Synthesis and Evaluation of a Beta Cyclodextrin-Based Molecularly Imprinted Copolymer

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ABSTRACT: A new molecularly imprinted copolymer of 2-hydroxy ethyl methacrylate (HEMA) and beta-cyclodextrin-coupled HEMA is synthesized. The copolymer is studied for its interaction with a pair of steroids, namely, cholesterol and testosterone. The molecularly imprinted copolymer is found to absorb the print molecule by several fold compared to an imprinted poly(HEMA). The enhanced absorption capacity is attributed to the presence of beta cyclodextrin moieties in the copolymer. The imprinted copolymer could be used as an adsorbent matrix. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 70: 15–18, 1998

Key words: molecular imprinting; beta cyclodextrin; copolymer

INTRODUCTION

Recent years have received considerable attention in molecular imprinting as an elegant and simple approach to create spatial memory of print (template) molecules in extensively crosslinked polymers like poly(methacrylic acid).[1–4] These types of imprinted polymers have been used in applications as diverse as the chromatographic separation of chiral species, antibody mimics, protein separation, and sensor components.[4–8]

To create recognition sites in synthetic polymers, commonly, 2 distinct approaches have been used. In one method, which is designed by Wulff et al.,[3] initially, a liable covalent bond is formed between the functional groups of the template monomers. After the polymerization, the template molecule is removed by processes like hydrolysis.

The approach of Mosbach et al.[1] is based on the prearrangement of the print molecule and functional monomers prior to the polymerization. After the polymerization, the print molecule is removed, and the resultant polymer contains sites complementary to the print molecule both in shape and size. Such polymers can bind the print molecules with remarkable degree of selectivity.

In the near future, molecularly imprinted polymers (MIPs) may replace antibody and enzyme-based assay systems. The remarkable advantages of MIPs compared to biosystems like antibodies, are their reusability, thermal stability, and compatibility with organic phases. In spite of all these advantages, MIPs are poor in certain aspects. The most important drawbacks of these systems is perhaps their relatively low absorption capacity.[9]

Appropriate modification of functional monomer prior to the imprinting could be useful in improving the adsorption capacity. Beta cyclodextrin and their derivatives are well known for their ability to form inclusion complexes with a variety of compounds.[10–13] Cyclodextrins containing MIPs may have enhanced absorption capacity in addition to the selectivity acquired by the imprinting. To the best of our knowledge, MIP based on cyclodextrin with a view to improve the adsorption capacity has not been reported. This article describes the synthesis and evaluation of a copoly-

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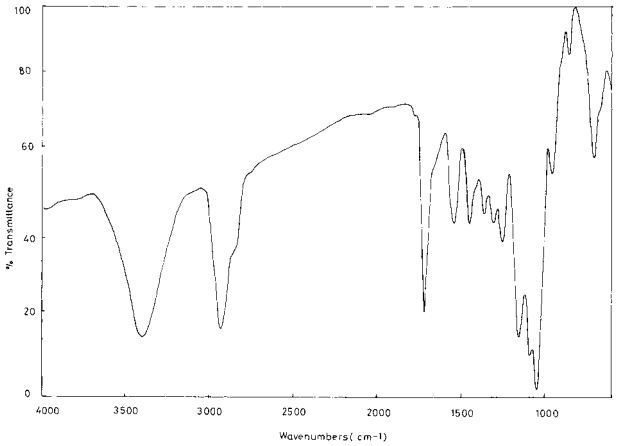

Figure 1 IR spectrum of the beta-cyclodextrin-based copolymer.

mer of HEMA and beta-cyclodextrin-coupled HEMA imprinted for cholesterol.

EXPERIMENTAL

Beta cyclodextrin, cholesterol, and testosterone were procured from Sigma Chemicals, St. Louis, MO and used as received 2-hydroxy ethyl methacrylate (HEMA) and ethylene glycol dimethacrylate, obtained from Fluka, AG, Germany were used after removing the stabilizer. Other reagents (analytical grade or chromatographic grade) were obtained from Spectrochem, Bombay, India.

Instrumental

The infrared (IR) spectra were recorded on a model impact 410 Fourier transform IR (FTIR) spectrophotometer (Nicolet Inc, Madison, WI) there were 50 scans. A Waters Assoc Inc (Milford, CT) consisting of a model 510 solvent delivery pump, a Reyodyne model 7725 I injector, and a model 486 tunable absorbance detector was used. The chromatographic estimation of cholesterol and testosterone were estimated as reported elsewhere.[14]

Synthesis of Molecularly Imprinted Copolymer

The synthesis and evaluation of beta-cyclodextrin-(BCD)-coupled HEMA has been detailed elsewhere.[15] The modified monomer (BCD–HEMA) is copolymerized with HEMA. 5 g of BCD–HEMA is mixed with 1 g of HEMA and 7 g of EGDMA. 450 mg of cholesterol was added to this mixture. Added about 5 mL of chloroform to the mixture to dissolve cholesterol. The mixture was then subjected to polymerization by thermal imitation in the presence of azo-bis-iso-butyro nitrile. The copolymer formed was powdered and extracted extensively with chloroform to remove the print molecule (cholesterol). The complete removal of the print molecule was ensured prior to further study using the polymer. In a similar fashion, a copolymer without imprinting was also prepared to serve as control.

Interaction with Steroids

About 50–60 mg of the polymers were placed in a mixture of cholesterol and testosterone in methanol at room temperature (30 ± 1°C) under static condition. After 2 h, the polymer was collected and then dried in an vacuum oven at 50°C. The polymers were then subsequently transferred into conical flasks containing 10 ml of hexane to extract the adsorbed steroids. 100 μL of these solutions were used for the chromatographic estimation. The experiments were performed in triplicate.

RESULTS AND DISCUSSION

Figure 1 shows the IR spectrum of the copolymer. The spectrum shows strong peaks around 3500, 1715, and 1020 cm^{-1}, respectively. Figures 2 and

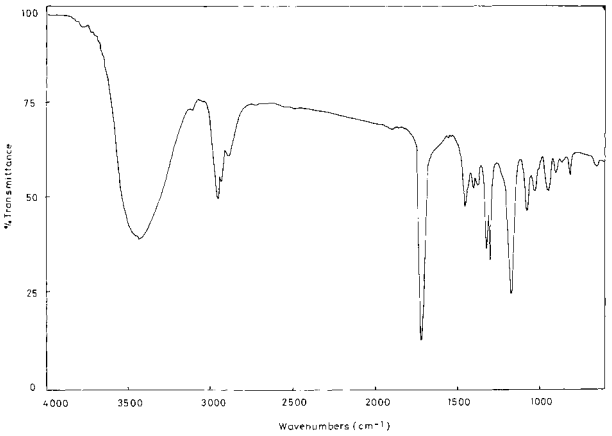

Figure 2 IR spectrum of poly(HEMA).

3 depict the spectra of poly(HEMA) and BCD. The typical feature of BCD is the strong peak centered around 1020 cm^{-1}. It can be seen that the spectrum shown in Figure 1 has all the features of the spectra depicted in Figures 2 and 3, indicating that the copolymer of HEMA and BCD–HEMA is formed.

MIPs are well known for their specificity in interaction with the print molecules. The efficiency of imprinting is evaluated by studying the interaction of MIPs with another molecule of close resemblance in geometry and structure with the print molecule. The uptake of this molecule should be less, compared to the extent of absorption of the print molecule. In this study, since the polymer is imprinted for cholesterol, testosterone is taken as molecule of structural similarity with that of cholesterol. To evaluate the efficiency in imprinting, the polymer is placed in a mixture of cholesterol and testosterone.

Table I summarizes the extent of uptake of these 2 molecules by the imprinted copolymer (MIP copolymer) and the control copolymer. The MIP copolymer absorbs a considerably higher amount of cholesterol compared to the control copolymer. The extent of uptake of testosterone is also in good proportion. We have recently synthesized a HEMA-based MIP imprinted for cholesterol.[14] The uptake of cholesterol by 100 mg of this polymer was about 0.4 mg, and the extent of absorption of testosterone was about 0.015 mg. In comparison with these figures, the absorption capacity of the new MIP copolymer is several fold more. Interestingly, the uptake of testosterone by the MIP copolymer is also relatively high. A striking feature is that the control copolymer also shows a more or less similar trend. The extent of

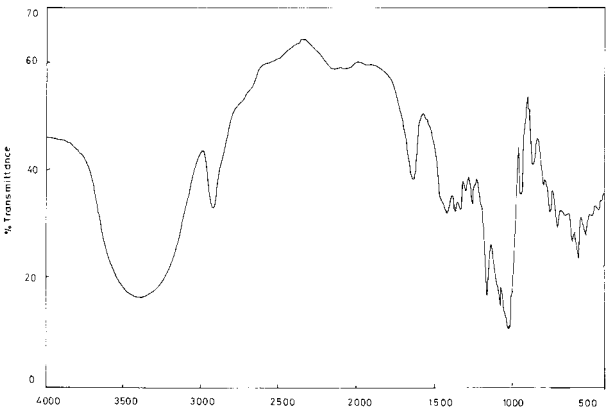

Figure 3 IR spectrum of beta cyclodextrin.

Table I Extent of Absorption of the Steroids by the Imprinted Copolymer and Control Copolymer

	Amount Absorbed (100 mg)	
Component	MIP Copolymer	Control Copolymer
Cholesterol	4.68 ± 0.05 mg	1.76 ± 0.06 mg
Testosterone	1.37 ± 0.04 mg	1.43 ± 0.03 mg

absorption of cholesterol by the control copolymer is 1.76 mg, which is several fold higher than the quantity absorbed by homopoly(HEMA).[14] The enhanced absorption of both cholesterol and testosterone by the control copolymer is attributed to the presence of BCD moieties in the copolymer.

It is well known that BCD forms inclusion complexes with steroids.[16,17] The extent of absorption of testosterone is nearly equal in the MIP copolymer and the control copolymer, reflecting that the absorption is due to the BCD entities. The nearly identical values show further, that imprinting does not created any affinity sites in the copolymer towards testosterone. On the other hand, the uptake of cholesterol by the MIP copolymer is considerably higher than the absorption capacity of control copolymer. The increased affinity towards cholesterol could be assigned to the imprinting.

It is apparent from the absorption data that the ability of MIP copolymer to discriminate 2 molecules of close resemblance is reduced. That is, the selectivity in absorption is less, compared to the poly(HEMA) imprinted for cholesterol.[14] This aspect, in fact, points out that the copolymer is not an ideal system for the construction of sensor's component in which selectivity is the prime factor. However, BCD is a molecule with the ability to interact with a wide class of compounds like amino acids, drugs, lipids, and steroids, and the BCD copolymer of the nature reported here could be tailored with improved absorption capacity. Such polymers can be used as an adsorbent matrices in several applications, including chromatography. These types of novel matrices would be ideal for sample recovery in a preparative scale.

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