Chiral ion-exchange chromatography

Correlation between solute retention and a theoretical ion-exchange model using imprinted polymers

Börje Sellergren[☆] and Kenneth J. Shea*

Department of Chemistry, University of California, Irvine, CA 92717-2025 (USA)

ABSTRACT

Mobile phase effects were studied in the separation of D- and L-phenylalanine anilide (D,L-PA) on an imprinted chiral stationary phase (CSP). Using an aqueous-organic mobile phase, an improved column performance was seen, reflected in a two-fold decrease in the reduced plate height and an almost doubling of the resolution as compared to when a pure organic mobile phase was used. A strong dependence of retention (k') and enantiomer selectivity (α) on mobile phase pH was observed. k' reached a maximum at a pH close to the pK_a value of the solute and α was high at low pH value but decreased when pH exceeded the solute pK_a. Potentiometric titration data allowed estimation of the state of protonation of both the carboxylic acid containing CSP and the amino group containing solutes. The data are analyzed using a simple cation-exchange model to allow simulation of the retention as a function of mobile phase pH. The close agreement between the simulated and experimental curves for retention *versus* pH suggests that a simple cation-exchange mechanism controls the retention in this system. Moreover, the slightly lower average pK_a of the imprinted polymer compared to that of a corresponding blank polymer explains the high selectivity seen at low pH values. Based on these findings, a model describing the events controlling binding and selectivity as a function of pH is proposed.

INTRODUCTION

During the last years several examples of the use of imprinted polymers for specific molecular recognition have been reported [1–12]. In one case [4–9] (Fig. 1) a chiral template molecule (L-phenylalanine anilide = L-PA) was used to preorganize functionalized monomers (methacrylic acid = MAA) in solution. Copolymerization of the template assemblies with a cross-linking monomer (ethylene glycol

dimethacrylate = EDMA) gave a network polymer which was freed from template by extraction. After crushing and sieving a selective chromatographic stationary phase was obtained.

With this technique resolutions of a variety of racemates have been successfully achieved [12]. Recently the technique has also been used to prepare affinity matrices for the DNA-bases [10] and as antibody mimics in a drug assay [11].

For the user of chiral stationary phases (CSPs) it is desirable that *predictable* separations of enantiomers as well as achiral compounds from several classes can be performed on a single column [13]. Although the imprinted CSPs usually have a high substrate selectivity, the similarity in composition between these CSPs and meth-

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* Corresponding author.
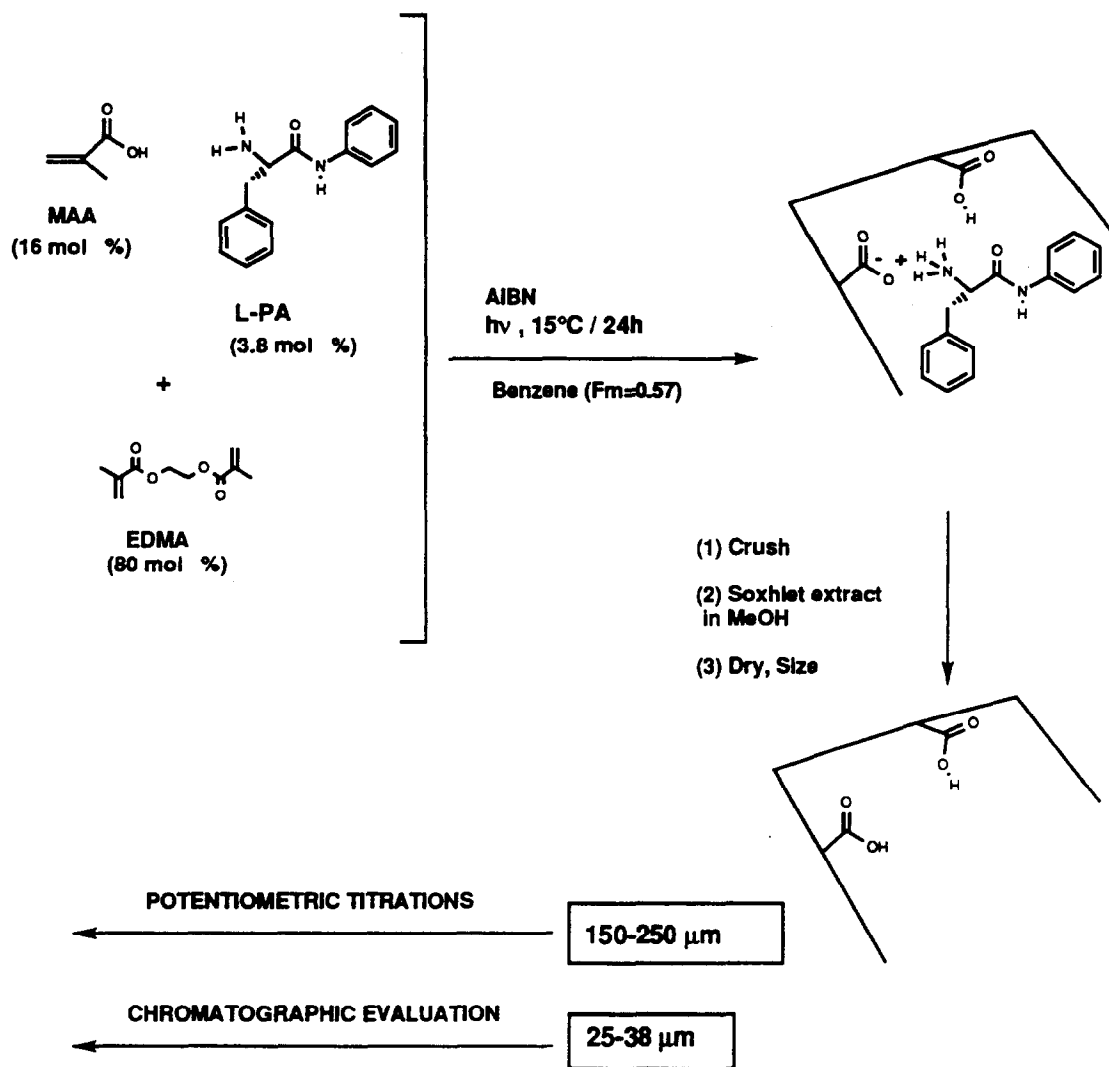☆ Present address: Bioherb Inc., Bifrostgatan 40, 25362 Helsingborg, Sweden.

Fig. 1. Schematic of the synthesis, processing and evaluation of imprinted polymers as chiral stationary phases. Fm refers to the volume fraction of polymerisable monomers to benzene solvent.

acrylate-based weak cation-exchange resins [14–16] suggests that they may be applied in simple cation-exchange separations as well. This therefore led us to investigate whether ion exchange is the main process controlling retention on these CSPs.

EXPERIMENTAL

General procedures

D- and L-PA were synthesized as described elsewhere [5,6]. The methacrylate monomers were obtained from Aldrich. EDMA was purified by extraction with 10% NaOH, brine, drying over anhydrous magnesium sulfate followed by distillation. MAA was purified by drying over anhydrous magnesium sulfate followed by distillation. The porogens were all distilled under a positive nitrogen atmosphere prior to use and all other reagents purified according to standard procedures. The pH was monitored with a standard pH electrode connected to a pH meter (Fischer Scientific, Accumet 910) standardized at pH 4, 7 and 10 using

buffer solutions. All chromatographic evaluations was done using a Waters 484 UV detector, Waters 501 pump equipped with a U6K injector and a Hewlett-Packard integrating recorder.

Polymerizations

The polymers were prepared as follows: The monomer mixture consisted of EDMA (3.8 ml, 20 mmol) and MAA (0.34 ml, 4 mmol) in benzene (5.6 ml) to which was added template and the initiator azo-bis-isobutyronitrile (AIBN; 40 mg, 0.25 mmol). The templates were L-PA (240 mg, 1 mmol) giving polymer P-L-PA or benzylamine (BA) (107 mg, 1 mmol) giving polymer PBA. No addition of template gave the blank polymer (PBL). The mixture was transferred to a 50-ml thick-walled glass tube. This was freeze thaw degassed three times and sealed under vacuum. The tubes were symmetrically placed at ca. 10 cm distance from a standard UV light source in a waterbath thermostatted at 15°C and turned at regular intervals for a symmetric exposure. After 24 h the tubes were broken and the polymers separated then ground in a mortar followed by Soxhlet extraction in methanol for 12 h. The polymers were dried overnight under vacuum at 50°C and then sieved to a 150–250 μm and a 25–38 μm particle size.

Chromatographic evaluation

The polymers with a 25–38 μm particle size were slurry packed into 100-mm stainless-steel columns [5 mm I.D. containing ca. 0.5 g (dry mass) of polymer after packing] using acetonitrile–water–acetic acid (92.5:2.5:5, v/v/v) as mobile phase. After passing ca. 50 ml at a flow-rate of 10 ml/min the column was equilibrated at 1 ml/min until a stable baseline was reached. The flow rate was 1 ml/min, the volume of injected solute 10 μl and the detection wavelength 260 nm unless otherwise stated. The capacity factor was calculated as $(t_R - t_0)/t_0$ where t_R is the peak maxima retention time of the solute and t_0 the retention time of a non-retained void marker ($NaNO_3$). The separation factor (α) measures the relative retention between the enantiomers ($\alpha = k'_L/k'_D$) and h the reduced plate height from the number of theoretical plates (N) as $h = L/(d_p N)$ where $L =$ column length ($=10$ cm), $d_p =$ average particle diameter ($=31.5$ μm) and $N = 5.55$ $(t_R/t_{1/2})^2$ where $t_{1/2}$ is the peak width at half height. The resolution factor R_s [3] and the asymmetry factor A_s [17] were obtained graphically as described elsewhere.

Potentiometric pH titrations

Conditioning. The polymers were initially converted to the acid form. Polymer (0.6 g, 150–250 μm) was washed by shaking with 2×25 ml 0.1 M HCl for 1 h each. After sedimentation the supernatant was removed with Pasteur pipette followed by several wash cycles with water until no change in pH was observed (pH ca. 4). In the second cycle the polymer was stirred by shaking for 1 h.

Titration. Polymer (0.5 g) was suspended in 20 ml of a CO_2-free 0.1 M NaCl solution by magnetic stirring. Titrations were performed under nitrogen atmosphere by addition of 25-μl increments of 0.5 M NaOH allowing ca. 30 min equilibration between additions. After titration of the polymers a blank solution (in absence of polymer) was titrated in presence and absence of an amount of acetic acid corresponding to the theoretical amount of carboxylic acid groups in the polymer. Following the above procedure another titration was done in acetonitrile–0.1 M NaCl (7:3, v/v). Standardization was again performed against the aqueous buffers [18,19]. During titration some mechanical breakdown of the polymer particles occurred due to the mechanical stirring. This caused the response time to decrease during the titration. Homogeneous titrations were carried out on L-PA and BA in the same solvent system. This resulted in apparent pK_a values of 6.4 and 8.6, respectively.

Calculations

The degree of ionization (α^*) was obtained from a combination of mass and charge balance equations as described by Dubin and Brant [18] in the reduced equation:

$$\alpha^* = [V + V_a - V_b]/V_{eq} \qquad (1)$$

where $V =$ volume added base titrant to achieve a certain pH, V_a and $V_b =$ volume of added acid

and base titrant to the blank solution to achieve the same pH. V_{eq} = volume of added titrant at the equivalence point. V_{eq} in the aqueous and organic–aqueous systems were determined from the maximum in the plot of dpH/dV. Since a correct determination of the equivalence point may be critical for the resulting shape of the graph $V_{eq} \pm 0.1$ ml was also used in the α^* calculations. This did not change the general shape of the curve. The pK_a was then determined at each pH from the equation $pK_a = pH - \log[\alpha^*/(1 - \alpha^*)]$ and the average pK_a ($\overline{pK_a}$) from the y-intercept of a plot of pH versus $\log[\alpha^*/(1 - \alpha^*)]$ according to the equation $\log[\alpha^*/(1 - \alpha^*)] = pH - pK_a$.

RESULTS AND DISCUSSION

Mobile phase effects

One of the drawbacks to the routine use of imprinted polymers in chromatography has been the extensive peak broadening and asymmetry. Although clear improvements were seen by either increasing the column temperature (up to 90°C) [6] or by using polymers prepared at lower temperature [20], the column efficiency was still poor compared to that of commercially available

chiral stationary phases. A mobile phase containing acetic acid as additive was routinely used. In view of the unusual isotherms, broad peaks and peak splitting associated with this mobile phase, we suspected that the poor performance was mobile phase related. Other mobile phases were therefore tried (see Table I).

By increasing the aqueous content of the mobile phase retention decreases, possibly due to the extensive solvation of ammonium ions by water [21]. In the absence of acetic acid a remarkably large retention is seen. The retention volume seems to be strongly dependent on the acetic acid concentration since by adding 1% acetic acid a low k' is again observed. This suggests that both retention and selectivity can be modulated by controlling the mobile phase pH. Thus, by using the same organic-aqueous volume ratio but adding potassium phosphate buffer salts, a clear dependence of k' and α on pH was seen with strong retention and high selectivity at low pH and weak retention and low selectivity at high pH. With interest we noted that these changes improved chromatographic performance. This can be seen in the almost *50% reduction in the reduced plate heights (h) of the retained solutes in the buffered system compared to those observed using the original acetic*

TABLE I

STEPWISE MOBILE PHASE OPTIMIZATION IN THE CHROMATOGRAPHIC ENANTIOMER SEPARATION OF D,L-PA ON POLYMER P-L-PA

At a flow-rate of 1 ml/min 10 μmol of D,L-PA in 10 μl was injected. MeCN = Acetonitrile; HOAc = acetic acid; KP = potassium phosphate. ND = Not determined; VB = very broad; NR = no enantiomeric resolution. h_D, h_L and h_0 are the reduced plate heights for the D and L enantiomers of PA and for a weakly retained compound (acetone), respectively.

Mobile phase	k'_L	α	h_D	h_L	h_0	R_s
MeCN–H₂O–HOAc (92.5:2.5:5, v/v/v)	14	5.4	118	397	6	1.1
MeCN–5% HOAc (7:3, v/v)	0.8	3.2	ND	ND	ND	<1
MeCN–H₂O (7:3, v/v)	64	13	167	VB	ND	ND
MeCN–1% HOAc (7:3, v/v)	3.6	4.1	53	244	ND	1.3
MeCN–0.05 M KP, pH 3.5 (7:3, v/v)	9.3	5.4	75	167	6	2.0
MeCN–0.05 M KP, pH 9 (7:3, v/v)	0.4	NR	11		ND	NR
MeCN–0.05 M KP, pH 3 (3:7, v/v)	10	2.8	70	1060	42	1.0
MeCN–0.05 M KP, pH 9 (3:7, v/v)	87	2.1	50	VB		53

acid containing system. In addition, there was a parallel increase in resolution (R_s) for a constant separation factor (α) (see elution profiles in Fig. 2). The column efficiency on the other hand does not change with the mobile phase as seen from the reduced plate height (h_0) for a weakly retained compound such as acetone. This suggests that the use of organic–acetic acid mobile phases results in slow mobile phase equilibria. When further increasing the aqueous content using the buffered mobile phases, k' increases while α decreases. The pH interval for separation is now extended to higher pH while the column efficiency, reflected in the reduced plate height (h_0), is considerably poorer (see Fig. 2). Since both k' and α appeared to respond to pH changes, information about the protonation state of the polymer and the solute in organic aqueous solvent systems is of primary interest. Potentiometric titrations were therefore carried out.

Potentiometric titration of the acid groups of the polymers

Potentiometric pH titrations on linear polymers containing carboxylic acid groups has provided information about conformational changes as well as estimates of the related energy barriers and electrostatic free energies [18,19]. Titrations of highly cross-linked carboxylic acid containing polymers (*i.e.* weak cation exchangers) are mostly used in order to determine ion-exchange capacity buffering range and the average pK_a of the polymer [14–16]. Fig. 3 shows pH-titration profiles in aqueous and organic–aqueous systems of imprinted and blank polymers. The ionic strength was kept constant by addition of 0.1 M NaCl. Obviously most of the carboxylic acid groups originally added as monomers have been titrated, indicating that they are accessible. A lower accessibility, 60–70%, is seen in the titration in the aqueous system compared to the aqueous–organic system, 75–85%. This may be related to the lower swelling observed in water [9] leading to a more compact structure and a lower accessibility. The accuracy of the titrations was checked by titrating an amount of acetic acid corresponding to one equivalent of carboxylic
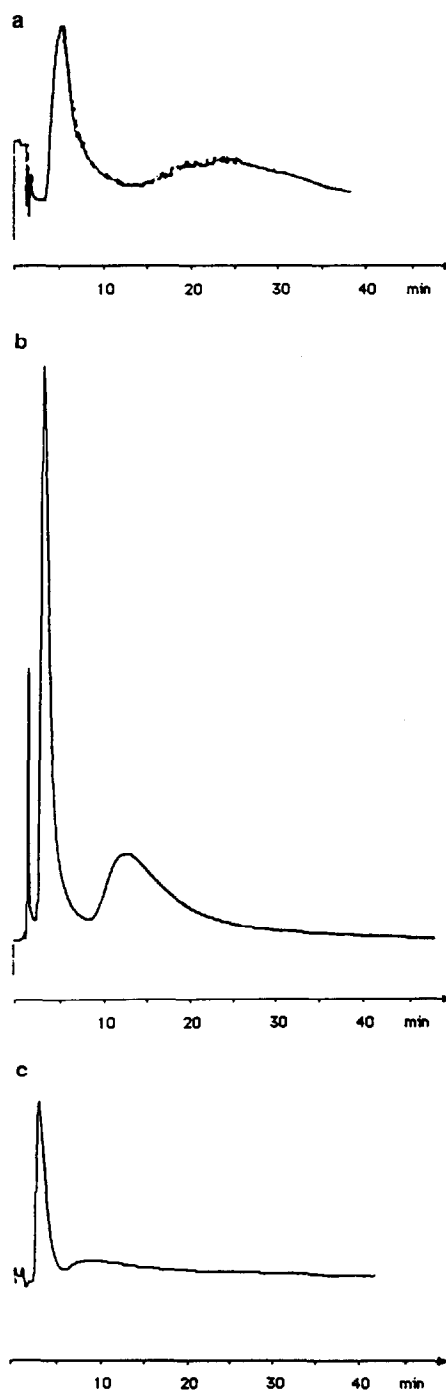

Fig. 2. Elution profiles from the experiment described in Table I using as mobile phase (a) MeCN–H_2O–HOAc (92.5:2.5:5, v/v/v), (b) MeCN–0.05 M potassium phosphate, pH 3.5 (7:3, v/v) and (c) MeCN–0.05 M potassium phosphate, pH 3 (3:7, v/v).
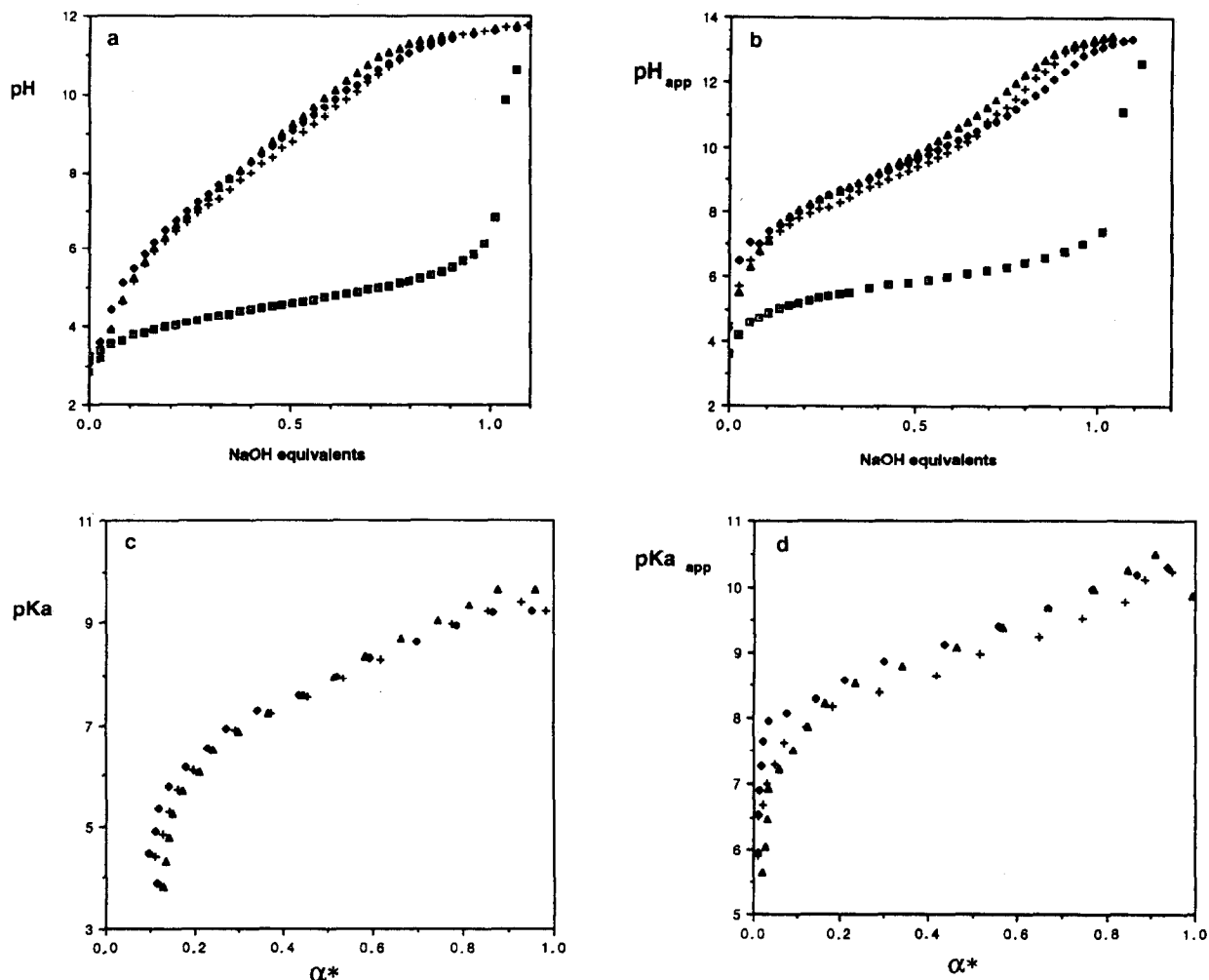
Fig. 3. Potentiometric titration curves on P-L-PA (+), PBA (▲), PBL (◆) and acetic acid (□) in (a) 0.1 M NaCl and (b) MeCN–0.1 M NaCl (7:3, v/v). The NaOH equivalents (x-axis) are calculated based on the theoretical amount of carboxylic acid groups present in the polymer. In (c) and (d) are seen the calculated pK_a distribution as a function of the degree of ionization (α^*). (c) $\overline{pK_a}$ = 8.0 (P-L-PA), 8.1 (PBA), 8.2 (PBL); (d) $\overline{pK_a}$ = 8.9 (P-L-PA), 9.2 (PBA), 9.3 (PBL). The polymer swelling [9] (ml/ml) in this solvent system was constant in the pH interval 3–12 and was for P-L-PA: 1.32 and for PBL: 1.26.

acid groups in the polymer. The polymers have a buffer capacity over a wide pH range and in the organic–aqueous system this range is found at a higher pH than in the aqueous system. The physical meaning of this difference is not clear since the method used to standardize the pH meter gives only apparent pH values.

In the titration of polyelectrolytes, neighboring group effects are a significant influence on the pH-titration profile [22]. For instance, in polyacrylic acid the ionization of the most acidic

group may be facilitated by hydrogen bonding to a neighboring carboxylic acid group. However at higher levels of ionization the net increase in negative charge of the polymer will make it more difficult to ionize the remaining carboxylic acid group. It has been shown that the apparent pK_a increases with the degree of ionization (α^*) may be represented by:

$$pK_a = pH - \log[\alpha^*/(1 - \alpha^*)] \qquad (2)$$

A plot of pK_a as a function of α^* can therefore

be regarded as a measure of the ease of proton removal from the polyion at a given degree of ionization. The average apparent pK_a was higher in the organic aqueous system but this may be due to the standardization procedure discussed above. However the differences exhibited by the polymers in the shape of the pK_a *versus* α^* plot is more interesting. Similar titrations of weak cation exchangers commonly exhibits buffering capacity at a lower pH [14,15]. The difference may reflect the influence of different charge densities. The more weakly cross-linked cation exchange resins exhibits higher swelling and can thus expand and reduce the buildup of electrostatic repulsion, leading to a titration curve that approaches that of the free acid. Swelling as a function of pH was measured in the organic aqueous system. *As seen in the legend to Fig. 3 the L-PA imprinted polymer P-L-PA showed significantly larger swelling than the corresponding blank non-imprinted polymer PBL. However no change in swelling was observed when the pH was varied.* In the organic–aqueous system the difference between the titration curves of PBL and P-L-PA can therefore be explained by their different swelling. Contribution from the structural organization of the carboxylic acid groups in the polymer is also possible (which *per se* may be the reason for the difference in swelling). In PBL the absence of template during polymerization will leave the carboxylic acid groups to interact mainly with themselves forming acid dimers. However in the presence of template, the acid groups will interact also with the template molecule. After freeing the polymers from template the acid groups in PBL will still have other acid groups to interact with while in P-L-PA the acid groups have lost their hydrogen bond partner and are thus in a more isolated environment. Such an arrangement would result in a lower pK_a for the more isolated acid groups compared to the pK_a of the associated groups. *Indeed the pK_a of P-L-PA is lower than that of PBL.* It should be noted however that the polymers exhibited very similar Fourier transform IR spectra [9], indicating that no large detectable difference exists in the extent of hydrogen bonding between the carboxylic acid groups of the polymers. However as discussed

below the chromatographic data support the above explanation for the observed pK_a difference between the polymers.

Mobile phase pH dependence

Using polymer P-L-PA and PBA as stationary phases 10 and 100 nmol amounts of D,L-PA and BA were injected at different mobile phase apparent pH values (pH_{app}) ranging from 3.5 to 10 (measured on the total mobile phase) at 0.5 pH unit intervals. In Fig. 4a and b the resulting pH–retention graphs are shown. For reasons of comparison the given pH is that of the net mobile phase mixture (measured and standardized as described for the potentiometric titrations in the Experimental section) and not that of the aqueous buffer. The reproducibility was confirmed by repeating the experiment at intervals of one pH unit. Moreover similar pH dependence was observed using other L-selective polymers. It should be noted that due to the weak retention at low and high pH values the α determination is highly dependent on an accurate determination of the void retention time. When increasing the aqueous content in the mobile phase the solvent peak (MeCN) eluted at a 20% shorter time while acetone, used as a void marker in the organic–acetic acid mobile phases, still eluted at the original void retention time. However sodium nitrate, a commonly used void marker in aqueous systems [23], coeluted with the solvent peak and was therefore used as void marker in this system. The possibility of a Donnan exclusion effect at high pH did not result in any significant change in the retention time. When further increasing the aqueous content (see Table I) a lower t_0 was observed. These effects may be related to the lower swelling observed in the aqueous solvents [9].

As seen in Fig. 4a and b maxima in retention occur at pH values corresponding to the apparent pK_a values of the solutes, measured potentiometrically in the same solvent system (Note that the salt concentration of the aqueous portion in the titrations was 0.1 M while in the LC experiments the buffer concentration was constant at 0.05 M. For solubility reasons no extra salt was added here. Although titration curves can be strongly dependent on ionic strength it is
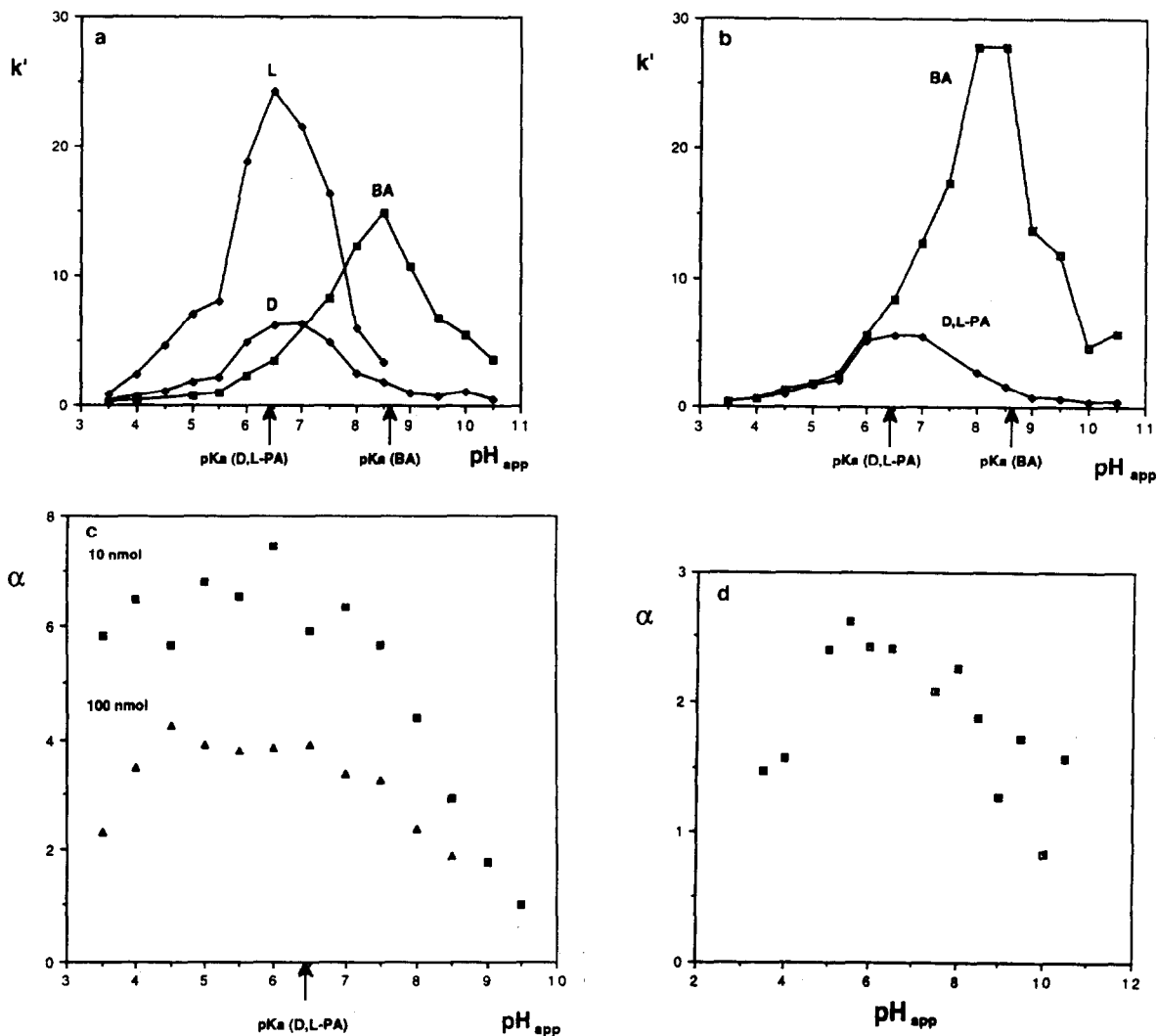
Fig. 4. Retention (k') of D- and L-PA and BA on (a) P-L-PA and (b) PBA injecting 100 nmol solute *versus* mobile phase pH_{app}. (c) and (d) show the corresponding separation factors (α) obtained at two different sample loads. The separation factor of D,L-PA (c) was calculated as $\alpha = k'_L/k'_D$ and of benzylamine (100 nmol) (d) as $\alpha = k'_{BA}$ (on PBA)/k'_{BA} (on P-L-PA).

unlikely that this difference will have a major influence on the position and relative height of the maxima in Fig. 4). Moreover Fig. 4c and d shows that each polymer binds preferentially the compound used as template. Thus the α versus pH plots of D,L-PA on P-L-PA show a stable high selectivity in the low pH region both at 10 and 100 nmol sample load. When pH_{app} exceeds pK_a of the solute, α drops off to approximately 1 at pH_{app} 9.5. The selectivity factor of BA on PBA was estimated as an α value calculated from the

ratio of k' on PBA to k' on P-L-PA[a]. This graph also shows a trend of decreasing α with increasing pH although here the falloff is observed over a larger pH range. *The fact that maximum retention is observed at a pH_{app} corresponding to the apparent pK_a of the solute suggested to us that*
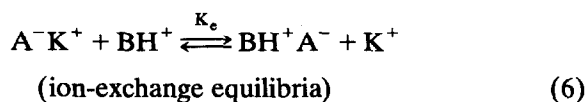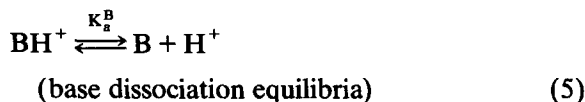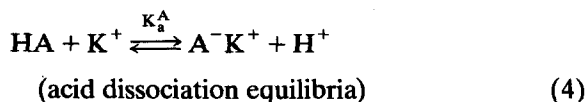
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[a] In this treatment P-L-PA has been taken as a blank polymer to BA. The absence of selective interaction of P-L-PA with BA is seen in the weak retention at low pH where L-PA is strongly retained.

the retention was controlled by a simple ion-exchange process. Thus the amino group containing solute B (D,L-PA or BA) is bound to the polymer containing carboxylic acid groups (HA) forming ion-pairs BH^+A^-. The capacity factor can now be expressed as:

$$k_B' = \phi[B]_b/[B]_f = \phi[BH^+A^-]/([B]+[B^+]) \quad (3)$$

where b and f indicates bound and free solute respectively and ϕ = phase volume ratio. Since potassium ions (K^+) are the counterions in the buffered system the following equilibria should be considered [24].

$$HA + K^+ \overset{K_a^A}{\rightleftharpoons} A^-K^+ + H^+$$

(acid dissociation equilibria) $\quad (4)$

$$BH^+ \overset{K_a^B}{\rightleftharpoons} B + H^+$$

(base dissociation equilibria) $\quad (5)$

$$A^-K^+ + BH^+ \overset{K_e}{\rightleftharpoons} BH^+A^- + K^+$$

(ion-exchange equilibria) $\quad (6)$

Eqn. 3 can now be rewritten as:

$$k_B' = \frac{\phi K_e[BH^+][A^-K^+]}{[K^+]([B]+[BH^+])}$$

$$= \phi(1/[K^+])K_e \alpha_B^* \alpha_A^*[A]_{tot} \quad (7)$$

where α_A^* and α_B^* are the degree of ionization of the acid (A) and the base (B) respectively, K_e = the ion-exchange equilibrium constant and $[A]_{tot}$ = the total concentration of A. Eqn. 7 can be even further simplified:

$$k_B' = K\alpha_B' \alpha_A^* \quad (8)$$

where K is a constant for a given column and ionic strength. In order to test this model α_A^* and α_B^* need to be determined as a function of pH_{app}. In Fig. 5 values for α^* for L-PA, BA and the polymer have been plotted *versus* pH_{app} and in Fig. 6 the product $\alpha_B^* \alpha_A^*$. *The agreement with the experimental chromatographic data, both in the pH region where the maxima are found and in the relative retention of the solutes at these maxima is striking*. Similar behavior is often observed for weak bases on weak cation exchangers
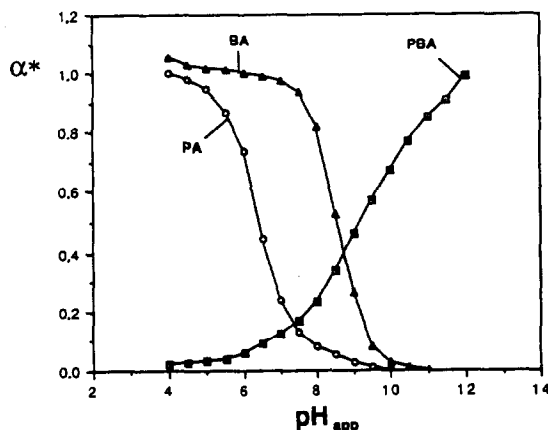

Fig. 5. Degree of ionization (α^*) as a function of mobile phase pH_{app} for the solutes BA and D,L-PA and for PBA. α^* was calculated from the corresponding potentiometric titration data as described in the experimental section.

[24]. It can therefore be concluded that cation exchange is the process controlling retention in this system. What about the selectivity? According to the retention model (eqn. 8) α can be expressed as:

$$\alpha = k_L'/k_D' = k_L\alpha_L^* \alpha_A^*/K_D\alpha_D^* \alpha_A^* = K_L/K_D \quad (9)$$

where K_L and K_D are the average ion-exchange equilibrium constants to the imprinted sites for the L and the D form respectively. Eqn. 9 follows from the fact that the degree of ionization of the D and the L form are the same at a given pH. In
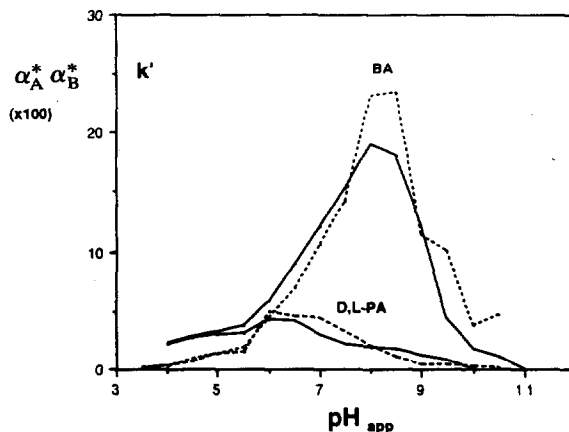

Fig. 6. Product of the degree of ionization of the solute (α_B^*) and the polymer PBA (α_A^*) ($\times100$) *versus* mobile phase pH_{app} (solid line). Overlayed are the experimental data from Fig. 4b (dashed line).

other words, α does not change with the protonation state of either the polymer or the solute as long as these changes affect K_L and K_D to the same degree [25]. This would be the case if the binding involved just one electrostatic interaction. We propose that the decrease in enantioselectivity upon an increase in mobile phase pH is due to either: (a) deprotonation of a second group in the site, leading to a loss of an enantioselective hydrogen bond interaction, or (b) deprotonation of additional non-selective sites. If K_{ns} in case (b) represents the average ion-exchange equilibrium constant to the non-selective sites (ns) α can here be expressed as:

$$\alpha = \frac{K_L \alpha_L^* \alpha_s^* + K_{ns} \alpha_L^* \alpha_{ns}^*}{K_D \alpha_D^* \alpha_s^* + K_{ns} \alpha_D^* \alpha_{ns}^*} \tag{10}$$

Since the second term in both the numerator and the denominator are identical an increase in α_{ns}^* will obviously lead to a decrease in α. An argument for this explanation is the sharp increase in non-specific binding above pH_{app} 6. This is clearly seen in the plot of k' versus pH_{app} for BA on P-L-PA (Fig. 4) and in the parallel increase in α^* for the latter (Fig. 5). This can also be seen from the plot of the estimated separation factor of benzylamine (α_{BA}) versus pH_{app} (Fig. 4d) where α is highest at low pH

values [below $pK_a(BA)$] and decreases over a large pH interval. Furthermore the potentiometric titrations showed that P-L-PA had a lower average pK_a than PBL (see Fig. 3). *This strongly suggests that the carboxylic acid groups of the selective sites have a lower average pK_a than those of the non-selective sites.* It could be argued that nonionic sorption could contribute to the observed retention. However such a process seems to be negligible in view of the parallel decrease in the degree of ionization and the retention of PA and BA. For instance k'_L decreases from 25 to less than 1 upon neutralization.

In Fig. 7 a model is presented, showing how the protonation states of D,L-PA and the polymer (P-L-PA) can account for the observed retention and selectivity. At pH_{app} 4 the solute D- and L-PA (two-dimensional representation) are protonated and bind differentially to enantioselective sites. At pH_{app} 5.5 the solute is partly deprotonated while a larger amount of selective sites are available. This will result in an increase in k' whereas α remains constant. At pH_{app} 6.5 (ca. pK_a for D,L-PA) half of the solute is protonated and non-selective sites are becoming deprotonated resulting in stronger non-selective interactions and a net increase in binding. At pH_{app} 8 the solute is only partly protonated while the negative charge of the polymer increases mainly

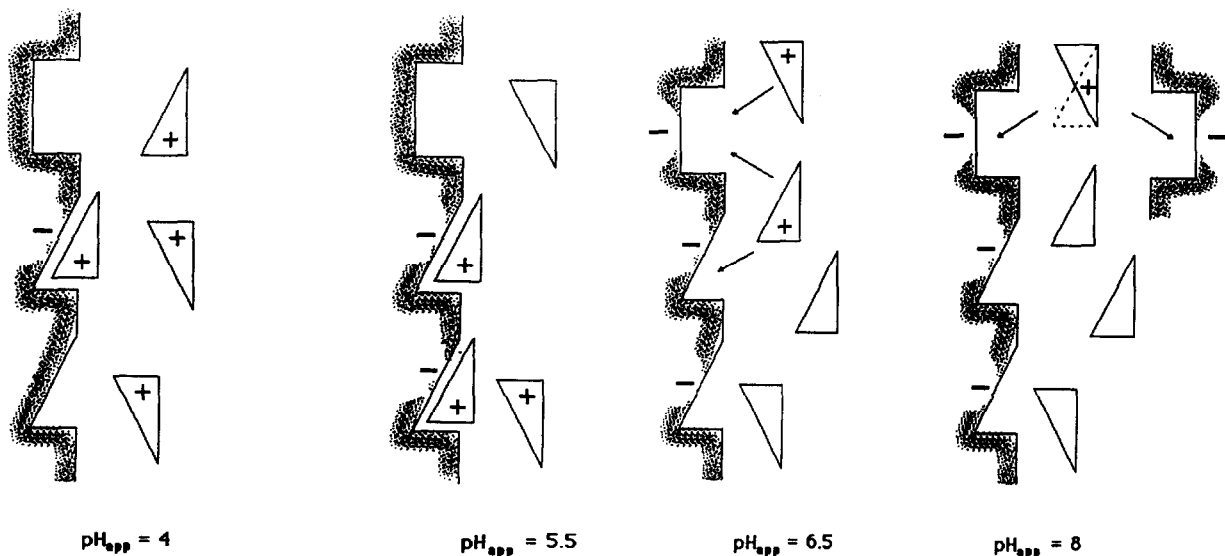pH$_{app}$ = 4   pH$_{app}$ = 5.5   pH$_{app}$ = 6.5   pH$_{app}$ = 8

Fig. 7. Proposed model for the influence of pH on the retention volume.

as non-selective sites are deprotonated. Thus the chance of non-selective binding increases while retention as a whole decreases.

As noted in the first proposed explanation (a) for the change in enantioselectivity, an increase in pH may *per se* lead to a decrease in the enantioselectivity of the imprinted sites. Contribution from this effect cannot be estimated and thus it cannot be excluded. Therefore it is not possible at this stage to draw any conclusions about the origin of the enantioselectivity at a molecular level.

CONCLUSIONS

During mobile phase optimization using imprinted CSPs a buffered aqueous–organic mobile phase was found to give a clear improvement in column performance. Since both enantiomer retention and selectivity were sensitive to mobile phase pH a study of the protonation state of both solute and polymer in the same solvent system was carried out. A potentiometric titration of the polymers revealed a high accessibility of the carboxylic acid groups and a buffering capacity on the basic side with an average pK_a in water of around 8. A slightly lower pK_a was observed for the imprinted polymers compared to the blank polymers prepared in the absence of template. A molecular level explanation was given based on the difference in the state of hydrogen bonding in the imprinted and the blank polymer respectively. This explanation was supported by the high selectivity found at low pH and a close correlation observed between the chromatographic experimental data and a weak cation exchange model.

The combination of the weak cation-exchange properties and the predictable enantioselectivity of the phase may have interesting applications in the separation of basic drugs containing stereogenic centers. For instance pH control may be used to switch chiral separation on and off or to speed up separations using pH gradient elutions. Furthermore, advantage may be taken of the difference in pK_a between the selective and the non-selective sites for selective inhibition of non-specific binding sites. This may lead to an improved column performance. In this context it
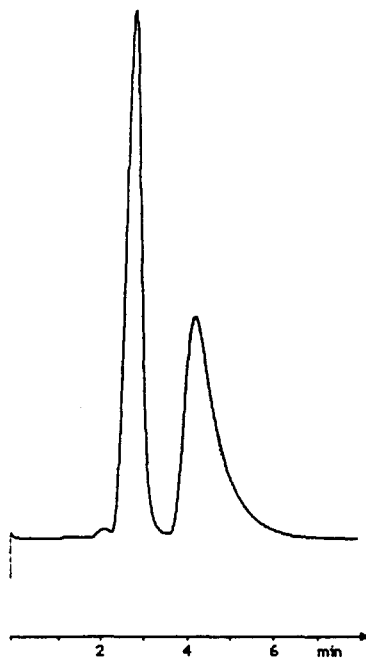

Fig. 8. Elution profile of D,L-PA (10 nmol) applied on a heat treated L-PA selective polymer. Mobile phase: MeCN–0.05 M KP, pH 4 (7:3, v/v). Flow-rate: 1 ml/min. Column temperature: 80°C. Reduced plate heights: $h_D = 9$, $h_L = 18$.

should be mentioned that heat-treated polymers run at elevated column temperatures (Fig. 8) have been found to give resolutions and reduced plate heights in the same order as those observed for some commercially available CSPs [9].

REFERENCES

1 G. Wulff, in W.T. Ford (Editor), *Polymeric Reagents and Catalysts* (*ACS Symposium Series*, No. 308), American Chemical Society, Washington, DC, 1986, pp. 186–230.

2 G. Wulff, in M. Zief and L.J. Crane (Editors), *Chromatographic Chiral Separations*, Marcel Dekker, New York, 1988, pp. 15–49.
3 G. Wulff, H.-G. Poll and M. Minarik, *J. Liq. Chromatogr.*, 9 (1986) 385.
4 L. Andersson, B. Sellergren and K. Mosbach, *Tetrahedron Lett.*, 25 (1984) 5211–5214.
5 B. Sellergren, B. Ekberg and K. Mosbach, *J. Chromatogr.*, 347 (1985) 1–10.
6 B. Sellergren, M. Lepistö and K. Mosbach, *J. Am. Chem. Soc.*, 110 (1988) 5853.
7 B. Sellergren, *Chirality*, 1 (1989) 63.
8 M. Lepistö and B. Sellergren, *J. Org. Chem.*, 54 (1989) 6010.
9 B. Sellergren and K.J. Shea, *J. Chromatogr.*, 635 (1993) 31–49.
10 K.J. Shea, D.A. Spivak and B. Sellergren, *J. Am. Chem. Soc.*, 115 (1993) 3368.
11 G. Vlatakis, L.I. Andersson, R. Muller and K. Mosbach, *Nature*, 361 (1993) 645.
12 B. Sellergren, in G. Subramanian (Editor), *Chiral Separations by Liquid Chromatography*, VCH, Weinheim, in press.
13 M. Zief and L.J. Crane (Editors), *Chromatographic Chiral Separations*, Marcel Dekker, New York, 1988.
14 F. Helfferich, *Ion Exchange*, McGraw-Hill, New York, 1962.
15 D.J. Pietrzyk, in K.K. Unger (Editor), *Packings and Stationary Phases in Chromatographic Techniques*, Marcel Dekker, New York, 1990, p. 585.
16 O. Mikeš, P. Štrop, M. Smrž and J. Čoupek, *J. Chromatogr.*, 192 (1980) 159.
17 G.S. Weber and P.W. Carr, in P.R. Brown and R.A. Hartwick (Editors), *High Performance Liquid Chromatography*, Wiley, New York, 1989.
18 P.L. Dubin and D.A. Brant, *Macromolecules*, 8 (1975) 831.
19 E.P. Serjeant, *Potentiometry and Potentiometric Titrations*, Wiley, New York, 1984.
20 D.J. O'Shannessy, B. Ekberg and K. Mosbach, *Anal. Biochem.*, 177 (1989) 144.
21 M. Meot-Ner, in J.F. Liebman and A. Greenberg (Editors), *Molecular Structure and Energetics*, Vol. 4, VCH, Weinheim, 1987.
22 P. Molyneux, *Water-Soluble Synthetic Polymers: Properties and Behaviour*, Vol. II, CRC Press, 1984.
23 C.F. Simpson (Editor), *Techniques in Liquid Chromatography*, Wiley–Heyden, Chichester, 1982.
24 P.J. Schoenmakers, *Optimization of Chromatographic Selectivity*, Elsevier, Amsterdam, 1986.
25 W.H. Pirkle and C.J.J. Welch, *J. Chromatogr.*, 589 (1992) 45–51.