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ION-EXCHANGE DERIVATIVES OF SPHERON

III. CARBOXYLIC CATION EXCHANGERS*

O. MIKEŠ and P. ŠTROP

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6 (Czechoslovakia)

M. SMRŽ

Research Institute of Pure Chemicals, Lachema, 621 33 Brno (Czechoslovakia)

and

J. ČOUPEK

Laboratory Instruments Works, VVCHP, 162 03 Prague 6 (Czechoslovakia)

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SUMMARY

Weakly acid cation-exchange derivatives of the glycol–methacrylate macroreticular gel Spheron 300 were prepared by carboxymethylation, succinylation and oxidation. The use of the latter method was also attempted in the preparation from the more macroporous Spheron 1000. Carboxymethyl derivatives were prepared with four nominal capacities for small ions: 0.26, 0.50, 1.02 and 2.20 mequiv./g. The ion-exchange samples thus prepared were characterized by the determination of the working volume, the specific non-penetratable volume and the specific weight of non-penetratatable mass in the swollen state, by titration curves and by the determination of the specific inner surface area and the magnitude of sorption for proteins (serum albumin). The samples were tested by chromatographic separation experiments using a natural mixture of egg proteins, a synthetic mixture of serum albumin, chymotrypsinogen and lysozyme; chromatography of some cyanogen bromide fragments of serum albumin was also tested. Equipment used in the medium-pressure chromatography of proteins and a dynamic method for the determination of protein sorption on the chromatographic column are described. The comparatively large differences between the determination of protein capacity using the dynamic and static (batch) methods are explained by means of a hypothesis assuming multiple sorption of proteins in batch experiments. The possibility of applying a high-performance liquid chromatograph in protein analysis is discussed.

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INTRODUCTION

Macroporous Spheron ion exchangers have been used successfully in the high-performance liquid chromatography (HPLC) of proteins and of their higher molecular weight fragments[1–7] and of oligonucleotides[8], and in the separation of monosaccharides[9] and oligosaccharides[10]. In Parts I and II we described the properties of the polymeric matrix of Spheron[3], and the preparation and properties of the diethylaminoethyl derivative[7]. This paper is concerned with the carboxylic derivatives of Spheron. Hydrophilic macroporous weakly to medium basic anion exchangers and weakly acid cation exchangers have been most frequently used in biochemistry, which is why these derivatives of Spheron were the first to attract our attention.

EXPERIMENTAL

Materials

Spheron 300*, bead size 20–40 μm, was a product of Lachema (Brno, Czechoslovakia); it was extracted before use as described in Part I[3]. Unextracted Spheron 1000, particle size 40–60 μm, was also a Lachema product. Human serum albumin, bovine chymotrypsinogen and chicken lysozyme were of the same origin as earlier[1,3]. Chicken egg proteins were prepared according Rhodes *et al.*[11]. Cyanogen bromide fragments of human serum albumin were obtained by courtesy of Dr. B. Meloun (Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague). All other chemicals (reagent grade) were supplied by Lachema.

Preparation of carboxymethyl-Spheron 300 (CM-Spheron 300) by carboxymethylation

Spheron 300 was mixed in the cold for at least 1 h with an aqueous solution containing a 2-fold weight amount of potassium hydroxide. Then a 2-fold weight amount of potassium iodide and a 1.5-fold weight amount of chloroacetic acid (with respect to the weight of Spheron) were added gradually and with cooling[12]. The temperature was gradually increased to 60°, 15% (w/w) of potassium hydroxide was added, and the mixture was digested at 60° for 15 min. All of the gel was washed with water on a fritted disc.

This substitution procedure was repeated three times, the ion exchanger was decanted in water and cyclized with 2 *M* sodium hydroxide solution and 2 *M* hydrochloric acid, and the H^+ form was then thoroughly washed with water, methanol and acetone, and eventually dried. The dependence of the degree of ionogenic substitution on the number of repetitions of the procedure is shown in Fig. 1.

Preparation of succinyl-Spheron 300 (Suc-Spheron 300) by succinylation

To Spheron 300, pre-swollen in pyridine, the same weight amount of succinic anhydride was added gradually with cooling (18°) and stirring[12]. The suspension was then heated to 60°. On cooling, the gel was washed on a fritted disc with acetone, methanol and water, and dried *in vacuo*. Fig. 2 illustrates the relationship between the

* An analogous series of sorbents based on copolymers of 2-hydroxyethylmethacrylate with ethylenedimethacrylate under the name of Separon H is also produced by Laboratory Instruments Works, Prague, Czechoslovakia.

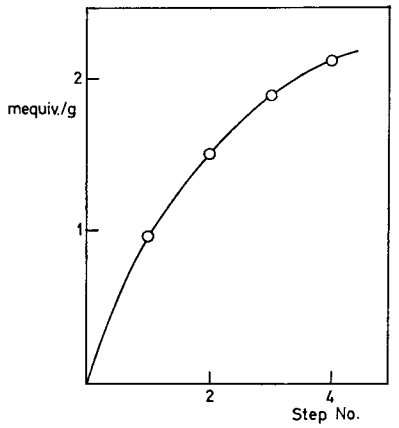


Fig. 1. Dependence of the nominal capacity of CM-Spheron 300 on the number of repetitions of the carboxymethylation process.

time of succinylation of Spheron 300 and the eventual nominal capacity of the cation exchanger thus formed. The substitution of pyridine with dimethylformamide or ethyl acetate with addition of a minor amount of pyridine or triethylamine did not result in any increase in the nominal capacity of the cation exchanger.

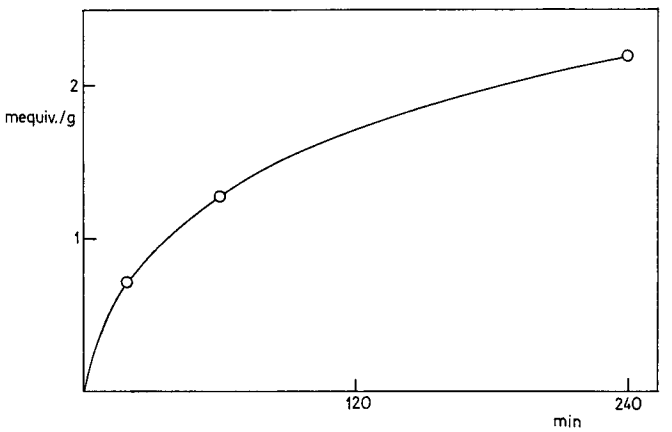


Fig. 2. Dependence of the resulting nominal cation-exchange capacity of Suc-Spheron 300 on the duration of succinylation under conditions described in the text.

Preparation of carboxylic derivatives (C-Spheron 300 and Spheron C 1000) by oxidation

To the suspension of Spheron 300 (or Spheron 1000) pre-swollen in 1 *M* sulphuric acid, a 2.75% solution of potassium permanganate was added with stirring at room temperature[13]. On completion of the reaction, the excess of permanganate was removed with several portions of oxalic acid, the ion exchanger was thoroughly washed with 2 *M* hydrochloric acid (at the beginning with oxalic acid added) and cyclized with 2 *M* sodium hydroxide solution and 2 *M* hydrochloric acid. The ion exchanger in the H^+ form was washed with water, methanol and acetone, and dried

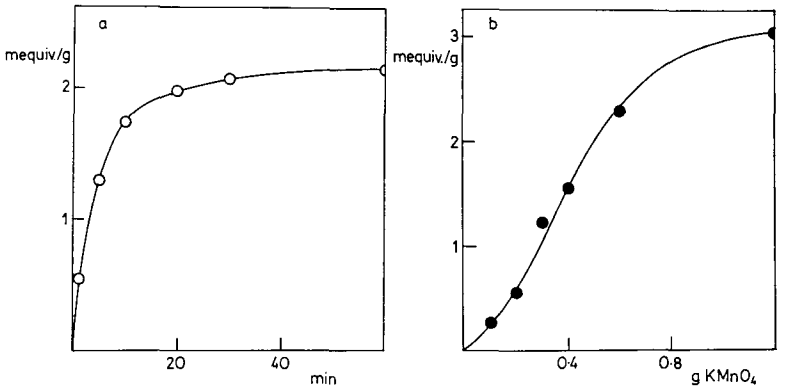


Fig. 3. Effect of time (a) and of the amount of oxidizer (b) on the resulting nominal capacity of Spheron C 1000. Oxidation was performed in acidic solution. (a) 0.55 g of potassium permanganate per gram of Spheron 1000; (b) oxidation lasted for 30 min with the given amount of potassium permanganate per gram of Spheron 1000.

under the vacuum of a water pump. The effect of reaction time and of the amount of oxidizing agent on the resulting capacity is illustrated in Fig. 3.

Equipment for the determination of the capacity of ion exchangers by the dynamic method and for the pressure chromatography of proteins

The equipment is shown in Fig. 4. A Plexiglass GM mixer provided with a side funnel made possible the continuous linking of linear gradients without inter-

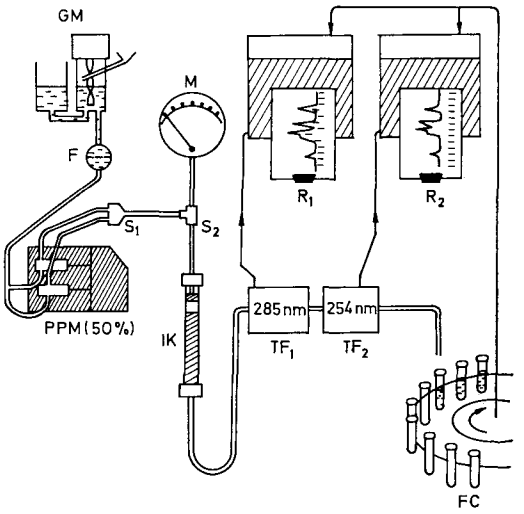


Fig. 4. Chromatographic device used also in the determination of the capacity of ion exchangers for proteins by the dynamic procedure. GM = gradient mixer; F = fritted disc, functioning both as a filter and a bubble collector; PPM = proportional programmable micropump with two reversely operating pistons, adjusted to the same output power for reduction of pulses; $S_{1,2}$ = fitting connections; IK = column with ion-exchanger column, 20 × 0.8 cm; M = manometer, 0–6 MPa (0–60 atm); $TF_{1,2}$ = flow photocells for absorbance measurements at 285 and 254 nm; $R_{1,2}$ = linear recorders; FC = fraction collector yielding recordable pulses.

ruption of the operation; it was built at the Institute of Organic Chemistry and Biochemistry. The proportional pump was manufactured in the Development Works of the Czechoslovak Academy of Sciences (henceforth referred to as Development Works). The ion-exchange column, its fittings and the manometer were those used in amino acid analysers (Development Works and Laboratory Instruments Works). Flow ultraviolet analysers (285 and 254 nm) and the line recorder (R_2) were from the Development Works. The recorder EZ 4 and fraction collector (denoted by FC) were manufactured at the Laboratory Instruments Works, Prague. The equipment was provided with a pulse generator (Institute of Organic Chemistry and Biochemistry), allowing fractions from the collector to be recorded with both recorders. In the collected fractions, pH was measured with a compensation pH meter (Development Works) and the electric conductivity was recorded with an OK-102/1 conductivity meter (Radelkis, Budapest, Hungary). In addition to measurements of capacity, all the pressure chromatographic analyses of proteins were also performed using this equipment.

Determination of the capacity of ion exchangers for proteins by the dynamic method (on a chromatographic column)

The determination was carried out using the equipment described in the preceding section and shown in Fig. 4. The ion exchanger of known volume in the column (IK) was equilibrated with a sorption buffer (of low ionic strength). The buffer was then replaced with a protein solution in the sorption buffer within the whole system including the head of the column.

Sorption. The protein solution was pumped on to the ion exchanger. As soon as breakthrough of the protein was observed, the elution volume was recorded. The protein solution was allowed to pass through the ion exchanger until its concentration in the effluent remained constant.

Washing. Only pure sorption buffer was pumped on to the ion exchanger, until the recorded absorbance line dropped to the baseline.

For desorption of the protein a linear gradient of the desorption buffer was applied (high ionic strength), which was followed by isocratic elution with the absorbance line dropped to the baseline. Then the effluent volume was measured in which the amount of eluted protein was determined spectrophotometrically. This procedure is illustrated in Fig. 5 by the determination of the capacities of four cation exchangers for serum albumin.

Other methods

The ion exchangers prepared in this work were characterized by the determination of the nominal capacity for small ions[3], by titration curves[7], by the non-penetratable volume[3] and by measurements of the specific inner surface area by the one-point method. The capacity for serum albumin was determined by the static (batch) method in a slowly rotating test-tube[7] using buffer A (0.01 *M* ammonia + acetic acid, pH 5.0) for sorption and buffer B (0.1 *M* ammonia + acetic acid, pH 7.0, 2 *M* with respect to sodium chloride) for desorption.

Before the first application and between the particular chromatographic experiments, the ion exchangers were regenerated and cyclized on a fritted disc; the ion exchanger was mixed with an approximately 3-fold volume of the regenerant, the

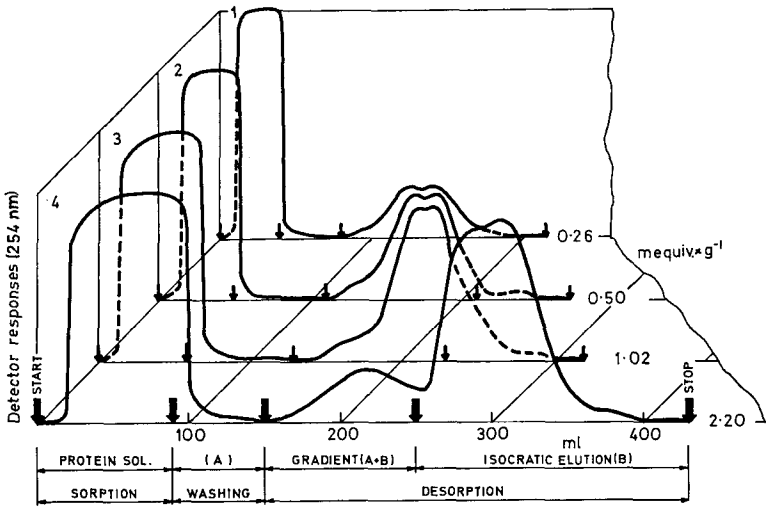


Fig. 5. Determination of the capacities of four samples of CM-Spheron 300 for serum albumin by the dynamic method. Samples of cation exchangers are denoted by numbers on the left (according to Table I), nominal capacities are given on the right. Ion-exchange column IK (*cf.*, Fig. 4) was equilibrated with sorption buffer A having a low ionic strength (0.01 *M* ammonia + acetic acid, pH 5.0). A 5% (w/w) solution of serum albumin in buffer A, centrifuged before use, was pumped on to the column. Pure buffer A was used for washing. Desorption was performed by using buffer B having a high ionic strength (0.1 *M* ammonia + acetic acid, pH 7.0, 2 *M* with respect to sodium chloride). Effluent from the whole desorption (*i.e.*, from gradient elution + isocratic elution) was collected, and the amount of washed-out serum albumin in it was determined and re-calculated to 1 ml of the bed of the ion-exchanger used. Calculated capacities for serum albumin are given in Table I. Minimal respective volumes of effluent needed for complete sorption and desorption depend on the nominal capacity of the cation exchanger and were adjusted in particular cases according to the record of absorbance.

solution was sucked off after 5–10 min, and the procedure was repeated. The regenerant was subsequently washed repeatedly with water in a similar manner. The order of regenerants for cation exchangers was 2 *M* sodium chloride solution, 2 *M* sodium hydroxide solution and 2 *M* hydrochloric acid.

Equilibration. Cation exchanger in the H^+ form was mixed on a fritted disc with the first elution buffer and the decreased pH value, measured by a glass electrode, was balanced using the base of the respective buffer. The ion exchanger was then repeatedly washed on the fritted disc by employing the procedure just described until equilibrium was established between the buffer and effluent (identical pH values, conductivities and absorbances).

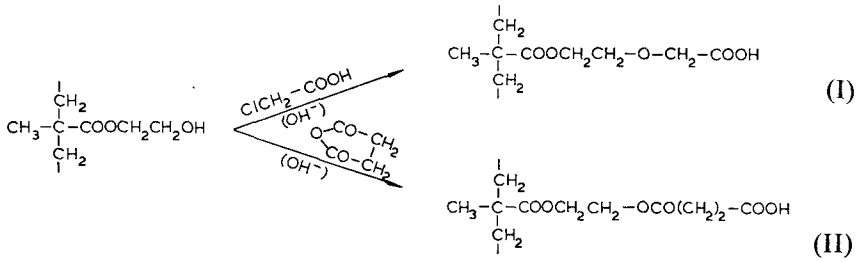
RESULTS AND DISCUSSION

Survey of cation exchangers prepared in this work

Weakly acidic cation exchangers of the macroreticular 2-hydroxyethyl methacrylate Spheron[1] can be prepared by direct copolymerization of inogenic components[14] (this procedure has not been examined here), or by additional substitution of

hydrogen atoms on numerous hydroxyl groups of Spheron with a suitable ionogenic group[12]:

a

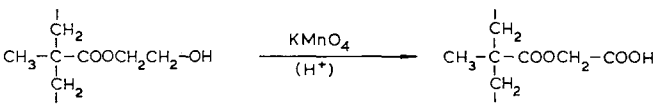


(I)

(II)

Finally, the third method[13] consists in oxidation of the copolymer with a strong oxidation agent:

b



(III)

All of these routes of preparation are possible owing to the extraordinary stability of the ester bond on the trimethylacetate group. The first procedure was employed in the preparation of CM-Spheron (I), the second led to Suc-Spheron (II), and the third yielded the carboxylic derivative with the oxidized matrix (C-Spheron) (III). The ion exchangers thus prepared are summarized in Table I.

There is essentially no difference between the titration curves of CM-Spheron and C-Spheron (Fig. 6). The working volume of cation exchangers decreases with increasing nominal capacity within the whole range (samples 1–4), in both acidic and basic solution. Hence, the same paradox that has already been commented upon for anion-exchange derivatives of DEAE-Spheron with different capacities[7] occurs also in this instance. The specific inner surface area of all of the derivatives of Spheron 300 usually varies between 50 and 100 m^2/g and is considerably dependent on the method of extraction and drying of the samples.

Relationship between the protein sorption values determined by the static and dynamic methods

The capacity values of carboxylic ion exchangers determined by the static (batch) and dynamic (column) methods differed considerably, as shown in Fig. 7. The curve of the dependence of the static sorption of serum albumin on nominal capacity (solid line) has a form very similar so that of the static sorption of proteins on the nominal capacity of DEAE-Spheron (*cf.*, Fig. 4 in ref. 7): there is a steep rise at the beginning, after which the curve remains almost horizontal. The curve determined by the dynamic method is not linear as was the relationship between the capacity for small ions and the degree of ionogenic substitution (*cf.*, Fig. 4 in ref. 7), but approaches this relationship. The difference between the two methods does not lie only in the form of the two dependences. Both methods also differ considerably in

TABLE I

SURVEY AND CHARACTERIZATION OF SPHERON 300 WEAKLY ACIDIC CATION EXCHANGERS PREPARED IN THIS WORK

Property	*Unit*	*Unsubstituted* Spheron 300*	*Sample No.*					
			1	*2*	*3*	*4*	*5*	*6*
Type of ion exchanger**			CM	CM	CM	CM	Suc	C
Nominal capacity	mequiv./g	0.035	0.26	0.50	1.02	2.20	0.51	2.12
Capacity for serum albumin:								
Sorption statically***	mg/g	2–3	88	247	307	305		
Desorption statically***	mg/g		104	256	313	298		
Desorption dynamically	mg/ml		4.5	10.6	15.8	24.9		
	mg/g		17.8	41.6	60.8	85.7		
Specific non-penetratable volume***	ml/g	0.752	0.754	0.731	0.679	0.752		0.746
Specific weight of non-penetratable mass in swollen state***	g/cm^3	1.330	1.326	1.367	1.473	1.330		1.341
Working volume***:								
In 2 *M* sodium hydroxide	ml/g		3.97	3.94	3.92	3.57	3.82	4.02
In 2 *M* hydrochloric acid	ml/g	3.95§	3.92	3.89	3.77	3.32	3.77	3.77
Specific inner surface area (by the Klyachko–Gurvich method)***	m^2/g	55	94	96	75	56		55

* For comparison with samples 1–6 some data[3] are given for unsubstituted Spheron 300, which was the initial raw material used in ionogenic substitutions.

** Types denote derivatives: CM = carboxymethyl; Suc = succinyl; C = carboxyl. In the text these types are denoted as structures I, II and III, respectively.

*** Methods were described in detail or cited in refs. 3 and 7.

§ Holds for distilled water.

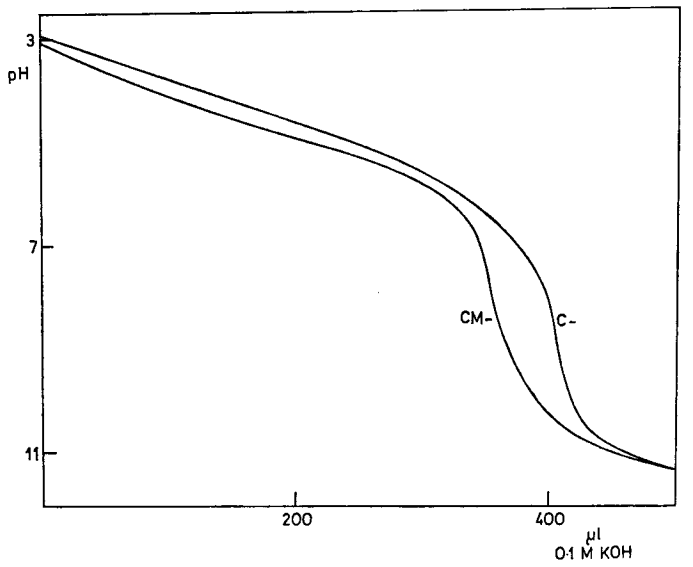


Fig. 6. Titration curves of CM-Spheron 300 with nominal capcity 1.02 mequiv./g and C-Spheron 300 with capacity 2.16 mequiv./g. Titrations performed with autotitrator in 1 *M* potassium chloride solution.

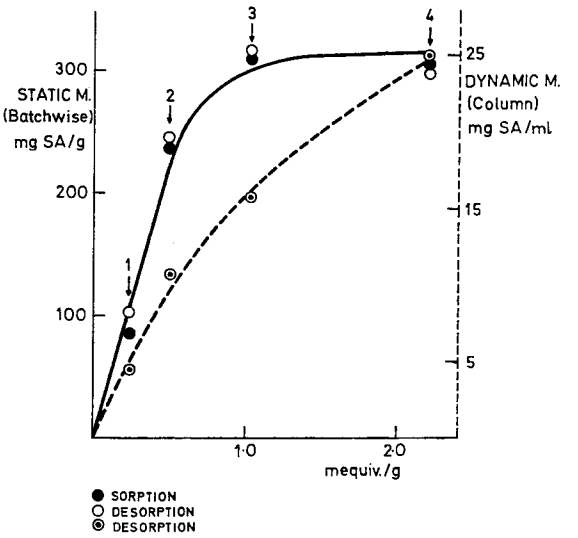


Fig. 7. Relationship between capacities of carboxymethyl derivatives of Spheron 300 for sorption of serum albumin determined by static and dynamic methods. Samples numbered as in Table I. Solid line and left-hand weight scale relate to results of the static method; broken line and right-hand volume scale relate to the dynamic method. Horizontal axis expresses nominal capacities of tested CM-Spherons for small ions (*e.g.*, Na^+).

the absolute values of their results, which becomes evident if they are converted into the same units, *e.g.*, weight units (*cf.*, Table I). The values measured by the static method are three to four times higher.

This may be explained by a hypothesis of multiple sorption under static conditions, as follows. With a protein bound on the ion exchanger its charge distribution is shifted owing to the effect of the functional groups present. For this reason, the layer of sorbed protein behaves as a cation exchanger and may sorb another layer. This effect may be transferred to yet another layer. Multiple sorption, based only on weak interactions, is due to the higher protein concentration in the neighbourhood, and the respective equilibrium needs a long time to be established. This equilibrium mostly does not hold under dynamic conditions, even if the same buffer is used as in the static method. On the outer parts of the beads it is perturbed hydrodynamically, while in the bulk of the macropores it is upset by the rapid change in the protein concentration. In the static method[7] we measure the final equilibrium of sorbed protein towards the protein solution in the mobile phase, while in the dynamic procedure the final equilibrium is evaluated towards the pure sorption buffer. The protein sorption values determined by the dynamic method more adequately describe the possibilities of loading the ion exchanger in chromatography. In contrast values ascertained by the static method give a better picture of the possibility of utilization of the ion exchanger for sorption using the batch method.

Testing of the chromatographic properties of cation exchangers prepared in this work

The chromatographic properties of the ion exchangers were tested by the separation of a natural mixture of egg proteins and of a synthetic artificial mixture of proteins. The dependence of the separation of a natural mixture of proteins on the nominal capacity of CM-Spheron 300 can be seen in Fig. 8. Cation exchangers with low nominal capacity are unable to retain the proteins, and the greatest part of the latter is eluted in a single large peak at the beginning of the chromatogram. With increasing capacity the retention increases, and some peaks are displaced. Similar comparative experiments were performed on the individual ion exchangers with increasing load (*e.g.*, 8, 15 and 30 mg of the protein mixture). At the same time it was observed, as expected, that ion exchangers of higher capacity give adequate separations also at a higher load. Fig. 9 illustrates the separation of 60 mg of egg proteins on a high-capacity cation exchanger. Comparison with the separation on a ion exchanger with the same high capacity, but at a lower load (2.20 mequiv./g, load only 8 mg; Fig. 8) leads to the conclusion that also at higher loads the positions of the peaks in the elution profile remain constant.

Tests with C-Spheron 300 yielded results similar to those with CM-Spheron, if subjected to regeneration (*i.e.*, used repeatedly). The first chromatographic analysis on C-Spheron usually did not proceed in the expected way. In repeated chromatographic analyses of the proteins, C-Spheron differed from CM-Spheron only insignificantly in the displacement of peaks. Both sorbents differed in their nominal capacities and in the length of the spacer carrying the functional group.

Further chromatographic tests on the cation exchangers were performed using a synthetic mixture of serum albumin (SA), chymotrypsinogen (CH) and lysozyme (LZ). An interesting effect of the pH of ammonium acetate buffers on the separation of SA and CH was observed, as illustrated in Fig. 10. The synthetic mixture could be adequately separated on CM-Spheron 300 with a capacity of 2.20 mequiv./g at pH 8 and 7, exhibiting three separate peaks, I, II and III. At pH 5 and 6, on the other hand, only two peaks were revealed by the chromatographic analysis. Tests involving pure

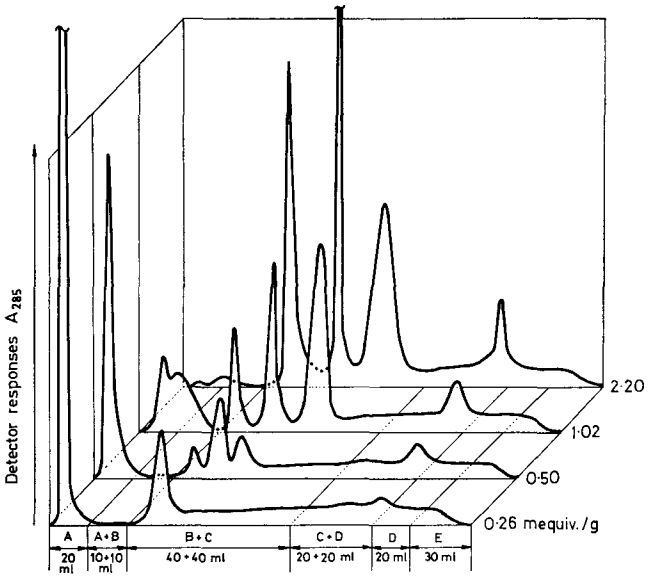


Fig. 8. Effect of nominal capacity of CM-Spheron 300 on separation of egg proteins. Dimensions of column 20 × 0.8 cm, load 8 mg in all instances. Capacities of tested ion exchangers are given on the right. Buffers used: A, 0.05 *M* ammonia + acetic acid, pH 5.0; B, 0.3 *M* Tris + acetic acid, pH 7.5; C, 0.5 *M* Tris + hydrochloric acid, pH 9.5; D, buffer C, 1 *M* with respect to sodium chloride. Short isocratic elution was followed by three linear gradients and by isocratic elution with buffer having a high ionic strength. The column was finally washed with 2 *M* sodium chloride solution (E).

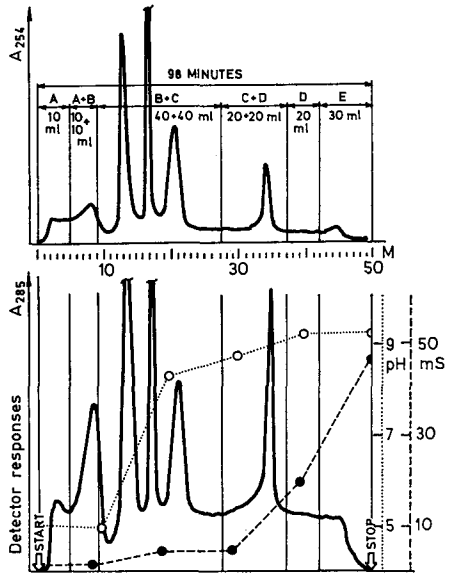


Fig. 9. Chromatographic analysis of 60 mg of a mixture of egg proteins on CM-Spheron 300 (column 20 × 0.8 cm), nominal capacity 2.20 mequiv./g. Detection performed with a tandem system of UV analysers (*cf.*, Fig. 4). Buffers used and the other parameters as in Fig. 8. Fractions 4.2 ml in volume were collected in intervals of 118 sec. M = step-marks of the fraction collector. Right-hand scales: for measurements of pH (open circles) and conductivity (full circles).

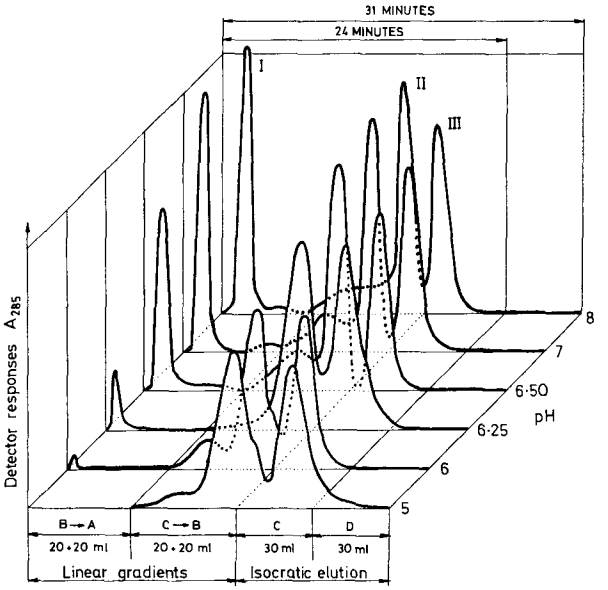


Fig. 10. Diagram illustrating separation of 8 mg of serum albumin (I), 4 mg of chymotrypsinogen (II) and 4 mg of lysozyme (III) on a 20 × 0.8 cm column of CM-Spheron 300, nominal capacity 2.20 mequiv./g, using buffers of different pH. Ion exchanger equilibrated with buffer A. Mixture of proteins was applied in 0.3 ml of buffer A. Arrows indicate mixing of buffers when linear gradients are formed. All buffers were prepared from ammonia solution (the resulting molarity of which is indicated below) acidified with acetic acid to the pH shown. Buffer A was 0.01 *M*, B was 0.1 *M*, C was buffer B which was 1 *M* with respect to sodium chloride, D was 2 *M* sodium chloride solution. Flow-rate, 4.5 ml/min; temperature, 25°; counter pressure, 5–7 atm. Peaks I–III contain the given proteins only at runs at pH 8 and 7. A further decrease in pH led to displacement of serum albumin from peak I to peak II (see text).

proteins alone at pH 5 showed that both SA and CH exhibit, under the conditions used, virtually identical retention volumes, in agreement with the effect of pH on the displacement of the peaks of these proteins. In experiments involving the separation of the above mixture of three proteins in the pH range 6–7, the peak of SA was shifted from position I to II, but not owing to a gradual change in the retention volume. Already at pH 6 traces of SA could be observed at position I, and the amount gradually increased as the pH increased to 6.25 and 6.50, reaching a maximum at pH 7. At the same time, the area of peak II gradually decreased. Similar results were recorded with C-Spheron 300 with a capacity of 2.12 mequiv./g. All of these phenomena can be explained by a hypothesis involving the interaction of both proteins under the experimental conditions, giving rise to a product that is not separated on the carboxylic derivatives of Spheron within the given range of pH.

CM-Spheron was also used in the testing of separations of higher molecular weight fragments of proteins. Fig. 11 illustrates such tests, aimed at establishing conditions for the chromatography of cyanogen bromide fragments of serum albumin[5]. Further chromatographic applications of this cation exchanger were described earlier[2], together with a description of its testing in the reversible sorption of proteolytic enzymes. In earlier work[1], CM-Spheron 300 was compared with CM-

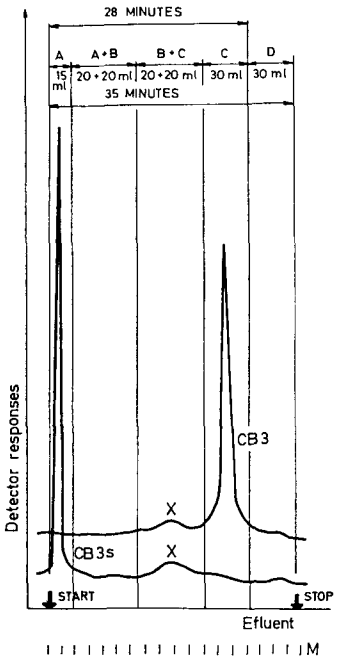


Fig. 11. Chromatographic analysis of cyanogen bromide fragment of serum albumin CB3 and of its succinylated derivative CB3s on a column of CM-Spheron 300, nominal capacity 2.20 mequiv./g. The figure is a superposition of records made with the apparatus shown in Fig. 4. Records at 285 and 254 nm were connected by averaging. Individual chromatograms are arranged one above another with intervals between blank lines. In uniformly performed experiments, 8 mg of fragment was applied each time in 0.2 ml of starting buffer. Short isocratic elution was followed by two linear gradients and by isocratic elution with the last buffer. The flow-rate was 4 ml/min. Fractions denoted by M were collected at 2-min intervals. Buffers used: A = 0.01 *M* ammonia + acetic acid, pH 7.0; B = 0.1 *M* ammonia + acetic acid, pH 7.0; C = 0.5 *M* ammonia + acetic acid, pH 7.0, 1 *M* with respect to sodium chloride. The column was finally washed with 2 *M* sodium chloride solution (D). Cyanogen bromide fragments have been characterized in detail in ref. 15; they contain 175 amino acid residues. X = unidentified peak, probably a residue of pyridine from preceding treatment. At the same time, the figure illustrates the effect of succinylation of polypeptide on the positions in the elution profile.

cellulose, and it was found that it did not exhibit irreversible sorption. The commercially produced weakly acid cation exchanger of type III, the so-called Spheron C 1000 (*cf.*, ref. 16, Table 5.6B, p. 260) yielded good results in a systematic study of the HPLC of pectolytic enzymes, which is now in progress.

CONCLUSIONS

Weakly acid carboxylic hydrophilic and macroporous cation exchangers are in general well suited for the separation of proteins and their higher molecular weight fragments. Samples of CM-Spheron 300 corresponding to this classification have been applied in many chromatographic experiments, in which they exhibited separation properties similar to those observed with carboxymethyl derivatives of cellulose or polydextran. With CM-Spheron or Spheron C, however, higher pressures could

be employed, which considerably accelerated the separation process. Spheron ion exchangers can be subjected to multiple regeneration without any change in their sorption properties.

Chromatographic analyses of various enzymes and other proteins and their fragments may be performed with Spheron derivatives of particle size 20–40 μm within a period of tens of minutes, using a moderate pressure not exceeding 15–20 atm. This makes possible the utilization of glass columns, pumps and connections used in amino acids analysers. Reduced particle size, together with a higher pressure applied in suitable devices equipped for the ion-exchange HPLC of proteins, leads to retention times measurable in minutes. Experiments carried out in this direction may lead to the construction of a high-speed protein analyser, which may be used not only in biochemistry and related fields, but also in fermentation technology (*e.g.*, in the investigation of the formation of bioproducts in large fermentors), in clinical diagnostics (*e.g.*, in the high-speed analysis of plasma proteins), in the production of sera and vaccines, technical enzymes and other proteins, in food technology and in agrochemical research. Spheron ion exchangers appear to be prospective packings for such an HPLC protein analyser.

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