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Influence of template basicity and hydrophobicity on the molecular recognition properties of molecularly imprinted polymers

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Abstract

Triazine herbicides were used as model templates for a basic study of the molecular recognition process in imprinted polymers. Five structurally related triazine herbicides (atrazine, ametryn, cyanazine, prometryn and terbutylazine) that differ in basicity and hydrophobicity were imprinted. Chromatographic evaluation of the resulting materials in an aqueous-poor mobile phase showed that the selectivity for the template increased with its Brönsted basicity whereas it did not correlate with template hydrophobicity. Thus, the highest and lowest affinity and selectivity for the template was observed using the ametryn-imprinted ($pK_a=4.1$) and the cyanazine-imprinted polymers ($pK_a=1.0$), respectively. In aqueous-rich mobile phases however affinity and selectivity correlated with template hydrophobicity. On a polymer imprinted with atrazine (log $P_{ow}=2.6$), the selectivity for atrazine over the more hydrophobic prometryn (log $P_{ow}=3.4$) decreased whereas the retention increased when going from a mobile phase containing 2.5% (v/v) water ($k'_{ATR}=1.7$, $k'_{PRO}=0.6$) to one containing 70% (v/v) water ($k'_{ATR}=15$, $k'_{PRO}=38$). A different trend was observed using a polymer imprinted with prometryn. In this case both the selectivity and affinity dramatically increased when changing the mobile phase in the same order ($k'_{PRO}=1.8$, $k'_{ATR}=1.2$ at 2.5% water, $k'_{PRO}=150$, $k'_{ATR}=21$ at 70% water). Depending on the hydrophobicity of the template, the selectivity in molecular imprinting may thus be enhanced by increasing the aqueous content of the medium used in the rebinding step.

Keywords: Pesticides; Triazines; Hydrophobicity; Basicity; Molecular recognition; Molecular imprinting; Pesticides; Triazines

1. Introduction

During the last years numerous reports of selective separations using materials prepared by molecular imprinting have appeared (for reviews, see [1–4]). These include chromatographic enantiomer separations [4] and discrimination between structurally related drugs [5], amino acid derivatives [6,7] as well as nucleotide base derivatives [8]. The high affinity and selectivity for the target analyte exhibited by the imprinted materials have, in some cases, justified a comparison with the corresponding immunoaffinity (IA) phases. Convincing examples of the antibody analogy are the previous competitive assays of drugs in biofluids, [5] selective sample enrichment techniques for direct drug determination [9] or imprinted polymers in capillary affinity electrophoresis. [10] The imprinted materials are particularly attractive with respect to their robustness and large sample load capacity compared to corresponding IA phases. [11] With the perspective of high affinity separations on a preparative scale, we have focused on achieving

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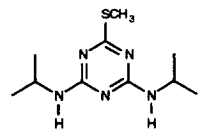
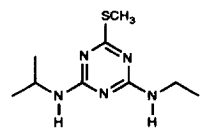
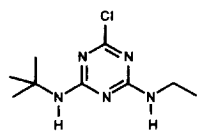
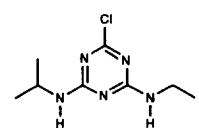
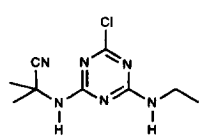
affinity and selectivity for other compound classes. This requires a systematic approach where each parameter involved in the preparation of materials is studied. These studies can be on the type of monomer–template interaction, the type of polymer matrix as well as on the stability of the template assemblies formed prior to polymerization. [12] Furthermore, in addition to the indirect techniques used so far, in situ techniques for binding site characterisation at a molecular level are of great interest.

Imprinted materials showing high selectivity for basic N-heterocycles can be prepared by copolymerization of methacrylic acid (MAA) and ethyleneglycol dimethacrylate (EDMA) in the presence of a template (the target N-heterocycle) and weakly-polar weakly-hydrogen-bonding solvents. [5,8] High affinity and selectivity in particular were reported when 9-ethyladenine was used as the template [8]. This was thought to be due to a combination of Watson and Crick- and Hoogsten-type hydrogen bonds between the base and the carboxylic acid (MAA). In order to better understand which structural parts are required to obtain high affinity rebinding, we decided to investigate a number of structurally related N-heterocycles. One particular advantage of using small aromatic templates is their conformational rigidity that allows ring substitutions to be made with minimal change in conformation. This is important when the influence of acidic or basic properties or the shape of the template on the rebinding properties are to be studied. Furthermore, spectroscopic characterisation of the imprinting or rebinding process is expected to be more straightforward. After having observed that materials selective for the herbicide atrazine can be prepared by molecular imprinting [13], we previously introduced the triazine family of herbicides as new model templates (Scheme 1) [14]. These are available in large numbers with minimal structural differences as well as with different known basicities and hydrophobicities. Their conformations and their interactions with small ligands in non-polar solvents are known [15]. More recently, they have also been successfully used by other research groups for the preparation of highly selective imprinted materials for triazines [16,17]. In this report we show that the selectivity and affinity of the polymers for their templates clearly relate to either template basicity or to template hydrophobicity, depending on the medium used during evaluation of the polymers. These materials may find practical use in selective sample enrichment of triazines from environmental samples [18,19] using the approach we introduced previously for the selective enrichment of drugs from biological samples [9].

	$\log P_{ow}$	pK_a
 Prometryn	3.4	4.1
 Ametryn	3.07	4.1
 Terbutylazine	3.04	2.0
 Atrazine	2.6	1.7
 Cyanazine	1.7	1.0

Scheme 1.

2. Experimental

2.1. Materials

Triazine standards of 98–99% purity [ametryn (AME) and cyanazine (CYA)] were obtained from Riedel-de Haen (Seelze, Germany) whereas tech-

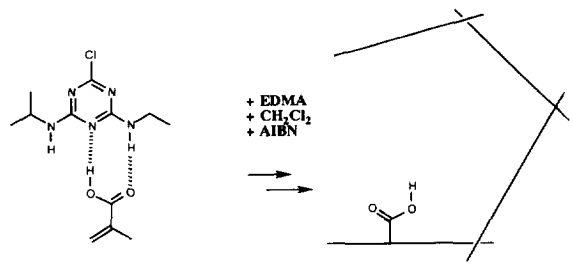
nical-grade prometryn (PRO) and terbutylazine (TER) were generously provided by Ciba-Geigy (Basel, Switzerland). Methacrylic acid (MAA) was purchased from Aldrich and ethyleneglycol dimethacrylate (EDMA) was from Fluka. The initiator azobisisobutyronitrile (AIBN) and atrazine (ATR, 98% purity) were obtained from Janssen. The UV lamp used in the photopolymerization was a medium-pressure mercury vapor lamp (Original Hanau 800). All chromatographic evaluations were done using a Bischoff HPLC pump, a Rheodyne injector, an LKB 2151 variable-wavelength monitor as the UV detector and a Kipp and Zonen DB41 plotter.

2.2. Polymer preparation

A modified version of a previously described procedure was followed [20]. To EDMA (20 mmol, 3.8 ml), MAA (4 mmole, 0.34 ml) and the triazine herbicide (1 mmol) in CH_2Cl_2 (5.6 ml) were added AIBN (0.25 mmol, 40 mg). The solution was transferred to a glass tube (14 mm I.D.). This was purged with nitrogen while cooled to −20°C in a thermostatted water–acetone bath and then sealed. The tubes were symmetrically placed at ca. a 10-cm distance from a UV light source, immersed in the water–acetone bath, and turned at regular intervals for symmetric exposure. After 18 h, the tubes were crushed and the polymer was ground and sieved under water to a particle size fraction of 25–50 μm. A reference material was prepared identically but using 3-(1-methyl-2-pyrrolidinyl)pyridine as the template.

2.3. Chromatographic evaluation

The sieved polymers were slurry packed in acetonitrile (MeCN)–water (1:1, v/v) into 100×4.6 mm I.D. stainless steel columns. After having passed ca. 50 ml through the columns at a flow-rate of 5 ml/min, the columns were equilibrated at 1 ml/min using the specified mobile phase until a stable baseline was reached. The flow-rate was 1 ml/min, the UV detector wavelength was 260 nm and the column temperature was 22–28°C, unless otherwise stated. The retention, k', was calculated as $k' = (t - t_0)/t_0$ where t_0 is the elution time of the void marker, acetone, which normally eluted as a sharp peak with a plate number, N, of approximately 3500 m^{-1}. The phosphate buffer mobile phases were prepared by adjusting the pH of a 0.05 M potassium phosphate (KP) solution and mixing this with acetonitrile to the desired proportion.



Scheme 2.

3. Results and discussion

3.1. Aqueous-poor mobile phases: effect of template basicity

Using triazines as templates, materials selective for triazines can be prepared by the previously developed non-covalent imprinting procedure (Scheme 2) [13,14,16,17]. As seen in Fig. 1, the

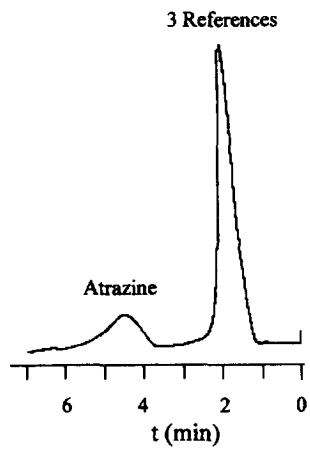


Fig. 1. Elution profile of atrazine (10 nmole) and of three reference bases [10 nmol each of 3-(1-methyl-2-pyrrolidinyl)pyridine, 3′-azido-3′-deoxythymidine and adrenaline] on an atrazine-selective column. Mobile phase: MeCN–HOAc–water (92.5:5:2.5, v/v).

solute used as the template was retained more strongly than three structurally unrelated bases and a comparison of the retention of the template on the imprinted material with that on a reference material, imprinted with a structurally unrelated base, shows that selective retention of the template occurs (Fig. 2). This is not surprising considering the relatively strong complexes known to form between atrazine and carboxylic acids [15]. Typically, the retention decreases with increasing sample load. We anticipated that a comparison of imprinted materials prepared against the five structurally related triazines seen in Scheme 1 would provide information on the structural and functional criteria important for the molecular recognition process. In Fig. 3 the capacity factors of the compounds injected separately on all of the triazine-selective columns in an aqueous-poor mobile phase are given. In spite of small structural differences between the templates, most of the materials showed pronounced selectivity for their template. The level of selectivity is, however, not the same on all polymers. Whereas the CYA-imprinted polymer (P-CYA) showed essentially no selectivity for CYA, the AME-imprinted polymer (P-AME) strongly and selectively retained AME. This difference can be discussed in terms of the different basic and hydrophobic characteristics of the template. Molecular recognition by imprinted polymers is the

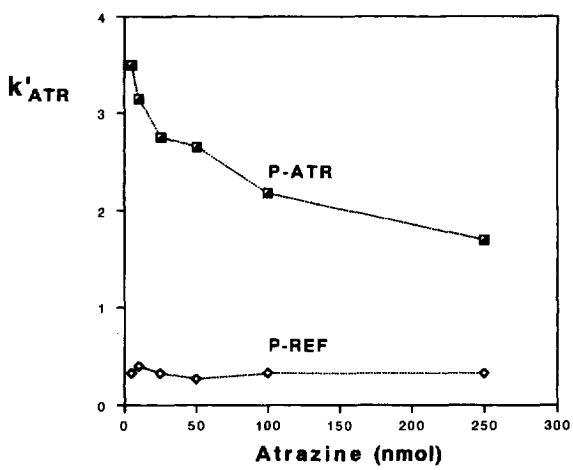


Fig. 2. Retention versus sample load of atrazine on a column packed with an atrazine-imprinted polymer (P-ATR) and on a reference column using a mobile phase consisting of MeCN–HOAc–water (92.5:5:2.5, v/v).

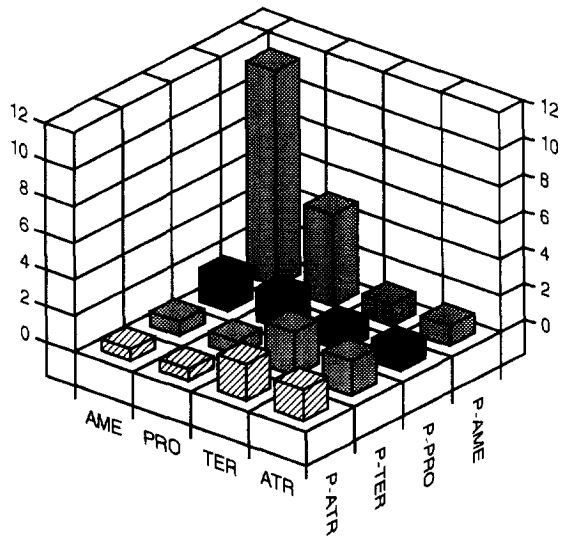


Fig. 3. Three-dimensional representation of the capacity factors measured after the separate injection of 10 nmole of four different triazines on four triazine-selective columns using MeCN–HOAc–water (92.5:5:2.5, v/v) as the mobile phase. The pK_a values increase from front to back. The capacity factors of the triazines on P-CYA were: CYA, 1.0; ATR, 1.7; TER, 2.3; PRO, 1.1 and AME, 1.1.

result of two processes in which the template plays an equally important role. During polymerisation, it interacts in non-polar medium with the functional monomers in order to provide the binding sites necessary for molecular recognition. In the chromatographic evaluation, it reversibly interacts with the functional groups of the same sites. When using a more polar solvent system in the chromatographic evaluation than that used during imprinting, the driving force for rebinding to the site can be widely different from the interactions occurring in the non-polar medium used in the polymerisation step [21,22]. In order to directly probe the interactions occuring during polymerisation, it is therefore preferable to evaluate the polymers in an aqueous-poor mobile phase resembling the medium used during polymerisation. The strongest interactions are expected to be those of a cooperative hydrogen bond between the nitrogen para to the chlorine substituent and an exocyclic amino group of the triazine and the carboxylic acid group of MAA, as indicated in Scheme 2 [15]. As concluded by Albrecht and Zundel [23], the association between substituted

pyridines and carboxylic acids in acetonitrile becomes stronger with increasing Brönsted basicity of the base. The strength of the monomer–template interaction is therefore expected to be strongly influenced by the basicity of the triazine. This means that more basic templates will interact more strongly with MAA promoting a larger population of sites, with optimal arrangement of carboxylic acid groups for binding of the template [24]. In fact, with the exception of the prometryn-imprinted material, a notable increase in affinity and selectivity (Figs. 3 and 4) with increasing pK_a (Scheme 1) of the template occurs. The low capacity factors obtained on the corresponding reference materials show that non-specific binding is negligible. Meanwhile, due to the aromatic nature, bringing structural rigidity, as well as the structural similarity of the templates, their conformations should be similar, resulting in only small differencies between the interaction sites presented towards MAA. Thus, the above comparison should reflect the influence of template basicity on the affinity and selectivity exhibited by the polymer binding sites. The chromatographic results using the ametryn polymer agree with the results previously obtained using a polymer imprinted with 9-ethyladenine having a similar pK_a value as ametryn [8]. A complex with a 2:1 stoichiometry between the

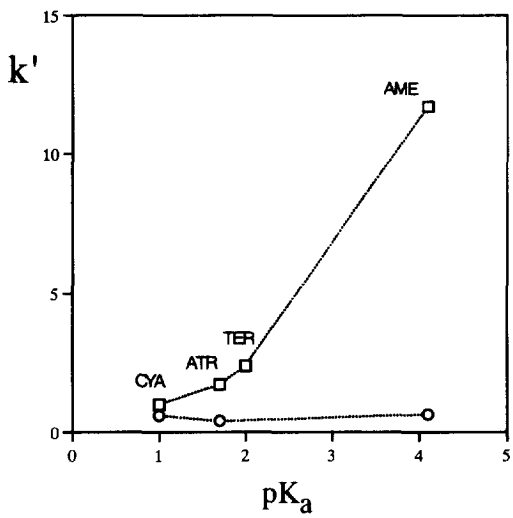
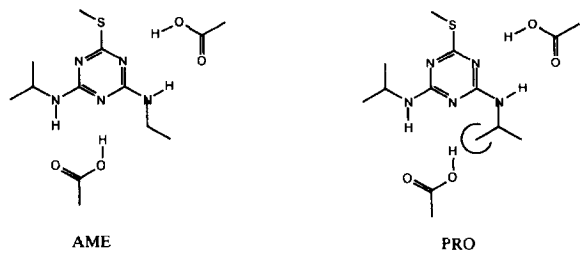
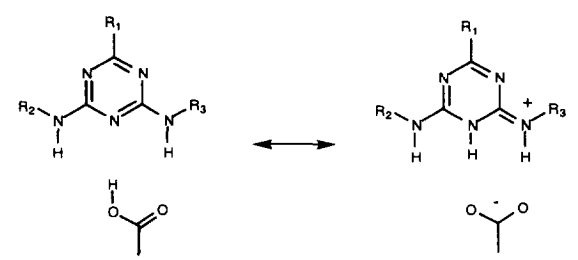


Fig. 4. Capacity factor versus pK_a of the triazines CYA, ATR, TER and AME on their complementary columns (squares) as well as on a reference column (circles).



Scheme 3.

monomer and the template was suggested as one possible explanation for the high selectivity observed. The similar selectivity observed using P-AME suggests that ametryn also forms a two-point interaction with MAA. Assuming this to be the case, molecular models show that prometryn, having the same pK_a as ametryn but with bulkier N-substituents, would by steric arguments be less prone to form a 2:1 complex with MAA (Scheme 3). This would explain the lower affinity and selectivity exhibited by P-PRO compared to P-AME. One puzzling result is the apparent class specificity, that is, the polymers imprinted with Cl-triazines preferentially bind Cl-triazines over the S-triazines and vice versa. Purely steric factors can only explain the weaker binding of the S-triazines to the Cl-triazine-selective columns, since the binding sites generated using the S-triazine templates also would be able to accommodate the smaller Cl-triazines. However, a reasonable explanation may be found by considering the amine–imine equilibrium. The extent of proton transfer between MAA and the base during polymerization will determine how much of the template will be imprinted in the imine form compared to the amine form (Scheme 4). Molecular models indicate that these tautomers, if imprinted, would result in binding



Scheme 4.

sites of different shapes. Another possible cause for the discrimination are interactions involving the thioether group. However, these should be weak compared to those involving the amino groups and the ring nitrogens [25]

3.2. Aqueous-rich mobile phases: effect of mobile phase pH

Depending on the nature of the solute, increasing the aqueous content of the mobile phase may introduce other mechanisms of retention, including ion-exchange and solvation–desolvation effects. [21,22] In Fig. 5, the influence of mobile phase pH on retention (k') is seen for an atrazine- and an ametryn-imprinted material in a 30% aqueous mobile phase. Whereas the atrazine polymer showed the highest selectivity at pH 2, the ametryn material exhibited a stable high selectivity in the pH range 3–5. How can these effects be explained? We previously showed that solute retention and selectivity on imprinted polymers based on MAA–EDMA follow a simple cation-exchange model [21]. Thus, for primary amines, k' reached a maximum at a mobile phase pH that was close to the pK_a value of the solute, whereas the separation factor was high at low pH and decreased to one when the pH value exceeded the pK_a value of the solute. The apparent pK_a of the solute, measured in the mobile phase (which was identical to the one used in this study), was approximately two units lower than the one measured in 0.1 *M* NaCl. This would mean that only ametryn can be partly protonated at the lowest pH value (pH 2). It is noted that at this pH value, AME is only weakly bound to the ametryn-selective polymer as well as to the atrazine reference. With increasing mobile phase pH values, the retention and selectivity should decrease, mainly as a result of the loss of hydrogen bond donor groups at the binding sites with the increase in the degree of ionization of the COOH groups [21]. This was observed using the atrazine-imprinted material (Fig. 5). A mobile phase pH of 4 was chosen for the following studies.

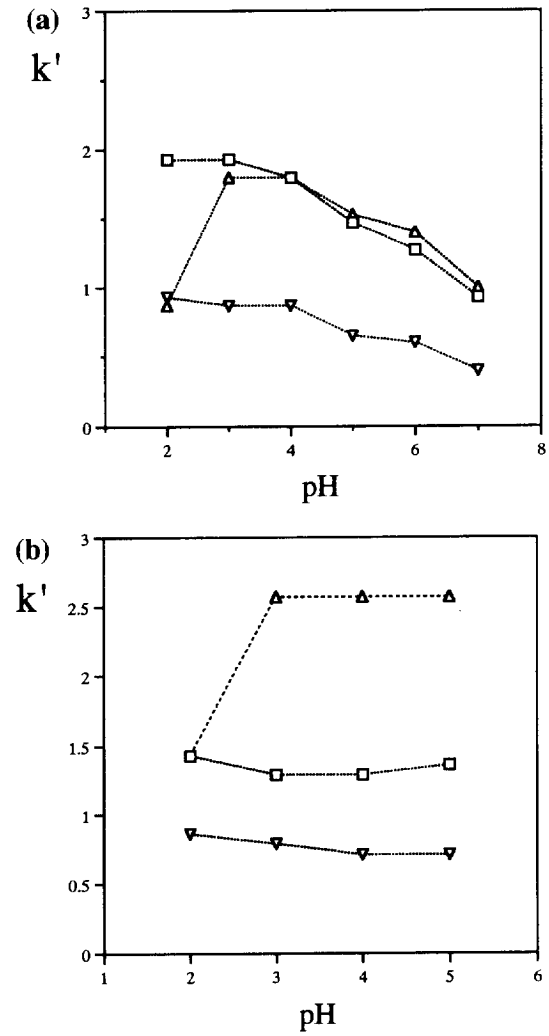


Fig. 5. Retention of atrazine, ametryn and cyanazine (10 nmole of each, injected separately) on an atrazine-imprinted material (a) and on an ametryn-imprinted material (b) as a function of the pH of the mobile phase. The mobile phase was MeCN–0.5 *M* KP (7:3, v/v), where the pH refers to the aqueous portion. KP=potassium phosphate. ▽=Cyanazine; □=atrazine and △=ametryn.

3.3. Effect of template hydrophobicity

Not only the basicity but also the hydrophobicity (reflected in the log P_{ow} values) [26] of the template will affect the capacity factor in aqueous-rich mobile phases. Considering the different hydrophobicities of the templates, we expected that an increase in the aqueous content of the mobile phase would show if the hydrophobic effect contributed specifically or non-specifically to the observed retention. We therefore compared three materials, one imprinted with the relatively hydrophobic triazine, prometryn (log

P_{ow}=3.4), one with the less hydrophobic triazine, atrazine (log P_{ow}=2.6) and one reference material imprinted with a structurally unrelated base [3-(1-methyl-2-pyrrolidinyl)pyridine]. Although the retention of all triazines increased when the aqueous content was increased from 2.5 to 70% (v/v), a marked difference between the columns was observed (Fig. 6). Considering first the reference column, the retention of the triazines increased in the order of increasing log P_{ow} values (Fig. 7a), in agreement with what is observed on reversed phase columns [27]. Typically, when the hydrophobic effect is the dominating retention mechanism, a linear dependence is seen between log k' and log P_{ow} as well as between log k' and the aqueous content in the mobile phase. The order of retention as well as

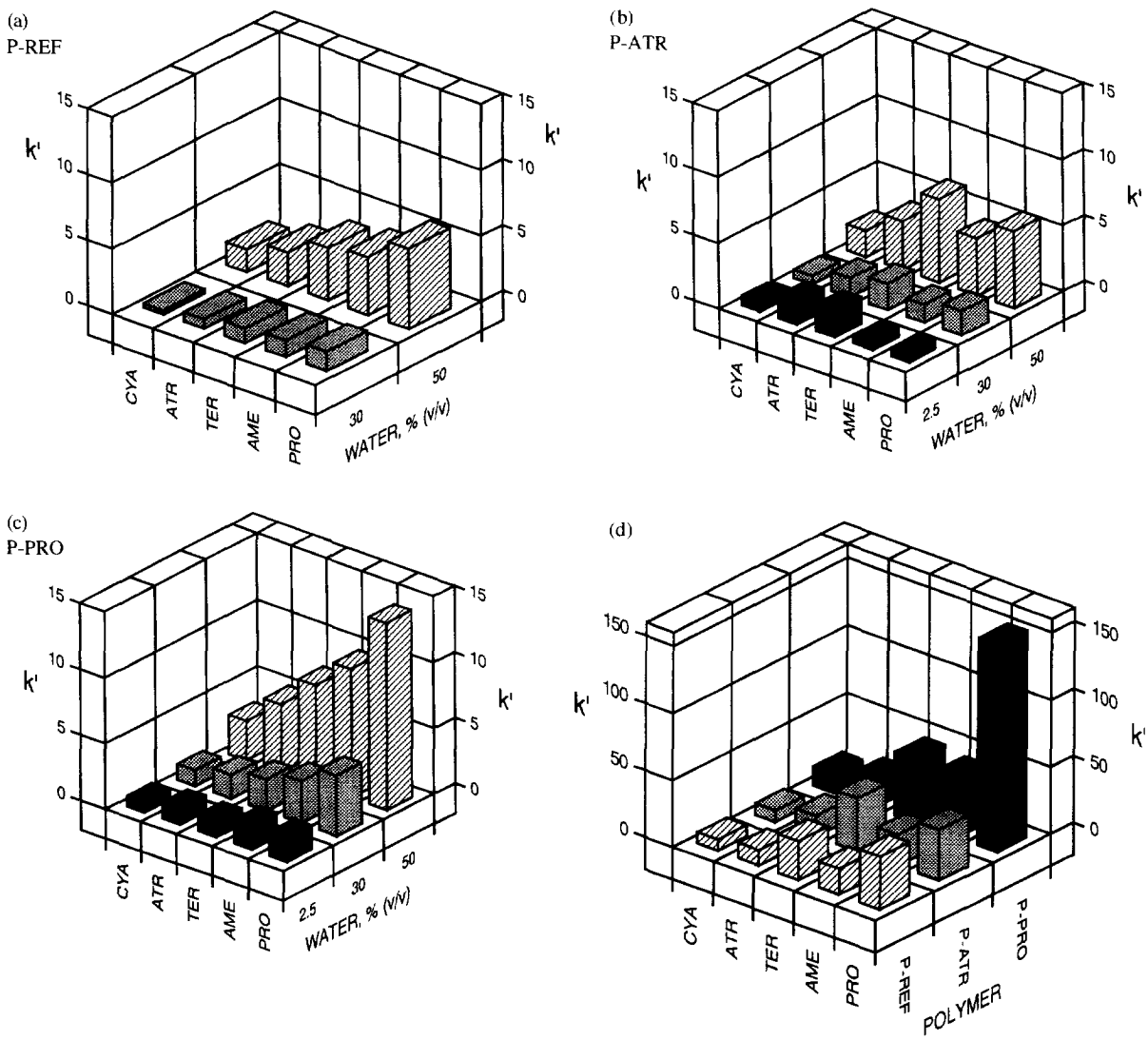


Fig. 6. Three-dimensional representation of the capacity factors of five different triazines injected separately (10 nmole) on (a) a reference column, (b) an atrazine-imprinted column and (c) a prometryn-imprinted column in mobile phases containing acetonitrile and different amounts of aqueous potassium phosphate buffer (0.05 *M*, pH 4). In (d) the three columns are compared using a mobile phase containing 70% (v/v) buffer. The amount of CYA injected on the reference column was 100 nmole in (d). The hydrophobicity of the triazines decreases from front to back of the graph.

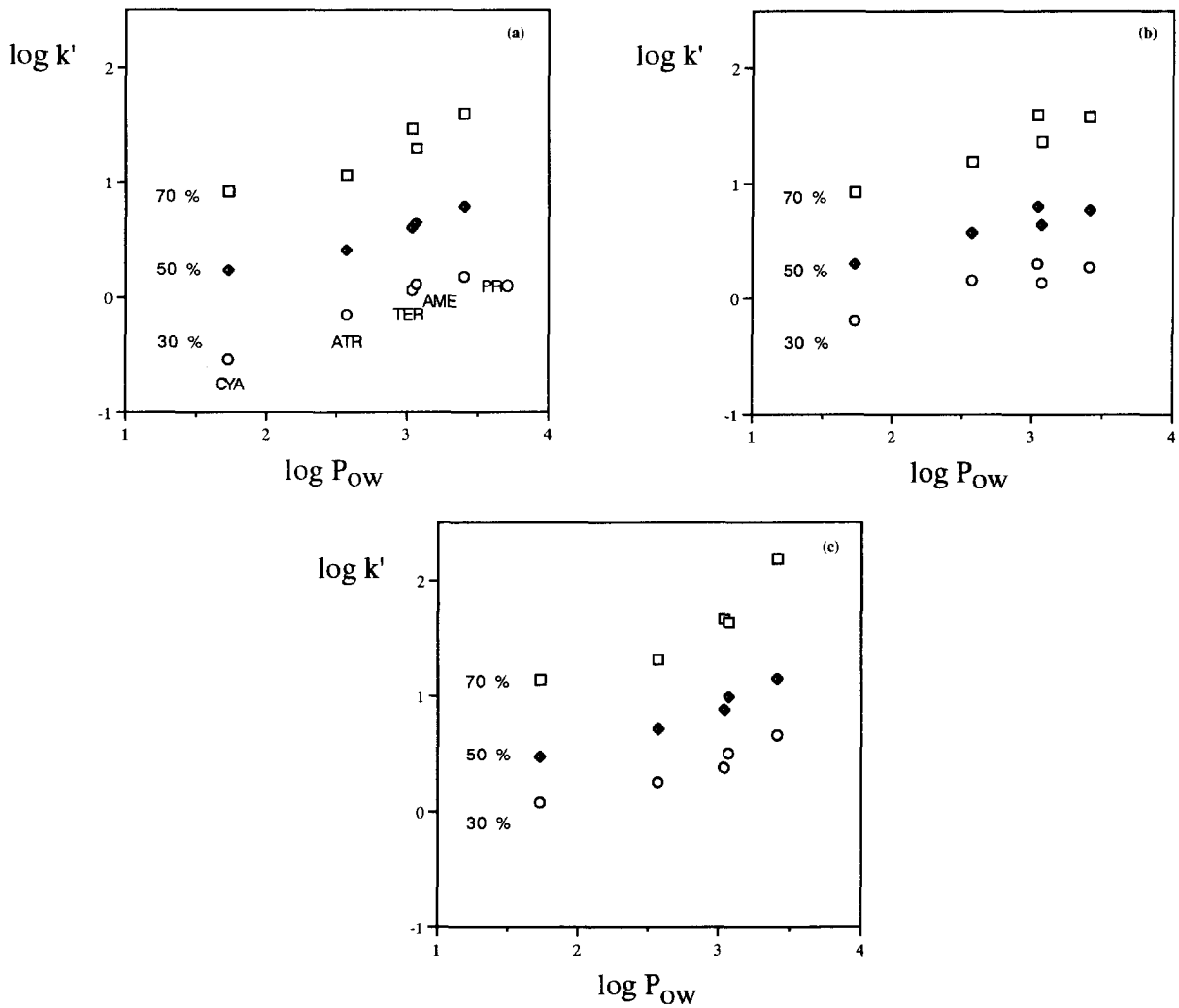


Fig. 7. Logarithmic plot of the capacity factors (k') versus the hydrophobicity values (log P_{ow}, see Scheme 1) of the different triazines measured on (a) the reference column, (b) the atrazine-imprinted column and (c) the prometryn-imprinted column, using mobile phases with different aqueous contents.

the regular displacement of the curves with increasing aqueous content provide evidence that the hydrophobic effect is the dominating retention mechanism of the triazines on the reference column. Considering the atrazine selective column, Fig. 2 shows that in an aqueous-poor mobile phase, atrazine is clearly more retained on the atrazine-selective column than on the reference column. As seen in Fig. 6, the structurally similar terbutylazine is also selectively retained on this column. Increasing the aqueous content, the Cl-triazines, atrazine and terbutylazine, are more retained on this column than on the reference column, although the selectivity over the S-triazines decreases due to the increasing non-specific hydrophobic contribution to retention (Fig. 7). At 70% water, only atrazine and terbutylazine are more retained on this column than on the reference column. Comparison of Fig. 7a and Fig. 7b shows

that the retention at this aqueous content is controlled by a non-specific hydrophobic driving force. Finally, considering the prometryn-selective column, the selectivity for prometryn in the aqueous-poor mobile phase is in the same order as that for atrazine using the atrazine-selective column in the same medium. By increasing the aqueous content, it is observed that all triazines are more retained on this column than on the reference column (Figs. 6 and 7). The retention pattern among the triazines is different from that observed using the atrazine-selective column. Thus, a pronounced preference for the S-triazines is seen. This appears most clearly at 70% water, where a k' of 150 was measured for prometryn. Obviously, depending on the hydrophobicity of the template, the hydrophobic effect can either enhance or attenuate the selectivity of the molecular recognition in imprinted polymers. The former effect is analogous to the hydrophobic binding of substrates to enzymes or antigens to antibodies [28]. Ascribing the observed phenomenon entirely to the hydrophobic effect assumes however that the protonation state of the triazines remains unchanged when the aqueous content is increased. The chosen pH of 4 ensures that they remain uncharged at least up to 50% water. This is supported by the similar retentions of terbutylazine, with a pK_a of 2.0, and ametryn, with a pK_a of 4.1, on the reference column (Fig. 7). At 70% water, however, a part of ametryn and prometryn may exist in a protonated form. An ion-exchange contribution to retention can therefore not be excluded in this mobile phase.

4. Conclusions

Highly crosslinked methacrylate-based weak cation-exchangers exhibiting pronounced affinity and selectivity for various triazine herbicides behave predictably with respect to rebinding affinity and selectivity for the various triazine analogues. This is an important result in view of the small structural differences between the templates, but also because of the current interest in finding selective analytical methods for these compounds. The correlation between affinity and selectivity in the rebinding step and the pK_a value of the template as well as the enhanced selectivity for hydrophobic templates at increased aqueous contents in the mobile phase indicate that molecular recognition in imprinted polymers may be fine-tuned by considering template basicity and hydrophobicity. A striking similarity is seen here with the molecular recognition principles operating in biological systems. [28]

Acknowledgments

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