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USE OF CHEMICALLY BONDED STATIONARY PHASES IN HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY. II*

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SUMMARY

Reversed-phase high-performance thin-layer chromatography (HPTLC) has been carried out on LiChrosorb RP-2, RP-8 and RP-18, and Whatman KC_{18} pre-coated thin-layer plates. With methanol or acetonitrile containing 0–30% water as the mobile phase, the KC_{18} and RP-type HPTLC plates perform equally well, the behaviour of the KC_{18} plates being very similar to that of the RP-8 plates. With mobile phases containing more than *ca.* 30% water, RP-coated HPTLC plates cannot be used conveniently due to non-wettability of the stationary phase. KC_{18} plates can be used irrespective of the proportion of water, provided 3% NaCl is added to the mobile-phase solvent mixture.

Various separations are shown, *e.g.*, of phthalate esters, chloroanilines, aromatic hydroxy compounds and polynuclear aromatic hydrocarbons.

INTRODUCTION

During the past 3 years, several new thin-layer chromatographic (TLC) techniques have been introduced or have gained acceptance. These include programmed multiple development[2], the use of TLC plates with concentrating zones[3], the propagation of simultaneous transmission–reflection measurements in densitometry[4] and the so-called high-performance thin-layer chromatography (HPTLC)[5]. In this paper we are concerned with HPTLC and refer to the literature cited for a definition of this new mode in liquid chromatography.

So far, most of the over 80 papers devoted to HPTLC have dealt with silica gel as the stationary phase. Recently, however, increased attention has been paid to the use of apolar chemically bonded stationary phases as coating materials for reversed-phase HPTLC. In 1976, Gilpin and Sisco[6] studied the preparation and characterization of C_n-bonded coatings for $n = 1, 2, 6, 12$ and 18 and noted the closely analogous behaviour of their dodecyl-modified silica gel thin-layer material and an octadecyl reversed-phase high-performance liquid chromatographic (HPLC) column. More

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[*] For Part I, see ref. 1.

TABLE I

SURVEY OF LITERATURE ON REVERSED-PHASE HPTLC ON CHEMICALLY BONDED STATIONARY PHASES

*Stationary phase**	*Mobile phase*	*Application*	*Reference*
C-1 to -18 (home-made)	Acetonitrile–0.01 *M* KH_2PO_4 (40:60) Acetonitrile–0.01 *M* $(NH_4)_2CO_3$ (30:70)	Hydroxybenzoates, substituted anilines and phenols	6
RP-8, -18 (M)	Acetonitrile Methanol–water (97:3) Methanol–acetonitrile (90:10)	Headache pill, air-particulate extracts	7
RP-8 (home-made)	Acetonitrile, methanol	Alkylpyridines, polynuclear aromatic hydrocarbons	8
C18 (W)	Methanol–water (70:30)	Zeranol and its diastereoisomer	9
C18 (Q)	Methanol–acetone–water (20:4:3 and 20:20:10)	Chloroplast pigments	10
KC_{18} (W)	Methanol–0.5 *M* NaCl (50:50)	Constituents of APC tablets	11
RP-8 (M)	Methanol–water (var.) Acetonitrile–water (var.)	Phthalate esters, chloroanilines, polynuclear hydrocarbons	1
RP-18 (M)	Methanol–water (85:15)	Polynuclear aromatic hydrocarbons	12
RP-2, -8 and -18 (M), KC_{18} (W)	Methanol–water (var.)	Barbiturates, alkylbenzenes	13
RP-2, -8 and -18 (M)	Ion-pair chromatography with tetraalkylammonium bromide	Food dyes	14
RP-2, -8 and -18 (M)	Various mixtures of methanol, ethanol and other organic solvents with aqueous solutions	Alkylphenones, steroids, organic acids, fluoranthene	15
KC_{18} (W)	Methanol–water (80:20)	Aromatic hydrocarbons	16

* Letters in parentheses denote manufacturers: M = Merck; Q = Quantum Industries; W = Whatman.

recently, Kaiser and Rieder[7] remarked on the use of LiChrosorb RP-8- and RP-18-coated TLC plates and, subsequently, a number of papers dealing with the characteristics of plates pre-coated with commercially available reversed-phase HPLC packing materials, and their application, have been published (Table I).

The extreme fragility of home-made reversed-phase thin layers noted by Siouffi *et al.*[8] to-day no longer poses a problem. However, the pre-coated RP-8 layer is a much softer material than is either HPTLC or conventional TLC silica gel, and it does not adhere to the glass as well as does silica gel[1]. Another noteworthy aspect is that for plates pre-coated with LiChrosorb RP-2, -8 or -18 the times of run are stated[1,13,15] to increase very rapidly with increasing water content of the mobile phase. Such problems do not arise to the same extent with KC_{18} plates[12,13].

In this study, work has mainly been directed at a comparison of the characteristics of the various pre-coated chemically bonded thin-layer materials and at an evaluation of the applicability of reversed-phase HPTLC for different classes of compounds.

MATERIALS AND METHODS

Commercially available 10 × 10 cm thin-layer plates pre-coated with LiChrosorb RP-2, -8 or -18 and containing a fluorescent indicator were obtained from Merck (product Nos. 13726, 13725 and 13724, respectively; Merck, Darmstadt, G.F.R.);

5 × 20 cm pre-coated KC_{18} thin-layer plates containing C_{18} carbon chains bonded to the silica gel via Si–O–Si bonds were supplied by Whatman ($KC_{18}F$ plates, product No. 4803600; Whatman, Springfield Mill, Great Britain). For comparative studies, 20 × 20 cm RPS Uniplates, which contain a layer of a long-chain hydrocarbon-impregnated support (Analtech, Newark, Del., U.S.A.) and 20 × 20 cm silanized silica gel thin-layer plates (Kieselgel 60 F_{254} silanisiert, product No. 5747; Merck) were used. All plates were used without pre-treatment.

Phthalate esters, aminophenols, chloroanilines, polynuclear aromatic hydrocarbons, aromatic oxy compounds and aromatic acids were selected as model compounds. These, and all solvents and further chemicals used, were of analytical-reagent grade.

Preliminary chromatographic experiments were routinely carried out on small rectangular thin-layer plates of about 6.5 × 1.5 cm; final series of experiments were performed using 10 × 3 cm pieces. Spotting was effected as described in the next section. Ascending development was carried out in a Hellendahl jar or a normal-sized rectangular tank, saturation of the chromatographic chamber being achieved by lining it with filter-paper impregnated with the mobile phase. Hellendahl jars containing a 1-mm layer of mobile phase were used for two 6.5 × 1.5 cm plates, which were not conditioned prior to development. In the rectangular tank, up to six of the larger thin-layer plates were conditioned for 30 min. Next, the plates were lowered into a narrow trough containing a layer of filter-paper ribbons soaked with the mobile phase, thus starting the chromatographic process. Development was carried out over distances of 5 or 9 cm. Detection was generally effected by viewing the plates under 254-nm UV light. Densitograms were recorded using a Zeiss PMQII spectrophotometer (Zeiss, Oberkochen, G.F.R.) with a conventional densitometer attachment.

RESULTS AND DISCUSSION

Thin-layer plates

Thin-layer plates of the appropriate size (see above) were obtained by placing the commercially available plates, with their pre-coated side down, on a flat, hard surface, and making continuous scores with a conventional glass-cutter along a thick wooden ruler[17]. The slides were broken off at the edge of a flat bench. Although the LiChrosorb RP chemically bonded stationary phases are relatively soft materials (this does not hold for the KC_{18}, and other types of plates used), the cutting procedure could conveniently be performed with less than a 5% loss of material.

Sample application

As was stated in the Introduction, a problem encountered with some of the reversed-phase materials in HPTLC is their non-wettability by mobile phases containing more than about 20–40% of water. As a consequence, the spotting of samples dissolved in the appropriate mobile phase occasionally also poses problems. In order to circumvent this situation, the model compounds were routinely dissolved in a pure organic solvent. Since for all preliminary experiments small-sized spots were applied using a pointed paper-wick partly impregnated with the sample solution, a relatively high-boiling solvent, *viz.*, ethanol, was selected instead of the more conventional, but too volatile, acetone or dichloromethane. For the sake of convenience, ethanol was also used as the solvent in the larger-scale experiments. Here, a home-made glass capillary (Fig. 1) was employed, which delivers a constant volume of *ca.* 200 nl, which is the upper limit of the volume range recommended for HPTLC work. Construction details are given in the legend to Fig. 1. With the above spotting device, a repeatability of better than 3% (relative standard deviation) ($n = 6$) was obtained.

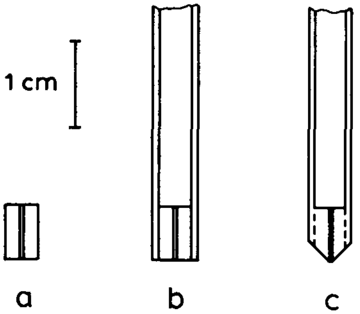

Fig. 1. Glass capillary for quantitative spotting of small sample volumes. A short piece of a very narrow glass capillary of appropriate dimensions (a) is welded to another, larger, glass tube which has an inner diameter slightly wider than the outer diameter of the glass capillary (b); as welding is done by gentle heating, the glass does not really melt and, consequently, the original shape of the capillary is maintained. Lastly, the conical shape of the spotting device (c) is obtained by means of grinding.

Comparison of bonded-phase materials

As a continuation of our previous work[1] on the use of plates pre-coated with LiChrosorb RP-8, some of the earlier series of experiments were repeated using LiChrosorb RP-2, -8 and -18 and KC_{18} as stationary phases. Table II summarizes data* on the dependence of the time of run on the mobile phase composition for both methanol–water and acetonitrile–water mixtures; the migration distance was 5 cm. It is clearly evident that, even when using a relatively non-viscous organic solvent such as acetonitrile, a proportion of 20–30% of water is the upper limit with all three LiChrosorb RP materials from the point of view of time of analysis. Although the mutual differences are small and insignificant, the time of run appears to increase in the order RP-18 < RP-2 < RP-8. This does not agree with the findings of Siouffi *et al.*[8], who reported the sequence RP-8 < RP-18. However, it should be noted that the RP-8 material used by us was ordered about 1 year earlier than were the RP-2- and -18-coated plates. Hence, a minor change in the production process or an ageing effect may explain the slightly surprising sequence observed by us.

More important, Table II indicates that with the KC_{18} plates the times of development typically are 3–4 times shorter than they are with the LiChrosorbs; a similar observation has recently been made by Gonnet and Marichy[13]. Unfortunately, however, with proportions of water of over about 40%, the pre-coated KC_{18} thin layers tend to swell and/or flake off, which prevents normal development of the

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* One should note that with acetonitrile (but not methanol) the times of run reported for RP-8 are considerably shorter than those recorded in ref. 1. This is due to the fact that in the earlier work, an unsaturated instead of a saturated chamber was used for development.

TABLE II

DEPENDENCE OF TIME OF RUN ON MOBILE PHASE COMPOSITION FOR VARIOUS CHEMICALLY BONDED STATIONARY PHASES

Conditions: 5-cm run; saturated chamber. Asterisks denote: $t_{run} > 50$ min.

Bonded-phase material	*t_{run} (min) for mobile phases having x vol.-% of organic solvent*					
	60	*75*	*80*	*85*	*90*	*95*
	Methanol					
RP-2	*	*	40	34	27	24
RP-8	*	*	48	34	28	19
RP-18	*	*	42	34	24	16
KC_{18}	30	14	11	9	8	6
	Acetonitrile					
RP-2	*	33	11	9	8	7
RP-8	*	*	12	10	9	6
RP-18	*	18	10	8	7	6
KC_{18}	15	4.5	4	3.5	3	2.5

mobile phase. On the basis of observations made by Sherma and Beim[11], we successfully overcame this problem by adding 3% of sodium chloride (resulting in a 0.5 *M* solution) to the solvent mixtures used as the mobile phase. The time of development was then virtually constant at between 10 and 15 min, irrespective of the use of methanol or acetonitrile as the organic solvent and the proportion of water added; similar results were obtained when sodium chloride was replaced with an equimolar amount of calcium chloride. The water content was varied from 5 to 80% with methanol and from 25 to 80% with acetonitrile, because with the latter solvent the presence of 3% of sodium chloride (and also sodium bromide, ammonium bromide, lithium chloride or sodium nitrate) results in demixing with mixtures that contain less than 25% of water. With the LiChrosorb RP bonded phases, the addition of sodium chloride did not lead to a significant increase in migration rate, irrespective of the mobile phase composition selected.

Next, the chromatographic behaviour of the various stationary phases was compared using di-*n*-butyl and di-(2-ethylhexyl) phthalate as model compounds. From the experimental R_F values, R_M was calculated using the relationship

$$R_M = \log (1 - R_F)/R_F \qquad (1)$$

The dependence of R_M on mobile-phase composition is shown in Fig. 2. As is to be expected, the retention of the relatively apolar model compounds increases with increasing chain length of the LiChrosorb RP material, with KC_{18} showing a behaviour closely similar to that of RP-8. For a single HPTLC system, *viz.*, LiChrosorb RP-8/methanol–water, the dependence of R_M on the carbon chain length of (five) phthalate esters is as shown in Fig. 3. For all four mobile-phase compositions tested, straight-line relationships are obtained, which indicates a constant contribution of the CH_2 group to retention. The slope of the lines and therefore the selectivity increase with increasing percentage of water. With acetonitrile–water mixtures as the

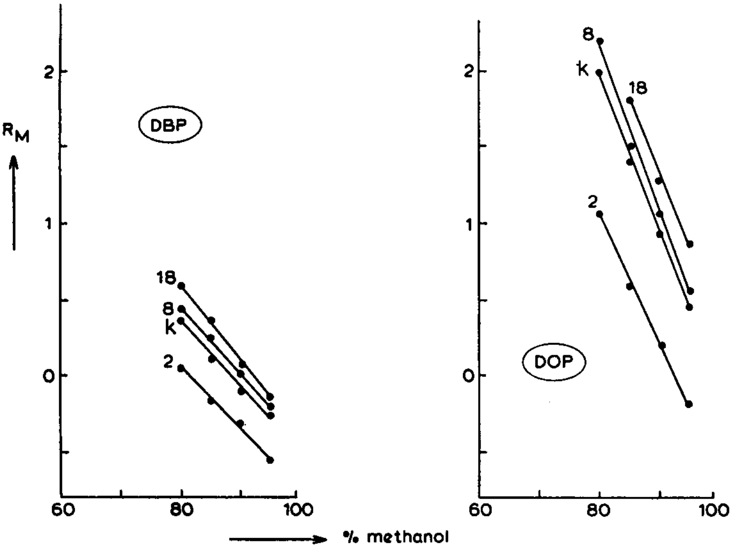

Fig. 2. Dependence of R_M on mobile-phase composition (methanol-water mixtures) for di-*n*-butyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DOP) using four different chemically bonded stationary phases.

mobile phase, straight-line relationships were also obtained, but the same slope was calculated irrespective of the proportion of water.

Thirdly, we should emphasize that, under comparable conditions, the spot sizes were similar with all four stationary-phase materials tested. For migration

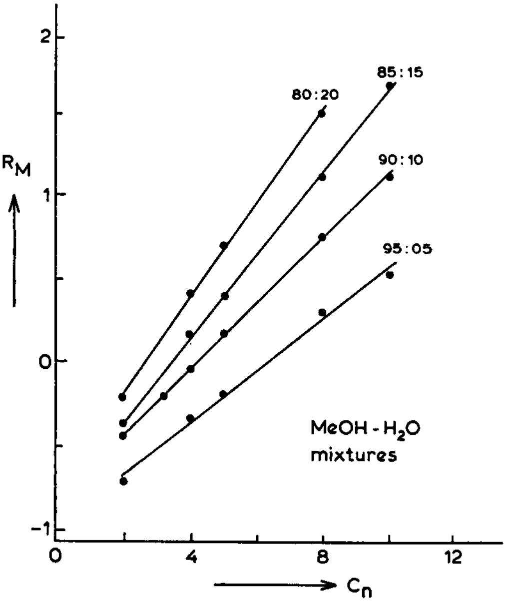

Fig. 3. Dependence of R_M on the carbon chain-length, C_n, for five phthalate esters in the system LiChrosorb RP-8/methanol-water. The ratios shown indicate the mobile-phase composition.

distances of between about 2 and 5 cm, plate heights (H), calculated from the relationship

$$H = \sigma_x^2 / z_x \tag{2}$$

where σ_x is the standard deviation of the spot peak and z_x the distance migrated by the spot, were typically 40–80 μm, without a correction for initial spot width having been applied.

Separations

As a demonstration of the potential of reversed-phase HPTLC, Fig. 4 shows the successful separation of a number of aromatic oxy compounds, aminophenols and aromatic acids. As all of these separations were performed using mobile phases containing only 20–30% of organic solvent, KC_{18} had to be used as the stationary phase material, and 3% of sodium chloride was added to the mobile phase. With mobile phases rich in organic solvent, KC_{18} can be replaced with LiChrosorb RP-8 or -18. This conclusion can also be drawn from Fig. 2 and is further confirmed by the separations of polynuclear aromatic hydrocarbons and chloroanilines shown in Fig. 5. It should be added that the retention on KC_{18} invariably was found to be lower than that on RP-18, and generally was closely analogous to that observed for RP-8. A similar conclusion can be read from the data reported in ref. 13.

Lastly, in Fig. 6, the separation of seven aromatic compounds by HPTLC on a KC_{18} plate is compared with that by HPLC on a 10-cm column packed with LiChrosorb RP-18. The orders of elution are identical and the overall resolution is about the

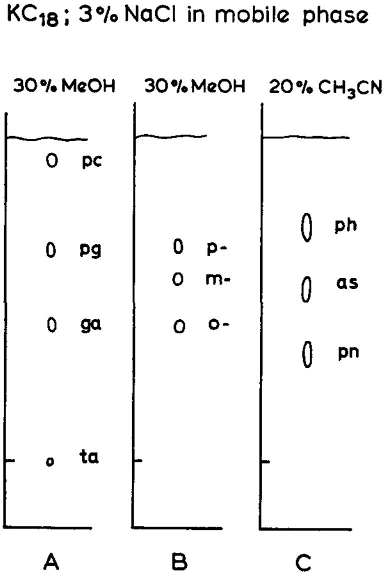

Fig. 4. HPTLC on KC_{18} plates using acetonitrile–water mixtures containing 3% of NaCl as mobile phase. Separation of (A) the aromatic oxy compounds pyrocatechol (pc), pyrogallol (pg), gallic acid (ga) and tannin (ta), (B) *o*-, *m*- and *p*-aminophenol (o-, m- and p-, respectively), and (C) the aromatic acids *p*-hydroxybenzoic acid (ph), acetylsalicylic acid (as) and *p*-nitrobenzoic acid (pn).

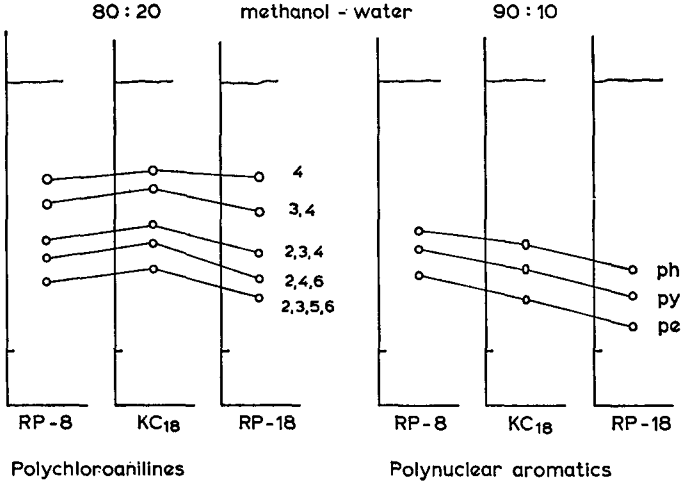

Fig. 5. HPTLC, on LiChrosorb RP-8 and -18 and KC_{18} plates, of chloroanilines and polynuclear aromatic hydrocarbons, using methanol–water (80:20) and methanol–water (90:10), respectively, as mobile phase. pe = Perylene; ph = phenanthrene; py = pyrene.

same in both systems, with biphenyl ether and naphthalene being slightly better separated in the HPTLC system, while the opposite is true for the pair biphenyl–biphenyl ether. Actually, from the experimental data a straight-line relationship

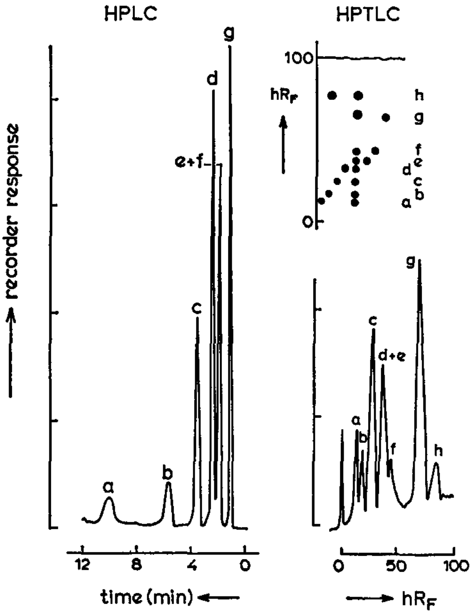

Fig. 6. HPLC (on 10-cm LiChrosorb RP-18 column) and HPTLC (9-cm run on KC_{18} plate) of seven aromatic compounds, using methanol–water (80:20) as mobile phase. Detection by means of 254-nm UV absorption (HPLC) or fluorescence diminution (HPTLC). Flow-rate in HPLC, 2 ml/min. a = Chrysene; b = pyrene; c = phenanthrene; d = biphenyl; e = biphenyl ether; f = naphthalene; g = *m*-dinitrobenzene; h = contaminant in pyrene.

between the capacity ratio in HPLC, k'_{HPLC}, and the capacity ratio in HPTLC, $k'_{\mathrm{HPTLC}} = (1 - R_F)/R_F$, could be calculated:

$$k'_{\mathrm{HPLC}} = 1.6\,k'_{\mathrm{HPTLC}} - 0.25 \qquad (3)$$

The regression coefficient was calculated to be $r = 0.990$ (all seven compounds) or $r = 0.9985$ (biphenyl ether omitted). Our R_F values were strikingly similar to those reported elsewhere for the same HPTLC system[16].

CONCLUSIONS

The results show the usefulness of reversed-phase HPTLC for the separation of various classes of both non-polar and polar compounds. With mobile phases (methanol–water or acetonitrile–water) containing 0–30% of water, thin-layer plates coated with a KC_{18} or a LiChrosorb RP chemically bonded stationary phase perform equally well. However, the KC_{18} plates will generally be favoured because of the relatively short times of run observed for systems involving their use. With mobile phases containing over about 30% of water, LiChrosorb RP-coated plates cannot conveniently be used owing to the non-wettability of the stationary phase. No such problem is encountered with the KC_{18} plates. They can be used irrespective of the proportion of water present in the mobile phase, provided that a small amount of sodium chloride or other salt is added in order to prevent dislodgement of the stationary phase from the glass backing.

For the sake of completeness, we should add that, in addition to the KC_{18} plates, two more types of pre-coated reversed-phase TLC plates are commercially available that can be used with mobile phases containing high proportions of water, *viz.*, Merck's silanized silica gel plates and Analtech's RPS Uniplates; however, these do not contain a high-performance chemically bonded stationary phase (see Materials

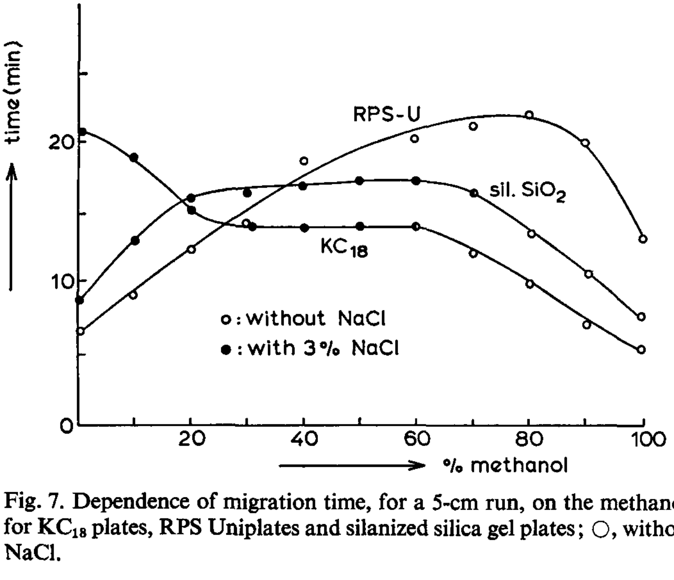

Fig. 7. Dependence of migration time, for a 5-cm run, on the methanol content of the mobile phase for KC_{18} plates, RPS Uniplates and silanized silica gel plates; ○, without added NaCl; ●, with 3% of NaCl.

and Methods). Plots of the dependence of development time, for a 5-cm run, on the methanol content of the mobile phase for all three types of plates are shown in Fig. 7. Three aspects are worthy of note. (1) Contrary to the information provided by the manufacturers[18], the silanized silica gel plates can be used even at high water contents, again provided 3% of sodium chloride is added. (2) With the recently introduced Uniplates, the addition of salt is superfluous; actually, the plate performance slightly deteriorated without the time of run decreasing. (3) With mobile phases containing over about 30% of methanol, use of the KC_{18} plates provides the highest speed of analysis.

Lastly, we mention that preliminary series of experiments with polynuclear aromatic hydrocarbons and aromatic oxy compounds indicate that the plate performance of the silanized silica gel plates is poorer than that of the KC_{18} plates and the RPS Uniplates. Current work in our laboratory is directed at the further evaluation of the characteristics of the latter two types of plates, which display about equal chromatographic efficiencies. However, already it can be stated that their introduction has allowed reversed-phase (HP)TLC to take a great step forward and that the major problem noted so far, the use of mobile phases with high water contents, has been adequately solved for at least one chemically bonded stationary phase material.

ACKNOWLEDGEMENTS

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