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Theophylline molecularly imprinted polymer dissociation kinetics: a novel sustained release drug dosage mechanism

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The template release kinetics of theophylline molecularly imprinted polymers has been examined with a view to determining their potential as a controlled release drug dosage form. The basis for the ligand selectivity of these polymers has been shown through the demonstration of pre-polymerization template–monomer complexation and HPLC studies of the product polymer ligand selectivities. The release kinetics shows a dependence upon template loading and pH. Small differences in release characteristics between imprinted and non-imprinted (reference) polymers have been observed. © **1998 John Wiley & Sons, Ltd.**

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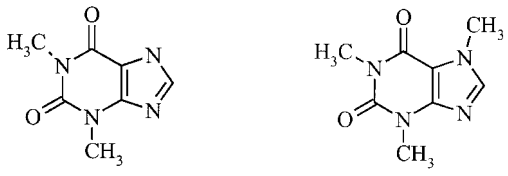
Introduction

Methyl xanthines have been employed in the treatment of asthma for more than a century, initially in the form of strong coffee (Rall, 1993). Today the xanthine usually employed in clinical medicine is theophylline (Rall, 1993; Rang *et al.*, 1995) (Fig. 1). Theophylline promotes diaphragmatic contractility and mucociliary clearance, aids cardiac functions and lowers pulmonary artery pressure. However, theophylline has the potential for significant adverse effects. Appropriate dosing is essential, as theophylline has a narrow therapeutic index which lies in the range 30–100 μmol l^{-1}, with toxic effects likely at concentrations greater than 110 μmol l^{-1}, (Berkow, 1987). Plasma concentrations in excess of 200 μmol l^{-1} result in serious cardiovascular and CNS effects, the most serious being arrhythmia, which can be fatal (Rang *et al.*, 1995). Theophylline is routinely administered in the form of oral sustained release preparations in order to maintain effective plasma concentrations for periods as long as 12 h. More efficient systems, however, are clinically desirable.



Theophylline **Caffeine**

Figure 1. Structures of theophylline and caffeine

Ligand-selective recognition by non-covalent molecularly imprinted polymers (MIPs) has found use in a diverse array of applications ranging from the preparation of artificial antibody combining site mimics and chiral chromatographic stationary phases (Steinke *et al.*, 1995; Wulff, 1995; Mosbach and Ramström, 1996; Andersson and Nicholls, 1997a; Vulfson *et al.*, 1997); to mediators of organic syntheses and enzyme mimics (Shea, 1994; Nicholls *et al.*, 1995; Davis *et al.*, 1996). The selectivity of these materials for a predetermined ligand makes them versatile systems for the study of molecular recognition phenomena (Nicholls, 1995). In addition, MIPs constitute an interesting complement to other rationally designed small-molecule recognition systems.

The principles underlying the preparation of non-covalent molecularly imprinted polymers entail the judicious selection of a monomer or monomer mixture to ensure chemical functionality complementary to that of a template (imprint) molecule. The complementarily interacting functionalities form predictable solution structures (Sellergren *et al.*, 1988; Andersson and Nicholls, 1997b), which after polymerization in the presence of a suitable crosslinking agent followed by extraction of the template species lead to the defining of recognition sites of complementarily steric and functional topography to the template molecule. Subsequent incubation of a mixture of the template and related chemical species results in the selective rebinding of the imprinted structure. This selectivity has been utilized in molecularly imprinted polymer-based chiral stationary phases (CSPs), which are distinguished from other CSPs by their predictable order of elution, such that application of a racemate

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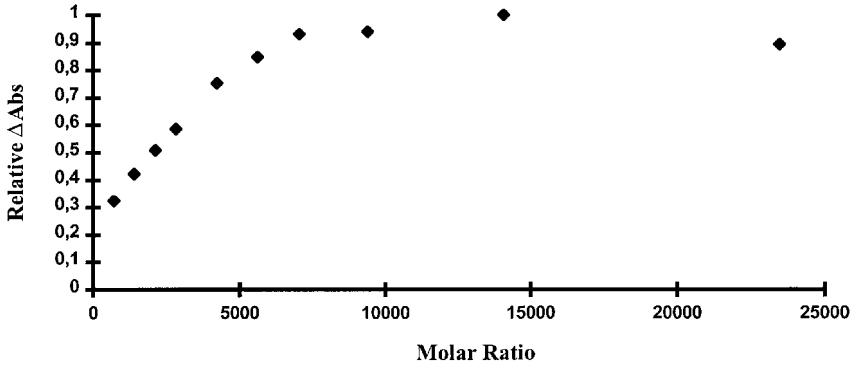

Figure 2. Plot showing the titration of theophylline with methacrylic acid (determined at 285 nm). The observed change in absorbance (ΔAbs) reaches a maximum corresponding to saturation of interaction between functional monomer and template structure.

of an imprint species always result in longer retention times for the imprinted enantiomer. This effect is reflected by the different binding affinities of enantiomers at MIP recognition sites (Kempe and Mosbach, 1991).

While many studies have utilized the selective adsorption characteristics of molecularly imprinted polymers, here we describe an investigation of the dissociation kinetics of template structures from molecularly imprinted polymers (Norell *et al.*, 1997).

Materials and Methods

All chemicals and solvents were of analytical or HPLC grade. UV spectra were recorded on a Hitachi U-2001 double-beam instrument. HPLC was performed using a Perkin Elmer series 200 LC pump and an Applied Biosystems 785A programmable absorbance detector.

Spectrophotometric evaluation of theophylline–functional monomer pre-polymerization mixtures

UV spectrophotometric titration studies were performed according to the method of Andersson and Nicholls (1997b). Chloroform was used as the reference and was titrated in the same way as the theophylline solution. Analyses were performed in triplicate at room temperature (20–22 °C). At each wavelength the relative ΔAbs (Abs/Abs_0) was plotted against MR (mol theophylline/mol methacrylic acid), allowing the estimation of an apparent dissociation constant (and ΔG values) as described earlier (Andersson and Nicholls, 1997b) via the construction of a Hill-type binding plot.

Polymer preparation

Theophylline-imprinted polymers were prepared according to the procedure of Vlatakis *et al.* (1993). A non-imprinted reference polymer, in the absence of theophylline, was analogously prepared. The polymers were packed into columns ($8 \times 370\ mm^2$) and washed with methanol/acetic acid (9:1, v/v) (flow 0.75 ml min^{-1}, total volume 1.0 l), then dried under vacuum. Fractions taken from the washing eluent were analysed to follow template extraction.

HPLC evaluation of molecularly imprinted polymer recognition characteristics

Polymer samples were packed in HPLC columns (inner diameter 4.6 mm, length 250 mm) with an air-driven fluid pump (Haskel Engineering, CA, USA) at 340 bar. Samples (10 μg) of theophylline, caffeine, theophylline/caffeine mixture and acetone (void marker) were injected in triplicate. Chromatographic analyses were performed with isocratic elution with acetonitrile/acetic acid (99.5:0.5, v/v) at a flow rate of 1.0 ml min^{-1}. Detection was carried out at 272 nm. Capacity factors (k') were calculated from the retention volumes (V_R) and the void volume (V_o) using the equation.

$$k' = {}^{(V_R - V_o)}/_{V_o} \quad (1)$$

Polymer–theophylline dissociation kinetics

Dried polymer samples (100 mg) were incubated with different concentrations (2.0, 10 and 50 mg theophylline/g dried polymer) of theophylline in chloroform for 17 h. The solvent was subsequently evaporated to produce a dry powder.

The polymers were transferred to membrane tubes (Spectra/Por® molecular porous membrane tubing, MWCO 6–8000, flat width 23 mm, diameter 14.6 mm, volume/length 1.7 ml cm^{-1}). The tubes were closed and transferred

Table 1. Apparent K_{diss} and $-\Delta G$ for the complex formation between methacrylic acid and theophylline

Wavelength (nm)	K_{diss} (M)	$-\Delta G$ (kJ mol^{-1})
295	4×10^{-5}	25.1
290	5×10^{-5}	24.5
285	8×10^{-5}	23.4

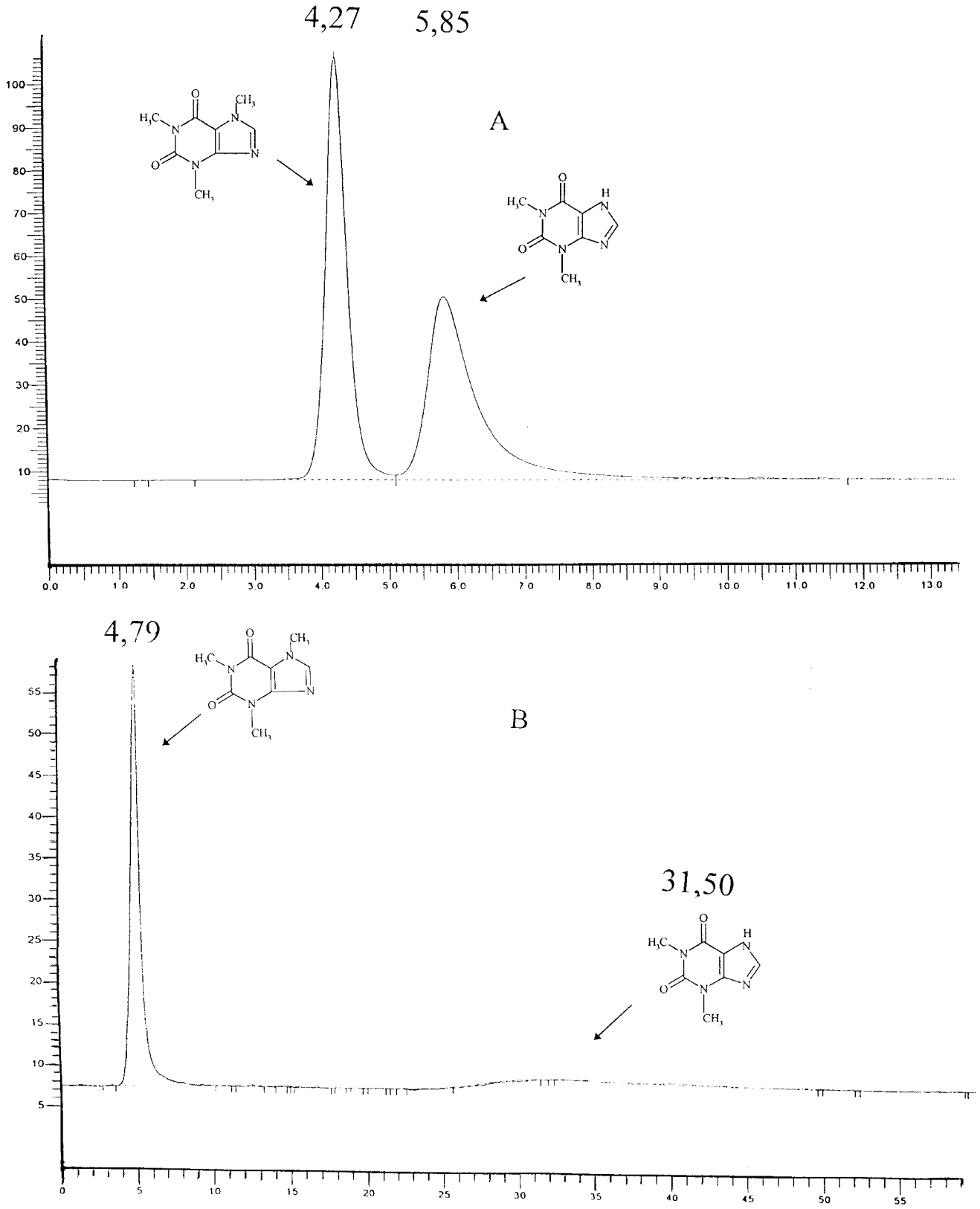

Figure 3. HPLC traces from the co-injection of theophylline and caffeine on (A) a reference polymer column (caffeine, 4.27 min; theophylline, 5.85 min; void, 3.40 min; α 2.8) and (B) a theophylline-imprinted polymer column (caffeine, 4.79 min; theophylline, 31.50 min; α 19.0).

Table 2. HPLC evaluation of theophylline-imprinted and reference polymers

Polymer	Analyte	Capacity factor k'	Separation factor α
Reference	Caffeine	0.26	
	Theophylline	0.72	2.0
Imprinted	Caffeine	0.45	
	Theophylline	8.55	18.0

to capped 100 ml Duran® flasks containing 70 ml of buffer (0.1 M at pH 6.0, 7.0 and 8.0, Na_2HPO_4/NaH_2PO_4). The extent of theophylline dissociation was determined by spectroscopic analysis of the external buffer ($A_{271.6\ nm}$); aliquots were replaced after measurement.

^{3}H-theophylline release studies

Radio labelled theophylline (^{3}H-theophylline, 18.6 Ci mmol^{-1}, Amersham) was used to increase sensitivity when analysing polymers incubated at lower concentrations. 400 mg of polymer was incubated in 6 ml of solution A (12 ml acetonitrile, 48 µl ^{3}H-theophylline, 64 µl stock solution (1 mg theophylline in 1 ml acetonitrile)) in a capped beaker for 17 h. The incubated polymers were evaporated in the beaker without cap for 36 h. Sample preparation was performed as described above. Analyses were initiated as 70 ml of buffer (0.1 M phosphate buffer, pH 7.0) was added to the Duran® flask. 100 µl fractions were transferred from the flask to a scintillation vial (Beckman Mini Poly-Q Vial, 6 ml) containing scintillator liquid (Beckman Ready Gel™, 1 ml). Samples were taken in duplicate and were analysed in scintillator (Beckman LS 6000 SE, each sample counted for 5 min).

Results and Discussion

Prior to polymer synthesis a spectroscopic evaluation of template–functional monomer interaction was performed to ascertain that monomer–template self-assembly does in fact take place and to determine the stability of the self-assembled complex. Although theophylline-imprinted polymers have been reported in several studies (Vlatakis *et al.*, 1993; Kobayashi *et al.*, 1995; Matsui *et al.*, 1995), no evidence has yet been presented for functional monomer–theophylline self-assembly during the pre-polymerization phase. Through monitoring changes in UV spectra of the template upon titration with methacrylic acid, plots of monomer concentration as a function of change in absorbance yield saturation isotherms from which dissociation constants may be calculated using binding plot analyses (Andersson and Nicholls, 1997b) (Fig. 2). These analyses were performed at three wavelengths, each yielding comparable calculated dissociation constants (Table 1).

Theophylline MIP and reference polymer were prepared according to the procedure of Vlatakis *et al.* (1993). To verify that theophylline-selective sites had been introduced into the polymers, an HPLC-based assay was performed comparing the retention characteristics of the template and caffeine on non-imprinted and theophylline-imprinted polymers (Fig. 3). A marked selectivity for the template was observed in the case of the theophylline-imprinted polymer (Table 2).

The release kinetics of theophylline from the imprinted and reference polymers was subsequently examined. Polymers were loaded with different quantities of template (50–0.1 mg theophylline/g polymer (dry weight)) and the dry charged polymer samples were then placed in dialysis tubing. The extent of release of template in a pH 7.0 phosphate buffer (22 °C) was followed by UV spectrophotometry, and by scintillation counting after loading with radioactive theophylline in the case of the 0.1 mg g^{-1} loaded polymers (Fig. 4). Superior controlled release characteristics were observed for the lower-loading samples, 2.0 and 0.1 mg g^{-1}. The release of 50% of loaded theophylline varies by up to a factor of four over the loading range studied. The difference in release rates observed at higher loadings is attributed to the utilization of non-specific, weak, binding modes in these polymers, a phenomenon illustrated in recent work (Andersson *et al.*, 1996). The differences in release rates as a function of polymer loading are indicative of the heterogeneity of theophylline receptor site populations.

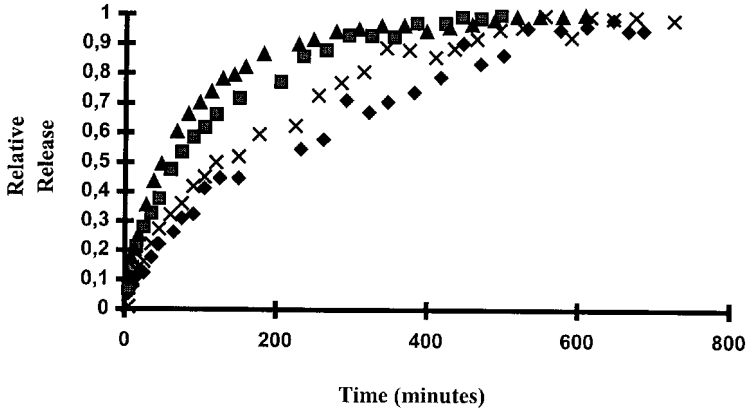

Figure 4. Release characteristics for theophylline-imprinted polymers. Polymers loaded with: ▲, 50 mg theophylline/g polymer; ■, 10 mg g^{-1}; ◆, 2.0 mg g^{-1}; ×, 0.1 mg g^{-1}.

Table 3. Release rates for theophylline-loaded polymers in phosphate buffer

Incubation (mg theophylline/g polymer)	Polymer	Release rate (mmol g^{-1} min^{-1} at pH 6.0	7.0	8.0
50	Reference	3×10^{-4}	–	8.4×10^{-3}
	Imprinted	5×10^{-4}	–	3.7×10^{-3}
10	Reference	1×10^{-4}	1×10^{-4}	7.4×10^{-3}
	Imprinted	1×10^{-4}	6×10^{-5}	3.7×10^{-3}
2	Reference	2×10^{-5}	2×10^{-5}	3.0×10^{-5}
	Imprinted	2×10^{-5}	1×10^{-5}	3.0×10^{-5}
0.1	Reference	–	4×10^{-5}	–
	Imprinted	–	3×10^{-5}	–

The release characteristics of imprinted and reference polymers were compared at pH 6.0, 7.0 and 8.0. Selectivity was most pronounced at pH 7.0. No discernible differences in release characteristics were observed at pH 6.0, however, and at pH 8.0 only at higher loadings (Table 3). Differences in release characteristics between imprinted and reference polymers may be attributed to differences in polymer structure, as reflected by differences in porosity, surface area and functionality distribution (Sellergren and Shea, 1993). Nonetheless, the variation in release rates as a function of polymer loading demonstrate the utilization of different binding modes at higher and lower loadings.

In this study we have demonstrated that the conceptual basis for preparation of theophylline molecularly imprinted polymers, namely the presence of pre-polymerization adducts between the functional monomer methacrylic acid and theophylline, is valid. Furthermore, such polymers show high degrees of selectivity for theophylline relative to the structurally related caffeine. The dissociation of template from theophylline molecularly imprinted polymers is loading-dependent and differences exist, albeit not great, between the release kinetics of imprinted and blank polymer systems. This study indicates that the selective binding characteristics of molecularly imprinted polymers offer promise for the preparation of novel controlled release drug dosage forms. Work focused on improving the homogeneity of recognition site populations should lead to enhanced performance.

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