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Communication

Pharmaceutical applications for molecularly imprinted polymers☆

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Abstract

Molecular imprinting is a means of introducing sites of specific molecular arrangement into an otherwise uniform polymeric matrix. This is achieved by formation of a pre-polymerisation complex between complementary monomers and the template molecule. Subsequent polymerisation in the presence of a crosslinker, in a porogenic environment, results in the production of a macroporous polymer capable of specific molecular recognition. This paper considers potential roles for molecularly imprinted polymers within a pharmaceutical remit. Applications including controlled release, drug monitoring devices and biological receptor mimetics are discussed. Histamine and ephedrine molecularly imprinted polymers (MIPs) were studied as potential biological receptor mimics whilst a propranolol MIP was investigated for its use as a rate attenuating selective excipient in a transdermal controlled release device. Preliminary studies concerning the preparation of a theophylline selective transcutaneous monitoring device, using a theophylline MIP, are also described.

Keywords: Molecularly imprinted polymer; Biomimetic; Molecular recognition

1. Introduction

Molecular imprinting is a technique for the introduction of regions of highly specific molecular arrangement into a polymeric matrix (Allender et al., 1999). Formation of a pre-polymerisation complex, between complementary monomers and the template molecule, followed by polymerisation in the presence of a crosslinker, in a porogenic environment, produces a molecularly imprinted polymer (MIP) capable of specific molecular recognition (Fig. 1).

The most common use of this technology has been resolution of racemates, but MIPs are increasingly used as molecularly selective components in other applications such as combinatorial screening (Bowman et al., 1998), pseudoimmunoassays (Vlatakis et al., 1993), heterogeneous catalysis (Matsui et al., 1996) and analyte enrich-

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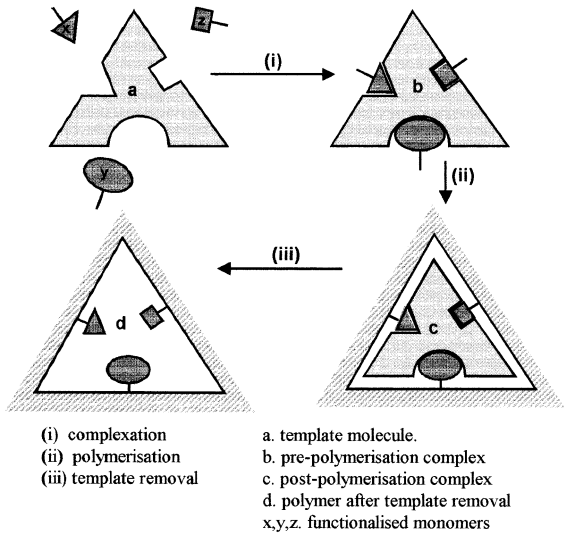

Fig. 1. Schematic representation of a molecular imprinting process.

ment (Martin et al., 1997). This paper describes some potential pharmaceutical applications. Histamine and ephedrine MIPs were studied as potential biological receptor mimics, a propranolol MIP was investigated as a rate attenuating selective excipient in a transdermal controlled release device, and initial studies on preparation of a theophylline selective transcutaneous monitoring device are also described. To evaluate histamine and ephedrine MIPs as potential biological receptor mimics, polymers were packed into HPLC columns and retention behavior of known antagonists and agonists studied. The major problem in development of a transdermal/MIP controlled release device was reduction in the water content of the environment around the MIP. In an aqueous environment, the highly polar, hydrogen-bonding, nature of water minimises interaction between MIP and ligand, resulting in loss of affinity and selectivity. The approach was to prevent water from associating with the imprinted binding site on the MIP by embedding MIP and drug within a secondary polymer matrix of commercially available non-polar transdermal adhesive. A batch binding protocol was used to investigate whether a theophylline MIP could selectively entrap theophylline in the presence of the analogue, caffeine, at concentrations below 1 μg/ml.

2. Materials and methods

2.1. Materials

Histamine, ephedrine, theophylline and propranolol were obtained from Sigma (Poole, UK), methacrylic acid (MAA) and ethyleneglycol dimethacrylate (EGMA) from Aldrich (Poole, UK) and azoisobutyronitrile (AIBN) from Jansen (Geel, Belgium). Chloroform and acetonitrile were of analytical and HPLC grade respectively and were obtained from Fisher (Loughborough, UK).

2.2. Preparation of molecularly imprinted polymers (MIPs)

Histamine, ephedrine, theophylline and propranolol MIPs were all prepared using MAA, EGMA, AIBN and chloroform (O'Shannessy et al., 1989) (Table 1). Solvent was dispensed into a 25-ml multi-injection vial and cooled on ice for 5 min before addition of the template. Once the template had dissolved the monomers and AIBN were added, the vial was thoroughly mixed, and crimp sealed. Dissolved oxygen was removed by sonicating under vacuum and sparging with nitro-

Table 1
MIP compositions

	Template	MAA (mmol)	EGMA (mmol)	AIBN (mmol)	Porogen (mmol)
Histamine	1	4	20	0.25	MeCN
Ephedrine	1	4	20	0.5	$CHC1_3$
Propranolol	1	6	30	0.5	$CHCl_3$
Theophylline	1	6	30	0.5	$CHCl_3$

Table 2
Retention data for analyses on histamine MIP and NIP-columns

Analyte	Therapeutic class	K' MIP	K' NIP
Histamine	Receptor agonist	5 64	1 29
Mepyramine	H_1 Antagonist	14.18	3 67
Diphenhydramine	H_1 Antagonist	4.43	0.64
Chlorpheniramine	H_1 Antagonist	16 53	3.31
Cimetidine	H_2 Antagonist	23.14	1.33
Ranitidine	H_2 Antagonist	11.40	2.95
Propranolol	β Receptor antagonist	7 43	1.78
Pindolol	β Receptor antagonis	11.07	2 63
Quinine	Antimalarial	5.90	1.6
Imidazole	Heterocycle	3.98	1.15
Prilocaine	Anaesthetic	8.5	2.59

Table 3
Retention data for analyses on ephedrine MIP and NIP columns

Analyte	Therapeutic class	K' MIP	K' NIP
1*R*, 2*S*-Ephedrine	β Agonist	25.70	0.10
2*R*,1*S*-Ephedrine	β Agonist	3.01	0.11
Salbutamol	β Agonist	3.57	0.24
Metaprolol	β Receptor antagonist	3.65	0.17
R-Proranolol	β Receptor antagonist	3.57	0.11
S-Propranolol	β Receptor antagonist	5.28	0.11
RS-Pindolol	β Receptor antagonist	3.95 ± 6.07	0.11
Nadolol	β Receptor antagonis	3.61	0.09
Labetolol	β Receptor antagonist	2.91	0.17
Adrenaline	α and β agonist	3 34	0 49
Noradrenaline	α and β agonist	3.08	0.24
D/L Amphetamine	CNS stimulant	0.66	0.03
Pseudoephedrine	Symapathomimetic	4.03	0.24
Tyramine	Symapathomimetic	0.68	0.06

gen for 5 min. The vial was returned to ice and irradiated with UV light at 366 nm for 6 h and the vials refrigerated (2–4°C) for a further 12 h. Control, non-imprinted polymers (NIPs) were also prepared in the absence of a template molecule.

2.3. Histamine and ephedrine MIPs as biological receptor mimics

Polymers were coarsely ground by hand, wet balled-milled in acetonitrile for 2.5 h, and sieved (45 μm). Material that passed the sieve was sedimented in acetonitrile for 3×3 h to remove fines. Sedimented material was packed into a 15-cm × 4.6-mm stainless steel HPLC column using a Jones Chromatography (Hengoed, UK) slurry column packer. MIP and NIP columns were eluted with 80% acetonitrile: 20% acetic acid until a stable base line was obtained. Detection was at 226 nm for histamine and 257 nm for ephedrine. Affinity of a range of antagonists, agonists and other structurally related analytes was examined in terms of peak shape and retention time. Injection amounts were 2–20 ng in 20-μl injection volumes.

2.4. Propranolol MIP as a selective excipient in transdermal controlled release devices.

MIP and NIP were ball-milled for 6 h and sieved (45 μm) but not sedimented as increased surface area resulting from inclusion of fines was potentially advantageous. Template was removed by sequential washing with acetonitrile containing 20% acetic acid. To prepare 1 cm (0.79 cm^2) diameter devices with a drug loading of 0.5 mg, 19.1 mg of propranolol was dissolved in 3 ml chloroform in a 20-ml snap top sample vial and 100, 300 or 500 mg propranolol MIP added. Control devices were prepared with 0 mg MIP. After 2 h equilibration, 4.5 ml of adhesive was added and thoroughly mixed, taking care not to introduce bubbles, and poured into an aluminium former (15×2 cm, area 30 cm^2) lined with aluminium foil. The former was covered and left overnight before 1-cm diameter discs were cut with a cork-borer. These were firmly pressed onto

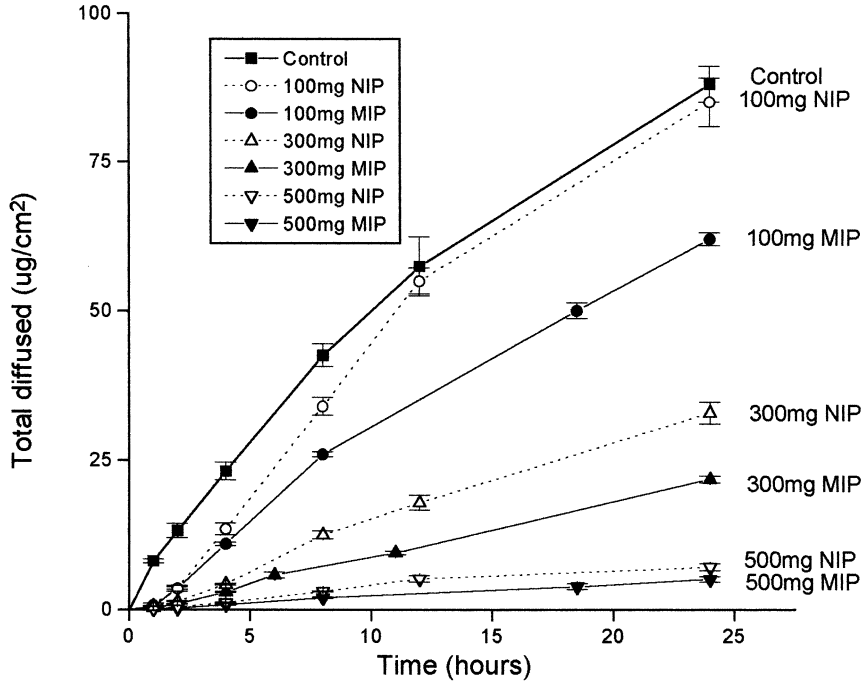

Fig. 2. Permeation profiles for the diffusion of propranolol from MIP and NIP transdermal devices.

the centre of 4-cm^2 silicone membranes and the membrane/devices mounted in Franz type diffusion cells placed in a water bath at 37°C and stirred continuously. Receptor phase was 50% ethanol and 400-μl samples were taken at 1, 2, 4, 8, 12 and 24 h and replaced with pre-equilibrated receptor solution. Samples were stored at −20°C prior to HPLC analysis [Apex C18 25 cm × 4.6 mm (Jones Chromatography, Hengoed, UK), mobile phase 70% MeCN:30% 100 mM sodium dodecasulphate, 10 mM Na_2HPO_4 (aq) adjusted to pH 2 with orthophosphoric acid, flow rate 1 ml/min, detection at 291 nm, injection vol. 20 μl].

2.5. Selective entrapment of theophylline

MIP and NIP were ball-milled for 6 h and sieved (45 μm) but not sedimented and template molecule removed by sequential washing in acetonitrile containing 20% acetic acid. A total of 25 mg of theophylline MIP and NIP and 1 ml of a theophylline or caffeine solution (0.2–1.0 μg/ml in MeCN containing 5% AcOH) were added to 2-ml Eppendorf tubes which were stirred overnight at room temperature. Polymer was spun down (1000 rpm for 5 min) and the concentration of analyte in the supernatant determined by HPLC (25 cm Apex C18 20% MeCN/80% 0.01 M acetate buffer

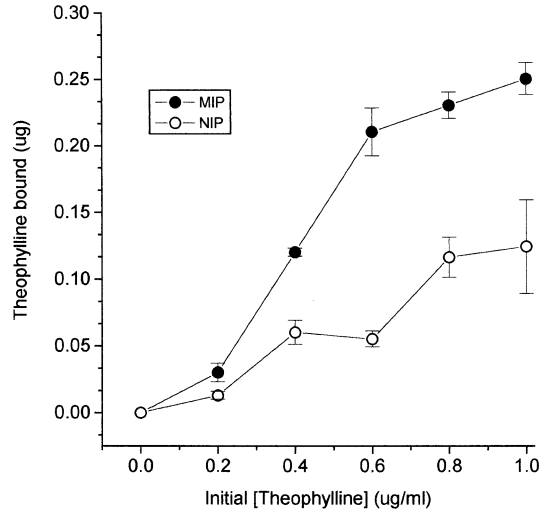

Fig. 3. Binding profile for theophylline (0.2–1.0 μg/ml) to a theophylline MIP and NIP.

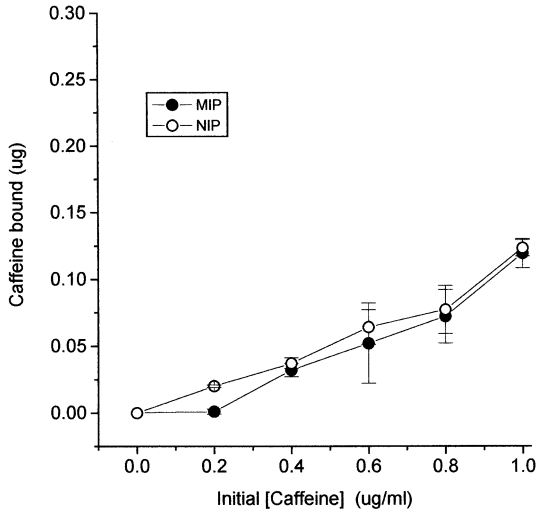

Fig. 4. Binding profile for caffeine (0.2–1.0 μg/ml) to a theophylline MIP and NIP.

pH 4, flow rate 1 ml/min, detection 270 nm, 20-μl injection volume).

3. Results

3.1. Histamine and ephedrine MIPs as biological receptor mimics

Tables 2 and 3 give capacity factors (K') for the histamine and the ephedrine column.

3.2. Propranolol MIP as a selective excipient in transdermal controlled release devices

Permeation profiles for the propranolol MIP and NIP devices are shown in Fig. 2.

3.3. Selective entrapment of theophylline

Figs. 3 and 4 shows the binding profiles of the theophylline MIP and NIP for caffeine and theophylline.

4. Discussion

Chromatographic data for both ephedrine and histamine MIP columns shows a significantly higher binding for their template molecules relative to the NIP column. In addition, binding of antagonistic, agonistic and structural relatives suggests that the imprinted materials possess biomimetic character. Of particular note was the ability to discriminate between 1*R* 2*S* ephedrine (template) and 1*S* 2*R* ephedrine and to separate the enantiomers of the β-antagonists propranolol and pindolol.

The difference in permeation of propranolol from NIP and MIP devices indicated that specific binding occurred. MIP/adhesive devices are therefore capable of controlling flux and could provide a useful means of extending the delivery profile of devices.

Theophylline binds to theophylline MIP to a greater extent than to the NIP but this difference was not observed with caffeine. Such materials could allow selective transcutaneous sequestration of analytes and provide a useful approach to non-invasive monitoring of systemic drug levels.

5. Conclusion

Molecular imprinting is an accessible solution to the problem of preparing synthetic molecular recognition systems. Results suggest that their use in pharmaceutical devices may provide significant advantage over some currently utilised approaches.

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