Effect of the Type of Monomers of Molecularly Imprinted Polymers on the Interaction with Steroids

K. SREENIVASAN

Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Poojapura, Trivandrum 695012 India

Received 12 July 1997; accepted 6 September 1997

ABSTRACT: The recognition ability of molecularly imprinted polymers (MIPs) is largely governed by factors such as polymerization conditions, nature of crosslinking agents, and degree of crosslinking. In addition to these factors, monomers could also influence the ability of the MIPs to recognize the print molecules. MIPs based on 2-hydroxy ethyl methacrylate and *N*-vinyl pyrolidone imprinted for cholesterol and testosterone are prepared. The results show that the recognition ability strongly depends on the characteristics of monomer also, in addition to the nature of the print molecule. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 68: 1863–1866, 1998

Key words: molecularly imprinted polymer; cholesterol; testosterone

INTRODUCTION

Molecular imprinting has emerged as a powerful technique for the preparation of polymers containing recognition sites of predetermined specificity.[1–4] Over these years, several molecularly imprinted polymers (MIPs) have been prepared and used for varied applications, such as chromatographic separations of enantiomers, in selective detection, and as antibody mimics.[5–7] Unlike biomolecules like enzymes, MIPs are stable and can be stored for a prolonged period of time at room temperature without any detectable loss in their recognition ability.[8] The ease in their preparation, the high degree of selectivity in binding, the low cost of components, and prolonged stability make MIPs extremely popular materials.

As is very well known, molecular imprinting entails polymerization of functional monomers in the presence of print molecules. The functional groups in the print molecules interact with the complementary functionalities present in the monomer. After the polymerization, the print molecules are removed, and it is said that the polymer contains memory sites for the print molecules, which can subsequently interact with print molecules specifically. Molecular imprinting is a simple and straightforward method to create synthetic polymers with predetermined selectivity.

The bulk of the literature on MIPs report the use of methacrylic acid and its derivatives for the preparation of MIPs.[8] This choice is largely due to the possibility of hydrogen bonding interaction between the carboxylic groups and the polar functional groups of several types of print molecules.

It is known that hydrophobic interactions between monomers and print molecules could also influence the recognition capabilities of the MIPs.[8] Interaction of this nature is more prominent among hydrophobic biomolecules like steroids. The nature of monomers particularly in the synthesis of MIPs imprinted for hydrophobic molecules may be important to optimize MIPs specifically designed as a substrate for the absorption of a typical hydrophobic molecule like cholesterol. Studies in this direction, as far as we know, have not been followed. This communication addresses, the synthesis of polymers based on monomers without carboxylic groups imprinted for testoster-

Journal of Applied Polymer Science, Vol. 68, 1863–1866 (1998)
 CCC 0021-8995/98/111863-04

Table I Composition of the Polymerization Mixture

	Amount of		Print Molecule	
Monomer	Monomer (mg)	Crosslinker (mg)	Cholesterol (mg)	Testosterone (mg)
HEMA	160	1650	260	240
NVP	150	1500	230	220

one and cholesterol, two steroids having close structural features.

EXPERIMENTAL

2-Hydroxy ethyl methacrylate (HEMA), *N*-vinyl pyrolidone (NVP), and ethylene dimethacrylate (EGA) were obtained from Fluka, Germany. These monomers were used as received. Testosterone and cholesterol were procured from Sigma Chemicals, St. Louis, MO, USA. These components were used without further purification. All other chromatographic grade solvents were obtained from E. Merck, Bombay, India.

Preparation of the Polymers

We have used the gamma irradiation method for the preparation of the polymers, as reported elsewhere.[9,10] The monomer, the crosslinking agent, and the print molecule in the ratios shown in Table I were dissolved in a test tube containing 10 mL of methanol, bubbled with nitrogen, and sealed. The samples were subjected to gamma irradiation from a ^{60}Co source (Panoramic batch irradiator, BARC, Bombay) to a total dose of 0.3 Mrad at a rate of 0.1 Mrad/h. Polymers without print molecules were also prepared in a similar fashion to serve as controls.

After the polymerization process, the test tubes were broken, and the polymers were collected. The powdered polymers were washed extensively with chloroform to remove the print molecules. The complete removal of the print molecules were ensured by chromatographic analysis prior to the use of these of polymers for further studies.

Instrumental Methods

A Hitachi model S-2400 scanning electron microscope was used for observing the surface features of the polymers. A thin layer of gold was coated onto the samples prior to the microscopic observations.

A Waters Associates Inc. liquid chromatographic system consisting of a Model 6000A solvent delivery pump, Model U6K injector, and a Model 486 tunable absorbance detector was employed for the chromatographic analysis. A μ-Bondapak C18 column (Waters Associates Inc., Milford, MA, USA) in conjunction with water–methanol (30 : 70 v/v) as mobile phase was used for the estimation of cholesterol and testosterone. The column effluents were monitored at 206 nm (for cholesterol) and at 241 nm (for testosterone).

Interaction Studies of MIPs with Steroids

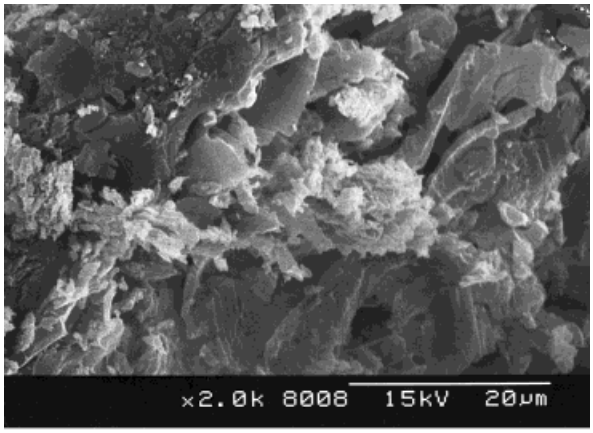
40 mg of the MIPs were placed in methanolic solutions of cholesterol and testosterone, respectively, for a period of 3 h. Exactly the same quantity of control polymers were also placed in these solutions. The polymers were collected by filtration, vacuum dried at 50°C, and then placed in 5 ml of methanol for 24 h. The amount of steroids extracted from the polymers were estimated by chromatographic method.

RESULTS AND DISCUSSION

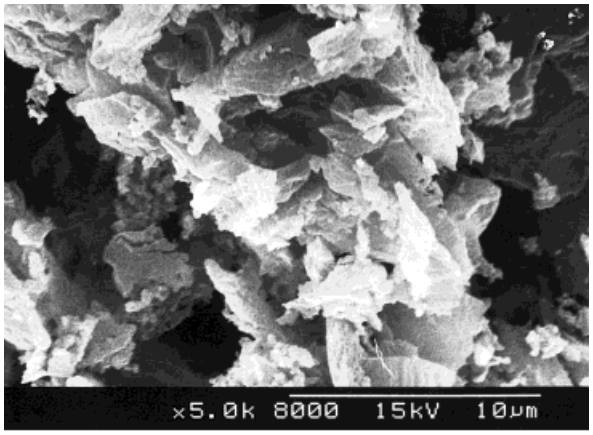
Table II summarizes the extent of uptake of cholesterol by MIPs based on NVP and HEMA. The

Table II Cholesterol Uptake by MIPs and the Respective Control Polymers

	Uptake of Cholesterol by 100 mg Polymer	
Polymer	MIP (μg)	Control (μg)
Poly(HEMA)	486 ± 6	16 ± 2
Poly(NVP)	306 ± 4	14 ± 3



A



B

Figure 1 Scanning electron micrographs of (A) MIP based on poly(HEMA) and (B) MIP based on poly(NVP).

amount of cholesterol absorbed by the respective control polymers are also shown in Table II. The extent of uptake of cholesterol by the control polymers is negligibly small compared to the extent of uptake by MIPs.

The two MIPs are prepared under similar conditions with the same molar ratios. The scanning electron photomicrographs shown in Figure 1(A) and (B) clearly indicate that two MIPs have identical texture. The water absorption capability of the two polymers are close (Table III), suggesting that the available volume in the matrices are nearly equal. Since the materials are highly crosslinked, the water uptake is considerably less. Based on these results it is quite reasonable to argue that the physical characteristics of the materials are identical. In that sense, since the polymers are imprinted for cholesterol, the extent of uptake should be identical or at least should be very close. However, the variation of cholesterol absorption by these polymers is 180 μg, reflecting that the number of recognition sites are not equal in these polymers based on NVP and HEMA.

Table III Equilibrium Water Absorption of the Polymers

Polymer	% Water Uptake by MIP	% Water Uptake by Control
Poly(HEMA)	4.2 ± 0.6	4.7 ± 0.4
Poly(NVP)	5.6 ± 0.8	5.3 ± 0.2

It is known that NVP is more polar than HEMA. In other words, hydrophobic interactions would be more in a cholesterol–HEMA system than in an NVP–cholesterol combination. The higher uptake of cholesterol by HEMA-based MIP may be attributed to the higher degree of hydrophobic interactions prior to polymerization, leading to the creation of more recognition sites in HEMA-based MIP for cholesterol.

Table IV shows the quantitative data of testosterone absorption by MIPs based on poly(NVP) and poly(HEMA), respectively. As stated above, these polymers are also prepared under similar conditions. Though identical uptake of testosterone is expected by these polymers, it is interesting to see that NVP-based MIP absorbs more testosterone than HEMA-based MIP.

Testosterone is relatively more polar than cholesterol, which, in fact, executes more polar–polar interaction with relatively more polar NVP. This additional factor, though minor, may be responsible for the increased uptake of testosterone by NVP-based MIP. Again, control polymers absorb very little testosterone, reflecting that the imprinting has a greater role in the absorption behavior of MIPs.

Table IV Extent of Absorption of Testosterone by MIPs and Control Polymers

	Uptake of Cholesterol by 100 mg Polymer	
Polymer	MIP (μg)	Control (μg)
Poly(HEMA)	625 ± 8	18 ± 3
Poly(NVP)	936 ± 7	21 ± 4

The efforts through these years have thrown much light on the mechanism of the recognition abilities of MIPs. Anderson et al. have described a model for recognition in noncovalent MIPs.[11] This mechanism is supported by studies using radiolabelled substrates and nuclear magnetic resonance investigations of complex formation in the prepolymerization mixtures.[12] The major factors governing the recognition process have been identified as H-bonding, ionic, dispersive, and hydrophobic interactions. The latter may be more important in the recognition process of typical hydrophobic molecules like steroids.

The data discussed here show that even the minute differences in the interaction potential largely affect the recognition process in MIPs. Additionally, the data summarized in this communication indicate that optimization in the recognition ability of MIPs to interact specifically with a given molecule largely depends on the nature of interaction between the monomers and print molecules in addition to the influence of crosslinking agent and solvents.

REFERENCES

1. K. Mosbach, *Trends Biochem. Sci.,* **19,** 9 (1994).
2. K. Shea, *Trends Polym. Sci.,* **2,** 166 (1994).
3. G. Wulf, *Angew. Chem., Int. Ed. Engl.,* **34,** 1812 (1995).
4. I. Nicholls, L. I. Anderson, K. Mosbach, and B. Ekberg, *Trends Biotechnol.,* **13,** 47 (1995).
5. L. I. Anderson and K. Mosbach, *J. Chromatogr.,* **516,** 313 (1990).
6. M. Kempe and K. Mosbach, *Anal. Letts.,* **34,** 1137 (1991).
7. G. Vlatakis, L. I. Anderson, R. Muller, and K. Mosbach, *Nature,* **361,** 645 (1993).
8. L. I. Anderson, I. A. Nicholls, and K. Mosbach, in *Highly Selective Separation in Biotechnology,* G. Street, Ed., Blackie Academic & Professional, London, 1994, p. 207.
9. K. Sreenivasan, Ind. Pat. Appl. 669, MAS (1995).
10. K. Sreenivasan, *Polym. Int.,* **42,** 169 (1997).
11. L. I. Anderson, D. J. O'Shannessy, and K. Mosbach, *J. Chromatogr.,* **513,** 167 (1990).
12. B. Sellegren, M. Lepisto, and K. Mosbach, *J. Am. Chem. Soc.,* **110,** 5853 (1988).