soit due à la présence dans la DEAE-cellulose d'un groupe aminé substitué. En effet, des colorations spécifiques sont également observées sur un support formé par l'épichlorhydrine triéthanolamine (ECTEOLA)-cellulose, alors qu'elles ne le sont pas dans le cas des supports cellulosiques ne contenant pas de groupes aminés (CM-cellulose) (résultats non publiés).

Laboratoire de Morphologic Expérimentale et Endocrinologie, Collège de France, Paris (France)

PEDRO DE LA LLOSA
COLETTE TERTRIN
MARIAN JUTISZ

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The use of Sephadex G-25 in partition column chromatography

Cellulose powder column chromatography has been used for the separation of a variety of materials, *i.e.* sugars and amino acids. However, it has not always been as successful in resolution as might have been predicted from the results obtained with paper sheet chromatography. The main difficulty has been the packing of a uniform column. Modifications have been made in the preparation of the cellulose, as well as the use of other column materials such as starch, but these also suffer from the problem of preparing columns which will give reproducible results.

Sephadex, a cross-linked dextran (obtained from Pharmacia, Inc. as Sephadex G-25), has been used very successfully in the fractionation of various biological materials according to their molecular size[1]. This cross-linked dextran has not been as useful for the fractionation of mixtures of individual monosaccharides or amino acids by what is essentially adsorption chromatography[2].

We have used Sephadex G-25 partition column chromatography to separate mixtures of sugars, amino acids and to some extent a purine and a nucleoside. The method utilizes various solvent mixtures previously used for paper partition chromatography of the materials to be separated.

Experimental

The solvents used were: solvent A, butanol-1–acetic acid–water, 62:15:25 (by volume) and solvent B, ethanol–1 *M* ammonium acetate pH 7.5, 7:3 (by volume). To prepare the columns, 5–10 g of dry Sephadex G-25 powder was stirred for 18–24 h with

100 ml of the solvent used for chromatography. The Sephadex G-25 slurry can be stored in the solvent until used. The Sephadex was then poured as a thick slurry into a glass chromatographic tube with a sintered glass disc bottom, containing about 5 ml of solvent. The slurry is allowed to settle to form the column. Depending on the solvent, 5 g of Sephadex powder will make a column of 5 to 10 ml volume. After the column is attached to an automatic fraction collector, the solvent is allowed to descend to the top of the column bed and the sample dissolved in the same solvent is applied. At this point collection of fractions is started; when the sample has all gone into the column, fresh solvent is added carefully so as not to disturb the column bed. Finally the column is connected to a solvent-containing reservoir.

In an experiment to separate 500 μg each of glucose, rhamnose, N-acetylglucosamine and glucosamine, 1 ml fractions were collected from a column with a bed volume of *ca.* 5 ml. Fractions were tested for the presence of sugar by spotting 25 μl aliquots of each tube on paper, and visualized by use of the alkaline silver nitrate reagent described by TREVELYAN[3]. The tubes containing sugar were then chromatographed in solvent A on Whatman No. 1 paper to identify the sugars present. Table I shows a comparison of the results obtained from a 5-ml column and a second column of greater volume (8–10 ml). The results indicate that the R_{Rh}* values obtained are reproducible.

TABLE I

COMPARISON OF SEPARATION WITH SEPHADEX COLUMNS

Sugar	*5 ml column*		*10 ml column*	
	Elution volume (ml)	R_{Rh}*	*Elution volume (ml)*	R_{Rh}*
Rhamnose	11	1.0	36.4	1.0
N-Acetylglucosamine	13	0.84	42.4	0.87
Glucose	21	0.52	64	0.52
Glucosamine·HCl	31	0.35	91.8	0.39

* $R_{Rh} = \frac{\text{Elution volume rhamnose}}{\text{Elution volume compound}}$

The main disadvantage is that the slower moving components, although well separated, tend to tail, giving more diffuse peaks than do the faster moving components.

The larger column (10 ml) was also used to fractionate a mixture of amino acids and amino sugars using solvent A. A mixture of muramic acid (755 μg), alanine (396 μg), glutamic acid (510 μg), glucosamine·HCl (750 μg), and lysine (505 μg) was applied to the column in a volume of 2.0 ml. Fractions of 3.5 ml were collected, and the tubes containing the materials were found by the use of ninhydrin on spots made on paper. The material in each tube was then identified by paper electrophoresis in 2 *N* acetic acid.

Table II gives the peak volumes in which the compounds were eluted from the column. Alanine and glutamic acid were not completely separated but might well

* $R_{Rh} = \frac{\text{Elution volume rhamnose}}{\text{Elution volume compound}}$

have been if smaller fractions had been taken. The volume required to elute glucosamine·HCl from this run and the volume needed in the run previously described in Table I were similar (87.5 and 91.8 ml respectively) indicating the reproducibility of these columns.

TABLE II

ELUTION VOLUMES OF AMINO COMPOUNDS FROM SEPHADEX COLUMN

Amino compound	*Elution volume (ml)*
Muramic acid	38.5
Alanine	45.5
Glutamic acid	49.0
Glucosamine·HCl	87.5
Lysine	119

The order of elution of the compounds from the columns is identical with that found on paper sheet chromatography in the same solvent. Using a column of 5 ml volume and solvent B, a mixture of adenosine and adenine can readily be separated.

The method described using Sephadex G-25 as a packing for a partition chromatographic column seems to be a useful and reproducible method for separation of sugars and amino acids. The capacity of the columns or the effects of other chromatographic solvents, or of varying the degree of cross linking of the Sephadex on the results and efficiency of the elutions have not been determined.

Research Department, CIBA Pharmaceutical Company, Summit, N.J. (U.S.A.) L. D. ZELEZNICK*

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* CIBA Fellow in Microbiology.