Fig. 2. Curves No. 1, 2, 3 and 4 show the incomplete removal of the last traces of sugars from the Florex–Celite mixture. Curve 5 for air blown Florex indicates (within experimental error) complete removal of glucose as can be seen by comparison of the area under this curve with that under curve 7 for a 2.1 × 100 cm. non-adsorbent column packed with Celite alone (100 g.). The areas under the fructose curves were not determined as they were found to be unreliable estimates of fructose because of the large variation of specific rotation of fructose with both temperature and concentration. The very gradual trailing off and smaller areas of glucose curves 1 and 3 can be explained by the presence of more strongly adsorbing[4] very finely divided particles. The removal of these by air blowing produces a more uniform adsorbent from which the sugars can be completely eluted by the proper solvent, *i.e.*, 90% EtOH for glucose and 80% for fructose. The incomplete elution of sugars from untreated Florex XXX has been mentioned in the literature.[5] It is evident that the air blown Florex (curves 5 and 6) offers a much better chance for separation of glucose and fructose than the untreated Florex–Celite mixture (curves 1–4) and at the same time yields a considerably improved flow rate. It also seems very likely that air blowing of other adsorbents would generally improve their behavior in chromatographic columns.

Theoretical Elution Curves.—Theoretical points were calculated according to equation (3) Mayer and Tompkins[6] for elution curve No. 5. C was calculated as 1.35 from the column free volume of 270 ml. and p was determined as 164 from the maximum of 1.22° and the total ml. degree area under the curve of 115 from the equation

$$p = 6.28 \times C \times (C + 1) \times \left(\frac{\text{max. rotation} \times \text{column free vol.}}{\text{ml. degree area under curve}}\right)^2$$

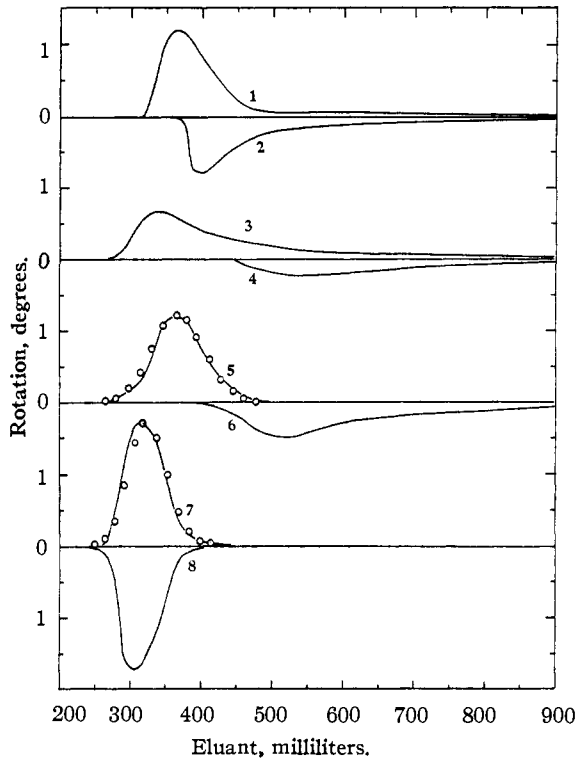

Fig. 2.

It is evident that the experimental curve is slightly steeper on the leading edge and more gradual on the trailing edge. This can be accounted for by a slightly smaller C (and faster movement through the column) for the more concentrated center portion of the band. Curves 7 and 8 illustrate the effect of a non-adsorbent on curve shape. As can be seen, they approximate the condition of $C=1$ (column free volume of 300 ml.) in terms of Mayer and Tompkin's theory. Points predicted for a p of 260 and a C of 1 are included with experimental curve No. 7.

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(4) Zechmeister and Cholnoky, "Principles and Practice of Chromatography," John Wiley and Sons, Inc., New York, N. Y., 1941, p. 44.
(5) W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, **72**, 4778 (1950).
(6) S. W. Mayer and E. R. Tompkins, *ibid.*, **69**, 2866 (1947).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, TRINITY COLLEGE]

Chromatographic Adsorption. II. The Separation of D-Glucose and D-Fructose

BY DWIGHT FAY MOWERY, JR.

Quantitative data are obtained for the chromatographic separation of D-glucose and D-fructose mixtures using air blown Florex XXX. Crystalline D-fructose is prepared in good yield from sucrose, and a rough calculation indicates 10 lb. of D-fructose might be produced per day from sucrose in a 1 × 6 ft. column.

Although the field of chromatography has expanded rapidly in the last ten years, there has been relatively little published on the separation, in any quantity, of unsubstituted sugars; and this, in spite of the fact that sugar mixtures are notoriously difficult to separate, and therefore provide worthy subjects for the chromatographic method. In particular, the separation of D-fructose and D-glucose has never been thoroughly studied, even though it might be advantageous industrially in the manufacture of D-fructose from sucrose *via* invert sugar.

Many investigators have reported a difference in the relative strengths of adsorption of sugars on charcoal,[1-8] particularly blood charcoal, and on

(1) R. O. Herzog and J. Adler, *Z. physiol. Chem.*, **60**, 79 (1909).
(2) Vasily Kniaseff, *J. Phys. Chem.*, **36**, 1191 (1932).
(3) Fujio Hayashi, *J. Biochem. (Japan)*, **16**, 1 (1932).
(4) B. P. Gyani, *J. Indian Chem. Soc.*, **21**, 79 (1944).
(5) A. Tiselius, *Kolloid. Z.*, **105**, 101 (1943).
(6) A. Tiselius and L. Hahn, *ibid.*, **105**, 177 (1943).
(7) S. Claesson, *Arkiv. Kemi Mineral Geol.*, **24A**, No. 16 (1947).
(8) R. L. Whistler and D. F. Durso, THIS JOURNAL, **72**, 677 (1950).

fuller's earth.[2,9,10] In quite a few cases[5-10] actual separations have been reported but only in one case[10] is the separation of D-glucose from D-fructose actually described. It appears from the literature that fuller's earth is more selective than charcoal for the adsorption of sugars. The solvent appears to have a profound effect upon the relative adsorptive strength on fuller's earth as Kniaseff reports D-glucose more strongly adsorbed than D-fructose from water solution, whereas Wolfrom, *et al.*, report D-fructose more strongly adsorbed than D-glucose from 95% EtOH (H_2O). The results of the present work, using 90 and 80% EtOH (H_2O) support the observations of the latter investigators in the case of D-glucose and D-fructose and also the intermediately adsorbed sucrose. Evidently adsorption data, which almost invariably have been obtained for water solutions of the sugars, cannot be applied even approximately to other solvents.

Experimental

Reagents.—These were the same as previously[11] described, with the addition of sucrose, National Sugar Refining, Jack Frost.

The Chromatographic Column.—The chromatographic runs were carried out as already described,[11] using a 2.1 × 100 cm. column of air blown Florex XXX and pressures up to 110 lb. per sq. inch. Polarimeter readings were taken every 25 ml. after rejecting the first 25 ml. (volume of exit tubing) plus $^1/_2$ the volume of the charge. The charges were made up by pipetting into the top of the column the appropriate quantity of a fructose–glucose solution made by dissolving 5.00 g. of each in 20 ml. of water and diluting to 100 ml. with 95% alcohol, producing an approximately 76% EtOH solution. In some cases further quantities of fructose, glucose or sucrose solutions made up in the same way as above were pipetted in to give unequal mixtures of the sugars. The solutions were all allowed to stand at least two days before use to ensure complete mutarotation.

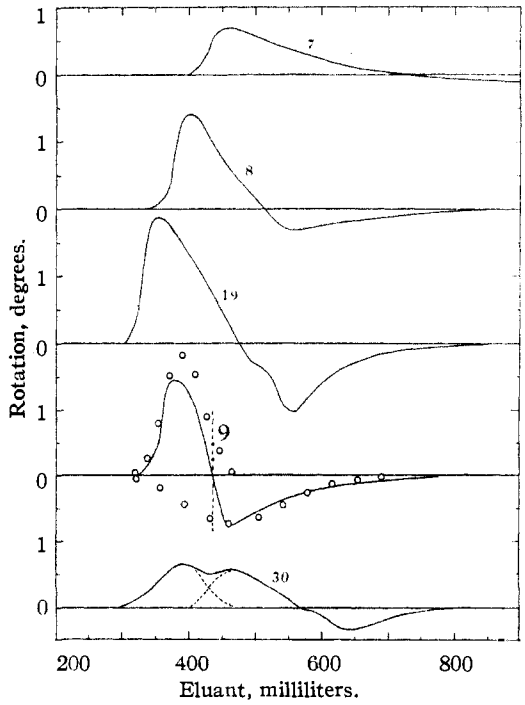

Fig. 1.

Calculation of Per Cent. Separation.—Elution curve number 9 of Fig. 1 was combined with the complete theoretical curve for fructose (circles) calculated from Mayer and Tompkins[12] equation (5), C being 1.72 and p, 73 (interstitial volume 270 ml.). The total glucose curve could then be added (C = 1.44) and was found to be best fitted not by the calculated p = 400 but by p = 300. By considering the areas of the diagram it can be shown that if the fraction is cut at the point of zero rotation (dashed line), the glucose cut will contain about 77% glucose while the fructose cut will contain about 94% fructose. If the cut is made at the point of minimum rotation, the glucose solution will contain about 69% glucose while the fructose cut will be essentially pure fructose. Obviously the degree of separation in a case of partial separation of this kind will be ambiguous. In this paper it has been thought desirable to use an arbitrary "per cent. separation" based on the grams of glucose "separated," as indicated by the area (or increase of area) of positive rotation. The negative area was found to be an unreliable measure of the amount of fructose present because of the wide variation of the specific rotation of fructose with concentration and temperature.

The positive areas of Table I were determined with a planimeter, accurate to better than 1%, by carefully plotting the rotations observed in the two decimeter polarimeter tube against milliliters of effluent, the scale being 0.4 degree and 25 ml. to the inch. The grams of glucose represented by these areas were calculated as area/(2 × 57.8). 57.8° was estimated as the average glucose specific rotation[13] for a run. The data upon which this estimation is based are shown in Fig. 2, curves A and B representing the change of specific rotation on fourfold dilution to 80 and 90% (or 95%) EtOH, respectively, of a standard glucose charge rotating initially +58.4° (C, 1.500; 76% EtOH). Curve C shows the change of specific rotation of glucose (C, 1.500) with % H_2O by volume in H_2O–EtOH mixtures, while curve D shows the change of specific rotation of glucose with concentration in 90% EtOH by volume (10 ml. H_2O made up to 100 ml. with absolute alcohol). The change of rotation (C, 1.5; 90% EtOH) between 20 and 30° was found to be negligible as was also the difference in rotation of a glucose solution kept at 20° and one cooled rapidly from 60 to 20° (runs 22–25) or heated rapidly from 0 to 20° (run 27).

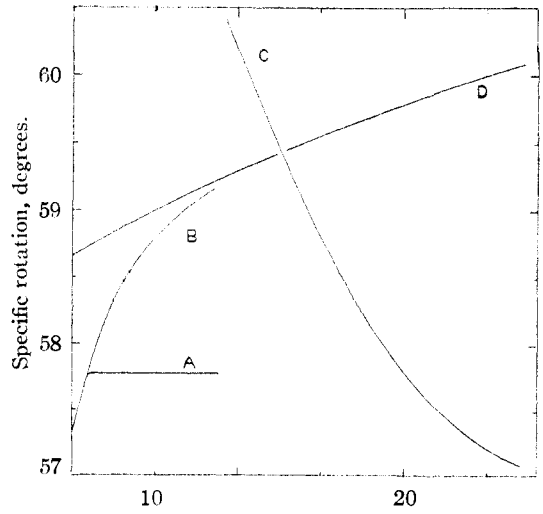

Fig. 2.

The figures for per cent. separation of the mixture, calculated in most cases as 100(grams of glucose recovered/grams of glucose charged) (Table I), agree very well with those calculated as 100(positive area in given separation/positive area of corresponding glucose charge). It should be borne in mind that incomplete separations may be in

(9) B. W. Lew, M. L. Wolfrom and R. M. Goepp, Jr., THIS JOURNAL, **68**, 1449 (1946).

(10) W. W. Binkley and M. L. Wolfrom, *ibid.*, **72**, 4778 (1950).

(11) Paper I, D. F. Mowery, Jr., *ibid.*, **73**, 5047 (1951).

(12) S. W. Mayer and E. R. Tompkins, *ibid.*, **69**, 2866 (1947).

(13) All specific rotations in this paper are given for the D line of sodium at 20°; C = g. per 100 ml.

TABLE I[a]

Run	Charge, g. Gluc.	Fruc.	EtOH,[b] ml.	Temp., °C.	Eluant %EtOH[c]	Average flow rate, ml./min.	Pressure lb./sq. in.	Positive area ml. × deg.	Glucose recovered, g.	Sepn.,[d] %
1	1.0	1.0	20	24	95	2.3	80	109.7	0.949	95[e]
2	1.5	1.5	30	24	95	1.7	100	128.0	1.107	74[e]
3	1.0	1.0	20	25	90	1.6	100	88.0	0.761	76[e]
4	0.5	0.0	10	24	90	5.6	80	59.0	.510	102
5	1.0	.0	20	23	90	3.5	55	115.0	.995	99
6	1.5	.0	30	22	90	5.0	80	174.5	1.510	101
7	1.0	1.0	20	24	95	6.5	40	108.8	0.941	94
8	1.0	1.0	20	24	90	6.3	50	117.8	1.019	102
9	1.0	1.0	20	22	85	6.0	60	86.4	0.747	75
10	0.5	0.5	10	22	80	4.1	100	50.8	0.439	88
11	1.25	1.25	25	25	90[f]	5.8	80	134.5	1.163	93
12	1.5	1.5	30	23	90[f]	4.3	60	147.0	1.272	85
13	1.5	1.5	30	26	90[f]	2.0	16	142.2	1.230	82
14	2.0	2.0	40	22	90[f]	5.0	70	154.9	1.340	67
15	2.0	1.0	40	26	90[f]	3.5	80	190.5	1.648	71[g]
16	1.0	2.0	40	23	90[f]	5.5	50	51.6	0.446	45
17	0.5	1.5	30	23	90[f]	5.2	100	46.7	.404	81
18	1.0	1.25	25	23	90[f]	4.8	100	107.2	.927	93
19	1.5	1.0	30	23	90[f]	4.6	100	174.7	1.511	101
20	2.0	0.5	40	23	90[f]	4.3	100	230.7	1.995	100
21	2.5	0.5	50	23	90[f]	4.3	110	231.4	2.002	17[g]
22	1.0	1.0	20	65	90[f]	5.0	70	65.4	0.566	57
23	1.0	1.0	20	50	90[f]	6.0	60	101.2	0.875	88
24	1.0	1.0	20	50	95[f]	4.8	70	94.2	0.815	81
25	1.0	1.0	20	35	90[f]	4.0	80	107.3	0.928	93
26	1.5	1.5	30	15	90[f]	4.3	90	141.8	1.227	82
27	1.5	1.5	30	2	90[f]	3.6	100	142.9	1.236	82
28	1.0	1.0[h]	20	23	90[f]	4.3	80	111.0	0.960	96
29	0.5 g. Sucrose		10	23	90	4.8	110	55.1		
30	Gl. Fr. Su[i]		20	25	90[j]	3.8	60	104.0		91[i]

[a] The adsorbent in all runs except 1, 2 and 3 is Florex XXX air blown from 740 to 185 g. (100 cm. height of packing in 2.1-cm. tube). [b] Calculated to be approximately 76% EtOH, 24% H_2O by volume. [c] By volume; made from 95% alcohol assuming no contraction in volume. [d] See calculation of per cent. separation for definition. [e] 110 g. Florex XXX, untreated–55 g. Celite 535 (100 cm. height of packing). [f] 250 ml. of 90% EtOH followed by 80% EtOH. [g] Calculation allows for original positive rotation of charge. [h] Invert sugar made from sucrose. [i] 0.5 g. each; % separation calculated from sum of glucose and sucrose areas. [j] 350 ml. of 90% EtOH followed by 80% EtOH.

some error because of the wide variation of fructose rotation with temperature and concentration.

Recovery of Crystalline D-Fructose and D-Glucose from Sucrose.—26.25 g. of sucrose was dissolved in 25 ml. of 0.4 *N* HCl and the solution allowed to stand 24 hours, at which time it had reached a minimum rotation. The solution was then introduced over a 2.3 × 28 cm. column of DeAcidite (Permutit Company) which had previously been thoroughly washed with 95% EtOH. The solution was run slowly into the adsorbent by gravity and then 25 ml. of water and finally 95% alcohol were run through. The effluent was passed through a polarimeter tube. A forerun of 60 ml. of 95% alcohol was discarded and the charge collected in 190 ml., showing a zero rotation at the start and finish of the run. The 190 ml., collected in a volumetric flask, was then made up to 250 ml. with 95% alcohol at 20°. This solution is calculated to contain 1.00 g. each of fructose and glucose in 20 ml. of approximately 76% EtOH. This solution (*p*H 7) was chromatographed (run 28) and gave a 96% recovery of glucose, based on the original sucrose weight. The column effluent was separated into three parts, the first cut being made at the initial appearance of glucose and the second at the point of zero rotation, so that the second fraction, containing glucose, was 150 ml. and the third, containing fructose, was 275 ml. These solutions were evaporated under vacuum at 40–50° with several additions of absolute alcohol to remove water. The final solutions then crystallized readily, producing 0.80 g. of glucose, rotating +51.8° (*C*, 2.740) and 0.76 g. of fructose, rotating −91.2° (*C* 2.582). 0.03 g. of glucose and 0.08 g. of fructose were recovered from mother liquors, making the total crystalline yields 83% for glucose and 84% for fructose. The final mother liquors showed polarimetrically the presence of further sugar which presumably, on a larger scale, could be recovered to give near quantitative yields of glucose and fructose from sucrose.

Discussion

D-Glucose–D-Fructose Separations.—While 95% EtOH is the most satisfactory developer for the separation of glucose and fructose using untreated Florex XXX–Celite (2:1 by weight) as adsorbent (runs 1, 2 and 3 of Fig. 1), 90% is best for Florex XXX air blown to 25% of its initial weight. Comparisons of runs 1 and 8, and 2 and 12 shows that the untreated Florex mixed with Celite is less effective, volume for volume, than air blown Florex in separating fructose and glucose. Runs No. 7, 8 and 9, using air blown Florex (Table I and Fig. 1), show the effect of increasing amounts of water on the separations. In all cases, 1 g. each of glucose and fructose were charged and the separation falls off from complete for 90% EtOH to 75% for 85% EtOH. The elution of the fructose, however, is slow and incomplete with 90% EtOH, but much more satisfactory with 85% EtOH. A method was therefore used in most of the runs which consisted of introducing 250 ml. of 90% EtOH immediately after the charge had run down into the adsorbent, and following it with 80% EtOH. The separation was effected and the fructose partly eluted by the 90% EtOH, the 80% then rapidly completing the

elution of the remaining fructose. Curve No. 19 of Fig. 1 is typical of this type of run, showing the sharp break produced in the fructose elution curve.

Various quantities of glucose and fructose (as shown in Table I) were separated in varying degrees, the % separation being calculated as described previously. From this table, it is evident that 1.0 g. each of the sugars is the maximum quantity that can be completely separated in an equimolar mixture by a 2.1 × 100 cm. (185 g.) column of air blown Florex XX (run 8). 1.25 g. each is separated only to the extent of 93%, 1.5 g. each 85%, and 2.0 g. each 67%. Increase of temperature above 30° results in a poorer separation (runs 22, 23, 24 and 25) and a decrease below 20° (runs 26 and 27) does not improve it. Also a slower flow rate (run 13) gives no better separation. With unequal quantities of the sugars, it is evident that larger quantities of glucose can be separated from fructose than fructose from glucose. For instance 1.5 g. of glucose can be completely separated from 1.0 g. of fructose (run 19), whereas 1.25 g. of fructose is separated from 1.0 g. of glucose to the extent of only 93%. 2.0 g. of glucose is completely separated from 0.5 g. of fructose, whereas 1.5 g. of fructose is only 81% removed from 0.5 g. of glucose. The condition for complete separation of glucose from fructose per gram of Florex XXX blown to 25% weight and using 90% EtOH, appears to be approximately

$$\text{g. glucose} + 2 \times \text{g. fructose} = 0.0162 \text{ g.}$$

Gluckauf's theory[14] predicts for optimum conditions of separation

$$\text{g. A} + \text{g. B} = \text{a constant}$$

Distribution Ratios and Theoretical Plates.—The distribution ratios, C, as defined by Mayer and Tompkins[12] varied from 1.3 to 1.7 for glucose, 1.6 to 2.1 for fructose, and were 2.2 for 0.5 g. of sucrose alone (run 29) and 1.7 for 0.5 g. of sucrose mixed with like quantities of glucose and fructose, the sucrose being eluted before the fructose, as shown in elution curve No. 30. In Table II are listed the distribution ratios, C, calculated from the column free volume of 270 ml., and the number of theoretical plates, calculated as described previously,[11] for several glucose and glucose–fructose runs in which complete separation allowed easy calculation.

From the table, it is apparent that C increases slightly in dilute solution (low maximum). This is in keeping with the skewness of many of the curves,

(14) E. Gluckauf, *Proc. Roy. Soc.* (*London*), **A186**, 35 (1946).

TABLE II

Run	Gluc., g.	Fruc., g.	EtOH, %	Max. pos. rot., deg.	Glucose C	Glucose p
4	0.5	0.0	90	0.56	1.61	173
5	1.0	0.0	90	1.22	1.35	164
6	1.5	0.0	90	1.42	1.33	94
8	1.0	1.0	90	1.44	1.48	251
19	1.5	1.0	90	1.91	1.31	166
20	2.0	0.5	90	1.86	1.28	87
7	1.0	1.0	95	0.72	1.68	90
9	1.0	1.0	85	1.85	1.44	400[a]

[a] 400 is the calculated value but actually 300 gives a better fit.

exemplified by No. 7, 8 and 19 of Fig. 1. The number of theoretical plates varies widely from run to run, dropping off with higher concentrations of glucose, increasing in going from 95 to 85% EtOH, and increasing when fructose is present. Unfortunately, advantage of the increase of theoretical plates (narrower bands) with larger concentrations of water cannot be utilized in the separation of fructose from glucose because at the same time C for fructose is considerably reduced while C for glucose is not changed much (runs 8 and 9).

Separation of D-Glucose, D-Fructose and Sucrose.—Curve 30 of Fig. 1 shows the separation of glucose, fructose and sucrose, using 350 ml. of 90% EtOH and then 80% EtOH. It is evident that even 0.5-g. quantities of these sugars are incompletely separated. If theoretical curves are drawn in for glucose, first positive hump, and sucrose, second positive hump, it can be seen that the separation of glucose and sucrose is in the neighborhood of 90%, while the separation of fructose from sucrose, determined from the total positive area and the sum of the positive areas for glucose and sucrose separately (runs 4 and 29) gives 91% as the separation of the fructose.

D-Fructose from Sucrose on a Larger Scale.—A rough calculation can be made for a continuously operating column, through which are run cycles of acid-free invert sugar in 76% EtOH, 90% EtOH, and 80% EtOH, the effluent fractions being concentrated to crystallization. Curve No. 19 shows that a typical glucose–fructose cycle requires about 500 ml. of solvent per gram of fructose recovered, which takes about 110 min. to flow through a 2.1 × 100 cm. column. About 10 lb. of fructose should be capable of production per 24 hr. day in a 1 × 6 ft. column, operating under 200 lb. pressure. This yield might be increased if it is found possible to combine crystallization and chromatography in the separation.

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