CHROM. 4432

IDENTIFICATION OF POLYGLYCEROLS BY THIN-LAYER CHROMATOGRAPHY OF THEIR ACETATES

M. S. J. DALLAS
Unilever Research Laboratory, The Frythe, Welwyn, Herts. (Great Britain)

SUMMARY

The procedure of DITTRICH for the estimation of the number of hydroxyl groups by thin-layer chromatography has been shown to apply satisfactorily to polyglycerols, and systems for the separation of the various acetates of some lower synthetic polyglycerols are described. A two-dimensional procedure involving *in situ* acetylation by acetic anhydride between developments has been developed; a characteristic pattern of spots is obtained with commercial polyglycerols, which helps in the detailed qualitative comparison of different samples.

INTRODUCTION

Commercial polyglycerol is a mixture of polymers which, since the glycerol units may link together in several ways, is fairly complex. Its detailed analysis has become important on account of its increasing use in esterified form as a food additive. Paper chromatography, gas chromatography[1,2] and thin-layer chromatography[2–4] have all been employed for the separation of the components of polyglycerol or their derivatives. In a previous paper[3] the separation of the free polyglycerols in one dimension was described and SAHASRABUDHE[2] has shown that some separation of the polyglycerol stearates also can be achieved on silica gel layers. The only separation of polyglycerol acetates, known to have been described, is that by BARRETT *et al.*[1], using gas chromatography.

The present paper describes TLC methods for aiding the identification of polyglycerol components and for further elucidating the composition of a complex commercial polyglycerol mixture.

DITTRICH[5] has described a general method for estimating the number of hydroxyl groups in polyalcohols by a technique involving partial acetylation followed by TLC of the reaction products. This method has been applied to model polyglycerol compounds and found to work satisfactorily; it has also been applied here in aiding the identification of some unidentified components of polyglycerol. The principle, described by DITTRICH[5], has now been employed in a new two-dimensional TLC technique, involving *in situ* partial acetylation of the polyglycerols after develop-

ment in the first direction. Examination of the complex two-dimensional pattern of spots obtained can help appreciably in qualitatively comparing polyglycerol samples and in identifying the components separated.

The acetylation of spots *in situ* on thin-layer chromatograms has been described by several workers[6–8] and the two-dimensional technique with *in situ* reaction between developments, as first described by STAHL[9], has been employed in several different ways recently[10–12]. It was probably MILLER *et al.*[13] in 1953, who first described how the pattern of spots after *in situ* reaction on thin layers could help in the identification of substances and the present paper extends this general principle to the particular case of polyglycerols.

EXPERIMENTAL

(I) Partial acetylation off the plate and one-dimensional chromatography (method of Dittrich)

Standard glass plates were coated with a nominally 0.25-mm layer of 1:1 Silica Gel G–Kieselguhr G, dried at 110° and stored over anhydrous calcium chloride. 1–10 mg of sample was dissolved in about 100 vol. of pyridine and 1 μl of this solution was applied to a plate. About 20 vol. of acetic anhydride were then mixed into the pyridine solution and 1 μl of this was applied to an adjacent position on the plate. The same solution was just brought to the boil over a microburner and, when cool, a further 1 μl was applied to the plate. Finally the solution was kept close to the boiling point for several minutes and a final 1-μl sample was applied alongside the previous three.

As expected, the developing solvent system had to be tailored, to some extent, to suit the particular sample under examination. An ordinary paper-lined developing tank was used and the solvent made from a mixture of cyclohexane–ether–ethanol–formic acid in each case; for glycerol and 1,1'-diglycerol the proportions were 30:82:6:2, for linear triglycerol 23:67:15:15 and for cyclic diglycerol 10:102:6:2. Both the polyglycerols and their acetates were satisfactorily detected by the method of ADACHI[14]; the reagent, made by dissolving 0.5 g of thymol in 5 ml of concentrated sulphuric acid and adding this to 95 ml of ethanol, was sprayed on fairly heavily and the chromatoplate then was heated to 120° for about 20 min to give permanent grey–purple spots on a pale mauve background.

(II) Two-dimensional chromatography with in situ acetylation between developments

The 20 × 20 cm glass plates were coated with a 0.25-mm layer of a slurry made from 15 g of Silica Gel G, 15 g of Kieselguhr G and 60 ml of 0.045 *M* calcium chloride[3], dried at 110° and stored over anhydrous calcium chloride. 300–500 μg of polyglycerol sample were applied as a spot in the bottom right corner 2 cm from each edge and the plate was developed twice in the first direction, over 15 cm in each case, in ethyl acetate–isopropanol–water (55:30.5:14.5).

The plate was allowed to dry in air for 30 min–1 h and a 1.5 × 20 cm band (the centre coincident with the centre of the original spot and the axis lying in the direction of the first development) was sprayed lightly with a fine spray of 60% v/v acetic anhydride in pyridine (the amount sprayed on should be controlled, the order of 1 ml being sufficient); during this spraying the remainder of the layer was covered by

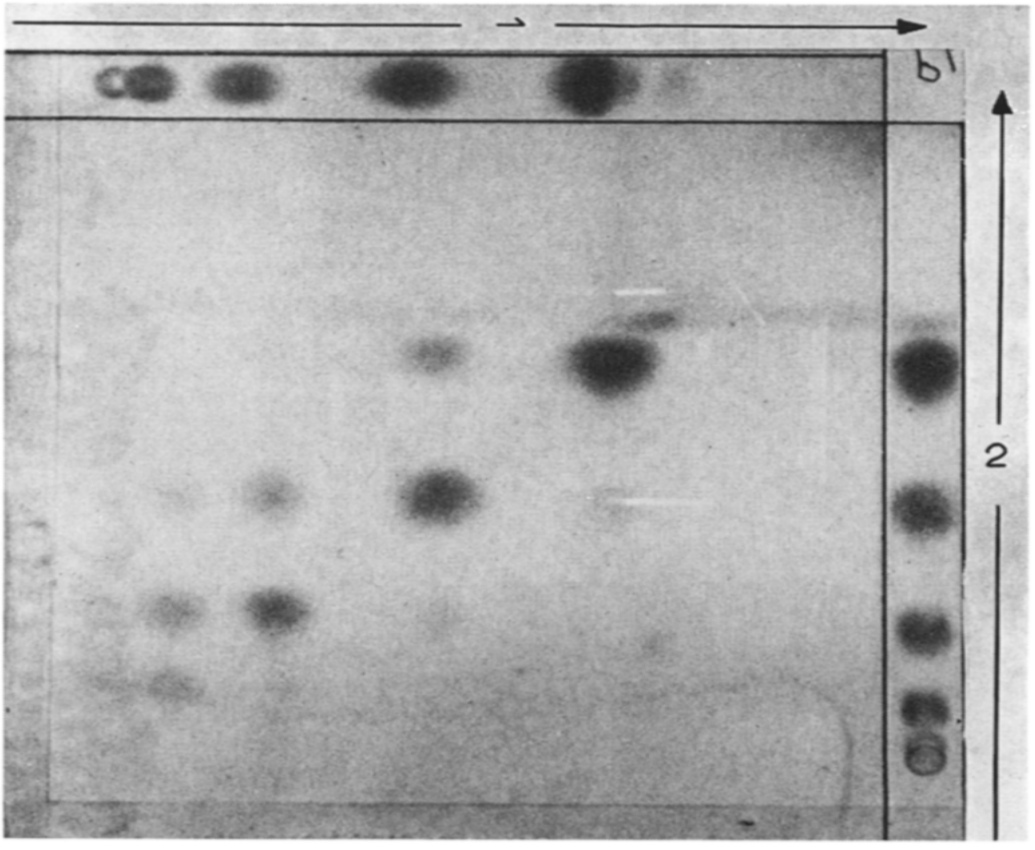

Fig. 1. Two-dimensional TLC of partially acetylated 1,1'-diglycerol with further *in situ* acetylation between developments. See EXPERIMENTAL, section II.

glass, which was then removed. A 20 × 2 cm strip of thin glass was placed over the freshly sprayed area and the whole plate, with the glass strip in place, was put upside down onto a flat electric hotplate at 95–100° for approximately 15 min and then allowed to cool in air (with the glass strip removed) for several hours or overnight to allow excess reagent to evaporate.

Development in the second direction at right angles to the first was done once, over 15 cm, in cyclohexane–ether–ethanol–formic acid (10:102:6:2) in the paper-lined tank. The spots were visualised by the thymol reagent[14] described under EXPERIMENTAL, section I.

RESULTS AND DISCUSSION

When a suitable developing solvent had been found the method of DITTRICH[5] was found quite straightforward and reliable, when applied to glycerol, to linear diglycerol, to linear triglycerol and to cyclic diglycerol. The R_F values for the various products are given in Table I. The synthesis of the pure polyglycerols has been described earlier[3].

There was negligible separation of the isomers of each derivative and, in each case examined, the number of hydroxyl groups correlated without any doubt with the number of spots obtained.

TABLE I

R_F VALUES OF PRODUCTS OF ACETYLATION

Solvents as described in EXPERIMENTAL, section I; temperature, 20 ± 1°.

Substance	*Glycerol*	*Diglycerol*	*Triglycerol*	*Cyclic diglycerol*
Original compound	0.05	0.01	0.07	0.26
Monoacetyl derivative	0.19	0.05	0.20	0.56
Diacetyl derivative	0.43	0.14	0.38	0.82
Triacetyl derivative	0.62	0.32	0.57	
Tetraacetyl derivative		0.55	0.73	
Pentaacetyl derivative			0.83	

R_M value differences were measured for each added acetyl group but were not found constant as might be expected in a pure partition system, the difference always being greatest for the first acetyl group added and decreasing for each successive acetyl group. It is quite probable, therefore, that adsorption plays an appreciable role in the chromatography in this case.

In the two-dimensional method described in Section II of EXPERIMENTAL the

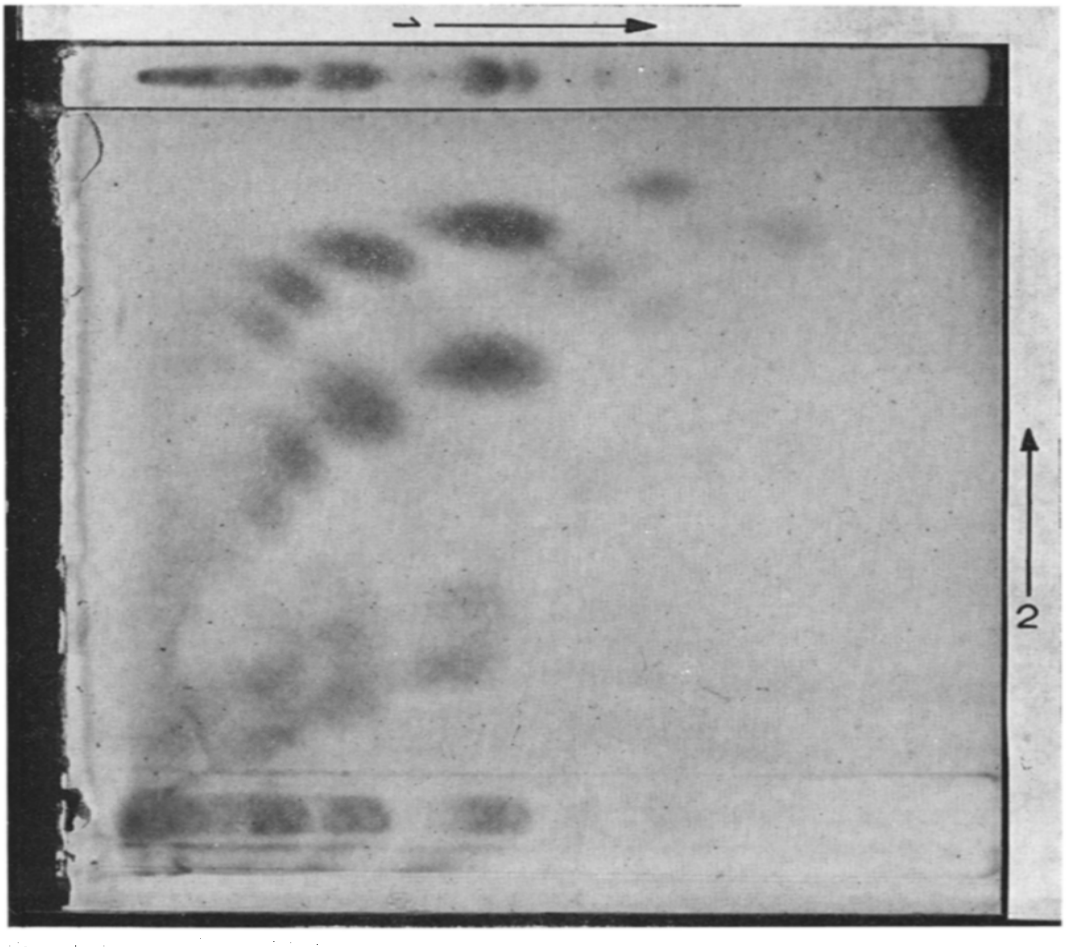

Fig. 2. Two-dimensional TLC of a commercial polyglycerol with partial *in situ* acetylation between developments. See EXPERIMENTAL, section II.

adsorbent and the solvent system for the development in the first direction were essentially similar to those employed in the one-dimensional method described in an earlier publication[3]. An acetic anhydride–pyridine mixture gave clearer and more reproducible chromatograms than pure acetyl chloride, though the latter led to a similar pattern of spots. The reproducibility of the acetylation stage is important and it was found essential to pay careful attention to the details of the application of the acetylating reagent.

A number of model experiments were conducted and the result of a typical one of these is shown in Fig. 1, the sample in this case being partially acetylated 1,1′-diglycerol (mixture from application of the DITTRICH method).

Application of this two-dimensional technique to a typical commercial polyglycerol sample (*ex* Food Industries Ltd., Great Britain) showed that a characteristic pattern was obtained with several important details (see Fig. 2). It may be noted that it is very helpful to have, as reference, a one-dimension separation of the original (unacetylated) sample on one side of the same plate as shown in Figs. 1 and 2. Fig. 3 illustrates the general pattern in a diagrammatic form and summarises the results of a number of chromatograms.

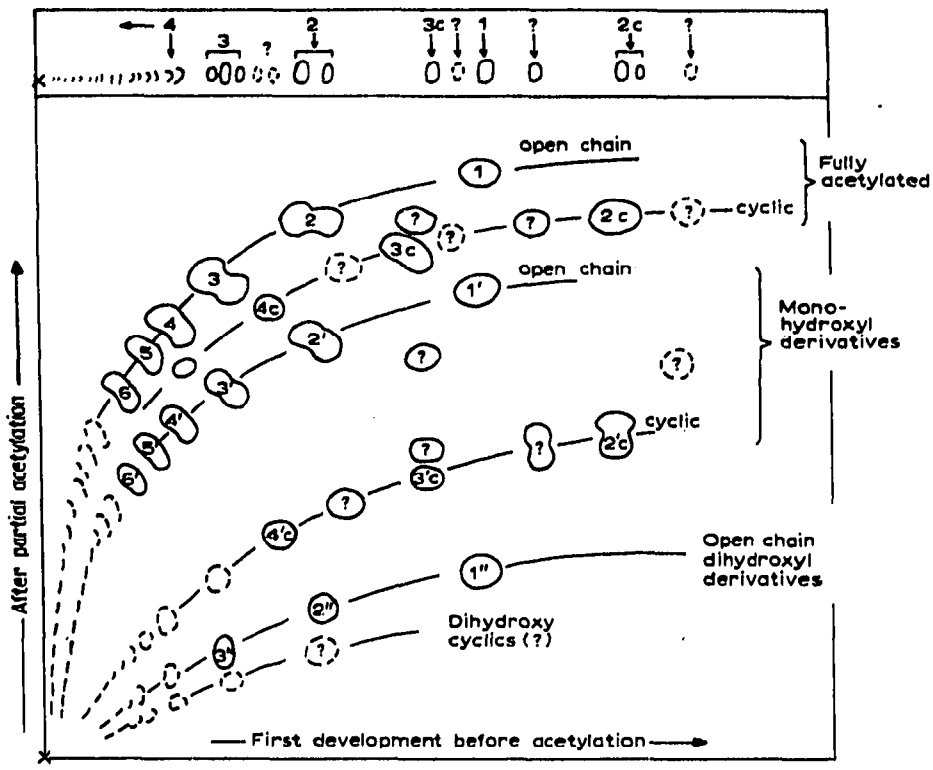

Fig. 3. Summary of the results of a number of chromatograms. Numbers refer to number of glycerol units per molecule; c = cyclic; ′ = one hydroxyl group unacetylated; ″ = two hydroxyl groups unacetylated. Example: 3′c = cyclic triglycerol with one free hydroxyl group.

CONCLUSIONS

The following conclusions have been drawn from results with the two-dimen-

sional technique: (1) Additional information regarding the detailed composition of a sample is obtained, which is not available from a normal one-dimension chromatogram; (2) the fully acetylated linear polyglycerols lie on a smooth curve and the mono-hydroxy linear polyglycerol acetates similarly lie on a smooth curve of lower R_F value; (3) the fully acetylated cyclic polyglycerols lie in the area between the curves mentioned in item 2 above and can thus be clearly distinguished from the linear polyglycerol acetates; (4) the unidentified polyglycerol spot, whose R_F value lies just below that of glycerol itself, appears to be a group of several components of similar properties; (5) the unidentified component just above glycerol appears likely, from its position, to be a cyclic rather than a linear polymer; (6) it is not possible to infer the number of hydroxyl groups in a component from the two-dimensional chromatogram, since detection and resolution of the acetates of low R_F values are not sufficiently good; (7) the technique has been found valuable for qualitatively comparing polyglycerol samples of apparently the same or very similar composition; and (8) the method could, in general, be applied to mixtures of homologues in a continuous series (*e.g.* other polymer series), where a distinct pattern of spots would be expected to be formed by this technique.

ACKNOWLEDGEMENTS

The author is indebted to Miss M. McMULLIN and Mr. C. WOOLSTON for much of the experimental work and to Dr. M. F. STEWART for providing samples of synthetic di- and triglycerols.

REFERENCES

1 C. B. BARRETT, N. SEN AND M. KEATING, *J. Gas Chromatog.*, 5 (1967) 269.
2 M. R. SAHASRABUDHE, *J. Am. Oil Chemists' Soc.*, 44 (1967) 376.
3 M. S. J. DALLAS AND M. F. STEWART, *Analyst*, 92 (1967) 634.
4 A. SEHER, *Fette Seifen Anstrichmittel*, 66 (1964) 371.
5 S. DITTRICH, *Mikrochim. Acta*, (1966) 447.
6 S. J. PURDY AND E. V. TRUTER, *Proc. Roy. Soc. (London)*, 158B (1963) 536.
7 F. JAMINET, *Farmaco (Pavia), Ed. Prat.*, 18 (1963) 633.
8 M. H. A. ELGAMAL AND M. B. E. FAYEZ, *Z. Anal. Chem.*, 226 (1967) 408.
9 E. STAHL, *Arch. Pharm.*, 293/65 (1960) 531.
10 C. V. VISWANATHAN, F. PHILLIPS AND W. O. LUNDBERG, *J. Chromatog.*, 35 (1968) 66.
11 G. PATAKI, J. BORKO, H. C. CURTIUS AND F. TANCREDI, *Chromatographia*, 1 (1968) 406.
12 L. A. HORROCKS, *J. Lipid Res.*, 9 (1968) 469.
13 J. M. MILLER AND J. G. KIRCHNER, *Anal. Chem.*, 25 (1953) 1107.
14 S. ADACHI, *J. Chromatog.*, 17 (1965) 295.