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Comparative study of chromatography on thin layers impregnated with organic stationary phases

Chromatographic separation of nitrophenols

J. GASPARIČ*
Faculty of Pharmacy, Charles University, CS-501 65 Hradec Králové (Czechoslovakia)
and
J. SKUTIL
East Bohemian Chemical Works, CS-532 17 Pardubice-Semtín (Czechoslovakia)
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ABSTRACT

The chromatographic behaviour of nitrophenols on thin layers of silica gel and cellulose was compared, both without impregnation and impregnated with non-aqueous polar stationary phases (formamide, dimethylformamide) and less polar stationary phases (liquid paraffin, octan-1-ol, 1-bromonaphthalene). Cellulose is preferred when using formamide or dimethylformamide if a pure partition process is required. For each particular analyte a certain amount of the stationary phase is always required to suppress the adsorption activity of silica gel. Separation by reversed-phase thin-layer chromatography is strongly affected by the type of stationary phase (the possibility of forming charge-transfer complexes with 1-bromonaphthalene) and its support (the acidic properties of silica gel and its adsorption activity), the mobile phase (content of organic modifier, pH, presence of salts) and the properties of the solutes (polarity, ionizability).

INTRODUCTION

Much work was carried out in the 1960s in which thin layers impregnated with an organic stationary phase were used for analytical separations (for reviews, see Grassini-Strazza *et al.* [1] and Gasparič [2]). These layers were later largely replaced in analytical work by chemically bonded phases [3]. However, reversed-phase thin-layer chromatography (RP-TLC) became a simple method of obtaining quantitative information about the hydrophobic character of biologically active compounds in the study of quantitative structure activity relationships (QSAR) [4], and impregnated layers continued to be routinely, and often mechanically, used by medicinal chemists without considering the possible complications that could affect the partition mechanism [5].

These studies were based on the determination of the R_M values of the tested compounds on thin layers impregnated with a less polar stationary phase (such as

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octan-1-ol, liquid paraffin or silicone oil). Aqueous methanol or acetone were used as mobile phases. Buffer solutions were sometimes used instead of pure water. Straight-phase chromatography on layers impregnated with a non-aqueous polar stationary phase (dimethylformamide) were also used for this purpose [6]. It has been shown that the conditions of a pure partition mechanism required for these measurements are often strongly affected by the properties of the support used (silica gel, cellulose, Kieselguhr), the stationary and mobile phases and the properties of the solute [2]. The interaction of polar solutes with active sites on the support can sometimes be stronger than with the non-polar stationary phases; this is observed with sulphonamides and sulphonated azo dyes [7]. These two groups of compounds migrate in an identical manner on silica gel and cellulose layers either without impregnation or impregnated with octan-1-ol.

This work investigated the behaviour of relatively polar model compounds and considered the problems encountered with ionisable compounds in RP-TLC on impregnated layers.

EXPERIMENTAL

Chemicals

The nitrophenols used in this study have been described previously [8,9]. All liquids used as stationary phases and the mobile phases components were of analytical-reagent grade. Britton–Robinson buffer solutions of pH 3, 8 and 10 were prepared according to Sýkora and Zátka [10].

Thin-layer chromatography

All experiments were carried out on Silufol (silica gel) and Lucefol (cellulose) ready-made 15 × 15 cm sheets (Kavalier, Votice, Czechoslovakia). Experiments were also carried out with Kieselgel 60 "Fertigplatten" (E. Merck, Darmstadt, Germany) for comparison. The layers were impregnated by the dipping technique. The following solutions were used for impregnation: 1–40% solutions of dimethylformamide in ethanol, 1–20% solutions of formamide in ethanol, a 5% solution of 1-bromonaphthalene in chloroform, a 10% solution of liquid paraffin in *n*-hexane and 10% solutions of octan-1-ol or dodecan-1-ol in ethanol. After immersing the layers into the solutions, the sheets were dried vertically by standing on a pack of filter paper (the edge with the starting line at the bottom) for 10 min so that any excess impregnating solution was removed and the auxiliary solvent evaporated. Nitrophenols were applied in 5–10 μg amounts dissolved in ethanol or benzene. The development was carried out in glass tanks. The mobile phases used in the RP systems were always saturated with the stationary phase. Detection was carried out by exposing the developed and dried chromatogram to ammonia vapour for several seconds (until the appearance of a yellow colour).

The R_f values obtained on layers impregnated with formamide and the literature pK_A values of the nitrophenols are given in Table I. The behaviour of nitrophenols in RP systems was represented as "profiles" to enable a fast optical evaluation.

TABLE I

R_F AND pK VALUES OF NITROPHENOLS

Mobile phase: benzene–acetic acid (95:5).

No.	Phenol	pK_A[a]	R_F		
			TL_1[b]	TL_2[b]	TL_3[b]
1	2-Nitrophenol	7.17	0.83	0.92	0.99
2	3-Nitrophenol	8.28	0.21	0.14	0.20
3	4-Nitrophenol	7.15	0.17	0.11	0.14
4	2,3-Dinitrophenol	4.96	0.24	0.10	0.14
5	2,4-Dinitrophenol	4.07	0.58	0.44	0.63
6	2,5-Dinitrophenol	5.2	0.68	0.70	0.92
7	2,6-Dinitrophenol	3.7	0.22	0.13	0.14
8	3,4-Dinitrophenol	5.42	0.11	0.09	0.10
9	3,5-Dinitrophenol	6.7	0.17	0.11	0.18
10	2,4,6-Trinitrophenol	0.38	0.33	0.04	0.00
11	2,4-Dinitro-6-methylphenol	4.70	0.76	0.85	0.97
12	2,4-Dinitro-3-methylphenol	4.0	0.20	0.11	0.18
13	2,4-Dinitro-6-*tert*.butylphenol	5.2	0.94	0.99	0.99
14	2,4-Dinitro-6-fluorophenol	2.40	0.54	0.10	0.09
15	2,4-Dinitro-6-chlorophenol	2.10	0.72	0.09	0.10
16	2,4-Dinitro-6-bromophenol	2.11	0.76	0.11	0.10
17	2,4-Dinitro-6-iodophenol	2.3	0.83	0.12	0.10
18	2,4-Dinitro-5,6-dimethylphenol	5.1	0.80	0.96	0.99
19	2,4-Dinitro-3,6-dimethylphenol	3.4	0.40	0.41	0.70
20	2,4-Dinitro-3,5-dimethylphenol	4.95	0.42	0.35	0.40
21	4-Nitro-2,6-dichlorophenol	3.1	0.31	0.13	0.30s
22	4-Nitro-2-chloro-6-bromophenol	3.1	0.35	0.14	0.25s
23	4-Nitro-2,6-dibromophenol	3.0	0.42	0.17	0.22
24	4-Nitro-2,6-diiodophenol	3.0	0.58	0.33	0.42s
25	2-Nitro-4,6-dichlorophenol	3.5	0.96	0.99	0.99
26	2-Nitro-4-chloro-6-bromophenol	4.8	0.97	0.99	0.99
27	2-Nitro-4,6-dibromophenol	5.4	0.97	0.99	0.99
28	2-Nitro-4,6-diiodophenol	2.4	0.98	0.99	0.99
29	2,4,6-Trinitro-3-methylphenol	–	0.35	0.06	0.02
30	2,4,6-Trinitro-3,5-dimethylphenol	–	0.41	0.09	0.10

[a] From Sergeant and Dempsey [11].

[b] TL_1 = Silica gel; TL_2 = silica gel impregnated with a 20% solution of formamide; TL_3 = cellulose impregnated with a 20% solution of formamide. s = Elongated spot.

RESULTS AND DISCUSSION

Straight-phase partition TLC with formamide as the stationary phase

This type of TLC using non-aqueous polar stationary phases was performed on layers of silica gel and cellulose impregnated with formamide. The impregnation was carried out with a 20% solution and established the conditions for the partition mechanism. Similar separations were obtained on both layers when benzene–acetic acid (90:5, v/v) was used as the mobile phase. The presence of acetic acid positively influenced the form of the spots of higher-acidity nitrophenols (tendency to tailing).

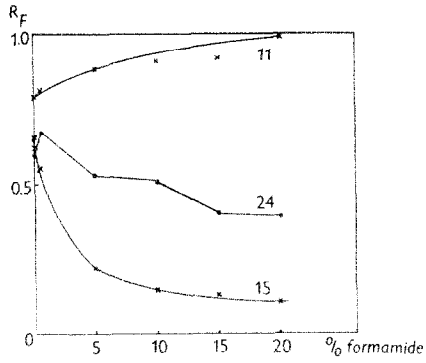

Fig. 1. Influence of degree of impregnation of silica gel with formamide on the mobility of nitrophenols. Mobile phase: benzene–acetic acid (95:5). See Table I for compound names.

The R_F values on silica gel were almost all lower than those on the cellulose layers. The same phenomenon had been observed previously for the phenoxyalkanoic acids and ascribed to adsorption [12]. Blanks on untreated layers using the same mobile phase showed that a very similar retention and separation of nitrophenols takes place on silica gel, whereas practically no interaction was observed with cellulose.

A certain amount of formamide is required in the layer to suppress the adsorption activity of the silica gel [13]. Therefore the response of individual nitrophenols to the gradual conversion of the adsorption mechanism into a partition mechanism was tested using chromatograms impregnated with 1–20% solutions of formamide. The results are summarized in Fig. 1. The R_F values of 2,4-dinitro-6-methylphenol increased due to deactivation of the silica gel, whereas a considerable decrease was seen in the R_F value of 2,4-dinitro-6-chlorophenol as a result of the increasing concentration of the stationary liquid. This must be taken into account if conditions for a pure partition system are to be established.

For comparison, 2-methoxyanthraquinone was chromatographed [14] on cellu-

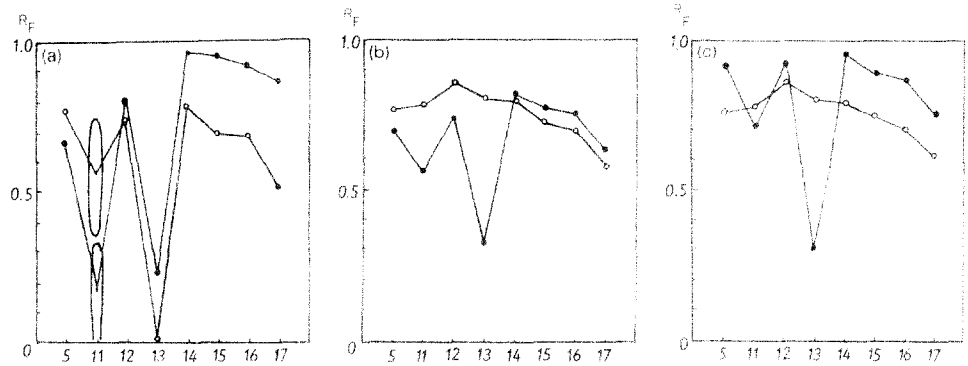

Fig. 2. Chromatograms on untreated layers of cellulose (○) or silica gel (●) using aqueous buffer solutions as mobile phases. (a) pH 3; (b) pH 8; (c) pH 10. See Table I for compound names.

lose and silica gel layers, both untreated and impregnated with 1–40% dimethylformamide using *n*-hexane as the mobile phase. The retention on untreated silica gel was strong (R_F 0.0) whereas on cellulose it was weak (R_F 0.70). A decrease of the R_F value was observed with increasing dimethylformamide content on cellulose owing to the gradual build-up of the partition system. On silica gel the R_F value increased under the same conditions as a result of successive deactivation of the sorbent and conversion to the partition system. At the highest degree of impregnation the R_F values were practically identical on both layers (0.25).

RP partition chromatography

Three types of less polar stationary phases were used to establish the conditions for RP chromatography: liquid paraffin, 1-bromonaphthalene and octan-1-ol (or dodecan-1-ol). Blank experiments were run for each mobile phase using untreated layers of both silica gel and cellulose to detect any interaction of the nitrophenols with the sorbent. Chromatograms using untreated layers and aqueous buffer solutions of pH 3, 8 and 10 as mobile phases gave results showing a strong interaction of some nitrophenols (especially of 6-alkyl-2,4-dinitrophenols) with silica gel over the whole pH range studied (see Fig. 2a–c). The sequence of spots for 2,4-dinitrophenol, its 6-methyl and 6-*tert.*-butyl derivatives as well as of the 6-fluoro, 6-chloro, 6-bromo and 6-iodo derivatives corresponds with that expected in RP chromatography. This is in agreement with the definition of RP chromatography of Bij *et al.* [15] according to which the use of silica gel layers with a neat aqueous or water-rich hydro-organic mobile phase also falls into the category of RP chromatography.

The interaction with cellulose at pH 3 is practically as strong as with silica gel; it is, however, only slight at higher pH values at which nitrophenols are supposed to be dissociated. This can be explained by the fact that only undissociated molecules are retained. The different behaviour on silica gel and cellulose can also be explained by the lower actual pH value on the silica gel layers. It was observed [16,17] that when using a buffer solution as the mobile phase the pH difference at the origin and near the mobile phase front can be two pH units as a result of sorption of the cations onto the Si–OH sites during the flow through the layer.

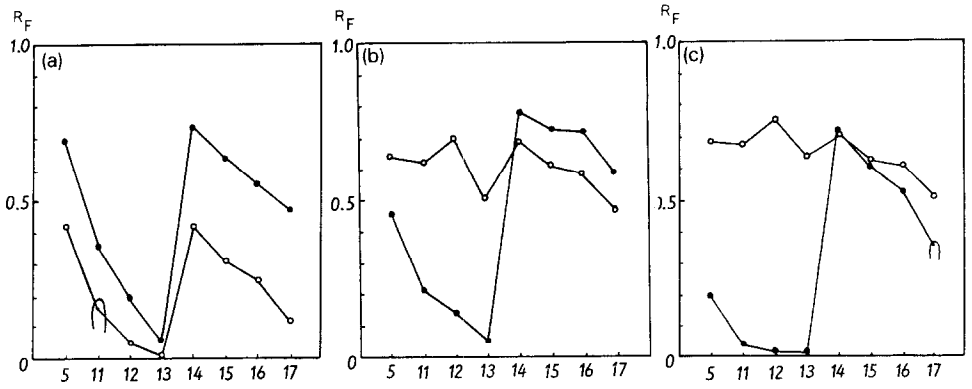

Fig. 3. Chromatograms on layers of cellulose (○) and silica gel (●) impregnated with octan-1-ol using aqueous buffer solutions as mobile phases. (a) pH 3; (b) pH 8; (c) pH 10. See Table I for compound names.

Impregnation with octan-1-ol brings about an increased retention on the silica gel layers which is practically identical at all three pH values studied. The retention on cellulose at pH 3 is stronger than that on the silica gel layers, but is very low at pH 8 and 10 (Fig. 3a–c).

Further experiments were carried out to compare liquid paraffin, 1-bromonaphthalene and octan-1-ol under the same conditions to determine whether the

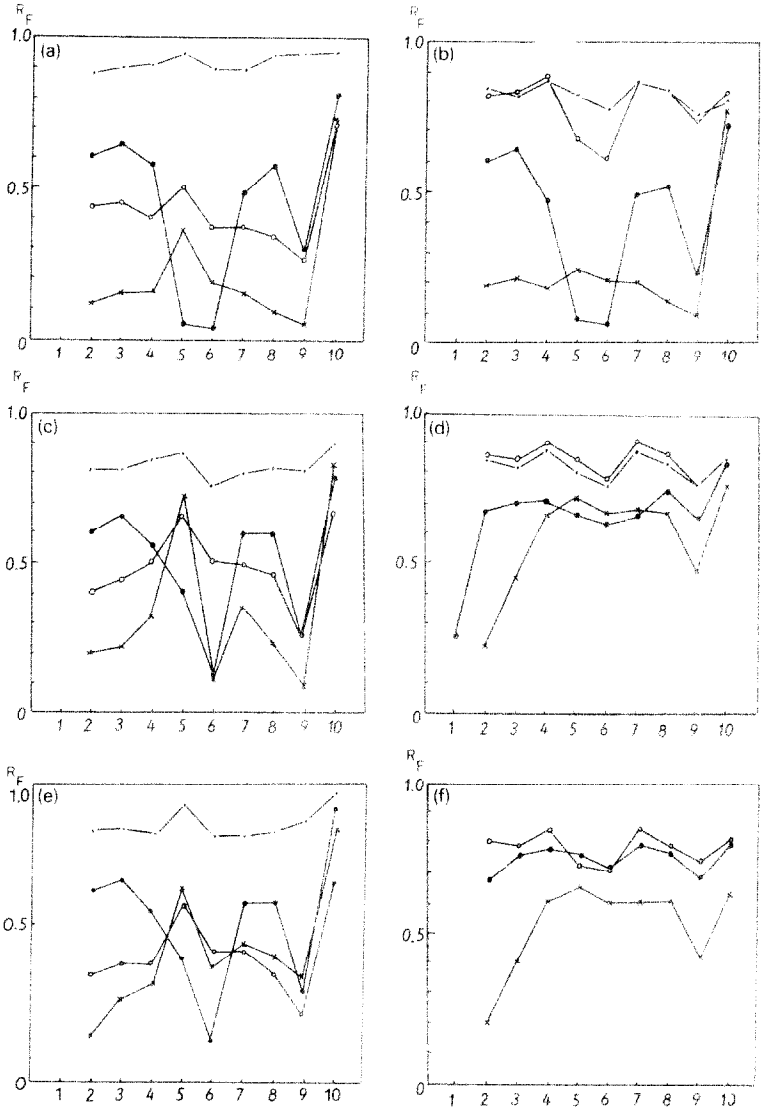

Fig. 4. Chromatograms on (—) untreated layers and impregnated layers with liquid paraffin (○), 1-bromonaphthalene (●) or octan-1-ol (x). Layers a, c, e = silica gel; b, d, f = cellulose. Mobile phases: a, b = buffer pH 3–ethanol (2:1); c, d = buffer pH 8–ethanol (2:1); e, f = buffer pH 10–ethanol (2:1). See Table I for compound names.

results can be influenced by the nature of the stationary phase. Ethanol was added to the mobile phase as an organic modifier to obtain reasonable R_F values. Mixtures (2:1) of the individual aqueous solutions with ethanol were always used saturated with the corresponding stationary phase. The addition of the organic modifier moved the spots on the untreated layers (blanks) to higher values. No significant difference between silica gel and cellulose was observed. The presence of the organic modifier suppressed the adsorption.

Results obtained for individual supports, stationary liquids and particular pH values of the mobile phases differ significantly (Fig. 4a–f). Thus, retention on layers impregnated with liquid paraffin is only observed on silica gel at pH 3, 8 and 10. On cellulose layers retention is observed only at pH 3; at pH 8 and 10 it is practically zero. The results on silica gel layers impregnated with octan-1-ol (dodecan-1-ol gave practically the same results) are similar to those of liquid paraffin. On cellulose layers there is a similar retention only at pH 3; there is practically no retention at pH 8 and 10 as seen with liquid paraffin.

The impregnation of silica gel layers with 1-bromoanphthalene and the mobile phase with pH 3 buffer gives a strong retention and the sequence of nitrophenols significantly differs from that obtained with other stationary phases. This indicates that another mechanism must be involved. This can be explained by charge-transfer complex formation, which is a known interaction between polycyclic hydrocarbons and nitro compounds often used in chromatography [18–20]. The change of mobile phase pH from 3 to 8 and 10 does not significantly change the behaviour of nitrophenols, with the exception of 2,4-dinitrophenol. The results for cellulose layers and the pH 3 mobile phase are practically identical with those obtained with silica gel; there is, however, decreased retention and separation efficiency at pH 8 and 10, similar to that seen with octan-1-ol and liquid paraffin as stationary phases. The application of 80% acetic acid as the mobile phase [8] results in analogous retention and separation on both silica gel and cellulose layers (see Fig. 5a and b).

A TLC system using impregnated layers is very complicated. Cellulose should be preferred when using formamide or dimethylformamide as the stationary phase if a pure partition process is required. For each particular analyte a certain amount of

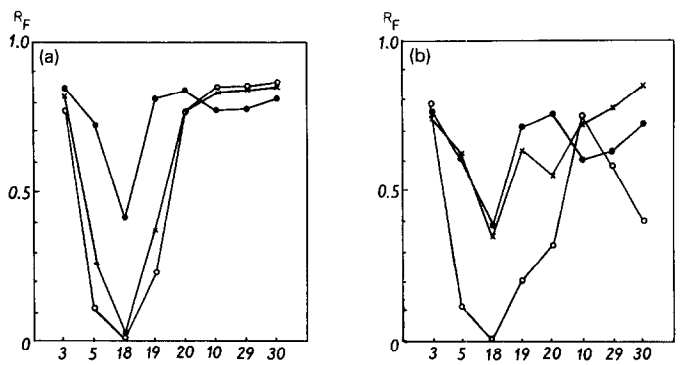

Fig. 5. Chromatograms on layers of (a) silica gel and (b) cellulose impregnated with 1-bromonaphthalene. Mobile phases: ● = 80% acetic acid; ○ = buffer pH 3–ethanol (2:1); x = buffer pH 8–ethanol (2:1). See Table 1 for compound names.

stationary phase is required to suppress the adsorption activity of the silica gel. RP-TLC results are strongly affected by the type of stationary phase (*e.g.* the possibility of charge-transfer complex formation with 1-bromonaphthalene) and its support (the acid properties of silica gel and its adsorption activity), the mobile phase (content of organic modifier, pH, presence of salts) and the properties of the solutes (polarity, ionizability). Mutual interactions between these constituents of the RP-TLC system can considerably affect the results.

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