On the Application of Molecularly Imprinted Poly(HEMA) as A Template Responsive Release System

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ABSTRACT: 2-Hydroxy-ethyl-methacrylate-based molecularly imprinted polymer imprinted for hydrocortisone was found to absorb a considerable amount of testosterone. The release of testosterone to water was found to be very slow. Its release was, however, rapid in the presence of the print molecule (hydrocortisone). This opens up the possibility of developing drug release systems capable of modulating the release with respect to the presence of specific molecules. Such systems could be used in the release of steroids and peptides. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 71: 1819–1821, 1999

INTRODUCTION

Molecular imprinting has received considerable attention as a simple and elegant method to create recognition sites for template molecules in crosslinked synthetic polymers. The imprinted polymers have been investigated in areas as diverse as chromatography, catalysis, and synthetic chemistry.[1–3] The recent report on the use of molecularly imprinted polymer (MIP) as a plastic antibody has evoked much interest in the design of sensors using MIPs.[4] Over these years, several MIPs having affinity sites towards drugs, steroids, amino acids, and sugars have been prepared and evaluated.[5–10] Interestingly, most of these studies deal with MIPs specifically designed to interact with a given molecule. It is perhaps possible to use MIP as a guest-responsive controlled releasing system. Often, MIP absorbs a considerable amount of molecules of close resemblance to the print molecule. This feature of MIP can be explored as a release matrix responsive to the concentration of the print molecule. This possibility is demonstrated in this communication using 2-hydroxy-ethyl-methacrylate-based MIP imprinted for hydrocortisone (Hy) and testosterone (T) as a molecule of close resemblance to Hy.

EXPERIMENTAL

Chemicals

2-hydroxy ethyl methacrylate (HEMA), ethyleneglycoldimethacrylate (EDMA), Hy, and T were obtained from Sigma Chemicals, St. Louis, MO, USA. Other chromatographic grade solvents were procured from Sisco Chemicals, Bombay, India.

Instrumental

A Waters Assoc Inc. (Milford, MA, USA) high-performance liquid chromatographic system consisting of a model 510 solvent delivery pump, a model 7725 thermodyne injector, and a model 486 tunable absorbance detector was used for the chromatographic estimation of the components. A μ-porasil column in conjunction with a mixture of chloroform and isopropanol (11 : 1 *V*/*V*) as mobile phase at a flow rate of 1 mL min was used for the separation of the components. The column effluents were monitored at 241 nm.

Preparation of the Polymer Imprinted for Hy

For the initiation of the polymerization, the gamma irradiation method used is one we have

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used extensively for the preparation of MIPs.[11] 1 g HEMA, 4 g EDMA, and 100 mg Hy were mixed in beaker. 6–8 mL of chloroform was added to dissolve the ingredients. The content was transferred to a polyethylene tube, flushed with nitrogen, and then subjected to gamma irradiation from a Co^{60} source to a total dose of 0.3 Mrad at the rate of 0.1 Mrad h. In similar fashion, a polymer without the print molecule (Hy) was also prepared to serve as a control. After the polymerization process, the contents were powered and extensively extracted with acetonitrile to remove the print molecule. The complete removal of the print molecule was ensured by analysing the extract chromatographically.

Interaction of the Polymers with Hy and T

MIP and control polymers were placed in solutions of Hy and T prepared in chloroform containing about 0.5 mg of the compounds. After 2 h, the polymers were collected by filteration, dried, and then extracted by keeping in 10 mL chloroform for 1 h at an elevated temperature (50°C). The solutions were then subjected to chromatographic analysis.

Release of T from the MIP to Water

50 mg of MIP collected from T solution was placed in 10 mL of triple-distilled water at room temperature (30°C). At regular time intervals, the solution was analyzed chromatographically to estimate the amount of T released.

Release of T to Water in the Presence of Hy

50 mg of MIP loaded with T was placed in 10 mL of the solution containing Hy (50 μg mL). At regular time intervals, the solution was analyzed chromatographically to estimate the T released.

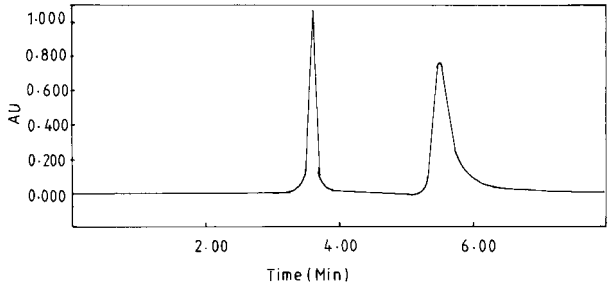

Figure 1 Chromatographic trace of a mixture of T and Hy.

Table I Extent of Uptake of T and Hy by MIP and the Control Polymer

Compound	Amount Absorbed by 100 mg MIP	Amount Absorbed by 100 mg of Control Polymer
Testosterone	175 ± 4 μg	36 ± 2 μg
Hydrocortisone	296 ± 5 μg	46 ± 3 μg

RESULTS AND DISCUSSION

Figure 1 illustrates the chromatogram of a mixture of T and Hy. Under the present chromatographic conditions, T elutes at 3.62 ± 0.05 min, and Hy has a retention time of 5.52 ± 0.06 min. The extent of uptake of these components by the polymers was estimated from the peak areas corresponding to these retention times.

Table I summarizes the extent of uptake of Hy and T by the MIP imprinted for Hy and the control, respectively. Compared to the control polymer, MIP absorbs a considerable amount of Hy, indicating the creation of recognition sites for Hy in the crosslinked polymer matrix. One striking feature is the extent of uptake of a remarkable quantity of T by the MIP, though it is not imprinted for it. The molecular size of T is close to Hy, which may be the factor for the higher uptake of this molecule by the MIP. Several other studies have also shown the enhanced absorption of species of close resemblance to the print molecules. For instance, Cheong et al. have shown a higher absorption of estradiol by MIP imprinted for testosterone.[12]

The time-dependent release of T from the MIP to water in the absence of Hy is summarized in Table II. It is apparent that even after 24 h, nearly 55% of the compound is retained in the MIP.

Table II Time-Dependent Release of T from the MIP into Water

Time (h)	Amount of T Released (per 100 mg of Polymer)
1	28 ± 3 μg
2	46 ± 2 μg
4	61 ± 1 μg
24	79 ± 2 μg

Table III shows the release of T from MIP in the presence of Hy. It can be seen that T is released rapidly in the presence of Hy and almost all T is released within a period of 4 h. The rapid release of T from the MIP in the presence of Hy can be attributed to the affinity of the polymer towards Hy since it is imprinted for Hy, and due to this, T is replaced by Hy.

The polymer is then collected and extracted with 10 mL of chloroform and subsequently subjected to chromatographic analysis. The chromatographic trace depicted in Figure 2 clearly shows the presence of Hy in the form of a peak centered around 5.5 min, the retention time of Hy (see Fig. 1). The peak area corresponds to 290 μg (per 100 mg of polymer) of Hy, which is close to the extent of uptake of Hy by the MIP (see Table I). Another notable aspect is the absence of any peak around 3.6 min, the retention time of T indicating the complete removal of T from the polymer. This data confirm the replacement of T by Hy. In the absence of Hy, the release is largely governed by thermodynamic features mainly the solubility of T in the medium as well as the extent of interaction of T with the polymer. In fact, T is absorbed in the recognition sites of Hy created in the polymer matrix by imprinting. These sites naturally have more affinity towards Hy; due to this, in the presence of Hy, T is rapidly replaced.

The data summarized here show that the release of T to the medium is slow in the absence of Hy. However, its release is relatively rapid in the presence of the print molecule (Hy), which can be assumed due to higher affinity of the polymer towards the print molecule. This aspect opens up the feasibility of using MIPs as a stimuli-responsive release systems. The stimuli can be the print molecules. The MIPs could be tailored for the release of components like steroids and peptides. The systems could also be used in the construction of simple sensors.

Table III Time-Dependent Release of T from MIP to Water in the Presence of Hy

Time (h)	Amount of T Released (per 100 mg of Polymer)
1	123 ± 3 μg
2	166 ± 4 μg
4	171 ± 3 μg

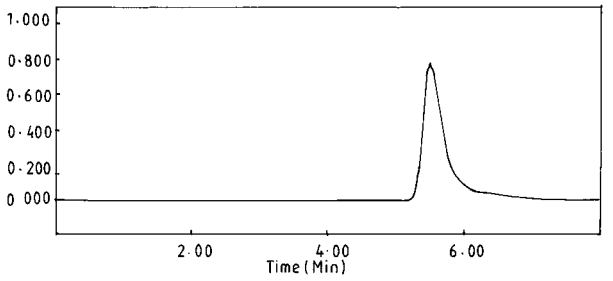

Figure 2 Chromatographic trace of the extract of T-loaded MIP collected from water : acetonitrile mixture containing Hy after 24 h.

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