α-POLY-L-LYSINE AS A POTENTIAL BIOSORBENT FOR REMOVAL OF HEXAVALENT CHROMIUM FROM INDUSTRIAL WASTE WATER

A THESIS

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By

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ABSTRACT

Remediation of heavy metals from industrial effluents and ground water sources poses a significant challenge. Hexavalent chromium is one such heavy metal, prevalent in industrial wastewaters, which has been proven to be toxic to humans and other living organisms. Most of the conventional methods available for dealing with chromium are either cost prohibitive or generate secondary effluents which are difficult to deal with. The idea of bioremediation has gained much momentum over the last few decades because of its potential low cost and minimum impact on the environment. This study explored the potential for hexavalent chromium bioremediation using a synthetic cationic biopolymer α-poly-l-lysine (α-PLL) as a biosorbent. In the present research work, equilibrium batch studies were performed in a specially designed dialysis apparatus to obtain preliminary information about the adsorption capacity of the polymer. Metal uptake by the polymer was found to be maximum when the pH of chromium solution (pH 4.6) and that of poly-lysine (pH 5.7) was not changed at the beginning of the experiment. Applying the Langmuir adsorption isotherm model showed that α-PLL has a maximum uptake capacity of 42.2 μg Cr/mg α-PLL, and a binding constant of 1.2 μg/mL $\pm$10%. The metal uptake performance of the polymer was also evaluated in a Polymer Enhanced Diafiltration (PEDF) system. The polymer-metal complex was retained and concentrated by the PEDF set up using a tangential flow filtration membrane, while the clean filtrate flowed through. When 3.4 L of 10 mg/L chromium solution in the $Cr_2O_7^{2-}$ form was processed using 300 mL of 2 gm/L PLL, the concentration of chromium in the permeate reached a maximum of 0.79 mg/L. When 30 mg/L chromium solution was used, 2 L

could be processed using 300 mL of 2gm/L PLL, and 7.8 mg/L chromium could be detected in the permeate in the end.

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LIST OF ABBREVIATIONS

Da	Daltons
dH_2O	Deionized water
EDTA	Ethylenediamine Tetraacetate
EPA	Environmental Protection Agency
MWCO	Molecular Weight Cut Off
PEDF	Polymer Enhanced Diafiltration System
α-PLL	α-Poly-L-Lysine
ε-PLL	ε-Poly-L-Lysine
γ-PGA	γ-Poly Glutamic Acid
TMP	Trans Membrane Pressure
TFF	Tangential Flow Filtartion
UF	Ultra Filtration

LIST OF SYMBOLS

Co	Cobalt
Cr (III)	Trivalent chromium
Cr(VI)	Hexavalent chromium
Cu	Copper
Pb	Lead
pKa	Negative logarithm of acid ionization constant
q	Metal uptake concentration (mg/g adsorbent)
qmax	Maximum metal uptake (mg/g adsorbent)
K_A	Equilibrium association constant (L/mg)
K_D	Equilibrium dissociation constant (mg/L)
K_F	Overall mass transfer coefficient (mh^{-1})
C	Bulk liquid phase metal concentration (mg/L)
Zn	Zinc

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1. INTRODUCTION

1.1 Problem Statement and Objective

Most industries such as manufacturing, defense, power generation, pharmaceutical use or produce chemicals in efforts to improve human living standards, but the unplanned intrusion of those chemicals into the environment can reverse the same standards of living that they are intended to foster (Dzantor, 1999).

The accumulation of non-biodegradable heavy metals in soil and ground water resources has reached alarming rates. In 1980, EPA (U.S. environmental protection agency) created the Superfund program for hazardous site identification and clean up. So far 70 % of all the sites listed on Superfund's priority list as hazardous, have heavy metal contamination of one kind or the other. Many of the heavy metals such as lead, arsenic, chromium and mercury are toxic and can cause severe damage to animals and plants alike. Hexavalent chromium is one such toxic heavy metal, which has widespread use in iron & steel, chrome plating, leather tanning, wood and textile industries. Effluents from industries like these are contaminated with hexavalent chromium and can find their way into ground water.

Traditional methods of clean up such as landfills and incineration do little to eliminate the threat of heavy metal contamination. Methods that are currently employed for this purpose include precipitation, ion exchange, chelation etc are ineffective at low metal concentrations, sometimes producing large amount of sludge, and are also cost prohibitive for handling large volumes (Wang and Chen, 2006). The need for alternative cost effective strategies for clean-up purposes has never been greater.

Bioremediation describes several technologies and strategies that take advantage of natural systems already in place for transformation, removal and stabilization of chemical pollutants. In that regard, the use of microbial biopolymers and other substances derived from microbes for heavy metal biosorption has gained considerable interest. Several such compounds have been tested or are currently being investigated for this purpose. The low cost combined with excellent selectivity for metal make biopolymers a lucrative choice for cleanup compared to conventional methods.

The candidate polymer used in this study was the homopolymer α-poly-l-lysine. Br. It is synthetically produced although another form of this polymer, ε-poly-l-lysine (ε-PLL) occurs in nature naturally. The high molecular weight of α-PLL makes it suitable for metal sorption applications. No research has so far been done to identify this polymer as a potential biosorbent for hexavalent chromium.

1.2 Main Objectives of Study

The main purpose of this study was to study if α-PLL can be successfully used as a biosorbent for hexavalent chromium. α-PLL served as a model biopolymer in these studies, and further research is needed to produce this polymer naturally which would drive costs down. The first goal was to establish via equilibrium batch mode studies, the feasibility of this polymer for metal uptake and the second goal was to assess the suitability of its use in a PEDF system.

The main objectives were as follows:

1. Determine the metal uptake capacity of the polymer in mg/g and also assess the optimum pH requirements using equilibrium batch mode studies.

2. Determine the efficiency of the polymer enhanced diafiltration system in removing chromium from aqueous solution.
3. Study the rheological characteristics of the metal- polymer complex.

2. BACKGROUND

The industrial revolution changed life on this planet forever. The rapid growth in industry following the revolution scaled new heights after World War II and has since continued, promising us a better tomorrow.

But tomorrow is also riddled with questions and concerns about health and well being, global warming and environmental pollution. Questions about the very sustainability of the planet are now being raised. The threat of pollution is very real, and some argue that the approaching danger is closer than we think.

As our industries have evolved and have become complex over time, so has the waste and toxic effluents they generate. Synthetic substances such as plastics, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), radioactive and metallic contaminants are not only toxic, but also non-biodegradable. Once they are released into the environment, they tend to accumulate over long periods of time and ultimately find their way into the ecological food chain. The mining, refining, electroplating, tanning and textile industries which generate a bulk of the hazardous waste usually discharge their waste into municipal sewerage which ultimately finds its way into the groundwater system. In fact some industrial areas have become so hazardous that they are no longer suitable for human and nonhuman inhabitation.

In 1980, the U.S. Environment Protection Agency created the Superfund program to hasten the identification, clean up and containment of hazardous waste sites. As of July 2008, there were 1255 sites listed on its National Priorities list (source: EPA *website*) that require immediate attention.

Approximately 70% of these sites are contaminated with heavy metals. Sites managed by Department of Defense and Department of Energy are also contaminated by heavy metals. It is estimated that federal, state and private industries will spend billions of dollars annually to clean up sites contaminated with hazardous waste. This investment validates the need to research newer and more cost effective methods to clean up contaminated sites.

2.1 Heavy Metal Contamination

Among toxic substances reaching hazardous levels are heavy metals (Vieira and Volesky, 2000). Some debate exists as to what constitutes a heavy metal and which elements should be properly classified as one. Most recently the term "heavy metal" has been used as a general term for those metals and semi-metals with potential human or environmental toxicity (Soghoian and Sinert, 2008). This definition includes a broad section of the periodic table with elements like arsenic, chromium, lead, mercury, zinc, iron and copper to name a few.

In trace amounts, some of these elements are essential to the proper functioning of the body, however above certain levels they pose significant health risks.

Heavy metals are major pollutants in marine, ground, industrial and even treated wastewaters (Demir and Arisoy 2006). Ingestion of some of these heavy metals such as copper, chromium, arsenic etc. beyond permissible quantities causes various chronic disorders in human beings. Heavy metals also tend to bioaccumulate in our bodies over a long period of time posing serious health risks. Major industrial activities such as mining, electroplating and power generation have problems with their effluents containing toxic heavy metals, often in anionic complex forms such as chromate (CrO_4^{2-}), vanadate (VO_4^{3-}), selenate ($Se0_4^{2-}$) (Niu and Volesky, 2004).

Although regulations are strictly enforced in many developed countries, these regulations are not usually followed in most developing countries. Many hazardous waste dumpsites are unlined, with no barrier between the waste and groundwater. (Dua, et al., 2002). Moreover, in many of these countries, these untreated waste-waters are used as sources for irrigation, whereby heavy metals often make their way into the food chain. Many studies have been carried out worldwide which singularly point to this fact. Studies carried out in Ethiopia to identify the effect of using waste-water in irrigation found elevated levels of Pb, Zn, Cu, Co in the soil. (Fitama, et al., 2006). Similar studies have been carried out in India, China, and Korea and have found much higher than recommended levels of heavy metals in rivers, soils and aquatic plants.

2.2 Hexavalent Chromium

Chromium is one such toxic heavy metal of widespread use. Extensive use of chromium in many industries such as electroplating, steel production, wood preservation and leather tanning results in releasing chromium containing effluents in to the environment making

it a severe threat to the ecological system (Demir and Arisoy, 2006). Industrial waste-water rich in heavy metal ions remain an important cause of concern. Approximately 142,000 metric tones of chromium are discharged into water bodies globally every year (Mohan and Pittman, 2006).

Chromium is a transition metal, and has oxidation states from Cr (-II) to Cr (+VI).

Chromium is the sixth most abundant element on earth but elemental chromium does not occur naturally on earth. It usually occurs as halides, oxides or sulphides. The two environmentally relevant valence states of chromium, Cr (III) and Cr (VI) have contrasting impacts on environment and health (Compton, et al., 2004). Trivalent chromium is relatively harmless and is an essential trace element in mammalian metabolism. It is involved in glucose metabolism. Hexavalent chromium on the other hand is much more toxic due to its high water solubility and mobility. Under normal physiological conditions, Cr^{6+} interacts spontaneously with the intracellular reductants (e.g. ascorbate and glutathione) to generate the short lived intermediates Cr^{5+}, Cr^{4+}, free radicals and end product Cr^{3+} (Costa 2003; Xu et al 2003, 2004; Cheung et al, 2006). The process produces reactive oxygen species (ROS) that easily combines with DNA- protein complexes (Cheung, et al., 2006).

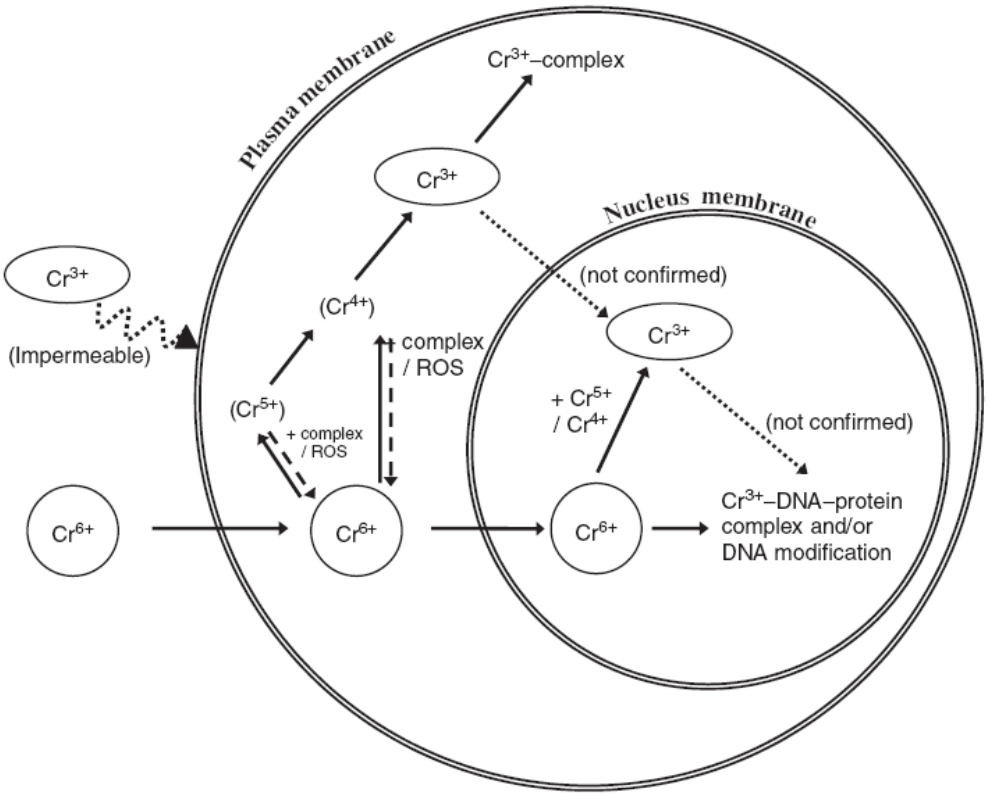

Figure 1. Schematic showing hexavalent chromium taken up by cell membrane and its reduction mechanism within the membrane. (Source: K.H Cheung, Ji-Dong Gu 2006, *Journal of Industrial biodevelopment*)

The highest exposure to hexavalent chromium occurs during chromate production, chrome pigment production, chrome plating and stainless steel welding.

Acute exposure to Cr (VI) causes nausea, diarrhea, liver and kidney damage, dermatitis, internal hemorrhage, and respiratory problems (Mohan, et al., 2006). In humans, exposure to hexavalent chromium salts for a period of 2 to 26 years has been implicated as a cause of cancer of the lungs and digestive tract (Costa and Klien, 2006).

The valence state of chromium in water depends on the pH and the redox potential in the solution it is in. Cr (VI) predominates under high oxidation conditions, such as shallow groundwater with pH 6-8, while trivalent chromium predominates under reducing

conditions like deep groundwater (Berceloux, 1999). The hexavalent species exists primarily as chromic acid (H_2CrO_4) and its salts, hydrogen chromate ion ($HCrO_4^-$) and chromate ion (CrO_4^{2-}), depending on the pH (Dionex Technical note). The dichromate ion ($Cr_2O_7^{2-}$) is a dimer of ($HCrO_4^-$), less a water molecule, which forms when the concentration of chromium exceeds approximately 1 gm/L (Dionex Technical note).

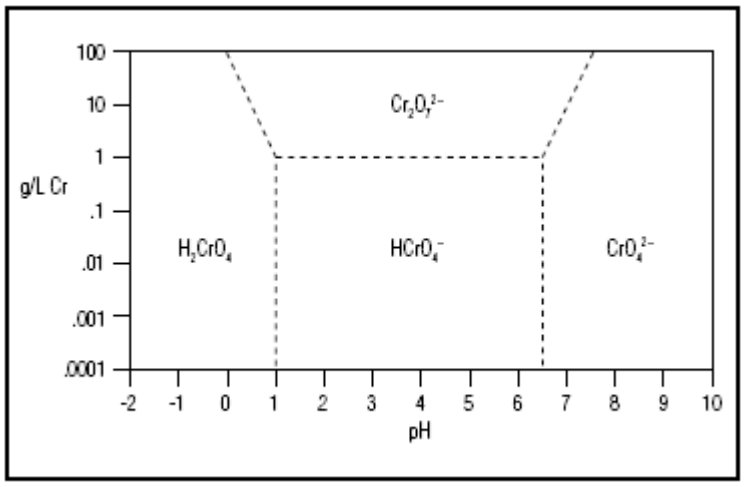

Figure 2. Speciation diagram of hexavalent chromium (Source Dionex technical note, Dionex Corp)

2.3 Traditional Methods of Chromium Remediation

Several treatment technologies have been developed in the past for removal of hexavalent chromium from water and wastewater. Some of these methods include chemical and electrochemical precipitation, adsorption, membrane filtration, ion exchange, reverse osmosis, air and steam stripping, flocculation and chelation. Though precipitation has been widely used in the past, the process creates sludge, and sludge disposal is a big problem. Ion exchange is a better alternative but at the same time is expensive. Most

remediation methods are effective in removing chromium from contaminated water if the initial concentration of chromium is high (>100mg/L) (Mohan and Pittman Jr, 2006). As a result of these and other shortcomings of physiochemical treatment processes, low cost and effective alternatives are actively being sought for the removal of trace concentrations of environmentally toxic metals (Mark, 2006).

2.3 Bioremediation

Put simply, bioremediation is the use of biological organisms to return the environment to its original state (Hunter, 2007). Organisms such as bacteria, fungi and plants are used directly or indirectly to process toxic products into less toxic or non toxic forms.

Bioremediation approaches are popular because they are natural, considerably less expensive and can be implemented on a mass scale. A good number of bioremediation strategies have been explored and successfully implemented.

For treating toxic wastewater containing heavy metals, there exists four basic bioremediation strategies. Most of these strategies aim to reduce the mobility and bioavailability of the metals: (1) Biosorption using living or nonliving biomass. In such cases metal uptake mostly occurs by adsorption or ion exchange. Seaweeds (Sargassum for uranium uptake), fungi (*Saccharomyces cerevisiae* for lead and uranium uptake) and bacterial cells (*E. coli* for copper, chromium and nickel) have been successfully studied. (2) Extra cellular precipitation caused by sulfate and phosphate reducing bacteria. When a microorganism oxidizes or reduces a heavy metal species, the reaction causes metals to precipitate (National Research Council, 1993). Mercury and chromium can be precipitated in this way (3). Microbial metal transformations wherein microbes are

employed to transform metals from toxic form to the less toxic, stable forms and (Pseudomonas *aeruginosa* converts hexavalent chromium to less toxic trivalent form) (Cheung and Gu, 2006) (4) biosorption using biopoylmers and other molecules secreted or derived from microbial cells (γ-PGA used for copper remediation) (Mark, 2006).

2.3.1 Biosorption

In the recent past adsorption has evolved as the frontline of defense for chromium removal, (Mohan and Pittman Jr, 2006). Selective adsorption by biological materials including viable as well as non-viable biomass has generated increasing excitement. Metal sequestering properties of non-viable biomass provide the basis for a new approach to remove heavy metals when they occur at low concentrations (Vieira and Volesky, 2000).

Heavy metal biosorption has been extensively investigated during the last several decades (Volesky, 1990). Several reports have been published that have looked into the various aspects of heavy metal bioremediation (Wang and Chen, 2006). From these reviews it is clear that the focus has mainly been on three areas of biosorption:

1. The search for efficient and cheap biosorbents.
2. The mechanism of metal uptake by biosobents and the various interactions involved on a micro level.
3. The large scale implementation of biosorption to make it an industrially viable option.

There are several chemical groups in biomass that could potentially attract and sequester metal ions: acetamido groups in chitin, amino and phosphate groups in nucleic acids,

amino, amido, sulfhydryl and carboxyl groups in amino acids and hydroxyls in polysaccharides (Holan and Volesky, 1995).

The importance of any given group for biosorption of a certain metal by a certain biomass depends on factors such as: number of sites in the biosorbent material, accessibility of the sites, the chemical state of the site and the affinity between site and metal (Vieira and Volesky, 2000).

The decision to use living biomass for metal removal vs. nonliving or biological molecules derived from living biomass depends on availability and ability of the living biomass to tolerate high toxic environment. Biopolymers or other nonviable biomaterials prove to be effective in places where bacteria and other species cannot be metabolically active either due to toxicity or other environmental conditions.

2.3.2 Biopolymers as Biosorbents

The use of microbial biopolymers for sequestering heavy metal from wastewater or any other metal of importance has been investigated for some time now. Polymers with a high concentration of ionic groups have the ability to chelate or exchange metal ions. This property facilitates their use in the recovery and separation of metal ions from aqueous solutions (Rivas, 2004).

Many biopolymers are known to bind metals strongly and the use of biopolymers as adsorbents for the recovery of metals has been studied (Baysal, 2006; Jeon, et al., 2002). For example chitin and chitosan are biopolymers which have interested researchers for their ability to form complexes with metal ions, particularly transition and post-transition metal ions. Chitin is a homopolymer of $\alpha(1 \rightarrow 4)$ linked N-acetyl –β-D-glucosamine

residues. It is a natural polysaccharide found in the shell of crabs and other crustaceans. The success of poly-γ-glutamic acid, an extracellular polyamino acid formed by *Bacillus licheniformis* for the removal of copper has added enough impetus to look for more such novel polymers (Mark, 2006)

The candidate polymer used in this study was the homopolymer α-PLL. A cationic polymer at neutral pH, α-PLL is ubiquitous in biotechnology labs as a cell adhesion agent for cell culturing experiments in plates and other solid substances (Krikorian, et al., 2002).

This study focuses on the biosorption of anionic metal complex of chromium, the chromate ion, by biopolymer, α-PLL.

2.4 Poly- L- Lysine

Poly-glutamic acid and Poly-lysine: a comparison

Poly amino acids are referred to a small group of polyamides that consist of only one type of amino acid linked by amide bonds (Shih, et al., 2004). They form an important class of biodegradable biopolymers which are being investigated for a variety of pharmaceutical applications. Both poly-glutamic acid and poly-lysine have two different structures, one that is naturally occurring and one that is chemically synthesized. Poly-α-glutamic acid and poly-α-lysine are synthesized chemically, by polymerization reaction having amide linkages between α-amino and α-carboxylic acid groups. Poly-γ-glutamic acid is a naturally occurring anionic polymer that is made of D- and L- glutamic acid units connected by amide linkages between α-amino and γ-carboxylic acid groups. In contrast ε-PLL is a naturally occurring cationic polymer made up of L-lysine residues connected

between ε-amino and α-carboxyl groups (Shih, et al., 2004). Both poly-glutamic acid and poly-lysine are water soluble, biodegradable and non-toxic towards humans and the environment (Shih, et al., 2004). Fig 3. shows the structure of poly-glutamic acid and polylysine.

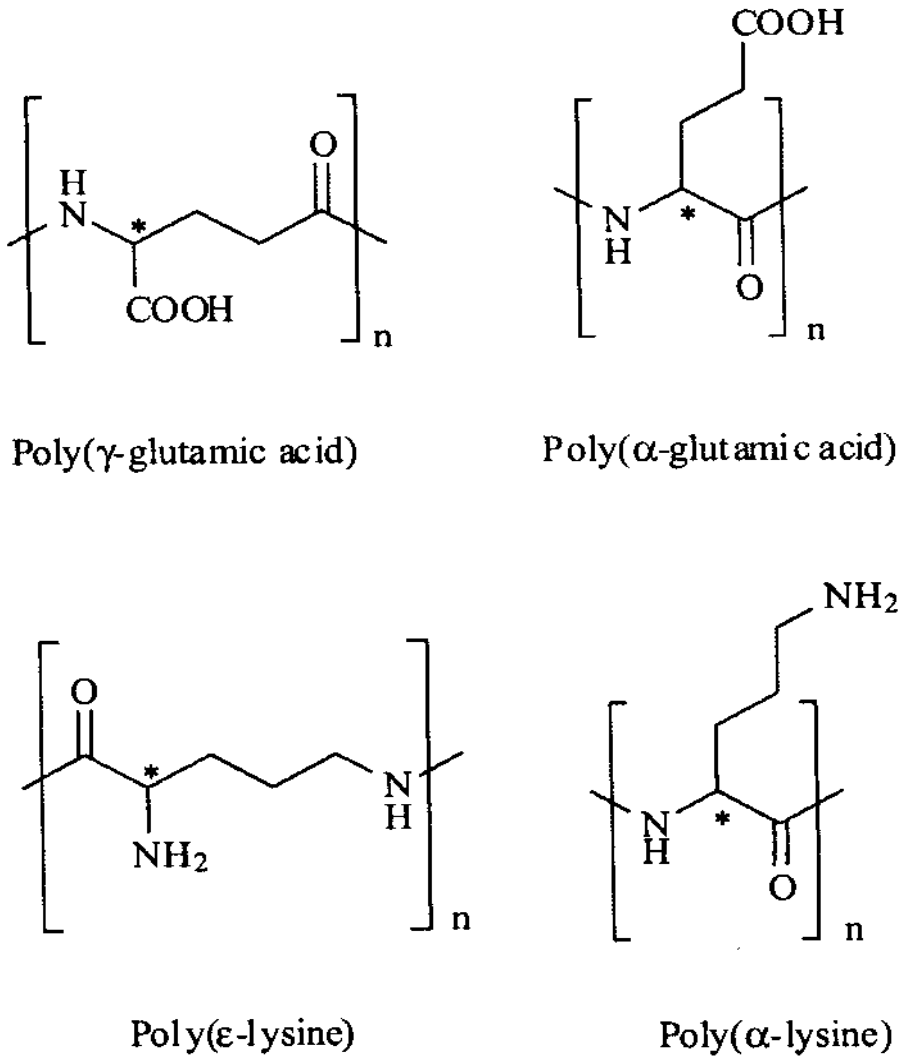

Figure 3. Structure of poly-glutamic and poly-lysine. (Source: Shih, et al., 2004, *Mini reviews in medicinal chemistry*)

ε-poly-l-lysine shows a wide range of antimicrobial and antiphage activities and is stable at high temperatures and under acidic and alkaline conditions (Ouyang, et al., 2006). It is used in the food industry as a food preservative. Several strains of *Streptomycetaceae* have been reported to secrete ε-PL into the medium, and the production of ε-PL by *Streptomyces albulus* has been studied most intensively (Ouyang, et al., 2006). However most strains produce a low molecular weight ε-PL composed of 36 to 40 lysine residues. Due to its low molecular weight, ε-poly-lysine is not suitable for metal sequestering applications. For metal adsorption, a high molecular weight polymer like poly-γ-glutamic (mol wt 10^6) acid is more useful as it has a higher number of sites to adsorb metal ions.

Here we focused on Sigma's synthetically manufactured α-PLL, with a molecular weight of greater than 300,000 Da. The lower molecular weight α-PLL (30,000 to 70,000 Da) makes this reagent easier to use, as it is less viscous in solution and also far less expensive. However, the higher molecular weight α-PLL (>300,000 Da), used for this project, provided more attachment sites per molecule.

Each lysine residue has molecular weight of 146.19 Da, hence this polymer has over 2000 lysine residues. Its pI is 9.59 and pKa's are at 2.2, 8.9 and 10.3.

It dissolves in water producing a viscous liquid. Its applications include glass coating to promote cellular adhesion, drug delivery, cell micro-encapsulation (e.g. Islet cells) and chromosomal preparations(Shih, et al., 2004). Polymers of both D- and L- lysine are used to coat slides. When adsorbed to the surface, poly-lysine increases the number of positively charged sites available for cell binding (Sigma catalogue). Here, the metal (anion) sequestering ability of the high molecular weight α-poly-l-lysine is being investigated. Although the binding of metal cations by α-poly-l-lysine and ε-poly-l-lysine

has been published by Shima & Sakai (1985), no major work has been done with regards to anions.

Efforts are ongoing to produce high molecular weight α-PLL from bacterial fermentation, for use in the future. For now, the synthetic α-poly-l-lysine serves as a suitable model for investigation of its use as a biosorbent for hexavalent chromium and other anionic complexes.

2.4.1 Mechanism of α-Poly-L-Lysine and Metal Interactions

The polymer metal ion interaction is mostly electrostatic in nature although sometimes it can include the formation of coordinative bonds. Variables both intrinsic and extrinsic to the polymer affect the interaction between the polymer and the metallic species. The former includes the polymer structure in terms of the composition and geometry, which affect the flexibility of the chains in the solution, the branches of the chain, the chemical nature of the functional groups, their distribution at the polymer chain and so forth (Rivas, 2006). Extrinsic factors include variables such as pH of the solution, ionic strength, the metallic ion itself, temperature and presence of other competitive ionic species etc. Not much has been investigated about the structure of α-poly-l-lysine in aqueous solution, where amide linkage exists between individual monomers. However it is known that at low and neutral pH, the polymers assumes a random coil structure (Fernandez-Barbero, et al., 2005). Some constraints exist due to the amide linkages and repulsion between the charged amine groups but in the case of α-PLL, where the amine groups are placed far away from one other, such repulsions are likely to be minimal. In a polar medium such as water, the amine groups on the polymer would be charged**,** the net

charge on the chain would depend on the ionic strength and pH of the surrounding medium. The interaction between the amine on the polymer and the chromate ion is electrostatic, although information about the exact interaction between the metal and the polymer is not known at this stage.

2.5 Equilibrium and Kinetic Batch Mode Studies

Preliminary testing of a candidate material for consideration as a potential biosorbent begins with batch mode sorption studies which involved both equilibrium and kinetic tests. If successful, this is followed by continuous flow sorption studies. The sorption study gives the very first insight into the adsorption performance of a polymer, and is mandatory for any new biosorbent to be used for this purpose.

The determination of metal uptake rate by the biosorbent is often based on the equilibrium state of the sorption system (Wang and Chen, 2006). In batch mode studies, the biosorbent and the metal species are brought in contact in solution until metal is sequestered by the sorbent in equilibrium with free metal left in the solution, temperature being constant.

The relationship between the metal uptake (q) by the biosorbent and the remaining free metal concentration (Ceq) is represented graphically with the help of adsorption isotherms. The resulting graph is mostly hyperbolic with the metal uptake increasing rapidly in the beginning and then leveling off as more and more metal ions adsorb on the available sites on the biosorbent as it nears saturation.

Metal uptake (U) in milligrams of metal per gram of biomass is given by

$$U = \frac{V(C1 - C2)}{M}$$

Where V = volume of metal bearing solution

C1 = initial metal concentration

C2 = final metal concentration

M = amount of biomass

The maximum metal uptake by the polymer is an important parameter for consideration as it reflects the maximum adsorption capacity of any given polymer, at a given concentration of the metal. The higher the value of q_{max}, which is the maximum metal uptake, the better the biosorbent. It is also preferred that the isotherm curve has a steep rise which indicates high affinity of the biosorbent for the metal.

Another important factor to consider is the time required to reach a state of equilibrium between the metal bound biosorbent and the free unbound metal in solution. A smaller time required to reach equilibrium translates into a faster process which can be significant for large scale operations.

2.5.1 Langmuir Adsorption Isotherm

There are two widely used adsorption isotherms, proposed by Langmuir and Freundlich respectively. These help to linearize the hyperbolic isotherm curves making it simpler to make quantitative deductions based on a linear plot of the data. For the purpose of this project, the Langmuir isotherm was followed. The Langmuir isotherm is based on certain assumptions:

1. The adsorbate covers the surface up to complete coverage as a monolayer on the adsorbent.
2. The adsorbed species only react with the adsorbent and not with each other.

3. On the substrate, all the binding sites are equivalent

The general form of the Langmuir isotherm is given by

$$q = \frac{q_{max} K_A C}{1 + K_A C}$$

Where q is the metal uptake concentration (mg/g adsorbent), q_{max} is the asymptotic maximum metal uptake (mg/g adsorbent), K_A is the equilibrium association constant (L/mg) and C is the bulk liquid phase metal concentration (mg/L solution). Another parameter which is closely related is K_D which is the dissociation constant and is the reciprocal of K_A. It is the metal concentration corresponding to half saturation of biosorbent (Fourest and Roux, 1992). The above equation can be written as follows, in the form of reciprocal equation.

$$\frac{1}{q} = \frac{1}{q_{max} K_A C} + \frac{1}{q_{max}}$$

The reciprocal plot of 1/q vs 1/C allows the hyperbolic graph to be linearized and hence the values of q_{max} and K_A can be easily obtained from the slope and intercept of straight line graph.

2.6 Polymer Enhanced Biosorption

Filtration is a pressure driven separation process that separates molecules based principally on size and sometimes also on charge. Membrane filtration is a widely used separation technique and the process can be successfully used for the separation of

inorganic species and for their enrichment from dilute solutions with the aid of water soluble polymers (Rivas, et al., 2000).

Ultrafiltration (UF) is a versatile technique in concentration, purification and separation processes. The ultra filtration of water soluble high molecular weight polymers with low molecular weight ionic species, allows for adequate interaction between the metal and the polymer.

The concept of using water soluble polymers to retain small ionic species was first discussed in the late 1960s by Michaels (Jarvinen, et al., 2000). Since then much work has been done to improve the type of polymers, allow for more polymer metal interaction and increase the efficiency of the process as a whole. A variety of terms and associated acronyms have been used in the literature for this technology including polymer supported ultrafiltration (Geckeler, 1996), liquid phase polymer based retention (LPR) (Spivakov, 1989), polyelectrolyte enhanced ultrafiltration (PEUF) (Scamehorn, 1990) and polymer assisted ultrafiltration (Smith, 1995). Some of the advantages of this technique as noted by Jarvinen et al include:

1. The reaction between the target species and the receptor sites occur in a single liquid phase which helps in rapid attainment of equilibrium.

2. Dissolving the polymer in solution also means that all of the polymer structure can be used for binding sites

3. A mixture of polymers can be used to target a group of metal/ionic species in solution and they can be separated in a single UF step

4. The scale up of a UF system is simple and well established

5. The UF system requires relatively low pressures, around 10 psig to operate

2.6.1 Polymer Enhanced Diafiltration Using Poly-L-Lysine

Broadly, PEDF involves two steps: binding of the polymer with the metal ion and retention of the polymer metal complex by the UF membrane. The pore size of the membrane is chosen so that it allows the polymer metal complex to be retained and other smaller moleules to pass. The metal is concentrated in the process and can be recovered later for subsequent use. In the current thesis work retention of hexavalent chromium (chromate) by α-poly-l-lysine was examined using PEDF. In the set up, a solution of hexavalent chromium was pumped into a reservoir containing the polymer. The interaction between the metal and the polymer took place in the reservoir and this solution was then circulated over the UF membrane until all the available sites on the polymer were saturated and chromium started showing in the filtrate. The necessary transmembrane pressure was created using a secondary pump to flow the feed into the UF membranes. A constant volume diafiltration was performed wherein the retentate volume was kept constant throughout the run.

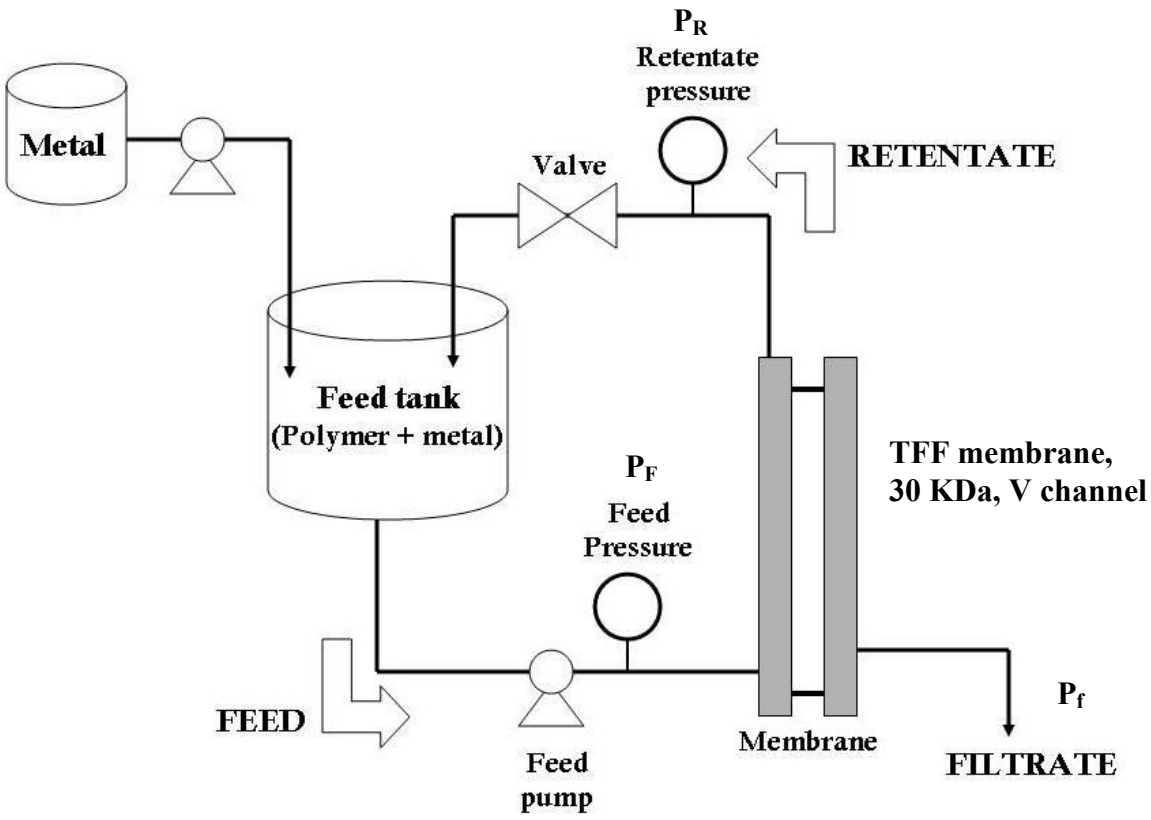

Figure 4. Schematic of PEDF unit (Source: Ameesha Shetty, *Metal Anion Removal from Wastewater Using Chitosan in a Polymer Enhanced Diafiltration System*)

2.6.2 Factors Effecting PEDF

Two of the important parameters affecting the performance of a tangential flow filtration system are trans-membrane pressure (TMP) and cross-flow rate. The flow of the feed along the length of the membrane causes a pressure drop from the feed to the retentate end of the channel; this is called the trans-membrane pressure. This is the principal force that drives the flow of the filtrate through the membrane. Additional pressure difference

can be created by applying pressure externally on the retentate side. The trans-membrane pressure or TMP is given by

$$TMP = [P_F + Pr/2] - P_f$$

Where, P_F is the feed pressure, Pr is the retentate pressure and P_f is the filtrate pressure. Cross-flow rate as the name suggests is the rate of flow of the feed along the membrane surface. Usually at constant trans-membrane pressure, higher the cross flow rate, higher the permeate flux. Higher flow rates also reduce membrane fouling due to the sweeping action of the feed. However, if the flow rate is too high it may exert unnecessary force on the polymer and cause it to shear. A low flow rate on the other hand can cause accumulation of molecules on the filter surface which can lead to membrane fouling. It therefore is important to strike a balance between cross-flow rate and trans-membrane pressure for optimum performance.

2.7 Rheological Properties of α-PLL

Rheology is the study of flow of matter and its deformation under applied stress and strain. Newtonian, non-Newtonian, viscoelastic etc are some of the attributes used to describe the rheological properties of fluid. Parameters such as viscosity, stress, shear stress, strain are used to quantify those attributes. Stress is defined as the force /area, shear stress is where the stress is parallel to the face of the material. Viscosity measures the resistance of a fluid to shear stress. Newton's equation states that the resulting shear of a fluid is directly proportional to the force applied and inversely proportional to the

force applied. Viscosity is measured in Pa.s (Pascal second) or centipoise. A fluid is termed Newtonian when it continues to flow regardless of the forces acting on it, whose stress versus rate of strain curve is linear. The viscosity of such liquids is constant. Non-Newtonian fluids are those, whose viscosity changes with changing stress, and hence cannot be defined by a single value of viscosity. Additionally some fluids have memory, i.e they flow under the slightest stress but revert back, the term viscoelastic is used to describe such fluids.

Rheometry refers to the set of techniques that are applied to determine the rheological properties of matter (solid/fluid). In this a rheometer is usually employed to exert torque/force on a material and its response over time is measured. The rheometer exerts a series of stress steps of increasing magnitude, in a ramp fashion on the fluid, sandwiched between the two plates of the rheometer, and the resulting deformation over time is used to determine the flow curve for that fluid. The flow curve can be plotted as a function of shear stress vs shear rate, bulk viscosity vs shear rate, or bulk viscosity as a function of stress. The later has been applied to plot the flow curve in this study.

Rheological behaviors of polymers are important to understand how a polymer behaves in solution and how its viscosity is related to its molecular weight. Many high molecular weight polymeric solutions are non-Newtonian. Most polymers exist in an entangled state governed by physical conditions like pH, temperature, ionic strength and concentration of the polymer. At neutral pH and room temperature PLL adopts a random coil structure, with some constraints due to peptide bond and repulsion between charges of amine groups. As metal is added to a PLL solution, its viscosity drops significantly, which indicates that as metals take up the free sites available on the polymer, the entanglements

are freed and the viscosity drops. It also suggests that metal-polymer interaction is intermolecular rather than intra-molecular in case of PLL and chromium.

2.8 Regeneration of Biosorbent

The cost-benefit analysis of any sorption process takes into account how well the sorbent can be reused for the purpose of metal remediation. For the sorbent to be reused, the metal-sorbent complex needs to be successfully decoupled, or the metal has to be desorbed from the sorbent, in this case the polymer. In some cases, the regeneration of the metal is just as important as the regeneration of the polymer. In cases where the metal is precious like gold and platinum, electrochemical methods can be applied to deposit the pure metal on a cathode/anode. However, such practices cannot be applied to everyday remediation practices, as they are very expensive. The sorption mechanism can help design a desorption strategy (Volesky, 2001). Sometimes increasing the pH can weaken the metal-polymer bonding, and the metal can be washed in a process similar to diafiltration. Similarly changing the ionic strength of the solution can cause bonding to weaken ultimately resulting in desorption. Chelating agents like EDTA can be used to desorp metal from polymer, and have been used in the case of chitosan-chromate complex. The desorption of chromium from α-PLL is beyond the scope of this study, and has not been pursued any further.

3. MATERIAL AND METHODS

3.1 Preparation of Chromium solution

Analytical grade $K_2Cr_2O_7$ (Mallinckordt Chemical Works, St.Louis, MO) was dissolved in deionized water (2 M Ohm) to make all the chromium solutions used throughout the experiment. 282.8 mg of $K_2Cr_2O_7$ was dissolved to 1 L of deionized water to yield the stock solution of chromium at 100 mg/L. The stock solution was appropriately diluted as needed.

3.2 Preparation of Poly-L-Lysine (PLL) solution

Poly-L-Lysine hydrobromide was purchased from Sigma (mol wt > 300,000). α-PLL was weighted and dissolved in dH_2O to make the solution. For a majority of experiments, 2 gm/L α-PLL solution was used. The solution was freshly prepared for each experiment. The pH of dissolved poly-lysine was 5.6 to 5.7.The dry polymer was stored at -20 C.

3.3 Spectrophotometric determination of Chromium

The concentration of chromium was determined using a colorimetric method using the diphenylcarbazide dye taken from *Standards Methods for the Examination of Water and Wastewater, method 7196A.* Samples containing hexavalent chromium (volume was usually below 2 ml) were mixed with 10% (v/v) H_2SO_4. The acid is required because the dye can bind to chromium more effectively in an acidic environment. Diphenylcarbazide (Sigma Aldrich) (0.5%) solution was made by dissolving it in acetone (HPLC grade 99%). To the acidified sample 200 µL of the dye was added to get a dark violet complex which was diluted to 10 mL with dH_2O. The absorbance of the solution was measured at

540 nm using as Beckman UV/VIS spectrophotometer. The dye was freshly prepared for each experiment, in 10 ml batches. Once prepared it was wrapped in foil to avoid exposure to sunlight and could be used for 2 -3 days. The dye was discarded if it turned yellowish.

3.4 Study of chromate Binding Properties of Poly-L-Lysine Using Equilibrium Dialysis

3.4.1 Construction of Dialysis Apparatus

The dialysis apparatus is made of polycarbonate which consists of two identical units. When the two units are attached using screws, they form set of eight cells or chambers which can hold liquid. The cells are separated using a semi-permeable membrane placed with the help of O rings between the two identical units. Each cell holds 2.5 ml solution, on each side of the membrane. The semi-permeable membrane is made of regenerated cellulose (Spectra/Por dialysis membrane, Spectrum laboratories, MW cut off 12-14 KDa).

Prior to use, the dialysis apparatus and the O rings were thoroughly rinsed first with 10% (v/v) HNO_3 to leach away any metals and then with dH_2O. The dialysis membranes were first cut in circular shapes 1cm in diameter and then soaked for 1 hr in a mixture of 0.01M Sodium bicarbonate and 0.001 M Na_2EDTA at 37C. The membranes were rinsed with 3 separate dH_2O washes of 30 min each at 37 C.

3.4.2 Equilibrium Batch Mode Studies

Two gm/L α-PLL and 100 mg/L chromium solutions were prepared as mentioned by the above methods. 2.5 mL of α-PLL solution (pH 5.7) was pipetted into one side of the dialysis chamber (recovery cell) and another 2.5 ml of chromium solution (pH 4.5) was pipetted into the other side of the chamber (feed cell). The top of the apparatus was sealed with screws to prevent leaks and put on a shaker at 250 rpm, 25 ^{o}C for 24 hours. At the end of 24 hours, samples (100 uL) were removed from the feed cell and the amount of free unbound chromium which was at equilibrium with free chromium on the other side of the membrane was determined using spectrophotometric analysis as discussed in section 3.3. A standard curve was drawn using known concentrations of chromium and the unknown amount was determined from the equation of the straight line.

3.4.3 Determination of Optimum pH for Chromium Uptake

The uptake of chromium was determined at pH 4, 8 and without changing the pH of Chromium solution and α-PLL solution at all. α-PLL has a pI of 9.59 and so it is protonated at pH 4 and 8. In this case, the pH of both chromium and α-PLL solutions were adjusted initially using 100 mM NaOH or 100 mM HCl. 2.5 mL of α-PLL was pipetted into one side of the dialysis chamber and 2.5 mL of chromium adjusted to the same pH was pipetted onto the other side. The entire dialysis apparatus was put on a shaker at 250 rpm, 25 C for 24 hours. Samples (100 μL) were periodically removed from the feed cell side, where chromium solution was added and the amount of free chromium was determined. Equilibrium was reached after 24 hours and no significant decrease in

the concentration of free chromium was observed after 48 hours. The uptake of chromium was also determined at native pH, in which no change to the pH of either of the solutions (α-PLL and Chromium) was done and was kept "as is". Chromium solution had a pH of 4.6 approximately and α-PLL was at pH 5.7 to start with. The pH of the solution during any of the experiments was not controlled. Control experiments with no α-PLL were carried out in the same apparatus to eliminate any experimental errors. The amount of metal bound to the polymer was determined by subtracting the free metal concentration from the free metal concentration in the control cells at equilibrium.

3.4.4 Equilibrium Adsorption Isotherm

Equilibrium isotherm studies were carried out using the dialysis apparatus with varying concentration of chromium solution, at 10, 20, 30, 40, 60, 80, 100 mg/L. The α-PLL solution was always at 2 gm/L. The same process was followed for the dialysis experiment, wherein 2.5 mL of α-PLL solution (pH 5.7) was pipetted into one side of the dialysis chamber (recovery cell) and another 2.5 ml of chromium solution (pH 4.5) was pipetted into the other side of the chamber (feed cell). However each cell had a different concentration of chromium. Controls without any α-PLL were also run. Samples were taken at regular intervals and the amount of free chromium was determined. The isotherm was plotted as uptake by polymer in mg/g versus concentration of free chromium at equilibrium.

3.5 Polymer Enhanced Diafiltration Setup

The experimental set up of the PEDF is shown in Fig 4. 300 mL of α-PLL at 2 gm/L was placed in a shake flask with outlet port. This acted as the feed tank. Another 1L shake flask was used as the reservoir, holding the chromium solution. A variable speed Millipore peristaltic pump with Cole Parmer pump head was used to pump fluid from the reservoir to the feed tank. Another pump (same model) was used as the inlet pump to push fluid over the TFF assembly. The pressure at the inlet and the retentate side was measured using pressure gauges. A hand valve was placed in the retentate line to adjust TMP. The filtrate was collected in a tall measuring cylinder to keep track of the volume being collected. The TFF membrane used was a Pellicon cassette from Millipore (MWCO 30K, V channel). All tubing (Masterflex 06485 14) used in the experiment was rinsed throughout in dH_2O.

3.5.1 Operation of PEDF Equipment

A constant volume diafiltration was performed during the experiment. The constant volume in the feed tank was maintained by having the chromium solution flow into the feed tank at the same rate as the filtrate flow rate. The feed tank was placed on top of a stir plate and its contents were continuously stirred to keep the solution homogeneous. The TMP was maintained between 13 to 14 psi using the hand valve on the retentate line. All PEDF experiments were conducted at room temperature.

3.6 Rhelogical Studies to Characterize α-PLL -- Chromium Complex

Rheological studies of the α-PLL chromium complex were studied using a Bohlin stress rheometer. 2 gm/L α-PLL solution containing 0, 1, 2, 10 mM chromium was placed on the lower plate of the instrument and the upper plate was lowered to apply stress on the liquid, sandwiched between the two plates. For each run, the instrument applied stress/force (0.75 to 1.5 mPa) on the liquid in an increasing order and the readings were stored in the computer. Stress viscometry software was used to manipulate the instrument and obtain readings. A graph of viscosity versus stress was plotted to determine the effect of stress on α-PLL solution, and how complexation with metal alters that.

4. RESULTS & DISCUSSION

4.1 Equilibrium Batch Mode Studies

As mentioned earlier, equilibrium batch mode experiments provide insight into the suitability of the polymer for adsorption purposes. Equilibrium batch mode studies were performed using the polycarbonate chamber separated by a semi-permeable membrane. Initial results were obtained with a α-PLL concentration of 2 gm/L and a chromium solution at 100 mg/L. Equilibrium was reached after 24 hours and samples were taken from the feed cell, to which the metal solution was initially added. The sample was analyzed for the presence of free residual chromium using the diphenycarbazide dye.

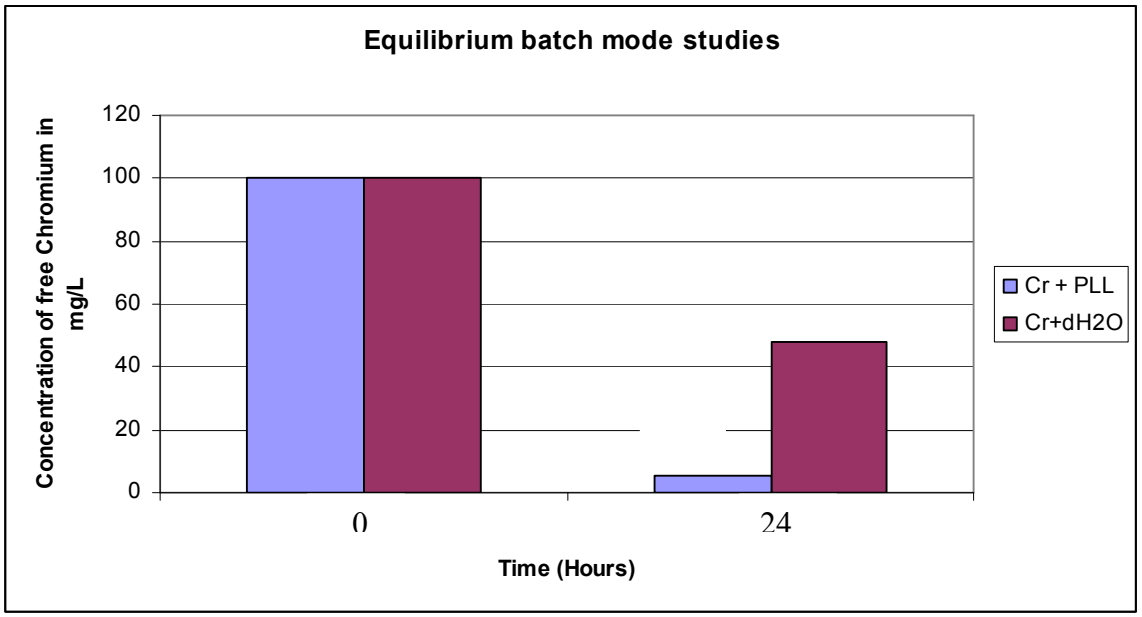

Figure 5. Equilibrium batch mode studies over 24 hours with 2 gm/L α-PLL. Starting concentration of chromium was 100mg/L, dH_20 used as negative control

Fig 5 shows the concentration of chromium in the feed cell after 24 hours. dH_20 was used as control to substitute for α-PLL. Without any polymer, the concentration of free chromium starting with 100 mg/L reached 48 mg/L in the control cell. In the presence of α-PLL (2gm/L), however, the concentration of chromium in the feed cell reached 5.7 mg/L. The pH in this experiment was not controlled or adjusted. The pH of the chromium solution at the start of the experiment was 4.67, and that of α-PLL was 5.5. The pH after the dialysis experiment was recorded at 4.96 in the feed cell with polymer in the recovery cell. This experiment gave the first insight into the performance of the polymer for biosorption. There was a significant decrease in the concentration of free chromium when α-PLL was present compared to no α-PLL present. This decrease in concentration of free chromium indicates a strong metal uptake by the polymer and helps to identify α-PLL as a potentially good biosorbent.

4.2 Optimum pH for Chromium Uptake

Figs 6 and 7 show the time course decrease in chromium concentration in the feed cell during equilibrium dialysis experiments at pH 4 and 8. The initial concentration of chromium was 100 mg/L and that of α-PLL was 2 gm/L. In the control cells where dH_20 was used instead of α-PLL, the equilibrium concentration of chromium after 24 hours was found to be 48 mg/L in case of pH 4. In the presence of 2 gm/L α-PLL, the equilibrium concentration of chromium was 9.5 mg/L. For pH 8, the concentration of chromium in the control cells was found to be 47.5 mg/L after 24 hours. In the presence of 2 gm/L α-PLL, the equilibrium concentration of chromium was 7.3 mg/L. In both

cases dialysis was continued for another 24 hours to determine if there is a further increase in uptake, but no significant change in values were observed.

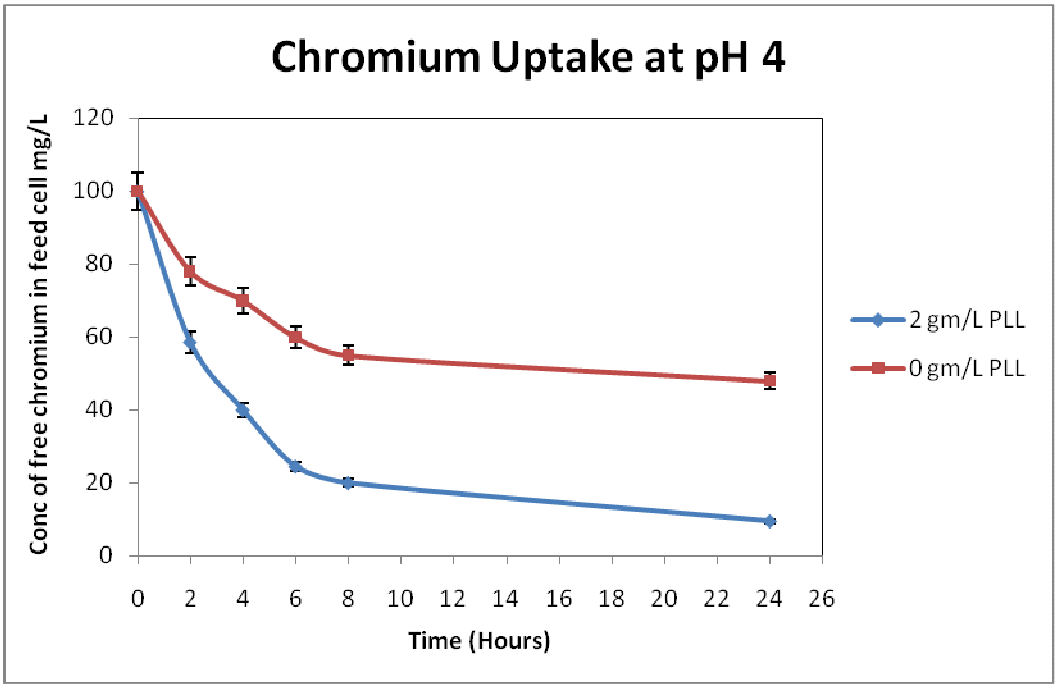

Figure 6. Chromium uptake by α-PLL at pH 4

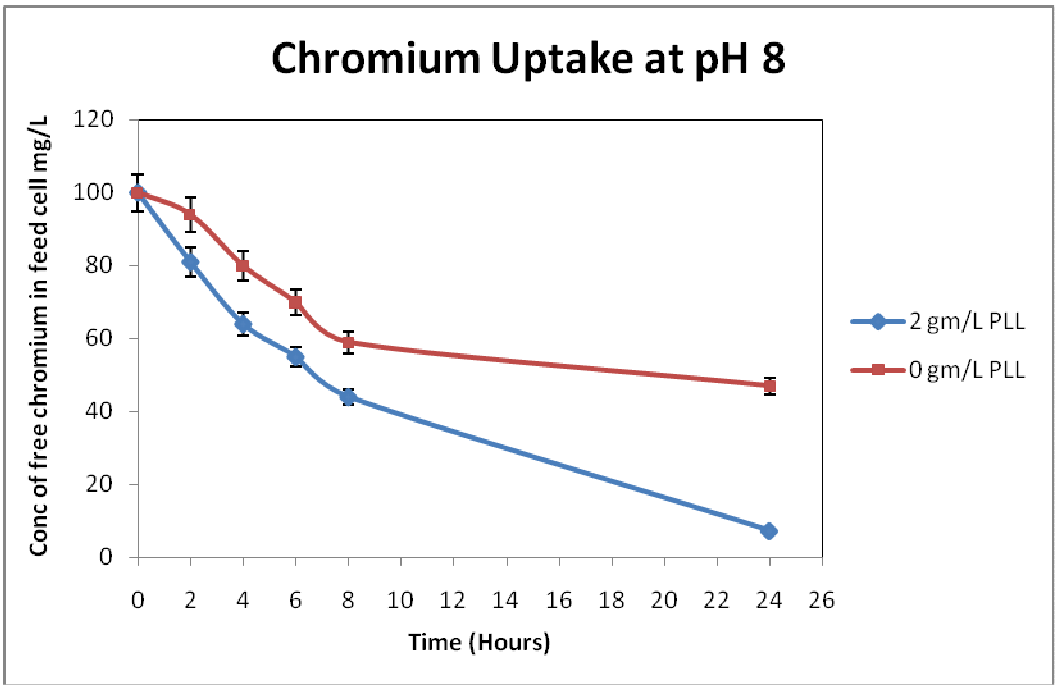

Figure 7. Chromium uptake by α-PLL at pH 8

Fig 8 shows the time course decrease in chromium concentration in the feed cell during equilibrium dialysis experiment at native pH, i.e the initial pH of the solutions were not adjusted. The pH of chromium solution at the start of the experiment was 4.6, and that of α-PLL was 5.7. The pH after the dialysis experiment was recorded at 4.9 in the feed cell with polymer in the recovery cell. The initial concentration of chromium was 100 mg/L and that of α-PLL was 2 gm/L. In the control cells where dH_20 was used instead of α-PLL the equilibrium concentration of chromium after 24 hours was found to be 47 mg/L. In the presence of 2 gm/L α-PLL, the equilibrium concentration of chromium was 5.7 mg/L.

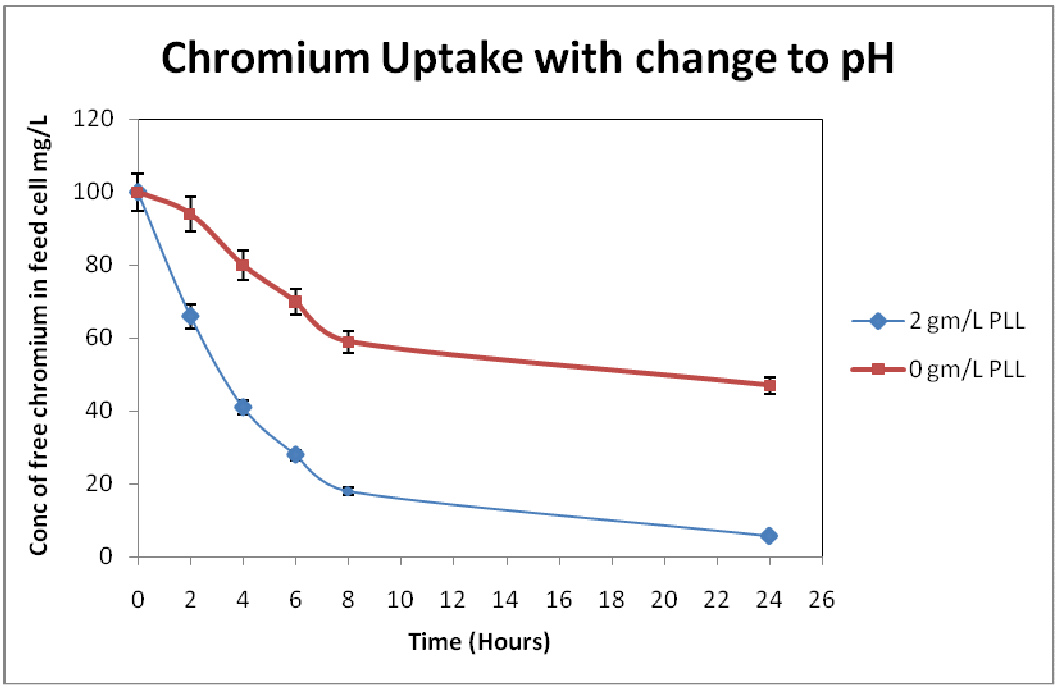

Figure 8. Chromium uptake by α-PLL with no change to pH

Table1 Effect of pH on uptake by Equilibrium Dialysis

Initial pH	Uptake (mg/gm)	Final pH
4	46.2	4.2
8	47.08	8.1
Cr 4.6, PLL 5.7	47.72	4.9

From the above plots and the table summarizing the uptake at different pH, it is evident that maximum uptake of chromium by α-PLL takes place when the pH of the solution is not adjusted and is kept as is. The concentration of free chromium in the feed cell decreased more rapidly in this case than observed previously. The pI of α-PLL is very high at 9.59, so at any pH lower than that, the amine groups on the polymer are protonated and can successfully attract the negatively charged chromate ions. However we do see that uptake is only slightly higher at pH 8 than it is at 4, a phenomenon which at this time could not be properly explained due to lack of any published literature dealing with the mechanism of poly-lysine-chromium binding.

4.3 Modeling of sorption kinetics:

The rate of change of metal concentration in the feed cell can be expressed approximately by the following equation (Tomida and Ikawa, 1993)

$$-\frac{dC1}{dt} = \frac{K_f A}{V_1}(C_1 - C_2) \qquad (1)$$

Where C_1 and C_2 are the concentrations of metal ions in the feed cell and the recovery cell respectively. K_f is the overall mass transfer coefficient, A is the effective membrane surface area and V1 is the solution volume in the feed cell. In the presence of water alone with no polymer present the equation can be written as

$$V_1 (C_0 - C_1) = V_2C_2 \quad (2)$$

With poly-lysine present in the recovery cell, the mass balance for the metal ions permeating into the recovery cell through the membrane is

$$V_1 (C_0 - C_1) = V_2C_2 + mq \quad (3)$$

Where m is the mass of PLL in the recovery cell and q is the uptake of chromium by α-PLL in mg/g of PLL. Assuming that binding of chromium to α-PLL follows Langmuir's isotherm model:

$$q = \frac{q_{max}K_AC}{1 + K_AC} \quad (4)$$

Where q_{max} is the maximum metal uptake by the polymer. K_A and C as stated earlier represent association constant and equilibrium bulk phase metal concentration in mg/L.

4.3.1 Equilibrium Adsorption Isotherm

As mentioned in the previous section, the uptake to chromium by α-PLL can be mathematically described in terms of the Langmuir adsorption isotherm. The isotherm is a plot of uptake of chromium by polymer in μg/mg *vs.* equilibrium concentration of free chromium in ug/ml. The experiment was carried out without any change to the initial pH of both chromium and α-PLL solutions. As shown in Fig 6, initially the uptake of free chromium increased with increasing availability of the metal ion. As the binding sites on the polymer became saturated, the uptake reached a plateau. The maximum adsorption is represented by $q_{max.}$ The pH was not controlled throughout the experiment and was found to be 5.1 at the end of the experiment.

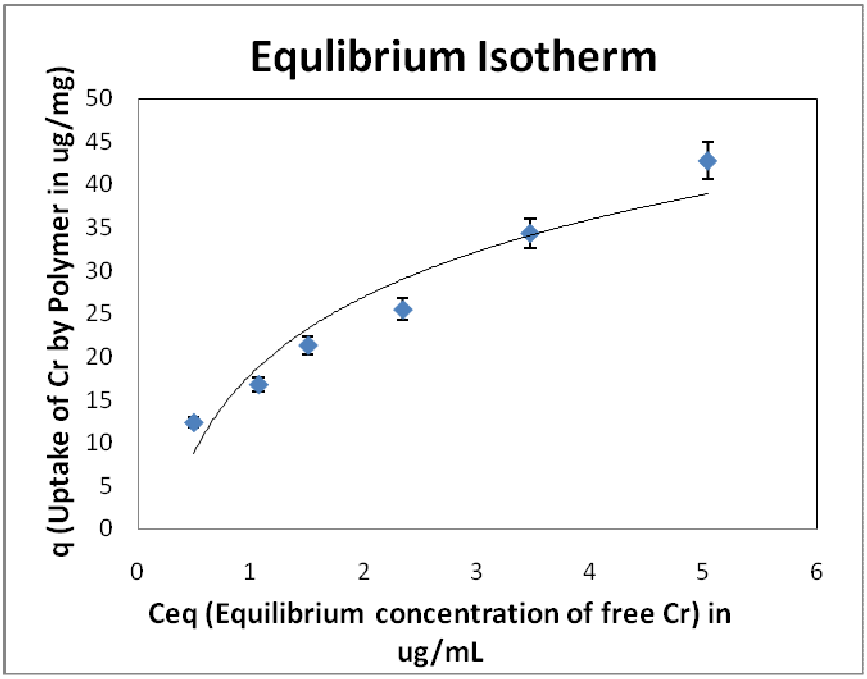

Figure 9. Equilibrium adsorption isotherm of chromium and α-PLL.

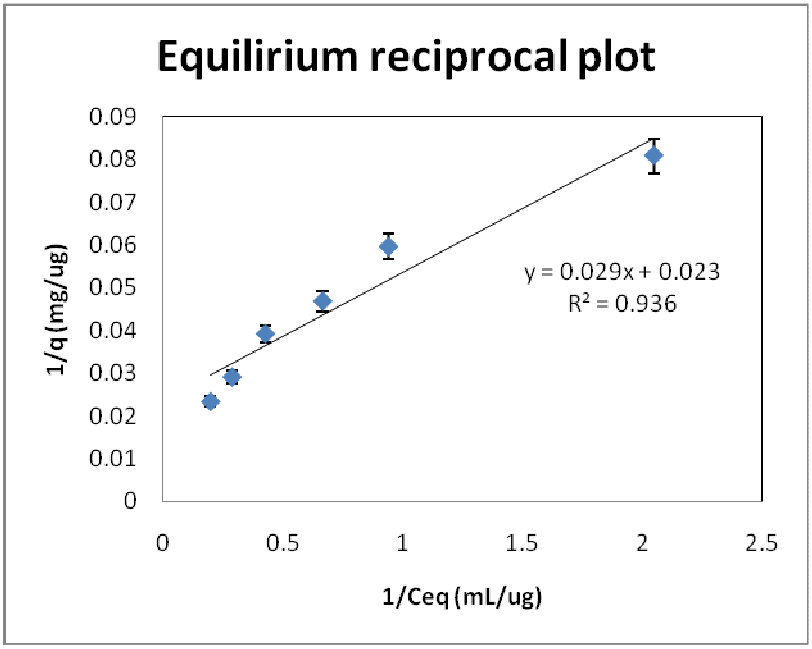

Figure 10. Double reciprocal plot of equilibrium isotherm.

Due to the non-linear nature of the isotherm plot, a reciprocal plot which is linear was used to find the values of K_A and q_{max} . Equation (4) can be modified as follows to find the values of K_A and q_{max} from the slope and the intercept of the straight line, plot of 1/q vs 1/C

$$\frac{1}{q} = \frac{1}{q_{max} K_A C} + \frac{1}{q_{max}}$$

From the equation, the value of q_{max} was calculated to be 42.2 ug/mg and K_A was 0.8 mL/ug $\pm$ 10%. The dissociation constant K_D ($K_D = 1/K_A$) was calculated as 1.2 ug/mL $\pm$ 10%. The uptake determined from the Langmuir isotherm is close to that obtained by equilibrium batch mode studies. Indeed the low value of the dissociation constant K_D suggests strong binding between PLL and chromate anion.

4.4 Dynamic heavy metal binding of chromium using α-PLL in a Polymer Enhanced Diafiltration system

PEDF experiments were performed with three different concentration of chromium keeping the concentration of α-PLL constant at 2 gm/L. The pH was not changed during any of the experiments, for both chromium and α-PLL solutions. The transmembrane pressure was maintained at 7.5 psi.

4.4.1 PEDF with no Polymer

An initial control PEDF was performed with chromium alone. Chromium solution having a concentration of 25 mg/L was pumped into the feed tank filled with 300 mL of dH_2O. The permeation curve of chromium into the filtrate is shown in Fig 11. It is a plot of concentration of chromium in the filtrate versus the volume of permeate collected in mL.

Samples of the filtrate were collected every 10 minutes until most of the chromium started to appear in the filtrate. As shown in Fig. 11, the curve is slightly asymptotic, the concentration of chromium in the filtrate reaches a maximum value of 19 mg/L and then leveled off. It can be assumed that 1 mg chromium is lost to the membrane and the set-up due to non-specific binding, and that is not significant. Mostly all the chromium that was added to the system, 20 gm/L was successfully retrieved and the PEDF set-up including the membrane were safe for analysis of metal uptake with poly-lysine.

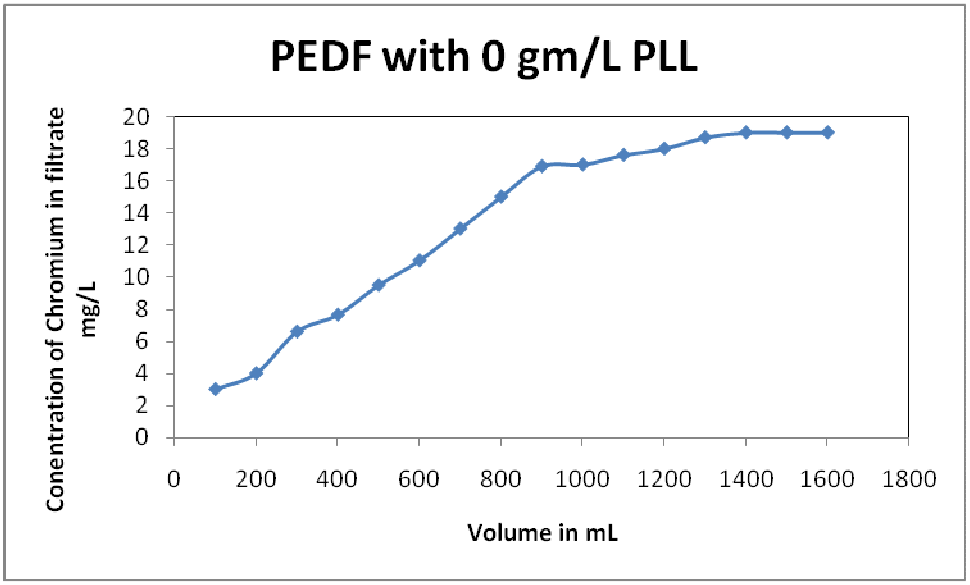

Figure 11. Permeation curve of 20 mg/L chromium in the absence of any polymer in a PEDF system.

4.4.2 Effect of chromium concentration on binding using PEDF

Figs. 12, 13, 14 show the effect of increasing concentration of chromium on binding in a PEDF system. At the beginning, only α-PLL solution was run through the TFF membranes in a closed loop, without adding any metal solution. This was done for about 15 minutes, to eliminate any low molecular weight fractions that may be present in the

polymer solution. After that, the metal solution from the reservoir was introduced into the feed tank via a peristaltic pump. A constant volume was maintained in the feed tank.

Fig. 12 shows the permeation curve of chromium in the filtrate with 2 gm/L α-PLL and 10 mg/L chromium solution. The TMP was maintained at 7.5 psi, and no change to pH of the solutions was done.

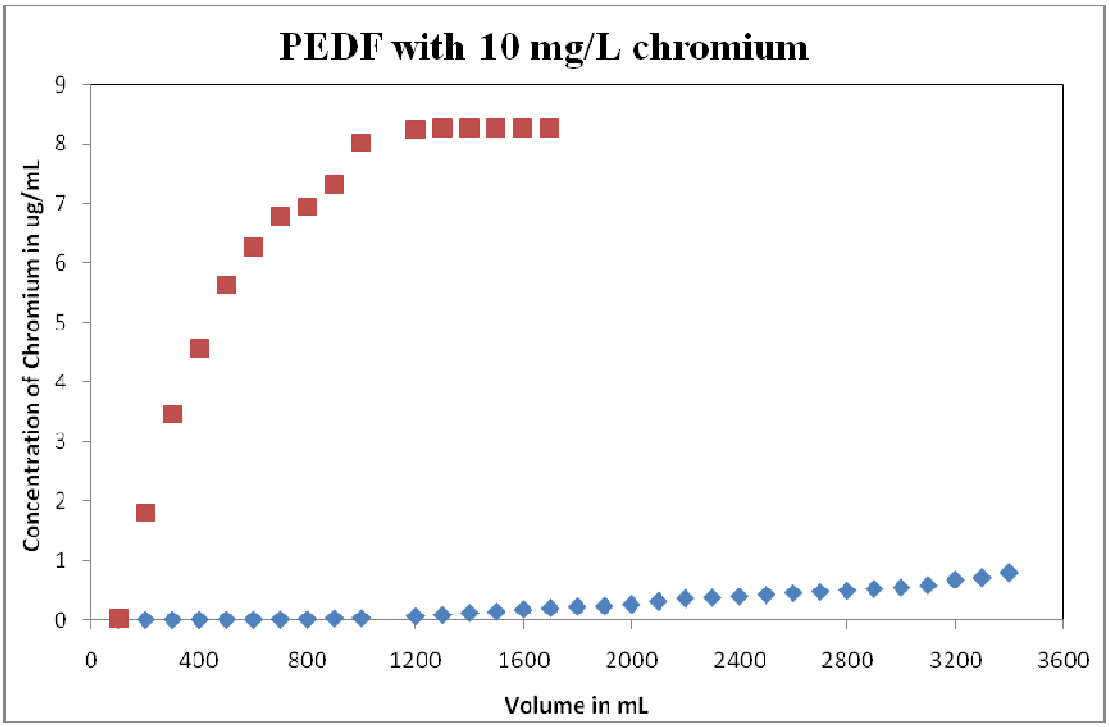

Figure 12 Permeation curve of 10 mg/L chromium in a PEDF system with 2 gm/L α-PLL in blue. Red dots – 0gm/L PLL

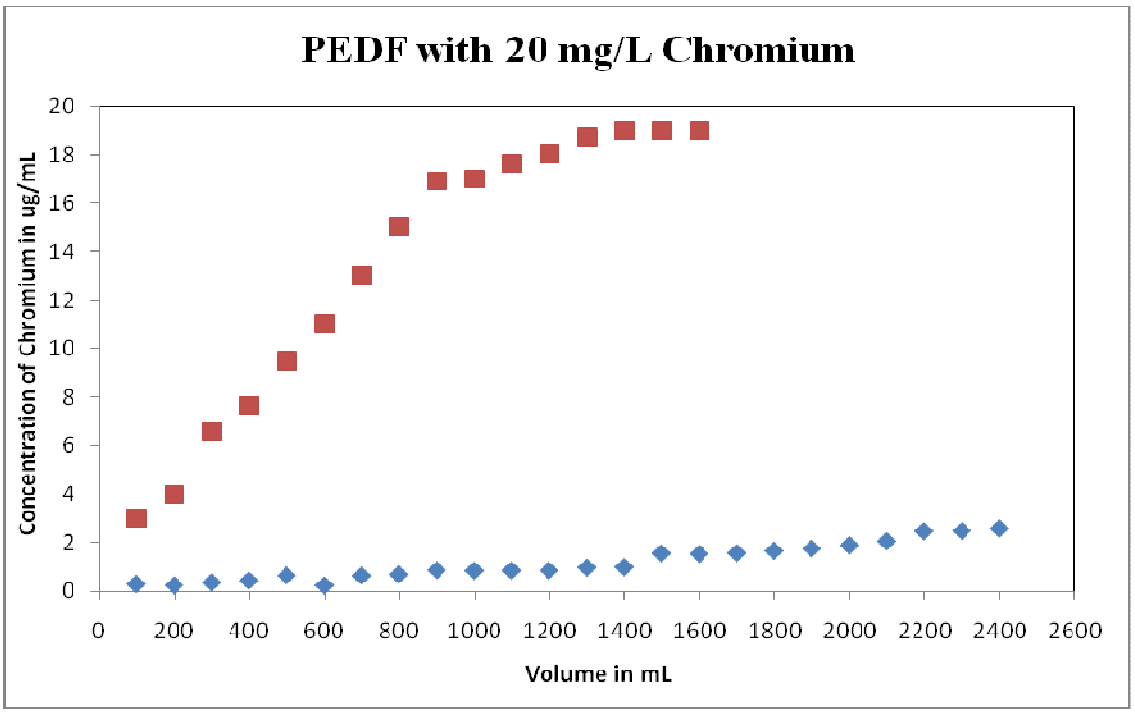

Figure 13 Permeation curve of 20 mg/L chromium in a PEDF system with 2 gm/L α-PLL in blue. Red dots – 0gm/L α-PLL

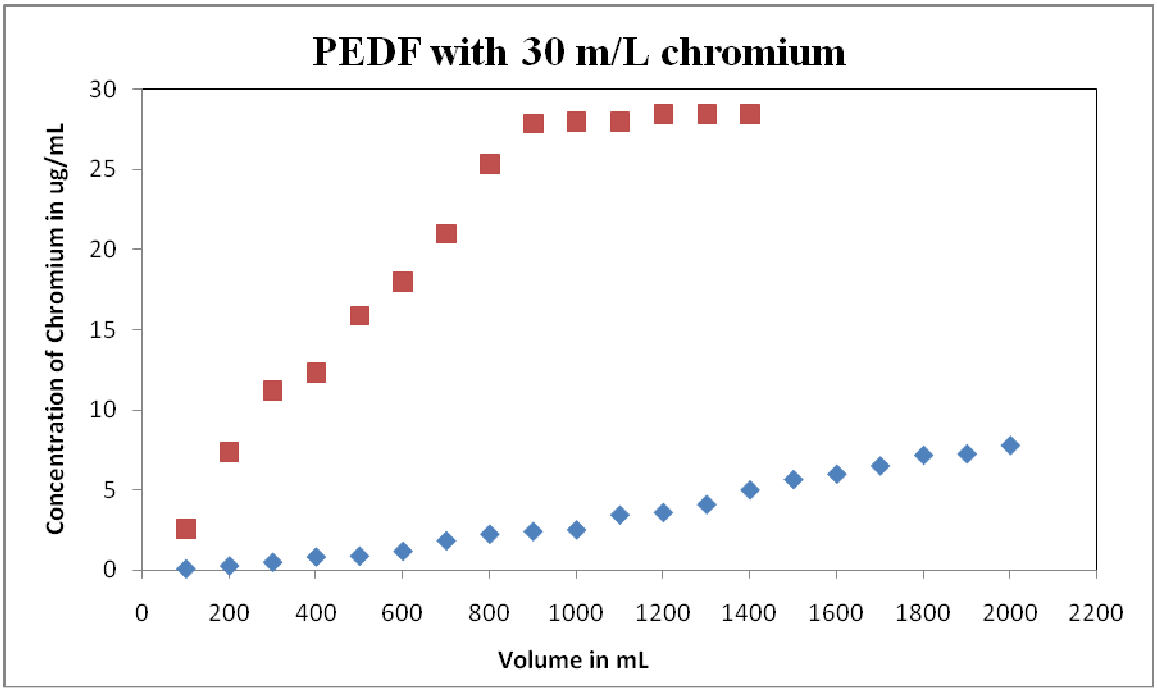

Figure 14. Permeation curve of 30 mg/L chromium in a PEDF system with 2 gm/L α-PLL in blue. Red dots – 0gm/L α-PLL

Figs. 13 and 14 show the permeation curve of chromium into the filtrate for a concentration of chromium at 20 mg/L and 30 m/L.

It is evident form the above plots that for the given concentration of PLL, 2 gm/L, the diafiltration step is effective in removing chromium from water at low concentrations. For 10 mg/L chromium concentration, almost 3.5 L of fluid could be processed with only 0.75 mg/L chromium appearing in the filtrate. For 30 mg/L chromium solution, 1.5 L of solution could be processed effectively before significant chromium (>5 mg/L) started appearing in the permeate. One reason for this could be that as more of the polymer complexed with the metal, its effective size decreased, and as a result the whole metal-polymer complex could be leaking through the membranes. A simple ninhydrin assay was performed to detect the presence of poly-lysine in the filtrate, and though some change in color was detected for the presence of PLL, the results were not very conclusive.

4.4.5 Modeling of PEDF Data

In modeling the sorption data for PEDF system, once again the Langmuir sorption model was used. In the case of PEDF, the kinetic model for biosorption is given by

$$V\frac{dC}{dt} = FCo - FC - \frac{dq}{dt}XV$$

V is the reaction volume (L), F is the inlet flow rate (L/h), and X is the biomass concentration in solution (g/L). Equation (4) and equation (7) can be combined and rearranged as follows

$$\frac{dC}{dt} = \frac{(Co - C)}{\tau\left[1 + \frac{qmaxKsX}{(C + Ks)^2}\right]}$$

τ is the residence time in the reactor (h), q_{max} is the maximum adsorption capacity of the polymer (mg/g) and Ks is the dissociation constant (mg/L). The values of q_{max} and Ks were already known form the Langmuir adsorption isotherm. This equation, integrated with the initial conditions: t = 0 and C = 0 was used to predict the ideal process performance.

Fig. 15 shows the permeation curve of 20 mg/L chromium, along with the predicted curve. As is evident, the observed level of chromium in the filtrate is much less than that predicted by the sorption model. One possible explanation for this discrepancy is the possible formation of a polarized gel layer on the membrane surface which obstructs the flow of chromium into the filtrate. However, this needs to be further investigated.

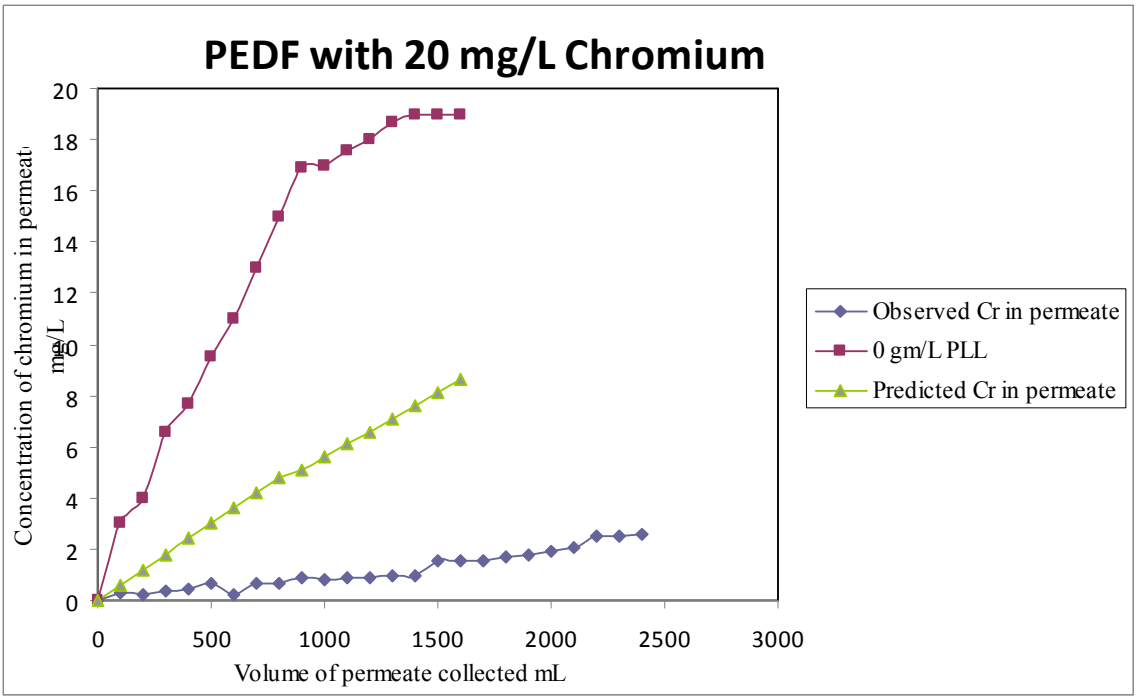

Figure 15. Permeation curve of 20 mg/LChromium , 2 mg/L α-PLL showing observed and theoretical values

4.5 Rheological Studies

The rheological properties of α-PLL-chromium complex were analyzed under different stress conditions. First, the viscosity versus stress relationship was determined for α-PLL to see if it behaved as shear thinning or shear thickening. Stress (0.75 to 1.5 mPa) was applied to 2 gm/L α-PLL without the addition of any metal. Later, 1mM, 2mM and 10 mM metal solution was added to 2 gm/L α-PLL keeping the total volume constant and the same stress was applied to determine the changes in viscosity stress relationship.

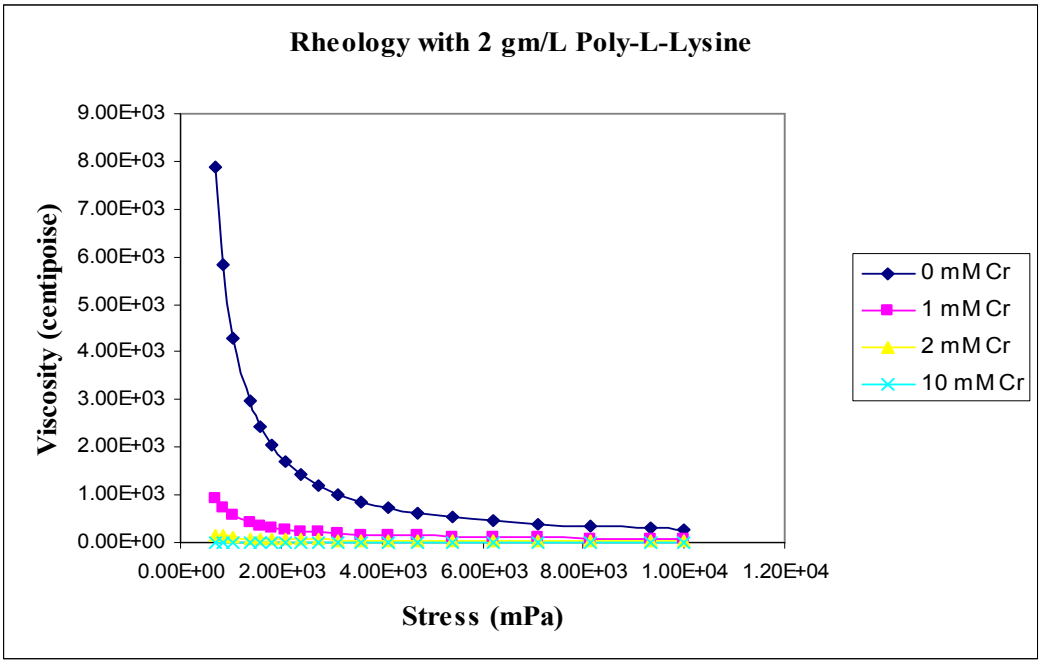

Figure 16. Rheological properties of PLL-Chromium complex

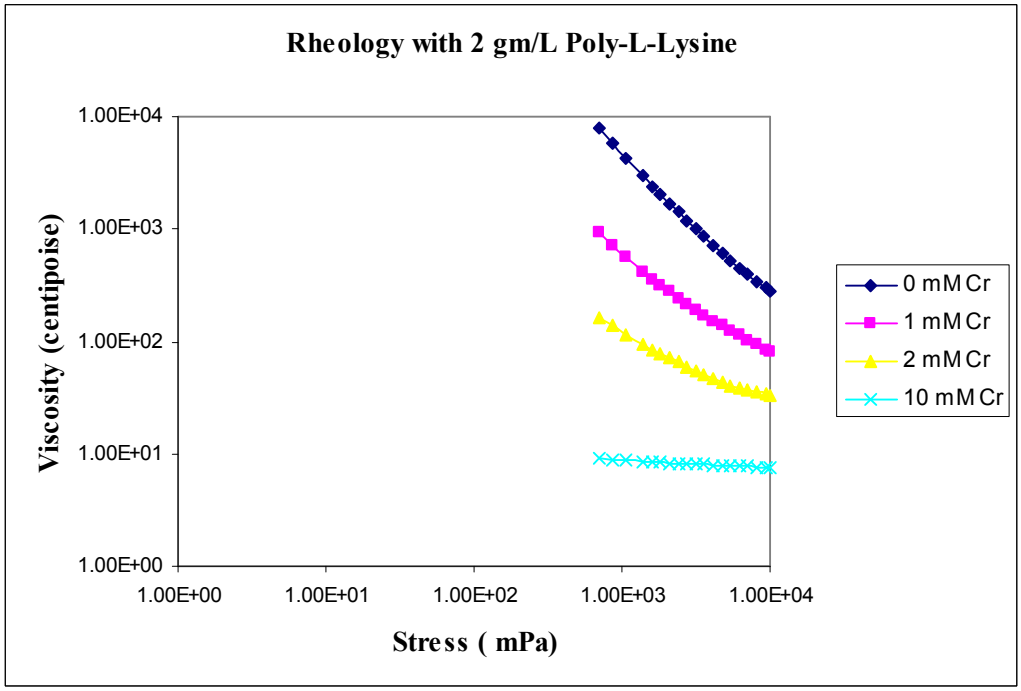

Figure 17. Rheological properties of α-PLL-Chromium complex (Log-Log plot)

As shown in Fig. 16, α-PLL acts as a shear thinning liquid when shear stress is applied. As stress is increased, the viscosity drops quite rapidly. In the presence of metal, the viscosity drops further, decreasing with addition of more metal. This can be explained as the polymer complexes with metal, it causes the polymer to dis-entangle, and hence the viscosity goes down. It also is indicative of the fact that the bonding between the metal and the polymer is inter-molecular as opposed to intra-molecular. Intra-molecular bonding would cause the polymer branches to come closer to form bonding with the metal, causing the viscosity to go up.

5. CONCLUSION

α-Poly-L-lysine can prove to be a good candidate for remediation of chromium. Equilibrium adsorption studies showed good metal uptake ability (42.2 mg/gm) and strong affinity for chromate anion as evident from low K_D value (1.2 ug/mL). Fairly high binding was also observed in our PEDF system, where the observed chromium in the filtrate was lower then that predicted. However the behavior of the polymer at higher metal concentrations needs to be studied further. If the polymer size reduces after forming a complex with metal, then some new strategy needs to be devised for effectively retaining the metal-polymer complex in a TFF system. Due to the high cost of the synthetic polymer, experiments such as effect of ionic strength, higher α-PLL concentration, temperature and other anions were not performed in this study. Those experiments need to be performed in the future. Attention also needs to be paid to effectively desorp the metal from the polymer, and recovery of the polymer.

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