Note

Dual retention mechanism on commercial acetylcellulose as a stationary phase in planar chromatography[*]

JIŘÍ GASPARIČ

Faculty of Pharmacy, Charles University, Heyrovsky 1203, 501 65 Hradec Králové (Czechoslovakia)

(Received July 11th, 1988)

Acetylated paper was originally introduced by Koštíř and Slavík[1] as a carrier of less polar stationary phases in reversed-phase paper chromatography. Later, reversed-phase separations were achieved using acetylated paper alone as a stationary phase. Similarly, layers of acetylated cellulose powders were found suitable for reversed-phase thin-layer chromatography (TLC). Both the papers and powders are prepared by converting cellulose into its acetate esters. Up to three hydroxyl groups per cellulose structural unit can be esterified. The acetyl content may vary from 6% up to a maximum of *ca.* 45%, the latter value corresponding to the triacetate. A gradual change from the "aqueous" to the "organic non-polar" stationary phase can thus be effected through variation of the extent of acetylation[2]. Thus, acetylated cellulose is in principle suitable for the separation of lipophilic substances and good separations have been achieved in the case of aromatic hydrocarbons[2,3], disperse dyes[2], antioxidants[2], etc.

Commercially available papers and powders are manufactured with an acetyl content up to 37%. Because of the incomplete acetylation it is supposed that the free hydroxy groups can also exhibit some interactions with the chromatographed compounds. A similar "dual retention mechanism" was observed in the case of less polar and polar organic compounds chromatographed on cellulose paper or on silica gel layers impregnated with less polar organic stationary phases: polar organic compounds behaved as if no stationary liquid were present, whereas non-polar substances were chromatographed according to the rules common for reversed-phase chromatography[4,5]. Therefore, we tried to show that the commercially available acetylcellulose materials represent a dual stationary phase, *i.e.*, acetylcellulose as the stationary phase for reversed-phase chromatography of lipophilic compounds and cellulose for normal-phase chromatography of hydrophilic compounds.

MATERIALS AND METHODS

All dyes chromatographed were standard substances from our collection. Solvents used as mobile phases were of current reagent grade purity. Paper chromatogra-

---

[*] Presented at the *6th Danube Symposium on Chromatography, Varna, October 12–17, 1987.* The majority of papers presented at this symposium have been published in *J. Chromatogr.*, Vol. 446 (1988).

phy was carried out using FN 7 paper (Papierfabrik Niederschlag, G.D.R.) and acetylated paper MN Cell 300Ac 10% (Macherey, Nagel & Co., Düren, F.R.G.). TLC was performed using commercial layers of microcrystalline cellulose Lucefol quick® (Sklárny Kavalier, Votice, Czechoslovakia) and of acetylated cellulose MN Polygram cel 300Ac-10 (Macherey, Nagel & Co). All chromatograms were developed by ascending development using C_1–C_4 alcohols in mixtures with water or ammonia, as the mobile phases.
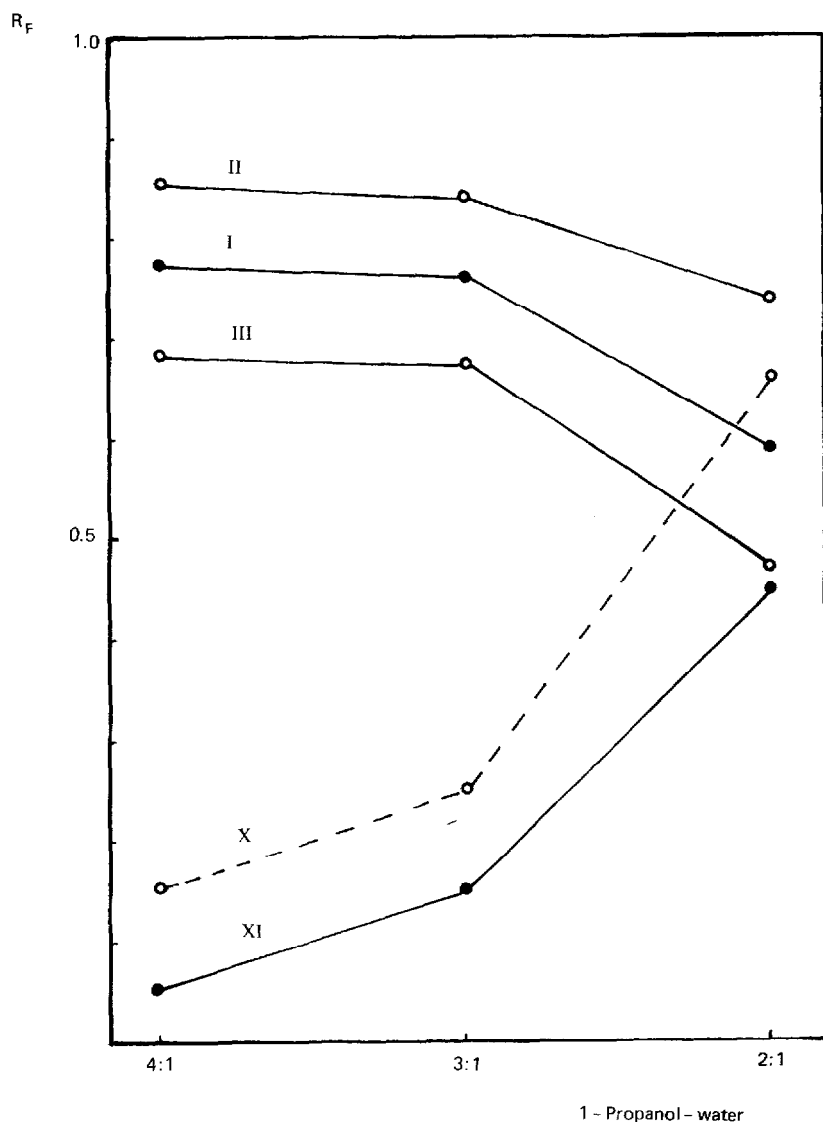

Fig. 1. Influence of the 1-propanol water ratio on the retention of lipophilic dyes I-III and hydrophilic dyes X and XI.

TABLE I

R_F VALUES OF MODEL DYES ON CELLULOSE AND ACETYLATED CELLULOSE MATERIALS

PC = Paper chromatography; ROH = aliphatic alcohol; C = cellulose; Ac = acetylated cellulose.

| | Substituent | | | | TLC/80% ROH | | | | | | | | TLC/1-propanol-water | | | | | | PC/ROH–NH₃ (2:1) | | | |
| | X_1 | X_2 | X_3 | X_4 | Methanol | | Ethanol | | 1-Propanol | | 1-Butanol | | 4:1 | | 3:1 | | 2:1 | | Ethanol | | 1-Butanol | |
					C	Ac	C	Ac	C	Ac	C	Ac	C	Ac	C	Ac	C	Ac	C	Ac	C	Ac
I	H	H	H	H	0.80	0.31	0.98	0.57	1.00	0.71	1.00	0.76	0.99	0.77	1.00	0.76	1.00	0.69	0.99	0.70	0.98	0.74
II	C₅H₁₁	H	H	H	0.58	0.16	0.90	0.51	1.00	0.84	1.00	0.84	1.00	0.85	1.00	0.84	1.00	0.74	0.99	0.84	0.98	0.86
III	C₆H₅	H	H	H	0.45	0.12	0.89	0.37	1.00	0.67	1.00	0.70	1.00	0.68	1.00	0.67	1.00	0.47	0.99	0.48	0.98	0.50
IV	H	H	SO₃H	H	0.80	0.87	0.92	0.96	0.92	0.89	0.80	0.74	0.92	0.92	0.89	0.95	0.96	0.93	0.86	0.81	0.83	0.72
V	C₅H₁₁	H	SO₃H	H	0.83	0.84	1.00	0.99	0.99	0.97	0.98	0.90	0.98	0.98	0.99	0.98	1.00	0.99	0.95	0.90	0.92	0.86
VI	C₆H₅	H	SO₃H	H	0.83	0.78	0.91	0.90	0.98	0.89	0.99	0.85	0.97	0.93	0.98	0.95	0.98	0.93	0.86	0.66	0.83	0.58
VII	H	SO₃H	SO₃H	H	0.80	0.84	0.69	0.80	0.35	0.50	0.05	0.12	0.32	0.43	0.39	0.50	0.65	0.84	0.72	0.89	0.62	0.82
VIII	C₅H₁₁	SO₃H	SO₃H	H	0.83	0.85	0.93	0.97	0.63	0.82	0.20	0.50	0.60	0.85	0.64	0.87	0.84	0.97	0.51	0.73	0.46	0.60
IX	C₆H₅	SO₃H	SO₃H	H	0.77	0.77	0.62	0.77	0.28	0.45	0.05	0.10	0.24	0.32	0.34	0.82	0.83	0.93	0.47	0.48	0.42	0.48
X	H	SO₃H	SO₃H	SO₃H	0.78	0.82	0.55	0.68	0.12	0.18	0.01	0.01	0.07	0.15	0.16	0.25	0.45	0.66	0.20	0.40	0.23	0.49
XI	SO₃H	SO₃H	SO₃H	SO₃H	0.70	0.77	0.38	0.60	0.05	0.07	0	0	0.04	0.05	0.10	0.15	0.36	0.45	0.15	0.30	0.09	0.44

RESULTS AND DISCUSSION

A series of eleven azo dyes of the basic structure I was used as model compounds. Dyes I–III represent compounds of lipophilic character, the lipophilicity of the basic compound I being increased by the presence of 1-pentyl or phenyl groups, in compounds II and III. Compounds IV–XI are hydrophilic. Their hydrophilicity increases with the number of sulpho groups, *i.e.*, in the sequence IV < VII < X < XI, whilst it is slightly decreased by the presence of the 1-pentyl or phenyl groups in compounds V, VI, VIII and IX. This series of compounds was simultaneously chromatographed on cellulose and acetylcellulose materials using the same mobile phases, *i.e.*, C_1–C_4 alcohols in mixtures with water or ammonia. The R_F values thus obtained are summarized in Table I.

The hydrophilic sulphonated compounds migrate on both materials in the same sequence, *i.e.*, their R_F values are decreased in the series IV > VII > X > XI. The sequence of the unsubstituted compound and that with the 1-pentyl and phenyl groups is also the same on both stationary phases. The increasing chain length of the alcohol in the mobile phase brings about a significant decrease in the R_F values with a reproducible exception in the case of methanol. The increasing water content of the mobile phase containing 1-propanol as the organic modifier is manifested by an increase in R_F values. All these phenomena are common to both the cellulose and acetylcellulose materials and are in accordance with the rules of normal (straight) phase chromatography. The absolute R_F values cannot reliably be compared since the corresponding cellulose and acetylcellulose materials are not from the same supplier. However, R_F values of higher sulphonated compounds are observably higher on acetylcellulose than on cellulose.

The lipophilic dyes I–III behave on cellulose and acetylcellulose as expected, in a quite different way. They migrate on cellulose with the solvent front in the case of all aqueous alcohols as mobile phases, with the exception of methanol. In 80% methanol as the mobile phase, however, their behaviour is similar to that in a reversed-phase system! Their retention on acetylated cellulose with 80% methanol or ethanol is as expected in reversed-phase chromatography, *i.e.*, it is decreased in the series I > II > III. On the other hand, the R_F values of these dyes decrease in the series II > I > III when 1-propanol or 1-butanol is used in the mobile phase. This sequence is not

TABLE II

R_F VALUES OF ALKYLPYRIDINIUM CHLORIDES USING 1-BUTANOL SATURATED WITH AMMONIA AS THE MOBILE PHASE

According to Kabil and Prey[6]. C = S + S 2043b paper; Ac = S + S 2043b acetylated paper.

Compound	R_F	
	C	Ac
Ethylpyridinium chloride	0.12	0.16
1-Butylpyridinium chloride	0.31	0.28
1-Octylpyridinium chloride	0.62	0.08
1-Dodecylpyridinium chloride	0.73	0.08
1-Cetylpyridinium chloride	0.89	0.06

ANALYTE

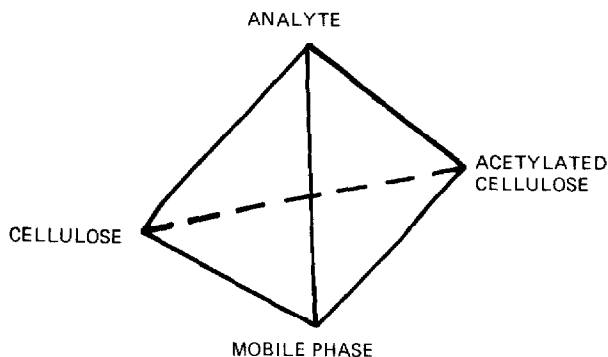ACETYLATED
CELLULOSE

CELLULOSE

MOBILE PHASE

Fig. 2. Schematic representation of the chromatographic system using acetylated cellulose materials.

characteristic for a reversed-phase system. The increasing water content in the mobile phase containing 1-propanol brings about a decrease in the R_F values of the lipophilic compounds. The comparison of the response of lipophilic and hydrophilic compounds to the increasing water content in the mobile phase is illustrated also in Fig. 1.

A very interesting example which is in good accordance with our results can be found in the literature though the authors did not comment it: in 1958 Kabil and Prey[6] chromatographed a homologous series of alkylpyridinium salts on cellulose and acetylcellulose papers. The R_F values of those compounds increased with increasing length of the alkyl group on cellulose paper, whereas on acetylated cellulose paper the R_F values increased only up to the $\sim C_6$ alkyl compound and began then to decrease with increasing length of the alkyl chain (see Table II).

It can be concluded from our results that, due to incomplete acetylation, two stationary phases must be considered in commercial acetylated materials: cellulose and acetylated cellulose. Using one mobile phase, the analytes prefer one of these materials according to their hydrophilicity or lipophilicity. Thus, the hydrophilic analytes are separated according to the rules of normal (straight) phase chromatography and the lipophilic ones according to the rules of reversed-phase chromatography. There is also some influence of the organic modifier in the mobile phase. Some anomalies were observed using 80% methanol on cellulose and 1-propanol and 1-butanol on acetylated cellulose. Therefore the system considered is a complex one as illustrated in Fig. 2. This phenomenon is quite analogous to the action of free silanol groups in silica-bonded hydrocarbonaceous stationary phases in reversed-phase high-performance liquid chromatography[7-9].

REFERENCES

1 J. V. Koštíř and K. Slavík, Collect. Czech. Chem. Commun., 15 (1950) 17.
2 P. Wollenweber, in E. Stahl (Editor), Thin-layer Chromatography. A Laboratory Handbook, Springer, Berlin, Heidelberg, New York, 2nd ed., 1961, p. 37.
3 J. Gasparič and J. Churáček, Laboratory Handbook of Paper and Thin-layer Chromatography, Ellis Horwood, Chichester, 1978, p. 89.
4 J. Gasparič, J. Chromatogr., 47 (1970) 51.
5 J. Gasparič, J. Chromatogr., 196 (1980) 391.
6 A. Kabil and V. Prey, Monatsh. Chem., 87 (1956) 625.
7 A. Nahum and Cs. Horváth, J. Chromatogr., 203 (1981) 53.
8 K. E. Bij, Cs. Horváth, W. R. Melander and A. Nahum, J. Chromatogr., 203 (1981) 65.
9 T. Welsh and H. Frank, Chromatographia, 19 (1984) 457.