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Recognition of ephedrine enantiomers by molecularly imprinted polymers designed using a computational approach†

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A new approach to the computational design of molecularly imprinted polymers (MIP) specific for ephedrine is presented. A virtual library of functional monomers was developed and screened against the template using molecular modelling software. The monomers giving the highest binding score were co-polymerized with a cross-linker in the presence of ephedrine. Control (blank) polymers were prepared under the same conditions but in the absence of the template. A good correlation was found between the modelling results and performance of the materials in an HPLC study. A MIP based on one of the selected monomers—hydroxyethyl methacrylate—gave a separation of ephedrine enantiomers with a separation factor α of 1.42–2.09 (depending on temperature). This figure is larger than the α values generally obtained with commercially available chiral phases. It is anticipated that the computational approach will be of use for the rational design of MIPs and the prediction of polymer affinity and specificity.

Introduction

The technology of molecular imprinting, originally developed in 1931,[1] and rediscovered twice in 1949[2] and 1972,[3] is in the process of exponential growth.[4] Essentially, this progress is a result of fundamental achievements by Mosbach and Wulff in the areas of non-covalent and reversible covalent imprinting.[5,6] The broad variety of functional monomers currently available makes it possible to design a molecularly imprinted polymer (MIP) specific for potentially any type of chemical compound. Currently, the selection of the best monomer for polymer preparation is one of the most crucial issues in molecular imprinting. Although thermodynamic calculations and combinatorial screening approaches offer a possible solution, and have already been used successfully in the past to predict polymer properties and to optimize polymer composition,[7–9] in practical terms, the application of these methods will always be limited to very specific cases and examples. The reason for this conclusion lies in the technical difficulty of performing thermodynamic calculations on real systems at this stage, and the amount of time and resources needed for the combinatorial screening of polymers. To check a simple two-component combination of 100 monomers, for example, one has to synthesize and test more than 5000 polymers—an enormous task.

In this paper, we describe an alternative approach to the design of MIPs, which is based on molecular modelling and the fact that it is significantly easier to perform a computational screen of a virtual monomer library than to perform a real one. The idea is to create a virtual library of functional monomers and screen them on all possible interactions with the molecular model of the template.

We hypothesize that the monomers giving the highest binding score with the template should give the polymer with higher affinity and specificity. In order to test this concept, a set of MIPs was designed, synthesized and tested using HPLC experiments to monitor the separation of ephedrine enantiomers.

Experimental

Materials

(−)-Ephedrine [(1*R*,2*S*)-(−)-α-(1-methylaminoethyl)benzyl alcohol] and (+)-ephedrine hydrochloride [(1*S*,2*R*)-(+)-α-(1-methylaminoethyl)benzyl alcohol hydrochloride] were supplied by Chemical Development, GlaxoSmithKline R&D, UK. The free base of (+)-ephedrine was prepared by neutralizing the hydrochloride salt with 1 N NaOH and extraction with chloroform. Ethylene glycol dimethacrylate (EGDMA), itaconic acid (IA), methacrylic acid (MA), hydroxyethyl methacrylate (HEM), 2-vinylpyridine (2-VP), acrylamide (AA), 1,1′-azobis(cyclohexanecarbonitrile), hexamethylenediamine (HMDA) and chloroform were purchased from Aldrich (UK). Tetrahydrofuran (THF) and dimethylformamide (DMF) were obtained from BDH (UK). All chemicals were of analytical or HPLC grade and were used without further purification.

Molecular modelling

The workstation used to simulate monomer–template interactions was a Silicon Graphics Octane running the IRIX 6.5 operating system. The workstation was configured with two 195 MHz reduced instruction set processors, 712 MB memory and

† Electronic Supplementary Information available. See http://www.rsc.org/suppdata/an/b1/b102426b/

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a 12 GB fixed drive. This system was used to execute the software package SYBYL 6.7 (Tripos Inc., St. Louis, MO, USA). The molecular model of (−)-ephedrine (template) and a virtual library of the 20 commonly used monomers were created (Fig. 1). In addition, molecular models of the formally charged template and formally charged acidic and basic monomers were generated and added to the library. All of these structures were then charged with the Gasteiger–Huckel computational method, and refined by the molecular mechanics method (often called force field method), applying an energy minimization using the MAXIMIN2 command.[10] This command enables one to optimize the geometry and to minimize the strain energy of a given molecule. In the second step, the LEAPFROG algorithm was used to screen the library of functional monomers on their possible interactions with the template. The program was applied for 30,000 iterations and the results of these were examined and the empirical binding energy score evaluated (Table 1). The interactions contributing to the binding energy score are ionic and hydrogen bonds, van der Waals' and dipole–dipole interactions and steric factors. The top four monomers giving the highest binding score and capability of forming the strongest complexes with the template molecule were selected for the polymer preparation: IA, MA, HEM and AA.

Preparation of MIPs

A set of polymers was synthesized using five monomers which showed the highest binding energy towards the template. The composition of the monomer mixture is presented in Table 2. A 10 : 1 molar ratio of functional monomers to template was used in order to saturate all functional binding sites in the template.[11] The amount of cross-linker (EGDMA) was calculated as 80% w/w of the functional monomer, template and solvent. The polymers were prepared using chloroform as a porogen, with the exception of IA- (P1) and AA-based (P4) polymers, which needed THF to solubilize the monomers. 1,1′-Azobis(cyclohexanecarbonitrile) (1%) was used to initiate the polymerization. Corresponding blank polymers (B1–B5) were prepared in the absence of the template. The reaction mixture was purged with nitrogen and then left to polymerize overnight at 80 °C. The bulk polymers were ground in methanol with an SL2 suspension

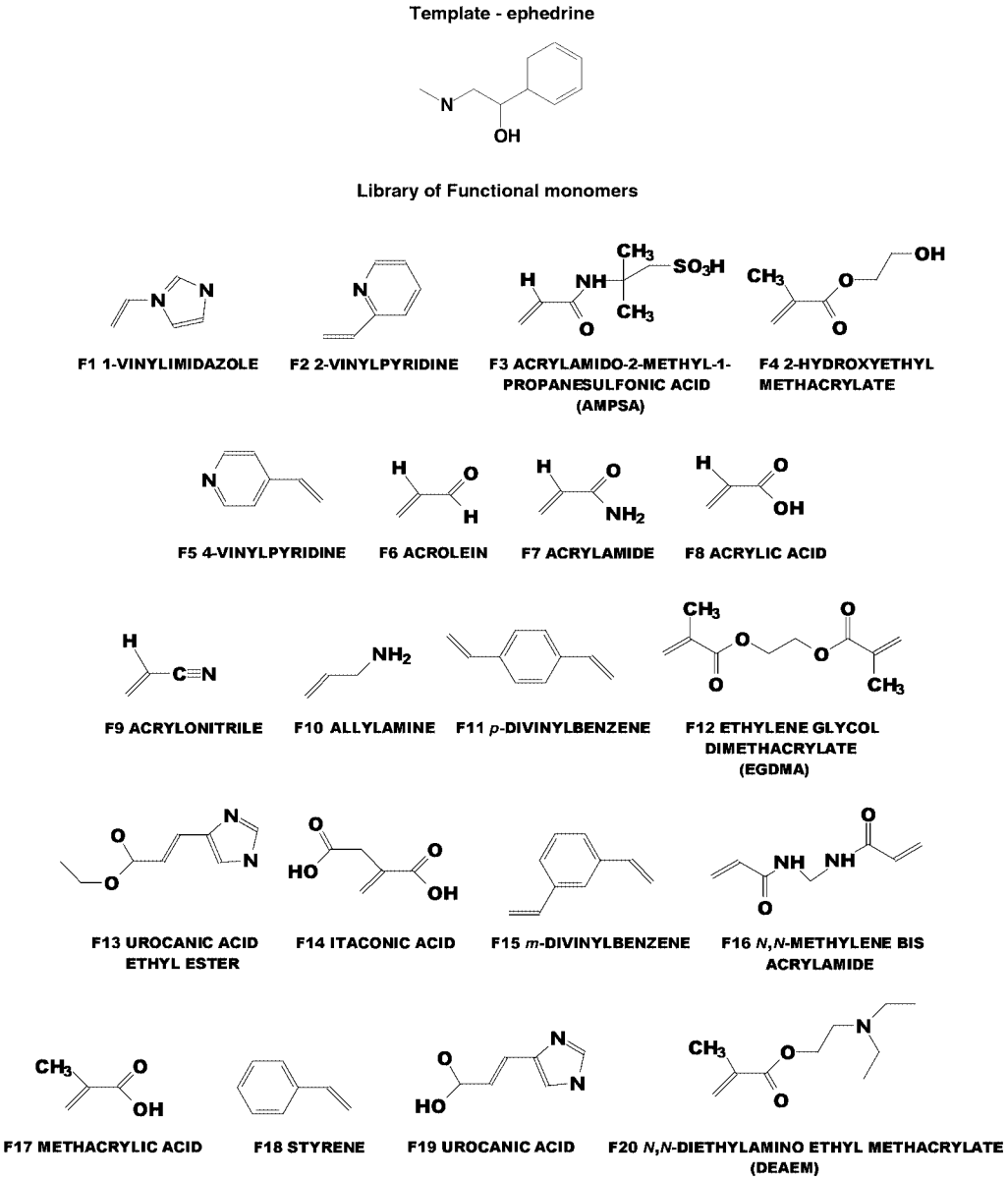

Fig. 1 The structure of the template and the functional monomers included in the virtual library and used in the computer simulation.

grinder (Silverson, UK) and mechanically wet-sieved through 106 and 38 μm sieves (Endecotts, UK). Polymer particles with a size range of 38–106 μm were collected, dried under vacuum and used for packing the HPLC columns.

HPLC analysis

For the analysis of MIP recognition properties, 1 g of polymer particles (size, 38–106 μm) was suspended in methanol and packed into an HPLC column (100 × 4.6 mm) under reduced pressure. Columns were washed (1 ml min^{-1}) with 20% acetic acid in acetonitrile, followed by water and acetonitrile to remove residual template. The evaluation experiments were carried out using an HPLC system, which included a Consta-Metric-3200 solvent delivery system (LDC Analytical, UK), Perkin-Elmer ISS-100 automatic injection system and a Waters Lambda-Max Model 481 LC detector (UK). The chromatographic conditions, such as eluent composition and flow rate, were optimized for each polymer in order to achieve the best enantiomer separation. Elution was monitored optically at 260 nm. All reported chromatographic data represent the results of at least three concordant experiments. The standard deviation in the experiments was below 5%. Capacity factors (k') were determined from $k' = (t - t_0)/t_0$, where t is the retention time of a given species and t_0 is the retention time of the void marker (acetone). Effective enantioseparation factors (α) were calculated from the relationship $\alpha = k'(-)/k'(+)$, where $k'(-)$ and $k'(+)$ are the capacity factors of (−)- and (+)-ephedrine, respectively. The imprinting factor (I) was calculated from the equation: $I = k'(-)_{MIP}/k'(-)_{Blank}$, where $k'(-)_{MIP}$ is the capacity factor of (−)-ephedrine calculated for MIP and $k'(-)_{Blank}$ is the capacity factor of (−)-ephedrine calculated for the corresponding blank polymer.

Table 1 Screening of a virtual library of functional monomers on their interaction with ephedrine

Number	Binding interaction	Binding/ kcal mol^{-1}
1	Itaconic acid (charged)–ephedrine (charged)	−64.01
2	Itaconic acid (neutral)–ephedrine (neutral)	−33.81
3	Itaconic acid (neutral)–ephedrine (charged)	−23.14
4	Itaconic acid (charged)–ephedrine (neutral)	−15.94
5	Methacrylic acid (charged)–ephedrine (charged)	−58.72
6	Methacrylic acid (charged)–ephedrine (neutral)	−26.71
7	Methacrylic acid (neutral)–ephedrine (charged)	−22.48
8	Methacrylic acid (neutral)–ephedrine (neutral)	−14.62
9	Hydroxyethyl methacrylate (neutral)–ephedrine (neutral)	−15.72
10	Acrylamide (neutral)–ephedrine (neutral)	−13.63
11	2-Vinylpyridine (neutral)–ephedrine (neutral)	−1.82

Table 2 Polymer composition. Corresponding blank polymers (B1–B5) had the same composition, with the exception of ephedrine which was absent from the monomer mixture

Polymer	P1	P2	P3	P4	P5
(−)-Ephedrine/g	0.20	0.20	0.20	0.20	0.20
IA/g	1.58	—	—	—	—
MA/g	—	1.04	—	—	—
HEM/g	—	—	1.57	—	—
AA/g	—	—	—	0.86	—
2-VP/g	—	—	—	—	1.27
EGDMA/g	7.10	4.96	7.10	4.24	5.89
Chloroform/g	—	6.20	8.87	—	7.36
THF/g	8.88	—	—	5.30	—
1,1′-Azobis(cyclohexanecarbonitrile)/g	0.18	0.12	0.18	0.11	0.15

Results and discussion

Calculated energy of the monomer–template interactions and polymer affinity

Two factors are important for the effective recognition of the template by MIP: the strength and quantity of the interactions between the monomers in the polymer network and the template. The computer simulation experiment identified acidic monomers (IA and MA) and neutral/weak basic monomers (AA and HEM) as the most promising, with the capability of forming strong interactions with the template. To provide diversity for the polymer systems, additional polymers (P5 and B5) were prepared using 2-VP. This monomer is found near the bottom of Table 1 with a binding energy of only −1.82 kcal mol^{-1} (see complete set of calculated values of monomer binding energies in the Electronic Supplementary Information†). The interactions between ephedrine and cross-linkers, such as EGDMA and divinylbenzene, included in the library were negligible, which is important for the minimization of non-specific interactions between the polymer and the template. By using blank polymers (B1–B5) in the HPLC study, we anticipated a correlation between the affinity of the polymer and the calculated binding energy for template–monomer interactions. Because it is highly unlikely that IA and MA will be charged under the conditions normally used for the chromatographic evaluation of MIPs (chloroform in the presence of a weak acid or base), the results of modelling with charged monomers were excluded from the evaluation. To mimic the conditions in which a neutral polymer interacts with a neutral and protonated (ionized) template, the free base and HCl salt of ephedrine were injected separately into the columns containing blank polymers. The experiment conducted with chloroform in the presence of 0.3% acetic acid indicated that a correlation between the calculated binding energy and the capacity factor of the analyte exists (Table 3). The monomers with the highest binding energy scores (IA and MA) produced polymers (B1 and B2) which had the strongest interaction with the template in the HPLC experiments. The only exception was found in the behaviour of the HEM-based polymer (B3) which demonstrated unusually low binding ability to ephedrine, with $k' = 0.3$, that did not

Table 3 Chromatographic evaluation of polymers performed in chloroform. Ten microlitres of sample (concentration, 1 mg ml^{-1}) were injected for analysis. The eluent was chloroform with 0.3% acetic acid. The flow rate was 1 ml min^{-1}

Monomer	Template condition	Monomer–template binding energy/ kcal mol^{-1}	$k'(-)_{Blank}$	$k'(-)_{MIP}$	Imprinting factor, I
IA	Neutral	−33.81	9.4	∝	∝
	Ionized	−23.14	7.2	—	—
MA	Neutral	−14.62	3.3	9.46	2.87
	Ionized	−22.48	7.3	—	—
HEM	Neutral	−15.72	0.3	0.8	2.67
AA	Neutral	−13.63	1.4	2.59	1.85
2-VP	Neutral	−1.82	0.1	0.1	1

match the calculated binding energy for these molecules (-15.72 kcal mol^{-1}). The explanation for this phenomenon could be the fact that HEM is quite hydrophobic, as can be seen from the separation experiment on a C_{18} column with a water–acetonitrile gradient (see data in Electronic Supplementary Information†). It is possible that, during the polymerization step and phase separation, a significant portion of this monomer is trapped in the hydrophobic core of the polymeric domains where it has very little access to the surface. The low proportion of the surface functional groups available for the interaction with ephedrine in the HEM-based blank polymer could be responsible for the unexpectedly low affinity of this material.

The second possibility is the different reactivity of the monomers in terms of their incorporation into the growing polymer chain. It is possible that HEM polymerizes less effectively than MA, for example, providing fewer numbers of functional groups exposed at the polymer surface. We are currently working on the development of an experimental procedure which will enable us to test these hypotheses. It will also be important for the future to be able to incorporate the polymerization rate of functional monomers into the modelling process for the selection of optimal monomers for MIP preparation.

It is interesting to note that the computer simulation can also be used to mimic real chromatographic experiments. For example, the interaction of the MA-based polymer (B2) with the HCl salt of ephedrine (binding energy of MA with ionized ephedrine is -22.48 kcal mol^{-1}) was much stronger, giving a capacity factor of $k' = 7.3$, than its interaction with neutral free base ephedrine (binding energy of MA with neutral ephedrine is -14.62 kcal mol^{-1}), where the capacity factor k' was 3.3. A similar correlation also existed in the performance of the IA-based polymer (B1) (Table 3). MIPs also demonstrated the same correlation between the calculated binding energy and the polymer affinity (Table 3). An even better correlation was found between the binding energy of the monomer–template interaction and the imprinting factor (I). Obviously, the strength of the monomer–template interaction is important for successful imprinting.

Calculated energy of the monomer–template interactions and polymer specificity (enantioseparation)

As anticipated, no separation of enantiomers was observed using blank polymers, which indicates that the polymer chirality originates from the imprinting effect. In contrast to affinity data for MIP and blank polymers, the analysis of polymer enantiospecificity proved to be more difficult to perform and to interpret. First of all, it was impossible to perform enantioseparation on MIPs using the same conditions due to a significant difference in binding of the template to the polymers. Thus, the elution of ephedrine from the IA-based MIP (P1) was only possible using at least 10% acetic acid in chloroform. These elution conditions were apparently too aggressive for the rest of the polymers. As a result, the experimental conditions were optimized for each polymer separately. The results of the evaluation are presented in Table 4.

Table 4 The separation of ephedrine enantiomers on computationally designed MIPs under optimized conditions. Ten microlitres of sample (concentration, 1 mg ml^{-1}) were injected for analysis. The flow rate was 1 ml min^{-1}

Polymer	Eluent	$k'(-)$	$k'(+)$	α
P1 (IA)	10% acetic acid in chloroform	3.25	2.76	1.18
P2 (MA)	1% acetic acid in chloroform	6.48	4.82	1.34
P3 (HEM)	0.1% HMDA in chloroform	1.09	0.77	1.42
P4 (AA)	0.1% HMDA in chloroform	1.1	0.92	1.2
P5 (2-VP)	0.1% HMDA in chloroform	0.1	0.1	1

It is possible to conclude that, in contrast to polymer affinity, the enantioselectivity does not depend directly on the strength of the monomer–template interaction. This conclusion is not totally surprising. For effective enantioseparation, a polymer should be able to form multiple interactions with the template (*e.g.* hydrogen bonds and van der Waals' interactions). The screening of the virtual library of functional monomers using the LEAPFROG algorithm gives information about the strength of template binding with one monomer molecule only. Further attempts were made to predict the polymer specificity using simulated annealing, a special molecular dynamics experiment, which reflects the influence of solvent and cross-linker on monomer–template interactions.

Simulated annealing of the monomer–template complexation

During this step, the space around the template in a virtual box was surrounded by multiple copies of monomer, cross-linker and corresponding solvent molecules (THF for IA and AA, chloroform for MA, HEM and 2-VP) using the XFIT solvation algorithm (integrated module of SYBYL 6.7), and the energy of the system was minimized.

The simulated annealing process was then applied to the box to analyse the arrangement of functional monomers around the template as it exists in the monomer mixture. Simulated annealing is a type of molecular dynamics experiment in which the temperature of the system is cycled over time with the goal of wide sampling of the conformational space around the template. The mechanism is to apply a higher temperature to allow the system to rearrange from its present state, and to lower the temperature to bring the system into a stable state. This method was used to obtain information about low-energy configurations of a system of interacting molecules and to study the possible influence of other factors, such as solvent and cross-linker, on monomer–template interactions.

The starting annealing temperature was fixed at 973 K and lowered to a final temperature of 273 K in seven successive 100 K steps (the dynamic equilibrium was reached in 2000 fs). After each step, the system was minimized to 0.01 kcal mol^{-1}. At the end of the program, when 273 K was reached, the number and position of the functional monomers were examined.

The annealing experiment showed that the solvent has a marked influence on the complexation process. Thus, only one molecule of IA and AA formed hydrogen bonds with ephedrine in the presence of THF. In chloroform, two molecules of each monomer, MA and HEM, interacted through hydrogen bonds with one template molecule (Fig. 2). No indication of hydrogen bond formation between 2-VP and ephedrine was observed. These results are in agreement with experiment, where MA and HEM were identified as the best monomers (Table 4). Superior enantioseparation was demonstrated by the HEM-based MIP (P3), which again could be related to the very low non-specific binding of (+)-ephedrine due to the low exposure of the functional groups at the P3 surface, as postulated previously.

Separation of ephedrine enantiomers with MIPs

The separation of enantiomers could be further improved by increasing the temperature (Table 5). The positive effect of increased temperature has been explained previously in terms of a general improvement in mass transfer kinetics,[12] and disproportional changes in the numbers of theoretical plates observed for two enantiomers at increased temperature.[13] Alternatively, we can assume that, with a subsequent increase in temperature, the thermal extension of the polymer approaches the level that existed during polymerization. As a result, the size and structure of the binding cavities should resemble more

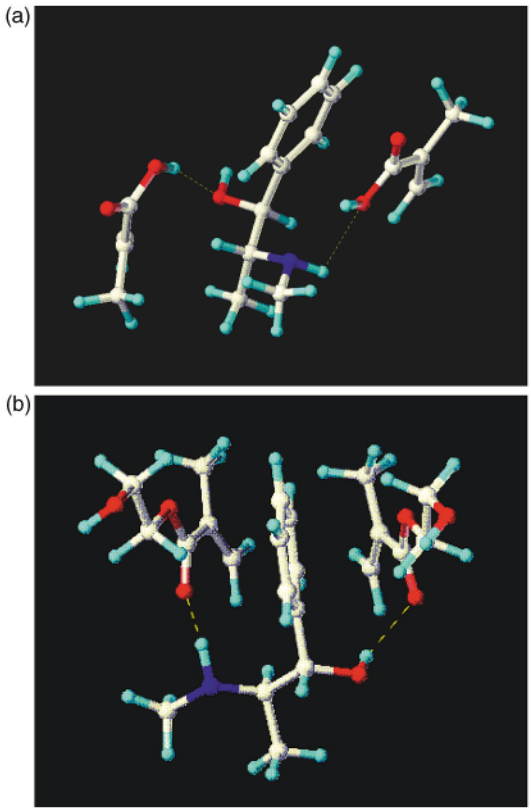

Fig. 2 The possible structure of the monomer–template complex formed between (−)-ephedrine and MA (a) or HEM (b).

Table 5 Influence of the temperature on the enantioseparation achieved for HEM-based MIP (P3) (eluent, 0.1% HMDA in chloroform). Ten microlitres of sample (concentration, 1 mg ml^{-1}) were injected for analysis. The flow rate was 1 ml min^{-1}

Temperature/°C	$k'(-)$	$k'(+)$	α
−10	0.47	0.39	1.21
+24	1.09	0.77	1.42
+40	1.08	0.62	1.74
+55	0.94	0.45	2.09

accurately the structure of the original imprints as they were created around the template molecules.

Although the efficiency of the enantiomer separation was poor (Fig. 3), we anticipate that, with further optimization of the solid phase format, *e.g.* by using larger columns packed with smaller particles (5–10 μm) and providing high-pressure packing, baseline separation of the enantiomers could be achieved.

The selectivity achieved in the separation of ephedrine enantiomers by P3 (α = 2.1) is better than the selectivity demonstrated previously using commercially available chiral phases. According to the literature (Chirobase.db/ISIS™/Base2.3 (MDL Information Systems Inc, USA), only two out of 20 commercially available chiral phases tested for the separation of ephedrine enantiomers demonstrated a separation factor higher than 1.3, with the absolute record achieved for an α-acid glycoprotein-based column (α = 1.8). It is important to note that, in addition to the separation factor, two other parameters, *i.e.* the resolution factor and binding capacity of the polymer, are important for the practical application of MIPs. In this respect, the imprinted polymers studied here are inferior to

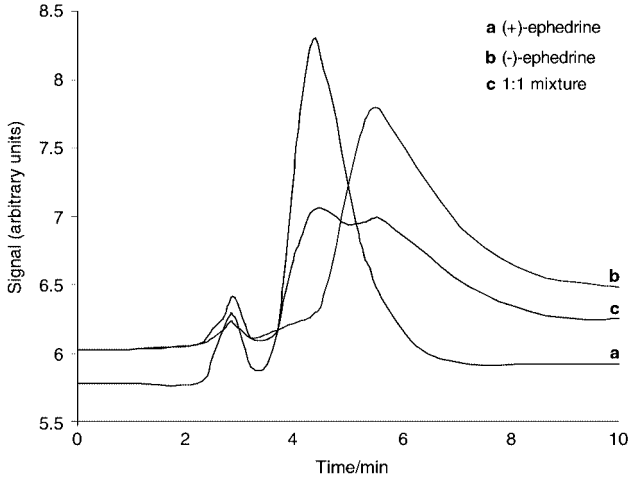

Fig. 3 The separation of ephedrine enantiomers using HEM-based MIP (P3) in 0.1% HMDA in chloroform at 55 °C. Ten microlitres of sample (concentration, 1 mg ml^{-1}) were injected for analysis. The flow rate was 1 ml min^{-1}.

commercially available chiral phases. More work needs to be performed to improve polymer performance before the full potential of MIPs in enantioseparation will be realized.

Conclusions

A new computationally designed MIP specific for (−)-ephedrine was prepared and tested for enantiomer separation. A clear correlation was found between the strength of the polymer–template interaction and the binding score obtained for the template–functional monomer in a molecular modelling study. Experiments indicate that the efficiency of enantioseparation has no direct correlation with the monomer–template binding strength and depends on the organization of the monomer–template and, perhaps, on the distribution of the functional monomers in the bulk polymer and its surface. Simulated annealing can be used to predict the influence of polymerization conditions (solvent, cross-linker, *etc.*) on the performance of imprinted polymers. The computational approach described here represents a first step towards the rational design (tailoring) of MIPs and the prediction of polymer properties.

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