Nanoporous polymeric adsorbents for blood purification

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Nanoporous polymeric adsorbents for blood purification.

By Iain Roche

A Doctoral Thesis submitted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy of Loughborough University.

Department of Chemical Engineering.

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Abstract

This thesis is concerned with applying engineering principles to the use of polymeric nanoporous adsorbents for use in blood purification to obtain original knowledge.

Styrene divinylbenzene copolymer nanoporous adsorbents offer a potential means to remove middle molecular (MM) sized molecules when in direct contact with blood. Three adsorbents produced from this material were selected based on their pore structures and characterised using nitrogen porosimetry (CW1, surface area accessible to MM's ~20m², pore cut off ~11nm, CW4, surface area accessible to MM's ~35m², pore cut off ~11nm, XAD4, surface area accessible to MM's ~100m², pore cut off ~30nm). The effects of pore structure, particle size and mixing conditions were investigated to help understand how these fundamental parameters affect solute uptake characteristics. It was shown that the larger pore cut off of the XAD4 material resulted in albumin accessing the internal pore structure (q* ~95mg/g for albumin), whilst CW1 effectively excluded albumin (q* ~2mg/g), CW4 showed some accessibility to its internal structure resulting in a albumin capacity of ~51mg/g. CW4 demonstrated the highest capacity for lysozyme (MM marker) with a q* of ~570mg/g, with XAD4 having a q* of ~415mg/g and CW1 q* equalling just ~ 60mg/g. External agitation had little effect on overall mass transfer resistance, whilst particle size and pore structure both had substantial effects. The internal mass transfer resistance (quantified as an effective diffusivity value) was an order of magnitude greater in CW1 (~3.6x10⁻¹³) compared with XAD4 ~32x10⁻¹³) due to its tighter pore size distribution.

Finally, a novel approach of dynamically scaling down a commercial haemoperfusion column resulted in a means to access biocompatibility in realistic flow conditions was completed. It was demonstrated that one of the adsorbents (CW4) possessed a pore structure suitable for blood purification, due to its high capacity and relatively open pore structure.

KEYWORDS : adsorption, blood purification, sorption, uremic toxins, Albumin
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I continue to be thoroughly gratefully to my parents for their emotional support over the last 3 years (and beyond !)
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<tr>
<th>Symbol</th>
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<th>Units</th>
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<tbody>
<tr>
<td>A</td>
<td>Area</td>
<td>(m²)</td>
</tr>
<tr>
<td>A(t)</td>
<td>Time dependent integration constant</td>
<td>(-)</td>
</tr>
<tr>
<td>Aₚ</td>
<td>External area of adsorbent particles</td>
<td>(m²)</td>
</tr>
<tr>
<td>Bi</td>
<td>Biot number (= kᵦ/Dₑ)</td>
<td>(-)</td>
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<tr>
<td>C</td>
<td>Bulk solute concentration in the stirred tank</td>
<td>(kg m⁻³)</td>
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<tr>
<td>C₀</td>
<td>Initial solute concentration (t = 0) in the stirred tank</td>
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<td>Cₘᵢₙₜ</td>
<td>Final solute concentration (t = ∞) in the stirred tank</td>
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<td>cᵦ</td>
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<td>c_e</td>
<td>Concentration of solute within the adsorbent pores</td>
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<tr>
<td>dₑ</td>
<td>Hydraulic channel diameter</td>
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</tr>
<tr>
<td>( \rho_s )</td>
<td>Polymer solid density</td>
<td>(kg m⁻³)</td>
</tr>
<tr>
<td>( q )</td>
<td>Adsorption uptake</td>
<td>(kg kg⁻¹)</td>
</tr>
<tr>
<td>( q^* )</td>
<td>Saturation adsorption capacity</td>
<td>(kg kg⁻¹)</td>
</tr>
<tr>
<td>( \bar{q} )</td>
<td>Average solid phase solute composition</td>
<td>(kg m⁻³)</td>
</tr>
<tr>
<td>( r )</td>
<td>Radial position within spherical adsorbent particle</td>
<td>(m)</td>
</tr>
<tr>
<td>( r_i )</td>
<td>Radial position of adsorption front within spherical adsorbent particle</td>
<td>(m)</td>
</tr>
<tr>
<td>( R )</td>
<td>Radius of adsorbent particle</td>
<td>(m)</td>
</tr>
<tr>
<td>( Re )</td>
<td>Reynolds number</td>
<td>(-)</td>
</tr>
<tr>
<td>( Sc )</td>
<td>Schmidt number ((= \mu/\rho D_v))</td>
<td>(-)</td>
</tr>
<tr>
<td>( Sh )</td>
<td>Sherwood number ((= k_R/\rho D_v))</td>
<td>(-)</td>
</tr>
<tr>
<td>( t )</td>
<td>Time</td>
<td>(s)</td>
</tr>
<tr>
<td>( u )</td>
<td>Superficial velocity</td>
<td>(ms⁻¹)</td>
</tr>
<tr>
<td>( \mu )</td>
<td>Solution viscosity</td>
<td>(Pa.s)</td>
</tr>
<tr>
<td>( V )</td>
<td>Volume of solution in the stirred tank</td>
<td>(m³)</td>
</tr>
<tr>
<td>( \mu_L )</td>
<td>Viscosity of fluid</td>
<td>(Pa.s)</td>
</tr>
<tr>
<td>( x )</td>
<td>Distance for diffusion</td>
<td>(m)</td>
</tr>
<tr>
<td>( \phi )</td>
<td>Reduced radius length scale ((= r/R))</td>
<td>(-)</td>
</tr>
</tbody>
</table>
This chapter offers an introduction to kidney function and failure. The current treatment modalities are discussed and their limitations identified. The solutes known to be retained at elevated concentrations in dialysis patients (due to their molecular size) are highlighted and some conditions related to their presence are identified. Therefore a case to augment dialysis by removing uraemic toxins by haemoperfusion emerges. Some current devices and adsorbent specifications are also included. The chapter is concluded by the identification of scientific objectives for the thesis.

1.1 Introduction to the clinical need.

Blood performs many essential functions within the human body. One such role is the transportation of waste products arising due to normal metabolic processes. The kidneys are the organs that filter the blood and are responsible for separating the waste products ready for excretion from the body via the urinary system.

The biological kidneys consist of millions of nephrons which are the functional units of the organ. Ultrafiltration occurs in the blind end of the nephron – the Bowman’s capsule (see Figure 1.1)
Figure 1.1 – *Schematic of kidney filtration mechanism.*

The Glomerulus has the function of producing the ultrafiltrate of the plasma which enters the proximal tubule. The ultrafiltrate passes along the proximal tubule providing opportunities for water and essential molecules to regain the bloodstream. Molecular size is the predominant determinant to capillary retention or filtration controlled by diffusion. The collecting tube is connected to the urinary system enabling the retained waste molecules and excess water to be removed from the body.

The efficiency of the kidneys function can be expressed as the glomerular filtration rate (GFR). GFR is expressed in terms of the volume of plasma which is cleared of creatinine per unit time. A useful marker to measure this is creatinine. Creatinine is a product of muscle metabolism and therefore as long as the patient remains at rest the plasma creatinine levels can be assumed to be constant. Although some creatinine can be excreted by the kidney, the assay used over estimates the value, and the two errors are said to cancel each other out. clearance is calculated by the following equation :-
Chapter 1: Introduction

\[ C_x = \frac{U_x V}{P_x} \]  
\text{(Equation 1.1)}

Where

\[ C_x = \text{The clearance of } x \text{ (i.e. creatinine)} \]
\[ U_x = \text{Urine concentration of } x \]
\[ P_x = \text{Plasma concentration of } x \]
\[ V = \text{Volume of urine per unit of time} \]

GFR is often used as an indicator of kidney function.

### 1.1.1 Kidney failure – when it all goes wrong.....

Kidney failure can be either chronic or acute in nature. The sudden and reversible loss of kidney function is termed acute renal failure. Acute renal failure can present itself for a number of reasons such as severe trauma, septic shock, or a multitude of underlying conditions.

If acute renal failure occurs, physicians must ensure correct electrolyte and fluid balance is obtained and act to minimize the build up of nitrogenous waste to prevent further damage. Despite improvements in renal technologies, the mortality rates have remained constant, primarily due to the increased age of affected patients and the related increased presence of co-morbid conditions.

Renal failure will present as the glomerular filtration rate (GFR) decreases. Typically this will occur over the course of days or weeks. Due to the reduction in GFR, the body's electrolyte balance changes and a build up of nitrogenous waste occurs due to the reduction of excretion.
The longer term progressive loss of renal function is termed chronic renal failure. Chronic renal failure requires long term treatment, usually for the remainder of the patient's life. If kidney function falls to 5-10% of normal, most patients will not survive for long without dialysis. The highest risk of death within this patient group is due to cardiovascular complications. In addition to dialysis itself, there are often a number of drugs which need to be administrated to complement the treatment and protect the patient from secondary complications such as anaemia. The failing kidneys are unable to increase appropriately the production of erythropoietin. The introduction of genetically engineered recombinant erythropoietin (EPO) has almost eliminated anaemia which was a major contributor to the malaise and poor health in renal failure patients (Eschbach et al, 1989).

However, irrespective of the cause, kidney failure will require some type of dialysis to carry out the function of the kidney. This intervention will last until the natural kidney has regained its function, or in the case of chronic kidney failure, until a transplant organ becomes available. The actual type of treatment a patient will receive will depend upon patient specific parameters in addition to accepted national practices.

There are two primary methods of renal replacement therapy (excluding transplant). Both methods rely on diffusion of toxins across a semi-permeable membrane. The semi-permeable membrane sits between the blood and a dialysate fluid. The composition of the fluid is such that it contains sodium and chloride at physiological concentrations to prevent their loss but provides a concentration gradient that favours removal of toxins.

Haemodialysis is a renal replacement strategy where the blood is mechanically pumped from the body, before passing through the membrane (surrounded by flowing dialysate). The cleansed blood is then returned to the patient and sessions are usually conducted 3 times a week for several hours. Although it is possible to have haemodialysis treatments at home, they are usually conducted in treatment centres.
Peritoneal dialysis (PD) is where the peritoneal membrane (inside the patients abdomen) is used as the semi-permeable membrane. Dialysate fluid is added to the abdominal cavity via a catheter. The diffusion occurs through the peritoneum and the dialysate fluid is subsequently drained and exchanged for fresh fluid. PD dialysis requires good patient cooperation and commitment. Infections are a common occurrence with PD patients.

With over a million people currently requiring ongoing dialysis treatment worldwide, typically 3 times per week, there is a huge demand for dialysis hardware. Lysaght (2002) estimated an increase in the need for patients requiring dialysis at 7% per year. In 2004 approximately 88% of those requiring dialysis were treated using haemodialysis, with the remainder on peritoneal dialysis (Grassman et al, 2005)

1.1.2 Haemodialysis Treatment and its limitations – identifying the clinical need

The molecules associated with dialysis treatments can be grouped according to their size. Low molecular weight molecules (<500Da), middle molecules (500 – 60000Da) and large molecules (>60000Da) see Table 1.1. These categories have been discussed in the literature over the years and the sizes stated here are as published by Winchester et al, (2006), which are slightly modified from an earlier classification of Vanholder et al, (2001). The small molecules are usually water soluble, or protein bound compounds. Dialysis membranes are generally effective in removing this group of molecules due to their physical size. However, the same cannot be said for the larger molecules. The middle molecular weight molecules (MM) are thought to be of peptidic origin (Winchester et al, 2001).
Chapter 1 Introduction

Haemodialysis is insufficient at removing uraemic solutes ranging from 500 to 60000 Da which may act as uraemic toxin causing ill health in patients (Vanholder et al, 2001). Therefore it follows that an augmented treatment could be one that combines dialysis with a means of removing the middle molecular sized molecules.

Table 1.1. Categorisation of molecular weight groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Size</th>
<th>Removed by standard haemodialysis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low molecular weight</td>
<td>&lt;500 Da</td>
<td>Yes</td>
</tr>
<tr>
<td>Middle molecular weight</td>
<td>500 – 60000 Da</td>
<td>No</td>
</tr>
<tr>
<td>Large molecular weight</td>
<td>&gt;60000 Da</td>
<td>No</td>
</tr>
</tbody>
</table>

Molecules of the middle molecular weight range have been shown to be present in uraemic blood (Kaplan et al, 2003). This study utilized sensitive protein microsequencing and mass spectrometry to chemically identify the uraemic peptides in the blood samples. Winchester et al, (2001) combined the more contemporary uraemic molecules with the traditional molecules identified in earlier papers. As Uremia is understood further, the identification of new molecules will continue. Recent discovery of molecules such as reactive oxygen speicies (ROS), Advanced lipoxygenation end products (ALEs) and C-reactive protein (CRP) have been shown by Miyata et al,(1998) as remaining in dialyzed uraemic patients.

Although dialysis is a successful treatment for kidney failure, its use, or more specifically, its extended use can lead to secondary complications affecting patient health. Due to the molecular cut off of the dialysis membrane the process is unable to remove larger molecules or any that exhibit larger molecule behaviour (i.e small protein bound molecules). Membranes often use molecular weight cut off as a means to express their pore structure. It is also possible to quote pore size as a means of estimating the size of molecules the membrane will retain. Table 1.2 tabulates some dialysis membrane pore sizes.
Table 1.2. Dialyser manufactures maximum pore size information. Adapted from (Hoenich N. A, 1996)

<table>
<thead>
<tr>
<th>Type</th>
<th>Model</th>
<th>Supplier</th>
<th>Membrane</th>
<th>Max pore size (microns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parallel plate</td>
<td>TPE</td>
<td>Cobe</td>
<td>polyvinylchloride</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Ps 4000</td>
<td>Cobe</td>
<td>Cellulose acetate</td>
<td>0.45</td>
</tr>
<tr>
<td>Hollow fibre</td>
<td>Plasmaflux P2</td>
<td>Fresenius</td>
<td>polypropylene</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Hemaplex BT 900</td>
<td>Didecco</td>
<td>polypropylene</td>
<td>0.5</td>
</tr>
</tbody>
</table>

The molecular cut off of the membranes is a useful parameter for identification of which molecules, it may be capable of removing. Although the data shown above is a maximum pore size, mean pore size distributions may be more useful for this evaluation. Table 1.3 shows the hydrated diameters of some of the molecules involved. It is worthy of note that although some molecules have greatly different molecular weights, their hydrated diameters are similar. Therefore, both values should be considered when selecting membrane pore size. Table 1.3 provides useful information as to what pore size distributions may be required in order to ensure targeted molecules are dialysed whilst larger molecules such as albumin remain in the patient. However, protein fouling of the pores occurs very quickly after contact, effectively reducing the molecular cut off of the membranes thus enabling only removal of small molecules. Most current dialysers have a molecular weight cut off of around 50kDa before fouling effects occur. It is accepted that dialysis is an effective way of removing small molecules but it’s efficacy at removing larger molecules or protein bound solutes (in the MM size range) is questionable due to the effective cut off of the fouled membranes being too small.
Therefore it is beginning to emerge that retention of MM molecules occurs in dialysis patients due to the effective pore cut off of dialysis membranes. Although dialysis offers an effective means of removing smaller molecular weight toxins and water, the retention of MM's could be causing health complications. Therefore a case for their removal via an additional strategy is being made.

### 1.1.3 The Uraemic Syndrome

Uraemic syndrome is the term associated with complications arising from the retention of solutes which are normally removed by the healthy kidney and not removed by current dialysis methods. The solutes which remain are often termed the “uraemic toxins” although the exact composition or effects of these molecules is not totally understood. These molecules are in the middle molecular size range. By controlling intake of precursors such as potassium and protein in a patient’s diet, the onset of uraemic syndrome may be suppressed. However, the long term accumulation of the “uraemic molecules” can lead to a number of morbid conditions.
This increase in concentration of middle molecular weight molecules within the body is attributed to the deterioration of the patient's health (Vanholder et al, 1996). In addition to the increase of toxins, uraemic syndrome is often a result of a malfunctioning hormonal and enzymatic homeostasis. It has been surmised that the fluctuations of toxin levels is more important than the mean level. This is supported by clinical observations that have demonstrated that it is patients that are undergoing intermittent hemodialysis that demonstrate a less satisfactory condition than those which undergo continuous ambulatory peritoneal dialysis (CAPD) (Vanholder et al, 1992)

The suppression of immune response in uraemia is often deemed counter intuitive. Although the circulating levels of cytokines and other molecules concerned with the expression of immune response are intrinsically high in uraemic patients, the immune response itself is often poor. Girndt et al, 2001 highlighted the functionality of T and B cells in uraemic patients. It was suggested that greatly reduced activation of T and B cells were apparent despite a global increase in serum levels of other markers. The effect of uraemia on the immune response is critical to health. Clinical problems observed include tuberculosis, presence of immunologic disorders, increased incidence of cancer and inadequate production of antibodies. All these factors suggest an immune deficiency in uraemic patients. Again these issues are not purely due to the presence of toxic solutes but also due to issues relating to the dialysis process itself. Biocompatibility, contamination and infection relating to catheter access (Chatzinikolaou, et al, 2004) and Vitamin D deficiency are all examples of non toxic factors associated with the immune system response.

Interestingly the detection of immune response change in uraemic patients is often subtle. The number of circulating white cells is often unchanged. Circulating phagocytes are also unaffected, but granulocytic and macrophagic quantities may differ (Ponassi et al, 1987).
Bacterial infection is said to be suppressed by actions carried out by neutrophils and monocytes (Lewis et al., 1987). The depression of phagocytic metabolism has been demonstrated once Creatinine is higher than 6mg/100mL. Indeed, the dialysis treatment furthers this response although the cause may be related to bioincompatibility of dialysis membranes and not the toxic effects of the uraemic syndrome (Vanholder et al., 1992). The precise reasons why uraemia causes depression of phagocytic function is not known. However many studies have highlighted potential causes and most interestingly a study by Haagweber et al., (1994) highlighted a peptidic structure that resembled part of the β2-microglobulin molecule.

The precise effects uraemic toxins have on patients' health is often difficult to evaluate due to the heterogeneous nature of the patient group. However, there is a body of evidence that supports the removal of these toxins. Quantification of uraemia is possible although due to the non-specific nature of the condition, confusion arises as to which markers are most relevant. Creatinine clearance is a common clinical measure used to assess the progression of kidney failure. Indeed, creatinine clearance was established by Bonomini et al., (1986) as a metric for deciding on treatment options, and a level of 5 ml/min was suggested as a limit before transplant would be required. Although creatinine clearance has been criticised by Lameire et al., (2005) and others it is still routinely quoted.

Studies have shown correlations between time on dialysis and onset of dialysis related amyloidosis (DRA) (Vraet et al., 1999). The retention of middle molecular weight toxins is suggested as being the cause for DRA in the dialysis patient population. This condition encompasses a wide range of symptoms such as osteolytic bone lesions and carpal tunnel syndrome.

Direct links with the presence and inadequate clearance of specific uraemic molecules such as β2-microglobulin have been associated with DRA. In addition, defective production of insulin like growth factor-1 has been linked by Ritz et al., (1992) with bone disease.
End stage renal disease (ESRD) has been linked to the presence of middle molecular weight toxins and Winchester et al, 2002 highlight the use of sorbents in this field.

Lindner et al, (1974) established a strong link between end stage renal disease (ESRD) and artherosclerotic cardiovascular disease (CVD). The HEMO study (Depner et al, 2004) furthers support for MM removal suggesting that greater clearances of small molecules didn't necessarily improve mortality rates. Therefore the case for further research into middle molecules and their effect on patient mortality is required and further promotes the need for their on line removal as a potential treatment strategy.

Hence, it is known that the uraemic syndrome is a complex and multifactorial condition. However, the body of evidence suggests that reduction in uraemic toxins could cause an improvement in patient health. Although work has been undertaken identifying specific molecules and their associated symptoms, the case is still strong for an attempt to non specifically removing all known uraemic toxins based upon their molecular size.

1.1.4 Identification of target molecules for the research

In order to validate any potential device's efficacy at removing middle molecular weight toxins, a suitable marker molecule should be identified. The literature contains details of the toxins identified to date and a brief overview of the most commonly studied molecules is included below.

β2 Microglobulin

Successful removal of β2-microglobulin with a commercial adsorbent column (Lixelle) demonstrated improved patient health (Hiyama et al, 2002).
addition strong links with the removal of β2-microglobulin on the effects of
dialysis related amyloidosis (DRA) have been demonstrated (Kazama et al,
2001). The augmented activation of β2-microglobulin has been linked by
Hakim et al, (2000) to the bioincompatibility of dialysis membranes, and
hence promotes the case for its removal.

It has also been demonstrated that although plasma levels of β2-microglobulin
don’t correlate directly with the onset of DRA, high values are always present
in dialysis patients (Gejyo et al, 1993). This fact therefore suggests that an
elevated level of β2-microglobulin leads to the onset of DRA. A long term
study (Lornoy et al, 2000) backed up these observations and supports the
removal of β2-microglobulin.

The Glycated form of β2-microglobulin has also been implicated by Koda et al
(1997) to deposit in amyloid fibrils producing bone cysts, severe carpal tunnel
syndrome and crippling arthritis.

Despite there being some evidence to suggest a survival advantage to
reduced β2-microglobulin concentrations in dialysis patients, it is not
effectively removed using cellulosic membranes and the reuse of PMMA
membranes reduces its mass transfer (Westhuyzen, J et al, 1992). Transplant patients demonstrate a dramatic reduction in β2-microglobulin
concentrations post operation.

Due to the overwhelming evidence supporting the need to remove β2-
microglobulin, many papers have used it as a marker for middle molecule
removal.

β2-microglobulin has been widely studied in literature and makes for a
suitable comparison between new novel adsorbent materials and current
products already reported in the literature.
Cytokines

Another important and increasingly well documented group of molecules are the cytokines. The cytokines act as intracellular communicators within the immune system and between the immune system and other systems. Their function is highly specific and due to their hormone type activities they can effect both local and systemic events. They have been linked to the symptoms of a plethora of physiological events such as propagation of immune response, pathology of infection as well as malignant disease. Their relevance within the dialysis treatment is important due to their involvement in response to biomaterials as well as their insufficient removal by the dialysis treatment itself. A sub group of the cytokine family, the interleukins, have been studied in dialysis treatments by a number of groups. The pro inflammatory interleukins (IL’s) have been linked to the pathogenesis of sepsis. Low concentrations of IL’s are normal, and any rise is usually controlled in healthy individuals. However, in uraemic patients significantly higher levels of IL’s have been identified. Cheung, (1990) demonstrated the role IL’s have in the assessment of biocompatibility. In addition Pereira et al, (1995) demonstrated how cytokines are involved in a multitude of responses to the dialysis process, furthering the importance of their study. Other studies have shown a spontaneous production of IL-1 in dialysis patients (Lonnemann et al, 1990).

Stenvinkel et al, (2005) highlighted that IL-10 (18 kDa) plays an important role as an anti-inflammatory immune regulating cytokine communications. IL-10 is commonly secreted following a release of pro-inflammatory factors and is therefore said to be responsible for the down regulation process observed. Although the precise mechanism is not known, the effect is to reduce the systemic inflammatory response. IL-10 is often released due to endotoxin production and / or complement activation due to the presence of bio-incompatible materials known to be used in renal replacement therapies.
Like many uraemic toxins, the presence of IL-6 (polypeptide, 20kDa) is attributed to multiple factors. IL-6 can be secreted by a multitude of different cells including monocytes and endothelial cells and is influenced by patient specific factors (age, medical history, genetics, comorbidity, oxidative stress, hypertension etc) and dialysis related factors (backfiltration, sterilisation issues, and bio-incompatible materials). Stenvinkel et al, (2002) discussed likely links with IL-6 and mortality and health in ESRD. The paper linked the inflammation state of ESRD and suggests that the known pro and anti inflammatory cytokines (IL-1, IL-10, CRP and their relevant receptors) are contributed to by the presence of IL-6. IL-6 is often elevated in uraemic patients although this is not always the case suggesting that there may be other factors involved. Existing strong links between C-reactive protein (CRP) and outcome, as reported by Zimmermann et al, (1999) could be due to the association of IL-6 production and levels (Ridker et al, 2000). The cause of raised IL-6 serum levels are numerous, but have been reported to be linked to regenerated cellulose membranes in addition to co morbid conditions related to aging.

IL-1 is a cytokine that has undergone much study in uraemia. IL-1 (17kDa) can be excreted by nearly all nucleated cells but is released primarily by mononuclear phagocytes. High concentrations of IL-1 can be assumed to be toxic. In addition, there are some links with IL-1 and hormonal responses, (Lonnemann et al, 1990) which could also result in decreased patient health. In a later study, Lonnemann et al, (1999) also highlighted the clinical relevance of cytokine activity in dialysis patients. Due to the relatively low serum concentrations of IL-1 (max of 500pg/ml in sepsis, (Teraoka et al, 2000)) the identification of such molecules is usually carried out by ELISA kits. The same study highlighted the limitations of some current hemoadsorbents suggesting there is still work to be done to further their removal.
AGEs

Another emerging group of uraemic toxins are advanced glycation end products. When proteins are exposed to carbohydrates usually in the form of glucose they can form advanced glycation end products (AGEs). Like other uraemic toxins, AGEs begin to accumulate due to the reduction of renal excretion. Interestingly, in addition to high concentrations of AGEs being reported in renal failure, they are also found in diabetic patients. Vlassara et al, (1988) suggested AGEs role in a host of biological responses including the promoted release of cytokines. The latter due to the AGEs ability to activate monocytes and macrophages in addition to endothelial cells although this has only been proved in vitro (Bierhaus et al, 1998). This leads to clinically important physiological changes and contributes to the increase in oxidative stress, inflammation and abnormal cell proliferation.

AGEs have also been linked to the modification of β2-M which is known to be a predominant component of dialysis related amyloidosis (DRA).

Many AGEs can be removed successfully by high flux membranes (MW < 10kDa). The removal when using a standard dialysis membrane was shown by Makita et al, (1994) as being less efficient. However, polysulphone dialysis has been shown to reduce pentosidine (protein bound AGE, MW <5000Da) levels (Jadoul et al, 1999).

Although AGEs have not been researched to the same extent as some of the more traditional MM markers, their involvement in propagation of many other important systems as described above makes them a potent and important group of toxins. A recent publication by Winchester et al, (2002) discusses the identification of ESRD as being an inflammatory state. Interestingly, alongside the traditional markers of inflammation such as cytokines, AGEs are also connected with the inflammatory response. The inflammatory response has been used by many as an indication of biocompatibility of haemodialysis membranes. In addition, AGEs have been confirmed by Kazama et al, (2001) as having direct involvement with DRA alongside beta-2 microglobulin. There
is therefore increasing interest in including AGEs as markers for patient health and comparison of different treatments to reduce their concentrations.

Lysozyme

Although Lysozyme has been identified by Winchester et al, (2001) as one of the “new” uraemic toxins, its prevalence in literature is limited. However, it is an inexpensive robust protein that lends itself to preliminary studies looking at the fundamentals of the use of sorbents in blood purification. Lysozyme has a molecular weight of 14kDa (hen egg white lysozyme used in this study, has a MW of 14.4kDa), and an isoelectric point of 10.7.

Albumin.

Albumin is produced in the liver and is the most abundant protein in plasma (typically between 30 – 50g/L). It is a large protein with a molecular weight of 67kDa. Albumin is a key protein linked to the regulation of osmotic pressure. Albumin is a vital protein and therefore should not be removed by any proposed haemoperfusion system. The use of any adsorbent based system is severely restricted by the fact that the albumin is present in blood at a concentration of 40g/l whereas the concentration of β2-M and many MM toxins is three orders of magnitude smaller. Hence, if the solutes have an equal affinity for the surface, albumin would swamp the adsorbent surface resulting in inefficient adsorption. Albumin has an isoelectric point of 4.8.

Marker molecule selection.

Selection of a suitable MM marker is not intuitive due to the number of potential molecules available. This fact, combined with cost of sourcing high purity molecules required for adsorption studies further reduces the number of potential possibilities. In order to achieve the scientific objectives of this work, the robust protein lysozyme was been chosen to act as a surrogate middle molecular weight toxin. Albumin was selected to validate whether the pore
structure is capable of allowing access to this larger molecule as it is understood this could inhibit the adsorbents ability to remove middle molecular weight toxins. As a final clinical study, beta 2 microglobulin was studied as it is a commonly used marker in literature and has many direct links with disease and death.

1.1.5 Novel / Current devices and their limitations

Protein adsorption (also known as fouling) is well known in membrane separation processes. Middle molecules (specifically β2- microglobulin) are known to be adsorbed to certain dialysis membranes (Winchester et al, 2001). The capacity of this mechanism is low and doesn't remove other uraemic toxins which have possible links to patient mortality. In addition, it has also been reported that the reuse of dialysers significantly reduces the efficiency of β2- microglobulin removal (Drueke et al, 2000). Odell et al, (1991) reported 23 – 37 percent reductions in plasma β2 Microglobulin levels by using high flux membranes. This builds on evidence that high flux membranes can retard DRA progression. Much of these types of studies do however, fail to identify the molecules they are able to remove, and hence any specific correlations between molecules and patient response are not available.

Although this suggests that high flux membranes offer a suitable treatment for middle molecule removal, capacity remains low and there use is not without potential problems. Davankov et al, (2000) highlighted a strong case for the use of both a traditional dialysis membrane in conjunction with an adsorbent based system that would ensure the removal of MM whilst the membrane continues the function of removal of excess water and smaller toxins. This combination has the potential to be both an efficient remover of uraemic toxins, water and other waste products, whilst remaining clinically viable in terms of both economics and hardware compatibility. A number of products have been developed with varied success.
This thesis is concerned with the use of a haemoperfusion device used in series with current haemodialysis to augment the treatment of kidney failure.

Hemoperfusion is defined as "the direct contact of blood from patients or animals with a sorbent system" (Winchester et al, 2002). This definition acts as a useful introduction into a plethora of varying technologies and techniques.

The discussion of sorbents in uraemia can be split into two distinct areas, namely the adsorption of target middle molecules within the adsorbent particle and the biocompatibility response partly due to blood contact. This statement supports the views of Denti et al, (1980) whose early studies of carbon adsorbents led to a specification outline as follows:

Adsorbents should be:
- Free from particle fines
- Resist attrition
- High adsorption capacity - fast adsorption kinetics
- Low micro particle generation
- High blood compatibility
- Reproducible
- Low toxicity
- Minimum elution of toxic ions
- Low pyrogenicity
- Smooth surface morphology

Although much work has been completed on the use of sorbents in uraemia and related conditions, there are still few viable commercial products which utilize this technology.
1.1.6 Adsorbent technologies in Renal failure patients

Davankov et al, (2000) discussed how hypercrosslinked networks form a special three dimensional polymer created in an excess of a thermodynamically suitable solvent. The resulting material (often polystyrene) had a homogeneous molecular structure with inherent low density and a suitably high accessibility to small molecules. The predominant limitation of such materials is their susceptibility to swelling. However, derivatives of this family of materials are being used successfully in industrial adsorption technologies as reported by Tsyurupa et al, (1995). The widespread application of these materials is due in part to the large surface areas produced (1000 – 1500m² / g). This process does however often form a microporous structure with a pore distribution of 1 -2 nm which is not suitable for human protein adsorption. Purolite Ltd. were successful in the production of mesoporous materials with the addition of larger transport pores on the materials surface in the region of 80 – 90nm. This modification made it ideal for the removal of proteins. These materials have been shown to remove β2-microglobulin by Davankov et al, (1997). This paper also suggested that a more suitable material may be one that possess mesopores in the region of 4-10nm which will result in a higher capacity for β2-M adsorption.

Mesoporous materials can be made by microphase separation during the formation of a polymeric network. One of the best documented materials containing mesopores by microphase separation is the copolymer of styrene and divinylbenzene (Belyakova et al, 1991).

Winchester et al, (2001) reported on the successful removal of solutes with molecular masses of between 60 and 21500Da using activated carbon adsorbents in direct contact with blood. The group experimented with surface coating materials to improve their biocompatibility as this was highlighted as a major issue with carbon adsorbents. The same study demonstrated that surface coating tended to reduce the adsorption characteristics of larger
weight molecules (>350 Da). This material also demonstrated the ability to adsorb physiologically important solutes and thus created the need for further study to understand the effects of pore size distributions on adsorption of uraemic molecules.

The clinical implications of the use of such devices has been studied. The study by Winchester et al, (1977) demonstrated a platelet loss of 30% suggesting potential biocompatibility issues. Coagulation factors didn’t change enough to be of significance, although the paper still recommended the pursuit of more biocompatible materials. These issues are discussed in chapter 4.

### 1.1.7 Betasorb / cytosorb

A material researched by Medasorb International, BetaSorb™ demonstrated β2-M removal in the region of 92% in a uraemic dog (Winchester et al, 2001). A further study using the same material demonstrated β2-M removal of 69% and 79% of β2-M respectively with two dialysis patients. The biocompatibility was considered acceptable as there was no change in the platelet counts or leukocyte count over the 3 hour treatment. Further work indicated that the material possessed the ability to remove some cytokines and thus provided a strong case for further studies into the material's potential commercial use.

Another material developed by Medasorb International, cytosorb™ is a polystyrene divinyl benzene copolymer with a polyvinylpyrrolidone coating to make it biocompatible. Kellum, (2004) reported experiments both ex vivo and in vivo using the cytosorb resin. The in vivo study looked at survival outcomes of animals undergoing severe septic shock against a control group. The results showed a large increase in survival (after 12 hours) for the group that was undergoing the cytosorb treatment. Ex vivo studies backed up this observation demonstrating rapid adsorption of tumor necrosis factor,
interleukin-6 and interleukin-10. No kinetic or precise capacity data is reported in these papers.

This cytosorb device received approval for clinical study for the treatment of sepsis in October 2007.

1.1.8 BioLogic DT/PF

This DT filter based sorbent haemoabsorption system utilizes a 2 litre sorbent suspension around a cellulosic or cuprophane plate dialyser to clear the dialysate of toxins. The suspension comprises of 140g of powdered charcoal (with surface area of 400m²/g). However, much of this surface area is within the micropore range (<2nm) and hence is not available for protein adsorption. The sorbent solution contains glucose, which is returned to the patient during the removal of toxins.

In order to overcome issues relating to the removal of larger weight molecules, Biologic introduced a secondary filtration system downstream of their DT system. This second filter utilises direct contact between charcoal and the plasma, resulting in successful removal of both protein bound and larger molecular weight molecules. This system uses a cartridge with free space to allow the adsorbent particles to circulate and help uptake kinetics.

The device suffered a set back due to financial problems but the technology has now been sold and research scientists are developing a new generation device which presently is still to arrive on the market.
1.1.9 Lixelle

The Lixelle device, developed by the Kaneka Corporation, Osaka, Japan is a direct hemoperfusion adsorbent device capable of removing β2-microglobulin from blood. The column contains cellulose beads with a mean diameter of 460 µm, which have covalently bound hexadecyl groups on their surface acting as ligands. This results in a surface that binds well with hydrophobic substances. The porous material is said to be of a size which should size exclude Albumin. Although the device is marketed towards the removal of β2-microglobulin, Namatani et al (1998), demonstrated that the device is capable of removing peptides and proteins in the molecular weight ranges 4,000 – 20,000Da. In addition, Tsuchida et al, (1998) demonstrated removal of cytokines IL-1β and IL-6.

The device has been used in Japan for the treatment of patients who presented with symptoms of DRA and pain. The device was tolerated clinically and large improvements in patient health were reported. The review by Kutsuki (2005) demonstrated that both subjective parameters (joint pain, stiffness etc) as well as objective parameters (number of nocturnal awakenings, grasping power, pinch strength, motor nerve latency, area of bone cyst, thickness of synovia) were all significantly reduced or normalised completely against a control group. Gejyo et al, (2004) researched the effect of augmented dialysis using a polysulfone membrane and the lixelle device in series against a control group. The two year study showed that 50% of the lixelle group demonstrated improvements of subjective parameters within 3 months of treatment. The control group (just dialysis) all tended to obtain worsening scores for the subjective parameters. In the patients treated by Lixelle, the β2-microglobulin clearance was correlated with the changes in joint stiffness and soreness suggesting a direct link between β2-microglobulin and the symptoms of DRA. Abe et al, (2003) made similar observations. Homma et al (2003), showed that after only 5 months, cystic areas on humeral heads reduced significantly, and that they began to increase when
treatment was stopped. Further studies have shown that patients treated with Lixelle over a 2 year period did not develop additional bone cysts, whereas a control group continued to develop them.

Clinical experience of Lixelle has also identified a number of potential issues. Main issues concerned temporary hypotension, decrease in hematocrit and hypovolemia. Most of these adverse effects were only seen in the Lixelle S-35 device and not during use of the the S-15 (S35 has a volume of 350ml, S15 just 150ml) suggesting the additional material or extra extracorporeal blood volume was to blame. Interestingly, the efficacy of both treatments measured by improving clinical symptoms were comparable.

Despite promising clinical evidence, the lixelle system has failed to obtain widespread commercial success probably due to its cost. In Japan, its use is limited to 2 years and its high cost could be one of the predominant limiting factors for its use elsewhere. However, its clinical success furthers the evidence that removal of β2-microglobulin is advantageous to patient health and survival.

1.2 Conclusions.

There is well documented evidence that middle molecules are present in elevated concentrations in patients with kidney failure. Although the uraemic milieu is not fully understood, there are a number of toxins which are well documented as being present and direct links with patient health and secondary conditions have been established. There is therefore good support for their removal. Although it has been reported in literature that some middle molecular weight toxins are removed by current membrane dialysers, their capacity for removal is known to be low.

A small number of devices designed to remove either middle molecular weight toxins non specifically or target specific uraemic molecules have become
available. Documented limitations of current devices seem to be associated with either cost or biocompatibility issues. Arguably the most efficient of the current devices is the Betasorb system although little is reported on its physical properties in the literature. In addition to the removal strategy, little has been reported concerning the optimisation of the device in terms of pore size distributions, material size, material quantity, in column packing density and flow conditions.

1.3 Scientific objectives.

This work aims at taking a multidiscipline approach to study the fundamental parameters effecting MM uptake onto nanoporous polymeric adsorbents suitable for blood purification. By applying chemical engineering principles new knowledge of the structure-properties relationship in porous material in liquid phase adsorption may emerge. This has the potential to offer new insight into the design of a medical device suitable for extracorporeal blood purification. In addition, an original approach to the design of a scaled down haemoperfusion column based upon dimensions from an existing commercial device is to be completed, aimed at providing a means of evaluating biocompatibility data in realistic flow conditions instead of stagnant pools.

1) Use physical characterisation techniques to demonstrate the suitable selection of a styrene divinylbenzene copolymer adsorbents capable of size excluding albumin whilst allowing access to Middle molecular weight size toxins. Comparison of these material with a similar styrene divinylbenzene copolymer adsorbent which possesses a pore structure capable of allowing access to albumin to identify the effects of this.

2) To evaluate the adsorption of lysozyme (surrogate middle molecular weight marker) and albumin on each of the materials to develop an understanding of the effects of pore surface area and pore volume on uptake kinetics by obtaining effective diffusivities for each molecule on each material. Also, the influence of particle size and boundary layer reduction on uptake
kinetics is to be studied in order to inform future haemoperfusion column designs. The kinetic uptake experiments will also be used to obtain capacity data for both albumin and lysozyme on each of the adsorbent materials.

3) To develop an understanding of the effects of a binary component system containing various concentrations of Albumin and lysozyme and measuring the uptake kinetics of lysozyme on a material that allows some access to albumin and one that effectively does not for direct comparison.

4) A batch reactor experiment containing biologically relevant solutions should be prepared and the uptake kinetics of a clinically relevant marker such as B2-M measured to act as an initial indicator of uptake performance relative to other devices.

5) It is important to consider the design of any proposed hemoperfusion column on its adsorption performance and any potential damage to blood cells. Therefore, evaluation of a mock commercial column using conventional dimensionless fluid dynamics parameters is to be completed and a scale down system for clinical development also investigated. The effects of packing, particle size and flow rate should be reported. In order to complete this work a suitable blood analogue will have to be developed and validated.

6) Finally, an adsorption study using the scale down column should be undertaken to highlight the efficacy of the finalised column design parameters chosen.
Chapter 2

Material Characterisation

This chapter is concerned with the characterisation of nanoporous adsorbents to help their selection for blood purification. Desirable pore size distributions are proposed. Various methods of characterisation are discussed and data obtained for the selected Styrene divinylbenzene polymer adsorbents are reported. The characterisation process produces a detailed insight into the physical properties of various adsorbents and highlights their potential use as haemodsorbents. The effects of pore size distributions when particles of different sizes are produced is also explored.

2.1 Introduction to method for physical characterisation of nanostructured adsorbents.

Adsorbents in their various forms have been successfully utilised for the removal of molecules from liquid media. The surface area and pore distribution influences their capacity and adsorption rate as does the particle size of the adsorbent itself. The material the adsorbent is made from may also influence the adsorption performance and specificity. Surface modification such as the addition of functional groups may influence the effects on adsorption characteristics and are commonly used to tailor an adsorbent for a specific application. The result is a great variety of potential materials capable of specifically or non-specifically adsorbing molecules from solution. This chapter identifies methods of investigating porous media using both optical and nitrogen adsorption techniques to identify adsorbents for the removal of MM toxins from blood.
Due to the plethora of different MM molecules retained in dialysis patients it is clear that any suitable removal strategy should be non-specific in nature. However, the use of any adsorbent based system is severely restricted by the fact that the blood protein, human serum albumin (MW 69kDa) is present in blood at a concentration of 40g/l whereas the concentration of the MM's is at least three orders of magnitude smaller. For example concentrations of Beta-2 microglobulin in dialysis patients is typically around 35mg/L (Winchester, 2002). Hence, if the solutes have an equal affinity for the surface, albumin would swamp the adsorbent surface resulting in low adsorption of MM's. By tailoring an adsorbent that effectively size excludes albumin and large blood proteins, an attempt to prevent such an occurrence may be possible.

A carefully selected method of characterising the adsorbents is required in order to compare various materials for their suitability at removing MM's efficiently whilst size excluding albumin. Therefore, methods of identifying pore size, surface area and pore volume distributions will offer insight into potential capacity and kinetic uptake performance of MM's. Work by Davankov (2000) suggested a pore size of between 4 and 10nm would be capable of removing molecules in the region of 4-30kDa whilst excluding albumin.

In order to investigate the scientific objectives of this work, a number of adsorbents were selected on the basis of their physical characteristics. All the adsorbents were prepared from styrene divinylbenzene but changes in the synthesis condition (eg monomer, porogen ratio, cross link degree etc) result in subtle changes in the pore structure. Styrene divinylbenzene polymer adsorbents were selected because they are well characterised non-specific adsorbents which have been used for similar applications in the past, (Winchester (2001). XAD4, a commercial styrene divinylbenzene copolymer (Rohm & Haas) was purchased to compare with in-house materials which were synthesised to have a molecular size cut off capable of excluding human serum albumin (CW1). The in house materials were prepared at
Loughborough University by fellow researchers; the method for which was published by Webb, (2007). XAD4 contains larger transport pores accessible by albumin. A derivative of CW1 was also produced thought to have the same cut off, but with a greater surface area and pore volume (CW4). This material was produced by Webb and reported in the same paper highlighted above.

The adsorbents studied consist of a mixture of monomers; styrene, divinylbenzene, ethyl-styrene and porogens; toluene and undecane. It is by manipulating the ratios of these ingredients which allows polymer scientists to produce differing pore structures.

Traditional suspension polymerisation involves adding a continuous phase to a stirred reactor, with a stirrer speed set to a pre-determined value. This value is dependent on reactor and stirrer geometries which are used to control the particle size distribution. The monomer phase is added to the reactor and the reactor is sealed. The monomer phase forms droplets due to the stirrer agitation. Once the droplets have been formed, the reactor is quickly heated to 80°C and polymerisation begins to take place. The reactor is left in these conditions for a period of 24 hours. This method although suitable for large scale production, typically results in a wide particle size distribution due to non homogenous mixing conditions.

Using a Micropore Technologies dispersion cell (see Figure 2.1) which utilises a patented membrane system it is possible to control the particle size distribution. The monomer is injected into the continuous phase through the membrane and the droplets are removed and their size controlled by the shear at the membrane surface created by the stirrer. Once the required quantity of monomer phase has been dispersed the dispersion is transferred to a conventional reactor, which is sealed before being heated to 80°C and the material allowed to polymerise for a period of 24 hours.
Chapter 2 Material characterisation

The in house polystyrene divinylbenzene adsorbents were generated using the traditional suspension polymerisation technique. XAD4 is prepared in a similar manor albeit on an industrial scale. In addition, a Micropore Technologies stirred cell capable of generating the dispersion before polymerisation resulting in a tight particle size distribution is also discussed. The membrane can be selected to produce a specific particle size.

2.1.1 Particle size analysis

Laser diffraction particle size analysis is a well known technique for obtaining particle size distributions. The method relies on the principle that when a particle travels through a laser beam, light is scattered at an angle that is directly related to the size of the particle. An increase in particle size will result in a decrease in the observed scattered angle. In addition to this observation, the light intensity also diminishes in relation to particle size; larger particles scattering light at low angles and high intensity where small...
particles scatter the light at larger angles but reduced intensity. Therefore laser diffraction particle size analysers usually consist of (i) a light source (the laser) (ii) a means of presenting the sample to the light source (a recirculating system ensuring homogenous mixing of the sample in water) and (iii) a series of detectors capable of measuring the light and feeding this back to the software to obtain the scattering profile and light intensities. The range of angles the instrument can measure dictates the particle size range that can be detected. A Malvern Mastersizer 2000 was used in this work. The instrument is capable of measuring particle sizes in the range of 2 – 2000\(\mu\)m. From the light scattering data obtained, the software uses a mathematical model to convert the raw data into a size distribution. This model assumes all particles are spherical, homogenous and the optical properties of the medium are well known. The particle population in the examination cell must be sufficiently dilute that the light scattering off one particle can be obtained before the next particle scatters the light.

Once the hardware has obtained a particle size distribution, a single value can be obtained to describe the entire population; the Sauter mean squared value.

In order to characterise the particle size of the adsorbents used, first the shape of the particles should be considered. The sphere is a readily understood geometric shape which can be characterised by one dimension; its diameter. In order to allow for particle shape Wadell's sphericity equation was used:

\[
\psi = \frac{\text{surface area of sphere with equal volume}}{\text{surface area of particle}}
\]

Equation 2.1
Table 2.1. *common particle shape descriptions – adapted from Holdich (1996)*

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>Wadell’s Sphericity</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spherical</td>
<td>1.000</td>
<td>Glass bead</td>
</tr>
<tr>
<td>Rounded</td>
<td>0.82</td>
<td>Atomised drops</td>
</tr>
<tr>
<td>Cubic</td>
<td>0.806</td>
<td>Sugar</td>
</tr>
<tr>
<td>Angular</td>
<td>0.66</td>
<td>Crushed minerals</td>
</tr>
<tr>
<td>Flaky</td>
<td>0.54</td>
<td>Talc</td>
</tr>
<tr>
<td>Platelet</td>
<td>0.22</td>
<td>graphite</td>
</tr>
</tbody>
</table>

There are a number of ways to describe the particle size distribution. A common approach is to categorise the distribution into different size grades from the minimum size to the maximum size and then identify how many particles within the distribution fit into each of these grades (or sometimes referred to as fractions). The cumulative number of particles below a size can be plotted against particle size. The resulting plot will show the number of particles within each of the grades. A histogram (instead of a cumulative distribution) is also commonly used. It is often useful to express the mass of particles within each grade. This conversion is possible using the following equation

\[
\text{Mass of a single particle} = K_v d^3 \rho_s \quad \text{(Equation 2.2)}
\]

Where:

- \( K_v \) = volume shape factor (\( \pi/6 \) for spheres)
- \( d \) = particle diameter.
- \( \rho_s \) = density of particle.

In order to obtain the mass of the grade the above equation is multiplied by the number of particles in the grade or fraction.
Mass of grade or fraction = $K_v d_i^3 \rho_s f_i$  \hspace{1cm} \text{(Equation 2.3)}

From the latter equation, a mass fraction can be calculated. This value is the mass of the fraction divided by the mass of the entire distribution.

Mass fraction ($M_i$) = \( \frac{K_v d_i^3 \rho_s f_i}{\sum k_i d_i^3 \rho_s f_i} \) \hspace{1cm} \text{(Equation 2.4)}

Which simplifies to \( M_i = \frac{d_i^3 f_i}{\sum d_i^3 f_i} \). \hspace{1cm} \text{(Equation 2.5)}

The above equation assumes that shape coefficients and density are constant throughout the distribution. The subscript "i" is the midpoint of each fraction. On review of this equation it can be seen that the resulting mass based distribution will be more biased towards the larger particles than a number based system. The terms volume fraction and mass fraction can be used interchangeably since the density cancels.

As mentioned earlier, the use of histograms instead of a cumulative total is commonly used and offers a good means for comparison between different materials. Hence, particle size distribution data has been presented as volume percentage in each size grade.

Although the distribution curves described above are useful for in depth comparison of different samples, this thesis is often dealing with very similar samples (which should have similar distribution profiles) but the mean particle size differs. Therefore the identification of an "average" particle size for a tight polydispersed sample would be advantageous.
The surface area of a single sphere is given by $\pi d^2$ and its volume, $\frac{\pi d^3}{6}$. Therefore it is possible to express a term for the specific surface area per unit volume as follows (and is referred to as the specific surface):

\[ s_v = \frac{6}{d} \quad \text{(Equation 2.6)} \]

Therefore the specific surface area of a distribution can also be obtained thus:

\[ s_v = \frac{\pi \sum d_i^2 f_i}{\pi / 6 (\sum d_i^3 f_i)} = \frac{6 \pi \sum d_i^2 f_i}{\pi (\sum d_i^3 f_i)} \quad \text{(Equation 2.7)} \]

The above equations can also be converted to mass terms by substituting in the mass fraction equation given above. Once rearranged the following is obtained:

\[ S_v = 6 \sum \frac{m_i}{d_i} \quad \text{(Equation 2.8)} \]

This allows the use of another equivalent spherical diameter to be derived that is suitable for use over the entire distribution. This value is essentially a spherical particle diameter that has the same specific surface as that of the entire distribution. It is called the Sauter mean diameter and is commonly used in particle technology when doing fluid flow calculations. It is given by

\[ x_{sv} = \frac{6}{S_v} \quad \text{(Equation 2.9)} \]

The Sauter mean is idea for use in kinetic modelling as it represents the average size of particle in the distribution based upon volume.
2.1.2 Pore size characterisation

The International Union of Pure and Applied Chemistry (IUPAC) recommend the following classifications for pore sizes. See Table 2.2

Table 2.2. IUPAC classification of pore sizes

<table>
<thead>
<tr>
<th>Name</th>
<th>Pore size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micropores</td>
<td>&lt;2nm</td>
</tr>
<tr>
<td>Mesopores</td>
<td>2nm - 50nm</td>
</tr>
<tr>
<td>Macropores</td>
<td>&gt;50nm</td>
</tr>
</tbody>
</table>

Techniques such as scanning electron microscopy are limited in their ability to characterise materials with length scales (e.g., pore size) below around 3nm. Nitrogen gas adsorption has become a standard technique to evaluate the pore structure of nanoporous materials.

The method of nitrogen adsorption utilises a model to calculate pore size distributions and surface area values. When a clean porous material is allowed to contact the probe gas (i.e., nitrogen) the gas will be attracted to the surface of the material in order to satisfy the imbalance of surface atom forces. The increased concentration of gas molecules at the material surface is referred to as adsorption. The movement of molecules away from the surface and into the bulk is termed desorption. The length of time a gas molecule remains on the surface of the solid material will depend upon many factors including the type of solid / gas molecule and the temperature of the solid surface. When the molecules collide more often it is due to either an increase in population or due to an increase of speed (due to an increase in gas temperature) and results in a pressure increase. Therefore it follows that if the concentration of gas molecules in the bulk decreases (due to an increase of concentration on the solid material) then a pressure decrease will be observed. By obtaining this pressure decrease in conjunction with the
temperature and the volume of the container it is possible to determine the number of molecules adsorbed onto the material. With this value, it is possible to calculate the surface area and pore volume of the material.

From the experimental data obtained (equilibrium pressure, relative pressure at room temperature) the volume of nitrogen adsorbed at each relative pressure can be calculated. When the adsorbed volume is plotted against the relative pressure of adsorbate the adsorption isotherm is produced.

**Adsorption Isotherms.**

There are five common types of adsorption isotherm. Type I isotherms are often referred to as Langmuir type and are obtained when adsorption is restricted to a monolayer. Type I isotherms are usually related to physical adsorption onto solids with extremely small pores. Type II isotherms usually represent multilayer physical adsorption on non porous solids or materials having relatively large pores. The type of hysteresis observed in the isotherms also offers insight into the material being analysed. deBoer (1958) suggested 5 different hysteresis loops, 3 of which were able to describe mesoporous materials. The IUPAC classification was developed in 1985 and it is based upon the work of deBoer and later Seaton (1991) who presented a technique to determine the connectivity of porous materials based upon the hysteresis observed during nitrogen adsorption. These models were developed and improved by Seaton and Liu (1992) to allow the prediction of effective diffusivities using effective medium theory.

The main limitation of adsorption porosimetry is that a model is used to obtain pore size distributions and surface areas from the isotherm data. Therefore, the results are sensitive to the type of model used and hence a brief overview of suitable models is included here. Both surface area and pore volumes can be derived from the isotherm data.
Langmuir Theory.

Langmuir (1916) first proposed a theory for mechanism of gas molecules adsorbing onto simple surfaces. The theory stated that when gas molecules hit a solid surface they do not rebound elastically and instead are held to the surface (adsorbed) by a force similar to that holding atoms. The amount of molecules adsorbed is a product of the strength of the attractive forces resulting in monolayer coverage of the surface if the strength of the attractive forces is sufficient. At high gas pressures, Langmuir demonstrated that the surfaces tend to become saturated with the adsorbed gas resulting in a monolayer coverage. Others who were working on porous materials used saturated vapours so that the condensation of liquid occurred in the capillary spaces.

BET Theory

Brunauer, Emmett and Teller (1938) continued the work started by deBoer and published isotherm equations for multilayer adsorption. It was thought that multilayer adsorption was a function of induced dipole interactions caused by the polarisation of the adsorbed layers.

The Brunauer group however, suggested that the forces required for multilayer adsorption were the same as those that are present during condensation. The resulting model, referred to as the BET successfully explained most van der Waals adsorption isotherms of molecules including nitrogen onto various materials. However, the BET model has not been without its critics and there are a number of papers published using slightly modified techniques. Halsey (1948) questioned the theory that a single molecule could adsorb a second one and subsequent layers to arrive at multilayer adsorption. However, despite others arriving at their own models,
the BET is still the most widely used and comparison of similar materials is possible and common practice.

Calculation of BET surface area

\[ S_m = \frac{n_m a_m N_A}{m} \]  

(Equation 2.10)

Where:
- \( N_A \) = Avogadro's number (6.023 \times 10^{23} \) molecules/mol
- \( m \) = mass of sample (g)
- \( s_m \) = specific surface area per unit mass (m²/g)
- \( n^*_m \) = uptake of gas corresponding to monomolecular coverage of the solid surface.
- \( a_m \) = average area occupied by each molecule (16.2 Å² for nitrogen).

Therefore for nitrogen,

\[ S_m = 9.76 \times 10^4 \frac{n^*_m}{m} \]  

(Equation 2.11)

The monolayer quantity, \( n^*_m \) is calculated by the adsorption quantity of \( n^* \) at a series of pressures and interpreting the data using the BET equation in the following form:

\[ \frac{x}{n^*(1-x)} = \frac{1}{n^*_m C} + \frac{(c-1)x}{n^*_m C} \]  

(Equation 2.12)

Where \( C \) is a constant. The plot of \( x / n^*(1-x) \) against \( x \) will produce a straight line with a gradient of \( (c-1)/n^*_m C \) and an intercept of \( 1/n^*_m C \). From this plot it is possible to extract values of \( n^*_m \) and \( C \).
Pore size distributions.

Models first became available to calculate pore volumes in 1951 when Barrett et al published their paper on nitrogen adsorption. This model was limited to pores above 15 angstrom as below this the model was inaccurate. deBoer published a number of papers from 1964 regarding the t-curve method for identification of pore surface area in the micropore region. He used the density of nitrogen in the adsorbed layer to be the same as that of liquid nitrogen. By plotting the adsorbed nitrogen against the calculated thickness, \( t \) enables an insight into the micropore region. Non porous materials will exhibit a linear t-plot going through the origin. However, t-plots for porous materials exhibited a curve due to the filling of the micropores with the nitrogen. It was deduced that the linear portion of the t-plot was a measure of the specific surface area of the adsorbent. This work was developed further by Mikhail et al, (1968) who determined the gradient at each point on the t-plot which enabled them to calculate the surface area required to allow access to the nitrogen molecules at a specific pressure. This enabled surface areas and volumes of microporous materials to be obtained.

Although other approaches followed since the earlier work by deBoer etc it wasn't until the 80's when some important models were developed. Horvath et al, 1983 developed a model (HK) for the calculation of the effective pore size distribution of molecular sieve carbons. Their approach was based upon that of Everett et al, 1976 who's work concentrated on a slit potential model. Although this technique is still in use today, it has been criticised for its accuracy in larger pores.

A newer technique derived from the HK model is called the density functionel theory (DFT). It is essentially the same as the HK model but it calculates the density of fluid near the walls of micropores where the HK model assumes it to be a constant. As with the HK model, it assumes slit pores with graphite interaction energies. The DFT model is accepted for pore sizes upto 4000
angstrom. However, the DFT model still assumes slit shaped pores with graphite interaction energies. Despite these limitations the DFT model is in common use and will be used for these studies in conjunction with the BET.

2.2 Experimental section

2.2.1 Material preparation.

Prior to characterisation, the adsorbent is washed using a Soxhlet extraction system using toluene as the solvent for 8 hours. Once the sample was removed from the Soxhlet it was dried in a vacuum oven to remove the toluene. If the material was made / purchased from a stirred cell manufacturing method, the dry resin was then sieved in order to obtain size fractions with a much tighter distribution. The size fractions obtained were 300-500 μm, 500 μm – 710 μm and 710-850 μm in addition to samples obtained by dry crushing the material. Any material produced using the membrane emulsification method did not require sieving as the method produced particles with a significantly tighter size distribution. The dry resin was wetted using acetone followed by displacement of acetone with water using 20 bed volumes of distilled water (flow rate approximately 10 bed volumes per hour). The adsorbent was subsequently stored in water. For adsorption work, wet adsorbent was centrifuged immediately prior to use (3000rpm, 5 mins) to remove any adherent water from the adsorbents particles. The centrifuged adsorbent was weighed using a 6 digit balance in the quantity required for each experiment. In order to obtain the correct dry mass of adsorbent, the moisture content of the centrifuged resin was previously evaluated so a correction could be made for each material to arrive at the correct dry mass.
2.2.2 Optical photographs / SEMs

The use of optical photographs demonstrate the spherical nature of the adsorbents and also give a visual demonstration of the range of particle sizes obtained from the suspension polymerisation method. Scanning electron micrographs utilise a high energy beam of electrons and measures the interaction they have with the surface of the sample that has been covered in gold. The energy exchange between the electron beam and the sample results in the emission of electrons which in turn can be detected and subsequently converted into an image. SEM's were obtained to demonstrate the internal pore structure of the adsorbents. A Jeol 100CX scanning electron microscope was used to obtain the images shown here.

2.2.3 Particle size distribution analysis

A Lasentec S400A instrument made by Malvern was used to analyse equivalent spherical diameter by calculation of area by light scattering properties. This type of instrument is capable of obtaining a full particle distribution for particle ranges of interest in this thesis. Each of the size fractions was pre wetted in water before being measured. The Sauter mean value, median and mode were obtained. Additionally, a measure of span is also reported, defined as

\[
\frac{d_{0.9} - d_{0.1}}{d_{0.5}} = \text{Span}
\]

(Equation 2.13)

Span values lower than 0.5 are considered as approximately monosized.
2.2.4 Polymer density.

The polymer density is simply the ratio of polymer mass to volume. The mass of each of the samples was easily measured using a 6 digit laboratory balance. The volume occupied by the adsorbent was obtained using a helium pycnometer. The pycnometer works by detecting the pressure changes resulting from displacement of gas by the polymer. The instrument obtains the skeletal volume of the porous adsorbent and its absolute density is obtained by knowing the mass of the adsorbent in the cell. Helium was used as the displacement gas as it is a small probe molecule and can diffuse into small micropores. The accuracy of the instrument is good providing the sampling gas is free from moisture. The sample must also not contain volatile substances so the samples were first vacuum dried in an oven prior to helium pycnometry measurement. 5 measurements of each material were made and the values reported in represent the mean average of those readings.

2.2.5 Surface area and pore size distributions.

Surface area and pore size distributions were obtained using a Micromeritics ASAP 2000 automatic analyser using high purity nitrogen as the probe molecule. Samples of each material and each size fraction were obtained, cleaned and dried in a vacuum oven prior to their study. The samples were prepared by degassing for a minimum of 24h in the degas port of the machine. The mass of adsorbent was carefully measured by subtracting the weight of the empty sample tube from that of the degassed tube including the sample.
2.2.6 Surface area and pore structure comparison.

The three materials, XAD4, CW1 and CW4 were characterised in order to evaluate their pore size distributions, pore size cut off, total pore volume and surface area using both the DFT and BET models. In order to demonstrate the effect pore cut off would have on potential uptake for the marker molecules used in this work, the pore structures are expressed in terms of surface area accessible to lysozyme and albumin based on pore sizes having a cut off of 6nm for Lysozyme and 14nm for albumin respectively. These values were obtained using size exclusion chromatography in previous studies.

2.2.7 Effect of particle size on pore structure.

Each material was fractionated using mechanical sieves so that discrete size fractions could be characterised to identify if samples within a particular particle size displayed differences in pore structures. In addition, adsorbents synthesised using the membrane emulsification process were evaluated to see if this variation in manufacturing technique had any effect on pore size distributions for the same material. This is of importance when considering the design a haemoperfusion column as one of the parameters which may be changed is the particle size. However, in order to ascertain how changing this parameter may affect the device's performance it is important to know if any other variables are changing such as the pore structure itself. In addition crushed samples were produced for kinetic studies and it was deemed important to characterise the effect of this practice on the resulting pore structure.
2.3 Results and discussion

2.3.1 Optical photographs / SEM

The optical photographs shown in Figure 2.2 demonstrate the spherical nature of the particles produced using the suspension polymerisation method. The image on the left of the figure show that large particles were produced using a slow stirrer speed, and that smaller particles were obtained using a higher stirrer rate. The photograph on the right shows evidence of smaller particles (fines) caused by areas of high shear within the reactor.

Figure 2.2. Optical photographs of CW1 particles.

Samples for SEM investigation consisted of both whole particles and crushed samples of XAD4, CW1 and CW4. The samples were taken from bulk (not fractionated and not sieved after crushing). Figure 2.3 shows a crushed sample of XAD4, demonstrating the amount of fines that result from this process. Figure 2.4 shows the surface of a non crushed XAD4 particle. The
surface appears to have irregularities that could indicate the presence of porosity, and were probed further at higher magnification. Figure 2.5 shows a high magnification image of a fracture face of XAD4, clearly showing porosity. The image shows evidence of nodules (25 – 100 nm) and a cauliflower like structure with mesopores between the nodules. The SEM image of CW1 shown in Figure 2.6 again shows the crushed non spherical material although less fines seem to be produced here than with the XAD4 sample. Evidence of potential porosity is again present in Figure 2.7. The porosity is visible along the fracture face shown in Figure 2.8.

The fractured particle of CW4 shown in Figure 2.9 shows the presence of porosity, clearly visible along the fracture faces. Figure 2.10 is a magnified image of a fracture face of CW4 showing even distribution of the nodules which make up the structure.

Figure 2.3. SEM of crushed fragments of XAD4.
Chapter 2

Material characterisation

Figure 2.4. SEM image of XAD4 surface (non fracture face).

Figure 2.5. SEM of fracture face of XAD4 showing porosity.
Figure 2.6. SEM of crushed sample of CW1 showing non spherical particles

Figure 2.7. SEM of CW1 surface structure (non fracture face).
Figure 2.8. *SEM of fracture face of crushed sample of CW1 showing porosity.*

Figure 2.9. *SEM of fractured CW4 fragment showing exterior surface and internal fracture face.*
Table 2.3 tabulates the particle size fractions obtained from the Malvern instrument. The mean diameter refers to the Sauter mean value. The sieved fractions resulted in reasonably tight distributions as characterised by low span values. Although effort was made to sieve the crushed fraction to remove fines, it appears some remained in the XAD4 fraction resulting in a high span value. The majority of the size fractions exhibit a reasonably wide range of particle sizes despite being sieved. The larger size fractions have a tendency to exhibit tighter distributions, as the sieves are more effective at removing the smaller particles. The smaller sized sieves have a tendency to clog easily with fines thereby preventing the passage of smaller particles. Although care was taken to sieve only small quantities at a time, it is clear this process has limitations.

**Figure 2.10.** *SEM of pore structure of CW4 taken along a fracture face.*

### 2.3.2 Particle size distributions.
Figure 2.11. Plot of particle size distributions for all adsorbents. Plotted as a percentage of distribution against size.

Table 2.3. Average particle sizes for adsorbents used in kinetics experiments.

<table>
<thead>
<tr>
<th>ID / Fraction</th>
<th>Particle Diameter (µm)</th>
<th>Span</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
</tr>
<tr>
<td>CW1 crushed</td>
<td>197</td>
<td>236</td>
</tr>
<tr>
<td>CW1 120*</td>
<td>120</td>
<td>112</td>
</tr>
<tr>
<td>CW1 300-500µ</td>
<td>430</td>
<td>449</td>
</tr>
<tr>
<td>CW1 500-710 µ</td>
<td>513</td>
<td>559</td>
</tr>
<tr>
<td>CW1 710-850 µ</td>
<td>780</td>
<td>820</td>
</tr>
<tr>
<td>CW4 20-50 µ</td>
<td>28</td>
<td>38</td>
</tr>
<tr>
<td>CW4 40-70 µ</td>
<td>49</td>
<td>66</td>
</tr>
<tr>
<td>XAD4 crushed</td>
<td>163</td>
<td>268</td>
</tr>
<tr>
<td>XAD4 300-500µ</td>
<td>400</td>
<td>368</td>
</tr>
<tr>
<td>XAD4 500-710µ</td>
<td>478</td>
<td>433</td>
</tr>
<tr>
<td>XAD4 710-850µ</td>
<td>716</td>
<td>693</td>
</tr>
</tbody>
</table>

* made by micropore membrane
The CW4 fractions were not sieved prior to use due to the small particle sizes and the span values were poor. The heterogeneous turbulence in the polymerisation reactor is likely to have caused the distribution of particle sizes. The CW1 sample made using the micropore membrane exhibits a low span value without the requirement to sieve the material. This demonstrates a commercially viable way of producing materials in bulk with a tight particle size distribution without the need of any post processing.

### 2.3.3 Surface area and pore size distributions

All the materials used in the study exhibit type 4 nitrogen adsorption isotherms (using the IUPAC classification, see Appendix 1). Type 4 isotherms are typical of mesoporous materials and are characterised by an initial monolayer coverage up to point B where monolayer to multilayer coverage is taking place. The limiting uptake at higher P/P₀ suggest total pore filling. The hysteresis observed is evidence of pore condensation.

The hysteresis observed in the isotherms offers insight into the material mesoporous structure. The XAD4 samples demonstrate a H2 hysteresis according to the IUPAC classification (see Appendix 1). This type of hysteresis is typical of a porous material containing small pores usually caused by agglomerates of spherical form and similar in size. The high magnification scanning electron micrographs support presence of such a structure. The hysteresis is more apparent for nitrogen adsorption isotherms of CW1 and CW4 samples. This is most likely due to the lower cut off of mesopores in these materials compared with XAD4 (see Figure 2.12- Figure 2.18).
XAD4 demonstrated a higher capacity for nitrogen adsorption that CW4 which in turn was higher than CW1. The increased adsorption volume is due to the higher total porosity of XAD4 due to large macropores.

### 2.3.4 Adsorption / desorption Isotherms.

![Adsorption / desorption isotherm for crushed fraction of XAD4](image)

**Figure 2.12.** Adsorption / desorption isotherm for crushed fraction of XAD4
Figure 2.13. Adsorption / desorption isotherm for 300 – 500μ fraction of XAD4

Figure 2.14. Adsorption / desorption isotherms for 500 - 710μ fraction of XAD4
Figure 2.15. Adsorption / desorption isotherms for 710-850μ fraction of XAD4

Figure 2.16. Adsorption / desorption isotherm for mixed fraction of CW1.
Figure 2.17. Adsorption / desorption isotherms for 20-50μ fraction of CW4.

Figure 2.18. Adsorption / desorption isotherm for 40-70μ fraction of CW4.
2.3.5 Comparison of materials.

The BET model doesn’t take into account the possibility of micropore filling or penetration, therefore it could falsify the results.

Using the DFT model it is possible to obtain plots of surface area and pore volume based upon the experimental data obtained from the nitrogen adsorption isotherms. Incremental pore volumes and pore surface areas were obtained in addition to cumulative pore volumes. The Incremental values are obtained by calculating the quantity of area or volume at a particular pore width. The cumulative values are obtained by summing the (area or volume) values for each pore width together. The sharp peaks in some of the data are likely to be an artefact of the model and not actual pore structure. When the plots of incremental surface area against pore width are overlaid as in Figure 2.19 the differences in pore structure become apparent. CW1 and CW4 have virtually identical pore cut off of around 11nm. XAD4 however, exhibits a considerably higher cut off of around 30nm. Figure 2.20, Figure 2.21 and Table 2.4 highlight the fact that XAD4 has considerably more pore volume than both CW4 and CW1. CW4 contains more pore volume and surface area than CW1. By normalising the cumulative pore volume data as in Figure 2.22 it can be shown that CW1 and CW4 have much of their pore structure in the mesopores region where as XAD4 contains a large quantity of larger mesopores as well as significant microporosity. Although CW1 and CW4 have the same cut off, CW4 contains a greater pore volume and surface area in the mesopore region compared to CW1. The surface area and pore volume in this region is still less than that found in XAD4 however. The pore structure data suggests that both CW1 and CW4 should size exclude albumin whilst allowing access to MM’s. XAD4 in contrast may allow access to human serum albumin due to the larger mesopores. Table 2.4 provides guidance on the potential amount of surface area accessible to the two bio markers on each of the materials based on DFT data. The characterisation of these
materials suggest that CW1 & CW4 posses suitable pore structure for removal of MMs and are were therefore chosen to evaluate MM adsorption. XAD4 with its more open pore mesopore structure was selected to compare with CW1 & CW4 and see whether albumin could result in competition of binding sites for MMs.

![Comparison of pore structure of materials CW1, CW4 and XAD4 with respect to incremental surface area against pore width.](image)

**Figure 2.19.** *Comparison of pore structure of materials CW1, CW4 and XAD4 with respect to incremental surface area against pore width.*
Figure 2.20. *Comparison of pore structure of materials CW1, CW4 and XAD4 with respect to incremental pore volume against pore width.*

Figure 2.21. *Comparison of pore structure of materials CW1, CW4 and XAD4 with respect to incremental pore volume against pore width.*
Figure 2.22. Comparison of pore structure of materials CW1, CW4 and XAD4 with respect to normalised cumulative pore volume against pore width.
Table 2.4. *Surface area and pore volume measurements for polymer adsorbents.*

<table>
<thead>
<tr>
<th>Sample i.d.</th>
<th>BET</th>
<th>DFT</th>
<th>DFT</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Surface area available to Lysozyme (m²/g)</td>
<td>Surface area available to Albumin (m²/g)</td>
<td>Polymer Density</td>
<td>Porosity</td>
<td></td>
</tr>
<tr>
<td>CW1 (emulsion)</td>
<td>120*</td>
<td>412</td>
<td>0.34</td>
<td>88</td>
<td>4.4</td>
<td>0.18</td>
<td>0.03</td>
<td>20</td>
</tr>
<tr>
<td>CW1 mixed</td>
<td>488</td>
<td>0.4</td>
<td>104</td>
<td>4.5</td>
<td>0.22</td>
<td>0.03</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>CW4 28</td>
<td>442</td>
<td>0.46</td>
<td>148</td>
<td>6.37</td>
<td>0.33</td>
<td>0.04</td>
<td>31</td>
<td>1</td>
</tr>
<tr>
<td>CW4 49</td>
<td>636</td>
<td>0.61</td>
<td>173</td>
<td>14</td>
<td>0.37</td>
<td>0.09</td>
<td>37</td>
<td>1</td>
</tr>
<tr>
<td>XAD4_crushed</td>
<td>826</td>
<td>1.07</td>
<td>229</td>
<td>44</td>
<td>0.54</td>
<td>0.33</td>
<td>103</td>
<td>21</td>
</tr>
<tr>
<td>XAD4 300-500</td>
<td>888</td>
<td>1.31</td>
<td>228</td>
<td>65</td>
<td>0.54</td>
<td>0.44</td>
<td>125</td>
<td>37</td>
</tr>
<tr>
<td>XAD4 500-710</td>
<td>853</td>
<td>1.22</td>
<td>228</td>
<td>64</td>
<td>0.55</td>
<td>0.44</td>
<td>128</td>
<td>40</td>
</tr>
<tr>
<td>XAD4 710-850</td>
<td>846</td>
<td>1.23</td>
<td>221</td>
<td>60</td>
<td>0.52</td>
<td>0.43</td>
<td>117</td>
<td>37</td>
</tr>
</tbody>
</table>

* made by Micropore membrane.
2.3.6 Effect of particle size on pore structure.

Although the pore structure of the materials appears well suited for use as a selective adsorbent for blood purification, it was important to ensure synthesis of adsorbents under identical conditions except for particle size doesn't have a great impact in the resulting pore structure. Figure 2.23 shows that there is little effect on pore structure with regard to different particle size fraction. The BET data for each of the size fractions of XAD4 (see Table 2.4) provides evidence that there is little difference in the surface areas when the particle size of the adsorbent is changed. The pore volume is reduced slightly in the crushed sample of XAD4 based on the DFT modelling data. This is most likely to be due to the fact the particles will fracture along the largest pores as these are likely to exhibit the lowest mechanical strength. The result is a relative reduction in pore volume especially in the larger pore region (10-50nm). Figure 2.23 shows a plot of incremental pore volume against pore width for all size fractions of XAD4 obtained by using the DFT model. The data from the different size fractions overlay with the exception of the crushed sample which has as stated previously a slightly lower pore volume. This observation is better illustrated by Figure 2.24 where cumulative pore volume is plotted against pore width. In addition Figure 2.25 where the same data is normalised against pore volume it becomes apparent that the pore volume distributions for all of the XAD4 size fractions are similar but the crushed fraction has a slight reduction in volume in the larger pores. This provides supporting evidence that the larger pores have been lost in the crushing process. Figure 2.26 demonstrates that there is a negligible difference in pore surface area in the crushed sample of XAD4 compared to the sieved whole particles. This suggests that the particles have fractured along the larger pores and hence although some pore volume is lost the surface area remains constant. Therefore, it is assumed crushed particles will have similar adsorption characteristics to whole particles.
In addition, the CW1 material made using the dispersion cell method demonstrated similar pore structure in the mesopore region. Therefore, it appears that the membrane emulsification process and the emulsion generation using a commercial stirred reactor does not effect the mesopore structure of the material. Thus control of particle size may be affected by the use of membrane emulsification techniques without influencing the pore structure development.

Figure 2.23. *plot of incremental pore volume against pore width for different size fractions of XAD4.*
Figure 2.24. *Plot of cumulative pore volume against pore width for different size fractions of XAD4*.

Figure 2.25. *Plot of normalised cumulative pore volume against pore width for various size fractions of XAD4.*
Figure 2.26. Plot of incremental surface area against pore width for different size fractions of XAD4.

2.4 Conclusions

Using a combination of physical characterisation techniques, data on the particle size, pore size and surface area for 3 different styrene divinylbenzene copolymer adsorbents have been obtained. The data suggests that both CW1 and CW4 should be capable of removing MM toxins whilst size excluding albumin. The commercial material, XAD4 may allow human serum albumin into some of its internal pore structure although it does possess the largest quantity of surface area available to MM's. CW1 has the lowest surface area available for MM removal with CW4 having around 50% more surface area whilst maintaining the same cut off (11nm). The materials were therefore selected for further adsorption studies to see whether the exclusion of human serum albumin may be demonstrated. In addition, the differing pore structures between CW1 and CW4 may highlight the effects of pore structure and surface areas on kinetics and uptake capacity. The use of a micropore
technologies dispersion cell has shown a commercially viable method of producing monosized particles with little effect on the mesopore structure. The traditional suspension polymerisation technique has been shown to produce wide particle size distributions that would require fractionating in order to increase the bed porosity in any proposed fixed bed type haemoperfusion device to prevent high pressure drop during treatment. The particle size has been shown to have little effect on pore structure.
Chapter 3

Protein adsorption studies

This chapter introduces the principles of mass transfer and how their understanding has the potential to inform the engineering of a suitable blood purification device. The resistance to mass transfer of simple single solute systems is explored on 3 adsorbent materials (characterised in chapter 2). Binary systems containing both lysozyme and albumin were studied in addition to a more complicated concentrated biological fluid (PD fluid) containing a plethora of MM’s and albumin at physiological concentrations. From these experiments a clearer understanding of the fundamental key parameters in uptake of MMs from blood emerge.

3.1 Introduction to mass transfer theory

Adsorbents are commonly used for purification applications. A solid material is used to separate soluble solutes from solution. The process relies on interaction of the solute molecules contained within the solution to the adsorbent material surface due to shot range Van der Waals forces that arise as a solute approaches an adsorbent surface. It is possible that the molecules could adhere to the material surface due to chemisorption, which is where a chemical bond is formed between the adsorbent and solute.

Adsorbents offer a suitable means of separating solutes from dilute solutions e.g. organic pollutants including herbicides and pesticides from water (Daignault et al (1988), Tsyurupa et al (1995). It is possible to alter the adsorption characteristics of the adsorbent by controlling the physicochemical properties. Adsorbents can be made to be highly specific in their adsorption of target molecules. For example, incorporation of surface functional groups
that form complexes between solutes in solution could significantly enhance the solute affinity for the adsorbent.

Identifying the key parameters influencing protein adsorption along with the role of the pore structure in terms of size exclusion may allow the design of an adsorbent suitable for the removal of middle molecules.

The adsorption process may be viewed as three distinct steps in series.

**External transport:** The transport of molecules to the surface of the adsorbent from the bulk solution. This process may be influenced by external mass transfer effects e.g. mixing.

**Internal transport:** The transport of molecules within tortuous channels (pores) of the adsorbent material e.g. via mesopores and macropores.

**Sorption:** The physical attachment of molecules to the surface within the adsorbent bead.

The diffusion transport processes described above can be modelled using Fick’s first law of diffusion:

\[ J = -D \frac{dc}{dx} \]  

(Equation 3.1)

The interaction of proteins at the adsorbent/solution interface depends on both the surface characteristics of the adsorbent and the solute chemistry influenced by the intimate solution environment (e.g. pH, ionic strength etc). The surface characteristics include surface charge, surface energies, surface hydrophobic properties and the presence of surface functional groups.

Ramsden (1997) suggested that non-specific hydrophobic interactions are likely to be the primary mechanism for protein adsorption onto polymer surfaces. It is well documented in the literature (Malik et al, 2008) that activated carbon materials exhibit larger adsorption capacity for proteins than their polymer counterparts suggesting that perhaps the increase in hydrophobicity results in this increased uptake.
Chapter 3 Protein adsorption studies

The mechanisms of adsorption of lysozyme and albumin onto polymer surfaces used in this work have not been studied in detail. Suggestions for how this may be done are included in chapter 5. The focus of the work presented here is the investigation of how the adsorbent pore structure affects the adsorption performance in terms of mass transport and protein size exclusion. The adsorbents studied are all copolymers of polystyrene-co-divinylbezene. These are known to be hydrophobic in nature, therefore protein-surface interactions with the adsorbent are thought to occur primarily due to hydrophobic interactions.

Equilibrium and the adsorption isotherm

The equilibrium adsorption isotherm relates the equilibrium partitioning of the solute between the solid/liquid phases. An adsorption isotherm may be obtained by undertaking batch adsorption experiments under controlled conditions (e.g. temperature). By contacting equal amounts of adsorbent with solute solution having a different initial solute concentration, the solute uptake (at equilibrium) may be plotted as a function of the equilibrium solute solution concentration. Usually, in physical adsorption processes, the equilibrium condition is one that is dynamic in nature. The rate of solute adsorption equals the rate of solute desorption.

Protein adsorption has however, been found to be concentration independent (Ramsden, 1997 and 2002), resulting in a rectangular adsorption isotherm (see Figure 3.1 below). Therefore if a site is vacant and there are solute molecules in solution available to fill the site, then the site shall eventually be filled. The filling will continue until the surface is saturated. Literature suggests that proteins are able to form a monolayer on the polymer surface (Ramsden, 2002)
3.1.1 Kinetics of adsorption

The rate of adsorption can be said to be governed by two main factors. The rate at which the molecules arrive at the adsorption surface (within the adsorbent particle) and the rate at which molecules at the surface undergo adsorption (e.g. if there is a barrier to adsorption so that the frequency of adsorption attempts is not the same as the rate of adsorption).

Understanding of the underlying mass transport mechanism is important in order to optimise both the adsorbent material and the design of the blood purification system. As discussed previously, the transport mechanism may be split into three distinct steps.
External Transport.

Diffusion of the solute occurs across a stagnant liquid film adherent to the external surface of the particle. The thickness of the liquid film (the stagnant boundary layer) is influenced by the degree of external agitation (empirical correlations are available that relate the dimensionless Sherwood number ($Sh$) as a function of Reynold's number ($Re$), Schmidt number ($Sc$) and power number ($Po$)). External agitation (e.g. using a stirrer and baffles) affects the film thickness (shown schematically in Figure 3.2).

![Boundary Layer Diagram](image)

**Figure 3.2.** *Schematic diagram showing stagnant film boundary layer around the adsorbent bead.*

Transport within the particle

It is suitable to think of a pore as a capillary and a pore size distribution in terms of a number of capillaries of various sizes (see Figure 3.3). Generally, adsorbents containing larger pore sizes will have a smaller surface area. Typically, microporous adsorbents (pore sizes less than 2nm) have surface areas in excess of several hundred square meters per gram of adsorbent. Adsorbents with small mesopores (2-10 nm) could result in the solute (MMW protein) interacting with the surface more often, thus causing a hindrance to
diffusion. This happens when the solute mean free path (distance the solute travels before it encounters another solute molecule and collides with it) is longer than the pore width. Therefore an adsorbent with large transport pores (10-50 nm) is likely to exhibit faster adsorption kinetics. However, the size of pores for blood purification is potentially limited due to the requirement to size exclude larger proteins (see Figure 3.3). For the molecule to be able to access the adsorbent internal surface, the transport pores must be large enough to allow the solute to enter into the adsorbent pore structure.

![Diagram](image)

**Figure 3.3.** *Schematic diagram of a size selective adsorbent bead, with a nanoporous structure suitable for size exclusion of HSA whilst permitting smaller proteins e.g. lysozyme, to diffuse within.*

**Adsorption on the pore wall**

Within the confines of the pore, the solute experiences Van der Waals forces with the likelihood of being retained at the surface (phenomenon known as adsorption).
Desorption from the pore surface.

The process of adsorption described above may be reversible. The adsorbed molecule may leave the surface and hence be desorbed. The phenomenon of desorption is less likely in protein adsorption due to the protein unfolding and attaching at multiple sites on the surface. The accompanying increase in system entropy and the exothermic nature of the adsorption process results in a significant decrease in the system free energy. If desorption does occur, it is possible for the molecule to leave the pore and subsequently pass through the film boundary layer at the particle surface and return to the bulk solution. (see Figure 3.4) However from simple solutions such as a single protein in buffer, it is often thought that once the protein adsorbs, the process is irreversible.

![Figure 3.4. Schematic of adsorption / desorption of molecules at the solid / liquid interface.](image)

Although literature suggests that protein adsorption onto polymer surfaces is often irreversible, Vroman (1962) suggested that in complex solutions there is a hierarchy of protein adsorption onto a material surface, this phenomenon is known as the "Vroman effect". Some proteins have the ability to dislodge a
preadsorbed protein if the affinity of the adsorbing protein for the surface is greater than that of the protein previously adsorbed at the surface. This is of particular interest for a blood purification device because if albumin is able to access the internal surface area within the adsorbent bead containing previously adsorbed MM's there could be an exchange between the faster diffusing smaller proteins eg MM's by albumin taking place due to its presence at the top of the hierarchy.

**Experimental Methodology**

Batch adsorption experiments carried out in an agitated tank offer a means to characterise the kinetic performance of the selected adsorbents at removing MM's. By measuring the time dependent reduction in solution concentration of a single marker protein (lysozyme, >99.9% purity, and human serum albumin, ~99% purity, both purchased from Sigma-Aldrich, UK.) in the bulk solution and applying a mass balance, the adsorption kinetics of each adsorbent may be studied.

All experiments utilised the methodology detailed below. Adsorbents XAD4, CW1 and CW4 with different mean particle sizes and various rates of agitation were studied. This enabled evaluation of the mass transfer resistance to MM adsorption within the nanoporous adsorbents. In addition binary solute systems containing both lysozyme and albumin in Hepes buffer solution were used. This was to evaluate if albumin access to the internal pore area of any proposed adsorbent bead may result in blocking of the pores and / or reduce the capacity for MM removal. Finally a clinically relevant multi-component system (concentrated peritoneal dialysis fluid) was used to study competitive adsorption effects. A matrix of the adsorption experimental work undertaken is presented in Table 3.3.

**Protein solution preparation**

A 0.1M Hepes buffer solution containing 100 mM NaCl (pH7.4) was used to prepare all protein solutions. A known weight of protein was carefully
dissolved in the solution. Care was taken during dissolution to prevent foaming of solution by gently agitating the protein solution.

**Batch adsorption experiments using the stirred tank reactor**

A 1 litre round bottomed glass reactor flask was placed in a temperature controlled water bath (temperature controlled at 37°C). A PTFE paddle (4 blades) connected via a variable rotational speed overhead stirrer (60-1500rpm) provided controlled agitation to study the influence of mixing. The speed of the stirrer was measured using a tachometer prior to each experiment. The PTFE paddle stirrer geometry was selected after it was noted that some geometries were capable of mechanically damaging the adsorbent at high agitation rates resulting in production of fines. The stirrer used in the study was selected following initial studies showing that agitation of the adsorbent beads caused no change to the particle size distribution of the sample after 24 hours agitation at the maximum agitation rate (826rpm). 4 stainless steel baffles (at right angles) were added to the reactor to reduce vortex formation and the subsequent entrapment of air. A sampling syringe equipped with an in-line filter was added to the system to aid sample collection. The reactor was filled with 500ml of buffered protein solution 12 hours prior to the addition of any adsorbent to ensure that any non-specific adsorption on the vessel walls was complete and no further change in solution concentration took place. Once the system was at equilibrium, a 1ml solution sample was withdrawn to measure the initial protein concentration ($C_{\text{initial}}$) prior to addition to the reactor of a known weight of pre-wetted adsorbent sample. A stop watch was started to follow the adsorption kinetics. The adsorbent was pre-wetted in ultra-pure water and centrifuged to remove all external particle moisture apart from that contained within the pore structure. This process ensured access of solution within the beads once they were added to the protein solution. Subsequent samples were then taken at time varying time intervals (typically after 1, 2, 5, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 360, 420, 1440 min). Solution samples were withdrawn using the syringe and in-line filter assembly to prevent removal of particles during sample collection. Each solution sample was filtered through a 0.45μm
filter and stored in a refrigerator until all samples for the experiment had been collected. Samples were taken out of the refrigerator and brought to room temperature prior to analysis using the HPLC method described below.

**Methodology for protein detection**

The separation of biological macromolecules such as proteins from aqueous solution is achieved by injecting a small quantity of the sample onto a chromatography column. Mobile phase is pumped through the column containing the stationary phase. The separation occurs within the column based on different affinities of the solutes with the column stationary phase. The solution coming out of the column passes through a cell equipped with a detector e.g. a UV detector. The resulting chromatogram contains peaks corresponding to the elution of various analytes. The area under the peak may be used to calculate the concentration of the analyte by relating the area via a calibration curve obtained using pure samples of known concentrations.

Initially, a size exclusion column, Aquagel OH-30 purchased from Polymer Laboratories (UK), was used to quantify albumin from buffer solution. Quantification of Lysozyme by SEC resulted in poor resolution hence, reverse phase HPLC was used for the separation and detection of both lysozyme and albumin. A linear solvent gradient starting with 2% A (ultrapure water containing 0.01% trifluoroacetic acid (TFA)) and 98% B (acetonitrile containing 0.01% TFA) and linearly changing the composition to 80% A and 20% B over 60 minutes was found to offer the best binary protein separation and peak resolution over a reasonable analysis time (~1hr). 0.01% trifluoroacetic acid was added to both phases to introduce a source of protons. Mobile phase flow rate was selected to give the best peak shape, and the column temperature was set to 35°C. A wavelength scan of single protein in buffer solution (limit of detection of lysozyme was 2 mg/l and HSA 3 mg/l) was used to decide the detection wavelength ($\lambda$).

The following is a summary of the HPLC method used for the work.
Chapter 3 Protein adsorption studies

Column type: Supelco C18 reverse phase column
Mobile phase flow rate: 1 ml/min
Solvent A: Ultrapure water containing 0.01% TFA
Solvent B: Acetonitrile containing 0.01% TFA
Gradient profile: 98% A and 2% B to 20% A and 80% B, over 60 mins, followed by a step change back to 98% A and 2% B with an equilibration time of 10 mins before the next sample was injected into the column.
Injection volume: depending on concentration (20 – 100 µl)
Column temp: 35°C.
Detection wavelength: 230 nm for Albumin, 280 nm for Lysozyme.

Figure 3.5 shows results of a typical calibration obtained by making serial dilutions from a concentrated stock solution containing 1 g/l of human serum albumin in 10 mM Hepes buffer. Each sample was injected 5 times and the mean and standard deviation (error bars) values were evaluated (these are included in Figure 3.5).

![Figure 3.5](image)

Figure 3.5. Calibration data for human serum albumin measured on a reverse phase C18 column; detection wavelength 230 nm, limit of detection 3 mg/l. 
Figure 3.6 presents sample calibration data for lysozyme in 0.1M Hepes buffer solution obtained in the same way as described previously for albumin.

![Figure 3.6. Calibration curve for Lysozyme measured on a C18 column at 280nm, limit of detection 2mg/l.](image)

**Figure 3.6.** *Calibration curve for Lysozyme measured on a C18 column at 280nm, limit of detection 2mg/l.*

### 3.1.2 Detection of Beta2-microglobulin and Albumin from concentrated peritoneal dialysis fluid.

Although HPLC offers a good method for quantifying marker proteins from simple synthetic protein solutions, it is not possible to measure single solutes from a highly complex biological fluid such as peritoneal dialysis fluid containing many unknown solute species.

There are a multitude of highly selective enzyme based immunoassay techniques available. Although some generic principles run through all the techniques the precise protocols for each immunoassay may be slightly different.
A two site sandwich immunoassay was used for the detection of human serum albumin and β₂-microglobulin from concentrated peritoneal dialysis fluid. The basic method is to immobilise a capture antibody on to a solid phase (usually the microplate wall) and then allow the sample solution to come into contact with the immobilised antibody. The equilibrium portioning of protein in solution and immobilised antibody is governed by the mass action law. After equilibration, a second antibody is attached to the bound target molecule and subsequently a molecule is attached to this which is capable of having an enzymatic effect on the substrate solution (a colour change) which is added in the next step. The colour intensity created is related to the quantity of the bound target molecule which is measured. Due to the fact two antibodies are used to bind to a single molecule the sandwich type assay is extremely specific. A more detailed explanation of the procedure is given in Figure 3.7 below.

The following is a generic overview of the sandwich ELISA technique (to be read in conjunction with Figure 3.7).

A capture antibody (in red) was added to each of the wells and incubated for a period of time depending on the capture antibody used (20 min – overnight) (1). This time allowed non-specific adsorption of the capture antibody on to the surface of the microplate walls.

The capture antibody solution was removed and the microplates wells were rinsed with a washing buffer. This process removed any unbound capture antibody solution but did not remove the immobilised capture antibody molecules adsorbed (bound) to the microplate wall (2).

A blocking solution (shown in orange (3)) was added to the microplate wells. The role of a blocking agent present in this solution was to non-specifically adsorb to the remaining wall area i.e. sites not already taken-up by the capture antibody molecules. This is an important step that ensures that the molecules from subsequent steps do not adsorb onto the microplate surface.
and thus affect results. The system was then left for a period of time to allow adsorption to take place and equilibrate.

The blocking solution was removed and the microplate wells were rinsed with a washing buffer. Again, this ensured the bulk solution was removed (unbound molecules), whilst not removing the molecules adsorbed to plate surface (4).

This step involved the addition of the standard (known concentration of protein in buffer solution) or the sample (containing the analyte i.e. unknown concentration of protein in buffer solution) followed by an incubation period (5). The protein attaches specifically to the antibody previously adsorbed to the plate surface.

Another wash step was carried out to remove the bulk solution from the microplate wells (6).

The next step in the process was the introduction of a detection antibody in buffer solution (shown in green in frame (7)). The antibody binds to the target molecule thus forming a sandwich. The binding process is extremely specific; precise details of cross reactivity may be found in the data sheets provided by the manufacturer of the ELISA kit.

A wash phase ensured the removal of all unbound molecules(8).

A solution containing the enzyme Horseradish peroxidase (HRP) (shown in black in frame 9) was added to the microplate wells. The incubation time required for HRP to bind to the detection antibody is dependent on the age of the kit, the molecules being studied and atmospheric conditions. HRP enzyme or conjugates are of little value on their own; its presence is made visible using a substrate that when oxidized by HRP using hydrogen peroxide as the oxidizing agent, yields a characteristic colour change that is detectable by a spectrophotometer. Some ELISA kits use a HRP conjugate system that includes the detection antibody hereby eliminating the previous 2 steps.

A wash step was used to remove all unbound molecules (10).

The next step was to add the substrate solution which is clear when added, but upon oxidation by HRP forms a coloured product (11). Due to the high turnover rate of HRP strong signals in a relatively short time are made possible. The colour intensity produced is in theory directly proportional to the amount of analyte bound to the microplate well.
Chapter 3  Protein adsorption studies

The reaction which occurs in step 8 can be “stopped” by adding a weak acidic solution (12). The intensity of the colour produced was then read at an appropriate wavelength (450nm for HRP systems).
ELISA method for the detection of Human Serum Albumin

Although ELISA's offer a suitable means of quantifying specific molecules they often require a degree of method development to maximise sensitivity. The albumin ELISA kit was purchased from R&D systems, UK. Initially, the kit instructions suggested a capture antibody concentration of 1:50,000 but when the kit was used at this concentration, saturation of the spectrophotometer detector occurred. By diluting the capture antibody concentration to 1:150,000 the sensitivity of the assay to discern albumin concentrations over a wider concentration range was improved, the signal intensity however was weak. Increasing the incubation time from 20 min to 25 min, amplified the signal (results are presented in Figure 3.8). Samples from adsorption experiments were diluted if the albumin concentration in the samples was greater than the detection range of the ELISA kit.

Figure 3.8 Results of albumin ELISA method development showing effects of varying capture antibody concentration and incubation time.
ELISA method for the detection of Beta2-Microglobulin

Beta2-microglobulin ELISA kits were purchased from BioSupply UK and required no optimisation prior to use. The kit included antibody precoated wells. All solution samples were diluted to ensure the concentration was within the detectable range of the kit (measurable concentration range 0.2 - 12 μg/l).

3.1.3 Mass transfer limitations on protein adsorption dynamics: Influence of film boundary layer.

The effect of turbulence in the bulk of the fluid (influenced by the stirrer agitation rate) and the effect this has on the reduction of the stagnant film boundary layer surrounding the adsorbent particle was studied. This was accomplished by studying the adsorption kinetics of a single protein (lysozyme or albumin) in buffer solution on a fixed particle size fraction of adsorbent material. A number of experiments were carried out under identical conditions (15g of adsorbent contacting 0.5l of single protein Hepes buffered solution) except for each experiment a different stirrer speed was used. As the agitation rate was increased, it was possible to study the effect of external mixing conditions on the mass transfer coefficient. If the external film boundary layer surrounding the particle has an appreciable affect on mass transfer of MM's its effect on the uptake kinetics would become apparent as a consequence of the controlled experimental conditions. The bulk solution concentration change during adsorption experiments was followed for a time period of 24 h. A low agitation rate (lowest stirrer speed required to just suspend particles) of 84 rpm, an intermediate agitation rate of 700 rpm and a high agitation rate of 826 rpm (highest stirrer speed possible whilst avoiding vortex formation) were selected for the study. Particle size fractions between 500-710 μm of XAD4 and CW1 copolymer samples were prepared (by sieving
the appropriate size fraction from a bulk sample) for the adsorption of the single protein lysozyme from 0.1M Hepes buffer solution. A 710-850 μm fraction was used for the adsorption of albumin on XAD4. 15g of dry adsorbent was contacted with 0.5l of protein solution ((commercial blood purification columns typically contain between 150 g and 300 g of adsorbent material to treat 5L of blood). Thus for the batch adsorption experiments a similar solid / liquid ratio was initially used. Physiological concentration of lysozyme (surrogate MM marker) was used for this study. In addition albumin at 200 mg/l was used to ensure the resulting change in concentration could be detected.

3.1.4 Intraparticle mass transfer effects on protein adsorption dynamics: influence of particle size.

Using the same copolymer adsorbent, but sieving the sample to obtain tight particle size fractions allowed the effect of particle size on the uptake kinetics of both albumin (initial concentration 200mg/l) and lysozyme (initial concentration 100mg/l) to be studied. Experiments were performed using a single solute dissolved in 0.1M Hepes buffer solution. For all experiments, the mass of adsorbent (15g), the initial concentration of protein and the stirrer speed (826 rpm) were kept constant. The stirrer speed was set to the highest value as this reduces external mass transfer resistance to solute diffusion. A reduction in the adsorbent particle size affects the diffusion path length. If intraparticle diffusion is the rate limiting step for the adsorption of the protein within the adsorbent bead then reducing the particle size should have a marked effect on the adsorption kinetics. As the diffusion path length is related to the radius of the particle whilst the time for diffusion is proportional to r², the time for the solute to diffuse from outside the particle to the centre of the particle is four times less when the particle size is halved.
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Particle sizes fractions of the adsorbent copolymer XAD4 were prepared as follows, a crushed sample (particle size < 300 µm), 500-710 µm and 710-850 µm. CW1 fractions studied were 300-500 µm, 500-710 µm, and 710-850 µm.

3.1.5 Adsorption dynamics in a binary solute system

Single solute adsorption experiments permit analysis of the effect of the adsorbent pore structure on the rate of adsorption of MM's under controlled conditions. Also, the maximum adsorption capacity of the solute may be evaluated and correlated with the available adsorbent area. However, blood is a highly complex mixture of molecules and the presence of other molecules may affect the uptake of specific solutes that are marker MM's (e.g. β2-M) due to competitive adsorption effects. Albumin is present in blood at concentrations around 40g/l and is the most prevalent blood protein. Its presence at concentrations several orders of magnitude greater; β2-M (renal blood concentration typically ~ 35 mg/l), may potentially result in swamping of the adsorbent surface resulting in a marked reduction in the uptake capacity of the adsorbent for the target MMs.

In order to study these effects, solutions containing 100 mg/l of lysozyme and either no albumin or 200mg/l albumin or 1000 mg/l of albumin (approximately equal number of albumin molecules to lysozyme) were prepared in 0.1M Hepes buffer. Adsorption experiments using the binary solute system were conducted using CW1 and XAD4. CW1 was synthesised with a tailored mesopore structure with the aim to size exclude proteins such as albumin. The DFT data presented in chapter 2 suggests CW1 possesses less that 1 m²/g of accessible surface area for albumin based upon size exclusion chromatography. XAD4 has larger mesopores and so may allow albumin molecules access to surface area within the adsorbent bead (~ 40 m²/g). 15 g
of the 500-710 µm fractions of each of the adsorbents were added to the reactor and experiments were carried out under the conditions described above.

3.1.6 Multicomponent protein adsorption dynamics from concentrated peritoneal dialysis fluid

Single solute adsorption experiments provide useful data for the characterisation of the adsorption potential (capacity and kinetics) of adsorbents for use in a haemoperfusion device. The introduction of a binary system is a controlled step-up in complexity offering insight into the effects of competitive adsorption. Blood is an extremely complicated fluid which contains a plethora of molecules (unknown composition) and it is difficult to know how their presence will affect the adsorption uptake of MM's in any potential adsorption device. *In vitro* experiments using biological fluids (e.g. readily available waste peritoneal dialysis fluid) is an economical and accessible means to measure the uptake of clinically relevant molecules on any proposed adsorbent material.

Although blood would naturally be the fluid of choice, its use *in vitro* presents a number of experimental difficulties. Obtaining sufficient volumes of blood (0.5l per adsorption experiment) is costly. Associated problems such as addition of blood anticoagulants, changes in blood composition arising from stressing blood cells outside the body for long periods of time (several hours in a stirred tank) pose formidable challenges to the experimentalist. However, peritoneal dialysis (PD) fluid offers ready access to a highly complex multicomponent mixture of uraemic toxins and human serum albumin but is inherently easier to use *in vitro*. Adsorption studies using PD fluid was considered a suitable precursor to future adsorption experiments with whole blood.
As previously stated (Chapter 1), beta2-microglobulin (β2-M) is a clinical marker often reported in scientific literature, its elevated blood concentration (typically ~ 35 mg/l) in patients on long term renal dialysis is often correlated with their poor health. Therefore, adsorption performance of the novel mesoporous copolymers in terms of effectiveness in reducing β2-M concentration in PD fluid was considered worthy of study.

The concentration of β2-M in peritoneal dialysis fluid was unknown but expected to be below the expected serum level of ~ 35mg/l in renal dialysis patients. Therefore a pilot study was undertaken to sample typical peritoneal dialysis fluid β2-M concentrations and develop a methodology for concentrating the solute from the fluid to increase the β2-M concentration to ~ 35 mg/l.

The concentration of β2-M in PD fluid was measured using ELISA (method outlined above) in its "as received" state and following a series of concentrations obtained using an ultrafiltration stirred cell device (see Figure 3.9 showing a schematic diagram of the stirred cell apparatus). Pressure in the cell was provided by a regulated nitrogen gas supply (A), since the use of compressed air could induce undesirable pH shifts. The magnetic stirrer (B) provided agitation within the cell and reduced concentration polarisation effects enhancing the filtration rate. The nitrogen supply was connected to a ball valve (C) and a pressure regulator (D) as well as a pressure gauge (E) in order to allow for controlled pressure adjustments. Retentate samples were taken after the volume of feed had been reduced to ~6% of the original volume thereby achieving a concentration of β2-M by a factor of 16.
Figure 3.9. Stirred cell ultrafiltration apparatus used to concentrate peritoneal dialysis fluid.

Preliminary trials used a 48mm diameter 5kDa Microdyne-Nadir ultrafiltration membrane. Initial measured concentration of β2-M was just below 2mg/l and by concentrating by a factor of 16, a final concentration of approximately 29mg/l was achieved (see Table 3.1)

Table 3.1 Concentration of PD fluid using a 48mm diameter 5kDa ultrafiltration membrane.

<table>
<thead>
<tr>
<th>Membrane cut off</th>
<th>Concentration factor</th>
<th>B2-M concentration (mg/l)</th>
<th>Albumin concentration (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5kDa</td>
<td>0</td>
<td>1.8</td>
<td>&lt;2</td>
</tr>
<tr>
<td>5kDa</td>
<td>2</td>
<td>3.6</td>
<td>na</td>
</tr>
<tr>
<td>5kDa</td>
<td>4</td>
<td>7.5</td>
<td>na</td>
</tr>
<tr>
<td>5kDa</td>
<td>8</td>
<td>14.5</td>
<td>na</td>
</tr>
<tr>
<td>5kDa</td>
<td>16</td>
<td>28.9</td>
<td>26</td>
</tr>
</tbody>
</table>
Complete retention of β2-M by the 5kDa membrane was observed. However, due primarily to the small surface area available for filtration, the time required to achieve 16x concentration was not practical as 0.5L of concentrated fluid was required for each adsorption experiment. The use of a hollow fibre polysulfone haemodialysis membrane module (Fresenius F60) was tested due to the considerably larger surface area (~1.25 m² per module). However, the large molecular weight cut-off of the dialyser (typically ~50kDa) meant the required concentration factor was not achievable using this device. The work indicated the potential of such a system as the filtration rate was significantly higher. Therefore, a hollow fibre device incorporating membranes with a smaller molecular weight cut off (MWCO ~ 10kDa) was secured from Microdyne-Nadir GmbH (Germany) and used to concentrate β2-M from PD fluid. Prior to purchasing the 10kDa hollow fibre module, preliminary concentration experiments were conducted to verify that UF membranes with a MWCO of 10kDa were capable of concentrating β2-M in PD fluid to the desired amount (~40 mg/l). Table 3.2 tabulates the results of the experiments using a different patient PD sample with a 10kDa cut-off flat sheet Microdyne-Nadir membrane in the stirred cell. The results showed that concentration of β2-M was possible whilst maintaining an acceptable filtration flux.

Table 3.2. Concentration of PD fluid attempt using Microdyne-Nadir 10kDa MWCO hollow fibre membrane module.

<table>
<thead>
<tr>
<th>Membrane Cut Off</th>
<th>Concentration Factor</th>
<th>B2-M Concentration (mg/l)</th>
<th>Albumin Concentration (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10kDa</td>
<td>0</td>
<td>2.5</td>
<td>&lt;2</td>
</tr>
<tr>
<td>10kDa</td>
<td>2</td>
<td>5.4</td>
<td>na</td>
</tr>
<tr>
<td>10kDa</td>
<td>4</td>
<td>10.6</td>
<td>na</td>
</tr>
<tr>
<td>10kDa</td>
<td>8</td>
<td>20</td>
<td>na</td>
</tr>
<tr>
<td>10kDa</td>
<td>16</td>
<td>40.3</td>
<td>25</td>
</tr>
</tbody>
</table>

The 10kDa hollow fibre membrane unit was used to concentrate the PD fluid using the experimental ultrafiltration rig shown in Figure 3.10. Based on the
pilot studies typical initial concentrations of β2-M in PD fluid were of the order of ~2 mg/l. Therefore, starting with an initial pooled volume of 10L of PD fluid volume reduction to 0.5L resulted in β2-M concentration of ~40mg/l.

\[\text{Figure 3.10 Schematic diagram of experimental hollow fibre ultrafiltration rig used for concentrating the peritoneal dialysis fluid.}\]

Once the peritoneal dialysis fluid had been concentrated, the solution was added to the stirred tank batch reactor and the adsorption experiment conducted following the methodology described earlier. 0.5g of adsorbent XAD4 was contacted with 0.5l of concentrated PD fluid and 0.5g of CW4 was contacted with 0.5l of concentrated PD. Adsorption experiments were carried out under identical conditions. Solution samples were withdrawn from the batch reactor over a period of 7h and the samples were analysed using the ELISA method (described previously).
## Table 3.3. Matrix summarising parameters used for batch adsorption experiments.

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>Protein</th>
<th>Objective</th>
<th>SS/Binary/PD</th>
<th>Sample mass (g)</th>
<th>Size (µm)</th>
<th>Conc (mg/l)</th>
<th>Stirrer (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XAD4</td>
<td>▲LYZ</td>
<td>▲BL reduction</td>
<td>▲SS</td>
<td>15</td>
<td>500 - 710</td>
<td>100</td>
<td>84</td>
</tr>
<tr>
<td>XAD4</td>
<td>LYZ</td>
<td>BL reduction</td>
<td>SS</td>
<td>15</td>
<td>500 - 710</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td>XAD4</td>
<td>LYZ</td>
<td>BL reduction</td>
<td>SS</td>
<td>15</td>
<td>500 - 710</td>
<td>100</td>
<td>700</td>
</tr>
<tr>
<td>XAD4</td>
<td>LYZ</td>
<td>BL reduction</td>
<td>SS</td>
<td>15</td>
<td>500 - 710</td>
<td>100</td>
<td>826</td>
</tr>
<tr>
<td>XAD4</td>
<td>LYZ</td>
<td>Effect of particle size</td>
<td>SS</td>
<td>15</td>
<td>&lt; 300</td>
<td>100</td>
<td>826</td>
</tr>
<tr>
<td>XAD4</td>
<td>LYZ</td>
<td>Effect of particle size</td>
<td>SS</td>
<td>15</td>
<td>&gt; 710</td>
<td>100</td>
<td>826</td>
</tr>
<tr>
<td>XAD4</td>
<td>LYZ</td>
<td>Effect of particle size</td>
<td>SS</td>
<td>15</td>
<td>Crushed</td>
<td>100</td>
<td>826</td>
</tr>
<tr>
<td>CW1</td>
<td>LYZ</td>
<td>BL reduction</td>
<td>SS</td>
<td>15</td>
<td>500 - 700</td>
<td>100</td>
<td>84</td>
</tr>
<tr>
<td>CW1</td>
<td>LYZ</td>
<td>BL reduction</td>
<td>SS</td>
<td>15</td>
<td>500 - 710</td>
<td>100</td>
<td>700</td>
</tr>
<tr>
<td>CW1</td>
<td>LYZ</td>
<td>BL reduction</td>
<td>SS</td>
<td>15</td>
<td>500 - 710</td>
<td>100</td>
<td>826</td>
</tr>
<tr>
<td>CW1</td>
<td>LYZ</td>
<td>Effect of particle size</td>
<td>SS</td>
<td>15</td>
<td>300 - 500</td>
<td>100</td>
<td>826</td>
</tr>
<tr>
<td>XAD4</td>
<td>HSA &amp; LYZ</td>
<td>Competitive adsorption</td>
<td>Binary</td>
<td>15</td>
<td>500 - 710</td>
<td>200 &amp; 100</td>
<td>826</td>
</tr>
<tr>
<td>CW1</td>
<td>HSA &amp; LYZ</td>
<td>Competitive adsorption</td>
<td>Binary</td>
<td>15</td>
<td>500 - 710</td>
<td>1000 &amp; 100</td>
<td>826</td>
</tr>
<tr>
<td>CW1</td>
<td>LYZ</td>
<td>Effect of particle size</td>
<td>SS</td>
<td>15</td>
<td>710 - 850</td>
<td>100</td>
<td>826</td>
</tr>
<tr>
<td>XAD4</td>
<td>HSA</td>
<td>BL reduction</td>
<td>SS</td>
<td>15</td>
<td>710 - 850</td>
<td>100</td>
<td>84</td>
</tr>
<tr>
<td>XAD4</td>
<td>HSA</td>
<td>BL reduction</td>
<td>SS</td>
<td>15</td>
<td>710 - 850</td>
<td>100</td>
<td>826</td>
</tr>
<tr>
<td>XAD4</td>
<td>HSA</td>
<td>BL reduction</td>
<td>SS</td>
<td>15</td>
<td>710 - 850</td>
<td>200</td>
<td>700</td>
</tr>
<tr>
<td>XAD4</td>
<td>HSA</td>
<td>Effect of particle size</td>
<td>SS</td>
<td>15</td>
<td>300 - 500</td>
<td>200</td>
<td>700</td>
</tr>
<tr>
<td>XAD4</td>
<td>HSA</td>
<td>Effect of particle size</td>
<td>SS</td>
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<td>500 - 710</td>
<td>200</td>
<td>700</td>
</tr>
<tr>
<td>XAD4</td>
<td>HSA &amp; LYZ</td>
<td>Competitive adsorption</td>
<td>Binary</td>
<td>15</td>
<td>500 - 710</td>
<td>200 &amp; 100</td>
<td>700</td>
</tr>
<tr>
<td>XAD4</td>
<td>HSA &amp; LYZ</td>
<td>Competitive adsorption</td>
<td>Binary</td>
<td>15</td>
<td>500 - 710</td>
<td>1000 &amp; 100</td>
<td>700</td>
</tr>
<tr>
<td>XAD4</td>
<td>HSA</td>
<td>Uptake kinetics</td>
<td>SS</td>
<td>15</td>
<td>710 - 850</td>
<td>200</td>
<td>700</td>
</tr>
<tr>
<td>XAD4</td>
<td>HSA</td>
<td>Uptake kinetics</td>
<td>SS</td>
<td>15</td>
<td>710 - 850</td>
<td>100</td>
<td>700</td>
</tr>
<tr>
<td>XAD4</td>
<td>βLM</td>
<td>Competitive adsorption</td>
<td>PD</td>
<td>0.5</td>
<td>114</td>
<td>58mg/l</td>
<td>Max</td>
</tr>
<tr>
<td>CW4</td>
<td>βLM</td>
<td>Competitive adsorption</td>
<td>PD</td>
<td>0.5</td>
<td>48</td>
<td>61mg/l</td>
<td>Max</td>
</tr>
</tbody>
</table>

Notes: ▲LYZ = lysozyme; ◆HSA = Human serum albumin; ♥BL = boundary layer; ▲SS = single solute system;
3.1.7 Modelling batch adsorption dynamics – irreversible adsorption

Characterising adsorbents in terms of adsorption capacity and adsorption kinetics is possible experimentally using a batch stirred tank reactor. The time to equilibrate/saturate the adsorbent with protein diffusing from the bulk solution to the interior of the adsorbent particle depends on the particle size of the adsorbent beads and the effective diffusivity of the protein in the interior of the adsorbent. A small number of experiments under carefully controlled conditions coupled with use of a mathematical model incorporating the necessary physics of the process allows parameter estimation of the effective diffusivity for the adsorbent-protein system. Subsequently, having experimentally validated the predictive capability of each adsorbent, the model may be used as tool to inform the design of a haemoperfusion device. This approach has a number of merits both in terms of cost and time spent investigating a large combination of experimental parameters. Modelling offers insight into the effect of the physical properties of the adsorbent and helps in highlighting the rate limiting parameters. By quantifying the effects of pore structure (eg, tortuosity & porosity), particle size and boundary layer reduction in terms of an effective diffusivity, comparison and selection of different adsorbents may be made more rationally.

The case of an irreversible adsorption isotherm has been modelled previously by Suzuki et al, (1973) and was used by the researchers to estimate the intraparticle diffusivities of organic solutes within carbon adsorbents. A detailed account of the model development is provided in the following section. For the development of the model the following assumptions were made:-

- Adsorption was considered permanent and irreversible.
- Rectangular isotherm; strong adsorption, limited by capacity.
The particles were considered homogeneous.
Any accumulation of solute within the liquid phase within the particle
was considered negligible.

These assumptions result in a case where the adsorption process was
considered to be taking place only at the adsorption front \( (r = r_i) \), (see Figure
3.11) where the concentration of the liquid phase was said to be zero. Within
the shell \( (r_i < r \leq R) \) the adsorbent was considered to be saturated by the
solute. The rate at which the adsorption front moved towards the centre of the
particle was considered to be dictated by the rate of adsorption of diffusion of
the solute in the liquid phase through the shell. Experimental design
undertaken during this study was such that the final concentration \( (C_{mf}) \) was
reasonably different from zero to ensure operation on the linear part of the
plot. Consideration of the initial solute concentration \( (C_0) \) was an important
experimental consideration to ensure that for a given solid-liquid ratio, the final
solution concentration was non-zero. Therefore low quantities of adsorbent
were required to satisfy this criterion.

Figure 3.11. Schematic diagram illustrating a shell balance used for the
diffusion model.
From the basic diffusion equation (Fick's Law):

\[- \frac{1}{4\eta r^2} \frac{\partial (JA)}{\partial r} = \frac{\partial}{\partial t} qLp(1-\epsilon) + \frac{\partial}{\partial t} c(\epsilon) \]  

(Equation 3.2)

\[ J = -D_e \frac{\partial c}{\partial r} \]  

(Equation 3.3)

\[ \frac{D_e}{q^2} \frac{\partial}{\partial r} \left[ r^2 \frac{\partial c}{\partial r} \right] = Lp(1-\epsilon) \frac{\partial q}{\partial t} + \epsilon \frac{\partial c}{\partial t} \]  

(Equation 3.4)

For irreversible adsorption:

\[ \frac{\partial q}{\partial t} = 0 \text{ and } \frac{\partial c}{\partial r} = 0 \text{ for } r_1 \leq r \leq R \]  

(Equation 3.5)

Therefore:

\[ \frac{\partial}{\partial r} \left[ r^2 \frac{\partial c}{\partial r} \right] = 0 \]  

(Equation 3.6)

Integrate once,

\[ r^2 \frac{\partial c}{\partial r} = A(t) \]  

(Equation 3.7)

At \( r = R \),

\[ \frac{\partial c}{\partial r} \bigg|_{r=R} = \frac{\partial c}{\partial r} \bigg|_{r=R} \]  

(Equation 3.8)

\[ De \frac{\partial c}{\partial r} \bigg|_{r=R} = k_f C - \tilde{c} \]  

(Equation 3.9)

\( k_f = \text{m/s (film mass transfer coefficient)} \)

Introduce the following into the above equation.
\[ \Phi = \frac{r}{R} = \left( \frac{C - C_\infty}{C_0 - C_\infty} \right)^{1/3} \]  
\[ \text{(Equation 3.10)} \]

By eliminating \( C \), the following reduction is reached

\[ - \frac{d\Phi}{dt} = \frac{D_e}{R^2} \left( \frac{C_0}{pq_0} \frac{1}{1 + a^3} \right) \times \left\{ \frac{Bi}{1 + Bi(1/\Phi - 1)} \right\} \left\{ \Phi + a^3 \frac{1}{\Phi^2} \right\} \]  
\[ \text{(Equation 3.11)} \]

Where \( Bi = \frac{K_f \times R}{D_e} \) and \( a = \left( \frac{C_\infty}{C_0 - C_\infty} \right)^{1/3} \)

Saturation concentration = \( C_\infty = \frac{q \times M}{V} \)  
\[ \text{(Equation 3.12)} \]

This can then be solved and rearranged to obtain

\[ - \frac{d\Phi}{dt} = \frac{D_e Bi}{R^2 (1 - e)Lpq} \left\{ \frac{\Phi}{Bi + \Phi (1 - Bi)} \right\} \left\{ C_0 + \frac{q \times M \rho}{V} [\Phi^3 - 1] \right\} \]  
\[ \text{(Equation 3.13)} \]

A full derivation can be found in appendix 2

The 1\textsuperscript{st} order differential equation was solved using Matlab software (Version 7.0). The value of the fitting parameter (the effective diffusivity) was varied using the software to minimise the sum of the squares error between the experimental data and the model prediction over the first 24h (explanation given below) and the resulting effective diffusivity value reported to 2s.f. Results reported below are normalised concentration \( (C(t) / C_0) \) plotted against time.

**Experimental data requirements**

The model may be used to predict the effects of changing adsorbent parameters e.g. amount of adsorbent, effect of variation of particle size, and
external agitation rates etc. The results of the simulation work presented below offer insight into the affect of pore structure on the adsorption kinetics of both albumin and lysozyme. Before the model was used as a predictive tool, preliminary experiments were undertaken to obtain the required parameters used in the subsequent simulation work. The protein adsorption capacity value ($q^*$) was required along with material characterisation data including solid density, porosity and mean particle size. An estimate of the value of $D_e$ was obtained using the following equation:

$$D_e = \varepsilon \times \frac{D}{4}$$  \hspace{1cm} (Equation 3.14)

The value of 4 is a tortuosity factor, as used by Suzuki, (1974)

### 3.1.8 Estimation of protein effective diffusivities within nanoporous copolymer adsorbents.

The effective diffusivity is a parameter that is unique to each solute / adsorbent pair. Therefore by directly comparing the effective diffusivities for each solute within the different materials it was possible to begin to quantify the effects of the changing pore structure on the protein adsorption dynamics.

Equilibrium uptake capacity data was required for the model. Physiological concentrations (~ 50 mg/l) of the uraemic toxins were found not to be suitable given the slow uptake kinetics experienced (solute diffusion flux is proportional to the concentration gradient between the bulk solution and the particle interior) in preliminary work resulting in equilibrium not being reached for hundreds of hours. Therefore, higher initial solute concentrations were used to increase the concentration gradient between the bulk solution and the adsorbent interior. The experimental methodology used was the same as described previously. The mass of adsorbent used was such that upon achieving equilibrium, measurable concentration of residual protein was still present in solution. Each experiment was carried out following the
methodology described in section 3.1.1 following samples taken within the first 24h, one sample was subsequently taken every 24 hours until no further change in solution concentration was measured over a 24h period. All samples were measured using the HPLC method described previously. The resulting change in concentration was then converted into a normalised concentration \((C(t)/C_0)\) and plotted against time. The model predictions were overlayed on the experimental data and the best fit value of the effective diffusivity \((D_e)\) was determined.

Capacity experiments were conducted with XAD4, CW1 and CW4 with both lysozyme and albumin in single solute systems. All these experiments were carried out at a stirrer speed of \((700\text{rpm})\) to minimise effects of external mass transfer resistance. After initial experiments it was clear that protein adsorption capacities were considerably higher than those quoted in literature by Davankov et al, (2000) and Winchester et al, (2004). Subsequently, 0.5g of adsorbent was used and this resulted in an equilibrium uptake value \((q^*)\) being evaluated where the initial solute concentration, \(C_0\) was sufficiently different from the final solute concentration, \(C_{\text{inf}}\) to allow quantification of \(q^*\) with confidence.

3.1.9 Influence of stirrer speed on protein uptake kinetics and evaluation of Biot number.

In order to quantify the effect of external mixing on the convective mass transfer of solute from the bulk to the surface of the particle the magnitude of the dimensionless Biot number \((Bi)\) was evaluated using an empirical correlation from literature to obtain the magnitude of the convective mass transfer coefficient. Two experiments were undertaken, one at the low agitation speed \((84\text{rpm})\) and the other at the intermediate agitation speed \((700\text{rpm})\). 0.5g of XAD4 was contacted with 0.5l of single protein lysozyme in 0.1M Hepes buffer solution.
The following mass transfer correlation by Kulov et al. (1983) was used to evaluate the magnitude of the film mass transfer coefficient:

\[ Sh = 0.267 \times Sc^{0.25} \times Re^{0.75} \times N_{sp}^{0.25} \times \frac{d_t}{(V \times d_t)^{0.25}} \]  

(Equation 3.15)

Where:

\[ Sc \equiv \text{Schmidt number}; \quad Sc = \frac{\mu}{\rho D_v} \]  

(Equation 3.16)

The rotational Reynolds number (Re) for the agitated tank may be defined as follows:

\[ Re = \frac{\rho \times N \times D_t^2}{\mu} \]  

(Equation 3.17)

The dimensionless Sherwood number is a measure of convective mass transport to diffusive mass transport.

\[ Sh = \frac{k_f d_t}{D_v} \]  

(Equation 3.18)

The film mass transfer coefficient was evaluated by multiplying the Sherwood number with the bulk diffusivity of the solute and divided by the tank diameter.

\[ k_f = Sh \times \frac{D_v}{D_t} \]  

(Equation 3.19)

The film mass transfer coefficient can then be used to evaluate the Biot number (Bi) as follows:

\[ Bi = \frac{k_f R}{D_t} \]  

(Equation 3.20)

For Biot numbers exceeding 100, the film boundary layer effect may be considered negligible (shown in Figure 3.). Therefore if the Biot number for
each of the adsorption experiments exceeds this value, the external mass transfer resistance may be ignored. If the magnitude of the effective diffusivity is known, simulations may be undertaken to show the influence of the Bi on the uptake kinetics. Bi values of 1, 2, 5, 10, 20, 50, 100 and 1000 were used and results are discussed later.

3.1.10 Mass transfer effects on protein adsorption dynamics: modelling influence of particle size.

In order to use the model to predict the effect of changing particle size, it was first necessary to complete experiments using the same adsorbent material and protein but varying only the particle size (thus keeping the effective diffusivity of the protein unchanged). If the effective diffusivity remains constant, any change in kinetics was due to the change in particle size. This may be presented by plotting the normalised concentration data for the various particle sizes on the same plot using a dimensionless time defined as

\[ \tau = \frac{D_z t}{r_p^2} \]

By plotting the uptake data using dimensionless time the effect of particle size is essentially normalised. If the pore structure of the two particle sizes is the same, the plots should overlay.

Two different mean particle sizes (28µm and 49µm) of CW4 were studied using an initial concentration of lysozyme of 600 mg/l. The stirrer speed was set at 700 rpm for the study to minimise the effect of film mass transfer resistance whilst not causing excessive foaming.

In addition to \( D_e \) parameter estimation from the experimental data, simulations were undertaken to show the effect of particle size (0.5, 10, 20, 40, 80, 160, 320, 400 µm beads) on lysozyme uptake kinetics for CW4.
Table 3.4. Matrix of experiments undertaken to obtain data for parameter estimation.

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>Protein</th>
<th>Mean Particle Size (μm)</th>
<th>Adsorbent Mass (g)</th>
<th>Initial Conc (mg/l)</th>
<th>Stirrer Speed (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XAD4</td>
<td>HSA</td>
<td>478</td>
<td>5</td>
<td>3000</td>
<td>700</td>
</tr>
<tr>
<td>XAD4</td>
<td>HSA</td>
<td>70</td>
<td>0.5</td>
<td>400</td>
<td>700</td>
</tr>
<tr>
<td>XAD4</td>
<td>LYZ</td>
<td>165</td>
<td>0.5</td>
<td>400</td>
<td>700</td>
</tr>
<tr>
<td>XAD4</td>
<td>LYZ</td>
<td>478</td>
<td>5</td>
<td>5000</td>
<td>700</td>
</tr>
<tr>
<td>XAD4</td>
<td>LYZ</td>
<td>114</td>
<td>0.5</td>
<td>600</td>
<td>84</td>
</tr>
<tr>
<td>CW1</td>
<td>HSA</td>
<td>197</td>
<td>22</td>
<td>500</td>
<td>700</td>
</tr>
<tr>
<td>CW1</td>
<td>LYZ</td>
<td>197</td>
<td>0.5</td>
<td>200</td>
<td>700</td>
</tr>
<tr>
<td>CW4</td>
<td>HSA</td>
<td>28</td>
<td>3</td>
<td>600</td>
<td>700</td>
</tr>
<tr>
<td>CW4</td>
<td>LYZ</td>
<td>28</td>
<td>0.5</td>
<td>600</td>
<td>700</td>
</tr>
<tr>
<td>CW4</td>
<td>LYZ</td>
<td>49</td>
<td>0.5</td>
<td>600</td>
<td>700</td>
</tr>
</tbody>
</table>
3.2 Results and discussion

3.2.1 Mass transfer effects on protein adsorption dynamics: Influence of film boundary layer.

The effect of external mass transfer resistance was studied using lysozyme and the adsorbent XAD4 (Sauter mean particle size of 478 μm). The reduction in bulk solution concentration of lysozyme was used to evaluate the adsorption uptake value \( q(t) \) at any given time (using the mass balance equation 3.21 below) and this was normalised (on the basis of the protein uptake after 24h) and plotted against time. Thus data presented here as normalised uptake \( \{q(t)/q(f)\} \) represent the uptake of the protein at a given time point \( q(t) \equiv \text{mg/g} \) compared with the uptake of the protein after 24h, \( q(f) \). Due to the low starting protein concentrations mimicking physiological concentrations of MMs (see Table 3.3 for details) and the low diffusivity values of proteins such as lysozyme \( (1 \times 10^{-10} \text{ m}^2/\text{s}) \) and albumin \( (6.1 \times 10^{-11} \text{ m}^2/\text{s}) \) the measured protein (lysozyme/albumin) uptake values \( q(t) \) after 24h for all samples were quite low and nowhere near the saturation uptake values (studied in detail and reported later in section 3.2.5. The solution concentrations after 24h were all very low (<4mg/l lysozyme for XAD4, <10mg/l lysozyme for CW1) showing that near complete removal of MM's from solution took place during this time period.

\[
q(t) = \frac{(C_o - C)V}{m} 
\]

(Equation 3.21)

Figure 3.12 shows the uptake of lysozyme onto XAD4 in a 24 hour period minutes. The plot has reached a plateau due to near complete removal of lysozyme from solution. It is difficult to ascertain if any uptake rate changes are due to stirrer speed or a slightly different \( q(f) \) value. Figure 3.13 shows
the same data plotted as normalised q(t)/q(f) against time and the plot shows negligible differences. Examining the data over the initial 100 minutes as in Figure 3.14 shows little change between the 700rpm and 826rpm speeds. The 84rpm stirrer speed appears to demonstrate a slightly slower uptake over this time period.

Figure 3.12 Kinetic uptake of lysozyme expressed in terms of q(t)/time onto XAD4 (particle size range 500-710 μm); uptake data for different stirrer speeds, initial concentration of lysozyme: 100mg/l.
Figure 3.13. Kinetic uptake of lysozyme expressed in terms of $q(t)/q(f)$ onto XAD4 (particle size range 500-710 μm); uptake data for different stirrer speeds, initial concentration of lysozyme : 100mg/l.

Figure 3.14 Kinetic uptake of lysozyme expressed in terms of $q(t)/q(f)$ onto XAD4 (particle size range 500-710 μm); uptake data for different stirrer speeds over $T = 0$-100mins, initial concentration of lysozyme : 100mg/l.
Uptake of lysozyme onto CW1 (see Figure 3.15) within the first 400 minutes of the experiment, external agitation does appear to influence the uptake kinetics. Initial uptake of lysozyme at the two higher speeds seem similar, but at the slowest stirrer speed (84 rpm) there is a marked reduction in the adsorption rate of lysozyme. After 400 minutes, the two higher stirrer speeds exhibit similar values of lysozyme uptake by CW1.

However, it would be expected that the two high speeds begin to overlap suggesting the boundary layer thickness is such that its impact on overall mass transfer is negligible. This can be seen in Figure 3.16 where the data is again plotted as normalised $q(t)/q(f)$. From 400mins to 24hours the two higher stirrer speeds show similar uptake suggesting external mass transfer resistance is similar.

The normalised uptake against time plot for the lowest stirrer speed exhibits a lower gradient up to 400 minutes suggesting a reduced uptake per unit time (see Figure 3.17). As the particle size and initial concentration are constant, the additional resistance to mass transfer could be a result of an increase in boundary layer thickness. However, after 400 minutes the kinetic uptake for all stirrer speeds is comparable for the two higher speeds and slightly reduced on the slowest speed suggesting a small increase in external mass transfer resistance.
Figure 3.15. Kinetic uptake of lysozyme expressed in terms of $q(t)/\text{time}$ onto CW1 (particle size range 500-710 µm) at different stirrer speeds, \textit{initial concentration of lysozyme: 100mg/l}.

Figure 3.16. \textit{Kinetic uptake of lysozyme expressed in terms of $q(t)/q(t)$ onto CW1 (particle size range 500-710 µm); uptake data for different stirrer speeds over $T = 0$-1440 mins, \textit{initial concentration of lysozyme: 100mg/l}.}
Figure 3.17. Kinetic uptake of lysozyme expressed in terms of $q(t)/q(f)$ onto CW1 (particle size range 500-710 μm); uptake data for different stirrer speeds over $T = 0$-400 mins, initial concentration of lysozyme : 100 mg/l.

The effect on uptake of albumin onto XAD4 with varying stirrer speeds is proved difficult to quantify due to the small uptakes ($q(f) < 2$). After the 24h period, there was still around 150 mg/l of albumin present in solution, however due to the slower diffusivity of this molecule, little uptake has occurred. Figure 3.18 shows $q(t)$ against time over a 24h period. It suggests that there is little difference in uptake rates between the various stirrer speeds. The normalised data (Figure 3.19) demonstrates that in fact the 700 rpm speed appears to exhibit the fastest kinetics over a time period of 0-400 mins. Figure 3.18 is the same data as the previous figure plotted over the time period of 0-400 mins. Although the 700 rpm appears to exhibit a reduction in kinetic uptake over this period, it is likely that this is experimental error due to the small changes in bulk concentration observed.
Figure 3.18. Kinetic uptake of albumin expressed in terms of $q(t)/time$ onto XAD4 (particle size range 500-710 $\mu$m) at different stirrer speeds. Initial concentration of albumin: 200 mg/l.

Figure 3.19. Kinetic uptake of albumin expressed in terms of $q(t)/q(f)$ onto XAD4 (particle size range 500-710 $\mu$m); uptake data for different stirrer speeds over $T = 0$-1440 mins, initial concentration of albumin: 200 mg/l.
Figure 3.20. *Kinetic uptake of albumin expressed in terms of q(t)/q(f) onto XAD4 (particle size range 500-710 μm); uptake data for different stirrer speeds over T = 0-400 mins, initial concentration of albumin 200mg/l.*

Albumin uptake onto CW1 very low (~0.6mg/g after 24 hours) (See Figure 3.21). Due to such low uptake values over this period, it wasn't possible to identify if agitation rates affected uptake kinetics using this methodology. The data does suggest that CW1 may be excluding albumin from its internal pore structure.
In summary, the influence of external agitation on protein uptake kinetics appears to be negligible for XAD4 with both marker proteins. Lysozyme uptake onto CW1 showed some differences in terms of uptake rate when agitation rate was varied. The particle size of CW1 (Sauter mean 513 μm) was larger than the XAD4 (Sauter mean 478 μm) and the expected hindrance to internal mass transfer of CW1 was likely to be higher than XAD4 (due to the pore structure identified in chapter 2). We would expect therefore the influence of external mass transfer effects would be less in CW1 than XAD4. The result could be due to error associated to the small amount of uptake measured over the 24-hour period and the fact \( q(f) \) may be greatly different from \( q^* \). At the physiological concentrations used, in conjunction with the low diffusivities of the solutes, it may be difficult to identify clearly external mass transfer effects. The modelling section (presented later in this chapter) approaches the issue of external mass transfer using a modified approach and offers additional insight into the influences of boundary layer thickness in this system.
3.2.2 Mass transfer effects on protein adsorption kinetics: influence of particle size.

The particle size of the adsorbent affects the path length for solute diffusion and therefore it is an important parameter that may be varied by the engineer in designing a haemoperfusion device. Experiments were undertaken using different particle size fractions of the same material in order to identify the effect of changing the particle size on the protein adsorption kinetics over a clinically relevant time period (typically ~ 6 h with a final adsorption data point at 24 h).

Uptake $q(t)$ of lysozyme (initial concentration 100mg/l) over a time scale of 0 – 400 mins with three different particle size fractions is shown in Figure 3.22. The $q(f)$ value is not a $q^*$ since the amount of adsorbent used in these experiments (15g) is such that even if all the protein is removed there is still available area for further adsorption.

![Figure 3.22](image)

**Figure 3.22.** Kinetic uptake of lysozyme expressed in terms of $q(t)$ against time onto XAD4 (particle size values show are Sauter means, stirrer speed set to 826rpm); effects of changing particle size; $T = 0$- 1440mins, initial concentration of lysozyme 100mg/l.
Figure 3.23 shows the normalised uptake \( \frac{q(t)}{q(f)} \) where \( q(f) \) is the adsorption uptake value after 24 h, of lysozyme onto different size fractions of XAD4 (the stirrer speed used was 826 rpm to minimise external mass transfer effects). The largest particle size (Sauter mean 716 µm) showed slightly slower kinetics when compared with the 478 µm sample. However, there was a marked difference in the lysozyme adsorption kinetics (significantly faster) displayed by the crushed (Sauter mean 163 µm) sample. The crushed sample removed all the lysozyme from the solution in less than 20 minutes, whereas the larger sized fractions took in excess of 300 minutes.

Figure 3.23. Kinetic uptake of lysozyme expressed in terms of normalised \( \frac{q(t)}{q(f)} \) onto XAD4 (particle size values show are Sauter means, stirrer speed set to 826rpm); effects of changing particle size; \( T = 0\, \text{to} \, 400 \text{mins}, \) initial concentration of lysozyme : 100mg/l.

The reader is referred to chapter 2 where characterisation of the pore structure of the different size fractions of XAD4 showed no real differences. The observed differences in the lysozyme adsorption kinetics reported here are due exclusively to the increased path length for diffusion in the larger particles. This is clearly shown by rescaling the experimental adsorption data.
by defining a dimensionless time \( \tau \) that accounts for the effect of particle size on the observed adsorption kinetics. Since the time for solute diffusion within the particle is proportional to the square of the particle radius, time may be scaled by the square of the radius of the particle, thus, a dimensionless time \( \tau \) may be defined as:

\[
\sqrt{\frac{D_L}{r^2}}
\]

(Equation 3.22)

Upon rescaling of the XAD4 lysozyme adsorption data to account for the increased path length for diffusion in the larger sized particles, all the data fell on a single curve (see Figure 3.24). These results support data obtained from the nitrogen porosimetry measurements (reported in chapter 2) that indicated that XAD4 particles of various sizes had similar internal pore structures.

Figure 3.24. Kinetic uptake of lysozyme expressed in terms of normalised \( q(t)/q(f) \) onto XAD4 plotted against dimensionless time \( \tau \) (particle size values show are Sauter means, stirrer speed set to 826rpm), initial concentration of lysozyme : 100mg/l.
Lysozyme adsorption kinetics on CW1 for different particle sizes (430 µm, 513 µm and 780 µm, using a high stirrer speed, 826 rpm, to minimise external particle film diffusion effects) is shown in Figure 3.25.

![Figure 3.25](image-url)

**Figure 3.25.** Kinetic uptake of lysozyme expressed in terms of \( q(t) \) against time onto CW1 (particle size values show are Sauter means, stirrer speed set to 826rpm); effects of changing particle size; \( T = 0-1440 \text{mins} \), initial concentration of lysozyme : 100mg/l.

The largest sized fraction (Sauter mean size 780µm) showed considerably slower lysozyme uptake and after 3h was approximately 20% lower in comparison with the 430 µm sized fraction. The adsorption kinetics of lysozyme onto CW1 was noticeably slower in comparison with XAD4 (compare data in Figure 3.22 and Figure 3.25).

Both of these experiments were conducted under the same conditions and it is apparent that for similar size fraction e.g. 478µm (XAD4) and 430µm (CW1) after 3h the uptake of lysozyme onto XAD4 is greater than CW1. This is likely to be due to the more open pore structure of XAD4 as reported in chapter 2.

The normalised uptake of lysozyme onto different size fractions of CW1 is shown in Figure 3.26. Again the largest particle size exhibits a noticeable difference in uptake kinetics compared to the smaller sizes. The 513µ fraction
is slightly slower that the 430μ fraction but the differences are small due to the relatively similar sizes.

Figure 3.26. Kinetic uptake of lysozyme expressed in terms of normalised $q(t)/q(f)$ onto CW1 plotted against time (particle size values show are Sauter means, stirrer speed set to 826rpm); effects of changing particle size; $T = 0-400$ mins, initial concentration of lysozyme : 100mg/L.

Upon rescaling of the adsorption data, all the data fell on a single curve. Figure 3.27 shows the dimensionless time plot for the uptake of lysozyme on CW1 suggesting that the internal pore structure of the adsorbents is the same for all particle sizes studied.
Figure 3.27. Kinetic uptake of lysozyme expressed in terms of normalised $q(t)/q(f)$ onto CW1 plotted against dimensionless time “$\tau$” (particle size values shown are Sauter means, stirrer speed set to 826rpm, initial concentration of lysozyme: 100mg/l.

Comparison of the lysozyme adsorption data for XAD4 and CW1 suggested that the uptake kinetics for CW1 appears considerably slower in comparison with XAD4. The reduced kinetic uptake may be attributed to the tighter mesopore structure of CW1 manifesting in a reduction in the magnitude of the effective diffusivity of the solute within the adsorbent particle (discussed in detail later in section 3.2.5.)

Albumin adsorption kinetic data for different size fractions of XAD4 (Sauter mean values 400 µm and 716 µm using stirrer speed 826 rpm) demonstrated notably slower adsorption of albumin (see Figure 3.28) The effect of changing particle size was more apparent with albumin than lysozyme because albumin has a lower diffusivity in solution due to its larger molecular size.
Figure 3.28. Kinetic uptake of albumin expressed in terms of $q(t)$ against time onto XAD4 (particle size values shown are Sauter means, stirrer speed set to 826rpm); effects of changing particle size; $T = 0 - 1440\text{mins}$. $q(f)$ for $400\mu$ fraction was 3.17mg/g and 2mg/g for the $716\mu$ fraction. Initial concentration of Albumin : 200mg/l.

When the data is normalised ($q(t)/q(f)$) and plotted against dimensionless time the data begins to fall on a single curve suggesting that again internal pore structure appears unaffected by particle size (see Figure 3.29).
Figure 3.29. Kinetic uptake of albumin expressed in terms of normalised $q(t)/q(f)$ onto CW1 plotted against dimensionless time “$\tau$” (particle size values shown are Sauter means, stirrer speed set to 826rpm), initial concentration of Albumin : 200mg/l.

Albumin adsorption on two different size fractions of CW1 (513µm and 780µm) was also studied (see Figure 3.30). However, due to the low total adsorption capacity for albumin (~0.5 mg/g over 24h) on this material, any differences in uptake kinetics with varying particle size could not be distinguished experimentally (possibly due to most of the adsorption taking place on the external surface of the adsorbent bead). The adsorption data for the first 120 min suggests that initial uptake kinetics is similar for both size fractions. After approximately 200 min albumin uptake by the 513µm size fraction seemed to be complete, $q^* \sim 0.3$mg/g. Albumin adsorption on the larger size fraction (780µm) continued beyond 2h and the adsorption capacity after 24h ($q^* \sim 0.5$ mg/g). The uptake capacity values measured for albumin were over a 24h period were low making it difficult to have confidence in the observed measured differences. Capacity calculations obtained from this data show a $q(f)$ value after 24h contact of adsorbent with albumin solution of around ~0.5mg/g.
Figure 3.30. Kinetic uptake of albumin expressed in terms of $q(t)$ against time onto CW1 (particle size values show are Sauter means, stirrer speed set to 826rpm); effects of changing particle size; $T = 0 - 1440$ mins, initial concentration of albumin: 200mg/l.

3.2.3 Adsorption kinetics in a binary system.

Figure 3.31 shows adsorption data for lysozyme on CW1 for a number of experiments where different concentrations of a competing protein (albumin) was present in solution. 15g of the adsorbent was used, with a stirrer speed of 826rpm. Adsorption data for lysozyme on CW1 from a single solute in buffer overlays on that obtained from two other experiments with 200 mg/l and 1000 mg/l of albumin present in buffer solution (in addition to 100mg/l of lysozyme in solution). The data suggests that the presence of albumin had little effect on the uptake kinetics of lysozyme. This may be due to size exclusion of albumin from the internal adsorption surface of the adsorbent particle. If lysozyme diffuses more quickly into the internal pore structure of the adsorbent (due to a larger diffusivity in comparison with albumin), it may
irreversibly adsorb on the internal surface of the adsorbent reducing the possibility of competition from albumin. As the concentration of albumin in solution rises, the driving force for diffusion increases and this may counter the smaller diffusivity of albumin. This effect is not noticed for the lysozyme adsorption data on CW1 obtained with 1000 mg/l background albumin in solution.

Figure 3.31. Adsorption uptake of lysozyme onto CW1 with varying concentrations of albumin present in a binary system. Stirrer speed 826rpm, initial concentration of lysozyme : 100mg/l

XAD4 however, has demonstrated its ability to allow albumin into its pore structure. Therefore when albumin is present in a binary system with lysozyme, it is possible the adsorption kinetics and capacity for lysozyme will reduce. However the experimental data shows that the presence of albumin at 200mg/l appears to have little effect on the uptake of lysozyme similar to the effects seen with CW1. When the concentration of albumin increases to 1g/l the uptake of lysozyme actually increases (see Figure 3.32). Although this is initially counter intuitive, the literature does offer potential explanation. Kandori et al (1999) published work concerning cooperative adsorption of albumin and lysozyme onto CaHap surfaces. They presented evidence that
suggested lysozyme and albumin formed agglomerates in solution, so the free lysozyme bound to albumin both in solution and on the CaHap surface. An increase in uptake of lysozyme was reported. It is therefore hypothesised that adsorbed albumin on XAD4 may offer additional sites for removing lysozyme from solution.

To examine this hypothesis further, a sample of XAD4 was "pre-coated" with albumin. The coated XAD4 was then centrifuged to remove extra particle liquid and then added to a reactor containing 100mg/I of lysozyme in a single solute system. If the adsorbed albumin is offering additional sites for lysozyme adsorption, it may be possible the precoated resin would augment the uptake of the lysozyme. Figure 3.33 shows plots of lysozyme uptake from a single solute system, a binary system containing 1g/I of albumin and the pre-coated XAD4 sample in a single solute solution. Lysozyme uptake onto the pre-coated adsorbent is notably faster than the virgin material suggesting the lysozyme molecules are attracted to the albumin adsorbed to the surface.

![Figure 3.32. Adsorption uptake of lysozyme on XAD4 (15g) with varying background concentrations of a competing protein albumin present in solution (particle size 478 µm; stirrer speed 826rpm. Initial concentration of lysozyme: 100mg/l.](image-url)
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Figure 3.33. Adsorption of lysozyme on XAD4, lysozyme present as a single protein with varying background concentrations of a competing protein albumin present in solution, and with a pre-coat of albumin on the adsorbent (particle size 478 μm; stirrer speed 826 rpm.

This data suggest that the Vroman effect does not appear to be occurring between lysozyme and albumin because uptake of lysozyme is consistent with single solute systems in CW1 and actually increased in XAD4. If the Vroman effect was occurring in this system, a decrease in lysozyme uptake would occur due to the displacement of the lysozyme molecules by albumin.

3.2.4 Competitive adsorption using a clinically relevant peritoneal dialysis fluid.

The adsorption performance of the adsorbent CW4 (as a potential middle molecule size selective adsorbent) was compared with XAD4 using peritoneal dialysis (PD) fluid. The PD fluid had been concentrated using ultrafiltration (method described previously) so as to contain ~50 mg/l β2-M and ~40 g/l albumin. Both materials were tested under identical conditions. The CW4
material showed fast adsorption kinetics for $\beta_2$-M with the concentration of the $\beta_2$-M falling below the detectable limit (measured by ELISA) within 3 hours whilst the XAD4 sample showed comparatively slower rate of removal of $\beta_2$-M from solution within the 7 hour period over which the adsorption experiment was conducted (data presented in Figure 3.34). The particle sizes of the two materials were slightly different, CW4 mean size 48 µm whereas XAD4 mean size 114 µm meant, the effect of particle size on $\beta_2$-M removal would need to be taken into account. When the experimental data is plotted against dimensionless time. (see Figure 3.35) the plot is essentially normalised against the particle size and the true effects of the differing pore structures become apparent. In order to non dimensionalise the time effective diffusivities for lysozyme in CW4 ($6.5 \times 10^{-12} \text{m}^2\text{s}^{-1}$) and XAD4 ($3.4 \times 10^{-12} \text{m}^2\text{s}^{-1}$) were used as an estimate. These values were obtained in the modelling section of this thesis (see section 3.2.5 below)

The data suggest that the kinetic uptake of $\beta_2$-M onto both materials is very similar even though the internal pore structure of the two materials are different. Due to the noise in the data it is not possible to quantify the differences in kinetics. Therefore from this data the influence of albumin adsorption on the uptake kinetics of $\beta_2$-M appears minimal assuming the effective diffusivity values for $\beta_2$-M is similar to lysozyme for both materials. However, it is likely that the capacity for $\beta_2$-M will have reduced due to some of the sites being occupied by albumin molecules on XAD4. Therefore it is clear that the size excluding principle results in extra capacity for MM’s due to reduced loss of this area because of albumin accessibility. In addition any issues relating to the effects of albumin reduction in the patient will be reduced in CW4 over XAD4 based on the data presented in the modelling section and the chapter 2. The data also suggest there is no augmentation of $\beta_2$-M removal due to the presence of albumin on the materials surface as seen with lysozyme.
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Figure 3.34. Kinetic uptake of β2-M expressed in terms of q(t) against time onto CW4 (mean size 48 μm) and XAD4 (mean size 114 μm) from a multicomponent system. Stirrer speed : 826rpm. Initial concentration of β2-M~50 mg/l.

Figure 3.35. Uptake of β2-M on CW4 (mean size 48 μm) and XAD4 (mean size 114 μm) plotted against dimensionless time obtained by estimation of β2-
3.2.5 Estimation of lysozyme and HSA effective diffusivities in nanoporous polymeric adsorbents.

The effective diffusivities for lysozyme and human serum albumin in single solute systems were obtained for each of the adsorbent materials XAD4, CW1 and CW4.

Experimental uptake of lysozyme on XAD4 (mean size 165 µm) was used to estimate the magnitude of the effective diffusivity of lysozyme within XAD4 using the mass transfer model (developed earlier) incorporating the irreversible adsorption isotherm (see Figure 3.1). The value of the effective diffusivity $D_e$ of lysozyme obtained from best fit of the model prediction to the experimental adsorption data was $D_e = 3.1 \times 10^{-12}$ m$^2$/s. (see Figure 3.36). The effective diffusivity value was used to predict the adsorption dynamics of lysozyme within larger particles of XAD4 (mean size 476 µm). The model prediction for the initial rate of change of concentration (over a period of 12 h following adsorbent-solution contact) matches up with the adsorption data quite well (see Figure 3.37). However, at long times, the experimental data and the model prediction are less well matched. There may be a number of explanations for such an observation including denaturing of the protein leading to lower confidence in the correct measurement of the solute concentration in solution at long time intervals (this was quite a common occurrence for experiments that were run over several days).
Figure 3.36. Adsorption uptake of lysozyme on XAD4 (mean size 165 μm; stirrer speed 700 rpm, effective diffusivity used for model prediction $D_e = 3.1 \times 10^{-12}$ m$^2$/s, initial lysozyme concentration : 400mg/l, adsorbent mass : 0.5g.

Figure 3.37. Adsorption uptake of lysozyme on XAD4 (mean size 478 μm; stirrer speed 700 rpm), effective diffusivity used for model prediction $D_e = 3.4 \times 10^{-12}$ m$^2$/s, initial lysozyme concentration 5g/l, adsorbent mass : 5g.
The value of the effective diffusivity $D_e$ of albumin obtained from best fit of the model prediction to the experimental adsorption data of human serum albumin on XAD4 (mean size 165 µm) resulted in estimation of the value of $D_e = 1.6 \times 10^{-13}$ m$^2$/s. The model prediction and experimental data were in good agreement for data obtained over 3 days however, data obtained thereafter showed a sudden observed decrease in the protein solution concentration (see Figure 3.38). This reduction in protein solution concentration may arise due to protein denaturing over long periods of time. It is also possible that bacterial growth could occur in solution. The bacteria could then feed on the proteins and reduce their concentration in the bulk.

![Figure 3.38](image_url)  
*Figure 3.38. Adsorption uptake of albumin on XAD4 (mean size 165 µm; stirrer speed 700 rpm, effective diffusivity used for model prediction $D_e = 1.6 \times 10^{-13}$ m$^2$/s, initial albumin concentration : 200mg/l, adsorbent mass 0.5g*)

In a similar manner, the value of the effective diffusivity $D_e$ of albumin obtained from best fit of the model prediction to the experimental adsorption data of human serum albumin on XAD4 (mean size 70 µm) resulted in estimation of the value of $D_e = 1.9 \times 10^{-13}$ m$^2$/s. The model prediction and experimental data were in good agreement (see Figure 3.39).
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Figure 3.39. Adsorption uptake of albumin on crushed sample of XAD4 (mean size 70 µm; stirrer speed 700 rpm), effective diffusivity used for model prediction $D_e = 1.9 \times 10^{-13}$ m²/s, initial concentration of albumin : 400mg/l, mass of adsorbent : 0.5g.

The value of the effective diffusivity $D_e$ of lysozyme obtained from best fit of the model prediction to the experimental adsorption data of lysozyme on CW1 (mean size 197 µm) resulted in estimation of the value of $D_e = 3.6 \times 10^{-13}$ m²/s. The model prediction and experimental data were in good agreement for the first 24 h (see Figure 3.40). The estimated effective diffusivity for lysozyme in the CW1 is considerably lower than that on XAD4 ($D_e = 3.5 \times 10^{-13}$ m²/s ) with the same molecule. This suggests the different pore structure is affecting the rate of adsorption.
Figure 3.40. Adsorption uptake of lysozyme on CW1 (mean size 197 µm; stirrer speed 700 rpm), effective diffusivity used for model prediction $D_e = 3.6 \times 10^{-13}$ m²/s. Initial concentration of lysozyme: 400 mg/l, mass of adsorbent: 2.5g

The value of the effective diffusivity $D_e$ of albumin obtained from best fit of the model prediction to the experimental adsorption data of albumin on CW1 (mean size 197 µm) resulted in estimation of the value of $D_e = 4 \times 10^{-13}$ m²/s. The model prediction and experimental data were in good agreement (see Figure 3.41).
Figure 3.41. Adsorption uptake of albumin on CW1 (mean size 197 µm; stirrer speed 700rpm, effective diffusivity used for model prediction $D_0 = 3.5 \times 10^{-13}$ m$^2$/s, initial albumin concentration 200mg/l, mass of adsorbent 22g.

The value of the effective diffusivity $D_0$ of lysozyme obtained from best fit of the model prediction to the experimental adsorption data of albumin on CW4 (mean size 49 µm) resulted in estimation of the value of $D_0 = 6.5 \times 10^{-12}$ m$^2$/s. The model prediction and experimental data were in good agreement (see Figure 2.42).
Figure 3.42. Adsorption uptake of lysozyme on CW4 (mean size 49 µm; stirrer speed 700 rpm, effective diffusivity used for model prediction $D_e = 6.5 \times 10^{-12}$ m²/s. Initial lysozyme concentration 600mg/l, mass of adsorbent 0.5g.

The value of the effective diffusivity $D_e$ of lysozyme obtained from best fit of the model prediction to the experimental adsorption data of albumin on CW4 (mean size 28 µm) resulted in estimation of the value of $D_e = 2.3 \times 10^{-12}$ m²/s. The model prediction and experimental data were in good agreement (see Figure 3.43).
Figure 3.43. Adsorption uptake of lysozyme on CW4 (mean size 28 μm; stirrer speed 700rpm), effective diffusivity used for model prediction $D_e = 2.3 \times 10^{-12}$ m$^2$/s. Initial lysozyme concentration 600mg/l, mass of adsorbent 0.5g.

The value of the effective diffusivity $D_e$ of albumin obtained from best fit of the model prediction to the experimental adsorption data of albumin on CW4 (mean size 28 μm) resulted in estimation of the value of $D_e = 3.6 \times 10^{-14}$ m$^2$/s. The model prediction and experimental data were in good agreement (see Figure 3.44).
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Figure 3.44. Adsorption uptake of albumin on CW4 (mean size 28 μm; stirrer speed 700 rpm), effective diffusivity used for model prediction \( D_e = 3.6 \times 10^{-14} \) m²/s. Initial albumin concentration 600mg/l, mass of adsorbent 3g.

Table 3.5 summarises the results of the estimated effective diffusivities (\( D_e \)) for lysozyme and human serum albumin in each of the materials XAD4, CW1 and CW4. The estimated effective diffusivity values for albumin were an order of magnitude smaller than for lysozyme in both XAD4 and CW4. This indicated hindered diffusion of albumin within the adsorbent particles. The effective diffusivity of lysozyme in CW1 was found to be smaller than that for XAD4. This may be attributed to the tighter mesopore structure of CW1. The magnitude of the lysozyme effective diffusivity in CW4 was comparable to that of lysozyme effective diffusivity in XAD4 suggesting that although the pore cut-off values were similar for CW1 and CW4, CW4 may possess a more open pore structure.
Table 3.5. Summary of parameters used for modelling studies and evaluated effective diffusivities.

<table>
<thead>
<tr>
<th>Material</th>
<th>Solute</th>
<th>Particle Diameter (µm)</th>
<th>Mass (g)</th>
<th>Stirrer Speed (rpm)</th>
<th>Effective Diffusivity ( (D_e \times 10^{-13}) ) ( \left( m^2 s^{-1} \right) )</th>
<th>Re ( \times 10^7 )</th>
<th>Bi ( \times 10^5 )</th>
<th>q* (mg g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>XAD4</td>
<td>LYZ</td>
<td>478</td>
<td>5</td>
<td>700</td>
<td>34</td>
<td>7</td>
<td>6.9</td>
<td>419</td>
</tr>
<tr>
<td>XAD4</td>
<td>LYZ</td>
<td>165</td>
<td>0.5</td>
<td>700</td>
<td>31</td>
<td>7</td>
<td>78.5</td>
<td>410</td>
</tr>
<tr>
<td>XAD4</td>
<td>LYZ</td>
<td>114</td>
<td>0.5</td>
<td>84</td>
<td>34</td>
<td>0.7</td>
<td>1.08</td>
<td>415</td>
</tr>
<tr>
<td>XAD4</td>
<td>HSA</td>
<td>73</td>
<td>0.5</td>
<td>700</td>
<td>1.9</td>
<td>7</td>
<td>60.0</td>
<td>105</td>
</tr>
<tr>
<td>XAD4</td>
<td>HSA</td>
<td>165</td>
<td>0.5</td>
<td>700</td>
<td>1.6</td>
<td>7</td>
<td>1360</td>
<td>95</td>
</tr>
<tr>
<td>CW1</td>
<td>LYZ</td>
<td>197</td>
<td>0.5</td>
<td>700</td>
<td>3.6</td>
<td>7</td>
<td>4.7</td>
<td>59</td>
</tr>
<tr>
<td>CW1</td>
<td>HSA</td>
<td>197</td>
<td>22</td>
<td>700</td>
<td>3.5</td>
<td>7</td>
<td>8.1</td>
<td>2</td>
</tr>
<tr>
<td>CW4</td>
<td>LYZ</td>
<td>28</td>
<td>0.5</td>
<td>700</td>
<td>23</td>
<td>7</td>
<td>22</td>
<td>567</td>
</tr>
<tr>
<td>CW4</td>
<td>LYZ</td>
<td>49</td>
<td>0.5</td>
<td>700</td>
<td>65</td>
<td>7</td>
<td>2.7</td>
<td>577</td>
</tr>
<tr>
<td>CW4</td>
<td>HSA</td>
<td>28</td>
<td>3</td>
<td>700</td>
<td>0.36</td>
<td>7</td>
<td>4.61</td>
<td>51</td>
</tr>
</tbody>
</table>

Table 3.6 summarises the saturation uptake data of lysozyme and albumin onto each of the adsorbent materials. Protein surface coverage values were evaluated using a theoretical monolayer coverage per square meter based on the area footprint of each of the molecules (-9 nm\(^2\) for lysozyme and ~49 nm\(^2\) for albumin). The surface area accessible to each molecule was based on the nitrogen porosimetry data (see Chapter 2). The theoretical calculations correlated well with measured experimental protein adsorption values for both CW1 and XAD4. Measured lysozyme adsorption on CW4 suggested a higher capacity indicating greater available surface area compared with measured surface area from nitrogen porosimetry data. CW4 also displayed a significantly higher adsorption capacity for albumin compared with CW1, around half that observed for XAD4. CW4 displayed adsorption capacity for lysozyme greater than that measured for XAD4. The lack of correlation of protein surface coverage for CW4 (with the dry surface area values reported in chapter 2) suggested that perhaps the adsorbent material pore structure...
may change when exposed to the buffer solution e.g. due to polymer segment swelling.

Table 3.6. Estimation of protein surface coverage based on experimental adsorption data and available surface area measurements.

<table>
<thead>
<tr>
<th>Material</th>
<th>Particle size (µm)</th>
<th>Protein adsorption capacity (mg g⁻¹)</th>
<th>Accessible surface area for LYZ (m² g⁻¹)</th>
<th>Accessible surface area available for HSA (m² g⁻¹)</th>
<th>Protein surface coverage (mg m⁻²)</th>
<th>*Monolayer coverage (mg m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XAD4</td>
<td>165</td>
<td>LYZ = 410</td>
<td>103</td>
<td>-</td>
<td>3.9</td>
<td>2.7</td>
</tr>
<tr>
<td>XAD4</td>
<td>430</td>
<td>LYZ = 415</td>
<td>125</td>
<td>-</td>
<td>3.3</td>
<td>2.7</td>
</tr>
<tr>
<td>XAD4</td>
<td>780</td>
<td>LYZ = 419</td>
<td>128</td>
<td>-</td>
<td>3.3</td>
<td>2.7</td>
</tr>
<tr>
<td>XAD4</td>
<td>165</td>
<td>HSA = 95</td>
<td>-</td>
<td>21</td>
<td>4.5</td>
<td>2.4</td>
</tr>
<tr>
<td>CW1</td>
<td>197</td>
<td>LYZ = 59</td>
<td>24</td>
<td>-</td>
<td>2.5</td>
<td>2.7</td>
</tr>
<tr>
<td>CW1</td>
<td>197</td>
<td>HSA = 2</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>2.4</td>
</tr>
<tr>
<td>CW4</td>
<td>28</td>
<td>LYZ = 567</td>
<td>31</td>
<td>-</td>
<td>18</td>
<td>2.7</td>
</tr>
<tr>
<td>CW4</td>
<td>28</td>
<td>HSA = 51</td>
<td>-</td>
<td>1</td>
<td>51</td>
<td>2.4</td>
</tr>
<tr>
<td>CW4</td>
<td>49</td>
<td>LYZ = 577</td>
<td>37</td>
<td>-</td>
<td>15.6</td>
<td>2.7</td>
</tr>
</tbody>
</table>

* theoretical coverage based upon footprint sizes of ~9 nm² for lysozyme (LYZ) and ~49 nm² for albumin (HSA).

The observed adsorption data allows rational assessment of the suitability of each of the adsorbent materials in terms of potential use in a blood purification device. Whilst CW1 showed good size exclusion properties, effectively eliminating albumin, the measured adsorption capacity for lysozyme was considerably lower than the other materials. Therefore, a larger quantity of CW1 would be required in order to reduce the concentration of MMs from blood in comparison with the other adsorbents studied here. This implies making the device larger (to accommodate more of the adsorbent) with the
consequence that the extracorporeal blood volume is increased. Also, the increased external bead surface area per unit volume may affect the biocompatibility of the device e.g. a larger bed height resulting in a greater overall pressure drop across the column, thus shear damage to blood cells may arise (explained further in chapter 4). In order to remove the same quantity of MM’s as CW4, 10 times more CW1 adsorbent material would be required. For example to cleanse 5l of blood, typical blood concentration of $\beta_2$-M $\sim$ 35 mg/l, $\sim$3 g of CW 1 would be required whereas less than $\sim$0.5 g of CW4 would be required for the same task. The higher albumin adsorption capacity displayed by CW4 would still only result in less than 25mg of human serum albumin which would not be clinically relevant given HSA blood concentration $\sim$ 40 g/l.

The considerably higher lysozyme adsorption capacity coupled with superior adsorption kinetics displayed by CW4 makes it a suitable adsorbent material for further clinical testing in a haemoperfusion device. To illustrate the effects of available surface area the model was used to compare the three materials (CW1, CW4 and XAD4). The mass of each adsorbent was selected to give a saturation uptake of 572mg. The particle size of each material was fixed at 400 µm, solution volume 0.5l, stirrer speed set to 826rpm (negligible external mass transfer effects) and the initial concentration of lysozyme fixed at 700mg/l. The effective diffusivities for lysozyme uptake and saturation capacities were taken as the average values for each material reported in Table 3.5. Figure 3.45 shows the model prediction for 24h of adsorbent-solution contact. The results clearly showed that despite XAD4 and CW4 having slightly different effective diffusivities, the uptake profile when the saturation uptake values were normalised were virtually identical. Despite a large quantity of the adsorbent CW1, the resulting uptake was still slower due to slower intraparticle solute diffusion. This data highlights the suitability of CW4 as a potential adsorbent material for use in blood purification. The adsorbent selectively removed MMs (similar $D_e$ as XAD4) whilst removing less human serum albumin and required a lower adsorbent mass for the task.
3.2.6 Influence of external agitation on the adsorption kinetics

As described previously, the magnitude of the Biot number was evaluated for each of the adsorption experiments undertaken. Table 3.5 summarises the data for each of the experiments conducted. The experiment conducted at 84 rpm had a Biot number an order of magnitude less than the experiments conducted at the maximum stirrer rate. As all of the experiments were conducted using a Biot number greater than 100 the external mass transfer resistance was negligible. To demonstrate the effect of external agitation (magnitude of Biot number) the mass transfer model was used to conduct simulations using different magnitudes of Biot number. Adsorption uptake of albumin onto CW4 was used to study the effect of external agitation on the resulting mass transfer rate (using an initial solute concentration of 600mg/l,
adsorbent mass 0.5g). When the Biot number was greater than approximately ~ 20 the plots virtually overlay on one another (see Figure 3.46) Therefore the predominant resistance to mass transfer under these conditions was within the adsorbent bead itself and not due to film boundary layer resistance.

![Figure 3.46. Influence of Biot number on uptake kinetics of albumin on CW4 (mean size 28 µm, initial concentration of albumin : 600mg/l), effective diffusivity used for model prediction $D_e = 3.6 \times 10^{-14}$ m²/s.]

### 3.2.7 Mass transfer effects on protein adsorption dynamics: modelling the effect of particle size.

By undertaking experiments using the same adsorbent material and solute molecule but with different particles size fractions, the effective diffusivity ($D_e$) of the solute in the particle would remain the same, hence it would be possible to investigate the effect of changing the particle size on protein adsorption dynamics. By using the previously estimated value of the effective diffusivity of the protein in a given adsorbent material, it was possible to change other parameters e.g. the particle size and observe the resulting solute adsorption
dynamics simply by using the model and undertaking simulations of such an exercise. However, in order to ensure the effective diffusivity was independent of the particle size it was first necessary to obtain experimental data for differing particle size fractions, fitting the $D_e$ parameter and ensuring that its magnitude did not vary with particle size for the given solute-sorbent system. Results of nitrogen porosimetry (see Chapter 2) supported the assumption that pore structure remained unaffected as a consequence of a change in the particle size. The $D_e$ values were fitted to a number of experiments and the results are tabulated in Table 3.7. The fitted $D_e$ for lysozyme adsorption using two different particle sizes of CW4 showed similar orders of magnitude, the variability likely to be due to the fact the two size fractions were from different batches. The effective diffusivity values for XAD4, Sauter mean 165 µm and 478 µm were similar suggesting that the intraparticle diffusivity may be taken to be particle size independent.

**Table 3.7.** Fitted $D_e$ values to CW4 and XAD4 adsorption data for different particle sizes.

<table>
<thead>
<tr>
<th>Material</th>
<th>Solute</th>
<th>Mean particle diameter (µm)</th>
<th>Effective diffusivity ($D_e \times 10^{12}$) (m² s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CW4</td>
<td>LYZ</td>
<td>28</td>
<td>2.3</td>
</tr>
<tr>
<td>CW4</td>
<td>LYZ</td>
<td>49</td>
<td>6.5</td>
</tr>
<tr>
<td>XAD4</td>
<td>LYZ</td>
<td>478</td>
<td>3.4</td>
</tr>
<tr>
<td>XAD4</td>
<td>LYZ</td>
<td>165</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Figure 3.47 shows a plot of the two size fractions of CW4 with respect to adsorption of lysozyme (initial concentration of 600mg/l, stirrer speed 826rpm). The x-axis is dimensionless time effectively normalising for the effect of particle size on diffusion time. The data appears to overlap suggesting the longer adsorption times for diffusion are simply due to the longer diffusion path length. The data suggests that once an effective diffusivity value has been obtained for a given adsorbent / solute combination,
the model may be used to simulate the dynamics of adsorption with respect to changing particle size. This is of particular importance when considering the design of a haemoperfusion device where the particle size is an engineering parameter. Although it is intuitive that a small particle size will result in faster adsorption kinetics (and so highly desirable) any proposed blood purification device imposes limitation on the actual particle size range that may be used due to consideration of blood pressure drop across the packed bed which increases as the particle size decreases. High shear may damage the blood cells and needs to be avoided. Therefore, this scenario leads to an optimisation problem, where the particle size may be minimised to maximise uptake kinetics, whilst not causing damage to the cells (this problem will be discussed in more detail in chapter 4). The discussion does however highlight the need for a predictive model to help quantify the effects of changing the adsorbents physical characteristics. Figure 3.48 shows a theoretical plot of adsorption uptake of lysozyme on CW4 and the effect of changing the adsorbent particle size. Clearly, the particle size has a significant effect on the adsorption kinetics particularly over a clinically relevant time period typically between 3 – 5 h. For an initial solute concentration of 600mg/l, the particles with a diameter larger than 300 µm showed a fractional uptake of less than 0.1 (adsorption time ~ 400m), whilst an adsorbent with a diameter 40 µm, reduced the concentration of the MM marker to less than half the initial concentration in the same time period.
Figure 3.47. Modelled adsorption uptake of lysozyme on to different particle size of CW4 fractions (49μ and 28μ) plotted against dimensionless time “τ”. stirrer speed : 700rpm. Initial concentration of lysozyme : 600mg/l.

Figure 3.48. Modelled adsorption uptake of lysozyme onto CW4 at various particle sizes. stirrer speed : 700rpm, initial concentration 600mg/l.
3.3 Conclusions

In this chapter three copolymer adsorbents have been characterised in terms of their adsorption capacity and kinetics. The effect of tailoring adsorbent pore structure on human serum albumin accessibility has been studied using simple binary systems as well as adsorption studies using clinically relevant peritoneal dialysis fluid. Experiments in combination with a theoretical model have allowed assessment of the effect of pore structure, particle size and external fluid mixing conditions on the adsorption dynamics. The lysozyme adsorption capacity of CW1 and XAD4 correlate well with the measured surface area values. The lysozyme and albumin adsorption capacity values of CW4 appear greater than those suggested from material surface area characterisation.

Albumin adsorption capacity values measured with CW1 suggested that the adsorbent pore structure effectively size excluded albumin \((q^* \sim 2 \text{ mg/g})\) whilst XAD4 pore structure was accessible to albumin \((q^* \sim 100 \text{ mg/g})\). The differences in the measured effective diffusivity values for lysozyme in CW1 and XAD4 highlights the effect of a tightly regulated mesopore structure \((\text{pores} < 10\text{nm})\) on the hindered diffusion of MMs in the adsorbent particle. Lysozyme diffusion in CW4 was considerably faster than in CW1 suggesting a more open mesopore structure accessible to MMs. Human serum albumin adsorption on CW4 was noticeable. CW4 had a greater adsorption capacity for lysozyme than the XAD4 material. The adsorption data using peritoneal dialysis fluid with CW4 and XAD4 suggested similar uptake rates for \(\beta_2\)-M.

The adsorption model allowed comparison of the MM adsorption dynamics for the three adsorbent materials. When the adsorption data was normalised for capacity, the MM uptake performance of XAD4 and CW4 were comparable. As CW4 demonstrated a reduced capacity for albumin and showed a higher saturation capacity \((\text{on a mg/g basis})\) than XAD4 it seems to offer potential as an adsorbent for use in a haemoperfusion device. The high adsorption capacity should result in a reduction in the required quantity of adsorbent material required to remove clinical quantities of MM's. Therefore, the
adsorption device may be made more compact with a lower pressure drop and less potential damage to blood cells. A reduction in the external adsorbent surface area coming into contact with blood components could benefit biocompatibility of the device.
Chapter 4

Dynamic scaling down of a fixed-bed haemoperfusion column

This chapter introduces the importance of considering the design of a haemoperfusion device for both its ability to adsorb MM's and any potential damage it could have on the cells as a result of the flow regime present. Models are developed to help understand the effects of changing key design parameters on the flow conditions. The principle of dynamically scaling down a commercial column is included and its potential use as a means to study biocompatibility of adsorbents is discussed. Uptake of lysozyme onto two adsorbents is conducted on a dynamically scaled down system.

4.1 Introduction to haemoperfusion column design.

Chapter 3 highlighted the importance of pore structure (solute size exclusion and intraparticle diffusion) and mixing (external mass transfer) on the uptake of uremic toxins. However, for nanostructured polymeric adsorbents to be successfully used as a treatment, the contacting system needs to be considered. Extracorporeal blood volume has to be minimised, shear damage to the blood cells is a concern as well as bioincompatible blood-surface interactions. A fixed-bed adsorber is a column packed with a bed of adsorbent material through which the fluid flows in a downward direction, usually with the aid of a pump. Often a diffuser is placed at the inlet of the column to promote good flow distribution and minimise flow channelling effects.

The flow dynamics within the clinical column may have a pronounced effect not only on the dynamics of solute removal but also the biocompatibility of the
device. The focus of this chapter is the examination of the flow conditions found in a clinical haemoperfusion device. Subsequently, a dimensional analysis to dynamically scale-down the experimental evaluation of adsorbents using a small-scale mini-column device is made. The use of such an approach has the potential of reducing the cost of testing adsorbent materials (using limited quantities of available donor blood) without compromising the validity of the testing protocols. Studies of both adsorption performance and biocompatibility may be possible.

4.1.1 Introduction to Biocompatibility

An important development in modern medicine is the introduction of foreign materials to replace biological matter or to aid the body's natural functions. In addition to using materials in intimate contact with the body, many extracorporeal devices have been developed to assist in surgical procedures, and sustain life when natural organs begin to fail.

The term biocompatibility refers to what effect a foreign material has on the body when it comes into contact with living tissue and body fluids. Perhaps the biggest influences on this reaction involve the specific material, the amount of trauma caused during its implantation, the host, and the location of the material. Normally, the body's immune response is directed at rejecting foreign bodies. There are many ways in which biocompatibility can be measured, and these will be discussed later in this chapter.

Early biomaterials tended to focus on methods of repairing failed mechanical structure within the body such as bone fractures and displacements. Early practice included use of metal screws and plates to assist in fracture fixation. Such methods date back to the late 1800's. It wasn't until the 1940's when materials were used for applications beyond mechanical support. Coinciding with the introduction of acrylics, scientists were beginning to develop materials
specifically designed to be biocompatible, and looked at furthering their use in biological systems.

Literature suggests that it is prudent to categorise "biomaterials" depending on whether their contact with blood will be long term (e.g. a joint implant) or short term (e.g. an extracorporeal device used as a life saving measure infrequently or for a short duration). Although haemodialysis may be viewed as short term exposure (typically a haemodialysis session last around 3-5 hr) of blood to the dialysis membrane, due to its repetitive nature (typically three times per week, potentially for several years) current clinical thinking is that blood-dialyser interactions should be viewed as though the device were permanent when evaluating biocompatibility.

The ISO standard 10993 helps to categorise the type of testing required of a biomaterial depending on its end use. Although the standard doesn't offer any specific protocols for biomaterial evaluation, it does offer a structured test simulation system. In addition, the standard cites various references with details of appropriate test methodologies.

This standard is concerned with all medical devices that come into contact with blood. Although much of it is generic in nature, section 4, 'selection of tests for interactions with blood (ISO 10993-4:2002) offers useful guidelines for the design of testing protocols. Any biocompatibility testing must adhere to the guidelines set out in this standard.

Biomaterial engineering aims to replicate biological functionality whilst minimising adverse side reactions. The reader is referred to a review article by Michanetzis et al. (2002) that further elaborates on the use of ISO standard 10993 for biocompatibility evaluation of biomedical devices. The review also discusses a number of biocompatibility testing protocols and evaluates them against both cost and efficiency. The multi-centre work highlighted the need for continuity in biocompatibility testing and commented on the potential for multifactorial influences of the effects demonstrated in each of the protocols. It was highlighted that the equipment used in the testing (e.g. piping and
Chapter 4 Dynamic scaling down of a fixed-bed haemoperfusion column.

tubing used for haemodialysis) could itself be responsible for a adverse biocompatibility response.

Michanetzis et al. (2002) used assays for platelet adhesion, activation, aggregability and activation of the coagulation system. This paper highlights the lack of well defined protocols in the field of biocompatibility testing. In addition to the plethora of potential experimental methods and their associated errors, the variability of blood sources may also impact on the data gathered. It is therefore prudent to focus initial biocompatible investigations on global responses such as changes in platelet counts and damage to red blood cells (e.g. due to shear damage), and then choose more subtle tests (e.g. release of cytokines) to undertake more detailed studies of suitable adsorbent materials that have undergone preliminary screening.

4.1.2 Measuring biocompatibility

Due to the chronic nature of renal disease, long term effects of haemodialysis treatment manifest in elevated immune response of patients due to the blood routinely coming in contact with artificial surfaces (haemodialysis membrane etc). Development of polymeric adsorbents for use in haemoperfusion requires evaluation of biocompatibility reactions. One approach is to look at the two predominant effects a foreign material (e.g. the adsorbents) may have on the blood post contact. These effects may be compartmentalised as affecting the complement and coagulation systems. The two systems also have the potential to interact once activated (i.e. the complement system can be activated due to thrombus production.)

There are a number of adverse biological responses when blood comes into contact with a foreign material. Protein pathways such as activation of the complement system are established ways to identify a response between the blood and artificial surface. Although complement activation is know to act via two pathways (classical and alternative) it is the alternative pathway which is
almost exclusively involved in contact with biomaterials. Each of the pathways involves different entities in the activation process, although they do share a terminal pathway. The propagation of the system involves a cascade not unlike that of the coagulation system. Each of the propagation steps are controlled by regulatory proteins and are either inhibitory or amplifying in nature. The alternative pathway can operate without antibody participation. 6 proteins (C3, B, D, H, I & P) are involved and these are able to initiate and control the response of the pathway. The result of the initiation is the formation of activator bound C3/C5 convertase (Pangburn et al, 1986).

After initiation, the propagation of the alternative pathway is continuous and begins with a spontaneous hydrolysis of the thioster in the native C3. C3 is then cleaved by C3 convertase. This process creates C3b, which is then free to attach to either host or non-host particles.

The initiation of many of these interactions (immune response, coagulation etc) is due to interactions at the material surface / blood interface (Turbill et al, 1996). It is therefore not surprising to find that a huge amount of research has been undertaken to study the behaviour of blood at the solid-liquid interface. Of particular relevance, is the Vroman effect which is the sequential displacement of proteins present in blood serum that adsorb to the material surface. It is these sequential events taking place at the interface that have been identified as the starting blocks of the coagulation cascade, and also contribute to the body's immune response. The reader is referred to the paper by Brash et al, (1984) for more details on this. The possibility of engineering surfaces that retard such events may hold the key to improved biocompatible surfaces. Since albumin is said to be the first protein to adsorb onto the material surface, research has focused on limiting its adsorption to the surface.

The reader is referred to a paper by Mao et al. (2004) who have summarised key factors involved in the propagation of a biocompatibility response. The paper divides two major approaches to engineering biomaterials. The first deals with the development of a material that prevents or in some way
suppresses the reactions with blood (an approach was that of interfering with the coagulation cascade). The second approach was to prepare polymers that were inert to the blood interaction. The former strategy has been argued to be the most important due to the knowledge that the resulting reactions from contact with an artificial material are associated with the materials surface and not its bulk characteristics (Sun et al, 2003). The secondary advantage of taking this approach is that the surface of materials can be modified in order to improve their properties, whilst the bulk material remains unchanged giving greater flexibility in controlling mechanical as well as biological characteristics. This approach may be appropriate for the polymeric adsorbents studied here where the functionality (size exclusion due to pore size control and surface area for adsorption) lies within the bead whereas the contact of blood cellular components is on the outside of the bead surface that may be modified post adsorbent synthesis.

4.1.3 Experimental design consideration for the study of biocompatibility.

What becomes apparent after reviewing relevant literature is the need to identify not only the correct markers to identify bio-incompatible responses but also minimising any effects associated with the experimental design. The concurrent analysis of a 'control' sample is a useful way to evaluate some of these issues, but concerns have been raised concerning the sensitivity of each method. Much work in current literature (eg Sandeman et al (2005) contacts the adsorbent with a stagnant pool of blood. The results of such work are then extrapolated to predict the behaviour in flow systems. Therefore, the design and use of a dynamic system for evaluating bio-incompatibility responses could offer additional insight. In addition, technologies employed to evaluate specific parameters (such as platelet activation) may require much validation to ensure any effects observed are due to the biomaterial being investigated. Therefore by identifying a robust
scale down system, all the relevant factors can be studied in 'real' flow conditions, ensuring accurate reproducible results with the clinical system whilst using small blood volumes.

4.1.4 Characterisation of column haemodynamics

Damage to cells

Chapter 3 showed that high shear rates are effective in reducing external mass transfer resistance, thus high blood flow rate though the packed-bed may be desirable. In addition, reduced particle size was shown to have a dramatic effect on the uptake kinetics of the target molecules. However, if a haemoperfusion device contains a packed-bed of particles, as the particle sizes decreases, the pressure drop across the bed increases. To achieve high blood flow rates, the increased shear in the system may damage blood cells. Figure 4.1 depicts potential effects of shear stress on blood cell damage. It is therefore important to consider the potential damage to cells that may occur as a result of high blood flow rates in a haemoperfusion device.
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Figure 4.1. Effect of shear rate on platelets and red blood cells. Adapted from Deutch et al (2006)

The combination of shear stress experienced by blood cells and exposure time to the stress within the column requires some consideration. The retention time of blood within the column may be evaluated from the column volume, bed porosity and flow rate quite easily. However, the fact that blood is re-circulated and each parcel of blood may make multiple passes makes analysis of the effect of exposure time vs shear stress more complicated. One way would be to multiply the contact time per pass by the number of passes per treatment to obtain the overall contact time.

4.1.5 Determining a blood analogue suitable for column studies

In order to be able to experimentally analyse the hydrodynamic effects within a haemoperfusion device, the selection of a blood analogue (in terms of rheological properties) was considered. For the work carried out here, use of whole blood was not possible due to unavailability of sufficient quantities of
donor blood, handling issues due to risk of infection transmission, blood coagulation etc. Since the main objective was to investigate the dynamic scaling of the fluid dynamics from a clinical-sized column to a small-scale column, the use of a blood analogue mimicking the rheological behaviour of blood as found in clinical systems was acceptable.

Blood is a complex multicomponent two phase mixture of salts, water, proteins and cells. Blood rheology shows considerable dependence on its many components, in particular, red cell and protein concentrations. The flow characteristics of blood at high shear rates (typically greater than $20 \, \text{s}^{-1}$ such as in arterial flow) can be considered as Newtonian. However at lower shear rates (less than $20 \, \text{s}^{-1}$), blood exhibits non-Newtonian characteristics.

Blood exhibits shear thinning behaviour mainly due to the presence of red blood cells. These are bi-concaved discs approximately $8 \, \mu\text{m}$ in diameter but are capable of severe deformation. Typically, these cells make up about $40\%$ of the total blood volume. Variation in red cell count (often referred to as haematocrit) has a pronounced effect on the blood viscosity. When blood is at rest, the red cells tend to aggregate together to form a structure resembling rouleaux. At low shear rates, the aggregates begin to break-up. During this phase, the viscoelasticity of blood is determined primarily by the aggregation tendency of the red cells only. The forces that are required to break-up the rouleaux bring about orientation changes resulting in elastic energy being stored within the blood. Any additional flow requires the sliding of the cellular structure, the energy from which is dissipated as viscous friction. As the shear rate is increased further, the cells de-aggregate and the resulting forces act to orientate the cells in the direction of flow. At higher rates of shear the cells deform and will form layers between plasma.

Platelets (typical size $2.5 \, \mu\text{m}$) are smaller than the red blood cells and have little effect on blood rheology, however they are important in clot formation. The fluid dynamics in the column may influence mass transfer of platelets to the surface of the adsorbent which subsequently influence the coagulation
cascade. Therefore, the fluid dynamics within the column could have a role to play with the resulting biocompatibility response.

It is also well documented in literature (Chien et al. (1977)) that changes in plasma protein concentrations have major effects on blood rheology. The protein content also influences the behaviour of the red cells (aggregation) and hence also influence its rheology via this mechanism. The three main serum proteins that affect blood viscosity are human serum albumin, fibrinogen and globulins. Large blood protein e.g. globulins appear to have more of an effect than both albumin and fibrinogen weight for weight, although the effects is disproportionately high at low shear rates (less than 30 s⁻¹).

From a hydrodynamic perspective, the key characteristics of blood are its apparent viscosity and the sedimentation rates of the red cells. Once blood is removed from the vascular system it quickly coagulates unless it is chemically altered by the addition of an anticoagulant.

Blood plasma exhibits a viscosity of around 18 mPa.s and the final characteristics of whole blood are defined by a number of other factors including cell and protein concentration. Shear rate also effects the viscosity of blood as it exhibits shear thinning characteristics at low shear rates. Figure 4.2 shows a plot of apparent viscosity against shear rate for whole blood (hematocrit 35-45%) The plot demonstrates that blood essentially behaves as a Newtonian fluid at shear rates above 20 s⁻¹ with an apparent viscosity of 8mPa.s. Therefore at shear rates likely to be found in a haemoperfusion device, blood flow behaviour may be described as Newtonian. It follows that any blood analogue designed for study of flow conditions in a haemoperfusion device may be Newtonian with a viscosity of 8mPa.s. The blood analogue must not demonstrate any hysteresis else the changes in the viscosity will change the behaviour exhibited in the column during experiments where the blood analogue volume is re-circulated through the adsorber bed.
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Figure 4.2. **Effects of shear rate on viscosity of whole blood.** Adapted from O'Callaghan (2005)

After consulting literature, it was found that blood analogues may be produced by mixing glycol with water at various concentrations. In order to obtain an analogue with a viscosity of 8mPa.s solutions of different concentrations of glycol in ultrapure water (10 – 90 wt.% ) were prepared. In addition to the glycol mixtures, a sample of the Hepes buffer solution (not containing any protein) was also made and analysed to identify its rheology.

Once the relevant concentration of glycol/water giving a viscosity of 8mPa.s was identified, additional testing was carried out to ensure its properties remain constant during studies of flow through a packed bed. If the solution displayed a hysteresis curve when the shear rate was progressively increased to a maximum and then decreased along the same range of values then, the solution would not be appropriate for the study. In addition, in order to identify if there was any plastic behaviour of the blood analogue, a step change analysis was undertaken. Thus, the shear rate was increased linearly to a maximum value (1400s⁻¹) and then suddenly reduced to zero following which immediately the linear increase in shear rate was repeated. If the gradient
(shear stress \( \tau \) versus shear rate \( \gamma \)) were found to remain unchanged following each of the step changes then the solution was found not to exhibit plastic behaviour. The range of shear rates used depended on the expected viscosity but values used were in the range of 0 - 2500 s\(^{-1}\).

**Effects of temperature**

The effect of temperature on fluid rheology was studied by measuring the viscosity of each of the solutions at three temperatures (18°C (room temperature), 25°C, and 37°C). Any fluid dynamics work subsequently carried out was undertaken at a fixed temperature.

**Hepes buffer evaluation**

In order to understand the flow characteristics of Hepes buffer solution through a packed column, the viscosity of the solution was measured.

**Measurement of viscosity**

In order to characterise the blood analogue a concentric cylinder viscometer was used (A Haake, VT 550). A bob and cup arrangement was used where the cup was stationary and the bob was rotated. A force transducer measured the torque required to maintain a constant rotational speed of the bob. Resistance to the rotation of the bob was caused by the fluid. The instrument was capable of varying shear rate and the resulting shear stress was measured. Shear stress was plotted against shear rate, the gradient of the resulting trend line (if the data showed a linear correlation) provided a measure of the viscosity of the fluid. A linear relationship indicated that the sample was Newtonian in nature. A non-linear profile would suggest that the fluid flow characteristics were non-Newtonian with the gradient at a particular shear rate corresponding to the apparent viscosity at the measured conditions. A number of different sensor (bob) and cup arrangements were available and the expected properties of the sample (range of viscosities)
dictated the selection of the appropriate sensor. For the study of the Newtonian glycol-water blood analogues, the "NV" sensor was selected (sensor details provided in Table 4.1.)

**Table 4.1. Properties of NV sensor.**

<table>
<thead>
<tr>
<th>Geometry</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity (mPas)</td>
<td>2-2000</td>
</tr>
<tr>
<td>Recommended Viscosity Range (mPas)</td>
<td>5-300</td>
</tr>
<tr>
<td>Shear Rate (s(^{-1}))</td>
<td>27-27,000</td>
</tr>
<tr>
<td>Sample Volume (cm(^3))</td>
<td>9</td>
</tr>
<tr>
<td>Gap (mm)</td>
<td>0.35</td>
</tr>
<tr>
<td>Radius; Length (mm)</td>
<td>20.1/60</td>
</tr>
<tr>
<td>Reproducibility (±%)</td>
<td>3</td>
</tr>
</tbody>
</table>

A number of assumptions are inherent in the mathematical relationships describing flow within the instrument:

- Steady state flow
- Laminar flow
- No end effects
- Fluid is incompressible.
- Constant temperature

In order to obtain the viscosity of the various concentrations of glycol in water and a hepes buffer sample, the NV geometry was utilised and a shear rate range of between 0 - 2500 s\(^{-1}\) was used.
4.1.6 Scale-down of haemoperfusion column design: scale down.

A scaled-down system has the potential benefit that it may be used to undertake \textit{in vitro} biocompatibility studies and/or for studying adsorption performance of MMs during fluid flow through a packed bed. The advantage of scaling down is the reduction in the required volume of donor blood whilst still exposing both the blood and adsorbent to clinically relevant flow conditions. The success of any small scale experiments relies on the dynamic scaling down of the system. Therefore, a thorough investigation into the validity of the proposed scale down system should be undertaken before any biocompatibility / adsorption studies commence.

Before discussion of how the scale down parameters were obtained, a discussion of the key hydrodynamic characteristics of flow through a packed bed will be presented.

A packed-bed contains both the packing material and voids created in the area between the packing material. The bed void fraction is the term used to describe the relative amount of voids a particular packing material creates within a particular packed bed. The volume fraction of voids is also known as the bed porosity ($\varepsilon$). $S_0$ represents the specific surface area per unit volume of the solid particles in the bed. The total surface area per unit volume of packed bed is given by the following equation:

$$\text{Total area} = (1 - \varepsilon)S_0 \quad \text{(Equation 4.1)}$$

The particles used as packing material were assumed to be spherical (refer to Chapter 2 showing optical photographs of spherical adsorbent beads), therefore $S_0$ is given by:
Chapter 4 Dynamic scaling down of a fixed-bed haemoperfusion column.

\[
\frac{4\pi (d_p / 2)^2}{4/3\pi (d_p / 2)^3} = \frac{\pi d_p^2}{4/6\pi (d_p / 6)^3} \frac{\pi d_p^2}{\pi d_p^2} = \frac{6}{d_p} \quad \text{(Equation 4.2)}
\]

\[
s_0 = \frac{6}{d_p} \quad \text{(Equation 4.3)}
\]

In order to develop a Reynolds number for flow through a packed bed, it is necessary to obtain an equivalent diameter available for flow in the packed bed. For random packing, this can be defined as follows:

\[
d_e = \frac{4\varepsilon}{(1 - \varepsilon)s_0} \quad \text{(Equation 4.4)}
\]

In addition to the above parameter, the fluid velocity in the bed was also required. This can be obtained by using the volumetric flow rate and dividing it by the empty cross sectional area of the bed. This is known as the superficial velocity \(u\). The mean velocity within the interstices of the bed can be calculated by the following equation.

\[
u_b = \frac{u}{\varepsilon \times A} \quad \text{(Equation 4.5)}
\]

From this, a Reynolds number for a fixed bed can be derived.

\[
Re_b = \frac{\rho u_b d_e}{\mu} \quad \text{(Equation 4.6)}
\]

For \(Re_b \leq 2\), flow is said to be laminar.

Resistance to fluid flowing through a packed bed, e.g. due to frictional drag results in pressure drop along the length of the bed. The resistance to flow is related to the properties of the packed-bed, e.g. particle diameter, bed porosity, bed-length etc.
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Force balance across a pipe

For a capillary, radius \( r_i \) and length \( L \), a force balance yields:

\[
P_1 - P_2 \times (\pi r_i^2) = \tau_{\text{wall}} \times (2\pi r_i L) \quad \text{(Equation 4.7)}
\]

Therefore:

\[
\tau_{\text{wall}} = \frac{|\Delta P| r_i}{2L} \quad \text{(Equation 4.8)}
\]

Since,

\[
\mu = \frac{\tau_{\text{wall}}}{\gamma_{\text{wall}}} \quad \text{(Equation 4.9)}
\]

Therefore:

\[
\gamma_{\text{wall}} = \frac{\tau_{\text{wall}}}{\mu} = \frac{|\Delta P| r_i}{2\mu L} \quad \text{(Equation 4.10)}
\]

The equivalent capillary model of a porous medium

The bed is considered to be composed of single-sized spheres of diameter \( d_p \), and the bed packing is taken to be random, rather than ordered. The specific surface of the particle is therefore defined as:

\[
S_o = \frac{\pi d_p^2}{6} = \frac{6}{d_p} \quad \text{(Equation 4.11)}
\]
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An element of fluid takes a convoluted path as it moves through a packed bed (hence we introduce the concept of an equivalent capillary). The equivalent diameter of such a capillary will be based on the "hydraulic radius" concept. If we assume that the characteristics of the packed bed are such that the wetted perimeter and the fraction of the cross section of the bed that is open to flow are uniform along the length of the bed (in a statistical sense) then it follows that we may write the hydraulic radius ($r_h$) as:

$$ r_h = \frac{\text{cross section open to flow}}{\text{wetted perimeter}} \quad \text{(Equation 4.12)} $$

Thus, the hydraulic radius takes the form:

$$ r_h = \frac{ed_p}{6(1 - \varepsilon)} \quad \text{(Equation 4.13)} $$

Detailed derivations may be found in fluid mechanics text books such as Middleman (1998).

We define the friction factor ($f$) for flow in a packed bed as:

$$ f = \frac{\tau}{\frac{1}{2} \rho u^2} \quad \text{(Equation 4.14)} $$

Taking the relation of shear stress ($\tau$) for a capillary and using the definition of the hydraulic radius (derived above), the friction factor, $f$ may be defined as:

$$ f = \frac{2 \left( \frac{\Delta P}{L} \right) r_h}{\rho u^2} = \frac{\left( \frac{\Delta P}{L} \right) \varepsilon^2 d_p}{3 \rho u_o^2 (1 - \varepsilon)} \quad \text{(Equation 4.15)} $$

Where:

$$ u_o = u \varepsilon \quad \text{(Equation 4.16)} $$
and $u$ is the average velocity (which we don't know) whereas the superficial velocity ($u_0 = Q/A_c$) we can measure. $Q$ is the volumetric flow rate and $A_c$ is the cross-sectional area of the empty column.

Equation 15 is simply a definition of $f$ in terms of bed characteristics. To relate pressure drop in the packed bed to flow rate we assume that in laminar flow the friction factor for flow through a packed bed will be similar to that given by the Hagen-Poiseuille law (we simply replace the pipe diameter with $4r_h$). The Hagen-Poiseuille law may be written in the following way:

$$f = \frac{16}{Re} \quad \text{(Equation 4.17)}$$

For a packed bed, the modified Reynolds number is:

$$Re_b = \frac{u(4r_h)p}{\mu} \quad \text{(Equation 4.18)}$$

Using the Hagen-Poiseuille relationship (above), we get the following correlation:

$$f = \frac{16}{\varepsilon Re_b} \quad \text{(Equation 4.19)}$$

The above equation doesn't fit available data for slow (laminar) flow of viscous fluids through packed beds however a simple empirical correction of the numerical coefficient results in the model fitting the data extremely well. This leads to the Carman-Kozeny equation (Carman 1938) which is often used to correlate pressure drop in packed beds for low flows, $Re_b < 2$.

$$u = \left( \frac{\Delta P_f}{L} \right) \left( \frac{1}{K_r \mu} \right) \left[ \frac{\varepsilon^3}{(1 - \varepsilon)^2 S_0^2} \right]$$

\( \text{(Equation 4.20)} \)
Which can be rearranged to yield:

$$\Delta P_f = (K_c, \mu L) \left[ \frac{(1 - \epsilon)^2 S_0^2}{\epsilon^2} \right] u$$  \hspace{1cm} \text{(Equation 4.21)}$$

As the adsorbent particles within the haemoperfusion column may be assumed to be spherical, the value of $K_c = 5$ therefore, the equation may be written as:

$$\Delta P_f = (180 \mu L) \left[ \frac{(1 - \epsilon)^2}{\epsilon^2 d_p^2} \right] u$$  \hspace{1cm} \text{(Equation 4.22)}$$

$K_c$ is a constant, the magnitude of which depends on the particle shape, the bed porosity and particle size range. This value is usually in the region of 3.5 – 5.5, but often a value of 5 is used when the particles are spherical.

Calculation of wall shear

$$\gamma_w = 8 \frac{u}{d_e}$$  \hspace{1cm} \text{(Equation 4.23)}$$

Where :

$$d_e = \frac{4 \epsilon}{(1 - \epsilon) \frac{d_p}{6}}$$  \hspace{1cm} \text{(Equation 4.24)}$$

and

$$u = \frac{Q}{\epsilon \cdot A_e}$$  \hspace{1cm} \text{(Equation 4.25)}$$
4.1.7 Experimental design.

Once a column design is obtained, effects of packing with different material, size fractions and varying bed porosities can be evaluated in terms of wall shear, Reynolds number or pressure drop, with a blood substitute fluid. In order to validate the scale down column design initial studies using water will be undertaken. For scale-down of the clinical system the following factors were considered:

- Wall effects (ratio of column diameter and particle size)
- Magnitude of Re
- Magnitude of Wall shear rate
- Contact area (adsorbent to fluid ratio)

For scaling down the column, the average particle size should be decreased by the same factor as the reduction in the column diameter. The flow rate through the column needs to be changed to keep the flow regime similar. This may be done by keeping the magnitude of the Reynolds number the same, or attempting to keep the wall shear rate similar. Geometric scaling of the aspect ratio (column diameter : particle size) results in an increase in the external surface area to blood volume in column due to the reduced particle size. The scaled down system then offers a worse case scenario in terms of biocompatibility of blood surface response due to the higher surface area contact with blood. However, due to the porous structure of the adsorbents the external surface area is negligible when compared with the internal structure therefore the effects of scale down on adsorption capacity is likely to be low. The volume of blood can be scaled in accordance with the reduction of column volume and the flow rate will dictate the time required to ensure the same number of passes each unit of blood through the column.
4.1.8 Scale down rig design.

A commercial haemoperfusion column (Medasorb device by Renaltech) was not available for flow characterisation experiments. Therefore, recourse to literature provided information on typical particle sizes, bed lengths (25cm), column diameters (5cm) and typical range of flow rates used in clinical haemoperfusion columns. This information was used to calculate typical Reynolds numbers found in clinical haemoperfusion columns. An adjustable (bed height) 26mm (ID) x 500mm (length) column was purchased from Sigma (hence forth called pilot-scale system). The flow dynamics found in a clinical sized column was replicated in the pilot scale system. Subsequently, a scaled-down mini-column (0.5cm X 4cm) was used to validate the scale down methodology. See Figure 4.4 for schematic of this approach.

The base of the columns contain a frit to prevent loss of particles. After some validation work using mechanical pressure gages, the final rig was designed using an electronic pressure transducer purchased from RS capable of pressure measurements from 0 – 1 bar. This was linked to a digital display unit purchased from Digitron, UK. The bottom of the column was open to atmosphere and the pressure recorded is therefore gauge pressure. A peristaltic pump capable of supplying flow rates between 50ml/min and 400ml/min was used. Initial studies highlighted the pulsing created from the peristaltic pump resulted in difficulties in accurately recording the pressure drop across the packed-bed for a given flow rate. A length of large bore tubing was added to the outlet of the pump which reduced these effects. In addition, a snubber was attached to the pressure transducer, which further reduced the pulsing effects (Figure 4.3 shows a schematic diagram of the rig). The pilot-scale column was used to study the effect of flow conditions on pressure drop and ascertain whether reduction in bed height resulted in increasing wall effects. Results from the experimental set-up were then used in conjunction with the equations above to identify flow conditions for the scaled-down mini-column. Once the Reynolds number or shear rate for the
commercial system are obtained at a given flow rate (range 200 – 450ml/min), by knowing the particle size and bed porosity for the other systems in addition to the column geometry, an estimate of the required flow rate can easily be obtained using the models described above. Table 4.2 shows an example of how scale down of the commercial column is possible based (in this case) on wall shear rate. The models were inputted into Excel and the various parameters of each system added. Then, the flow rate of the pilot study and micro columns was adjusted until the resulting wall shear rate was approximately equal to the commercial column.

**Figure 4.3** Schematic of Rig design. (1) fluid reservoir (2) peristaltic pump (3) large bore tubing to help reduce effects of pulsing (4) Pressure transducer (5) Diffuser to dissipate flow evenly over bed (6) frit at bottom of column to prevent loss of adsorbent.
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Calculate wall shear based on values calculated on commercial column.

Figure 4.4. Schematic of scale down strategy from commercial column to mini column.
Table 4.2. Properties of the 3 systems at minimum and maximum flow rates.

<table>
<thead>
<tr>
<th></th>
<th>CC</th>
<th>PS</th>
<th>MC</th>
<th></th>
<th>CC</th>
<th>PS</th>
<th>MC</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td>value</td>
<td>value</td>
<td>value</td>
</tr>
<tr>
<td>Flow rate</td>
<td>200ml/m</td>
<td>57ml/m</td>
<td>0.19ml/m</td>
<td></td>
<td>450ml/m</td>
<td>134ml/m</td>
<td>0.43ml/m</td>
</tr>
<tr>
<td>Porosity of bed</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td></td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Diameter of column (D)</td>
<td>0.05m</td>
<td>0.026m</td>
<td>0.005m</td>
<td></td>
<td>0.05m</td>
<td>0.026m</td>
<td>0.005m</td>
</tr>
<tr>
<td>Area of column</td>
<td>0.00196m²</td>
<td>0.00053m²</td>
<td>1.96E-05m²</td>
<td></td>
<td>0.00196m²</td>
<td>0.00053m²</td>
<td>1.96E-05m²</td>
</tr>
<tr>
<td>Height of column</td>
<td>0.25m</td>
<td>0.25m</td>
<td>0.04m</td>
<td></td>
<td>0.25m</td>
<td>0.25m</td>
<td>0.04m</td>
</tr>
<tr>
<td>Vol. of column</td>
<td>0.00049 m³</td>
<td>0.00013 m³</td>
<td>7.85E-07 m³</td>
<td></td>
<td>0.00049 m³</td>
<td>0.00013 m³</td>
<td>7.85E-07 m³</td>
</tr>
<tr>
<td>Vol. of polymer</td>
<td>0.00029 m³</td>
<td>8E-05 m³</td>
<td>4.71E-07 m³</td>
<td></td>
<td>0.00029 m³</td>
<td>8E-05 m³</td>
<td>4.71E-07 m³</td>
</tr>
<tr>
<td>Vol. of blood in column</td>
<td>0.0002 m³</td>
<td>5.3E-05 m³</td>
<td>3.14E-07 m³</td>
<td></td>
<td>0.0002 m³</td>
<td>5.3E-05 m³</td>
<td>3.14E-07 m³</td>
</tr>
<tr>
<td>Particle diameter, dp</td>
<td>0.0005m</td>
<td>0.00056m</td>
<td>0.000048m</td>
<td></td>
<td>0.0005m</td>
<td>0.00056m</td>
<td>0.000048m</td>
</tr>
<tr>
<td>Superficial velocity (u')</td>
<td>0.00424m/s</td>
<td>0.00463m/s</td>
<td>0.000403m/s</td>
<td></td>
<td>0.00955m/s</td>
<td>0.01052m/s</td>
<td>0.000919m/s</td>
</tr>
<tr>
<td>Channel diameter (dc)</td>
<td>0.00022m</td>
<td>0.00024m</td>
<td>2.13E-05m</td>
<td></td>
<td>0.00022m</td>
<td>0.00024m</td>
<td>2.13E-05m</td>
</tr>
<tr>
<td>Wall shear rate</td>
<td>152.789s⁻¹</td>
<td>151.535 s⁻¹</td>
<td>151.1972 s⁻¹</td>
<td></td>
<td>343.775 s⁻¹</td>
<td>344.422 s⁻¹</td>
<td>344.5705 s⁻¹</td>
</tr>
<tr>
<td>Apparent viscosity</td>
<td>0.008 Pa.s</td>
<td>0.008 Pa.s</td>
<td>0.001 Pa.s</td>
<td></td>
<td>0.008 Pa.s</td>
<td>0.008 Pa.s</td>
<td>0.001 Pa.s</td>
</tr>
<tr>
<td>Density of blood</td>
<td>1107kg/m³</td>
<td>1107 kg/m³</td>
<td>1107 kg/m³</td>
<td></td>
<td>1107kg/m³</td>
<td>1107 kg/m³</td>
<td>1107 kg/m³</td>
</tr>
<tr>
<td>Reynolds No. (Re')</td>
<td>0.13051</td>
<td>0.14581</td>
<td>0.009522</td>
<td></td>
<td>0.29364</td>
<td>0.35597</td>
<td>0.0217</td>
</tr>
<tr>
<td>Aspect Ratio (D/dp)</td>
<td>100</td>
<td>49.0566</td>
<td>104.1667</td>
<td></td>
<td>100</td>
<td>47.2727</td>
<td>104.1667</td>
</tr>
<tr>
<td>Blood residence time</td>
<td>58.9049s</td>
<td>55.8873s</td>
<td>99.20819s</td>
<td></td>
<td>26.1799s</td>
<td>23.7552s</td>
<td>43.53246s</td>
</tr>
<tr>
<td>No. of beads in column</td>
<td>45000000</td>
<td>1021649</td>
<td>8138021</td>
<td></td>
<td>45000000</td>
<td>914200</td>
<td>8138021</td>
</tr>
<tr>
<td>Area of beads in column</td>
<td>3.53429m²</td>
<td>0.90158 m²</td>
<td>0.058905 m²</td>
<td></td>
<td>3.53429 m²</td>
<td>0.86879 m²</td>
<td>0.058905 m²</td>
</tr>
<tr>
<td>Surface area/volume</td>
<td>18000m²/m³</td>
<td>16981.1 m²/m³</td>
<td>187500 m²/m³</td>
<td></td>
<td>18000m²/m³</td>
<td>16363.6 m²/m³</td>
<td>187500 m²/m³</td>
</tr>
</tbody>
</table>
Chapter 4 Dynamic scaling down of a fixed-bed haemoperfusion column.

The bed packing material used for the large column experiments was a styrene-DVB copolymer MN200 (provided courtesy of Purolite International (UK) Limited). The polymeric material was available in a large quantity (for use in the pilot scale system). The adsorbent pore structure is unsuitable for use in the removal of middle molecules from blood, however, the material possesses similar packing properties to the materials characterised in this work. The material was used “as received”. It was characterised using the methods developed in chapter 2 and a summary of the important properties is presented in Table 4.3

Table 4.3 Particle size data for materials used in packed-column experiments

<table>
<thead>
<tr>
<th>Material</th>
<th>Sauter mean (µm)</th>
<th>Medium (µm)</th>
<th>Mode (µm)</th>
<th>Span</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN200</td>
<td>550</td>
<td>575</td>
<td>601</td>
<td>0.5</td>
</tr>
<tr>
<td>XAD4</td>
<td>130</td>
<td>231</td>
<td>250</td>
<td>0.6</td>
</tr>
<tr>
<td>CW4</td>
<td>49</td>
<td>66</td>
<td>72</td>
<td>1.4</td>
</tr>
</tbody>
</table>

The pilot-scale system was first characterised in terms of pressure drop versus flow rate using the 8mPa.s blood analogue to acquire the relevant flow characteristic data within the system. The particle size was reduced by the same factor as the reduction in column diameter as close as possible (x5). Table 4.3 highlights the larger span of the CW4 material compared to the other materials. Considerable effort was spent to obtain a tight particle size distribution but for the smaller sized particles (~50 µm) the larger span was the best that could be achieved using the sieve fractions available. The CW4 material was used for the initial studies into the validity of the scale down procedure.

Pre Testing validation

The bed was packed by free settling of a slurry. Upon commencement of flow the bed height reduced resulting in compression of the bed and changes in
Chapter 4 Dynamic scaling down of a fixed-bed haemoperfusion column.

the bed porosity. In order to ensure reproducibility of results taken for the pilot-scale system, measurements were taken once the bed had settled. A simple experimental procedure was established by undertaking experiments to evaluate the time taken for a freshly packed bed to settle. Figure 4.5 shows data for a 44cm bed height with a fluid (viscosity 8mPa.s) pumped through the bed at a flow rate of 400ml/min. The data suggests that 30 min was required for the bed to settle under the conditions of the experiment. For all column flow experiments, 30 min time was allowed to elapse before experimental data on pressure drop at a particular flow rate was recorded.

![Figure 4.5](image)

**Figure 4.5** Pressure drop across a 30cm bed in the pilot scale column with respect to time.

**Calculation of bed porosity (ε)**

In order to obtain the bed porosity (ε), the following procedure was followed. A bed of adsorbent particles was packed into the pilot scale column as a slurry. The 8mPa.s fluid was pumped through the bed using a peristaltic pump and the system was allowed to reach steady state by leaving it running for 30 mins. After this time, the flow was stopped and the valve at the base of the column was shut. The valve was opened to allow liquid to drain until the top
of the liquid was level with the top of the adsorbent bed, at this point the valve was closed. In order to collect the liquid between the particles, the bed contents were centrifuged and the mass of the liquid collected was converted into volume to give the porosity ($\varepsilon$) of the packed bed using the following equation:

$$\varepsilon = \frac{V_{\text{interstitial}}}{V_{\text{bed}}}$$  \hspace{1cm} (Equation 4.26)

The values of $\varepsilon$ were obtained for bed heights of 10cm using the pilot-scale column. The measurements were repeated 3 times for each packing material and an average value of $\varepsilon$ was obtained. The method was repeated using the small scale mini-column with materials XAD4 and CW4.

**Pressure drop measurements**

The pressure drop across the entire system comprises of frictional drag in the pipe work and fittings as well as across the bed itself. To minimise the pressure drop of the fittings etc., the pipe length between the pump discharge and the entrance to the column was kept as short as possible. The pressure drop associated with the adapter connecting the feed pipe to the column via the fluid distributor had to be modified resulting in virtually no detectable pressure drop across the empty column (filled with fluid) when the system was operated at clinically relevant flow rates (250-400 ml/min).

**4.1.9 Validation of wall effects**

Fluid flow through a packed bed follows the path of least resistance, e.g. in channels and along the walls of the column where the local porosity is high. This may be minimised by using a high column diameter to particle size aspect ratio (usually around 50) so for the pilot scale column (i.d. 25mm), the particle size would need to be around 500μm. For a given column diameter, accurate measurement of flow characteristics through the bed i.e. pressure drop versus flow rate data requires high bed height to column diameter ratios
(greater than ~10). In this study, the pressure transducer provided a linear response between 0-1 bar(g) with accuracy of ±0.01 bar(g).

To evaluate when (with the smallest bed height) wall effects became dominant in the pilot-scale system (\( \Delta P/L \)) vs Q (flow rate) data was obtained at different bed heights. Deviation of the plotted data from the universal trend line suggests domination of wall effects as the bed height was reduced. The experiments were conducted at clinically relevant flow rates (150 – 450 ml/m). The column was carefully filled with adsorbent material (MN200) and pressure drop measurements taken as described previously. Once measurements over the desired flow rates were completed, the bed was emptied and additional material was added to increase the bed height and the method repeated.

For the small scale mini-column system, experiments of pressure drop across the column were conducted and compared with the pilot-scale column system. To maintain dynamic similarity, reduced flow rates were required and these were provided by employing a Masterflex type peristaltic pump. As the measured pressure drops were also considerably smaller (around 0.01 Bar) a U-tube manometer was used to accurately obtain the pressure drop values. This method was employed using the small scale mini-column system at two packed bed heights (2cm & 4cm). The result were plotted as friction factor (f) versus modified Reynolds number (Re_b). In the case of effective dynamic scaling, the data for the pilot-scale and mini-column systems should lie on one universal curve.

4.1.10 Modelling of adsorber column parameters

Once an adsorbent material has been selected on the basis of middle molecule adsorption capacity and size exclusion characteristics, there are still
a number of parameters that may be changed in order to optimise the haemoperfusion treatment. By changing the size & quantity of the adsorbent, and the packing density the resulting flow conditions vary greatly. In order to study the effect of these parameters on the adsorption performance of the device and to ensure that any proposed device is unlikely to cause shear damage to the blood cells, analysis of the effect of these parameters was undertaken.

4.1.11 Effects of changing bed porosity

The porosity of the bed affects shear within the packed-bed of a commercially sized column. The porosity of the bed is a parameter that can be changed for a given particle size by adjusting the span of the particle size distribution. If monosized particles are manufactured (e.g. using the membrane emulsification system) the resulting bed porosity may be much higher than for a bed packed with particles having the same mean size but a wider size distribution. The increase in porosity may result in lower shear facilitating the use of higher flow rates and/or a reduction in particle size for the same wall shear. In Chapter 2, it was shown that using the membrane emulsification technique polymeric adsorbents with a tight particle size distribution may be synthesised with no apparent change in internal pore structure. This would be of considerable benefit for use in a packed-bed haemoperfusion column.

In order to identify the effect of changing bed porosity for a given particle size some simulations were carried out. The flow rate was varied within the clinical range (200 – 450ml/min) and the resulting wall shear experienced by blood cells was calculated for values of bed porosity ranging from 0.2 – 0.6. Calculation of the resulting wall shear was done using the following equation:

\[ \text{wall shear rate} = 8 \times \frac{u}{d_r} \]  

(Equation 4.27)
Where:-
\[ d_r = \frac{4 \times \varepsilon}{(1 - \varepsilon) \times \frac{d_p}{6}} \]  
(Equation 4.28)

and \[ u = \frac{\text{flow rate}}{\varepsilon \times \text{area of column}} \]  
(Equation 4.29)

4.1.12 Effects of changing particle size

In order to quantify the effects of different particle sizes, a set of simulations were conducted for a bed with porosity set to 0.4 and changing the flow rates within the clinical range. This is of some importance because minimising the particle size greatly enhances the kinetic uptake of MM molecules. The effects were modelled by calculating the wall shear for a fixed bed porosity and varying only the particle size. The commercial sized column was used for this evaluation. Minimum and maximum flow rates (200ml/m & 450ml/m) were used for these calculations.

4.1.13 Scale down column evaluation of uptake kinetics of lysozyme by XAD4 and CW4

Chapter 3 contained a number of empirical and theoretical approaches to characterise the adsorption characteristics of polymer nanoporous adsorbents at removing MM's and size excluding albumin. This section discusses how a suitable scaled down haemoperfusion column may be used for evaluating adsorbent biocompatibility as well as studying the adsorption kinetics from clinical fluids including blood. In order to validate the system and highlight any changes in adsorption capacity due to the fixed bed system preliminary adsorption studies were completed.
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The system was scaled down based upon the Reynolds number to ensure the flow dynamics in the small-scale column mimicked those in a commercial system. The Reynolds number for the commercial column was calculated and the flow rate adjusted for the scaled down column system so that the Re was similar in magnitude.

The scaled down system may be used for the concurrent study of biocompatibility and adsorption. Experiments were conducted using CW4 and XAD4 and the uptake kinetics of lysozyme was evaluated under dynamically scaled flow conditions. Both these materials had been previously characterised (using well mixed batch adsorption experiments in chapter 3). The use of a fixed-bed contacting arrangement instead of the well-mixed stirred reactor system would provide insight into the effect of the contacting arrangement on external mass transfer and hence kinetics of the adsorption process.

Bed height was fixed at 4cm and the amount of adsorbent required for the packing of the column was carefully weighed to allow calculation of uptake capacity. Reynolds values were obtained by calculation for the commercial column and then the flow rate for the scale down calculated based upon the particle size of the adsorbent (measured using the Malvern mastersizer as described in Chapter 2) and the geometry of the mini-column. Initial concentration of the lysozyme in Hepes buffer was 1g/l and the volume of the fluid reactor was 0.5l.

The stock solution was contained in a 1L stirred vessel placed in a thermostated water bath (temperature maintained at 37°C). A short length of insulated piping was run through the peristaltic pump and into the adsorber column which was allowed to flow back into the reservoir. See Figure 4.6
In order to obtain low flow rates (0.48 -1.09 ml/min) required to obtain the required Re number, a smaller capacity peristaltic pump was used. The pump was equipped with 12 rollers to minimise pulsing. The desired flow rate was set by calibrating the pump speed and measuring the volumetric output. The flow rate was checked once the column was connected to the rig as the additional resistance created by the packed bed affects the resulting volumetric flow rate. The Re of the commercial column was obtained using the following equation:

$$\text{Re}_b = \frac{\rho u_b d_z}{\mu}$$  \hspace{1cm} (Equation 4.30)

and,

$$u_b = \frac{u}{\epsilon \times A}$$  \hspace{1cm} (Equation 4.31)
Samples from the bulk solution (in the reactor) were taken and the resulting concentrations measured using the HPLC method outlined previously (see chapter 3). An initial sample was taken \((t = 0)\) before the pump was started and then subsequent samples were taken at regular intervals to follow the concentration change in the bulk.

### 4.2 Results and discussion

#### 4.2.1. Blood analogue

The glycol water mixtures were analysed using the method described above and the resulting viscosities plotted as in Figure 4.7. It is apparent that the fluid viscosity changes with increasing concentration of glycol and the change is non-linear. Therefore an exponential form of the equation was fitted to the data and is included in the figure 4.7.

From the figure, a glycol concentration of 41% should give a solution with a viscosity of 8 mPa.s at room temperature. This solution was prepared and analysed to ensure it did exhibit the required viscosity of 8 mPa.s. (see Figure 4.8)

The rheology of the solution was then measured to see if the solution exhibited any hysteresis effects when sheared. To further validate its suitability for use as a blood analogue in packed bed experiments, a number of shear rate step changes were made to see if the solution exhibited any plastic behaviour. The results (see figure 4.9) clearly show that no hysteresis was observed therefore the analogue appeared suitable for use in the study. 2L of solution was used for the packed bed experiments to reduce the potential effect of temperature increase caused by prolonged flow through the pump during experimentation.
4.2.2 Effects of temperature change on blood analogue.

Each of the samples were run at 3 differing temperatures to identify any change in viscosity that may occur. The temperature of the fluid was maintained by circulating pre-heated water around the jacket of the concentric cylinder viscometer and an internal probe recorded the actual temperature during analysis. Again, exponential fits were used to describe the relationship between solution viscosity and the concentration of glycol in water.

Figure 4.7 shows that solution viscosity increased as the temperature decreases. The effect of temperature was more apparent as the concentration of glycol increased. Therefore, during experiments the temperature was controlled to minimise changes in solution viscosity.

Figure 4.8 shows that at room temperature, a 41% glycol solution had a viscosity of 8 mPa.s. In order to ensure this value was correct, 2L of the sample was prepared and viscosity measured. Figure 4.8 shows considerable scatter, however, the gradient suggests that the fluid viscosity is 8 mPa.s. Figure 4.9 highlight that no hysteresis was shown by the fluid as the shear rate was increased and decreased.

Hepes buffer

Hepes buffer was found to exhibit Newtonian behaviour and had a viscosity of 1.6 mPa.s at 37°C.
Figure 4.7 Effects of changing concentration of glycol and temperature on viscosity

Figure 4.8. Plot of final blood analogue (41% glycol), $\mu=8\text{mPa.s.}$
Chapter 4 Dynamic scaling down of a fixed-bed haemoperfusion column.

Figure 4.9. Hysteresis plot of 41% glycol solution

4.2.3 Validation of wall effects

The packed bed height in the pilot scale column was varied and the pressure drop across each bed height at a range of clinically relevant flow rates was measured. Figure 4.10 shows results of pressure drop per unit length of bed against flow rate. The data falls on a straight line suggesting that the packing characteristics of the bed don't particularly change as the bed height is varied between 10-30cm.

When the same data is plotted as frictional factor against the Reynolds number (Re), again, the data appears to fall on a universal line. Figure 4.11 shows this data in addition to data obtained for the small scale mini-column with bed height 2 cm and 4cm over a range of flow rates. The plot shows that the fluid dynamics in the mini-column system and the pilot scale system are similar for the same $Re_b$. Data for the mini-column system at the two bed heights suggests wall effects may be ignored.
Figure 4.10. Effects of changing bed height on pressure drop per unit length in the pilot study sized column.

Figure 4.11. Plot of friction factor against Re for both size columns with various bed heights (solid markers are mini column, hollow markers are pilot scale sized column).
The Carman-Kozeny equation can be used to correlate pressure drop in packed columns.

The experimentally obtained pressure drop measurements across the packed bed of the pilot-scale column were compared with those predicted by the Carman-Kozeny equation. The following equation was used and various values of $K$ substituted to fit the model to the experimental data.

$$\Delta P_f = (36K\mu L) \left[ \frac{(1 - \varepsilon)^2}{\varepsilon^3 d_p^2} \right] u$$  \hspace{1cm} (Equation 4.32)

Expected values of $K$ are normally in the region of 3 – 5.5. The pressure drop across each of the bed heights was obtained at different flow rates and compared with the predicted values obtained when using the Carman-Kozeny equation. As the bed height increased the accuracy of the equation increased. However, there was still a fair discrepancy between the empirical data and the estimated values (with $K$ in the region of 3 – 5.5). Much of this error could be associated with difficulties encountered in experimentally obtaining the bed porosity values. The Carman-Kozeny equation is sensitive to the magnitude of $\varepsilon$. The values for the mini-column system did not correlate well with the equation. Accurate measurement of the bed porosity was difficult for the mini-column system which is a likely source of error.

### 4.2.4 Effects of changing packing porosity on wall shear.

It is known that bed porosity influences the resulting shear stress experienced by blood cells during haemoperfusion. Although this value is linked directly to the particle size, it is also affected by the particle size distribution. Therefore, by being able to quantify the effects of changing the bed porosity on pressure
drop, it is possible to predict the effects of both the particle size and distribution. As expected the results of the calculation (see Figure 4.12) demonstrate that at the higher flow rate the effects of changing porosity are more apparent. As any inline treatment requires the haemoperfusion column to cope with the dialyser flow rates, the final column design must ensure that the shear rates at the upper clinical flow rates are acceptable.

![Figure 4.12 Effects of changing packing porosity on wall shear rate](image)

**Figure 4.12 Effects of changing packing porosity on wall shear rate**

### 4.2.5 Effect of changing particle size on pressure drop

In order to optimise kinetic uptake of MM's, chapter 3 demonstrated the dramatic effect of particle size on solute diffusion time. However, it is also clear that as particle size decreases, pressure drop and wall shear increase. Therefore, there is benefit in being able to determine the minimum particle size that may be successfully used in a haemoperfusion device. Calculations were made to look at the effects of varying particle size with a constant bed porosity of 0.4 and flow rates of 250ml/min and 450ml/min (see Figure 4.13).
It is interesting to note that as the particle size increases, the effects on wall shear become less apparent.

![Graph](image)

**Figure 4.13.** Effect of changing particle size on the wall shear rate (Porosity of bed 0.4)

### 4.2.6 Potential optimisation of a commercially sized haemoperfusion device in terms of particle size.

Although there are several parameters that have the capacity to be simultaneously changed, it is possible to make an attempt to optimise the haemoperfusion column. The particle size distribution is the primary parameter effecting packing porosity. By selecting a number of porosity values, a minimum particle size can be obtained for each porosity value using the equations described previously.

As an estimate of exposure time, the contact time of each parcel of blood through the column can be multiplied by the total number of passes during a 6 hour dialysis session. It is assumed the blood volume is 5L. Blood flow rate was set at the max value of 450ml/m. The commercial column dimensions
are 5cm x 25cm. The contact time can be obtained by dividing the volume of blood in the column by the blood flow rate (450ml/m). The volume of blood in the column will vary depending on the porosity. In order to obtain the maximum shear rate, the total exposure time was estimated at each porosity value and then multiplied by 4 to provide a safety factor. Total exposure time $T_e$ was calculated by

$$T_e = \left( \frac{V_c}{F_s} \right) C$$  \hspace{1cm} (Equation 4.33)

Where $V_c$ is the volume of blood in the column, $F_s$ is the system flow rate and $C$ is the amount of times each parcel of blood will pass through the column in the treatment time ($T$). Hence the $C$ value can be obtained:

$$C = \left( \frac{F_s \times T}{V_b} \right)$$  \hspace{1cm} (Equation 4.34)

Where $V_b$ is the blood volume of the patient (assumed to be 5L).

The results of these calculations over a range of bed porosity values is shown in Table 4.4.

**Table 4.4. Effects of changing porosity on the potential minimum particle size based on the criteria suggested by Deutch et al (2006).**

<table>
<thead>
<tr>
<th>Porosity</th>
<th>Volume of blood in column (cm$^3$)</th>
<th>Contact time for 6 hour treatment (sec)</th>
<th>Estimate of max shear rate permissible (s$^{-1}$)</th>
<th>Min Particle size ($\mu$m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>98</td>
<td>416</td>
<td>600</td>
<td>1500</td>
</tr>
<tr>
<td>0.3</td>
<td>147</td>
<td>640</td>
<td>560</td>
<td>630</td>
</tr>
<tr>
<td>0.4</td>
<td>196</td>
<td>832</td>
<td>500</td>
<td>345</td>
</tr>
<tr>
<td>0.5</td>
<td>245</td>
<td>1024</td>
<td>490</td>
<td>185</td>
</tr>
<tr>
<td>0.6</td>
<td>295</td>
<td>1248</td>
<td>480</td>
<td>105</td>
</tr>
<tr>
<td>0.7</td>
<td>343</td>
<td>1472</td>
<td>420</td>
<td>65</td>
</tr>
</tbody>
</table>
Chapter 4 Dynamic scaling down of a fixed-bed haemoperfusion column.

By using a Micropore Technologies Ltd dispersion cell system, it is possible to produce particles of a tight size distribution as discussed in chapter 2. The resulting particles have the potential to have a packing porosity of around 0.6 - 0.7. As discussed in chapter 3, the smaller particle size will offer substantial benefits in terms of uptake kinetics. Therefore by utilising such a technique for the production of the adsorbent beads, it may be possible to use particle sizes in the region of 60 - 100\(\mu m\) in a commercially sized column. In addition, by using a material similar to CW4, it has been demonstrated that a considerably smaller amount of material is required. Therefore, the pressure drop across the bed will likely to be considerably smaller than current devices.

4.2.7 Uptake in mini-column system.

The Sauter mean value for the XAD4 particle size used in this study was 130\(\mu m\). The CW4 adsorbent had a mean particle size of 48\(\mu m\). Calculations for flow rates of 325ml/min (mid-point of clinical blood flow rate range of 200 - 450 ml/min) suggests Re ~ 0.21. In order to obtain a Re of 0.21 a flow rate of 1.2 ml/min was required for the XAD4 packed column and 3.1 ml/min for the CW4 system. The pump speed was adjusted to produce these flow rates. Figure 4.14 shows the resulting decrease in concentration with respect to time for the XAD4 system. A final saturation uptake value of 358mg/g was observed. This value is of similar magnitude to saturation values reported in chapter 3 (410mg/l) for the idealised well-mixed system. Figure 4.15 shows the reduction in lysozyme concentration due to adsorption onto CW4 using the scaled down mini-column system. The final saturation uptake was 492mg/g, again similar in magnitude to the data reported in chapter 3 (577mg/g). Slight differences may be attributed to batch-to-batch variability.

During a 3 hour dialysis session with the commercial sized column, each parcel of blood will pass through the column around 12 times. Based on the volume of fluid used for this experiment (0.5l) and the flow rates used, it was
calculated that it would take 3.4 days for 12 passes of each parcel of blood to pass through the XAD4 mini-column and 1.3 days in the CW4 system. Looking at both the plots it is clear that the material has been saturated by this time. The efficacy of the CW4 material does however suggest that there is no need to use a column with the quantity of adsorbent used in the current commercial columns. However, much of the uptake rate is due to the small particle size and it is likely that an excess of material would be required to reduce the concentration of toxins to a sufficient level within a normal dialysis treatment time if a larger particle size was used. However this may be a reasonable approach to use for studies of biocompatibility.

![Graph](image)

**Figure 4.14.** Uptake of lysozyme onto XAD4 (130µm), 1 g/l initial concentration in a dynamically scaled down micro column.
Chapter 4 Dynamic scaling down of a fixed-bed haemoperfusion column.

Figure 4.15. Uptake of lysozyme onto CW4, particle size 48μm, 1.6 g/l initial concentration in a dynamically scaled down micro column.

4.3. Conclusions

This chapter has outlined an approach for the dynamic scaling down of a clinical-sized haemoperfusion column to that of a small scale mini-column system that may be used for investigations of biocompatibility and adsorption performance. The chapter outlined the lack of continuity in current biocompatibility studies and therefore provides a case for standardisation. Much of the current biocompatibility work is carried out under flow conditions too dissimilar to how the device is likely to be used and the scale down approach hopes to negate much of this disparity. It is possible to scale down based on an important flow parameter such as the Reynolds number or wall shear rate. By ensuring such parameters are constant, the performance of the device may be studied under “clinically relevant” flow conditions without the need for large quantities of donor blood. The scale down procedure inevitably results in some compromises. This study used a scaled down particle size which resulted in an increase in the external surface area to
column volume ratio. Therefore a “worse case” situation arises in terms of biocompatibility response.

This chapter highlights the important role that flow has on any proposed haemoperfusion device. The effects of changing critical parameters such as particle size and packing porosity have been quantified and related to the potential damage they could have on cells. The resulting understanding provides a vital platform to inform a commercial column design and for fundamental studies into biocompatibility.

By using a Micropore Technologies Ltd dispersion cell system it could be possible to use a particle size approximately 4 times smaller than those used in some existing commercial columns.
Chapter 5

Conclusions and future work

The objectives of this research were to select divinylbenzene polymer adsorbents based upon pore structure so that an evaluation of their suitability for blood purification could be assessed. Initial investigation highlighted a group of middle molecular sized molecules that literature suggested would benefit patient health if they were removed. A surrogate marker (lysozyme) was then selected to study the efficacy of different adsorbents. A potential strategy of size excluding human serum albumin molecules to allow access to the internal pore structure to MM size molecules was considered. An attempt at understanding the fundamental influences of pore structure, particle size and external mixing conditions on uptake of lysozyme (MM surrogate marker) and human serum albumin (large blood protein) was made. The effects of competitive adsorption on uptake of MM markers was also studied in both simple and clinically relevant multi-component solutions (PD fluid). A model was obtained to help predict adsorbent performance once an effective diffusivity value for a particular molecule / adsorbent pair had been obtained experimentally. Finally an insight into the flow conditions within a haemoperfusion device were obtained using a scale down system having the potential to be used as a method of studying biocompatibility responses. A model was used to help predict the influence of particle size, flow rate and bed porosity on shear rate.

The work contained within this thesis has contributed to original knowledge. By applying engineering principles new knowledge of the structure-properties relationship in porous material in liquid phase adsorption has been demonstrated. This knowledge can be applied to the design of an augmented haemoperfusion device which could have important application in clinical
renal care. In addition, an original approach to the design of a scaled down haemoperfusion column based upon dimensions from an existing commercial device was completed. This technique has provided a means of evaluating biocompatibility of haemoadsorbents in realistic flow conditions.

5.1 Selection and characterisation of materials

Various styrene divinylbenzene polymer adsorbents were obtained and characterised using a number of techniques. Nitrogen porosimetry was carried out to obtain estimates of the pore structure for each of the selected adsorbents. Materials were selected based upon their structures. Adsorbents allowing access to larger blood proteins such as albumin (XAD4) in addition to materials that were unlikely to allow passage of these larger molecules (CW1 & CW4) were chosen.

Micrograph and SEM images demonstrated all particles were spherical and had a cauliflower type internal pore structure.

The nitrogen porosimetry data suggested likely surface areas available for adsorption of lysozyme and albumin. From this data XAD4 appeared to have the highest capacity for lysozyme adsorption, followed by CW4 and CW1. However, XAD4 had a pore structure that based upon the porosimetry data was likely to allow access to albumin, whilst the other two adsorbents were likely to size exclude it.

Particle size distributions were obtained for each of the sieved size fractions. This data suggested that even when carefully sieved, fines were still present and it was difficult to obtain tight particle size distributions using this method. A novel membrane based system capable of producing particles with a tight particle size distribution was also investigated to see if the process affected the resulting pore structure. The particle size analysis confirmed that the
resulting span was tight and the porosimetry data suggest little change in the pore structure when compared to suspension polymerisation produced adsorbents.

5.1.1 Influence of pore structure and mixing conditions on mass transfer of MM’s

Each of the adsorbents were evaluated using a batch reactor for uptake kinetics and capacity of lysozyme and human serum albumin. Adsorption capacity correlated well with pore distributions obtained from nitrogen porosimetry with the exception of CW4 which demonstrated a slightly greater capacity for adsorption then the porosimetry data suggested. Internal mass transfer resistance appeared predominant and external mass transfer resistance appeared negligible. Particle size influenced kinetic uptake of lysozyme and albumin greatly and it is clear this is a vital parameter to consider in the design of a hemoperfusion column.

CW1 effectively size excluded albumin, whilst both CW4 and XAD4 allowed it access to some of their internal pore structure. XAD4 had a capacity for albumin around double that of CW4 although CW4 exhibited a greater capacity for lysozyme molecules.

A model was used to predict behaviour of adsorbents once a small number of batch experiments had been completed to obtain vital parameters. By using a fitting parameter (the effective diffusivity) the model can also be used to identify the possible effects of pore structure on uptake kinetics. The model demonstrated that pore size distributions did influence the resulting effective diffusivities for Lysozyme and albumin. CW1, which effectively size excluded albumin possessed a lysozyme effective diffusivity an order of magnitude smaller than that of XAD4. CW4 appeared to have the highest effective
diffusivity for lysozyme. Clearly a more open pore structure results in improved uptake kinetics for the molecules able to access the internal structure.

In addition to single solute systems, binary solutions were also prepared and studied using the batch reactor methodology on XAD4 and CW1. Binary systems containing albumin and lysozyme demonstrated an augmentation of lysozyme uptake on XAD4 thought to be due to cooperative adsorption. Therefore this offered limited insight into the effects of competitive adsorption.

However, experiments conducted at Leicester General hospital using concentrated peritoneal dialysis fluid (a complex fluid containing both MM's and albumin at clinical concentrations) offered additional insight into effects of competitive adsorption. XAD4 and CW4 were compared for their ability to remove β2-M in a batch reactor from the concentrated PD fluid. XAD4 demonstrated that the kinetic uptake of β2-M did not vary significantly from CW4, which possesses a pore structure that allows much less albumin into its internal structure. Therefore, it is likely that MM capacity will be higher on CW4 as less of its surface area can be swamped by albumin.

5.1.2 Potential influence of column design on flow conditions

From the literature it became apparent that there is a need for a standardised procedure for the evaluation of biocompatibility in blood purification devices. Not only does there appear to be a lack of continuity but also biocompatibility appears to be rarely studied under clinical flow conditions. It is believed that flow is an important consideration for the biocompatibility performance of any device and could be an important component of its evaluation.
Study of a proposed scaled down hemoperfusion system was presented, thus allowing study of adsorbents in clinically relevant flow conditions without the necessity for large volumes of donor blood. The scale down procedure was based upon dimensionless parameters obtained from an existing clinical device. It was demonstrated that wall effects were negligible and that dynamic scale down of a commercial sized column is possible. However, it was noted that the ratio of surface area of the adsorbent to column size in the scale down column was greater than in the full size column. Therefore, it represents a 'worse case' scenario in terms of biocompatibility.

The scale down parameters were then used as a predicative tool to model effects of changing some key engineering parameters (particle size, packing porosity, flow rate). It was shown that changing particle size had the greatest effect on the resulting wall shear rate (within the clinical flow rate range of 200 – 450ml/m). Changing packing porosity also made substantial differences to the wall shear rate. As particle size has been shown to have a great influence on uptake kinetics of MM's, the flow model was used to try to identify the smallest possible particle size whist not creating conditions likely to damage the cells. By using a Micropores Technologies dispersion cell it is possible to create adsorbents with a tight particle size distribution. The nitrogen porosity data suggests use of such a method has little effect on the resulting pore structure. Therefore by utilising such a technology it has been shown that high bed porosities can be obtained, allowing the use of adsorbents around 5 times smaller than those currently employed in commercial columns. The capacity data would suggest that a material like CW4 could be used in smaller quantities than current commercial adsorbents due to its high capacity. Therefore it may be possible to use a much smaller device still capable of out performing current technologies. This offers a clinical advantage in terms of minimising extracorporeal blood volume and reducing the cost of the device. This may also present an advantage in terms of biocompatibility due to the reduced quantity of adsorbent.
5.2 Future work.

Insight has been gained concerning the influence of particle size, pore structure and mixing conditions on the performance of a blood purification adsorbent. With the exception of preliminary batch reactor studies using PD fluid, the majority of this work has been done using simple solutions. Therefore further investigation into more complex solutions may offer additional insight into how MM's may interact with other molecules within the blood and effect uptake.

An obvious next step would be to evaluate the uptake kinetics and capacity of other MM's (as documented in chapter 1) from a complex clinical fluid such as concentrated PD fluid. Following such an exercise the scale down column could be used to measure uptake of the same markers from whole blood.

Perhaps the biggest area requiring further study is the biocompatibility of the selected adsorbents. Although it has been shown that a smaller quantity of adsorbent than current commercial columns may be required it has not been demonstrated if this result in an acceptably biocompatible device. The use of the scale down column proposed in this work would prove useful in this evaluation. Initial studies should be concerned with global markers such as platelet counts, in addition to more specific investigations into things like concentration of C3b.

The adsorbent CW4 may be suitable for use in a device to augment current heamodialysis. However, a study of other application such as sepsis may provide further applications and therefore increase the likelihood of commercialisation. If the device was to be used as a stand alone treatment, it may also be desirable to study the potential of use in conjunction with a plasma separation module. If the plasma could be separated, and the cells bypass the adsorbent device, the biocompatibility of the device may be irrelevant.


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Appendix 1

IUPAC Classification of adsorption isotherms

Figure 1. Types of physisorption isotherms (adapted from Sing et al, 1985)

Figure 2. Types of hysteresis loops (adapted from Sing et al, 1985)
Appendix 2

Derivation of irreversible adsorption model by Suzuki et al (1973)

The usual shell balance for mass transfer within the adsorbent particle:

$$J|_r^- - J|_r^+ = \frac{\partial q(1-\epsilon) \rho_s 4 \pi^2 \delta r}{\partial t} + \frac{\partial |c \rho_s 4 \pi^2 \delta r|}{\partial t}$$  \hspace{1cm} (1)

Applying Fick's Law:

$$J = -D_e \frac{\partial c}{\partial r}$$

For irreversible adsorption, the solute uptake at equilibrium, $q^*$, is independent of the solute solution concentration $\tilde{c}$, i.e. if there is solute in solution, adsorption will continue until the solute runs out or the adsorbent gets saturated.

Between $r_i \leq r \leq R$:

$$\frac{\partial q}{\partial t} = 0$$

Ignoring solute hold-up in the adsorbent pores, eq. 1 simplifies to:

$$\frac{\partial}{\partial r} \left[ r^2 \frac{\partial \tilde{c}}{\partial r} \right] = 0$$  \hspace{1cm} (2)
Integrating eq. 2 once gives:

\[ r^2 \frac{\partial \tilde{c}}{\partial r} = A(t) \quad (3) \]

A boundary condition may be as follows:

At \( r = R \);

\[ \frac{\partial \tilde{c}}{\partial r} \bigg|_{r=R} = \frac{\partial \tilde{c}}{\partial r} \bigg|_{r=R} \quad (4) \]

Where:

\[ D \frac{\partial \tilde{c}}{\partial r} \bigg|_{r=R} = k_f \left( C - \bar{c}_R \right) \quad (5) \]

Therefore:

\[ \frac{\partial \tilde{c}}{\partial r} \bigg|_{r=R} = \frac{k_f}{D_e} \left( C - \bar{c}_R \right) \]

And hence:

\[ A(t) = \frac{R^2 k_f}{D_e} \left( C - \bar{c}_R \right) \quad (6) \]

Integrating eq. 3 requires a second boundary condition which is as follows:

At \( r = r_i, \bar{c} = 0, \) thus:
\[
\int_{c=0}^{c=r} \frac{r^n A(t)}{r^2} dr = 0
\]  
(7)

Yielding:

\[
\bar{c} = A(t) \left[ \frac{1}{r_i} - \frac{1}{r} \right]
\]
(8)

Therefore, combining the results in [6] and [8]:

\[
\bar{c} = \frac{R^2 k_f}{D_e} \left[ C - \bar{c}_r \left( \frac{r - r_i}{rr_i} \right) \right]
\]
(9a)

Hence at \( r = R \):

\[
\bar{c}_r = \frac{Rk_f}{D_e} \left[ C - \bar{c}_r \left( \frac{R - r_i}{r_i} \right) \right]
\]
(9b)

Define the following variables:

\[
\phi = \frac{r_i}{R} \quad \text{and} \quad Bi = \frac{k_f R}{D_e} \;
\]

Therefore, [9b] may be represented as:

\[
\bar{c}_r = Bi \left[ C - \bar{c}_r \left( \frac{1 - \phi}{\phi} \right) \right]
\]
(10)

Express \( \bar{c}_r \) in terms of \( C \):

\[
\bar{c}_r = C \left[ \frac{Bi (1 - \phi)}{Bi + \phi (1 - Bi)} \right]
\]
(11)
Applying a mass balance on the solute in the stirred vessel:

\[-V \frac{dC}{dt} = \frac{d}{dt} \left[ \dot{q} \rho_s (1 - \epsilon) \left( \frac{4}{3} \pi R^3 \right) N_p \right] = \Lambda_p k_f (C - \bar{c}_s) \tag{12}\]

The average solute composition in the adsorbent is:

\[-\dot{q} = \frac{\frac{4}{3} \pi [R_3 - i_3^3] q^*}{\frac{4}{3} \pi R^3} = q^* (1 - \phi^3) \tag{13}\]

And:

\[q^* = \frac{(C_o - C_{inf}) V}{m_p} \tag{14a}\]

\[-\dot{q} = \frac{(C_o - C) V}{m_p} \tag{14b}\]

Dividing eq. 14b by eq. 14a and using the result in eq. 13:

\[-\frac{\dot{q}}{q^*} = \frac{C_o - C}{C_o - C_{inf}} = 1 - \phi^3 \tag{15}\]

Thus:

\[C = C_o - \left\{ (1 - \phi^3) (C_o - C_{inf}) \right\} \tag{16}\]

From eq. 14a:
\[ C_o - C_{\text{inf}} = \frac{q^* m_p}{V} \]  

(17)

Replacing eq. 17 in eq. 16:

\[ C = C_o - \left[(1 - \phi^3) \left(\frac{q^* m_p}{V}\right)\right] \]  

(18)

Combining [11], [12], [13] and [18] yields a first order ordinary differential equation:

\[ -\frac{d\phi}{dt} = \frac{D_s B_1}{\rho (1 - \epsilon) \phi q^* R^2} \left[C_o + \left(\frac{(\phi^3 - 1) q^* m_p}{V}\right) \phi \left(Bi + \phi (1 - Bi)\right)\right] \]  

(19)

The o.d.e. in eq. 19 was solved numerically using an explicit 4th order Runge-Kutta method in Matlab (release 2007b) using the initial condition:

At \( t = 0; \phi = 1 \)

The value of the film mass transfer coefficient was evaluated using the correlation in Middleman (1998):

\[ Sh = 0.418 Sc^{0.25} Re^{0.75} \left(\frac{D_i^4}{VD_i}\right)^{\frac{1}{4}} \]  

(20)