

Superintendent of the Admiralty Mining Establishment for the loan of the hydrophones and other apparatus, is gratefully acknowledged.

M. N. HILL
P. L. WILLMORE

Department of Geodesy and Geophysics,
Downing Place,
Cambridge.
March 28.

¹ Bullard, E. C., and Gaskell, T. F., *Proc. Roy. Soc., A*, **177**, 498 (1941).

A New Method for the Observation of Zones of Colourless Substances on a Chromatographic Column

WHEN chromatographic adsorption analysis is used for colourless substances (or on strongly coloured adsorbents, for example, activated carbon) it is impossible to see the zones of the different solutes adsorbed on the column. Some special arrangement is then needed in order to be able to locate the zones. In principle, two methods are available: (1) no observation is made on the column, but instead the concentration of the solution leaving the column is followed in a small cell; (2) the positions of the zones on the column are determined by using some special physical or chemical properties of the solutes. The first method, which was introduced by Tiselius¹ and improved by Tiselius and me^{2,3}, has also the advantage that the separation between two adjacent zones is greater in the filtrate than on the column. However, in many cases it is of importance to follow the separation directly on the column, and a number of different arrangements have been proposed⁴. In most of these cases the solutes either are converted into coloured compounds by means of suitable reagents, or indicators are applied before or after the chromatogram is finished, or use is made of fluorescence methods. These methods are, however, limited in use and unsuitable when zones are in direct contact with each other.

The new procedure to be described can be applied quite generally. The zones are detected on the column by observing the changes in refractive index of the solution in contact with the particles of the adsorbent. It is quite clear that this can be followed by any method for the determination of refractive index which uses the total reflexion of light entering through the glass wall of the tube in which the adsorbent is packed.

One of the simplest arrangements for the observation is the following. A tube with rectangular cross-section is used for the adsorbent (Fig. 1). Such a tube can easily be made by clamping two glass windows against a U-shaped plate of metal. One of the windows is rather thick and has polished edges. If the adsorbent is observed at the proper angle (Fig. 1) from a suitable distance (c. 1 metre), the boundary of the region of total reflexion is easily seen, and its position depends on the refractive index of the solution wetting the glass. Its shape will, therefore, be as in Fig. 2a in the case of an ordinary chromatogram with separate zones, and as in Fig. 2b if frontal analysis or displacement development is used. With strongly coloured adsorbents it may sometimes be useful to place a strip of thin white paper between the adsorbent and the glass window.

The operation of this method is in principle equivalent to that of a Pulfrich refractometer. It is

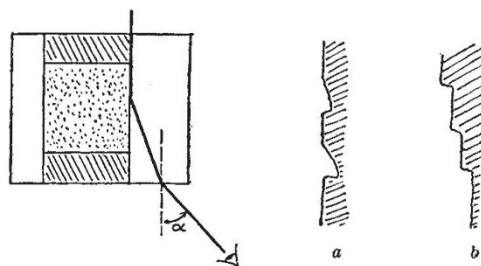


Fig. 1

Fig. 2

Fig. 1. TUBE FOR CHROMATOGRAPHY SEEN FROM ABOVE
Fig. 2. SHAPE OF TOTAL REFLECTING REGION FOR (a) ELUTION ANALYSIS, (b) FRONTAL ANALYSIS

clear that the sensitivity is improved by using monochromatic light during the observation. If necessary, the method can also be made quantitative by measuring the angle α with a telescope, since

$$n_0^2 - n_1^2 = \sin^2 \alpha,$$

where n_0 is the refractive index of the glass and n_1 that of the solution. The accuracy can, of course, also be increased by using more elaborate methods for the observation; for example, an arrangement equivalent to a Hallwachs's cuvette⁵.

This method has the advantages that it can be applied quite generally and is independent of the specific properties of the solutes. Furthermore, it can be used when zones containing very similar substances with increasing concentration are in contact with each other, as in frontal analysis and displacement development. It seems also to be quite useful for inorganic chromatography. In the case of very large columns it is only necessary to place a strip of plane parallel glass in the wall of the container. It is also worth while to point out that different areas with different refractive indices can be detected in the same way when paper chromatography^{6,7} is used, by placing the paper under the glass.

Although the method has the disadvantage that it is not so sensitive as most of the colour reactions, it does not exclude other kinds of observation, since the column can be observed in the usual way through the windows. Some experimental applications of the above principle will be reported later.

STIG CLAESSION

Gates and Crellin Laboratories of Chemistry,
California Institute of Technology.

Feb. 28.

¹ Tiselius, A., *Arkiv. Kem. Min. Geol.*, **14B**, No. 22 (1940).

² Tiselius, A., *Arkiv. Kem. Min. Geol.*, **16A**, No. 18 (1943).

³ Claesson, S., *Arkiv. Kem. Min. Geol.*, **23A**, No. 1 (1946).

⁴ See, for example, Zechmeister, L., and Cholnoky, L., "Principles and Practice of Chromatography" (London, 1943).

⁵ Hallwachs, W., *Wied. Ann.*, **50**, 577 (1893).

⁶ Schoenbein, C. F., *Verh. Naturf. Ges. Basel*, **3**, 249 (1861).

⁷ Conden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **38**, 224 (1944).

Set Time and Particle Size

IN dissolving high-molecular weight compounds in hydrocarbons to give viscous solutions, it is convenient to stir or otherwise agitate the mixture until the viscosity of the solvent is raised to such a degree as to prevent any settling of the partially dissolved particles on standing. After such a 'set time' is reached, the material can be left to dissolve on its own accord. Homogeneity may be attained by a final shaking or mixing.