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REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY OF NITROGEN BASES USING ALKYL SULPHONATES AS ION-PAIR REAGENTS

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SUMMARY

The retention behaviour of a number of nitrogen bases on reversed-phase pre-coated thin-layer chromatographic layers in the presence of different ion-pair reagents was investigated. Sodium hydrogen sulphate and sodium alkyl sulphonates with chain lengths from 1 to 12 carbon atoms were used as counter ions. The dependence of R_F values on the eluent composition, the chain length of the ion-pair reagent and the ratio of two ion-pair reagents in the eluent is described.

INTRODUCTION

Reversed-phase ion-pair chromatography is used increasingly for the separation of polar and charged substances by high-performance liquid chromatography (HPLC). In this method, an organic salt, the ion-pair reagent, also known as a counter ion, is added to the usually mainly aqueous eluent. The substances to be investigated form ion pairs with the ion-pair reagent, which are retained more strongly by the stationary phase. Tetraalkylammonium compounds[1–3] have proved useful as ion-pair reagents for the separation of acids, alkyl sulphonates[4–6] and alkyl sulphates[7,8] have been successful for bases. By altering the type[6,8] and concentration of the ion-pair reagents[1–3,7,8] and the composition of the eluent[4,9], it is possible to vary the retention of the sample substances and the selectivity of the chromatographic system.

In thin-layer chromatography (TLC), the method has so far found only limited use. One of the first investigations was carried out by Lepri *et al.*[10–12] with silanized silica gel layers which were impregnated with sodium lauryl ether sulphate or triethanolamine dodecylbenzene sulphonate. This was followed by work by Volkman[13,14] and Gonnet *et al.*[15] with HPTLC and TLC reversed-phase pre-coated layers. For the investigation of the sulphoxides of some phenothiazine bases and various alkaloids[13,14], heptanesulphonic acid proved a suitable ion-pair reagent. Hydrophilic dyes were successfully separated with high selectivity on HPTLC reversed-phase pre-coated plates using tetraalkylammonium compounds[15]. Investigations into the retention behaviour of benzo[*a*]pyrene metabolites were carried out by Marshall *et al.*[16].

This paper demonstrates the relationship between the retention of a number of

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nitrogen bases and the type of ion-pair reagent as well as the eluent composition on TLC RP-18 pre-coated plates and shows, with the aid of some chromatograms, the selectivity of this chromatographic method.

EXPERIMENTAL

Plates and solvents

TLC pre-coated RP-18 F_{254s} plates (Cat. No. 15423) from E. Merck (Darmstadt, G.F.R.) were used. Prior to use the pre-coated plates were heated at 130°C for 15 min. The solvents used were LiChrosolv-grade acetone (E. Merck) and water.

Ion-pair reagents

The following substances were used as ion-pair reagents: sodium hydrogen sulphate monohydrate (GR; E. Merck), methanesulphonic acid (GR; E. Merck), butane-1-sulphonic acid sodium salt (C_4SO_3Na) for surfactant testing (E. Merck), 1-pentanesulphonic acid sodium salt monohydrate (C_5SO_3Na) for ion-pair chromatography (purum; Fluka, Buchs, Switzerland), hexanesulphonic acid sodium salt monohydrate (C_6SO_3Na) for ion-pair chromatography (purum; Fluka); heptanesulphonic acid sodium salt monohydrate (C_7SO_3Na) for ion-pair chromatography (purum; Fluka); octane-1-sulphonic acid sodium salt (C_8SO_3Na) for surfactant testing (E. Merck) and dodecane-1-sulphonic acid sodium salt ($C_{12}SO_3Na$) for surfactant testing (E. Merck). Sodium hydroxide pellets (GR; E. Merck) were also used. When sodium methane sulphonate (C_1SO_3Na) was used as an ion-pair reagent, 1 molar equivalent of methanesulphonic acid and sodium hydroxide was added to the eluent in each instance.

Chromatographic measurements

The test substances used (atropine, codeine, caffeine, eupaverin and papaverine) were dissolved in methanol at concentrations of 0.1–1.0%. For the measurements, 300–500 nl of the individual sample substances or synthetic mixtures were applied to the plate with a Hamilton syringe and subsequently eluted by ascending linear chromatography in a normal chamber without chamber saturation. The chromatograms were evaluated *in situ* at 254 nm, using a TLC/HPTLC scanner with a monochromator from Camag (Muttenz, Switzerland).

RESULTS AND DISCUSSION

Relationship between the R_F values of the nitrogen bases and the water content of the eluent

In reversed-phase chromatography, the R_F values can be influenced by changes in the water content of the eluent. This is also possible in the presence of inorganic salts or ion-pair reagents. Fig. 1 shows the relationship between the R_F values of several nitrogen bases and the water content of the eluent in the acetone–water system on RP-18 F_{254s} pre-coated TLC plates. In each instance 0.1 mol/l of ion-pair reagent was added to the mobile phase. The sodium salts of butane-, hexane- and octanesulphonic acids were used as counter ions.

It can be seen from Fig. 1 that the R_F values of the bases investigated, independent of the type of ion-pair reagent used, decline with increasing water content

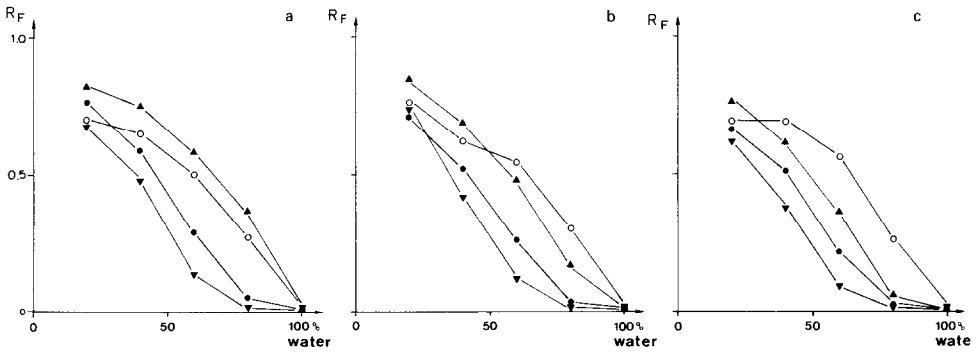

Fig. 1. Relationship between the R_F values of the nitrogen bases and the water content of the eluent. Plate: pre-coated RP-18 F_{254s} TLC plate. Eluent: water–acetone (20:80 to 100:0, v/v) with the addition of 0.1 mol/l of ion-pair reagent: (a) butanesulphonic acid, sodium salt; (b) hexanesulphonic acid, sodium salt; (c) octanesulphonic acid, sodium salt. Migration distance: z_f = 7 cm. Normal chamber without chamber saturation. Detection: UV (254 nm). Test substances: (▲) codeine; (○) caffeine; (▼) eupaverin; (●) papaverine.

in the eluent and reach zero when pure water is used as the eluent. The change in the water content of the mobile phase not only influences the magnitude of the R_F values but also the relationship between the R_F values of the individual substance spots. With a decreasing proportion of water in the eluent, the substance spots initially move further apart, so that the best selectivity is reached in the range 40–60 % of water in acetone. With lower proportions of water in the eluent, the substance spots come closer together and the selectivity of the system is poorer.

In the presence of different ion-pair reagents, the variation of the eluent composition leads to an additional change in the retention sequence of the nitrogen compounds. If butanesulphonic acid sodium salt is used as the counter ion, an R_F inversion for papaverine and caffeine occurs with water contents of 30 % (Fig. 1a). In the remaining range investigated, the elution sequence is eupaverin, papaverine, caffeine and codeine in order of increasing R_F values. If sodium butyl sulphonate is replaced by another alkanesulphonic acid sodium salt, an R_F inversion of caffeine and codeine occurs with the use of sodium hexyl sulphonate (Fig. 1b) with a water content of 50 % in acetone and with the use of octanesulphonic acid sodium salt (Fig. 1c) at a water content of 30 %.

Relationship between the R_F values of the nitrogen bases and alkyl chain length of the ion-pair reagents

Reversed-phase ion-pair chromatography offers not only the possibility of varying the eluent composition but also the choice of suitable ion-pair reagents to adjust the retention values of the substances investigated into particular desired ranges. HPLC investigations have shown that with this method, the hydrophobic character of the ion-pair reagent[9,10,17,18] is of great importance. The hydrophobic characteristics of an ion-pair reagent are generally determined by the number of carbon atoms or by the chain length of its alkyl groups. Fig. 2 shows the relationship between the R_F values of the nitrogen bases investigated and the chain length of the R group of the ion-pair reagents used with the general formula RSO_3Na. When R was

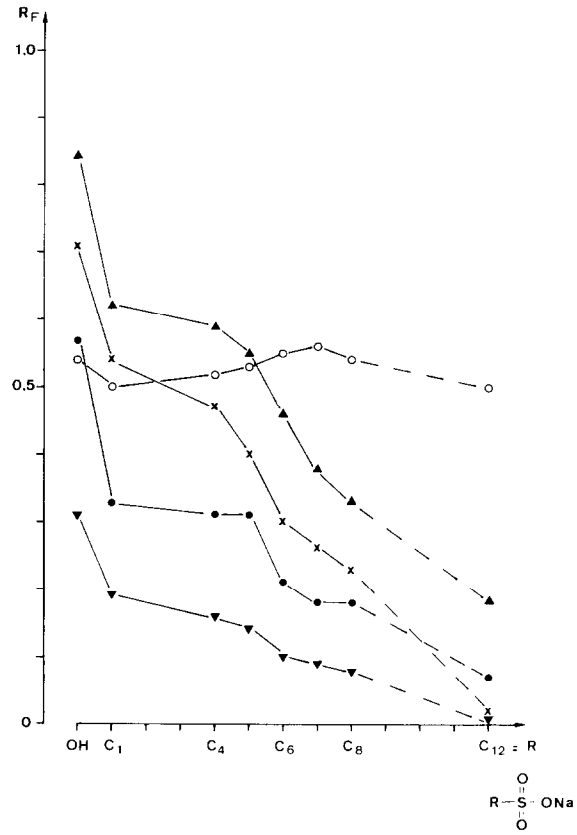

Fig. 2. Relationship between the R_F values of the nitrogen bases and the chain length of the ion-pair reagents used with the general formula RSO_3Na (R = OH, CH_3, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{12}H_{25}$). Plate: pre-coated RP-18 F_{254s} TLC plate. Eluent: water–acetone (60:40, v/v) with addition of 0.1 mol/l of ion-pair reagent ($C_{12}SO_3Na$ saturated solution). Migration distance: z_f = 7 cm. Normal chamber without chamber saturation. Detection: UV (254 nm). Test substances: (×) atropine; (▲) codeine; (○) caffeine; (▼) eupaverin; (●) papaverine.

OH, sodium hydrogen sulphate was used as the counter ion. In all other instances R was an *n*-alkyl group, from C_1 (methyl) to C_{12} (dodecyl). The concentration of the ion-pair reagents added to the eluent (water–acetone, 60:40) was 0.1 mol/l except for dodecanesulphonic acid sodium salt; because of the limited solubility of sodium dodecyl sulphonate in water–acetone (60:40), a cold saturated solution (*ca.* 0.05 mol/l) was used.

For atropine, codeine, eupaverin and papaverine, the R_F values decrease with increasing chain length of the ion-pair reagent used. A particularly clear reduction in R_F value can be found when the inorganic sodium hydrogen sulphate is replaced by the smallest alkyl sulphonate, sodium methyl sulphonate.

In the series of alkyl sulfonates from C_4SO_3Na to C_8SO_3Na the curves of atropine, codeine and eupaverin demonstrate an almost linear relationship. Papaverine does not show such linearity. In contrast to these four bases, caffeine shows no significant change in its R_F values when different ion-pair reagents are used. The R_F

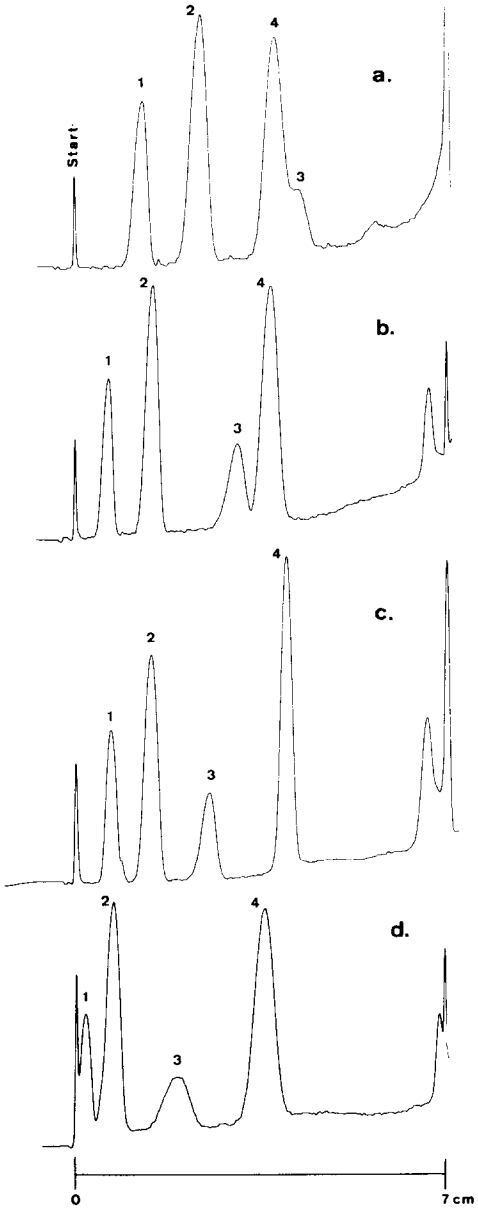

Fig. 3. Separation of eupaverin (1), papaverine (2), codeine (3) and caffeine (4). Plate: pre-coated RP-18 F_{254s} TLC plate. Eluent: water–acetone (60:40, v/v), with the addition of 0.1 mol/l of ion-pair reagent: (a) butanesulphonic acid, sodium salt; (b) hexanesulphonic acid, sodium salt; (c) octanesulphonic acid, sodium salt; (d) dodecanesulphonic acid, sodium salt (saturated solution). Migration distance: z_f = 7 cm. Normal chamber without chamber saturation. Detection: *in situ* evaluation with a TLC/HPTLC scanner (Camag), UV (254 nm).

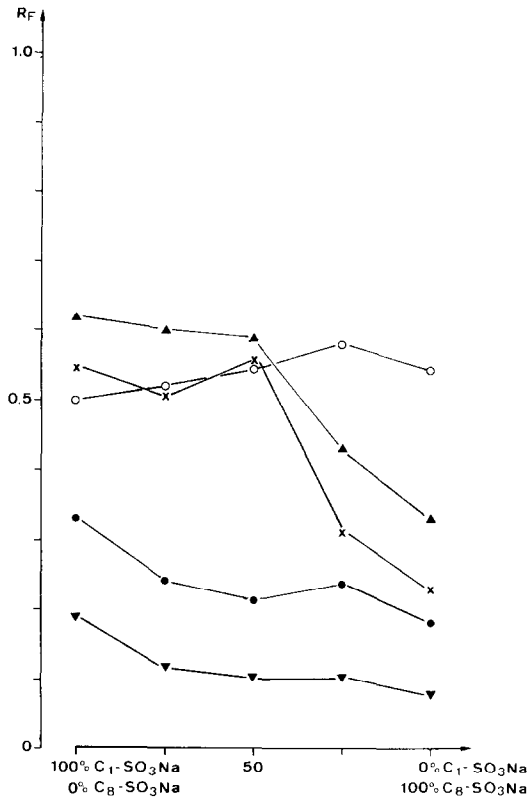

Fig. 4. Relationship between the R_F values of the nitrogen bases and the ratio of the ion-pair reagents C_1SO_3Na and C_8SO_3Na added to the eluent. Plate: pre-coated RP-18 F_{254s} TLC plate. Eluent: water–acetone (60:40, v/v) with the addition of a total of 0.1 mol/l of ion-pair reagent. Migration distance: z_f = 7 cm. Normal chamber without chamber saturation. Detection: UV (254 nm). Test substances: (×) atropine; (▲) codeine; (○) caffeine; (▼) eupaverin; (●) papaverine.

value of caffeine fluctuates within the range 0.5–0.56 (Fig. 2). This effect can be explained by the fact that caffeine is a neutral molecule under the chromatographic conditions used and therefore, in contrast to the other four bases investigated, caffeine is not able to form ion pairs.

Because of this chromatographic behaviour of caffeine, the R_F sequence of the five compounds investigated changes in the presence of different ion-pair reagents in the eluent. Thus, for instance, codeine and atropine are less strongly retained in the presence of sodium methyl sulphonate than caffeine, and papaverine and eupaverin are retained more strongly than caffeine.

The R_F values of caffeine and codeine coincide when pentylsulphonate is used as the counter ion. If longer chain alkyl sulphonates are added to the eluent, all of the test substances have lower R_F values than caffeine. Fig. 3 shows the change in R_F values and sequence in the separation of caffeine, codeine, eupaverin and papaverine when different ion-pair reagents with different chain lengths were used.

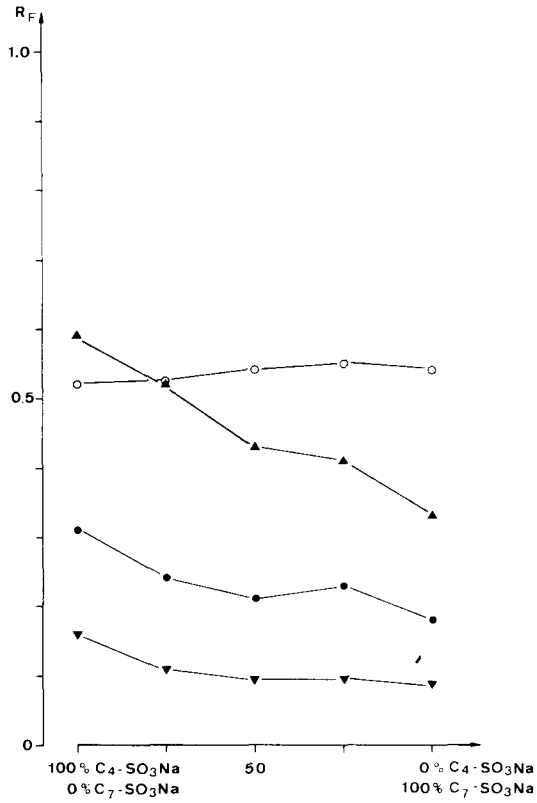

Fig. 5. Relationship between the R_F values of the nitrogen bases and the ratio of the ion-pair reagents C_4SO_3Na and C_7SO_3Na added to the eluent. Plate: pre-coated RP-18 F_{254s} TLC plate. Eluent: water–acetone (60:40, v/v), with the addition of a total of 0.1 mol/l of ion-pair reagent. Migration distance: $z_f = 7$ cm. Normal chamber without chamber saturation. Detection: UV (254 nm). Test substances: (▲) codeine; (○) caffeine; (▼) eupaverin; (●) papaverine.

Mixtures of ion pair reagents

In most instances it is sufficient to add one ion-pair reagent to the eluent in sufficient amount to achieve a desired R_F value. However, it is also possible to influence the selectivity of the chromatographic system in a particular direction[19,20] by combining two ion-pair reagents in a certain ratio. To investigate this, two series of tests were conducted, in one of which methyl and octyl sulphonate and in the other butyl and heptyl sulphonate were added to the eluent (water–acetone, 60:40) in different ratios. The total concentration of ion-pair reagents in the eluent in each instance was 0.1 mol/l.

In Figs. 4 and 5, the change in the R_F values of the bases investigated is shown as a function of the ratios of ion-pair reagents added to the eluent. Fig. 4 shows an almost linear decrease in R_F values for papaverine and eupaverin with increasing proportions of C_8SO_3Na. The retention of codeine with increasing proportions of longer chain ion-pair reagents initially increases slowly then very strongly from 50% of C_8SO_3Na. Atropine from pure sodium methyl sulphonate up to 50% C_8SO_3Na shows no significant change, but a substantial reduction in R_F value with a further increase in the porportion of sodium octyl sulphonate. In contrast, a slight increase in R_F value for caffeine from 100% C_1SO_3Na to 100% C_8SO_3Na can be observed. With

a ratio of ion-pair reagents of C_1SO_3Na:C_8SO_3Na = 25:75, all the nitrogen bases investigated have the same R_F values as when pure C_6SO_3Na is used (*cf.*, Figs. 2 and 4). For the mixture C_1SO_3Na–C_8SO_3Na (15:85), R_F values similar to those for C_7SO_3Na are obtained. Comparisons of this type between mixtures of C_1SO_3Na and C_8SO_3Na with a pure ion-pair reagent with a chain length of less than six carbon atoms in this system are possible only for individual substances and not for the whole series of bases investigated.

Fig. 5 shows the relationship between the R_F values of the test substances and the ratio of ion-pair reagents C_4SO_3Na and C_7SO_3Na. In this system also similar retention behaviour of the nitrogen compounds can be seen to that shown in Fig. 4. The R_F values of codeine, papaverine and eupaverin decrease with increasing proportions of C_7SO_3Na. Caffeine shows a slight increase in R_F value with an increase in proportion of the longer chain ion-pair reagent in the eluent. Here too it is possible, with certain combinations of ion-pair reagents, to obtain R_F values similar to those which can be obtained with a pure ion-pair reagent. Thus, for the combination C_4SO_3Na–C_7SO_3Na (75:25) almost identical R_F values are obtained for caffeine, papaverine and eupaverin to those obtained with pure C_6SO_3Na as the counter ion at the same concentration (*cf.*, Figs. 2 and 5).

These investigations demonstrate that it is possible, by using a suitable mixture of ion-pair reagents with different alkyl chain lengths, to obtain R_F values identical with those which would be obtained with a pure ion-pair reagent whose chain length lies between those of the mixed ion-pair reagents.

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