ELSEVIER

Journal of Chromatography A, 848 (1999) 39–49

JOURNAL OF CHROMATOGRAPHY A

Study of the nature of recognition in molecularly imprinted polymers, II [1] Influence of monomer–template ratio and sample load on retention and selectivity

Håkan S. Andersson[a], Jesper G. Karlsson[a], Sergey A. Piletsky[a,b], Ann-Christin Koch-Schmidt[a], Klaus Mosbach[c], Ian A. Nicholls[a,*]

[a]*Bioorganic Chemistry Laboratory, Institute of Natural Sciences, University of Kalmar, P.O. Box 905, S-391 29 Kalmar, Sweden*
[b]*Institute of Molecular Biology and Genetics, Academy of Science of Ukraine, Zabolotnago 150, Kiev-143, Ukraine*
[c]*Department of Pure and Applied Biochemistry, Lund University, P.O. Box 104, S-221 00 Lund, Sweden*

Received 17 December 1998; received in revised form 15 March 1999; accepted 31 March 1999

Abstract

To investigate the extent and nature of the monomer–template association of molecularly imprinted polymer pre-polymerisation mixtures, a series of (−)-nicotine molecularly imprinted acrylic polymers was prepared using a range of monomer–template molar ratios. Load capacity studies performed in the chromatographic mode showed an unexpected increase in retention at higher nicotine sample loads. Further analyses of this effect indicate that solvation effects, and potentially the presence of higher order template complexes, may explain this behaviour. The implications of these results are discussed in terms of the current model for molecular imprinting, and it is suggested that the possibility of template self-association should be included in this model.

Keywords: Molecularly imprinted polymers; Monomer–template ratio; Sample load; Retention behaviour; Selectivity; Stationary phases, LC; Nicotine

1. Introduction

The preparation of molecularly imprinted polymers (MIPs) has gained acceptance as a means for constructing binding sites of predetermined ligand selectivity [2–7]. This selectivity is attributed to the formation either of reversible covalent or non-covalent complexes in solution between a template molecule (the compound for which recognition is desired) and functionalised monomers, which are subsequently fixed through polymerisation using an excess of cross-linker to yield a rigid polymer network. Extraction of the template species leaves a material with the ability to bind the template in preference to structurally related compounds [8].

The practical applications of MIPs based on non-covalent rebinding have been extensively explored over recent years. However, relatively little effort has

*Corresponding author. Tel.: +46-480-446258; fax: +46-480-446262.
E-mail address: ian.nicholls@ng.hik.se (I.A. Nicholls)

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00483-5

thus far been devoted to understanding the mechanisms of the non-covalent imprinting process per se. This is contrasted by an extensive series of studies on the nature of ligand recognition in covalent MIPs [9,10]. Although the presence of monomer–template solution complexes in non-covalent MIP systems has been verified both by NMR [11,12] and UV spectroscopy [13], few direct physical studies of MIP-ligand complexes have been reported. The current perception of MIP-ligand binding has been deduced from the results of either batchwise [14] or chromatographic [15,16] studies.

The present study is part of ongoing research in our laboratories [1], aiming to clarify the mechanisms underlying the molecular imprinting process and MIP-ligand recognition. In this study, we have examined the influence of the functional monomer–template molar ratio (M/T) in the pre-polymerisation mixture on the recognition characteristics of the resultant acrylic polymers. A series of (−)-nicotine imprinted and reference polymers has been prepared and their recognition characteristics have been evaluated extensively in the chromatographic mode, with an emphasis on MIP-ligand selectivity as a function of sample load. The results are discussed in relation to the anticipated complex states in the pre-polymerisation mixture and in the eluent, and it is suggested that template self-association may play a role in MIP-ligand selectivity and in the molecular imprinting process.

2. Experimental

2.1. Chemicals

2,2′-Azo bis(2-methylpropionitrile) (AIBN) and methacrylic acid (MAA) were purchased from Acros Chimica, Belgium. 4,4′-Bipyridyl, ethylene glycol dimethacrylate (EGDMA) and (+/−)-nicotine were obtained from Fluka (Buchs, Switzerland). 3-Picoline was supplied by Lancaster, UK. (−)-Nicotine (>98% ee) was purchased from Merck, Germany. All solvents were of analytical grade and obtained from commercial sources.

2.2. Polymer preparation

Two series of polymers (**P0–P6**, **P7–P9**) were prepared following the general method of O'Shannessy et al. [17], and a third series (**P10–P12**) was prepared according to Andersson et al. [1], Table 1. A mixture of MAA, EGDMA and AIBN was dissolved in the porogen (chloroform or acetonitrile) in a 50-ml glass ampoule together with the template molecule [4,4′-bipyridyl or (−)-nicotine], Table 1. The monomer mixture was cooled on ice and degassed in a sonicating bath, then sparged for 15 min with N_2. The ampoule was then sealed and placed under a UV source (366 nm) at 4°C, followed by heating (**P0–P9**) at 75°C overnight, Table 1. The reference (non-imprinted) polymers (**P0**, **P7**, **P10**)

Table 1
Polymer compositions and methods for preparation

Polymer	P0	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
Nicotine (mmol)	–	1.9	3.8	7.5	22.5	22.5	75	–	1.9	22.5	–	1.9	–
4,4′-Bipyridyl (mmol)	–	–	–	–	–	–	–	–	–	–	–	–	1.9
HOAc (mmol)	–	–	–	–	–	3.8	–	–	–	–	–	–	–
MAA (mmol)	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	15	15	15
EGDMA (mmol)	39.3	39.3	39.3	39.3	39.3	39.3	39.3	39.3	39.3	39.3	78.6	78.6	78.6
MeCN (ml)	–	–	–	–	–	–	–	12.2	11.9	8.6	–	–	–
$CHCl_3$ (ml)	12.2	11.9	11.6	11.0	8.6	8.4	–	–	–	–	24	24	24
AIBN (mmol)	0.57	0.57	0.57	0.57	0.57	0.57	0.57[a]	0.57	0.57	0.57	1.14	1.14	1.14
M/T ratio	1/0	4/1	2/1	1/1	1/3	1/3[b]	1/10	1/0	4/1	1/3	1/0	8/1	8/1
UV at 366 nm, 4°C	24 h	24 h	24 h	24 h	24 h	24 h	24 h	24 h	24 h	24 h	44 h	44 h	44 h
Heat, 75°C	20 h	20 h	20 h	20 h	20 h	20 h	20 h	20 h	20 h	20 h	–	–	–

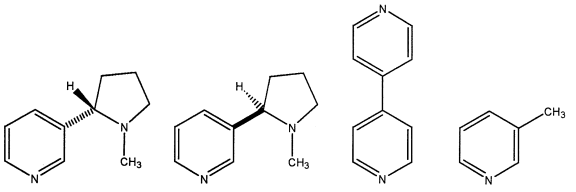
[a] Heating in a water bath (60°C, 1 min) was performed under vigourous shaking, prior to UV illumination, to allow for the solubilisation of AIBN in this mixture.

[b] This figure does not account for the use of HOAc as a monomer competitor, which renders an M/T ratio of 1/2.

were prepared identically, although in the absence of the template species. The bulk polymers were ground in short repeated cycles with a mechanical mortar (Retsch, Germany). Between each grinding cycle, the products were wet-sieved in acetone through 63 μm sieves (Endecotts, UK). Sieved fractions were pooled and sedimented from acetone (6×60 min), to yield a packing material that was suitable for HPLC.

2.3. Chromatographic evaluation

A 1.5-g amount of polymer was subsequently suspended in 80 ml of chloroform–acetonitrile (85:15, v/v). The suspensions were sonicated for 15 min, placed in a slurry reservoir and packed into stainless steel HPLC columns (100×4.6 mm) at 340 bar with a single action reciprocating plunger pump (Haskel Engineering Supply Co., USA) using 250 ml of acetone as the packing solvent. The polymer content of each column was approximately 0.7 g. The columns were washed with 250 ml of methanol–HOAc (9:1, v/v) to remove residual template from the polymers. The procedure was monitored spectroscopically at 260 nm. Subsequent evaluations were carried out at 25°C using an HPLC system comprising a thermostated column oven (Croco-cil, C.I.L., France), a Series 200 LC pump (Perkin Elmer, Norwalk, CT, USA), a 20-μl injection loop (Valco, UK) and a 785A programmable absorbance detector (Applied Biosystems, Roissy, France). All analyses were performed in triplicate. Elution was monitored at fixed wavelengths ranging between 250 and 300 nm, depending on sample and load. Acetone was used as the void marker (V_0) in all systems. All analyte solutions were prepared by dilution of the stock solutions in their corresponding mobile phases. The retention volumes (V_R) were assigned to the point of the peak where 50% of the sample had been eluted.



The influence of the M/T ratio in the pre-polymerisation mixture on the chromatographic performance of the polymers was examined using polymers **P0–P6**, which cover an M/T ratio range from 4:1 to 1:10, Table 1. (−)-Nicotine samples in the range 0.03–1.85 μmol, 4,4′-bipyridyl (0.18 μmol) and 3-picoline (0.18 μmol) were analysed using MeCN–HOAc (94:6, v/v) as the buffer at a flow-rate of 1.5 ml/min. The same sample concentrations were also used for **P2** in chloroform–HOAc (94:6, v/v) and in MeCN–TEA (99:1, v/v). A second series of (−)-nicotine injections (0.03–6.2 μmol) and (+/−)-nicotine (0.03–6.2 μmol) was subsequently performed on **P0**, **P3**, **P4** and **P6** in MeCN–HOAc (95:5, v/v). **P7–P9** were analysed in MeCN–HOAc (94:6, v/v) using (−)-nicotine in the range 0.03–1.85 μmol, 4,4′-bipyridyl (1.85 μmol) and 3-picoline (1.85 μmol). **P8** was also analysed in MeCN–TEA (99:1, v/v). Analyses of **P10–P12** were carried out in MeCN–HOAc (94:6, v/v) using (−)-nicotine samples (0.015–1.85 μmol), 3-picoline (0.03–1.85 μmol) and 4,4′-bipyridyl (0.03–1.85 μmol).

3. Results and discussion

The opportunity to assess the effects of high concentrations of template, i.e. low M/T ratios, on MIP recognition arises from the very high solubility of nicotine in these polymerisation systems. It is noteworthy that one polymer, **P6**, was prepared whereby the porogen (chloroform) was completely replaced by template, resulting in an M/T ratio of 1:10. A softer polymer texture was observed for some of the polymers prepared in the presence of high concentrations of template (especially **P6**), thus motivating heat treatment of **P0–P9** (75°C, 20 h) to yield sufficient rigidities to allow their use in subsequent high-performance liquid chromatographic (HPLC) analyses. Slight discoloration was observed for polymers prepared with the highest excesses of nicotine (**P4–P6**, **P9**).

3.1. Selectivity versus M/T ratio

The molar relationship between the functional monomer and template has been found to be important with respect to the number and quality of

MIP recognition sites [1]. Low M/T ratios afford less than optimal complexation on account of insufficient functional monomer. Too high an M/T ratio, the extreme case being a non-imprinted polymer, yields non-selective binding [18].

Fig. 1 shows the selectivity (k'_{NIC}/k'_{BIPY}) of nicotine relative to 4,4′-bipyridyl for **P0–P6**, which clearly indicates that an excess of template during polymerisation is unfavourable with regard to selectivity. This is interpreted as arising from shifts in equilibria between the anticipated complex states, Fig. 2. An excess of the nicotine template in the pre-polymerisation mixture can be expected to favour the formation of complex states **II–III** over **IV**. This conclusion is supported by the relative selectivities of **P4** and **P5**, which differ only by the presence of a small amount of HOAc added during the preparation of **P5**. The presence of the carboxylic acid led to a significantly lower selectivity for **P5** than for **P4**, which is likely to arise from competition between HOAc and MAA for template binding. Consequently, an excess of monomer in relation to the template would favour the opposite situation, where the complexes formed would be of type **IV,** but the excess of monomer would yield high numbers of non-complexed, randomly distributed monomer (state **I**), which contribute to non-specific binding, as indicated by earlier work [13].

As selectivity is considered to arise from the preorganisation of monomer by the template prior to polymerisation, we should expect complex state **IV** to be the one that is responsible for the production of higher affinity, more selective, binding sites. States **I–III** would lead to the formation of site populations of lower selectivity, although additional, weaker interactions to the cross-linker EGDMA may partly compensate for the absence of MAA. The possibility of complexes containing more than one template also exists [11], as discussed below, and this group is designated as state **V**.

3.2. Selectivity versus sample load

As high-affinity binding sites are present in limiting numbers in MIPs [14], a decrease in retention and selectivity would be expected to result from higher sample loads. Although the effects of different sample loads of (−)-nicotine on **P0–P6** in MeCN–HOAc (94:6, v/v), Fig. 3, agree with the discussion of M/T ratio variations above, they indicate that additional mechanisms may be involved. Selectivity *increases* as a function of sample load in all systems, up to approximately 1–2 μmol of analyte, where a plateau is reached. Similar effects, albeit less pronounced, have previously been observed by Sellergren [19] in the case of template

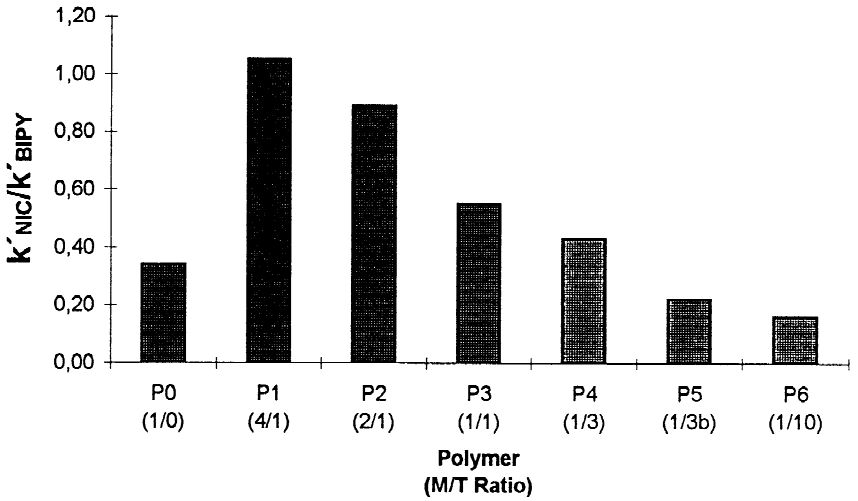


Fig. 1. Selectivities (k'_{NIC}/k'_{BIPY}) for (−)-nicotine as related to 4,4′-bipyridyl on **P0–P6** at a sample load of 0.18 μmol. Buffer: MeCN–HOAc (94:6, v/v).

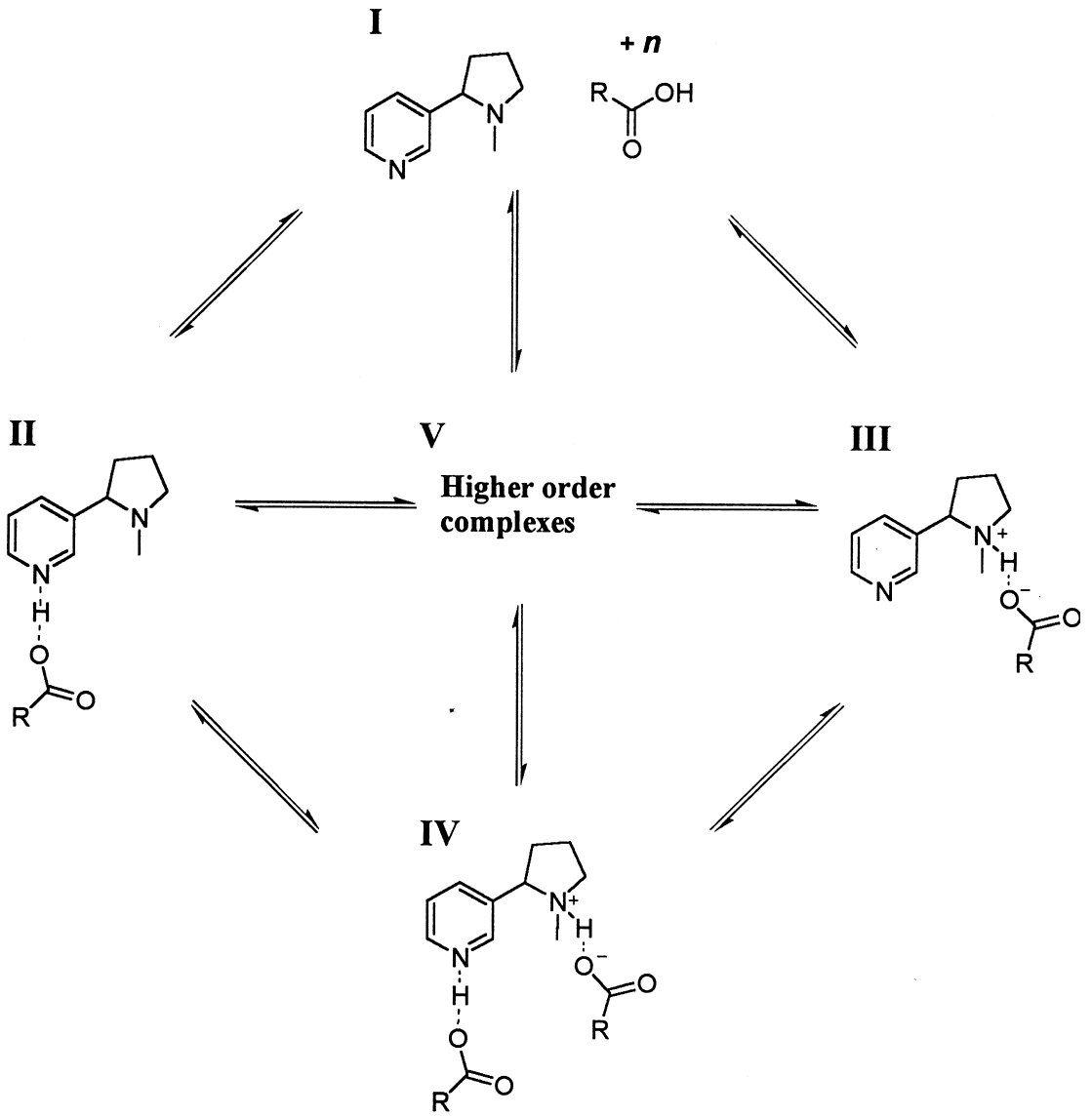


Fig. 2. Hypothetical model of the anticipated carboxylic acid (MAA or HOAc)–nicotine complex states in the pre-polymerisation mixture and in the eluent.

rebinding to L-phenylalanine anilide MIPs at sample loads below 0.5 μmol (recalculated to a comparable column format). This observation was suggested to arise, at least partly, from differences in template solvation by HOAc [20]. A recent examination of the mass-transfer kinetics in the L-phenylalanine anilide MIP system, indicating lower mass-transfer rates at low analyte concentrations, offers support for this suggestion [21]. The complex states illustrated in Fig. 2 serve as a good general illustration of such a mechanism; at sufficiently low analyte concentrations in the eluent, a major fraction will be complexed by analogy to state **IV**, whereas relatively little will be in non-complexed (state **I**) or partially complexed (state **II** or **III**) form. The larger rotation diameter of state **IV** may limit its access to the

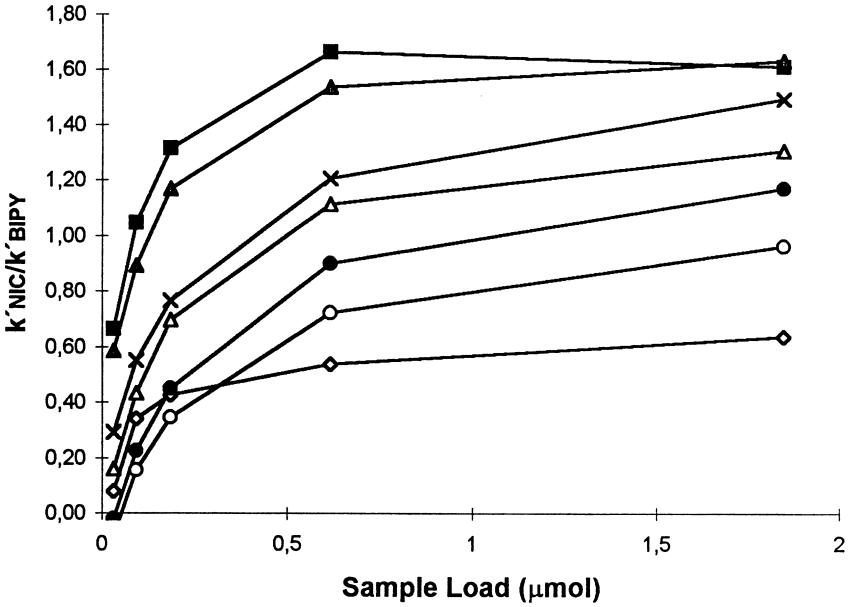


Fig. 3. Selectivities (k'_{NIC}/k'_{BIPY}) for (−)-nicotine (0.03, 0.09, 018, 0.62 and 1.85 μmol) as related to 4,4′-bipyridyl (0.18 μmol) as a function of (−)-nicotine sample load on **P0** (☆), **P1** (■), **P2**, (▲), **P3** (×), **P4** (△), **P5** (●) and **P6** (○) in MeCN–HOAc (94:6, v/v). Flow-rate: 1.0 ml/min.

binding sites as compared to states **I–III**. A gradual increase in analyte concentration will shift the equilibrium towards the 'smaller' states **II** and **III**, and subsequently to state **I**, and may thus cause an increase in chromatographic retention.

To shed light on the template complexation states in the eluent, **P2** was analysed in MeCN–TEA (99:1, v/v). The use of TEA was necessary as the retentions in pure MeCN were very long and peak-broadening was significant. No increase in retention following higher sample loads was observed in this environment (Fig. 4), suggesting that protonation of the nicotine molecule plays a role, indicating a direct involvement of acetic acid in complexation.

Further investigations were performed in MeCN–HOAc (95:5, v/v), where the retention profiles of (−)-nicotine and (+/−)-nicotine were compared on **P0**, **P3**, **P4** and **P6**. Whereas **P0** and **P6** did not exhibit any enantioselectivity, Fig. 5 shows the retention profile of (+/−)-nicotine on **P3**, which exhibits a maximum in selectivity in the racemate sample load range of 0.1–0.4 μmol. A similar effect was seen on **P4** ($\alpha = 1.25$), although no difference in retention was observed between the two enantiomers for any other sample load on this system. Fig. 6 shows the same series of analyses on **P3** with regard to resolution, (f/g)[1]. It is noteworthy that although resolution is low, the sample load exhibiting the best resolution concurs with the maxima in selectivity observed on **P3** and **P4**. These observations are important, as differences in the solvation of enantiomers are highly unlikely. Although this single experiment does not allow too far-reaching conclusions, an alternative or additional mechanism than solvation appears plausible.

From analyses of **P10–P12** (Fig. 7a–c), it is evident that higher 4,4′-bipyridyl loads lead to significantly *reduced* retentions. This reduction is especially pronounced on the 4,4′-bipyridyl imprinted polymer (**P12**). The behaviour of 3-picoline is analogous to that of 4,4′-bipyridyl. Higher capacity factors with increasing sample loads are, however,

[1]Resolution according to Meyer [22]. A line is drawn perpendicular to the baseline through the valley (the minimum) between the peaks to a line that connects the maxima of the peaks. This distance is defined as *g*. The distance from the intersection of the two lines is defined as *f*. The resolution value ranges from 0 to 1, where 1.0 represents complete baseline resolution.

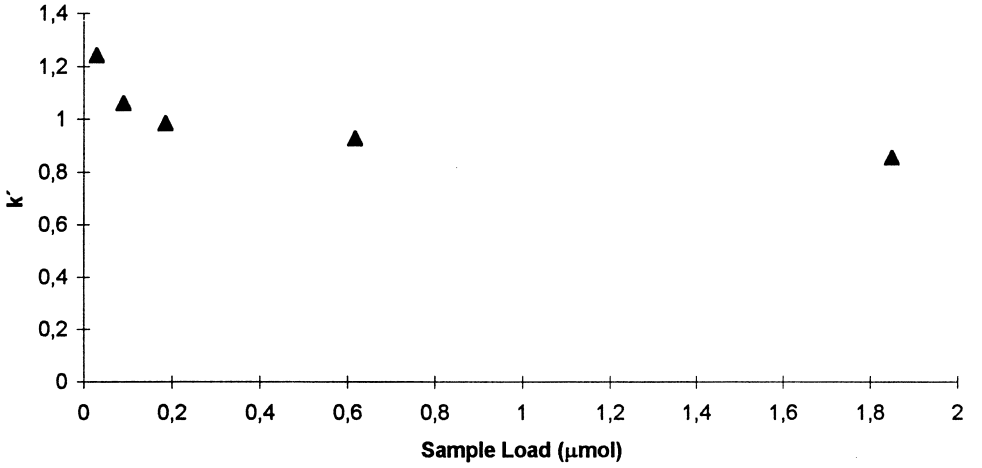


Fig. 4. Capacity factors (k') as a function of sample load for (−)-nicotine on **P2** in MeCN–TEA (99:1, v/v). Chromatographic conditions: 1.5 ml/min; 25°C

exhibited by (−)-nicotine in all polymers, although it is most significant on the (−)-nicotine imprinted system (**P11**). Moreover, as the increase in retention is also evident on **P0**, which was prepared in the absence of the nicotine template, the bulk of the effect does not appear to be a consequence of molecular imprinting. Consequently, template-induced polymer conformational changes [23] are an unlikely explanation for the chromatographic behaviour of (−)-nicotine. It is noteworthy that, both on the **P10** and **P11** polymers, an increase in sample load leads to a reversed order of elution between (−)-nicotine and 4,4′-bipyridyl.

Based on the observed maximum in enantioselectivity as a function of sample load, we point to the possible presence of higher order template–template complexes during the imprinting process and in the eluent, c.f. state V, Fig. 2, which allows for a type of cooperative binding. The results presented thusfar are in good agreement with such a model. Moreover, the shapes of the chromatographic peaks concur with

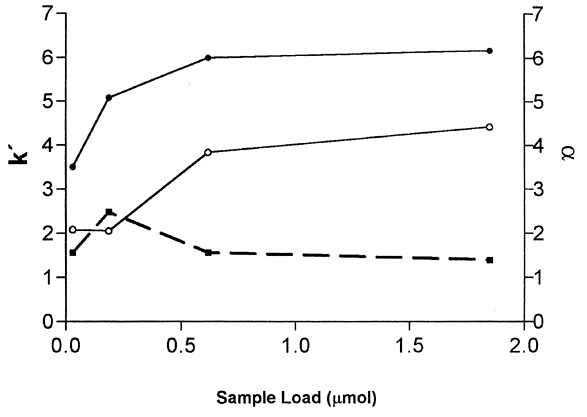


Fig. 5. Selectivity factors, α (dashed line, ■) for (−)-nicotine as related to (+)-nicotine, and capacity factors (k') of (−)-nicotine (solid line, ○) and (+)-nicotine (solid line, ●), as functions of sample load on **P3** in MeCN–HOAc (95:5, v/v). Chromatographic conditions: 1.5 ml/min; 25°C

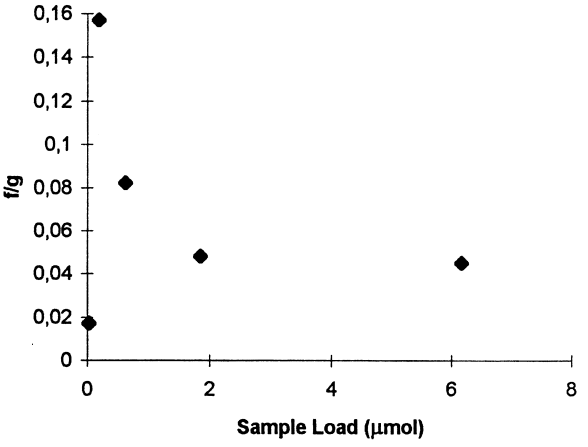


Fig. 6. Resolution (f/g) [27] of (−)-nicotine from (+)-nicotine as a function of sample load on **P3** in MeCN–HOAc (95:5, v/v). Chromatographic conditions: 1.5 ml/min; 25°C.

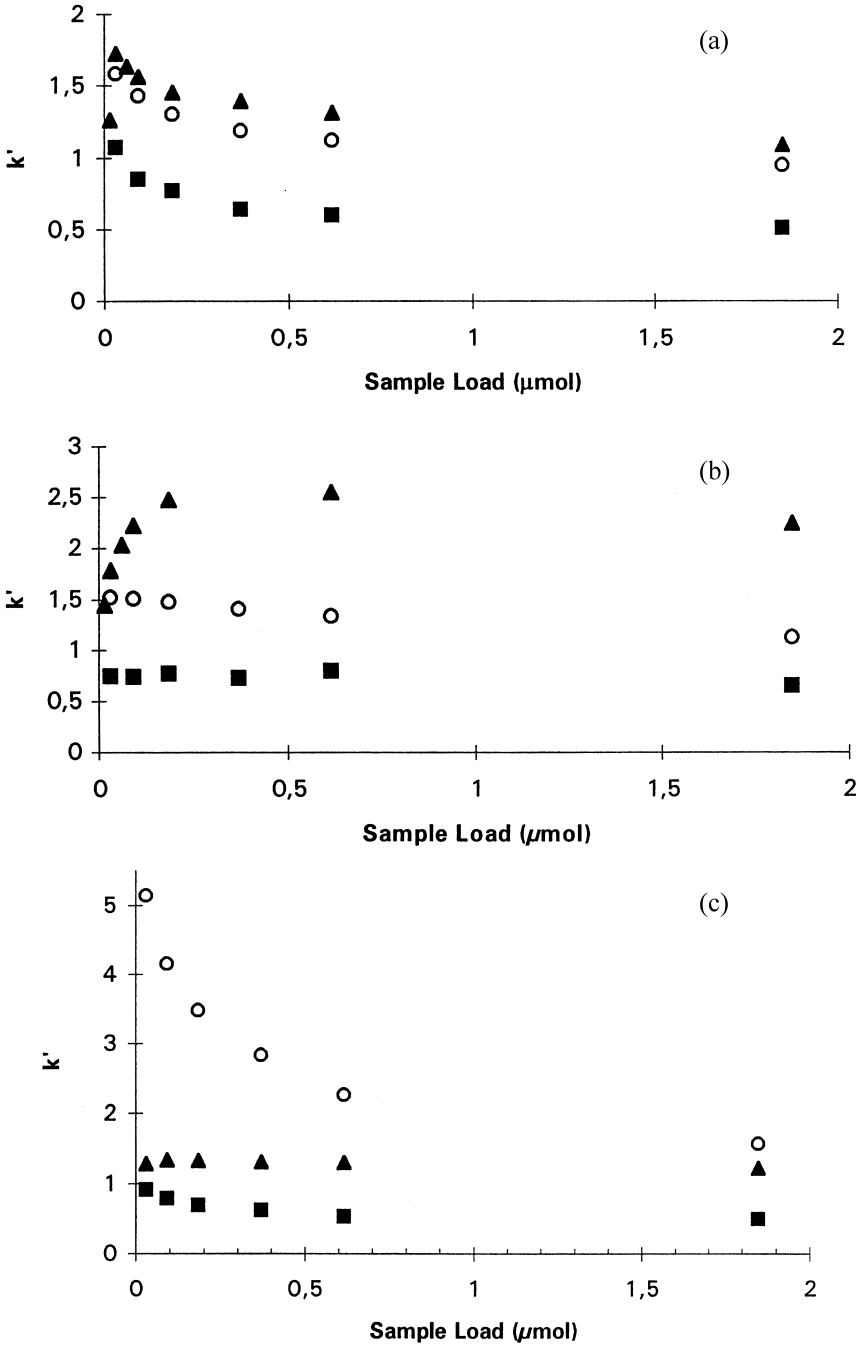


Fig. 7. a–c: Capacity factors (k') as a function of sample load for 3-picoline (■; 0.03, 0.09, 0.18, 0.37, 0.62 and 1.85 μmol), 4,4′-bipyridyl (○; 0.03, 0.09, 0.18, 0.37, 0.62 and 1.85 μmol) and (−)-nicotine (▲; 0.015, 0.03, 0.09, 0.18, 0.37, 0.62 and 1.85 μmol) on (a) **P10**, (b) **P11** and (c) **P12** in MeCN–HOAc (94:6, v/v). Flow-rate: 1.5 ml/min.

descriptions of cooperativity in chromatographic systems from earlier work [24–26]. Any self-complexation process is concentration-dependent and, assuming a Gaussian concentration distribution resulting from dilution of the injected sample, self-association would be most pronounced in the sample fraction where the analyte concentration reaches a maximum. If a significant extent of multimerisation occurs on the solid phase, this may distort the Gaussian distribution of analyte in the system towards a longer retention of this fraction. As illustrated by Fig. 8, increased sample loads reduce the Gaussian nature of peak shape through marked fronting and rear edge tailing, which, at even higher loads in some cases, lead to the appearance of split peaks.

However, the validity of the proposed self-association model is not entirely clear, as we were unable to observe the retention effect when evaluating these polymers in $CHCl_3$–HOAc (94:6, v/v) (data not shown). These results are more conveniently explained by the solvation model. To further explore the question of solvent effects, polymers **P7–P9** were prepared. The procedures for their preparation and compositions were identical to those for **P0**, **P1** and **P4**, apart from the use of acetonitrile as the polymerisation solvent, Table 1. **P8** was evaluated in MeCN–TEA (99:1, v/v), but, as expected, the retention effect was not evident in this study (data not shown). In addition, **P7–P9** were subject to evaluation in MeCN–HOAc (94:6, v/v) and were expected to show the retention phenomenon, but, although they do, as can be seen in Fig. 9, it is only in the case of **P8** that a maximum is evident. **P7** and **P9** show an initial decrease in retention as a consequence of higher sample load, and the following increase is very weak and appears to be of non-selective (not imprinting-related) origin. In a co-operative model, it is implicit that large amounts of nicotine present during polymerisation would favour the formation of template–template imprints, but a comparison between **P8** and **P9** indicates an adverse

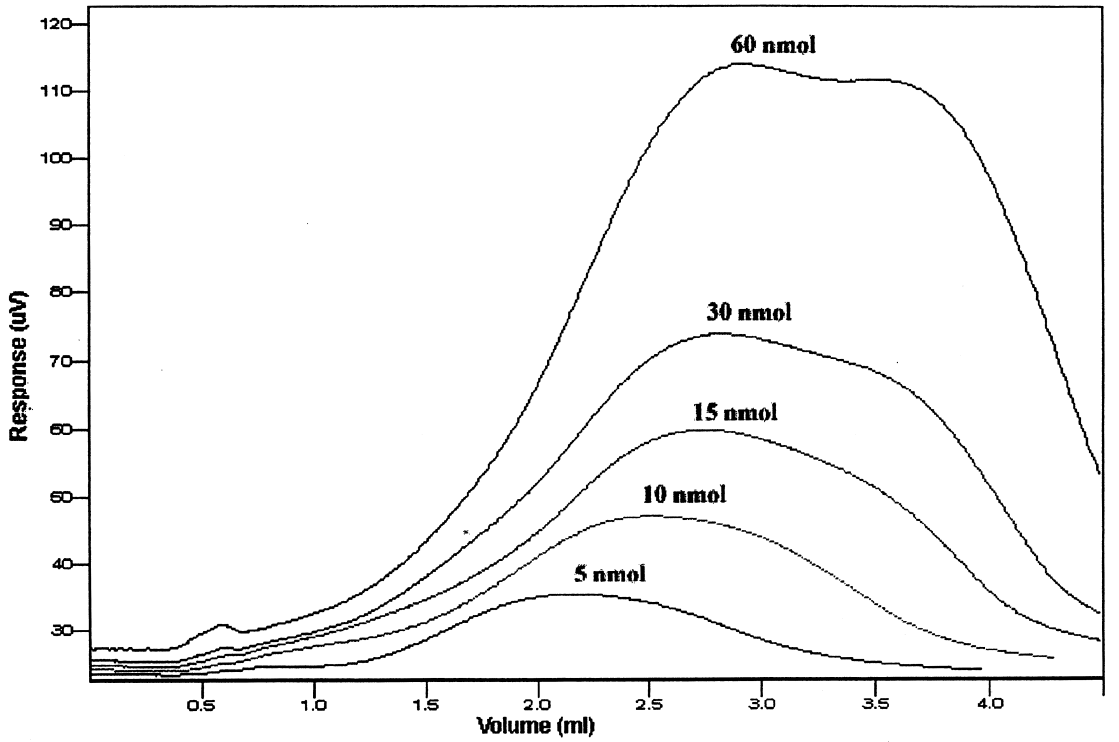


Fig. 8. Overlay of chromatograms illustrating the retention of 5, 10, 15, 30 and 60 nmol injections of (−)-nicotine on the **P11** column. Chromatographic conditions: MeCN–HOAc (94:6, v/v); 1.5 ml/min; 25°C. A response of 1000 μV on the ordinate corresponds to 1 a.u.

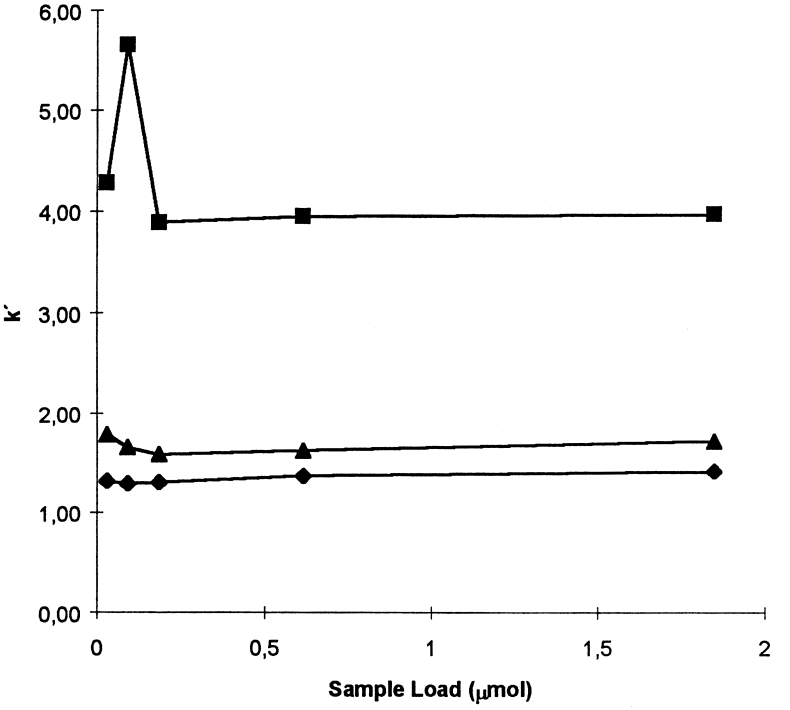


Fig. 9. Capacity factors of (−)-nicotine as a function of sample load on **P7** (♦), **P8** (■) and **P9** (▲) in MeCN–HOAc (94:6, v/v). Chromatographic conditions: 1.0 ml/min; 25°C.

dependence within the interval. Solvation effects, however, are consistent with these results.

3.3. Proposed mechanisms underlying the retention phenomenon

To summarise, the increase in nicotine retention following higher sample loads cannot be fully explained in the present study, but the results indicate that several mechanisms are likely to be acting in concert. The model based upon differences in solvation [20], as described above, is consistent with most of these results, but is not so for the resolution of (+/−)-nicotine. The hypothesis of higher-order complex states, on the other hand, is in agreement with the non-selective nature of the effect, due to the possibility of higher order complexes in the eluent and on the polymer surface. It may also explain the sample load optimum for enantiomer resolution, if imprints generated against template–template complexes are present. However, this cooperative model is inconsistent with the results obtained in $CHCl_3$–HOAc mixtures and does not fit well with analyses of **P7–P9**. Moreover, the model indicates that the carboxylate moieties of HOAc and/or MAA should be involved in the formation of the proposed higher order complexes. Whereas the results obtained in the MeCN–TEA mixture support this idea, the limited literature data available on nicotine–carboxylate complexes from infrared [27–29] and crystallographic [30] studies do not provide an adequate structural basis for such complexes. Co-existence of the above two models would better explain the results of this study, although it is not unlikely that additional mechanisms may be involved.

Nicotine has previously been employed as a template for molecular imprinting [1,31–34], although the retention phenomenon described here was not discussed. This suggests that it is possible that systems other than nicotine and L-phenylalanine anilide MIPs might exhibit characteristics similar to those reported here. We propose that the complexation model illustrated in Fig. 2 might also be valid in other MIP systems, although the nature and extent

of template–template higher order complexes must clearly be highly system-dependent. The change in elution order of nicotine and 4,4′-bipyridyl as a function of the sample load, regardless of the underlying mechanisms, suggests the need for closer examination of other MIP systems.

4. Conclusions

In conclusion, the results of this study imply that working with higher M/T ratios should lead to more homogeneous receptor populations. They also show that the retention of nicotine as a function of sample load in these MIP systems leads to unexpected effects, where an increased sample load yields longer retention. Solvation and cooperative binding effects are proposed as explanations for this phenomenon. Furthermore, experimental results suggesting the presence of MIP binding sites that are selective for template–template complexes are presented. Further work will be required to assess the possible generality and relevance of these observations to the imprinting process and to MIP-ligand recognition. These results do not pose a need for a novel hypothesis to explain molecular imprinted polymer selectivity, but indicate that the existing model might not be sufficiently broad. We propose that the recognition properties of MIPs reflect the complexes existing in the pre-polymerisation mixture, *including* potential complexes composed of template–template interactions.

Acknowledgements

This work was financed by the Swedish Engineering Sciences Research Council (TFR, grant no. 230-97-831) and the University of Kalmar Research Fund. We wish to express our sincere gratitude to Drs. A.G. Mayes and R.J. Ansell (Institute of Biotechnology, Cambridge University, UK) and Dr. B. Sellergren (Department of Inorganic Chemistry and Analytical Chemistry, University of Mainz, Germany) for valuable discussions.

References

[1] H.S. Andersson, A.-C. Koch-Schmidt, S. Ohlson, K. Mosbach, J. Mol. Recogn. 9 (1996) 675.
[2] K. Haupt, K. Mosbach, Trends Biotechnol. 16 (1998) 468.
[3] E.N. Vulfson, C. Alexander, M.J. Whitcombe, Chem. Britain 33 (1997) 23.
[4] H.S. Andersson, I.A. Nicholls, Recent Res. Dev. Pure Appl. Chem. 1 (1997) 133.
[5] K. Mosbach, O. Ramström, Bio/Technology 14 (1996) 163.
[6] G. Wulff, Angew. Chem. Int. Ed. Engl. 34 (1995) 1812.
[7] J.H.G. Steinke, D.C. Sherrington, I.R. Dunkin, Adv. Polym. Sci. 123 (1995) 80.
[8] R. Arshady, K. Mosbach, Makromol. Chem. 182 (1981) 687.
[9] G. Wulff, ACS Symp. Ser. 308 (1986) 186.
[10] K.J. Shea, Trends Polym. Sci. 2 (1994) 166.
[11] B. Sellergren, M. Lepistö, K. Mosbach, J. Am. Chem. Soc. 110 (1988) 5853.
[12] M.J. Whitcombe, L. Martin, E.N. Vulfson, Chromatographia 47 (1998) 457.
[13] H.S. Andersson, I.A. Nicholls, Bioorg. Chem. 25 (1997) 205.
[14] G. Vlatakis, L.I. Andersson, R. Müller, K. Mosbach, Nature 361 (1993) 645.
[15] L.I. Andersson, K. Mosbach, J. Chromatogr. 516 (1990) 313.
[16] K.J. Shea, D.A. Spivak, B. Sellergren, J. Am. Chem. Soc. 115 (1993) 3368.
[17] D.J. O'Shannessy, B. Ekberg, K. Mosbach, Anal. Biochem. 177 (1989) 144.
[18] I.A. Nicholls, Chem. Lett. (1995) 1035.
[19] B. Sellergren, Chirality 1 (1989) 63.
[20] B. Sellergren, K.J. Shea, J. Chromatogr. A 690 (1995) 29.
[21] P. Sajonz, M. Kele, G. Zhong, B. Sellergren, G. Guiochon, J. Chromatogr. A 810 (1998) 1.
[22] V.R. Meyer, Chromatographia 24 (1987) 639.
[23] M. Watanabe, T. Akahoshi, Y. Tabata, D. Nakayama, J. Am. Chem. Soc. 120 (1998) 5577.
[24] S. Okada, Y. Husimi, S. Tanabe, A. Wada, Biopolymers 14 (1975) 33.
[25] J. Hubble, Biotechnol. Bioeng. 30 (1987) 208.
[26] R. Lemque, A. Jaulmes, B. Sébille, C. Vidal-Madjar, J. Chromatogr. 599 (1992) 255.
[27] G. Barrow, J. Am. Chem. Soc. 78 (1956) 5802.
[28] D. Hadzi, Z. Elektrochem. 62 (1958) 1157.
[29] M. Dezelic, B. Nikolin, Spectrochim. Acta 23A (1967) 1149.
[30] K.-I. Fukawa, S. Harada, N. Kasai, M. Toda, K. Mori, F. Toda, Bull. Chem. Soc. Jpn. 62 (1989) 2714.
[31] J. Matsui, A. Kaneko, Y. Miyoshi, K. Yokoyama, E. Tamiya, T. Takeuchi, Anal. Lett. 29 (1996) 2071.
[32] J. Matsui, O. Doblhoff-Dier, T. Takeuchi, Anal. Chim. Acta 343 (1997) 1.
[33] J. Matsui, T. Takeuchi, Anal. Commun. 34 (1997) 199.
[34] A. Zander, P. Findlay, T. Renner, B. Sellergren, A. Swetlow, Anal. Chem. 70 (1998) 3304.