PAPER CHROMATOGRAPHY OF STEROIDS IN SYSTEMS WITH ETHYLENE GLYCOL AS THE STATIONARY PHASE

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In paper chromatography of corticosteroids and 17-ketosteroids, the systems with more polar organic solvents, such as propylene glycol, formamide, dimethylformamide, phenylcellosolve, cellosolve, 1,3-butanediol, etc., in the stationary phase are routinely used.

Ethylene glycol in the stationary phase (ethylene dichloride as mobile phase) has been used as early as 1951 by BOSCOTT[1] for paper-chromatographic separation of urinary phenols and œstrogens. The chloroform/ethylene glycol system has been described for paper chromatography of cardenolides[2]. Ethylene glycol in the stationary phase for the separation of corticosteroids was first proposed for partition chromatography on columns[3,4], while recently it has been used for effective separations, especially of aldosterone, by paper chromatography, toluene being the mobile phase[5].

Systems with ethylene glycol have also been successfully used for the separation of 17-ketosteroids[6], œstrogens and corticosteroids[7]; their use will, therefore, be dealt with in some detail.

EXPERIMENTAL

Steroids in chloroform solution were applied to sheets of Whatman No. 1 paper (15 × 48 cm). After the application of the solution the strips were soaked in a 30% solution of ethylene glycol in methanol, blotted between filter paper and exposed to air at room temperature for 20 minutes. The chromatograms were then transferred to the chromatography tanks, saturated with vapours of the mobile phase, and developed by the descending technique at 17–20°.

For chromatographic separation of corticosteroids, toluene or carbon tetrachloride saturated with ethylene glycol, and for 17-ketosteroids petroleum ether (b.p. 45–65°) were used.

The steroids were detected in the usual ways, corticosteroids by U.V. photocopying or by treatment with a solution of blue tetrazolium, 17-ketosteroids with *m*-dinitrobenzene reagent.

RESULTS AND DISCUSSION

Ethylene glycol is only slightly soluble in carbon tetrachloride and toluene, and in comparison with propylene glycol only sparingly miscible with chloroform; consequently systems with ethylene glycol as the stationary phase may even be used for

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the separation of very polar steroids[1,2,5]. On the other hand it may be possible, by using an appropriate mobile phase, *e.g.* hexane or petroleum ether, to separate even less polar C_{19}-steroids.

The R_F values of some corticosteroids in the carbon tetrachloride/ethylene glycol and the toluene/ethylene glycol systems are shown in Table I, and those of some 17-ketosteroids in the petroleum ether/ethylene glycol system in Table II. In these

TABLE I

R_F VALUES AND MOBILITIES OF SOME CORTICOSTEROIDS AND ŒSTROGENS IN ETHYLENE GLYCOL SYSTEMS

Steroid	*Toluene/ethylene glycol*		*Carbon tetrachloride/ethylene glycol*	
	R_F	*Mobility (cm/20 h)*	R_F	*Mobility (cm/20 h)*
Tetrahydrocortisol		4.5		0.1
Tetrahydrocortisone		8.2		0.5
Cortisol	0.03	13.2	0.01	1.6
Aldosterone	0.07	18.9	0.08	19.0
Cortisone	0.10	25.3	0.10	26.2
11-Desoxycortisone	0.43		0.15	
Corticosterone	0.51		0.20	
11-Desoxycorticosterone	0.88		0.88	
Cortisone acetate	0.70		0.26	
11-Desoxycortisone acetate	0.85		0.40	
11-Desoxycorticosterone acetate	0.96		0.98	
Œstrone			0.50	
Œstradiol			0.12	

TABLE II

R_F VALUES OF KETOSTEROIDS IN A PETROLEUM ETHER/ETHYLENE GLYCOL SYSTEM

Steroid	R_F
Androstane-3α,11β-diol-17-one	0.06
Ætiocholan-3α-ol-11,17-dione	0.13
Δ^4-Androsten-11β-ol-3,17-dione	0.20
Δ^4-Androstene-3,11,17-trione	0.24
Δ^5-Androsten-3β-ol-17-one	0.47
Androstan-3β-ol-17-one	0.56
Ætiocholan-3α-ol-17-one	0.54
Androstan-3α-ol-17-one	0.67
3,5-Cycloandrostan-6β-ol-17-one	0.76
Androstane-3,17-dione	0.85
3β-Chloro-Δ^5-androsten-17-one	0.94
Testosterone	0.22
17-Methyltestosterone	0.43
Pregnane-3,20-dione	0.92
Progesterone	0.84

determinations the sheets were always impregnated with a 30% solution of ethylene glycol in methanol. Impregnation with this solution gives the highest rate of flow of the mobile phase with the most sharp separation of steroids that are frequently

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estimated. The influence of concentration of the impregnating solution on the R_F values of some 17-ketosteroids is apparent in Fig. 1.

The influence of the saturation rate of the tank and variations of temperature on the rate of flow of the mobile phase is especially marked when very volatile solvents,

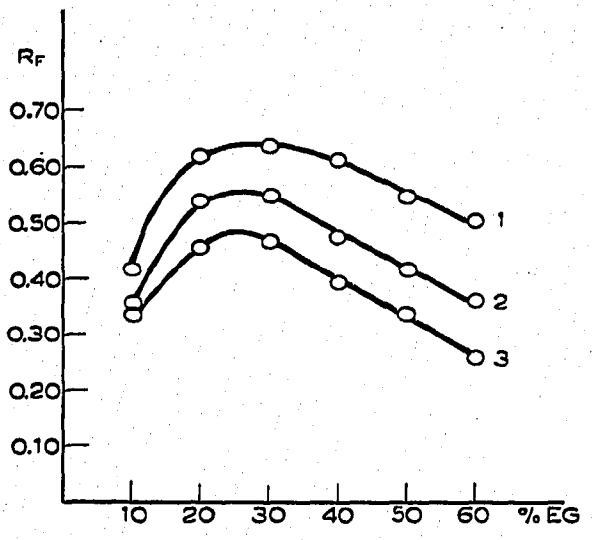

Fig. 1. Effect of the ethylene glycol (EG) concentration on the R_F values of some 17-ketosteroids. 1 = androsterone; 2 = epiandrosterone; 3 = dehydroepiandrosterone. Mobile phase: petroleum ether.

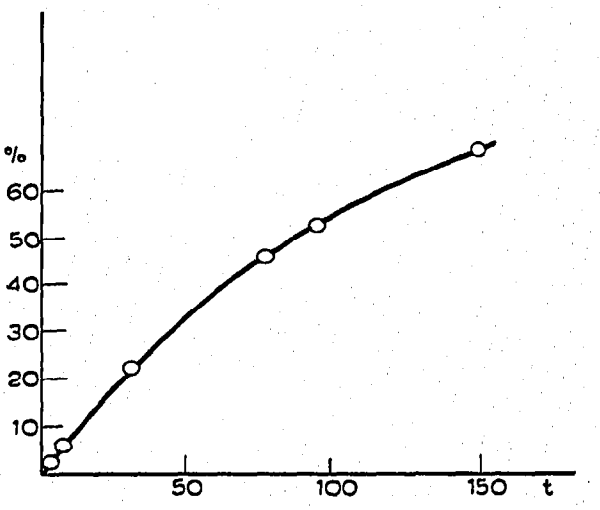

Fig. 2. Effect of increase of time of evaporation on ethylene glycol loss from the impregnated paper. Time t in hours. % = percentage of ethylene glycol lost.

e.g. petroleum ether, are used. In chromatography of 17-ketosteroids the optimal rate of flow is 30–35 cm in 2 ½ hours. Within this velocity range the R_F values are readily reproducible. In solvent systems with carbon tetrachloride or toluene the liquid front reaches 35 cm in 3–4 hours. For the separation of steroids the use of ethylene glycol has the advantage that a high velocity of partition is obtained.

The capacity of Whatman No. 1 paper, on which the separated steroids appear as round or oval spots, depends to a certain extent on the nature of the investigated substance, on the amount of ethylene glycol in the stationary phase, and on the

TABLE III

CAPACITY OF WHATMAN NO. 1 PAPER FOR SOME STEROIDS. EFFECT OF ETHYLENE GLYCOL CONCENTRATION IN THE IMPREGNATING SOLUTION

Results are expressed as μg steroid/spot

Steroid	*Conc. of ethylene glycol*			
	10%	*30%*	*50%*	*70%*
Cortisol*	50	100	100	150
Cortisone*	40	350	380	420
Δ^5-Androsten-3β-ol-17-one**	150	350	400	450

* Mobile phase: toluene.
** Mobile phase: petroleum ether.

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mobile phase. Values obtained for some steroids are shown in Table III. The capacity of Whatman No. 3 paper exceeds by 50% the capacity of Whatman No. 1 paper.

In the case of identically impregnated sheets, the amount of ethylene glycol in the stationary phase depends on the concentration of the solution of ethylene glycol in methanol. During the impregnation with a 10% solution the stationary phase of ethylene glycol amounts to about 7% of the weight of the paper, with a 30% solution it amounts to 28% and with 50% solution to 45%.

Ethylene glycol evaporates from the paper strips more quickly than formamide. This facilitates the removal of the impregnating agent from the sheets. However, during the development of the chromatogram the evaporation from the sheet is never so marked that the effect on the R_F values of the loss of ethylene glycol is significant. The evaporation of ethylene glycol from the paper strips is illustrated in Fig. 2, which shows the influence of time on ethylene glycol loss at room temperature in a closed chromatography tank.

Ethylene glycol systems have been successfully used not only for the separation of pure steroids, but also for the separation of corticosteroids and 17-ketosteroids from biological material, especially extracts from urine.

Ethylene glycol is available in a grade of satisfactory quality and it does not influence the majority of color reactions used for the quantitative colorimetric determination of steroids in urine.

SUMMARY

The use of ethylene glycol as the stationary phase for the separation of corticosteroids and of 17-ketosteroids by paper chromatography is proposed. Some characteristics of systems with ethylene glycol in the stationary phase are described.

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Received September 11th, 1958