SYNTHESIS AND ENGINEERING OF POLYMERIC LATEX PARTICLES FOR
MEDICAL APPLICATIONS

By

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by

Sangyup Kim
To all who made this work possible.
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Latex particles with well-defined colloidal and surface characteristics have received increasing attention due to their useful applications in many areas, especially as solid phase supports in numerous biological applications such as immunoassay, DNA diagnostic, cell separation, and drug delivery carrier. Hemodialysis membrane using these particles would be another potential application for the advanced separation treatment for patients with end stage renal disease (ESRD). It is desirable to remove middle molecular weight proteins with minimal removal of other proteins such as albumin. Thus, it is necessary to understand the fundamental interactions between the particles and blood proteins to maximize the performance of these membranes. This improvement will have significant economic and health impact.

The objective of this study is to synthesize polymeric latex particles of specific functionality to achieve the desired selective separation of target proteins from the human blood. Semi-continuous seed emulsion polymerization was used to prepare monodisperse
polystyrene seed particles ranging from 126±7.5 to 216±5.3 nm in size, which are then enlarged by about 800nm. Surfactant amount played a key role in controlling the latex particle size. Negatively charged latex particles with a different hydrophobicity were prepared by introduction of a sodium persulfate initiator and hydrophilic acrylic acid monomer. The prepared polymeric particles include bare polystyrene (PS) particles, less hydrophobic PS core and PMMA shell particles, and more hydrophilic PS core and PMMA-co-PAA shell latex particles with a 370nm mean diameter. SEM, light scattering, and zeta potential measurements were used to characterize particle size and surface properties. Adsorption isotherms of two proteins, bovine serum albumin (BSA) and β2-microglobulin (β2M), on latex particles were obtained as a function of pH and ionic strength using the bicinchoninic acid (BCA) assay method. The Langmuir-Freundlich adsorption model was used to determine the adsorption amount of protein at equilibrium. The thickness of adsorbed BSA layer on latex particles was obtained in order to investigate the adsorption orientation such as end-on or side-on mode. Adsorption kinetics experiments for both proteins and all latex particles were also performed. The adsorption kinetic constant determined from the Langmuir-Freundlich adsorption isotherm model was used to calculate Gibbs free energy of adsorption to compare the competitive adsorption of BSA and β2M. Hemolysis tests were performed to investigate the blood compatibility of synthesized latex particles. PS/PMMA\textsubscript{90}PAA\textsubscript{10} and PS/PMMA\textsubscript{75}PAA\textsubscript{25} core shell latex particles had desirable material properties with, not only a large amount and high rate of selective β2M adsorption over BSA but also high blood compatibility showing less than 3% hemolysis.
CHAPTER 1
INTRODUCTION

End stage renal disease (ESRD) is a chronic condition in which kidney function is impaired to the extent that the patient’s survival requires removal of toxins from the blood by dialysis therapy or kidney transplantation. The National Kidney Foundation estimates that over 20 million Americans had chronic kidney disease in 2002 [NKF]. The number of people with ESRD is rapidly increasing in the United States with approximately 96, 295 incidents and 406, 081 prevalent patients, including 292, 215 on dialysis and 113, 866 with a functioning graft in 2001. It is projected that there will be more than 2.2 million ESRD patients by 2030 [USRDS 2003]. The expenditure for the ESRD treatment program had reached $22.8 billion, 6.4% of the Medicare budget in 2001. Due in part to the limited availability of kidneys for transplantation, hemodialysis is the primary clinical treatment for the patients with ESRD.

The central element of a hemodialysis instrument is the semipermeable membrane that allows for selective transport of low molecular weight biological metabolites less than 5,000 Da such as urea and creatinine as well as excess water and electrolytes [Baker 2004] from the blood. One limitation of current dialysis technologies is the inability to efficiently remove middle molecular weight toxins such as β2-Microglobulin (β2M) and interleukin 6 (IL-6).

β2M is a causative protein of dialysis-related amyloidosis (DRA), a disease arising in patients with chronic kidney failure as a serious complication of long-term hemodialysis treatment [Gejyo et al. 1985]. β2M deposition in tissue is the primary cause
of destructive arthritis and carpal tunnel syndrome [Vincent et al. 1992; Drueke 2000].

The $\beta_2$M structure is shown in Figure 1-2.

![Stereo drawing of the $\alpha$–carbon backbone of $\beta_2$M](image)

Figure 1-2. Stereo drawing of the $\alpha$–carbon backbone of $\beta_2$M [Becker et al. 1985].

Although attempts have been made to increase the efficiency of middle molecular weight toxin removal by changes in the membrane pore size and the use of innovative materials to adsorb these toxins [Samtleben et al. 1996; Ronco et al. 2001], removal efficiency is not as high as those achieved by a normal healthy kidney. Traditional membranes have a number of processing and performance limitations [Westhuyzen et al. 1992; Leypoldt et al. 1998], such as a restricted choice of surface chemistries and limited control of porosity. The development of novel engineering membrane technology is needed to remove middle molecule toxins.

Polymeric latex particles have received increasing attention in medical application areas, especially as solid phase supports in biological applications [Piskin et al. 1994]. Examples of these applications include immunoassay [Chen et al. 2003; Radomske-Galant et al. 2003], DNA diagnostic [Elaïssari et al. 1998], cell separation, drug delivery carrier [Luck et al. 1998; Kurisawa et al. 1995; Yang et al. 2000], etc. This is because of
the well-defined colloidal and surface characteristics of the particles. By using a seed emulsion polymerization method, it is possible to synthesize monodisperse latex particles with various particle size ranges and surface chemistry. Functionalized core-shell latex particles can be introduced by multi-step emulsion polymerization. Core particles are synthesized in the first stage of the polymerization and the functional monomer is added in the second stage. This is done without any emulsifier addition to prevent the production of new homopolymer particles [Keusch et al. 1973]. The core-shell particles are useful in a broad range of applications because of their improved physical and chemical properties over their single-component counterparts [Lu et al. 1996; Nelliappan et al. 1997]. Through the development of a hemodialysis membrane using monodisperse latex particles, improvements in advanced separation treatment for patients with end stage renal disease (ESRD) can be realized. This requires the maximum removal of middle molecular weight proteins with minimal removal of other beneficial proteins such as albumin. Thus, an understanding of the fundamental interactions between the particles and biopolymers is vital to maximize the performance of this membrane technology.

The field of material science and biotechnology is based on fundamental chemistry has developed over the past three decades into today’s powerful discipline that enables the development of advanced technical devices for pharmaceutical and biomedical applications. This novel and highly interdisciplinary field is closely associated with both the physical and chemical properties of organic and inorganic particles [Niemeyer 2001]. Hemodialysis membrane using these particles would lead to improvements in the advanced separation treatment for patients with end stage renal disease (ESRD). The interdisciplinary nature of this approach enables a more complete understanding of the
phenomena of protein adsorption and the material properties necessary for selective separation. This innovative approach to membrane fabrication has the potential of making inexpensive, highly efficient membranes for both industrial and specialized separation processes.

The goal of this study is to prepare polymeric latex particles with tailored properties to maximize separation of toxin molecules and to investigate the fundamental interactions between the particles and molecules in the biological system in order to optimize the performance of a membrane material for these applications.

Polymeric latex particles were synthesized with specific functionality in an attempt to achieve selective separation. Seeded emulsion polymerization was used to synthesize functionalized monodisperse latex particles in various sizes. Negatively charged hydrophobic polystyrene latex particles were synthesized by the same method. Core shell latex particles, PS/PMMA_{100}, PS/PMMA_{90}PAA_{10}, PS/PMMA_{75}PAA_{25}, were also synthesized to differentiate the degree of hydrophobicity of particles. Scanning Electron Micrograph (SEM) and light scattering measurements were used to characterize particle size and shape, and zeta potential measurements were conducted to measure the electrical surface property of synthesized particles. Adsorption isotherms of target proteins, bovine serum albumin (BSA), and $\beta_2$M on latex particles were obtained as a function of pH, ionic strength, and protein concentrations using the BCA assay method. Adsorption kinetics for both proteins on the latex particles were also measured. Finally, hemolysis tests were run to determine the biocompatibility of polymer latex particles with human blood. This research will be described in more detail in chapters 3 and 4.
CHAPTER 2
BACKGROUND AND LITERATURE SURVEY

2.1 The Significance of End Stage Renal Disease (ESRD)

The kidneys are responsible for removing excess fluid, minerals, and wastes from the blood regulating electrolyte balance and blood pressure and the stimulation of red blood cell production. They also produce hormones such as erythropoietin (EPO) and calcitriol that keep bones strong and blood healthy [Casadevall and Rossert 2005; Kurbel et al. 2003]. EPO acts on the bone marrow to increase the production of red blood cell in case of bleeding or moving to high altitudes. Calcitriol mainly acts on both the cells of the intestine to promote the absorption of calcium from food, and also bone to mobilize calcium from the bone to the blood. When kidneys fail, harmful waste builds up, blood pressure rises, and body retains excess fluid. The body also does not make enough red blood cells. When this happens, treatment is needed to replace the function of failed kidneys.

Figure 2-1. An image of the location and cross section of a human kidney.
The National Kidney Foundation estimates that over 20 million Americans had chronic kidney disease in 2002 [NKF]. Chronic renal disease is a gradual and progressive loss of the function, unlike acute renal disease where sudden reversible failure of kidney function occurs. Chronic renal failure usually takes place over a number of years as the internal structures of the kidney are slowly damaged. In the early stages, there can be no symptoms. In fact, progression may be so gradual that symptoms do not occur until kidney function is less than one-tenth that of a normal health kidney. If left untreated, chronic kidney disease may ultimately lead to kidney failure known as End Stage Renal Disease (ESRD). ESRD is a rapidly growing health-care problem in the United States. In 2001, approximately 96,295 incidents and 406,081 prevalent patients were diagnosed with ESRD, including 292,215 patients on dialysis and 113,866 patients with a functioning graft [USRDS 2003]. The projected number of patients with ESRD is expected to exceed 2.2 million patients by 2030 with much of this growth being driven by the increasing prevalence of major contributing factors such as diabetes and high blood pressure [USRDS 2003]. A great extent of ESRD program cost and Medicare budget have been spent in 2001. Due in part to a limited availability of kidneys for transplantation, hemodialysis (HD) is the primary method of treatment for ESRD and is currently used for approximately 61% of U.S. ESRD patients.

### 2.2 Hemodialysis (HD) Treatment

HD removes toxins from the body by extracorporeal circulation of the blood through a semipermeable membrane, referred to as a dialyzer. The toxins are removed primarily by diffusion across the membrane to a dialysate solution which is circulated on the opposite side of the membrane. The cleaned blood is then returned to the blood stream. A surgically constructed vascular access connects the extracorporeal circuit to the
patient’s system. Treatments are almost always performed three times per week in specially equipped dialysis facilities for 3-4 hours per each treatment. Figure 2-2 shows the scheme of the hemodialysis route. The critical element of a HD instrument is the semipermeable membrane, which allows for selective transport of low molecular weight biological metabolites from blood.

Figure 2-2. A schematic draw of the hemodialysis route

ESRD patients who undergo dialysis therapy often experience several other problems associated with the treatment such as anemia, fatigue, bone problems, joint problems, itching, sleep disorders, and restless legs. Anemia is common in patient with kidney disease because the kidneys produce the hormone erythropoietin (EPO), which stimulates the bone marrow to produce red blood cells. Diseased kidneys often do not produce enough EPO to stimulate the bone marrow to make a sufficient amount of red blood cells. This leads to bone disease, referred to as renal osteodystrophy and causes bones to become thin and weak or malformed and can affect both children and adults. Older patients and women who have gone through menopause are at greater risk for this disease. Uremic toxins, which cannot be remove from the blood by the current dialyzer membranes can lead to itching. This problem can also be related to high levels of
parathyroid hormone (PTH). Dialysis patients can also experience day-night reversal, that is, they have insomnia at night and sleep during the day. This can be related to possible nerve damage in the body and a chemical imbalance in the blood due to the excess toxins. Oxidative stress is a problem for patients on maintenance dialysis. This problem is due to an imbalance between pro- and antioxidant factors [Roselaar et al. 1995; Cristol et al. 1994]. Oxidative stress affects oxidation of low-density lipoproteins which are the main factor for atherogenesis [Huysmans et al. 1998] and is also involved in the development of malignancies and diabetes mellitus [Rice-Evans et al. 1993]. In order to reduce antioxidant defense, dialysis is needed to contribute to help stimulate free radical production or eliminate antioxidants. Dialysis-related amyloidosis (DRA) is also a common and serious problem for people who have been on dialysis for more than 5 years. DRA develops when proteins in the blood deposit on joints and tendons, causing pain, stiffness, and fluid in the joint, as is the case with arthritis. Normally the healthy kidneys can filter out these proteins, but dialysis filters are not as effective.

2.3 Advances in Membrane Technology

Dialysis for blood purification is widely used in the treatment of ESRD. Hemodialysis (HD) techniques use a semi-permeable membrane to replace the filtration role of the kidney. The membranes used in HD can be broadly classified into those based on cellulose and those manufactured from synthetic copolymers. These membranes come in various shapes such as sheets, tubular structures or hollow fiber arrangements. The hollow fiber type is the most popular and is incorporated into over 800 different devices in world wide [Ronco et al. 2001].

The first attempt at blood dialysis using a cellulose based membrane occurred 1913. John Abel [1990] from the Johns Hopkins Medical School, described a method
whereby the blood of a living animal may be submitted to dialysis outside the body using a membrane based on cellulose and returned to the natural circulation without exposure to air, infection by microorganisms or any alteration that would necessarily be prejudicial to life. This same technique is still used to today, however the device used has been modified over the years as better membranes were developed and the anti-coagulant, heparin, has become available.

Cellulose membranes have been widely used for the treatment of renal failure from 1928, when the first human dialysis was performed, until the mid 1960. The basic molecular structure of cellulose is made of a long chain containing hydroxyl (OH) groups. The realization that such groups imparted undesirable qualities on the material in respect to blood contact behavior was discovered in the early 1970s and since has been the focus of development. These modified cellulose membranes used the partial substitution of benzyl groups to replace the proton of the hydroxyl groups in an attempt to reduce their negative effect. The result is a molecular mosaic of hydrophobic (benzyl) and hydrophilic (hydroxyl and cellulose) regions.

Kolff [Van Noordwijk 2001] studied the rotating drum artificial kidney for patients with acute renal failure in 1943. Cellophane tubing was used for the membrane with heparin as the anticoagulant. For the next 17 years, hemodialysis therapy was performed by this method but only for the patients with acute reversible renal failure. Vascular access required repeated surgical insertions of cannulas (slender tubes) into an artery and vein, and limited the number of treatments for a patient could receive in order to minimize the amount of vascular damage.
Initially, the need for dialysis in patients with acute renal failure was determined mainly by the development of signs and symptoms of uremia. After dialysis, some time might elapse before uremic manifestations returned to warrant a sequential treatment of dialysis. Many patients with acute renal failure, secondary to accidental or surgical trauma were hypercatabolic, but the interdialytic interval might be prolonged because of anorexia or use of a low-protein diet. However, Teschan and his coworkers [Obrien et al. 1959] showed that patient well-being and survival were improved by what they termed prophylactic daily hemodiaylsis, or administration of the treatment before the patient again became sick with uremia. Their report in 1959 was the first description of daily hemodialysis.

Development of membrane accessories such as a shunt has also been an area of focus for treatment improvement. In 1960, the development of a shunt [Quinton et al. 1960], a flexible polytetrafluoroethylene (PTFE or Teflon®) tubing, made many more hemodialysis treatments possible for chronic kidney failure patients. PTFE has a non-stick surface and is relative biocompatibility leading to minimized blood clotting in the shunt.

Synthetic membranes are prepared from engineered thermoplastics such as polysulfone (PSf), polyamide (PA), and polyacrylonitrile (PAN) by phase inversion or precipitation of a blended mixture resulting in the formation of asymmetric and anisotropic structures. Figure 2-3 shows a fiber type of the Polyflux S membrane consisting of poluamide (PA), polyacrylethersulfone (PAES), and polyvinylpyrrolidone (PVP) with the integral three-layer structure. The skin layer on the inside fiber type membrane contacts blood and has a very high surface porosity and a narrow pore size...
distribution. This layer constitutes the discriminating barrier deciding the permeability and solute retention properties of the membrane. The skin layer is supported by thick sponge-type structure larger pores, providing mechanical strength and very low hydrodynamic resistance.

Figure 2-3. Cross-sectional SEM image view of the Polyflux S (polyamide + polyacrylethersulfone + polyvinpyrrolidone) dialysis membranes [Deppisch et al. 1998]

PSf is a widely used membrane material for the hemodialysis application [Malchesky 2004], because of its thermal stability, mechanical strength, and chemical inertness. According to a report from the National Surveillance of Dialysis-Associated Disease (NSDAD) in the US, over 70% of hemodialysis membranes were PSf based [Bowry 2002]. This is most likely because PSf has many advantages over other materials. This synthetic polymer is one of few materials that can withstand sterilization by steam, ethylene oxide, and γ-radiation. PSf membrane can be prepared by conventional immersion precipitation methods into many different shapes including porous hollow
fiber or flat sheet hemodialysis membranes. The material also has a high permeability to low molecular weight proteins and solute, and high endotoxin retention. The chemical structure of PSf is shown in Figure 2-4.

![Chemical structure of polysulfone (PSf)](image)

**Figure 2-4.** The chemical structure of polysulfone (PSf).

There is one major disadvantage to PSf. The hydrophobic nature of the PSf causes serious complications through the activation of the complement alternative pathway leading to the adsorption of serum proteins onto the membranes [Singh et al. 2003]. Anticoagulants are added during dialysis therapy to avoid blood clotting, but this does not completely eliminate the problem. In order to overcome this disadvantage of the PSf membrane, various studies have been performed to change the material’s surface properties. These investigations include hydrophilic polymer coating [Brink et al. 1993; Kim et al. 1988; Higuchi et al. 2003], layer grafting onto PSf membrane [Wavhal et al. 2002; Song et al. 2000; Pieracci et al. 2002; Mok et al. 1994], and chemical reaction of hydrophilic components onto the membrane surface [Higuchi et al.; 1990; 1991; 1993; Blanco et al. 2001; Nabe et al. 1997; Guiver et al. 1993]. Hydrophilic monomers, 2-hydroxy-ethylmethacrylate (HEMA), acrylic acid (AA), and methacrylic acid (MMA), have also been grafted onto PSf membrane to increase flux and Bovine Serum Albumin (BSA) retention [Ulbricht et al. 1996]. Hancock et al [2000] synthesized polysulfone/poly(ethylene oxide) (PEO) block copolymers to improve the resistance to platelet adhesion. Kim et al. [2003] also studied blending a sulfonated PEO acrylate diblock copolymer into PSf in order to reduce platelet adhesion and enhance
biocompatibility. PEO is a commonly used biomaterial due to its excellent resistance to protein adsorption and inherent biocompatibility [Harris 1992]. Kim et al. [2005] studied a self-transformable copolymer to enhance the hydrophilicity of an asymmetric PSf membrane with an ultra-thin skin layer. The polymer had an entrapped diblock copolymer containing a hydrophilic block of poly (ethylene glycol) (PEG)-SO₃ acrylate and a hydrophobic block of octadecylacrylate (OA). Molecular dynamic (MD) simulations were performed as a function of copolymer density to optimize interfacial structure information. McMurry [2004] developed a strategy using an amphiphilic graft copolymer added to PSf membranes by introducing polysulfone-g-poly (ethylene glycol). When compared to unmodified PSf, these graft copolymer and resulting blend membranes are found to hold promise for biomedical device applications.

Polyamide (PA) membranes have also been used for hemodialysis because of thier mechanical strength in both wet and dry conditions. Polyamide consists of aromatic or/and aliphatic monomers with amide bonding (-CONH-), also known as a peptide bond. The basic amide bond in polyamide is shown in Figure 2-5. R₁ and R₂ can be either aromatic or aliphatic linkage group.

\[ \begin{align*}
\text{O} \\
\sim & \quad \text{C} - \text{N} - \text{R}_2 \sim \\
| & \\
\text{H} 
\end{align*} \]

Figure 2-5. The chemical structure of polyamide (PA).

Panichi and co-workers [1998] evaluated the biocompatibility of the PA membrane and concluded that PA hemofiltration was a highly biocompatible technique due to the use of a synthetic membrane with a sterile re-infusion fluid and the convective removal of
the activated anaphylatoxins and \( \beta_2 \)-Microglobulin (\( \beta_2 \)M). The PA based membrane, Polyflux\textsuperscript{®} (manufactured by Gambro GmbH, Germany) blended with polyamide, polyarylethersulfone and polyvinylpyrrolidone (PVP), was able to clean small molecules such as urea, dreatinine, and phosphate, as well as decrease \( \beta_2 \)M amount by 50.2% [Hoenich et al. 2002]. Due to the non-selectivity of the membrane removal of these unwanted materials also led to the undesirable loss of beneficial proteins during therapy. Meier et al. [2000] evaluated different immune parameters using a modified cellulose low-flux hemophan and synthetic high-flux PA membrane during a 1 year period in chronic hemodialysis patients. They found that the 1-year immunological evaluation of hemodiaysis membrane biocompatibility was associated with changes in the pattern of chronic T-cell actiovation.

Polyacrylonitrile (PAN) is another commonly used membrane material because it is inherently hydrophilic and has been commercialized for ultrafiltration and microfiltration [Scharnagl et al. 2001]. PAN is a semi-crystalline polymer and the mechanical properties strongly depend on the crystalline structures. The chemical structure of PAN is shown in figure 2-6.

\[
\left( \text{CH}_2 - \text{CH}_\text{n} \right)_\text{CN}
\]

Figure 2-6. Chemical structure of polyacrylonitrile (PAN).

The addition of additives such as polyvinylpyrrolidone (PVP) as a pore forming agent, gives PAN membranes more flexible processing parameters and increased performance [Jung et al. 2005]. PAN membrane performance has been optimized through copolymerization with many other vinyl monomers including glycidyl methacrylate
[Godjevargova et al. 1999; Hicke et al. 2002], N-vinylimidazole [Godjevargova et al. 2000], hydroxyl ethyl methacrylate [Ray et al. 1999; Bhat et al. 2000], methacrylic acid [Ray et al. 1999], vinyl pyrrolidone [Ray et al. 1999], acrylic acid [Trotta et al. 2002], acrylamide [Musale et al. 1997], and vinylchloride [Broadhead et al. 1998]. These monomers provide a reactive group for enzyme immobilization, improved mechanical strength, solvent-resistance, pervaporation, permeation flux, anti-fouling and biocompatibility. Because of this, PAN-based copolymer membranes have great potential for the treatment of hemodialysis in an artificial kidney. This material can also be used for other applications like the treatment of wastewater, the production of ultra-pure water, biocatalysis together with separation, and methanol separation by pervaporation.

Lin and his coworker [2004] studied the modification of PAN based dialyzer membranes to increase the hemocompatibility by the immobilization of chitosan and heparin conjugates on the surface of the PAN membrane. When a foreign material is exposed to blood, plasma proteins are adsorbed, clotting factors are activated, and a non-soluble fibrin network, or thrombus, is formatted [Goosen et al. 1980]. The result of this research was that the biocompatible chitosan polymer and a blood anticoagulant heparin prevented blood clotting. They showed prolonged coagulation time, reduced platelet adsorption, thrombus formation, and protein adsorption.

Nie et al. [2004] studied PAN-based ultrafiltration hollow-fiber membranes (UHMFs). In order to improve the membrane performance, acrylonitrile (AN) was copolymerized with other functional monomers such as maleic anhydride and  α-allyl glucoside. They found that the number and size of macrovoid underneath the inner surface of membrane decreased by increasing the amount of solvent DMSA in the
The water flux of the UHFMs also decreased while the bovine serum albumin rejection increased minutely. Godjevargova et al. [1992] modified the PAN based membrane with hydroxylamine and diethylaminoethylmethacrylate to improve membrane dialysis properties. Formed functional groups like primary amine, oxime, and tertiary amine groups, provided the membrane with more hydrophilic properties and a substantial increase in the permeability of the membranes.

The wide use of filtration in practice is limited by membrane fouling. Solute molecules deposit on and in the membrane in the process of filtration causing dramatic reduction in flux through the membrane. Fouling occurs mostly in the filtration of proteins. Three kinetic steps are involved in the fouling of UF membranes according to Nisson [1990]. The first step is the transfer of solute to the surface. The second step is the transfer of solute into the membrane until it either finally adsorbs or passes through after a set of adsorption-desorption events. The third step includes surface binding accompanied by structural rearrangement in the adsorbed state [Ko et al. 1993]. Bryjak et al. [1998] studied the surface modification of a commercially available PAN membrane to develop superior filtration properties with less fouling by proteins. The PAN membrane was immersed in excess NaOH solution to convert some of the surface nitrile groups into carboxylic groups by the hydrolysis process. This modified PAN membrane was not so severely fouled in the Bovine Serum Albumin (BSA) filtration test. The pore size, however, decreased during the hydrolysis process leading to a significant reduction in flux and made the membrane less productive in the ultrafiltration (UF) mode.

2.4 Sorbent Technology

Over the last three decades, sorbent technology [Castino et al, 1976; Korshak et al. 1978; Malchesky et al. 1978] has been further developed to increase the efficiency of
dialysis, or replace it, for the treatment of ESRD. Sorbents remove solutes from solution through specific or nonspecific adsorption depending on both the nature of the solute and the sorbent. Specific adsorption contains tailored ligands, or antibodies, with high selectivity for target molecules. Specific adsorbents have been used in autoimmune disorders such as idiopathic thrombocytopenic purpura [Snyder et al. 1992] and for the removal of lipids in familial hypercholesterolemia [Bosch et al. 1999]. Nonspecific adsorbents, such as charcoal and resins, attract target molecules through various forces including hydrophobic interactions, ionic (or electrostatic) attraction, hydrogen bonding, and van der Waals interactions.

New dialysate with sorbents has become an accepted modification of dialysis, and sorbent hemoperfusion is gaining ground as a valuable addition to dialysis, especially as new sorbents are developed [Winchester et al. 2001]. Hemoperfusion is defined as the removal of toxins or metabolites from circulation by the passing of blood, within a suitable extracorporeal circuit, over semipermeable microcapsules containing adsorbents such as activated charcoal [Samtleben et al. 1996], various resins [Ronco et al. 2001], albumin-conjugated agarose etc. Novel adsorptive carbons with larger pore diameters have been synthesized for potential clinical use [Mikhalovsky 1989]. Newly recognized uremic toxins [Dhondt et al. 2000; Haag-Weber et al. 2000] have resulted in several investigations on alternatives to standard, or high-flux, hemodialysis to remove these molecules. These methods include hemodiafiltration with [de Francisco et al. 2000] or without [Ward et al. 2000; Takenaka et al. 2001] dialysate regeneration using sorbents, as well as hemoperfusion using such adsorbents as charcoal and resins.
Kolarz et al. [1989; 1995] studied the hyper-crosslinked sorbent prepared from styrene and divinylbenzene (DVB) for a hemoperfusion application. They found that the pore structure of a swelling sorbent was changed by additional crosslinking with \( \alpha, \alpha' \)-dichloro-p-xylene in the presence of a tin chloride catalyst and in a dichloroethane solution. They also realized that the hemocompatibility was useful for nemoperfusion and could be imparted to the sorbents by introducing sulfonyl groups at a concentration of about 0.2mmol/g.

A special polymeric adsorbing material (BM-010 from Kaneka, Japan) has been investigated by another group [Furuyoshi et al. 1991] for the selective removal of \( \beta 2M \) from the blood of dialysis partients. The adsorbent consists of porous cellulose beads modified with hexadecyl groups that attract \( \beta 2M \) through a hydrophobic interaction. The adsorption capacity of this material is 1mg of \( \beta 2M \) per 1ml of adsorbent. Using a hemoperfusion cartridge containing 350ml of these cellulose beads in sequence with a high-flux hemodialyzer, several small clinical trials were performed. During 4-5 hours of treatment, about 210mg of \( \beta 2M \) were removed, thus reducing the concentration in the blood by 60-70% of the initial level [Nakazawa et al. 1993; Gejyo et al. 1993; 1995].

RenalTech developed a hemoperfusion device, BetaSorb\textsuperscript{®}, containing the hydrated cross-linked polystyrene (PS) divinylbenzene (DVB) resin sorbents with a pore structure designed to remove molecules between 4 and 30 kDa [Winchester et al. 2002]. In this case, solute molecules are separated according to their size based on their ability to penetrate the porous network of the beaded sorbents. The resin beads were prepared with a blood compatible coating, and confirmed to be biocompatible in vivo in animals [Cowgill et al. 2001].
2.5 Limitation of Current Hemodialysis Treatment

Hemodialysis is a widely used life-sustaining treatment for patients with ESRD. However, it does not replace all of the complex functions of a normal healthy kidney. As a result, patients on dialysis still suffer from a range of problems including infection, accelerated cardiovascular disease, high blood pressure, chronic malnutrition, anemia, chronic joint and back pain, and a considerably shortened life span. One significant limitation of the current dialysis technology is the inability to efficiently remove larger toxic molecules. This is mainly because of the broad pore size distribution reducing the selective removal of toxins, and unsatisfied biocompatibility causing lots of complications such as inflammation, blood clotting, calcification, infection, etc.

Dialysis purifies the patient's blood by efficiently removing small molecules, like salts, urea, and excess water. However, as toxic molecules increase in size, their removal rate by hemodialysis substantially declines. Typically, only 10% - 40% of these middle molecular weight toxins (300-15,000 Da) are removed from the blood during a dialysis session [Vanholder et al. 1995]. These toxins then reach an abnormally high level and begin to damage the body. One such toxin, $\beta_2$M, causes destructive arthritis and carpal tunnel syndrome, by joining together like the links of chain to form a few very large molecules and deposit damaging the surrounding tissues [Lonnemann et al. 2002]. This is also a main cause of mortality for long-term dialysis patients. Other middle molecule toxins appear to inhibit the immune system and may play a significant role in the high susceptibility to infections in dialysis patients. Still others are believed to impair the functioning of several other body systems, such as the hematopoietic and other endocrine systems. This may contribute to accelerated cardiovascular disease, the leading cause of
death among dialysis patients, as well as clinical malnutrition, which affects up to 50% of this patient population.

Over the last decade, polymeric dialysis membranes have been developed to increase the capacity for removing middle molecular weight toxins by changing the pore size of dialyzer membranes and using new materials that adsorb these toxins for improved removal characteristics. However, removal efficiency is not as high as those achieved by a normal healthy kidney.

### 2.6 Latex Particles

The first synthetic polymer synthesized using emulsion polymerization was a rubber composed of 1,3-butadiene and styrene made during World War II in the United States. The Dow chemical company has been a major manufacturer of polystyrene, including latex, which they used in paint formulations. The theory of emulsion polymerization, in which a surfactant is used, was established by Harkins [1948] and by Smith and Ewart [1948]. By 1956 the technology was complete, including the method of building larger diameter particles from smaller ones. The product by this emulsion polymerization is referred to as latex, a colloidal dispersion of polymer particles in water medium [Odian 1991]. Latexes are currently undergoing extensive research and development for a broad range of areas including adhesives, inks, paints, coatings, drug delivery systems, medical assay kits, gloves, paper coatings, floor polish, films, carpet backing and foam mattresses to cosmetics. The relatively well known and easy control of the emulsion process is one of main advantages for these applications. Therefore, polymeric latex particle prepared by emulsion polymerization can be a candidate for the medical applications because of the easy control of the particle size and morphology as well as flexible surface chemistry to be required.
2.6.1 The Components for Emulsion Polymerization

The main components for emulsion polymerization process are the monomer, a dispersing medium, a surfactant, and an initiator. Available monomers are styrene, butadiene, methylmethacrylate, acryl acid, etc. The dispersing medium is usually water, which will maintain a low solution viscosity, provide good heat transfer, and allow transfer of the monomers from the monomer droplets into micelles and growing particles surrounded by surfactants, respectively. The surfactant (or emulsifier) has both hydrophilic and hydrophobic segments. Its main functions is to provide the nucleation sites for particles and aid in the colloidal stability of the growing particles. Initiators are water-soluble inorganic salts, which dissociate into radicals to initiate polymerization. To control the molecular weight, a chain transfer agent such as mercaptan, may be present.

2.6.2 Particle Nucleation

Free radicals are produced by dissociation of initiators at the rate on the order of $10^{13}$ radicals per milliliter per second in the water phase. The location of the polymerization is not in the monomer droplets but in micelles because the initiators are insoluble in the organic monomer droplets. Such initiators are referred to as oil-insoluble initiators. This is one of the big differences between emulsion polymerization and suspension polymerization where initiators are oil-soluble and the reaction occurs in the monomer droplets. Because the monomer droplets have a much smaller total surface area, they do not compete effectively with micelles to capture the radicals produced in solution. It is in the micelles that the oil soluble monomer and water soluble initiator meet, and is favored as the reaction site because of the high monomer concentration compared to that in the monomer droplets. As polymerization proceeds, the micelles grow by the addition of monomer from the aqueous solution whose concentration is refilled by dissolution of
monomer from the monomer droplets. There are three types of particles in the emulsion system: monomer droplets; inactive micelles in which polymerization is not occurring; and active micelles in which polymerization is occurring, referred to as growing polymer particles.

The mechanism of particle nucleation occurs by two simultaneous processes: micellar nucleation and homogeneous nucleation. Micellar nucleation is the entry of radicals, either primary or oligomeric radicals formed by solution polymerization, from the aqueous phase into the micelles. In homogeneous nucleation, solution-polymerized oligomeric radicals are becoming insoluble and precipitating onto themselves or onto the oligomers whose propagation has ended [Fitch et al. 1969]. The relative levels of micellar and homogeneous nucleation are variable with the water solubility of the monomer and the surfactant concentration. Homogeneous nucleation is favored for monomers with higher water solubility and low surfactant concentration and micellar nucleation is favored for monomers with low water solubility and high surfactant concentration. It has also been shown that homogeneous nucleation occurs in systems where the surfactant concentration is below CMC [Roe 1968]. A highly water insoluble monomer such as styrene [Hansen et al. 1979; Ugelstad et al. 1979] has probably created by micellar nucleation, while a water soluble monomer such as vinyl acetate [Zollars 1979] has been formed by homogeneous nucleation.

A third latex formation reaction mechanism has been proposed, referred to as coagulative nucleation. In this reaction, the major growth process for the first-formed polymer particles (precursor particles) is coagulation with other particles rather than polymerization of monomer. The driving force for coagulation of precursor particles,
several nanometers in size, is their relative instability compared to larger sized particles. The small size of a precursor particle with its high curvature of the electrical double layer permits the low surface charge density and high colloidal instability. Once the particles become large enough in size, maintaining the high colloidal stability, there is no longer a driving force for coagulation and further growth of particles takes place only by the polymerization process.

2.6.3 Types of Processes for Emulsion Polymerization

There are three types of production processes used in emulsion polymerization: batch, semi-continuous (or semi-batch), and continuous. In the batch type process, all components are added at the beginning of the polymerization. As soon as the initiator is added and the temperature is increased, polymerization begins with the formation and growth of latex particles at the same time. There is no further process control possible once the polymerization is started. In the semi-continuous emulsion polymerization process, one or more components can be added continuously. Various profiles of particle nucleation and growth can be generated from different orders of component addition during polymerization. There are advantages to this process such as control of the polymerization rate, the particle number, colloidal stability, copolymer composition, and particle morphology. In the continuous process, the emulsion polymerization components are fed continuously into the reaction vessel while the product is simultaneously removed at the same rate. High production rate, steady heat removal, and uniform quality of latexes are advantages of the continuous polymerization processes.

Other available methods include the intermittent addition and shot addition of one or more of the components. In the shot addition process, the additional components are added at one time, during the later stages of the polymerization, prior to complete
conversion of the main monomer. This method has been used successfully to develop water-soluble functional monomers such as sodium styrene sulfonate [Kim et al. 1989].

### 2.6.4 Chemistry of Emulsion Polymerization

Emulsion polymerization is one type of free radical polymerization and can be divided into three distinct stages: initiation, propagation, and termination. The emulsion polymerization system is shown in Figure 2-7.

![Emulsion polymerization system](image)

Figure 2-7. Emulsion polymerization system. [Radicals (R·) are created from initiators (I). Monomer is transferred from larger monomer droplet into micelles by emulsifier. Initiated polymer particle by radicals is keep growing until monomers are all consumed. The reaction is performed in aqueous media]

In the initiation stage, free radicals are created from an initiator by heat or an ultraviolet radiation source. The initiator with either peroxides groups (-O-O-), such as sodium persulfate, or azo groups (-N=N-) such as azobisisobutyronitrile, is commonly used for emulsion polymerization. The primary free radicals created from initiator react with the monomer for initiation of polymerization. In the propagation stage, the polymer chain grows by monomer addition to the active center, a free radical reactive site. There are two possible modes of propagations, head-to-head addition and head-to-tail addition.
The head-to-tail mode is the predominant configuration of the polymer chain, a result of steric hindrance of the substitute bulky group. In the termination stage, polymer chain growth is terminated by either coupling of two growing chains forming one polymer molecule or transferring a hydrogen atom (disproportionation) from one growing chain to another forming two polymer molecules, one having a saturated end group and the other with an unsaturated end-group.

**2.6.5 Seed Emulsion Polymerization**

Polymer latex particles have received increasing interest because of the versatility of the many applications heterophase polymerization processes like emulsion, dispersion, micro-emulsion, seeded emulsion, precipitation, etc. Especially, to prepare well-defined microspheres having monodisperse and various particle sizes as well as surface group functionalities, it is necessary to use seed particles prepared by emulsion polymerization and enlarge them to a desired size in the further stages of reactions.

Polymeric particles are required to have a uniform particle size in many applications, such as chromatography, where they are used as a packing material. Morphological control of latex particle is also important for many practical applications [Schmidt 1972]. Seed emulsion polymerization (or two-stage emulsion polymerization) is a useful method to achieve both monodisperse particle size and morphological design. In seeded emulsion polymerization [Gilbert 1995], preformed ‘seed’ latex is used to control the number of particles present in the final latex. The advantage of seeded emulsion polymerization is that the poorly reproducible process of particle nucleation can be bypassed, so that the number concentration of particles is constant and known. Various mechanisms have been proposed for the growth of latex particles [Ugelstad et al. 1980; Okubo et al. 1992] using this polymerization technique. The initial seed particle
preparation step is well known, and relatively easy to perform because, at the relatively small particle sizes (0.2 to 0.5 micron), the particle growth process can be readily controlled by the use of an emulsifier. Enough emulsifier is used to prevent coagulation but not enough to cause the formation of new particles. As the particles are grown to larger sizes in successive seeding steps it becomes increasingly difficult to maintain a stable, uncoagulated emulsion without forming new particles and thereby destroying monodisperisty of the latex. Recently, there have been several reports concerning the reaction kinetics of seed emulsion polymerization and the development of latex morphologies over the course of the reaction [Chern et al. 1990; Delacal, et al. 1990; Lee et al. 1995; Lee 2000; 2002].

2.6.6 Polystyrene Latex Particles

A number of papers have described the synthesis of the polystyrene latex particles bearing various functional surface groups such as carboxyl [Lee et al. 2000; Tuncel et al. 2002; Reb et al. 2000], hydroxyl [Tamai et al. 1989], marcapto [Nilson 1989], epoxy [Shimizu et al. 2000; Luo et al. 2004], acetal [Izquierdo et al. 2004; Santos et al. 1997], thymine [Dahman et al. 2003], chloromethyl [Izquierdo et al. 2004; Park et al. 2001; Sarobe et al. 1998], amine [Counsin et al. 1994; Ganachaud et al. 1997; Ganachaud et al. 1995; Miraballes-Martinez et al. 2000; 2001; Anna et al. 2005], ester [Nagai et al. 1999], etc. In order to produce these functionalized particles, different methods for particle preparation must be used. The polymer emulsion with core-shell morphology of latex particles is one of them. This is a multistep emulsion polymerization process in which the polystyrene “core” particle is synthesized in the first stage and the functional monomer is added in the second stage of the polymerization without any emulsifier postfeeding to prevent the production of new homopolymer particles, thus forming the functionalized
polymer “shell” on the “core” particle [Keusch et al. 1973]. There are requirements to limit secondary nucleation and encourage core-shell formation in seeded emulsion polymerization including the addition of smaller seed particles at high solid content to increase particle surface area; low surfactant concentration to prevent formation of micelles; and the semi-continuous addition of monomer to create a starved-feed condition and keep the monomer concentration low. There are some advantages [Hergeth et al. 1989] of dispersions with polymeric core-shell particles: First, it is possible to modify the interfacial properties of polymer particles in the aqueous phase by the addition of only very small amounts of a modifying agent during the last period of the reaction. Thus, these core-shell particles are useful in a broad range of applications since they always exhibit improved physical and chemical properties over their single-component counterparts [Lu et al. 1996; Nelliappan et al. 1997]. In this way, the improvement of surface properties of such dispersions is straightforward and inexpensive. The other is that polymers with a core-shell structure are perfect model systems for investigating the material properties of polymer blends and composites because of their regular distribution of one polymer inside a matrix polymer and because of the simple spherical geometry of the system.

Their properties usually depend on the structures of latex particles. Chen and his coworkers [Chen et al. 1991; 1992; 1993] reported the morphological development of core shell latex particles of polystyrene/poly(methyl methacrylate) during polymerization. Before the research by Chen and his coworker, Min et al. [Min et al. 1983] reported the morphological development of core shell latex of polystyrene (PS)/polybutyl acrylateat (PBA) by seeded emulsion polymerization as a function of the
addition method of PS. They found that the percentage of grafting PS to the PBA was greatest for the batch reaction, and the PBA-PS core-shell particles with a high degree of grafting remained spherical upon aging test because of the emulsifying ability of graft copolymer.

2.6.7 Various Applications of Latex Particles

Latex particles are applicable to a wide range of areas such as biomedical applications [Piskin et al. 1994], especially as the solid phase such as in immunoassays [Chern et al. 2003; Radomske-Galant et al. 2003], DNA diagnostic, drug delivery carriers [Luck et al. 1998; Kurisawa et al. 1995; Yang et al. 2000], blood cell separations, and column packing reagents. Thus protein adsorption on polymeric solid surfaces has become a center of attention. Of particular interest are microspheres, defined as “fine polymer particles having diameters in the range of 0.1 to several microns”, which can be used as functional tools by themselves or by coupling with biocompounds. Singer and Plotz [1956] firstly studied microsphere, or latex agglutination test (LAT’s), by using monodisperse polystyrene (PS) and polyvinyltoluene polymer particles as the support on which the biomolecules were going to adsorb. The biomolecule adsorption, however, was limited by possible desorption of the adsorbed species or loss of specific activity of the complex formed. Since this work was published, the latex particle applications for immunoassay have been rapidly and widely studied and developed.

Latex agglutination test, or latex immunoassays, start with tiny, spherical latex particles with a uniform diameter and similar surface properties. The particles are coated with antibodies (sensitized) through the hydrophobic interaction of portions of the protein with the PS surface of the particles. If sensitized particles are mixed with a sample containing antigen, urine, serum, etc. The latex will become agglutinated and visibly
agglomerate. Latex tests are inexpensive as compared with the other techniques [Bangs 1988].

Unipath [Percival 1996] has manufactured a range of immunoassay materials based on a chromatographic principle and deeply colored latex particles for use in the home and clinical environments. The latex particles, which are already sensitized with a monoclonal antibody, can detect any antigens that bound to the surface of the latex particles. Some detectable pollutants include estrogen mimics, which induce abnormalities in the reproductive system of male fishes and lead to a total or partial male feminization. Rheumatoid factor (RF) in different age subpopulations has also been evaluated according to a patient’s clinical status by using a rapid slide latex agglutination test for qualitative and semiquantitative measurement in human serum along with latex immunoassay method [Onen et al. 1998]. Magalhaes and his coworkers [2004] studied a diagnostic method of contamination of male fishes by estrogen mimics, using the production of vitellogenin (VTG) as a biomarker. This was based on a reverse latex agglutination test, developed with monoclonal antibodies specific to this biomarker. Premstaller and his coworkers [Premstaller et al. 2000; 2001] have prepared a porous poly(styrene-divinylbenzene) (PS-DVB) polymer monolith to use for highly efficient chromatographic separation of biomolecules such as proteins and nucleic acids. They used a porogen, a mixture of tetrahydrofuran and decanol, to fabricate a micropellicular PS-DVB backbone. Legido-Quigley and his colleagues [2004] have developed the monolith column to obtain further chromatographic functionality to the column by introducing chloromethylstyrene in place of styrene into the polymer mixture.
Core shell type monodisperse polymer colloids have been synthesized by Sarobe and Forcada [1998] with chloromethyl functionality in order to improve the biomolecule adsorption through a two-step emulsion polymerization process. They investigated the functionalized particles by optimizing the experimental parameters of the functional monomer including reaction temperature, the amount and type of redox initiator system used, the type of addition of the initiator system, and the use of washing. They concluded that the relation between the amount of iron sulfate and the persulfate/bisulfite system added should be controlled to obtain monodisperse particles and prevent the premature coagulation of the polymer particles during the polymerization.

A semi-continuous emulsion polymerization technique for latex particle synthesis was performed by McDonald and other researchers [Ramakrishnan et al. 2004; Steve et al. 1999]. They introduced a variety of particles sizes, compositions, morphologies, and surface modifications to fabricate latex composite membranes (LCMs). Arraying and stabilizing latex particles on the surface of a microporous substrate form narrowly distributed interstitial pores formed between the particles, which serve as separation channels. They investigated the membrane performance using gas fluxes, water permeability, and the retention characterization of dextran molecules. From these tests they concluded that the narrow, discriminating layer made of the latex particles leads to a highly efficient composite membrane.

### 2.7 Proteins

Proteins are natural polyamides comprised of about 20 different α-amino acids of varying hydrophobicity [Norde 1998]. Proteins are more or less amphiphilic and usually highly surface active because of the number of amino acid residues in the side groups along the polypeptide chain which contain positive or negative charges [Norde 1998].
Proteins are polymers of L-α-amino acids [Solomon and Fryhle 2000]. The α refers to a carbon with a primary amine, a carboxylic acid, a hydrogen and a variable side-chain group designated as R. Carbon atoms with four different groups are asymmetric and can exhibit two different spatial arrangements (L and D configurations) due to the tetrahedral nature of the bonds. The L refers to one of these two possible configurations. Amino acids of the D-configuration are not found in natural proteins and do not participate in biological reactions. Figure 2-8 shows the chiral carbon in 3-D as the L isomer. Proteins consist of twenty different amino acids differentiated by their side-chain groups [Norde 1998]. The side-chain groups have different chemical properties such as polarity, charge, and size, and influence the chemical properties of proteins as well as determine the overall structure of the protein. For instance, the polar amino acids tend to be on the outside of the protein when they interact with water and the nonpolar amino acids are on the inside forming a hydrophobic core.

![L-α-amino acid](image)

Figure 2-8. L-α-amino acid.

The covalent linkage between two amino acids is known as a peptide bond. A peptide bond is formed when the amino group of one amino acid reacts with the carboxyl group of another amino acid to form an amide bond through the elimination of water. This arrangement gives the protein chain a polarity such that one end will have a free amino group, called the N-terminus, and the other end will have a free carboxyl group, called the C-terminus [Solomon and Fryhle 2000]. Peptide bonds tend to be planar and
give the polypeptide backbone rigidity. Rotation can still occur around both of the $\alpha$-carbon bonds resulting in a polypeptide backbone with different potential conformations relative to the positions of the R groups. Although many conformations are theoretically possible, interactions between the R-groups will limit the number of potential conformations and proteins tend to form a single functional conformation. In other words, the conformation, or shape of the protein, is due to the interactions of the chain side groups with one another and with the polypeptide backbone. The interactions can be between amino acids that are close together, as in a poly-peptide; between groups that are further apart, as in amino acids; or even on between groups on different polypeptides all together. These different types of interactions are often discussed in terms of primary, secondary, tertiary and quaternary protein structure.

The primary amino acid sequence and positions of disulfide bonds strongly influence the overall structure of protein [Norde 1986]. For example, certain side-chains will promote hydrogen-bonding between neighboring amino acids of the polypeptide backbone resulting in secondary structures such as $\beta$-sheets or $\alpha$-helices. In the $\alpha$-helix conformation, the peptide backbone takes on a 'spiral staircase' shape that is stabilized by H-bonds between carbonyl and amide groups of every fourth amino acid residue. This restricts the rotation of the bonds in the peptide backbone resulting in a rigid structure. Certain amino acids promote the formation of either $\alpha$-helices or $\beta$-sheets due to the nature of the side-chain groups. Some side chain groups may prevent the formation of secondary structures and result in a more flexible polypeptide backbone, which is often called the random coil conformation. These secondary structures can interact with other secondary structures within the same polypeptide to form motifs or domains (i.e., a
tertiary structure). A motif is a common combination of secondary structures and a domain is a portion of a protein that folds independently. Many proteins are composed of multiple subunits and therefore exhibit quaternary structures.

2.7.1 Interaction Forces between Proteins

Proteins in aqueous solution acquire compact, ordered conformations. In such a compact conformation, the movement along the polypeptide chain is severely restricted, implying a low conformational entropy. The compact structure is possible only if interactions within the protein molecule and interactions between the protein molecule and its environment are sufficiently favorable to compensate for the low conformational entropy [Malmsten 1998]. Protein adsorption study is often focused on structural rearrangements in the protein molecules because of its significance to the biological functioning of the molecules and the important role such rearrangements play in the mechanism of the adsorption process. Knowledge of the major interaction forces that act between protein chains and control the protein structures helps to understand the behavior of proteins at interface. These forces include Coulomb interaction, hydrogen bonds, hydrophobic interaction, and van der Waals interactions.

Coulombic interaction. Most of the amino acid residues carrying electric charge are located at the aqueous boundary of the protein molecule. An the isoelectric point (IEP) of the protein, where the positive and negative charges are more or less evenly distributed over the protein molecule, intramolecular electrostatic attraction makes a compact structure favorable to proteins. Deviation to either more positive or more negative charge, however, leads to intramolecular repulsion and encourages an expanded structure. Tanford [1967] calculated the electrostatic Gibbs energy for both a compact impenetrable spherical molecule (protein) and a loose solvent-permeated spherical
molecule (protein) over which the charge is spread out. From the results, he found that the repulsion force was reduced at higher ionic strength due to the screening action of ion.

**Hydrogen bond.** Most hydrogen bonds in proteins form between amide and carbonyl groups of the polypeptide backbone [Malmsten 1998]. The number of available hydrogen bonds involving peptide units is therefore far greater than that involving side chains. Because α-helices and β-sheets are aligned more or less parallel to each other, the interchain hydrogen bonds enforce each other. Kresheck and Klotz [1969] examined the role of peptide-peptide hydrogen bonds and concluded that hydrogen bonds between peptide units do not stabilize a compact structure of protein. However, because the peptide chain is shielded from water due to other interactions, hydrogen bonding between peptide groups do stabilize α-helical and β-sheet structures.

**Hydrophobic interaction.** Hydrophobic interaction refers to the spontaneous dehydration and subsequent aggregation of non-polar components in an aqueous environment. In aqueous solutions of proteins, the various non-polar amino acid residues will be found in the interior of the molecule, thus shielded from water. The intermolecular hydrophobic interaction for the stability of a compact protein structure was first recognized by Kauzmann [1959]. If all the hydrophobic residues are buried in the interior and all the hydrophilic residues are at the outermost border of the molecule, intramolecular hydrophobic interaction would cause a compact protein structure. However, geometrical and other types of interactions generally cause a fraction of the hydrophobic residues to be exposed to the aqueous environment.

**Van der Waals interaction.** The mutual interaction between ionic groups, dipoles and induced dipoles in a protein molecule cannot be established as long as the protein
structure is not known in great detail. Moreover, the surrounding aqueous medium also contains dipoles and ions that compete for participation in the interactions with groups of the protein molecule. Dispersion interactions favor a compact structure. However, because the Hamaker constant for proteins is only a little larger than that of water, the resulting effect is relatively small [Nir 1977].

2.7.2 β2-Microglobulin (β2M)

The protein β2M is of particular interest because it is involved in the human disorder dialysis-related amyloidosis (DRA) [Geiyo et al. 1985; Argiles et al. 1996; Floege 2001]. DRA is a complication in end stage renal failure patients who have been on dialysis for more than 5 years [Bardin et al. 1986; Drueke 2000]. DRA develops when proteins in the blood deposit on joints and tendons, causing pain, stiffness, and fluid in the joints, as is the case with arthritis. In vivo, β2M is present as the non-polymorphic light chain of the class I major histocompatibility complex (MHC-I). As part of its normal catabolic cycle, β2M dissociates from the MHC-I complex and is transported in the serum to the kidney where the majority (95%) is degraded [Floege 2001]. Renal failure disrupts the clearance of β2M from the serum, resulting in an increase in β2M concentration by up to 60-fold [Floege 2001]. By a mechanism that is currently not well understood, β2M then self-associates into amyloid fibrils and typically accumulates in the musculoskeletal system [Homma et al. 1989]. Analysis of ex vivo material has shown that the majority of amyloid fibrils in patients with Dialysis Related Amyloidosis (DRA) is present as of full-length wild-type β2M, although significant amounts (~20-30%) of truncated or modified forms of the protein are also present [Floege 2001; Bellotti et al. 1998]. Figure 2-9 shows the ribbon diagram of human β2M. Native β2M consists of a single chain of 100 amino acid residues and has a seven stranded β-sandwich fold, typical
of the immunoglobulin superfamily [Saper et al. 1991; Trinh et al. 2002]. \( \beta_2 \)M was first isolated from human urine and characterized by Berggard et al. [1980] in 1968. The normal serum concentration of \( \beta_2 \)M is 1.0 to 2.5 mg/L. It is a small globular protein with a molecular weight of 11.8 kDa, a Strokes radius of 16 Å, and a negative charge under physiological conditions (isoelectric point, IEP = 5.7). \( \beta_2 \)M contains two \( \beta \)-sheets that are held together by a single disulphide bridge between the cysteines in positions 25 and 81 [Berggard et al. 1980; Parker et al. 1982; Cunningham et al. 1973]. \( \beta_2 \)M cannot be removed completely by current dialysis techniques but through a better understanding of the structure and interaction forces that lead to this structure, it will be possible to more efficiently remove this problematic protein.

![Ribbon diagram of human \( \beta_2 \)M](image)

Figure 2-9. Ribbon diagram of human \( \beta_2 \)M taken from the crystal structure of the protein bound to the heavy chain of the MHC class I complex (PDB 1DUZ) [Khan et al. 2000]

### 2.7.3 Serum Albumin

Serum albumin is the most abundant protein found in plasma and is typically present in the blood at a concentration of 35~50 g/L. According to extensive studies about its physiological and pharmacological properties, albumin has a high affinity to a very wide range of materials such as electrolytes (\( \text{Cu}^{+2}, \text{Zn}^{+2} \)), fatty acids, amino acids, metabolites, and many drug compounds [Fehske et al. 1981; Kraghansen 1981; Putnam
The most important physiological role of the protein is therefore to bring such solutes in the bloodstream to their target organs, as well as to maintain the pH and osmotic pressure of the plasma. Bovine serum albumin (BSA) is an ellipsoidal protein with the dimensions of 140 X 40 X 40Å [Peter 1985]. The primary structure is a single helical polypeptide of 66 kDa (IEP = 4.7) with 585 residues containing 17 pairs of disulfide bridges and one free cysteine [Dugaiczyk et al. 1982]. BSA has been classified as a soft and flexible protein because it has a great tendency to change its conformation on adsorption to solid surfaces [Kondo et al. 1991; Norde et al. 1992; Soderquist et al. 1980; Carter and Ho, 1994] and consists of three homologous domains (I-III) most likely derived through gene multiplication [Brown 1976]. Each domain is composed of A and B sub-domains [He et al. 1992]. The secondary structure of human serum albumin (HSA) is shown in Figure 2-10.

Figure 2-10. Secondary structure of human serum albumin (HSA) with sub-domains [Zunszain et al. 2003].

HSA has the same structure domains with the serum albumin from other species such as BSA [Brown 1976]. Although all three domains of the albumin molecule have similar three-dimensional structures, their assembly is highly asymmetric [Sugio et al.
1999]. Domains I and II are almost perpendicular to each other to form a T-shaped assembly in which the tail of subdomain IIA is attached to the interface region between sub-domains IA and IB by hydrophobic interactions and hydrogen bonds. In contrast, domain III protrudes from sub-domain IIB at a 45° angle to form the Y-shaped assembly for domains II and III. Domain III interacts only with sub-domain IIB. These features make the albumin molecule heart-shaped.

2.8 Protein Adsorption

Protein adsorption studies date back to the 1930’s. At the beginning, these studies mainly focused on the determination of the molecular weight, electrophoretic and chromatographic applications. Later, the adsorption mechanism, especially the structural rearrangements was studied. Recently, the studies of the relation between protein adsorption and biocompatibility of the sorbent materials were investigated [Norde 1986].

2.8.1 Interaction between Protein Molecule and Latex Particle

There are interaction forces at the interfaces between protein molecules and latex particles. These forces are mainly divided into the following groups: hydrophobic interaction, ionic interaction, hydrogen bonding, and van der Waals interaction [Andrade, 1985].

**Hydrophobic interaction.** It is known that hydrophobic interaction has a major role in protein adsorption phenomena. The adsorption of proteins on the low charged latex particles occurs by this interaction force. Generally, monomers such as styrene offer hydrophobic surfaces that protein molecules adsorb to. The amount of adsorbed protein by this interaction force is maximum at the isoelectric point (IEP) of the protein, and the pH at maximum adsorption shifts to a more acidic region with an increase in ionic strength [Suzawa et al. 1980; 1982; Shirahama et al. 1989; Kondo et al. 1992]. By the
reports [Suzawa et al. 1980; 1982; Lee et al. 1988], protein adsorption was greater on a hydrophobic surface than on a hydrophilic one, implying that hydrophobic interaction is one of the most dominant forces in protein adsorption.

**Ionic interaction.** Negatively charged latex particles have ionic functional groups on their surfaces, such as salts of sulfonic and carboxylic acid. Sulfate groups originate from an initiator such as sodium persulfate, and carboxylic groups originate from a hydrophilic compound such as acrylic acid (AA) or methacrylic acid (MAA). Ionic bonds are formed between the negative charges of these latex particles and positive surface charges of protein molecules. The conventional low-charged latex particles rarely form these ionic bonds.

**Hydrogen bonding.** Hydrogen bond is a strong secondary interatomic bond that exists between a bound hydrogen atom (its unscreened proton) and the electrons of adjacent atoms [Callister 1999]. Protein can be adsorbed on hydrophilic polar surfaces through hydrogen bonding. Hydrogen bonds are frequently formed between hydroxyl-carbonyl or amide-hydroxyl. Hydroxyl-hydroxyl or amide-hydroxyl bonds are also formed in protein adsorption.

**Van der Waals interaction.** This interaction force is operative over small distances, only when water has been excluded and the two non-polar groups come close to each other. Lewin’s calculation showed that the van der Waals interaction is negligible compared with the forces involved in the entropy increases, i.e. hydrophobic interaction [Lewin 1974].

### 2.9 Hypothesis for Toxin Removal

As mentioned earlier, insufficient removal of middle molecular range toxins is a major drawback of current dialysis membrane therapy for end stage renal disease (ESRD)
patients. This may cause destructive arthritis and carpal tunnel syndrome, inhibit the immune system, and accelerate cardiovascular disease leading to death among dialysis patients. In order to overcome these complications, artificial dialysis membranes have been developed to increase the capacity for removing middle molecular weight toxins by changing the pore size of dialysis membranes and using new materials that adsorb these toxins for improved removal characteristics over the last decade. However, removal efficiency is not as high as those achieved by a normal healthy kidney.

With the knowledge obtained from all above literatures, the following hypothesis has been established for the development of a membrane for the successful removal of the target protein, β₂-Microglobulin (β₂M), without the removal of serum albumin. This will be done using a novel membrane design composed of an assembly of engineered polymeric latex particles synthesized to predetermined specifications. Important factors will include pore size and surface chemistry.

2.9.1 Toxin Removal by Size Sieving Based on Monodispersed Pore Size

The packing of monodispersed spherical particles can lead to the formation of porous layers suitable for use as filters and membranes [Hsieh et al. 1991]. The pore size is defined as the largest spherical particles that can pass through the interstitial spaces.

It is well known that monodispersed spherical particles can be obtained from the seed emulsion polymerization method. Many commercially available latex particles are synthesized by this technique and are inexpensive. When the defect free membrane with a monodisperse pore size distribution based on a particular particle array is established, it is expected to outperform the traditional hemodialysis membrane limitation, which is a broad range of pore size distribution. When spherical particles pack in regular crystalline arrays, a number of packing geometries, such as hexagonal closest packing, cubic closest
packing, and body-centered cubic packing, are possible. The pore size of the array depends on these packing geometries as well as particle size. Theoretical pore size can be calculated from the simple geometry of the array. Figure 2-11 shows the relationship between particle size and pore size.

![Figure 2-11](image)

Figure 2-11. The relationship between pore size and particle size in body-centered cubic and closed packed arrays [Steve et al. 1999].

McDonald and his coworkers [Ramakrishnan et al. 2004: Steve et al. 1999] have described the fabrication of latex composite membranes and their performance in previous papers. It is from this prior research, and knowledge gained from conversations with Dr. McDonald, that this current research has been initiated [McDonald 2003]. The focus of this thesis is the extension of McDonald’s research in the area of composite membranes to medical application such as dialysis membranes.

### 2.9.2 Toxin Removal by Selective Adsorption on Engineered Latex Particles

The flexibility in latex surface properties is a significant advantage to this material’s ability to separate proteins since the selectivity is strongly dependent on the charge interactions of both the protein and the latex particles under the conditions of
separation [Menon et al. 1999; Chun et al. 2002]. First, all sorbents were designed to have negative surface properties because most plasma proteins in blood are negative and should not be removed with charge interaction between proteins and sorbents at physiological condition (pH7.4). It was reported that a negatively charged surface was more blood compatible than a positive one [Srinivasan et al. 1971]. The toxin protein, $\beta_2$-Microglobulin ($\beta_2$M) can be selectively adsorbed on the surface of latex particles by the design of a suitable hydrophobic portioned surface where only $\beta_2$M protein can be anchored with hydrophobic interactions. Albumin adsorption on the latex particle is not allowed because charge repulsion is more dominant than hydrophobic interaction with side-on mode. The figure 2-12 shows the schematic representation of the selective adsorption of $\beta_2$M protein on the engineered latex particles.

Figure 2-12. Schematic representation of the protein adsorption on the core shell latex particles at pH 7.4
Hydrophilic/hydrophobic microdomain structures were proven to be more blood compatible [Mori et al. 1982; Higuchi et al. 1993; Deppisch et al. 1998]. The optimization of suitable hydrophobic to hydrophilic ratio is also important for the biocompatibility. The monomers, such as styrene (St), methyl methacrylate (MMA) and acrylic acid (AA) are widely used hydrophobic and hydrophilic monomer models in emulsion polymerization process.

In summary, the background and fundamental literature survey about the history, material properties and limitation of hemodialysis membrane; latex particle preparation, surface chemistry, and manufacturing process; target proteins and protein adsorption; and finally the hypothesis for a toxin removal with high separation efficiency have been suggested. This research focuses on such hypothesis and the materials and characterization methodology for achievement of suggested hypotheses are described in the next chapter.
CHAPTER 3
EXPERIMENTAL AND CHARACTERIZATION METHODOLOGY

As mentioned earlier, the goal of this study is to prepare polymeric latex particles with tailored properties to maximize separation of toxin molecules and to investigate the fundamental interactions between the applied particles and molecules in the biological system to optimize the membrane performance for hemodialysis applications. Polymeric latex particles with monodisperse size distribution to obtain uniform pore size and various size ranges to utilize membrane construction are necessary. Surface engineering with various combination of hydrophobic/hydrophilic domain on the surface of latex particles is expected to affect the removal of target protein by selective adsorption, and to improve the biocompatibility of membrane. Therefore, the materials and characterization methodology are addressed in this chapter.

3.1 Materials

Styrene (St) monomer used for a seed and a core particle, was purchased from Fisher Scientific and used without any other purification process. Acrylic acid (AA), and methyl methacrylate (MMA) monomers introduced for shell formation, were purchased from Fisher Scientific and used without any other purification process. Sodium persulfate (SPS) and sodium bicarbonate (SBC) were obtained from Fishers Scientific and used as received. Divinylbenzene (DVB) crosslinking agent was purchased from Aldrich. The anionic surfactant, Aerosol ®MA80-I [sodium di(1,3-dimethylbutyl) sulfosuccinate], was kindly donated by Cytec. 78-80% Aerosol ®MA80-I is mixed with isopropanol and water. Its critical micelle concentration (CMC) is 24.5 mM (1.19%). Ion exchange resin,
AG® 501-x8 Resin (20-50 mesh), was purchased from Bio-Rad Laboratories, Inc. It is a mixed-bed resin with \( H^+ \) and \( OH^- \) as the ionic forms and is 8% crosslinked. Bovine serum albumin (BSA) (heat shock treated) was purchased from Fisher Scientific. The isoelectric point of the BSA is 4.7-4.9 and the molecular weight is 66,300Da. \( \beta_2 \)-Microglobulin (\( \beta_2 \)M) was purchase from ICN Biomedicals, Inc., and was separated from patients with chronic renal disease, and lyophilized from ammonium bicarbonate. The molecular weight by SDS-PAGE was approximately 12,000Da. BSA and \( \beta_2 \)M proteins were used as received without further purification process. In order to investigate the protein adsorption evaluation, bicinchorinic acid protein assay kits were purchased from Sigma (Cat # BCA-1) and Pierce Biotechnology (Cat # 23225). The chemical structures for the main chemicals are shown in Figure 3-1.

![Chemical structures of main chemicals](image)

Figure 3-1. Chemical structure of main chemicals.
3.2 Latex Particle Preparation

The experimental set up and the particle preparation schemes for various types and size ranges of latex particles are shown in Figure 3-2 and 3-3. In the experimental setup, a mechanical glass stirrer is connected from a motor and Teflon stirrer bar is located at the end of glass stirrer. The agitation rate is precisely adjusted by controller motor. Paar glass reactor with 1L volume is partially in silicon oil bath on hot plate. The reactor has four openings for a stirrer, a thermometer, a nitrogen gas inlet, and a reactant feeding inlet. The reactants for latex polymerization are fed by precisely controlled by metering pump.

The particle preparation is described in each latex preparation section.

Figure 3-2. Experimental setup for semi-continuous emulsion polymerization.
3.2.1 Preparation of Seed Latex Particles

Polystyrene seed particles were synthesized through a typical semi-continuous emulsion polymerization. Polystyrene latex particles were prepared in a four-necked Paar glass vessel equipped with a mechanical glass stirrer, thermometer, glass funnel for nitrogen gas purge, and a tube for monomer feeding. The reactor was placed in a silicon oil bath on a hot plate for homogenous and stable temperature control. The vessel was firstly charged with de-ionized water, the emulsifier, sodium persulfate (SPS) initiator, sodium bicarbonate (SBC), styrene (St) monomer, divinylbenzene (DVB) monomer, under nitrogen gas atmosphere, and then heated to 72±2 °C for 1h. The reaction was allowed to continue for another hour. Second, reactants St and DVB monomers, SPS initiator, and SBC were fed separately using fine fluid metering pumps (Model RHSY, Fluid metering INC, NY) under a nitrogen gas atmosphere for 2h. After complete feeding
of all reactants, the reaction was continued for another hour at elevated temperature, 80±1 °C and then cooled to room temperature by removal of the oil bath.

3.2.2 Preparation of Seeded Latex Particles

The particle growth was achieved by seeded continuous emulsion polymerization. The vessel was charged with the polystyrene seeds and de-ionized water to make desired solid content, and heated up to 72±2 °C for 1h under nitrogen gas atmosphere. Then, St and DVB monomers and solution of SPS and SBC in de-ionized water were continuously added using metering pump with a precisely controlled feed rate. After feeding all reactants, the reaction was continued for three hours keeping temperature of 80±2 °C and then the reaction vessel was cooled down to room temperature.

3.2.3 Preparation of Core Shell Latex Particles

Monodisperse, spherical polystyrene seeds, with a 280nm mean diameter were used for core shell structured latex particle. The pre-polymerized polystyrene seeded emulsion was charged in a 500ml glass flask under a nitrogen gas atmosphere. De-ionized water was added to achieve the desired solid content. The emulsion was heated to 80±2°C for 1.0 hour. Monomers, initiator, and SBC, to form the shell, were added by fluid metering pumps (LAB PUMP JR.,RHSY model, Fluid metering Inc. USA) with fine controlled feed rate. Flow rate can be adjusted by control ring graduated in 450 division from 0 to 100% flow which is in the range of 10ml/min respectively. The reaction was sustained for additional 3.0 hours at 90±2°C, then cooled down to room temperature.

3.2.4 Purification of Synthesized Latex Particles

Synthesized latex particles were purified with an ion exchange resin. 200g (10% w/w polystyrene solid content) of emulsion were mixed with 4g of ion exchange resin and stirred for 40 minute to remove unreacted monomers, initiators and other impurities.
Then the emulsion was diluted with DI water to a specific degree of solid content as needed.

### 3.3 Characterization

#### 3.3.1 Degree of Conversion

The degree of monomer to polymer conversion was determined gravimetrically following the procedure described by Lee et al. [1995] before the latex cleaning process. The synthesized emulsion raw materials were weighed and dried in a conventional oven at 120°C for 30min to evaporate any unreacted monomers and water. The remaining solid was weighed and the degree of conversion was determined. This process was repeated three times and a mean value of the degree of conversion was determined.

#### 3.3.2 Fourier Transform Infrared (FTIR) Spectroscopy

A molecule can absorb only select frequencies (energy) of infrared radiation which match the natural vibration frequencies. The energy absorbed serves to increase the amplitude of the vibrational motions of the bonds in the molecule [Pavin et al. 1996]. Only those bonds which have a dipole moment that changes as a function of time can absorb the infrared radiation. The range of wavelengths for infrared radiation is between 2.5 µm (4000 cm\(^{-1}\)) and 25 µm (400cm\(^{-1}\)).

The synthesis of the latex particles such as polystyrene (PS) homopolymer and PS/PMMA, PS/PMMA\(_{90}\)PAA\(_{10}\), PS/PMMA\(_{75}\)PAA\(_{25}\) core-shell copolymers, were verified with FTIR. Purified latex particles were dried in vacuum oven at 40°C for 24 hours before analysis in the FTIR (Nicolet Magma, USA). Dried latex (10mg) was mixed with 250 mg of KBr, which had also been dried in vacuum oven at 120°C for 3h. Transmission spectra using the drift mode were plotted with 128 scans and 4cm\(^{-1}\) resolution.
3.3.3 Quasielastic Light Scattering (QELS)

Latex particle size was measured by the Brookhaven ZetaPlus particle size analyzer. The solid concentration of latex particles used was 0.001\% (w/v). This instrument uses photon correlation spectroscopy (PCS) of quasielastically scattered light. For this technique, time dependent interference patterns of light scattered from particles in the sample cell are analyzed. The interference pattern changes due to Brownian motion, giving rise to fluctuations in scattering intensity. The frequency of these fluctuations depends on the particle’s diffusion constant that is inversely proportional to the particle diameter.

3.3.4 Field Emission-Scanning Electron Microscopy (FE-SEM)

Particle size and surface morphology were characterized using a FE-SEM (JEOL JSM-6335F, Japan). A diluted suspension of latex particles in water was dropped onto a silicon wafer at room temperature and allowed to dry. The sample was then coated with the thinnest layer of carbon needed to obtain the required conductivity for this instrument. Secondary electron image mode was used with a 15KV of accelerating voltage. The magnification range used was between 10,000X and 70,000X.

3.3.5 Zeta Potential Measurement

Synthesized and purified latex particles were diluted with de-ionized water to 0.01 wt \% and adjusted six different pH values: 2.05, 3.45, 4.81, 5.65, 6.43, and 7.47. These particle suspensions were then transferred to standard cuvettes for zeta potential measurement. At least two runs of ten measurements were taken for each sample and averaged.

The zeta potential measurement was carried out using Brookhaven ZetaPlus zeta-potential analyzer. It measures the electrophoretic mobility, velocity of charged, colloidal
particles in solution, and calculate zeta-potential by using the Smoluchowski equation.

The frequency of laser light passing through the sample and is compared to the frequency of a reference beam. The shift in the frequency, called a Doppler shift, and the magnitude of the shift correspond to the polarity and the magnitude of the electrophoretic mobility, respectively. The zeta potential is calculated from the solution conditions and the measured mobility.

3.3.6 Protein Adsorption

Protein adsorption experiments of the synthesized latex particles were performed with bovine serum albumin (BSA) as a standard model protein and β2-Microglobulin (β2M) as a target protein in two types of buffer solution, phosphate buffer (PB) and phosphate buffered saline (PBS). To make 5 mM of PB solution, 0.345g of sodium phosphate monobasic (NaH$_2$PO$_4$·H$_2$O) was dissolve in 500ml of de-ionized water. To make PBS solution, 0.345g of sodium phosphate monobasic (NaH$_2$PO$_4$·H$_2$O) and 4.178g (143mM) of sodium chloride (NaCl) were dissolved in 500ml of de-ionized water. The synthesized latex particles were diluted with each buffer to have a solids content of 0.5% (w/w), adjusted to pH values of 3.2, 4.8, and 7.4, and mixed with the selected protein. BSA concentrations were chosen to be 0.05, 0.1, 0.3, 0.5, and 0.7 mg/ml and those for β2M were 0.015, 0.030, 0.045, and 0.060mg/ml. The mixture was gently rotated using the shaker, Labquake® (Barnstead/Thermolyne, Model #4002110, USA), with 8 RPM in the incubator at 37°C for 12 hours before the latex-protein mixture was centrifuged at 13,000 rpm for 15min. The amount of protein adsorbed was determined by quantifying the free proteins in the supernatant after the centrifugation process using the bicinchoninic acid (BCA) assay method [Lowry et al. 1951; Smith et al. 1985; Baptista et al. 2003; Wiehelman et al. 1988; Brown et al. 1989]. BCA assay kits consist of Reagent
A, containing bicinchoninic acid, sodium carbonate, sodium tartrate, and sodium bicarbonate in 0.1N NaOH with pH=11.25 and Reagent B containing 4% (w/v) copper (II) sulfate pentahydrate. The BCA working reagent was prepared by mixing 50 parts of Reagent A with 1 part of Reagent B. 100µl of protein supernatant was then mixed with 2ml of BCA working reagent in a UV cuvette. Incubation was allowed to continue further at room temperature until color developed about 2h. The absorbance at 562nm was measured using a UV-VIS spectrophotometer (Perkin-Elmer Lambda 800, USA). Unknown concentration of sample protein was determined by comparison to a standard of known protein concentrations. The adsorbed amount per unit surface area was determined by the mass balance of the protein after adsorption process. The simple schematic of the procedure for a protein adsorption test is shown in Figure 3-4.

Figure 3-4. Schematic of the procedure for a protein adsorption test.
3.3.7 Blood Biocompatibility by Hemolysis Test

Hemolysis is the destruction of red blood cells, which leads to the release of hemoglobin from within the red blood cells into the blood plasma. Hemolysis testing is used to evaluate blood compatibility of the latex particles and the damaged to the red blood cells can be determined by monitoring amount of hemoglobin released. The red blood cells (RBCs) were separated by centrifuging blood at 1500 rpm for 15 minutes. Figure 3-5 shows the phase separated blood with plasma, white blood cells, and red blood cells after centrifugation process. Separated RBSs were then washed with isotonic phosphate buffer solution (PBS) at pH 7.4 to remove debris and serum protein. This process was repeated 3 times.

![Figure 3-5. Separation of RBC from whole blood by centrifuge process.](image)

Prepared latex particles were re-dispersed in PBS by sonification to obtain homogeneously dispersed latex particles. 100µl of the mixture of red blood cell (3 parts) and PBS (11 parts) was added to 1ml of 0.5% (w/w) particle suspension. PBS was used as a negative control resulting 0% hemolysis and DI water used as a positive control to produce 100% hemoglobin released by completely destroyed RBCs. The mixture was incubated in water bath with gentle shaking for 30 minutes at 37°C and then centrifuged at 1500 rpm for 15 minutes. 100µl of the supernatant was mixed with 2ml of the mixture of ethanol (99%) and hydrochloric acid (37%) (EtOH/HCl = 200/5, w/w) to prevent the
precipitation of hemoglobin. In order to remove remaining particles, the mixture was centrifuged again with 13,000 RPM at room temperature. The supernatant was then transferred into the UV cuvette. The amount of hemoglobin release was determined by monitoring the UV absorbance at a wavelength of 397nm. The schematic of the procedure for hemolysis test is shown in Figure 3-6.

Figure 3-6. Schematic of the procedure for hemolysis test.

In summary, a description of the experimental materials and characterization methodology has been explained. In the next chapter, the results and discussion of the data obtained from these experiments is addressed.
CHAPTER 4
RESULTS AND DISCUSSION

4.1 Polymerization of Latex Particles

4.1.1 Polystyrene Seed Latex Particles

Polymer particles have many different applications including spacer particles, lubricants, packing materials for chromatography, standard particles and diagnostic drugs. All applications strongly require these particles to have a uniform particle size. Uniform particle size is the first requirement for a latex composite membrane formed by particle arrays with the interstitial spaces serving as pores for size discriminations [Jons et al. 1999; Ramakrishnan et al. 2004]. Although suspension polymerization has been mainly used as one of conventional polymerization methods to prepare uniform particles, the uniformity of the recovered particles is insufficient for membrane use. There is another method known as seed emulsion polymerization where a vinyl monomer is absorbed into fine monodisperse seed particles and polymerization causes the monomer to increase the sizes of the seed particles uniformly. In order to obtain monodisperse larger particles by this method, the procedure of absorption of monomer into fine polymer particles and polymerization of the monomer is repeated.

In this study, cross-linked polystyrene seed latex particles were firstly synthesized in the presence of styrene monomer, divinylbenzene crosslink agent, sodium persulfate initiator, sodium bicarbonate buffer, and the Aerosol® MA80-I [sodium di(1,3-dimethylbutyl) sulfosuccinate] surfactant using a batch type semi-continuous emulsion polymerization process, in which one or more components can be added continuously.
Styrene (St) monomer and divinylbenzene (DVB) crosslink agent as well as initiator and buffer are continuously added to enlarge the seed latex particles. Various profiles of particle nucleation and growth can be generated from different orders of component addition during polymerization. There are several advantages of this process such as easy control of the polymerization rate, monodisperse particle size and particle number, colloidal stability, copolymer composition, and particle morphology. The main goal in this process is to avoid secondary particle formation leading to a monodisperse particle size distribution. Figure 4-1 shows the schematic representation of seed latex particle preparation and growth.

![Figure 4-1: Schematic representation of semi-continuous seed latex particles preparation and growth.](image)

After the first synthesis of a small size of crosslinked polystyrene (PS) seed particles stabilized by surfactants, styrene, DVB, and initiator are continuously fed into the system to enlarge the seed latex particles maintaining a narrow size distribution. The recipes and reaction conditions for synthesizing these seed particles are summarized in
Table 4-1. The original recipe for latex particle preparation from McDonald was modified for this emulsion system [McDonald 2003].

Table 4-1. The polymerization recipe of polystyrene (PS) seed particles.

<table>
<thead>
<tr>
<th>Latex label</th>
<th>( \text{PS}_{S2.59} )</th>
<th>( \text{PS}_{S2.33} )</th>
<th>( \text{PS}_{S2.07} )</th>
<th>( \text{PS}_{S1.81} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>75 ± 5</td>
<td>75 ± 5</td>
<td>75 ± 5</td>
<td>75 ± 5</td>
</tr>
<tr>
<td>Water(^a) (g)</td>
<td>131.5</td>
<td>131.5</td>
<td>131.5</td>
<td>131.5</td>
</tr>
<tr>
<td>Styrene (g)</td>
<td>99.48</td>
<td>99.48</td>
<td>99.48</td>
<td>99.48</td>
</tr>
<tr>
<td>Divinylbenzene (g)</td>
<td>0.52</td>
<td>0.52</td>
<td>0.52</td>
<td>0.52</td>
</tr>
<tr>
<td>MA 80 (g)</td>
<td>2.59</td>
<td>2.33</td>
<td>2.07</td>
<td>1.81</td>
</tr>
<tr>
<td>NaHCO(_3) (g)</td>
<td>1.12</td>
<td>1.12</td>
<td>1.12</td>
<td>1.12</td>
</tr>
<tr>
<td>Na(_2)S(_2)O(_8) (g)</td>
<td>1.90</td>
<td>1.90</td>
<td>1.90</td>
<td>1.90</td>
</tr>
<tr>
<td>Water(^b) (g)</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Reaction time (min)</td>
<td>140</td>
<td>140</td>
<td>140</td>
<td>140</td>
</tr>
<tr>
<td>% Solids content</td>
<td>39.6</td>
<td>39.6</td>
<td>39.6</td>
<td>39.6</td>
</tr>
<tr>
<td>Mean particle diameter (nm)</td>
<td>126±7.5</td>
<td>171±3.9</td>
<td>182±4.1</td>
<td>216±5.3</td>
</tr>
<tr>
<td>Conversion (%)</td>
<td>96.5</td>
<td>97.9</td>
<td>95.7</td>
<td>97.1</td>
</tr>
</tbody>
</table>

Four different seed latex particles were synthesized based on surfactant amount in order to determine the optimum condition for stable and monodisperse latex particles. The emulsion polymerization of latex particles has to be carried out in a narrow range of surfactant concentration, where particles are stable [Nestor et al. 2005]. A wide range of surfactant concentrations causes flocculation or phase separation leading to broad particle size distribution and an unstable emulsion system. Therefore, the range of surfactant amount was varied from 1.81g to 2.59g in increments of 0.26g in each latex label. Other reactants amounts were kept constant. Water\(^a\) is the initial charge amount and water\(^b\) represents the amount of aqueous solution containing dissolved initiator and buffer,
which are fed continuously after the seed particle creation. The extent of monomer to polymer conversion was obtained by gravimetric calculation and was more than 95% at all seed particles labeled PS\textsubscript{S2.59}, PS\textsubscript{S2.33}, PS\textsubscript{S2.07}, and PS\textsubscript{S1.81}. Labeled subscripts, such as S2.59, S2.33, S2.07 and S1.81, indicate the surfactant amount added for the seed latex preparation. The conversion indicates the percentage of experimentally obtained solid content divided by the theoretically estimated solid content and was calculated by the following formula:

\[
\text{Conversion} \ (\%) = \frac{\text{Experimental solid content}}{\text{Estimated solid content}} \times 100 \quad (4-1)
\]

In this equation, the experimental solid content is the weight of the solid remaining after evaporation of emulsion at 120°C for 30min and the estimated solid content is the weight of monomers calculated from the recipe.

As expected, the seed particle size was different for each surfactant loading in seed emulsion system. A sample of each suspension was diluted by 0.001% (w/w) with deionized water and the mean particle diameter was determined using the Brookhaven ZetaPlus particle size analyzer. From these results, the seed latex particle size increased as the amount of surfactant decreased. The amount of emulsifier also affects the polymerization process by changing the number of micelles and their size. It is known that large amount of emulsifier produces larger numbers of smaller sized particles [Odian 1991] and was corroborated in the current work.

These synthesized seed latex particles should be of narrow size distribution in order to obtain monodisperse larger particles. The SEM image showing the particle morphology and size of a sample of polystyrene seed particles can be seen in the Figure 4-2.
Figure 4-2. Scanning Electron Micrograph (SEM) of polystyrene seed latex particles (A) PS_{S2.59} (B) PS_{S2.33} (C) PS_{S2.07} (D) PS_{S1.81} (subscripts indicate the amount of surfactant added)
Figure 4-2. Continued.
As seen in the image, the seed particles are very smooth and spherical. The uniformity in the particle size distribution of these PS seed latex particles can also be seen in the SEM image. From this data, we concluded that seed particles synthesized in the presence of a surfactant with the chosen amounts are suitable for use to prepare larger particles with high size uniformity and smooth, spherical morphology.

4.1.2 The Growth of Polystyrene (PS) Seed Latex Particles

Seeded emulsion polymerization has been conducted for several decades, and various mechanisms have been proposed. Gracio et al. [1970] suggested that the growing polymer particles consist of an expanding polymer-rich core surrounded by a monomer-rich shell, with the outer shell providing a major locus of polymerization. Seeded emulsion polymerization is commonly used for preparing latex particles less than 1µm in size [Cha et al. 1995; Zou et al. 1990; 1992; Gandhi et al. 1990; Park et al. 1990]. Such latexes can be obtained from the growth of pre-prepared seed particles. The seeds introduced in this process serve as nucleation sites for particle growth. By first swelling the seed latex particles with additional monomer to the desired size, polymerization can then be initiated. Several size ranges of monodisperse polystyrene (PS) latex particles were prepared by this multistep seeded emulsion polymerization method. For the fabrication of particle-based membranes, monodisperse latex particles with a variety of size ranges are required.

In this study, seed particles from 171nm to 470nm in mean diameter and with a highly uniform size distribution were prepared and used. Water\textsuperscript{a} is the initial charge amount and water\textsuperscript{b} represents the amount aqueous solution containing dissolved initiator and buffer that is continuously fed into system. Feed time is precisely controlled by metering pump. The % solid contents were maintained between 19.9% and 30.0%. After
polymerization under these conditions, the conversions of the latex particles labeled as PS\textsubscript{258}, PS\textsubscript{320}, PS\textsubscript{370}, and PS\textsubscript{410}, were 95.2, 95.7, 94.6 and 96.4%, respectively. The recipes used in seeded emulsion polymerization to form the particles less than 500nm in mean diameter are shown at the Table 4-2. The conversions of the latex particles larger than 500nm in mean diameter and labeled as PS\textsubscript{525}, PS\textsubscript{585}, PS\textsubscript{640}, and PS\textsubscript{790}, were 92.3, 84.02, 72.3 and 96.9%, respectively. The recipes used in the preparation of these latex particles are shown at the Table 4-3.

A simple calculation can be used to predict the ultimate size that a latex particle can reach after ending the polymerization. Let’s suppose that seed density ($\rho_s$) and grown particle density ($\rho_{gp}$) are the same. The following equation is obtained from the mass balance ($m = \rho V$), where $m$ and $V$ are mass and volume of a particle, respectively.

$$\frac{m_s}{V_s} = \frac{m_{gp}}{V_{gp}} \tag{4-2}$$

In this equation, $m_s$ and $m_{gp}$ are the weight of seed and total weight of seed and added monomers, respectively; and $V_s$ and $V_{gp}$ are the volume of seed and grown particle, respectively.

If we know the mass of monomer to be added, the ultimate particle size can be calculated from the equation 4-3.

$$\frac{m_s}{m_s + \chi} = \left( \frac{D_1}{D_2} \right)^3 \tag{4-3}$$

where $D_s$ and $D_{gp}$ are the diameters for seed and grown particle, respectively, and $\chi$ is the weight of the monomer added. Crosslinked PS latex particles with a size range from 258±11.2 ~ 410±11.7 in diameter, polymerized by the recipes in Table 4-2 are shown in the SEM images in Figure 4-3.
Table 4-2. Continuous addition emulsion polymerization recipe for growing polystyrene (PS) latex particles less than 500nm in size.

<table>
<thead>
<tr>
<th>Latex label</th>
<th>PS\textsubscript{258}</th>
<th>PS\textsubscript{320}</th>
<th>PS\textsubscript{370}</th>
<th>PS\textsubscript{410}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial charge</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Seed latex (g)</td>
<td>100.0\textsuperscript{171}</td>
<td>100.0\textsuperscript{216}</td>
<td>100.0\textsuperscript{216}</td>
<td>100.0\textsuperscript{320}</td>
</tr>
<tr>
<td>Water\textsuperscript{a} (g)</td>
<td>147.0</td>
<td>250.0</td>
<td>198.4</td>
<td>260.0</td>
</tr>
<tr>
<td>Monomer continuous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Styrene (g)</td>
<td>80.0</td>
<td>120.0</td>
<td>80.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Divinylbenzene (g)</td>
<td>0.6</td>
<td>0.7</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Feed time (min)</td>
<td>0-163</td>
<td>0-110</td>
<td>0-75</td>
<td>0-82</td>
</tr>
<tr>
<td>Initiator stream</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na\textsubscript{2}S\textsubscript{2}O\textsubscript{8} (g)</td>
<td>0.7</td>
<td>0.65</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>NaHCO\textsubscript{3} (g)</td>
<td>0.7</td>
<td>0.65</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Water\textsuperscript{b} (g)</td>
<td>75.0</td>
<td>120.0</td>
<td>20.0</td>
<td>37.0</td>
</tr>
<tr>
<td>Feed time (min)</td>
<td>0-167</td>
<td>0-140</td>
<td>0-100</td>
<td>0-92</td>
</tr>
<tr>
<td>% Solid content</td>
<td>28.4</td>
<td>30.0</td>
<td>30.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Mean particle diameter (nm)</td>
<td>258±11.2</td>
<td>320±15.4</td>
<td>182±7.1</td>
<td>410±11.7</td>
</tr>
<tr>
<td>Conversion (%)</td>
<td>95.2</td>
<td>95.7</td>
<td>94.6</td>
<td>96.4</td>
</tr>
</tbody>
</table>

* The solid content of seed latex for PS\textsubscript{258}, PS\textsubscript{320}, PS\textsubscript{370}, and PS\textsubscript{410} are 39.2, 39.9, 39.9, and 30.0\%, respectively.
Table 4-3. Continuous addition emulsion polymerization recipe for growing polystyrene (PS) latex particles larger than 500nm in size.

<table>
<thead>
<tr>
<th>Latex label</th>
<th>PS$_{525}$</th>
<th>PS$_{585}$</th>
<th>PS$_{640}$</th>
<th>PS$_{790}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial charge</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Seed latex (g)</td>
<td>100.0$^{390}$</td>
<td>100.0$^{470}$</td>
<td>100.0$^{470}$</td>
<td>100.0$^{320}$</td>
</tr>
<tr>
<td>Water$^a$ (g)</td>
<td>100.0</td>
<td>52.0</td>
<td>120.0</td>
<td>36.0</td>
</tr>
<tr>
<td>Monomer continuous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Styrene (g)</td>
<td>29.0</td>
<td>20.0</td>
<td>40.0</td>
<td>17.3</td>
</tr>
<tr>
<td>Divinylbenzene (g)</td>
<td>0.2</td>
<td>0.14</td>
<td>0.3</td>
<td>0.09</td>
</tr>
<tr>
<td>Feed time (min)</td>
<td>0-80</td>
<td>0-40</td>
<td>10-76</td>
<td>5-65</td>
</tr>
<tr>
<td>Initiator stream</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na$_2$S$_2$O$_8$ (g)</td>
<td>0.2</td>
<td>0.14</td>
<td>0.3</td>
<td>0.12</td>
</tr>
<tr>
<td>NaHCO$_3$ (g)</td>
<td>0.2</td>
<td>0.14</td>
<td>0.3</td>
<td>0.09</td>
</tr>
<tr>
<td>Water$^b$ (g)</td>
<td>20.0</td>
<td>120.0</td>
<td>40.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Feed time (min)</td>
<td>0-85</td>
<td>0-46</td>
<td>0-77</td>
<td>0-75</td>
</tr>
<tr>
<td>% Solids content</td>
<td>19.9</td>
<td>20.0</td>
<td>20.0</td>
<td>20.1</td>
</tr>
<tr>
<td>Mean particle diameter (nm)</td>
<td>525±15.1</td>
<td>585±18.6</td>
<td>640±17.8</td>
<td>790±69.3</td>
</tr>
<tr>
<td>Conversion (%)</td>
<td>92.3</td>
<td>84.02</td>
<td>72.3</td>
<td>96.9</td>
</tr>
</tbody>
</table>

* The solid content of seed latex for PS$_{525}$, PS$_{585}$, PS$_{640}$, and PS$_{790}$ are 20.0, 18.2, 19.3, and 17.3%, respectively.
Figure 4-3. SEM of PS latex particles less than 500nm in size (A) 258nm (B) 320nm (C) 370nm (D) 410nm
Figure 4-3. Continued.
Particle size was well matched to that estimated by the geometrical calculation. From this it can be concluded that the added monomer was consumed for polymer particle growth with a high degree of conversion. From the SEM characterization, newly nucleated particles were not seen, as indicated by the narrow particle size distribution. The particles less than 500nm in mean diameter were very spherical in shape with a highly uniform particle size distribution. As the particles were grown larger than 500nm mean diameter, however, they became of non-spherical shape with an uneven surface. This irregularity of particle surface can be attributed to the non-homogeneous monomer swelling into the shell of a growing polymer, which can be controlled by the factors such as temperature, agitation speed, initiator feeding rate, and surfactant amount required to stabilize the colloidal system. The SEM image of latex particles larger than 500nm mean diameter is shown in Figure 4-4. The particle size distribution is broader than that of the smaller particles. These particles can, however, still be used for membrane construction as a support layer rather than skin layer. The support layer does not necessarily have to be as highly monodisperse as the skin layer, which require pores of high uniformity for the selective removal of toxins.

As mentioned earlier, the amount of surfactant as well as monomer in an emulsion system is the primary determinant of the particle diameter [Odian 1991]. Anionic surfactants are generally recommended at the level of 0.2-3.0 wt % based on the amount of water [Odian 1991]. The critical micelle concentration (CMC) of Aerosol® MA 80-I anionic surfactant is 1.19 %.The surfactant loading in this emulsion system ranged from the 1.21 % for $S_{S1.81}$ to 1.71% for $S_{S2.59}$. 
Figure 4-4. SEM of PS latex particles larger than 500nm in size (A) 525nm (B) 585nm (C) 640nm (D) 790nm
Figure 4-4. Continued.
The surfactant to monomer ratio refers to the value of the surfactant amount divided by monomer amount after polymerization ends. The surfactant amount is the weight of the surfactant contained in emulsion and the monomer amount is the total weight of the monomer added for polymerization to form the ultimate polymer particles in emulsion. As the surfactant to monomer ratio decreases, mean particle diameter increases, as described by Odian [1991]. Generally, lower surfactant concentration forms fewer micelles resulting in larger particle size [Evans et al. 1999]. However, there is a plateau in particle mean diameter between a surfactant to monomer ratio of 0.005 and 0.013. This is due to low polymerization conversion of latex particles from monomer leading to a smaller particle size than expected. This is not a factor of surfactant amount. The dependence of the particle size on the amount of surfactant is shown in Figure 4-5.

Figure 4-5. Dependence of the particle size on the surfactant to monomer ratio.
4.1.3 Core Shell Latex Particles

The latex particles shown in Figure 4-3 (A) and labeled as $S_{258}$ in Table 4-2 were used as seeds to prepare core-shell latex particles because of their high particle uniformity and smooth surface morphology. Methyl methacrylate (MMA) and acrylic acid (AA) monomers were introduced to increase surface hydrophilicity over that of bare polystyrene particles. AA is a more hydrophilic monomer than MMA because of the carboxyl acid group in AA is more favorable for hydrogen bonding with water. The surface carboxyl groups on PAA may have many promising applications in biomedical and biochemical fields [Kang et al. 2005]. MMA monomer is less hydrophobic than styrene. PMMA is known to more biocompatible than PS but still PMMA is hydrophobic. The optimization of suitable hydrophobic to hydrophilic ratios and control of microdomain structures is important for biocompatibility [Mori et al. 1982; Higuchi et al. 1993; Deppisch et al. 1998]. Figure 4-6 shows the schematic of core shell latex particle structure. The recipe for core shell structures is shown in Table 4-4.

![Core shell latex particles](image)

Figure 4-6. Schematic of core shell latex particle structures.
Table 4-4. The preparation recipe of PS core with various shell latex particles.

<table>
<thead>
<tr>
<th>Latex label</th>
<th>PS/PMMA$_{100}$</th>
<th>PS/PMMA$<em>{90}$PAA$</em>{10}$</th>
<th>PS/PMMA$<em>{75}$PAA$</em>{25}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial charge</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS seed latex (g)</td>
<td>50.0$^{258}$</td>
<td>50.0$^{258}$</td>
<td>50.0$^{258}$</td>
</tr>
<tr>
<td>Water$^a$ (g)</td>
<td>157.0</td>
<td>157.0</td>
<td>157.0</td>
</tr>
<tr>
<td><strong>Monomer continuous</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methly methacrylate (g)</td>
<td>32.0</td>
<td>28.8</td>
<td>24.0</td>
</tr>
<tr>
<td>Acrylic acid (g)</td>
<td>0.0</td>
<td>3.2</td>
<td>8.0</td>
</tr>
<tr>
<td>Feed time (min)</td>
<td>0-120</td>
<td>0-50</td>
<td>0-60</td>
</tr>
<tr>
<td><strong>Initiator stream</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na$_2$S$_2$O$_8$ (g)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>NaHCO$_3$ (g)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Water$^b$ (g)</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Feed time (min)</td>
<td>0-122</td>
<td>0-60</td>
<td>0-65</td>
</tr>
<tr>
<td>% Solids content</td>
<td>18.3</td>
<td>18.3</td>
<td>18.3</td>
</tr>
<tr>
<td>Mean particle diameter (nm)</td>
<td>370±18.6</td>
<td>370±17.8</td>
<td>370±19.1</td>
</tr>
<tr>
<td>Conversion (%)</td>
<td>95.5</td>
<td>92.4</td>
<td>95.2</td>
</tr>
</tbody>
</table>

Three types of core shell structures were prepared. PMMA$_{75}$PAA$_{25}$ shell is a copolymer consisting of the PMMA to PAA ratio of 75% to 25% by weight. PMMA$_{90}$PAA$_{10}$ shell is a copolymer with the PMMA to PAA ratio of 90% to 10% by weight. PMMA100 is the PMMA homopolymer shell on PS core. The particle size of these core-shell particles as well as PS latex particle was prepared to be about 370nm in mean diameter and are characterized to determine their zeta potential, protein adsorption, and biocompatibility. The image of SEM of PS and core shell particles is shown at Figure 4-7. The synthesized latex particles had a high uniformity in size and a smooth, spherical surface morphology.
Figure 4-7. Scanning Electron Micrograph of latex particles (A) PS (B) PS/PMMA_{100} (C) PS/PMMA_{90}PAA_{10} (D) PS/PMMA_{75}PAA_{25}
Figure 4-7. Continued.
4.2 Characterization of Latex Particles

4.2.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy has proven to be a powerful tool to characterize polymeric materials. A molecule can absorb only select frequencies (energy) of infrared radiation which match the natural vibration frequencies. The energy absorbed serves to increase the amplitude of the vibrational motions of the bonds in the molecule [Pavin et al. 1996]. Only those bonds which have a dipole moment that changes as a function of time can absorb infrared radiation. The FTIR spectra of polymerized latex particles made during this work are shown in Figure 4-8. There are number of characteristic peaks for PS latex particles [Bhutto et al. 2003; Li at al. 2005]. Aromatic C-H stretching vibration is shown at 3002 cm\(^{-1}\) and 3103 cm\(^{-1}\), and aliphatic C-H asymmetrical and symmetrical stretching is shown at 2900 cm\(^{-1}\) and 2850 cm\(^{-1}\), respectively. There is an additional carbonyl (C=O) adsorption at 1730 cm\(^{-1}\) wavenumber for all core shell latex particles containing some amount of either or both PMMA and PAA. The broad OH group peak at 3400 cm\(^{-1}\) appear for particles containing some amount of PAA and has intensity dependent on the amount of PAA in the shell. For example, the peak intensity of OH groups for PS/PMMA\(_{75}\)PAA\(_{25}\) is greater than that of PS/PMMA\(_{90}\)PAA\(_{10}\) since there is a higher AA monomer content in PS/PMMA\(_{75}\)PAA\(_{25}\) than in PS/PMMA\(_{90}\)PAA\(_{10}\). This OH peak is not seen for PS and PS core PMMA shell particles because of the absence of OH groups on PS and PMMA polymer chains.

4.2.2 Zeta Potential Measurements

The electrical surface property was characterized by zeta potential measurements of latex particles. Zeta potential is an important and useful indicator of surface charge which can be used to predict and control the stability of colloidal systems. The greater the zeta
Figure 4-8. FTIR spectra of polymerized latex particles. (A) bare polystyrene (PS) (B) PS/PMMA$_{100}$ (C) PS/PMMA$_{90}$PAA$_{10}$ (D) PS/PMMA$_{75}$PAA$_{25}$
potential the more likely the suspension is to be stable because the charged particles repel one another and thus overcome the natural tendency to aggregate. The measurement of zeta potential is often the key to understanding and control of the interaction of proteins with solid surfaces such as latex particles.

Zeta potential ($\zeta$) is the electrical potential that exists at the shear plane of a particle, which is some small distance from the surface [Myers 1999]. The zeta potential of the particles can be determined by measuring the mobility of the particles in an applied electric field, termed electrophoretic mobility, or its response to an alternating electric field. Colloidal particles dispersed in a solution are electrically charged due to their ionic characteristics and dipolar attributes. The development of a net charge at the particle surface affects the distribution of ions in the neighboring interfacial region, resulting in an increased concentration of counter ions to the surface. Each particle dispersed in a solution is surrounded by oppositely charged ions called fixed layer or Stern layer. Outside the Stern layer, there are varying compositions of ions of opposite polarities, forming a cloud-like area. Thus an electrical double layer is formed in the region of the particle-liquid interface. This double layer may be considered to consist of two parts: an inner region which includes ions bound relatively strongly to the surface and an outer, or diffuse region in which the ion distribution is determined by a balance of electrostatic forces and random thermal motion. The potential in this region, therefore, decays with the distance from the surface, until at a certain distance it becomes zero. Figure 4-9 shows a schematic representation of a typical ion distribution near a positively charged surface. The Stern surface (also referred to as the outer Helmholtz plane) is drawn through the center of those ions, which are effectively adsorbed to the surface [Hunter 1981]. The
extent of ion adsorption is determined by electrical and other long-range interactions between the individual ions and surface of particles. The ions outside of the Stern layer form the diffuse double layer, also referred to as the Gouy-Chapman layer [Burns and Zydney 2000].

![Schematic representation of ion distribution near a positively charged surface.](image)

Zeta potential is a function of the surface charge of a particle, any adsorbed layer at the interface and the nature and composition of the surrounding medium in which the particle is suspended. The principle of determining zeta potential is very simple. A controlled electric field is applied via electrodes immersed in a sample suspension and
this causes the charged particles to move towards the electrode of opposite polarity. Viscous forces acting upon the moving particle tend to oppose this motion and the equilibrium is rapidly established between the effects of the electrostatic attraction and the viscosity drag. The particle therefore, reaches a constant terminal velocity.

Figure 4-10. Schematic representation of zeta potential measurement.(source: http://nition.com/en/products/zeecom_s.htm)

Because protein adsorption mechanisms are very complex and hard to explain in biological systems, protein adsorption is generally studied by using more ideal systems consisting of one or more well characterized proteins, a well-characterized adsorbent, and a well-defined aqueous solution. Even so, small changes in the experimental conditions, such as pH, ionic strength, and temperature generate totally different results. Therefore, two media systems, phosphate buffer (PB) and phosphate buffered saline (PBS), were chosen to be used for the zeta potential measurements and protein adsorption study. PB is a simple aqueous system of 5 mM monobasic sodium phosphate (NaH$_2$PO$_4$·H$_2$O) to maintain a constant pH. PBS is used to keep the water concentration inside and outside of the cell balanced. If the water concentration is unbalanced, the cell risks bursting, or shriveling up because of a phenomenon called osmosis. PBS is a solution with the 5 mM of monobasic sodium phosphate (NaH$_2$PO$_4$·H$_2$O) and 143 mM of sodium chloride (NaCl) in water.
The results of zeta potential measurement at room temperature (25±1°C) as function of pH and ionic strength (different media, PB and PBS) for synthesized latex particles are seen in Figure 4-11. Zeta potential values of polystyrene (PS) latex particles at the Figure 4-9 are negative between -29.1 mV and -59.9 mV in PB and between -20.3 mV and -27.8 mV in PBS at all pH ranges. These negative zeta potential values of PS latex particles are due to the sulfate groups [Vandenhu et al. 1970] originated from persulfate initiators attached via an oxygen bridge (-C-O-SO$_3^-$) at the end of polymer chain where polymerization was initiated.

![Figure 4-11. Zeta potential of PS latex particles at 25°C.](image)

The zeta potential measurements of PS/PMMA$_{100}$ core shell latex particles, as seen in Figure 4-12, were also negative with a range of -28 and -50.5 mV in PB and between -14.3 mV and -18.6 mV in PBS at the pH 2.1-7.8. These negative values are also due to sulfate groups on the particle surface.
Figure 4-12. Zeta potential of PS/PMMA\textsubscript{100} latex particles at 25°C.

The zeta potential for PS/PMMA\textsubscript{90}PAA\textsubscript{10} and PS/PMMA\textsubscript{75}PAA\textsubscript{25} was also negative, as seen in Figure 4-13 and 4-14. The zeta potential value for the PS/PMMA\textsubscript{90}PAA\textsubscript{10} were between -36.7 mV and -67.8 mV in PB medium and between -14.7 mV and -19.3 mV in PBS both at 25°C. In case of the PS/PMMA\textsubscript{75}PAA\textsubscript{25} core shell particles, zeta potential values were between -29.1 mV and -52.0 mV in PB and between -11.5 mV and -21.0 mV in PBS media. Sulfate groups in PS/PMMA\textsubscript{75}PAA\textsubscript{25} and PS/PMMA\textsubscript{90}PAA\textsubscript{10} core shell particles contribute to the negative zeta potential values. Carboxylic groups from acrylic acid monomer also would participate in forming a negative surface charge of the latex particles at a pH greater than 7.0 because the pK\textsubscript{a} value for the carboxylic acid is between 4.0 and 6.0 pH range [Ottewill et al. 1967]. This indicates that carboxylic acid dissociation begins at a pH between pH 4.6-6.0 and increases with pH. The strength of an acid is expressed by the term pK\textsubscript{a}, which is the pH at which an acid is 50% dissociated. However, the contribution of the carboxylic acid...
group to negative zeta potential values of PS/PMMA-PAA core shell particles was not
detectable because there was no significant difference in zeta potential values for
synthesized latex particles at a pH greater than 7.0.

Figure 4-13. Zeta potential of PS/PMMA$_{90}$PAA$_{10}$ latex particles at 25°C.

Figure 4-14. Zeta potential of PS/PMMA$_{75}$PAA$_{25}$ latex particles at 25°C.
The zeta potential plots and values were similar for all latex particles. This may be due to the similar initiator density on the latex surface. The initiator concentrations to monomers for shell polymerization were the same for all core shell latex particles (0.62% w/w from Table 4-4). A similar concentration (0.55% w/w) of initiator to monomer was added to prepare bare PS latex particles used in zeta potential measurements.

Since the zeta potential values of all synthesized latex particles were negative between pH=2.0 and pH=7.8, the isoelectric point (IEP) of these latex particles would be less than pH=2.0. Sulfates have a $pK_a$ in the range of 1.0 to 2.0 [James 1985], indicating that the sulfate group, the conjugate base of a strong acid, is protonated (-C-OSO$_3$H) at less than pH 2.0. This means that the synthesized latex particles are all negative at the physiological pH. Most serum proteins have a negative surface charge in blood, therefore, when the negative latex particles are applied to blood stream, fatal complication by coagulation between applied latex particles and serum proteins is avoided by charge repulsion in the blood. Table 4-5 shows the common blood proteins and their isoelectric points (IEP).

<table>
<thead>
<tr>
<th>Serum proteins</th>
<th>Isoelectric point (IEP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>4.8</td>
</tr>
<tr>
<td>$\alpha_1$-Globulin</td>
<td>2.0</td>
</tr>
<tr>
<td>$\alpha_2$-Microglobulin</td>
<td>5.4</td>
</tr>
<tr>
<td>$\gamma_1$-Globulin</td>
<td>5.8</td>
</tr>
<tr>
<td>Heptoglobin</td>
<td>4.1</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>7.2</td>
</tr>
<tr>
<td>$\beta_1$-lipoprotein</td>
<td>5.4</td>
</tr>
<tr>
<td>Immunoglobulin G (Ig G)</td>
<td>7.3</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>5.8</td>
</tr>
</tbody>
</table>

(source: http://www.fleshandbones.com/readingroom/pdf/1085.pdf; http://www.medal.org/visitor/www%5CActive%5Cch13%5Cch13.01%5Cch13.01.05.aspx)
The absolute zeta potential values of the latex particles in phosphate buffer (PB) was higher than that in phosphate buffered saline (PBS). This is because high concentration of sodium and chloride electrolytes in PBS compressed the electrical double layer causing rapid decay of surface potential. Figure 4-15 shows that the thickness of the electrical double layer is inversely proportional to the concentration of electrolyte in the system.

Figure 4-15. The decay of surface potential with distance from surface in various electrolyte concentrations: (1) low (2) intermediate (3) high [Myers 1999].

The electrical potential in the solution surrounding the particle surface falls off exponentially with distance (Z) from the surface according to the Debye-Huckel approximation [Myers 1999]

\[ \psi = \psi_0 \exp(-\kappa Z) \]  

(4-4)

where \( \kappa \) is the reciprocal of the thickness of the electrical double layer, referred to as the Debye length. The potential fallen off by a factor of \( 1/e \). The theoretical equation for the double layer thickness, \( 1/\kappa \), is

\[ \frac{1}{\kappa} = \left( \frac{\varepsilon_0 \varepsilon kT}{e^2 \sum c_i z_i^2} \right)^{1/2} \]  

(4-5)
where $\varepsilon_0$ is the permittivity of a vacuum of free space, $\varepsilon$ is the permittivity of a medium, $\epsilon$ is the relative permittivity or dielectric constant of the medium, $c$ is the concentration of an ion $(i)$, $z$ is the valency of an ion $(i)$, $k$ is Boltzmann’s constant, $T$ is absolute temperature (K).

4.3 Protein Adsorption Study

The adsorption study was performed to measure the protein adsorption on the synthesized latex particles. Many physical and chemical processes occur at the boundary between two phases and adsorption is one of the fundamental surface phenomena [Oscik 1982]. This phenomenon is driven by the change in the concentration of the components at the interface with respect to their concentrations in the bulk phase. There are two adsorption processes, physical adsorption occurring when non-balanced physical forces appear at the boundary of the phases, and chemical adsorption or chemisorption occurring when atoms and molecules from adjacent phases form chemical bonds at the interface [Jaroniec et al. 1988].

Flexible proteins in solution possess high conformational entropy as a result of the various states each of the many segments in the protein chain can have. Adsorption leads to a reduction of this conformational entropy, hence, adsorption takes place only if the loss in conformational entropy is compensated by sufficient attraction between polymer segment and solid surface [Martin 1998]. Isotherm shape of high affinity adsorption between polymer and solid is shown in Figure 4-16 where the adsorbed mass, $\Gamma$ is plotted against the polymer concentration in solution after adsorption, $C_p$. The initial part of the isotherm merges with the $\Gamma$-axis because at low polymer concentration, the protein has a high affinity for the surface and all of the polymer is adsorbed until the solid surface is saturated.
Figure 4-16. High-affinity adsorption isotherm of typical flexible polymer on solid surface.

In order to investigate the adsorption of blood proteins, latex particle properties such as electrical surface charge, hydrophobicity, and environmental conditions like pH, ionic strength, and temperature are considered as experimental factors [Arai and Norde 1990]. Figure 4-17 shows the overall schematic representation of the protein adsorption on the synthesized latex particles with the functional groups such as sulfate and carboxyl groups.

Figure 4-17. Overall schematic representation of the protein adsorption on the synthesized latex particles.

The surface functional groups as well as suitable surface hydrophobicity / hydrophilicity of latex particles are closely related to protein adsorption property. As indicated at Figure 4-17, various interaction forces such as hydrophobic interaction, ionic
interaction, hydrogen interaction, and van der Waals interaction [Andrade 1985] exist between protein molecule and the solid surface of latex particle.

### 4.3.1 Adsorption Isotherm

The protein adsorption isotherm is generally defined as the relationship between the amount of protein adsorbed by a solid (latex particle) at a constant temperature and as a function of the concentration of protein. Adsorption isotherms were plotted to determine the amount of protein adsorbed onto latex particles. To evaluate the equilibrium concentration of target proteins, a calibration curve is firstly needed. Figure 4-18 shows the UV absorbance as a factor of bovine serum albumin (BSA) concentrations. The UV absorbance of the known protein concentration was measured at 562nm, which is the $\lambda_{\text{max}}$ for a protein treated by a bicinchoninic acid (BCA) assay.

![Figure 4-18. The dependence of UV absorbance on BSA concentration. (A) 1.0mg/ml (B) 0.6mg/ml (C) 0.2mg/ml](image)

Figure 4-19 shows the standard calibration curve used in adsorption isotherm experiment of protein onto latex particles. The net absorbance of each known protein
concentration was plotted and then a linear equation passing through a concentration of zero protein concentration was obtained. This equation is applied to determine the unknown protein amount adsorbed on the synthesized latex particles. The amount of free protein was also determined using UV light at the 280nm wavelength, however, a distinguishable UV intensity was too low to determine a precise value of protein adsorption.

The bicinchninic acid (BCA) assay technique [Smith et al. 1985] has been reported for high efficiency of protein evaluation and was used for determination of protein adsorption [Tangpasuthadol et al. 2003; Williams et al. 2003]. This technique has many advantages, such as high sensitivity, easy of use, color complex stability, and less susceptibility to any detergents. Five different BSA concentrations (0.05, 0.1, 0.3, 0.5, and 0.7mg/ml) were used to generate this curve as well as the adsorption isotherm.

![Figure 4-19. Standard curve of net absorbance vs BSA sample concentration.](image)
All isotherms were fitted to the Langmuir-Freundlich isotherms using the nonlinear regression method. Two adsorption isotherm models, the Langmuir type isotherm and the Langmuir-Freundlich combination type isotherm, were compared. As seen in Figure 4-20, the Langmuir isotherm model deviated further from the experimental data. Therefore, all isotherms were fitted to the Langmuir-Freundlich isotherms [Yoon et al. 1996; Lee et al. 1998] using the nonlinear regression method with the following equation,

\[
q = q_m \frac{kC_{eq}^{\frac{1}{n}}}{1 + kC_{eq}^{\frac{1}{n}}}
\]  

(4-6)

where \( q \) is the amount of adsorbed BSA per unit surface area, \( q_m \) is the adsorbed amount in equilibrium, \( C_{eq} \) is the equilibrium concentration of BSA, \( k \) is the adsorption constant, and \( n \) is the exponential factor for a heterogeneous system. \( n \) values are empirical and usually from zero to no more than unity [Jaekel 2002]. The values of \( n \) in our system were between 0.45 and 0.55, and hence \( n \) was fixed to its average 0.5. Actual \( q_m \) values were evaluated after fixing the \( n \) value.

![Figure 4-20. Fitted models for adsorption isotherm of bovine serum albumin (BSA) on polystyrene latex particles at 37°C in phosphate buffer media.](image-url)
The BSA protein adsorption on the synthesized latex particles was performed at three pH levels, 3.2, 4.8, 7.4, in both phosphate buffer (PB) and phosphate buffered saline (PBS), at physiological temperature, 37°C. These pH levels correspond to an acidic pH lower than the isoelectric point (IEP) of BSA, the pH at the IEP, and an alkaline pH higher than the IEP of BSA, respectively. The IEP corresponds to the pH at which the amino acid becomes a zwitterion. Here its overall electrostatic charge is neutral and the charge expressed by the basic amino group (-NH₂, -NH₃⁺) of the amino acid is equal to the charge expressed by the acidic carboxyl group (-COOH, -COO⁻). The IEP of BSA is pH 4.8, and indicates that the side chain of the amino acids of BSA contains a greater amount of acidic functional groups than that of basic functional groups. At pH 3.2 and 7.4, BSA has a positive and negative charge, respectively.

Figure 4-21 and 4-22, show adsorption isotherms of BSA onto polystyrene (PS) latex particles.

Figure 4-21. Adsorption isotherm of BSA on polystyrene (PS) latex particles in phosphate buffer (PB) at 37°C.
At the IEP of BSA, the adsorbed amount ($q_{\text{max}}$) of BSA onto PS at equilibrium was maximum, indicating that hydrophobic interaction between latex particle and BSA protein is maximum at the IEP of BSA. This is because the BSA molecules form the most compact structures at the IEP eliminating the repulsion force between proteins. A higher number of BSA molecules can adsorb on a given surface area by hydrophobic interaction when in this compact structure. This observation agrees well with reported data by other researchers who also indicated that the maximum adsorption from aqueous protein solution was observed at the IEP [Mularen 1954; Watkins et al. 1977; Oh et al. 2000].

There was no significant difference of adsorbed BSA amount in PB and in PBS media at pH 4.8. The conformational alteration of BSA was not affected significantly by the ionic strength of the two solutions. There was evidence of the effect of the electrostatic interaction to some extent, even at the IEP, since the amount of BSA adsorbed in PBS is slightly higher than in PB at this pH.
The adsorbed amount of protein is shown to decrease at a pH 3.2 and 7.4. The decrease in adsorption efficiency when deviating from IEP of BSA is a result of the increase in conformational size of the protein molecules and the lateral electrostatic repulsions between adjacent adsorbed BSA molecules. Figure 4-23 shows a schematic of possible conformational changes at each pH, where hydrophobic interaction always exists between non-polar portion of a protein and hydrophobic portion of solid surface.

**Figure 4-23.** Conformation of bovine serum albumin (BSA) on latex particles (Assuming that charge repulsion reduces the effect of the hydrophobic interaction at pH 7.4).

In addition, the adsorbed amount of BSA at pH 3.2 was larger than that at pH 7.4 and still less than that at 4.8. This is because there is the electrostatic interaction between the positive BSA molecules and negative PS latex particles at pH 3.2, and electrostatic repulsion is dominant between negative BSA and negative PS latex particles at pH 7.4. The amount adsorbed increased with increasing ionic strength in pH 3.2 and 7.4. This is because the high concentration of electrolytes reduces the electrostatic repulsion between BSA and PS latex particle. It should be noted that the BSA concentration used in these experiments was lower than that found in normal human blood. This is because the equilibrium BSA adsorption onto the latex particles is actually reached at a much lower concentration than that found in normal blood. Table 4-6 shows the calculated results of
adsorbed BSA amount at equilibrium from the Langmuir-Freundlich adsorption isotherm equation.

In summary, the hydrophobic interaction between PS latex particle and BSA protein exists and is the dominant mechanism for the adsorption process. Conformational changes of BSA at each pH also affected the adsorption amount. Adsorption was highest at pH 4.8 because at this pH there is the least amount of ionic repulsion in the protein leading to a compact conformation of BSA. There are two main forces affecting adsorption amount at an acidic pH of 3.2. They include the ionic attraction between the negative latex particle and the positive protein, and lateral repulsion between positive proteins. The smallest amount of BSA adsorption was observed at a pH of 7.4. At this point the charge repulsion between negatively charged particles and protein molecules, and the lateral repulsion between proteins dominant adsorption even though there always exists the hydrophobic interaction between the solid latex particle and protein. The effect of charge repulsion was reduced by the high concentration of electrolytes in PBS medium.

Table 4-6. The equilibrium concentration values of BSA adsorption on polystyrene (PS) latex particles calculated the Langmuir-Freundlich isotherm model.

<table>
<thead>
<tr>
<th>Media</th>
<th>pH</th>
<th>qw (mg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate buffer</td>
<td>3.2</td>
<td>1.74</td>
</tr>
<tr>
<td></td>
<td>4.8</td>
<td>2.99</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>1.36</td>
</tr>
<tr>
<td>Phosphate buffered saline</td>
<td>3.2</td>
<td>2.60</td>
</tr>
<tr>
<td></td>
<td>4.8</td>
<td>2.83</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>2.07</td>
</tr>
</tbody>
</table>

The adsorption isotherms of BSA on PS/PMMA₁₀₀ core shell particles are shown in Figure 4-24 in phosphate buffer (PB) and in Figure 4-25 in phosphate buffered saline (PBS). The adsorbed BSA amount was calculated using the Langmuir-Freundlich
adsorption isotherm equation and the amount listed in Table 4-7. The trend seen in the adsorption results was very similar to the case of PS latex. The overall adsorption process was dominated by hydrophobic interactions between the PS/PMMA<sub>100</sub> core shell particle and BSA proteins. At pH 4.8, the adsorbed amount (q<sub>m</sub>) of BSA on PS/PMMA<sub>100</sub> core shell particles was 2.21 mg/m<sup>2</sup> in PB and 2.37 mg/m<sup>2</sup> in PBS, and are the maximum values recorded among the three pH levels, 3.2, 4.8, and 7.4. Suzawa and his co-workers [1982] have obtained similar results. This can be also explained by the compact conformation of BSA molecules at the IEP of BSA, which lead to a high protein population on the solid surface. At a pH of 3.2 and 7.4, BSA adsorption was less than that at pH 4.8, because of the protein conformation change. There was a small increase in adsorbed BSA at pH 3.2 because of the charge attraction between negative PS/PMMA<sub>100</sub> core shell particles and positive BSA. At pH 7.4, however, the adsorption of BSA was further reduced by charge repulsion between the negative PS/PMMA<sub>100</sub> core shell particles and the negative BSA proteins.

Figure 4-24. Adsorption isotherm of BSA 37°C in PB on PS/PMMA<sub>100</sub> core shell latex particles.
The adsorbed amount of BSA increased more rapidly in PBS with increasing ionic strength at pH 7.4. This shows that the low concentration of electrolytes promotes electrostatic repulsion between negative BSA and negative PS/PMMA_{100} core shell particle. As the ion strength of PBS is increased, however, the high concentration of electrolyte leads to less electrostatic repulsion, promoting more rapid protein adsorption. By comparing the amount of adsorbed protein between bare PS latex particles and PS/PMMA_{100} core shell particles, the amount of protein adsorption on the PS latex particles is greater than that on the PS/PMMA_{100} core-shell particles [Suzawa et al. 1980; 1982; Lee et al. 1988].

![Figure 4-25. Adsorption isotherm of BSA at 37°C in PBS on PS/PMMA_{100} core shell latex particles.](image-url)
Table 4-7. The equilibrium concentration values ($q_m$) of BSA adsorption on PS/PMMA$_{100}$ particles calculated the Langmuir-Freundlich isotherm model.

<table>
<thead>
<tr>
<th>Media</th>
<th>pH</th>
<th>$q_m$ (mg/m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.2</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td>4.8</td>
<td>2.21</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>1.30</td>
</tr>
<tr>
<td>Phosphate buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.2</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td>4.8</td>
<td>2.37</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>1.62</td>
</tr>
<tr>
<td>Phosphate buffered saline</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The BSA adsorption mechanism and trend on PS/PMMA core shell particles was very similar to that of the PS latex particle. Unlike the case of PS and PS/PMMA$_{100}$ latex particles, the amount of BSA adsorbed onto PS/PMMA$_{90}$PAA$_{10}$ and PS/PMMA$_{75}$PAA$_{25}$ core shell particles was higher at pH 3.2 than pH 4.8 and 7.4. Figure 4-26 through 4-29 shows the adsorption isotherms of BSA on PS/PMMA$_{90}$PAA$_{10}$ and PS/PMMA$_{75}$PAA$_{25}$ core shell particles. The amount of BSA adsorbed increased at pH 3.2 and pH 4.8 as the hydrophilicity was developed by the addition of PAA. On the contrary, the adsorbed amount of BSA was dramatically decreased at pH 7.4. The adsorption amount of BSA on PS/PMMA$_{90}$PAA$_{10}$ and PS/PMMA$_{75}$PAA$_{25}$ core shell particles at equilibrium is listed in Table 4-8 and 4-9, respectively.

Carboxylic acid as well as sulfate groups are distributed on the surface of PS/PMMA$_{90}$PAA$_{10}$ and PS/PMMA$_{75}$PAA$_{25}$ core shell particles and seem to largely affect the BSA adsorption. The isotherms showed very strong pH dependence to the BSA adsorption onto these core shell latex particles. The carboxylic groups (COOH) may especially enhance the BSA adsorption prominently at pH 3.2 by hydrogen bonding with protein molecules. The $pK_a$ value for the PAA is 4.0-6.0 and a good indication that the
carboxylic acid groups are protonated at pH 3.2 [Gebhardt et al. 1983]. The main adsorption interaction force at pH 3.2 was generated by hydrogen bonding as well as charge attraction between latex particle and protein even though the conformational change and charge repulsion between proteins results in a reduction of the protein adsorption at this pH. The amount of BSA adsorbed also increased as the PAA amount in the core shell particles increased. The adsorption of BSA was minimal, however, less than 0.7 mg/m² at pH 7.4. Here the carboxylic groups are ionized, which increases the charge repulsion by ionized carboxylic groups (COO⁻) resulting in PS/PMMA-PAA latex particles with much lower protein adsorption.

Figure 4-26. Adsorption isotherm of BSA at 37°C in PB on PS/PMMA₉₀PAA₁₀ core shell latex particles.
Figure 4-27. Adsorption isotherm of BSA at 37°C in PBS on PS/PMMA$_{90}$PAA$_{10}$ core shell latex particles.

Table 4-8. The equilibrium concentration values of BSA adsorption on PS/PMMA$_{90}$PAA$_{10}$ particles calculated the Langmuir-Freundlich isotherm model.

<table>
<thead>
<tr>
<th>Media</th>
<th>pH</th>
<th>$q_m$ (mg/m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.2</td>
<td>3.61</td>
</tr>
<tr>
<td>Phosphate buffer</td>
<td>4.8</td>
<td>2.83</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>3.2</td>
<td>5.19</td>
</tr>
<tr>
<td>Phosphate buffered saline</td>
<td>4.8</td>
<td>2.26</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>0.43</td>
</tr>
</tbody>
</table>
Figure 4-28. Adsorption isotherm of BSA 37°C in PB on PS/PMMA_75PAA_25 latex particles.

Figure 4-29. Adsorption isotherm of BSA 37°C in PBS on PS/PMMA_75PAA_25 core shell latex particles.
Table 4-9. The equilibrium concentration values of BSA adsorption on PS/PMMA_{75}PAA_{25} particles calculated the Langmuir-Freundlich isotherm model.

<table>
<thead>
<tr>
<th>Media</th>
<th>pH</th>
<th>( q_{\text{m}} ) (mg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate buffer (PB)</td>
<td>3.2</td>
<td>10.83</td>
</tr>
<tr>
<td></td>
<td>4.8</td>
<td>6.62</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>0.75</td>
</tr>
<tr>
<td>Phosphate buffered saline (PBS)</td>
<td>3.2</td>
<td>10.77</td>
</tr>
<tr>
<td></td>
<td>4.8</td>
<td>6.81</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>0.57</td>
</tr>
</tbody>
</table>

The adsorption of \( \beta_2 \)-microglobulin (\( \beta_2 \)M) was determined under one set of conditions, in phosphate buffer at 37°C and pH 7.4 because of the limited amount of \( \beta_2 \)M protein. The adsorption isotherms of the \( \beta_2 \)M protein on the synthesized latex particles are shown in Figure 4-30, 4-31 and 4-32. The \( \beta_2 \)M protein concentrations for adsorption isotherm experiment were chosen to be 0.015, 0.030, 0.045, and 0.060mg/ml since the \( \beta_2 \)M concentration for the patients with renal failure can be elevated to similar concentrations, about 0.05mg/ml or more, up to 50 times the normal level [Nissenson et al. 1995]. There were steep initial slopes of the adsorption isotherm curves of \( \beta_2 \)M on latex particles in all isotherms, indicating a high affinity adsorption type. It seems that the hydrophobic interaction is enough for the adsorption process of \( \beta_2 \)M proteins to occur on the latex particles, even though charge repulsion exists between protein and latex particle at pH 7.4. The complete plateau regions were not seen for all isotherms, indicating that \( \beta_2 \)M proteins were not saturated at this level and there are still available sites for \( \beta_2 \)M adsorption on latex particles. The initial slope of the \( \beta_2 \)M isotherms for PS was steeper.
than that of any of the other latex particle, PS/PMMA$_{100}$, PS/PMMA$_{90}$PAA$_{10}$, and PS/PMMA$_{75}$PAA$_{25}$.

Figure 4-30. Adsorption isotherm of $\beta_2M$ onto PS and PS/PMMA$_{100}$ latex particles in PB at 37°C and pH 7.4.

Figure 4-31. Adsorption isotherm of $\beta_2M$ onto PS and PS/PMMA$_{90}$PAA$_{10}$ latex particles in PB at 37°C and pH 7.4.
Figure 4-32. Adsorption isotherm of $\beta_2$M onto PS and PS/PMMA$_{75}$PAA$_{25}$ latex particles in PB at 37°C and pH 7.4.

The equilibrium concentration values ($q_m$) of $\beta_2$M adsorption on latex particles are calculated and listed in Table 4-10. The difference between the maximum amounts of $\beta_2$M adsorption on all the latex particles was not significantly different and their values were between 0.69 and 0.8mg/m$^2$.

Table 4-10. The equilibrium concentration values of $\beta_2$M adsorption calculated the Langmuir-Freundlich isotherm model.

<table>
<thead>
<tr>
<th>Latex particles</th>
<th>$q_m$ (mg/m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>0.80</td>
</tr>
<tr>
<td>PS/PMMA$_{100}$</td>
<td>0.69</td>
</tr>
<tr>
<td>PS/PMMA$<em>{90}$PAA$</em>{10}$</td>
<td>0.72</td>
</tr>
<tr>
<td>PS/PMMA$<em>{75}$PAA$</em>{25}$</td>
<td>0.72</td>
</tr>
</tbody>
</table>
4.3.2 Adsorbed Layer Thickness

There are several possible explanations for large amount of BSA adsorbed at acidic pH levels, including the end-on adsorption pattern of BSA, the flexibility of BSA molecules, the tilting of protein molecules due to the asymmetry of the charge distribution, or multilayer formation [Peula et al. 1993]. The adsorbed BSA thickness (δ) can be calculated by the following equation [Chiu et al. 1978]. This equation was also cited again by other researchers [Shirahama et al. 1985]

\[ \delta = \frac{3\sqrt{3}}{\pi} q_m \cdot \rho_{BSA} \]  \hspace{1cm} (4-7)

where \( \frac{3\sqrt{3}}{\pi} \) is the packing factor, \( q_m \) is the plateau value of the adsorbed amount, and \( \rho_{BSA} \) is the density of BSA molecule which corresponds to the reciprocal of its known partial specific volume. The layer thickness (δ) of BSA proteins on synthesized latex particles in phosphate buffer (PB) ranged from 27 - 81 Å at 37°C and pH 4.8. These calculated values are listed in Table 4-11. Only δ values at pH 4.8, the isoelectric point (IEP) of BSA are important, because intra- and intermolecular electrostatic repulsion of protein molecules is minimized at the IEP [Shirahama et al., 1985]. Compared to the hydrodynamic dimensions of BSA, 140 × 40 × 40 Å³ [Fair et al, 1980; Peter 1985], these values of layer thickness indicate that the BSA molecules adsorb by side-on (40Å) and end-on (140Å) mode. It is unnecessary to consider multilayering because thickness do not exceed 140 Å. The layer thickness (δ) of BSA proteins on synthesized latex particles in phosphate buffered saline (PBS) was ranged of 28 ~ 83 Å at 37°C and pH 4.8. These calculated values are also listed in Table 4-12.
Table 4-11. Absorbed BSA layer thickness (Å) of BSA in phosphate buffer (PB) at 37°C.

<table>
<thead>
<tr>
<th>pH</th>
<th>PS</th>
<th>PS/PMMA₁₀₀</th>
<th>PS/PMMA₉₀PAA₁₀</th>
<th>PS/PMMA₇₅PAA₂₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>21</td>
<td>17</td>
<td>44</td>
<td>133</td>
</tr>
<tr>
<td>4.8</td>
<td>37</td>
<td>27</td>
<td>35</td>
<td>81</td>
</tr>
<tr>
<td>7.4</td>
<td>17</td>
<td>16</td>
<td>5</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 4-12. Absorbed BSA layer thickness (Å) of BSA in phosphate buffered saline (PBS) at 37°C.

<table>
<thead>
<tr>
<th>pH</th>
<th>PS</th>
<th>PS/PMMA₁₀₀</th>
<th>PS/PMMA₉₀PAA₁₀</th>
<th>PS/PMMA₇₅PAA₂₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>32</td>
<td>21</td>
<td>64</td>
<td>132</td>
</tr>
<tr>
<td>4.8</td>
<td>35</td>
<td>29</td>
<td>28</td>
<td>83</td>
</tr>
<tr>
<td>7.4</td>
<td>26</td>
<td>20</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

### 4.3.3 Gibbs Free Energy of Protein Adsorption

In the Langmuir isotherm model, k represents the ratio of adsorption to the desorption rate constants. This definition is not applicable to the Langmuir-Freundlich isotherm model but its meaning is similar [Yoon et al. 1996]. In this sense, k is closely related to the affinity between protein molecules and latex particles. When the equilibrium concentration, $C_{eq}$, reaches zero, the value of $C_{eq}^{1/n}$ will be very small ($1 \gg kC_{eq}^{1/n}$) and then the equation 4-6 becomes

$$q = q_m k C_{eq}^{1/n}$$ (4-8)

So, k is closely related to the initial slope of the isotherm showing the affinity between BSA and latex particles [Yoon et al. 1996]. A key point in the characterization of adsorption processes on solid-liquid interfaces is to determine the change in Gibbs’s free energy ($\Delta G^*$), one of the corresponding parameters for the thermodynamic driving forces, during adsorption. The traditional method to evaluate adsorption thermodynamic
constants is based on measurement of adsorption isotherms under static conditions [Malmsten 1998]. The Langmuir-Freundlich adsorption equilibrium constant, \( k_{if} \), can be easily found from a slope of the isotherm. Then, this enables calculation of the free-energy change, \( \Delta G_{ads}^\circ \), during adsorption expressed as:

\[
\Delta G_{ads}^\circ = -RT \ln k_{if}
\]

(4-9)

where \( R \) is the general gas constant (\( R = 8.314 \text{J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1} \)) and \( T \) is the absolute temperature. Gibbs free energy change of BSA adsorption values in PB and PBS are listed in Table 4-13 and 4-14, respectively. As the \( \Delta G_{ads}^\circ \) values are more negative, the protein adsorption on a solid substrate will be more favorable.

Table 4-13. The values of Gibbs free energy change of BSA adsorption in phosphate buffer (PB) at 37°C.

<table>
<thead>
<tr>
<th>pH</th>
<th>PS</th>
<th>PS/PMMA100</th>
<th>PS/PMMA90PAA10</th>
<th>PS/PMMA75PAA25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.2</td>
<td>-24.11</td>
<td>-27.26</td>
<td>-28.46</td>
<td>-28.05</td>
</tr>
<tr>
<td>4.8</td>
<td>-23.52</td>
<td>-23.83</td>
<td>-21.98</td>
<td>-19.72</td>
</tr>
<tr>
<td>7.4</td>
<td>-24.52</td>
<td>-8.88</td>
<td>-3.24</td>
<td>-2.80</td>
</tr>
</tbody>
</table>

Table 4-14. The values of Gibbs free energy change of BSA adsorption in phosphate buffered saline (PBS) at 37°C.

<table>
<thead>
<tr>
<th>pH</th>
<th>PS</th>
<th>PS/PMMA100</th>
<th>PS/PMMA90PAA10</th>
<th>PS/PMMA75PAA25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.2</td>
<td>-23.15</td>
<td>-26.20</td>
<td>-23.12</td>
<td>-26.83</td>
</tr>
<tr>
<td>7.4</td>
<td>-24.30</td>
<td>-19.92</td>
<td>-2.58</td>
<td>-2.63</td>
</tr>
</tbody>
</table>

Table 4-15 shows the calculated values of Gibbs free energy change of \( \beta_2 \)M adsorption on synthesized latex particles. PS/PMMA75PAA25 core shell latex particle has
largest negative Gibbs free energy of $\beta_2$M adsorption, indicating largest affinity between $\beta_2$M and latex particle.

Table 4-15. The values of Gibbs free energy change of $\beta_2$M adsorption in phosphate buffer (PB) at $37^\circ$C and pH 7.4.

<table>
<thead>
<tr>
<th></th>
<th>PS</th>
<th>PS/PMMA$_{100}$</th>
<th>PS/PMMA$<em>{90}$PAA$</em>{10}$</th>
<th>PS/PMMA$<em>{75}$PAA$</em>{25}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta G^o_{ads}$ (kJ/mol)</td>
<td>-36.26</td>
<td>-34.66</td>
<td>-36.59</td>
<td>-39.03</td>
</tr>
</tbody>
</table>

The Gibbs free energy of $\beta_2$M was much more negative in phosphate buffer (PB) than that of BSA in physiological condition ($37^\circ$C and pH 7.4) from the Table 4-13 and the Figure 4-31. This indicates that $\beta_2$M molecules more readily adsorb on latex particles than BSA, and that the affinity of $\beta_2$M for the latex particle is much larger than that of BSA. This can be explained by the rate of diffusion and accessibility of $\beta_2$M molecules to the latex particle surface. It is easier and faster for $\beta_2$M molecules than BSA because of the smaller size (molecular weight) and spherical shape of $\beta_2$M molecules. It may also be due to the higher hydrophobic force, and less electrostatic repulsion, that contribute to the adsorption of $\beta_2$M molecules not as prevalent in the case of BSA; because the IEP of $\beta_2$M (5.7) is closer to physiological condition (pH 7.4) than that of BSA (4.8), causing $\beta_2$M to be less electrostatically repelled from the latex particles than BSA. From Figure 4-33, it can be seen that the $\Delta G^o_{ads}$ of $\beta_2$M adsorption onto the synthesized latex particles is increasing with the hydrophilicity of the latex particles whereas the $\Delta G^o_{ads}$ of the BSA molecules is decreasing or constant. This phenomenon supports the theory that the selective adsorption of $\beta_2$M molecules over BSA molecules can be enhanced by utilizing more hydrophilic PS/PMMA$_{90}$PAA$_{10}$ and PS/PMMA$_{75}$PAA$_{25}$ core shell latex particles.
4.3.4 Kinetics of Adsorption

Protein adsorption is a complex process, known to have a large biological impact and is currently not well understood quantitatively because of extreme sensitivity to pH, the concentration of other electrolytes and molecules present, temperature, and many other factors that can change in the physiological system. Accurate knowledge of the adsorption kinetics under a given set of conditions is a prerequisite for elucidating the mechanisms of many fundamental biological processes to be predicted at the molecular level [Regner 1987]. This adsorption kinetics approach is used in protein adsorption studies because of the uncertainty related to the time needed for the interfacial proteins to reach the equilibrium. It is generally accepted that the process of protein adsorption is comprised of the following steps: a) transport toward the interface, b) attachment at the interface, c) eventual structural rearrangements in the adsorbed state, d) detachment from
the interface and transport away from the interface. Each of these steps can affect the overall rate of the adsorption process.

The quantitative analysis of the protein adsorption kinetics requires that the protein amount adsorbed is known as a function of time. The kinetics tests were carried out in phosphate buffer (PB) at 37°C and pH 7.4, a condition similar to physiological condition. The protein concentrations chosen were 0.7mg/ml for BSA and 0.06mg/ml for \( \beta_2 \)M, which are the maximal concentrations used in an adsorption isotherm test. A time-based evaluation of the adsorption was made by measuring the concentration of protein in solution at different incubation times. The results are shown below from Figure 4-34 to 4-37. The adsorption kinetics of proteins onto the latex particles is expressed as a second-order model [Özacar 2003] shown by the following equation:

\[
\frac{dq_t}{dt} = k(q_e - q_t)^2
\]  

(4-10)

Integrating above equation and applying the boundary conditions, yields

\[
q_t = \frac{t}{\frac{1}{kq_e^2} + \frac{t}{q_e}}
\]  

(4-11)

where \( q_t \) and \( q_e \) stand for the amount (mg/m²) of protein adsorbed at time \( t \) and at equilibrium respectively, \( k \) is the equilibrium rate constant of second-order adsorption (m²/mg·hr), and \( t \) is the incubation time (hr).

The fitting parameters calculated by using the equation 4-11 are listed in table 4-16. From this data it is seen that the amount of BSA adsorbed at equilibrium was higher than that of \( \beta_2 \)M on PS and PS/PMMA₁₀₀ latex particles but less than that of \( \beta_2 \)M on PS/PMMA₀₉₀PAA₁₀ and PS/PMMA₇₅PAA₂₅ latex particles. These results are similar to the previous adsorption isotherm results representing good reproducibility of adsorption
Figure 4-34. The kinetics of protein adsorption in PB on PS latex particles at 37°C and pH 7.4.

Figure 4-35. The kinetics of protein adsorption in PB on PS/PMMA\textsubscript{100} latex particles at 37°C and pH 7.4.
Figure 4-36. The kinetics of protein adsorption in PB on PS/PMMA\textsubscript{90}PAA\textsubscript{10} latex particles at 37°C and pH 7.4.

Figure 4-37. The kinetics of protein adsorption in PB on PS/PMMA\textsubscript{75}PAA\textsubscript{25} latex particles at 37°C and pH 7.4.
test. The variation of $\beta_2$M equilibrium adsorption amount was nearly negligible for all latex particles. The plateau region is reached earlier for $\beta_2$M than BSA on all latex particles and the K values (equilibrium rate constant of second order adsorption) for the $\beta_2$M adsorption process onto latex particles are much larger than for BSA adsorption. These values indicate that the $\beta_2$M molecules have a higher adsorption rate onto the latex particles than the BSA molecules.

Table 4-16. Fitting parameters of second-order kinetic model.

<table>
<thead>
<tr>
<th>Latex particles</th>
<th>BSA</th>
<th>$\beta_2$M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$q_e$ (mg/m²)</td>
<td>$K$ (hr⁻¹)</td>
</tr>
<tr>
<td>PS</td>
<td>1.44</td>
<td>6.7</td>
</tr>
<tr>
<td>PS/PMMA$_{100}$</td>
<td>1.75</td>
<td>35.3</td>
</tr>
<tr>
<td>PS/PMMA$<em>{90}$PAA$</em>{10}$</td>
<td>0.46</td>
<td>9.7</td>
</tr>
<tr>
<td>PS/PMMA$<em>{75}$PAA$</em>{25}$</td>
<td>0.60</td>
<td>17.9</td>
</tr>
</tbody>
</table>

* $K = (k \times q_e)$, hr⁻¹

There are several explanations for the phenomena observed in these experiments. First, the smaller sizes of $\beta_2$M molecules allow easier diffusion and a close approach to the latex substrate. Second, a reduced number of hydrophobic domains on the latex particle surface, replaced by sulfate groups (-OSO$_3$⁻) and ionized carboxylic groups (COO⁻) are not favorable for larger BSA molecules. Third, the conformational change by intra- and inter-molecular repulsion would be faster in $\beta_2$M due to smaller molecular weight. Finally, the ionic repulsion between $\beta_2$M and the latex particles is less than that between BSA and latex particles because IEP (5.7) of $\beta_2$M is closer to the tested condition (pH 7.4) than IEP (4.8) of BSA.
4.4 Blood Compatibility

Blood compatibility can be defined as the property of a material or device that permits it to function in contact with blood without inducing any adverse reactions. Successful functioning of blood contacting biomedical materials or devices in contact with blood strongly depends on the extent to which they damage blood during operation. High shear stresses, turbulence, relatively long contact times, and cellular impact with artificial surfaces are most common events experienced by blood in contact with artificial devices and can cause hemolysis or thrombosis [Bernhard et al. 1978; Winslow and et al. 1993]. Therefore, before any materials can be placed into the human body, biocompatibility testing must be the first performed in the view of safety. The hemolysis test serves as a method for preliminary screening of biomaterials to determine the biocompatibility with blood and other circulating body fluid. The tests estimate the concentration of hemoglobin released from the red blood cell damage induced by a foreign material.

There are several types of blood cells in our body, but the ones that make blood red are erythrocytes, or red blood cells (RBCs). Red blood cells (erythrocytes) are the most numerous type in the blood. These cells are average about 4.8-5.4 million per cubic millimeter (mm³ : same as a microliter (µl)) of blood but these values can vary over a larger range depending on such factors as health and altitude. RBCs are red because they contain hemoglobin, which is a protein that contains iron. When blood circulates through lungs, oxygen binds to the hemoglobin and is then delivered to all the parts of body. RBCs are made in the bone marrow. They start out as a nucleus, like other cells, but when they fill up with hemoglobin the nucleus becomes smaller until it disappears. Without a nucleus, RBCs are fragile and live only about 120 days. In a normal healthy
about 2 million red blood cells die per second. But bone marrow produces new ones just as fast. RBCs are about 7.5 micrometer across, and are responsible for carrying oxygen, salts, and other organic substances to the cells and tissues throughout our body.

In humans and most mammals the cells are concave (indented in the middle) and flexible enough to squeeze through even the tiniest of blood vessels, the capillaries.

Figure 4-38. Image of red blood cells.

(Source:http://www.pbrc.hawaii.edu/bemf/microangela/rbc1.htm)

RBCs are among the first cells that come into contact with a foreign material injected in the blood. Therefore, a hemolysis assay gives information about the biocompatibility in the case of an in vivo application. The concentrations of prepared latex particles used for the tests in this research are 0.5, 2.5, and 5.0% (w/w) and the incubation time under moderate shaking was 30 minutes at 37°C. Test results are shown in Figure 4-39. The amount of blood cells that ruptured when in contact with the PS latex particles was largely increased from about 30% to 91% as the solid content of PS increased from 0.5% to 5.0%. However, hemolysis by the core shell latex particles, such as PS/PMMA$_{100}$, PS/PMMA$_{90}$PAA$_{10}$, and PS/PMMA$_{75}$PAA$_{25}$ was less than 3.5%. Of
particular interest was that hemolysis caused by PS/PMMA$_{90}$PAA$_{10}$ particles at 5.0% solid concentration was less than 0.2% hemolysis, the lowest value among the latex particles. The low hemolysis value indicates that the PS/PMMA$_{90}$PAA$_{10}$ core shell latex particles are the most blood compatible at all solid contents. Other core shell particles such as PS/PMMA$_{100}$ and PS/PMMA$_{75}$PAA$_{25}$ also had excellent blood compatibility with low RBS breakage.

![Hemolysis caused by latex particles, n=5.](image)

Figure 4-39. Hemolysis caused by latex particles, n=5.
5.1 Summary of Results

The goal of this study was to synthesize monodisperse polymeric latex particles with tailored properties, and to investigate the fundamental interactions between the synthesized latex particles and target proteins. An understanding of the fundamental mechanism of selective adsorption is strongly required in order to maximize the separation performance of membranes made of these materials so that it may applicable to hemodialysis therapy for end stage renal disease (ESRD) patients. For the achievement of above research goal, several investigations have been performed. Analysis of the obtained data leads to several conclusions as discussed below.

Monodisperse polystyrene seed latex particles were synthesized with the size ranges between 126±7.5 and 216±5.3 nm. Divinylbenzene (DVB) was used as a crosslinking agent to make the latex particles more hydrodynamically stable. The conversion of monomer to polymer was over 95% and seed latex particles are spherical in shape and have smooth surface. From these small seeds, bigger latex particles as large as 800nm were synthesized using a semi-continuous seeded emulsion polymerization process. The particles with a mean diameter less than 500nm are very spherical in shape and highly uniform in particle size distribution. However, as the particles grow larger than 500nm in mean diameter, they became non-spherical with an uneven surface. This irregularity of particle surface can be attributed to the non-homogeneous monomer
swelling into the shell of a growing polymer, which can be controlled by the factors such as temperature, agitation speed, initiator feeding rate, and surfactant amount required to stabilize the colloidal system. It was determined that as the surfactant to monomer ratio decreased, the mean particle diameter increased. Methyl methacrylate (MMA) and acrylic acid (AA) monomers were introduced to make the latex particle surfaces more hydrophilic than bare polystyrene particles. The prepared core shell latex particles were made of PMMA$_{100}$, PMMA$_{90}$PAA$_{10}$, and PMMA$_{75}$PAA$_{25}$. PMMA$_{100}$ is the PMMA homopolymer shell on a PS core. PMMA$_{90}$PAA$_{10}$ shell is a copolymer with a PMMA to PAA ratio of 90% to 10% by weight. PMMA$_{75}$PAA$_{25}$ shell is a copolymer consisting of a PMMA to PAA ratio of 75% to 25% by weight. The particle size of these core-shell particles were prepared to be about 370 nm in mean diameter.

The successful synthesis of latex particles was confirmed using Fourier Transform Infrared spectroscopy (FTIR). The C-H aromatic stretching vibration is seen between 3002 cm$^{-1}$ and 3103 cm$^{-1}$ for PS latex particles. The C-H asymmetrical stretching and symmetrical stretching vibration peaks of CH$_2$ for all latex particles is seen at 2900 cm$^{-1}$ and 2850 cm$^{-1}$, respectively. There is a carbonyl (C=O) characteristic band at 1730 cm$^{-1}$ wavenumber for PS/PMMA$_{100}$ core-shell latex particles. The broad OH group peak at 3400 cm$^{-1}$ can be seen in the spectroscopy of the particle with PMMA/PAA shell (PS/PMMA$_{90}$PAA$_{10}$ and PS/PMMA$_{75}$PAA$_{25}$). The peak intensity of OH group of PS/PMMA$_{75}$PAA$_{25}$ was larger than that of PS/PMMA$_{90}$PAA$_{10}$ relatively due to more AA monomer content in PS/PMMA$_{75}$PAA$_{25}$ than in PS/PMMA$_{90}$PAA$_{10}$.

In the zeta potential measurements, polystyrene (PS) latex particles had negative values between -29.1 mV and -59.9 mV in the phosphate buffer (PB) and between -20.3
mV and -27.8 mV in the phosphate buffered saline (PBS) at 25°C and pH 2.1-7.8. These negative zeta potential values for PS latex particles are due to ionization of the sulfate groups (-OSO$_3^-$) originated from the initiator. Zeta potential values of the PS/PMMA$_{100}$ core shell latex particles were also negative, between -28 mV and -50.5 mV in PB and between -14.3 mV and -18.6 mV in PBS at pH 2.1-7.8. Negative values are also generated by dissociation of sulfate groups on a particle surface. The zeta potential values of PS/PMMA$_{90}$PAA$_{10}$ and PS/PMMA$_{75}$PAA$_{25}$ were of negative values between -36.7 mV and -67.8 mV in PB medium and -14.7 mV and -19.3 mV in PBS medium at 25°C. For PS/PMMA$_{75}$PAA$_{25}$ core shell particles, zeta potential values were between -29.1 mV and -52.0 mV in PB media and between -11.5 mV and -21.0 mV in PBS media. Sulfate groups contributed to the negative zeta potential values in PS/PMMA$_{75}$PAA$_{25}$ and PS/PMMA$_{90}$PAA$_{10}$ core shell particles. Carboxylic groups from polyacrylic acid also contribute to the negative surface charge for the latex particles at greater than pH 7.0, however, the degree of their contribution was not detectable from the zeta potential measurements. The absolute zeta potential values of the synthesized latex particles in PB were higher than that in PBS. This is because of the high concentration of sodium chloride electrolyte in PBS that compress electrical double layer thickness.

The bicinchoninic acid (BCA) assay technique was used to determine the protein adsorption and all isotherms were fitted to the Langmuir-Freundlich isotherms using the nonlinear regression method. The hydrophobic interaction between PS latex particle and BSA protein was dominant for the adsorption process. Conformational change of BSA at each pH also affected the adsorption amount, which was highest at pH 4.8 because of low ionic repulsion making compact conformation of BSA. The next larger amount of BSA
adsorbed onto PS was at pH 3.2. There were two main forces affecting the amount of
adsorption at acidic pH values. One is ionic attraction between negative latex particle and
positive protein. The other is lateral repulsion between positive proteins. The smallest
amount of BSA adsorption was at pH 7.4 where charge repulsion between negative
particles and proteins is present at this pH. Lateral repulsion between proteins was
dominant for protein adsorption even though there always exists the hydrophobic
interaction between solid latex particle and protein. The charge repulsion was reduced by
the high concentration of electrolytes in the PBS medium. The BSA adsorption process
on PS/PMMA core shell particles was very similar to the case of PS latex particle where
hydrophobic interaction between latex particle and protein was dominant. However the
adsorbed BSA amount was less than that of PS in PBS. The adsorbed amount of BSA on
PS/PMMA$_{90}$PAA$_{10}$ and PS/PMMA$_{75}$PAA$_{25}$ core shell particles was higher at pH 3.2 than
pH 4.8 and 7.4. The adsorbed BSA amount became higher at pH 3.2 and pH 4.8 as the
hydrophilicity was increased by the higher PAA amount. On the contrary, the adsorbed
amount of BSA was dramatically decreased at pH 7.4. Carboxylic acid groups as well as
the sulfate groups are distributed on the surface of PS/PMMA$_{90}$PAA$_{10}$ and
PS/PMMA$_{75}$PAA$_{25}$ core shell particles and largely affect the BSA adsorption. The main
adsorption interaction forces at pH 3.2 was generated by hydrogen bonding as well as
charge attraction between latex particle and protein even when the conformational change
and charge repulsion between proteins reduce the adsorption of protein at this pH. The
adsorbed BSA amount was also increased as the amount of PAA increases. However, the
adsorption of BSA was minimal (less than 0.7 mg/m$^2$) at pH 7.4, where the carboxylic
groups are ionized leading to more enhancement of charge repulsion by ionization of the
carboxylic groups (COO\(^{-}\)). Because of this, the PS/PMMA-PAA latex particles have a much lower protein adsorption.

There were steep initial slopes for adsorption isotherms of \(\beta_2M\) on latex particles in all isotherms, indicating a high affinity type adsorption. The complete plateau regions were not seen for all isotherms, indicating that \(\beta_2M\) proteins were not saturated and there are still available sites for \(\beta_2M\) adsorption on latex particles. The initial slope of \(\beta_2M\) isotherms for PS was steeper than for the other latex particles, PS/PMMA\(_{100}\), PS/PMMA\(_{90}\)PAA\(_{10}\), and PS/PMMA\(_{75}\)PAA\(_{25}\).

The layer thickness (\(\delta\)) of BSA proteins on synthesized latex particles in phosphate buffer (PB) was in the range of 27 - 81 Å at 37°C and pH 4.8. Only the \(\delta\) values at pH 4.8, the isoelectric point (IEP) of BSA, are important because intra- and inter-molecular electrostatic repulsion of the protein molecules is minimized at the IEP. Compared to the hydrodynamic dimensions of BSA, 140 \(\times\) 40 \(\times\) 40 Å\(^3\), these values of the adsorbed layer thickness indicates that the BSA molecules adsorb by the side-on (40Å) and end-on mode (140Å). Consideration of the multi-layering is unnecessary because the adsorbed thickness of BSA do not exceed 140 Å.

The Gibbs free energy of \(\beta_2M\) was more negative in phosphate buffer (PB) than that of BSA in physiological condition (37°C and pH 7.4), indicating that \(\beta_2M\) molecules are more favorably adsorbed on the latex particles than BSA, and that the affinity of \(\beta_2M\) was much larger than BSA. This may be explained by the rate of diffusion and accessibility of \(\beta_2M\) molecules being easier and faster than that for BSA because of smaller size (or weight) and spherical shape of \(\beta_2M\) molecules. It may also be possible to explain that more hydrophobic forces (less electrostatic repulsion) contributes to
adsorption of \( \beta_2 \text{M} \) than to that of BSA because the IEP of \( \beta_2 \text{M} \) (5.7) is closer to physiological condition (pH 7.4) than that of BSA (pH 4.8). The selective adsorption of \( \beta_2 \text{M} \) molecules over BSA molecules can be enhanced by developing more hydrophilic PS/PMMA\(_{90}\)PAA\(_{25} \) and PS/PMMA\(_{75}\)PAA\(_{25} \) core shell latex particles.

The adsorption kinetics of proteins on latex particles was expressed as a second-order model. The plateau region for \( \beta_2 \text{M} \) was reached earlier than BSA on all latex particles and the K values (equilibrium rate constant of 2nd order adsorption) in \( \beta_2 \text{M} \) adsorption process on latex particles were much larger than in BSA adsorption, indicating that faster adsorption rate of \( \beta_2 \text{M} \) molecules than that of the BSA molecules. There are several explanations for these phenomena. First, the smaller size of \( \beta_2 \text{M} \) molecules can more easily diffuse and approach to the latex substrate. Second, enhanced hydrophilic domain on the latex particle surface by sulfate groups (SO\(_4^\)\(^-\)) and ionized carboxylic groups (COO\(^-\)) is not favorable for larger BSA molecules but is for smaller \( \beta_2 \text{M} \) to be anchored. Third, the conformational change by intra- and inter-molecular repulsion would be faster in \( \beta_2 \text{M} \) due to lower molecular weight. Finally, the ionic repulsion between \( \beta_2 \text{M} \) and latex particles is less than that between BSA and latex particles because IEP (5.7) of \( \beta_2 \text{M} \) is closer to the tested condition (pH 7.4) than IEP (4.8) of BSA.

In the biocompatibility test, the number of red blood cells (RBCs) ruptured by PS latex particles ranged from 30% to 91% as the solid content of PS increased from 0.5% to 5.0%, respectively. However, hemolysis of the other core shell latex particles, such as PS/PMMA\(_{100} \), PS/PMMA\(_{90}\)PAA\(_{10} \), and PS/PMMA\(_{75}\)PAA\(_{25} \) were less than 3.5%. The PS/PMMA\(_{90}\)PAA\(_{10} \) particles at all applied solid concentrations had less than 0.2% hemolysis and was the most blood compatible among the prepared latex particles. Other
core shell particles such as PS/PMMA\(_{100}\) and PS/PMMA\(_{75}\)PAA\(_{25}\) also had excellent blood compatibility with low RBC breakage.

5.2 Conclusions

The major conclusions from this study include:

- Using engineered core shell latex particles, selective adsorption of \(\beta_2\) microglobulin (\(\beta_2\)M) over that of the bovine serum albumin (BSA) at pH 7.4, physiological condition, was demonstrated.

- The rate of adsorption of the \(\beta_2\)M was also much higher than that of BSA. This was achieved by controlling the PMMA and PAA ratio, to optimize hydrophobic/hydrophilic microdomain size.

- The core shell particles also exhibited excellent biocompatibility with blood. These particles posses the most desirable requirements for use in a human dialysis membrane.

5.3 Recommendation for Future Work

With the results and conclusions obtained through this study, several suggestions may be given for future work as follows:

- Competitive adsorption tests of whole blood proteins on synthesized latex particles under physiological condition

- Preparation of latex particles with optimum hydrophobic/ hydrophilic microdomains to maximize selective adsorption and removal of toxin molecules.

- Membrane fabrication by suitable pore size design to discriminate a toxin molecule by size sieving as a function of particle layers.

- Evaluation of membrane performance such as flow rate in water and in simulated blood fluid (SBF).

- Application for hemodialysis as well as other technologies such as a water remediation.
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Sangyup Kim was born and raised in the small town of Sung-Joo, Kyungbook, Korea, on August 7, 1968. He obtained a Bachelor of Science degree in Chemistry from Keimyung University (Daegu, Korea) in 1993. He entered graduate school and earned a Master of Science degree in Chemical engineering in 1995. He worked as a student researcher for 2 years at Textile Polymer Division in Korea Institute of Science and Technology (KIST) in Seoul Korea. Next he worked over a year at the Automobile Coating Division in DongJoo-PPG in Korea. In August 2001, he began studying for the degree of Doctor of Philosophy in materials science and engineering at the University of Florida, in Gainesville. After getting his Ph.D., he plans to work at the Digital Media Business Division of Samsung Electronics in Korea, starting in January 2006.