

**Optimization of Polymer Enhanced Diafiltration system
by studying copper removal from aqueous solutions
using Lambda-Carrageenan**

A Thesis

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By

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ABSTRACT

Excessive discharge of heavy metals has been one of the major causes of water pollution worldwide. Various traditional methods of heavy metal removal have been devised but certain drawbacks like high cost, high energy requirement, and the production of toxic sludge have limited their use. Hence, biosorption is one of the alternative methodologies. This study combined biosorption and diafiltration in an attempt to optimize Polymer Enhanced Diafiltration to study copper removal from aqueous solutions by the use of a biopolymer, lambda-carrageenan. Lambda-carrageenan was studied as a biosorbent owing to properties such as low cost, good water solubility, non-gelling nature and the presence of sulfate groups which can sequester cations. Conditions for binding such as pH, temperature and concentration of copper and lambda-carrageenan were studied. Equilibrium dialysis experiments were performed to study the metal ion membrane transport kinetics and to determine the metal ion binding capacity and strength of the copper-biopolymer association. Rheological measurements were performed to determine how the viscosity of lambda-carrageenan changes with increase in shear stress and with increase in metal concentration. The solution was found to be shear thinning. However, with increase in metal concentration, viscosity was found to increase when high concentrations of polymer (8 g/L and 12 g/L) were used. Solution viscosity was found to decrease with increase in metal concentration when 4 g/L of polymer was used. Polymer Enhanced Diafiltration studies showed no leakage of the polymer through the membrane and no significant binding elsewhere in the PEDF system. It also showed an impressive retention of copper inspite of a rather high metal ion-polymer dissociation constant suggesting a yet not understood series of events occurring on the membrane of the PEDF system. Lambda-carrageenan is a linear polysaccharide, which might be stacking up on the membrane after forming layers, and not allowing any free metal ion to escape. Other reasons could be the sieving effect, degradation of the polymer due to shear and compaction of molecule on metal binding such that the polymer is not itself escaping through the membrane, but also not allowing the free metal ion to escape. Hence, this study suggests the need for more information on the metal-polymer interactions on the surface of the membrane by designing a direct observation experiment with a mini-tangential flow filtration system.

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INTRODUCTION :

1. OBJECTIVES OF THE STUDY :

“Heavy metal” is a term which refers to a wide variety of elements that include an important class of pollutants (Vieira & Volesky 2000). Metals are referred to as heavy metals if they have a specific gravity of more than 5g/cm^3 in their standard state (Jarup 2003) . Many heavy metals are essential in biochemical processes and many have found importance in medical applications. However, excess heavy metal in a living organism can lead to poisoning. It represents an uncommon, yet clinically significant medical condition (Shanab 2007). There have been various instances where heavy metal toxicity has lead to morbidity and mortality (Shrivastav 2001).

Bismuth, gold, gallium, lithium, aluminum may lead to iatrogenic metal toxicity. Metal fume fever is an occupational heavy metal toxicity seen in workers exposed to metal oxide fumes of zinc, copper, magnesium, cobalt, characterized by fever, headache, cough etc (American Welding Society 2002). Chronic exposure of cobalt may lead to pulmonary fibrosis. Chronic inhalation of cadmium may lead to fibrotic and emphysematous lung damage, as well as affecting bones and kidneys. Excess copper exposure may lead to it's deposition in liver, brain, kidney, cornea and may lead to Wilson's disease and childhood cirrhosis. Excess of an inorganic form of lead may affect CNS, peripheral nervous system, cardiovascular and reproductive systems (Lin 2003). Various other heavy metals leading to toxicity are chromium, mercury and arsenic.

Copper is a red-colored heavy metal found in rock, soil, water, sediments and air. It is an essential nutrient but may result in health problems if taken in large quantities. Ground water and surface water usually contain very low amount of copper, about $4\ \mu\text{g/L}$. However, its concentration is generally higher in drinking water. When corrosive water comes in contact with copper plumbing, copper dissolves from the plumbing and levels in drinking water increase. It has been found that if corrosive water remains stationary in plumbing systems for 6 hours or more, copper levels may exceed $1000\ \mu\text{g/L}$. According

to USEPA, copper levels in drinking water should not exceed 1300 µg/L. However, World Health Organization (WHO) has adopted the copper level in drinking water as 2000 µg/L (Fitzgerald 1998, www.epa.gov/safewater).

Traditional methods of heavy metal removal from water include chemical precipitation, ion exchange, reverse osmosis and solvent extraction (Arpa 2000, Goksungur 2003, Esposito 2001). However, high costs, generation of toxic wastes and other disadvantages led scientists to look for more economical methods of heavy metal removal. Biosorption is one such economical method involving the use of biopolymers. A novel method termed as Polymer Enhanced Diafiltration was applied for heavy metal removal using biopolymers. This method has been traditionally used for clarifying, concentrating and purifying proteins. (Millipore 2003)

The aim of this study was the optimization of our polymer enhanced diafiltration (PEDF) system to study the uptake of copper by the biopolymer lambda-carrageenan. This system used an ultrafiltration membrane with a certain molecular weight cut off which retained both the polymer molecules and the metal-polymer complex, allowing any unbound metal to pass through. The metal in the filtrate was then assayed and the amount of metal bound to the polymer determined. In order to achieve the main goal of PEDF optimization, the following objectives were:

1. To perform equilibrium dialysis experiments to determine thermodynamics of binding and to estimate the uptake of metal in mg/g of polymer.
2. To determine binding characteristics of metal to the polymer by Polymer Enhanced Diafiltration System.
3. To study change in viscosity of the polymer upon metal ion binding in the presence of metal by rheological measurements

2. LITERATURE REVIEW:

2.1. Heavy Metal Pollution:

Heavy metals are naturally found in the earth's crust. They cannot be degraded or destroyed (Shanab 2007). They might enter our bodies through food, drinking water and air. A few heavy metals like copper, zinc, selenium, in trace amounts, are necessary to maintain the normal metabolism of the human body. However, at higher concentrations, they may lead to metal poisoning.

Heavy metals are often dangerous because they tend to accumulate in our bodies by a phenomenon called bioaccumulation which can be defined as the increase in concentration of a chemical entity in a biological organism over time as compared to its concentration in the environment. A threat to the environment arises due to accumulation of heavy metals in air (for example, during combustion), soil (in groundwater) and water due to human activities (Jarup 2003). The release of heavy metals into the environment, in most developed countries, is well regulated (requiring controlled or treated industrial emissions) or accidental (chemical spillage). Various sources of heavy metal pollution include intensive industry, roadways, automobiles etc. Effluents discharged from such sources may contain relatively high levels of heavy metals such as U, Cd, Hg, Cu, Zn and Pb which may have adverse effects on the environment. Their continuous accumulation in the food chain and presence in nature may have undesirable and serious impacts.

The factors contributing to heavy metal pollution are the chemical nature of metals, soil and sediment particles and pH of the surrounding environment. Soil and dust particles also have charged sites on their surfaces, some positive and some negative. They tend to attract and bind the oppositely charged metal ions and prevent their dissolution in water. However, the soluble form of these heavy metals is more dangerous as it can be readily transported to various plants and animals. pH also plays a very important role in heavy metal pollution.

2.2 Traditional Methods of Heavy Metal Removal:

The traditional methods of removing metal ions are chemical precipitation, ion exchange, reverse osmosis and solvent extraction (Arpa 2000, Goksungur 2003, Esposito 2001).

Chemical precipitation involves precipitating out the metals by addition of coagulants like alum, lime, iron salts, organic polymers etc. The main disadvantage associated with this process is the production of sludges containing toxic compounds which also must be treated or disposed of (Tomko 2006). Ion exchange involves the exchange of metal ions from dilute solutions with the ions held by electrostatic forces on the exchange resin. The disadvantages of this process are the high cost and partial removal of certain ions (Tomko 2006). Reverse osmosis involves the separation of heavy metals by a semi-permeable membrane at a pressure greater than osmotic pressure caused by dissolved solids in wastewater. High cost is the main disadvantage associated with this process. Electrodialysis involves the separation of heavy metal ions through the use of semi-permeable ion-selective membranes. On applying electrical potential between the two electrodes, cations and anions migrate towards their respective electrodes. Due to the alternate spacing of cation and anion permeable membranes, cells of concentrated and dilute salts are formed. However, this process is associated with a disadvantage of formation of metal hydroxides, which might clog the membrane. Ultrafiltration is an irreversible pressure driven membrane operation that uses a porous membrane for removal of heavy metals. Generation of sludge is the disadvantage of this process. Phytoremediation is another evolving method that involves the use of certain plants to clean up soil, sediment and water contaminated with metals. The main disadvantage of this process is that it is a time-consuming process (as plants take years to grow) and the biomass may in fact concentrate the metal ions to levels which are unacceptable for traditional disposal processes (Barros 2006, Tomko 2006).

Hence, the disadvantages associated with traditional methods of heavy metal removal like incomplete metal removal, high energy requirement, generation of toxic sludge and other waste products have made it important to look of other cost-effective treatment methods

(Barros 2006). Moreover, traditional methods of heavy metal removal are not so efficient when metals are present in concentrations less than 100mg/L (Tomko 2006, Mark 2006). Hence, attention is being focused to the development of alternative methods of heavy metal removal like bioremediation. One of such processes is biosorption.

2.3 Biosorption

This method is based on the metal binding capacities of various biological materials. It can further be defined as the ability of biological materials to accumulate heavy metals from dilute solutions through metabolically mediated or physico-chemical pathways of uptake (Goksungu 2003). Hence, it is a process which may include physical or chemical adsorption, ion-exchange, coordination, complexation, chelation, microprecipitation (Goksungu 2003). The major advantages of biosorption over traditional methods of heavy metal removal are economy, good efficiency, minimized sludge production, regeneration of biosorbent and the possibility of metal recovery (Veglio 2002).

The biosorption process includes the interaction between a solid phase (the biosorbent, which is a biological material) and a liquid phase (a solvent) containing a dissolved species that is to be sorbed (called sorbate, like metal ions). Hence, the principle behind the biosorption process is the binding of sorbate to the sorbent due to its higher affinity. This process continues until an equilibrium is reached between the amount of sorbate bound to the biosorbent and its portion remaining in the solution. The degree of sorbent affinity for the sorbate is indicative of its distribution between the solid and liquid phases. Various mechanisms active in biosorption are : chemisorption, physical adsorption, microprecipitation and oxidation/reduction.

Biosorbents can be obtained from various sources like – seaweeds, microorganisms, activated sludge, fermentation wastes etc. There are different types of biosorbents available. Some are broad range biosorbents, capable of binding various heavy metals with no specific activity, while other biosorbents are specific to certain metals (Vieira & Volesky 2000). The affinity of a biosorbent to any heavy metal depends on the presence

of several chemical groups. These chemical groups attract and sequester heavy metals like the acetamido groups of chitin, amino and phosphate groups in nucleic acids, amino, sulphhydryl and both the carboxyl groups in proteins, hydroxyl group in polysaccharide, carboxyl and sulfate groups in polysaccharides of various marine algae (Goksungur 2003).

In the case of covalent metal binding, an occupied site is also theoretically available. However, the extent to which this site can be used by a metal is dependent on its binding strength and concentration as compared to the metal already occupying the site. In case of electrostatic metal binding, a site is available only if the metal is ionized (Vieira and Volesky 2000). There are various factors which might also affect biosorption. pH affects the solubility of metals and activity of functional groups in the biosorbent. Temperature does not normally influence biosorption in the range 20-35°C. Concentration of biosorbent also affects biosorption. An increase in biosorbent concentration leads to increase in interference between the binding sites. However, there is still some conflict regarding this factor. Presence of other metals might also lead to an increase in uptake of a specific metal. However, presence of other metals may lead to increase in specific metal uptake.

2.4. Biosorption Equilibrium models:

A general formula used for calculating the uptake of metal in mg per g of polymer is represented by the following equation :

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

Where U = uptake of metal in mg/g of the polymer

V = volume of metal containing solution (ml)

C1 = Initial metal concentration (mg/L)

C2 = Final metal concentration (mg/L)

M = Amount of biosorbent (g)

In order to determine the maximum adsorption of metal to the polymer, various adsorption models have been used (Mark 2006). They were used for the assessment of sorption performance. The experimental points are fitted in these models to describe the equilibrium of biosorption process. The two linearised equilibrium adsorption isotherm models for single solute system are – the Langmuir and Freundlich adsorption isotherm models (Goksungur 2003). They are used for determining the sorption capacity of a biosorbent (Gupta 2000). Both the models are well suited at constant pH. For proper evaluation of the equilibrium sorption performance, it needs to be supplemented with kinetic studies.

Various assumptions in Langmuir model are:

1. Surface sites have same adsorption energy for the sorbate.
2. Adsorption at one site does not interfere with adsorption by the adjacent site.
3. Adsorption at one site does not affect the energy of adsorption of adjacent sites.
4. Activity of sorbate is directly proportional to its concentration.

Hence, the Langmuir model equation is denoted as

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

Where q = uptake of species (mg/g)

q_{\max} = maximum uptake of species (mg/g)

C = final concentration in solution (mg/L)

K_A = equilibrium association constant (L/mg)

(Mark 2006)

K_A is related to the affinity between biosorbent and sorbate. Reciprocal of association constant (K_A) is the dissociation constant (K_D) which is the measure of metal concentration corresponding to half saturation of sorbent (Mark 2006).

The Langmuir model was initially developed to study physical adsorption. It is now a useful tool to interpret biosorption profiles (Goksungur 2003).

For a good biosorbent, high q_{\max} and low K_D are desirable. The values of q_{\max} and K_A can be determined by double reciprocal plot of the Langmuir equation, also analogous to the so-called Lineweaver-Burke equation used in enzyme kinetic analysis.

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

A plot of $1/q$ and $1/C$ would give q_{\max} , corresponding to the intercept, and K_D , which can be determined from the slope (Goksungur 2003) .

Another equilibrium adsorption model is the Freundlich adsorption model which determine heterogenous surface adsorption (Goksungur 2003), given by:

$$q = kC^{1/n}$$

Where q = metal concentration (mg/g)

k = related to adsorbent capacity

$1/n$ = heterogeneity factor

C = bulk liquid phase metal concentration

$1/n$ = “heterogeneity factor” ranging from 0 to 1

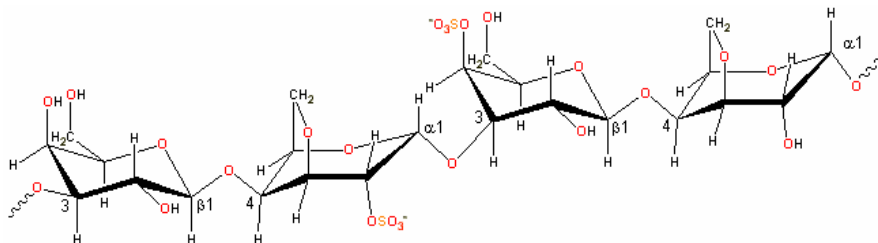
This can also be linearized

$$\ln q = \ln k + 1/n \ln C$$

$\ln k$ is the measure of adsorbent capacity, corresponding the intercept, and $1/n$ is the intensity of adsorption, corresponding to the slope (Goksungur 2003). Monolayer adsorption and constant adsorption energy are a few assumptions of the Langmuir model whereas the Freundlich equation deals with heterogenous surface adsorption (Goksungur 2003). Moreover, the Freundlich model was not used here as it does not predict physical saturation of a given biosorbent, whereas in the Langmuir equation, q_{\max} directly determines the maximum binding capacity of the adsorption system (Mark 2006).

2.5 Carrageenan as Biosorbent:

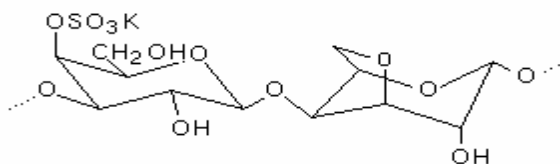
Carrageenans are the linear polymers of about 25000 galactose derivatives, obtained from red seaweed (*Rhodophyceae*), mostly belonging to the genus *Chondrus*, *Eucheuma*, *Gigartina* and *Iridaea*. Different types of carrageenans are produced from different seaweeds. The general structure of carrageenan consists of alternating 3-linked- β -D-galactopyranose and 4-linked- α -D-galactopyranose units.



(<http://www.lsbu.ac.uk/water/hycar.html>)

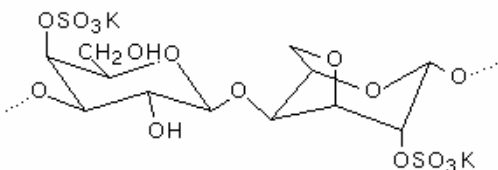
There are mainly three types of carrageenans that have commercial applications, namely, kappa-carrageenan, iota-carrageenan and lambda-carrageenan.

Kappa-Carrageenan is mostly obtained from tropical seaweed, *Kappaphycus alvarezii*.



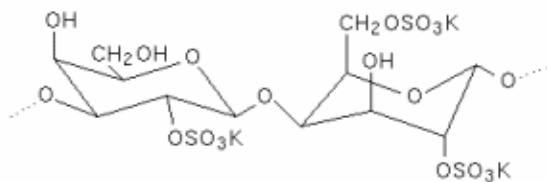
(<http://www.cybercolloids.net/library/carrageenan/structure.php>)

Iota-Carrageenan is mostly obtained from Philippines seaweed, *Eucheuma denticulatum*.



(<http://www.cybercolloids.net/library/carrageenan/structure.php>)

Lambda-Carrageenan is mostly obtained from *Chondrus crispus*.



(<http://www.cybercolloids.net/library/carrageenan/structure.php>)

Commercially available carrageenan is in the form of powder with a color ranging from white to brownish depending on the raw material used and the process used to obtain or manufacture it. Carrageenans are insoluble in organic solvents such as alcohol, ether and oils. Carrageenans may be of gelling or non-gelling types. Gelling carrageenans need to be heated to dissolve in water whereas non-gelling carrageenans are soluble in cold water too. They can be used in concentrations varying between 0.005%-3% (McHugh 1987).

The chemical reactivity of the carrageenans is due to their half ester sulfate groups that are strongly anionic. The functional properties of carrageenan, to a large extent, depend on their rheological properties. They form highly viscous aqueous solutions due to their unbranched, linear macromolecular structure and polyelectrolyte nature. The highly extended structure of this molecule is due to the mutual repulsion of the negatively charged half ester sulfate groups along the chain of the polymer but due to its hydrophilic nature, it is surrounded by a sheath of immobilized water molecules. Both these properties contribute to the resistance to flow (McHugh 1987).

Temperature and pH play a very important role in determining the stability of carrageenans. There might be loss of functionality at high temperature and low pH (McHugh 1987).

Carrageenan has been chosen for this study because it is a cheaply available anionic polymer, a property that makes it ideal for studying copper binding. Amongst the available carrageenans, lambda-carrageenan has been further chosen due to the

availability of more sulfate groups per monomeric unit. Kappa-carrageenan contains only one sulfate group while and iota-carrageenan consists of two sulfate groups per monomer. and lambda-carrageenan consists of three sulfate groups per monomer. Hence, the greater the number of sulfate groups, the more are the opportunities for copper ion binding. However, there are more factors that favored lambda-carrageenan over the other two carrageenans with respect to the solubility profile of lambda-carrageenan. It is also a non-gelling polymer and hence, more favorable in the kind of application sought in the project. Some of the properties of the three carrageenans are:

Solubility	λ-Carrageenan	κ-Carrageenan	i-Carrageenan
Hot water	Soluble	Soluble at 70°C	Soluble at 70°C
Cold water	Soluble	Na salts soluble	Na salts soluble
Gelification			
In water			
i) Cation effects	No gel	With K	With Ca
ii) Texture	No gel	Strong & brittle	Elastic
iii) Freeze/unfreeze	Stable	Unstable	Stable

(<http://www.cpkelco.com/carrageenan/solubility.html>)

Lambda-carrageenan was a gift from FMC Biopolymer, Philadelphia. It is a free-flowing powder, forming non-gelling, pseudoplastic solution in water. It is in the weight range of 100-500 KDa. It is compatible with water-miscible solvents and insoluble in organic solvents. It has approximatey 35% ester sulfate and very little or no anhydro-galactose.

(<http://www.fmcbiopolymer.com/Ingredients/Carrageenan/Introduction/tabid/2391/Default.aspx>)

2.6 POLYMER ENHANCED DIAFILTRATION (PEDF):

Filtration is a separation process across a membrane which uses pressure to separate components in an aqueous solution on the basis of differences in size and charge. There are two modes of operation – Normal Flow Filtration and Tangential Flow Filtration.

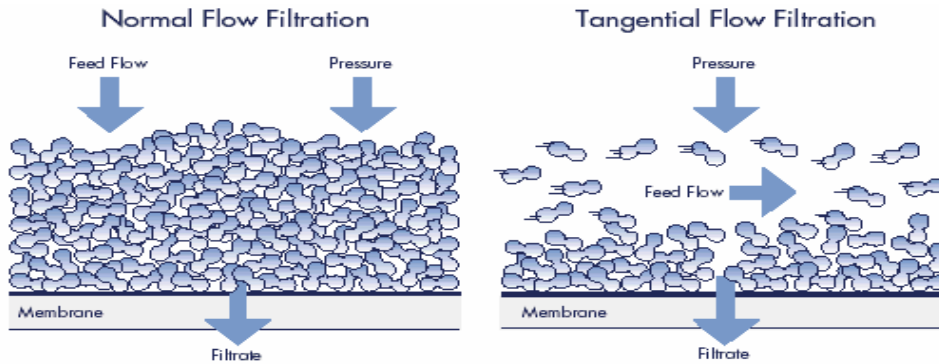


Fig. 1. Representation of normal flow filtration and tangential flow filtration (Millipore 2003)

In normal flow filtration, fluid flow occurs normal to the membrane under pressure. Molecules that are larger than the pores of the membrane tend to accumulate on the membrane surface whereas smaller molecules pass through. This type of filtration is also called dead-end filtration.

In tangential flow filtration, fluid flow occurs tangential to the surface of the membrane under pressure. This applied pressure enables a portion of the fluid to move across the membrane to the filtrate side. The molecules that are larger than the pores of the membrane are retained and smaller molecules pass through. As opposed to the normal flow filtration, the retained molecules do not accumulate on the surface of the membrane, rather they move along with the tangential flow of the fluid. It is also called cross-flow filtration (Iverson 2003).

Microfiltration and ultrafiltration are the most widely employed forms of tangential flow filtration. The membrane pore size cutoffs for microfiltration are in the range of 0.05-1 μm whereas for ultrafiltration, it is 1-1000 KDa. Reverse osmosis and nanofiltration are

the other types of TFF that have very tight membranes of molecular weight cutoffs of 1 KDa and lower. Diafiltration is a TFF process that uses any of these separation processes to enhance product yield or quality (Millipore 2003). Polymer Enhanced Diafiltration is a combination of diafiltration and biosorption.

PEDF also involves binding of the metal ions to the polymer and retention of the metal-polymer complex on the membrane. Pore sizes of the membrane are so chosen such that polymer cannot pass through the membrane whereas any unbound metal ion can pass through. Hence, when a metal ion binds to the polymer, a macromolecular complex is formed which is retained by the membrane (Iverson 2003).

2.6.1 : Working of PEDF unit :

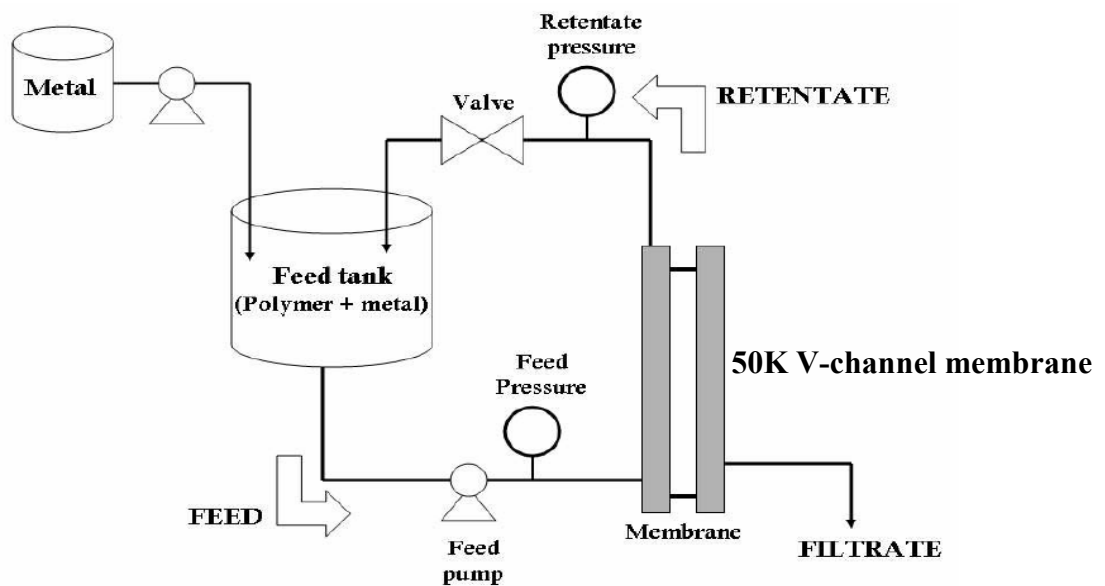


Fig 2: Diagrammatic representation of general working of PEDF system

(Modified from MS thesis by Ameesha R. Shetty, 2006)

The desired metal is placed in the metal reservoir which is pumped into the feed tank containing the polymer. The rate at which the metal is added to the feed tank is the same as the rate at which the filtrate is collected. Hence, the volume in the feed tank is always kept constant. The metal-polymer complex which is formed is retained and it is returned to the feed tank as retentate and any unbound metal goes out in the filtrate. There are two

pumps attached to the system, one which controls the metal addition to the feed tank and the other which controls the metal-polymer circulation in the system. There are two pressure gauges attached, one which shows the feed pressure and the other shows the retentate pressure. A valve controls both the pressure and amount of retentate. A 50K V-channel ultrafiltration membrane is attached which allows small molecules such as water and copper ions to pass through but retains the much higher molecular weight lambda-carrageenan and any metal ion-polymer complex formed.

2.6.2. Key Process Parameters:

Transmembrane Pressure is a key process parameter in PEDF. It is the pressure difference across the membrane generated due to the flow which is the driving force of the separation process. Hence, TMP can be defined as the average of the pressure from the feed side and retentate side of the membrane, subtracting any pressure to the filtrate side. Hence,

$$\text{TMP} = \frac{P_F + P_R}{2} - P_f$$

Where P_F = Feed Pressure

P_R = Retentate Pressure

P_f = Filtrate Pressure

(Iverson 2003)

Permeate flux is an important parameter defined as the permeate flow rate that has been normalized for the area of the membrane. It is denoted as :

Permeate Flux = Permeate Flow rate/Membrane area

The greater the pressure difference, the greater will be the permeate flux. A very high permeate flux is undesirable as it may lead to deformation of the polymer chains, enabling them to pass through the pores of the membrane. Hence, it is important to choose an optimum pressure for efficient retention (Iverson 2003).

Crossflow rate is another parameter. It is the flow rate leading to an increase in the sweeping action across the membrane and reducing the concentration gradient towards the membrane surface. Normally, a higher crossflow rate gives higher flux at equal TMP. A higher crossflow rate causes the product to pass through the pump more times in a given time period. This may lead to degradation of the product quality. Hence, a crossflow rate should be chosen so that a balance is maintained between the increase in flux, the number of pump passes and holdup volume (Iverson 2003).

Concentration polarization is another important parameter that needs to be considered. It can be defined as the retention of solute particles on the surface of the membrane. The phenomenon arises when transport of materials towards the membrane is greater than the movement back to the feed reservoir. The layer of solute formed on the membrane surface leads to increase in osmotic pressure and decrease in permeate flux. Increased feed flow rates lead to increase in permeate flux and hence, prevent membrane fouling and limit the effect of concentration polarization at the membrane due to increase in back-diffusion of retained particles to the feed reservoir (Millipore 2003).

Polymers have differing molecular weight distribution, referred to as average molecular weights. Hence, polymer solutions might have low molecular weight fractions which might leak through the membrane. Hence, it is common to perform a diafiltration run before addition of metal, that is by adding only water to the polymer as diafiltration proceeds. This enables the washing out of any low molecular weight polymer fractions. All the available ultrafiltration membranes have a Molecular Weight Cut-Off (MWCO) limit. This corresponds to a minimum molecular mass of the solute that can be retained by the membrane (Shetty 2006).

2.6.3. Choice Of Polymer :

Choice of the polymer is an important factor in a PEDF process. The polymer chosen for the run should possess the following properties:

- 1 Good affinity for the metal under study
- 2 Have an oppositely charged functional group for binding the metal
- 3 High molecular weight
- 4 Stable
- 5 Low toxicity
- 6 Cheap (Vieira & Volesky 2000)
- 7 Possibility of regeneration

(Gupta 2000)

The polymer chosen should have a high molecular weight so that it can be retained by the membrane on the basis of the molecular weight cut-off of the membrane.

However, a very high molecular weight of the polymer might lead to an increase in viscosity, thereby increasing concentration polarization and reducing permeate flux. A polymer should be stable when in solution as there are chances of the polymer undergoing degradation due to mechanical shearing by the pump (Gupta 2000).

2.7 RHEOLOGICAL STUDIES :

Rheology can be defined as study of deformation and flow of matter under the influence of a shear stress. It represents a relation between the flow behavior of a material and its internal structure. A branch of rheology that shows the flow or deformations due to a simple shear stress field is called as shear rheometry. Shear stress (τ) is a state in which stress is parallel or tangential to the face of the material and shear rate ($\dot{\gamma}$) is the measure of rate of shear deformation. In a Newtonian fluid, it can be related to shear stress as $\tau = \mu \cdot \dot{\gamma}$, where μ is the viscosity of the fluid. Fluids such as water, ethanol, air and benzene are Newtonian. All the low molecular weight liquids and solutions of low molecular weight substances in liquids are Newtonian. On plotting a graph between shear

stress and shear rate at a given temperature, a straight line with a constant slope is obtained. This slope is the viscosity of the fluid. For defining a non-Newtonian fluid, there are other ways of explaining it as it cannot be described by a single constant viscosity. In this, viscosity changes on application of shear stress. Many high molecular weight polymer solutions are non-Newtonian. In this, on plotting a graph between shear stress and shear rate, a straight line is not obtained. The fluid is said to be shear-thinning when viscosity decreases with increase in shear rate and it is said to be shear-thickening when viscosity increases with increase in shear rate (<http://en.wikipedia.org/wiki/Rheology>). The rheological behavior of lambda-carrageenan was studied on addition of different concentrations of copper.

2.7.1 Rheological properties of Carrageenan :

Viscosity of carrageenan depends on concentration, temperature, presence of other solutes, type of carrageenan and its molecular weight. Viscosity increases exponentially with increase in concentration due to increased interaction between polymer chains. With increase in salts, viscosity undergoes a reduction due to decreased repulsion among the sulfate groups. However, at low temperatures and high salt concentrations, there might be an increase in apparent viscosity. Viscosity decreases with increase in temperature. There is an increase in viscosity with increase in molecular weight, according to the Mark-Houwink equation:

$$[\eta] = KM^\alpha$$

Where $[\eta]$ = Intrinsic viscosity

M = Molecular weight of carrageenan

K and α = constants

Carrageenan solutions having viscosities less than 100centipoise have flow properties very close to Newtonian. However, at higher viscosities, they tend to exhibit shear-thinning behavior (McHugh 1987).

The viscosity measurements are usually carried out at higher temperatures ($\sim 75^\circ\text{C}$) for gelling type of carrageenans to avoid the effects of gelation. However, viscosity measurements for non-gelling carrageenans can be carried out at room temperature ($\sim 25^\circ\text{C}$) (<http://www.fao.org/docrep/field/003/AB730E/AB730E03.htm>).

MATERIALS AND METHODS

1. METAL BINDING STUDIES USING CARRAGEENAN:

The protocols were obtained from the works of Mark. S.S., 2006 and Shetty. A.R., 2006. Lambda-carrageenan was used as the biosorbent to study the adsorption of copper.

1.1 Preparation Of Lambda-Carrageenan Solution:

Lambda-carrageenan, a gift from FMC Biopolymer, Philadelphia was used in this study. It is readily soluble in water and does not form a gel in water. Solutions of 4 g/L and 8 g/L lambda-carrageenan were prepared by dissolving powdered carrageenan in water. pH adjustments with 1M HCl were made depending on the experiment.

1.2 Preparation Of Copper Solution :

Analytical grade $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (Sigma) was used to make all the solutions. A stock solution of 1000 mg/L was prepared by dissolving it in water. The stock solution was diluted to make working standards. The pH was adjusted using 1M HCl and 1M NaOH.

1.3 Spectrophotometric determination of Copper :

Copper concentration was determined using the Bathocuproine method from Standard Methods for Examination of Water and Wastewater (Eaton 1995, Viviana 2002). Copper samples were diluted with water to 5 ml total volume. 0.1ml of 1:1 hydrochloric acid was added to release copper at pH values lower than 1. 0.5 ml of 30% sodium citrate was added to neutralize the solution to pH 7.5. 0.5 ml of 10% hydroxylamine was added to reduce the copper which could then be chelated by addition of 0.5 ml of 0.1% Bathocuproine disulfonate to give an orange-colored complex which was then detected at 484nm in a Perkin Elmer UV/VIS spectrophotometer (Viviana 2002) . All the glassware used was rinsed properly with 6N HCl to leach out any trace metals that might be present and then thoroughly rinsed with deionized water. A standard curve of copper was

determined by plotting 6 different concentrations (namely 2, 4, 6, 8, 10, 20 μg of copper) of copper and performing the standard assay of copper as described below and determining the OD at 484nm. A blank was also assayed containing no copper.

1.4 Copper Binding To Lambda-Carrageenan Using Equilibrium Dialysis :

1.4.1. Construction of Equilibrium Dialysis Apparatus:

The equilibrium dialysis method was the same one described in the study of Mark.S., Crusberg.T.C., Dilorio. A.A., 2006. The dialysis apparatus is made up of polycarbonate. It contains 2 identical units joined together with screws. A semi-permeable regenerated cellulose membrane (Spectra/Por dialysis pre-cut discs obtained from Spectrum labs. MW cut off 12-14KDa) was placed to give rise to 8 chambers on both the sides of the apparatus. Rubber O-rings were used around each chamber to hold the membrane in place. Before putting in the O-rings, they were leached in 1M HCl for 8 hrs to remove any trace metals and then, rinsed in distilled water for 8 hours and then, air dried. The apparatus (depicted below) was further sealed with Parafilm to prevent any evaporation during the experiment.

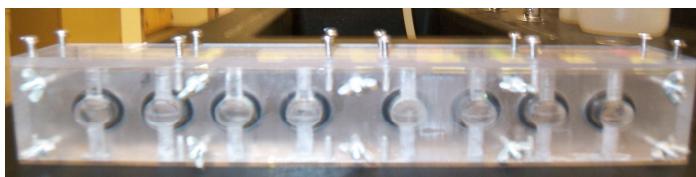


Fig 3 : Equilibrium Dialysis apparatus

The dialysis membranes were also washed before setting up the system with the following protocol. The membranes were cut to the desired size and soaked in a mixture of 0.01M sodium bicarbonate and 0.001M disodium-EDTA at 37° C for about an hour. The solution was then decanted and more was added and membranes were soaked further for another 30 min at 37° C, It was again decanted and membranes were then rinsed with deionized water. Three washes with distilled water

for 30 min at 37° C were then carried out. The membranes could then be stored in the refrigerator until use.

1.4.2. Time for establishment of equilibrium for Copper-lambda-Carrageenan across the membrane in dialysis apparatus:

Four g/L of a lambda-carrageenan solution was prepared by dissolving lambda-carrageenan in deionized water at pH values of 2, 3, 4. Copper solutions were prepared using stock solution of 1000 mg/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ diluting it to 320 mg/L. The pH was adjusted to 2, 3, 4 using 1M HCl and 1M NaOH. Two ml of 4 g/L lambda-carrageenan was added on one side of the dialysis apparatus and 2 ml of 320 mg/L of copper solution was added to the other side. 0.4 M KCl (1 ml of 2 M KCl added to total of 5 ml of copper solution to give 0.4 M KCl) was also added to the metal side of the apparatus to prevent non-specific metal ion binding to the membrane. The entire dialysis apparatus was kept in a shaker incubator at 250 rpm at 25° C. Samples were taken after every 2 hrs from the feed cell (metal containing cell) and estimated for copper using the Bathocuproine assay. This was continued until equilibrium was reached. A control experiment was also set up along with the actual experiment in which distilled water was added to the recovery cell (polymer side of the apparatus) instead of lambda-carrageenan. This experiment was performed at all the three pH values 2, 3, 4.

1.4.3. Determination of Optimum pH for uptake using equilibrium dialysis:

The effect of pH on copper uptake was studied at pH values of 2, 3, 4. Four g/L of lambda-carrageenan was prepared in deionized water at pH values of 2, 3, 4. A 320 mg/L solution of copper was prepared and its pH was adjusted to 2, 3, 4 using 1M HCl and 1M NaOH. Two ml of a lambda-carrageenan solution was added on one side of the apparatus and 2 ml copper solution along with 0.4 M KCl (1 ml of 2 M KCl added to total of 5 ml of copper solution to give 0.4 M KCl) was added on the opposite side. The same pH was

maintained on the polymer and metal side of the apparatus. Initial metal conc. was determined by means of the Bathocuproine assay. The dialysis apparatus was kept in the shaker incubator at 250 rpm at 25° C. In the kinetic studies, it was established that it takes ~48hrs for equilibrium to be reached. Hence, samples were taken after 48 hrs and estimated for residual copper using the Bathocuproine assay. Controls were also set by adding only water to the polymer side of the apparatus.

1.4.4. Study Of Equilibrium Isotherm and Determination Of Optimum Temperature For Uptake Using Equilibrium Dialysis:

Four g/L solution of lambda-carrageenan at optimum pH 3 was prepared and 2 ml was added to one side of the dialysis apparatus. Two ml of varying concentrations of copper i.e. 100,120,160, 200,240,280,320 mg/L at pH 3 was added to the other side of the dialysis apparatus. The apparatus was kept in the shaker incubator at 25° C and 37° C at 250rpm for 48 hrs. A volume of solution that would theoretically give 10µg copper (in the complete absence of binding by polymer) was withdrawn from each feed cell and the concentration of free copper was determined by the Bathocuproine assay. The copper bound to lambda-carrageenan was determined from the initial and final copper concentrations corrected for any copper bound to the membrane or elsewhere in the system. An equilibrium adsorption isotherm graph was plotted taking the equilibrium concentration of copper (in mg/L) and copper bound in mg/g of the polymer. This was used to determine the maximum copper bound (q_{max}) and the dissociation constant (K_D) by studying the reciprocal plot of equilibrium isotherm.

1.5 Polymer Enhanced Diafiltration System (PEDF) :

1.5.1. Elimination of low molecular weight fractions :

Low molecular weight fractions of polymer were eliminated from the bulk polymer solution by running a PEDF with polymer but no metal for 30 min. Instead of metal, water was added to the feed tank containing the polymer. After 30 min of wash, metal addition was begun. This was noted earlier by Mark (2006). Permeate samples were

collected and residual copper determined spectrophotometrically. It was compared with permeate samples without this wash.

1.5.2. Qualitative Carrageenan Assay Using Chitosan :

Carrageenan which may have been in the permeate was determined using a light scattering method. In the presence of a cationic polymer, negatively charged carrageenan will bind and cause the neutral polymer complex to precipitate. In this assay, 0.1 ml-1 ml of 0.04% lambda-carrageenan were prepared and mixed with 1 ml of 0.04% chitosan. The total volume was adjusted to 5 ml with water and OD values were recorded at 600 nm. However, this served as the basis for the qualitative or presence/absence test for carrageenan. In the actual PEDF, 1 ml permeate sample was mixed with 1 ml of 0.04% chitosan and the volume made until 5 ml and absorbance was measured at 600 nm. The absorbance obtained was used to determine the concentration of any lambda-carrageenan polymer leaking out of the membrane before the addition of copper into the feed tank and after copper started appearing in the permeate.

1.5.3. PEDF Set up:

Three hundred ml of 4 g/L and 8 g/L lambda-carrageenan solutions were placed in the feed tank respectively. Ten mg/L, 20 mg/L and 50 mg/L of copper solution respectively was added from the metal reservoir to the feed tank, at a rate equal to the permeate flow rate. A pump (Millipore pump with Cole Parmer pump head) was used to feed the metal into the feed tank. Another pump (Millipore pump with Cole Parmer pump head) was used to feed the metal-polymer complex to the UF membrane. The membrane used was Pellicon 2 Mini ultrafiltration Module Biomax – 50 V 0.1m² cassette and tubing used was Masterflex Pharmed 6485-15 manufactured by Norton. Pressure gauges were fitted at the inlet and outlet to the UF membrane indicating feed pressure and retentate pressure respectively. A hand valve was also fitted to control the feed and retentate pressures. The cross-flow rate was adjusted to about 120 ml/min and permeate flow rate was then 20-22 ml/min.

1.5.4. PEDF operation :

The volume of polymer-metal complex was maintained constant throughout the run. In order to do so, the inflow rate of the metal-containing solution into the feed tank was manually adjusted such that it was equal to the permeate outflow rate. The feed and retentate pressures were adjusted to give average pressures between 7-8 psi. The hand valve was used to maintain the pressure throughout the run. 1ml samples of permeate were collected after regular time intervals and assayed for presence of copper by the Bathocuproine assay. The permeate flow rate was also determined at regular time intervals. The entire PEDF run was carried out at room temperature (~ 25°C). A control PEDF was also run in the absence of lambda-carrageenan to determine non-specific copper binding to the membrane or anywhere else in the system. Water was used instead of polymer in this run.

2. RHEOLOGICAL STUDIES FOR CHARACTERIZING CARRAGEENAN-COPPER COMPLEX :

Rheological studies were carried out to study the effect of different concentrations of copper on the viscosity of lambda-carrageenan on application of varying degrees of shear stress. A Bohlin Rheometer DSR-F was used for the study. Stress viscometry in the software menu was selected to study the change in viscosity with increase in shear stress. 4 g/L, 8 g/L and 12 g/L solutions of lambda-carrageenan containing 10 mM, 20 mM and 30 mM copper at pH 3 were used in the study (1mM copper ion corresponds to 63mg/L copper ions). About 2 ml of this solution was placed on the lower fixed plate of the rheometer and the upper movable plate was lowered after unlocking the shaft. The change in viscosity was recorded for varying shear stress and a graph was plotted between viscosity and shear stress. A control was also run using only lambda-carrageenan.

RESULTS:

1. DETERMINING COPPER BINDING BY IAMBDA-CARRAGEENAN USING EQUILIBRIUM DIALYSIS:

1.1 Standard curve for copper :

Fig 4 shows the standard curve of copper taking 6 different concentrations of copper (namely 2, 4, 6, 8, 10, 20 µg) and plotting the graph against OD at 484nm after the Bathocuproine assay for copper determination.

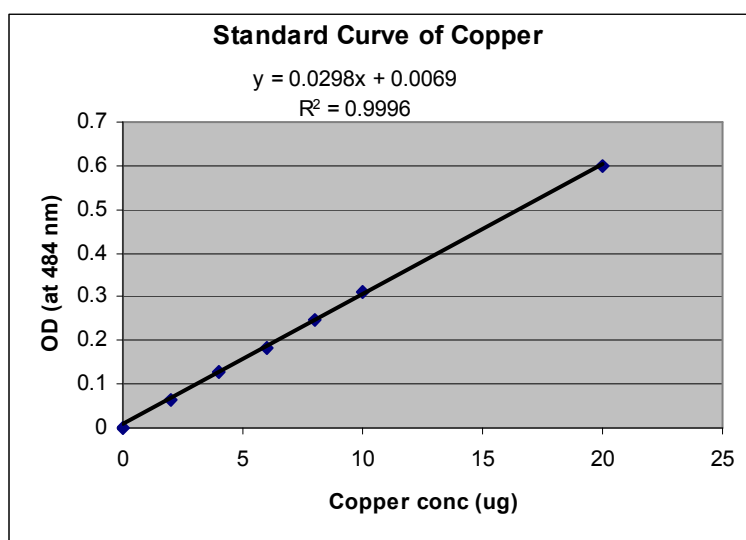


Fig 4 : Standard curve of copper

A straight line was obtained on plotting the graph. A standard curve for copper was plotted each time an experiment was performed.

1.2 Adsorption Isotherm Models For Determining Biosorption :

Respective samples were taken from the feed cell of the equilibrium dialysis apparatus into which metal containing solution was added and analyzed for the presence of any residual copper. The following equation was used to determine the change in concentration in the feed cell :

$$-(dC_1/dt) = (K_f A/V_1) (C_1 - C_2) \quad (1)$$

Where C_1, C_2 = Concentrations of metal ions in feed and recovery cells respectively

K_f = Overall mass transfer coefficient

A = Surface area

V_1 = Volume in the feed cell

In the control, where water was added in place of the polymer, equation 1 can be solved as follows:

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

Where C_0 = Initial concentration of metal ions in the feed cell

In the presence of the polymer in the recovery cell, following equation was used :

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

Where m = Mass of the polymer present in the recovery cell

q = Uptake of copper by the polymer in mg/g of polymer

In equation 3, it is assumed that C_2 is in equilibrium with the polymer-metal complex.

Solving for the Langmuir adsorption model, the binding can be depicted as :

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

Where q_{\max} = Maximum binding of the metal to the polymer

K_A = Equilibrium association constant (L/mg)

C = Equilibrium metal concentration (mg/L)

1.3 Time taken for the establishment of equilibrium for Copper-lambda-Carrageenan across the membrane in dialysis apparatus :

Fig 5 shows the decrease in copper concentration in the feed cell of the equilibrium dialysis apparatus over a period of 48 hrs at pH 2. In the absence of lambda-carrageenan, starting with an initial copper concentration of 328.48 mg/L, an equilibrium concentration of 160.39 mg/L was obtained after 48 hours. In the presence of 4 g/L lambda-carrageenan, starting with an initial copper concentration of 328.48 mg/L, an equilibrium concentration of 143.8 mg/L was obtained after 48 hours.

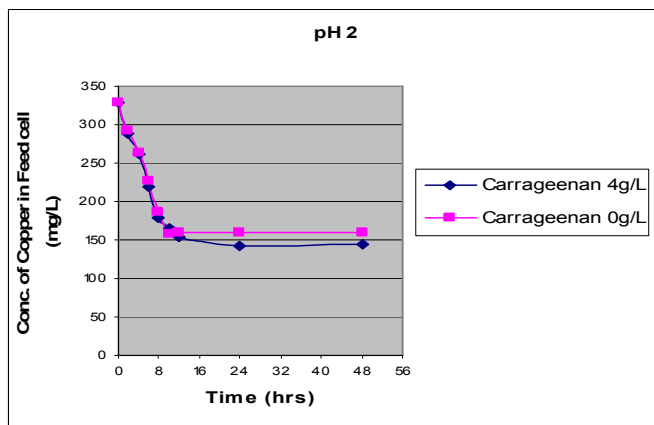


Figure 5 : Diffusion of copper across the membrane and uptake by lambda-Carrageenan at pH 2

Fig 6 shows the decrease in copper concentration in the feed cell of the equilibrium dialysis apparatus over a period of 48 hrs at pH 3. In the absence of lambda-carrageenan, starting with an initial copper concentration of 338.99 mg/L, an equilibrium concentration of 165.08 mg/L was obtained after 48 hours. In the presence of 4 g/L lambda-carrageenan, starting with an initial copper concentration of 338.99 mg/L, an equilibrium concentration of 128.39 mg/L was obtained after 48 hours.

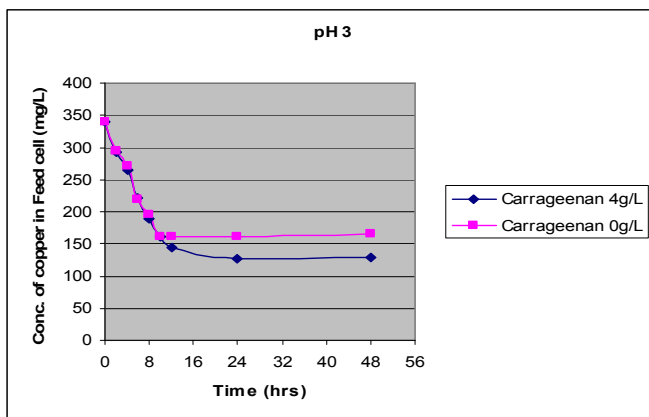


Figure 6: Diffusion of copper across the membrane and uptake by lambda-Carrageenan at pH 3

Fig 7 shows the decrease in copper concentration in the feed cell of the equilibrium dialysis apparatus over a period of 48 hrs at pH 4. In the absence of lambda-carrageenan, starting with an initial copper concentration of 338.23 mg/L, an equilibrium concentration of 165.9 mg/L was obtained after 48 hours. In the presence of 4 g/L

lambda-carrageenan, starting with an initial concentration of 338.23 mg/L, an equilibrium concentration of 132.58 mg/L was obtained after 48 hours.

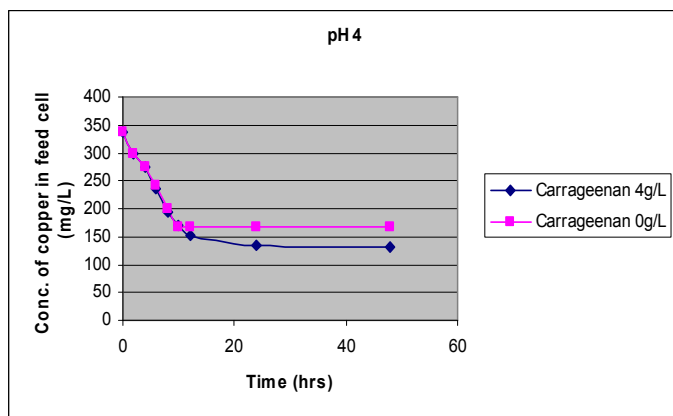


Figure 7: Diffusion of copper across the membrane and uptake by lambda-Carrageenan at pH 4

Hence, from Figs. 5, 6, 7, it becomes quite evident that lambda-carrageenan is an adsorbent of copper, as can be seen with a decrease in free concentration of copper in the presence of lambda-carrageenan. The binding between copper ions and lambda-carrageenan can occur in about a millisecond, i.e. as soon as the metal ion comes in contact with the polymer. This indicates the reaction time of binding between the metal ion and the polymer. However, these incubation times, after regular intervals, refer to the time taken by copper ion to diffuse through the membrane and then, bind to the polymer and eventually, attain equilibrium. Therefore, for the equilibrium to be reached, an incubation time of 24-48 hours was needed. On the basis of data obtained by sorption studies, an equilibration time of 48hours was taken for all further equilibrium dialysis studies.

1.4 Effect Of pH On Copper Uptake :

Table 1 shows the effect of pH on the uptake of copper by lambda-carrageenan. The uptake of copper was determined as mg of copper/g of the lambda-carrageenan at pH 2, 3 and 4. Higher pH values were not considered as copper tends to precipitate as $\text{Cu}(\text{OH})_2$ at higher pH values and the precipitate tends to block the pores of the membrane (Kopecky

2005). Although carrageenan at pH values of 3 and 4 was found to have similar adsorption characteristics, yet pH 3 was considered in all further studies due to the better adsorption of copper to the polymer observed in static experiments.

Table 1 : Effect of pH on adsorption of copper by lambda-carrageenan. 4 g/L of lambda-carrageenan was used for the study and 320 mg/L of copper adjusted to pH 2,3,4 was used in the feed cell respectively.

pH	Uptake (mg/g)
2	3.8
3	12.8
4	9.8

1.5 Equilibrium Adsorption Isotherm :

An equilibrium adsorption isotherm is a plot of copper uptake in mg/g of lambda-carrageenan vs. concentration of free copper in solution. From kinetics experiments, an equilibrium adsorption experiment was carried out for 48 hrs at pH 3. Fig 8 shows an equilibrium isotherm for lambda-carrageenan at pH 3 at 25°C. In an ideal case, uptake increases with increase in free copper ions, until the time all the binding sites on the polymer (in this case, lambda-carrageenan) are completely saturated with the copper ions and no more adsorption is possible. This represents q_{max} or the theoretical maximum amount of copper bound to the biosorbent. Since q_{max} could not be experimentally determined, a reciprocal plot was used to determine q_{max} and K_D . The reciprocal plot was linear and hence, estimation of these two parameters was simpler and more reliable. Fig 9 was used to determine the parameters q_{max} and K_D . They could be determined from the following equation:

$$1/q = [1/(q_{max}K_D C)] + 1/q_{max} \quad (5)$$

The slope and intercept gave q_{max} and K_D respectively using equation 5. The predicted line in Fig. 9 and Fig. 11 respectively was determined by MS Excel and the equation of the line was also obtained from it. From Fig 8 and Fig 9, q_{max} at 25°C was found to be 39.9 mg of copper / g of lambda carrageenan and K_D was found to be 28.01 mM.

Similar experiment was carried out at 37°C to determine the effect of temperature on q_{\max} and K_D as shown in Fig 10 and Fig 11. q_{\max} was found to be 36 mg of copper/g of lambda-carrageenan and K_D was found to be 21.3 mM. There was not a significant difference in both the results. Hence, 25°C or the room temperature was considered in all subsequent experiments.

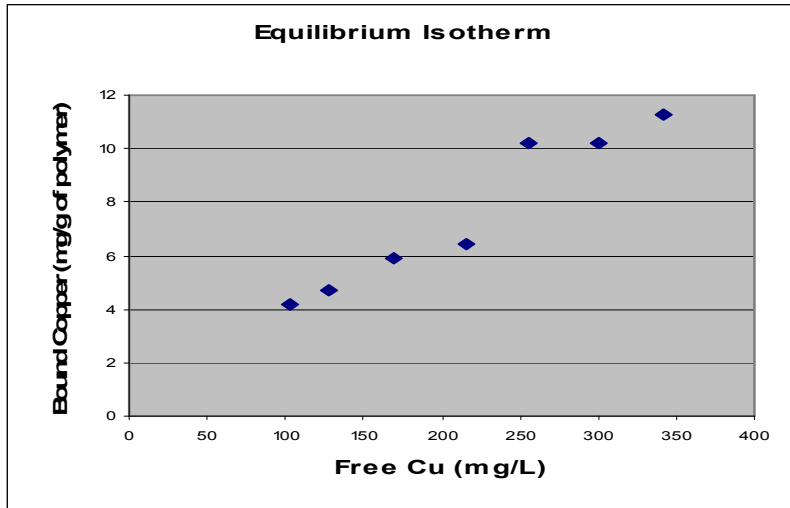


Fig 8 : Equilibrium adsorption isotherm of copper by lambda-carrageenan at pH 3 at 25°C. Each of the readings are an average of three OD measurements.

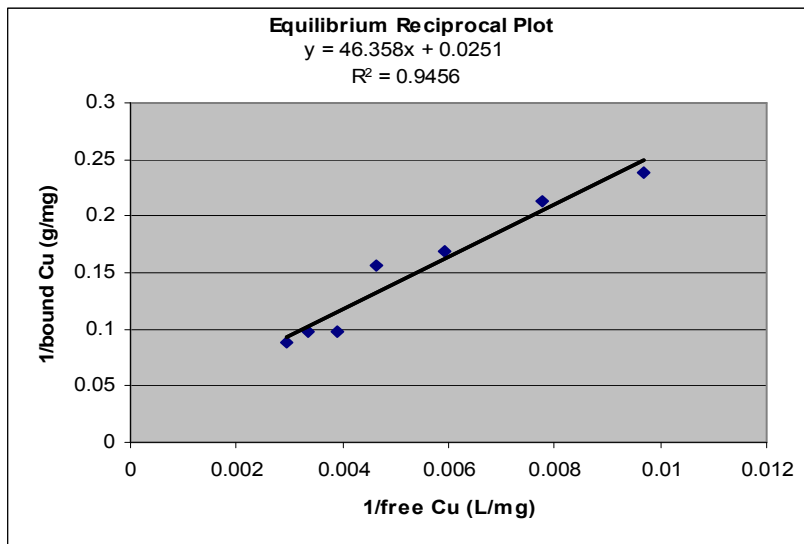


Fig 9 : This represents the reciprocal of equilibrium adsorption isotherm plot (reciprocal of Fig 8). The slope determines K_D and intercept gives q_{\max} .

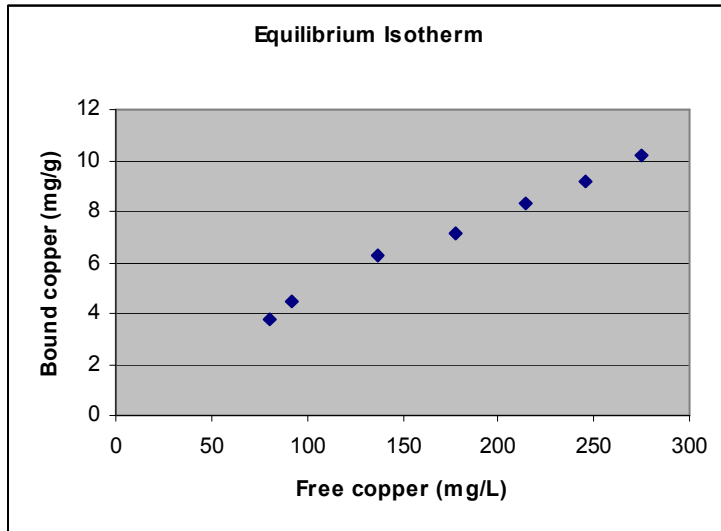


Fig 10 : Equilibrium adsorption isotherm of copper by lambda-carrageenan at pH 3 at 37°C. Each of the readings are an average of three OD measurements.

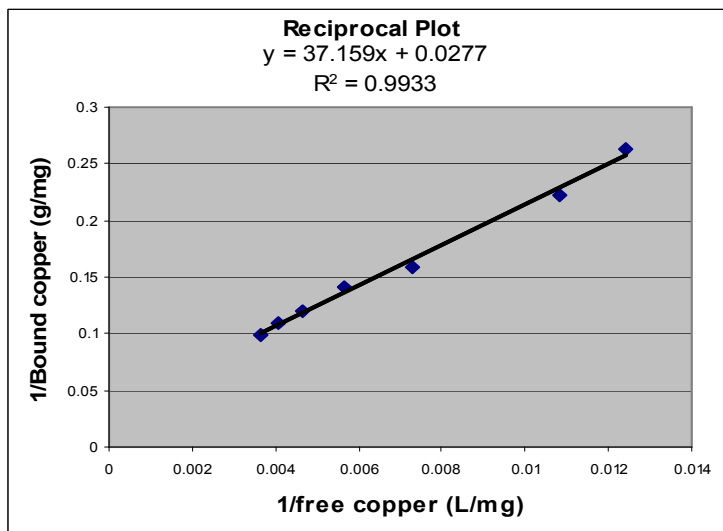


Fig 11 : This represents the reciprocal of equilibrium adsorption isotherm plot (reciprocal of Fig 7). The slope determines K_D and intercept gives q_{max} .

2. DETERMINING BINDING CHARACTERISTICS OF COPPER IN A POLYMER ENHANCED DIAFILTRATION SYSTEM

2.1 Control Polymer-Enhanced Diafiltration With No lambda-Carrageenan:

A control experiment was carried out in the absence of lambda-carrageenan in the feed tank using a feed copper concentration of 10 mg/L. Three hundred ml of deionized water was placed in the feed tank instead of lambda carrageenan. Table 2 and Fig. 12 shows the copper appearing in the permeate when no polymer was used. This is a plot of concentration of copper in the permeate versus the volume of permeate collected. One liter was passed through the system. Starting with an initial copper concentration of 10 mg/L, 9.86 mg/L of copper passed into the permeate, showing that there was almost no copper ion binding to the membrane or anywhere else in the system.

Table 2 : PEDF measurements of volume of permeate collected and concentration of copper in the permeate when no polymer was used.

Volume of permeate collected (ml)	Concentration of copper in the permeate (mg/L)
0	0
100	0.20
200	2.58
300	4.58
400	5.66
500	6.96
600	7.99
700	9.08
800	9.68
900	9.81
1000	9.86

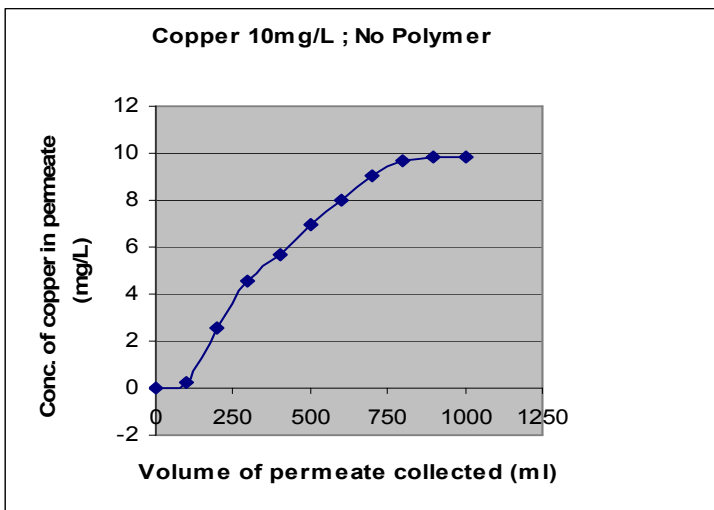


Figure 12 : Control permeation curve in the absence of lambda-carrageenan with a feed copper concentration of 10mg/L. This determines non-specific binding of copper to the membrane or anywhere else in the PEDF system.

2.2 Buffer Diafiltration To Remove Low Molecular Weight Fractions Of lambda-Carrageenan :

Buffer diafiltration was carried out to rule out any possibility of leakage of the low molecular weight fractions of the polymer. Water from the metal reservoir was added to the desired concentration of the polymer in the feed tank and PEDF run was carried out for 30-45 min. Any low molecular weight fractions were removed from the polymer mix, after which the desired concentration of metal was pumped in.

2.3 Qualitative Carrageenan Assay Using Chitosan:

A qualitative assay was performed to determine if any carrageenan was passing in to the permeate. In order to do that, a positively charged polymer, chitosan, was taken. When it was made to react with different concentrations of lambda-carrageenan, different intensities of white turbidity could be seen which was spectrophotometrically measured at 600nm. Table 3 shows the concentrations and volume of lambda carrageenan and chitosan giving those absorbances. By means of this qualitative assay, lambda-carrageenan as low as 0.008 mg/ml could be determined by measuring the turbidity. Before the start of the experiment, a solution of lambda-carrageenan was run in PEDF mode for about 30-45 min to get rid of any lower molecular weight fractions. Before

addition of any copper to the feed tank containing lambda-carrageenan, a qualitative assay with chitosan was performed to check for the leakage. It was also performed after copper started coming out in the permeate. No turbidity was ever seen with any permeate samples indicating the lack of low molecular weight components in the preparation.

Table 3 : Assay for lambda-carrageenan using chitosan. Permeate samples were collected from all the PEDF runs and no leakage of the polymer was observed.

0.04% Lambda Carrageenan (ml)	0.04% Chitosan (ml)	Water(ml)	OD (600nm)	Conc. Of Lambda-Carrageenan (mg/ml)
0.1	1	3.9	0.004	0.008
0.2	1	3.8	0.013	0.016
0.3	1	3.7	0.02	0.024
0.4	1	3.6	0.024	0.032
0.5	1	3.5	0.028	0.04
0.6	1	3.4	0.035	0.048
0.7	1	3.3	0.043	0.056
0.8	1	3.2	0.052	0.064
0.9	1	3.1	0.062	0.072
1.0	1	3	0.081	0.08
Permeate (1ml)	1	3	~ 0.000	~ No polymer in the permeate

2.4 Polymer Enhanced Diafiltration With 4g/L lambda- Carrageenan :

PEDF was run with lambda-carrageenan concentration as 4 g/L and copper concentrations 10 mg/L, 20 mg/L and 50 mg/L. The feed volume was 300 ml and the pH of the polymer in the feed tank and copper in the metal reservoir was 3. The pH in the feed tank was maintained by regular additions of 1 M NaOH. The transmembrane pressure was manually kept constant at 7.5 psi. Copper concentrations were determined

after every 30 minutes. Table 4 and Fig. 13 represent PEDF when initial concentrations of 4 g/L of lambda-Carrageenan and 10 mg/L copper were used.

Table 4 : PEDF measurements of volume of permeate collected, concentration of copper in the permeate, transmembrane pressure and pH when 4 g/L lambda-carrageenan and 10 mg/L copper was used. Permeate samples were collected after 30 minutes

Volume of permeate collected (ml)	Concentration of copper in the permeate (mg/L)	Transmebrane pressure (psi)	pH
0	0	7.5	3
700	0	7.5	3.01
1200	0	7.5	2.98
1800	0	7.5	2.99
2400	0	7.75	3.02
3000	0	7.5	3
3600	0	7.75	3.01
4250	0	8	2.96
4900	0	7.5	2.99
5700	0.17	7.5	3.01
6300	0.58	7.5	3
6900	0.87	7.5	3
7600	1.77	7.5	3.01
8400	2.73	7.75	3.01
8950	3.85	7.5	3.01

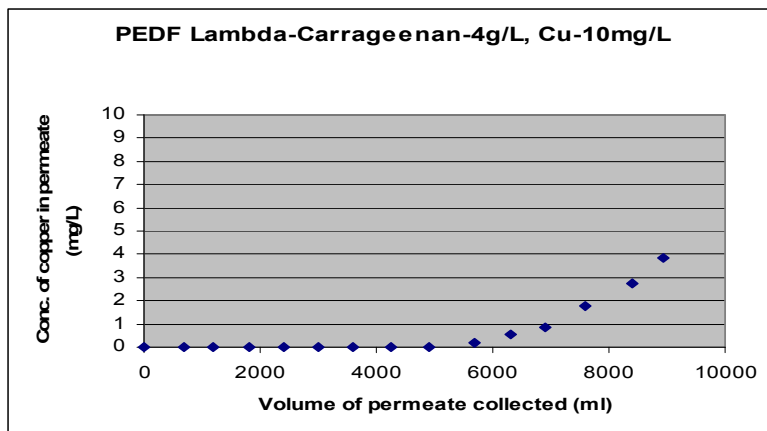


Figure 13 : Permeation curve of copper in Polymer Enhanced Diafiltration when 4 g/L lambda-Carrageenan and 10 mg/L copper were used.

Table 5 and Fig 14 represent PEDF when 4 g/L of lambda-Carrageenan and 20 mg/L copper were taken.

Table 5 : PEDF measurements of volume of permeate collected, concentration of copper in the permeate, transmembrane pressure and pH when 4 g/L lambda-carrageenan and 20 mg/L copper was used. Permeate samples were collected after 30 minutes

Volume of permeate collected (ml)	Concentration of copper in the permeate (mg/L)	Transmebrane pressure (psi)	pH
0	0	7.5	3
600	0.14	7.5	3
1400	0.20	7.75	2.95
1800	0.42	7.5	3.02
2350	0.45	7.5	3.01
2950	0.69	7.5	3
3550	0.94	7.5	2.99
4200	1.16	7.75	2.99
4900	1.78	7.75	3
5500	2.36	7.5	2.99
6150	3.32	7.5	3
6700	4.43	7.5	3

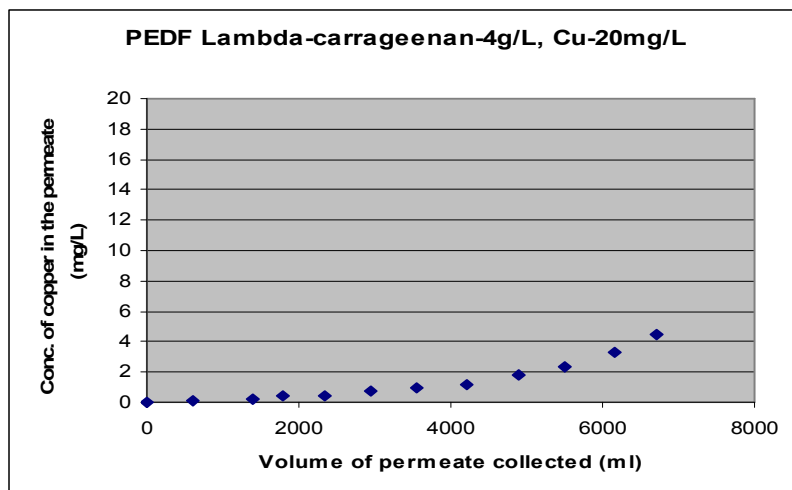


Figure 14 : Permeation curve of copper in Polymer Enhanced Diafiltration when 4 g/L lambda-Carrageenan and 20 mg/L copper was used.

Table 6 and Fig 15 represent PEDF when 4 g/L of lambda-Carrageenan and 50 mg/L copper were used.

Table 6 : PEDF measurements of volume of permeate collected, concentration of copper in the permeate, transmembrane pressure and pH when 4 g/L lambda-carrageenan and 50 mg/L copper was used. Permeate samples were collected after 30 minutes

Volume of permeate collected (ml)	Concentration of copper in the permeate (mg/L)	Transmebrane pressure (psi)	pH
0	0	7.5	3
600	0.56	7.5	3
1200	1.81	7.5	3
1800	4.76	7.5	3
2450	9.67	7.5	2.99
3000	18.25	7.5	3

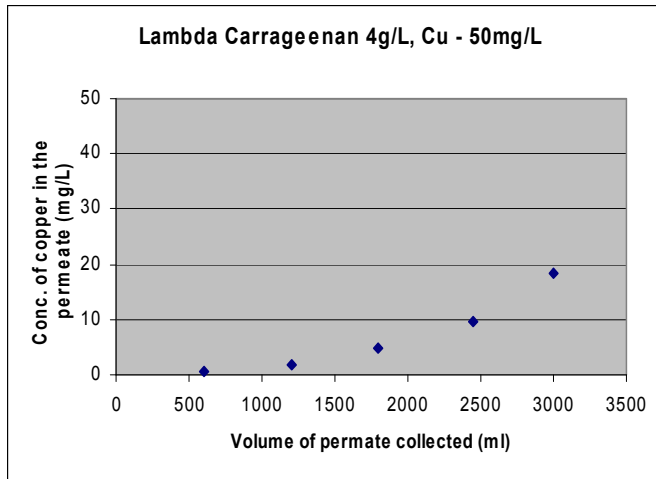


Figure 15 : Permeation curve of copper in Polymer Enhanced Diafiltration when 4 g/L lambda-Carrageenan and 50 mg/L copper was used.

2.4 Polymer Enhanced Diafiltration With 8g/L lambda-Carrageenan :

PEDF was run with the lambda-carrageenan concentration at 4 g/L and copper concentrations as 20 mg/L and 50 mg/L. The feed volume was 300 ml and the pH of the polymer in the feed tank and copper in the metal reservoir was 3. The pH in the feed tank was maintained by regular additions of NaOH. The transmembrane pressure was kept constant at 7.5psi. Copper concentration were determined after every 60 minutes. Table 7 and Fig 16 represent PEDF when 8g/L of lambda-Carrageenan and 20mg/L copper were used.

Table 7 : PEDF measurements of volume of permeate collected, concentration of copper in the permeate, transmembrane pressure and pH when 8 g/L lambda-carrageenan and 20 mg/L copper was used. Permeate samples were collected after 60 minutes.

Volume of permeate collected (ml)	Concentration of copper in the permeate (mg/L)	Transmebrane pressure (psi)	Ph
0	0	7.5	3
1000	0	7.5	3
1750	0	7.75	2.95
2350	0	7.5	3.02
2900	0	7.5	3.01
3500	0	7.5	3
4100	0	7.5	2.99
4800	0	7.75	2.99
5500	0.03	7.75	3
6150	0.11	7.5	2.99
6800	0.30	7.5	3
7450	0.68	7.5	3
8150	1.44	7.5	3

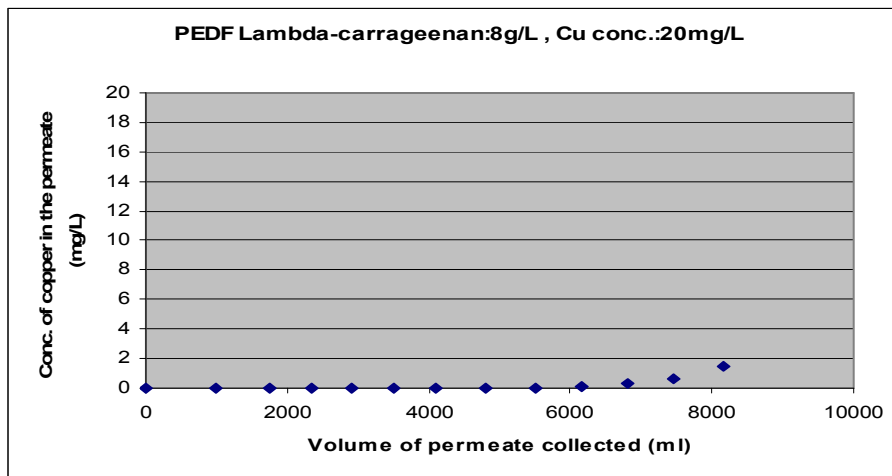


Figure 16 : Permeation curve of copper in Polymer Enhanced Diafiltration when 8 g/L lambda-Carrageenan and 20 mg/L copper was used.

Table 8 and Fig 17 represent PEDF when 8 g/L of lambda-Carrageenan and 50 mg/L copper were used.

Table 8 : PEDF measurements of volume of permeate collected, concentration of copper in the permeate, transmembrane pressure and pH when 8g/L lambda-carrageenan and 50mg/L copper was used. Permeate samples were collected after 30 minutes.

Volume of permeate collected (ml)	Concentration of copper in the permeate (mg/L)	Transmembrane pressure (psi)	pH
0	0	7.5	3
400	0	7.5	3
800	0.07	7.75	2.95
1150	0.14	7.5	3.02
1500	0.21	7.5	3.01
1900	0.45	7.5	3
2250	0.58	7.5	2.99
2600	0.75	7.75	2.99
3000	0.96	7.75	3
3400	1.16	7.5	2.99
3850	1.75	7.5	3

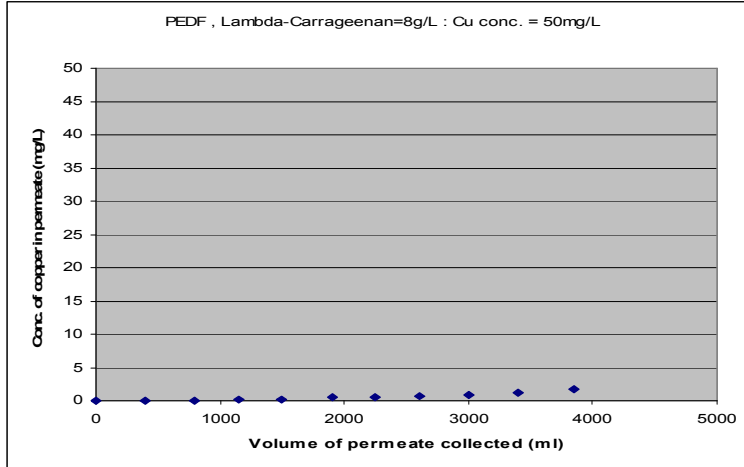


Figure 17 : Permeation curve of copper in Polymer Enhanced Diafiltration when 8 g/L lambda-Carrageenan and 50 mg/L copper was used.

2.6 Mathematical Modeling and PEDF Data Analysis :

The metal-specific uptake (q) was determined from the following equation.

$$q = \frac{C_0 - C_{eq}}{X} \quad (5)$$

Where q = metal-specific uptake (mg of metal/g of polymer)

C_0 = Initial metal concentration (mg/L)

C_{eq} = Metal equilibrium concentration (mg/L)

X = Concentration of polymer (g/L)

In order to simulate the experimental results, a material balance model of the membrane bioreactor has been developed. The model of the biosorption process could be evaluated by coupling the material balance for the heavy metal in a transitory regime to the equilibrium equation for a particular metal. Considering one heavy metal :

$$V \frac{dC}{dt} = FC_0 - PC - \frac{dq}{dt}X \quad (6)$$

$$F = P$$

Where P = Permeate flow rate (L/min)

(Veglio 2000)

Coupling the metal material balance and Langmuir Adsorption model, the following first order differential equation could be devised for copper concentration variation during time.

$$\frac{dC}{dt} = \frac{F \cdot (C_0 - C)}{V \cdot \left(1 + \frac{q_{\max} \cdot K_S \cdot X}{(C + K_S)^2} \right)} \quad (7)$$

Where C = Actual copper concentration in the reactor and in the permeate flux (mg/L)

C_0 = Copper concentration in the feeding stream (mg/L)

t = Time (min)

F = Feed flow rate (L/min)

V = Reactor volume (L)

X = Polymer concentration (g/L)

$K_S = 1/b$ where b is Langmuir model parameter

(Beolchini 2001)

Moreover, this equation is valid if copper has a rejection coefficient equal to zero, i.e. if it is not retained by the membrane (Veglio 2000).

Since there appeared to be some retention of copper by the membrane (due to unexplained reasons), a different derivation of the equation was done for determination of actual copper in the permeate/filtrate.

Moreover, the system used was a homogenous system, as opposed to the heterogenous system used by Veglio *et. al.*, who had derived the equation, another equation was derived.

Another kinetic model for biosorption can be developed by using the following equations:

$$dc/dt = (F/V) (C_0 - C) \quad (8)$$

Solving for the C, theoretical concentration of copper in the permeate :

$$\frac{C_0 - C}{C_0 - C_R} = e^{-(F/V)t} \quad (9)$$

Where C_0 = Initial copper concentration = 10 mg/L

C_R = Experimental concentration of copper in the permeate (mg/L)

F = Filtrate flow rate ~ 20 ml/min = 0.020 L/min

V = Volume in the feed tank = 300 ml = 0.3 L

t = time when copper starts to appear in the permeate (min)

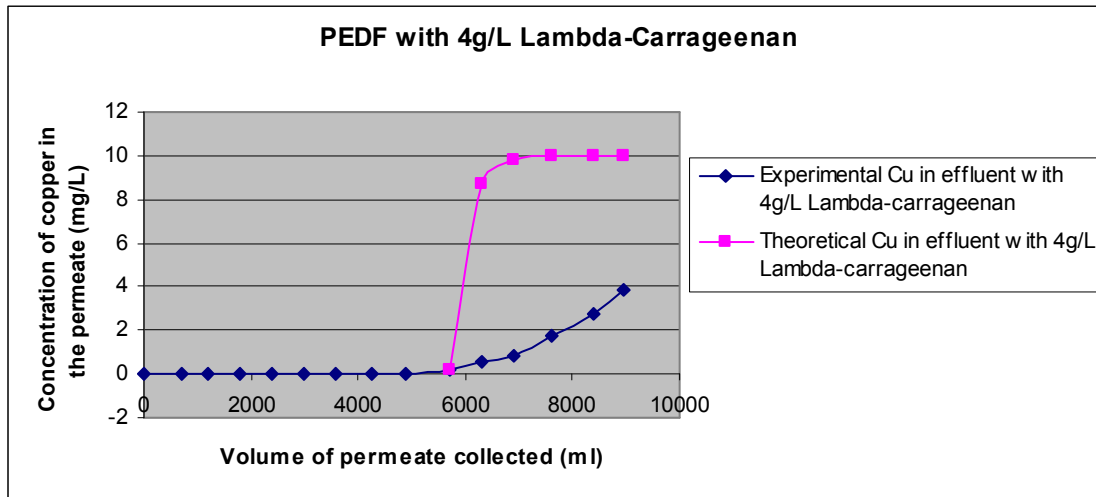


Figure 18 : Permeation curve of lambda-carrageenan at 4 g/L showing theoretical copper in the effluent. The theoretical curve is obtained by solving eq. 9. All these data are obtained at copper feed concentration of 10 mg/L

3. RHEOLOGICAL STUDIES FOR CHARACTERIZATION OF LAMBDA-CARRAGEENAN-COPPER COMPLEX:

Viscosity measurements of lambda-carrageenan were carried out when 10, 20 and 30 mM copper was added and varying degrees of shear stress was applied. A graph of viscosity versus shear stress was plotted. Three concentrations of lambda-carrageenan were used, namely 4 g/L, 8 g/L and 12 g/L. For each concentration of the polymer, a control with no metal was also employed. Fig 19 shows the effect of varying degrees of shear stress on the viscosity of the 4 g/L lambda-carrageenan in the presence of three different concentrations of metals, namely 10, 20, 30 mM (630, 1260, 1890 mg/L respectively). At this polymer concentration, a decrease in viscosity was observed on addition of copper as compared to control with no metal. However there was no difference in the viscosities of the polymer when different metal concentrations were used.

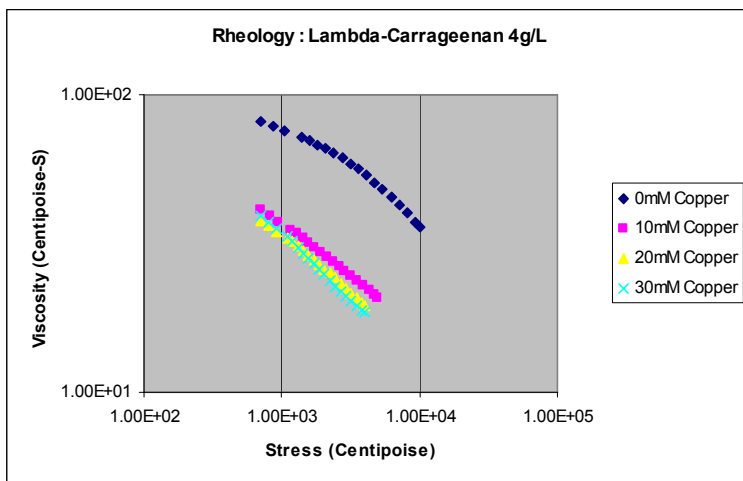


Figure 19: Log-log plot showing rheological behavior of 4 g/L lambda-Carrageenan in absence of copper and presence of 10, 20, 30 mM copper. Viscosity was measured with increase in shear stress in the presence of 10, 20, 30 mM copper.

Fig 20 and Fig 21 show that the viscosities of 8 g/L and 12 g/L solutions of lambda-Carrageenan increase on adding increased concentrations of metals, that is 10mM, 20mM and 30mM copper. However, on application of varying shear stress, there was a gradual decline in viscosities.

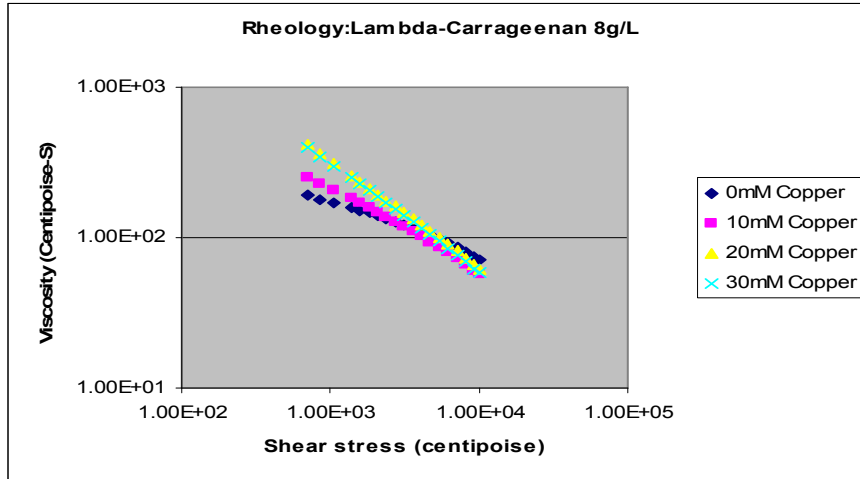


Figure 20: Log-log plot showing rheological behavior of 8 g/L lambda-Carrageenan in absence of copper and presence of 10, 20, 30 mM copper. Viscosity was measured with increase in shear stress in the presence of 10, 20, 30 mM copper.

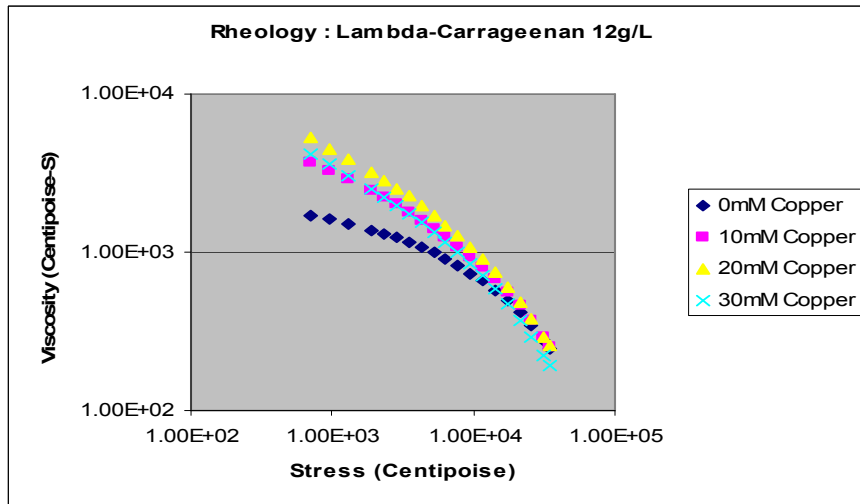


Figure 21: Log-log plot showing rheological behavior of 12 g/L lambda-Carrageenan in absence of copper and presence of 10, 20, 30 mM copper. Viscosity was measured with increase in shear stress in the presence of 10, 20, 30 mM copper.

DISCUSSION :

1. Time taken for the establishment of equilibrium for Copper-lambda-Carrageenan across the semi-permeable membrane :

Studying sorption kinetics using the equilibrium dialysis method helps in determining the basic qualities of an adsorbent. Fig 5, 6, 7 show copper uptake at pH 2, 3, 4 respectively over a period of 48 hours. A decrease in copper concentration in the feed cell indicates binding to the polymer, as compared with the “polymer-free” control. Sorption kinetics also helps in determining the optimum time taken by a particular polymer to adsorb the metal and reach equilibrium. The rate of polymer-metal binding was most likely rapid here. The true rate limiting step is the diffusion of metal ion across the membrane. In the adsorption of copper by lambda-carrageenan, a contact time of 24-48 hours was found to be suitable. In order to ensure that the metal ion-polymer equilibrium was reached, 48 hours of contact time was considered for subsequent studies.

2. Optimum pH :

Table 1 shows that maximum binding of copper to lambda-carrageenan occurred at pH 3. Though pH 3 and pH 4 gave comparable binding, yet due to the minor difference in binding capacity, pH 3 was considered for all subsequent experiments. The poorest binding occurred at pH 2. A higher pH was not considered as copper tends to precipitate as copper hydroxide and might block the pores of the dialysis membrane, making the study difficult and give erroneous results. (Kopecky 2005)

3. Equilibrium Adsorption Isotherm :

In the phenomenon of adsorption, metal ions are adsorbed onto the binding sites on the sorbent. In this, there was a gradual decrease in free concentration of copper and increase in copper adsorbed to lambda-carrageenan, until equilibrium was reached. This was expressed by an isotherm. Fig 8 is as adsorption isotherm at 25°C at pH 3. In order to determine the maximum adsorption of copper to lambda-carrageenan

(q_{\max}), a reciprocal plot was designed as shown in Fig 9. It was also used to determine K_D . Equilibrium adsorption isotherms were also determined at 37 °C to study the effect of temperature shown in Fig 10 and 11. There was no significant difference in the thermodynamic parameters observed at either temperatures. Hence, room temperature or 25 °C was considered for all subsequent experiments. It was found that the maximum adsorption of copper to lambda-carrageenan was 39.9 mg/g of polymer at 25 °C and 36 mg of copper/g of polymer at 37 °C. Though q_{\max} was not very high and K_D was quite high, yet there was some binding observed.

4. Rheological Studies Of Copper-lambda-Carrageenan Complex :

Rheological properties were studied to characterize the copper-lambda-carrageenan complex. Viscosity increases with increase in concentration in lambda-carrageenan. Hence, there was an exponential increase in viscosity with 4 g/L, 8 g/L and 12 g/L lambda-carrageenan concentrations. Addition of different concentrations of copper to 4 g/L lambda-carrageenan caused a reduction in viscosity. This could be due to reduction in electrostatic repulsion between the sulfate groups of the polymer on addition of copper ions. However, viscosity was found to increase when different concentrations of copper were added to 8 g/L and 12 g/L lambda-carrageenan, suggesting that intramolecular binding could be strong enough to be overcome by electrostatic repulsion. Moreover, viscosity was found to decrease in all the cases with increase in stress, suggesting that the fluid system behaves as shear thinning when copper ions were added to the polymer solution. This study was important to understand the behavior of different concentrations of the polymer in the absence of metal or in the presence of different concentrations of metal.

5. Polymer Enhanced Diafiltration :

The goal of this project was the optimization of the PEDF system to study metal removal using a biosorbent, as shown in studies by Mark. S.S., 2006 and Shetty. A.R., 2006. In order to do so, two concentrations of lambda-carrageenan were used and three concentrations of copper in the system were studied as shown in Fig 13-17.

There was no binding of copper to the membrane or anywhere else in the system as shown in Fig. 12. There was no leakage of the polymer through the membrane as was shown in table 4. No polymer leakage was observed even when copper started appearing in the filtrate, suggesting that copper binding to the polymer, if any, does not cause configurational changes in the polymer molecule by rounding up. This property is similar to that exhibited by chitosan. There is no macromolecular deformation of chitosan in presence of metal and hence, it does not leak out of the membrane. Hence, it could be suggestive of the fact that after saturation of all the sites on lambda-carrageenan, free copper started coming out in the filtrate. Appearance of copper in the filtrate occurred sooner when 50mg/L copper solution was used over 20 mg/L or 10 mg/L. However, from the evaluation of the dissociation constant, there was almost negligible binding of copper to lambda-carrageenan. Hence, appearance of copper in the filtrate did not indicate that the copper not coming out in the filtrate was getting adsorbed onto lambda-carrageenan. On plotting a graph showing theoretical copper in the effluent in Fig 18, most of the copper was in the permeate suggesting that there was hardly any binding between copper and the polymer. Hence, there was some peculiar phenomenon going on inside the PEDF system which was preventing any free copper from leaving the system. A possible explanation of this phenomenon could be that lambda-carrageenan is a linear polymer. During various runs in the system, polymer layers could be getting stacked by means of intramolecular bonding. This intramolecular binding between polymer molecules prevented copper binding to the polymer and also prevented it from passing through and getting eluted in the filtrate. Similar observation was made when higher concentration of polymer (8g/L lambda-carrageenan) was used. Copper started to appear sooner when 50 mg/L copper solution was used than when 20 mg/L or 10 mg/L copper solutions were used. This early appearance of copper in the filtrate could be due to increased electrostatic repulsion between copper ions forcing them to leave the system. Other reasons could be the sieving effect, degradation of the polymer and compaction of the molecule on metal binding. However, this compaction was not enough to enable polymer escape through the pore of the membrane. Hence, in order to understand the interaction between copper-polymer, there is a need to study events

happening on the surface of the membrane of the PEDF system. Since, it is not feasible to take the membrane out and study, there is a need to develop a mini-tangential flow filtration to study the interaction between metal and the polymer.

CONCLUSIONS :

Optimization of the Polymer Enhanced Diafiltration could be accomplished by studying copper adsorption using lambda-Carrageenan.

For carrying out adsorption studies, optimum pH was found to be 3.0. In equilibrium dialysis studies, binding of copper to lambda-carrageenan was observed. During the initial stages of a PEDF run, no polymer leakage or copper was observed in the filtrate. However, after collecting a few liters of filtrate, copper started appearing in the filtrate. A qualitative assay of lambda-carrageenan with chitosan confirmed no leakage of the polymer with the copper ions. Hence, the copper coming out in the filtrate was unbound copper ions. This could have suggested that after saturation of all the binding sites of lambda-carrageenan with copper ions, any copper that was free started coming out of the filtrate. But, on evaluation of copper binding to lambda-Carrageenan by means of dissociation constant, it was found that there were many more free copper ions that should have come out in the filtrate, indicating that there was a formation of metal-polymer or polymer-polymer complex which was preventing free copper ions from escaping into the filtrate. This interaction could not necessarily be copper binding to the polymer. Sieving effect, degradation of the polymer and compaction of molecule on metal binding were other few possibilities of such a behavior.

In order to determine any such interaction, rheological examination of polymer-copper was carried out. It was found that viscosity of lambda-carrageenan increased on addition of copper ions for 8 g/L and 12 g/L lambda-carrageenan. However, at 4g/L of lambda-carrageenan, viscosity of the polymer decreased suggesting earlier escape of free copper ions in the filtrate. However, at higher concentrations of lambda carrageenan, viscosity was found to increase with increase in copper concentration and appearance of copper in the filtrate was found to get delayed. Hence, on increasing the polymer concentration, formation of intramolecular complexes accumulating prevented any free copper from passing into the filtrate. However, as more copper was pumped in, some free copper escaped into the filtrate, which was detected by Bathocuproine assay. Hence, from the

amount of copper coming out in the filtrate, binding capacity of lambda-carrageenan could not be accounted for. On doing mathematical calculations, it was found that there was a lot of free copper inside the system which could not come out in the filtrate, suggesting that formation of layer(s) of intramolecular complexes prevented free copper from passing through them and coming out in the filtrate.

However, in order to fully understand this phenomenon, a thorough examination of the membrane and metal-polymer interaction is desired. Future work in this area will include designing a mini-Tangential Flow Filtration system to study the metal-polymer interaction on the surface of the membrane.

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