Journal of Chromatography, 346 (1985) 463–467
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 000

Note

Separation of organic quaternary salts by reversed-phase thin-layer chromatography

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(Received June 18th, 1985)

During the past five years, reversed-phase thin-layer chromatography (RP-TLC) has received considerable attention in the separation of a wide variety of compounds[1–4]. Its popularity is mainly due to the fact that the results obtained with RP-TLC substantially correlate with high-performance liquid chromatography (HPLC)[5,6]. RP-TLC is particularly suited for the separation and analysis of polar compounds which do not migrate on ordinary TLC plates. Also the resolution achieved by RP-TLC is far superior to that obtained on regular silica gel plates. The ease of manipulation of the solvent systems, the commercial availability of the bonded plates and the use of aqueous media have further enhanced the usefulness of RP-TLC. The preparation and properties of commercially bonded phases have been well ddocumented[7,8]. Silanes are used to modify the hydroxy groups of the stationary phase. These modifications endow the layers with a very high degree of hydrophobic character, retention, capacity, selectivity and stability. The selectivity of RP-TLC layers has been shown to increase with the corresponding increase in the water content of the solvent system[9]. However, the use of increasing amounts of water itself causes problems[10].

One of the advantages of RP-HPLC is the simplicity involved in the selection of a suitable solvent system to give satisfactory resolution. Thc conditions for optimizing solvent systems of RP-HPLC[11], have been found to be applicable to RP-TLC[12]. The polarity of the sample to be separated increases, with increasing water content of the solvent system[9]. Contrary to conventional TLC, the compounds migrate in order of decreasing polarity on the RP-TLC plates; the most polar moving the farthest and least polar migrating the least[13].

The addition of various ion-pairing salts to the mobile phase has been reported to considerably enhance the resolution capability of the RP-TLC by influencing the surface characteristics and properties of the silanized groups[14,15]. Although the mechanism by which this is brought about is not yet completely understood, it may either be due to an ion-exchange reaction or liquid–liquid partition of the neutralized

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0021-9673/85/$03.30

ion pair on the silanized layers. The formation of a zwitter ion of the type has been suggested[10]:

$$\text{solute}^{\pm} + \text{counter ion}^{\pm} \rightleftarrows (\text{solute}^{\pm})$$

Ion-pairing salts such as sodium chloride, ammonium chloride, ammonium acetate, at a concentration of 0.5 *M* permit the use of up to 50% of water in the solvent without disrupting the bonding properties of the layers and without affecting the separation. The separation of organic quaternary salts by conventional TLC is beset with many problems. It imposes certain limitations on the choice and use of aqueous solvent systems. Most often, adequate resolution on ordinary TLC plates cannot be achieved by using conventional solvent systems. Because of the almost unlimited wettability of the RP-TLC plates [recently Woelm has introduced RPTLC plates with 250 micron thickness of the coating that can withstand the use of 100% water (Catalog number 92101)], RP-TLC is particularly suited for use in reversed-phase ion chromatography.

In connection with our work on the synthesis of 4,4′-bipyridyl derivatives[16], we encountered complex mixtures containing variously quaternized 4,4′-bipyridyl quaternary salts. These salts carried 1 to 6 positive charges. The separation of these salts by conventional TLC proved fruitless. This led us to explore the use of RP-TLC to separate organic quaternary salts.

EXPERIMENTAL

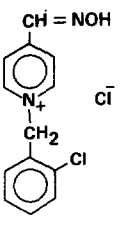
Watman $MKC_{18}F$ chemically bonded reversed-phase 7.5 × 2.5 cm (lot number 000230) and 20 × 10 cm RP-TLC plates were used. Individual solutions were prepared at 2–4 μg/μl in distilled water. The standardized mixtures were prepared from the individual solutions. Initial spots were applied 2 cm away from the bottom edge of the plates. Development was carried out at room temperature in circular glass jars containing 2–3 ml of the respective solvent. The solvent was introduced into the developing chamber with no paper lining and allowed to saturate the chamber before the introduction of the RP-TLC plates. The development required 20–30 min in the case of small plates and 2–3 h in the case of longer plates. The plates were air dried and spots were detected under ultraviolet light (254 nm).

Chemicals

N-(2-Chlorobenzyl)- (I), N-(4-chlorobenzyl)- (II), N-(2,4-dichlorobenzyl)- (III), N-(3,4-chlorobenzyl)-4-aldoxime pyridinium chloride (IV) were prepared by refluxing an alcoholic solution of 4-pyridinealdoxime and the respective benzyl-chloride and filtering the solid on cooling and/or on evaporating the solvent under reduced pressure. The remaining three compounds, 4,4′-(4-bromobutyl)-bipyridinium- (V); 1,4-(bis-4,4′-bipyridinium) butane- (VI) and 4,4′-(bis-4-bromobutyl)-bipyridiniumbromide (VII) were prepared by refluxing a solution of 1,4-dibromobutane and 4,4′-bipyridyl in N-methylpyrrolidone.

RESULTS AND DISCUSSION

Prior to attempting the separation of the quaternary salts, optimum conditions



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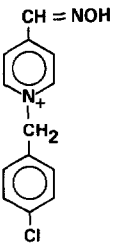

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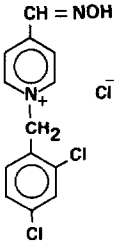

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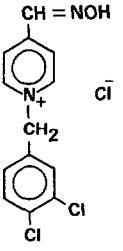

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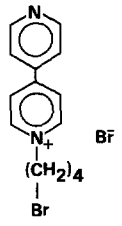

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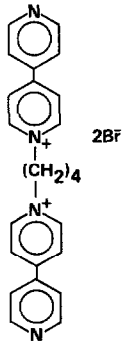

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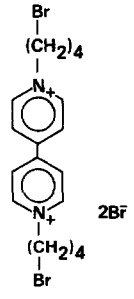

VII

were determined for the satisfactory separation of the Analtech Test Dye 30-03. In this quest, we explored some 40 odd solvent systems. Best resolution was obtained using a solvent consisting of methanol–0.45 *M* ammonium acetate (7:3). We also examined the effect of salt concentration on the resolution of the quaternary salts. In general, we observed that increasing the salt concentration reduced trailing and gave better resolution and that salt concentrations greater than 0.5 *M* had no pronounced effect on the resolution. The use of ammonium hydroxide and sodium phosphate caused excessive trailing.

Fig. 1 shows the separation of mono- and di-substituted N-benzylpyridinium salts. When these conditions were applied for the separation of the multiply charged 4,4′-bipyridinium quaternary salts, poor resolution was observed. Excessive trailing was also seen. Attempts to improve the separation by substituting solvents within the selectivity groups were not successful. However, the combination of hydrochloric acid and acetic acid with ethanol and methanol resulted in satisfactory separation. Acetic acid did cause some trailing. Fig. 2 shows the separation of variously quaternized 4,4′-bipyridinium derivatives on longer RP-TLC plates.

The addition of ammonium hydroxide and ammonium chloride in place of hydrogen chloride and acetic acid gave poor separation. Better resolution of the spots was achieved with solvent A (Fig. 2). Attempts to separate these multiply-charged quaternary salts by SDS gel electrophoresis were not successful.

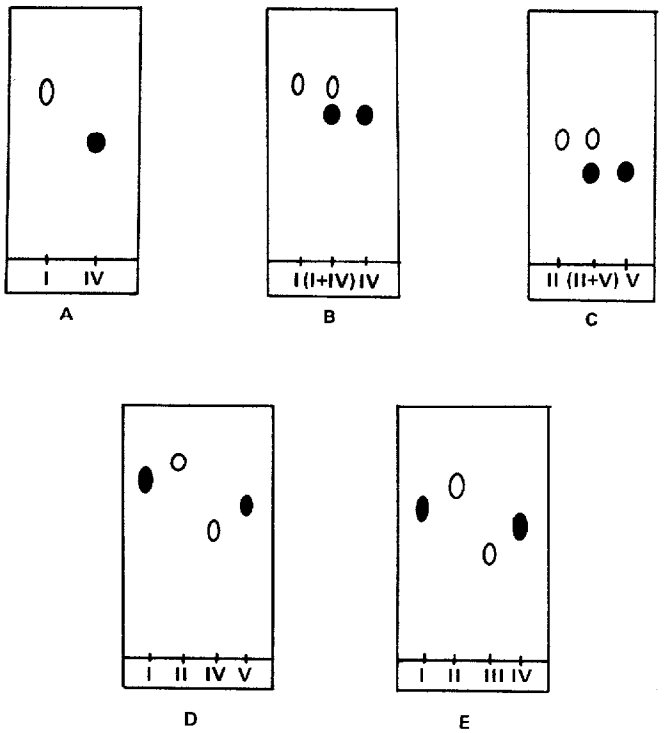

Fig. 1. Separation of N-benzylpyridinium-4-aldoxime salts. (A) I and IV; solvent, methanol–0.5 *M* ammonium acetate (7:3). (B) I, IV and I–IV mixture; solvent, methanol–0.38 *M* ammonium acetate (7:3). (C) II, V and II–V mixture; solvent, methanol–0.2 *M* sodium chloride (7:3). (D) I, II, III and IV; solvent, methanol–0.45 *M* ammonium acetate (6:4), (E) I, II, III and IV; solvent, dimethyl sulfoxide–0.2 *M* sodium chloride (3:2).

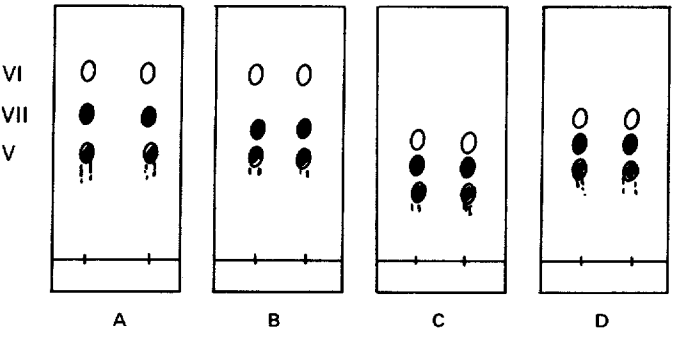

Fig. 2. Separation of variously quaternized 4,4'-bipyridyl derivatives on longer plates (20 × 10 cm), with (A) methanol–water–concentrated hydrochloric acid (7.5:2.45:0.05); (B) ethanol–water–concentrated hydrochloric acid (7.5:2.45:0.05); (C) methanol–water–glacial acetic acid (7.5:2.45:0.05); (D) ethanol–water–glacial acetic acid (7.5:2.45:0.05).

REFERENCES

1 M. Faupel and E. von Arx, *J. Chromatogr.*, 211 (1981) 262.
2 W. Meier and J. F. Conscience, Anal. Chem., 105 (1980) 334.
3 H. R. Pfaendler, J. Costel and R. B. Woodward, *J. Am. Chem. Soc.*, 102 (1980) 2039.
4 J. Sherma, R. Krywicki and T. E. Regan, *Am. Lab.*, 1 (1981) 117.
5 R. K. Gilpin and W. R. Sisco, *J. Chromatogr.*, 124 (1976) 257.
6 E. von Arx and M. Faupel, *J. Chromatogr.*, 154 (1978) 68.
7 E. Grushka and E. J. Kikta, Jr., Anal. Chem., 50 (1978) 1048A.
8 M. C. Hennion, C. Picard and M. Caude, *J. Chromatogr.*, 166 (1978) 21.
9 U. A. Th. Brinkman and G. de Vries, *J. Chromatogr.*, 192 (1980) 331.
10 A. M. Siouffi, T. Wawrzynowicz, F. Bressolle and G. Guiochon, *J. Chromatogr.*, 186 (1979) 563.
11 R. Lehrer, *Am. Lab.*, 10 (1981) 113.
12 J. Sherma and S. Charvat, *Am. Lab.*, 2 (1983) 140.
13 I. Halasz, Anal. Chem., 52 (1980) 1393A.
14 H. H. W. Thijssen, *J. Chromatogr.*, 133 (1977) 355.
15 H. E. Hauck and W. Jost, *Am. Lab.*, 8 (1983) 72.
16 S. Munavalli and E. J. Poziomek, unpublished results.