## Separation of aliphatic dicarboxylic acids by thin-layer chromatography (TLC)

For the determination of the position of the double bond in monoethenoid fatty acids, the most commonly employed method is oxidation with permanganate–periodate reagent and subsequent identification of the dicarboxylic acid fragment. Melting point determinations are not often sharp even after several recrystallizations. PHATAK *et al.*[1] and SUBBARAM[2] among others[3–5] used paper chromatographic methods for this purpose, but these are rather slow and tedious, and recently quicker TLC procedures have been used. Thus BRAUN AND GEENEN[6] separated the dicarboxylic acids from oxalic to sebacic on silica gel plates using an alcohol–ammonia–water mixture for development. PETROWITZ AND PASTUSKA[7] used two different solvent systems, consisting of benzene–dioxane–acetic acid and benzene–methanol–acetic acid. Separations obtained by these two methods are satisfactory for $C_2$–$C_5$ acids but only marginal for $C_6$–$C_{10}$ acids. The use of polyethylene glycol (molecular weight 1000) as stationary phase, sodium diethyldithiocarbaminate as antioxidant and a mixture of di-isopropyl ether–formic acid–water as developing solvent gave good separations[8]. Both the stationary phase and antioxidant are not readily available. In this note we describe a TLC method on a silica gel G plate for the separation of aliphatic dicarboxylic acids. The developing solvent used here is a composite of various solvent mixtures earlier employed by KALBE[4] in paper chromatographic separations. The present procedure has been in routine use in this laboratory for two years for structure determinations of unsaturated fatty acids, and for location of hydroxyl group position in a carbon chain following catalytic hydrogenation of epoxy fatty acids.

*Experimental*

All compounds were of high purity. The acids in either methanol or methanol–water (1:1) solution were spotted on a 20 × 20 cm glass plate coated with silica gel G (250 $\mu$) and developed (1 h) with a solvent system consisting of *n*-butanol, xylene, phenol, formic acid and water (10:70:30:8:2, v/v). The plates were then removed, dried at 150° for 30 min, cooled and sprayed with an alcoholic solution of bromocresol

TABLE I

TLC OF DICARBOXYLIC ACIDS OF $C_2$–$C_{10}$ CHAIN LENGTH

Solvent system: Xylene–phenol–butanol–formic acid–water (70:30:10:8:2, v/v).

| *Acid* | *Carbon chain length* | $R_F \times 100$ *observed* |
|---|---|---|
| Oxalic | 2 | 0 |
| Succinic | 4 | 15 |
| Glutaric | 5 | 20 |
| Adipic | 6 | 25 |
| Pimelic | 7 | 31 |
| Suberic | 8 | 38 |
| Azelaic | 9 | 44 |
| Sebacic | 10 | 49 |

purple containing a little alkali, when the acids appeared immediately as yellow spots on a purple background. The exact position of the spots was outlined quickly before the colour contrast lessened by fading.

*Results*

Table I shows the results. The dicarboxylic acids separate well with the solvent system mentioned (Table I), the $R_F \times 100$ values increasing from the lower to the higher homologues in a regular manner with a difference of about 6 units between adjacent members. For specific separation of dicarboxylic acids of $C_8$–$C_{13}$ chainlength, which are the most commonly encountered, a better spread of $R_F$ values was sought by alterations in the proportions of the solvent components. The proportions of formic acid and water in the combined solvent system were kept constant, and the effect of

TABLE II

TLC OF HIGHER DICARBOXYLIC ACIDS WITH VARYING SOLVENT PROPORTIONS

| | | *Solvent system, v/v* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *1* | *2* | *3* | *4* | *5* | *6* |
| Xylene | | 70 | 70 | 70 | 90 | 65 | 65 |
| Phenol | | 30 | 30 | 70 | 20 | 50 | 50 |
| Butanol | | 10 | 20 | 50 | 10 | 15 | 15 |
| Formic acid | | 8 | 8 | 8 | 8 | 8 | 8 |
| Water | | 2 | 2 | 2 | 2 | 2 | 2 |
| Ether | | — | — | — | — | — | 7 |
| *Acid* | | *$R_F \times 100$ values* | | | | | |
| | | *1* | *2* | *3* | *4* | *5* | *6* |
| Suberic | $C_8$ | 38 | 56 | 57 | 42 | 48 | 48 |
| Azelaic | $C_9$ | 44 | 62 | 64 | 45 | 56 | 56 |
| Sebacic | $C_{10}$ | 49 | 67 | 70 | 50 | 62 | 63 |
| Brassylic | $C_{13}$ | — | — | — | — | 75 | 76 |

alterations in the proportions of the remaining solvents examined (Table II). Increase in the proportion of either butanol or phenol, especially the former, caused a strong forward movement. Increase of phenol and butanol together rather surprisingly had only a small effect. Increase of xylene and addition of ether as an extra component had little effect on movement. Solvent systems 5 or 6 yielded the best spread in the spots.

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1 S. S. PHATAK, A. P. MAHADEVAN AND V. N. PATWARDHAN, *Current Sci. (India)*, 21 (1952) 162.
2 M. R. SUBBARAM, *Investigations on the Composition and Nutritive Value of Vanaspati*, Vol. 2, Council of Scientific and Industrial Research, New Delhi, 1958, pp. 25–47.

3 F. A. ISHERWOOD AND C. S. HANES, *Biochem. J.*, 55 (1953) 824.
4 H. KALBE, *Z. Physiol. Chem.*, 297 (1954) 19.
5 J. R. HOWE, *J. Chromatog.*, 3 (1960) 389.
6 D. BRAUN AND H. GEENEN, *J. Chromatog.*, 7 (1962) 56.
7 H. J. PETROWITZ AND G. PASTUSKA, *J. Chromatog.*, 7 (1962) 128.
8 E. KNAPE AND D. PETERI, *Z. Anal. Chem.*, 188 (1962) 184.

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## Quantitative Bestimmung von Harnstoff in Serum

## Spektralphotometrische Bestimmung nach dünnschichtchromatographischer Abtrennung auf Celluloseschichten

In einer früheren Arbeit[1] beschrieben wir eine quantitative Bestimmung des Harnstoffgehaltes im Harn. Die Methode kann auch auf Serum angewandt werden.

Hierzu wird das Serum zunächst enteiweisst. In einem Zentrifugenglas werden 2.00 ml Serum mit 4 ml 96 %igem Äthanol gemischt und zentrifugiert. Die überstehende Flüssigkeit wird in ein Glasschälchen abgegossen und das Zentrifugenglas mit etwas Äthanol nachgespült. Die vereinigten Flüssigkeiten werden vorsichtig abgedampft. Der Rückstand wird in 0.2 ml Wasser (genau abgemessen mit der Agla-Pipette) aufgenommen. 0.03 ml dieser Lösung und ebenso 0.03 ml Standardlösung werden in der gleichen Weise behandelt wie bei der Bestimmung des Harnstoffgehaltes im Harn beschrieben.

Der Gehalt wird entweder mit Hilfe der Standardlösung bestimmt oder der Eichkurve entnommen, die man erhält, wenn man jeweils 0.03 ml verschiedener Verdünnungen einer wässrigen Lösung von 1.8000 g Harnstoff/100.00 ml aufträgt. Die weitere Bestimmung erfolgt gemäss den in der oben zitierten Arbeit gemachten Angaben.

Die wiedergefundene Menge betrug 90.2 % ($\pm$ 1.39 %).

Harnstoff-Standardlösung: 0.1000 g Harnstoff werden in Wasser zu 100.00 ml gelöst.

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1 M. RINK UND A. GEHL, *J. Chromatog.*, 20 (1965) 415.

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