# Imparting Recognition Sites in Poly(HEMA) for Two Compounds Through Molecular Imprinting

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**ABSTRACT:** So far, molecularly imprinted polymers (MIPs) have been synthesized and evaluated as selective matrices for single components. It may be possible to impart multiple recognition sites in MIPs, which could be used as elements capable of binding more than one component. Such systems could advantageously be used in the design of sensors having the ability to sense more than one compound at a time. This communication discusses such a possibility by imprinting sites for two model compounds, namely, salicylic acid and hydrocortisone in poly(2-hydroxy ethyl methacrylate). © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 71: 1823–1826, 1999

**Key words:** molecular imprinting; poly(2-hydroxyl ethyl methacrylate); hydrocortisone; salicylic acid

# **INTRODUCTION**

Molecular imprinting has received considerable interest recently as an elegant approach to impart recognition ability in highly crosslinked polymers towards molecules of interest. Molecular imprinting entails the polymerization of functional monomers in the presence of print (template) molecules.<sup>1–3</sup> After the polymerization, the print molecules are removed, and the resultant polymers show the ability to recognize the substrate molecules with remarkable degree of selectivity. Over these years, several molecularly imprinted polymers (MIPs) have been prepared as column packing for chromatography, for the chiral separation of drugs, and absorption matrices for biomolecules like amino acids, peptides, steroids, proteins, and sugars.<sup>4–8</sup> MIPs possess severable advantages compared to biosystem like enzymes and antibodies in temperature stability, compatibility with organic solvents, reusability, and low cost of preparation. The demonstration of MIP as a plastic antibody by Mosbach et al. has accelerated the interest in MIPs as alternative systems in the design of sensors.<sup>9</sup>

To date, MIPs have been synthesized and evaluated as matrices to recognize and bind single components. A synthetic polymer capable of binding more than one component with a high degree of selectivity would be useful in the design of sensor element for the detection of multi components. The use of MIP as a matrix for binding more than one component, as far as we know, has not been reported. This communication addresses the evaluation of a 2-hydroxy ethyl methacrylate (HEMA)-based MIP capable of binding two model compounds, namely, salicylic acid and hydrocortisone.

## **EXPERIMENTAL**

2-hydroxy ethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDA), hydrocortisone, and cholesterol were obtained from Sigma Chemicals, St. Louis, USA. Salicylic acid, aspirin, and other

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chromatographic solvents were from Emerck, Bombay, India.

#### Instrumental

A Waters Assoc Inc. (Milford, MA, USA) HPLC system consisting of a model 510 solvent delivery pump, model 7725 reodyne injector, and a model 486 tunable absorbance detector was used for the chromatographic studies. A  $\mu$ -bondapack C<sub>18</sub> column in conjunction with acetonitrile and water mixture (90: 10 V/V) as mobile phase at a flow rate of 1 mL/min was used for the separation and estimation of salicylic acid and hydrocortisone. Under the present chromatographic conditions, hydrocortisone (Hy) eluted at 4 min, salicylic acid (SA) had a retention time of 6 min, aspirin had a retention time of 7.2 min, and cholesterol had a retention time of 8.7 min, respectively. By injecting varied concentrations of these compounds, calibration plots were constructed between peak height and concentrations. These plots were used for estimating the amount of Hy and SA in the solutions.

# Preparation of the MIPs

We used gamma irradiation for the preparation of the polymers, as reported elsewhere.<sup>10,11</sup> This route was chosen particularly considering the fact that extra additives like initiators are not needed. Subsequently, Uezu et al. have also reported the use of gamma irradiation for improving the efficiency of MIPs.<sup>12</sup>

1 g of HEMA, 3 g of EGDA, and 50 mg of SA were mixed. 5-6 mL of chloroform was added to the mixture to dissolve the ingredients. The content was then transferred to a polyethylene tube, flushed with nitrogen, and sealed. In a similar fashion, 1 g HEMA, 3 g EGDA, and 131 mg hydrocortisone were mixed to prepare the MIP imprinted for hydrocortisone. For the preparation of MIP imprinted for SA and hydrocortisone, HEMA, EGDA, SA, and Hy were mixed as in the previous cases. The molar ratios of SA and Hy in the mixture were similar to the composition of the polymer imprinted for SA and Hy. These solutions were subjected to gamma irradiation from a Co<sup>60</sup> source (Panoramic batch irradiator, BARC, Bombay, India) to a total dose of 0.3 Mrad at a rate of 0.1 Mrad h at room temperature (30°C). Polymer without the print molecules was also prepared to serve as a control. After the polymerization process, the polymers were crushed into

Table I	The	<b>Extent of Uptake of Print</b>	
Molecules by the Polymers			

Polymer	Amount of SA Absorbed by 100 mg of Polymer	Amount of Hy Absorbed by 100 mg of Polymer
Pc PSA PHy PM	$25 \pm 2 \ \mu { m g} \ 337 \pm 3 \ \mu { m g} \ 23 \pm 1 \ \mu { m g} \ 182 \pm 2 \ \mu { m g}$	$egin{array}{c} 15 \pm 1 \ \mu { m g} \ 17 \pm 2 \ \mu { m g} \ 155 \pm 3 \ \mu { m g} \ 72 \pm 3 \ \mu { m g} \end{array}$

powder. The powdered polymers were then extracted with chloroform to remove the print molecules. The complete removal of the print molecules were ensured prior to further studies using the polymers.

# Interaction of the Polymers with the Print Molecules

40 mg of the polymers imprinted for SA (hereafter PSA), Hy (PHy), Hy and SA (PM), and the control (PC) were placed in acetonitrile solutions of SA, Hy, and their mixture, respectively, at room temperature (30°C) at static condition. After 2 h, the polymers from the solutions were collected by filteration. The imprinted polymer (PM) and the control polymer were also placed in the solution containing aspirin and cholesterol to estimate the uptake of these compounds. The dried polymers were then transferred into 10 mL each of acetonitrile and kept for 24 h at elevated temperature (45°C) to extract the absorbed print molecules by the polymers. The solutions were then analyzed chromatographically to estimate the amount of SA and Hy.

# **RESULTS AND DISCUSSION**

Table I summarizes the extent of uptake of the print molecules by the four polymers. The imprinted polymers absorb the respective print molecules to a considerable extent compared to the control polymer. The uptake of SA by the polymer imprinted for Hy, as well as the extent of absorption of Hy by the polymer imprinted for SA, are close to the extent of uptake of these molecules by the control, indicating that these polymers did not have recognition sites to molecules other than the print molecules. The polymer imprinted for both

Compound	Recognition Sites (%)
SA	50.32
Hy	40.71

Table IIRecognition Sites in PM for SAand Hy

Hy and SA (PM) absorb these two molecules to an appreciable level compared to the control. This parameter sufficiently points out the presence of recognition sites in PM for both Hy and SA.

It is quiet reasonable to presume that the number of recognition sites in the imprinted polymers is directly related to the quantity of print molecules absorbed. In that sense, the fraction of recognition sites for each of the print molecules in PM can be expressed as

$$f_{\rm SA} = \frac{C_1 - C_0}{C_{\rm SA} - C_0} \tag{1}$$

$$f_{\rm Hy} = \frac{C_2 - C_0^1}{C_{\rm hy} - C_0^1} \tag{2}$$

where  $f_{\rm SA}$  is the fraction of recognition sites for SA in PM,  $C_1$  is the amount of SA absorbed by PM,  $C_0$ is the amount of SA absorbed by PC, and  $C_{\rm SA}$  is the quantity of SA absorbed by  $P_{\rm SA}$ . Similarly,  $f_{\rm Hy}$  is the fraction of recognition sites of Hy in PM,  $C_2$  is the amount of Hy absorbed by PM,  $C_0^1$  is the quantity of Hy absorbed by PC, and  $C_{\rm Hy}$  is the quantity of Hy absorbed by PHy. The fractions estimated by this procedure is summarized in Table II. It is quiet interesting to see that  $f_{\rm SA}$  is nearly 50%, while  $f_{\rm Hy}$  is relatively a less value of 41%.

It is apparent that SA has more recognition sites in PM. The —OH and —CO— groups in SA could form hydrogen bonds with the monomers prior to the polymerization. In that sense, monomers preferably prearrange around SA compared to Hy, and such improved interac-

Table IIICorrelation Between MolecularWeight of Print Molecules and the Extent ofUptake in PM

Compound	Molecular Weight (M)	Amount Absorbed $(C - C_0)$	$MX(C - C_0)$
SA Hy	$\frac{138}{362}$	$\begin{array}{c} 157 \\ 57 \end{array}$	$21666 \\ 20634$

tions would be responsible for the creation of more sites for SA in the PM. However, it is worth mentioning that Hy is relatively more polar, and it is quite reasonable to presume that Hy could execute more polar interactions with monomers more effectively. In spite of this possibility, however, the MIP shows reduced recognition towards Hy. Hy is relatively more bulky, and this aspect could lead to reduced probability for prearrangement of monomers around the print molecule compared to the probability of such an arrangement around SA.

The primary requirement of a system designed to function as sensor is its ability to recognize the molecule of interest from a mixture of structurally close molecules. In this study, aspirin and cholesterol are used as molecules of resemblance with SA and HY. Table III summarizes the extent of uptake of these molecules by the polymer imprinted for SA and Hy (PM). The extent of absorption of these compounds by the control polymer is also shown in Table IV. It can be seen that absorption of aspirin by the imprinted polymer is higher than the amount absorbed by the control polymer. However, the quantity is certainly much less than the extent of absorption of SA by PM. The imprinted polymer (PM) absorbs a negligibly small amount of cholesterol, which is almost comparable to the amount absorbed by the control polymer. The data support that PM contains mainly the recognition sites for the print molecules, namely, SA and Hy.

Table IVExtent of Absorption of Aspirin and Cholesterol by PM and<br/>Control Polymer

Polymer	Amount of Aspirin Absorbed by 100 mg of Polymer	Amount of Cholesterol Absorbed by 100 mg of Polymer
PM Pc	$56\pm4~\mu\mathrm{g}$ $22\pm3~\mu\mathrm{g}$	$egin{array}{llllllllllllllllllllllllllllllllllll$

Hydrogen bonding has been shown to be a major factor for creating the memory sites in molecularly imprinted polymers towards a template molecule. The feasibility of participating in Hbonding with monomers may be the reason for the creation of more recognition sites in PM for SA. Another interesting aspect that has emerged from the present study is the inverse correlation between the molecular weight and the extent of uptake of the print molecules by PM (see Table III). This aspect seems to be associated with the diffusion of the print molecules in the recipe before the polymerization. The monomers could prearrange more effectively around SA compared to the relatively bulkier Hy.

From the preceding discussions, it is apparent that recognition sites can be created in synthetic polymers for more than one component. The data further indicate the feasibility of optimising MIPs capable of recognising more than one compound.

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