Detection of Meprobamate on Paper Chromatograms

IN a recent communication, Drs. A. Fiori and M. Marigo¹ described the identification of meprobamate by adsorption chromatography on chromatoplates. Their method is both complex and laborious compared with the technique of paper chromatography, but was advocated because meprobamate appeared to be insoluble in water and because there were no available methods for detecting it on paper.

Paper chromatography can, however, be used in the identification of this substance. Meprobamate is slightly soluble in water, and we have found that it may be detected on paper chromatograms by the hypochlorite modification² of the chlorination method first described by H. N. Rydon and P. W. G. Smith³. The paper is sprayed when dry, first with 1 per cent sodium hypochlorite, then with ethanol and finally with a mixture of equal volumes of 1 per cent starch and *M*/20 potassium iodide.

This method has been used for identifying meprobamate in the urine of a patient in coma, in whom the diagnosis had previously been in doubt. A report of this case, with details of the chemical methods by which the diagnosis was established, is being published elsewhere⁴. In this context, it is of interest that the chlorination method, which has numerous applications, has recently been used to detect barbiturates on paper chromatograms⁵.

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¹ Fiori, A., and Marigo, M., *Nature*, **182**, 943 (1958).

² Pan, S. C., and Dutcher, J. D., *Anal. Chem.*, **28**, 836 (1956).

³ Rydon, H. N., and Smith, P. W. G., *Nature*, **169**, 922 (1952).

⁴ Bedson, H. S., *Lancet* (in the press).

⁵ Rentsch, G., *Naturwiss.*, **45**, 314 (1958).

BIOCHEMISTRY and PHYSIOLOGY

Cellulose Acetate Membranes suitable for Osmotic Measurements

IN a recent paper¹ it has been shown that 'Ultrafein filters allerfeinst', which the manufacturers state are composed of an unnamed cellulose ester, can be used to measure quite low number average molecular weights ($\bar{M}_n$). We have now found that membranes

having similar osmotic properties can, in fact, be prepared from cellulose acetate.

The membranes are prepared by casting acetone solutions of cellulose acetate, containing *n*-amyl alcohol as precipitant², and allowing them to evaporate until nearly dry. The actual conditions for carrying out this operation appear to be rather critical, and so far a procedure giving absolutely reproducible membranes has not been evolved. However, by using the following method, membranes which show similar variations in osmotic properties to those found with the 'Ultrafein filters allerfeinst' can be prepared.

The following solution is made up: 5 per cent (w/w) cellulose acetate; 5 per cent (w/w) *n*-amyl alcohol; 90 per cent (w/w) acetone, and a volume containing enough cellulose acetate to give a 'membrane density' (that is, weight per unit area) of 0.005 gm./cm.² is poured into a level glass dish and allowed to evaporate at room temperature in a cabinet free from draughts. As the membrane dries out, the surface first of all looks like orange peel and then goes opaque. For osmotic purposes the structure is fixed just before the membrane becomes opaque by pouring on ethanol. In a typical membrane, when the ethanol is poured on, three distinct zones appear: (1) a clear zone; (2) a white zone; (3) a white zone with dark spots. The effective part of the membrane is the white portion, since the clear areas (opaque before the addition of ethanol) have dried out too much and are usually impermeable, while the dark spots indicate pinholes which occur when the membrane has not dried out enough. The membranes are left in ethanol for at least 1 hr. to remove any residual amyl alcohol and are then conditioned to the required solvent; for example, in the case of benzene the membranes are left for periods of at least 1 hr. in (a) a 50 ethanol/50 benzene mixture and then (b) benzene.

The membranes used in the experiments described here were assembled in standard Zimm-Myerson type osmometers³ and characterized by: (a) the solvent permeability, as measured by the rate of fall of solvent in the measuring capillary from a 10-cm. head; and (b) the solute selectivity as measured by the maximum osmotic pressures developed with a 1 per cent (w/w) solution of a 'commercial' polystyrene and/or a 0.025 per cent (w/v) solution of sucrose octaacetate (molecular weight 679).

In Table 1 results with typical and very selective membranes of both cellulose acetate and 'Ultrafein filters allerfeinst' are shown. It will be seen that the cellulose acetate membranes compare very favourably with the 'Ultrafein filters allerfeinst', having similar solute selectivities combined with slightly

Table 1. COMPARISON OF 'ULTRAFEIN FILTERS ALLERFEINST' AND CELLULOSE ACETATE MEMBRANES; BENZENE SOLUTIONS AT 30° C.

Membrane		Permeability Fall from 10-cm. head of benzene (cm./5 min.)	Selectivity							
Type	Reference No.		1.0 per cent (w/w) commercial P.S.				0.025 per cent (w/v) sucrose octaacetate*			
			Rising $\bar{h}$ (cm.)	Time (hr.)	Falling $\bar{h}$ (cm.)	Time (hr.)	Rising $\bar{h}$ (cm.)	Time (hr.)	Falling $\bar{h}$ (cm.)	Time (hr.)
Typical membranes	<i>UFF/AF</i> 257/2	0.22	8.95	23	8.81	46	0.34	47	—	—
'Ultrafein filter allerfeinst'		0.81	8.61	16.5	8.65	4	—	—	—	—
Cellulose acetate	<i>CA</i> 2/58									
Very selective membranes	<i>UFF/AF</i> 1257/2	0.062	—	—	—	—	5.30	48.5	5.23	30
'Ultrafein filter allerfeinst'		0.175	11.15	43	—	—	5.11	30	5.44	65
Cellulose acetate	<i>CA</i> 1/68									

* $\bar{h}_{\text{theor.}} = 10.86$ cm.