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Binding Studies on Resins Imprinted with (*S*)-naproxen

Yue Hu and Robert A. Orwoll
Departments of Applied Science and Chemistry
College of William and Mary
Williamsburg, VA 23187-8795, U.S.A.

ABSTRACT

Resins were prepared in a free-radical polymerization of 4-vinylpyridine and ethylene glycol dimethacrylate in the presence of (*S*)-(+)-6-methoxy-α-methyl-2-naphtaleneacetic acid ((*S*)-naproxen). Initially (*S*)-naproxen, the imprinted molecule template, was assembled with the monomer 4-vinylpyridine by non-covalent interactions. After the polymerization, stepwise removal of the template left binding sites that retain complementary specificity and affinity. Binding parameters including the maximum number of binding sites and dissociation constant were calculated from the amount of template removed using a two-site Scatchard equation. The results are typical of other systems reported in the literature.

INTRODUCTION

Molecularly imprinted polymers (MIPs) are synthetic polymers having tailor-made selectivity for a particular template species. They are prepared by self-assembly with a template bound to a monomer in the presence of a crosslinking monomer. Polymerization of the monomers typically results in a macroporous support with the binding monomer positioned for interaction with the template. After extraction of the template, molecular sites are positioned to readsorb the template.

Molecular imprinting has been a very active field since 1990. Although attempts have been made to acquire MIP particles dimensionally and morphologically homogeneous, the heterogeneity in the binding has been a significant problem to date in the synthesis of MIPs. The heterogeneity not only affects MIP's analytical applications, but also complicates MIP's characterization. Studying how the template molecule is captured by and released from the MIP during the prearrangement procedure, before the polymerization, and during the extraction procedures will help to better understand the heterogeneity in the binding sites. A known MIP system was used: (*S*)-naproxen as the template and 4-vinylpyridine as the functional monomer.

In an early study using (*S*)-naproxen as a template, Mosbach *et al.* [1] prepared MIP by a free radical polymerization followed by crushing, grinding, and sieving to produce packing material for high-performance liquid chromatography (HPLC). They found good retentivity and enantioselectivity. Then between 1997 and 2001 Haginaka *et al.* studied the similar system but developed a multi-step swelling and thermal polymerization method with water as a suspension medium followed by hydrophilic surface modification techniques to make uniformly sized MIP particles [2]. By their method, the separation factor for the enantioselectivity of (*S*)-naproxen (1.74) was obtained, an improvement over that of Mosbach (1.65).

In this study, we employed Mosbach's method to synthesize MIP. Washing analysis of the template from the MIPs yielded data for a Scatchard plot [3] and the determination

of binding parameters. They were compared with parameters reported in the literature for other systems.

EXPERIMENTAL

Imprinted polymer preparation and washing

(*S*)-(+)-6-methoxy-α-methyl-2-naphtaleneacetic acid ((*S*)-naproxen), 4-vinylpyridine (4-VP), ethylene glycol dimethacrylate (EGDMA) and 2,2´-azobisisobutyronitrile (AIBN) were purchased from Aldrich. All organic solvents were of analytical or HPLC grade. 4-VP and EGDMA were purified before using.

0.4600 g (*S*)-naproxen (2 mmol), 1.30 mL 4-VP (12 mmol) and 11.32 mL EGDMA (60 mmol) were dissolved in 8 mL tetrahydrofuran (THF). After stirring in a 4°C bath for 1 hour, 0.1150 g AIBN dissolved in 8 mL THF was added. This mixture was immediately purged with nitrogen for 15 minutes and then irradiated for 48 hours with UV light from an ACE 7825-34 mercury vapor UV lamp (450 watts). The resulting polymer was ground in a ball mill (SPEX 8000) and dry sieved to sizes between 63 and 125 μm.

Approximately 1 g MIP was stirred with 20 mL THF for 24 hours, thereby removing some template from the resin to the THF. After centrifuging, the supernatant was filtered through 0.45 μm syringe filter. Its UV absorbance was measured at 363.5 nm using a Perkin-Elmer Lambda 35 UV/VIS spectrophotometer. Washing was repeated three more times. The UV absorbance for the solutions was measured after calibrating the absorbance as a function of concentration over the range for 0.02 to 0.25 mg/mL. A background absorbance was determined in washings of a resin that had been prepared in the absence of template. This correction, which in no case exceeded 4%, was subtracted from the absorbance of the washings in order to eliminate the effects of any non-template UV-absorbing material. These washing experiments were performed on three different MIP-(*S*)-naproxen samples.

A reference non-imprinted polymer was prepared using the same procedure without addition of the template.

UV/VIS and Fourier transformation infrared spectra (FT-IR)

A series of solutions was prepared with a fixed concentration of (*S*)-naproxen (0.04 μmol/L) and varying amounts of 4-VP in THF. The UV/VIS absorption spectra of these solutions were compared with corresponding (*S*)-naproxen or 4-VP.

Resins with and without the template were prepared as KBr disks. The IR spectra were measured between 1000 and 4000 cm^{-1} with a 4 cm^{-1} resolution using a Perkin-Elmer Spectrum 1600 FT- IR instrument.

DISCUSSION

Interaction between monomer and template

The interactions between (*S*)-naproxen and 4-VP are important for understanding imprinting and recognition phenomena in MIP.

The UV spectrum in Figure 1 shows the prearrangement interaction between 4-VP and (*S*)-naproxen. As the concentration of 4-VP was increased, the wavelength λ_{max} of maximum adsorption by (*S*)-naproxen shifted to greater λ, and the maximum absorbance at the maximum wavelength increases with the addition of 4-VP.

The interaction of the template with the polymer causes a shift in the infrared absorption for the carbonyl stretching in the EGDMA units of the resin. Figure 2 shows the spectrum of the 4-VP and EGDMA resin (A) in the absence of (*S*)-naproxen, (B) with the naproxen template in place after polymerization, and (C) after extraction of (*S*)-naproxen. The $\tilde{\nu}_{max}$ moves from (A) 1739 to (B) 1726 to (C) 1734 cm^{-1}. Such transitions to lower $\tilde{\nu}$ are characteristic of carbonyl oxygen in a hydrogen bond. This suggests that the template molecule may interact through hydrogen bonding in this MIP network and the hydrogen bonding may be more responsible for the selectivity of the template.

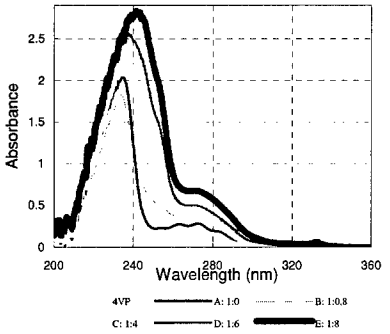

Figure 1. UV adsorption spectra of (*S*)-naproxen and 4-VP system in THF. Concentration of (*S*)-naproxen is 0.04 μmol/L; concentration of 4-VP (μmol/L): B: 0.032; C: 0.16; D: 0.24; E: 0.32.

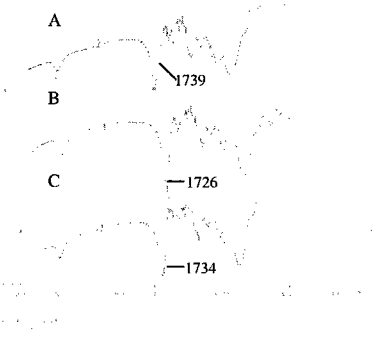

Figure 2. FT-IR Spectra for (A) MIP without (*S*)-naproxen as the template; (B) MIP before (*S*)-naproxen extraction; (C) MIP after (*S*)-naproxen extraction. Wave numbers are reported in cm^{-1}.

Washing analysis by Scatchard equation

Washing the template from the resin is an important step in preparing MIP systems for analytical applications. In this study, (*S*)-naproxen was extracted from the template-containing resin using several washings with THF. Although others [1] reported washings using THF with acetic acid added to promote the release of the (*S*)-naproxen, we found no difference with or without the acid. The molar concentration of template [T] in the wash solutions was measured directly from the UV absorbance of those solutions. The amount of (*S*)-naproxen remaining in the resin, [MT] measured in mmoles per gram of resin, was determined by difference from the amount of (*S*)-naproxen initially present in the polymerizing mixture. Four successive washings of each of three resin samples yielded 90-94% recovery of the template initially present. The data were fit to the two-

site Scatchard Equation $[MT] = \frac{n_{max,H}[T]}{K_{d,H}+[T]} + \frac{n_{max,L}[T]}{K_{d,L}+[T]}$ using an iterative spreadsheet procedure that minimized the sum of the square of the difference between the measured and calculated concentrations [MT] of bound (*S*)-naproxen by adjusting the parameters for the maximum number of binding sites $n_{max,H}$ and $n_{max,L}$ and dissociation constants $K_{d,H}$ and $K_{d,L}$. Here the subscripts H and L refer to high- and low-affinity binding sites.

Two of these sets of four washings yielded good fits. The third required a negative $K_{d,H}$ to fit the four data points to the four parameters. The mean values and their spreads for the two good data sets are $n_{max,H}$=13±1 μmoles of sites per gram of resin, $n_{max,L}$=110±30 μmol/g, $K_{d,H}$=0.003±0.002 mmol/L, and $K_{d,L}$=14±5 mmol/L. The data for one set of washings are depicted in Figure 3. The two-site Sactchard analysis shows the strong binding sites to be saturated at solution concentrations [T]>0.4 mmol/L. A more precise value for $K_{d,H}$ would require more data at concentrations below saturation than were obtained in these washing experiments.

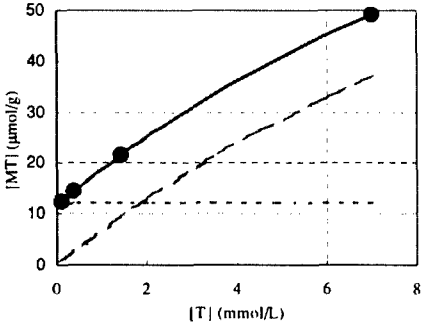

Figure 3. Two-site Scatchard plot showing the quantity of (*S*)-naproxen absorbed at the weak (dashed line) and strong (dotted line) binding sites. Measured values for the four washings are shown. The solid line is the calculated fit for the total amount bound.

Literature review

Researchers have interpreted binding in MIP systems in terms of the Scatchard equation. We summarize these studies and compare them with the results of the washing measurements of the present study.

The most common method for investigating the capacity of MIPs to absorb template from solutions is the rebinding experiment. After the template has been extracted, the MIP is immersed in solutions of varying concentration of the template. The change in concentration of the solution as the template is reabsorbed is measured, typically using HPLC or UV spectroscopy. Two kinds of binding sites with differing strengths were observed in the ten studies summarized in the beginning of Table 1. As described above, this behavior is consistent with a small equilibrium dissociation constant K_d for the template-specific binding sites and a large K_d for more general adsorption of template onto the resin.

In Table 1 the identities of the template, functional monomers, and crosslinking reagents are listed. The moles of monomer and of crosslinker relative to the number of moles of template is in the fourth column. The solvents used in the polymerization, the extraction of the template from the MIP, and the rebinding are identified in columns 5-7. The dissociation constants and concentrations of binding sites in the resins are tabulated in columns 8-11. In the six studies listed at the bottom of Table 1, only a single line was obtained in the Scatchard plot and so only single values are listed for K_d and n_{max}.

Because of the variability of chemical environments around the binding sites in an MIP resin, the dissociation constants associated with those binding sites should also vary. It is remarkable then that so many systems exhibit a bimodal distribution of binding constants such that the binding behavior can be represented accurately with double Scatchard plots. A continuous distribution function has been proposed [19] for more detailed analyses of bindings.

CONCLUSIONS

The 4-VP+EGDMA resin prepared in the presence of (*S*)-naproxen yielded K_ds for high-affinity (0.04 mmol/L) and low affinity (3 mmol/L) sites that are comparable to those reported in other systems, *i.e.*, 10^{-3}-1 mmol/L (high affinity) and 0.1-3 mmol/L (low affinity). Spectroscopic evidence obtained on this system is consistent with hydrogen bonding between the template and functional monomer.

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Table 1. Summary of Scatchard analyses from the literature.

Template	MIP Synthesis				Extraction Solvent[d]	Rebinding Solvent[d], conc (mM)	High Affinity		Low Affinity		Ref
	Mono.[a]	Xlink[b]	M:X:T[c]	Solvent[d]			K_d (mmol/L)	n_{max}, (μmol/g)	K_d (mmol/L)	n_{max}, (μmol/g)	
4-aminopyridine	maa	egdma	4:20:1	dmf	ma/aa	membrane; w, 0.1-4.0	0.54	49	3.2	50	[4]
4-hydroxybenzoic acid	a	egdma	6:30:1	acn	ma/aa	acn, 0-2.5	0.18	9	1.4	22	[5]
cefalexin	tfmaa	egdma	4:20:1	ma	ma/aa	w, 0.1-4.5	0.14	30	2.4	130	[6]
cefalexin	tfmaa+ 4vp	egdma	12:30:1	ma	ma/w	w, 0-4.5	0.14	28	2.8	157	[7]
norfloxacin	maa	egdma	6:30:1	c/dmf	ma/aa	c/dmf, 0-4.5	0.09	23	1.9	128	[8]
(5R)-5-benzylhydantoin	a	egdma	4:20:1	acn	ma/aa	acn, 0-30	0.05	40	0.13	48	[9]
4-aminopyridine	maa	egdma	4:30:1	c	ma/aa	acn, 0-4.5	6.2×10^{-3}	78	0.43	223	[10]
5,5-diphenylhydantoin	a	egdma	4:20:1	thf	ma/aa	acn, 0.05-4.0	2.1×10^{-3}	17	1.6	104	[11]
cortisol	maa	egdma	13:65:1	thf	ma/aa	thf, 0.03-20 mg/mL	0.6	0.2	1.6	280	[12]
corticosterone	maa	egdma	13:65:1	thf	ma/aa	thf, 0.03-20 mg/mL	1.2	0.4	0.8	130	
2-aminopyridine	maa	egdma	4:30:1	c	ma/aa	acn, 0-4.0	2.6	136	--	--	[13]
paracetamol	a	egdma	4:40:1	acn	ma/aa	acn, 0-4.5	2.3	126	--	--	[14]
testosterone	maa	egdma	8:25:1	c	acn	acn, 0-1.5	1.1 (UV), 0.8 (HPLC)	1.6 (UV), 2.5 (HPLC)	--	--	[15]
trimethoprim	maa	egdma	6:30:1	c	acn/aa	c, 0.5-9.0	0.2	202	--	--	[16]
trimethoprim	*maa*	*trim*	4:4:1	acn	acn	membrane; acn, 0-4.5	0.05	290	--	--	[17]
(-)-cinchonidine	maa	egdma	2.5:75:1	c	acn/ma	dcm; 0-2.0	1.5×10^{-4}	4.0	--	--	[18]
	zp	egdma	2.5:75:1	c	acn/ma	dcm; 0-2.0	6.9×10^{-4}	3.8	--	--	
	maa +zp	egdma	3.5:75:1	c	acn/ma	dcm; 0-2.0	8.8×10^{-5}	5.3	--	--	

[a]Monomer abbreviations: a, acrylamide; maa, methacrylic acid; tfmaa, 2-(trifluoromethyl)acrylic acid; 4vp, 4-vinylpyridine; zp, zinc porphyrin.
[b]Crosslinker abbreviations: egdma, ethylene glycol dimethacrylate; trim, tris(hydroxymethyl) propane trimethacrylate.
[c]Molar proportions of monomer:crosslinker:template.
[d]Solvent abbreviations: aa, acetic acid; acn, acetonitrile; c, chloroform; dmf, dimethyl formamide; dcm, dichloromethane; ma, methyl alcohol; thf, tetrahydrofuran; w, water