

**METAL ANION REMOVAL FROM WASTEWATER USING
CHITOSAN IN A POLYMER ENHANCED DIAFILTRATION
SYSTEM**

A Thesis

Submitted to the Faculty of

WORCESTER POLYTECHNIC INSTITUTE

in partial fulfillment of the requirements for the

Degree of Master of Science

in

Biotechnology

by

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May 2006

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ABSTRACT

Discharge of metal containing effluents into water has been a cause of major concern. Traditional treatment methods are proving to be ineffective and expensive. Chitosan was studied as a potential biosorbent due to its positive charge and relatively low cost. The study involves evaluating the metal binding performance of chitosan in a Polymer Enhanced Diafiltration (PEDF) system which uses an ultrafiltration membrane to retain the chitosan which, in turn, binds the metal, thereby preventing passage into the permeate stream. Conditions for binding such as pH, concentration of polymer and chromium were studied. Optimal performance was obtained when the system was operated at pH values lower than the pK_a of chitosan i.e. 6.3. Using 6 g/L chitosan at pH 4.0, chromium concentration was reduced to less than 1mg/L from a feed concentration of 20 mg/L. Equilibrium dialysis experiments were done to study the kinetics of binding and the uptake of metal per gram of polymer. Rheological measurements demonstrated that in the presence of 1-100 mM chromate, chitosan was found to be slightly shear-thickening at low concentrations such as 4 g/L and 6 g/L whereas it was slightly shear-thinning at higher concentrations like 12 g/L and 20 g/L This suggests that neutralization of chromium anions is due to the interaction of multiple chitosan molecules. This result is consistent with the relatively stiff nature of the polysaccharide. Overall, this study suggests that some modification of the native polymer would be required to improve uptake and make it an industrially workable process.

ACKNOWLEDGEMENTS

I would like to thank Dr. Alexander Di Iorio, major advisor on this project, for his cooperation and constant support, even when things did not go as expected. One attribute that I am most grateful for, is his accessibility, when I had any doubts concerning my experiments or results. I would also like to thank Dr. Theodore Crusberg, my co-advisor, for his expertise and help, especially with dialysis experiments and standardization assays. Their constant backing made it possible to finish this project without any setbacks. I am gratified to have had such excellent mentors. In addition, I would like to thank Dr. Daniel Gibson III for being a valuable part of the committee. A special thanks to Christopher DaCunha, lab coordinator at the Bioprocess laboratory, for his assistance with the diafiltration experiments. I would also like to thank Sonny Mark, whose Masters Thesis work on heavy metal removal using poly- γ -glutamic acid, has been an invaluable resource. Finally I would like to thank my parents and friends, especially Ms. Arzu Atabek, with whom I have had long discussions about my experiments.

TABLE OF CONTENTS

Abstract.....	ii
Acknowledgements.....	iii
List of Figures.....	v
List of Tables.....	vii
INTRODUCTION.....	1
MATERIALS AND METHODS.....	30
RESULTS.....	37
DISCUSSION.....	64
CONCLUSIONS.....	72
REFERENCES.....	73
Appendix.....	79

TABLE OF FIGURES

Figure 1: Polymer Enhanced Diafiltration unit.....	17
Figure 2: Kinetics of chromate uptake by chitosan at pH 3.0.....	39
Figure 3: Kinetics of chromate uptake by chitosan at pH 4.0.....	39
Figure 4: Equilibrium isotherm for adsorption of chromium by chitosan at pH 4.0.	42
Figure 5: Equilibrium isotherm for adsorption of chromium at higher equilibrium concentrations.....	42
Figure 6: A reciprocal of the equilibrium isotherm plot (Figure 3). It represents $1/q$ versus $1/C_{eq}$	43
Figure 7: Permeation curve of chromium in polymer enhanced diafiltration using a feed concentration of 10 mg/L of chromium.....	46
Figure 8: Permeation curve in the absence of chitosan with a feed concentration of 20 mg/L chromium. This serves as a control to determine binding to the membrane...	47
Figure 9: Permeation curve for 2 g/L chitosan in a polymer enhanced diafiltration system.....	49
Figure 10: Permeation curve for 4 g/L chitosan in a polymer enhanced diafiltration system.....	50
Figure 11: Permeation curve for 5 g/L chitosan in a polymer enhanced diafiltration system.....	51
Figure 12: Permeation curve for 6 g/L chitosan in a polymer enhanced diafiltration system.....	52
Figure 13: Permeation curve showing PEDF of 6 g/L chitosan for a process volume of about 3 L.....	55
Figure 14: Permeation curve showing PEDF at 20 g/L chitosan.....	55
Figure 15: Permeation curve with 4 g/L chitosan without buffer diafiltration.....	58
Figure 16: Permeation curve with 4 g/L chitosan with buffer diafiltration.....	58
Figure 17: Permeation curve of chitosan at 6 g/L with predicted curve.....	60
Figure 18: Rheological behavior of 6 g/L chitosan. Viscosity was measured with increase in shear stress in the presence of 5-80 mM chromium.....	62
Figure 19: Rheological behavior of 4 g/L chitosan. Viscosity was measured with increase in shear stress in the presence of 5-80 mM chromium.....	62

Figure 20: Rheological behavior of 12 g/L chitosan. Viscosity was measured with increase in shear stress in the presence of 1-100 mM chromium. 63

Figure 21: Rheological behavior of 20 g/L chitosan. Viscosity was measured with increase in shear stress in the presence of 1-100 mM chromium.....63

LIST OF TABLES

Table 1: Effect of pH on uptake by equilibrium dialysis.....	44
Table 2: Polymer Enhanced Diafiltration (PEDF) measurements for 10 mg/L Chromium and 4 g/L chitosan.....	46
Table 3: PEDF measurements in the absence if chitosan.....	47
Table 4: PEDF measurements for 2 g/L chitosan.....	49
Table 5: PEDF measurements for 4 g/L chitosan.....	50
Table 6: PEDF measurements for 5 g/L chitosan.....	51
Table 7: PEDF measurements for 6 g/L chitosan.....	52
Table 8: PEDF measurements for processing larger volumes with 6 g/L chitosan.....	54
Table 9: Assay for chitosan using poly- γ -glutamic acid.....	59
Table 10: Adsorption capacities of different chitosan based sorbents for chromium.....	65

INTRODUCTION

1. Problem statement and Objectives.

In the past century there has been a rapid expansion in the chemical industry. This has led to an increase in the complexity of toxic effluents. Several industrial processes generate metal containing wastes. Heavy metal contamination has been a critical problem mainly because metals tend to persist and accumulate in the environment. (Förstner, U., 1983). Chromium is one such toxic metal which is being widely used. Chromium is generated by electroplating, tanning and textile industry and is potentially toxic to humans. (Palanisamy, 2005). Therefore the effluents being generated by these industries are rich in chromium.

The current physico-chemical processes for chromium removal like precipitation, reduction, ion-exchange etc. are expensive and inefficient in treating large quantities. They also cause metal bearing sludges which are difficult to dispose of. More stringent rules by the government and media and public pressure regarding effluent discharges have necessitated the search for newer methods of treatment. (Volesky, 2001, Udaybhaskar, 1990).

Biosorption, which involves active and non-active uptake by biomass, is a good alternative to traditional processes. Widely available biopolymers are also being used for sorption mainly because they are a cheap resource. (Niu, Volesky, 2003).

Chitosan is obtained from the deacetylation of chitin. Chitin is one of the most abundantly available polymers after cellulose. Chitosan is a copolymer of glucosamine and N-acetylglucosamine and it has an amine functional group which is strongly reactive

with metal ions. Considerable research has been done on the uptake of metal cations by chitosan. (Rhazi, 2002) However, there are some metals which exist in aqueous solutions as anions, for example chromium. The amine groups on chitosan bind metal cations at pH close to neutral. At low pH, chitosan is more protonated; and therefore it is able to bind anions by electrostatic attraction. (Guibal, 2004). Chitosan is chosen as the biosorbent because it is a cheaply available cationic polymer; a characteristic ideal for the binding of metal anions such as chromate.

The aim of this study was demonstrating the use of chitosan as a biosorbent for uptake of chromate anions using polymer enhanced diafiltration (PEDF) system. The PEDF system uses an ultrafiltration membrane that retains the polymer-metal complex and allows any unbound metal to pass through into the filtrate. The filtrate can be analyzed for the presence of chromium. The concentration of residual chromium will elucidate the binding ability of chitosan. Some of the other objectives of this research were:

- To characterize optimum conditions, such as pH and polymer concentrations using PEDF
- To perform equilibrium dialysis experiments in order to determine kinetics of binding and estimate the uptake of metal in milligram per gram of polymer.
- To study the metal-polymer interaction using rheological measurements. The metal- polymer complex can be characterized by studying the change in viscosity of the polymer in the presence of the metal.

2. Literature review:

In the past century, there has been a rapid expansion in the chemical industry. As the use of chemicals increases there will be an increase in the complexity of toxic effluents. Traditional methods of effluent treatment have to be supplemented with modern and more effective means of treatment. Bioremediation provides a technique for cleaning up pollutants by means of biological tools.

2.1 Metal contamination

Heavy metals are natural constituents of the earth's crust. Human activities have drastically altered the biochemical, geochemical cycles and balance of some of the heavy metals. Heavy metals are stable and tend to persist and accumulate in the environment. They cannot be degraded or destroyed. Aquatic systems are particularly sensitive to pollution possibly due to the structure of their food chains. In many cases harmful substances enter the food chain and are concentrated in fish and other edible organisms. (Förstner, 1983). As they move from one ecological trophic level to another, metallic species start damaging the ecosystem. They also become difficult to track as they move up trophic levels. They accumulate in living tissues throughout the food chain. Due to biomagnifications, humans receive the maximum impact, since they are at the top of the food chain. (Volesky, 2001).

Many metallic elements play an essential role in the functioning of living organisms; they constitute a nutritional requirement in some. However, overabundance of the essential trace elements can cause toxicity symptoms or death. (Volesky, 1990). There are many sources through which metal pollution of the environment occurs which include

geological weathering, industrial processing of ores and metals, leaching of metals from garbage and solid waste dumps. (Förstner, 1983). There is tremendous need to control heavy metal emissions into to the environment. The use of biological materials, including living and non-living micro-organisms, in the removal and possibly recovery of toxic substances from industrial wastes, has gained important credibility during recent years, because of the good performance and low cost of these materials. The natural affinity of biological compounds for metallic elements could contribute to the purification of metal-loaded wastewater. (Şahin. Y., Öztürk. A, 2005). There are several metals which are studied mainly due to their toxicity and pollution concerns. Mercury as a pollutant has received extensive exposure because of its toxicity to humans. In 1953, people died in the fishing villages along Minamata bay in Japan due to mercury poisoning. High levels of mercury were discharged by the nearby plastics factory and were accumulated in the shellfish eaten by the villagers. Lead pollution from mining activities and smelting operations is of concern because inorganic and organic lead is considered extremely toxic. Cadmium is the third of the “big three” of heavy metals with greatest potential hazard in addition to lead and mercury. A disease known as “Itai Itai” in Japan is associated with cadmium poisoning, which results in multiple fractures in the body. Chromium, cobalt, nickel, molybdenum, arsenic are other metals whose growing levels in the environment has caused major concern. (Volesky, 1990).

2.2 Chromium contamination.

Textiles, electroplating, leather tanning and metallurgy industries extensively use chromium and therefore the wastes generated by these industries are rich in hexavalent or

trivalent forms of chromium. Leakage, poor storage and improper disposal at the manufacturing and ore processing facilities have lead to release of chromium into the environment. This has lead to contamination of both ground and surface water. (Dantas, 2001, Yeal-lee, 2005).

The processes that are generally used for the removal of chromium are physical and chemical processes such as adsorption by activated carbon, reverse osmosis, reduction of hexavalent chromium into trivalent form and precipitation as chromium hydroxide. These treatments are expensive and not completely effective. (Sag, Aktay, 2001. Rojas, 2004).

Chromium exists in oxidation states ranging from 0 to +6. It occurs mainly in three forms. Metallic chromium (Cr[0]) is a steel gray solid used to make steel and other alloys. This form does not occur naturally but is produced from chrome ore. In nature it exists in two oxidation states; Cr(III) and Cr(VI). Cr(III) occurs naturally in rocks, soils, plants and animals. Hexavalent chromium Cr(VI) is produced industrially when Cr(III) is heated in the presence of atmospheric oxygen. Cr(VI) is more toxic than Cr(III) and has therefore lead greater environmental concern. (Booker, 2000; Hernandez and Castillo, 1995). Chromium(VI) exists in aqueous solutions as $\text{Cr}_2\text{O}_7^{2-}$, HCrO_4^- CrO_4^{2-} and HCr_2O_7^- . (Yeal-lee, 2005). The fraction of any particular species depends upon chromium concentration and pH. (Sag, Aktay, 2001. Udaybhaskar, 1990). At neutral pH Cr(VI) exists as HCrO_4^- , CrO_4^{2-} and H_2CrO_4^0 . Under acidic conditions HCrO_4^- polymerizes to form dichromate, $\text{Cr}_2\text{O}_7^{2-}$. Chromate imparts a yellow color to water in concentrations above 1 mg/L. (Palmer, Wittbrodt, 1991).

Cr(III) is an essential trace element in human nutrition. It is involved in glucose metabolism. Dietary deficiency of trivalent chromium has been associated with faulty sugar metabolism. The required dietary amount has been reported from 50 to 200 µg per day. (Katz, 1991, Shupack,1991).

Cr(VI) can enter the body on breathing air, consuming food or water contaminated with it. Short term exposure due to inhalation at high levels can cause nosebleeds, ulcers, irritation of the nasal mucosa and holes in the nasal septum. Dermal exposure can also cause skin irritation and allergies. (Booker, 2000). Chromium is potentially toxic to humans as it is considered a carcinogen. It is also known to cause kidney failure, metabolic acidosis, and oral ulcers. (EPA report).

The U.S. Environmental Protection Agency (EPA) has set a Maximum Contaminant Level (MCL) in drinking water at 0.1 mg/L or 100 µg/L. Soil concentrations are not strictly regulated by the EPA. There is an exposure risk among workers since it is used in many different industries. The Occupational Safety and Health Administration (OSHA) establish permissible exposure limits (PELs). The PEL for Cr(VI) in workplace air during a 40 hour work week is 100µg/m³. (Booker, 2000).

2.3 Need for new methods of removal.

There is pressure by the public and media with regards to environmental discharges of toxic effluents, which has lead to stricter rules by the government, especially with those concerning metal discharges by industrial operations. The toxic effects of heavy metals and their impact on human health are being understood. There is also awareness that the current technologies used for metal effluent treatment are inadequate due the

quantities of these wastes. It leads to problems such as metal bearing sludges which are difficult to dispose of. Some of these traditional methods are also extremely expensive, thereby proving uneconomical, especially for developing countries, where large volumes of these wastes are generated. (Volesky, 2001). Therefore there is a requirement for newer and effective methods which are also cost-effective. Biosorption is a feasible option because it is both efficient and cheap. Compared with conventional methods for removing toxic metals from effluents, the biosorption process has the advantages of low operating cost, minimization of volume of chemicals and biological sludge to be disposed off and high efficiency in detoxifying very dilute effluents.

2.4 Biosorption of heavy metals

The term biosorption refers to many modes of non-active metal uptake by biomass. There are many different types of biosorbents like:

- Active biomass belonging to algae, bacteria or fungi.
- Non active kind of biosorbent which is essentially a waste product or a byproduct of a fermentation process.
- Abundant natural materials or polymers. (Holan, Volesky, 1995).

The advantage of biosorption is that the biomass used, could be a raw material which is either abundant, a waste from another industrial operation or could be cheaply available. There are certain broad range biosorbents which can collect all heavy metals from the solution with a small degree of selectivity. Metal sequestration can occur by complexation, chelation, ion-exchange or coordination. Other mechanisms are physical such as adsorption or precipitation. Any of these mechanisms may be important in

immobilizing the metal on the biosorbent. Since the biomaterials that are used for sorption are complex a number of these mechanisms could be occurring simultaneously. (Volesky,2001). 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 proteins and hydroxyls in polysaccharides. (Holan, Volesky, 1995).

The biosorptive metal uptake can be quantitated by experimental biosorption equilibrium isotherms. Uptake of species by the sorbent involves several steps that transfer the solute from the bulk of the liquid phase to certain sites on the sorbent. (Guibal, 2004). On contact between the sorbent material and the sorbed species, equilibrium is established at a given temperature, where a certain amount of the metal species sequestered by the sorbent is in equilibrium with the residue left free in the solution. The affinity of the sorbent material for the sorbate determines its distribution between solid and liquid phases. The quality of the sorbent is judged by how much sorbate it can attract and retain in an immobilized form. The isotherm graphical expression is a plot of the metal uptake (q) by the biosorbent (weight/ weight or mole/ weight units) against the residual concentration (C_{eq}). This graph is mostly hyperbolic because the uptake increases gradually and then levels off as it nears complete saturation at high concentration of the sorbate (metal). (Kratochvil, Volesky, 1998)

Uptake of the metal (U) in milligrams of metal per gram of biomass is calculated by the formula:

$$U = \frac{V(C_1 - C_2)}{M}$$

Where V= volume of metal bearing solution

C_1 = initial metal concentration

C_2 = final metal concentration

M= amount of biomass.

The maximum uptake value gives an estimate of the performance of the biosorbent at high residual concentrations of metal. The isotherm mainly represents an equilibrium process where the metal bound to the sorbent is in equilibrium with the metal that is free. Some time is required for this equilibrium to be reached. Therefore sufficient contact time needs to be provided for the system to reach equilibrium. An isotherm which is steep at low residual sorbate concentrations is highly desirable because it indicates high affinity of the sorbent for the given sorbate species. Such a sorbent will perform well at low concentrations of the sorbate. Also, the initial concentration of the sorbate (metal) does not play a significant role in the equilibrium characterization of the sorbent because the final equilibrium concentration is of importance. Equilibrium sorption studies with simple one metal sorption systems are usually completed first and then extended into examination of multimetal biosorbent behavior along with the selected biosorbent.

Kinetics studies are also important in addition to equilibrium data because it helps one determine the time course of metal uptake. A rapid uptake would mean shorter contact times required between biosorbent and metal, and therefore smaller equipment and reduced costs. Both equilibrium and kinetics data are important for process design.

(Volesky, 1990)

2.4.1 Isotherm models for adsorption

There are two widely accepted and easily linearized adsorption isotherm models proposed by Langmuir and Freundlich respectively. The assumptions of the Langmuir model are:

- The surface consists of adsorption sites.
- All adsorbed species interact only with the sites and not with each other.
- Adsorption is limited to a monolayer.
- Adsorption energy of all sites is identical and independent of the presence of adsorbed species on the other sites.

A general form of the Langmuir model equation is

$$q = \frac{q_{\max} b C_{\text{eq}}}{1 + b C_{\text{eq}}} \quad (\text{Equation 1})$$

Where q = uptake of species (mg/g), q_{\max} = maximum uptake (mg/g), C_{eq} = equilibrium (final) concentration in solution (mg/L), and b = constant related to energy of adsorption. b is the equilibrium or association constant (L/mg) and is related to the affinity between sorbent and sorbate. It is also represented as K_A . Dissociation constant or K_D is another constant which represents the metal concentration corresponding to half-saturation of sorbent. Low values of b are reflected in the steep initial slope of a sorption isotherm, indicating a desirable high affinity. Thus for “good” sorbents, a high q_{\max} and a steep initial isotherm slope is desired.

The values of q_{\max} and K_A can be found using the linear reciprocal plot of the equilibrium isotherm.

$$\frac{1}{q} = \frac{1}{q_{\max} K_A C} + \frac{1}{q_{\max}} \quad (\text{Equation 2})$$

This is a plot of $1/q$ vs. $1/C_{eq}$, where q_{max} and K_A can be found from the slope and intercept. (Volesky, 1990; Barba.D, 2001; Tomida, Ikawa, 1993; Mark.S, DiIorio.A, 2006; Kratochvil, Volesky, 1998).

The form of the Freundlich adsorption model is

$$q = kC^{1/n} \quad (\text{Equation 3})$$

where q = solid phase metal concentration (mg/g), k = related to adsorbent capacity, $1/n$ = “heterogeneity factor” ranging from 0 to 1 and C = bulk liquid phase metal concentration. This can be linearized by taking the natural logarithm of both sides of the equation to give:

$$\ln q = \ln k + 1/n \ln C \quad (\text{Equation 4})$$

The intercept $\ln k$ gives a measure of the adsorbent capacity and the slope $1/n$ gives the intensity of adsorption.

Both of these models are able to describe many data effectively. Since the parameters of these models do not have a physical interpretation to them, their use is limited. Fitting the model to the process behavior does not necessarily imply that a “pure” adsorption phenomenon has taken place. (Mark.S, 2006; Volesky, 1990).

Another aspect of biosorption is to determine the strategy for releasing the sequestered metal from the biosorbent. The kinetics of the desorption cycle can be established as well as the regeneration of the biosorbent. The desorption process should yield the metal in a concentrated form and also restore the biosorbent as close to the original condition as possible, so that its metal uptake capacity is undiminished and there is no physical change or damage to the biosorbent. (Volesky, 2001, 1990).

2.4.2 Modeling of biosorption data

Mathematical modeling and computer simulation is an extremely powerful tool for biosorption studies. It is essential for process design and optimization. It allows a combination of equilibrium and dynamic studies information which cannot be appropriately handled without modeling. When reaction kinetics is combined with mass transfer studies which in turn are dependent on fluid flow properties, modeling allows simple comprehension of the data. Biosorption process modeling is also important in predicting the process performance under different conditions. Computer simulations can replace numerous tedious tests. However, the simulations are only as good as the model behind them. Improved knowledge of mathematical modeling and simulations can allow this area to be dependable. Biosorption modeling can then be used to guide experimental research, optimize a given process and provide a basis for process control strategies.

(Volesky, 2001).

2.4.3 Effect of waste-water composition on biosorption

The efficiency of a biosorption process depends not only on the binding properties of the biosorbents, but also on the composition of the waste-water that it will be used for treating. After pilot scale tests are carried out with a specific industrial effluent, only effluents that can be treated efficiently should be selected for extended testing of the biosorption process. There are certain limits that might be imposed on the biosorption process by the composition of waste-water. Majority of industrial effluents contain more than one toxic metal. For example, effluents produced at a copper mine would contain a high level of Cu^{2+} and traces of Cd^{2+} , Ni^{2+} and Mn^{2+} . If the biosorption involves ion exchange, it will lead to competition among the metal species for a limited number of

binding sites on the biosorbent. Conversely, the industrial effluent might also contain certain non-toxic species that may have a greater affinity for the binding sites on the biosorbent than the toxic target metal. For example, a variety of ionic species of iron are a matter of concern for biosorption processes treating mining effluents like acid mine drainage. In this case, it is important to establish the selectivity of the biosorbent for toxic metals over iron. (Kratochvil, Volesky, 1998)

2.5 Polymer Enhanced Diafiltration (PEDF).

Filtration is a process that uses membranes to separate components based on their size and charge differences. It can be composed of two different operational modes – normal flow filtration and tangential flow filtration. In normal flow filtration, fluid is directed towards the membrane under an applied pressure. Molecules that are too large to pass through the pores of the membrane accumulate at the surface of the membrane whereas smaller molecules pass through in the filtrate. This type of filtration is also called dead-end filtration. In tangential flow filtration, the fluid is pumped tangentially along the surface of the membrane. Pressure is applied directly towards the membrane which forces a portion of the fluid through the membrane. Larger molecules will be retained on the membrane but in this case the retained molecules will not build up on the surface since they get swept along by tangential flow.

Polymer Enhanced Diafiltration involves two steps – binding of the polymer to the metal ion and retention of the metal-polymer complex on the membrane. Pore size of diafiltration membranes are large enough to allow retention of complexed metallic ions, the small sized metal ions will pass through the membrane when uncomplexed. Therefore using a polymer enables the metal ion to bind to it and form a macromolecular complex

that is held back by the membrane. The molecular weight cut-off of the membrane is chosen so as to allow retention of the polymer and passage of non-complexed ions into permeate. It is a feasible process to separate or concentrate metal ions from dilute aqueous solutions. (Llorens.J, 2004; Mark.S, 2006). A similar principle is also used in Micellar Enhanced ultrafiltration (MEUF) which involves the addition of a surfactant in order to trap ionic solutes in solution. (Baek. K., 2003).

2.5.1 Working of the PEDF unit

In a PEDF unit operation, a pump is used to add the metal into the feed tank or reservoir which contains the polymer. Another pump is used to generate flow of the feed stream from this tank to the membrane. The applied pressure will force a portion of the fluid through the membrane generating the filtrate/permeate. The filtrate is collected at regular intervals to analyze the residual metal concentration. Since the flow of the feed is tangential to the membrane surface, the macromolecular polymer-metal ion complex will be returned to the feed tank as the retentate. There is a pressure gauge at the inlet of the unit as well at the outlet, indicating the feed pressure and retentate pressure respectively. There is also a pressure control hand valve to adjust the pressure. If a constant-volume diafiltration is performed, where the retentate volume is kept constant throughout the run, the metal solution will enter the feed tank at the same rate that filtrate leaves. (Millipore manual, Müslehiddinoğlu, J. *et al*, 1997).

2.5.2 Parameters affecting membrane performance

The flow generates a pressure difference across the membrane. This is given by transmembrane pressure (TMP) or the average applied pressure from the feed side to the filtrate side of the membrane.

$$\text{TMP} = [P_F + P_R/2] - P_f$$

Where P_F = feed pressure

P_R = Retentate pressure

P_f = Filtrate pressure

(Millipore manual, Müslehiddinoğlu, J *et al*, 1997).

It is this pressure difference that is the driving force of the separation process. Permeate flux is another important parameter. It is defined as the filtrate flow rate normalized for the area of the membrane.

$$\text{Permeate flux} = \text{Permeate flow rate} / \text{membrane area.}$$

The higher the pressure difference, the greater is the permeate flux. However, a very high flux may cause the polymer chains to be deformed, thereby being able to pass through the pores of the membrane. Therefore, an optimum pressure must be chosen so as to have efficient retention. (Volchek *et al*, 1996). In a PEDF unit operation, permeate flux increases with increase in TMP up to a point then it levels off. The first part of the curve, where the flux increases with pressure is the pressure dependent regime. Here the primary factor limiting flux is the fouled membrane resistance. The second part of the curve is the pressure independent regime. As the feed flow rate decreases, the TMP at which the flux plateau decreases. (Millipore manual).

Crossflow rate is also an important parameter. It increases the sweeping action across the membrane. In general, a higher crossflow rate gives higher flux at equal TMP. However, too high crossflow rates might cause the polymer to go through many passes through the pump which can lead to degradation of the polymer.

As the fluid is passed through the membrane, accumulation of the retained particles of solute on the membrane surface is sometimes seen. This phenomenon is called concentration polarization. This is observed when the transport of material towards the membrane is greater than the diffusion of material back to the bulk stream. The thin layer formed on the membrane leads to increasing osmotic pressure. Thus permeate flux through the membrane declines. (Katsikaris, 2005). When polymers are used in diafiltration systems, solute rejection as well as membrane fouling could occur, depending on the properties of the polymer and the conditions at which the unit is run. Membrane fouling is a combination of initial pore blocking by the polymer as well as the formation of a fouling, second layer on the surface of the membrane. (Juang *et al*, 2000). At elevated feed flow rates, permeate flux tends to increase. This is due to the limited effect of concentration polarization at the membrane-solution interface due to enhancement of back-diffusion of the retained particles into the reservoir. (Katsikaris *et al*, 2005). Also, polymers have differing molecular weight distributions, represented by an average molecular weight. Therefore, the polymeric solutions might have low molecular weight fractions. This might lead to a loss of some amount of the polymer through the membrane as well as of some metal ions bound to them. Therefore it is important to perform a diafiltration step with only buffer, before the addition of metal, so that all low molecular weight molecules are washed out before the metal is added. This will eliminate the possibility of a leak of the metals bound to low molecular mass fractions. All commercially available ultrafiltration membranes have a characteristic called nominal molecular mass exclusion limit (MEL) or molecular weight cut-off (MWCO). This feature corresponds to the minimum molecular mass of a solute that can

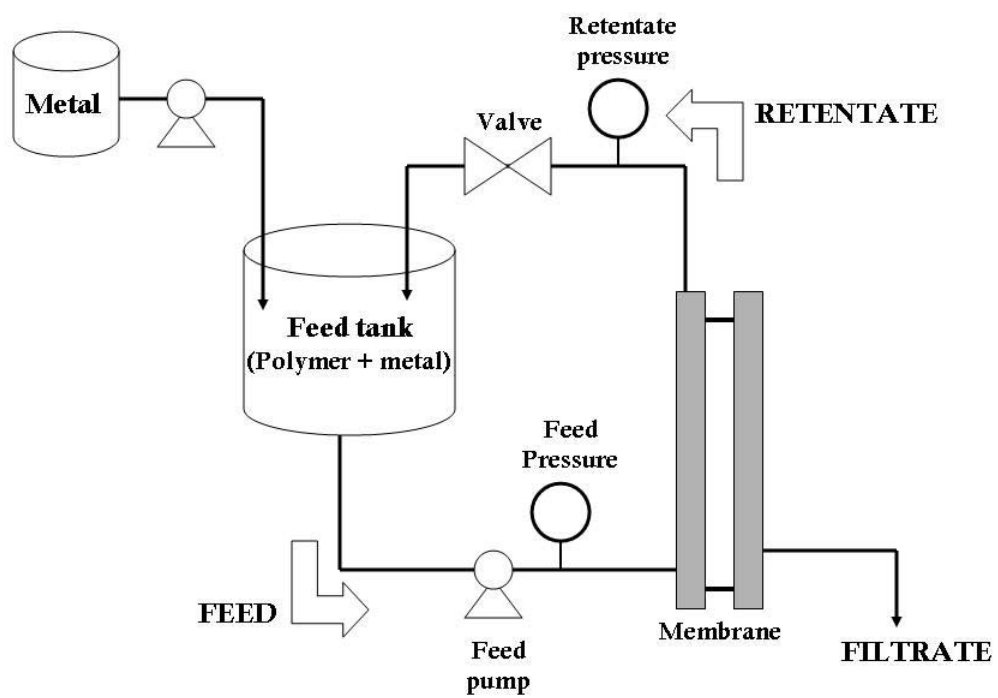


Figure 1: Polymer Enhanced Diafiltration unit.

be retained by the membrane. (Volchek *et al*, 1996).

2.6 Choice of the polymer

The polymer to be used in PEDF process needs to have the following properties: it should have a good affinity for the metal under study, preferably have an oppositely charged functional group for binding, it should have a high molecular mass, chemical and mechanical stability, low toxicity, low cost and possibility of regeneration. The polymer should have a large enough molecular mass such that it can be retained by the membrane depending on its molecular weight cutoff. However, the molecular weight should not be too high which might cause an increase in viscosity of the polymeric solution, leading to concentration polarization and reduction in permeate flux. The binding capacity of the polymer for the metal ion being studied should be high. The higher the capacity, the greater will be the retention of the metal by the membrane. Therefore, having a higher concentration will help, as it will lead to greater number of binding sites for the metal. However, too high a concentration could lead to concentration polarization. Therefore polymer concentration should be chosen such that there is high binding capacity without a decrease in flux. Degradation of soluble polymers e.g. by hydrolysis and mechanical shearing by pumps can occur if the polymers are not stable. Therefore, chemical and mechanical stability of the polymer are also important criteria. Molecules with longer chains have a high risk of being broken due to pump shearing. Some polymers possess a flexible structure and at certain hydrodynamic conditions, the shape of such polymers can change so that they will pass through the membrane even though their mass is more than the MWCO of the membrane. (Volchek *et al*, 1996).

2.7 Chitosan as a biosorbent

Chitin is an abundant organic material found mostly in crustaceans, mollusks and insects where it forms a constituent of the exoskeleton. It is also represented in fungal cell walls. (Guibal, 2004). Chitin can be obtained from fungi, insect, lobster, shrimp and krill. Crabs obtained from seafood processing waste are an important commercial source. (Volesky, 2003). According to some reports, 5000-8000 tons of crab shell material is disposed off by the seafood industry annually. Chitosan is derived from chitin, by a deacetylation reaction using an alkali. Chitosan is therefore a copolymer of glucosamine and N-acetyl glucosamine. (Rojas, 2005). It is composed of β -(1 \rightarrow 4)-2- amino-2-deoxy-D-glucopyranose (glucosamine units) and β -(1 \rightarrow 4)-2-acetamido-2-deoxy-D-glucopyranose (acetylglucosamine units). (Hamdine, 2005). The term “chitosan” refers to chitin that has been deacetylated to greater than 60%. Chitosan has many properties that have generated interest in its use such as biodegradability, biocompatibility and its non-toxic nature. (Varma, 2004).

The deacetylated product, chitosan, has an amine functional group, which is strongly reactive with metal ions. This has initiated research into the use of chitosan in metal uptake. The deacetylation degree will control the content of glucosamine and therefore the fraction of free amine groups available for metal binding. These groups are more reactive than the acetamide groups present on chitin. Also their solubility in acidic solutions differs, chitosan being soluble. The physico-chemical properties of chitosan depend (properties is plural and depends is singular) on various parameters such as degree of deacetylation, polymer weight, etc. (Guibal, 2004).

These reactive amine groups interact with the metal ions in different ways, such as by chelation or electrostatic attraction depending on parameters such as pH, and total composition of the solution. At pH values close to neutrality or in the non-protonated form, the free electron doublet on nitrogen may be used to form donor bonds with coordination transition metals such as Cu, Ni, Zn etc. At low pH, where protonation of the amine group takes place, the polymer attains cationic groups which can bind anions through electrostatic interactions. (Guibal, 2004; Juang.R.S, 2000).

There are many theories as far as the structure of chitosan-metal ion complex is concerned. One theory is that, two or more amino groups bind to the same metal ion; the bridge model. According to the bridge model, inter- or intermolecular complexation may occur between the metal ion and amine groups from the same or different chains. Some other experiments suggest that only one amino group is involved in the binding and the metal ion is bound to the amino group like a pendant; called the pendant model. (Vold, 2003; Domard, 1998).

2.7.1 Parameters affecting metal sorption by chitosan

There are several parameters affecting the complexation of metal ion to chitosan. One of them is the fraction of acetylated units. This determines the number of free amine groups available for binding. To be good sorbent, the polymer needs to have a high degree of deacetylation, conversely a low fraction of acetylated units. (Guibal, 2004, Vold, 2003). A study conducted showed that chitin, which was obtained by N-acetylation of chitosan, did not form complexes with metallic ions such as Cu(II), Co(II) or Ni(II). (Rhazi, 2002). In addition to the number of free amine groups available for binding, it is important to take into account the number of groups accessible to the metal ion. Some of

the amine sites are sometimes involved in some kind of inter- and intra-molecular bonds. The crystallinity of the polymer may make some groups inaccessible to the metal ions. (Guibal, 2004). Another important parameter is the chain length or the degree of polymerization. Also the degree of mixing of the metal-chitosan complex and the physical state of the chitosan affected the capacity of chitosan. (Rhazi, 2002). Chitosan solubility has been widely investigated. It is an important property which can be controlled by polymer weight, type and concentration of the acid used for dissolving the polymer and the presence of metal ions in the solution. Metals present in the solution can bind to the polymer and affect its solubility. (Guibal, 2004). Chitosan was found to be soluble in nearly all monovalent and multivalent acids. Chitosan is soluble in formic, acetic, citric, pyruvic and lactic acids. It does not dissolve in other organic acids. (Hamdine, 2005).

2.7.2 Binding of metal anions

Several studies have been done on the electrostatic attraction between chitosan and anions and anionic dyes. This mainly occurs at low pH. At neutral pH, about 50% of the total amine groups are protonated and therefore are theoretically available for binding of anions. The optimum pH is therefore 2-4. pH lower than this, does not give better uptake because a competitor effect comes in to play. At extremely low pH there is a large excess of competitor anions from the dissociated acid. (Guibal, 2004). Uptake by anions such as selenate (SeO_4^{2-}), vanadate (VO_4^{3-}) and molybdate (MoO_4^{2-}) has been studied. In the case of molybdate, a marked preference of chitosan for complex poly-nuclear and poly-anionic species was seen. Poly-nuclear species such as heptamolybdate was found to be highly adsorbable on protonated chitosan. (Chassary, Guibal, 2004).

In the case of chromium, the Cr(VI) ions are present in solution in the form of dichromate ions which are negatively charged. (Sag, Aktay, 2001). These can easily bind the cationic amine groups of chitosan at acidic pH. The pK_a of these amine groups is 6.3, therefore, at pH values lower than that, most of the chitosan will be protonated and cationic. Therefore at acidic pH, anion removal would mostly be by electrostatic attraction and cation removal would be by chelation or covalent binding. (Rojas, 2005, Guibal, 2004.). Above the pK_a , the sorbent undergoes deprotonation, is negatively charged and therefore the adsorption capacity decreases, and so chromium removal gets reduced. There is increased adsorption of chromium at lower pH. (Veera Boddu, 2003, Udaybhaskar *et al*, 1990). Also at higher pH, there are fewer protonated positively charged groups in chitosan and therefore there are fewer repulsion forces between the molecules leading to aggregation of the polymer. (Juang *et al*, 2000).

2.7.3. Effect of ionic strength on binding of anions.

Uptake of metal anions is reduced by increase in ionic strength. Changing ionic strength affects adsorption by affecting the activity of electrolyte ions and causing competition between the electrolyte ions and the adsorbing anions for the available sorption sites. The sorption of selenate and chromate is greatly affected at high ionic strengths thereby fortifying the idea that sorption of these anions occurs through electrostatic attraction. (Volesky, Niu, 2003). The removal of arsenate using different cationic polymers was studied. The removal was almost complete when arsenate solutions in deionized water were used, but the removal deteriorated in the presence of other anions such as sulfate, chloride and nitrate. (Volchek, 1996). It is also seen that chloride at low concentrations allows the formation of chloro-anionic species for

platinum and palladium, and improves its sorption capacities. However, at high concentration it greatly reduced metal recovery on cross-linked chitosan. The polynuclear species interacts with different monomer units of chitosan either on the same chain or adjacent chains. (Chassary, Guibal, 2004).

2.7.4 Binding of cations

The binding of metal cations to chitosan occurs through a different mechanism. The binding site in this case is also the amine group, which initiates a coordinate bond with the metallic ions. The bond formed is between the free electron pairs of the nitrogen in the amine group and the void orbitals of the metal. The affinity of chitosan for copper has been studied. The optimal pH for copper binding is in the range 5-7. (Rhazi *et al*, 2002). Since low pH favors the protonation of the amine groups, the charge reversal would affect the ability to chelate metal cations. This suggests that binding of cations would take place at a more neutral pH. At lower pH, chelation also gets reduced by the competitor effect of protons. (Chassary, Guibal, 2004). It is known that chitosan forms chelates with metals by releasing hydrogen ions. Hence adsorption of metal cations with chitosan leads to a decrease in pH. (Schmuhl. R., 2001). A high equilibrium sorption capacity for cadmium was also seen. It is seen that uptake process generally involves pore diffusion through the solid matrix followed by adsorption. (Evans *et al*, 2002). Cadmium uptake was also studied in conjunction with ultrafiltration. Chitosan was found a good candidate for binding cadmium at neutral pH. When the pH was reduced to 4, Cd^{2+} was released and chitosan was regenerated by alkali solution. The formation of two complexes was considered, $\text{Cd}(\text{R-NH}_2)^{2+}$ and $\text{Cd}(\text{R-NH}_2)_2^{2+}$. Computations showed that the complex where Cd^{2+} is bound to two amine groups i.e. $\text{Cd}(\text{R-NH}_2)_2^{2+}$ is most

abundant. (Llorens.J, 2004). According to a study, in the case of copper, a unique complex is formed with chitosan. Copper binds to three oxygen atoms and one nitrogen, with square-planar and tetrahedral geometry. (Guibal, 2004). Zinc uptake was studied at different pH. Optimum binding by chitosan derivatives occurred at pH 6.0, since higher pH allowed adsorption of Zn(II) to non-protonated amine groups. Uptake was reduced at pH 7.0 since precipitation of Zn(II) occurred at that pH. (Ding. P., 2006)

2.7.5 Modification of chitosan

The versatility of chitosan allows it to be easily modified, in order to change its properties, depending on the field it needs to be applied to. It can be physically modified into gels, beads and membranes. Chemical modification is more common, which involves either grafting a specific group onto the chitosan backbone or cross-linking. This is done to increase metal sorption capacities or improve selectivity of the polymer for a certain species. Modification is also done to prevent dissolving of the polymer when soluble chitosan is not required and the sorption is performed at acidic solutions. Cross-linking can be done using cross-linking agents such as glutaraldehyde, cyclodextrin and epichlorohydrin. However cross-linking step might reduce the uptake efficiency of chitosan especially in the case of glutaraldehyde. Reaction of the chitosan amine groups with glutaraldehyde leads to the formation of imine functional groups thereby reducing the number of amine groups. Epichlorohydrin and ethylene glycol can also be used since they react with -OH groups of chitosan, therefore they should not affect the residual amine functional groups for binding. (Tianwei et al, 2001). The adsorption capacity depends on the extent of cross-linking and generally decreases with increase in the extent of cross-linking. According to a study by Rojas et al, adsorption of Cr(VI) onto cross-

linked chitosan was found to be 215mg/g. (Rojas et al, 2004). A novel diethylene triamine derivative of chitosan was studied with regards to its binding properties with palladium, silver, copper and cadmium ions. This introduction of diethylene triamine into the chitosan backbone prevents the loss of amine groups due to cross-linking reaction with aldehyde. This modification enhanced the adsorption ability and selectivity of chitosan for silver ions. (Yi Yang et al, 2006). New functional groups are also grafted to increase the density of the sorption sites, to broaden the optimum pH range and to increase the selectivity of the target metal. According to a study by Chassary et al, Polyethyleneimine (PEI) was grafted onto chitosan and tested for platinum and palladium uptake. Grafting of PEI gives chitosan supplementary amine functions, primary, secondary and tertiary amines. It was found that this grafting improved sorption capacity but had limited effect on sorption selectivity. The grafting of carboxylic functions is also done mainly to design chelating derivatives for sorption of metal cations. (Guibal, 2004. Chassary and Guibal, 2004, Varma *et al*, 2004,)

2.7.6 Recycling of chitosan and desorption of metal.

The study of metal desorption and polymer recycling is important to improve the cost-effectiveness of the sorption process. The cost of the polymer can be a limiting factor except in the case of precious metal sorption. The recovery of metal also determines the efficiency of the process. In desorbing processes, regeneration of the biosorbent may be accomplished by washing the metal laden biosorbent with an appropriate solution, the type and strength of which would be determined by how the metal is bound to the biosorbent. The sorption mechanism can help design a desorption strategy. (Volesky, 2001). Since pH is an important parameter in binding, a simple

change in pH can allow the metal to desorb. Chelating agents such as EDTA can also be used, especially for desorption of metal cations. When pH is used for desorption of metal anions, increasing the pH might allow the reverse reaction, since binding occurs at low pH for anions. Molybdate and vanadate have been recovered using sodium hydroxide to increase pH. (Guibal, 2004). Successful desorption of chromate from chitosan was carried out using 10-100 mM EDTA. The sorbents were regenerated and could be used again to adsorb heavy metal ions. (Baran *et al*, 2006). However, continued recycling of chitosan may not be possible due to its biodegradability. Recycling is less important in the case of precious metal sorption because the cost of the target metal would allow single use of the polymer. Also since chitosan is essentially derived from a waste product of the food-industry, its production costs are not too high. Also, metal loaded chitosan can also be used for other sorption processes, taking advantage of the affinity of the metal loaded on chitosan for other solutes. For example, the chelating affinity of molybdate for arsenic has been used. Molybdate loaded chitosan beads have been used for uptake of As(V) and As(III) from dilute solutions. This makes use of the knowledge that chitosan has a strong affinity for molybdate and molybdate is known to form strong complexes with arsenate ions. (Dambies, Guibal, 2002).

An important aspect to be studied here is, during a desorbing process, the desorbed sorbate (metal) stays in solution and a new equilibrium is established between that and the metal still bound to the biosorbent. This leads to the concept of a desorption isotherm where the equilibrium is strongly shifted towards the sorbate dissolved in solution. Recovery of the metal from these concentrated desorption solutions can be carried out by using electroplating procedures. (Volesky, 2001).

2.8 Rheological studies

The study of rheology is the study of the deformation of matter resulting from the application of a force. The type of deformation depends on the type of matter. Gases and liquids will flow when a force is applied while solids will be deformed by a fixed amount. The stress is defined as the force divided by the area over which it is applied. When a stress is applied to a material, a deformation will occur. When a fluid system is studied by the application of a stress, motion is produced until the stress is removed. Consider two surfaces separated by a small gap containing a liquid. A constant shear stress must be applied on the upper surface for it to move a constant velocity, u . The rate of strain or shear rate is given by velocity/ displacement i.e. u/x . When a plot of shear stress versus shear rate is linear, the liquid behavior is simple and the liquid is Newtonian, the constant slope of the straight line being the viscosity of the fluid. Fluids such as water and ethanol are Newtonian. Any fluid that does not obey the Newtonian relationship between the shear stress and shear rate is called non-Newtonian. High molecular weight liquids are usually non-Newtonian. When the viscosity decreases with increase in shear rate, the fluid is called shear thinning whereas if the viscosity increases with increase in shear rate, the fluid is called shear thickening. The backbones of most polymers are made up of carbon chains. The bonds are usually single but there may be a significant number of double bonds. The side groups or side chains are important because these control the rotation around the bonds. This bond rotation gives the polymer its flexibility. (Goodwin, Hughes, 2000).

2.8.1 Rheological properties of chitosan

Polymers exist as an intermolecular entangled state in concentrated domain, in which accurate rheological characterization helps in determining the processibility and activity of the polymer. The physicochemical properties of dilute solutions of chitosan are governed by pH, ionic strength, degree of deacetylation, concentration and molecular weight. The effect of concentration of chitosan has been studied and it was found that viscosity increases with increase in concentration of chitosan. (Nyström.B, 1999, Cho.J, 2006). For low concentrations, the chitosan solutions exhibit a Newtonian behavior, but increasing the polymer concentration leads to the appearance of a non-Newtonian behavior. Also at low shear, Newtonian behavior can be maintained but not at high shear rates. At low shear rate, a constant zero shear viscosity can be maintained, since the rate of intermolecular disentanglements brought about by shear force exerted is nearly the same as that of newly formed entanglements. Conversely, the viscosity decreases with increasing shear rates, because the rate of disentanglements is higher than that of entanglements. (Hwang, J.K, 2000, Desbrieres, 2002). The polymer starts to behave shear thinning with increase in concentration. As the polymer concentration is increased, the freedom of movement of the individual chains becomes restricted due to the increased number of entanglements. Therefore there is an increase in time required to form new entanglements to replace those disrupted by deformation. Thus, the shear rate at which Newtonian behavior is lost progressively moves towards lower values. (Hwang, J.K, 2000). Hydrophobic chitosan were prepared by modification of raw chitosan and its rheological properties were studied. The hydrophobic chitosan shows unusual rheological characteristics which arise due to intermolecular associations of neighboring hydrophobic

substituents. It was shown that intermolecular association phenomenon was promoted by increasing hydrophobicity. (Nyström.B, 1999).

MATERIALS AND METHODS

1. Dynamic metal binding studies using chitosan in a polymer enhanced diafiltration system.

This method was obtained from the work of Mark.S.S., 2006. Chitosan was used as the biosorbent, the preparation of which is given below.

1.1 Preparation of chitosan solution

Chitosan (90%) from DNP International (city and state) was used to make all chitosan solutions. Chitosan solutions from 2 to 20 g/L were used for the experiments. The powdered chitosan was weighed and dissolved in 0.05M or 0.01M acetic acid, depending on the pH required. Slight adjustments in pH were made using 1M acetic acid.

1.2 Preparation of chromium solution

Analytical grade $K_2Cr_2O_7$ (Mallinckrodt Chemicals Works, St.Louis, MO) was used to make all chromium standard solutions used in the experiments. A stock solution of 1000 mg/L was prepared by dissolving the powder in reagent grade water. Working standards ranging from 10 mg/L to 100 mg/L were then prepared by appropriately diluting the stock solution. The pH of the metal solution was adjusted to that required by the experiment using 1M HCl and 1M NaOH.

1.3 Polymer enhanced diafiltration set-up

The set-up (**Figure 1**) consisted of a glass feed tank or reservoir. 500 ml of chitosan solution made in acetic acid was placed in the reservoir. A pump (Millipore pump with Cole Parmer pump head, Model no 7016-20) was used to feed the metal into the reservoir containing chitosan solution. Another pump (Millipore pump with Cole Parmer pump

head, model 7015-21) was used to feed the metal-chitosan complex onto the ultrafiltration membrane. The solution in the reservoir was constantly stirred using a Magnetic stir plate. The membrane used was a Pellicon cassette from Millipore (10,000 and 30,000 MW cutoff, V channel). The tubing used was Masterflex (Masterflex 06485-15 and 06485-16). There were pressure gauges at both inlet and outlet indicating feed and retentate pressure respectively. There was a hand valve to control the feed and retentate pressure. The crossflow rate was maintained at 240ml/min.

1.4 PEDF operation

A constant volume diafiltration was run. For this, the flow rate of the metal feed was maintained at the same rate that filtrate leaves the system. The transmembrane pressure was maintained throughout the run at 7 – 10 psi. The hand valve was used to maintain pressure during the run. Samples (2 ml) of permeate were collected at regular intervals during the run and were analyzed for the presence of chromium using a spectrophotometric assay. The permeate flow rate in mL/min was also measured at regular times. All systems were run at room temperature (25°C). A control was run in the absence of chitosan in order to determine binding to the membrane. Instead of the polymer 0.05M acetic acid was used in the run.

1.5 Determination of optimum concentration of chitosan using PEDF

Different concentrations of chitosan were tested in PEDF. 2.0, 4.0, 5.0, 6.0 and 20.0 g/L chitosan were used in separate runs. The starting feed concentration of the chromium in these runs was 20 mg/L. The residual chromium in the permeate was estimated in each case in order to determine the binding by the polymer. A pH probe was inserted in to the feed tank during the run such that pH could be maintained continuously. pH was

maintained at 4.0 during each run using 1 M acetic acid. Each run was performed at a transmembrane pressure of about 7 to 7.5 psi. The molecular weight cutoff of the membrane was 30K. The volume of permeate collected was measured and recorded throughout the run.

1.6 Buffer diafiltration to eliminate low molecular fractions

PEDF was performed along with a buffer diafiltration in order to get rid of low molecular weight fractions of chitosan if any. This was done by adding 0.05 M acetate buffer into the polymer containing reservoir for one hour. The PEDF was run circulating only buffer through the system without any addition of metal. After the buffer wash was done, metal solution (20 mg/L) was fed into the system. The PEDF was run at 4 g/L chitosan at pH 4.0. Permeate samples were collected once the addition of metal was begun and estimated for residual chromium. Any changes in the permeate chromium concentrations were recorded in comparison with a run without buffer diafiltration. A 30K MW cutoff membrane was used.

1.7 Spectrophotometric determination of chromium. .

The concentration of chromium was determined using the diphenylcarbazide method from *Standard Methods for the Examination of Water and Wastewater* (Prepared **and** published jointly by American Public Health Assoc., American **Water** Works Assoc. [and] **Water** Pollution Control Federation, 13th edition, 1971). The collected samples (0.5 mL) were mixed with 1 mL of 0.2 N sulphuric acid in order to create an acidic environment for the diphenylcarbazide to react. 0.5 % diphenylcarbazide (Sigma-Aldrich) was made with acetone as the solvent. 200 μ L of this was then added to the acidified samples to get a red-violet colored complex. This was diluted up to 10 mL with

distilled water in a volumetric flask. The absorbance of the resulting colored solution was measured at 540nm in a Perkin Elmer UV/VIS spectrophotometer. Some of the samples were accordingly diluted with distilled water in order to be within the range of the assay. All glassware used for this experiment was initially rinsed with 6N HCl in order to leach out any trace metals that might be present.

2. Qualitative chitosan assay using poly- γ - glutamic acid

In order to determine if there was any chitosan leaking out into the permeate, a qualitative assay for chitosan was performed using γ -PGA. 0.1 mL to 1.0 mL of 0.05% chitosan solution (concentration of chitosan varied from 0.01 to 0.1 mg/ml) was mixed with 1.0 ml of 0.05% γ - PGA solution. This was diluted up to 5.0 ml with distilled water. A white turbidity was formed. The turbidity was measured at 600 nm in a spectrophotometer. The relationship between concentration and absorbance was not linear, but turbidity was obtained at chitosan concentrations as low as 0.02 mg/mL. Therefore this serves as a qualitative or presence/ absence test for chitosan. Using this as a basis, the permeates collected from PEDF were tested with γ -PGA (both 0.05% and 0.1%) and absorbance was measured to look for the presence of chitosan in the permeate.

3. Study of chromate binding properties of chitosan using Equilibrium dialysis

3.1 Construction of dialysis apparatus.

This method was obtained from the study of Mark.S., Crusberg.T.C., DiIorio.A.A.,2006. The dialysis chambers are made of polycarbonate. Each dialysis

apparatus consisting of two identical units were joined together with screws. Each unit consisted of 4 cells of 40 mL capacity each. When the units were joined together, the cell on each side was separated by a semi-permeable regenerated cellulose membrane (Spectra/Por dialysis pre-cut discs of 100 mm diameter obtained from Spectrum laboratories, MW cutoff 12-14 KDa) to give 80 mL chambers. Rubber O-rings were used around each cell to hold the membrane in position. Rubber stoppers were used to close the opening on the top of the chamber during the experiment to prevent evaporation issues. Before the experiment the dialysis units, stoppers and O-rings are leached in 1M HCl for 8 hours to remove any trace metals. They are then rinsed in distilled water for 8 hours and then air dried.

The dialysis membranes were washed prior to use with the following protocol. The membranes cut to size were soaked for an hour in a mixture of 0.01M Sodium Bicarbonate and 0.001M Na₂EDTA at 37°C. After one hour, the solution was decanted and some more was added to be soaked for another 30 minutes at 37°C. This was then washed off and the membranes were rinsed with distilled water. This was followed by three changes of distilled water for 30 minutes each at 37°C. The membranes could be stored like this in the refrigerator until use.

3.2 Determination of kinetics of binding using equilibrium dialysis

Chitosan solution of 2 g/L was prepared by dissolving chitosan in 0.05 M acetic acid at pH 4.0. Chromium solutions were made using 1000 mg/L stock by diluting it to 100 mg/L. The pH of this solution was adjusted to 4.0 using 1M HCl and 1M NaOH. The chitosan solution (40 mL) is added to the cell on side of the dialysis membrane and 40 mL of metal solution was added to the cell on the other side. The entire dialysis unit was

kept in a shaker at 250 rpm at 25°C. Samples (100 µL) were taken every 2 hours from the feed cell into which the metal was initially added. These samples were then estimated for chromium using diphenylcarbazide method. This was continued until equilibrium was reached. A simultaneous control experiment was done in the absence of chitosan where 0.05 M acetic acid was added to the recovery cell instead of chitosan. Kinetics studies were also done at pH 3.0

3.3 Determination of optimum pH for uptake using equilibrium dialysis

The effect of pH on uptake was studied by studying binding at pH's 2.0, 3.0, 4.0 and 5.0. Chitosan solutions were prepared by mixing chitosan in acetate solutions at pH 2.0, 3.0, 4.0 and 5.0. 100 mg/L chromium working solution was prepared and pH was accordingly adjusted from 2.0 – 5.0 using 1M HCl and 1M NaOH. 40 ml of chitosan solution was added to the cell on one side and 40 ml of chromium solution was added on the other side. The solutions on either side of the membrane were at the same pH. Samples were taken initially to determine the initial metal concentration. The dialysis unit was kept under shaking conditions at 250 rpm at 25°C. Equilibrium was allowed to be established and samples (100 µL) were taken after 24 hours to estimate the residual chromium using diphenylcarbazide.

3.4 Study of Equilibrium isotherm.

For the study of equilibrium isotherms, the dialysis was done at varying concentrations of the metal i.e. 10, 30, 50, 60, 80, 100 and 120 mg/L chromium. Chitosan used was at 2.0 g/L at pH 4.0. The metal solutions were also adjusted at pH 4.0. Forty ml of metal solutions was added to the feed cell on one side and 40 ml chitosan solution was added to the recovery cell on the other side. The unit was placed under shaking

conditions at 250 rpm at 25°C. Samples (100 µL) were taken from the feed cell into which metal was added and estimated for chromium using diphenylcarbazide. A control run was performed in the absence of chitosan, using 0.05 M acetic acid in the recovery cell. 30 mg/L chromium was used in the feed cell of the control chamber. This would help determine if there was any binding to the membrane. After the final chromium concentrations were estimated the equilibrium isotherm was prepared by plotting uptake in mg/g versus the equilibrium concentration of chromium in mg/L.

4. Rheological studies to characterize chitosan-chromium complex.

Viscosity of chitosan-chromium complex was studied on applying shear stress. A Bohlin controlled stress rheometer (Bohlin Instruments) was used. Stress viscometry is selected in the software menu. Chitosan solution at pH 4.0 containing 1-100 mM chromium was placed on the lower fixed plate of the rheometer and the upper movable plate was lowered after unlocking the shaft. The range of stress to be applied was selected (0.75 to 1.5 mPa). The delay time and integration time selected was 30 seconds and 60 seconds respectively. The viscosity is then recorded for the range of shear of shear stresses. A graph of viscosity in mPas versus shear stress in mPa can then be plotted. Different concentrations of chitosan i.e. 4.0 g/L, 6.0 g/L, 12.0 g/L and 20.0 g/L was tested in order to study the effect of polymer concentration on rheological behavior in the presence of chromium. A control was run by studying the viscosity of only chitosan without any chromium.

RESULTS

1. Characterization of chromate binding by chitosan using Equilibrium Dialysis

1.1 Modeling of sorption

Samples are taken from the feed cell in to which the metal containing solution was added and are analyzed for the presence of residual chromium. The concentration change in the feed cell is expressed by the following equation:

$$-\frac{dC_1}{dt} = \frac{K_f A}{V_1} (C_1 - C_2) \quad (1)$$

where C_1 and C_2 are the concentrations of metal ions in the feed cell and recovery cell respectively, and K_f , A and V_1 are the overall mass transfer coefficient, effective surface area and solution volume in the feed cell respectively. In the presence of water alone, with no chitosan present, Eq (1) is solved using the following equation:

$$V_1(C_0 - C_1) = V_2 C_2 \quad (2)$$

With chitosan present in the recovery cell, it is assumed that concentration C_2 is in equilibrium with the concentration of the polymer-metal ion complex. The mass balance for the metal ions permeating through the membrane from the feed cell is

$$V_1(C_0 - C_1) = V_2 C_2 + mq \quad (3)$$

where m is the mass of the chitosan present in the recovery cell and q is the uptake of metal by chitosan in mg/g of biosorbent. Assuming that binding follows the Langmuir isotherm model:

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

where q_{\max} is the asymptotic maximum solid phase metal concentration, K_A is the equilibrium association constant in L/mg and C is the equilibrium bulk liquid phase metal concentration in mg/L. Assuming the Langmuir model, the following is derived:

$$-V_1 \frac{dC_1}{dt} = m \frac{dq}{dt} + V_2 \frac{dC_2}{dt} = \left\{ \frac{mq_0K}{1+KC_2} + V_2 \right\} \frac{dC_2}{dt} \quad (5)$$

Therefore, variation in concentration in the feed cell is obtained by numerical solution of equations (1), (2), (4) and (5) for a given value of K_f . (Tomida, Ikawa, 1993; (I think that it would be best to set off multiple references using a semicolon ;) Mark, DiIorio, 2006).

1.2 Kinetics data

Figure 2 shows the time course of decrease in chromium concentration in the feed cell during an equilibrium dialysis at pH 3.0. In the absence of any chitosan, starting with a concentration of 115.2 mg/L of chromium, an equilibrium concentration of 55.8 mg/L is reached after 12 hours of dialysis. In the presence of 2 g/L chitosan in the recovery cell, starting with a concentration of 115.2 mg/L of chromium, an equilibrium concentration of 16.2 mg/L is obtained after 12 hours. A reaction time of 10-12 hours is required to reach equilibrium.

Figure 3 shows the time course of decrease in chromium concentration in the feed cell during an equilibrium dialysis at pH 4.0. In the absence of any chitosan, starting with a concentration of 126 mg/L of chromium, an equilibrium concentration of 60 mg/L is reached after 12 hours of dialysis. In the presence of 2 g/L chitosan in the recovery cell, starting with a concentration of 126 mg/L of chromium, an equilibrium concentration of 14.2 mg/L is obtained after 12 hours. From Figures 1 and 2, it can be seen that chitosan functions as a sorbent for chromate. This is evident from the decrease in free concentration of chromium in the presence of chitosan. A reaction time of 10-12

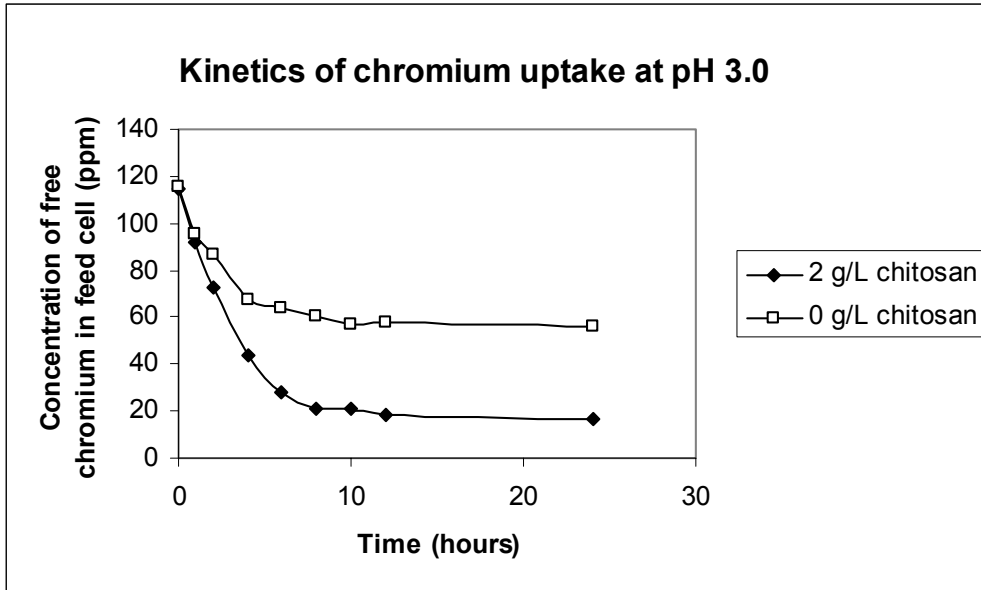


Figure 2: Kinetics of chromate uptake by chitosan at pH 3.0.

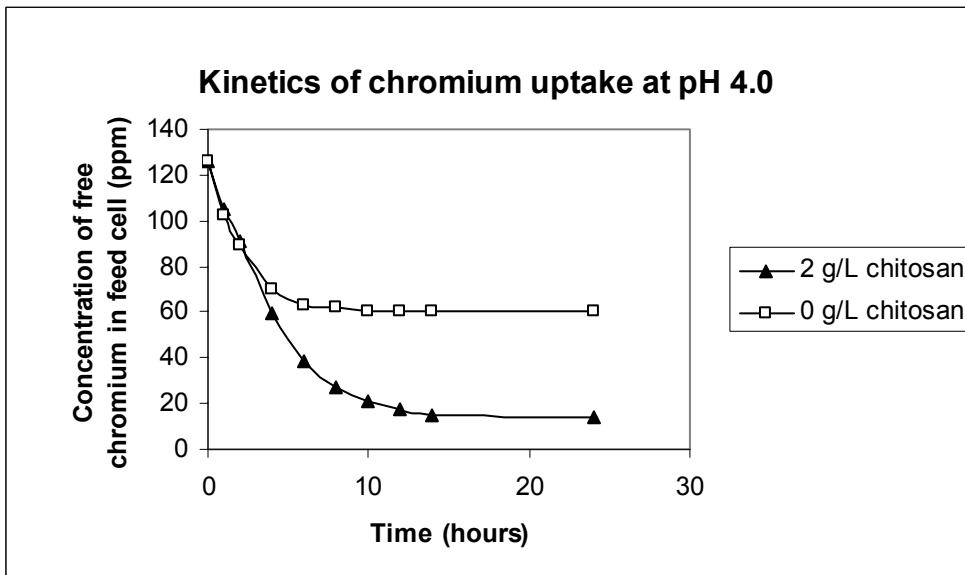


Figure 3: Kinetics of chromate uptake by chitosan at pH 4.0.

hours is required to reach equilibrium. Based on this data an equilibrium time of 24 hours was allowed for all further equilibrium tests

1.3 Equilibrium Isotherm data.

An equilibrium isotherm is a plot of uptake in mg of chromate/g of polymer. **Figure 4** shows an equilibrium isotherm for chitosan at pH 4.0. The Y axis represents the equilibrium concentration of free chromium after 24 hours at 25°C. Ideally, with increase in equilibrium free chromium concentration, uptake increases, until all the binding sites on the sorbent are saturated with no further increase in uptake. This represents q_{\max} or maximum adsorption capacity of the sorbent. As seen in **Figure 4**, q_{\max} could not be experimentally reached and no plateau in uptake was observed. Therefore higher concentrations of chromium were tested as shown in **Figure 5**. At equilibrium concentrations as high as 350 mg/L, there is still further increase in uptake. Therefore from **Figures 4 and 5**, we can deduce that the q_{\max} is much higher than can be experimentally reached. This might lead to estimating an asymptotic q_{\max} from the K_D value. Since the isotherm plot is non-linear, a reciprocal plot, which is linear, is used to calculate the K_d and q_{\max} values from the experimental data. **Figure 6** shows the linear reciprocal plot of $1/q$ versus $1/C_{eq}$. The parameters K_D and q_{\max} may be found by the following equation: (Tomida, Ikawa, 1993)

$$\frac{1}{q} = \frac{1}{q_{\max} K_D C_{eq}} + \frac{1}{q_{\max}} \quad (6)$$

From the slope and intercept of the straight line, the values of q_{\max} and K_D can be found. The predicted line in **Figure 6** is estimated by linear regression analysis by MS Excel from the experimental data. Excel was also used to obtain the equation of the line. From the equation, the values of K_D was found to be 153 mg/L.

1.4 Effect of pH on chromium uptake

The method used here to estimate the optimum pH did not involve control of pH throughout the dialysis. The pH was adjusted initially and then tested again after the experiment. **Table 1** shows the uptake in terms of mg chromium/g of chitosan at the different pH tested. The pH, before and after the dialysis experiment is also recorded. The optimum pH was found to be 4.0, followed by pH 3.0. All the subsequent experiments, including the Polymer Enhanced Diafiltration and the rheology studies, were performed at pH 4.0 in accordance with this data.

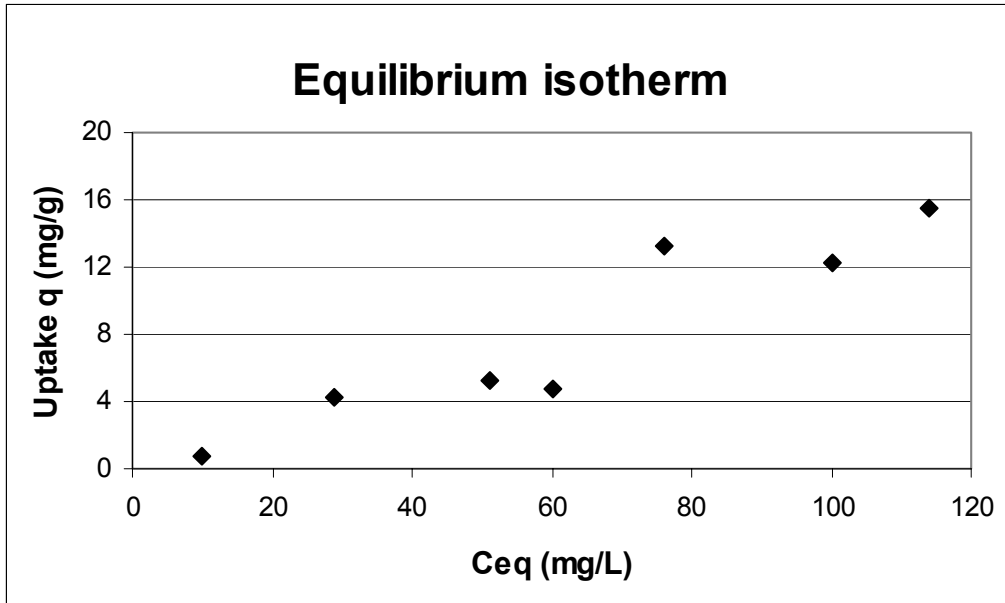


Figure 4: Equilibrium isotherm for adsorption of chromium by chitosan at pH 4.0. Each point represents a mean of triplicate measurements.

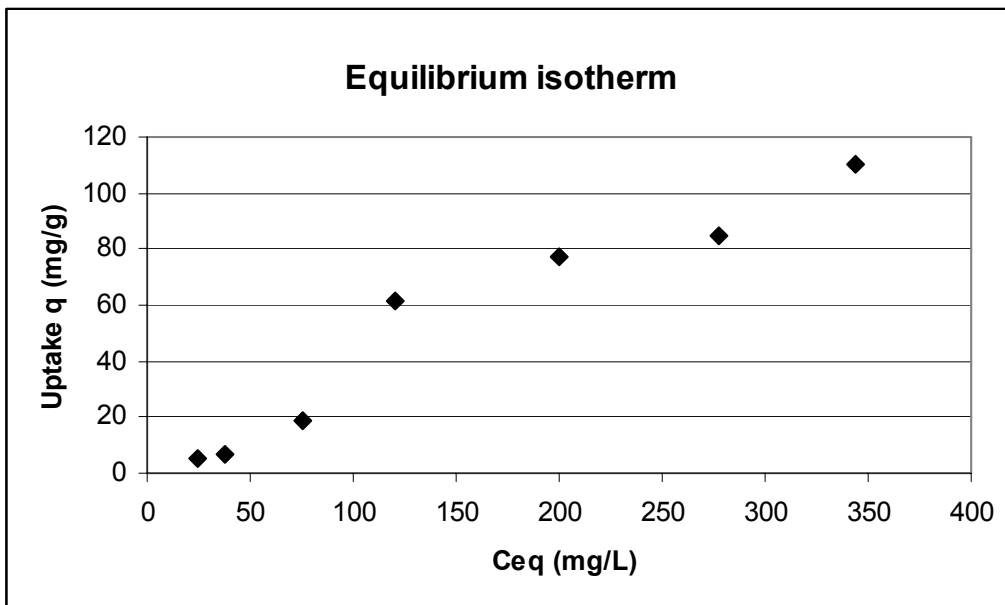


Figure 5: Equilibrium isotherm for adsorption of chromium at higher equilibrium concentrations. Each point represents a mean of triplicate measurements.

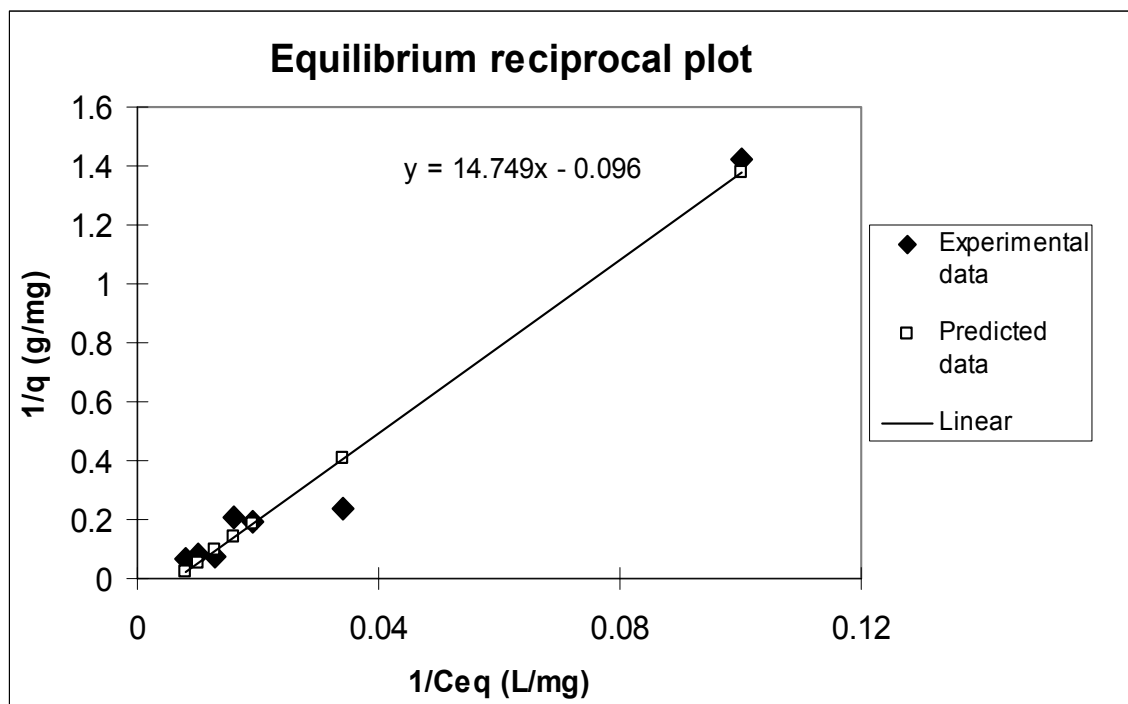


Figure 6: This is a reciprocal of the equilibrium isotherm plot (Figure 3). It represents $1/q$ versus $1/C_{eq}$.

Table 1. Effect of pH on uptake by equilibrium dialysis. Experiments were carried out using 2 g/L chitosan adjusted to different pH. 100 mg/L chromium adjusted to the required pH was added to the feed cell. Uptake values are a mean of triplicates. Temperature - 25°C.

<i>Initial pH</i>	<i>Uptake (mg/g)</i>	<i>Final pH</i>
2.00	28.5	2.08
3.00	32.4	3.09
4.00	47.8	4.00
5.00	4.3	5.12

2. Dynamic heavy metal binding studies using chitosan in a Polymer-Enhanced Diafiltration.

2.1 Polymer-Enhanced Diafiltration with a feed chromium concentration of 10 mg/L

PEDF was initially run with a feed concentration of 10 mg/L Cr. During the run, the concentration of chitosan in the reservoir was at 4 g/L and the working volume was 500 mL. The pH was maintained at 4.0 throughout. The transmembrane pressure was kept constant at 7.5 psi. **Figure 7** represents the PEDF data using a 10 mg/L feed Cr concentration. The measurements of pressure and filtrate flow rate during the experiment are shown in **Table 2**.

2.2 Control Polymer-Enhanced diafiltration with no chitosan.

A control experiment was run in the absence of chitosan in the reservoir using a feed Cr concentration of 20 mg/L. Five hundred mL of 0.05M acetic acid was placed in the reservoir instead of chitosan. **Figure 8** is a representation of that data. It is a plot of concentration of chromium in the permeate versus the volume of permeate collected. The volume passed through the system was 2200 mL. With a starting concentration of 20.4 mg/L, 20 mg/L passed into the permeate, indicating that no binding to the membrane took place.

The measurements of transmembrane pressure and filtrate flow rate during the experiments are shown in **Table 3**.

2.3 Effect of chitosan concentration on binding using PEDF.

In order to determine the effect of polymer concentration on PEDF, different concentrations of chitosan i.e. 2g/L – 6 g/L chitosan were used. Each of these experiments was run with a feed concentration of 20 mg/L chromium.

Table 2 PEDF measurements like transmembrane pressure, permeate flow rate throughout the run with 10 mg/L feed Cr and 4 g/L chitosan.

<i>Volume of permeate collected (mL)</i>	<i>Transmembrane pressure (psi)</i>	<i>Permeate flow rate (mL/min)</i>	<i>pH</i>
0	7.5	22	4.01
800	7.75	24	4.01
1650	7.75	28	4.01
2400	7.5	26	4.00
3200	7.5	26	4.00
4000	7.5	26	4.01

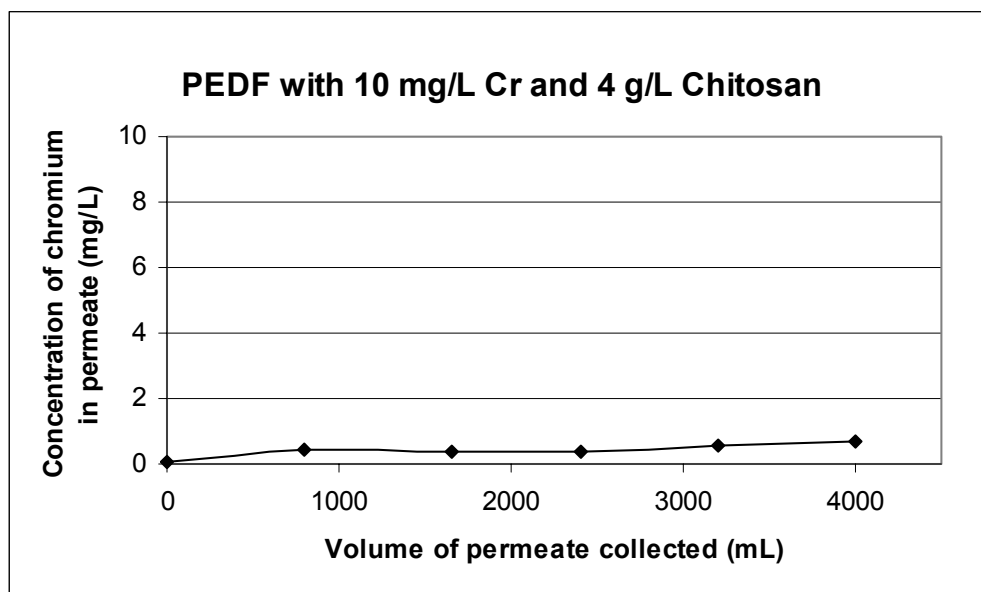


Figure 7: Permeation curve of chromium in polymer enhanced diafiltration using a feed concentration of 10 mg/L of chromium.

Table 3 PEDF measurements of transmembrane pressure, permeate flow rate and pH during the control run in the absence of chitosan.

<i>Volume of permeate collected (mL)</i>	<i>Transmembrane pressure (psi)</i>	<i>Permeate flow rate (mL/min)</i>	<i>pH</i>
0	4.0	14	4.00
500	4.0	14	4.00
950	4.5	14	4.00
1400	4.5	14	4.00
1850	4.5	14	4.00
2200	4.5	14	4.00

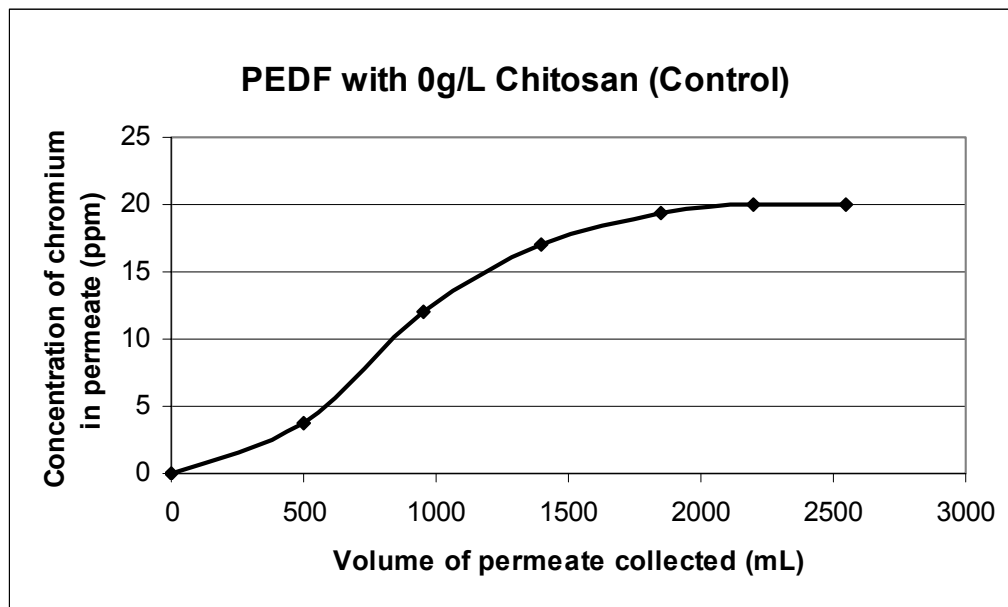


Figure 8: Permeation curve in the absence of chitosan with a feed concentration of 20 mg/L chromium. This serves as a control to determine binding to the membrane.

Table 4, 5, 6 and 7 show the transmembrane pressure, permeate flow rate and pH measurements during the 2 g/L, 4g/L, 5 g/L and 6 g/L chitosan runs respectively. In each of the experiments the permeation curve was very different from that obtained in the control experiment (**Figure 8**). Much less chromium passed into the permeate as compared to the control where most of the chromium was in the permeate eventually. The difference between the control and the experimental data indicates the binding occurred between the chromate ions and chitosan. As the concentration of chitosan was increased, the concentration of chromium passing into the permeate decreased. With 6 g/L chitosan, less than 1 mg/L chromium passed into the permeate. **Figure 9** shows the permeation curve for 2 g/L chitosan with a feed concentration of 20 mg/L Cr. **Figure 10** shows the permeation curve for 4 g/L chitosan with a feed concentration of 20 mg/L Cr. **Figure 11** shows the permeation curve for 5 g/L chitosan with a feed concentration of 20 mg/L Cr. **Figure 12** shows the permeation curve for 6 g/L chitosan with a feed concentration of 20 mg/L Cr. The volume of chitosan in the reservoir was 500 mL. The pH was maintained at 4.0 during each of the runs.

2.4 Polymer enhanced diafiltration using a 6 g/L chitosan processing larger volume of chromium solution.

PEDF was then performed at 6 g/L chitosan since that concentration showed a good performance in the previous PEDF runs (Figure 10). Larger volumes were run through the system as compared to the previous run where 1700 mL was processed. (**Table 7, Figure 12**). In this experiment, about 3000 mL chromium solution at 20 mg/L was processed for about 5 hours. A permeate concentration of 1.64 mg/L chromium was obtained after 3000 mL was processed. The pH was maintained at 4.0 throughout the run.

Table 4 Transmembrane pressures, permeate flow rate and pH measurements during PEDF with 2 g/L chitosan and 20 mg/L chromium.

<i>Volume of permeate collected (mL)</i>	<i>Transmembrane pressure (psi)</i>	<i>Permeate flow rate (mL/min)</i>	<i>pH</i>
0	6.75	28	4.04
900	7.00	28	4.00
1700	7.25	28	4.01
2550	7.25	30	4.00
3450	7.25	30	4.00

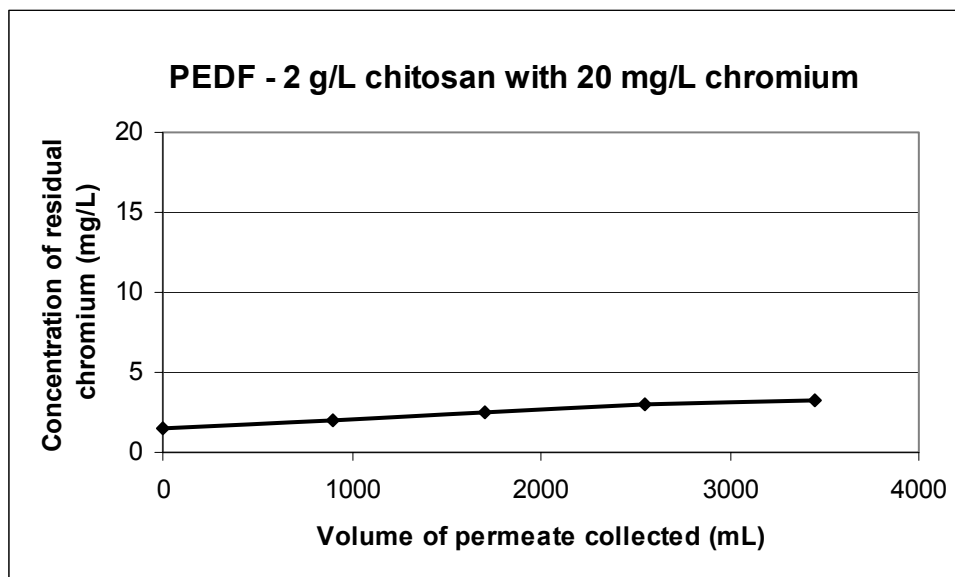


Figure 9: Permeation curve for 2 g/L chitosan in a polymer enhanced diafiltration system. PEDF was carried out with a feed concentration of 20 mg/L chromium. pH was maintained at 4.0 throughout.

Table 5 Transmembrane pressures, permeate flow rate and pH measurements during PEDF with 4 g/L chitosan and 20 mg/L chromium.

<i>Volume of permeate collected (mL)</i>	<i>Transmembrane pressure (psi)</i>	<i>Permeate flow rate (mL/min)</i>	<i>pH</i>
0	7.0	22	4.1
700	7.0	22	4.09
1400	7.25	22	4.05
2100	7.25	22	4.05
2750	7.25	22	4.06
3350	7.25	22	4.05

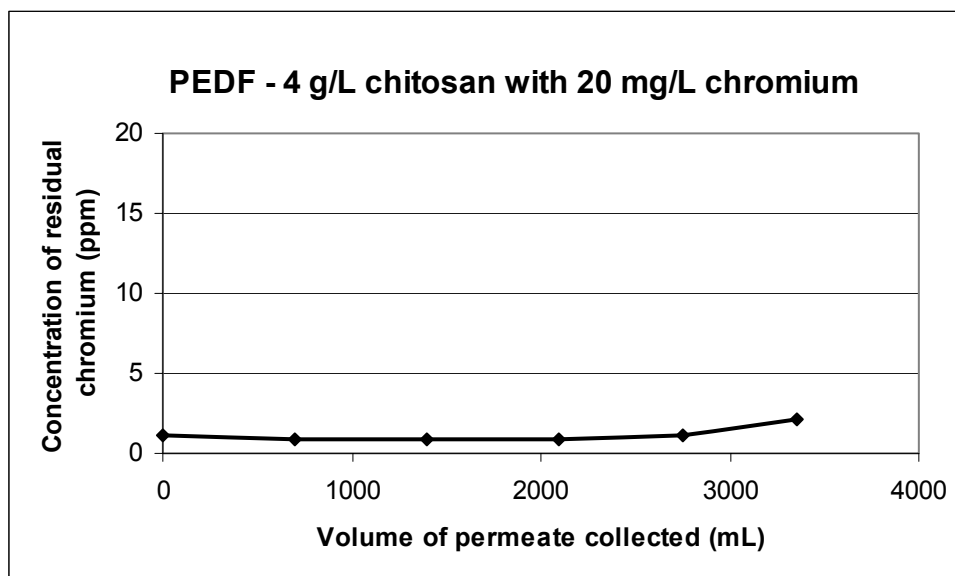


Figure 10: Permeation curve for 4 g/L chitosan in a polymer enhanced diafiltration system. PEDF was carried out with a feed concentration of 20 mg/L chromium. pH was maintained at 4.0.

Table 6 Transmembrane pressures, permeate flow rate and pH measurements during PEDF with 5 g/L chitosan and 20 mg/L chromium.

<i>Volume of permeate collected (mL)</i>	<i>Transmembrane pressure (psi)</i>	<i>Permeate flow rate (mL/min)</i>	<i>pH</i>
0	6.75	20	4.00
600	6.75	20	4.01
1100	7.00	18	4.00
1500	7.00	15	4.00
1900	7.00	15	4.00
2250	7.00	15	4.00

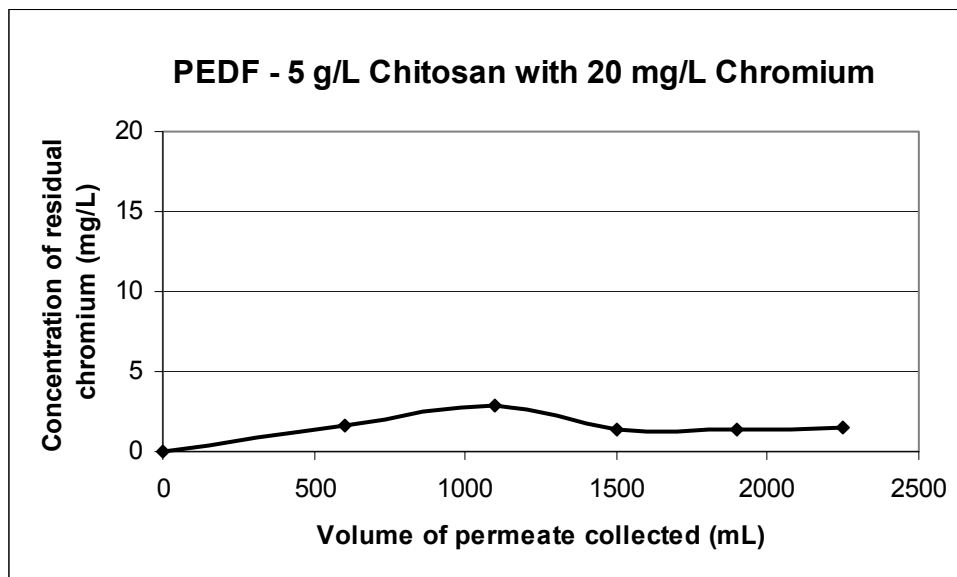


Figure 11: Permeation curve for 5 g/L chitosan in a polymer enhanced diafiltration system. PEDF was carried out with a feed concentration of 20 mg/L chromium. pH was maintained at 4.0.

Table 7 Transmembrane pressures, permeate flow rate and pH measurements during PEDF with 6 g/L chitosan and 20 mg/L chromium.

<i>Volume of permeate collected (mL)</i>	<i>Transmembrane pressure (psi)</i>	<i>Permeate flow rate (mL/min)</i>	<i>pH</i>
0	7.5	13	4.00
400	7.75	13	4.00
750	7.75	13	4.00
1050	7.75	13	4.01
1400	7.5	13	4.01
1700	7.5	13	4.00

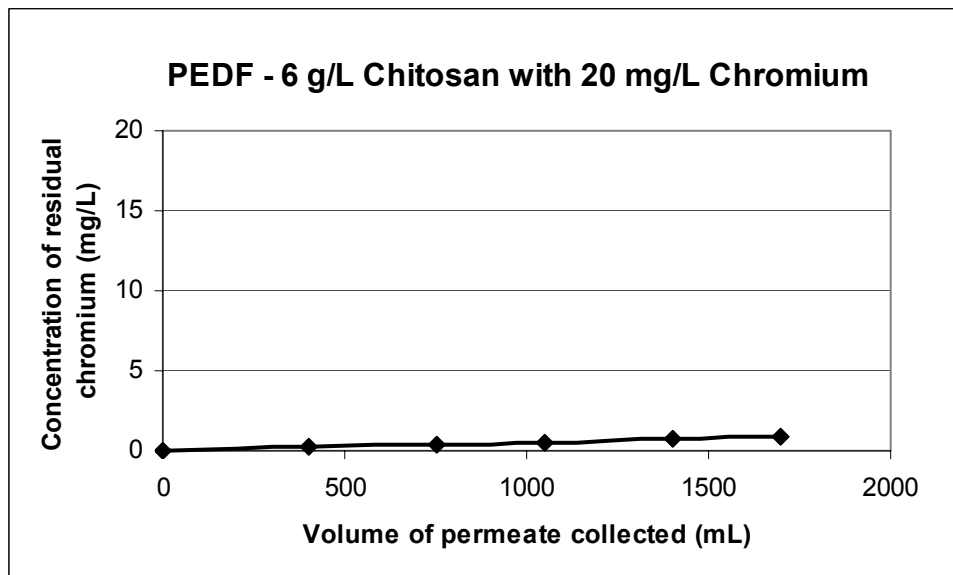


Figure 12: Permeation curve for 6 g/L chitosan in a polymer enhanced diafiltration system. PEDF was carried out with a feed concentration of 20 mg/L chromium. pH was maintained at 4.0.

The volume of chitosan was maintained at 500 mL. The transmembrane pressure, permeate flow rate and pH measurements during the run are depicted in **Table 8**. Throughout the run transmembrane pressure was maintained at 7.5 – 8.0 psi. **Figure 13** is the permeation curve for the PEDF with 6 g/L chitosan processing larger volumes (about 3 L) of chromium.

2.5 PEDF with 20 g/L chitosan at pH 2.5

This run was done in order to test the effect of very high concentration of chitosan on PEDF and binding of chromate. In order to be able to dissolve high concentrations of chitosan in acetic acid, a lower pH i.e. 2.5 was maintained. **Figure 14** depicts the data of the PEDF with 20 g/L chitosan. Very low concentration i.e. almost 0 mg/L was found in the permeate. However, the volume of permeate collected was low, which means the volume of chromium solution processed was low too. After 3 hours, only 300 mL was processed. This is due to a reduction in permeate flux due to the high viscosity of chitosan solution. There was also a large pressure drop feed from the feed side to the retentate side. The transmembrane pressure was maintained at 10 psi throughout the run.

2.6 PEDF with buffer diafiltration.

Buffer diafiltration was done in order to rule out the possibility of leakage of low molecular weight fractions of chitosan through the membrane and into the permeate. 0.05 M acetate buffer was added into the reservoir and the PEDF was run for an hour before the addition of metal was begun. This PEDF was run at the same conditions as the previous ones at 4 g/L chitosan and 20 mg/L chromium. pH was maintained at 4.0. **Figure 16** shows the PEDF data after buffer diafiltration was done. On comparing this

Table 8 Transmembrane pressures, permeate flow rate and pH measurements during the PEDF with 6 g/L chitosan.

<i>Volume of permeate collected (mL)</i>	<i>Transmembrane pressure (psi)</i>	<i>Permeate flow rate (mL/min)</i>	<i>pH</i>
0	7.5	12	4.00
350	7.25	12	4.01
600	7.5	12	4.01
900	7.5	12	4.00
1200	7.75	12	4.01
1500	7.75	12	4.00
1800	8.0	11	4.00
2100	8.0	12	4.00
2400	8.0	12	4.00
2750	8.0	12	4.01
3050	8.0	12	4.00

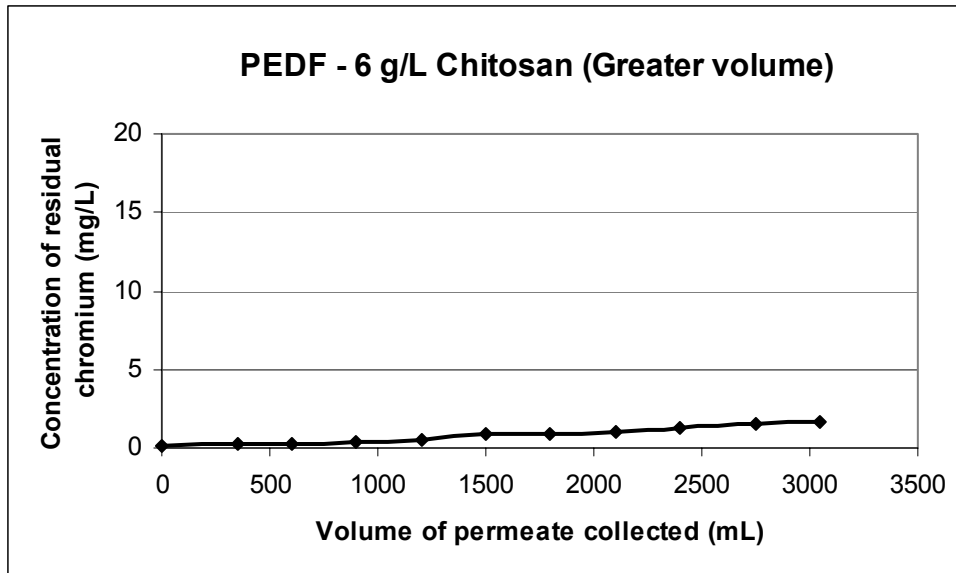


Figure 13: Permeation curve showing PEDF of 6 g/L chitosan. A process volume of about 3 L of 20 mg/L chromium was run through the system. pH was maintained at 4.0.

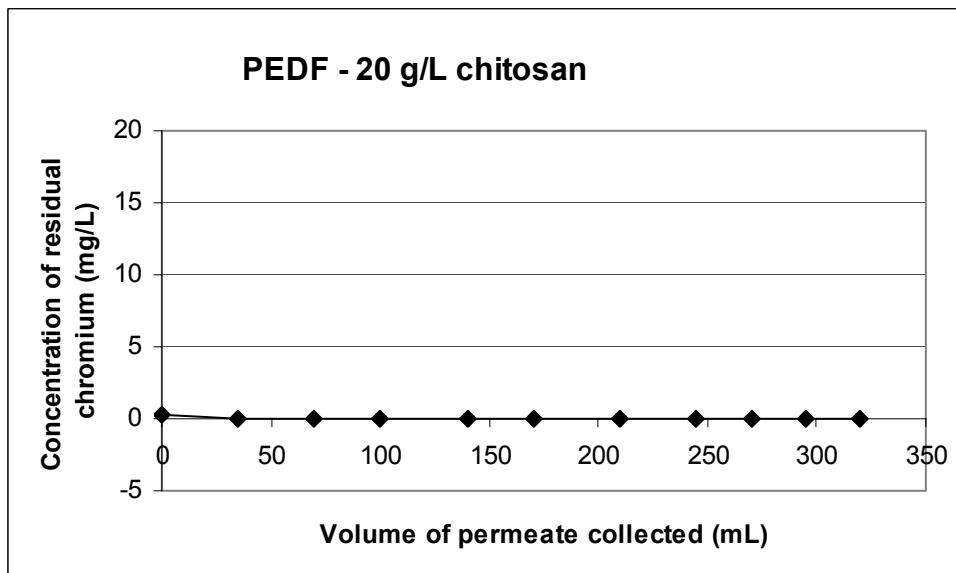


Figure 14: Permeation curve showing PEDF at 20 g/L chitosan. The chitosan was extremely viscous. Low volumes of chromium solution could be processed through the system. The concentration of chromium in the permeate was 0 mg/L. pH was maintained at 2.5.

figure with **Figure 15**, which is the PEDF data under the same conditions (pH 4.0, 4 g/L chitosan, 20 mg/L chromium feed concentration) but without buffer diafiltration, there is no decrease observed in the concentration of chromium in permeate in **Figure 16**. Since buffer diafiltration did not lead to a decrease in the chromium passing into the permeate, leakage of low molecular weight fractions is not taking place.

2.7 Chitosan assay using Poly- γ -glutamic acid.

In order to determine if there is any chitosan passing in to the permeate, the permeate was assayed for the presence of chitosan. Poly- γ -glutamic acid, being a negatively charged polymer, was made to react with different concentrations of chitosan. When the two polymers were mixed, a white turbidity was formed which was measured spectrophotometrically at 600 nm. **Table 9** shows the concentration and volume of chitosan and γ -PGA used and the measured absorbance. The relationship between concentration and absorbance was not linear. This assay could serve as a qualitative assay for presence of chitosan. A concentration as low as 0.02 mg/L of chitosan, could be detected as a measurable turbidity. The permeate samples collected were also tested with γ -PGA. No turbidity was seen with the permeate samples. This is also shown in **Table 9**

2.8 Modeling of PEDF data.

The Langmuir sorption model was chosen for fitting the experimental data. The equation of the Langmuir model is given as Equation (4). Depending on the functioning of the PEDF system, the kinetic model for biosorption can be developed by performing a material balance:

$$V \frac{dC}{dt} = FC_o - FC - \frac{dq}{dt} XV \quad (7)$$

Where V is the reaction volume (L), F is the inlet flow rate (L/h), X is the biomass concentration in solution (g/L). Now, introducing equation (4) into equation (7) and rearranging, the following differential equation can be obtained:

$$\frac{dC}{dt} = \frac{(C_o - C)}{\tau \left[1 + \frac{q_{max} K_S X}{(C + K_S)^2} \right]} \quad (8)$$

where τ is the residence time in the reactor (h), q_{max} is the maximum adsorption capacity of the polymer (mg/g) and K_S is the dissociation constant (mg/L). q_{max} and K_S are the Langmuir parameters. (K_S is equal to $1/K_A$). Equation (8) can be integrated with the following initial conditions: $t = 0$ and $C = 0$. (Barba. D, 2001). This equation can be used to predict the process performance.

Using equation (8) and the following conditions; 6 g/L biosorbent, $q_{max} = 310$ mg/g (asymptotic estimated value), $K_S = 153$ mg/L (experimental value), $C_o = 20$ mg/L Cr, a predicted theoretical concentration of chromium in the effluent was obtained as is depicted in **Figure 17**. A differential equation solver was used to predict the concentration of chromium in the permeate with time. A slight discrepancy is seen between the predicted values and the experimental data in **Figure 17**.

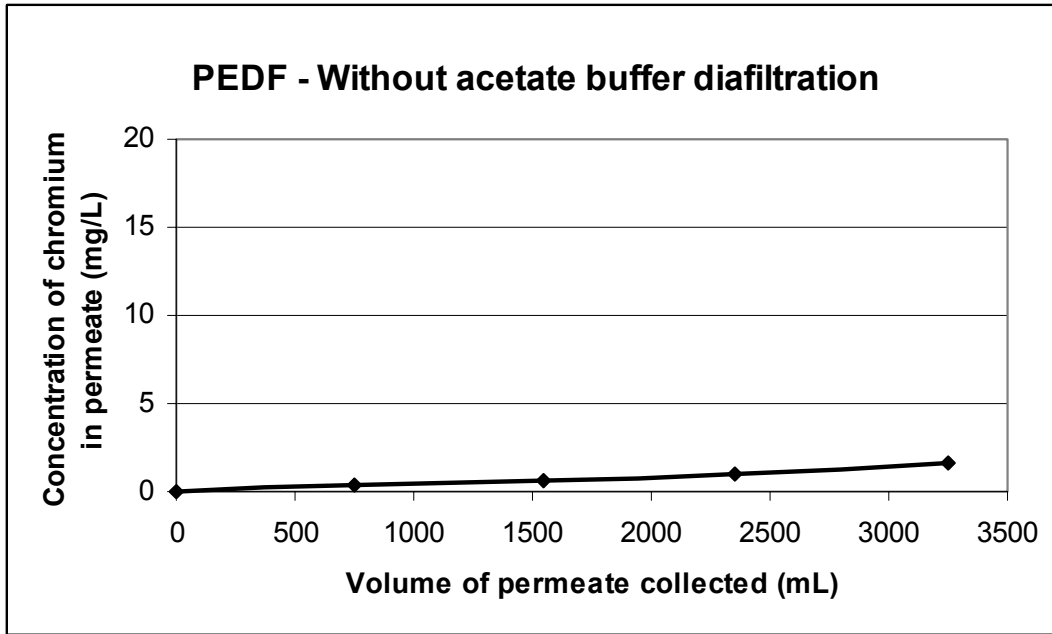


Figure 15: Permeation curve with 4 g/L chitosan without buffer diafiltration. Starting feed concentration of chromium was 20 mg/L. pH was maintained at 4.0.

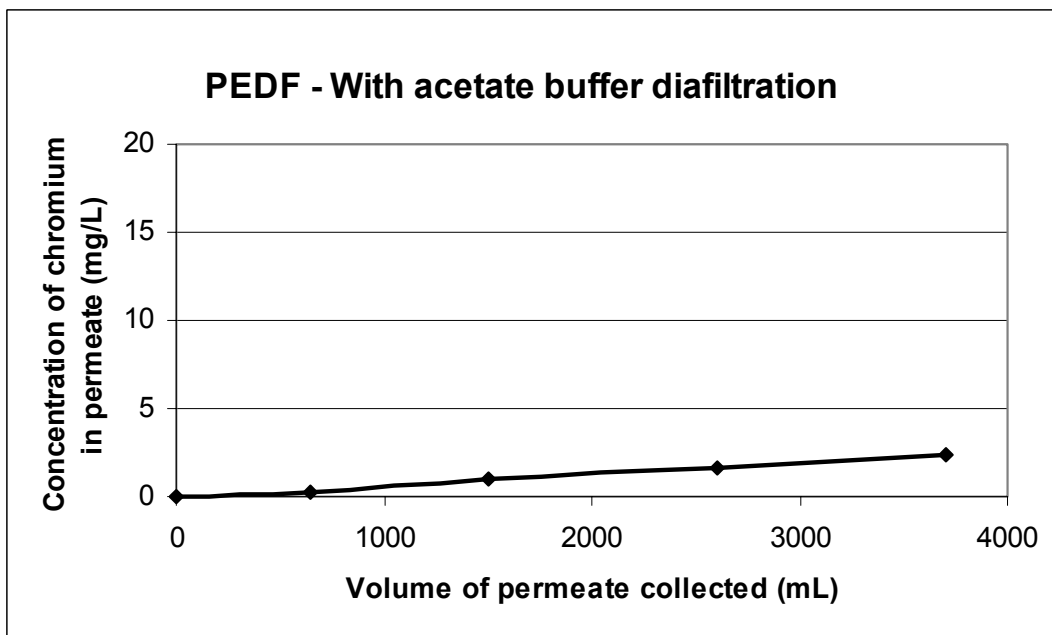


Figure 16: Permeation curve with 4 g/L chitosan with buffer diafiltration. Starting feed concentration of chromium was 20 mg/L. pH was maintained at 4.0. There was no decrease observed in chromium passing into the permeate as compared with Figure 13, which is the control without diafiltration.

Table 9: Assay for chitosan using poly- γ -glutamic acid. Assay results for permeate samples are also depicted.

<i>0.05% Chitosan (mL)</i>	<i>0.05% γ-PGA (mL)</i>	<i>Distilled Water (mL)</i>	<i>Concentration of chitosan (mg/L)</i>	<i>Absorbance (600 nm)</i>
0.1	1.0	3.9	0.01	0.00
0.2	1.0	3.8	0.02	0.003
0.3	1.0	3.7	0.03	0.016
0.4	1.0	3.6	0.04	0.045
0.5	1.0	3.5	0.05	0.076
0.6	1.0	3.4	0.06	0.09
0.7	1.0	3.3	0.07	0.116
0.8	1.0	3.2	0.08	0.202
0.9	1.0	3.1	0.09	0.240
1.0	1.0	3.0	0.1	0.247
<i>Permeate A[*] (1.0 mL)</i>	1.0	3.0		0.00
<i>Permeate B^{**} (1.0 mL)</i>	1.0	3.0		0.00

* - Permeate sample from 2 g/L chitosan run.

** - Permeate sample from 4 g/L chitosan run

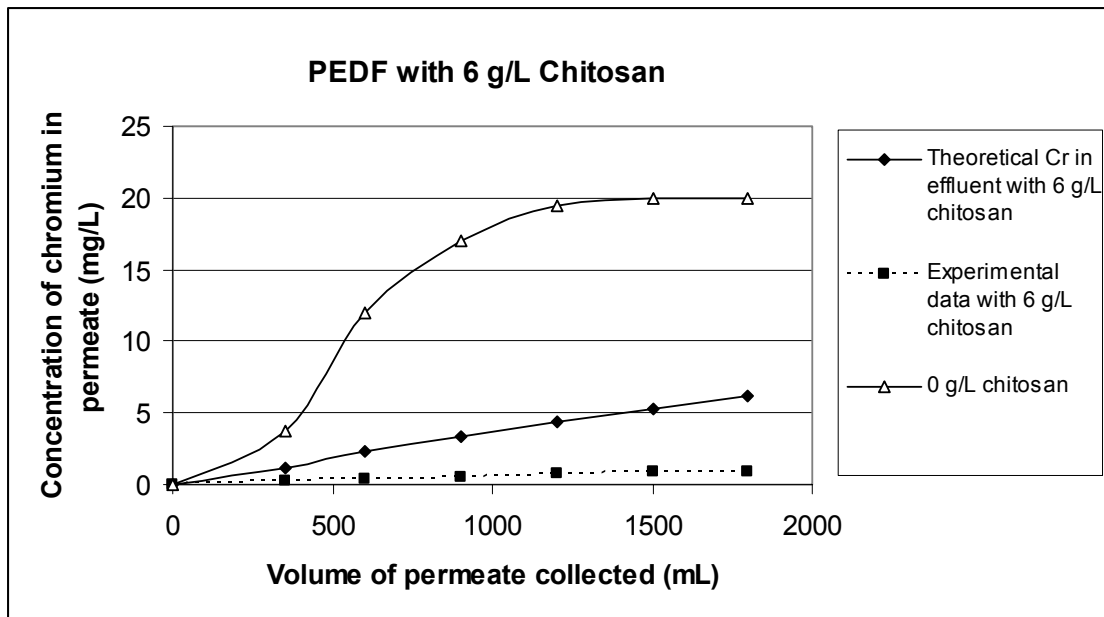


Figure 17: Permeation curve of chitosan at 6 g/L with predicted curve. The white triangles represent the experimental data with no chitosan. The black diamonds represent the predicted values of Chromium in the effluent with 6 g/L chitosan. The predicted values are obtained from solving equation (8). The back squares are the experimental data obtained for PEDF with 6 g/L chitosan. All of these data are at a feed concentration of 20 mg/L chromium.

3. Rheological studies to characterize chitosan-chromate complex.

Viscosity of chitosan-chromate complex was studied on application of varying degrees of shear stress. The viscosity was plotted against the shear stress applied. This was used to determine if the complex behaved as shear-thinning or shear-thickening. It was observed that at lower concentrations of chitosan i.e. 4 g/L and 6 g/L, when 5-80 mM chromate was added, the complex behaved slightly shear-thickening. The viscosity showed an upward trend with increase in shear stress as can be seen in **Figure 18** and **Figure 19**. **Figure 18** shows the rheological behavior of 6 g/L chitosan in the presence of 5-80 mM chromium. A control was also tested which was only 6 g/L chitosan without any metal. **Figure 19** shows the rheological behavior of 4 g/L chitosan in the presence of 5-80 mM chromium. A control was also tested which was only 4 g/L chitosan without any metal. The control in both the cases showed more or less Newtonian behavior i.e. viscosity did not change greatly with increase in shear stress. Whereas, at higher concentrations of chitosan i.e. 12 g/L and 20 g/L, when 1-100 mM chromium was added, the complex behaved slightly shear-thinning. The viscosity showed a downward trend with increase in shear stress. This is evident in **Figures 20 and 21**. **Figure 20** shows the rheological behavior of 12 g/L chitosan in the presence of 1-100 mM chromium. **Figure 21** shows the rheological behavior of 20 g/L chitosan in the presence of 1-100 mM chromium. The control in each experiment was chitosan without the presence of any metal. The control for 12 g/L was Newtonian but the control at a much higher concentration of 20 g/L also showed a shear-thinning behavior.

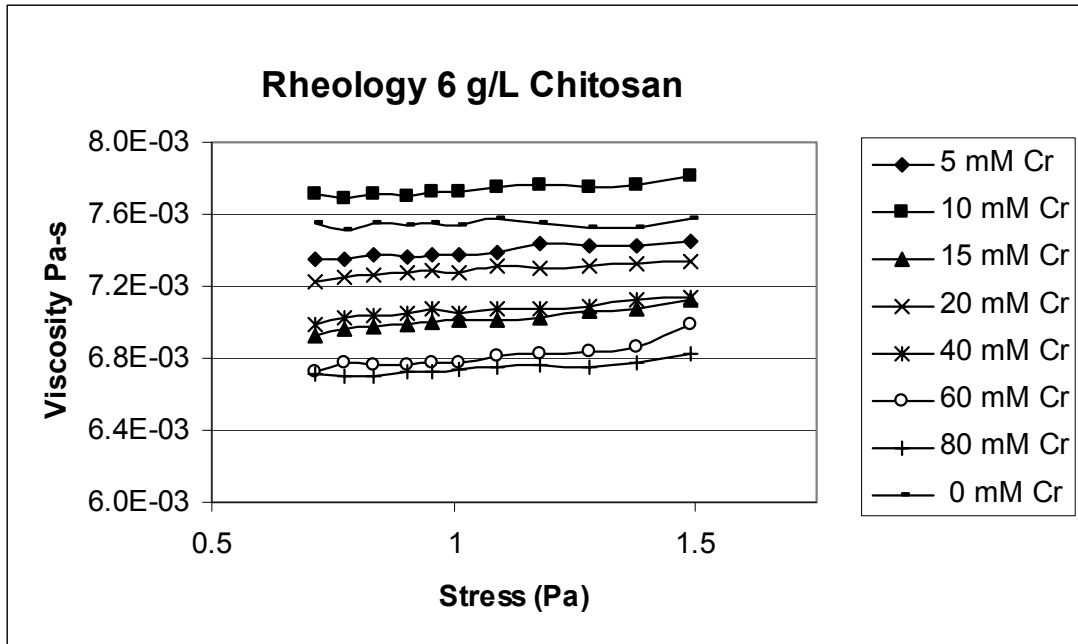


Figure 18: Rheological behavior of 6 g/L chitosan. Viscosity was measured with increase in shear stress in the presence of 5-80 mM chromium.

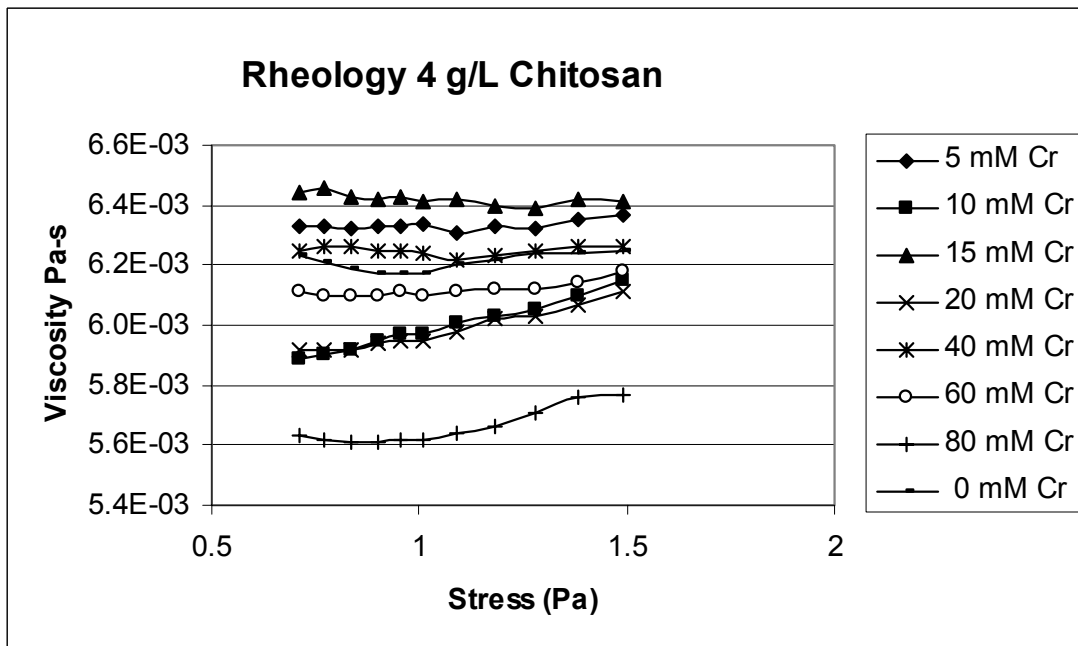


Figure 19: Rheological behavior of 4 g/L chitosan. Viscosity was measured with increase in shear stress in the presence of 5-80 mM chromium.

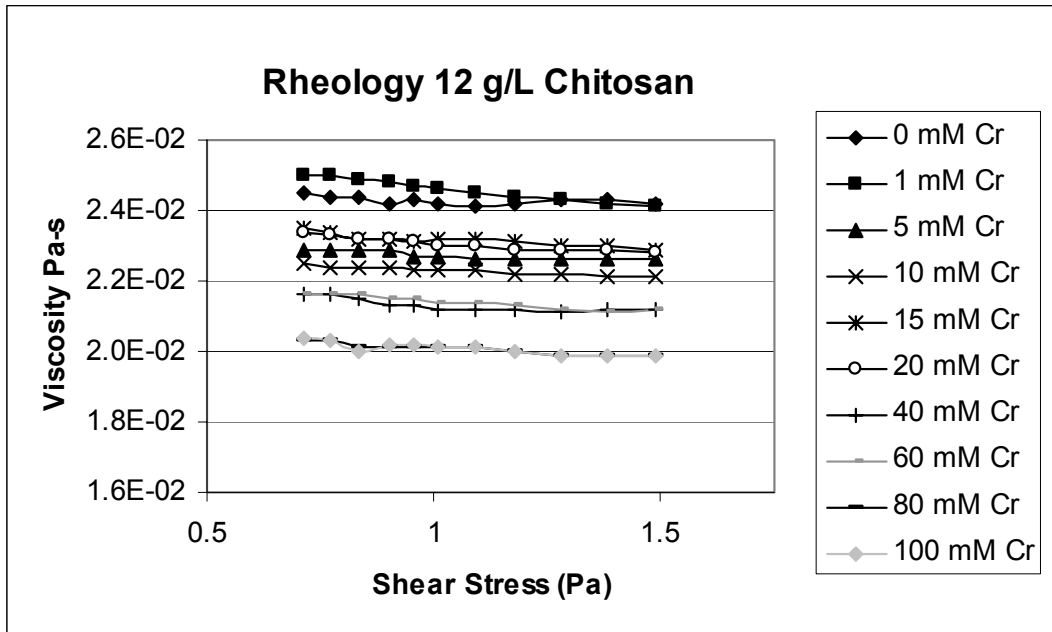


Figure 20: Rheological behavior of 12 g/L chitosan. Viscosity was measured with increase in shear stress in the presence of 1-100 mM chromium.

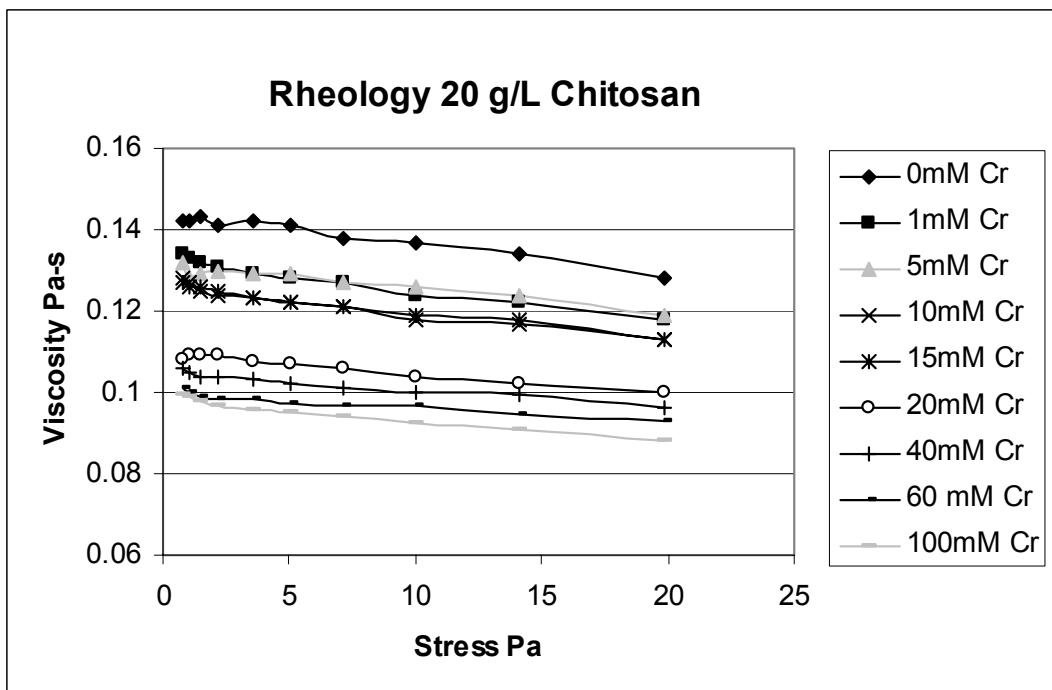


Figure 21: Rheological behavior of 20 g/L chitosan. Viscosity was measured with increase in shear stress in the presence of 1-100 mM chromium

DISCUSSION

Sorption kinetics

Sorption kinetics is an important parameter to evaluate the basic qualities of a good sorbent. It helps us determine whether or not the sorbent adsorbs metals and to what extent. (Schmuhl et al, 2001). Removal of Cr (VI) at pH 3.0 as a function of time is seen in **Figure 2**. **Figure 3** shows the Cr (VI) removal with time at pH 4. It can be seen that there is a decrease in the final free concentration of chromium when chitosan is present when compared to a 'no polymer' control. This indicates that fairly good binding of chromium occurs in the presence of chitosan. This also gives an indication of the time required by the metal-polymer reaction to reach equilibrium. A contact time of 10-12 hours is sufficient to reach equilibrium.

Adsorption isotherm

In a sorbent-sorbate system, sorption results in removal of the metal from the bulk solution as it gets adsorbed onto binding sites on the sorbent. This leads to a decrease in free chromium solution and an increase in chromium adsorbed on the sorbent, until equilibrium is reached between the uptake of chromium by chitosan and the equilibrium concentration of chromium in solution. At this equilibrium there is a defined distribution of the metal between the solid and liquid phases. This can be expressed by an isotherm. **Figures 4** and **Figure 5** represent these isotherms for chromium and chitosan. Unlike, most isotherms, these did not reach a plateau phase, in other words q_{\max} could not be experimentally reached. A reason for this could be that maximum adsorption for this sorbent-sorbate was higher than what could be experimentally determined. q_{\max} cannot be theoretically reached because it is an asymptotic limit. Therefore from the equilibrium

isotherm plot, a linear reciprocal plot was obtained. Using this linear plot and the Langmuir equation, the value for K_d or dissociation constant was found to be 153 mg/L. Since q_{max} was too high and could not be experimentally determined, an estimate of q_{max} was made based on the dissociation constant value. This estimated q_{max} is about 300 mg/g. Even though this is an estimated value, we can safely say that the uptake is definitely higher than 100 mg/g as can be seen from Figure 4, where at 350 mg/L equilibrium concentration, the uptake is 110.5 mg/g and the plot is still linear. Other studies involving adsorption of chromium onto differently modified chitosan have shown the following adsorption capacities:

Table 10: Adsorption capacities of different chitosan based sorbents for chromium.

<i>Type of adsorbent</i>	<i>Maximum adsorption capacity</i>	<i>Reference</i>
Cross-linked chitosan	215 mg/g	Rojas et al, 2005
Non cross-linked chitosan	> 80 mg/g	Schmuhl et al,2001
Raw chitosan	34.9 mg/g	Udaybhaskar et al,1990
Quaternized chitosan resin	> 32 mg/g	Qin et al, 2003
Composite chitosan (chitosan coated on alumina)	153 mg/g	Boddu et al, 2003
Chitosan based polymeric surfactants	180 mg/g	Yeal lee et al, 2004

Optimum pH

From **Table 1**, it can be seen that pH 4 gave best chromate uptake, followed by pH 3. The effect of pH on uptake can be explained on the basis of pKa of the amine groups of chitosan, which is 6.3. (Udaybhaskar.P, 1990). Below the pKa value of chitosan, the sorbent is positively charged, while the chromium anions in solutions are negatively

charged. This leads to an electrostatic attraction between the two. Above the pK_a , the chitosan will be less protonated or neutral and hence Cr (VI) removal is reduced at pH higher than the pK_a .

Yeal-lee et al (2001) found that for hexavalent chromium bound to chitosan based polymeric surfactants, uptake increased as pH increased from 2 to 5.5. Their pH optimum was around pH 6.0. At pH 8.0 the removal was only about 12.1% as compared to 65.6% at pH 5.0. Also because the protonation constant pK_a of the amine group is 6.3, they calculated that the extent of protonation would be 9, 50, 91 and 99% at pH values of 7.3, 6.3, 5.3 and 4.3 respectively.

According to Boddu et al (2003) it was observed that absorption was greater at lower pH and that it decreased with increasing pH. Cr (VI) can exist in several forms such as $Cr_2O_7^{2-}$, $HCr_2O_7^-$, $HCrO_4^-$ and CrO_4^{2-} . The relative abundance of any species depends on the concentration of chromium ion and the pH of the solution. Schmuhl et al (2001) also found that uptake decreased with increase in pH. According to their study maximum uptake occurred at pH 5.0. Above pH 7, a plateau was observed. This was also supported Qin et al (2003) where the maximum adsorption capacity for a epichlorohydrin cross-linked chitosan resin was seen at pH 4.0 and removal was lower than 30% at pH 8. In a study by Rojas et al (2005) chitosan cross-linked using glutaraldehyde was studying for it adsorption of chromium. pH 4.0 was found to be optimum; also supporting that 99% of the active sites of chitosan are protonated at pH 4. Dantas et al (2001) studied a chitosan impregnated microemulsion for uptake of chromium and found that maximum adsorption occurred at pH values between 2.5 and 3.5. In general the profile of uptake over different

pH suggested that uptake was low at extremely low pH such as 2, then peaked at pH 3 -4 and decreased again at pH higher than 6.

Polymer Enhanced Diafiltration.

Effect of concentration of chitosan.

The concept of continuous diafiltration has been studied as an effective method for the binding of heavy metal ions to natural lecithin and their separation. (Ahmadi, S, 1994).

The removal efficiency of divalent ions like Cu(II), Co(II), Ni(II) and Zn(II) from dilute solutions was studied using chitosan-enhanced ultrafiltration. (Juang, R.S., 2000). A similar principle of diafiltration was used in this study. From the PEDF data, it can be seen that chitosan is able to bind chromate in a dynamic system. High retention values were seen in the PEDF runs. In the experiment that was performed in the absence of chitosan, it was clear that no binding to the PEDF membrane took place. (**Figure 8**). The membrane chosen had a molecular weight cutoff of 30,000 which would be able to retain chitosan with an average molecular weight 125,000. When compared to **Figure 8**, the permeation curve in the presence of chitosan (**Figures 9-12**) has an almost flat slope. There was a marked difference from the curve in the absence of chitosan. This indicates that there is some binding taking place between chitosan and chromium, thus supporting the inference made from equilibrium data. The effect of concentration of chitosan on binding was then studied. From **Figures 9-12** it can be seen that increase in concentration of chitosan lead to a better uptake in the PEDF system. However, as seen in **Figure 12**, with more volume processed through the system, there is a steady increase in chromium passing in to the permeate. Even though the binding capacity of chitosan is high, this

might be occurring because probably not all binding sites on the polymer are accessible to the metal ions. Therefore there are always some unbound ions during PEDF. Chitosan is a copolymer of N-acetylglucosamine and glucosamine. (Guibal, E. 2004) The binding capacity depends on the extent of deacetylation and consequently on the number of glucosamine groups. Commercially available chitosan is always partially deacetylated so there are always some N-acetylglucosamine groups on it. This could explain the unbound ions passing into the permeate.

Diafiltration Data, γ -PGA Assay.

As was evident from **Figures 9-12**, some amount of chromium was always passing into (into is one word) the permeate. It was necessary to determine that the chromium in the permeate was not chromium that was bound to low molecular weight fractions of chitosan leaking through the membrane. A buffer diafiltration helps to negate that effect by washing out all the low molecular weight fractions, before the addition of metal is begun. If leakage of chitosan was in fact taking place then after buffer diafiltration, there would have been lesser chromium in the permeate. This was not observed (**Figure 15**, **Figure 16**) and so the phenomenon of low molecular weight leakage could be ruled out. This kind of diafiltration was also done to overcome the leakage of the polymer, when binding of cadmium to chitosan was studied. (Llorens et al, 2004). Then the γ -PGA assay was done to find out if there was any chitosan present in the permeate. This was an important study because it has been shown in the case of polymers; deformation of the macromolecules is common. Under certain hydrodynamic conditions the polymer can change its shape, such that it can pass through the membrane. (Volchek et al, 1996) γ -PGA, a negatively charged polymer was used to qualitatively assay the presence of

chitosan. **Table 9** shows that no chitosan was detected in the permeate. Therefore, it can be said that the chromium found in the permeate, is unbound chromium and not something that is bound to chitosan; either low molecular weight or otherwise.

PEDF at High Concentration of Chitosan.

PEDF with 20 g/L chitosan was done in order to estimate the effect of highly concentrated chitosan on binding and PEDF performance. It was seen that when employing 20g/L chitosan, which yields a very viscous solution, allowed only a very low volume of chromium solution to be processed. (**Figure 14**). Uptake of chromium was high, since there was almost 0 mg/L chromium detected in the permeate. However, there was a great reduction in permeate flux due to high viscosities. The molecular mass of the polymer should be chosen so as to ensure efficient separation of the ions and complete retention. However, it has been seen that high molecular masses reduce permeate flux and increase process cost. (Volchek et al, 1996). Therefore it becomes necessary to choose an optimum concentration of polymer for PEDF without affecting its performance.

When PEDF data is predicted using the Langmuir model, a slight disagreement is seen between the theoretical and experimental results. (**Figure 15**). This necessitates the modification of the model to better represent what is happening in the chitosan-chromate PEDF process.

Rheological studies of Chitosan-Chromium Complex.

In order to characterize the chitosan-chromate complex, its rheological properties were studied. The complex was subjected to various shear stresses and the change in viscosity was studied. It was found that at low concentrations of chitosan, the chitosan-

chromate complex behaved as a slightly shear-thickening fluid. (**Figure 18, Figure 19**). In contrast, at higher concentrations, the chitosan-chromate complex behaved like a shear-thinning fluid. The viscosity increased with increase in shear stress. (**Figure 20, Figure 21**). An explanation for this behavior could be that, in dilute polymer solutions there is more space available for the molecules to move independently of each other. Therefore, when chromate is added, amine groups from different molecules are able to come together to neutralize it. Due to this intermolecular interaction, different molecules of chitosan come closer, leading to the slight shear-thickening behavior observed at low polymer concentrations. Since chitosan is a rigid polysaccharide, intramolecular neutralization of chromium is not as likely.

Polymers exist in an intermolecular entangled state in concentrated domain. It has been seen that at low concentrations and low shear rates, chitosan behaves more or less Newtonian with no change in viscosity. This is because the rate of intermolecular disentanglements brought about by shear is the same as that of entanglements newly formed. (Hwang. J.K, 2000). This behavior of chitosan was observed in the control study done in the absence of chromate. (**Figure 18, Figure 20**). Hwang et al (2000) also showed that at higher shear rates, viscosity of chitosan goes down with increasing shear rates. Technically, chitosan can behave as a shear thinning fluid (in the absence of chromate), however, the degree of shear thinning is slight, again, indicative of the rigid nature of the polysaccharide. Chitosan behaves more pseudoplastic with increasing chitosan concentration. The shear-thickening behavior seen on the addition of chromate has not been seen in any of the studies on the rheological studies involving only chitosan. (Hwang, J.K 2000; Nyström, B. 1999). Therefore it can be said that the shear thickening

is an effect of chromate neutralization by chitosan molecules and an intermolecular interaction. At higher concentrations of chitosan, a shear thinning behavior is seen due to intramolecular interaction. This part of the study was important to provide information about the nature of the chitosan-chromate interaction.

CONCLUSIONS

Chitosan is a good candidate for biosorption of chromium. Fairly high binding of chromate occurs as seen in the Polymer Enhanced Diafiltration (PEDF) runs. A higher concentration of chitosan gave a better uptake. Less than 1 mg/L chromium passed into the permeate when 6 g/L chitosan was used. However, a high concentration, such as 20 g/L, led to reduction in permeate flux. It is, therefore, necessary to choose an optimum chitosan concentration without affecting the permeate flux. Higher concentrations mean high viscosities, thereby causing concentration polarization and affecting membrane performance. PEDF is a good process to concentrate metals from dilute aqueous solutions. It was also seen that chromium in the permeate is unbound chromium as there is no chitosan detected in the permeate. pH is an important parameter for uptake. Best uptake occurred at pH 4 which is lower than the pKa of chitosan i.e. 6.3. At low pH chitosan has a greater number of protonated amine groups for binding. pH affects the speciation of the metal ion as well as the solubility of the polymer, which is important for a process like PEDF. The rheological studies helped to characterize the chitosan-chromate interaction. The shear thickening behavior at low chitosan concentrations suggests that neutralization of chromate at low chitosan concentration is due to interaction of amine groups from multiple chitosan molecules. This behavior is not observed at high chitosan concentration. Future work in this area would involve the study of regeneration of the polymer, recovery of chromium from the chitosan-chromate complex and scale-up of the process for industrial applicability.

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APPENDIX

Standard curve for determination of chromium using Diphenylcarbazide assay

