Molecularly Imprinted Polymeric Beads for Decaffeination

Chin-I Lin Wen-Ping Chu[1] K Abraham Joseph Yu-Chi Wong
Chao-Kang Chang Yu Der Lee[1, *]

Intelligent Composites Department, Industrial Technology Research Institute, Hsin-Chu, Taiwan, 300, ROC
[1]Department of Chemical Engineering, National Tsing Hua University Hospital, Hsin-Chu, Taiwan, 300, ROC

Received 2 May 2003; Accepted 10 June 2003

Abstract

Caffeine, which is found in beverages like coffee, tea and cocoa etc., is the substance that keeps us awake and pronounced drug having many side effects. Unfortunately, the existing technology for caffeine removal will sweep away the pleasant aromas and flavors. A new methodology for molecular imprinting of caffeine has been developed successfully based on suspension polymerization. Most imprinted polymers are prepared by bulk polymerization that are then ground and sieved to produce particles of the desired dimensions. In this study, Methacrylic acid (MAA)-Ethylene glycol dimethacrylate (EDMA) copolymer beads imprinted with caffeine have been prepared by suspension polymerization using water as a dispersing medium. We have taken excess amounts of caffeine and MAA in order to compensate its lose due to the high solubility in water. We could obtain beads having average size of 96 μm with an adsorption amount of 0.32 μmol/g, which is relatively a high value. Unlike MIPs made by other methods the beads obtained in this technique has the advantages such as no complicated grinding and sieving, no use of expensive fluorocarbon solvent and high reaction temperature. The as made imprinted beads do have significant potential for future applications.

Keywords: Caffeine, Molecular imprinting, Suspension polymerization

Introduction

Molecular imprinting is a technique for creating recognition sites for an analyte molecules in a synthetic polymeric substrate. These artificially-generated recognition sites have their shapes, sizes and functionalities complementary to the analyte, and are capable of rebinding the analyte molecules in preference to other closely related structures. Molecular imprinting technology can be used in many different fields, like color chromatography, antibody/receptor complex simulant, catalysis/synthesis or biosensor, etc. As of today, the most popular method to produce imprinting polymer is bulk polymerization. The product has to go through grinding and sieving before use. When the product is used in color chromatography, further consideration is put on granule product shape. Bulk polymerization process has some disadvantages. The obtained product has relatively irregular shape. The process takes more time and efforts. Therefore, we hope to use suspension polymerization to directly prepare microbead products.

Molecular imprinting process has to start with reversible covalent or non-covalent reaction to distribute functional monomers around the template molecules. It is followed by addition of crosslinking agents for polymerization and highly crosslinked product is obtained finally. When imprinting molecule is removed, position with special identification characteristic is created.

For suspension polymerization or dispersion polymerization [1], the dispersed phase almost always contains water or highly polar solvent and hydrophobic monomers. However, such solvents are not suitable for some covalent and non-covalent imprinting mixtures, as they compete against monomers to react with template molecule [2]. Apart from this, high solubility for acidic monomer in water also affects polymerization. Water-soluble template molecule also affects the effect of imprinting polymer in use. Recently, researchers conducted unstable dispersion polymerization in polar solvents and template molecules [3,4]. This method results in irregular precipitates, but not regular microbeads. This method is only suitable for highly charged imprinted polymer as the highly charged template molecule has better competitiveness than solvent.

It was reported that replacement of the original polar solvent or water for dispersed phase with perfluoronated solvents [2,5]. Due to the inertness of perfluorocarbon, it is incompatible with organic solvent and does not compete with template molecule. But the high crosslinking density will affect the characterization of materials. Besides, it is hard to extract template molecule out of polymer.

* Corresponding author: Yu Der Lee
Tel: +886-3-713204; Fax: +886-3-5715408
E-mail: ydlee@che.nthu.edu.tw

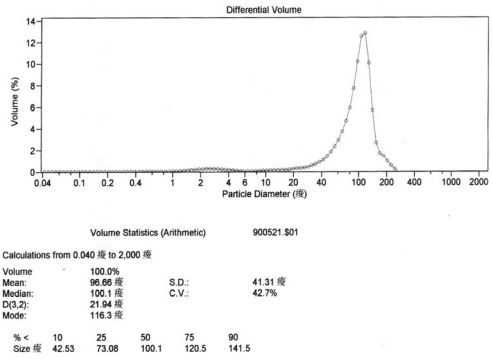


Figure 1. The suspension analysis result of MIP granule

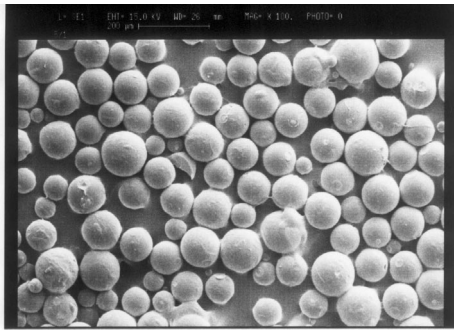


Figure 2. The MIP SEM obtained from suspension polymerization.

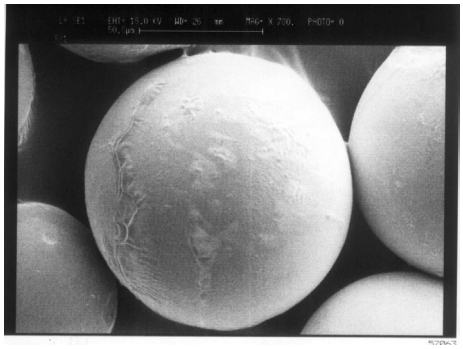


Figure3. The MIP SEM obtained from suspension polymerization. (700X)

Perfluorocarbon solvents and surfactants are harmful and expensive, so they are only suitable for use in laboratory research. They are not suitable for use in industrial production. Moreover, the availability of surfactants is also limited. Thus, we used water as solvent and polyethylene as stabilizer [6].

Experiment

Material

Caffeine (99 + %, Lancaster), theophylline and theobromine (3,7-dimethyl xanthine) were of technical grade purchased from TCI (Japan). methacrylic acid (MAA), ethylene glycol dimethacrylate (EDMA), 2,2'-Azobis-isobutryronitrile (AIBN), and polyvinyl alcohol (PVA) were purchased from TCI (Japan) and used as received. Methanol, acetonitrile, chloroform and acetic acid were purchased from Mallinckrodt.

Suspension Polymerization

Dissolved 0.6 grams of caffeine in 42 ml chloroform. Mixed 5.0 g of MAA, 21.54 g of EDMA and 250 mg of AIBN and to this solution was added 308 ml of water containing 1.43 grams of PVA. The solution mixture was kept at 60℃ under agitation of 300 rpm for 24 hours (MIP-S03). Other experiments have variables like：increasing agitation to 550 rpm and doubling caffeine (MIP-S04), reaction without caffeine (MIP-S05), 425 grams of initiator (MIP-S06). Distilled water and chloroform was used to rinse the sieved product. The template was extracted using a mixture of acetic acid and methanol (1:9 (v/v)) in a Soxhelet extractor for 24hr. The product was dried under vacuum.

Granule Size Distribution

Added appropriate amount of MIP into distilled water. Agitated the solution into suspension. Use BECKMAN COULTER LS200 to measure particle size distribution.

Adsorption Test

Prepared 5 ml of aqueous solution containing 200 ppm caffeine. Added 100 mg of MIP into the aqueous solution under ambient temperature then agitated the solution for 30 minutes. Use HPLC (Column: Alltima C18, Mobile phase: 85:15 (v/v) (DI Water: Acetonitrile), Flow rate: 1ml/min) to analyze concentration variation for caffeine solution. These results were used to calculate MIP adsorption effect. The calculation is carried out as follows:

Selectivity Experiment

Prepared an aqueous solution of caffeine, theobromine and theophylline each of 200 ppm. 15 ml of each solution was mixed together. Added 100 mg of MIP into 5 ml of this solution mixture and stirred the solution under ambient temperature for 60 minutes. HPLC was used to analyze concentration variations. The calculation method is as above.

SEM

Placed polymer powder in an alumna plate and used sputtering process to coat a 15 nm thin film of gold. S360 & AN10/85S were employed under 25 kV to obtain images to compare difference in size and surface porosity under different reaction conditions.

Results

Granule Size

From Figure 1, the MIP granule obtained from suspension polymerization is widely distributed. The average size is about 96 μm. Figure 2 and Figure 3 are the SEM graph of suspension-MIP. They show the bead shape granules. The size is about 100 μm. This observation is anastomotic to the analysis result.

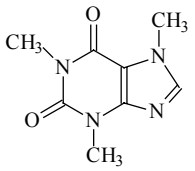
Comparison of different methods of polymerization

Table 1 is the comparison Figure of MIP adsorption to caffeine and its analogs (Theobromine, Theophylline) obtained through different polymerization methods. From the table, it is evident that for the bulk polymerization caffeine adsorption is slightly higher than suspension polymerization. As for the

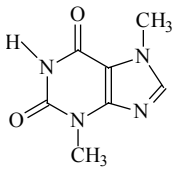
Table 1: The comparison Figure of MIP adsorption to caffeine and its analogs (Theobromine, Theophylline) obtained through different polymerization methods.

Method	Absorb [S] μmol/g			Selectivity (β)	
	CAF	Theobromin	Theophylline	Theobromine	Theophylline
Bulk†	0.336 ± 0.009	0.099 ± 0.011	0.131 ± 0.008	0.295	0.390
Suspension	0.320 ± 0.001	0.069 ± 0.006	0.187 ± 0.007	0.216	0.584

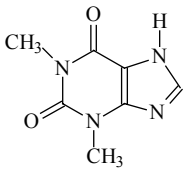
Selectivity (β) = $[S]_{(analogous)}$ / $[S]_{(imprint)}$



caffeine



theobromine



theophylline

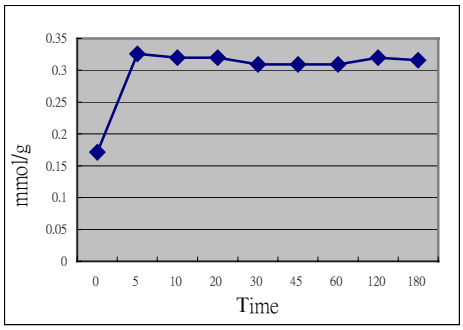


Figure 4. Adsorption amount vs. Time

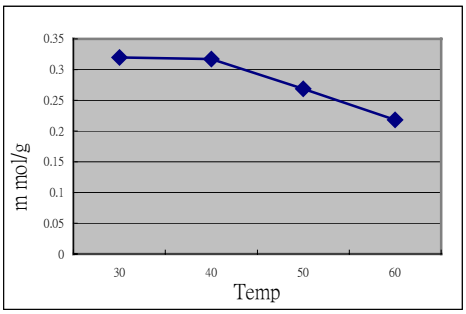


Figure 5. Adsorption amount vs. Temperature

selectivity test, both polymerizations have their fortes and flaws. However, the interesting matter is that the difference between granule structure or size of Theobromine and Theophylline are not very much. They are differentiated only by the position of N-methyl group. Yet, the experiment result shows that Theophylline has a higher adsorption rate than that of Theobromine in both methods. It is very possible because Theophylline has a higher K_b value than that of Theobromine. For slightly acidic monomers, the affinity is higher.

Adsorption Time & Adsorption Temperature

From Figure 4, the adsorption equilibrium is reached at 5 min. This is due to the fact that adsorption only happens at the MIP surface. The affinity adsorption of caffeine is dependent on the hydrogen bond formation inside the MIP network. When the ambient temperature rises, the hydrogen bond will be destructed and weakened. It will lower the adsorption quantity (Figure 5).

Conclusion

We have maintained the conditions for suspension polymerization by taking excess amounts of caffeine and MAA in spite of its high solubility in water. We could obtain beads having average size of 96 μm. Rebinding experiments using 200ppm caffeine solution showed that the adsorption balance is reached within 5 min. and the adsorption amount was found to be 0.32 μmol/g. When the ambient temperature rises, the hydrogen bond between the template and MIP is weakened and caused a lower adsorption rate. Unlike MIPs made by other methods the beads derived from suspension polymerization has the advantages such as no complicated grinding and sieving, no use of expensive fluorocarbon solvent and high reaction temperature. These advantages do have significant potential for future applications.

References

[1] Rimmer S., "Synthesis of Molecular Imprinted Polymer Networks", *Chromatographia*, 46: 470-474, 1998.

[2] Andrew G. M. and Klaus M., "Molecularly Imprinted Polymer Beads: Suspension Polymerization Using a Liquid Perfluorocarbon as the Dispersing Phase", *Anal. Chem.*, 68(21): 3769-3774, 1996.

[3] Sellergren, B., *Chromatogr. A*, 673: 133-141, 1994.

[4] Sellergren, B., *Anal. Chem.*, 66:1578-1582, 1994.

[5] Richard J. A. and Klaus M., "Molecularly imprinted polymers by suspension polymerisation in perfluorocarbon liquids, with emphasis on the influence of the porogenic solvent", *Journal of Chromatography A*, 787:55-66, 1997.

[6] Matsui J., Fujiwara K., Ugata S. and Takeuchi T., "Solid-phase extraction with a dibutylmelamine-imprinted polymer as triazine herbicide-selective sorbent", *Journal of Chromatography A*, 889: 25-31, 2000.