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Note

Sulphated Sepharose —a strong cation exchanger

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Cellulose-, silica- and Sephadex-based ion exchangers are frequently used for the separation of large molecules. During the last decade, ion exchangers based on other carriers such as Sepharose have also become commercially available. In this paper we report a simple method for preparing cation exchangers by sulphation of cross-linked polysaccharides, developed about 15 years ago but which we believe is still useful. The preliminary characterization of a sulphated Sepharose product and a comparison with the capacity of the commercially available cation exchangers[1] SE-Sephadex C-25[a] and SP-Sephadex C-50 are described.

EXPERIMENTAL

Sepharose 6B and 2B, Sephadex G-50, SE-Sephadex C-25 and SP-Sephadex C-50 were obtained from Pharmacia (Uppsala, Sweden). Epichlorohydrin cross-linked Sepharose (ECD-Sepharose) was prepared as described by Porath *et al.*[2]. Sulphur trioxide and pyridine were purchased from Kebo (Stockholm, Sweden), cytochrome *c* from Serva (Heidelberg, F.R.G.) and *Phaseolus vulgaris* (red kidney beans, type Stella Y/500) from Weibullsholm (Landskrona, Sweden). The agglutination activity (titre) was measured according to Salk[3] using a 2% phosphate-buffered saline suspension of three-times washed human red cells of A-type.

Preparation of sulphur trioxide–pyridine complex

A three-necked flask containing 150 ml of pyridine was equipped with a thermometer, a reflux condenser, a dropping funnel, and placed in an ice-bath on a magnetic stirrer. A volume of 20 ml of sulphur trioxide was added to the chilled pyridine, dropwise and with vigorous stirring. After the completed reaction, the flask was stoppered and the product was stored at 5°C.

Sulphation reaction

A 20-ml volume of the gravity-settled ECD-Sepharose 6B was washed on a glass filter funnel with 200 ml of distilled water, 400 ml of ethanol and 400 ml of pyridine.

[a] From January 1970 SP-Sephadex replaces the earlier SE-Sephadex. The two types have very similar properties (see Ref. 1, footnote to Table I).

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The suction-dried material was transferred into a 100-ml flask by means of a few millilitres of pyridine and allowed to swell. After addition of 20 ml of the suspension of the sulphur trioxide–pyridine complex, the flask was stoppered and the mixture was gently shaken at room temperature overnight. The sulphation of ECD-Sepharose 2B and of Sephadex G-50 was achieved in a similar way. The resulting product was washed on a glass filter funnel with about 0.6 l each of distilled water, ethanol, 1 *M* acetic acid containing 2 *M* sodium chloride, 1 *M* sodium carbonate containing 2 *M* sodium chloride and, finally, distilled water. For sulphur analyses[4,5], about 1 ml of the product was washed on a small glass filter funnel with 20 ml of ethanol and 20 ml of acetone and was lyophilized. The products were kept swollen in 0.1 *M* ammonium formate buffer (pH 3) containing 1 *M* sodium chloride and 0.1% sodium azide. Equilibration and regeneration were carried out according to the manufacturer's instructions[1] for equilibration and regeneration of SP-Sephadex (counter ion: ammonium).

Titration

Potentiometric titrations were performed principally as described by Porath and Fornstedt[6]. A gel volume sufficient to give at least 5 ml of sediment was allowed to settle in a cylinder and 5 ml of the sediment (corresponding to about 0.4 g of sulphated ECD-Sepharose 6B) were washed on a glass filter funnel with 50 ml of 0.5 *M* hydrochloric acid and then with 0.5 l of distilled water. The suction-dried material was transferred into a laboratory-made titration vessel[6] and 25 ml of 1.0 *M* sodium chloride were added. After adjusting of pH to below 2.0 with a few drops of 1.0 *M* hydrochloric acid, titration was effected by 0.05-ml portions of 0.1 *M* sodium hydroxide solution.

Preparation of kidney-bean extract

The extraction technique described by Rigas and Johnsson[7] was used with some modification. A 1-kg amount of finely ground red beans was extracted at pH 1 overnight with 5 l of 0.1 *M* hydrochloric acid. After readjusting the pH, the suspension was centrifuged and the clear supernatant dialysed against 0.1 *M* ammonium formate buffer (pH 4). The inactive precipitate was removed by centrifugation and the clear liquid lyophilized, giving about 35 g of material.

Adsorption capacity

Three columns of 3 cm × 10 mm I.D. were packed with the ion exchangers SE-Sephadex C-25, SP-Sephadex C-50 and sulphated ECD-Sepharose 6B. The available capacity for cytochrome *c* and kidney-bean proteins was determined by frontal analysis as follows. The packings were equilibrated with 0.1 *M* ammonium formate buffer (pH 3). A solution of 1 mg/ml of cytochrome *c* in the buffer was continuously pumped through at a flow-rate of 10 ml/h until the effluent showed the same absorbance (A_{254}) as the introduced solution. After thoroughly washing with the buffer, the adsorbed material was eluted with 0.1 *M* ammonium formate buffer (pH 8). UV-positive desorption fractions were pooled, concentrated and lyophilized. The available capacity for the kidney-bean proteins was determined as described for cytochrome *c* with the following modifications: two columns of 8 cm × 10 mm I.D. were packed with the SP-Sephadex C-50 and sulphated ECD-Sepharose 6B and the pack-

TABLE I

SULPHUR CONTENTS OF SULPHATED PRODUCTS

Product	*S (mg/g)*[a]	*Total capacity*[b] *(mequiv./g)*[a]
Sulphated ECD-Sepharose 6B	149	4.6[c]
Sulphated ECD-Sepharose 2B	114	3.5
Sulphated Sephadex G-50	140	4.4

[a] Dry weight.
[b] For the total capacities of the sulphopropyl substituted Sephadex types, see Table II in Ref. 1.
[c] To be compared with 4.1 mequiv./g as calculated from the titration curve (Fig. 1).

ings were equilibrated with 0.1 *M* ammonium formate buffer (pH 4) and saturated with a solution of 10 mg/ml of lyophilized kidney-bean extract in the starting buffer.

RESULTS AND DISCUSSION

The sulphur concentrations of the products are given in Table I. The capacity of the sulphated ECD-Sepharose 6B was almost constant over a fairly wide pH range (Fig. 1). As shown in Table II, the rate of the sulphating reaction is highest within the first 5 min. Likewise, a 2-h incubation time provides 80% yield. The sulphated gels withstood exposure to strongly alkaline conditions, which is of importance for the regeneration procedure. Sulphated ECD-Sepharose 6B seems to possess a higher capacity for small than for larger proteins and to show properties similar to those of SE-Sephadex C-25 with respect to small proteins (Table III). Sulphated products of the cross-linked polysaccharides are strongly acidic cation exchangers; the functional groups consist of monovalent ions.

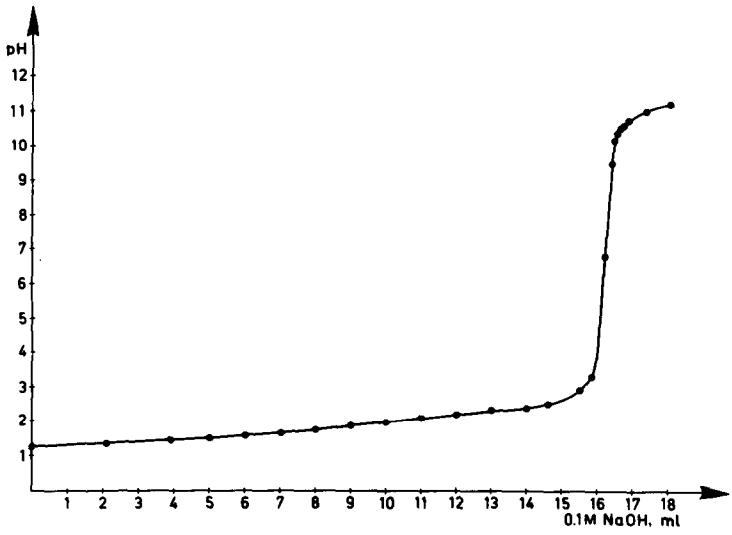

Fig. 1. Titration curve for sulphated ECD-Sepharose 6B.

TABLE II

EFFECT OF INCUBATION TIME ON THE SULPHATION OF ECD-SEPHAROSE 6B

Incubation time (min)	*S (mg/g)*[a]
0	0.4
5	60.5
15	77.0
30	89.5
120	128.0
900	151.0

[a] Dry weight.

TABLE III

AVAILABLE CAPACITY

Ion exchanger	*Cytochrome c (mg/ml)*	*Red bean proteins (mg/ml)*
Sulphated ECD-Sepharose 6B	260	220[a]
SE-Sephadex C-25	270	–
SP-Sephadex	40	940[b]

[a] Total titre: 4300.
[b] Total titre: 18800.

The procedure for the preparation of the sulphur trioxide–pyridine complex is simple. The sulphating reaction and the equilibration stage are not laborious and do not require the use of special equipment. As can be seen in Table I, the general approach can also be utilized for the sulphation of cross-linked dextrans. Further, desorption with concentrated salt solutions does not cause any shrinking. Moreover, the possibility of preparing ion exchangers with different matrices, porosities and ionic densities offers flexibility in the choice of the most suitable ion exchanger for an appropriate separation problem.

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