768. *The Distribution of Water and Butan-1-ol on Chromatograms Made by the Ascending Technique.*

By E. C. MARTIN.

It is shown that in ascending paper chromatography with butan-1-ol–water changes in composition of the solvent occur owing to volatisation and to absorption by the paper (particularly when swollen).

LITTLE research has been done on the distribution of solvent and solute on paper chromatograms since the work of Krulla [1] in 1909. Since then Gaye and Saini,[2] Wood and Strain,[3] and Takahashi [4] have measured the quantity of solvent as a function of height on chromatograms made by ascending techniques. They have thus provided an overall picture of the distribution of a number of solvents. Fujita and Hiroshi [5] have derived a mathematical

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[1] Krulla, *Z. phys. Chem.*, 1909, **66**, 307.
[2] Gaye and Saini, *Helv. Phys. Acta*, 1929, **2**, 445.
[3] Strain, *J. Phys. Chem.*, 1953, **57**, 638; Wood and Strain, *Analyt. Chem.*, 1954, **26**, 260.
[4] Takahashi, quoted in reference 5.
[5] Fujita and Hiroshi, *J. Phys. Chem.*, 1952, **56**, 625.

expression describing such distributions, using, with moderate success, Takahashi's data to test it.

None of these workers studied the separation of mixed solvents which occurs during chromatography. Horner and his co-workers[6] did however study the separation of a butanol–water and a phenol–water solvent mixture as shown by the variation in composition of drops of solvent leaving the end of a descending chromatogram when compared with the original. They found a varying composition of solvent, lower in water content than the original, but increasing and eventually equalling that concentration. They concluded that the separation of the components occurred in the manner of Tiselius's frontal analysis.[7]

Burma,[8] by calculations based on the adsorption isotherms of Urquhart and Williams,[9] showed that a distribution of butanol and water would occur on paper in a manner which, in essence, was the same as that found by Horner and his co-workers.

The present paper is concerned with the distribution of water and butanol on chromatograms made by the ascending technique of Williams and Kirby.[10] Solvents of varying water content were used and the effects of this variation on distribution, solvent–vapour equilibrium, and the swelling of the paper were studied.

EXPERIMENTAL

A " fast " paper, Schleicher and Schull No. 598, was used in all tests. The paper was cut into strips (8 × 20 cm.), the long side cut in the machine direction.

The solvents were prepared by mixing commercial butan-1-ol and water, both freshly distilled. It was found that the composition varied over several days even when stored in bottles with well-fitting ground-glass stoppers. Consequently the solvents were checked before use by a density determination which was compared with known values.[11]

The development jar, in which the chromatograms were made, was of glass, about 14 cm. in diameter and 30 cm. high. The edge had been ground flat and was closed with a glass plate through which a hooked glass rod, held by a rubber bung, protruded to support the paper. About 70 ml. of solvent was held in a 10 cm. Petri dish cover which rested on the bottom of the development jar.

All chromatograms were made at 25° ± 0·1°. The papers were dried at 110° and then humidified in the solvent vapour overnight, at 25°, in a jar similar to that described above. In order to saturate the atmosphere with the vapour of the solvent, some of the latter was placed in the bottom of the jar outside the Petri dish. Fresh solvent was poured into the dish immediately before each test.

To perform the test, the humidified paper was transferred rapidly to the devel pment jar and lowered into the solvent to a depth of 1 cm., all within a few seconds. The solvent was allowed to rise 17 cm. during 80—90 min. The paper was then removed from the jar and cut rapidly into sections, 1—2 cm. wide. Each section was placed in a weighing bottle or a specially dried jar for determination of total solvent and of water.

Cutting of the paper required 3—3·5 min., a little solvent probably being lost, but no correction was made for this.

The above method is essentially that used by Krulla.[1]

For each solvent, of which there were 13 including replicates, two runs were made, one to determine the total solvent, the other to determine the water content. Total solvent was determined as loss at 110°; water was determined by the Karl Fischer technique[12] modified

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[6] Horner, Emrich, and Kirschner, *Z. Electrochem.*, 1952, **56**, 987.

[7] Tiselius, *Arkiv Kemi, Min., Geol.*, No. 2, 1940, **14**.

[8] Burma, *Analyt. Chem.*, 1953, **25**, 549.

[9] Urquhart and Williams, *J. Textile Chem.*, 1924, **15**, T138, T433, T550; 1925, **16**, T155; 1926, **17**, T38.

[10] Williams and Kirby, *Science*, 1948, **107**, 481.

[11] Martin, *Analyt. Chim. Acta*, 1959, **21**, 588.

[12] " The Determination of Water by the Karl Fischer Method," British Standard Specification 2511: 1954.

to give a higher water equivalent than usual.[13] The strips on which the water had been determined were washed, dried, and weighed to give the water : paper ratio.

Results.—Thirteen series were run covering the following range of solvents: 0·082, 0·150, 0·166, 0·273, 0·278, 0·327, 0·343, 0·347, 0·362, 0·439, and 0·474 mole fraction of water (referred to below as m.f. H_2O) in butan-1-ol; and two determinations were made on butan-1-ol alone.

As the distribution on the paper showed little variation between solvents of similar composition, only six sets of results have been shown in Fig. 1. The curve for butan-1-ol is the mean of the two replicates. Those for solvents of composition 0·082, 0·166, 0·273, 0·327, 0·343, and 0·362 m.f. H_2O have been omitted to avoid confusion. For the most part these curves fall in their expected places in relation to the others.

The curves have been smoothed but it is worth noting that irregularities appear between the 5 cm. and 9 cm. heights for the solvents of lower concentration. It is not known whether this is experimental error or whether it reflects some property of the paper which disappears with solvents of higher concentration.

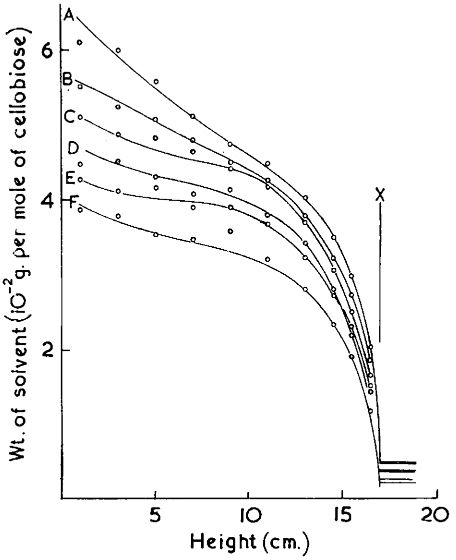

FIG. 1. *The distribution of solvent on chromatograms at various heights above the level of the solvent pool. Solvents* A—E *represent* 0·474, 0·439, 0·347, 0·278, 0·150 *mole fraction of water; and* F *anhydrous butan-*1*-ol. The vertical line* X *represents the liquid front. The horizontal lines beyond this represent the levels of solvent on the end strip of the chromatogram above the liquid front. The vertical axis is in terms of moles of cellobiose* (324 g. *of paper*).

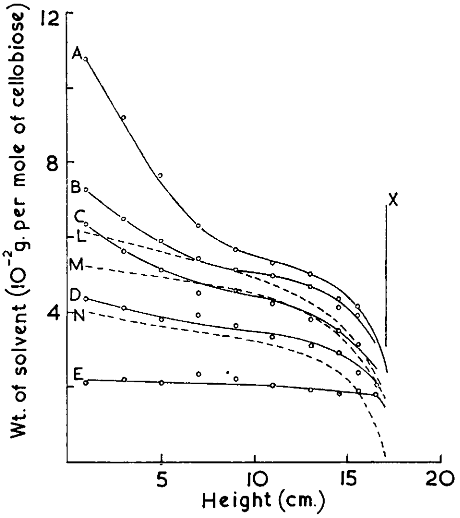

FIG. 2. *Lines* A—E *represent the distribution of water for the solutions with* 0·474, 0·439, 0·347, 0·278 *and* 0·150 *mole fraction of water respectively. Lines* L *and* M *refer to the butan-*1*-ol distribution for solutions with* 0·474 *and* 0·150 *mole fraction of water. The line* N *refers to the distribution of anhydrous butanol. The vertical line* X *represents the liquid front of the chromatogram.*

Fig. 2 shows only five of the curves for water distribution; the others, omitted for clarity, would fall in their appropriate places. The distribution of butanol for the lowest and the highest concentration, and for the anhydrous butanol series, is given for comparison

DISCUSSION

Distribution of Solvent.—The general form of the curves showing the distribution of solvent on the paper as a function of height follow the findings of Gaye and Saini, and Takahashi. An attempt to fit the butanol curve to Fujita and Hiroshi's formula did not succeed. The curves showing the water distribution are interesting in that they show a marked increase in the water : paper ratio with high concentrations of water in the

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[13] Bryson and Pickering, *J. Inst. Fuel*, 1952, **25**, 28.

solvent. At the same time the butanol : paper ratios also increase but not at the same rate. It seems that two things may happen: first, the paper absorbs water from the solvent to reach an equilibrium; secondly, the cellulose fibres and the paper swell considerably. Evidence of swelling is found when the quantity of total solvent imbibed by the paper at a particular height is compared with the volume of butanol occupying the same weight of paper at an equivalent height. The swelling is quite considerable where the water content is high. At low water concentrations (less than 0·3 m.f. H_2O) the swelling appears to be linearly related to the water content of the solvent. Combined, these phenomena will affect the equilibrium between the solvent on the paper and the vapour in the development jar.

Equilibrium between Solvent and Paper.—Randall and Weber[14] studied the vapour-pressure relation of water–butan-1-ol mixtures and showed that in the butanol-rich range a maximum solubility occurs at 30°; at about 0·52 mole fraction of water, the vapour pressures of the components were equal. Below 0·28 mole fraction the butanol had a higher vapour pressure and from 0·28 to 0·52 mole fraction water was the more volatile component. Thus the tendency is for the solvent to lose butanol by evaporation during chromatography. At concentrations less than 0·28 m.f. H_2O this loss is because of its higher vapour pressure. At higher concentrations the loss is by the adsorption of water by the cellulose, thus reducing its effective concentration in the moving solvent.

The figures given below for the gross composition of the solvent on the paper for two different solvents illustrate this point. With the more concentrated solvent, the gross composition exceeded the normal solubility of water in butanol to a significant extent and in both cases the ratio is higher than in the original solvent.

Height (cm.):	1	3	5	7	9
0·150—0·166 m.f. H_2O	0·31	0·32	0·32	0·34	0·33
0·474 m.f. H_2O	0·64	0·62	0·58	0·53	0·53

The height refers to the height above the solvent pool at which the sample was taken.

Solvents of water concentration above 0·3 m.f. H_2O showed the same trend as the higher of the series quoted. Those below 0·3 m.f. H_2O maintained a fairly constant ratio up to a height of about 11 cm. All solvents showed an increase above this height and at the liquid front all gave values which lay between 0·45 and 0·56 m.f. H_2O. However, the sample from which the latter figures were obtained had been exposed for a longer period during sampling and probably lost butanol more rapidly than water in the process. The results from solvents of lower concentration than 0·166 m.f. H_2O showed the same trends as the others but were more erratic.

Clearly, if the object of having a " humidifying " atmosphere in the container is to prevent loss of solvent from the paper during chromatography, the differential loss of solvent acts largely to nullify this aim. Further, the effect will vary depending on the volume of the container. It might be better to run the chromatogram in an atmosphere of the constituent most likely to be lost, in these cases butanol. This is particularly the case where the water is present in quantities larger than can be absorbed by the paper and thus leads to " flooding " of the chromatogram.

School of Applied Chemistry,
University of New South Wales,
Kensington, N.S.W., Australia.

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[14] Randall and Weber, *J. Phys. Chem.*, 1940, **44**, 917.