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A Bridge Unit for Filter-paper Chromatography

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In chemical pathology filter-paper chromatography is becoming an analytical tool of increasing importance. In this particular field well established methods now allow for accurate identification of urinary sugars (Partridge, 1948), amino-acids (Dent, 1946) and porphyrins (Sveinsson, Rimington & Barnes, 1949).

In the present communication an apparatus is described that has been tested in this laboratory over the last nine months with satisfactory results. Some modifications in analytical technique are given here by way of example; others are the subject of further research.

Apparatus

The novel feature of this all-glass unit for filter-paper chromatography is the arrangement of the paper strip in the form of a bridge from one flask to another by passing it through a glass sleeve. All parts are provided with standard ground-glass joints, the whole unit being made to the author's specification by Quickfit and Quartz, Stone, Staffordshire.

The unit is easily assembled, and all parts are readily accessible for cleaning. It occupies little space on the laboratory bench, having an overall length of approximately 50 cm. The unit can be accommodated in the incubator or the refrigerator as the analytical procedure may demand, and since it is quite airtight it is highly suited for work with volatile solvents.

Fig. 1 shows the general appearance and mode of assembly of the bridge unit. The two 500 ml. flasks (*D*) form, as it were,

the supporting pillars of the bridge. In practice, therefore, the glass bridge can be made to connect any one pair of flasks and no time need be lost in cleaning out the apparatus when changing from one method of analysis to another.

Parts *A* are T-shaped connecting pieces, which sit with their vertical limb on the flasks, the horizontal limb having one open and one slotted end (slot: 22 × 4 mm.); this latter end connects with part *C* or *B* of the bridge. The drawing of the assembly line of the bridge shows how parts *B* and *C* are arranged. Part *B* is a straight glass tube with cone and socket ends, the cone bearing a 9–10 mm. diameter hole in the ground-glass surface. Part *C* is also a glass tube, but here both ends form sockets and carry holes of the same size which locate with the holes in part *B*. One end of part *C* connects with part *A* to complete the bridge. The filter paper is introduced by opening the unit between parts *B* and *C* passing it either way through the slots into the flasks (Fig. 1), the paper just being held at small points of contact. When wet it sags, but even then it does not make contact with the glass along its broad surface.

The free ends of parts *A* are closed with caps *E* (standard size B34). These wide additional openings (internal diameter 28 mm.) to the unit were incorporated in the design for two reasons: first, they allow for the introduction of solutions and reagents to the unit after the filter-paper strip has been put in position and without disturbing it (a funnel with a semi-S-bent outlet is used for the purpose); secondly, this wide opening allows for the introduction of a pair of crucible tongs with which the filter-paper strip can be guided.

Since the holes in parts *B* and *C* are located in the ground-glass joints, the tubes may be rotated either to close or open them. In the former case ('closed' unit) the apparatus is

airtight and partition chromatography is carried out under these conditions. In the latter case ('open' unit) the holes are opened to allow evaporation. This procedure has been adopted in a novel form of chromatography in which the filter-paper strip forms a complete bridge between two

were selected as test material. The ordinary Whatman no. 1 filter paper commonly employed in this work did not seem quite suitable on account of its great thinness; a thicker paper was found in the fat-free strips of filter paper, Whatman no. 100, as used for milk-fat analysis. These strips measure

Fig. 1. Assembly of the bridge unit. (Note: the ventilators are not shown in the top drawing.)

different liquids, one of them being a solution of the substance to be analysed. The two liquids meet somewhere in the centre of the strip and continuation of flow is made possible by evaporation. Kawerau (1950) has given a preliminary report of chromatography of pathological urines carried out under these conditions, which will be published later in fuller detail.

Tests with the closed unit

In the first instance it was of importance to ascertain whether the change in the position of the filter paper affected R_F values to any significant extent. Carbohydrates

56 x 6 cm.; for use in the unit they are divided each into three strips of 2 cm. width. Washing by descending 'chromatography' was employed as it was found that distilled water removed small amounts of fluorescent and ninhydrin-reactive material, and, in agreement with the findings of Wynn (1949), the washed paper was also found to be faster.

R_F values were determined with A.R. samples of a number of sugars of pathological occurrence. The sugars were dissolved in distilled water or normal urine (0.25-1 mg./ml.) and 20 μ l. of each solution were used for chromatography. The chromatogram was developed with the *n*-butanol-acetic acid-water system of Partridge & Westall (1948), the butanol

Table 1. R_F values of some common carbohydrates (*n*-butanol-acetic acid-water 4:1:5 by vol.)

(Values show the range observed; temperature was not controlled.)

Substance	Dissolving medium	R_F	
		With the closed bridge unit (Whatman no. 100 paper)	By either ascending or descending chromatography (see Horrocks & Manning, 1949)
D-Glucose	Urine	0.15-0.21	—
	Water	0.19-0.21	0.15-0.19
Lactose	Urine	0.04-0.08	—
	Water	0.05-0.07	0.05-0.09
D-Galactose	Water	0.19	0.12-0.17
D-Xylose	Urine	0.30	—
	Water	0.28-0.32	0.25-0.28
D-Galacturonic acid	Water	0.14-0.16	0.14
D-Arabinose	Water	0.18-0.24	0.21-0.22

layer being in the left-hand flask (Fig. 1), whilst the aqueous layer was in the right-hand flask. After chromatography, the strips were air dried and the sugar spots revealed with aniline hydrogen phthalate (Partridge, 1949). After removal from the oven the strips may be treated with a saturated solution of 'Perspex' polymethacrylate resin in chloroform. This serves to retard the development of background colour which would otherwise obscure the spots after a day or so. This treatment does not hinder the spots from fluorescing strongly in ultraviolet light. It does not, however, prevent ninhydrin-amino-acid spots from fading.

RESULTS

The results of thirty tests are summarized in Table 1, which gives the range of R_F values for each individual sugar. Comparison of the bridge-unit values with

those recorded in the literature (Horrocks & Manning, 1949) show very close agreement, and the inference may be drawn that the modification in technique and the change in the position and nature of the filter-paper strip in no significant way affect R_F values.

SUMMARY

1. An all-glass bridge unit for filter-paper strip partition chromatography is described.
2. The unit can be used for any of the standard methods of one-dimensional partition chromatography; examples for carbohydrates are quoted.
3. The unit is supplied with ventilators, allowing a method of analysis in which continuous evaporation takes place from the paper.

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The Effect of Temperature on the Interference of Potassium in the Determination of Sodium

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In most of the microchemical methods for the determination of sodium in biological material the metal is precipitated as sodium zinc or magnesium uranyl acetate, and subsequently the uranium is estimated colorimetrically with potassium ferrocyanide or the acetic acid by titration. Phosphates are also precipitated by uranium salts, and must therefore first be removed with lime or ethanolic zinc sludge (McCance & Shipp, 1931*a*). Potassium as well as sodium forms a sparingly soluble zinc uranyl acetate. McCance & Shipp (1931*a*) considered that this would not interfere at 0°, provided the solution in which sodium was to be determined contained less than 0.3 mg. potassium/ml. Using the method of McCance & Shipp (1931*a, b*) for the determination of sodium in liver, unexpectedly high values were obtained. These could not be accounted for by sodium in the reagents, and the same high values were obtained however the phosphates had been removed. Since, moreover, no phosphate could be detected in the solutions after treating them with lime, it seemed improbable that phosphate was interfering.

As these high results were obtained both by colorimetry and titration, it was concluded that some potassium was being precipitated with sodium as the triple salt. Although the concentrations of potassium were well within the limits laid down by McCance & Shipp (1931*a*), the effect of temperature and time upon the precipitation of potassium was therefore reinvestigated using both acid extracts of liver tissue and pure solutions containing known amounts of sodium and potassium.

METHODS

For the precipitation and colorimetric determination of the triple salt, the method of McCance & Shipp (1931*a*) was used, with the following modifications. The precipitate of triple salt was dissolved in 5.0 ml. 10% (v/v) acetic acid, transferred to a 100 ml. flask and diluted almost to volume before adding 2.0 ml. 20% (w/v) $K_4Fe(CN)_6 \cdot 3H_2O$. The optical density of the coloured, colloidal solution of uranium ferrocyanide was determined in the Spekker Absorptiometer using a 1 cm. cuvette and Ilford filter 604.

The amount of K present in the triple acetate precipitate was determined by dissolving the triple salt in water and