis of copolymer particles made from the temperature responsive poly(N-
isopropylacrylamide) (pNIPAAm) and the sulfobetaine poly(3-dimethyl(methacryloyloxyethyl)ammonium propane sulfonate (pDMAPS), in which the DMAPS was used in an attempt to infer antipolyelectrolyte behaviour to the pNIPAAm particles. They report that the size of the microgels increased with increasing DMAPS content, but incorporation of more than 7.3 wt% DMAPS resulted in precipitation of the particles.\(^6\) The synthesis of copolymer particles of vinyl acetate (VAc) and DMAPS for use in drug delivery matrices was reported by Kostova et al., using an emulsifier free emulsion polymerisation. Particles of \(\sim 250\) nm with 20 mol % DMAPS were formed. The amphiphilic DMAPS is thought to act as an emulsifier for the hydrophobic VAc, adsorbing onto the surface of the droplets during polymerisation. However, no detailed analysis on the particle morphology or size dispersity was reported.\(^7\) Membranes containing betaine copolymer colloid particles (synthesised from hydroxyethyl acrylate (HEA) and DMAPS) have been reported to display tuneable properties and reduced phase separation (and therefore higher membrane stability) when compared to membranes containing inorganic nanoparticles, highlighting the benefits of using such polybetaine colloids.\(^8\) Again, little characterisation data for the particles was given in this case. Supercritical carbon dioxide (scCO\(_2\)) has attracted significant attention as an alternative “green” reaction medium for polymerisations owing to its readily accessible critical point (T\(_c\) = 31.1 °C and p\(_c\) = 7.38 MPa), its unique combination of liquid-like density and gas-like diffusivity and the fact that it is inert to radical reactions. Whilst monomers are generally soluble, polymers tend to be insoluble, making scCO\(_2\) an ideal solvent for performing heterogeneous polymerisations.\(^9\) There are several well-established routes for producing particles using supercritical carbon dioxide, either as a reaction solvent or via a range of polymer processing methods.\(^10\) Dispersion polymerisation in scCO\(_2\) has been used for the synthesis of well-defined block copolymers,\(^11\) and is also generally the method employed to produce well-defined spherical particles; their formation often aided by the addition of a polymeric stabiliser and allowing production of particles in the size range of 0.1 – 15 μm.\(^12\) Another common approach is to use precipitation polymerisation in scCO\(_2\). For example, acrylic acid (AA) has been polymerised in both batch\(^13\) and semi-continuous\(^14\) systems, to prepare polymer particles. Generally, the particles produced in these systems are irregular and often highly agglomerated, with reaction temperature an important factor in determining particulate morphology, especially since the scCO\(_2\) can plasticise and significantly lower the polymer T\(_g\). For example, in scCO\(_2\) the T\(_g\) of PAA is depressed to ca. 75 °C and it was observed that working above this temperature yielded agglomerated morphologies whereas more discrete primary particles were produced below this. Partially neutralised AA (i.e. a mixture of the acid and sodium salt) has been polymerised in a suspension process, using a water/CO\(_2\) solvent mixture. Water was required because sodium acrylate is insoluble in both CO\(_2\) and AA, and a PDMS-b-PEO stabiliser was used to prevent particle coagulation.\(^15\)

The addition of a cross-linker can have a significant influence on the particle morphology in heterogeneous polymerisations, as the initial particle nucleation and growth, and overall colloidal stability are very sensitive to cross-linker addition.\(^16\) A major advantage of scCO\(_2\) here is that its low viscosity and high diffusivity swells polymers, improving monomer and cross-linker diffusion into the particles. Cooper first demonstrated success with divinyl benzene (DVB) and ethylvinylbenzene (EVB) in scCO\(_2\).\(^17,18\) Commercial grades of DVB/EVB were polymerised to form well-defined cross-linked spherical particles in scCO\(_2\) to high yields (90%), both with and without a fluorinated stabiliser. The surprising observation of spherical particles without stabiliser was rationalised by formation of rigid cross-linked surfaces which were unable to aggregate when collisions occurred. Similar systems of DVB polymerisation in scCO\(_2\) have since been studied, with different surfactants,\(^19\) controlled using RAFT polymerisation,\(^20\) and using acetone as a co-solvent.\(^21\) Interestingly, in pure scCO\(_2\) the particles were highly agglomerated, but with increasing acetone concentration the particles became more discrete and also more uniform in size (around 2 μm). This was attributed to the enhanced solubility of the initial oligomers that would otherwise precipitate out in the pure scCO\(_2\) system, thus demonstrating that in some cases a co-solvent may be required to aid solubility and subsequent particle formation. Thermoresponsive cross-linked pNIPAAm particles have been synthesised by several groups using scCO\(_2\) precipitation polymerisation. The first report by Temtem et al. used N,N-methylenebisacrylamide (MBAC) as the cross-linker up to 4.5 wt%.\(^22\) As shown earlier by Cooper,\(^23,24\) higher cross-linker concentrations led to more discrete particles, with the rigid surfaces apparently overcoming agglomeration. Others have also shown very high cross-linker concentrations to be advantageous in precipitation polymerisations of PNIPAAm. For example, Cao\(^25\) et al. used MBAM at concentrations up to 20 wt%, and Hu and co-workers utilised ethylene glycol dimethacrylate (EGDMA) at 26.4 wt%.\(^26\)

We have previously reported the synthesis of small ca. 20 nm particles of the polysulfobetaine pDMAPS and copolymers with polyethylene glycol methacrylate (PEGMA) by RAFT polymerisation directly in aqueous solution. These branched polymers were shown to be discrete, well defined particles, which could be readily dispersed in aqueous solution, showing high salt tolerance and significantly lower upper critical solution temperature (UCST) cloud points compared to their linear counterparts.\(^27\) We have also shown that pDMAPS can be incorporated into micellar structures with controlled disassembly\(^28\) and swelling.\(^29\) However, except for the example of carboxybetaine particles (~100 nm) by Jiang et al., made in an inverse emulsion system, the synthesis of larger (>100 nm) well-defined polybetaine particles has not been widely reported. The method used by Jiang requires very low monomer concentration (115 mg in 20.5 mL of solution) thus limiting its commercial scalability, and the redispersibility of the particles is not described in detail.\(^30,31\) Here we describe the simple, one-pot synthesis of well-defined polybetaine particles on the micron scale. We demonstrate

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how the unique solvent properties of scCO₂ and use of a cosolvent can overcome process limitations and provide a new route to access cross-linked pDMAPS particles in a larger size regime than has previously been reported. Such materials may find applications as stabilisers, delivery vehicles or in non-protein fouling membranes and surface coatings.

Experimental

Materials

2,2’-azobis(isobutyronitrile) (AIBN, Sigma-Aldrich, 97%) was used as initiator and purified by recrystallization from methanol prior to use. 3-dimethyl(methacryloyl oxyethyl) ammonium propane sulfonate (DMAPS, Sigma-Aldrich, 97%), ethylene glycol dimethacrylate (EGDMA, Sigma-Aldrich, 98%), polyethylene glycol dimethacrylate $M_\text{w} = 330$ (pEGDMA, Sigma-Aldrich), methylene bisacrylamide (MBAc, Sigma Aldrich, 99%), sodium dodecyl sulfate (SDS, Sigma Aldrich, 98%) methanol (VWR, reagent grade) and dry CO₂ (BOC Gases, 99.99%) were used as received.

Equipment

Mastersizer: A Malvern Mastersizer 2000 with a Hydro 2000S accessory, using full power agitation and sonication was used to obtain particle size in solution.

SEM: A Zeiss SupraSVP was used to acquire the SEM images, operated at an accelerating voltage of 5 kV. The samples were prepared by drop deposition on glass (followed by sputter coating with gold).

Synthetic procedures

General procedure for aqueous inverse emulsion polymerisation.

Surfactant, DMAPS monomer, polyethylene glycol dimethacrylate (pEGDMA – $M_\text{w} = 330$) and initiator (for amounts see SI – Table S1) were dissolved in water (in the order listed) by stirring. The mixture was sonicated in an ice bath for 10 minutes. The resultant emulsion was purged with nitrogen for 30 minutes and heated in an oil bath with stirring (600 rpm) at 65 °C for 16 hours.

General procedure for aqueous dispersion polymerisation.

Surfactant, DMAPS monomer, MBAC and initiator (for amounts see SI – Table S3) were dissolved in water (in the order listed) by stirring. The mixture was purged with nitrogen for 30 minutes and heated in an oil bath with stirring (600 rpm) at 65 °C for 16 hours. High conversion (>90%) was confirmed by $^1\text{H}$ NMR spectroscopy (remaining monomer concentration was compared to that of a standard – dimethylformamide (DMF)).

General procedure for solubility measurements in High Pressure View Cell.

Solubility measurements of monomer in scCO₂ were visually determined using a 100 mL static volume view cell equipped with two sapphire windows 40 and an overhead stirrer. A known amount of DMAPS was added into the view cell body, and CO₂ was pumped in until a pressure of 5 MPa was reached. The vessel was then heated to the reaction temperature of 65 °C, and the pressure increased to 27.6 MPa through further addition of CO₂. This was repeated with the addition of methanol, 2,2’-azobis(2-methylpropionitrile) (AIBN) and EGDMA, maintaining concentrations equal to those used during the synthesis in the 60 mL vessels.

General procedure for precipitation polymerisation in scCO₂.

Polymerisations were performed in a 60 mL autoclave equipped with a magnetically driven overhead stirrer (maximum operating temperature 150 °C, maximum operating pressure 30.0 MPa). 41 DMAPS monomer (0.5 g) and methanol (4.5 mL) were separately degassed by purging with argon for 15 minutes. AIBN (0.025 g, 5 wt% with respect to monomer) and EGDMA (47.5 μL, 10 wt% with respect to monomer) were introduced into the autoclave and oxygen removed by purging the vessel with CO₂ at 0.2 MPa for 15 minutes. DMAPS monomer was dissolved in methanol and transferred into the autoclave, which was sealed and the pressure raised to 5 MPa through CO₂ addition. The vessel was then heated to 65 °C, and the pressure raised to 27.6 MPa through further CO₂ addition. The polymerisations were conducted for 2 hours. Upon completion, the autoclave was cooled to 45 °C, and CO₂ flowed through the vessel at 27.6 MPa for 30 minutes to remove the methanol (no filter used on exit line). Finally, the vessel was cooled to ambient temperature before being vented slowly. The product was recovered as a white, free-flowing powder.

General procedure for the redispersion of particles in water.

PDMAPS synthesised by scCO₂ precipitation polymerisation (75 mg) was added to stirred DI water (50 mL) with SDS (75 mg) if required. Three cycles of stirring (500 rpm) and sonication (15 minutes each) were performed to achieve a cloudy dispersion. These were kept stirring to avoid sedimentation.

General procedure for the casting of films.

One drop of PDMAPS dispersed in water was added to a glass slide and allowed to dry in ambient conditions.

Results and discussion

Attempted synthesis using traditional techniques

Firstly, two conventional methods – inverse emulsion polymerisation and dispersion polymerisation – were used in an attempt to synthesise large (c.a. >100 nm) PDMAPS particles. A range of inverse emulsion polymerisation conditions were explored and in all cases resulted in destabilisation or reversal of the inverse emulsion (see SI – Figure S2 top left). Polybetaines are highly soluble in aqueous salt solutions, and their temperature responsive behaviour has been shown to be both molecular weight and concentration dependent. 44 However, they can also be swollen by organic solvents due to their hydrophobic backbone (see SI – Figure S2 top right). Whilst this complicated amphiphilic behaviour allows polybetaines to find use as stabilisers of polymer colloids 45 and nano-objects in polymerisation induced self-assembly (PISA), 43 it also means that they do not reside within a single phase of the inverse emulsion systems, causes extensive aggregation occurring during the attempted dispersion polymerisation, and therefore ill-defined particles (see SI – Figure S2 bottom). Because the standard synthesis attempts were unsuccessful, a new route was sought for the synthesis of PDMAPS particles.
Synthesis in supercritical carbon dioxide

Whilst precipitation polymerisations in scCO\textsubscript{2} have proven effective in the production of cross-linked microparticles of other monomers, the synthesis of polybetaine particles using scCO\textsubscript{2} as a solvent has not previously been investigated. Initial attempts to polymerise DMAPS in pure scCO\textsubscript{2} failed, as the DMAPS was found to be completely insoluble at the conditions tested (up to 65 °C, 27.6 MPa, Figure 1). Adamsky and Beckman showed that another poorly scCO\textsubscript{2} soluble monomer (acrylamide) could be polymerised in a scCO\textsubscript{2}/water inverse-emulsion system.\textsuperscript{44} We first attempted to replicate this approach for the synthesis of polybetaine particles using DMAPS and EGDMA. Polymer was certainly formed, but swelled within the reaction vessel causing blockages in the pressure release outlets. In addition, the end product after removing CO\textsubscript{2} was obtained as a water-swollen gel with no evidence of particle morphology (see SI – Figure S4).

As DMAPS is known to exhibit solubility in highly polar solvents such as acetone, methanol, water and fluorinated alcohols,\textsuperscript{13} we therefore introduced methanol as a scCO\textsubscript{2} miscible co-solvent to aid dissolution. CO\textsubscript{2}/methanol mixtures have been studied in the literature by a number of groups, showing good miscibility at a range of concentrations.\textsuperscript{40, 45} The phase behaviour studies of DMAPS, methanol and the DMAPS/methanol mixture (Figure 1) shows that DMAPS is insoluble in scCO\textsubscript{2} (a) and methanol is completely miscible at the reaction conditions of 65 °C and 27.6 MPa (b). A mixture of 3.33 g DMAPS with 7.5 mL methanol in the 100 mL volume view cell reactor at 65 °C and 27.6 MPa (c) results in a two phase system, but it was clear that a significant portion of the DMAPS is dissolved in the CO\textsubscript{2}-rich upper phase. Ideally a single phase system is required for an efficient precipitation polymerisation, but our experiment demonstrated that to achieve this would require a very low DMAPS concentration (too low to allow effective polymerisation) and a much higher pressure. Thus, experiments were performed in the two phase regime at 65 °C and 27.6 MPa.

Having established these parameters we repeated the experiment in the 60 mL volume reaction autoclave, and reactant amounts were scaled down from the 100 mL view cell, to 1 g DMAPS and 9 mL methanol. Initiator AIBN (5 wt% wrt DMAPS), which has good solubility in scCO\textsubscript{2}, and cross-linker EGDMA (10 wt% wrt DMAPS) were also added to the autoclave. AIBN was used at a relatively high loading to compensate for its slower decomposition in scCO\textsubscript{2} compared to conventional solvents, so to achieve high crosslink densities.\textsuperscript{46} After 2 hours of polymerisation at 65 °C and 27.6 MPa the autoclave was cooled to room temperature and the CO\textsubscript{2} released. The product was obtained as a methanol soaked wet solid. The methanol could then be removed in vacuo, to give a high yield (87%) of powdered product, but further inspection of the polymer using SEM (Figure 2) showed the majority of the sample consisted of highly agglomerated particles. However, there were small regions of the sample where discrete spherical microparticles were evident. These observations strongly suggest that particles were formed, but after venting the scCO\textsubscript{2}, the residual methanol caused agglomeration. The methanol penetrates into the cross-linked network, swells the polymer aided by the scCO\textsubscript{2}, and leads to agglomeration of the particles as scCO\textsubscript{2} is vented (or immediately after venting) and the particles “collapse”.

Supercritical fluid extraction. In order to prevent the softening, swelling and subsequent agglomeration of the particles, it is necessary to remove the MeOH quickly from the reaction system. We realised that scCO\textsubscript{2} extraction (a step typically used to remove residual monomer post-reaction)\textsuperscript{34, 47} could be employed immediately after the end of the polymerisation reaction to flush the methanol before depressurisation. Through optimisation, we found that lowering the temperature to 45 °C (and maintaining constant pressure of 27.6 MPa) before the extraction, which increased the CO\textsubscript{2} density, allowed enhanced removal. This methodology proved highly effective, and products were obtained as free flowing white powders with yields typically above 80% when using an extraction time of 30 minutes. Any residual monomer was also flushed out of the reaction mixture due to its high solubility in MeOH. \textsuperscript{1}H NMR spectroscopy of the products after flushing showed no evidence of residual monomer. The SEM

Figure 1: View cell images of reactants at reaction conditions of 65 °C and 27.6 MPa CO\textsubscript{2}. (a) Solid DMAPS monomer is not soluble in scCO\textsubscript{2}, (b) Methanol and scCO\textsubscript{2} are miscible (c) DMAPS/methanol mixture – a small lower phase containing some DMAPS is clearly visible.

Figure 2: SEM image of the reaction performed with DMAPS (1 g), AIBN (50 mg, 5 wt% with respect to monomer) and 9 mL methanol in a 60 mL autoclave at 65 °C and 27.6 MPa for 2 h. Methanol was removed in vacuo post-reaction after venting and removal of reaction mixture from the autoclave. Spherical particles highlighted.
image (Figure 3 top left) shows well-defined spherical particles, around 1 μm in size, throughout the sample, with minimal agglomeration. These particles were readily redispersed in water (1.5 mg/mL), with and without SDS (1.5 mg/mL) as a stabiliser, by repeated cycles of stirring and sonication to form cloudy solutions (Figure 3 top right). Resettling does occur over time (periods of hours to days); however this can be avoided by stirring or agitation of the dispersions. Light scattering analysis of the dispersed samples obtained with a Mastersizer instrument (using both stirring and sonication) reveals their low size dispersity and the narrow particle size distribution (PSD, Figure 3 bottom) highlights the lack of aggregation.

**Cross-linker concentration.** It has been reported that in precipitation polymerisations in scCO₂, the cross-linking density is vital to the successful formation of discrete particles, with crosslinking agent initial concentrations up to 20 wt% often required. In precipitation polymerisations, all reactants should be soluble at the beginning of the reaction, with the polymer chains precipitating once they reach a critical weight. Usually these particles would agglomerate, but through the addition of a crosslinking agent the particle surfaces become harder and if collisions occur, agglomeration is prevented. The effect of increasing the crosslinking agent initial concentration is shown in Table 1, with associated SEM images in Figure 4. A clear trend is seen, with low crosslinking agent initial concentrations (below 5 wt%) leading to highly agglomerated morphologies. As crosslinking agent initial concentration increases, the particles become more discrete, until at 10 wt% the SEM image shows very uniform micron-sized particles (Figure 4). When redispersed in water particles with lower than 5 wt% crosslinking agent initial concentration formed clear solutions, and particle sizes were not able to be measured by light scattering (see SI Figure S5). The crosslinking agent chemistry was also varied; when using a short chain hydrophilic crosslinking agent (MBAc) – well-defined spherical particles were obtained, however when using a long chain hydrophilic crosslinking agent (pEGDMA), poorly-defined

<table>
<thead>
<tr>
<th>Sample</th>
<th>MeOH / mL</th>
<th>EGDMA / wt%</th>
<th>Yield / %</th>
<th>Particle Size / μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.5</td>
<td>0.0</td>
<td>64</td>
<td>Highly agglomerated microparticles</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>0.5</td>
<td>85</td>
<td>Highly agglomerated microparticles</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>1.0</td>
<td>90</td>
<td>Highly agglomerated microparticles</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>2.5</td>
<td>87</td>
<td>Agglomerated microparticles</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>5.0</td>
<td>84</td>
<td>Agglomerated microparticles</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>10.0</td>
<td>84</td>
<td>0.92</td>
</tr>
</tbody>
</table>

*Reactions performed with DMAPS (1 g), AIBN (50 mg, 5 wt% with respect to monomer) and 9 mL methanol in a 60 mL autoclave at 65 °C and 27.6 MPa for 2 h, followed by supercritical fluid extraction of methanol at 45 °C and 27.6 MPa. *Cross-linker concentration with respect to monomer. YIELD determined gravimetrically after drying in vacuo. *Determined by SEM, average particle sized based on measurement of 100 microparticles. n.b. It was noted that the efficiency of the removal of methanol was variable, and residual amounts often remained in the polymer. For this reason the amount of methanol was reduced from 9 to 4.5 mL for the remaining reactions, which did not affect the phase behaviour significantly, but provided the benefit of easier removal post reaction.
particles with a high degree of agglomeration were observed (see SI – Figure S6). When redispersed in water these poorly-defined particles formed swollen gel-like masses rather than cloudy solutions, and particle sizes were again not able to be measured by light scattering.

Initiator concentration. Another variable affecting the cross-linking efficiency and particle formation of the polymers is the initiator concentration; for the previous examples 5 wt% AIBN was used. Variations between 1 – 10 wt% showed that lower concentrations were less successful, while 5 wt% was optimal for achieving discrete crosslinked particles (see SI – Table S7 and Figure S8). The crosslinking must occur rapidly, as once the particles precipitate they must be hard enough to not agglomerate during collisions. At low AIBN concentrations, the radical concentration will be lower, thus resulting in a slower reaction rate, meaning the particles may be softer and more likely to agglomerate. At higher than 10 wt% AIBN, the reaction will proceed too quickly, potentially consuming the EGDMA too early in the reaction; therefore not all the particles will be evenly crosslinked, again leading to agglomeration. Hence, for the remaining polymerisations, 5 wt% AIBN was used, combined with an EGDMA initial concentration of 10 wt%.

Monomer Loading. Whilst the view cell experiments showed evidence of two phases at all monomer loadings, to probe the effect of this on the polymerisation, a range of loadings were studied at constant methanol concentrations (Table 2). Highly agglomerated particles were obtained at low monomer concentrations; too low for efficient cross-linking/polymerisation to occur quickly enough. Micron-sized particles were obtained at loadings between 3.0x10^{-2} mol/L and 1.8x10^{-1} mol/L monomer (Sample 8 to Sample 10), with little difference in the particle sizes between samples (Figure 5). Above this loading, the products began to agglomerate. In this case, the polymer will precipitate at lower conversions/reaction durations, and the cross-linking may not fully occur prior to this. Another issue was that the removal of methanol became more difficult at the higher monomer loadings, as the cross-linked networks retained more of the co-solvent. This is evident from Sample 12, where the obtained yield was calculated to be 112% and the product appeared wet and clumped together, likely due to residual methanol. Upon attempted redispersion, the agglomerated particles formed cloudy solutions that displayed wide PSDs as measured by LS (see Figure S9).

Casting of films. The monodisperse nature of the discrete particles allows the simple casting of uniform films (see SI – Figure S10), giving them the potential to be used as antifouling coatings with unique nanostructured surfaces.

Conclusions

We present here the first example of the synthesis of micron-sized, discrete, cross-linked particles made entirely from polybetaines. The particles were synthesised by scCO₂ precipitation polymerisation using methanol as a co-solvent.

Table 2: Effect of varying the monomer concentration on the polymerisation of DMAPS in scCO₂/MeOH.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mass monomer/g</th>
<th>[Monomer]/mol L⁻¹</th>
<th>Yield/ %</th>
<th>Morphology and Particle Size/µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.1</td>
<td>6.0 x 10⁻¹</td>
<td>N/A</td>
<td>Highly agglomerated microparticles</td>
</tr>
<tr>
<td>8</td>
<td>0.5</td>
<td>3.0 x 10⁻²</td>
<td>85</td>
<td>1.05</td>
</tr>
<tr>
<td>9*</td>
<td>1.0</td>
<td>6.0 x 10⁻²</td>
<td>84</td>
<td>Agglomerated microparticles</td>
</tr>
<tr>
<td>10</td>
<td>2.0</td>
<td>1.2 x 10⁻¹</td>
<td>84</td>
<td>0.94</td>
</tr>
<tr>
<td>11</td>
<td>3.0</td>
<td>1.8 x 10⁻¹</td>
<td>90</td>
<td>Agglomerated microparticles</td>
</tr>
<tr>
<td>12</td>
<td>4.5</td>
<td>2.7 x 10⁻¹</td>
<td>112</td>
<td>Agglomerated microparticles</td>
</tr>
</tbody>
</table>

* Reactions performed with DMAPS, AIBN (5 wt% with respect to monomer), EGDMA (10 wt% with respect to monomer), 4.5 mL methanol in a 60 mL autoclave at 65 °C and 27.6 MPa for 2 h, followed by supercritical fluid extraction of methanol at 45 °C and 27.6 MPa; b Yield determined gravimetrically after drying in vacuo. Determined by SEM, average particle size based on measurement of 100 particles. *4.5 mL methanol used except for Sample 9 (9 mL used).

Figure 5: SEM images showing the effect of varying the concentration of DMAPS (60 mL autoclave with 4.5 mL of methanol as co-solvent) (a) 8 (3.0 x 10⁻² mol/L), (b) 10 (1.2 x 10⁻¹ mol/L), (c) 11 (1.8 x 10⁻¹ mol/L), (d) 12 (2.7 x 10⁻¹ mol/L).

Observations using a view cell revealed that the polymerisation does take place in a two-phase system, and minimising the second phase results in well-defined spherical particles. Variation of the initiator and monomer concentration, as well as crosslinking agent initial concentration, revealed the optimum conditions for the particle synthesis. Monomer concentrations less than 3.0x10⁻² mol/L result in poorly defined particles, whereas greater than 1.8x10⁻¹ mol/L cause the methanol removal to be inefficient leading to aggregation. Crosslinking agent initial concentration,
concentrations below 5 wt% result in ill-defined particles that swell in water to form gel-like masses rather than dispersing as discrete particles, whereas at 10 wt% well-defined spherical particles of ~1 micron in size can be observed by dry state SEM and light scattering in aqueous solution. In-situ removal of the methanol by scCO₂ extraction was shown to be an important step, and whilst there is clearly opportunity for further optimisation, this method could be used for the industrially scalable synthesis of well-defined polybetaine particles. This opens up countless possibilities for their use and applications, which were previously unachievable by standard emulsion and dispersion techniques due to the complex amphiphilic behaviour of polybetaines.

Acknowledgements

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References

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Well defined micron-sized polybetaine particles have been synthesised for the first time by precipitation polymerisation in a scCO₂/methanol mixture.
Supporting information for: One-pot synthesis of micron-sized polybetaine particles; innovative use of supercritical carbon dioxide

Simon P. Bassett, Natasha A. Birkin, James Jennings, Emma Chapman, Rachel K. O’Reilly, Steven M. Howdle, Helen Willcock

S1 – Overview of conditions attempted for inverse emulsion polymerisations...............................................2
S2 - Images of destabilised inverse emulsion after polymerisation (top left), dispersion of product in water rather than oil phase (top right) and wetting of polymer by the oil phase (right), SEM image of “best” irregular particles formed in EMUL 8 (scale bar 200 nm, bottom)...............................................3
S3 - Overview of conditions used for dispersion polymerisations......................................................................4
S4 – Image of water swollen gel formed when scCO₂/water inverse-emulsion system was attempted showing issues with this method.................................................................................................5
S5 – Image of redispersions of particles with varying crosslinker densities (left to right) – 6 (10 wt%), 5 (5 wt%), 4 (2.5 wt%), 3 (1 wt%), 2 (0.5 wt%). ......................................................................................5
S6 – SEM images of particles formed with 10wt% MBAc (left) and PEGDMA (right) as crosslinker in the place of EGDMA. .........................................................................................................................5
S7 - Effect of varying the concentration of initiator in the polymerisation of DMAPS in scCO₂..............6
S8 - Effect of varying the concentration of initiator (1-10 wt% with respect to monomer – samples Init 1 – Init 10 in table S7) in the polymerisation of DMAPS in scCO₂...........................................................7
S9 – Broad PSD of Sample 11 dispersed in water with SDS, d(0.5)volume = 2.85 µm.................................7
S10 - SEM images of cast film of particles, sample 6 – Table 1 (scale bar 20 µm left, 1 µm right)............8
<table>
<thead>
<tr>
<th>Sample #</th>
<th>Conditions</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Particle size (nm)</td>
</tr>
<tr>
<td></td>
<td>Monomer (wt% of aq phase)</td>
<td>Crosslinker (wt% of aq phase)</td>
</tr>
<tr>
<td>EMUL 1</td>
<td>DMAPS (9.5)</td>
<td>PEGDMA (0.5)</td>
</tr>
<tr>
<td>EMUL 2</td>
<td>DMAPS (41.3)</td>
<td>PEGDMA (2.2)</td>
</tr>
<tr>
<td>EMUL 3</td>
<td>DMAPS (21.9)</td>
<td>PEGDMA (1.1)</td>
</tr>
<tr>
<td>EMUL 4</td>
<td>DMAPS (21.9)</td>
<td>PEGDMA (1.1)</td>
</tr>
<tr>
<td>EMUL 5</td>
<td>DMAPS (21.9)</td>
<td>PEGDMA (1.1)</td>
</tr>
<tr>
<td>EMUL 6</td>
<td>DMAPS (21.9)</td>
<td>PEGDMA (1.1)</td>
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<tr>
<td>EMUL 7</td>
<td>DMAPS (21.9)</td>
<td>PEGDMA (1.1)</td>
</tr>
<tr>
<td>EMUL 8</td>
<td>DMAPS (21.9)</td>
<td>PEGDMA (1.1)</td>
</tr>
<tr>
<td>EMUL 9</td>
<td>DMAPS (21.9)</td>
<td>PEGDMA (1.1)</td>
</tr>
<tr>
<td>EMUL 10</td>
<td>DMAPS (21.9)</td>
<td>PEGDMA (1.1)</td>
</tr>
</tbody>
</table>
S2 - Images of destabilised inverse emulsion after polymerisation (top left), dispersion of product in water rather than oil phase (top right) and wetting of polymer by the oil phase (right), SEM image of “best” irregular particles formed in EMUL 8 (scale bar 200 nm, bottom).
**S3 - Overview of conditions used for dispersion polymerisations.**

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Monomer (wt%)</th>
<th>Crosslinker (wt% wrt mon)</th>
<th>Surfactant (wt%)</th>
<th>Solvent</th>
<th>Initiator (wt%)</th>
<th>Reaction temperature (°C)</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>DISP 1</td>
<td>DMAPS (5)</td>
<td>MBAc (0.5)</td>
<td>TWEEN 80 (1)</td>
<td>H₂O</td>
<td>V50 (0.04)</td>
<td>50</td>
<td>Aggregation</td>
</tr>
<tr>
<td>DISP 2</td>
<td>DMAPS (5)</td>
<td>MBAc (0.5)</td>
<td>TWEEN 80 (2)</td>
<td>H₂O</td>
<td>V50 (0.04)</td>
<td>50</td>
<td>Aggregation</td>
</tr>
<tr>
<td>DISP 3</td>
<td>DMAPS (1)</td>
<td>MBAc (0.5)</td>
<td>TWEEN 80 (0.2)</td>
<td>H₂O</td>
<td>V50 (0.008)</td>
<td>50</td>
<td>Less aggregation occurs erratic data, 500-900nm</td>
</tr>
<tr>
<td>DISP 4</td>
<td>DMAPS (1)</td>
<td>MBAc (2)</td>
<td>PEGMA (0.2)</td>
<td>H₂O</td>
<td>V50 (0.008)</td>
<td>50</td>
<td>Clear solution</td>
</tr>
<tr>
<td>DISP 5</td>
<td>DMAPS (1)</td>
<td>MBAc (2)</td>
<td>TWEEN 80 (0.2)</td>
<td>H₂O</td>
<td>V50 (0.008)</td>
<td>50</td>
<td>Aggregation</td>
</tr>
<tr>
<td>DISP 7</td>
<td>DMAPS (2)</td>
<td>MBAc (2)</td>
<td>SDS (0.04)</td>
<td>H₂O</td>
<td>KPS (0.02)</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>DISP 8</td>
<td>DMAPS (2)</td>
<td>MBAc (2)</td>
<td>SDS (0.04)</td>
<td>H₂O</td>
<td>KPS/TEMED (0.02)</td>
<td>25</td>
<td>Aggregation</td>
</tr>
<tr>
<td>DISP 9</td>
<td>DMAPS (1)</td>
<td>EGDMA (2)</td>
<td>TWEEN 80 (0.2)</td>
<td>H₂O</td>
<td>V50 (0.008)</td>
<td>50</td>
<td>Clear solution</td>
</tr>
<tr>
<td>DISP 10</td>
<td>DMAPS (1)</td>
<td>MBAc (0.5)</td>
<td>TWEEN 80 (0.2)</td>
<td>80% H₂O, 20% MeOH</td>
<td>V50 (0.008)</td>
<td>50</td>
<td>Aggregation</td>
</tr>
<tr>
<td>DISP 11</td>
<td>DMAPS (2)</td>
<td>MBAc (2)</td>
<td>SDS (0.04)</td>
<td>80% H₂O, 20% MeOH</td>
<td>KPS/TEMED (0.02)</td>
<td>25</td>
<td>Aggregation</td>
</tr>
<tr>
<td>DISP 12</td>
<td>DMAPS (1)</td>
<td>MBAc (2)</td>
<td>PEGMA (0.02)</td>
<td>H₂O</td>
<td>KPS/TEMED (0.02)</td>
<td>25</td>
<td>Aggregation</td>
</tr>
<tr>
<td>DISP 13</td>
<td>DMAPS (1)</td>
<td>MBAc (2)</td>
<td>TWEEN 80 (0.2)</td>
<td>95% H₂O, 5% MeOH</td>
<td>KPS/TEMED (0.02)</td>
<td>25</td>
<td>Aggregation</td>
</tr>
<tr>
<td>DISP 14</td>
<td>DMAPS (2)</td>
<td>MBAc (2)</td>
<td>SDS (0.04)</td>
<td>H₂O</td>
<td>KPS/TEMED (0.02)</td>
<td>4</td>
<td>Aggregation</td>
</tr>
</tbody>
</table>

*S4 – Image of water swollen gel formed when scCO₂/water inverse-emulsion system was attempted showing issues with*
S5 – Image of redispersions of particles with varying crosslinker densities (left to right) – 6 (10 wt%), 5 (5 wt%), 4 (2.5 wt%), 3 (1 wt%), 2 (0.5 wt%).

S6 – SEM images of particles formed with 10wt% MBAc (left) and PEGDMA (right) as crosslinker in the place of EGDMA.
S7 - Effect of varying the concentration of initiator in the polymerisation of DMAPS in scCO$_2$.

<table>
<thead>
<tr>
<th>Sample</th>
<th>AIBN / wt%$^b$</th>
<th>Obtained Yield / g$^c$</th>
<th>Morphology$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Init 1</td>
<td>1</td>
<td>93</td>
<td>Highly agglomerated microparticles</td>
</tr>
<tr>
<td>Init 2.5</td>
<td>2.5</td>
<td>94</td>
<td>Well defined spherical microparticles</td>
</tr>
<tr>
<td>Init 5</td>
<td>5</td>
<td>84</td>
<td>Well defined spherical particles</td>
</tr>
<tr>
<td>Init 10</td>
<td>10</td>
<td>87</td>
<td>Highly agglomerated microparticles</td>
</tr>
</tbody>
</table>

$^a$Reactions performed with DMAPS (2 g), EGDMA (190 μL, 10 wt% with respect to monomer), 4.5 mL methanol in a 60 mL autoclave at 65 °C and 27.6 MPa for 2 h, followed by supercritical fluid extraction of methanol at 45 °C and 27.6 MPa; $^b$Initiator concentration with respect to monomer; $^c$Yield determined gravimetrically after drying in vacuo; $^d$Determined by SEM

S8 - Effect of varying the concentration of initiator in the polymerisation of DMAPS in scCO$_2$. 

1 wt% AIBN

2.5 wt% AIBN

5 wt% AIBN

10 wt% AIBN
S9 – Broad PSD of Sample 11 dispersed in water with SDS, $d_{(0.5)\text{volume}} = 2.85 \, \mu m$.

![Particle Size Distribution Graph]

S10 - SEM images of cast film of particles, sample 6 – Table 1 (scale bar 20 \, \mu m left, 1 \, \mu m right).