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Optimization of the separation of enantiomers of basic drugs Retention mechanisms and dynamic modification of the chiral bonding properties on an α_1-acid glycoprotein column

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Abstract

The chromatographic properties of 29 basic drugs were studied by varying the pH and the concentration of inorganic ions in the mobile phase. It was observed that the chromatographic performance of most hydrophobic basic drug compounds could be strongly enhanced by decreasing the pH in the mobile phase from 7 to 4–6. The enantioselectivity increased and a much faster resolution was obtained. The results indicate that ion exchange and ion-pair distribution may be involved in the retention process of cationic drug enantiomers. Increasing the concentration of acetate and phosphate increases the retention of the enantiomers of the drug compounds. The relative contribution of the two retention processes can be affected by the pH and the nature and the concentration of the ions in the mobile phase. Decreasing the pH reduces the influence of the ion-exchange process since the negative charge of the protein is decreased. The enantioselectivity is also greatly affected by increasing salt concentration.

1. Introduction

In many publications it has been demonstrated that α_1-acid glycoprotein (AGP) has the ability to achieve stereoselective binding of enantiomers of widely different character [1–10]. Drug compounds can be bound to the binding domain of the protein by interaction with uncharged or charged groups or a combination of both types of interactions. An interesting property of immobilized α_1-acid glycoprotein (AGP) is that the character of this chiral selector can be changed by a simple change of the mobile phase composition, such as the nature and the concentration of uncharged modifier or by changing the pH. By such changes drastic effects can be obtained on the enantioselectivity and the retention [4,6]. The fact that the enantioselectivity can be induced by simple changes of the mobile phase composition is one of the reasons for the extremely broad applicability of the AGP column. By changing the pH of the mobile phase the degree of charge of the amino acids, containing free acidic or basic groups, are affected, which can, reversibly, influence the conformation of the protein and also the way the solutes and the mobile phase ions and additives are bound to the protein. In a recent paper the retention mechanisms of anionic drug compounds were discussed [4]. The aim of the present study was to obtain a deeper understanding of the mechanisms in-

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volved in the binding of cationic drug compounds to immobilized AGP.

2. Experimental

2.1. Chemicals and reagents

2-Propanol of HPLC grade was obtained from Lab-Scan (Dublin, Ireland). All other chemicals were of analytical-reagent grade. The drug compounds were gifts from the manufacturers. Structures of the compounds are shown in Fig. 1A and B.

2.2. Apparatus

The chromatographic system consisted of an LKB Model 2150 pump (Pharmacia–LKB Biotechnology, Uppsala, Sweden), a Kontron (Eching/Munich, Germany) Model 360 autosampler equipped with a 20-μl injection loop and a Spectra 100 variable-wavelength UV detector (Spectra-Physics, San Jose, CA, USA). The experimental data were collected and analysed on a Kontron Model 450 MT2 data system, which also controlled the autosampler. CHIRAL-AGP columns (100 × 4.0 mm I.D., 5 μm) were obtained from ChromTech (Hägersten, Sweden).

2.3. Chromatographic conditions

The experiments were carried out in a thermostated room at 23°C. A flow-rate of 0.9 ml/min was used. The UV detector was set at 225 nm. The sample concentrations were in the range 0.02–0.03 mg/ml. The void volume (V_0) was determined by injection of distilled water or mobile phase with a different composition.

2.4. Preparation of mobile phases

Mobile phases containing phosphate buffer at pH 6–7 were prepared from sodium dihydrogenphosphate. The phosphate salt was dissolved in 200 ml of distilled water, followed by adjustment of the pH with 2.0 *M* sodium hydroxide solution. When approaching the final pH, 0.1 *M* sodium hydroxide was used, 2-propanol was added and the volume was adjusted to 250.0 ml with distilled water.

The phosphate buffers used at pH 2.1 were prepared from concentrated phosphoric acid dissolved in 440 ml of distilled water. The pH was adjusted with 2.0 *M* sodium hydroxide solution. When approaching the final pH, 0.1 *M* sodium hydroxide was used. Distilled water was added to 500.0 ml. The phosphate concentrations given in the tables refer to the total phosphate concentration.

Acetate buffers were prepared from sodium or ammonium acetate. The acetate salt was dissolved in 200 ml of distilled water. The pH was adjusted with 3.0 *M* acetic acid solution, giving the higher total acetate concentration given in the tables. Then 2-propanol was added before addition of distilled water to 250.0 ml.

3. Results and discussion

3.1. Binding of solute molecules to α_1-acid glycoprotein

AGP is built up of a single peptide chain containing 183 amino acids [11]. Five carbohydrate units are linked to the peptide chain via the asparagine residues and the carbohydrate content is about 45%. At least two different binding sites have been demonstrated on AGP [12]. The main binding site is most likely a hydrophobic pocket which is formed by an enrichment of hydrophobic amino acid residues such as tryptophan, phenylalanine, tyrosine, leucine and isoleucine [13]. In addition to hydrophobic amino acids, the binding site contains numerous hydrogen bonding groups such as the amides and also numerous protolytic groups, both acidic and basic. Schmid [13] determined the amino acid composition and found 26 basic amino acid residues (13 lysine, 3 histidine and 10 arginine) and 51 acidic amino acids (21 asparagine and 30 glutamic acid). The pK_a values for the protolytic groups of AGP have been determined [14]. Two different carboxylic acid groups were observed

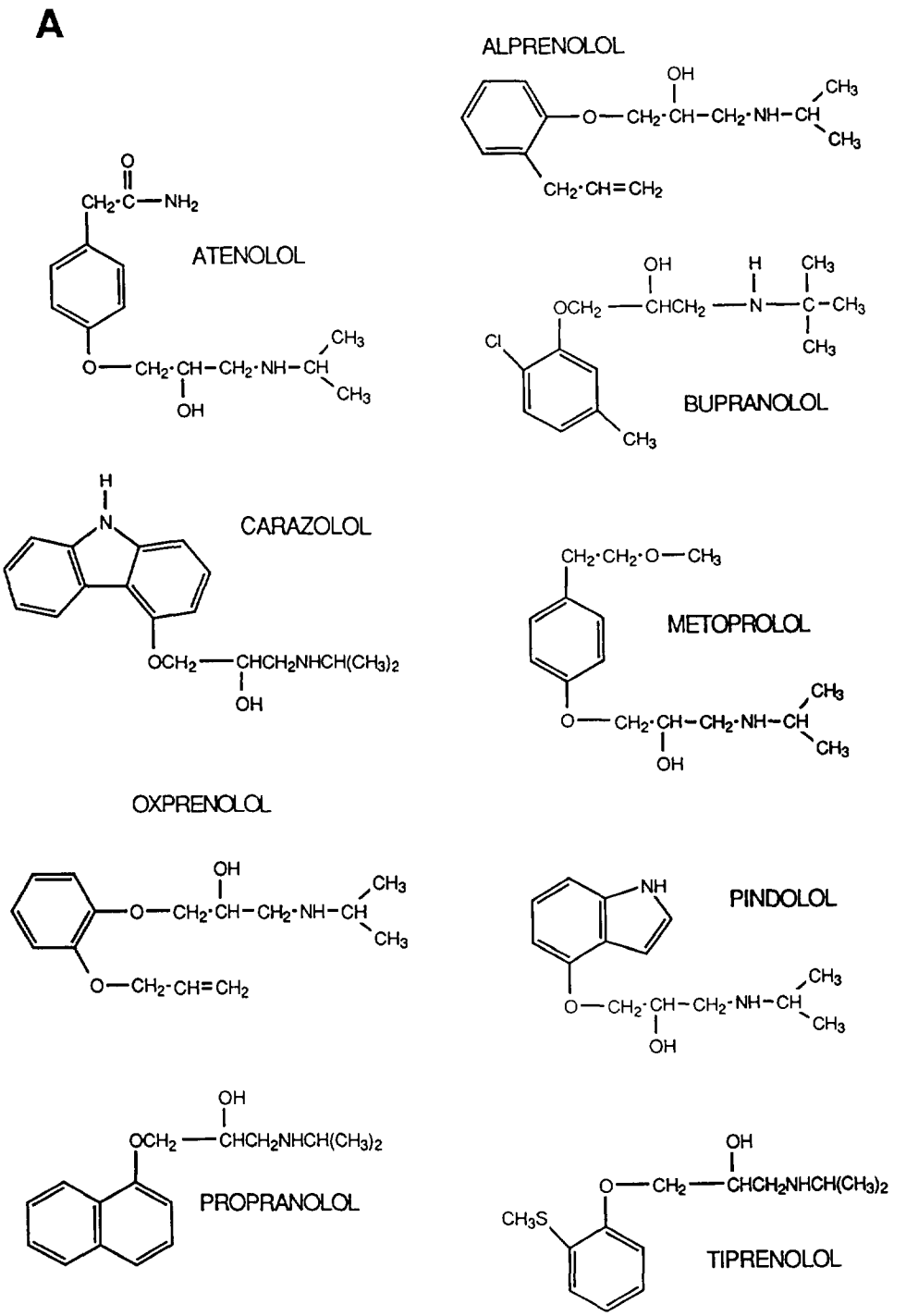
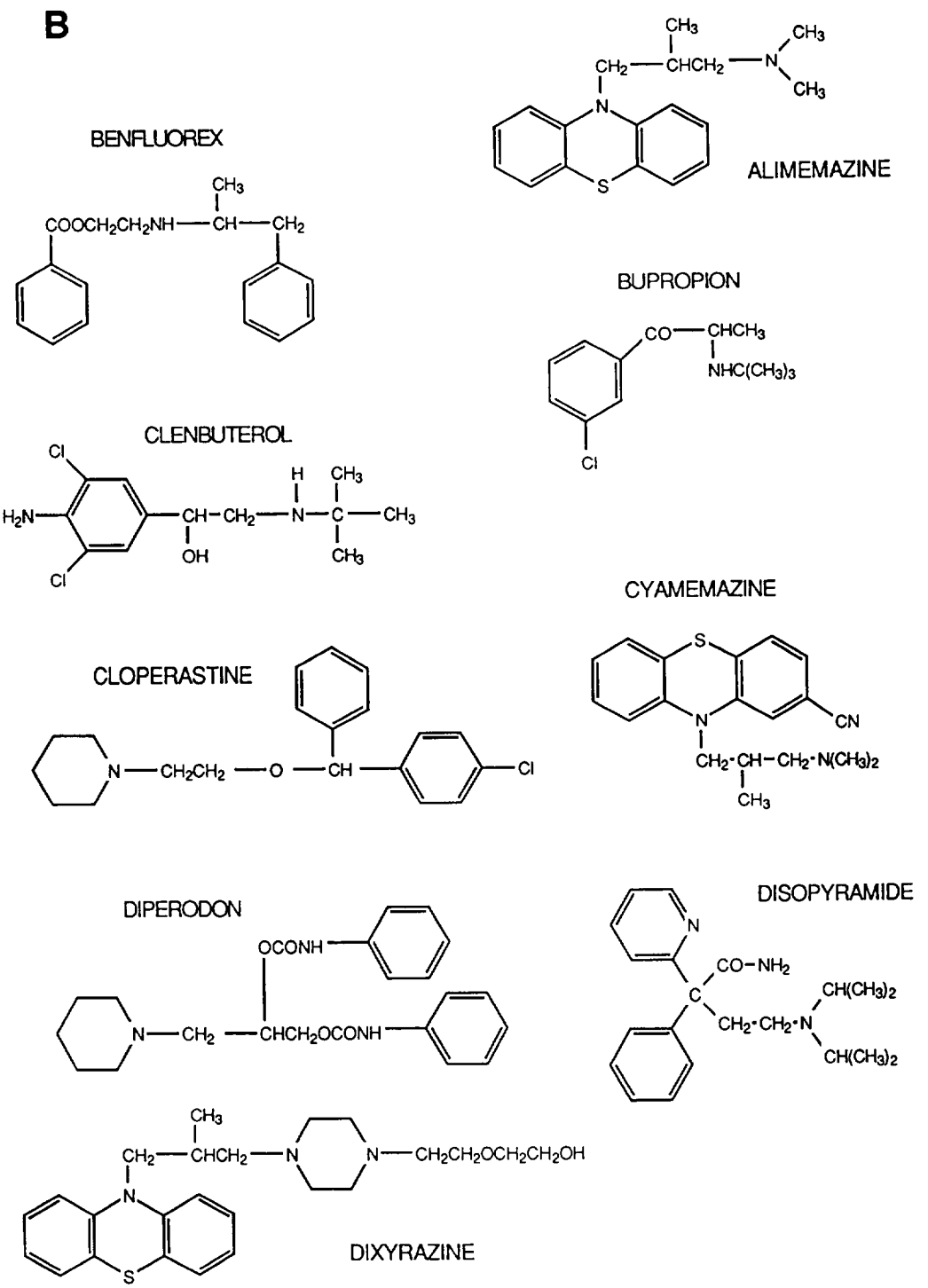


Fig. 1 (continued on p. 60).



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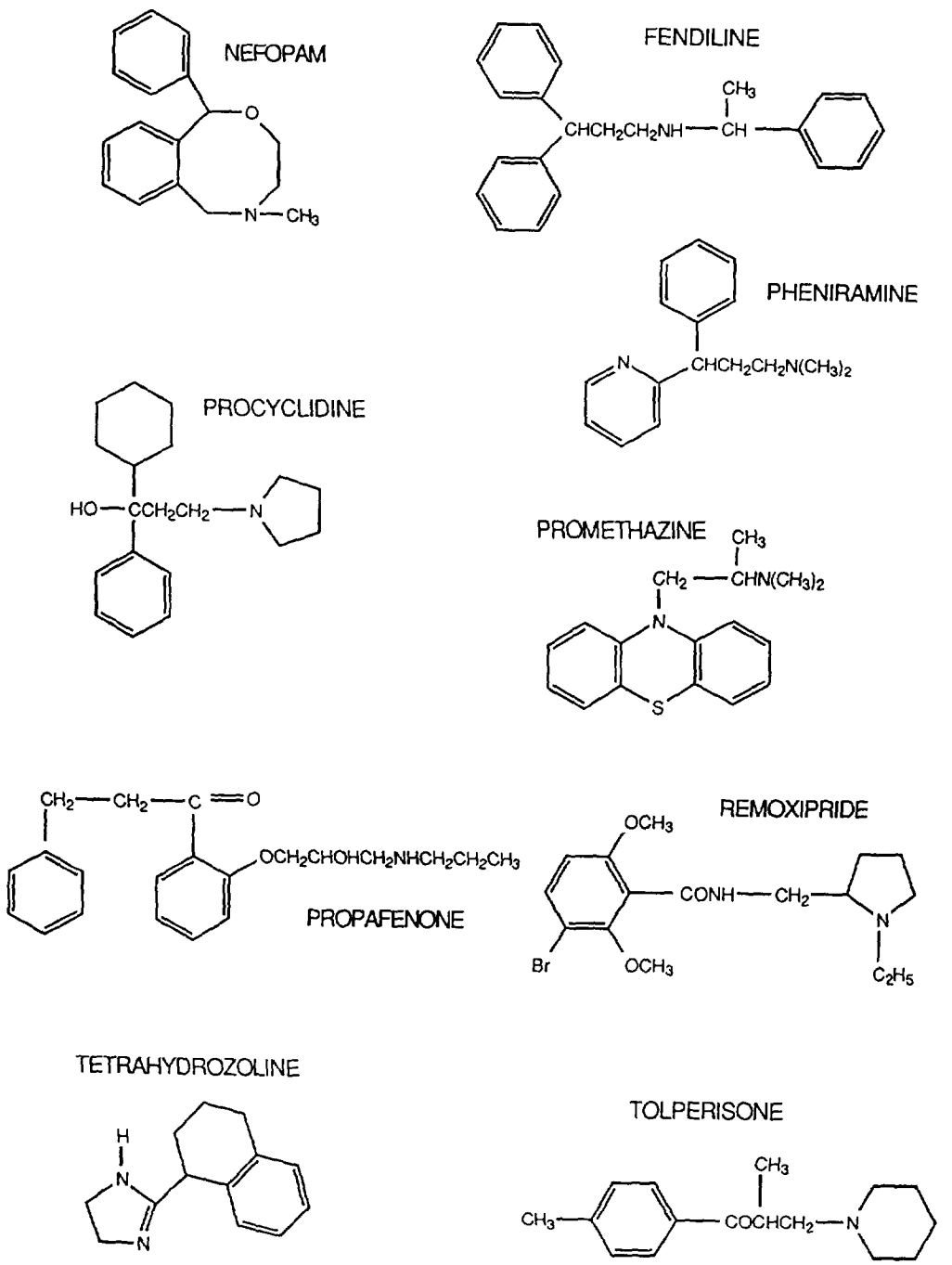

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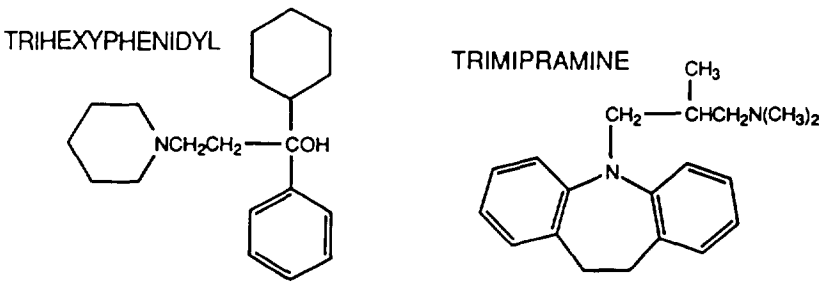

Fig. 1. Structures of (A) β-blockers and (B) other drug compounds.

with pK_a values of 2.95 and 4.14. The pK_a values for the basic groups, imidazole of histidine and the ε-amino groups of lysine, were determined to be 6.57 and 9.70, respectively [14]. The isoelectric point of AGP is 2.5 [13]. Many of the protolytic amino acid residues can most likely be found in the binding sites, allowing ionic binding between the solute and this kind of binding group in the binding sites. Such indications were found in chromatrographic and adsorption isotherm studies on the AGP column, using terodiline, a relatively hydrophobic secondary amine, as a model compound [12]. Normally chromatographic experiments are performed in the pH range 4–7, which means that the protein has a net negative charge. Thus, cationic solutes can be retained by interaction with negatively charged groups in the binding sites. This is supported by the strong effects on the capacity factors, obtained for basic and acidic solutes, by changing the pH of the mobile phase [2,15]. Chromatography of non-protolytic compounds at different pH values results in very small effects on the retention, but the enantioselectvity can be strongly affected [15]. A solute can also be retained by interaction with hydrogen bonding groups and with hydrophobic amino acid residues located in the binding sites. Hence, the solutes can be bound to the binding sites by, in principle, two different kinds of interactions, ionic binding and binding to uncharged groups.

3.2. Influence of pH on chromatographic properties of cationic solutes

Cationic drugs have traditionally been resolved on the AGP column using a pH of 6–7 [1,2]. The background for using mobile phases with pH in that range for resolution of cationic drugs was due to results of earlier studies where it was observed that the enantioselectivity increases for basic solutes with increasing pH. For example, the enantioselectivity for metoprolol increases from 1.25 to 1.48 on increasing the pH from 4.5 to 7.5 without a modifier in the mobile phase [1]. However, chromatography of cationic solutes at $pH \approx 7$ also results in high capacity factors and long retention times and the more hydrophobic the solutes the higher is the retention. Thus, chromatography of hydrophobic basic compounds at pH 7 requires the addition of an uncharged modifier in order to be able to elute the enantiomers within a reasonable time. Addition of uncharged modifiers such as 2-propanol or acetonitrile decreases the enantioselectivity for cationic compounds. This means that the concentration of modifier must be so low that the enantioselectivity is not reduced to such an extent that the resolution is incomplete, i.e., $R_s < 1.5$. The major reason for the high retention obtained for cationic solutes at pH 7 is that the protein has a high degree of negative charge at pH 7 since this is 4.5 pH units higher than the p*I* value of AGP. Chromatography of basic drug compounds with pK_a values higher than 9 at pH 7 means that the enantiomers are fully ionized and can be strongly retained by ionic bonding to the anionic groups in the binding sites of the protein. A decrease in the pH of the mobile phase towards the isoelectric point of the protein gives a lower degree of negative charge of the protein and thus lower retention of cationic drugs.

Table 1 gives results for five different cationic

Table 1
Influence of pH on the chromatographic properties of cationic drugs

Compound	pH 7.0[a]				pH 4.1[b]			
	k'_1	k'_2	α	R_s	k'_1	k'_2	α	R_s
Disopyramide	18.5	53.9	2.91	5.93	2.78	8.79	3.41	3.72
Diperodon	48.3	59.9	1.24	1.68	5.70	8.51	1.49	2.50
Carazolol	48.9	55.6	1.14	0.90	4.64	5.98	1.29	1.63
Bupranolol	25.0	31.4	1.25	1.58	3.04	3.81	1.25	1.39
Propranolol	48.9	56.1	1.15	1.08	7.04	10.7	1.52	2.62

For the preparation of the mobile phases, see Experimental.
[a] Mobile phase: 6% 2-propanol in 10 m*M* sodium phosphate buffer (pH 7.0).
[b] Mobile phase: 0.5% 2-propanol in 20 m*M* ammonium acetate buffer (pH 4.1) (total acetate concentration 96 m*M*).

drug compounds chromatographed at two pH values, 7.0 and 4.1. The mobile phase at pH 7 also contained 6% 2-propanol in order to be able to elute the most retained enantiomer within a reasonable time. However, despite this, the capacity factors for the last-eluted enatiomers of the solutes were in the range 31.4–59.9, which are fairly high. It can also be noted that relatively low separation factors, with one exception (disopyramide), were obtained for the compounds at pH 7. On decreasing the pH to 4.1 and the concentration of modifier to 0.5%, 2-propanol gives a higher enantioselectivity for all compounds, except bupranolol, with a separation factor of 1.25 at both pH values. The retention is also greatly reduced on decreasing the pH to 4.1, with capacity factors ranging from 3.8 to 10.7. For example, the capacity factors for the enantiomers of carazolol are reduced about tenfold on decreasing the pH from 7 to 4.1, despite the fact that the 2-propanol concentration was also substantially lowered. The resolution, R_s, of carazolol is 0.9 at pH 7 and 1.63 at pH 4.1.

The general rule concerning the uncharged modifier concentration in the mobile phase is that the retention increases with decrease in the concentration of uncharged modifier. From this it can be concluded that the strong decrease in the retention obtained by decreasing the pH is most likely caused by a decrease in the net negative charge of the protein. Figs. 2 and 3 demonstrate the dramatic improvement of the chromatographic performance and the strong decrease in retention time obtained at pH 4.1 compared with pH 7 for two other compounds included in Table 1, diperodon and propranolol.

Hydrophobic amines containing tricyclic ring structures, such as trimipramine, alimemazine, promethazine cyamemazine and dixyrazine, are very difficult to resolve with good chromatographic performance and low retention. Normally these compounds are chromatographed at a pH around 7. However, chromatography of these compounds at pH 7 gives very high capacity factors even if the mobile phase also contains

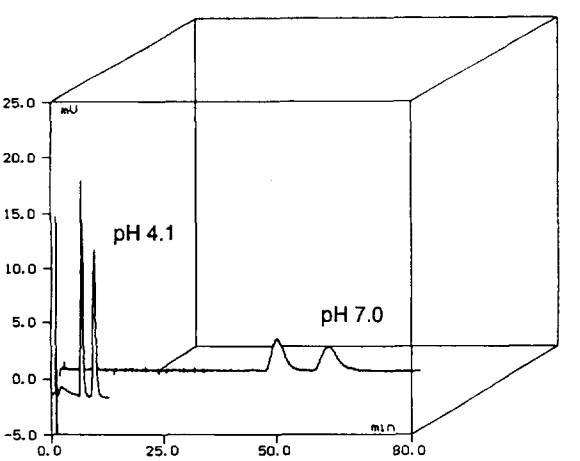

Fig. 2. Effect of pH on diperodon. Mobile phases: pH 4.1, 0.5% 2-propanol in 20 m*M* ammonium acetate buffer (total acetate concentration 96 m*M*); pH 7.0, 6% 2-propanol in 10 m*M* sodium phosphate buffer. For the preparation of mobile phases, see Experimental Column, CHIRAL-AGP (100 × 4.0 mm I.D.); flow-rate, 0.9 ml/min; detection, UV at 225 nm.

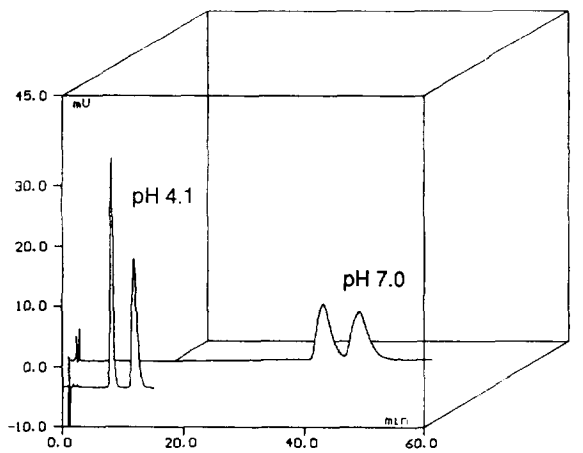

Fig. 3. Effect of pH on propranolol. Mobile phases: pH 4.1, 0.5% 2-propanol in 20 m*M* ammonium acetate buffer (total acetate concentration 96 m*M*); pH 7.0, 6% 2-propanol in 10 m*M* sodium phosphate buffer. For the preparation of mobile phases, see Experimental. Conditions, as in Fig. 2.

15% 2-propanol, as is demonstrated in Table 2. Two of the compounds, alimemazine and trimipramine, have capacity factors >60 under these conditions. The other compounds, cyamemazine, dixyrazine and promethazine, were eluted with capacity factors of the first-eluted enantiomer between 16.4 and 23.5. The enantioselectivity was also low and promethazine and dixyrazine have separation factors of 1.0 and 1.07, respectively. Cyamemazine was the only compound that could be resolved with a relatively high separation factor, 1.22, in the mobile phase with a pH of 7.0 and containing 15% 2-propanol. Decreasing the pH of the mobile phase from 7.0 to 4.0 and the content of organic modifier (acetonitrile or 2-propanol) to 1% results in drastic improvements in the enantioselectivity, as can be seen from Table 2. Separation factors between 1.31 and 1.84 were obtained. All compounds were baseline resolved with R_s ranging from 1.55 to 3.03. The retention was also strongly reduced.

Tables 1 and 2 show examples of compounds where the enantioselectivity and the resolution have been strongly improved by decreasing the pH and the modifier concentration in the mobile phase. It was also observed that similar improvements can be obtained for a very large number of basic compounds that previously have been resolved at pH ≈ 7. Some examples of such compounds are given in Table 3, which summarizes the capacity factors, the separation factors and the resolution of the drug compounds chromatographed at optimum conditions at low pH. Nine different β-blockers were also used as model compounds to test the influence of the pH of the mobile phase on the chromatographic performance. They were chromatographed with mobile phases with pH between 4 and 7 with the purpose of finding the optimum separation conditions, i.e., as low a retention as possible with baseline resolution. From Table 4 it can be seen

Table 2
Influence of pH on the chromatographic properties of hydrophobic basic drugs

Compound	pH 7.0					pH 4.0				
	Mobile phase[a]	k'_1	k'_2	α	R_s	Mobile phase[a]	k'_1	k'_2	α	R_s
Dixyrazine	1	21.3	22.8	1.07	–	2	8.29	12.0	1.45	1.81
Trimipramine	1	n.e.[b]	–	–	–	3	4.43	7.32	1.65	2.55
Cyamemazine	1	16.4	19.9	1.22	1.72	3	3.18	5.86	1.84	3.03
Promethazine	1	23.5	23.5	1	–	2	7.68	10.1	1.32	1.56
Alimemazine	1	n.e.[b]	–	–	–	2	10.7	14.0	1.31	1.55

[a] For the preparation of the mobile phases, see Experimental. Mobile phases: 1 = 15% 2-propanol in 10 m*M* sodium phosphate buffer (pH 7.0); 2 = 1% acetonitrile in 10 m*M* sodium acetate buffer (pH 4.0) (total acetate concentration 59 m*M*); 3 = 1% 2-propanol in 10 m*M* sodium acetate buffer (pH 4.0) (total acetate concentration 59 m*M*).
[b] Not eluted within 60 min.

Table 3
Separation of enantiomers of cationic drugs using low pH

Compound	Mobile phase[a]	k'_1	α	R_s
Benfluorex	1	5.72	1.42	2.80
Bupropion	2	1.61	1.40	1.59
Clenbuterol	10	2.27	1.55	2.93
Cloperastine	3	7.60	1.40	1.69
Diperodon	4	5.70	1.49	2.50
Fendiline	5	10.1	1.43	2.03
Nefopam	6	1.50	1.49	2.02
Pheniramine	7	4.04	1.46	2.12
Procyclidine	8	2.11	1.79	2.88
Remoxipride	9	2.03	1.63	2.50
Tetrahydrozoline	12	1.68	1.46	1.63
Tolperisone	10	2.06	1.40	1.69
Trihexyphenidyl	11	6.89	1.33	1.52

[a] For the preparation of the mobile phases, see Experimental. Mobile phases: 1 = 4% 2-propanol in 10 m*M* ammonium acetate buffer (pH 5.0) (total acetate concentration 15 m*M*); 2 = 0.5% 2-propanol in 10 m*M* ammonium acetate buffer (pH 5.0) (total acetate concentration 15 m*M*); 3 = 1% acetonitrile in sodium acetate buffer (pH 4.0) (total acetate concentration 59 m*M*); 4 = 0.5% 2-propanol in 10 m*M* ammonium acetate buffer (pH 4.1) (total acetate concentration 49 m*M*); 5 = 3% acetonitrile in 10 m*M* sodium acetate buffer (pH 4.1) (total acetate concentration 49 m*M*); 6 = 1% 2-propanol in 10 m*M* sodium acetate buffer (pH 4.5) (total acetate concentration 25 m*M*); 7 = 1% acetonitrile in 10 m*M* sodium acetate buffer (pH 5.0) (total acetate concentration 15 m*M*); 8 = 5% acetonitrile in 10 m*M* sodium acetate buffer (pH 4.1) (total acetate concentration 49 m*M*); 9 = 30 m*M* sodium acetate buffer (pH 4.0) (total acetate concentration 59 m*M*); 10 = 1% 2-propanol in 10 m*M* sodium acetate buffer (pH 5.0) (total acetate concentration 15 m*M*); 11 = 3% acetonitrile in 10 m*M* sodium acetate buffer (pH 4.1) (total acetate concentration 49 m*M*); 12 = 10 mM sodium acetate buffer (pH 5.0) (total acetate concentration 15 m*M*).

Table 4
Chromatographic properties of β-blockers

Compound	Mobile phase[a]	k'_1	k'_2	α	R_s
Carazolol	1	4.34	5.99	1.38	1.83
Propranolol	2	7.04	10.7	1.52	2.62
Bupranolol	3	2.90	3.77	1.30	1.65
Oxprenolol	4	4.03	5.16	1.28	1.61
Alprenolol	5	1.34	2.05	1.53	1.93
Tiprenolol	6	11.0	15.1	1.37	3.13
Pindolol	7	7.67	11.1	1.45	1.61
Atenolol	8	3.75	4.62	1.23	1.46
Metoprolol	9	10.5	13.2	1.26	1.71

[a] For the preparation of the mobile phases, see Experimental. Mobile phases: 1 = 0.5% 2-propanol in 5 m*M* ammonium acetate buffer (pH 4.1) (total acetate concentration 25 m*M*); 2 = 0.5% 2-propanol in 20 m*M* ammonium acetate buffer (pH 4.1) (total acetate concentration 96 m*M*); 3 = 0.5% 2-propanol in 39 m*M* ammonium acetate buffer (pH 4.1) (total acetate concentration 186 m*M*); 4 = 1% 2-propanol in 10 m*M* ammonium acetate buffer (pH 4.5) (total acetate concentration 25 m*M*); 5 = 3% acetonitrile in 10 m*M* ammonium acetate buffer (pH 4.0) (total acetate concentration 59 m*M*); 6 = 3% 2-propanol in 10 m*M* sodium phosphate buffer (pH 6.0); 7 = 10% acetonitrile in 10 m*M* sodium phosphate buffer (pH 7.0); 8 = 10 m*M* sodium phosphate buffer (pH 7.0); 9 = 0.5% 2-propanol in 10 m*M* sodium phosphate buffer (pH 7.0).

that six of the nine β-blockers were best resolved at a pH lower than 7. Figs. 4 and 5 show chromatograms of clenbuterol and cyamemazine using mobile phases with a low pH and a low content of organic modifier.

3.3. Effect of the buffer concentration on the retention and the enantioselectivity

As has been discussed above, the pH is a very important tool in optimizing a chiral separation on the AGP column. Another way to affect the retention and the enantioselectivity is to utilize the buffer concentration. It has been demonstrated previously that the retention and the enantioselectivity of solutes of different character could be strongly affected by the concentration of inorganic [4] and organic [4,15,16,17] mobile phase additives. In order to establish whether the buffer ion concentration could affect the retention and the enantioselectivity of cationic solutes, a series of experiments were performed using acetate buffers with total acetate concentrations between 12 and 186 m*M* at pH 4.1. Three basic drugs were used as model compounds, i.e., bupranolol, diperodon and propranolol, with one nitrogen atom in the molecule. The results of the study are summarized in Table 5. As can be seen, the enantioselectivity increases for bupranolol and propranolol with increasing acetate concentration. The

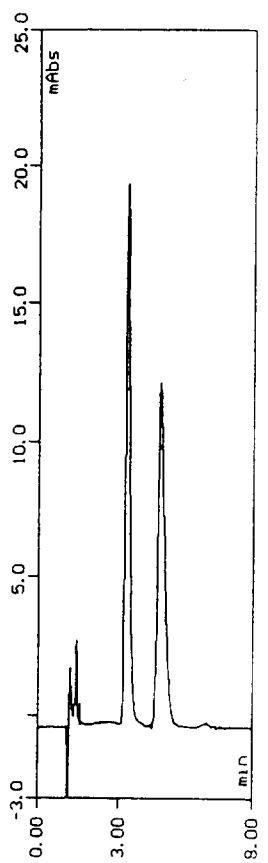

Fig. 4. Separation of the enantiomers of clenbuterol. Mobile phase: 1% 2-propanol in 10 m*M* sodium acetate buffer (pH 5.0) (total acetate concentration 15 m*M*). For preparation of mobile phase, see Experimental. Conditions, as in Fig. 2.

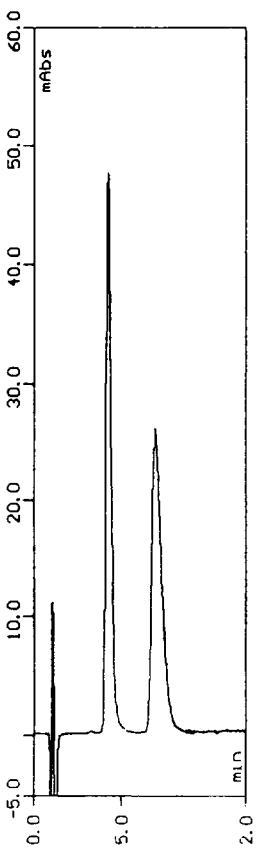

Fig. 5. Separation of the enantiomers of cyamemazine. Mobile phase: 1% 2-propanol in 10 m*M* sodium acetate buffer (pH 4.0) (total acetate concentration 59 m*M*). For preparation of mobile phase, see Experimental. Conditions, as in Fig. 2.

Table 5
Influence of acetate concentration on the retention and the enantioselectivity of cationic drugs containing one or two charged nitrogen atoms.

Acetate (mM)[a]	Bupranolol			Diperodon			Propranolol			Disopyramide		
	k_1'	k_2'	α	k_1'	k_2'	α	k_1'	k_2'	α	k_1'	k_2'	α
12	2.30	2.58	1.12	4.27	6.46	1.51	5.73	7.29	1.27	2.16	10.0	4.64
25	2.65	3.14	1.18	4.92	7.30	1.48	6.54	8.79	1.34	2.41	10.3	4.29
96	3.04	3.81	1.25	5.70	8.51	1.49	7.04	10.7	1.52	2.78	8.79	3.16
186	2.90	3.77	1.30	5.56	8.32	1.50	6.26	9.77	1.56	2.65	9.04	3.41

[a] Mobile phase: 0.5% 2-propanol in acetate buffers (pH 4.1) of different concentration. The mobile phases were prepared from 2.5, 4.9, 19.7 and 39.5 m*M* ammonium acetate, respectively. The pH was adjusted to 4.1 with acetic acid.

enantioselectivity for diperodon was almost constant. It is also very interesting that increasing the acetate concentration increases the retention of both enantiomers of bupranolol, diperodon and propranolol. This kind of effect has not been observed previously for cationic solutes. However, it has been reported that the retention of anionic solutes, i.e., anti-inflammatory drugs of the arylpropionic acid type, could be increased by increasing the concentration of, for example, sodium, potassium and ammonium in the mobile phase [4]. The increase in retention observed for the compounds listed in Table 5 with increasing acetate concentration, indicates that ion-pair distribution may be involved in the retention process of this kind of solute. It seems likely that this mechanism is favoured by a decrease in pH, as the net negative charge is reduced, which will suppress retention caused by ionic binding. This is supported by the fact that no increase in retention was observed on increasing the concentration of acetate at higher pH, where the protein has a higher degree of net negative charge and the ionic binding strongly affects the retention. It has also been demonstrated, for acidic drug compounds of the arylpropionic acid type, that the capacity factors increase on increasing the sodium concentration at pH 7, where the acids are negatively charged [4]. However, when performing a similar experiment at pH 2.1 where the acids are uncharged, and with no possibility of being retained as ion pairs, the result is a decrease in retention [4]. The weak acid hexobarbital, which is uncharged at pH 2.1 with no possibility of being distributed as an ion pair at this pH, demonstrated the same behaviour as the carboxylic acids on increasing the buffer concentration. These results support the assumption that ion-pair distribution might be a retention mechanism involved in the retention of both cationic and anionic solutes.

Studies with increasing buffer concentration were also performed with disopyramide, containing two charged nitrogens at pH 4.1. Most likely this kind of compound is retained according to the same mechanism as the compounds containing one charged nitrogen, by ionic binding and ion-pair adsorption. As can be seen from Table 5, chromatography of disopyramide with increasing acetate concentration at pH 4.1 results in relatively small changes in the capacity factors at total acetate concentrations >25 m*M*. This is most likely the result of a strong influence of the ion-exchange mechanism on the retention at pH 4.1. At this pH the protein has a relatively high degree of negative charge and the solute has two positively charged nitrogens. From Table 5 it can also be seen that the enantioselectivity increases on reducing the acetate concentration in the mobile phase.

In order to decrease the influence of the ion-exchange mechanism on the retention of cationic compounds the pH must be decreased to below 4. A decrease in the pH decreases the total ion-exchange binding capacity for cations, giving a larger influence of ion-pair adsorption. The nature and the concentration of the ions in the mobile phase also affect the relative influence of the two binding processes. Therefore, experiments were performed at pH 2.1 using phos-

Table 6
Influence of phosphate concentration on the retention (k') of cationic drugs containing one or two charged nitrogen atoms.

Phosphate (mM)[a]	Cloperastine, k'	Propafenone, k'	Diperodon, k'	Propranolol, k'	Disopyramide	
					k'_1	k'_2
25.0	3.93	2.90	1.24	0.61	0.94	2.68
50.0	5.64	3.55	1.56	0.90	1.30	3.00
100.0	8.09	4.68	2.54	1.41	1.82	4.29

[a] For preparation, see Experimental. Mobile phase: phosphate buffer (pH 2.1) of different concentrations.

phate buffers with increasing total phosphate concentrations between 25 and 100 mM. As demonstrated in Table 6, the capacity factors for diperodon, propranolol, cloperastine and propafenoe, with one charged nitrogen, increase with increasing phosphate concentration, indicating that ion-pair adsorption dominates the retention process at this pH. The capacity factors increase more than 100% on increasing the total phosphate concentration from 25 to 100 mM. The level of the capacity factors is, however, much lower than that at pH 4.1, probably because the retention caused by ionic binding has been reduced. However, to a limited extent ionic binding is most likely still involved in the retention at pH 2.1, as carboxylic acid residues with a pK_a value of 2.95 have been detected [14], which means that they are partially charged at pH 2.1. It can also be noted that the chiral selectivity is lost for these compounds.

As was mentioned above for disopyramide with two basic nitrogens, there is no increase in retention on increasing the acetate concentration at pH 4.1, indicating that ionic binding influences the retention to a high degree. However, chromatography of disopyramide at pH 2.1 means that the ionic binding of disopyramide is decreased. Increasing the phosphate concentration in the mobile phase at this pH increases the retention of disopyramide, as demonstrated in Table 6. It can also be noted that high separation factors can be obtained for disopyramide even at this very low pH. At the lowest phosphate concentration a separation factor of 2.87 was obtained for disopyramide. Increasing the phosphate concentration to 100 mM decreased the separation factor to 2.31. The above findings indicate that the ionic binding of the solutes is decreased at pH 2.1 to such an extent that ion-pair adsorption, with phosphate as counter ion, strongly contributes to the retention.

4. Conclusions

It has been demonstrated that the cationic solutes are retained according to two mechanisms on the AGP column, ionic binding and ion-pair distribution with the anionic buffer ions acetate and phosphate as counter ions. Increasing the buffer concentration increases the retention of the enantiomers of the drugs. The relative influence of the two processes can be affected by changing the pH and the nature and concentration of the ions in the mobile phase. A decrease in the pH of the mobile phase lowers the degree of negative charge of the protein, which decreases the influence of the ionic binding. From the above it follows that the pH of the mobile phase is a very important parameter when optimizing the separation of basic drugs. The chromatographic performance of most hydrophobic basic drugs could be strongly improved by using a pH between 4 and 6 and decreasing the content of uncharged modifier in the mobile phase. The explanation of this finding is that a decrease in pH lowers the influence of ionic binding on the retention, resulting in a lower retention. A lower retention means that it is not necessary to add an uncharged modifier to the mobile phase in order to elute the enantiomers within a reasonable time. No or low modi-

fier concentrations in the mobile phase give a higher enantioselectivity, with the result that high resolution and low retention can be obtained.

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