AN IMPROVED TECHNIQUE FOR RECORDING CHROMATOGRAMS ANALYSIS OF CRESOL MIXTURES

F. ALDERWEIRELDT

Laboratory for Organic Chemistry, University of Ghent (Belgium)

(Received May 20th, 1960)

In the course of an investigation of new counter current procedures, a rapid method suitable for series analyses of cresol mixtures was required. Since *o*- and *m*-cresol cannot be determined by U.V. spectrometry, because their spectra are too similar, a chromatographic analysis method was developed, which was an improvement of already known procedures[1-3].

Composition of the column

The celite column method described by WHITE AND VAUGHAN[2] gives excellent separation, but the elution takes too long (one hour) for our purpose. Increasing the flow rate by applying pressure finally reduced both the degree of separation and the lifetime of the column. This disadvantage of celite does not occur with silicagel (0.08–0.1 mm) and a phosphate buffer as stationary phase. A narrow grade in particle size is important for high column efficiency.

10 g of silicagel are suspended in about 50 ml of buffer (0.06 *M* sec. phosphate and 0.14 *M* tert. phosphate for cresol mixtures), and boiled a few minutes with rapid stirring. The suspension is allowed to cool, the bulk of supernatant buffer decanted to eliminate the fines and the gel is collected on a suction filter. The silicagel is again suspended in 50 ml of fresh buffer and the described procedure is repeated twice more. Finally the silicagel is collected on a fritted glass filter which can be placed in a centrifuge. The centrifuge is run at 1500 r.p.m. to remove the excess of buffer. The silicagel is then stirred into a slurry in 2,2,4-trimethylpentane and packed in a column (9 mm inner diam.) according to the method of MARTIN[3].

Column and flow-cell

The recording chromatographic procedure developed in our laboratory[1] was improved. The new sample introduction assembly is shown in Fig. 1.

The introduction of the samples (0.01–1 mg of the mixture in 0.1–1 ml solvent) is carried out as in gas–liquid chromatography with a hypodermic syringe through a rubber capsule without interrupting the flow of eluant (Fig. 1). The purpose of tube C (7 × 2 cm) filled with silicagel impregnated with stationary phase is to equilibrate the eluting solvent with the buffer phase before it reaches the separating column.

This device increases the lifetime of the column and acts as a buffer against pressure changes when samples are injected.

To introduce the sample in a minimum volume, stopcock A is closed, some air is injected and when the eluant-level just reaches the top of the silicagel column the sample is injected. The capillary tube B is rinsed by injecting fresh solvent (about 0.2 ml) and stopcock A is opened when the level again reaches the silicagel surface. The excess of pressure is automatically released through stopcock A. All these actions must be carried out rapidly since suction of air into the silicagel column must be avoided.

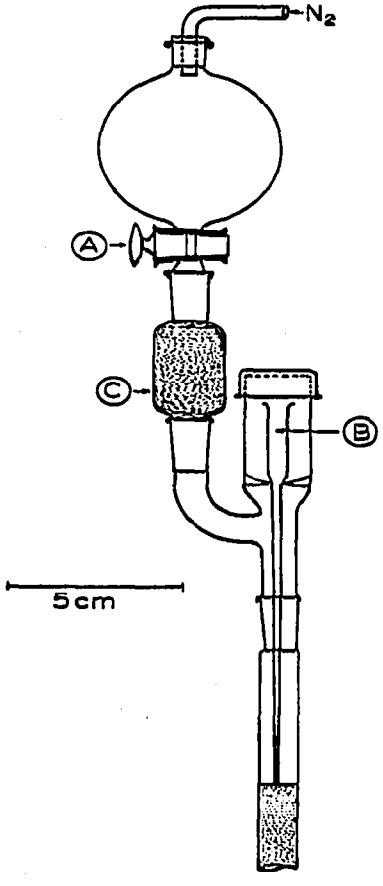

Fig. 1. Sample introduction. See text.

For series work the eluant is maintained at a level of 1–2 cm above the packing and the sample is injected as a "plug" without interrupting the flow of eluant. The eluant-level can be adjusted by pressing or sucking air with a syringe through the rubber capsule. The injection of sample and rinsing can be done simultaneously by carefully filling up the syringe first with fresh eluant and then with the sample. The required dexterity can be obtained by repeating all these actions with a coloured solution.

By using coloured solutions we could see that material could escape detection in the flow-cell, unless all parts of the cell are accessible to the light beam during the photometric measurement. To this end the cell is made as small as possible, the

quartz windows being actually somewhat smaller than the light beam of the photometer in the U.V. region. This does not affect the results if light scattering is avoided by blackening all cell parts, except of course the windows. The improved flow-cell is shown in Fig. 2. The quartz windows are fixed with an appropriate glue. For the solvent 2,2,4-trimethylpentane, a polyepoxyester (Stabilit) is satisfactory. The connec-

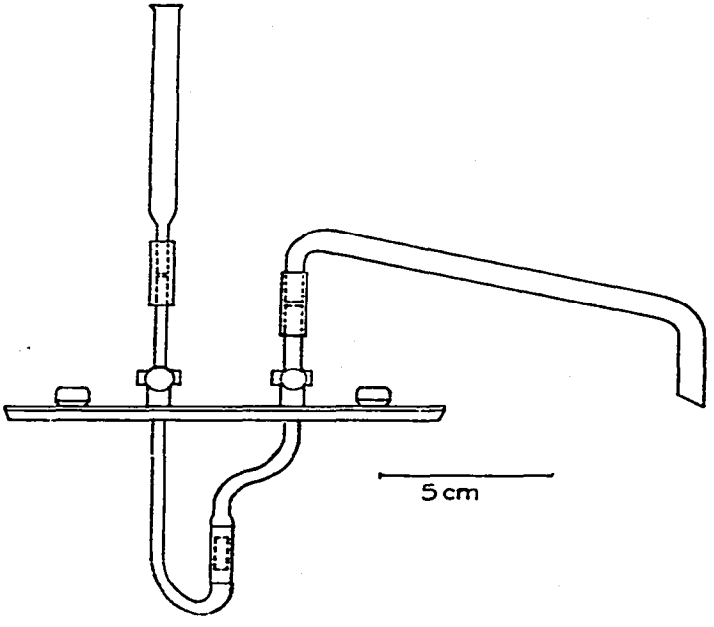

Fig. 2. Flow-cell showing introducing funnel, perforated photometer box lid, quartz windows etc.

tion tubes as shown are made of polyvinyl chloride. After a very short time all extractable material interfering with the light absorption is eliminated and no difficulties arise from the use of this connecting material.

Recording of the chromatograms

A Beckman D.U. spectrophotometer, mains fed by a Beckman Power Supply unit and provided with an energy-recording adapter (ERA 5800) and a 10 mV graphic recorder (G. 10 Varian Associates California), was used[1].

Transmission is recorded and the band surface is not proportional to the amount of material. However, the maximum extinction of each peak can be found by conversion of the transmission-extinction as found on the scale of the spectrometer. The relative concentrations can then be calculated by a modified triangle method, the base of the triangle being multiplied by the maximum extinction instead of the height of the triangle.

Of course the most reliable method consists in collecting each separated fraction with the recorder as a guide, and measuring its volume and extinction.

When the chromatography is run under a pressure of 50 cm water, the flow rate is about 150 ml/h for a column of 8 cm length. *o*- and *m*-Cresol mixtures are then separated in 10 to 20 minutes time (Fig. 3). With flow speeds of 300 ml/h no appreciable difference is noted in the chromatogram. The three cresols can be separated on a

column of 12 cm length (Fig. 4). These columns are suitable for up to fifty chromatograms.

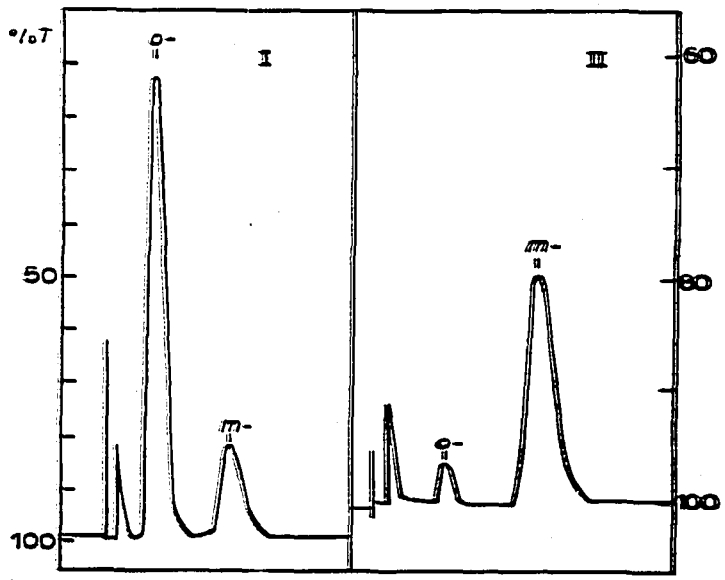

Fig. 3. Recording of the chromatographic separation of *o*- and *m*-cresol. Column length 8 cm, wave-length 276 mμ. The sample in II is about ten times smaller than in I. For other details see text.

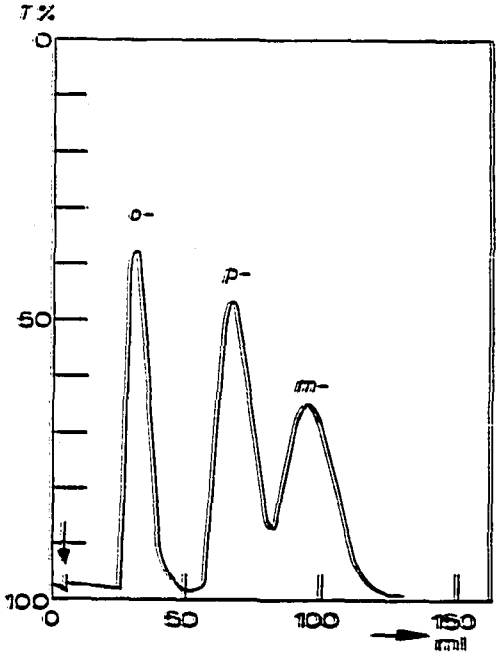

Fig. 4. Recording of the chromatographic separation of *o*-, *m*- and *p*-cresol (0.5 mg of each). Column length 12 cm, wave-length 276 mμ.

The sensitivity of the method can be increased by adjusting the amplification of the phototube-current until about 30 mV difference between "dark-current" and "open-slit" is obtained. In this way the recorder-pen goes off scale at a transmission of about 70 and relatively weak signals can be detected (Fig. 3 II).

As can be calculated from Fig. 3 the HETP for these columns is around 1 mm^4.

SUMMARY

A new device for the introduction of samples without interruption of the eluant flow in liquid chromatography is described. This, together with the described continuous U.V. recording technique of the outflow, makes rapid series analysis by partition chromatography as convenient as in gas chromatography.

REFERENCES

[1] M. VERZELE, *Nature*, 183 (1959) 604.
[2] D. WHITE AND S. A. VAUGHAN, *Anal. Chim. Acta*, 16 (1957) 439.
[3] A. J. P. MARTIN, *Symposium on Partition Chromatography*, Biochemical Soc., London, 1949, p. 11.
[4] M. VERZELE AND F. ALDERWEIRELDT, *Bull. soc. chim. Belges*, 64 (1955) 579; 66 (1957) 570.