Molecularly Imprinted Polyacrylic Acid Containing Multiple Recognition Sites for Steroids

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ABSTRACT: The preparation and evaluation of a molecularly imprinted polymer (MIP) based on acrylic acid and ethylene glycol dimethacrylate containing multiple recognition sites for steroids is discussed. The ability of the polymer to bind simultaneously three steroids, namely cholesterol, testosterone, and hydrocortisone, is demonstrated. These types of novel MIPs with multiple binding sites could be useful in the design of sensing elements capable of detecting more components at a time. Such polymers could also be used as stationary phases in chromatography with broad separation potential as well as sorbents in solid-phase extraction. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 82: 889–893, 2001

Key words: MIP; steroids; multiple recognition sites

INTRODUCTION

Molecular[1] imprinting has received considerable attention recently as a simple and elegant approach to create memory sites of print molecules in extensively crosslinked synthetic polymers.[1–3] Two essentially different approaches, namely covalent and noncovalent, have been reported for the preparation of molecularly imprinted polymers.[1–3] The first method uses reversible covalent binding of the functional groups of the print molecule and those of the complementary monomers that become fixed in their spatial arrangement on polymerization. The second method involves the self-assembly of monomers and the template by noncovalent interactions that on polymerization form the basis of the recognition sites. Over the years, several molecularly imprinted polymers (MIPs) were synthesized and used in areas as diverse as in chromatography, catalysis, synthetic chemistry, antibody mimics, sensors, etc.[4–10] The most widely studied area in the utilization of MIPs is in chromatography. In recent years, remarkable stereo- and regeioselective separation was achieved by using MIPs.

So far, reported MIPs contain memory sites for only a single compound. The separation matrices developed on the basis of these types of polymers can have only restricted utility. A MIP containing a memory site for more than one compound would be useful in addressing the separation and detection of more compounds at a time. Recently, we reported the preparation and evaluation of a MIP-containing recognition site for two compounds.[11] This polymer was found to bind these two compounds at one time. This report is a further extension of our work on the preparation and evaluation of acrylic acid based MIP based on noncovalent interaction-containing recognition sites for three compounds, namely cholesterol (CH), testosterone (T), and hydrocortisone (HY).

EXPERIMENTAL

Acrylic acid (AA) and ethylene glycol dimethacrylate (EGDMA) were obtained from Fluka, Buchs,

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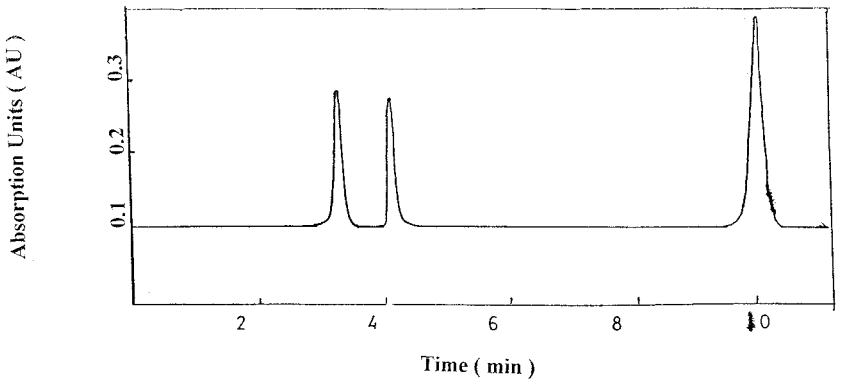

Figure 1 Chromatogram of a mixture of hydrocortisone, testosterone, and cholesterol.

Switzerland. CH, T, and HY were procured from Sigma Chemicals, St. Louis, MO. Other chromatographic grade solvents were from SD Fine Chemicals, Bombay, India.

Preparation of the Polymers

One gram of AA, 4 g EGDMA, and 250 mg cholesterol were dissolved in 5 mL dichloromethane. The contents were then transferred into a polyethylene tube, flushed with nitrogen, and sealed. The polymerization was initiated with gamma rays as reported elsewhere[12] (Panoramic Batch irradiator, BARC, Bombay, India). The mixture was irradiated to a total dose of 0.5 Mrad at a rate of 0.2 Mrad/h. In a similar fashion, polymers were prepared in the presence of testosterone and hydrocortisone. MIP containing the memory sites for CH, T, and HY was prepared similarly by dissolving appropriate amounts of the print molecules. A polymer without adding the print molecules was also prepared to serve as control. After the polymerization, the polymers were collected and crushed into powder. Particles (200–300 u sizes) were extracted with dichloromethane to remove the print molecules. Extracts were periodically subjected to chromatographic analysis. Extraction was repeated until there was no absorption corresponding to CH, T, and HY.

Interaction Studies of the Polymers with the Print Molecules

MIPs (50 mg) imprinted for CH (PCH), T (PT), HY (PHY), and their mixtures (PM) were placed in dichloromethane solutions of the print molecules (concentration of the solution was 0.1 mg/ml). A control polymer was also placed in the solution containing the components. After 90 min, the polymers were collected from the solutions by filtration. The polymers were then each placed in 10 mL dichloromethane and heated to boiling, and the dichloromethane was collected. The extraction was continued for at least three times and the combined extract was evaporated to dryness. The residue was dissolved in acetonitrile (10 mL)

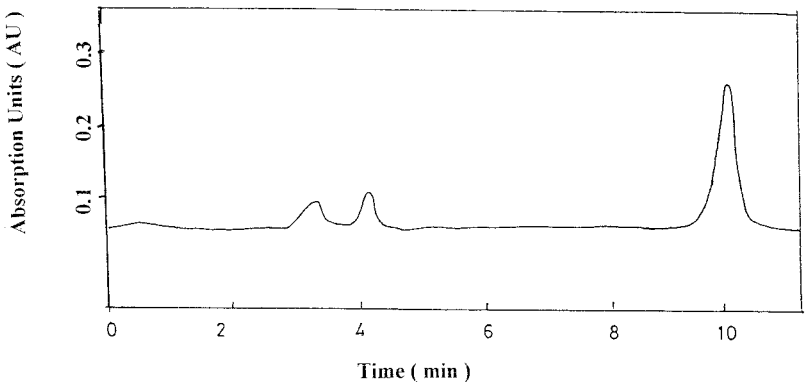

Figure 2 Chromatogram of the extract of MIP imprinted for cholesterol.

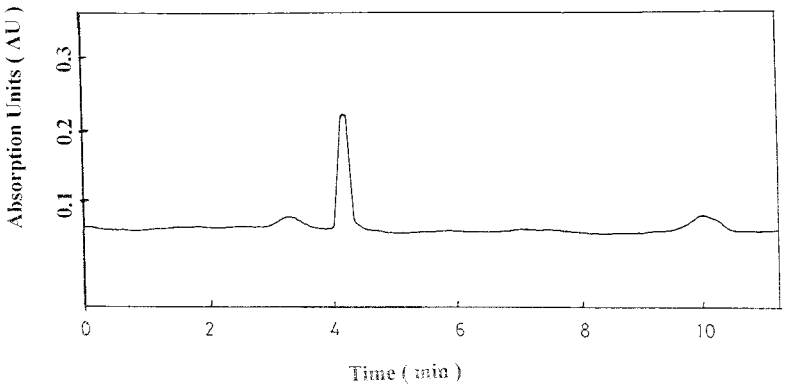

Figure 3 Chromatogram of the extract of MIP imprinted for testosterone.

and subjected to chromatographic analysis. The injection volume was 100 μl.

Instrumental

An HPLC system consisting of a Waters Assoc. Inc. Model 510 solvent delivery pump, Model 2725 Reodyene injector, and Model 486 tunable absorbance detector was used for the analysis. A μ-bondapak C_{18} column (Waters Assoc.) in conjunction with acetonitrile as mobile phase was used for the separation of the components. HY and T were detected at 241 nm, whereas CH was detected at 206 nm. The calibration plots were constructed for each of these compounds between the concentration and respective peak heights. These plots were used for the quantification.

RESULTS AND DISCUSSION

Figure 1 depicts a typical chromatogram of a standard mixture of the three steroids. Under the present chromatographic conditions, HY has a retention time of 3.4 min, the retention time of T is 4.3 min, whereas CH elutes at 10 min. Figures 2, 3, 4 and 5 show the chromatograms of the extracts of PCH, PT, PHY, and PM, respectively. It is apparent that PCH, PT, and PHY absorb predominantly the respective print molecules from the mixture. Interestingly, PM absorbs all three compounds to an appreciable level. Table I summarizes the amount of the steroids absorbed by the polymers, including the control. It can be seen that PT absorbs more HY than the control polymer. Similarly, PHY also absorbs higher amounts of T than the extent of absorption of this molecule by the control polymer. This aspect may be attributed to the resemblance of these molecules, particularly in their shape and sizes. PCH absorbs less T and HY and these values are comparable to the respective amounts absorbed by the control polymer. This similarity possibly indicates that the absorption of the molecules other than the print molecules is due to nonspecific interactions.

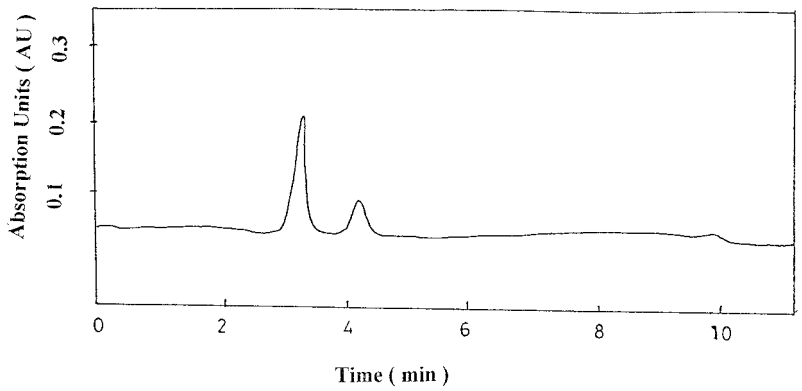

Figure 4 Chromatogram of the extract of MIP imprinted for hydrocortisone.

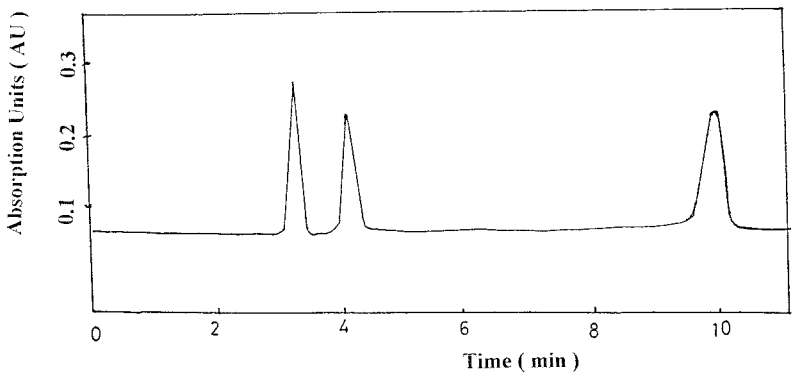

Figure 5 Chromatogram of the extract of MIP imprinted for cholesterol, testosterone, and hydrocortisone simultaneously.

The extent of uptake of the steroids by the polymers varies on the order of HY > T > CH. HY is relatively more polar. This could lead to more interaction between HY and monomers. Such enhanced interaction would create more affinity sites in the polymer. The same mechanism may be operative in the case of T also, considering the fact that T is relatively more polar than CH. PM also follows the same trend shown by the polymers imprinted for a single compound. In other words, the extent of absorption of the steroids by PM is also on the order of HY > T > CH.

It is reasonable to presume that equilibrium absorption can be considered as a measure of the number of recognition sites. In that sense, the population of memory sites of the steroids in PM may be estimated by using the equilibrium absorption parameters of HY, T, and CH. The percentage of memory sites in PM for HY, T, and CH may be represented as N_1, N_2, and N_3, respectively. These parameters in terms of equilibrium absorptions may be expressed as

$$N_1 = Q_1/Q_2 \quad (1)$$

Table I Extent of Uptake of Steroids by the Polymers

	Amount Adsorbed by 100 mg Polymers (μg)		
Polymer	HY	T	CH
Control	36 ± 3	28 ± 2	22 ± 2
PCH	48 ± 4	40 ± 3	298 ± 6
PT	60 ± 3	331 ± 4	38 ± 2
PHY	360 ± 7	65 ± 3	42 ± 2
PM	166 ± 4	127 ± 5	94 ± 4

$$N_2 = Q_3/Q_4 \quad (2)$$

$$N_3 = Q_5/Q_6 \quad (3)$$

where Q_1 is the corrected equilibrium absorption of hydrocortisone in PM (corrected absorption = quantity absorbed by MIP − quantity absorbed by control); Q_2 is the corrected equilibrium absorption of HY in MIP imprinted for HY; and Q_3 and Q_4 are the corrected equilibrium uptake of T by PM and the MIP imprinted for T. Similarly, Q_5 and Q_6 are the absorption of CH in PM and the MIP imprinted for CH. The population of affinity sites of the steroids in PM based on the above equations are summarized in Table II. PM has more affinity sites for HY than T and CH, which can be attributed to the enhanced interaction between HY and functional monomers in the prepolymerization mixture as mentioned earlier.

Table III shows the equilibrium water uptake by the polymers. This parameter is more or less equal for all polymers, including the control polymer. It is expected in the sense that the polymers are highly crosslinked and largely due to this higher water uptake cannot be expected as in less crosslinked hydrogel. The extent of water uptake, however, indicates that structural features of the

Table II Affinity Sites in PM Estimated Using Equilibrium Adsorption

Compound	Number of Sites (%)
CH	26
T	33
HY	40

Table III Equilibrium Water Content in the Polymers

Polymer	Quantity of Water Adsorbed (%)
Control	2.33 ± 0.02
PCH	2.41 ± 0.01
PT	2.39 ± 0.02
PHY	2.51 ± 0.03
PM	2.49 ± 0.04

polymers, particularly the extent of crosslinking, is nearly identical.

The biological and clinical importance of steroids are well known. These compounds are used in hormone therapy and as contraceptives. Steroids have also been used illegally in sports. Considering the importance of these molecules, the development of rapid, simple, inexpensive, and sensitive techniques have been emphasized. Few studies have been reported using steroids as substrates in molecular imprinting.[11,13,14] However, all these studies report the use of a single steroid as print molecule. The techniques capable of detecting more steroids simultaneously would be highly advantageous in this regard. The procedure based on the MIP discussed here is interesting.

The impact made by the MIPs in separation is largely due to their selectivity, which is essential in the isolation of a specific molecule in the pure form. However, the utility of such matrices is severely restricted considering the fact that it can be used in the separation or isolation of a single compound. Matrices containing multiple sites could be used in addressing the separation or isolation of more than a compound at a time. MIPs of this nature could also be useful in the design of sensing elements capable of detecting more components simultaneously.

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