Interaction of Molecularly Imprinted Polymers with Creatinine

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ABSTRACT: Memory sites toward clinically relevant creatinine have been imparted in polymers based on methacrylic acid, *N*-vinyl pyrrolidine, and 2-hydroxy ethyl methacrylate by the technique of molecular imprinting. The polymers are subjected to interaction with creatinine and creatine, a molecule of close resemblance with creatinine. The results show that selectivity is largely governed by the nature of the monomer. The reusability of the polymer is also demonstrated. © 1997 John Wiley & Sons, Inc. J Appl Polym Sci **66:** 2539–2542, 1997

Key words: molecularly imprinted polymer; creatinine; creatine

INTRODUCTION

Through the years, considerable efforts have been made to create synthetic materials for mimicking biological recognition systems. Molecular imprinting has emerged as a simple and elegant method to impart recognition sites in synthetic polymers to interact with molecules of interest with specificity comparable to that of biological entities like antibody, enzymes, and so forth.[1–4]

Molecular imprinting entails the polymerization of functional monomers in the presence of print molecules and other ingredients like initiators. After the polymerization, the print molecules are removed, leaving sites with induced molecular memory in the polymer matrix capable of recognizing the print molecules.

Over the years, the molecularly imprinted polymers (MIPs) have been used for a wide range of applications ranging from structural studies of ligand-receptor interaction to detection, separation, and purification.[5] Creatinine is an end product of nitrogen metabolism. It is transported to the kidneys from blood to urine. Blood level of creatinine is an indicator of renal function.[6] Many methodologies have been developed to detect and estimate this important molecule.[7] However, several of these methods suffer from serious drawbacks, which have been highlighted in a recent publication.[8] Efforts to use MIPs as an adsorbent for creatinine, as far as we know, have not been reported. This communication describes the interaction of creatinine with different types of MIPs and highlights the salient features of the MIP for the selective adsorption of creatinine.

EXPERIMENTAL

Analytical-grade *N*-vinyl pyrolidone (NVP), 2-hydroxy ethyl methacrylate (HEMA), methacrylic acid (MA), and ethylene glycol dimethacrylate were obtained from Fluka, AG, Daramstadt, Germany. These monomers were used after the stabilizers were removed. Creatine and creatinine, procured from CDH, New Delhi, India, were used as received. The amounts of monomers used for the preparation of MIPs are shown in Table I. MIPs were prepared by γ irradiation method as reported elsewhere.[10] Briefly, the monomers, the cross-linker, and the print molecule (creatinine)

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Table I Composition of the Polymerization Mixture

Monomer	Amount of Monomer (mg)	Crosslinker (EGDA) (mg)	Print Molecule (mg)
HEMA	110	1300	80
MA	86	860	53
NVP	110	1100	68

in the ratio shown in Table I were mixed in a test tube. Water : methanol mixture (1 : 1 v/v) was added to the test tubes drop by drop to get clear solutions. The test tubes were flushed with nitrogen, sealed, and subjected to γ irradiation with a Panaromic batch irradiator (BARC, Bombay, India) to a total dose of 0.6 Mrads at a rate of 0.2 mrad/hour. In a similar fashion, polymers without creatinine were also prepared to serve as controls. The polymers were cleaned by extensive washing with water followed by methanol. The complete removal of creatinine from the polymers was ensured by monitoring the absorption of the washing at 236 nm.

A sample of 100 mg of the powered polymers was kept in creatinine solution (0.2 mg/mL) under static condition at room temperature (30 ± 1°C). The polymers kept in the solution, for varied periods of time, were collected by filtration and then extracted with water by being kept in 20 mL of triple-distilled water at elevated temperature (45–50°C) for 1 hour. The amount of creatinine extracted from the polymers was estimated using a high-performance liquid chromatographic procedure. In a similar way, the adsorption of creatine by the polymers was also estimated.

A Waters Assoc. Inc. HPLC system consisting of a model 6000A solvent delivery pump, a model U6K injector, and a model 486 tunable absorbance detector was used for the chromatographic analysis. A μ-Bondapak C_{18} column (Waters Assoc. Inc.) in conjunction with methanol : water (40 : 60 v/v) was used for the estimation of both creatine and creatinine. The detection wavelength was 236 nm.

RESULTS AND DISCUSSION

Methacrylic acid is the monomer of choice for the preparation of MIPs intended for varied applications ranging from chromatographic packing to antibody mimics.[5] Monomers like itaconic acid, *p*-vinyl benzoic acid, and others have also been used for the synthesis of MIPs.[11–13] Recently, we have shown that HEMA can be used for the synthesis of MIPs, particularly for executing specific interactions with hydrophobic molecules like steroids.[9,10,14]

Table II Extent of Uptake of Creatinine by Various MIPs and the Respective Control Polymers

Polymer	Amount of Creatinine Adsorber by 100 mg of Polymer: Control Polymer (μg)	MIP (μg)
Poly (MA)	598 ± 12	968 ± 9
Poly (NVP)	276 ± 6	638 ± 10
Poly (HEMA)	12 ± 2	108 ± 4

The polymers were kept in the solutions for 1 hour.

Creatinine is a polar molecule, and therefore, improved interaction could be expected with MIPs synthesized from highly hydrophilic monomers like MA. Table II summarizes the extent of adsorption of creatinine by the three MIPs based on MA, NVP, and HEMA. The quantity adsorbed by the respective control polymers is also shown in Table II. Indeed, the uptake of creatinine by MA-based MIP is more, as expected, and it is least in MIP synthesized from HEMA, the least polar monomer among the three monomers used. The adsorption of creatinine by NVP-based MIP is between the amount adsorbed by MA-based MIP and HEMA-based MIP. Interestingly, the data summarized in Table II reveal that the uptake of creatinine by MA-based control polymer is also remarkably high, indicating that the polarity of both the polymer and the print molecule has a major role in governing the adsorption. It appears

Table III Interaction of Creatine by the MIPs and the Control Polymers

Polymer	Amount Adsorbed by 100 mg of Polymer: Control Polymer (μg)	MIP (μg)
Poly (MA)	460 ± 8	510 ± 11
Poly (NVP)	260 ± 3	276 ± 6
Poly (HEMA)	10 ± 2	18 ± 4

Polymers were kept for 1 hour in creatine solution.

Table IV The Selectivity Factor in Creatinine Adsorption

Polymer	Amount Adsorbed by 100 mg of Control Polymer ($C1$) (μg)	Amount Adsorbed by 100 mg of MIP ($C2$) (μg)	Ratio ($C2/C1$)
Poly (MA)	598	968	1.62
Poly (NVP)	278	638	2.29
Poly (HEMA)	12	108	9.00

that, even though MA-based MIP has a higher uptake of creatinine, its selectivity toward creatinine is rather poor. In other words, MA-based MIP could also adsorb other polar molecules. The content of Table III substantiates this view. Creatine, a molecule of close resemblance to creatinine, interacts with MA-based MIP and control polymer to an appreciable level. A notable aspect is that the polymers are not imprinted for creatine. The considerable adsorption of this molecule by the polymers shows that nonspecific interactions particularly polar-polar interactions are responsible for the adsorption. Again, HEMA-based polymers adsorb creatine the least.

On the basis of the uptake of creatinine and creatine by control polymers, it is reasonable to assume that polar interaction is playing a major role in deciding the extent of adsorption. It is well known that the polar nature of monomers used in this study increases in the order MA > NVP > HEMA. The extent of adsorption of both creatinine and creatine follows exactly the same order.

The ratio of the adsorption of creatinine by MIP and respective control polymer could be considered as a rough measure of selectivity. Table IV provides this parameter. It seems that HEMA-based MIP has a better specificity toward creatinine.

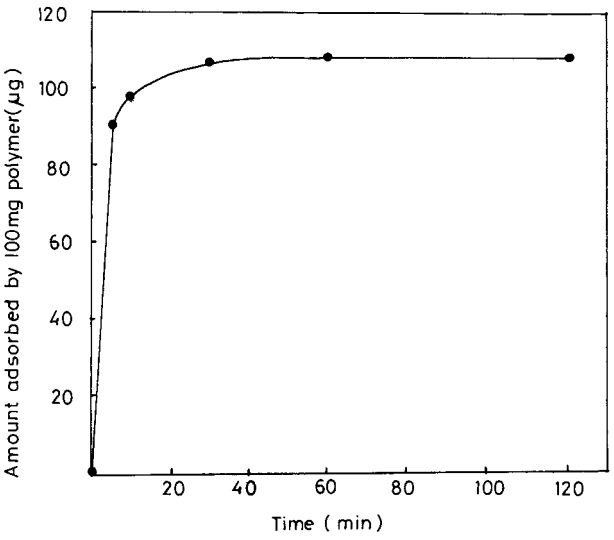

Figure 1 Time-dependent adsorption of creatinine by HEMA-based MIP.

It is true that MA-based MIP adsorbs a higher quantity of creatinine compared with HEMA-based MIP. However, to use MIP in selective applications (for example, in sensor to sense a given molecule from a mixture of components having comparable molecular structures), HEMA-based MIP seems to be a better choice rather than using the conventional MA-based MIP.

Figure 1 depicts the time-dependent adsorption of creatinine by HEMA-based MIP. It is apparent that within 5 min, MIP adsorbs about 90 μg of creatinine, which is 84% of the maximum adsorption capacity of the MIP. The interaction is fairly rapid, indicating the feasibility of using the MIP for sensor application in which the response time is a major factor.

MIPs are well known for their reusability. The polymer retains its memory toward the molecule of interest after treatment with organic solvents, even at elevated temperature. HEMA-based MIP, after removal of the adsorbed creatinine, was subjected to further interaction with creatinine by being placed in creatinine solution. Table V shows the adsorption of creatinine by the MIP in each cycle. In each cycle, the amount adsorbed is nearly identical, indicating that the memory sites in the MIP are intact. This opens up the possibility of

Table V Effect of Repeated Extraction on the Adsorption of Creatinine by the HEMA-Based MIP

Extraction Cycle	Amount Adsorbed by 100 mg of MIP (μg)
0	108 ± 4
1	105 ± 2
2	99 ± 1
3	102 ± 3

reusing the MIP in a continuous manner, which may be useful when using this polymer as a selective adsorbent for creatinine.

The data discussed in this article demonstrate that MIPs are interesting matrices for selectively removing clinically relevant molecules like creatinine. The data also show that selectivity, to a large extent, depends on the nature of the monomer.

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