The Use of Metal-Containing Monomer in the Preparation of Molecularly Imprinted Polymer to Increase the Adsorption Capacity

K. SREENIVASAN

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

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ABSTRACT: Molecular imprinting is an elegant approach to induce antibody like recognition ability in synthetic polymers. The technique of molecular imprinting has been used extensively in the preparation of tailor-made stationary phases in chromatography, sorbents in solid phase extraction, sensor elements, etc. Though several of the reported molecularly imprinted polymers (MIPs) possess substrate selectivity comparable to antibodies, they are poor in adsorption capacity. The adsorption capacity could be improved presumably through enhanced interaction between the functionalities of the monomers and the print molecule. A simple approach to improve the interaction is perhaps the use of chemically modified monomers in the synthesis of the MIPs. This article explores this possibility by using a metal-containing monomer in the synthesis of MIP. The data obtained using a copper acrylate based MIP and cholesterol as substrate indicates the adsorption capacity can be improved considerably through the simple chemical modification of the functional monomer. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 80: 2795–2799, 2001

Key words: molecular imprinting; metal-containing monomer; cholesterol

INTRODUCTION

Considerable attention has been directed to find synthetic systems capable of mimicking naturally occurring entities like antibodies and enzymes. Molecular imprinting is one strategy that has been evoked much attention to impart the antibody-like affinity in synthetic polymers.[1–3] The technique of molecular imprinting involves the arranging of polymerizable functional monomers around a print (template) molecule in a solution containing higher quantity of a crosslinker. After the polymerization process, the print molecule is removed and resultant highly crosslinked matrix exhibits ability to rebind the print molecule from a mixture.

Molecularly imprinted polymers (MIPs) possess several advantages compared to the fragile biomolecules such as enzymes and antibodies. MIPs are stable at elevated temperature, and are compatible with aqueous and nonaqueous phases. Additionally, they can be prepared easily and are reusable. The wide utility of molecular imprinting is reflected in its applications in areas as diverse as the preparation of chiral chromatography stationary phases, antibody and receptor mimics, catalysis, synthetic chemistry, and sensors.[4–8] The method of molecular imprinting is now well established and it is showing great potential in addressing—in particular, the problem of enantiomeric separation. In recent years, MIPs have ef-

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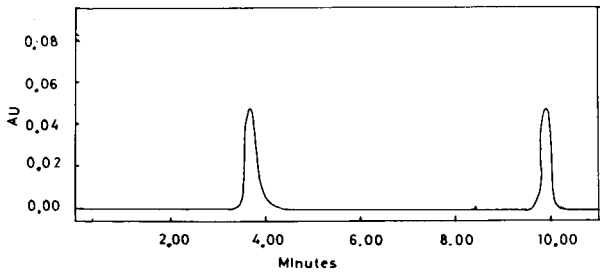

Figure 1 Chromatogram of a mixture of testosterone and cholesterol.

fectively been used to separate a wide range of racemic compounds.

In spite of all these exciting possibilities, relatively the low adsorption capacity of MIPs remains a problem to be addressed.[9] The adsorption capacity of MIPs is indeed a matter of serious concern with respect to their application in the large-scale separation and purification of specific analyte. Use of chemically modified monomers seems to be an interesting route to enhance the adsorption capacity of MIPs.

Metal ion mediated interactions involve in several processes in nature. In fact, metal ion based synthetic systems have been employed in chromatography and protein purification.[10,11] Considering the great role of metal ions in selective interactions, recently metal ions have been incorporated in the preparation of MIPs to exploit metal ion mediated ligand binding with improved selectivity and affinity.[12] To a large extent, metal ion containing systems have been studied to develop MIPs for amino acids and proteins.[13] So far, it is not known to what extent the presence of a metal ion influences the adsorption capacity of a MIP. This article discusses the preparation and evaluation of a MIP based on copper acrylate as an adsorbent for cholesterol.

EXPERIMENTAL

Chemicals

Acrylic acid (AA), ethylene glycol dimethacryalte (EGDMA), cholesterol, and testosterone were obtained from Fluka, Switzerland. Other chromatographic grade solvents and reagents were from SD Fine chemicals, Bombay, India.

Modification of the Monomer

Copper(11) acrylate was synthesized as reported elsewhere.[14] Briefly, copper carbonate was added to acrylic acid in dichloromethane. Monomer and copper carbonate were mixed in stoichiometric ratio and placed at room temperature overnight.

Preparation of the Polymer

Three milliliters of Cu(11) acrylate in dichloromethane (contains 1.5 g of Cu acrylate), 4 g of EGDMA, and 250 mg of cholesterol were placed in a beaker containing 5 mL dichloromethane. The content was stirred magnetically to get a clear solution and then transferred into a polyethylene tube, flushed with nitrogen, and sealed. The polymerization was initiated by γ irradiation and subjected to a total dose of 0.5 Mrad at a rate of 0.2 Mrad/h. This route of MIP preparation has been demonstrated in our earlier publications as an effective approach.[15,16] Polymer was also prepared without the addition of print molecule to serve as control. MIP based on acrylic acid as well as the respective control were also prepared. The polymers were crushed into powder and particle of 100–150 μ sizes were collected and extracted with dichloromethane to remove the print molecule. The complete removal of the print molecule was ensured prior to the further studies using the polymers. The presence of metal was confirmed by the energy dispersive X-ray analysis method.

Instrumental

A Waters Associates, Inc., high performance liquid chromatographic system consisting of a model 510 solvent delivery pump, A Reodyne model 2725 injector, and a model 486 tunable absorbance detector was employed for the chromatographic analysis. A μ-bondapak C_8 symmetry column in conjunction with methanol : acetonitrile (50 : 50 v/v) as mobile phase at a flow rate of 1 mL/min was used for the separation and estimation of the compounds. Testosterone was moni-

Table I Extent of Uptake of the Steroids by the Polymers

	Quantity Adsorbed by 100 mg Polymer (μg)	
Polymer	Testosterone	Cholesterol
MIPCu	122 ± 4	1217 ± 6
MIPAA	47 ± 2	440 ± 5
AACu (control)	102 ± 3	64 ± 4
AAC (control)	34 ± 4	39 ± 3

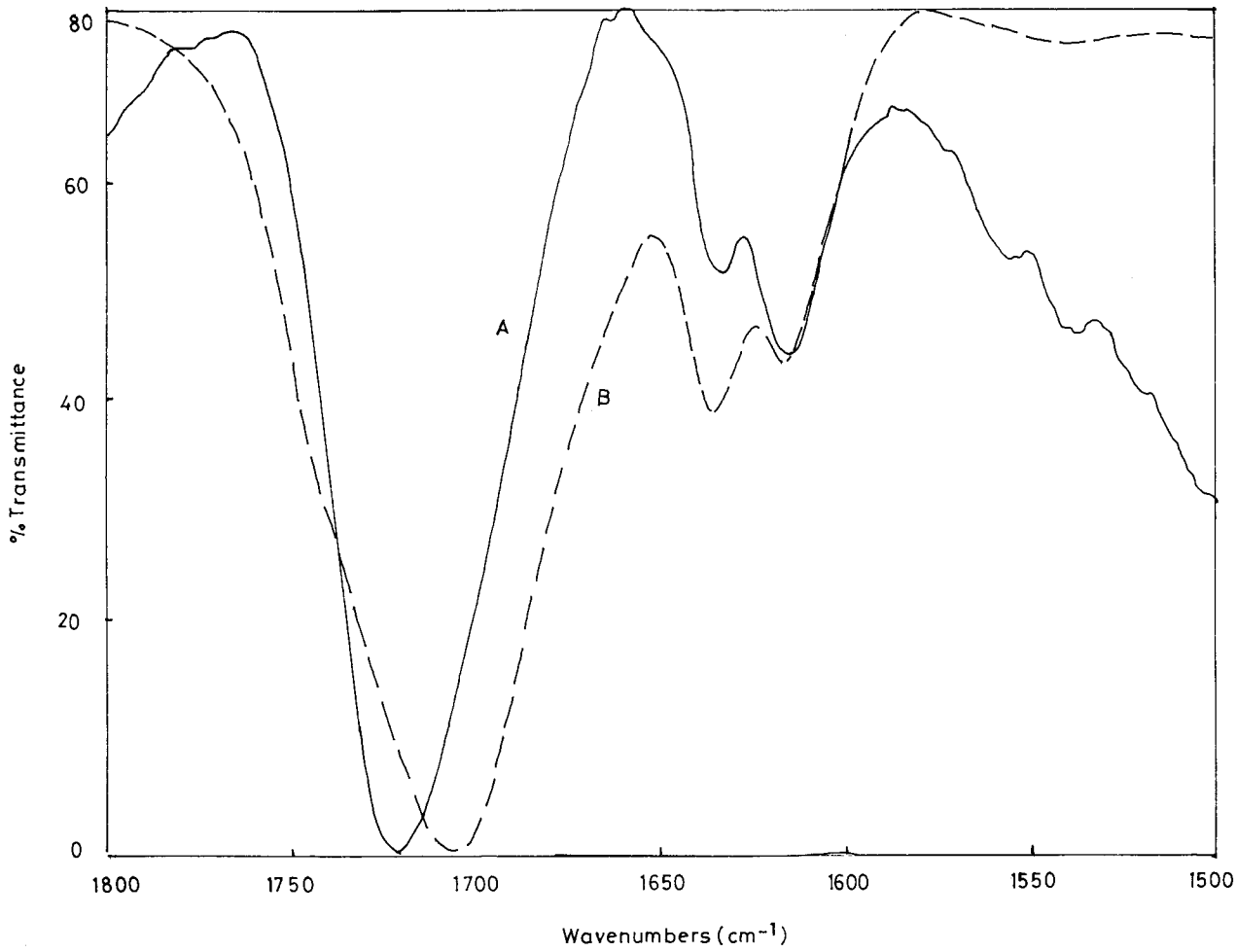

Figure 2 Peak maximum of the —CO— band of acrylic acid (A) in the absence of cholesterol and (B) in the presence of cholesterol.

tored at 241 nm while cholesterol was monitored at 206 nm.

A model Impact 410 Fourier transform infrared (FTIR) spectrophotometer (Nicolet, Inc., Madison, WI, USA) was used for recording the infrared spectra.

Interaction of the Polymers with Cholesterol and Testosterone

The specificity in interaction of a MIP with print molecule is normally evaluated by studying the interaction of the MIP with molecules of close resemblance with the print molecule. In this study, testosterone was used as a molecule of close resemblance with cholesterol, the print molecule.

Fifty milligrams of the MIP based on Cu(11) acrylate (MIPCu) was placed in mixture of cholesterol and testosterone in dichloromethane at room temperature (30°C) with occasional stirring. The concentration of the compounds in the solution was 0.1 mg/mL. After 1 h, the polymer was collected by filtration, dried, and then placed in 10 mL dichloromethane and heated to boiling. The solution was filtered and the filtrate was collected. The MIP was again extracted twice in a similar fashion and the combined filtrate was evaporated to dryness and the residue was dissolved in 5 mL of a acetonitrile : methanol mixture (same as the mobile phase). One hundred microliters of this solution was injected onto the column to estimate the quantity of the two steroids. Similarly extracts of the MIP based on acrylic acid (MIPAA) and the respective control polymers based on acrylic acid (AAC) and Cu acrylate (AACu) was prepared.

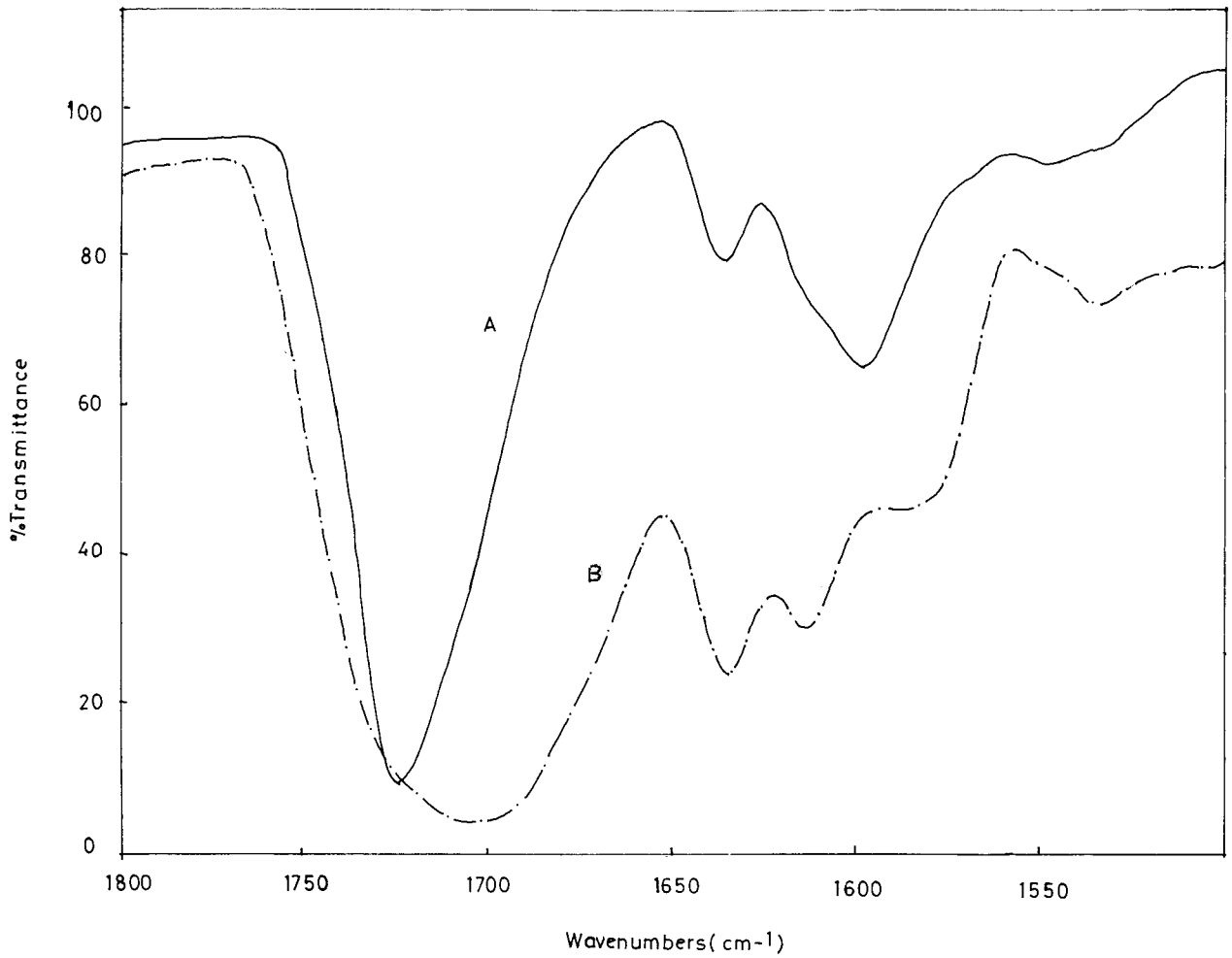

Figure 3 Peak maximum of the —CO— band of Cu acrylate (A) in the absence of cholesterol and (B) in the presence of cholesterol.

RESULTS AND DISCUSSION

Figure 1 illustrates the chromatographic trace of a mixture of testosterone and cholesterol. Under the present chromatographic conditions, testosterone has a retention time of 4 min, while cholesterol elutes at 10 min. The quantity of the steroids adsorbed by the polymers was estimated from the heights of the peaks at 4 min and 10 min of the respective chromatograms. The quantitative data are summarized in Table I. MIPCu adsorbs considerable quantity of cholesterol comparing to the control polymers as well as the MIPAA. MIPCu adsorbs about 3 times more cholesterol than the MIPAA.

MIPCu adsorbs 122 μg testosterone. Interestingly the respective control (AACu) also adsorbs more or less same amount of testosterone indicating the enhanced nonspecific interactions as result of the incorporation of Cu in the polymers. The extent of uptake of cholesterol and testosterone by AAC is negligibly small. The quantitative data indeed indicate the creation of affinity sites in both MIPs based on Cu acrylate and acrylic acid. The remarkable feature emerged from the study is the significant increase in the adsorption capacity of the MIP based on Cu acrylate.

Considerable efforts have been expended to understand the mechanism governing the creation of affinity sites in MIPs toward a specific analyte. Undoubtedly the creation of recognition sites in MIP based on the noncovalent strategy heavily depend on the complexation between the print molecule and the functional monomers during the polymerization as well as between the analyte and the prearranged polymer functionalities in the later binding process. In the noncovalent approach of molecular imprinting, complex forma-

tion is based on the noncovalent interactions between the print molecule and the functional monomers. Among the most often involved are hydrogen bonds and ionic interactions.[9]

It is interesting to probe to what extent the hydrogen bonding is affected, as a result of the monomer modification—namely, the introduction of the Cu ions. Figure 2 depicts the —CO— absorption band of acrylic acid in the absence and presence of cholesterol (print molecule). It is apparent that, peak maximum is shifted from 1721 to 1706 cm^{-1} in the presence of cholesterol indicating the hydrogen-bond formation between the —CO— group and —OH group. Figure 3 shows the —CO— absorption peak of Cu acrylate in the absence and presence of cholesterol. Apparently, the shift in the absorption maximum is more (shifted from 1729 to 1703 cm^{-1}), indicating enhanced interaction in the Cu acrylate–cholesterol system.

The major aspect that has enhanced the binding capacity of MIPCu could possibly be attributed to the improved interactions between the functionalities of the polymer and the print molecule. The presence of Cu atoms may result in the formation of more rigid networks in which the shape and size of the microcavities are retained, even after the exhaustive extractions to remove the print molecule after the polymerization, allowing the receptor to bind the template more effectively.

The binding capacity of the MIP is enhanced using Cu acrylate instead of the widely used acrylic acid. The present study points out that the use of metal-containing monomer could enhance the interaction between the polymer and print molecule. Such MIPs based on metal containing monomers can be envisaged for use in applications where higher uptake of the analytes is expected.

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