[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, TRINITY COLLEGE]

Chromatographic Adsorption. I. An Efficient Utilization of Finely Powdered Adsorbents

BY DWIGHT FAY MOWERY, JR.

The construction and operation of a laboratory chromatographic column at pressures up to 120 lb. per square inch is described. A method of air blowing adsorbents has been adopted which improves flow rate and allows complete elution of adsorbed sugars from Florex XXX. Elution curves for glucose and fructose from air blown Florex XXX, Florex–Celite, and Celite columns were obtained and two were compared with the theoretical curves of Mayer and Tompkins.

Numerous authors[1,2] in the past have advocated the use of moderate pressures in the operation of chromatographic columns, but the majority of chromatographic work carried out at present involves the use of either vacuum or pressures not exceeding one atmosphere. This produces a very slow flow rate for the 200-up mesh particles found most satisfactory for chromatographic work. One object of the present work was to design and test a laboratory column capable of operating at pressures up to 120 lb. per square inch. At the same time it was thought desirable to test a new method for preparation of adsorbents for chromatographic work which it was hoped would increase the flow rate and homogeneity of adsorption without causing a serious loss in capacity or resolution. This method consists in blowing air through the adsorbent to remove the finer particles.

Experimental

Reagents.—The D-fructose used was Pfanstiehl Levulose, C.P. special. The D-glucose was Eastman Kodak Dextrose (Anhydrous). The Florex XXX was obtained from the Floridin Co., Warren Pa.

Air Blowing of Florex XXX.—Diagram A of Fig. 1 shows the apparatus used for the air blowing of adsorbents. It consists of a 4.8 × 122 cm. glass tube over the lower end of which is taped a circle of 100 mesh copper screen and over this an ordinary 2 inch funnel, the tape (not shown) extending several inches up the tube. A slow speed stirring motor fitted with a long glass rod, the lower end of which is fashioned into a small propeller, is mounted so that the glass rod hangs down into the large glass tube and almost touches the screen. It was not found necessary to provide a guide to keep this agitator in the center of the tube. The top of the tube is fitted with a tray to catch most of the dust blown off. In operation, which should be carried out in a well-ventilated hood, compressed air is introduced at the bottom and then 200 g. of finely powdered adsorbent (usually 200-up mesh) is added. The agitator is started in order to prevent channeling of the adsorbent and the air blowing is continued for various lengths of time at rates of between 5 and 10 liters per min. At the end of a run the adsorbent is removed, weighed, and tested for flow rate, and either stored or returned for further air blowing.

Measurement of Flow Rate.—The adsorbent was introduced in a slurry in the selected solvent into a 6.5 × 400 mm. tube, the bottom of which was closed securely by a piece of filter paper supported on a perforated clay plate. This was easily accomplished by forcing two rubber stoppers, one carrying a short outlet tube and the other the 400 mm. tube, into opposite ends of a short length of large glass tubing so that the perforated clay plate and filter paper were held firmly between them. Fresh solvent was introduced and run through the tube by applying about one atm. of compressed air at the top of the tube. As soon as the adsorbent had settled completely, the tube was filled up with solvent and the time required for 5 ml. to flow through was measured with a stopwatch. The height of the adsorbent in the column (usually 20–25 cm.) and the exact pressure of the compressed air were recorded. No attempt was made to control the temperature, which, however, probably did not vary by more than 2° from 25°. From these data the specific flow rate (Table I) was calculated as

$$\frac{4{,}300 \times \text{mm. of adsorbent}}{\text{seconds} \times \text{mm. of Hg}} = \text{ml. per min. per cm.}^2 \text{ (cross sect.) per cm. (height) per lb. per square inch}$$

Specific flow rates determined in this small tube were found to predict approximately the actual flow rates that could be obtained in the larger chromatographic tubes.[3]

TABLE I

Florex XXX	Loss in wt., %	Liquid	Specific flow rate[a]
Untreated	0	95% EtOH	0.32
With Celite[b]	0	95% EtOH	1.3
Air blown 8 1/3 hr.	45	95% EtOH	0.80
5 3/4	63	95% EtOH	5.3
18 3/4	72	95% EtOH	7.4
20 1/4	75	95% EtOH	8.1
20 1/4	75	90% EtOH[c]	7.1
20 1/4	75	80% EtOH[c]	5.4
22	79	95% EtOH	9.4
14	97	95% EtOH	12.9

[a] Ml. per minute per cm.² cross section per cm. height of adsorbent per lb. per sq. inch pressure. [b] 2 parts Florex XXX to 1 part Celite 535 by wt. [c] By volume; made from 95% alcohol assuming no contraction in volume.

Chromatographic Apparatus.—The chromatographic apparatus is illustrated by parts B and C of Fig. 1. B is the chromatographic column, the inside glass tube, 2.1 × 122 cm., being a heavy-walled Pyrex tube. These dimensions were selected for the present work as it would, with relatively small solvent volumes, provide data on a 100-cm. bed of adsorbent. In general a ratio of diameter to length of column of one to four to one to ten is considered satisfactory.[1] The bottom of the chromatographic tube is closed by means of a perforated porcelain plate fitting inside the tube and supporting a small circle of filter paper. The procelain plate is held in place by a neoprene stopper with a large hole directly under the plate about 1/8 inch deep, and a small hole the rest of the way through to fit the copper outlet tube tightly. The adsorbent was introduced in a slurry in 95% ethanol, using one atmosphere pressure to remove excess solvent. The end plates of the column, to which the exit tubes were soldered, are made of 1/4 inch brass and the three tension rods (only two are shown) are 1/4 inch brass rods tightened at the ends by thumb screws. The chromatographic column is glass jacketed for operation at other than room temperature as are also the copper inlet and outlet tubes, the jackets in these cases being copper and soldered directly to the center tubes for better heat transfer. Water is circulated through these jackets from a constant temperature bath maintaining the temperature to 0.1°. The effluent from the bottom of the column was run through a two decimeter polarimeter tube as future separations of sugar mixtures were contemplated. From the polarimeter tube the effluent was run into a graduated cylinder, all connections being short lengths of neoprene tubing. A stainless steel tube one inch long was placed in the brass polarimeter tube and could be moved back and forth by a strong Alnico

(1) H. M. Strain, "Chromatographic Adsorption Analysis," Interscience Publishers, Inc., New York, N. Y., 1945, pp. 32–41.

(2) E. M. Titov, *Zavodskaya Lab.*, **13**, 1359 (1947).

(3) A. L. LeRosen, *Anal. Chem.*, **19**, 189 (1947).

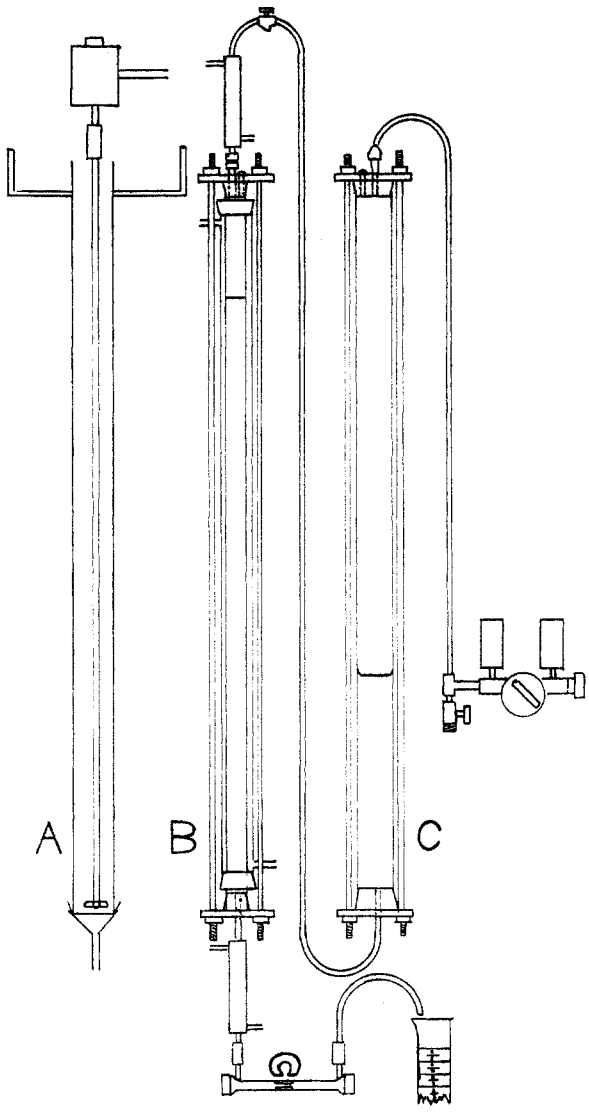

Fig. 1.

magnet to ensure homogeneity of the solution and clear polarimeter readings. The developing solvent is introduced into the top of the chromatographic column by means of a copper tube from the bottom of the 3.6 × 122 cm. solvent reservoir, C, a heavy walled Pyrex tube. The solvent is forced through the system by nitrogen pressure from a cylinder equipped with a reduction valve capable of passing 125 lb. per sq. inch. Both columns were well shielded although no trouble was experienced at pressures up to 120 lb. per sq. inch.

Operation of the Column.—In a typical run, the coupling at the top of the chromatographic tube was disconnected, any residual solvent in the reservoir blown out, and the liquid above the adsorbent syphoned down to the level of the adsorbent. After opening the air vent (by removal of the closure screw), the charge was pipetted in over the adsorbent, the coupling reconnected, and the vent screw replaced. The pressure was slowly increased to about 20 lb. and the top of the charge run down to within $^1/_2$ inch of the adsorbent. The shut-off valve in the copper tube at the top of the column (Fig. 1) was then closed and the tube disconnected from the top of the solvent reservoir. The air vent was opened, the desired solvent introduced, the tube reconnected, and the air vent closed. Pressure of 20 lb. was put on the reservoir and the shut-off valve above the chromatographic column slowly opened. The pressure was then slowly increased to produce the desired flow rate. A change of solvent was made by allowing the original solvent to run down to within about one inch of the top of the adsorbent (for high pressures), closing the shut-off valve, reducing the pressure on the reservoir, introducing the new solvent, increasing the reservoir pressure to the original, and slowly opening the shut-off valve. This does not subject the chromatographic column to rapidly changing pressure, which was found to cause a separation of the adsorbent mass or excessive packing and resultant decrease of flow rate. In order to slowly reduce the pressure at the finish of a run, the space above the adsorbent is allowed to fill completely with solvent by momentarily loosening the vent screw. The shut-off valve is then closed, the pressure on the reservoir reduced, and the pressure in the chromatographic column allowed to bleed slowly away in the course of several hours or overnight.

The charges were made by dissolving 5.00 g. of glucose or fructose in 20 ml. of water and diluting to 100 ml. with 95% EtOH, producing an approximately 76% EtOH solution. They were introduced from a 20 ml. pipet, after standing at least two days to ensure complete mutarotation. Polarimeter readings were taken every 25 ml. after discarding the first 25 ml. (volume of rubber tube and polarimeter tube) plus one-half the volume of the charge.

Discussion

Air Blowing of Adsorbents.—In order to improve the flow rate, it was found that air blown through the adsorbent to remove the finer particles was a more satisfactory treatment than the usual practice of mixing the adsorbent with a filter aid. Table I gives the results of air blowing Florex XXX for various lengths of time. From these data, obtained with varying air flow rates, it is evident that the final solvent flow rate through the adsorbent depends principally on the per cent. loss in weight of the adsorbent and very little on the air flow rate. The Florex XXX used in this paper, however, was air blown overnight (18 hours) for convenience sake. The specific flow rate is approximately equal to

$$^1/_4\%\ \text{loss in wt.} - 11$$

for losses in weight from 45 to 97%. From Table I, it is obvious that the flow rate of Florex XXX can be increased approximately 4-fold by admixture with 50% of its weight of Celite 535. By air blowing to 25% of its original weight, however, its flow rate can be increased about 25-fold. In 2.1 × 100 cm. (adsorbent depth) columns the flow rate for air blown Florex (185 g.) is about 4 times that for the Florex–Celite mixture (110:55 g.) under comparable pressures (Table II).

TABLE II[a]

Run no.	Adsorbent	Developer % EtOH	Flow rate ml./min.	Pressure lb./sq. in.	Pos. area[b] ml. × deg.
1	Florex, 110 g. Celite, 55 g.	90	2.3	100	101[c]
2		90	2.0	100	
3		95	1.9	120	105
4		95	1.7	120	
5	Air blown Florex, 185 g.	90	3.5	55	115
6		90	4.0	60	
7	Celite, 100 g.	95	2.6	2.2	118
8	Celite, 100 g.	95	2.3	2.2	

[a] Temperatures 23–25°. [b] Determined with a planimeter from a large scale plot. [c] Not including the long "tail."

Elution Curves.—Several elution curves were run for 1 g. quantities of glucose and fructose introduced in 20 ml. of 76% EtOH and developed with the designated % EtOH (made from 95% EtOH, assuming no contraction of volume on adding water). The results are given in Table II and

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

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

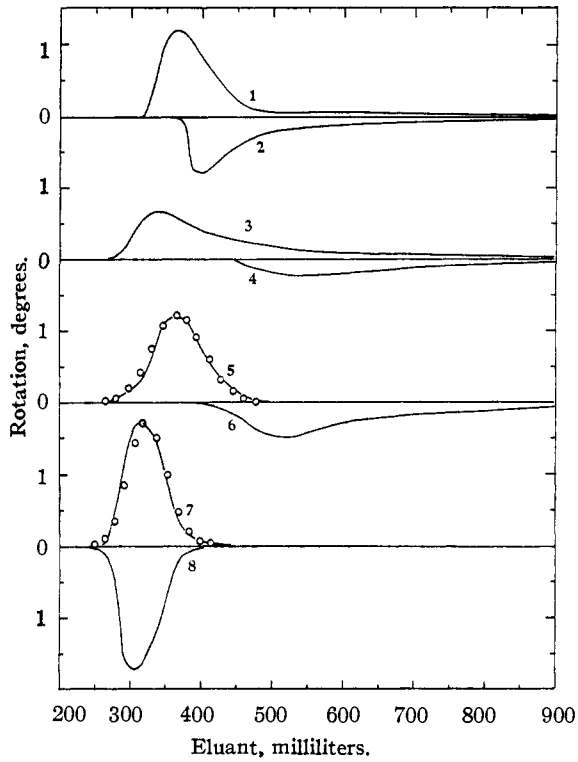

Fig. 2.

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).

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

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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).