EXTRUSION COLUMN CHROMATOGRAPHY ON CELLULOSE

M. L. WOLFROM, D. H. BUSCH, ROSA M. DE LEDERKREMER, SHARON C. VERGEZ AND J. R. VERCELLOTTI
The Ohio State University, Columbus, Ohio (U.S.A.)
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INTRODUCTION

Extrusion column chromatography has long been successfully applied to adsorption systems[1]. The qualitative correlation of thin-layer techniques with extrusion column chromatography has been effectively applied in this laboratory for the adsorbent Magnesol[2]. An improved thin-layer partition chromatography on microcrystalline cellulose was recently reported[3]. This paper describes the extension of thin-layer chromatography on microcrystalline cellulose (Avirin*) to a cellulose extrusive technique which can be correlated qualitatively with the thin-layer technique.

Extrusion column chromatography offers an analytical as well as preparative tool which is both compact and rapid, provided the proper conditions can be found for separating compounds and locating zones. The extrusive technique reported herein is a modification of that reviewed by THOMPSON[1]. It was the purpose of the present work to apply the extrusive technique to a representative range of organic and inorganic compounds containing polar functional groups which permit good partitioning effects.

EXPERIMENTAL

Adsorbent

Avirin is a microcrystalline cellulose obtained from the Avicel Sales Division of American Viscose Division, FMC Corp., Marcus Hook, Pa. Avicel is the pharmaceutical grade of the same material. We found that this grade possessed no advantage over our samples of Avirin, but either grade is satisfactory.

Column preparation

The ZECHMEISTER[1,4] type column was packed by pouring, onto a cotton filter pad, a blended suspension of cellulose (Avirin) in the development solvent (1:4, w/v) and permitting the solvent to filter through the cotton pad with about one-third of an atmosphere pressure (provided by bleeding air into the trap of a water aspirator with stopcock open). This pressure was maintained during washing, elution, and drying of the column to assure an even partitioning surface inside the column.

When the desired amount of developing solvent (determined by experimentation) had passed through the column and free dripping from the bottom of the

* Now designated Avicel-Technical Grade.

column had ceased, the cellulose was assumed dry enough for extrusion and was extruded in the usual manner. The surface of the extruded column was dried with a stream of warm air in preparation for the location of the zones.

Sugars were located by the silver nitrate–sodium hydroxide method[5]. A strip 0.5 cm wide was formed by spraying through a slit in a plastic shield, equal in length to the column, followed by spraying with *M* sodium thiosulfate. Sugars were also detected by streaking with a solution containing four parts of 2 % aqueous sodium metaperiodate and one part of 1 % potassium permanganate in 2 % aqueous sodium carbonate[6]. Compounds containing the primary amino group were located by spraying through the shield with 0.2 % ninhydrin in ethanol. The colored iron complexes were easily visible during all phases of the separation.

Procedure

A typical separation was that effected between D-galactose and D-xylose. A column (34 × 145 mm) was prepared as described above by blending together cellulose (60 g) and 1-butanol–ethanol–water (8:1:1, 240 ml, developing solvent). After washing for 1 h with about 200 ml of the same solvent, the solvent level above the column was permitted to come to a point where the top of the cellulose was just uncovered. D-Galactose (80 mg) and D-xylose (80 mg) dissolved in 1-butanol–ethanol–water (4:1:1, 5 ml) were poured on the column top. When the solution of sugars had settled into the column, a filter paper was placed on the column top and the developing solvent, 1-butanol–ethanol–water (8:1:1, 200 ml) was poured through the column over a 2-h period under the same vacuum conditions as used for packing the column. When no more solvent dripped from the bottom of the column, the column was extruded, partially dried with a stream of warm air, and streaked with permanganate–periodate reagent[6] as described above. Two zones appeared (see Table I), one zone 25 mm from the top of the column and 25 mm wide, the second zone 75 mm from the top of the column and 35 mm wide. The zones were excised, eluted three times with 30 ml of water, centrifuged, and fractions concentrated to about 5 ml, and filtered through a 4-mm Celite pad on a sintered glass funnel. Thin-layer chromatography[3] on cellulose showed chromatographic homogeneity for the fractions on developing with 1-butanol–ethanol–water (4:1:1). The fractions were concentrated to sirups, taken up in hot methanol, and filtered to remove insoluble residues. Crystals formed on concentrating both solutions; yield, 65 mg (81 %) of D-xylose and 62 mg (78 %) of D-galactose, m.p. identical with literature values.

Other separations are recorded in Table I.

The colored iron compounds constitute the crude mixture of complexes formed by self-condensation of *o*-aminobenzaldehyde in the presence of iron(II) salts[7]. These are the subject of continuing investigations. Elution of the excised zones containing the complexes was most effectively carried out by blending the zone into a slurry of the developing solvent and pouring into a column which was then eluted further with several more volumes of the developing solvent.

DISCUSSION OF RESULTS

A great asset of this extrusion technique is that it apparently can be applied to compounds usually separable by paper chromatography (see range of compounds in

TABLE I

EXTRUSION COLUMN ZONE MOBILITIES

Expt.	*Compounds*	*Mg applied*	*Column (mm)*	*Developer*	*Solvent (ml)*	*Time (h)*	*Indicator**	*Zone dimensions (mm)***	
1	D-Galactose	80	34 × 145	BuOH–EtOH–H_2O	200	2.0	A	25	25
	D-Xylose	80		(8:1:1)				75	35
2	(+)-Tartaric acid	100	34 × 145	BuOH–HOAc–H_2O	50	0.5	A	50	30
	Oxalic acid	100		(4:1:1)				83	20
3	Gentiobiose	20	40 × 200	BuOH–EtOH–H_2O	300	2.8	A, B	31	28
	Maltose	50		(4:1.2:1.2)				31	28
	D-Glucose	50						108	62
4	Gentiobiose	20	40 × 200	BuOH–EtOH–H_2O	1000	9.0	A, B	12	66
	Maltose	50		(8:1:1)				25	31
	D-Glucose	50						108	63
5	D,L-Valine	10	34 × 145	BuOH–HOAc–H_2O	200	2.0	C	90	10
	L-Leucine	80		(8:1:1)				105	20
6	2-Amino-2-deoxy-D-galactose·HCl		40 × 184	EtOAc–Pyr–H_2O–HOAc	1000	8.0	B, C	107	65
	D-Glucose			(5:5:3:1) (Ref. 8)				effluent	
7	Condensation products of *o*-amino-benzaldehyde and iron(II) salts		40 × 200	BuOH–EtOH–H_2O (4:1.2:1.2)	400	3.0	Colored complexes		
	Orange baseline***							0	20
	Orange-yellow							45	25
	Green							121	31
	Brown							197	12
	Yellow-brown							effluent	

* Reagent A: $KMnO_4$–$NaIO_4$; reagent B: $AgNO_3$–NaOH–$Na_2S_2O_3$; reagent C: ninhydrin.

** Distance in mm from top of column (1st column of numerals) and width of zone (2nd column).

*** Zone color.

Table I). The microcrystalline cellulose needs no filter-aid and permits easy packing of the ZECHMEISTER[1,4] column.

Applying the mixture of compounds to be separated to the top of the column is done effectively and evenly on this medium. The relative proportions of polar components in solvent mixtures and the amount of developing solvent necessary were effectively patterned on the known solvent systems and developing times for thin-layer partition chromatography on cellulose (see Table I).

After development, the column was permitted to drip solvent with the same vacuum as during elution until a relatively solid mass formed. The column was then easily extruded intact. This is in contrast to the Magnesol–Celite column which requires thorough drying before extrusion. After some surface solvent was removed from the column core with the aid of a warm air stream, no further drying was necessary before streaking to locate zones, excising the zones, and eluting the separated products from the cellulose.

It would appear that a less polar solvent which moves the zones quite slowly on a cellulose thin-layer plate brings about a more efficient partitioning effect between the cellulose, solvent, and compounds being separated at the flow rate of the developing solvent employed. Thus, comparing Table I, in Expt. 3 and Expt. 4, D-glucose moved the same distance on the column with both solvents, but in one case the mobility noted was effected in 2 h and in the other in 8 h. However, maltose and gentiobiose failed to separate in Expt. 3, whereas with the longer elution time in the less polar solvent (Expt. 4), effective separation occurred.

When the developed column was dried rapidly with vacuum instead of dripping until enough solvent was removed to permit extrusion, the zones moved quickly from their location at the end of the desired development time and smeared badly over the cellulose. This was avoided by the suggested technique.

The colored iron complexes[7] provided an interesting system with which to study development time. These possessed mobilities similar to mono- and disaccharide derivatives with butanol–ethanol–water solvent systems. The elution of the excised zones from the iron complexes was best carried out by placing the zone in a Waring blender with the same solvent used on the column and pouring this slurry into a column fitted with a cotton filter pad and stopcock and then passing several column volumes of the developing solvent through the filter. The iron complexes evidently adhere to the cellulose more tenaciously than do the sugars and amino acids, which were easily eluted by simpler washings of the cellulose.

Although a more complex mixture of amino acids (Expt. 5) was not investigated, the results obtained here indicated that solvent systems and developing conditions for a wide range of amino acids could be found.

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SUMMARY

Sugars, amino acids and transition metal complexes were shown to be separable

by cellulose extrusion column chromatography. The technique may be correlated with cellulose thin-layer chromatography, as well as with paper methods.

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