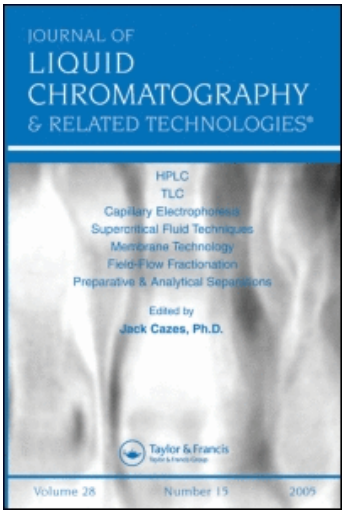

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Chiral Separation of Cationic Drugs on an α_1-Acid Glycoprotein Bonded Stationary Phase

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CHIRAL SEPARATION OF CATIONIC DRUGS ON AN α_1-ACID GLYCOPROTEIN BONDED STATIONARY PHASE

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ABSTRACT

A commercially available chiral stationary phase containing α_1-acid glycoprotein on silica (EnantioPac, LKB) was applied to the resolution of a number of pharmacologically important enantiomeric ammonium compounds. The optimization of retention and selectivity by cationic, anionic and neutral modifiers in the mobile phase was studied. The results suggest that the solutes are retained according to an ion-pair distribution model. Compounds of widely different structures were studied, and high separation factors were achieved for a majority.

INTRODUCTION

In the development and use of drugs that exist as stereoisomers, it is often important to treat each enantiomer as a separate entity, because of the fact that the enantiomers often

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differ in potency, pharmacological action or plasma disposition.

Until recently, the study of the pharmacological differences between enantiomers was hampered by difficult and often inexact analytical methods. This situation has been changed by the introduction of a number of chromatographic chiral stationary phases (CSPs).

One highly promising phase is the α_1-acid glycoprotein (AGP) CSP developed by Hermansson (1,2). This CSP appears to have a wide applicability to molecules of pharmacological interest (2,3), and its use has the added advantages of requiring no precolumn derivatization of the analytes and of allowing the use of aqueous mobile phases that are compatible with biological fluids.

Human AGP has a molecular weight of about 41,000. It is composed of a single 181-unit peptide chain which accounts for approximately 55% of the molecular weight and five carbohydrate units which comprise the rest. Included in these latter moieties are 14 residues of sialic acid. The protein has an isoelectric point of 2.7 in phosphate buffer (4).

Although a great deal is known about the structure of this protein, little is known about how AGP binds cationic molecules. This process appears to involve the sialic acid, to be sensitive to uncharged competitors and to have some stereospecificity. Pike et al. (5) demonstrated that enzymatic desialylation of AGP reduces the binding affinity for cationic drugs, whereas the

binding of neutral and acidic drugs is unaffected. The binding of propranolol and oxprenolol to AGP can also be reduced by noncharged competitors such as tris(2-butoxyethyl)phosphate (5,6). In addition, the stereoselective binding of cationic molecules to unbound AGP has been observed in vitro by using propranolol (7,8).

The AGP-CSP is prepared, according to the manufacturer (9), in the following manner. A monomolecular layer of protein is ionically bound to a diethylaminoethyl silica (about 180 mg/g; 4.4×10^{-6} moles/g). The protein is then oxidized with periodate to form aldehyde moieties and is cross-linked through Schiff base formation. The resulting enamines are reduced to secondary amines with cyanoborohydride.

This process results in a stable, immobilized protein which retains an affinity for cationic molecules. However, unlike the unbound AGP, a number of the carboxylic acid moieties of the bound protein are tied up in the ionic bonds to the silica and, therefore, are unavailable for binding to the cationic site of the solute. Thus, the binding properties of the AGP-CSP should differ from those of the native protein.

This paper presents the results of an investigation of the applicability of this CSP to a broad spectrum of pharmacologically important ammonium compounds. We studied the effect of a number of ionic and uncharged mobile phase additives on capacity and stereoselectivity in order to make the separation system

applicable to compounds of different hydrophobicity. The results indicate that retention is due to an ion-pairing mechanism and that it can be regulated within a wide range. It was possible to achieve better than 95% resolution for a majority of the racemates studied.

MATERIALS AND METHODS

Apparatus

The chromatography was performed with Spectra-Physics (Santa Clara, CA, U.S.A.) Models 8000 and 8700 liquid chromatographs, each equipped with a Spectra-Physics Model 770 variable wavelength UV detector. The temperature of the columns and the mobile phase reservoirs was regulated by using thermostatically controlled circulating water.

The separations were performed on commercially available EnantioPac columns (length 100 mm, i.d. 4.0 mm) containing cross-linked AGP silica (180 mg/g). The columns were generously supplied by LKB (Bromma, Sweden).

Materials

The tocainide and metoprolol analogs were supplied by Haessle (Molndal, Sweden), nadolol A and B were supplied by E.R. Squibb (Princeton, NJ, U.S.A.), and d- and l-cocaine were supplied by the Drug Enforcement Agency (Washington, DC, U.S.A.). All other solutes were obtained from the stores of the U.S. Food and Drug

Administration (Washington, DC, U.S.A.). The structures of some compounds of particular interest are given in Figure 1.

The ionic modifiers used in the study, N,N-dimethyloctyl amine, tetrapropylammonium and tetrabutylammonium bromide and butyric and octanoic acids were from Aldrich (Milwaukee, WI, U.S.A.). The 2-propanol was HPLC grade from Burdick & Jackson (Muskegon, MI, U.S.A.). All other chemicals were reagent grade and used without further purification.

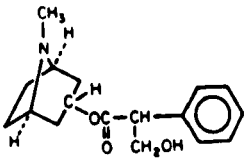
General Chromatographic Conditions

The standard conditions used during the chromatography were a flow rate of 0.30 ml/min and a column and mobile phase temperature of 20.0°C. The mobile phases were 0.02M sodium phosphate buffers to which modifiers were added; the pH was adjusted to the desired level by adding sodium hydroxide or phosphoric acid. A UV absorption maximum of the solute was chosen as the wavelength of detection.

RESULTS AND DISCUSSION

Binding Properties of the Chiral Stationary Phase

Our chromatographic studies of a broad variety of cationic molecules on the AGP-CSP indicate that retention and stereoselectivity are usually dependent on the presence of hydrogen bonding (HB) group(s) and large substituents or cyclic



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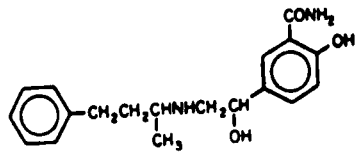

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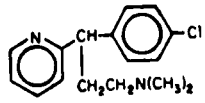

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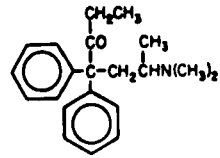

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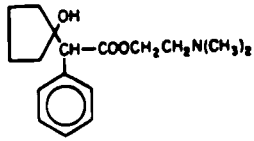

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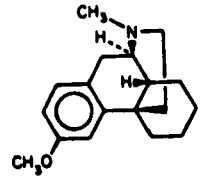
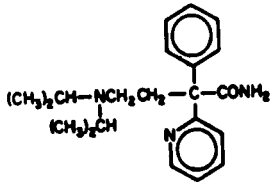


Methorphan



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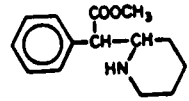

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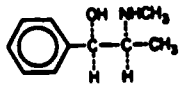
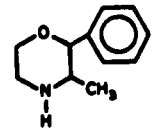
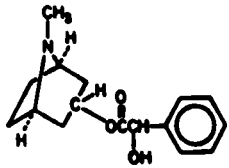


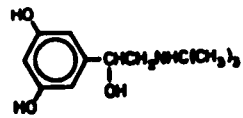
Ephedrine



Phenmetrazine



Homatropine



Terbutaline

FIGURE 1. Structures of some of the compounds used in this study.

TABLE 1

Separation of Enantiomeric Ions

Mobile phase: 0.02M Phosphate Buffer, pH 7.0, + 2-Propanol

H-Bonding Group	Solute	k_1'[a]	α	2-Propanol in Mobile Phase (%)
Alcohol	Labetalol A	8.8	1.25	2
Ester	Propoxyphene	3.4	2.4	8
Ester-alcohol	Cyclopentolate	14.0	1.92	8
Oxo	Methadone	4.8	1.53	8
Amide	Disopyramide	3.4	2.65	8
2-Pyridine	Brompheniramine	13.8	1.05	4
Ether	Bromodiphenhydramine	17.9	1.13	2
Phenothiazine	Promethazine	33.8	1.09	2
Unspecified	Methorphan	13.8	1.15	2

[a]Capacity factor of first eluted enantiomer.

structures at the ammonium ion, N^+, and/or the HB group. The results also suggest that the magnitude of the enantiomeric resolution is affected by the strength of the HB substituent. Some examples covering different kinds of HB groups are given in Table 1.

Previous studies of separation of enantiomeric ions by binding to the HB group and the charged site of a chiral reagent indicate that the distance between these binding sites is of vital importance (10). General relationships of this kind were not found in this study, which includes compounds of widely different structures (Figure 1). However, the importance of

the distance between the HB group and N^+ in certain kinds of molecules is clearly demonstrated in some of the results presented here.

Chromatographic Properties of the Chiral Phase

The capacity factors (k') and separation factors (α) on the AGP-CSP appear to be sensitive to both temperature and the pH of the mobile phase. A decrease in temperature leads to an increase in k' and α. An increase in the pH of the mobile phase has the same effect on k'. However, the magnitude of these changes varies from solute to solute. Some examples are presented in Table 2.

The plate height (H) of a resolved solute is affected by temperature and flow rate. A decrease in temperature results in peak broadening, whereas a decrease in flow rate has the opposite effect (Table 3). The strong effect of mobile phase speed, which is an indication of a slow mass transfer between phases, usually makes a flow rate higher than 0.3 ml/min unsuitable.

Since the molar concentration of the chiral binding agent, AGP, is fairly low, overloading effects can appear at rather low sample concentrations. The influence of solute concentration on retention time, α and peak symmetry is illustrated in Table 4. For methylhomatropine, an 8-fold increase in molar concentration resulted in a decrease in the retention time of about 10% and an increase in the tailing of both peaks. This effect is not pH

TABLE 2

Influence of pH and Temperature on Capacity Factor and Separation Factor

Mobile Phase: 0.1M NaCl in 0.02M Phosphate Buffer + 2-Propanol

Solute	2-Propanol in Mobile Phase (%)	pH	Temp. (°C)	k_1'	α
Cyclopentolate	2	6.5	10	3.9	1.94
			20	4.8	1.79
		7.0	10	10.0	1.96
			20	8.9	1.89
		7.5	10	15.0	2.02
			20	14.0	1.96
Methylhomatropine	0	6.5	10	3.3	2.82
			20	2.4	2.38
		7.0	10	5.5	3.00
			20	4.5	2.47
		7.5	10	5.2	3.10
			20	5.0	2.64

TABLE 3

Influence of Temperature and Flow Speed on Separation Factor and Plate Height[a]

Mobile Phase: 8% 2-Propanol in 0.02M Phosphate Buffer, pH 7.0

Solute	Temp. (°C)	Flow (ml/m)	α	H_1 (mm)	H_2 (mm)
Dimethindene	20	0.3	1.53	0.30	0.23
	5	0.3	1.62	0.40	0.35
	5	0.1	1.64	0.27	0.23
Cyclopentolate	20	0.3	1.41	0.28	0.27
	5	0.3	1.49	0.32	0.30
	5	0.1	1.51	0.19	0.25

[a]H_1 and H_2 = plate height of first and second eluted enantiomer, respectively.

TABLE 4

Influence of Sample Loading on Retention Time, Separation Factor and Peak Symmetry[a]

Mobile Phase: 0.1M NaCl in 0.02M Phosphate Buffer, pH 7.5

Flow: 0.30 ml/min Sample Volume: 20 µl

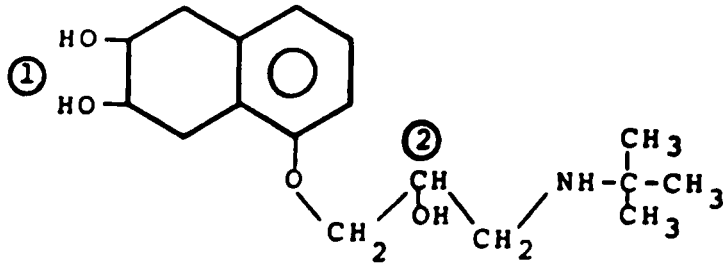
Solute: Methylhomatropine

Solute Concentration ($M \times 10^4$)	Retention Time of Peak 1 (sec)	α	asf_1	asf_2
1.4	1022	2.12	1.2	1.2
2.9	1007	2.11	1.2	1.5
5.8	964	2.11	1.7	2.5
9.7	950	2.07	2.0	2.3
11.6	935	2.06	2.0	2.4

[a]asf_1 and asf_2 = ratio of back half of peak area to front half of peak area (estimated by baseline measurement) for first and second peak, respectively.

dependent and does not significantly affect the enantiomeric selectivity. However, when optimal separation is needed, it is hardly advisable to use an amount of solute greater than 2-3 nmol.

The symmetry of the peaks corresponding to the resolved enantiomers of a solute is normally good with an asymmetry factor in the range of 0.9-1.2, as demonstrated by the chromatograms of nadolol A and B in Figure 2. However, some exceptions were observed, and the most striking of these are illustrated in Table 5. In the case of disopyramide, the first peak has a leading edge. This asymmetry can be partially corrected by raising the



Nadolol A (R,S ; S,R) Nadolol B (R,R ; S,S)

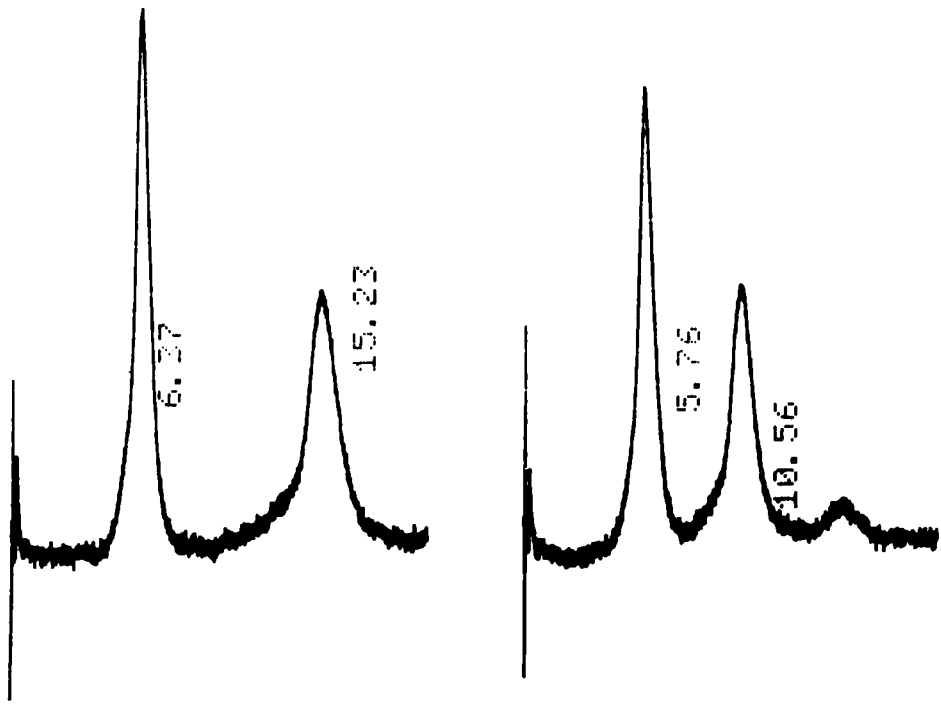


FIGURE 2. Resolution of nadolol enantiomers. Mobile phase: 0.001M tetrabutylammonium bromide in 0.02M phosphate buffer, pH 6.0.

TABLE 5

Peak Symmetry Deviations

Mobile Phase: 0.1M NaCl in 0.02M Phosphate Buffer + 8% 2-Propanol

Loading: 2 nmol

Solute	pH	k_1'	α	asf_1	asf_2
Disopyramide	6.5	1.9	2.77	0.3	1.0
	7.0	2.7	2.70	0.6	1.1
	7.5	3.3	2.67	0.6	1.1
Propoxyphene	6.5	3.2	1.52	1.2	0.8
	7.0	4.4	2.3	0.9	8.0
	7.5	5.1	2.4	1.0	--[a]
Methadone	6.5	5.0	1.51	0.9	2.0
	7.0	6.5	1.59	1.0	1.7
	7.5	7.4	1.57	1.5	2.0

[a]Unmeasurable.

pH. For propoxyphene, there is extreme tailing in the second peak at pH 7.0 and 7.5. However, this peak is almost symmetrical at pH 6.5. The first methadone peak has good symmetry, whereas the second displays significant tailing. This phenomenon is unaffected by a change in pH.

Regulation of Capacity Factor by Mobile Phase Modifiers

The use of the AGP-CSP with a mobile phase of phosphate buffer (0.02M, pH 7.0) with or without sodium chloride often results in an unacceptably high k' of 10 or greater, even for

moderately hydrophobic solutes. As a general rule, k' appears to depend on the number of carbon atoms (aliphatic or aromatic) and the nature and number of hydrophilic groups. For molecules which contain an alcohol function, k' often exceeds 10 when the number of carbon atoms is 15 or more. For molecules containing esters and alcohols, the critical number of carbons can be as high as 17 or 18, whereas for others containing a carbonyl function (an amide, for example) or an ether moiety, the limiting number of carbons appears to be in the range of 11-13.

Three classes of mobile phase modifiers -- uncharged, anionic and cationic -- were studied for their ability to optimize k'. The modifiers used in detailed studies were the following:

A. Uncharged -- 2-propanol;

B. Cationic -- tetrapropylammonium and tetrabutylammonium ions as bromides;

C. Anionic -- butyric and octanoic acids.

The effect of these modifiers on α and k' is presented in Table 6.

The results demonstrate that k' is affected not only by uncharged and cationic modifiers, but by anionic modifiers as well. This indicates that retention is not a function of binding to anionic sites on the AGP, since anionic modifiers have no influence on this process. Furthermore, the binding of the AGP to the silica support ties up anionic binding sites, thereby reducing the probability that they are involved in solute binding.

TABLE 6

Influence of Modifier on Retention and Chiral Selectivity for Several Solutes

Mobile Phase: Modifier in 0.02M Phosphate Buffer

Modifier	Atropine		Ephedrine		Labetalol A		Methorphan		Methylhomatropine		Metoprolol		Phenmetrazine	
	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α
NaCl														
0.1M, pH 7.0	2.8	1.10	1.4	1.24	--	--	--	--	4.7	2.65	6.1	1.64	8.3	1.0
2-Propanol														
0.33M, pH 7.0	2.7	1.0	--	--	9.7	1.19	14	1.15	1.0	2.42	--	--	--	--
TPrABr[a]														
0.003M, pH 7.0	4.0	1.0	0.8	1.15	--	--	--	--	2.1	4.17	2.4	1.44	2.5	1.25
TBuABr[b]														
0.003M, pH 6.0	0.9	1.0	0.2	1.0	7.5	1.46	5.2	2.69	0.6	3.10	0.5	1.0	0.6	1.0
Butyric Acid														
0.05M, pH 7.0	5.1	1.23	0.9	1.83	23	1.69	--	--	2.2	2.49	2.5	1.48	3.0	1.42
Octanoic Acid														
0.01M, pH 7.0	6.3	1.64	0.9	1.71	11.8	1.35	--	--	1.4	1.27	1.7	1.0	2.9	1.57

[a]TPrABr = tetrapropylammonium bromide.

[b]TBuABr = tetrabutylammonium bromide.

The retention seems to be based on an ion-pairing mechanism in which the counter ion has a synergistic effect on the binding to the CSP and competition exists for the same binding sites from other ion-pairing agents in the mobile phase. If it is assumed that there is only one kind of binding site, the following equation, Eqn. 1, can be used to describe this phenomenon (11):

$$k'_{HA} = \frac{q \times K^{o} \times K_{HAX} \times [X^{-}]}{1 + (K_{QX} \times [Q^{+}] \times [X^{-}])} \qquad (1)$$

where HA is the solute, X^{-} and Q^{+} are the mobile phase ions (modifiers), q is the phase ratio in the column, K^{o} is the binding capacity of the solid phase, and K_{QX} and K_{HAX} are the distribution constants of the ion pairs.

This equation can be rearranged to Eqn. 2:

$$\frac{[X^{-}]}{k'_{HA}} = \frac{1}{q \times K^{o} \times K_{HAX}} + \frac{K_{QX} \times [Q^{+}] \times [X^{-}]}{q \times K^{o} \times K_{HAX}} \qquad (2)$$

A plot in accordance with this equation for the two enantiomers of metoprolol is given in Figure 3. The linearity of the plot supports the assumption that the retention is the result of an ion-pair binding process. Further support is given by the fact that the quotient between slope and intercept, which is equal to K_{QX}, is the same for the two lines.

The retention model in Eqn. 1 can also be applied to anionic modifiers. Figure 4 shows plots in accordance with Eqn. 2 for

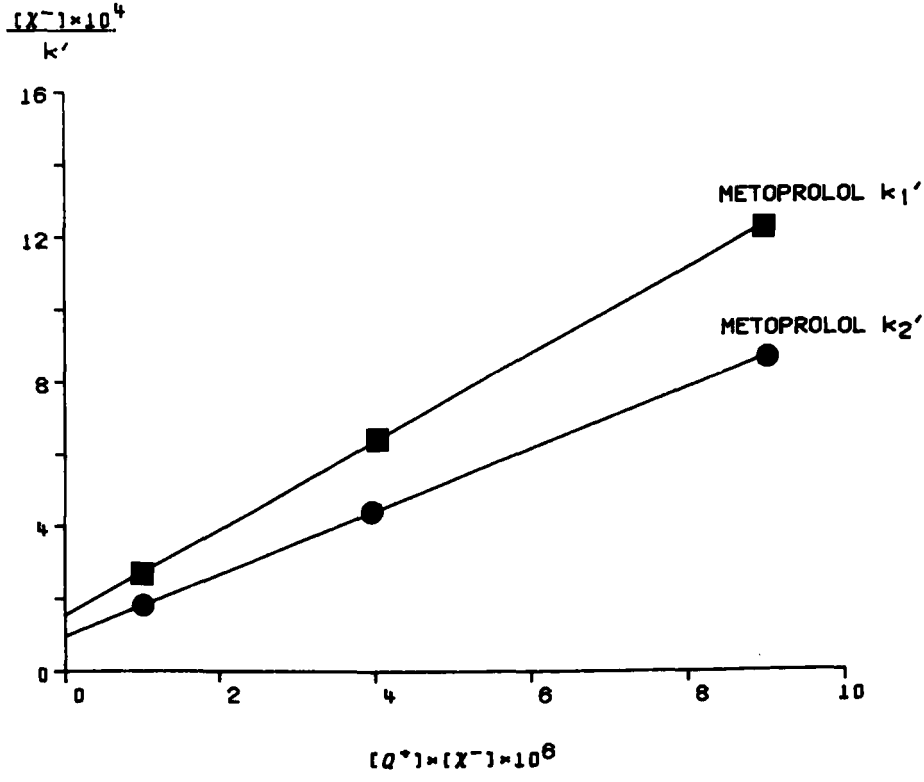


FIGURE 3. Application of metoprolol retention to the ion-pair distribution model. Mobile phase: tetrapropylammonium bromide in 0.02M phosphate buffer, pH 7.0.

atropine, homatropine and other cations in a system with octanoate as the modifier ($[X^-]$ in Eqn. 2) and sodium as the mobile phase cation ($[Q^+]$ in Eqn. 2).

The good linearity of these plots indicates the validity of this model. It also supports the assumption that, in the concentration ranges used in this study, retention is dominated by binding to one type of site.

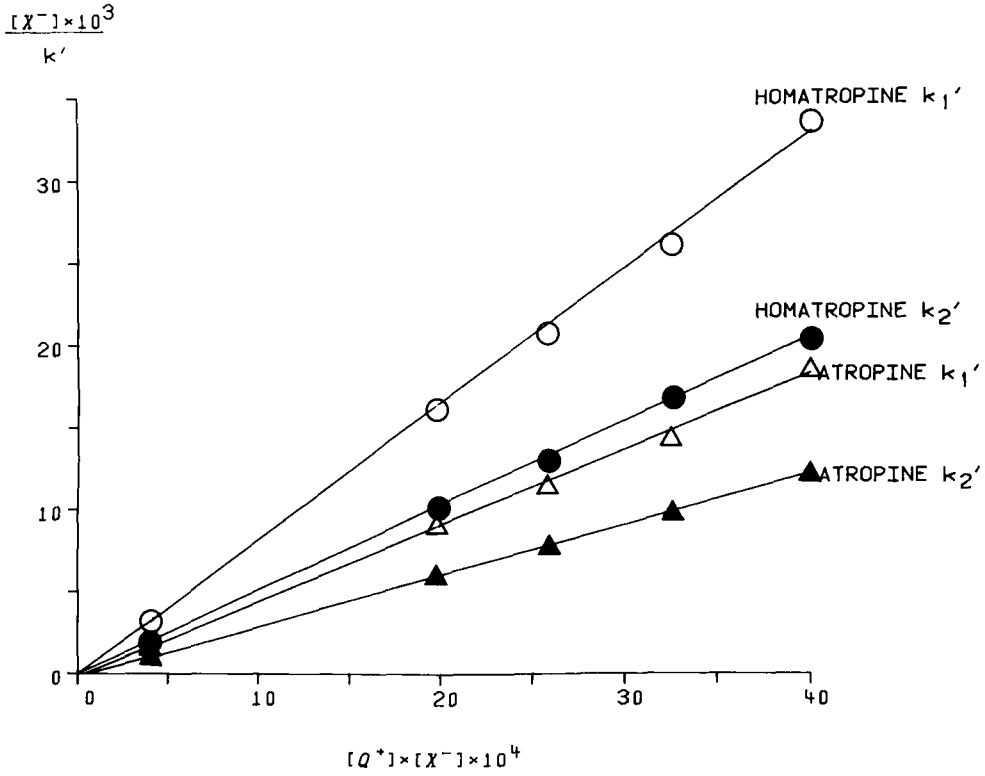


FIGURE 4. Application of atropine and homatropine retention to the ion-pair distribution model. Mobile phase: octanoic acid in 0.02M phosphate buffer, pH 7.0.

The assumption of an ion-pair distribution process is further supported by the fact that anionic racemates can be retained and resolved. This is illustrated by the resolution of 2-phenylbutyric acid, which is presented in Figure 5.

The Effect of Mobile Phase Additives on Selectivity

Some examples of the effect of mobile phase modifiers on stereoselectivity are illustrated in Table 6. Compared to the

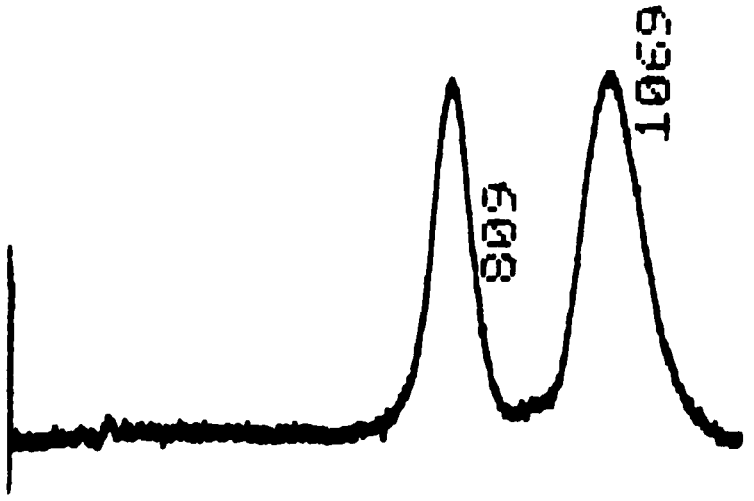


FIGURE 5. Resolution of enantiomers of 2-phenylbutyric acid. Mobile phase: 0.10M 4-hydroxybutyric acid in 0.02M phosphate buffer, pH 7.0.

selectivity obtained with a phosphate-sodium chloride mobile phase, the effects of the uncharged, anionic and cationic modifiers vary from dramatic improvement to total loss of stereo selectivity. The addition of an uncharged modifier, 2-propanol, uniformly resulted in a decrease in selectivity for the compounds used in this study, whereas the effect of the charged modifiers varied.

The addition of charged modifiers can sometimes lead to dramatic improvements in stereoselectivity. For example, the addition of tetrabutylammonium bromide increases the separation factor for methorphan from 1.15 (in the presence of 2-propanol) to 2.96; for methylhomatropine, α increases to a value greater than 4.0 in the presence of tetrapropylammonium bromide.

Dramatic increases also occur after the addition of anionic modifiers. The separation factor for ephedrine is 1.83 with butyric acid in the mobile phase and 1.61 when octanoic acid is used. Particularly significant is the resolution of atropine (α = 1.64) obtained with octanoic acid in the mobile phase.

The drastic effects of the carboxylate modifiers, particularly octanoic acid, on the selectivity might be due to the fairly low polarity of the ion pair between the cationic solute and the carboxylate counter ion, arising from hydrogen bonding between the ions (12). This change in polarity may promote stereoselectivity through an alteration in the binding properties of the solute.

To better understand the nature of the chiral binding site, we studied the relationship between selectivity and concentration of the charged modifiers. If the enantiomers are retained by a single type of site in accordance with Eqn. 1, a change in modifier concentration should not change the stereoselectivity. On the other hand, if the retaining phase has several kinds of sites with chiral and nonchiral binding properties, a change in the concentration of a modifier should result in a change in selectivity, if the modifier is used in appropriate concentrations. Some examples of such changes are presented in Table 7.

The results indicate that the assumption of one type of binding site is valid when cationic modifiers are used. However, when octanoic acid is used as a modifier, an increase in

TABLE 7

Influence of Modifier on Separation Factor

Mobile Phase: Modifier in 0.02M Phosphate Buffer

Solute	Octanoic Acid, pH 7.0				
	0.010M	0.032M	0.038M	0.044M	0.050M
Atropine	1.64	1.50	1.49	1.45	1.51
Cyclopentolate	1.82	1.54	1.52	1.45	1.50
Homatropine	1.63	1.60	1.59	1.53	1.63
Labetalol A	1.35	1.17	1.16	1.12	1.14
Methylphenidate	1.89	1.98	1.91	1.91	2.05

Solute	Tetrapropylammonium Bromide, pH 7.0		
	0.001M	0.002M	0.003M
Metoprolol	1.49	1.44	1.41
Phenmetrazine	1.29	1.30	1.25
Tocainide	1.30	1.28	1.30

selectivity occurs at the lowest concentration in some cases. This could be due to the existence of several kinds of binding sites or to an excess of solute in relation to the concentration of complexing agent (counter ion) present.

Structural Effects on Selectivity

The stereoselectivity of the CSP appears to be highly sensitive to structural differences in the solute. Alterations in the distance between the N^+ and HB moieties, in the substitution at N^+ and in the spatial relationship between N^+ and the HB group often result in dramatic changes in selectivity.

The change in selectivity within a series of compounds related to metoprolol illustrates this effect (Table 8). When the number of alkyl carbons in the chain between N^+ and the HB group is increased from 1 to 3, α is reduced from 1.49 to 1.00. A reduction in the steric bulk at N^+ has the same effect. There is a steady decline in α (1.73 to 1.00) from a t-butyl substituent to a methyl substituent. However, the primary amine is resolved with an α of 1.17.

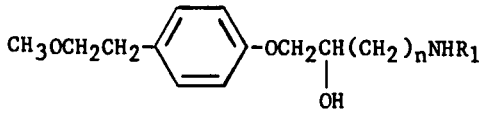
The sensitivity of stereoselectivity to the bulk at N^+ is also illustrated by the difference in resolution between terbutaline and metaproterenol. Terbutaline and metaproterenol differ only in the substitution at the N^+ site, i.e., t-butyl and isopropyl moieties, respectively. When chromatographed on the AGP-CSP with 0.003M tetrapropylammonium bromide as the modifier, terbutaline is resolved with a separation factor of 1.22, whereas metaproterenol is unresolved.

A dramatic change in selectivity was also observed for a series of substances related to tocainide (Table 9). The total loss of resolution following the removal of one or both of the ortho-methyl groups and the effect on selectivity following subtle changes in the alkyl substituents indicate that chiral recognition is extremely sensitive to structural variations in the molecules. Lengthening the alkyl chain between the amide group and N^+ increases the stereoselectivity as does increasing the bulk of the alkyl group at the asymmetric carbon atom.

TABLE 8

Separation of Enantiomeric Compounds
Related to Metoprolol[a]

Mobile Phase: 0.001M Tetrapropylammonium Bromide in
0.02M Phosphate Buffer, pH 7.0



R_1	n	α
i-propyl	1	1.49
i-propyl	2	1.14
i-propyl	3	1.0
t-butyl	1	1.73
n-propyl	1	1.23
ethyl	1	1.12
methyl	1	1.0
H	1	1.17

[a]Metoprolol is the first compound shown.

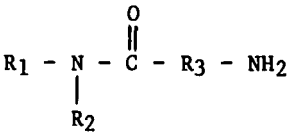
However, no chiral resolution is obtained when the asymmetric carbon atom is in a beta-position relative to the amide.

Differences in stereoselectivity also appear between diastereomers. The R,S;S,R enantiomers of some α,ß-aminoalcohols, e.g., labetalol A and ephedrine, display a higher selectivity than the corresponding R,R;S,S enantiomers, labetalol B and pseudoephedrine. Nadolol displays the same tendency when chromatographed with 0.001M tetrabutylammonium bromide as the modifier (Figure 2). However, when 0.025M butyric

TABLE 9

Separation of Enantiomeric Compounds Related to Tocainide[a]

Mobile Phase: 0.002M Tetrabutylammonium Bromide in 0.02M Phosphate Buffer, pH 6.0



R_1	R_2	R_3	α
2,6-xylyl	H	$CH(CH_3)$	1.45
2,4,6-mesityl	H	$CH(CH_3)$	1.24
2-tolyl	H	$CH(CH_3)$	1.0
phenyl	H	$CH(CH_3)$	1.0
benzyl	H	$CH(CH_3)$	1.0
2,6-xylyl	H	$CH(C_2H_5)$	3.31
2,6-xylyl	H	$CH(CH_3)CH_2$	2.30
2,6-xylyl	H	$CH_2CH(CH_3)$	1.0
2,6-xylyl	CH_3	$CH(CH_3)$	1.0

[a]Tocainide is the first compound shown.

acid is used as the modifier, the R,R;S,S enantiomeric pair, nadolol B, has a higher selectivity, i.e., 2.30, compared to 2.16 for nadolol A.

CONCLUSION

This study shows that retention and stereoselectivity of the AGP bonded on silica are based on an ion-pairing mechanism. Retention and selectivity can be altered through the addition of

TABLE 10

Highest Separation Factors with a Selection of Mobile Phases

Mobile Phase: Modifier in 0.02M Phosphate Buffer

Solute	α	Modifier[a]	Solute	α	Modifier[a]
Atropine	1.64	8	Mepivicaine	1.25	3
Bromodiphenhydramine	1.17	5	Methadone	1.59	6
Brompheniramine	1.50	14	Methorphan	2.54	12
Bupivicaine	1.41	3	Methylatropine	1.27	9
Butorphanol[b]	1.99	2	Methylhomatropine	4.2	11
Carbinoxamine	1.33	16	Methylphenidate[b]	1.70	14
Chlorpheniramine	2.26	14	Metoprolol	1.64	4
Clidinium	1.21	1	Nadolol A	3.98	12
Cocaine[b]	1.46	15	Nadolol B	3.03	12
Cyclopentolate	3.86	11	Oxyphencyclimine	1.42	6
Dimethindene	1.53	6	Oxprenolol	1.25	5
Diperodone	1.47	17	Phenmetrazine[b]	1.57	8
Disopyramide	2.70	6	Phenoxybenzamine	1.37	16
Doxylamine	1.37	13	Promethazine	1.25	5
Ephedrine	1.83	7	Pronethalol	1.26	16
Ephedrine, pseudo-	1.34	7	Propoxyphene[b]	2.3	6

Homatropine	1.63	8	Propranolol	1.13	17
Labetalol A	2.10	14	Terbutaline	1.22	11
Labetalol B	1.36	7	Tocainide	1.44	10
Mepensolate	1.32	5	Tridihexethyl	1.64	17

[a]Modifiers: (H_3PO_4 or NaOH added to give the indicated pH)
1 = 0.33M 2-propanol, pH 7.0;
2 = 0.67M 2-propanol, pH 7.0;
3 = 1.33M 2-propanol, pH 7.0;
4 = 0.1M NaCl, pH 7.0;
5 = 0.1M NaCl + 1.74M ethanol, pH 7.0;
6 = 0.1M NaCl + 1.33M 2-propanol, pH 7.0;
7 = 0.05M butyric acid, pH 7.0;
8 = 0.01M octanoic acid, pH 7.0;
9 = 0.25M cyclohexylsulfamic acid, pH 7.0;
10 = 0.001M tetrapropylammonium bromide (TPrABr), pH 6.0;
11 = 0.003M TPrABr, pH 7.0;
12 = 0.001M tetrabutylammonium bromide (TBuABr), pH 6.0;
13 = 0.003M TBuABr, pH 6.0;
14 = 0.003M TBuABr, pH 7.0;
15 = 0.001M dimethyloctylamine (DMOA), pH 7.0;
16 = 0.001M DMOA + 0.17M 2-propanol, pH 7.0;
17 = 0.002M DMOA + 0.33M 2-propanol, pH 7.0.

[b]One of the diastereomers (for which there are no generally accepted names).

uncharged and charged mobile phase additives, leading to a wide applicability of the AGP-CSP. The breadth of these applications is presented in Table 10, which lists the best separation factors obtained at 20.0°C with a selection of mobile phases.

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