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Fabrication of Porous Chitosan Affinity Membranes for Adsorption of Copper(II): A Kinetic Study

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The affinity of membranes with surface functional groups can be used as adaptive sites for separation, which are of great interest in many industrial and environmental applications. Among the various reactive functional groups, amino-group is very reactive than hydroxyl groups and can therefore be used directly as affinity adsorption sites. Recently, chitosan polymer has been increasingly studied as an absorptive material for various applications in the form of powders, flakes or gel beads. In the present study, novel semipermeable affinity membranes were fabricated from chitosan to be used in the adsorption of Cu(II) ions from aqueous solution. Porogens including polyethylene glycol and sodium chloride were tested for their effect on the membrane affinity for Cu(II) ions from aqueous solutions. Batch shake flask tests were conducted at different temperatures and the equilibrium-, Freudlich- and Langmuir-isotherms were constructed. The chitosan-NaCl membrane adsorbed almost 500 mg/g of chitosan. The kinetics of adsorption were determined according to Lagergren's models and the adsorption process was best described by the pseudo-second-order kinetic equation. The activation energy and thermodynamic parameters were also determined and the negative value of ΔG° suggested the feasibility of adsorption.

Keywords: Affinity membrane, Chitosan, Copper, Adsorption, Kinetics.

INTRODUCTION

Chitosan is the deacetylated form of chitin, which is the second most abundantly available biopolymer in nature after cellulose. It is an extremely hydrophilic material for which the reactive amino and hydroxyl groups owe their hydrophilic nature and endows it with a great capability in sorption of heavy metal ions [1]. Contamination of water and soil with heavy metals is detrimental to human beings and the environment and is a major concern worldwide [2]. Technologies for heavy metal removal from water and wastewater treatment in particular, have bloomed in the last few decades, for which adsorption appears to be an effective way by which heavy metal ions are removed from aqueous solutions. Absorptive membranes are a type of porous membranes bearing specific functional groups on its surfaces, which include $-NH_2$, $-SO_3$, –OH or –COOH, that can bond with the heavy metal ions either through ion exchange or surface complexation.

Chitosan is biodegradable, low-cost and available from the shells of crustaceans such as shrimps in seafood processing waste [3]. Amino groups make chitosan a cationic polyelectrolyte, that is soluble in aqueous acidic media and when dissolved possesses a high positive charge on $–NH_3$ groups, that adheres to negatively charged surfaces and chelates heavy metal ions with excellent gel-forming properties [4-14]. Preparation of pure chitosan membranes has been largely limited due to the poor mechanical strength and chemical stability of chitosan. However, Naim [15] has prepared adoptive membranes made from chitosan, which efficiently adsorbed Cu(II) ions from aqueous copper sulfate solution.

Chitosan has been coated on supports such as flat PES membranes [16] and on cellulose membranes [17,18] to make composite chitosan membranes. More recently, blending of chitosan with other polymers has been found to be an effective way to overcome the shortcomings of chitosan [19-24] because blending may form additional chemical bonds at the microscopic level due to chemical interactions. Chitosan/cellulose blend membranes suitable to be used as a wound dressing with anti-bacterial properties were prepared by Wu *et al.* [25]. Yang *et al.* [17] prepared a composite chitosan-cellulose membrane by coating chitosan on a filter paper and examined it as an affinity membrane. Wan *et al.* [26] prepared chitosan-based immobilized electrolyte porous composite membranes using glutaraldehyde as cross-linking agent. Ren *et al.* [27] reviewed

the chitosan binary blend membranes fabricated by solvent casting of chitosan solution containing highly deacetylated chitosan and moderately deacetylated chitosan with different ratio. Carvers and Arnal [28] evaluated the removal of heavy metals from wastewater by using chitosan.

Beppu *et al.* [29] reviewed the ability of chitosan to form complexes with bivalent metal ions. Zheng and Ruckenstein [30] described a procedure for the preparation of membranes with tailored pore size using a silica porogen. Guibal [3] reviewed the adsorption of metal cations by chelation on amino groups of chitosan in near neutral solutions in which sorption proceeds by electrostatic attraction on protonated amine groups. Shi *et al.* [31] prepared affinity membranes by coating chitosan on nylon membranes. Chitosan was employed as an excellent adsorbent for sorption of phenols and polychlorinated biphenyls [25] and proteins [32,33] and in pollution control as a chelating polymer for binding harmful metal ions [14,30,34,35].

In the present work, porous chitosan membranes are prepared *via* the addition of NaCl or PEG (as Persians) to chitosan and are compared to chitosan membrane. A blend of cellulose acetate/chitosan (CA/CS) is prepared for comparison. The membrane test for their ability to adsorb Cu(II) ions from aqueous solution. Different isotherms are constructed and the kinetics of adsorption are also determined, at different temperatures, then the activation energy and the thermodynamic parameters are determined.

EXPERIMENTAL

AnalaR chitosan powder (Alpha Chemica, India) and cellulose acetate (CA) flakes (Panreac, Egypt) were used in membrane fabrication and glacial acetic acid (AA) (Chemica jet, Egypt) was used as solvent. PEG 4000 (Carbowax 4000 SRL, India) and sodium chloride (Alpha, India) were used as porogens and sodium hydroxide (Chemica jet, Egypt) was used for neutralization.

Preparation of casting solution: The aqueous acetic acid (3 %) was added to 4 g chitosan and stirred while heating until complete dissolution. The solution was poured into several petri-dishes to a certain level, then left to completely air-dry for 48 h. Aqueous sodium hydroxide solution (4 %) was poured on the air-dried membrane in order to neutralize the acetic acid and prevent the resolution of the membrane in water. The membrane was washed efficiently with distilled water until the salt formed upon neutralization was totally removed.

Polyethylene glycol and sodium chloride were used as porogens. Each porogen was added to the chitosan solution separately and the method continued as aforementioned.

Batch adsorption experiments: Adsorption experiments were conducted batches using shake flask tests in order to evaluate the adsorption capacity of the membranes in adsorbing Cu(II) ions from aqueous solution. The membrane was cut into very tiny pieces before adsorption tests. 20 mL of $CuSO_4$ solution was added to each of numerous conical flasks which contained different weights of chitosan, then shaken mechanically at different temperatures for 2 h. Initially, adsorption experiments were conducted and analyzed for Cu(II) ions at different time intervals for determination of the minimum time required for equilibrium adsorption to take place.

Determination of adsorption kinetics: The adsorption kinetics and thermodynamic parameters were determined. An exact amount of finely cut chitosan membrane was weighed in a stoppered conical flask. 100 mL of 1 g Cu(II)/L solution were added to the membrane fragments and the flask shaken in an automatic shaker equipped with a thermostatically controlled water bath. 1 mL of the clear supernatant solution was pipetted, at different time intervals and analyzed for Cu(II) ions in standard phosphate solution, using KI to liberate the iodine and starch as indicator. The pseudo-first and pseudo-second order Lagergren's diagrams relating $\ln(q_e-q_t)$ *versus* t and t/q_t *versus* time it in respective order were constructed, where q_t is the mass of Cu(II) in mg biosorbed per gram of chitosan at time t (min) and q_e is the quantity biosorbed at equilibrium.

RESULTS AND DISCUSSION

Adsorption isotherms: The graphs indicating the equilibrium isotherms for the different membranes are presented in Figs. 1 and 2. The values of constants pertaining to both Langmuir and Freundlich models [eqns. 1 and 2] are clarified in Tables 1 and 2. Fig. 1 illustrates the equilibrium isotherms for adsorption of Cu(II) ions with a chitosan membrane at different temperatures shown on the figure. It is clear that chitosan membrane chelates Cu(II) significantly, reaching almost 250 mg/g of chitosan and that adsorption is exothermic since heating decreases x/m.

TABLE-1
ADSORPTION ISOTHERM CONSTANTS FOR ADSORPTION OF Cu(II) IONS ON CHITOSAN AT DIFFERENT TEMPERATURES

Temp. (K)	Langmuir isotherm parameters			Freundlich isotherm parameters		
	q_m (mg g^{-1})	K_L (L mg^{-1})	R^2	K_F (mg g^{-1}) (L mg^{-1})$^{1/n}$	1/n	R^2
295	243.8301	2.41248	0.9602	7.65773	0.5147	0.8765
313	213.7864	2.22741	0.9686	7.33669	0.5071	0.8054
323	192.2605	1.92639	0.9306	10.1976	0.4423	0.7586

TABLE-2
ADSORPTION ISOTHERM CONSTANTS FOR ADSORPTION OF Cu(II) IONS ON DIFFERENT CHITOSAN MEMBRANE BLENDS

Membrane	Langmuir isotherm parameters			Freundlich isotherm parameters		
	q_m (mg g^{-1})	K_L (L mg^{-1})	R^2	K_F (mg g^{-1}) (L mg^{-1})$^{1/n}$	1/n	R^2
Chitosan-NaCl	492.8886	0.96612	0.9716	30.78931	0.3539	0.9877
Chitosan	243.8301	2.41248	0.9602	7.65773	0.5147	0.8765
Chitosan-PEG	145.0000	1.16891	0.9011	44.84355	0.1999	0.7675

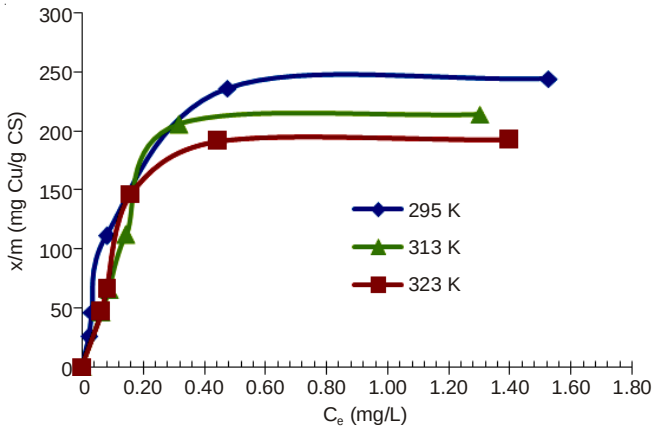

Fig. 1. Equilibrium isotherm for adsorption of Cu(II) ions (1 g/L) with chitosan membrane

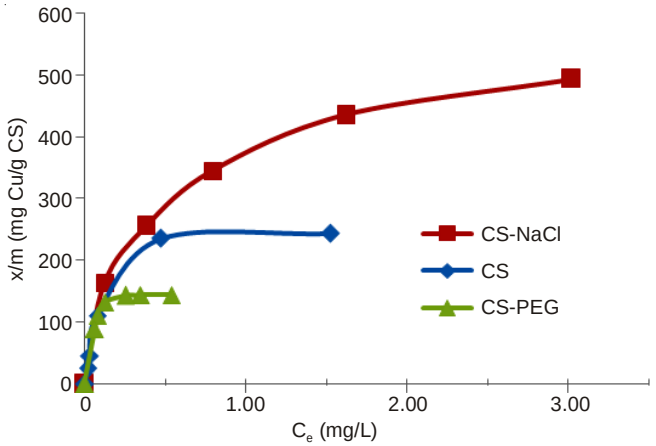

Fig. 2. Equilibrium Isotherm for adsorption of Cu(II) ions (1 g/L) with membrane blends at 22 °C

Langmuir:

$$\frac{C_e}{q} = \frac{C_e}{q_m} + \left(\frac{1}{K_L q_m}\right) \quad (1)$$

Freundlich:

$$\log q_e = \log K_F + \left(\frac{1}{n}\right)\log C_e \quad (2)$$

Table-1 presents the adsorption isotherms' constants for adsorption of Cu(II) ions on chitosan at different temperatures. It is obvious that the Langmuir isotherm model has applied strictly since the correlation coefficient is always higher than 0.93. This indicates that monolayer adsorption takes place and that the binding energy on the whole surface of the membrane was uniform. In other words chelation of the Cu(II) ions to the $-NH_2$ functional groups takes place due to the lone pair of electrons available on the nitrogen atom. Moreover, it is observed that q_m (maximum adsorption capacity) and K_L (Langmuir constant) vary inversely with temperature *i.e.* adsorption is exothermic. On the other hand, Freundlich model does not fit the experimental data; however, despite that, 1/n is less than one, which denotes favourable adsorption.

Fig. 2 presents the equilibrium isotherm for adsorption of Cu(II) ions with chitosan membrane blends with NaCl and PEG and for chitosan alone for comparison. It is clear that the chitosan-NaCl blend membrane gave the highest pickup of Cu(II) ions, which is double than chitosan membrane without purging and about quadruple than chitosan-PEG membrane. These results have been explained by examining the SEM micrographs, since the chitosan-NaCl membrane exhibited nano-pores covering the entire matrix, that led to this exceptional adsorptive capacity.

Table-2 clarifies that Langmuir isotherm model is obeyed for the blend membranes as well, since R^2 varies from 0.90 to 0.97. However, the chitosan-NaCl membrane only, follows the Freundlich model.

Fig. 3 indicates that adsorption of blend chitosan-cellulose acetate membrane gives type 2 (S-shaped) equilibrium isotherm, which signifies that Cu(II) ions slowly penetrate within the pores. As expected, neither Langmuir nor Freundlich adsorption models were obeyed, since the relationships were far from linear and the correlation coefficients were both less than 0.5.

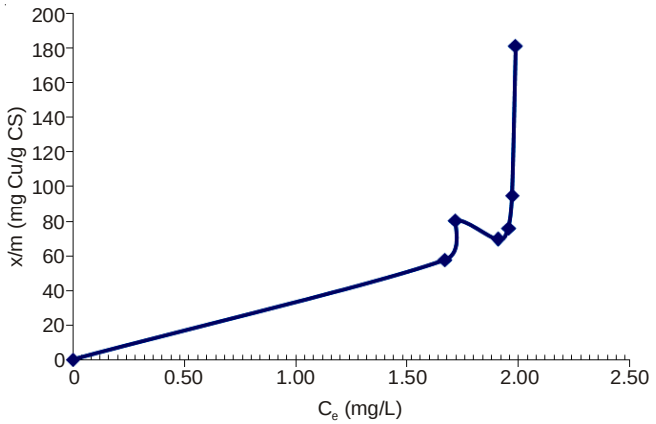

Fig. 3. Equilibrium isotherm for adsorption of Cu(II) ions from 1 g Cu/L with chitosan/cellulose acetate in AA (chitosan:cellulose acetate ≈ 1:20 b.w.) membrane at 22 °C

Adsorption kinetics: The kinetics of the adsorption process were studied by applying Lagergren's pseudo-first-order [36,37], pseudo-second-order [36,38] kinetic models.

Pseudo-first-order:

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303}t \quad (3)$$

Pseudo-second-order:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} \quad (4)$$

They were applied in order to obtain the rate constants, equilibrium adsorption capacity and the adsorption mechanism at different temperatures. The pseudo-first-order rate constant (k_1) and the equilibrium adsorption capacity (q_e) at different temperatures were computed from the slope and intercept of the plots of log ($q_e - q_t$) *versus* t. However, the correlation coefficient (R^2) was far from unity indicating poor fitting of the pseudo-first order kinetic model to the results.

The kinetic data were further analyzed using the pseudo-second-order equation (eqn. 4), of which the constants were determined from the slope and intercept of the plot of t/q_t *versus* t. The latter is illustrated at different temperatures in Fig. 4 and their values are presented in Table-3.

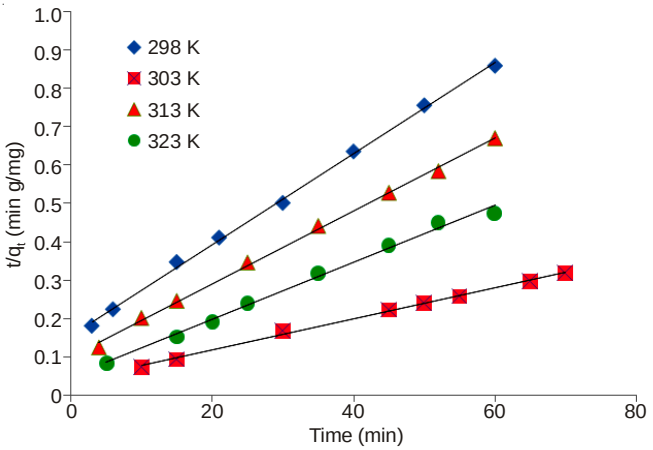

Fig. 4. Pseudo-second order kinetics plot for biosorption of Cu(II) ions (1 g/L) using chitosan membrane at different temperatures

TABLE-3
KINETIC PARAMETERS FOR ADSORPTION OF Cu(II) IONS ONTO CHITOSAN MEMBRANE

Temp. (K)	Pseudo-second-order kinetic model			
	q_e, cal (mg g^{-1})	k_2 (g mg^{-1} min^{-1})	h (mg g^{-1} min^{-1})	R^2
298	84.745	0.000898	6.451613	0.9986
313	105.263	0.000906	10.04016	0.9982
323	133.333	0.001202	21.36752	0.9937
303	243.902	0.000487	28.98551	0.9936

The fitting of the kinetic data showed good linearity with high R^2 over the studied temperature range. Accordingly, the data can be explained accurately by the pseudo-second-order kinetic model. It is also observed from Table-3 that the rate constant (k_2) varied directly with temperature indicating adsorption is endothermic in the range 298 to 323 K, except at 303 K, which suggests that the rate-limiting step of the adsorption process is physical adsorption. However, this anomaly (related to temperature) was verified and confirmed by conducting the experiments in duplicates.

The initial adsorption rate (h), was calculated at different temperatures using (eqn. 5) [38] and are presented in Table-3.

$$h = k_2 q_e^2 \quad (5)$$

It is clear that h increased with increase in temperature, suggesting that adsorption was favourable at high temperature. However, it was maximum at 303 K (Table-3), then decreased as the temperature increased.

Activation energy: From the pseudo-second-order rate constant k_2 (Table-3), the activation energy E_a for adsorption of Cu(II) ions onto chitosan was determined using the Arrhenius equation (eqn. 6) [39,40]:

$$\ln(k_2) = \ln A - \frac{E_a}{RT} \quad (6)$$

By plotting ln (k_2) *versus* 1/T (Fig. 5), E_a was obtained from the slope of the linear plot, of which the value of E_a for adsorption of Cu(II) on chitosan was found to be 36.88 kJ/mol. The value of E_a gives an idea about the type of sorption, which is either physical or chemical. The value of E_a for physical adsorption is less than 40 kJ/mol, since the forces involved in physical adsorption are weak. On the other hand, higher values

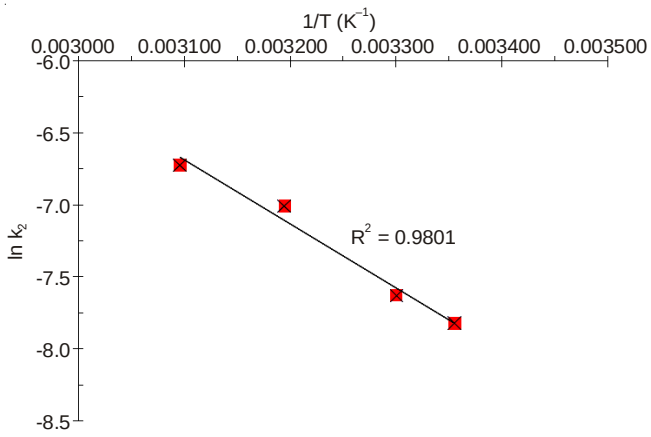

Fig. 5. Arrhenius equation plot for adsorption of Cu(II) ions onto chitosan

represent a chemical reaction since chemisorption is specific and involves much stronger force than physical adsorption does [41]. Accordingly, the value of E_a in the present work confirms that the nature of adsorption onto chitosan is physical adsorption, which indicates that the chitosan can be easily regenerated for reuse.

Thermodynamic parameters: Thermodynamic behaviour of adsorption of Cu(II) ions on chitosan membrane was evaluated by the thermodynamic parameters – Gibbs free energy change (ΔG°), enthalpy (ΔH°) and entropy (ΔS°), which were calculated using the following equations [42,43]:

$$\Delta G^\circ = -RT \ln K_C \quad (7)$$

$$K_C = \frac{C_a}{C_e} \quad (8)$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (9)$$

where K_C is the distribution coefficient for adsorption and cellulose acetate and C_e are the equilibrium concentrations (mg/L), on the chitosan fragments and in solution, respectively. A plot of ΔG° *versus* temperature T (Fig. 6), is linear, from which ΔS° and ΔH° were computed from the slope and intercept and were equal to -0.0715 (kJ/mol K) and 5.316 (kJ/mol), in respective order. Negative values of ΔG° indicate the feasibility nature of the adsorption process and decrease in its value with the increase in temperature suggests that higher temperature makes adsorption easier. The positive value of ΔH° implies that adsorption phenomenon is endothermic. The magnitude of ΔH° may give an idea about the type of sorption. The heat evolved during physical adsorption is of the same order of magnitude as the heats of condensation, *i.e.* 2.1-20.9 kJ/mol, while the heats of chemisorption generally fall into a range of 80-200 kJ/mol. Moreover, the negative value of ΔS° suggests that the process is enthalpy-driven and reflects the affinity of the chitosan towards Cu(II) ions [44].

TABLE-4
ACTIVATION ENERGY AND THERMODYNAMIC PARAMETERS FOR ADSORPTION OF Cu(II) IONS ONTO CHITOSAN

E_a (kJ/mol)	ΔG° (kJ/mol)			ΔH° (kJ/mol)	ΔS° (kJ/mol K)
	298	313	323		
36.89	-15.9023	-17.2834	-17.6457	5.316	-0.0715

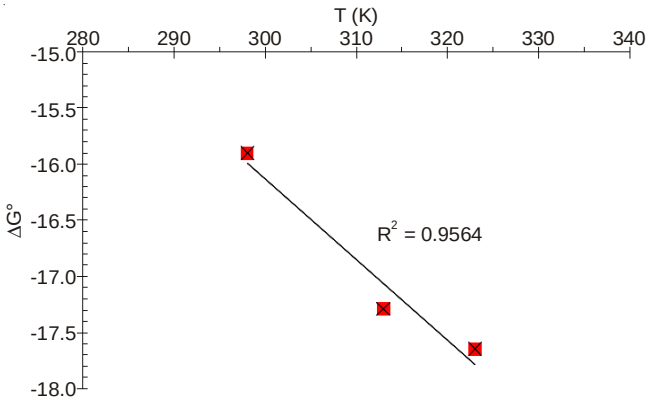

Fig. 6. Plot of Gibb's free energy change *versus* temperature for adsorption of Cu(II) ions onto chitosan

Conclusion

Porous chitosan membranes having exceptional affinity to Cu(II) ions have been successfully prepared. Polyethylene glycol and sodium chloride were used as porogens for the chitosan membranes. Batch adsorption studies proved that NaCl provided the best adsorption capacity compared to chitosan-PEG and chitosan membranes, for which q_m approached 500 mg/g which is much higher than values reported in the literature for different adsorbents. It was confirmed that monolayer adsorption took place, since the Langmuir model was obeyed. Adsorption increased with temperature until 303 K then decreased to further increase in temperature. Adsorption kinetics followed the pseudo-second order Lagergren's model and the rate constant was found to decrease with increase in temperature above 303 K, indicating exothermic adsorption. This was also proven from batch adsorption experiments of chitosan membrane. The value of E_a indicated physical adsorption, which allows reuse of the membrane after regeneration with acid. Negative values of $\Delta G°$ indicated the feasibility nature of the adsorption process and decrease in its value with the increase in temperature suggests that lower temperature makes adsorption easier. The positive value of $\Delta H°$ implied that adsorption phenomenon is exothermic. Moreover, the negative value of $\Delta S°$ suggests that the process is enthalpy-driven and reflects the affinity of the chitosan towards Cu(II) ions.

REFERENCES

1. A.G. Boricha and Z.V.P. Murthy, *J. Membr. Sci.*, **339**, 239 (2009).
2. L. Zhang, Y.-H. Zhao and R. Bai, *J. Membr. Sci.*, **379**, 69 (2011).
3. E. Guibal, *Sep. Purif. Technol.*, **38**, 43 (2004).
4. M. Morimoto, H. Saimoto and Y. Shigemasa, *Trends Glycosci. Glycotechnol.*, **14**, 205 (2002).
5. O.A.C. Monteiro Jr. and C. Airoldi, *Int. J. Biol. Macromol.*, **26**, 119 (1999).
6. F.L. Mi, C.Y. Kuan, S.S. Shyu, S.T. Lee and S.F. Chang, *Carbohydr. Polym.*, **41**, 389 (2000).
7. C. Peniche, W. Argüelles-Monal, H. Peniche and N. Acosta, *Macromol. Biosci.*, **3**, 511 (2003).
8. H.S. Kas, *J. Microencapsul.*, **14**, 689 (1997).
9. O. Felt, P. Buri and R. Gurny, *Drug Dev. Ind. Pharm.*, **24**, 979 (1998).
10. L. Ilium, *Pharm. Res.*, **15**, 1326 (1998).
11. S.V. Madihally and H.W.T. Matthew, *Biomaterials*, **20**, 1133 (1999).
12. B. Krajewska, *React. Funct. Polym.*, **47**, 37 (2001).
13. Z. Modrzejewska and W. Eckstein, *Biopolymers*, **73**, 61 (2004).
14. X. Zeng and E. Ruckenstein, *J. Membr. Sci.*, **117**, 271 (1996).
15. M.M. Naim, Preparation of an Adsorptive Membrane from Crude Chitosan obtained from Shrimp Shells, 6th International Conference on Role of Engineering Towards a Better Environment, RETBE, Alexandria, Egypt (2006).
16. X. Zeng and E. Ruckenstein, *J. Membr. Sci.*, **148**, 195 (1998).
17. L. Yang, W.W. Hsiao and P. Chen, *J. Membr. Sci.*, **197**, 185 (2002).
18. C. Liu and R. Bai, *J. Membr. Sci.*, **267**, 68 (2005).
19. A. Dufresne, J.Y. Cavaille, D. Dupeyre, M. Garcia-Ramirez and J. Romero, *Polymer*, **40**, 1657 (1999).
20. A. Isogai and R.H. Atalla, *Carbohydr. Polym.*, **19**, 25 (1992).
21. M. Hasegawa, A. Isogai, S. Kuga and F. Onabe, *Polymer*, **35**, 983 (1994).
22. S.Z. Rogovina, T.A. Akopova, G.A. Vikhoreva and I.N. Gorbacheva, *Polym. Degrad. Stab.*, **73**, 557 (2001).
23. Y.K. Twu, H.I. Huang, S.Y. Chang and S.L. Wang, *Carbohydr. Polym.*, **54**, 425 (2003).
24. L. Jin and R.B. Bai, *Langmuir*, **18**, 9765 (2002).
25. Y.-B. Wu, S.-H. Yu, F.-L. Mi, C.-W. Wu, S.-S. Shyu, C.-K. Peng and A.-C. Chao, *Carbohydr. Polym.*, **57**, 435 (2004).
26. Y. Wan, K.A.M. Creber, B. Peppley and V.-T. Bui, *J. Membr. Sci.*, **284**, 331 (2006).
27. D. Ren, H. Yi, H. Zhang, W. Xie, W. Wang and X. Ma, *J. Membr. Sci.*, **280**, 99 (2006).
28. M.L. Cervera, M.C. Arnal and M. de la Guardia, *Anal. Bioanal. Chem.*, **375**, 820 (2003).
29. M.M. Beppu, E.J. Arruda, R.-S. Vieira and N.N. Santos, *J. Membr. Sci.*, **240**, 227 (2004).
30. X. Zeng and E. Ruckenstein, *Ind. Eng. Chem. Res.*, **35**, 4169 (1996).
31. W. Shi, F. Zhang and G. Zhang, *J. Chromatogr. A*, **1081**, 156 (2005).
32. W.Q. Sun, G.F. Payne, M.S.G.L. Moas, J.H. Chu and K.K. Wallace, *Biotechnol. Prog.*, **8**, 179 (1992).
33. J.M.C.S. Magalhaes and A.A.S.C. Machado, *Talanta*, **47**, 183 (1998).
34. H.K. No and S.P. Meyers, *Rev. Environ. Contam. Toxicol.*, **163**, 1 (2000).
35. R.S. Juang, F.C. Wu and R.L. Tseng, *Bioresour. Technol.*, **80**, 187 (2001).
36. D.H.K. Reddy, Y. Harinath, K. Seshaiah and A.V.R. Reddy, *Chem. Eng. J.*, **162**, 626 (2010).
37. M.E. Russo, F. Di Natale, V. Prigione, V. Tigini, A. Marzocchella and G.C. Varese, *Chem. Eng. J.*, **162**, 537 (2010).
38. A. Sari, D. Citak and M. Tuzen, *Chem. Eng. J.*, **162**, 521 (2010).
39. S. Chowdhury and P. Saha, *Chem. Eng. J.*, **164**, 168 (2010).
40. M. Mohapatra, S. Khatun and S. Anand, *Chem. Eng. J.*, **155**, 184 (2009).
41. T.S. Anirudhan and P.G. Radhakrishnan, *J. Chem. Thermodyn.*, **40**, 702 (2008).
42. H.B. Senturk, D. Ozdes and C. Duran, *Desalination*, **252**, 81 (2010).
43. W.-T. Tsai and H.-R. Chen, *J. Hazard. Mater.*, **175**, 844 (2010).
44. C.X. Liu and R.B. Bai, Recovery of Bovine Serum Albumin (BSA) by Tripolyphosphate (TPP) Cross-Linked Chitosan Membrane, Presented at Recent Advances in Separation & Pharmaceutical Products Development, Nanyang Technological University, Singapore, 23 February 2005.