Molecularly Imprinted Polymers Chitosan-Glutaraldehyde for Monosodium Glutamate

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Abstract. Chitosan has been used as a functional monomer in the synthesis of molecularly imprinted polymers (MIP) for monosodium glutamate (MSG). MIP is made from a mixture of 5 g chitosan, 50 mg glutaraldehyde and 2 g MSG. MIP is formed as flakes and beads. MIPs are identified by the FTIR spectrum, SEM image and their adsorption capabilities. MIP flakes and beads have no structural differences if they are based on FTIR or SEM spectra, but MIP adsorption capacity of beads higher than flakes. Adsorption capacity of MIP flakes is 548 mg/g and MIP beads 627 mg/g.

1. Introduction

MIP is a technique for designing an artificial receptor that is a hollow polymer or molecular trace due to the disposal of mold molecules. Molecular Imprinting Technology (MIT) MIT is based on the formation of a complex between an analyte (template) and a functional monomer. In the presence of a large excess of a cross-linking agent, a three-dimensional polymer network is formed. After polymerization process, the template is removed from the polymer leaving specific recognition sites complementary in shape, size and chemical functionality to the template molecule. Usually, intermolecular interactions like hydrogen bonds, dipole–dipole and ionic interactions between the template molecule and functional groups present in the polymer matrix drive the molecular recognition phenomena. Thus, the resultant polymer recognizes and binds selectively only the template molecules [1].

MIP has a very high selectivity that can be applied to the extraction (separation) and the development of sensors. Solid Phase Extraction (SPE) is another important area of application of MIPs in analytical chemistry [2-7]. MIP for Solid-phase extraction (MISPE) has been applied both in on-line and off-line procedures. MIPs particles, used as selective sorbent materials, can be packed in an HPLC pre-column for the on-line mode and in a cartridge between two frits for the off-line mode [8].

One of the potentially functional materials is chitosan. Chitosan, a natural muco-polysaccharide with similar structure characteristics to cellulose, has been considered as one of thermo promising materials due to its biodegradability, biocompatible and non-toxicity [8–11]. Being a natural chiral compound, chitosan is also a multifunctional polymer containing large number of amine groups together with hydroxyl groups capable of assembling with template molecules such as amino acids through forming hydrogen bonds [12–14]. However, the poor physical/mechanical properties and high degree of swelling of chitosan in aqueous system limits its practical application in MIPs preparations. One way to overcome this limitation is the chemical cross-linking. Many of bi-functional reagents are...
frequently used as cross-linking agents such as glutaraldehyde [10], epichlorohydrin [15], sulfuric acid [16] and glyoxal [17]. In this study we described the preparation of new enantio-selective chitosan/glutaraldehyde molecularly imprinted resin in aqueous system with monosodium glutamate as a template molecule. The microscopic structure and the surface area of the resin were characterized. The method of determining MSG is done indirectly as ammonium (NH$_4^+$) by spectrophotometric.

2. Methods

2.1 Preparation of molecularly imprinted [19]
The molecularly imprinted MSG chitosan/glutaraldehyde was prepared as follows: 10 g of the previously prepared chitosan was dissolved in 100ml of 2% acetic acid aqueous solution at 60°C. After removal of the insoluble impurities by filtration, a 50ml of 0.5M MSG were added into the chitosan solution. The mixture was stirred for 2 h, and then 25ml of glutaraldehyde solution was added to the above solution and mechanically stirred for another 1 h. At the end of this period the resulted gelatinous resin was collected, washed with 0.01M HCl and dried in an oven at 60°C for 24 h. Then, the dry resin was ground in a mortar and sieved to get particles with a size about 120 mesh. To prepare the desired adsorbent for MSG separation from dilute aqueous solutions, the imprint template MSG molecules were leached out by stirring 3 g of MIP particles in 1000 ml of 0.1M HCl solution for 24 h. This step was repeated for three times to assure maximum extraction of imprint MSG molecules which may cross-link with the chitosan based network during the reaction to avoid its leaching in subsequent adsorption and extraction experiments.

2.2 Adsorption and extraction steps
In adsorption step, 0.1 g of imprinted polymer particles were added into a 25 ml aqueous solution containing 7.5 mg of MSG, the pH was adjusted to 6; 7; 8 value and stirred for 90 minutes. The solution was filtered and remaining particles on the filter were washed with a 50 ml aqueous solution at the same pH to remove the solution trapped within the polymer particles and non-specifically adsorbed solute. Extraction step was performed by replacing the washed polymer particles into a 100ml HCl (0.1–0.001M) solution with an adjusted pH, stirred for 90 minutes, at 150 rpm

3. Result and Discussion
MIPs are made by the same method but differ in their form, MIP A is a flake while MIP B is a form of beads. Structurally MIP A and B have no chemical structure difference, this can be seen from the FT-IR spectrum in Figure 1.

Figure 1. FT-IR MIP flakes (a) and MIP beads (b)

Based on the morphology of microscopic scanning results both MIPs also have no significant differences. This is evident from the SEM image in Figure 2. Therefore, MIP surface morphology
testing adsorptive MSG is only performed on MIP B alone, as shown in Figure 4.2, (c) and (d). From the SEM results it can be seen that the adsorption of MIP to MSG has certain patterns according to the shape of the MSG molecule.

![MIP morphology of Scanning Electron Microscopic (SEM) images for MIP flakes (a); MIP beads (b) and MIP beads that adsorb MSG (c) and (d).](image)

**Figure 2.** MIP morphology of Scanning Electron Microscopic (SEM) images for MIP flakes (a); MIP beads (b) and MIP beads that adsorb MSG (c) and (d).

The determination of the optimum contact time on MSG adsorption in MIP was done without pH adjusting, the contact time are 30, 60, 90 and 120 minutes. The purpose of this experiment is to determine the time required by MIP and MSG to reach adsorption equilibrium, it is the optimum time of MSG adsorption by MIP.
Based on the results shown in Figure 3, it is known that an increase in the number of MSG molecules adsorbed by MIP ranges from 60 to 90 minutes. This is due to the increasing diffusion of MSG molecules into the pores of MIP. The adsorption of MSG in the range of 90 to 120 minutes tends to decrease. This is caused by the desorption velocity is greater than the adsorption speed so that there is a decrease in the adsorption of MSG. In this experiment the equilibrium occurred at a contact time of 90 minutes with a 100% adsorption, equilibrium reach when the adsorption rate was equal to desorption.

The pH of the solution is an important factor in determining the optimum conditions of adsorption. The degree of acidity affects the solubility of the compound as well as the active groups present in MIP. So an experiment was conducted to determine the optimum condition of MSG molecule adsorption by MIP of chitosan-glutaraldehyde carried out at pH 6, 7 and 8 using MIP type A, B and C. MIP type C is MIP beads whose contain of glutaraldehyde more. Based on the results shown curve in Figure 4, it is known that both MIP A and B result in significant adsorption increase from pH 6 to pH 8. This indicates that the pH effect on MSG adsorption by both types of MIPs. Based on the results it is known that the optimum pH of MSG adsorption by both types of MIP is pH 8 with adsorption percentage of 89.91% (flakes) and 100% (beads). At a pH greater than pKₐ, of 4.25 to 9.10 monosodium glutamate will be negatively charged while chitosan as a functional monomer tends to be positively charged, so monosodium glutamate will tend to be absorbed in positively charged chitosan pores.
Figure 4. The curve of the relationship between pH and MSG mass is extracted by MIP types A, B and C.

Figure 5. The curve of the relationship between MSG concentration (ppm) to the extracted MSG mass (a) and % E (b).

The determination of MIP adsorption capacity on MSG is done by studying the effect of MSG concentration. This is done on the optimum conditions of adsorption at pH 8 and contact time 90 minutes. The adsorption capacity is expressed in mg of MSG per g MIP. For this, extraction for some MSG concentrations was then calculated by the extracted MSG mass or % E. In Figure 5 there was shown a relationship curve between the MSG concentrations to the MSG mass of the extraction. The extracted MSG mass would have remained if the adsorption capacity had been achieved, although MSG concentrations were increased and vice versa for % E, would decrease with increasing MSG concentrations. Based on Figure 5, the adsorption capacity can be determined that is 585 mg / g for MIP flakes and 627 mg / g for MIP beads. Thus MIP beads have a higher adsorption capacity than MIP flakes.

4. Conclusion

Selective MIP of MSG can be made from chitosan with glutaraldehyde as a crosslinker. Differences in the making of MIP textually ie flakes and beads do not produce the chemical structure difference based on FT-IR and SEM images. MIP flake adsorption capacity of 585 mg / g and MIP beads 627 mg/g at adsorption time 90 minutes at pH 8.
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References
1. Ramstrom, O., Mosbach, K., 1999, Curr. Opin. Chem. Biol., 3, 759–764.
2. Tamayo, F.G., Turiel, E., Martin-Esteban, A., 2007, J. Chromatogr. A, 1152, 32–40.
3. Lasáková, M., Jandera, P., 2009, J. Sep. Sci., 32, 788–812.
4. Pichon, V., 2007, J. Chromatogr. A, 1152, 41–53.
5. Caro, E., Marce, R.M., Borrull, F., Cormack, P.A.G., Sherrington, D.C., 2006, Trends Anal. Chem., 25, 143–154.
6. He, C., Long, Y., Pan, J., Li, K., Liu, F., 2007, J. Biochem. Biophys. Meth., 70, 133–150.
7. Turiel, E., Martin-Esteban, A., 2010, Anal. Chim. Acta, 668, 87–99.
8. Pichon, V., Chapuis-Hugon, F, 2008, Anal. Chim. Acta, 622, 48–61.
9. Lee, H.S., Hong, J., 2000, Chromatogr. A, 868, 189–196.
10. Kumar, M.N.V.R., Muzzarelli, R.A.A., Muzzarelli, C., Sashiwa, H., Domb, A.J., 2004, Chem. Rev., 104, 6017–6084.
11. Jameela, S.R., Jayakrishnan, A., 1995, Biomaterials, 16, 769–775.
12. Kim, J.H., Kim, J.H., Jegal, J., Lee, K.H., 2003, J. Membr. Sci. 213, 273–283.
13. Aburto, J., Borgne, S.L., 2004, Macromolecules, 37, 2938–2943.
14. Xia, Y.Q., Guo, T.Y., Song, M.D., Zhang, B.H., Zhang, B.L., Biomacromolecules, 6, 2601–2606
15. Su, H., Wang, Z.X., Tan, T.W., 2005, J. Chem. Technol. Biotechnol., 80, 439–444.
16. Tan, T.W., He, X.J., Du, W.X., 2001, J. Chem. Technol. Biotechnol., 76, 191–195.
17. Mukoma, P., Jooste, B.R., Vosloo, H.C.M., 2004, J. Power Sources, 136, 16–23.
18. Guinessi, L.S., Cavalheiro, E.T.G., 2006, Carbohydr. Polym., 65, 557–561.
19. Monier, M., El-Sokkary, A.M.A., 2010, Inter. J. Biol. Macromolecules, 47, 207–213.