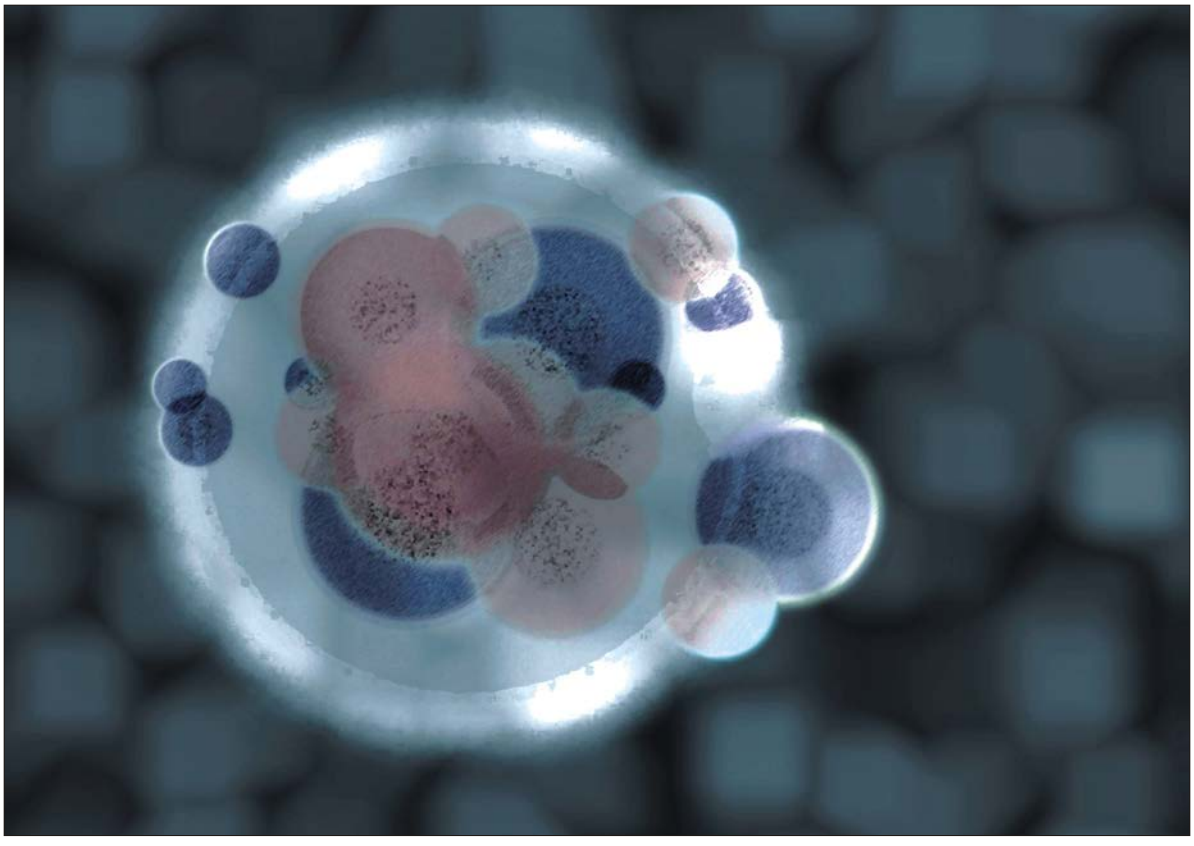

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A Multipurpose Biopolymer Membrane for Heavy Metal Preconcentration and Enzyme Immobilization

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Abstract: Rapid urbanization of our world has lead to accumulation of enormous number of contaminants in our environment. Heavy metals hold a superlative position in that list and are responsible of contaminating soil, water and all food stuffs taken up by the humans. The presented work was an effort to develop a biopolymer membrane which efficiently bonded and removed heavy metals like lead from the water sample. It was also found to be a very good matrix for enzyme immobilization in developing heavy metal biosensor. The membrane bonded to the positively charged heavy metal cations and lead to their preconcentration from the samples. The synthesized membrane was a hybrid of hydrosol-gel and alginate in a ratio which lead to the formation of a thin film that acted as the preconcentration medium in the study. It had been found to be an efficient electron transfer facilitator for application in electrochemical biosensor.

Key words: Alginate-hydrosol gel composite, metal removal, electron transfer facilitator, electrochemical biosensor

INTRODUCTION

Membrane synthesis has been a very less worked out area of research since 1920, after that enormous work has been done on development of membranes commonly for dialysis purpose (Henne and Dunweg, 1986; Klein *et al*., 1977; Dunweg *et al*., 1995; Diamantoglou and Lemke, 1992; Diamantoglou *et al*., 1995; Idris *et al*., 2005). The present status of biological research demands a much more emphasis on efficient natural polymers with multiple applications. Among the various biopolymers alginate is the most studied matrix for biomedical application because of its biocompatibility and low cost (Smidsord and Skjak-Break, 1990; Barbotin and Saucedo, 1998). Hirst and Rees (1965) postulated for the first time that alginate is a polymer of mannuronic acid and guluronic acid having 1,4 linkage. According to Baardseth (1996) alginates occur in both the intracellular regions and cell walls. Alginic acid prepared from whole plants show wide ranges in proportions of mannuronic acid and guluronic acid. Composition is not only species dependent but also tissue dependent. X-ray diffraction examination of several alginic acid preparations obtained by selective selection from several seaweed species had been done. Frei and Peterson (1962) proposed that alginic acid rich in polymannuronic acid is characteristic of young cell wall tissue and intracellular regions, whereas polyglucuronic rich alginic acid appears to be located in the cell wall proper. Haug *et al*. (1969) revealed that by changing the composition alginic acid could be prepared from 97% mannuronic acid (in the intracellular fluid of Ascophyllum) to about 30% mannuronic acid in the cortex of *L. hyperborean*.

Several microbial polysaccharide which resemble algal alginic acid are also known (Gorin and Spencer, 1966; Linker and Jones, 1966). The known difference between the algal and bacterial alginic acid is the presence of O-acetyl group in the latter polysaccharide (Davidson *et al*., 1977), also the bacterial alginic acid contains smaller proportion of homopolymeric sequence than algal alginic acid. Alginate has been a common matrix for enzyme immobilization (Youssef and Al-Omair, 2008; Panesar, 2007a, b; El-Enshasy *et al*., 2007; Borgio, 2011) and heavy metal adsorption. It is a well known fact that alginates have high affinity for divalent ions because of its guluronic acid content which forms the basis of its application in heavy metal removal from water.

There are previous reports of heavy metal adsorption by alginic acid, it is postulated that the heavy metal binding efficiency of the alginic acid is directly proportional to the carboxyl group content of the alginic acid (Jeon *et al*., 2002a). The binding efficiency is also dependent on the electronegativity of the investigated elements like Sr^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} (Fourest and

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Volesky, 1997) and it increases at higher temperature (Jeon *et al*., 2002b). A kind of magnetic modification of alginic acid has been reported by Nqomsik *et al*. (2006) and Jeon *et al*. (2007), to increase the adsorption efficiency towards lead and nickel. Now introduction of sol-gel derived biomaterials has attracted the attention of researchers towards its applicability with alginate to form a biocomposite. The application of alginate alone as the immobilization matrix to form enzyme immobilized beads poses limitations like, inhomogeneous beads, cavities and fractures over entire bead volume, decreased gel strength from the capsule to its surface and uncontrolled semi-permeability (Cordin *et al*., 2003). The silicate matrix causes brittleness and its narrow mesopore network imposes diffusional limitations in macroscopic catalyst particles (Chen and Hwang, 1998). So to overcome the limitations of both the matrices, both were combined to get an alginate-silicate composite which has advantage of strengthened matrix, increased thermal and mechanical stability of the immobilized biocomponent, increased activity of some enzymes and suitable diffusional properties along with reusability. The sol-gel/alginate composite also prevents cracking of the film (Lei *et al*., 2005). Various studies of alginate-TMOS and TEOS sol-gel hybrid for enzyme immobilization are known (Chen and Hwang, 1998; Lei *et al*., 2005; Mohidem and Mat, 2009) but the application of alginate-TEOS hybrid for enzyme immobilization and heavy metal preconcentration has not been reported yet. The presented work was intended to prepare a membrane which could serve multiple applications including enzyme immobilization and heavy metal preconcentration.

MATERIALS AND METHODS

All the reagents used in the study were procured from Sigma- Aldrich, Fluka and Hi-Media Pvt. Ltd.

Preparation of membrane: A stock of TEOS was prepared with 600 μL ethanol, 50 μL TEOS, 10 μL NaOH (5 mM) and 60 μL water. For enzyme immobilization 100 μL sodium alginate (3% w/v) was mixed with one-fifth volume of urease enzyme solution and then this mixture was dispensed on a watch glass. This droplet was covered properly with TEOS stock in a 2:1 ratio and left for air drying for 2 h. The prepared very thin film was scrapped out from the watch glass and used for enzyme assay. The membrane prepared in the same way but lacking the enzyme part was used for electrochemical and heavy metal preconcentration studies.

Characterization of the prepared membrane: The prepared membrane was subjected to Scanning Electron Microscopy (SEM) to reveal the surface properties and the interaction of alginate with TEOS. Membrane was principally prepared as the immobilization matrix in biosensor application. To check its compatibility with the electrochemical transducer, the electro conductivity studies were conducted on CH Instruments, Electrochemical Workstation. Membrane's impedance was studied at different potentials (0.1-0.5 V) and its effect on polarization of Para-Nitrophenol (PNP) in enzymatic and non- enzymatic system was deduced by cyclic voltammetry. For this purpose a Carbon Paste Electrode (CPE) was prepared by mixing graphite powder and mineral oil in 2:1 ratio and then alginate-TEOS hybrid was dropped at the tip of the electrode. Direct polarization studies of PNP was conducted with various concentrations of PNP in Tris HCl/KCl-$MgCl_2$ buffer system at a scan rate of 50 mV s^{-1}, in a potential range of 0.5-1.25 V.

In enzymatic system, OPH enzyme was immobilized in the lower 1/3 part of the CPE and the hydrolysis of methyl parathione pesticide which has deleterious health effects was studied. The OPH catalysed hydrolysis of methyl parathione produces PNP whose electrochemical polarization was studied as per the direct method. The suitability of this alginate-sol gel film as electron transfer facilitator was studied.

Application of the prepared membrane: Application of the membrane for enzyme immobilization was studied by immobilizing the urease enzyme in the alginate-TEOS composite. The prepared membrane was used in the standard Nessler's assay for urease and its immobilization efficiency was observed with respect to the free system. For heavy metal preconcentration, the prepared membrane was attached at the tip of the glassy carbon electrode in the electrochemical workstation with Ag/$AgCl_2$ as reference electrode. Lead oxidation in the presence and absence of the membrane was studied.

The study has been conducted from 15th August 2010 to 30rd March 2011 at Biosensor Technology Laboratory, Department of Biotechnology, Punjabi University, Patiala, India.

Data analysis: The data had been analyzed using statistical tools for calculating standard deviation. Electrochemical studies results had been analyzed by the in house software of CH instruments model 660 electrochemical workstation, using which studies had been conducted.

RESULTS AND DISCUSSION

The alginate-TEOS hybrid was able to form a thin membrane (Fig. 1) capable of serving multiple applications. The SEM studies of the membrane surface

Fig. 1: Alginate-TEOS membrane

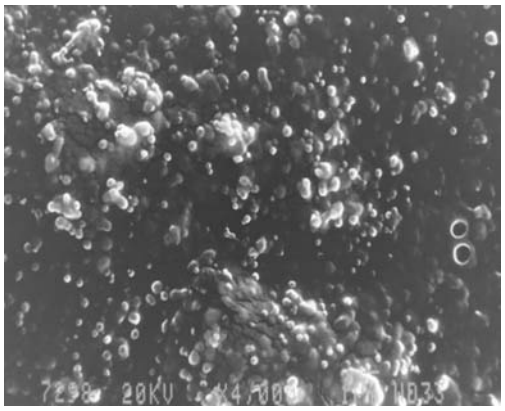

Fig. 2: SEM of the prepared membrane

Table 1: Comparison of urease activity in free and membrane immobilized state

Parameter	Free urease	Urease immobilized in alginate-TEOS membrane
Enzyme activity (IU mL^{-1})	53.87±1.41	50.27±1.03
K_m (mM)	7.69±0.6	5.93±0.13

(Fig. 2) revealed microstructures showing alginate-silicate interactions that results in small vesicles with increased surface area which forms the platform for enzyme immobilization and metal adsorption. The prepared membrane was used for urease immobilization. The enzyme activity and kinetics in immobilized state was comparable to the free system (Table 1). The immobilization in alginate-TEOS matrix resulted in decreased K_m value, showing that the sol-gel matrix provided a hydrophobic environment to the enzyme which increased its affinity towards the substrate as observed by Chen and Hwang (1998). It was observed that the immobilization of urease in alginate-TEOS matrix slightly decreased the enzyme activity of the free enzyme from 53.87 to 50.270±1.03 IU mL^{-1} which was not a dire deal taking up the advantage of reusability of the membrane. Although, due to lower mechanical strength the membrane could not be reused until modified with $CaCl_2$. The mechanical strength was improved by dropping $CaCl_2$ over the alginate-TEOS droplet that increased the reusability of the membrane to 5 times. The alginate- silicate composite proved to be a safe medium for retaining enzyme activity and could be used for biosensor construction.

As an electron mediator it was observed that the alginate- silicate hybrid membrane mediates electron transfer when used in conjunction with a redox couple like ferricyanide system (Fig. 3). The effect of membrane on PNP oxidation was studied and it was observed that the membrane provide lesser resistance to electron transfer and mediates faster electron transfer. As an immobilization matrix in electrochemical biosensor, conductivity studies of the membrane also shows that the membrane provides less resistance at higher potentials (Fig. 4). According to the AC impedance studies of potassium ferricynate at different potentials, higher potential (0.2-0.5 V) offered less resistance to the current flow suggesting the applicability of the membrane in higher potential range studies.

The successful application of the prepared membrane in electrochemical biosensor was confirmed by studying the polarization of standard PNP and enzymatically produced PNP. In case of standard PNP polarization studies by cyclic voltammetry, it was observed that the application of the membrane at the tip of the CPE resulted in current enhancement as compared to the membrane deficient system (Fig. 5). So, it is suggested that the alginate silicate composite facilitates electron transfer thus enhancement in the polarization current as observed by Lei *et al.* (2005) in case of HRP entrapped in sol-gel/alginate hybrid. The membrane was also applied to study the polarization of PNP produced after the enzymatic hydrolysis of methyl parathione and similar results were obtained. These results confirm that the prepared membrane has a potential to be used in electrochemical biosensor for enzyme immobilization, as electron facilitator and may serve as a blocking film to prevent enzyme leakage from CPE.

Promising results were obtained in heavy metal preconcentration studies of the membrane. As per the electrochemical studies, the membrane applied at the tip of the GC electrode was able to preconcentrate almost 99% lead from the solution. The oxidation peak of 10 ppm

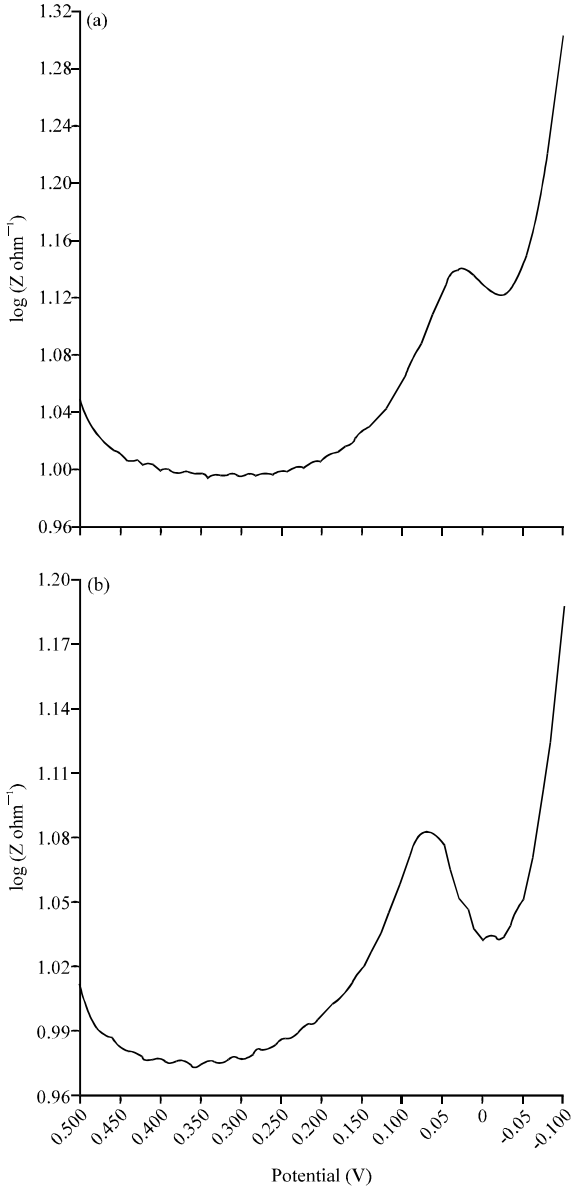

Fig. 3: Effect of alginate-silicate hybrid membrane on electron transfer (a) Impedance study of PNP oxidation in the absence of membrane and (b) Impedance study of PNP oxidation in the presence of membrane

lead got completely suppressed after the application of the membrane (Fig. 6) and the membrane's morphology changed after complexation with the metal. Affinity of alginic acid towards metal cations like lead has already been postulated by Jeon *et al.* (2002a). A report on 99.8% lead removal from water by immobilizing chitosan onto carbon nanotobes also facilitate the fact that alginate and chitosan like polymers are efficient

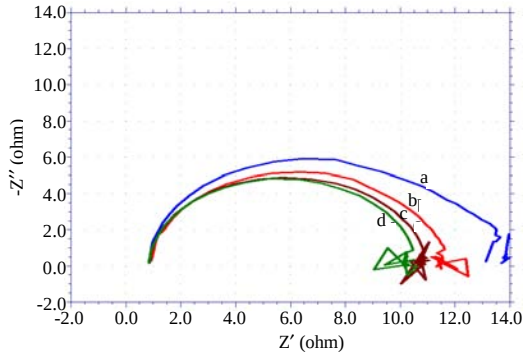

Fig. 4: AC Impedance studies of potassium ferricynate at different potentials (a) 0.1 V (b) 0.2 V (c) 0.3 V and (d) 0.5 V

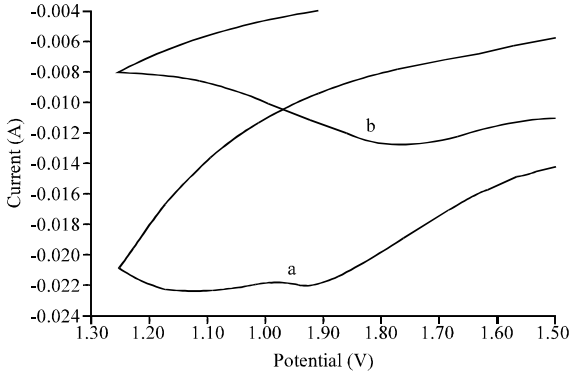

Fig. 5: Cyclic voltammetry of PNP polarization in non enzymatic system (a) with membrane and (b) without membrane

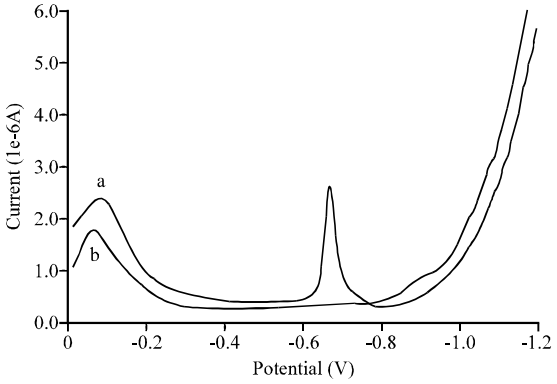

Fig. 6: Lead preconcentration on GC Electrode (a) without membrane and (b) with membrane

matrices for lead removal (Alkhatib *et al.*, 2010). Lead removal from city effluent had also been studied with different microbes individually and in consortium by

Chowdhury *et al.* (2011) and maximum efficiency obtained was 94.4%. The lead adsorption efficiency has been improved using magnetically modified alginic acid by Jeon *et al.* (2007). The observed interaction of the membrane with lead is also advantageous in developing urease immobilized membrane for heavy metal detection as the membrane will be able to concentrate cations close to the enzyme and hence enhanced inhibition could be observed towards heavy metal cations. The present work will help to decrease the detection limit of electrochemical biosensors for lead developed by Verma *et al.* (2011). As per the studies conducted, it is suggested that the prepared alginate-TEOS sol gel membrane is a very promising matrix for heavy metal preconcentration and its removal from the wastewaters.

CONCLUSION

The present study has been focused on the preparation and application of an alginate-TEOS membrane for enzyme immobilization and heavy metal preconcentration. The membrane proved to be a suitable matrix for enzyme immobilization with enhanced electron transfer ability as compared to the membrane free system. Heavy metal adsorption capability of the membrane was found to be promising for their removal from the wastewaters. The developed membrane proves to be a biocompatible and cost effective matrix for multiple applications.

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