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Retention processes on α_1-acid glycoprotein-bonded stationary phase

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ABSTRACT

Stereoselective separations of charged enantiomers on CHIRAL-AGP can be controlled by varying the pH and adding charged and uncharged additives to the mobile phase. The interaction with the selector, α_1-acid glycoprotein, was studied by monitoring the effects of the variables on retention and by indirect detection, in part using a simple multivariate design. The stereoselectivity is due to simultaneous retention processes involving ion-exchange and ion-pairing mechanisms. The predominant mode of interaction for different solutes was elucidated from variables that promote or counteract either of the processes. Considerable improvements in the stereoselectivity were achieved with chiral or achiral anionic and cationic additives that act in a synergistic or competitive mode.

INTRODUCTION

The dramatic development of chromatographic separation methods for enantiomeric compounds during the last decade has been achieved by different techniques. In many of them, such as with silica-bonded α_1-acid glycoprotein (Chiral-AGP), the character of the chiral binding process is incompletely known and the choice of conditions for stereoselective separation is more or less empirical. It is apparent from the separation of enantiomeric solutes of different structures that Chiral-AGP has binding sites of different character. By varying the properties of the mobile phase, a basis for the optimization of stereoselective separations and an insight into the binding mechanisms are obtained [1].

Chiral-AGP can be applied to the separation of both charged and uncharged enantiomers. The selector, α_1-acid glycoprotein (AGP), is an acidic protein with negatively charged groups in the aspartic acid residues and in the terminal serine group, whereas positively charged groups are present in the arginine, lysine and histidine residues. Uncharged, hydrogen-bonding chiral sites appear in the peptide chain and in the carbohydrate units that constitute 45% of the molecular weight [2]. Advanced studies of the chiral recognition properties by, *e.g.*, binding experiments with partly modified AGP have not been reported.

It has been found that the retention and stereoselectivity on Chiral-AGP can be varied over a wide range by changing the pH of the aqueous mobile phase and the content of charged and uncharged organic components [1]. The effects of these changes are highly dependent on the structure of the solute and bear no simple relationship to general properties such as charge or hydrophobicity. The complex nature of the binding process was illustrated in a previous paper by the effects of charged additives on retention and indirect detection [3]. The relationships were partly analysed by a two-level experimen-

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tal design with three variables using β-values to describe the effects of the variables, which is a systematic way to collect information. Enantiomers were affected differently, which indicates binding to several sites. The reversal of the retention order of the enantiomers of pseudoephedrine on addition of the counter ion octanoate [4] has the same basis.

The aim of this study was to develop models for the binding process to the chiral AGP-selector which can be used in the optimization of the stereoselective separation of charged enantiomers. The interaction between the solute and selector was studied by monitoring the effects of 2-propanol, pH and cationic and anionic additives on retention, selectivity and indirect detection. Solutes having an asymmetric centre in an aliphatic chain were included in the study.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a Model 2150 liquid chromatographic pump (LKB, Bromma, Sweden), a Rheodyne (Cotati, CA, USA) Model 7010 injector with a 20-μl loop and a Kratos (Ramsey, NJ, USA) Spectroflow 783 variable-wavelength detector. The temperature of the analytical column, injector and connecting tubes was controlled by immersing the system in a thermostated bath (RMS, Lauda-Königshofen, Germany). The chromatograms were recorded on a Perkin-Elmer (Norwalk, CT, USA) Model 56 instrument or a Spectra-Physics (San José, CA, USA) SP 4270 integrator.

Chromatographic conditions and chemicals

The separation column was Chiral-AGP (100 × 4.0 mm I.D.; 5 μm) from ChromTech (Norsborg, Sweden). The flow-rate was 0.4 ml/min and the system was thermostated at 22°C. If not stated otherwise, the mobile phases were phosphate buffers (ionic strength $I = 0.05\ M$) to which modifiers were added. The wavelength of detection was the UV absorption maximum or, when studying indirect detection, 220 nm. Atropine sulphate, homatropine bromide and sodium octanoate were purchased from Merck (Darmstadt, Germany). Metoprolol succinate was synthesized at Astra Hässle (Mölndal, Sweden). 1-(2-Hydroxyphenyl)-2-(*tert.*-butylamino)-ethanol (2HPE), 1-(4-hydroxyphenyl)-2-(*tert.*-butylamino)ethanol (4HPE) and ipratropine were kindly supplied by Astra Draco (Lund, Sweden). 4-Phenylbutyric acid, (*R*)- and (*S*)-3-phenylbutyric acid and racemic 2-phenylbutyric acid were purchased from Fluka (Buchs, Switzerland) and (*S*)- and (*R*)-2-phenylbutyric acid from Sigma (St. Louis, MO, USA).

Factorial design

Factorial design allows in a systematic way conclusions to be drawn about retention mechanisms from β-coefficients. The effects of pH, octanoate and 2-propanol on the retention were studied. A full-factorial design was made with 2^3 experiments, giving possibilities of calculating both the individual and combined effects of the three variables on retention and stereoselectivity. Calculations of effects expressed as β-values at different compositions of the mobile phases were made as described previously [3]. The magnitude of the effects of the variables depends on both the range studied and the magnitude of the response, which limits the use of the β-coefficients to a quantitative comparison of a certain variable on different solutes. The three variables were studied at two levels: pH in the mobile phase, 6.0 (−1) and 7.5 (+1); concentration of 2-propanol, 0% (−1) and 3% (+1); and concentration of octanoate, 0 mM (−1) and 4 mM (+1).

Indirect detection

A solute without an inherent detector response can be detected and quantified by an indirect detection technique. In a reversed-phase system the principle is as follows [5]: a detectable compound with moderate retention is included in the aqueous mobile phase. When a solution deviating in composition from the mobile phase is injected, the established equilibria are disturbed. Each retained component will form a zone which travels through the column with constant composition. When eluted, the zones appear as peaks as the concentration of the detectable compound deviates from that in the bulk of the mobile phase. The peaks can be positive or negative and they are given both by injected solutes and by mobile phase components (system peaks). The nature of the retention processes appears from the directions and relative retention of the solute and system peaks as shown previously [5]. Studies of system peaks, peak direction and solute peak size

combined with retention and selectivity data can give valuable information about retention principles.

RESULTS AND DISCUSSION

Binding processes

Two major retention principles have to be considered, *viz.*, binding to charged and uncharged sites. If the binding site is charged, the solid phase can act as an ion exchanger. The capacity factor of a solute HA^+ retained on a cation-exchanging site can be expressed as

$$k'_{HA,ie} = \frac{qC_R K_{HA}}{[H^+] + K_Q[Q^+]} \quad (1)$$

where q is the phase volume ratio [6], H^+ and Q^+ are mobile phase ions, K_{HA} and K_Q are equilibrium constants expressing the exchange of H^+ for HA^+ and Q^+, respectively, and C_R is the total binding capacity for cations.

AGP has an isoelectric point of 2.7 and its cation-exchanging capacity, C_R, increases with increasing pH. The retention of cations is dependent not only on $[H^+]$ but also on the concentration of other mobile phase cations, represented in eqn. 1 by Q^+. The effect of Q^+ is highly dependent on its affinity for the charged site.

The retention of an anionic solute is in an analogous way dependent on the OH^- concentration and the concentration and type of other mobile phase anions. The total binding capacity for anions decreases with increasing pH.

Eqn. 1 shows that an ion with a charge opposite to that of the solute has no direct influence on the retention. However, the presence of anion-exchanging sites might give rise to special retention effects. If a divalent anion, *e.g.*, HPO_4^{2-}, is bound to a monovalent site it will give rise to a negative charge that might retain cations.

An uncharged site can bind a charged solute as an ion pair with a counter ion. Assuming Langmuir adsorption, the retention of a cation, HA^+, can be expressed as

$$k'_{HA,ip} = \frac{qK_0 K_{HAX}[X^-]}{1 + K_{QX}[Q^+][X^-]} \quad (2)$$

where X^- and Q^+ are ions added to the mobile phase [7], K_0 is the total binding capacity and K_{HAX} and K_{QX} are the ion-pair distribution constants [6].

Eqn. 2 shows that additives of the same and opposite charge as the solute affect the retention. For any charged solute, both ion-exchange and ion-pairing processes are possible. An additive of the same charge as the solute has the same kind of effect on both processes whereas a counter ion to the solute directly affects only the ion-pair binding process. It should also be remembered that weak protolytes can be distributed to the solid phase in uncharged form, carboxylic acids usually at pH below 6 and aliphatic amines at pH above 8.

pH and mobile phase additives can affect both unspecific and stereoselective retention processes or sites, using a simple approach. A stereoselective site can bind two enantiomeric solutes with different strength. When applied to eqns. 1 and 2 this means that the equilibrium constants of the solutes are different whereas the remainder of the interaction pattern remains the same. If a single stereoselective site is responsible for the retention, the separation factor, $\alpha = k'_2/k'_1$, will be independent of the concentration of a mobile phase variable. If α changes with a mobile phase variable, more than one site must be involved in the retention process. The effect of the variable on k' and α can give information of the character of the binding sites. If the variable has a dominant influence on a stereoselective site, k' of the two enantiomers and α change in the same direction. If a reversal of the retention order is obtained [4], at least two stereoselective sites with different retention order are involved.

Retention studies

A rational way of approaching the complex influence of mobile phase components using a factorial design [8] was briefly demonstrated in a previous study [3]. Application of the method to analytes of different structures and charge can be based on conclusions on the properties of the binding sites in the chiral selector.

The effects of the three variables, pH, content of 2-propanol and the hydrophobic anion of octanoic acid, are expressed as β-coefficients and presented in Table I. The β-coefficients illustrate changes in retention and selectivity. The solutes are five cationic compounds. Atropine and homatropine have an ester and an alcohol group in the vicinity of the

TABLE I

CALCULATED EFFECTS OF pH, 2-PROPANOL AND OCTANOATE FOR FIVE SOLUTES

Solute	Parameter	β_{pH}	β_{pr}[a]	β_{oc}[a]
Atropine	k'_1	6.4	−5.8	−0.6
	k'_2	8.9	−8.3	1.8
	α	0.06	−0.10	0.20
Homatropine	k'_1	5.0	−3.5	−0.1[b]
	k'_2	7.1	−5.4	−1.2
	α	0.04	−0.11	−0.15
Metoprolol	k'_1	3.5	−3.1	−1.2
	k'_2	4.7	−4.5	−2.1
	α	0.06	−0.12	−0.08
4HPE	k'_1	1.4	−1.0	0.1[b]
	k'_2	1.9	−1.5	0.8
	α	0.13	−0.14	0.13
2HPE	k'_1	6.7	−6.8	−3.6
	k'_2	21	−22	−11.6
	α	0.39	−0.42	−0.19

[a] pr = 2-Propanol; oc = octanoate.
[b] Non-significant effect.

chiral centre, 4HPE and 2HPE have a 4-hydroxyphenyl or a 2-hydroxyphenyl group at the chiral carbon whereas metoprolol is an alkanolamine with a hydroxyl group at the chiral centre.

The experimental variation was determined using higher order interactions [9] and effects lower than this interaction effect are indicated in Table I as non-significant. Comparative calculations using a computer program, RS-Discover (BBN Software Products, Cambridge, MA, USA), confirmed our manual calculations. It should also be emphasized that the model did not completely describe the response, possibly owing to a lack of quadratic terms in the model. However, the aim was to use β-coefficients as a simple tool to visualize the dominant effects of mobile phase components.

Effects of pH

β_{pH}-values for k', $\beta_{pH}(k')$, of all the cationic enantiomers are positive, which shows that the dominant effect of increasing pH is an increase in k'. This indicates retention on a cation-exchanging site in accordance with eqn. 1. The β_{pH}-values for α, $\beta_{pH}(\alpha)$, are positive for all the cationic solutes, which indicates that the cation-exchange sites have stereoselective binding properties.

The cation-exchanging properties of the protein dominate in the pH range used and the anion-exchange capacity must be limited. However, the influence of anion-exchange groups is indicated by the significant retention of dihydrogenphosphate [10]. The effects of pH on a chiral anion, 2-phenylbutyrate, are discussed below in connection with the studies of influences of hydrophobic, cationic components in the mobile phase.

Effects of 2-propanol

$\beta_{pr}(k')$ is negative for all the enantiomers, indicating that the dominant effect of addition of 2-propanol is a decrease in the retention. This may be due to increased solvation of the enantiomers in the mobile phase, *i.e.*, a process that affects chiral and non-chiral binding to the same extent and is without effect on the stereoselectivity. 2-Propanol can also block the binding at uncharged sites on the solid phase and as $\beta_{pr}(\alpha)$ is negative for all the solutes it seems that blocking of a stereoselective uncharged site is one of the effects of 2-propanol.

Effect of octanoate

The $\beta_{oc}(k')$-values are fairly small in most instances. Positive effects are obtained for k'_2 of atropine and 4HPE whereas negative effects are found for k'_2 of homatropine and for k'_1 and k'_2 for metoprolol and 2HPE. This indicates that octanoate affects different sites or retention processes for the two enantiomers of atropine, homatropine and 4HPE whereas the same kind of site dominates the retention of both enantiomers of metoprolol and 2HPE.

$\beta_{oc}(\alpha)$ is positive for atropine and 4HPE and negative for the other solutes. If both $\beta_{oc}(k')$ and $\beta_{oc}(\alpha)$ are taken into account, it is obvious that octanoate influences stereoselective processes for all the cations.

The positive effects of octanoate on k'_2 are obviously due to an ion-pair binding process in accordance with eqn. 2. Octanoate can compete for the binding to uncharged sites as an ion pair with mobile phase cations or, at least at pH 6.0, in uncharged form.

The combined effects were generally smaller than the individual effects and hence more uncertain. However, the combined effects support the conclusions above.

Influence of anionic buffers

Not only hydrophobic anions such as octanoate but also the highly hydrophilic anions in the buffer can affect retention and stereoselectivity, as shown in Table II. This effect may be due to ion-pair binding to the uncharged site. However, it is also possible that the difference between phosphate and acetate is due to influences from anion-exchange sites as they can give rise to negative charges on the solid phase on binding of HPO_4^{2-} whereas the binding of acetate gives a neutral product. The difference between the buffers is maintained in the presence of a hydrophobic additive, which shows that all the anions participate in the retention process.

The buffer anions can give rise to major effects: homatropine has a high separation factor when the mobile phase contains (*S*)-2-phenylbutyric acid in phosphate buffer but the stereoselectivity is lost when the buffering is made with acetate. The retention decreases on increase in the buffer concentration. It might be due to competing effects in an ion-pair binding process but the increase in $[Na^+]$ has the same kind of effect on the cation-exchange site.

The system peaks achieved by use of the principle of indirect detection technique can give information of the retention process. The presence of a system peak shows the existence of an interaction between the solute and mobile phase components. The direction of the system peaks shows the type of interaction, *e.g.*, competitive or synergistic, and the number of system peaks indicates the number of mobile phase additives retained on the solid phase and interacting with the solute. Fig. 1 shows a separation of the enantiomers of homatropine using a mobile phase containing the detectable (*S*)-2-phenylbutyrate in phosphate buffer. The two negative system peaks show that the interaction is synergistic, *i.e.*, ion-pair retention, and that two mobile phase ions, the detectable (*S*)-2-phenylbutyrate and the buffer anion, are involved in the retention process.

The participation of all the anions in the retention process is further demonstrated by the retention of one of the system peaks being altered when phosphate is exchanged for acetate as buffering component. With racemic 2-phenylbutyrate as additive three system peaks appear as (*S*)- and (*R*)-2-phenylbutyrate have different retentions.

Retention effects of cationic additives

Mobile phase cations can affect retention both by interaction at a cation-exchanging site and as a counter ion in ion-pair interactions at an uncharged site. Two studies each with two variables, pH + tetrabutylammonium (TBA) and pH + dimethyloctylamine (DMOA), respectively, are presented in Table III.

For the anionic 2-phenylbutyrate the increase in pH gives a decrease in k' and an increase in the stereoselectivity. The considerable decrease in the retention with pH shows that the process involves

TABLE II

INFLUENCE OF BUFFERING ANIONS AND IONIC STRENGTH ON RETENTION AND STEREOSELECTIVITY FOR ATROPINE AND HOMATROPINE

Mobile phase: 0.5 m*M* additive and 1% 2-propanol in buffer of pH 6.0.

Buffer	Ionic strength	Additive[a]	Atropine		Homatropine	
			k'_1	α	k'_1	α
Acetate	0.05	–	2.1	1.2	1.9	1.4
Phosphate	0.05	–	2.8[b]	–	2.7	1.3
Acetate	0.05	(*S*)-2-phBA	2.7	3.9	4.3	1.0
Phosphate	0.05	(*S*)-2-phBA	3.2	3.0	3.0	1.4
Phosphate	0.05	2-phBA (racemic)	3.2	3.2	3.2	1.5
Phosphate	0.10	2-phBA (racemic)	2.6	3.4	2.4	1.6

[a] phBA = Phenylbutyric acid.
[b] Only (–)-atropine injected.

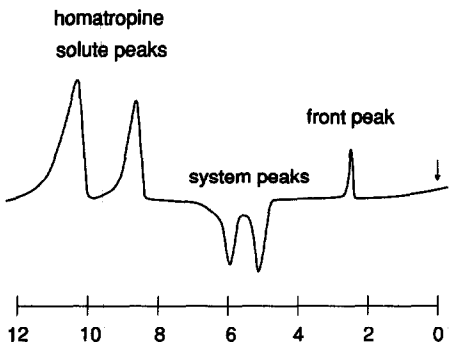

Fig. 1. Resolution of homatropine enantiomers. Mobile phase: 0.5 m*M* (*S*)-2-phenylbutyric acid + 1% 2-propanol in phosphate buffer (pH 6.0). Detection wavelength: 220 nm.

anion exchange or/and binding of 2-phenylbutyrate as an acid. The fact that k' and α change in opposite directions implies that pH influences the binding to a site with low or no stereoselectivity. It was further found that addition of 2-propanol gave an increase in α in addition to a decrease in k'. As 2-propanol mainly decreases the binding to uncharged sites, this means that it is the binding of 2-phenylbutyrate as acid that has no or low stereoselectivity.

The effects of TBA and DMOA on 2-phenylbutyrate are different. TBA gives a decrease in k' with a minor change in α. DMOA increases k' of the second-eluted enantiomer, which indicates ion-pair binding to an uncharged site. The k' value of the first-eluted enantiomer is almost unaffected and there is an increase in the stereoselectivity, which is particularly strong at pH 7.5. The substantially different effects of DMOA on the two enantiomers of 2-phenylbutyrate imply different retention processes. One of them is obviously an ion-pairing process. The different influences of DMOA and TBA might imply that ion pairs of quaternary ammonium ions are too polar for an interaction with the uncharged chiral sites (*cf.*, Table V).

The retention increases with increasing pH for the two cations, homatropine and 2HPE (Table III). The simultaneous increase in α shows that the cationic solutes are retained by a stereoselective cation-exchanging site, which is in agreement with the results presented in Table I.

The aprotic TBA and the basic DMOA have slightly different effects on the cationic enantiomers but k' decreases in accordance with the relationships for retention by ion-exchange (eqn. 1) or ion-pair binding (eqn. 2). There is no significant change in the stereoselectivity for homatropine but a decrease in α for 2HPE.

Influence of enantiomeric and isomeric ionic additives

The effect of the counter ions is dependent on their configuration, as shown in Table IV. This implies binding to an uncharged site with chiral character.

It is of special interest that atropine and its quaternized derivatives interact differently with chiral counter ions, as shown in Table V. (*S*)-2-Phenylbutyrate does not give rise to stereoselective separation of methylatropine and isopropylatropine,

TABLE III

EFFECT OF CATIONIC MOBILE PHASE ADDITIVES ON RETENTION AND STEREOSELECTIVITY

Mobile phase: 3 m*M* additive and 1% 2-propanol in phosphate buffer.

Solute	Parameter	Variable					
		pH 6.0			pH 7.5		
		–	TBA	DMOA	–	TBA	DMOA
2-Phenylbutyrate	k'_1	1.28	0.62	1.34	0.15	0.12	0.15
	α	1.52	1.59	1.97	1.97	1.84	5.70
2HPE	k'_1	2.42	1.13	1.56	11.6	3.24	5.70
	α	1.80	1.62	1.79	2.58	1.87	2.28
Homatropine	k'_1	3.14	1.52	2.04	16.2	4.66	8.30
	α	1.24	1.24	1.27	1.41	1.40	1.48

TABLE IV

EFFECT OF COUNTER ION ON RETENTION AND STEREOSELECTIVITY

Mobile phase: 0.5 m*M* additive and 1% 2-propanol in phosphate buffer of pH 6.0.

Additive[a]	Atropine		Homatropine	
	k'_1	α	k'_1	α
–	2.24	1.00	1.97	1.34
(*S*)-2-phBA	2.71	3.34	2.46	1.58
(*R*)-2-phBA	1.81	1.57	1.94	1.08
(*S*)-3-phBA	3.27	1.38	3.24	1.05
(*R*)-3-phBA	3.17	1.15	2.98	1.16
4-phBA	2.24	1.16	2.28	1.17

[a] phBA = Phenylbutyric acid.

only a competitive interaction. As shown above, ion-pair binding is necessary for the chiral separation of atropine and it might be that ion pairs of quaternary ammonium ions are too polar for an interaction with the uncharged chiral site. Homatropine, which has its main stereoselective interaction with the cation-exchanging site, is easily separated in quaternized form.

Even counter ions with small structural differences can give different effects, as shown in two examples giving the retention as a function of the

TABLE V

RETENTION AND STEREOSELECTIVITY OF ATROPINE, HOMATROPINE AND QUATERNIZED DERIVATIVES WITH AND WITHOUT (*S*)-2-PHENYLBUTYRATE AS MOBILE PHASE ADDITIVE

Mobile phase: 0.5 m*M* additive and 1% 2-propanol in phosphate buffer of pH 6.0. Performed on a Chiral-AGP column with a different batch number to that used in Table IV.

Solute	Additive			
	–		(*S*)-2-Phenylbutyrate	
	k'_1	α	k'_1	α
Atopine	2.5	1.0	2.7	3.2
Methylatropine	2.9	1.0	1.8	1.0
Ipratropine	4.6	1.0	3.1	1.0
Homatropine	2.7	1.3	2.5	1.6
Methylhomatropine	2.8	1.7	–	–

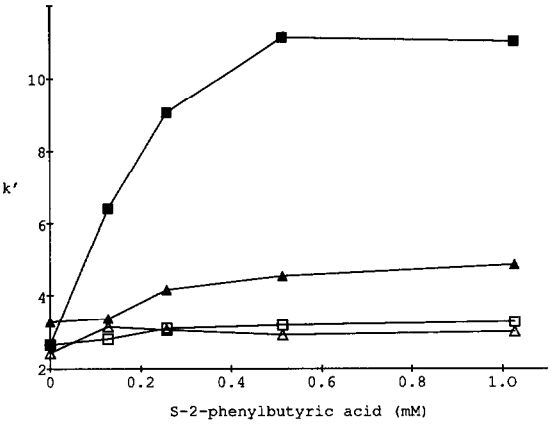

Fig. 2. Influence of (*S*)-2-phenylbutyric acid on capacity factors. Mobile phase: (*S*)-2-phenylbutyric acid + 1% 2-propanol in phosphate buffer (pH 6.0). Detection wavelength: 220 nm. □ = Atropine; △ = homatropine. Open symbols = k'_1; filled symbols = k'_2.

counter ion concentration. (*S*)-2-Phenylbutyrate (Fig. 2) gives with increasing concentration a retention increase for the second-eluted enantiomers that is typical of an ion-pair binding process, whereas the first-eluted enantiomers are almost unaffected. This indicates different retention processes for the two enantiomers, in accordance with the results obtained with octanoate as counter ion. The assumption of a synergistic process is supported by the chromatogram in Fig. 1. The system peaks are negative, which is one of the characteristics of an ion-pair distribution.

4-Phenylbutyrate gives a different pattern: the retention decreases for all the enantiomers, as is illustrated in Fig. 3. This might be due to competition at the uncharged site by an ion pair between the additive and a mobile phase cation. The chromatogram in Fig. 4 supports this view. The positive system peak before the solute peak is typical of a competitive interaction between solutes and an additive.

Chiral cations as additives can give large improvements in the stereoselectivity for cationic solutes. The effect of (–)-atropine is demonstrated in Table VI. The retention of all the cationic solutes decreases but the enantiomers are affected to a different extent as they are probably retained by different retention processes. This gives rise to a considerable improvement in the chiral separation of ipratropine, a quaternized atropine, whereas the

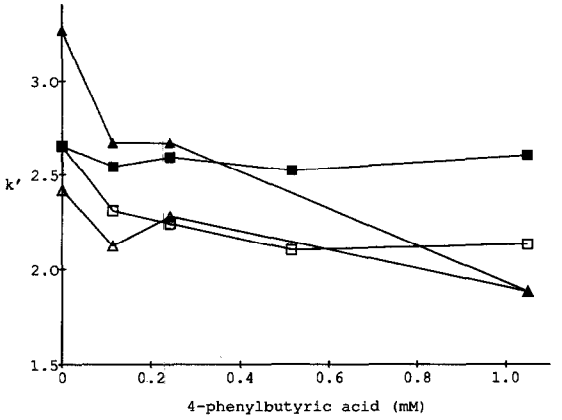

Fig. 3. Influence of 4-phenylbutyric acid on capacity factors. Mobile phase: 4-phenylbutyric acid + 1% 2-propanol in phosphate buffer (pH 6.0). Detection wavelength: 220 nm. Symbols as in Fig. 2.

effect on homatropine is the opposite, *i.e.*, a decrease in α. The retention of the (+)-atropine is also decreased when the (−)-antipode is present in the mobile phase.

The character of the site for the interaction of (−)-atropine cannot be elucidated from the results in Table VI, but some indications are given by studies with the enantiomers of uncharged methyl mandelate as mobile phase additive. These enantiomers, which have the same groups at the chiral carbon as homatropine, are easily separated on the AGP phase with $\alpha = 1.5$. As mobile phase additives they give rise to a decrease in the retention of

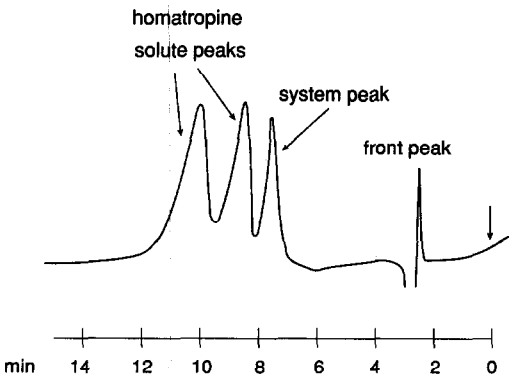

Fig. 4. Resolution of homatropine enantiomers. Mobile phase: 0.5 m*M* 4-phenylbutyric acid + 1% 2-propanol in phosphate buffer (pH 6.0). Detection wavelength: 220 nm.

TABLE VI

EFFECT OF RETENTION AND STEREOSELECTIVITY USING (−)-ATROPINE AS MOBILE PHASE ADDITIVE

Mobile phase: additive and 1% 2-propanol in phosphate buffer of pH 6.0.

Solute	Additive			
	−		(−)-Atropine (0.55 m*M*)	
	k'_1	α	k'_1	α
Ipratropine	4.6	1.0	2.4	1.5
Homatropine	2.7	1.3	2.0	1.3
Atropine (racemic)	2.8	1.0	1.6[a]	1.3

[a] System peak.

homatropine, atropine and their quaternized derivatives, but no chiral separation for atropine and its derivative is achieved and only a decrease in α for the other solutes. It seems that an additive with a competitive effect must interact at a charged chiral site to give rise to improvements in the stereoselectivity for this group of compounds.

Indirect detection of enantiomers

A detectable mobile phase additive can give rise to indirect detection effects that provide information about the retention processes. Fig. 5 shows the effects on cations in a system with the counter ion, (*S*)-2-phenylbutyrate, as detectable component. The

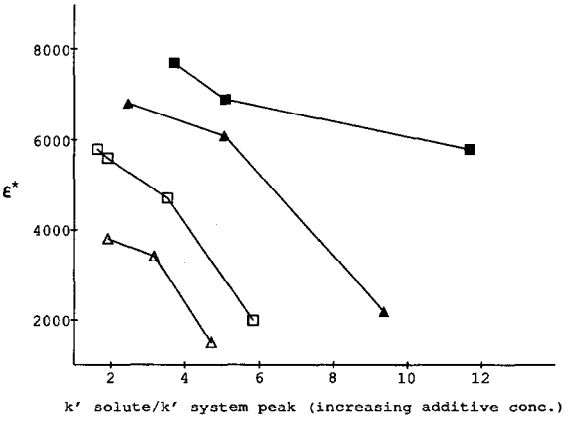

Fig. 5. Effects of (*S*)-2-phenylbutyrate on the apparent molar absorptivity for atropine and homatropine. Symbols, mobile phase and detection wavelength as in Fig. 2.

apparent molar absorptivity, ε^*, is calculated from the peak area and the amount of solute injected [11]. It is given as a function of the increase in relative retention, $k'_{\text{enantiomer}}/k'_{\text{main system peak}}$ occurring with an increase in the counter ion concentration.

Fig. 5 shows that ε^* decreases with increasing counter ion concentration, which is in accordance with the theory of ion-pairing systems with an adsorbing stationary phase [5]. However, on the AGP-phase the last-eluted enantiomer has a significantly higher ε^* than the first-eluted enantiomer. This is an important deviation from the effects appearing in a system with an adsorbing stationary phase, where the component eluted closer to the system peak is more influenced by interaction effects and has a higher ε^*. The results in Fig. 5 give further support to the view that the enantiomers of a solute are bound by different processes, *e.g.*, to sites with different fractional loadings of an anionic additive.

CONCLUSIONS

The stereoselectivity on silica-bonded AGP is due to ion-exchanging and ion-pairing processes and the effect is highly dependent on the structure of the solute. Small structural differences can alter the dominant chiral interaction, as shown by the solute pairs atropine–homatropine and 2HPE–4HPE. The retention and stereoselectivity are affected by different kinds of ions from hydrophilic buffers to hydrophobic cations and anions. Counter ions to the solute can improve the stereoselectivity by both increasing and decreasing the retention. Enantiomers of charged chiral additives can give stereoselectivity for new classes of compounds, *e.g.*, quaternized atropine derivatives, but their effect is also highly dependent on the configuration.

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