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Controlling the retention of clopenthixol and other basic drug substances by reversed-phase ion-pair chromatography on bonded-phase materials using two counter-ions of opposite charge

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ABSTRACT

In the reversed-phase chromatography of nitrogen-containing bases on chemically bonded ODS-silica, peak tailing and prolonged retention are often considerable problems. These effects are due to residual silanols on the surface of the column material and may be remedied by adding suitable amines or quaternary ammonium ions to the eluent as anti-tailing agents. However, further addition of an anionic compound is often needed to achieve a suitable retention. The retention mechanism in such systems is complex as interaction takes place between the anionic compound and the solute molecules, anti-tailing agent and column packing material. The influence of the nature of the anti-tailing agent and anionic counter-ion on the retention of *cis*- and *trans*-clopenthixol and of other basic drug substances was investigated and it was found that both the retention and the selectivity were greatly affected.

INTRODUCTION

Nitrogen-containing basic compounds constitute a major proportion of the drug substances used in modern therapy. Therefore, the determination of such compounds is an important topic in drug analysis. The relatively polar characteristics of such substances, which are readily soluble in *e.g.*, aqueous methanol, makes high-performance liquid chromatography (HPLC) on chemically bonded materials an obvious possibility. However, it was shown in 1978 by Wahlund and Sokolowski [1] that peak tailing and even prolonged retention are often seen when chromatographing nitrogen-containing bases on bonded-phase materials. This problem is probably due to the presence of residual silanol groups on the surface of the column material [2,3]. The peak tailing may be prevented by the addition to the eluent of a suitable anti-tailing agent to mask the residual silanols. The most efficient anti-tailing agents were shown to be long-chain dimethylalkylamines and trimethylalkylammonium compounds [*e.g.*, 1, 4–8]. By removing totally the effect of residual silanols, the retention of nitrogen-containing compounds will be strongly dependent on the pH of the eluent, as the ionized form of the solute will, at the concentration of methanol used in

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this study, have only a minor affinity to the apolar stationary phase. This means that at low pH such compounds can be expected to elute close to the void volume and the same situation will take place for quaternary ammonium compounds independent of pH. To achieve a suitable retention, it is possible to use a further additive to the eluent, an anionic compound suitable for the formation of ion pairs with the ionized amine or quaternary ammonium compound. This technique has previously been shown to result in efficient separations and good peak shapes [6–10].

In a previous investigation [7], several basic drug substances were investigated with a view to comparing he possible standardization of reversed-phase HPLC methods using bonded-phase materials and two counter ions of opposite charge in the eluent relative to systems based on dynamically modified silica. Several antidepressant and neuroleptic drugs were investigated, including *cis*- and *trans*-clopenthixol, imipramines and structurally related compounds. For all compounds it was found that an efficient separation with excellent peak shape was obtainable. However, the separation of *cis*- *trans*-clopenthixol required an analysis time of about 30 min (capacity factor, k', for the *trans* compound being *ca.* 20). The aim of this work was to investigate the influence of variations in methanol concentration and in the nature (alkyl chain length) of the two counter ions of opposite charge on the retention and selectivity of the basic drug substances previously investigated.

EXPERIMENTAL

Chemicals

The drug substances were of pharmacopoeial quality. Decyltrimethylammonium (DeTMA) bromide was obtained from Pfaltz & Bauer (Stamford, CT, U.S.A.), dodecyltrimethylammonium (DTMA) bromide and tetradecyltrimethylammonium (TTMA) bromide from Sigma (St. Louis, MO, U.S.A.) and octadecyltrimethylammonium (STMA) bromide and all sodium alkanesulphonates from Fluka (Buchs, Switzerland). All other chemicals, including cetyltrimethylammonium (CTMA) bromide, were of analytical-reagent grade from E. Merck (Darmstadt, F.R.G.).

Apparatus

The individual chromatographic systems were tested on a liquid chromatograph consisting of a Kontron Model 410 LC pump, a Kontron Model 735 LC UV detector (operated at 254 nm) and a Rheodyne Model 7125 injection valve. Chromatograms were recorded on a Kipp & Zonen Model BD-8 recorder. Retention data were collected on a Hewlett-Packard Model 3359A laboratory data system.

Chromatography

All experiments were performed on a 120 × 4.6 mm I.D. column from Knauer (Berlin, F.R.G.) psacked by the dilute slurry technique with chemically bonded LiChrosorb RP-18 ODS-silica (5 μm) (E. Merck).

The eluents used were methanol–water–0.2 *M* potassium phosphate buffer (pH 4.0) (60:35:5) with the addition of 2.5 m*M* alkyltrimethylammonium bromide and 5 m*M* sodium alkanesulphonate and systems with various amounts of methanol.

The chromatographic system, including column, eluent, pump and injection valve, was thermostated at 30°C.

RESULTS AND DISCUSSION

The test substances investigated were *cis*- and *trans*-clopenthixol and several other neuroleptic and antidepressant drug substances which had been examined in a previous investigation [7]. In particular, the separation of the former substances was found to be important as the use in therapy has recently changed from the mixture of the two compounds to the pure *cis* compound, which almost solely possesses the biological effect. Therefore, a method that is able to detect a small amount of *trans*-clopenthixol in *cis*-clopenthixol (*e.g.*, less than 1%) is needed. In a previous investigation [7] a fully satisfactory separation was achieved, the resolution between the two compounds being 1.6. However, a drawback was the time of analysis, which was *ca.* 30 min ($k' \approx 20$). The eluent used for that separation was methanol–water–0.2 *M* potassium phosphate buffer (pH 4.0) (60:35:5) with the addition of 2.5 m*M* DTMA bromide and 5 m*M* sodium decanesulphonate. An obvious possibility for improving the method was to adjust the retention by increasing the concentration of methanol in the eluent to achieve a time of analysis of, *e.g.*, *ca.* 10 min ($k' \approx 6$). This could be expected to work without problems from experience with reversed-phase chromatography in general, as the resolution would be expected to be sufficient provided that the separation factor is not affected. Calculating from the values of k' and assuming a constant separation factor (α), the resolution in an improved system should be *ca.* 1.45 [11]. However, by increasing the percentage of methanol to 65% the time of analysis was reduced to *ca.* 15 min and the resoltuion in this separation was 1.3. Another possibility for reducing the retention in a reversed-phase ion-pair chromatographic separation would be to reduce the carbon chain length in the alkanesul-

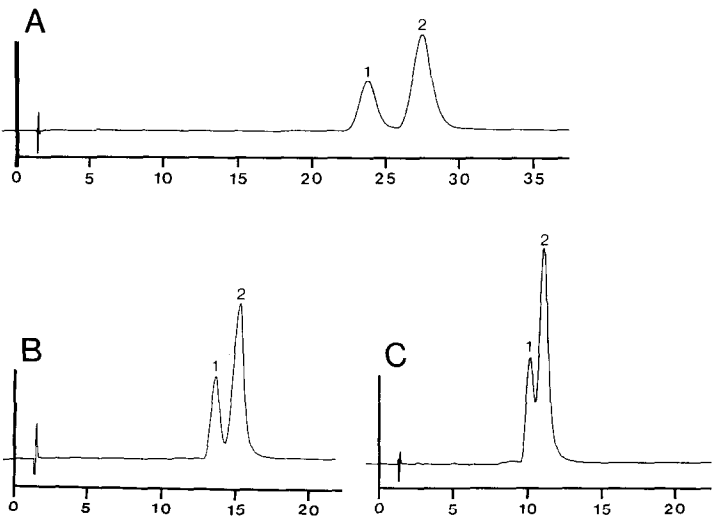

Fig. 1. Separation of (1) *cis* and (2) *trans*-clopenthixol using three different eluents. Column: LiChrosorb RP-18 (120 × 4.6 mm I.D.). Eluents: methanol–water–0.2 *M* potassium phosphate buffer (pH 4.0), A and C (60:35:5), B (65:30:5), with the addition of 2.5 m*M* DTMA bromide and 5 m*M* sodium decanesulphonate (A and B) or sodium hexanesulphonate (C). Flow-rate: 1 ml/min. Detection: UV, 254 nm, 0.32 a.u.f.s. Time scales in min.

phonate used as the counter ion. By using the original methanol concentration of 60% and substituting hexanesulphonate for decanesulphonate as the counter ion, a time of analysis of *ca.* 15 min was achieved but in this instance the resoltuion had been reduced to 0.7. The chromatograms are shown in Fig. 1.

The retention mechanism in these chromatographic systems is complex. The alkanesulphonate interacts both with the anti-tailing quaternary ammonium ions and with solute ions. Further, the two surfactants may be co-adsorbed on to the column material [12,13]. As the concentration of alkanesulphonate is twice that of the quaternary ammonium compound, there will always be counter ions present to form ion pairs with solute ions and the effect of variations in the nature of the counter ions can be expected to be as in reversed-phase ion-pair chromatography in general. The affinity of the quaternary ammonium ions to residual silanols is even more pronounced than that to alkanesulphonates, as it appears that the anti-tailing effect is not reduced by their presence [7]. The effect on retention and selectivity of variations in the nature of both quaternary ammonium ions and alkanesulphonates was investigated and a study was also made of the influence of variations in the concentration of methanol in the eluent.

Nature of quaternary ammonium compound and alkanesulphonate

The effect of alkyltrimethylammonium compounds with alkyl carbon chain lengths varying from 10 to 18 and of alkanesulphonates with carbon chain lengths varying from 4 to 12 were investigated. Some of the results obtained are given in Table I, which shows the effects of variations in the quaternary ammonium compound using 5 m*M* decanesulphonate as the anionic counter ion. Correspondingly, variations in the nature of the alkanesulphonates were investigated using 2.5 m*M* DTMA bromide as the anti-tailing agent.

From the variations in the quaternary ammonium compounds it appears that the retention of the basic compounds decreases with increasing alkyl chain length. This occurs because the affinity of the anti-tailing agent to silanol groups increases with increasing apolar moiety in the molecule, an effect which is well known from chromatography on dynamically modified silica [8]. The influence of the residual silanols is never totally removed, as shown by the results when no alkanesulphonate is present in the eluent. In such a case the retention of imipramine when, *e.g.*, DeTMA is used as the anti-tailing agent is $k' = 3.66$ and an asymmetry fractor of 3.7, whereas the results when using STMA is $k' = 0.28$ and asymmetry factor of 1.6. The tailing has thus been reduced to a level which is acceptable by increasing the alkyl chain length of the quaternary ammonium compound; *cf.*, previous investigations discussing the anti-tailing effects in reversed-phase chromatography using eluents containing two counter ions [7,8]. However, the retention is still considerable, taking into account that the substance is considerably ionized. The retention and the remaining tendency for tailing are due to a remaining interaction with silanol groups.

An increase in the alkyl chain length of the alkanesulphonate has the effect, well known in regular reversed-phase ion-pair chromatography, that the retention of cationic solutes increases [e.g., 14]. A linear relationship in ion-pair chromatography between log k' and carbon number in the counter ion has previously been predicted from theory and shown by experiments [15,16]. From the results of this study, it appears that such a linear relationship also seems to exist, as shown in Fig. 2. It is

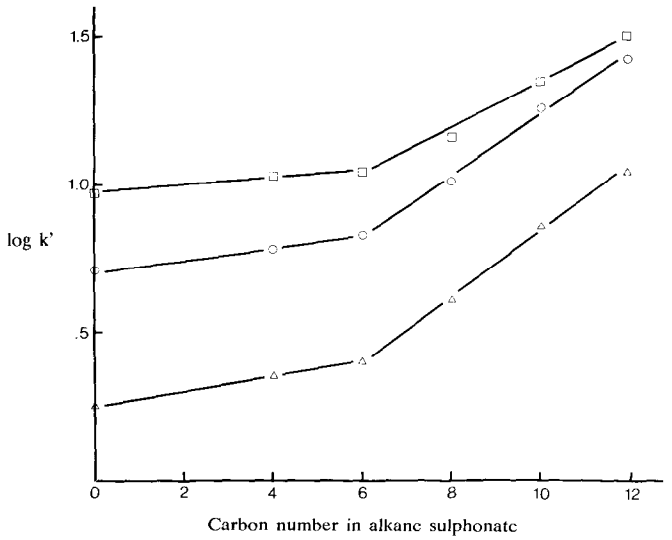

Fig. 2. Relationship between retention (log k') of (△) imipramine, (○) *cis*-clopenthixol and (□) prochlorperazine and carbon number in the alkanesulphonate added to the eluent. Column: LiChrosorb RP-18 (120 × 4.6 mm I.D.). Eluent: methanol–water–0.2 *M* potassium phosphate buffer (pH 4.0) (60:35:5) with the addition of 2.5 m*M* DTMA bromide and 5 m*M* sodium alkanesulphonate with carbon number 4–12.

seen, however, that a change in the linearity appears at six carbon atoms, or alternatively a curved plot approximation might be drawn. The background for this change relative to previously reported results [15,16] has not yet been elucidated. A possible explanation might be that sulphonate ions containing eight carbon atoms and more are adsorbed to some extent on the surface of the column material, thereby enhancing the retention when forming ion pairs with solute molecules. It has been shown by Knox and Hartwick [17] that a change in chain length or in concentration of a sulphonate counter ion will cause a change in surface concentration and, thereby, in surface charge. Further, such adsorbed sulphonate ions will also be able to form ion pairs with DTMA ions, thus increasing the amount of apolar stationary phase, as discussed below.

As mentioned previously, the additives in the eluent may interact with each other, forming ion pairs. The interaction is more pronounced the longer the chain length of the two counter ions. For the most lipophilic counter ions it appears that such ion pairs are adsorbed on the surface of the column packing material, thereby increasing the amount of apolar stationary phase. From Table I it is seen that this effect is reflected by an increased retention of the neutral solute benzene. The strongly lipophilic nature of the ion pairs between quaternary ammonium ions and alkanesulphonates was also demonstrated by solubility problems when using both types with long alkyl chains. Thus it was not possible to perform a planned experiment using STMA bromide and dodecanesulphonate as the ion pairs caused precipitation in the eluent. For other combinations it was found that only by using a chromatographic system which was thermostated at 30°C was it possible to prevent the precipitation which occurred in the eluents at room temperature.

TABLE I

RETENTION AND SELECTIVITY (SEPARATION FACTOR, α, RELATIVE TO ONE COMPOUND IN EACH GROUP) OF TEN TEST SUBSTANCES AND OF BENZENE

Eluent: methanol–water–0.2 m*M* potassium phosphate buffer (pH 4.0) with the addition of 2.5 m*M* alkyltrimethylammonium bromide and 5 m*M* alkanesulphonate as indicated.

Compound	Parameter	Quaternary ammonium compound, 2.5 *M* (5 m*M* decanesulphonate in eluent)					Alkanesulphonate, 5 m*M* (2.5 m*M* DTMA in eluent)					
		C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_0	C_4	C_6	C_8	C_{10}	C_{12}
Imipramine	k'	10.85	7.16	3.68	2.07	1.03	1.80	2.22	2.50	4.06	7.16	11.05
Desipramine	α	1.03	1.08	1.11	1.13	1.24	0.71	0.77	0.86	0.97	1.08	1.20
Imipramine N-oxide	α	0.68	0.78	1.02	1.14	1.96	2.65	2.25	1.85	1.23	0.78	0.60
cis-Clopenthixol	k'	25.73	17.61	8.89	4.57	2.41	5.18	5.98	6.80	10.11	17.61	26.69
trans-Clopenthixol	α	1.14	1.16	1.20	1.25	1.33	1.09	1.10	1.10	1.12	1.16	1.21
Chlorpromazine	k'	20.28	14.01	7.68	4.37	2.90	4.02	4.85	5.29	7.54	14.01	22.64
Prochlorperazine	α	1.64	1.60	1.63	1.57	1.53	2.33	2.20	2.11	1.92	1.60	1.40
Perphenazine	α	1.13	1.07	1.03	0.95	0.78	0.99	1.06	1.11	1.10	1.07	1.04
Amitriptyline	k'	13.33	8.83	4.48	2.43	1.23	1.89	2.49	3.00	4.75	8.83	13.94
Nortriptyline	α	0.97	0.91	0.89	0.84	0.79	1.17	1.26	1.15	1.01	0.91	0.84
Benzene	k'	3.04	3.06	3.04	3.16	3.58	2.89	3.01	2.94	2.97	3.06	3.09

It appears from Table I that not only the retention is greatly influenced by the nature of the two counter ions of opposite charge in the eluent. Large variations in selectivity are also seen, *e.g.*, for *cis*- and *trans*-clopenthixol it appears that an increased separation is obtained by increasing the alkyl chain length of both types of additives. However, the effects of the two additives on retention are opposite. The purpose of a separation method for these two compounds, as mentioned above, was to achieve an effective separation within *ca.* 10 min. It was found that the optimum combinations of additives for this particular separation was the addition to the eluent of 2.5 m*M* CTMA bromide and 5 m*M* sodium dodecanesulphonate. Fig. 3 shows the separation of *ca.* 1% of *trans*-clopenthixol in *cis*-clopenthixol.

Methanol concentration

As mentioned previously, the influence of variations in the concentration of methanol in the eluent is not as simple as expected for plain reversed-phase chromatographic systems. Examples of such a relationship are shown in Table II. It appears that the mean retention of the compounds decreases as expected with an increase in the amount of methanol in the eluent. However, the selectivity towards the individual groups of compounds is influenced to different extents and several pairs of substances reverse the order of elution during the series of experiments. For *cis*- and *trans*-clopenthixol it is seen that there is a general decrease in separation with increasing modifier concentration and this excludes the possibility, as indicated also by the preliminary investigations, of reducing the time of analysis by increasing the percentage of methanol in the eluent.

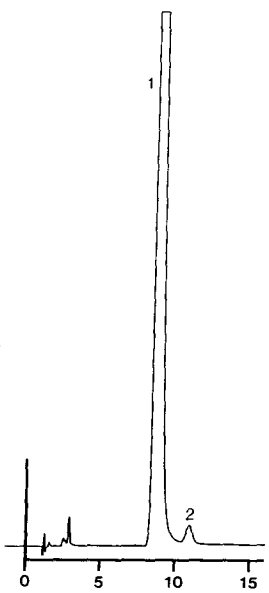

Fig. 3. Separation of 1% *trans*-clopenthixol (2) in *cis*-clopenthixol (1). Column: LiChrosorb RP-18 (120 × 4.6 mm I.D.). Eluent: methanol–water–0.2 *M* potassium phosphate buffer (pH 4.0) (60:35:5) with the addition of 2.5 m*M* CTMA bromide and 5 m*M* sodium dodecanesulphonate. Flow-rate 1 ml/min. Detection: UV, 254 nm, 0.32 a.u.f.s. Time scale in min.

TABLE II

RETENTION AND SELECTIVITY (SEPARATION FACTOR, α, RELATIVE TO ONE COMPOUND IN EACH GROUP) OF TEN TEST SUBSTANCES

Eluent: methanol–water–0.2 *M* potassium phosphate buffer (pH 4.0) with the addition of 2.5 m*M* alkyltrimethylammonium bromide and 5 m*M* alkanesulphonate as indicated. Methanol concentration varied from 50% to 70%.

Compound	Parameter	Additive									
		DTMA + C_8-sulphonate					STMA + C_8-sulphonate				
		50%	55%	60%	65%	70%	50%	55%	60%	65%	70%
Imipramine	k'	8.09	6.17	4.27	3.24	2.53	0.81	0.82	0.84	0.88	0.86
Desipramine	α	1.08	0.97	0.90	0.81	0.72	1.26	1.10	0.95	0.83	0.74
Imipramine N-oxide	α	1.76	1.82	1.88	1.83	1.76	3.89	3.61	3.44	3.14	2.88
cis-Clopenthixol	k'	26.46	17.75	10.77	7.24	4.81	2.67	2.29	2.28	2.21	1.99
trans-Clopenthixol	α	1.23	1.19	1.15	1.12	1.10	1.34	1.30	1.24	1.18	1.14
Chlorpromazine	k'	19.86	14.07	8.74	6.56	4.93	2.71	2.42	2.29	2.14	1.90
Prochloroperazine	α	2.04	2.13	2.23	2.31	2.27	2.54	2.03	2.19	2.28	2.38
Perphenazine	α	1.14	1.11	1.08	0.97	0.90	0.82	0.85	0.90	0.93	0.95
Amitriptyline	k'	10.36	7.50	4.92	3.57	2.66	1.07	0.98	0.97	0.98	0.86
Nortriptyline	α	1.09	0.99	0.93	0.84	0.77	1.26	1.12	1.00	0.87	0.83

CONCLUSION

Reversed-phase ion-pair liquid chromatographic separations of nitrogen-containing basic drug substances using two counter ions of opposite charge in the eluent were investigated. It was found that both the retention and selectivity are greatly influenced by the nature of the two additives and by the concentration of methanol in the eluent. It was shown that by choosing a suitable combination of the two types of additive it is possible to obtain an efficient separation within a reasonable time. This was demonstrated on the two closely related compounds *cis*- and *trans*-clopenthixol.

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