Short Communication

Methylated Sephadex as support in reversed phase partition chromatography

A number of different supports for the stationary phase have been used in reversed phase chromatography, *e.g.* rubber[1], siliconized kieselguhr[2], acylated or siliconized cellulose[3,4] and polyethylene powder[5]. During a study of the use of methylated Sephadex in gel filtration of lipids and lipid-soluble compounds[6] we also tested the ability of this material to carry a non-polar stationary phase in reversed phase partition chromatography. This Sephadex derivative was found to be very useful for solvent systems of medium polarity. The present paper describes this property using bile acids as model substances for the separations. Some preliminary results have been reported[7].

Experimental

Sephadex G 25, fine, in bead form, was purchased from AB Pharmacia, Uppsala, Sweden, and was methylated with dimethyl sulfate in alkali[8]. The content of methoxyl groups was 35.2–36.5 %.

The solvent systems tried were those used previously for bile acid separations[9]. Solvent system F 2 consisted of methanol–water–chloroform–heptane (180:120:45:5, v/v) and solvent system C consisted of methanol–water–chloroform–isooctanol (150:150:15:15).

Six ml of the stationary phase were mixed thoroughly with 4.5 g dried methylated Sephadex. Twenty-five ml of the mobile phase were added and the resulting slurry was poured into a chromatography tube having a diameter of 12 mm. After light homogenization with a perforated plunger the column was allowed to settle by gravity with free solvent flow. The sample was dissolved in 2 ml of the mobile phase and applied to the top of the column. When the sample had been rinsed into the column, mobile phase was added and 1–4 ml fractions collected. The flow rate was 20–40 ml/h. The fractions were titrated with 0.02 *N* methanolic NaOH.

When the chromatography was completed the column packing was emptied into a sintered glass funnel and was washed with a suitable solvent (*e.g.* ethanol). After drying at about 50° the methylated Sephadex can be used again.

Results and discussion

The capacity of methylated Sephadex to carry the stationary phase was tested by packing the support in a column with a large excess of stationary phase and then rinsing with mobile phase until no droplets of stationary phase appeared in the effluent. With the solvent systems tested, 4.5 g methylated Sephadex could carry 6 ml of stationary phase.

Fig. 1 shows the separation of cholic and deoxycholic acids with phase system F 2. The fractions were collected on a time basis. It was observed that the solvent flow decreased during the chromatography. This may be due to a slow change in the

composition of the stationary phase. No stationary phase, however, appeared as a separate phase in the effluent. The separations were not affected by the change in solvent flow and could be easily reproduced provided that the columns were made as described above.

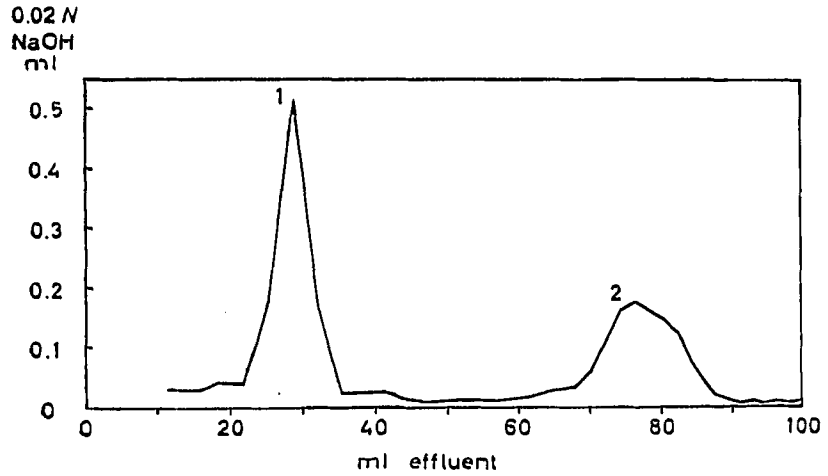

Fig. 1. Reversed phase partition chromatography of (1) cholic and (2) deoxycholic acids with phase system F 2, using methylated Sephadex G 25 as support for the stationary phase.

Glycocholic and cholic acids were separated with phase system C. With this system solvent flow changes were very small. Table I shows a comparison between effluent volumes on columns with siliconized Hyflo Supercel[9] and methylated Sephadex as support. It is seen that retention volumes are larger with the methylated Sephadex columns. In the case of phase system C this is probably due mainly to the fact that the methylated Sephadex can carry 50% more stationary phase. With phase system F 2 it is possible that the stationary phase on the more polar methylated Sephadex has a lower proportion of heptane than the original solvent mixture causing a retardation of the bile acids. In contrast to siliconized Hyflo Supercel the methylated Sephadex cannot carry heptane as stationary phase and it does not swell in this solvent. It is therefore reasonable to assume that methylated Sephadex cannot be used as support in reversed phase chromatography with less polar solvent systems where the stationary phase causes little or no swelling of the Sephadex (*e.g.* benzene, toluene)[6].

TABLE I

COMPARISON BETWEEN SEPARATIONS OBTAINED ON COLUMNS WITH 4.5 g SILICONIZED HYFLO SUPERCEL CARRYING 4 ml STATIONARY PHASE AND COLUMNS WITH 4.5 g METHYLATED SEPHADEX G 25 CARRYING 6 ml OF STATIONARY PHASE

Bile acid	*ml effluent at peak fraction*			
	Phase system F 2		*Phase system C*	
	G 25	*Hyflo*	*G 25*	*Hyflo*
Glycocholic acid	—	—	45	35
Cholic acid	30	12	130	100
Deoxycholic acid	75	35	—	—

The titration values showed that quantitative recoveries of the bile acids were obtained.

The present study indicates that methylated Sephadex is a useful support in reversed phase chromatography with solvent systems of medium polarity. It is probably not suitable for systems less polar than F 2 used in this investigation but further experiments may show that it is of value in more polar solvents. Since the partially methylated Sephadex swells in water it might also be useful as a support for aqueous stationary phases in "straight" partition chromatography. The high capacity and the ease with which it can be regenerated are important advantages of this support.

Acknowledgement

The technical assistance of Miss AIRA MATTSSON is gratefully acknowledged. This work was supported by a grant from the Swedish Medical Research Council.

Department of Chemistry, Karolinska Institutet, Stockholm (Sweden)

ERNST NYSTRÖM
JAN SJÖVALL

1 J. BOLDINGH, *Experientia*, 4 (1948) 270.
2 G. A. HOWARD AND A. J. P. MARTIN, *Biochem. J.*, 46 (1950) 532.
3 J. V. KOSTIR AND K. SLAVIK, *Collection Czech. Chem. Commun.*, 15 (1950) 17.
4 T. H. KRITCHEVSKY AND A. TISELIUS, *Science*, 114 (1951) 299.
5 O. WISS AND U. GLOOR, *Z. Physiol. Chem.*, 310 (1958) 260.
6 E. NYSTRÖM AND J. SJÖVALL, *Anal. Biochem.*, (1964), submitted for publication.
7 E. NYSTRÖM AND J. SJÖVALL, *Biochem. J.*, 92 (1964) 10P.
8 E. NYSTRÖM AND J. SJÖVALL, to be published.
9 A. NORMAN AND J. SJÖVALL, *J. Biol. Chem.*, (1958).

Received December 10th, 1964

J. Chromatog., 17 (1965) 574–576

Notes

Separation of amino acid *n*-butyl esters by means of thin-layer chromatography

During previous work concerning the gas-chromatographic separation of amino acid derivatives, the purity of amino acid *n*-butyl esters was checked by means of thin-layer chromatography. The method used for the preparation of these esters is described elsewhere.[1]

Glass plates (20 × 20 cm) were covered with Kieselgel G (Merck) in layers of 0.25 mm. The solvent used for the separation of the butyl esters was a mixture of