

PAPER CHROMATOGRAPHY OF NITRATE AND TOSYLATE ESTERS

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Several paper chromatographic solvent systems containing methanol have been reported for the separation of hydrophobic substances. RICE, KELLER AND KIRCHNER¹ described the paper chromatography of 3,5-dinitrobenzoates of various alcohols using 2% methanol in petroleum ether. MEIGH² followed up this work but used *n*-heptane saturated with methanol. BUSH³, apparently independently, used methanol-containing solvents for the paper chromatography of steroids. These procedures used conventional filter paper without pretreatment as the support medium and the solvent was irrigated downwards after allowing the spotted paper to equilibrate in the vapours of the two-phase organic solvent for 4 to 18 hours. In the present work several nitrate and tosylate esters of polyhydroxy compounds were found to chromatograph well in this type of system. Although neither of these two types of compounds could be chromatographed successfully on paper with aqueous-organic solvents it was possible to obtain good separation within a group of diastereoisomers with the methanol-alkane system.

Paper chromatography, as generally practised, is only suitable for substances which are more or less hydrophilic. The stationary phase in these systems consists of the aqueous part of the solvent sorbed on the cellulose and will be hydrophilic. A compound must be at least partially soluble in the stationary phase in order for it to move with an R_F value of less than 1.0. In the case of the solvent systems containing methanol, hydrocarbon, and very little water it is probable that the stationary phase will consist mainly of methanol and hence will be less hydrophilic.

Several methanol-hydrocarbon solvent systems (Table I) were tested with the nitrate and tosylate esters of the three diastereoisomeric 1,4:3,6-dianhydrohexitols (isohexides). *n*-Hexane or petroleum ether, b.p. 60–75°, saturated with methanol was found to be the most useful system for the chromatography of the isohexide dinitrates and clear separations were obtained. The corresponding tosylate esters did not move from the starting line in this solvent but could be separated when benzene was added to the system. Three series of variations in the solvent system were tested:

(a) Variation of the hydrocarbon in the methanol-alkane system was found to affect the R_F values of the nitrate esters (Table II). An increase in the length of the

TABLE I
COMPOSITIONS OF SOLVENT SYSTEMS (v/v) *

System	Alkane**	Benzene	Methanol	Water
A	20	-	10	-
B	20	-	20	-
C	20	-	20	-
D	20	-	9	1
E	20	-	8	2
F	20	-	7	3
G	18	2	8	2
H	16	4	8	2
I	16	4	9	1

* The proportions of hydrocarbon to methanol-water are not critical provided two layers are obtained. The proportions of alkane to benzene and of methanol to water are critical.

** *n*-Hexane or petroleum ether, b.p. 60-75°, except for B (*n*-heptane) and C (*n*-octane).

TABLE II
EFFECT OF HYDROCARBON ON R_F VALUES OF ISOHEXIDE DINITRATES

Solvent system	Alkane	Isomannide dinitrate	Isosorbide dinitrate	Isoidide dinitrate
A	<i>n</i> -Hexane	0.20	0.26	0.42
B	<i>n</i> -Heptane	0.12	0.175	0.31
C	<i>n</i> -Octane	0.07	0.10	0.20

alkane chain caused a decrease in R_F value although similar separation patterns for the isomers were obtained in each solvent.

(b) Absolute methanol was completely miscible with petroleum ether but addition of very small amounts of water caused separation into two layers. Since it was more convenient to have water present in the system a series of runs was made to determine the effect of the addition of different amounts of water (Table III). As the proportion of water in the methanol was increased, the R_F values of the nitrate esters were increased.

TABLE III
EFFECT OF WATER ON R_F VALUES OF ISOHEXIDE DINITRATES

Solvent system	Ratio water: methanol	Isomannide dinitrate	Isosorbide dinitrate	Isoidide dinitrate
A	0:1	0.20	0.26	0.42
D	1:9	0.18	0.26	0.43
E	2:8	0.25	0.34	0.54
F	3:7	0.50	0.59	0.72

(c) Addition of benzene to the solvent system increased the R_F values for both types of esters (Table IV). While the tosylate esters did not chromatograph in the methanol-alkane system, the addition of benzene brought about a clear separation. The effect of water on the benzene-containing system was the same as for the methanol-alkane system.

TABLE IV
EFFECT OF ADDED BENZENE ON R_F VALUES

Ratio water: methanol	Ratio benzene: alkane	Solvent system	Isomannide dinitrate	Isosorbide dinitrate	Isoidide dinitrate	Isomannide ditosylate	Isosorbide ditosylate	Isoidide ditosylate
0:1	0:1	A	0.20	0.26	0.42	0.0	0.0	0.0
2:8	1:9	G	0.34	0.45	0.60	0.18	0.36	0.58
2:8	2:8	H	0.49	0.57	0.68	0.43	0.58	0.66
1:9	2:8	I	0.44	0.51	0.63	0.24	0.40	0.55

The effects of the variations in the solvent system described above could be readily explained in terms of the solubilities of the esters in the stationary and mobile phases. The nitrate esters were less soluble in the higher alkanes and so less soluble in the mobile phase and a lower R_F was found. Addition of water, which would go mainly to the stationary phase, decreased the solubility in this phase and thus increased the R_F value. Benzene increased the solubility in the mobile phase and again the R_F was increased.

It is apparent that this type of solvent system is capable of being varied to suit a fairly wide range of hydrophobic substances. Since standard filter paper may be used without pretreatment or impregnation, a considerable saving in time and material may be realised. For the nitrate esters solvent systems D and E (Table I) are recommended, and for tosylate esters system H. The R_F values were reproducible to ± 0.02 with these systems.

KITCHEN⁴ has studied the petroleum ether-methanol system using ascending irrigation. This method gave lower R_F values than the descending method and poorer separation; however, variations in the solvent system were not studied.

The two groups of compounds were detected on the developed and dried chromatograms by spraying with 1% alcoholic solution of diphenylamine, drying and exposing to short wave u.v. light for 10 min. The nitrate esters were observed as yellow spots in daylight or black spots under the u.v. light⁷. The tosylate esters were observed as strongly fluorescent spots under the u.v. light. The nitrate esters could also be detected by spreading a solution of diphenylamine in concentrated sulphuric acid over the paper supported on a glass plate. Deep blue spots⁸ were readily observed at nitrate locations but the paper disintegrated after about 15 min. In contrast, the photochemical detection method provided a permanent record.

The photochemical reaction may also be used to detect nitrate ion⁹ and diphenylamine and is being further investigated.

EXPERIMENTAL

Materials

All solvents were C.P. grade and were used without further purification. The nitrate and tosylate esters were identical with samples described previously^{5,6}. Whatman No. 1 filter paper was used throughout.

Procedure

Dishes containing the two layers of the solvent mixture were placed at the bottom of the chromatographic tank and the top trough was filled with the hydrocarbon layer. The filter paper sheet was spotted with the materials to be tested and fastened to a glass rod which was then suspended directly below the lid of the tank by strings passing through a hole in the lid or by a glass rod in a device similar to that described by POTTER, LINDAY AND CHAYEN¹⁰. The paper was held over the top trough for at least 4 h, then lowered into the solvent so that descending irrigation could take place. The solvent was fast running (about 20 cm/h) and after a suitable development time the paper was removed and the solvent front marked quickly before the solvent evaporated.

The paper was allowed to dry, sprayed with 1% alcoholic diphenylamine, dried and exposed to u.v. light for 5 to 10 min. The nitrate esters were detected as yellow spots on a white background in daylight or black on white under u.v. light. The tosylate esters were strongly fluorescent under the u.v. light.

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SUMMARY

Three water-insoluble diastereoisomeric nitrate esters were cleanly separated on untreated filter paper by the use of alkane-methanol solvents. Addition of benzene to the solvent system permitted a similar separation of the corresponding ditosylates. The effects on the R_F values of variations in the solvent compositions were readily explained on the basis of the ester solubilities. The method should be applicable to a wide range of hydrophobic substances.

A photochemical reaction of diphenylamine with the esters has been developed as a convenient method for detecting them on chromatograms.

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