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Spectroscopic Evaluation of Molecular Imprinting Polymerization Systems

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An evaluation of the extent of self-assembly of functional monomer–template complexes in molecularly imprinted polymer prepolymerization mixtures has been performed. The results provide general insights into the nature of the prepolymerisation self-assembly phase. Furthermore, the method allows for the estimation of the number of medium- to high-affinity recognition sites in the product polymer and a means for the rapid evaluation of molecular imprinting systems.

Key Words: molecular imprinting; molecular recognition; UV spectroscopy; synthetic receptor.

Since the early work of Cram (*1*), Lehn (*2*), and Pedersen (*3*), significant effort has been directed toward the design and synthesis of chemical structures capable of selective molecular recognition. While many of the works in the area of *supramolecular chemistry* have justifiably been hailed as milestones of contemporary organic synthesis, the lengths and yields of syntheses of many synthetic receptors limit their practical use. In parallel to these efforts, a technique has been developed for the preparation of synthetic polymers containing recognition sites of predetermined selectivity, namely, *molecular imprinting* (Fig. 1). Ligand selectivities and affinities of molecularly imprinted polymers (MIPs) comparable to those of biological receptors, e.g., antibodies, have been observed, making these polymers useful models of their biological counterparts. This methodology is currently the focus of intense research interest and is being used in a wide range of application areas, e.g., the preparation of selective separation materials, artificial antibodies, and synthetic enzymes (*4–10*). This has prompted efforts to better understand these systems to allow for their rational design.

Molecular imprinting relies upon the presence of complementary interactions, noncovalent or reversible covalent, between sites in the template molecules and the functional monomer(s) used in the polymerization process. The thermodynamic concepts underlying the ''prearrangement'' of functional monomers and template have previously been discussed (*11*), and NMR (*12, 13*) and chromatographic (*14–17*) studies have demonstrated their presence. The noncovalent interactions generally employed in these polymers are polar in nature, e.g., hydrogen bonding, ion pairing, ion-dipole, and metal ion chelation. Thus, the strengths of these interac-

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tions are accentuated by the relatively nonpolar solvent conditions generally employed during the polymerization process.

The stoichiometry of a noncovalent imprinting polymerization mixture dictates the quantity and quality of recognition sites in the resultant polymer. An examina-

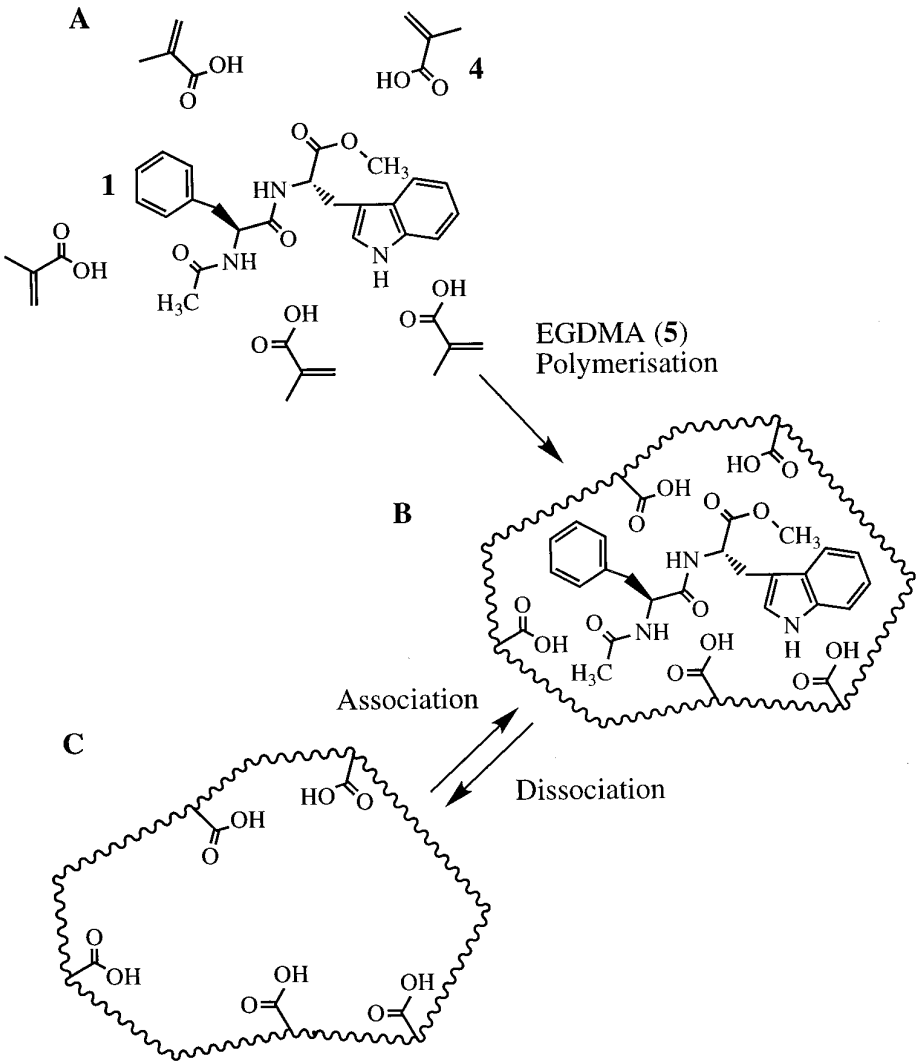
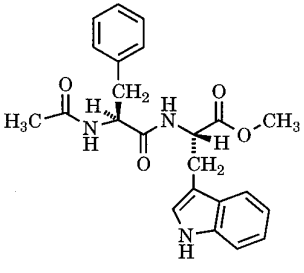


FIG. 1. Idealized representation of the molecular imprinting technique. (**A**) Judicious selection of a monomer or monomer mixture, with chemical functionality complementary to that of the imprint species, that is mixed with the template. The complementarily interacting functionalities form predictable solution structures. (**B**) Polymerization in the presence of a suitable cross-linking agent yields a rigid bulk polymer. (**C**) Removal of the template, leads to the defining of a recognition site of complementary steric and functional topography to the template molecule.

tion of published evaluations of stereoselective MIPs shows that too high a functional monomer–template ratio renders polymers with high nonspecific binding, due to an over abundance of polar functional groups distributed randomly throughout the polymer matrix, and reduced selectivity. Too low a ratio yields polymers with insufficient extents of template complexation and thus low site numbers and selectivity. *Optimal* functional monomer–template ratios should yield polymers which demonstrate selectivity of binding and relatively low nonspecific binding. To this point in time, the optimization of molecularly imprinted polymer systems has been performed by the preparation, processing, and evaluation of a range of polymers, which is both time consuming and tedious.

In this paper we describe a uv spectroscopic analysis of the molecular imprinting "prepolymerization stage," where interactions between the template and functional monomers are under thermodynamic control. This method was applied to the investigation of three MIP template systems, namely, *N*-acetyl-L-phenylalaninyl-L-tryptophanyl methyl ester (**1**) (*18, 19*), yohimbine (**2**) (*20*), and cinchonidine (**3**) (*21*), which have been imprinted in methacrylic acid (**4**)–ethyleneglycol dimethacrylate (**5**) crosslinked network copolymers. The analysis presented provides a means for determining the extent of template complexation and allows a semiempirical estimation of the number of selective recognition sites in a polymer prepared with a given monomer/template composition. This method should provide a valuable tool for the rapid investigation of new molecular imprinting systems.



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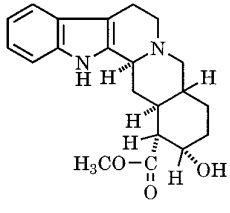

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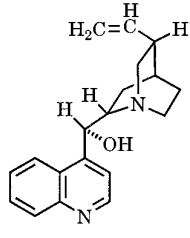

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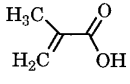

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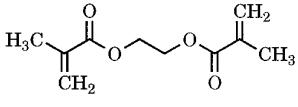

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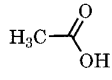

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MATERIALS AND METHODS

Chemicals were of analytical grade and all solvents of HPLC grade. Chloroform and all monomers were purified prior to use (*22*).

TABLE 1
Results from Titration of Template Structures with Functional Monomers and Functional Monomer Analogues

T	M	Solvent	K_{diss} (M) apparent	$-\Delta G$ (kJ/mol) apparent	Polymer M : T[a]	% Cxd T[b]
1	4	$CHCl_3$	0.06	6.8	5[18]	0.6
1	4	$CHCl_3$/**5**	0.07	6.5	5	0.5
1	6	$CHCl_3$	0.11	5.4	5	0.4
1	6	$CHCl_3$/**5**	0.13	5.1	5	0.4
2	4	CH_3CN	0.06	6.8	4.6[20]	0.5
2	6	CH_3CN	0.10	5.7	4.6	0.3
3	4	$CHCl_3$	0.06	7.0	4[21]	0.4
3	6	$CHCl_3$	0.10	5.6	4	0.4

Note. Results from uv titration experiments. T, template molecule; M, monomer or monomer analogue.
[a] Functional monomer : template ratios used in preparation of the corresponding polymers.
[b] Percentage adduct formation corresponding to the molar ratios used in polymer preparations calculated from saturation plot regressions. Monitored wavelengths and binding plot correlation coefficients: **1/6** λ 258.4 nm, R^2 0.98; **1/6/5** λ 294.5 nm, R^2 0.98; **1/4** λ 300.0 nm, R^2 0.96; **1/4/5** λ 297.0 nm, R^2 0.96; **2/4** λ 293.1 nm, R^2 0.90; **2/4** λ 300.3 nm, R^2 0.97; **3/6** λ 315.7 nm, R^2 0.93; **3/4** λ 310.0 nm, R^2 0.97.

Titration Experiments

A 1-ml solution of **1** (99 μM), **2** (106 μM), or **3** (161 μM) was titrated with consecutive 3-μl injections of **4** or **6** (Table 1) at 23°C. The solution was equilibrated for 3 min after each injection, prior to recording of the uv spectrum. Spectra were recorded on a Beckman DU640 single-beam spectrophotometer. Results represent the average values of a minimum of three sets of experiments for each system studied.

RESULTS AND DISCUSSION

The titration of the functional monomer, methacrylic acid (**4**), into a solution of the dipeptide **1** in chloroform containing the crosslinker **5** at concentrations comparable to those used in polymer preparation studies resulted in complex formation which can be observed by uv difference spectroscopy (Table 1). The observed shift reaches a maximum corresponding to saturation of interaction between functional monomer and template structure. Hill-type binding plots (*23*) were then prepared from the saturation plots, allowing calculation of apparent K_{diss} values.

The interaction of a crosslinking monomer with a template in molecular imprinting should ideally be small or negligible to reduce the nonspecific binding modes available in the product polymer. Titration of **5** into chloroform solutions of **1** revealed no appreciable change in the absorption spectra of **1,** implying that