An Improved Molecularly Imprinted Polymer Film for Recognition of Amino Acids

N. Sneshkoff, K. Crabb, J. J. BelBruno

Department of Chemistry, Dartmouth College, Hanover, New Hampshire 03755

Received 17 October 2001; accepted 4 March 2002.

ABSTRACT: A highly selective imprinted film was produced from a formic acid solution of nylon. Amino acids were used as template molecules and spin cast films typically of the order of 1 μm, but as thin as 500 nm, were routinely prepared. The films were shown to be specific to the identity and chirality of the template amino acid, and were shown to be effective after as many as five cycles of template extraction and reintroduction. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 86: 3611–3615, 2002

INTRODUCTION

Molecular imprinting is a chemical technique for the production of molecule-specific cavities that mimic the behavior of natural receptor binding sites, without the temperature sensitivity and high cost of the natural systems.[1–3] Polymers are prepared in the presence of a template molecule that interacts with the polymer network, for example, via ionic, covalent or hydrogen bonding interactions. After polymerization, the template is removed and the polymer exhibits the ability to recognize the template with a high degree of selectivity. Molecularly imprinted polymers (MIPs) have been widely used in chromatographic separations of drugs and biological products.[4,5] Typical MIP systems involve polymerization of a methacrylate monomer with photoinitiators in the presence of the template.[6,7] These systems operate with organic solvents, both for polymerization and for implementation of the MIP. Most MIPs are developed as powders for later applications as separation agents. Our interests lie in the application of MIPs as sensor components, for which thin films would be more appropriate. Only a few selected studies have involved the use of such films. Recently, a brief report[8] appeared describing a nylon-based MIP that employed formic acid as the solvent. In the reported procedure, the time frames for film production, template extraction, and template reintroduction were quite long. In addition, as is generally true in MIP studies, films were not the focus of the report. The MIP films that were produced were drop cast, and measured approximately 100 μm in thickness. However, this MIP system is unique in that the material is created from polymer pellets. That is, the polymerization step is eliminated. The template molecule is incorporated into the polymer matrix in solution, and the film is cast from this solution.

In this report, we describe a series of improvements on this nylon MIP system and provide some details on the mechanism of the process. Polymer solutions are prepared at room temperature, spin cast films as thin as 500 nm are produced, and template extraction/reintroduction is accomplished on a relatively short time scale. Quantitative spectroscopic measurements provide characterization data on the nature of the MIP films.

EXPERIMENTAL

Nylon-6 was obtained from Aldrich as 3 mm pellets with T_m of 276°C. Formic acid (99%) was reagent grade from Fisher Scientific, and the amino acids, of highest available purity, were purchased from Sigma Chemicals. Films were spin cast from formic acid onto 18-mm square glass microscope coverslips. Typically, the slides were cleaned with spectroscopic grade isopropanol and acetone prior to polymer deposition. In some cases, the slides were prewashed in concentrated nitric acid. Performance, determined by qualitative film adhesion, was not improved by nitric acid cleaning, which was subsequently halted. The rotor was operated at 4000 rpm for 30, with negligible rampup time. Infrared spectra were recorded, after nitrogen purging of the sample compartment, on a Perkin-Elmer 1605 FTIR. A total of 16 scans, at 2 cm^{-1} resolution, were averaged for the final spectrum. Typically, spectra were recorded over a narrow region, 3700–2800 cm^{-1} or 1750–1550 cm^{-1}, of interest. Film thickness was measured using a Tencuf Instruments Alpha Step 500 stylus profilometer. Optical micros-

Correspondence to: J. J. BelBruno (jjbeham@dartmouth.edu).
Contract grant sponsor: National Science Foundation; contract grant number: DMR 9820408.

copy measurements of film thickness were in agreement with those from the profilometer.

Preparation/testing of the MIPs

Polymer solutions, 10 or 20% by weight, were mixed in formic acid. The imprinted polymer solutions contained from 2 to 8% of an amino acid template, typically L-glutamine. Solutions were placed into 50-mL flasks, covered and stirred at room temperature for 24 h. Spin-cast films were produced immediately following this incubation time. Incubation times between 1 and 12 h were tested and found to be unsatisfactory. Most of these solutions have a postpreparation lifetime of approximately 2 days. Degradation of the amino acid template molecule was observed for longer times. Tryptophan-templated MIP solutions have a lifetime of only 1 day before significant degradation, evidenced by development of a purple color in the solution. Once the films are cast, however, they are quite stable and may be stored or used for an indefinite time. Spectra were obtained for films immediately after preparation, but as a check on film stability, spectra were obtained on the same films (in each state of the preparation/extraction/reintroduction process) over a period of 6 weeks, with no change in either the blank or the MIP film. All experiments were conducted with spin cast films. IR spectra were used to detect the presence or note the absence of the template molecule.

Extraction/reintroduction of the template

The template extraction process was conducted in acetic acid. Concentrations of 2, 5, 10, and 20% were tested to optimize conditions. At the low end of this range, 2 or 5%, the time frame for removal was of the order of 9–30 days. At the high end, the films were consistently damaged. We found that the template molecule could be completely removed, as determined by IR spectroscopy, in 10 min using a 10% acetic acid solution at room temperature, which became our standard method. Films are washed with distilled water after removal from the extraction solution and allowed to dry. Spectra are subsequently recorded.

Reintroduction of the template molecule followed a similar procedure. The amino acid could be reintroduced, with no apparent effect on the film quality, in 30 min or less using either a 10% acetic acid or 5% formic acid solution with 2% of the amino acid used as the template. Most of the studies reported here involved the 5% formic acid solution. Films were subsequently washed with distilled water and allowed to dry. The dried films were again analyzed by IR spectroscopy. The extraction/reintroduction process was repeated for five cycles with reproducible results for each reintroduction. Film degradation due to the formic acid reintroduction solution was the limiting lifetime factor.

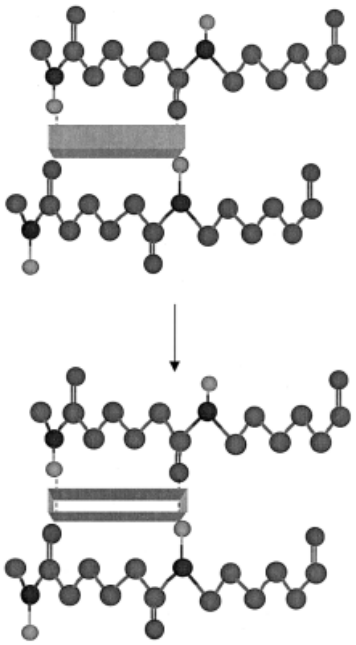

Figure 1 Schematic representation of the MIP formation process. The nylon is dissolved in formic acid in the presence of the template molecule forming a hydrogen-bonded network. After the film is spin cast, the template is removed, leaving the recognition site in its place.

RESULTS AND DISCUSSION

Nylon MIPs differ from the typical templating system in that the films are cast from polymer solutions of the template rather than polymerized from monomers in the presence of the template.[8] The polymer network and the templated polymer network have intermolecular hydrogen bonds. Hydrogen bonding has been shown to be very effective in the creation of recognition sites.[9] In a mixed template study, the presence of hydrogen bonds, formed prior to polymerization, was cited as the reason for the creation of a greater number of recognition sites for one of two competing templates.[10] These bonds are used to create the recognition sites that later recognize the template molecule during reintroduction. In formic acid solution, the crystalline polymer is separated into elongated chains. The template molecules are added to this solution and hydrogen bond with the nylon chains forming linked linear chains. Spin coating removes the solvent, forming a film that is a mixture of crystalline and amorphous nylon.

A schematic view of the overall MIP process is shown in Figure 1. Dissolution of the polymer in formic acid with amino acid present, results in a network of amino acid-and self-linked nylon. The extraction process removes the amino acid, but the cavity within

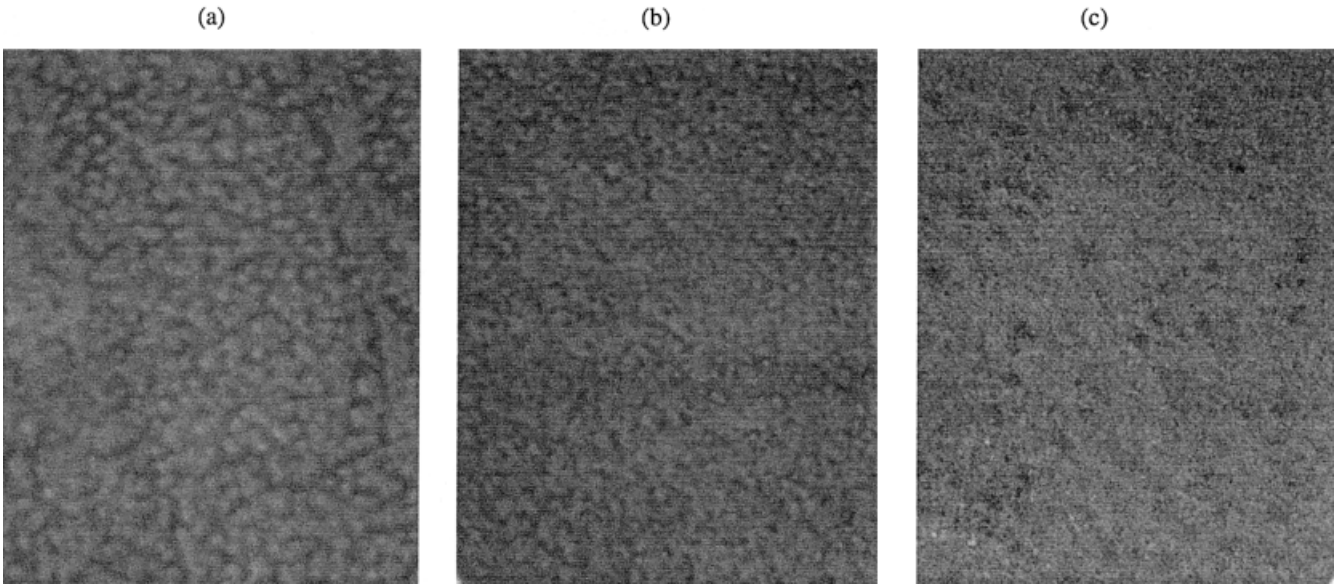

Figure 2 Optical microscopy (50× magnification) of (a) 0%; (b) 2%; and (c) 8% L-glutamine templated nylon films. Films were spin cast from 10% nylon in formic acid and are approximately 1 μm thick.

the polymer network remains. The amino acids appear to be a better linking agents than the nylon. Figure 2 shows three different films, containing 0, 2, and 8% amino acid, under 50 times magnification from an optical microscope. The morphologies are reproducible and clearly different, with a much finer length scale as the concentration of templating molecule is increased. We have interpreted this behavior as indicative of both a greater number as well as stronger hydrogen bonds as the volume percent of amino acid is increased. The particular granular features in the films are most likely a result of nucleation that begins as the solvent evaporates during spin casting. This evaporation increases the effective concentration of nylon and, similar to the phenomenon observed in concentrated bulk solutions, a type of solution semicrystallization occurs with large regions of amorphous material within each granule. The morphology of the films was unchanged after extraction of the template. That is, the film structure, once the film is cast, is independent of the presence of template molecule.

Film thickness

It is crucial that the film production technique be reproducible both in thickness and structure. To approach the problem, profilometry was used to measure the thickness of films produced under a range of conditions. In a study to determine the effect of amino acid concentration, six different concentrations were employed with 10% nylon casting solutions. The results are shown in Table I, where it may be observed that film thickness was slightly dependent on the template molecule concentration above 5% and that the 10% nylon solutions result in 1–1.5 μm films. As expected, the concentration of nylon in the casting solution was a critical variable. Additional studies indicated that the solutions of 20 and 5% nylon produced films of 20 μm and 500 nm, respectively. Unfortunately, the thinnest films were not sufficiently rugged for practical use.

Spectroscopic characterization

The interaction of amino acid and MIP was characterized by IR spectroscopy. The films are sufficiently thin so that narrow absorption bands are observed and the transitions due to the amino acids may easily be resolved from those of the nylon host, as determined by comparison to spectra of pure samples.[11] The amino acids are essentially isolated molecules, equivalent to matrix isolated or low pressure gas phase spectroscopic systems. In particular, analysis was focused on

TABLE I
Average[a] Film Thickness Measured by Profilometry for 10% Nylon Films Produced with Varying Concentrations of Template Molecule (L-Glutamine)

Volume–Percent L-Glutamine	Thickness, μm
0	1.24 ± 0.04
2	1.21 ± 0.01
3	1.29 ± 0.02
4	1.25 ± 0.03
5	1.41 ± 0.02
6	1.44 ± 0.02
8	1.68 ± 0.04

[a] Average of five films, each of which was measured at three different points.

the N—H region near 3500 cm^{-1} and the C=O region near 1600 cm^{-1}. Figure 3 shows a plot of absorption in the C=O region as a function of the percentage of template molecule in the polymer solution. As expected, the absorption, and by implication the number of recognition sites created in the polymer, increases as the amount of template increases. The ordinate is proportional to the concentration of L-glutamine in the solid films. The concentration of amino acid in the film was estimated by using the extinction coefficient reported for acidic solutions of the amino acid.[12] The absorption maximum and its extinction coefficient are shifted in moving from the solution to the solid phase, however, a reasonable *estimate* of the concentration is provided by using these parameters. We find that the ratio of the concentration of amino acid in the films to that in the templating solutions is not dependent upon the concentration. All films exhibit template concentration ratios of approximately 0.35; the amino concentration in the final product film is 35% of that in the solution used to produce that film. The presence of L-glutamine could be detected after templating with solutions as dilute as 0.2%. Although the use of additional scans in the FTIR could improve the signal-to-noise and the detection limits, the results in this study must be considered to have error bars of approximately this magnitude. Figure 4 shows the FTIR spectra of MIP films: as produced with 3% L-glutamine template, postextraction of the template and after reintroduction of the template using a 3% L-glutamine solution. The spectra clearly support the feasibility of this system for amino acid analysis. To the level detectable by the spectroscopic technique, the template may be removed and reintroduced with certainty. The

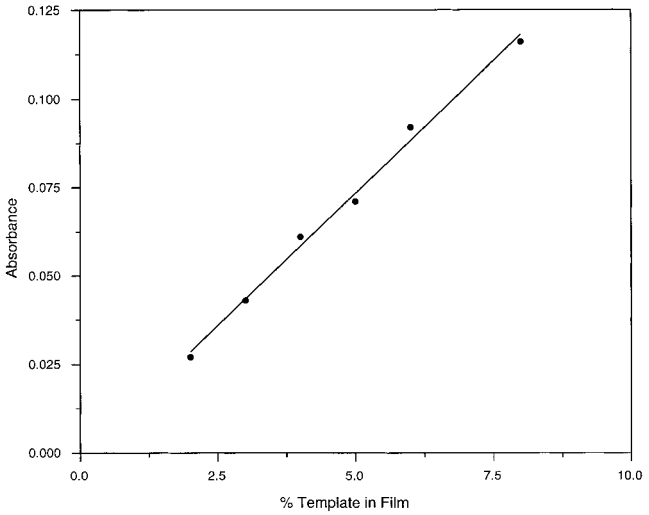

Figure 3 IR absorbance (proportional to the number of recognition sites) as a function of template concentration. Transmittance was recorded at 1688 cm^{-1}, in the region of C=O absorption. Values shown are averages for five different films.

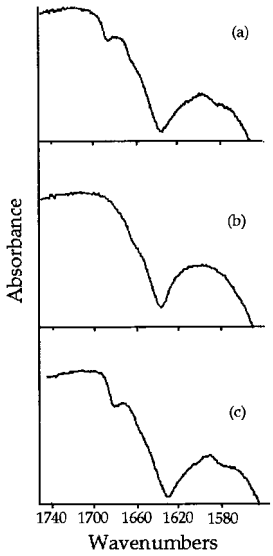

Figure 4 IR spectra in the C=O region for (a) MIP film as produced with 3% L-glutamine template; (b) after extraction of the template; and (c) after reintroduction of L-glutamine from a 3% L-glutamine in 5% formic acid aqueous solution.

absorption peak for the reintroduced sample was in good agreement with the absorption observed from a film produced from a 3% template solution. No L-glutamine was detected in control samples run through the reintroduction process.

Molecular specificity

The specificity of the MIPs was tested by attempting to introduce five other amino acids, including R-glutamine, into the L-glutamine-template extracted films. None of the foreign molecules was observed, by IR spectroscopy, to be incorporated into the films, while at any time, L-glutamine, at concentrations as low as 0.2%, could be reintroduced. This experiment also served to confirm that the source of the amino acid spectra was from the recognition sites created in the polymer rather than from molecules adsorbed onto the surface of the film. If the spectra were from the latter source, any amino acid would have been spectroscopically detected from any nylon film. Films produced from any of the other five amino acids: R-glutamine, histidine, tryptophan, alanine, or aspartic acid, provided performance, in terms of film thickness and molecular specificity, similar to that reported here for the L-glutamine templated MIPs.

CONCLUSION

Imprinted polymer films are readily produced from polymer solutions of nylon. The amino acid templates are incorporated in to the solution phase nylon network and, after removal of the template, recognition sites remain in the polymer. The level of imprinting

can be estimated, quantitatively, from IR spectroscopy. The preparation of the films is relatively rapid. Template extraction and reintroduction were shown to be possible in less than 30 mins and at concentrations of template as low as 0.2%. The reproducibility of the films, both chemically and physically, makes this system ideal for future studies on the molecular level, mechanistic details of the imprinting process.

This work was supported by the National Science Foundation through its Research Experience for Undergraduates program, Grant No. DMR-9820408 to the Dartmouth Molecular Materials Group. The authors wish to thank Dr. Chris Levey for help with the profilometry and Professor Ursula Gibson for helpful discussions and advice.

References

1. Shea, K. J. Trends Polym Sci 1994, 2, 166.
2. Wulff, G. Angew Chem Int Ed. 1995, 34, 1812.
3. Mosbach, K.; Ramstrom, O. Biotechnology 1996, 14, 163.
4. Owens, P. K.; Karlsson, L.; Lutz, E. S. M.; Anderson, L. I. Trends Anal Chem 1999, 18, 146.
5. Ensing, K.; deBoer, T. Trends Anal Chem 1999, 18, 138.
6. Das, K.; Duff, D. J.; Hsu, S. L.; Penelle, J.; Rotello, V. M. Polym Preprints 2000, 41, 1173.
7. Yan, M.; Kapua, A. Polym Preprints 2000, 41, 264.
8. Reddy, P. S.; Kobayashi, T.; Fujii, N. Chem Lett 1999, 293.
9. Sreenivasan, K.; Sivakumar, R. J Appl Polym Sci 1999, 71, 1823.
10. Yoshida, M.; Uezo, K.; Goto, M.; Furusaki, S. J Appl Polym Sci. 2000, 78, 695.
11. Aldrich Chemical Library of FTIR Spectra, Aldrich Chemical Co.
12. Venyaminov, S. Y.; Kalnin, N. N. Biopolymers 1999, 30, 1243.