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CHROMATOGRAPHY OF BIOPOLYMERS AND THEIR FRAGMENTS ON ION-EXCHANGE DERIVATIVES OF THE HYDROPHILIC MACROPOROUS SYNTHETIC GEL SPHERON*

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SUMMARY

New ion-exchange materials have been developed from the synthetic glycol methacrylate gel Spheron: carboxymethyl-Spheron (weakly acidic); phosphonyl-Spheron (medium acidic); sulphonyl-Spheron (strongly acidic); diethylaminoethyl-Spheron (Type 1, weakly basic; Type 2, medium to strongly basic). Information is presented on the chromatographic characteristics of these new, macroporous, hydrophilic and rigid ion-exchangers as determined by experiments on the separation of mixtures of proteins, peptides, amino acids, nucleic acids, oligonucleotides and nucleotides. The possibility is discussed of the use of ion-exchangers of this type in high-performance liquid chromatography of biopolymers.

INTRODUCTION

High-resolution liquid chromatography using high flow-rates and high pressures has developed rapidy in recent years. This has been made possible by the introduction of efficient supports composed of rigid particles of very small diameter and characterized by a short diffusion time[1]. Short elution times and low heights equivalent to a theoretical plate (HETP) have thus been achieved. Although a few papers dealing with these problems have been published concerning the separation of natural products of high molecular weight (*cf.* ref. 2), the use of high-resolution liquid chromatography for the analysis of biopolymers has been hampered by unfavourable properties of the supports. Ion-exchange resins based on a polystyrene matrix and a sufficiently rigid macromolecular matrix often show hydrophobic sorption leading to

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the inactivation of the bioproducts as a result of their denaturation. On the other hand, hydrophilic derivatives of fibrous cellulose introduced by Peterson and Sober[3,4] (*cf.* ref. 5) or hydrophilic polydextran derivatives, introduced by Porath and Lindner[6], which are capable of efficient separation of biopolymers and have been frequently used for this purpose (*cf.* ref. 7), are severely limited in high-resolution liquid chromatography where higher flow-rates and higher pressures are required.

Ion-exchangers based on a polyacrylate or a polymethacrylate matrix show less of the undesired adsorption effects in the chromatography of proteins than ion exchangers of the polystyrene type. The high density of cross-linking of the primary matrix provides a better mechanical stability which is necessary for the higher flow-rates; at the same time, however, the penetration of macromolecules into the inner structure of the matrix is impossible and thus only functional groups located on the pore surface can participate in the separation process. A decrease of the degree of cross-linking results in an increase in the number of functional groups which are accessible, but at the cost of a considerable decrease of rigidity; simultaneously, the flow characteristics of the resin become rather unfavourable.

This paper describes ion-exchange materials consisting of synthetic macroreticular and hydrophilic gels derived from hydroxyalkyl methacrylates (Spheron™). The mechanical rigidity and macroporosity of these materials is ensured by a special method of copolymerization in suspension (see next paragraph), and the hydrophilic character of the outer and inner surfaces of the polymer beads is ensured by a high number of hydroxyl groups. This paper is intended to provide an introduction to a series of more detailed communications on the individual ion-exchanger types which are in the course of development.

The macroporous hydrophilic gel Spheron (Lachema, Brno, Czechoslovakia), developed in the Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences[8,9], is prepared by precipitation copolymerization of hydroxyethyl methacrylate and ethyleneglycol bis(methacrylate). The sub-microscopic particles formed during the polymerization inside the suspended sphere contain inert organic solvents in addition to the monomer, copolymer and initiator. These particles aggregate during their formation giving rise to the macroporous structure of the beads (Fig. 1). The hydroxyl groups of the hydrophilic monomer permit additional modifications of the gel to be made and ion-exchangers to be obtained, similar to cellulose derivatives or to polydextran. The gel has a high content of the cross-linking agent which is responsible for minimum swelling and a satisfactory rigidity permitting the use of high flow-rates and high pressures. The porosity of the gel can be varied over a wide range with exclusion limits from $4 \cdot 10^4$ to 10^7 daltons (for polydextran, *cf.* Table I); some of the gels have a large surface and large pore volume. Gels have been employed successfully as supports in gel chromatography[10], in the immobilization of enzymes and affinity chromatography[13–15] and in covalent chromatography[16,17]. The favourable physical and chemical characteristics of gels stimulated our interest in the gel as a prospective matrix for ion-exchange materials.

EXPERIMENTAL

Materials

The Spheron starting material was prepared by the procedures described

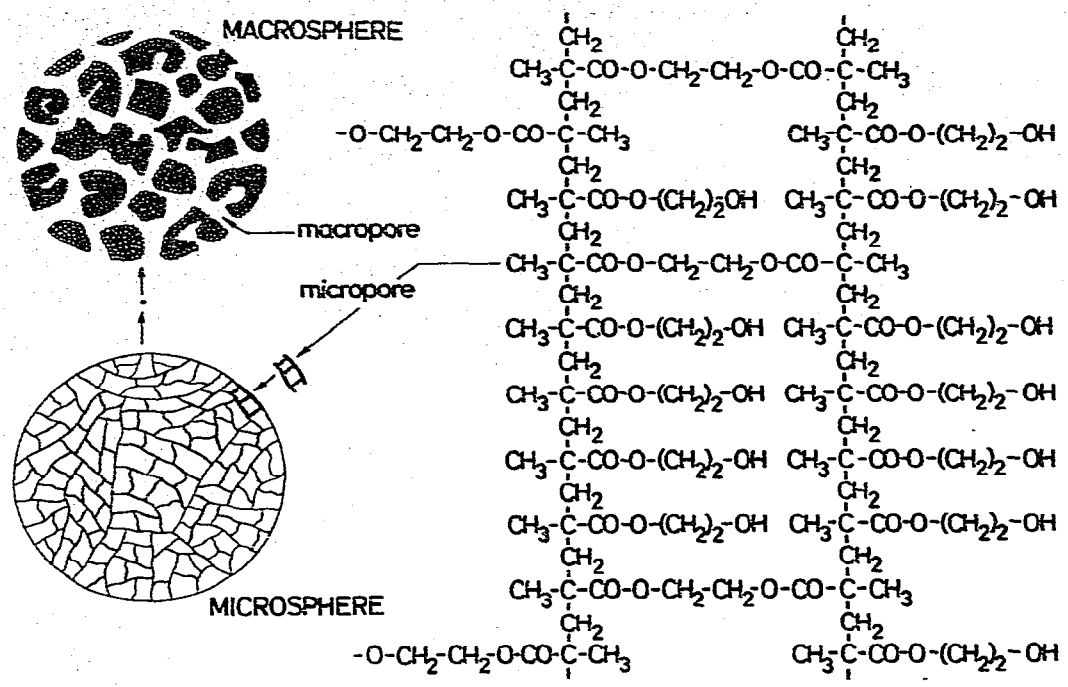

Fig. 1. Representation of the structure of spheroids of heterogeneous, hydrophilic, macroporous Spheron gel. The size of the micropores can be controlled by altering the ratio of hydroxyalkyl methacrylate to alkene dimethacrylate during copolymerization; the micropores are so small that they do not permit larger molecules to penetrate. The high content of the cross-linking agent in the polymerizing mixture ensures mechanical rigidity of the particles. The macroporosity (important for the penetration of biopolymers), the inner surface area and the number of groups which can be additionally chemically modified (important for the preparation of ion-exchangers and for the binding of affinants) can be controlled over a wide range by varying the ratio of the inert solvents in the suspension copolymerization.

above[8,9] or it was purchased from Lachema. Chicken egg-white proteins were prepared as described elsewhere[18]. Bovine chymotrypsinogen was prepared in our laboratory and recrystallized five times. Human serum albumin was obtained from Imuna (Šarišské Michalany, Czechoslovakia), human plasma from the Institute for Sera and Vaccines (Prague, Czechoslovakia), hog pepsinogen and egg-white lysozyme from Worthington (Freehold, N.J., U.S.A.), technical glucose oxidase from Boehringer (Mannheim, G.F.R.), and adenosine mono-, di- and triphosphate were purchased from Lachema, P-L Biochemicals (Milwaukee, Wisc., U.S.A.) and Calbiochem (San Diego, Calif., U.S.A.), respectively. The synthetic peptides were obtained from Dr. K. Jošt, and the preparation of calf-thymus nucleic acid[19] was a gift from Dr. J. Šponar of the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Sciences. The specific hydrolysate of *Bacillus subtilis* deoxyribonucleic acid[20] was kindly provided by Dr. J. Šatava.

Preparation of ion-exchange materials

Ion exchangers of the Spheron type can be prepared either by direct copoly-

TABLE I

SPHERON GELS

Hydroxyalkyl methacrylate gels (Lachema, Brno, Czechoslovakia); data from ref. 10.

*Spheron**	*Gel type***	*Specific surface area (m^2/g)****
P-1	Homogeneous	—
P-40	Semi-heterogeneous	142.3
P-100	Heterogeneous	1.3
P-200	Heterogeneous	3.1
P-300	Heterogeneous	77.7
P-500	Heterogeneous	131.5
P-700	Heterogeneous	15.3
P-1000	Heterogeneous	22.5
P-10,000	Heterogeneous	5.0

* The numerical designation of Spheron multiplied by 10^3 indicates the exclusion limit in daltons. The exclusion limit was tested chromatographically by the sieving effect using polydextrans.

** The gel type corresponds to the classification according to Heitz[11].

*** The inner surface area was determined by chromatographic desorption according to [12]. The P-1 gel does not contain macropores and is listed for reasons of comparison only. The P-40 gel was prepared under the conditions of polymerization characterized by phase separation; the P-100 to P-10 000 gels were prepared under the conditions of precipitating copolymerization in suspension. See ref. 10 for a detailed explanation.

merization from ionogenic monomers[21], by analogous reactions based on the reactivity of the hydroxyl groups[22,23], or by additional modifications of the ion-exchangers and matrix[24,25].

The ion-exchange derivatives described in this study are summarized in Table II. The weakly acidic carboxymethyl (CM)-Spheron was prepared from Spheron P-300 (bead size, 25–32 μm) by the reaction with sodium chloroacetate in a solution

TABLE II

ION-EXCHANGE DERIVATIVES OF SPHERON

Ion-exchange derivative	*Functional group*	*Capacity**	
		(mequiv./g)	*mg of protein per 100 mg of ion exchanger*
Spheron P-300	Non-ionogenic gel; exclusion limit, $3 \cdot 10^5$ daltons	—	—
Cation-exchangers			
CM-Spheron	$-COOH$	1.5	30, chymotrypsinogen
P-Spheron	$-PO(OH)_2$	4.0	40, chymotrypsinogen
S-Spheron	$-SO_3H$	1.0	20, chymotrypsinogen
Anion-exchangers			
DEAE-Spheron Type 1	$-N(C_2H_5)_2$	0.9	60, pepsin; 12, serum albumin
DEAE-Spheron Type 2	$-\overset{+}{N}(C_2H_5)_2-C_2H_4-N(C_2H_5)_2$	2.0	12, serum albumin

* The capacity determination will be discussed in detail in subsequent papers.

of sodium hydroxide according to ref. 22. The medium acidic phosphonyl(P)-Spheron was prepared from Spheron P-300 (bead size, 25–32 μm) by the reaction with phosphoryl chloride in ethyl acetate according to ref. 26. The strongly acidic sulphonyl(S)-Spheron was prepared[22] from Spheron P-300 (bead size, 20–40 μm) by the reaction with chlorosulfonic acid in ethyl acetate. The weakly basic diethylaminoethyl(DEAE)-Spheron Type 1 was obtained by copolymerization of hydroxyethyl methacrylate, ethylene dimethacrylate and 2-(diethylamino)ethyl methacrylate according to ref. 21. The medium basic DEAE-Spheron Type 2 was prepared from Spheron P-300 (bead size, 60–100 μm) by the reaction with (2-chloroethyl)diethylamine according to ref. 23. The ion-exchange materials were repeatedly extracted and equilibrated as described below. The capacities of the ion-exchangers for small ions were calculated from titration graphs and are given in Table II. More detailed characteristics of the individual ion-exchangers will be described in subsequent papers.

Ion-exchange chromatography

The ion exchangers were washed with 2 *M* sodium chloride and distilled water before use. Cation exchangers were then treated five times with 2 *M* NaOH, water, 2 *M* HCl and water. Anion exchangers were similarly treated with 2 *M* HCl, water, 2 *M* NaOH and water. The ion exchangers were then equilibrated with the first buffer to be used for chromatography until the pH and conductivity were constant.

The ion exchangers used were regenerated by washing with 2 *M* NaCl and treated as described above. The absorbance, pH and conductivity of effluent fractions of the separated compounds were determined; alternatively, the separation was recorded by a UV detector at 254 nm. Some of the experiments were made in a Beckman-Spinco Model 120B amino acid analyzer. The chromatographic profiles obtained with the individual ion-exchangers are described below in the order given in Table II.

RESULTS

Retention of proteins by non-ionogenic carrier gel

The elution profile of chymotrypsinogen from a column of unmodified Spheron P-300 gel is shown in Fig. 2. Solutions of pepsinogen and human serum albumin were eluted from the same column. The recoveries were determined spectrophotometrically and were consistently 100%. No unspecific adsorption was observed with acidic pepsinogen, with basic chymotrypsinogen or with serum albumin which is known for its strong adsorption ability. The retention of these proteins by the column is caused by the molecular sieving effect of the gel pores. The material thus tested, which was devoid of undesirable adsorption properties, was used for ionogenic substitutions.

CM-Spheron

The chromatography of chicken egg-white proteins (Fig. 3) was used to test the characteristics of CM-Spheron in comparison with CM-cellulose. A simple linear gradient of increasing pH and ionic strength and analogous column types were used. The differences in the relative positions of the peaks are due to the lower capacity of CM-cellulose for ions of low molecular weight which affects the course of the gradient

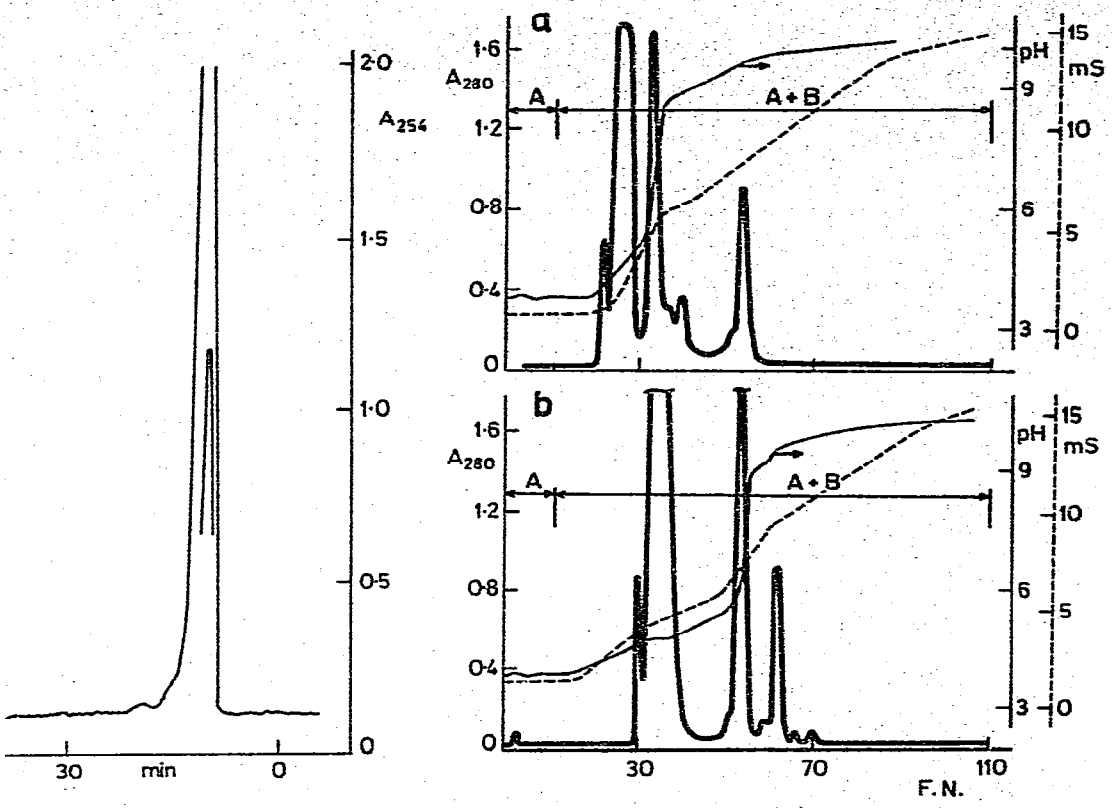

Fig. 2. Chromatography of chymotrypsinogen on a neutral Spheron carrier. Chymotrypsinogen (15 mg) dissolved in 0.5 ml of water was chromatographed on a column (25 × 0.8 cm) of Spheron P-300 (bead size, 25–32 μm). The column was eluted with water at a flow-rate of 45 ml/h. The elution profile was continuously recorded by means of a UV absorption meter at 254 nm.

Fig. 3. Comparative chromatography of chicken egg-white proteins on columns of CM-cellulose and CM-Spheron. In both cases, 200 mg of proteins dissolved in 1 ml of buffer A were placed on to a column (23 × 0.8 cm). The latter was eluted by buffer A up to fraction number (F.N.) 10. A linear elution gradient was then applied with 250 ml of buffer A and 250 ml of buffer B. Buffers: A, 0.03 *M* ammonia–acetic acid (pH 3.88); B, 0.1 *M* ammonia–0.2 *M* sodium carbonate–acetic acid (pH 10.9). Flow-rate 30 ml/h. Fraction volume, 5 ml. a, Column of microgranular CM-cellulose; b, column of CM-Spheron (bead size, 25–32 μm).

in the column. Comparison of the two supports shows that an elution profile similar to that obtained with CM-cellulose can be achieved with CM-Spheron, however, the yields of some proteins were higher in the latter case because some of the material chromatographed is probably irreversibly adsorbed on the cellulose.

P-Spheron

A mixture of proteins was fractionated on P-Spheron in the order of their isoelectric points (Fig. 4). A double linear gradient was used. Individual proteins were chromatographed independently with analogous results. Their desorption was practically quantitative. The chromatography of a more complicated mixture, human

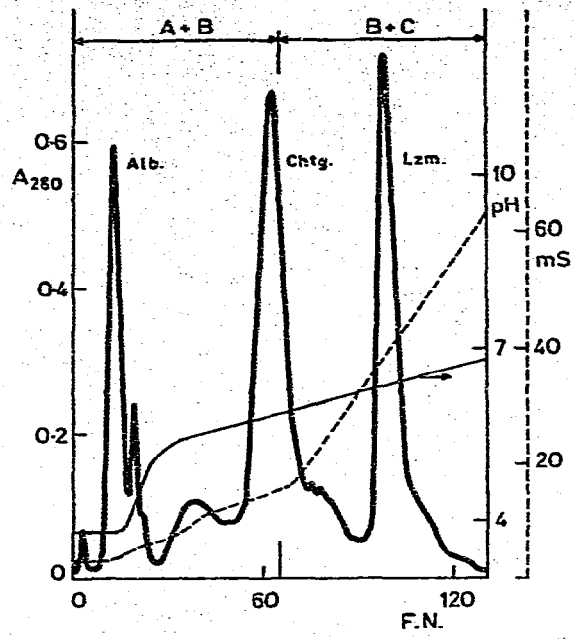

Fig. 4. Fractionation of an artificial mixture of proteins on P-Spheron. A mixture of serum albumin (Alb., 30 mg), chymotrypsinogen (Chtg., 20 mg) and lysozyme (Lzm., 10 mg) dissolved in 0.6 ml of water was chromatographed on a column (19 × 0.8 cm) of P-Spheron (bead size, 20–40 μm). Two linear elution gradients were used: first, 125 ml of buffer A + 125 ml of buffer B; second, 125 ml of buffer B + 125 ml of buffer C. Buffers: A, 0.05 *M* ammonia–formic acid (pH 3.5); B, 0.3 *M* ammonia–acetic acid (pH 6.0); C, 1 *M* ammonia–acetic acid (pH 8.0), 0.5 *M* in KCl. Fractions, 4.2 ml per 5 min.

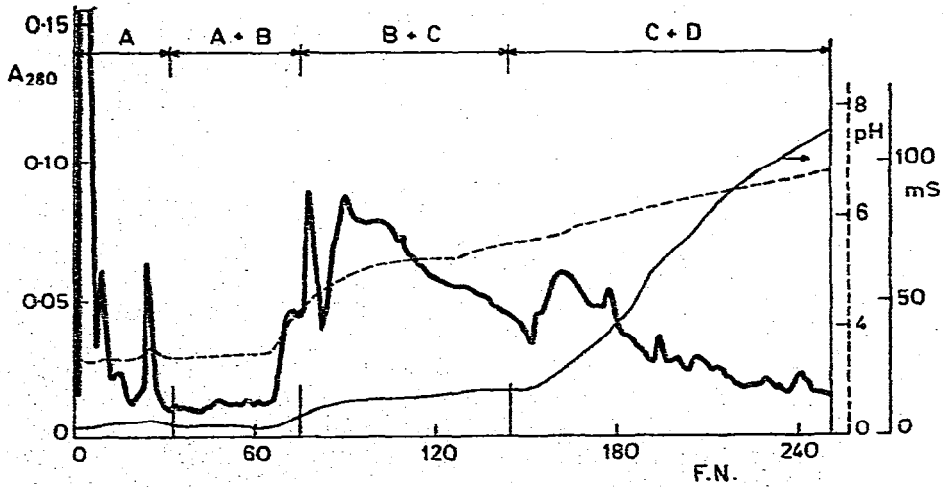

Fig. 5. Chromatographic profile of human plasma on P-Spheron. 2 ml of plasma (centrifuged before use) was applied to a column (24 × 0.8 cm) of P-Spheron (bead size, 20–40 μm). Buffers: A, 0.05 *M* ammonia–formic acid (pH 3.5); B, 0.2 *M* ammonia–acetic acid (pH 5.0); C, 0.2 *M* ammonia–acetic acid (pH 6.0); D, 0.3 *M* ammonia–acetic acid (pH 8.0), 1.5 *M* in KCl. Fractions, 4.3 ml per 8 min.

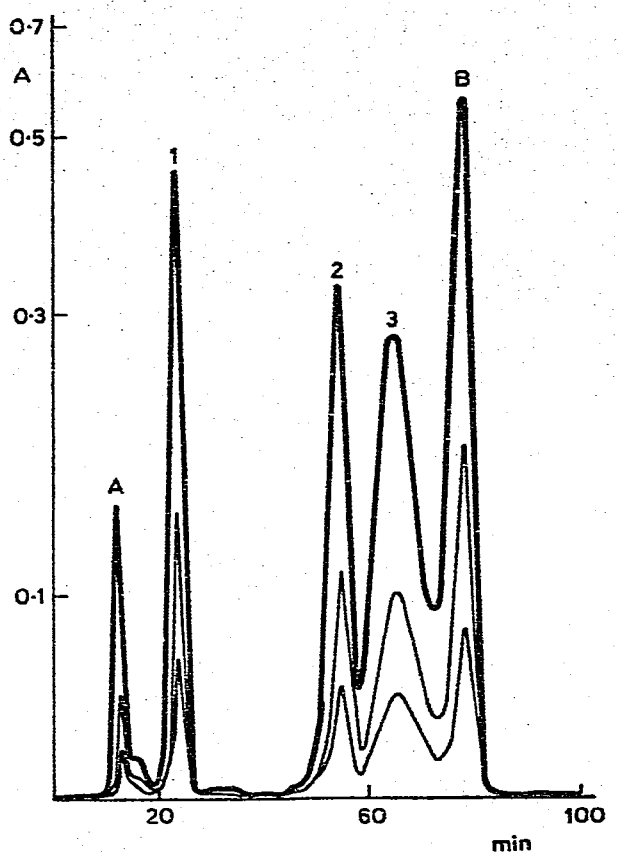

Fig. 6. Separation of a synthetic mixture of peptides on a column (24 × 0.8 cm) of P-Spheron (bead size, 20–40 μm) in the amino acid analyzer. Buffer, 0.1 *M* sodium hydroxide–citric acid (pH 4.0). Temperature, 55°. Pressure, 4 atm. Flow-rate, 68 ml/h (ninhydrin, 34 ml/h). The sample was dissolved in 0.5 ml of the buffer (pH 3.5). Direct ninhydrin colorimetry. Fractions: A, B = chains of oxidized insulin; 1 = triglycine; 2 = glycylglycinamide; 3 = tyrosylglycine.

plasma, effected by a combination of simple elution with three gradients, is illustrated in Fig. 5.

The macroporous ion-exchange derivatives of Spheron having an exclusion limit of $3 \cdot 10^5$ daltons can easily fractionate low-molecular-weight protein fragments and amino acids. The separation of a peptide mixture in the amino acid analyzer, evaluated by direct ninhydrin colorimetry without alkaline hydrolysis, is shown in Fig. 6. The same ion-exchange material was used in the analyzer to separate basic amino acids. Fig. 7 shows the interesting position of the ammonia peak which considerably differs from that observed on sulphonic acid cation-exchangers.

S-Spheron

As in P-Spheron also the strongly acidic S-Spheron is a suitable sorbent for the separation of mixtures of proteins which can be resolved in the order of their isoelectric

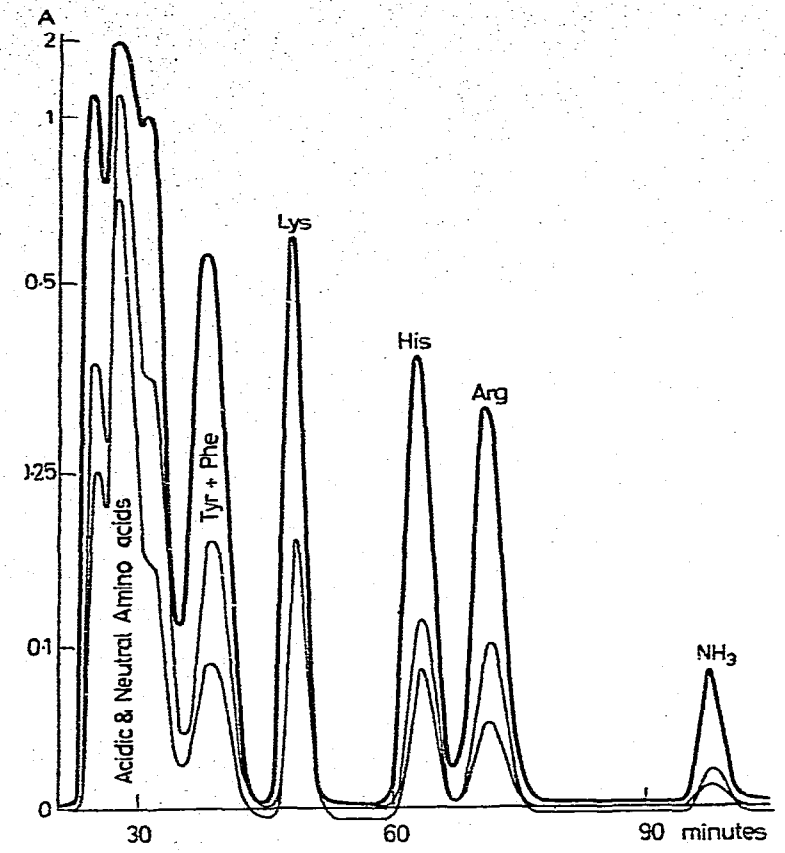

Fig. 7. Separation of basic amino acids on a column (58 × 0.9 cm) of P-Spheron (bead size, 20–40 μm) in the amino acid analyzer. Elution by buffer, 1.35 *N* sodium citrate (pH 5.28). Pressure 5 atm. Temperature 55°. Flow-rate, 68 ml/h.

points. In this respect, the ion-exchange derivatives of Spheron resemble the well known hydrophilic ion-exchangers, *i.e.* cellulose and polydextran derivatives. The separation of components of a technical enzyme preparation containing glucose oxidase, catalase and amylase is shown in Fig. 8.

DEAE-Spheron

Two variants of DEAE-Spheron were prepared. Type 1 was prepared by ternary copolymerization of 2-(diethylamino)ethyl methacrylate, 2-hydroxyethyl methacrylate and ethylenedimethacrylate. This material has only weakly basic tertiary amino groups and there are no quarternary basic groups (see Table II). Acidic proteins, such as pepsin, pepsinogen and serum albumin, were adsorbed well on Type 1. In general, Type 2, containing quarternary basic groups in addition to the DEAE groups (Table II), was more suitable for the analysis of biopolymers. The elution profiles obtained for plasma (Fig. 9) are similar to those reported by Sober and Peterson[27] on DEAE-cellulose. Although the nominal exclusion limit for Spheron P-300 is

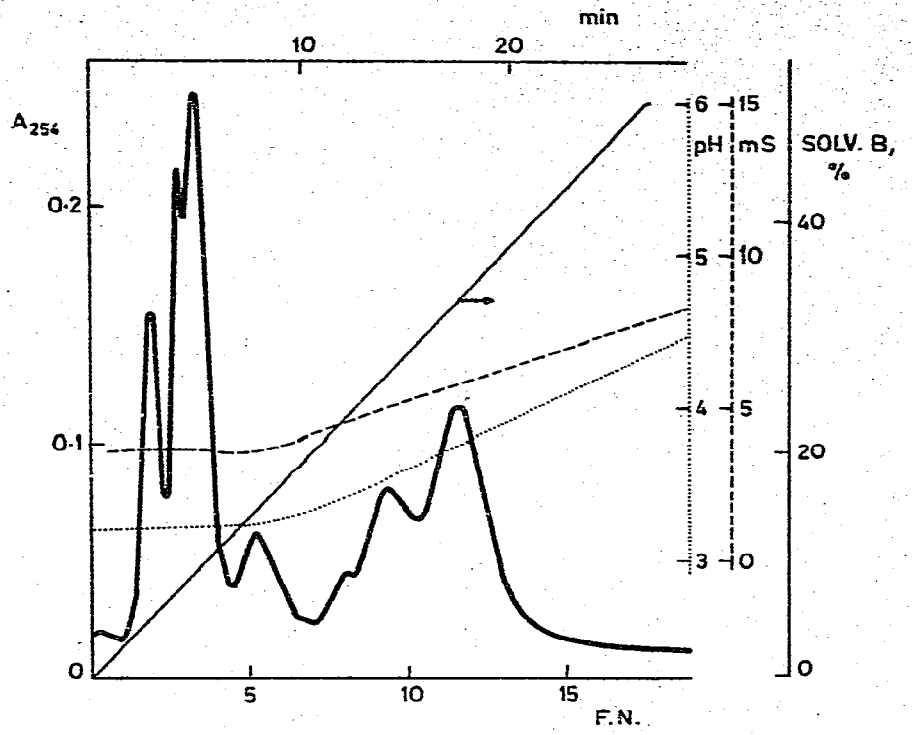

Fig. 8. Chromatography of a commercial glucose oxidase preparation on a column (25 × 0.8 cm) of S-Spheron (bead size, 25–40 μm). Loading, 10 mg in 1 ml of starting buffer. Linear gradient, 100 ml of A + 100 ml of B. Flow-rate, 240 ml/h. Fractions, 6 ml per 1.5 min. Buffers: A, 0.05 *M* ammonia–formic acid (pH 3.35); B, 0.3 *M* ammonia–acetic acid (pH 6.0).

$3 \cdot 10^5$ daltons, DEAE-Spheron showed very favourable characteristics as regards the chromatography of a highly viscous preparation[19] of calf-thymus deoxyribonucleic acid (Fig. 10), whose molecular weight exceeded 10^6 daltons. It is possible that only the functional groups located at the surface of the gel particles participate in this process, or that the exclusion limit obtained by testing with polydextran derivatives does not hold for nucleic acids.

Nucleic acid fragments could also be fractionated with relatively good results on DEAE-Spheron, as illustrated by the chromatogram of pyrimidine oligoribonucleotides (Fig. 11). The result is comparable with chromatography of the same hydrolyzate on DEAE-cellulose[20]. Spheron derivatives can be used to separate, similarly to proteins, peptides and amino acids, not only nucleic acids and their fragments of medium molecular weight but also their low-molecular-weight structural units, nucleotides. The fractionation of a mixture of commercial adenosine phosphates is shown in Fig. 12.

DISCUSSION

Modern and rapid ion-exchange chromatography of high-molecular-weight biopolymers at medium or high pressures requires column supports having the fol-

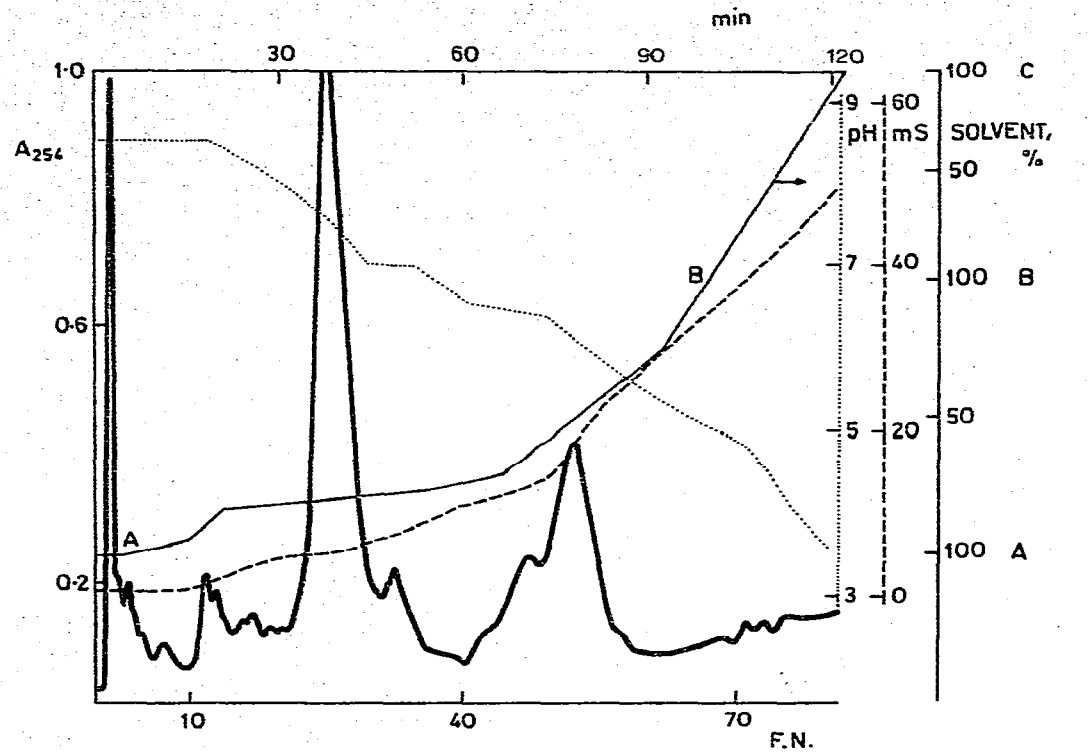

Fig. 9. Chromatography of human plasma on a column (19 × 0.8 cm) of DEAE-Spheron Type 2 (bead size, 25–32 μm). Loading 2 ml of plasma, centrifuged before application. A compound gradient (thin full line) was effected by automatic proportional pumps and buffers. Buffers: A, 0.025 *M* phosphoric acid–Tris (pH 8.50); B, 0.5 *M* phosphoric acid–Tris (pH 3.50); C, buffer B 1.0 *M* in KCl. Flow-rate, 225 ml/h. Fractions collected at 1.5-min intervals.

lowing properties: (1) a hydrophilic surface which does not lead to denaturation of the products separated; (2) large pores with a sufficiently high exclusion limit; (3) an appropriate microenvironment of the functional groups; (4) a large inner surface area; (5) sufficient capacity; (6) chemical stability; (7) resistance to microbes and enzyme activity; (8) mechanical rigidity; (9) a minimal swelling tendency; (10) minimal volume changes with pH and ionic strength; (11) good flow characteristics at high flow-rates and (12) a resistance to high pressure.

The classical materials available, such as the derivatives of cellulose or polydextran, fulfill the requirements 1–5. They have a high capacity in columns or when used in batches, and their functional groups have an appropriate microenvironment. Their limitations become apparent when high flow-rates or high pressures are used, at extreme pH values (especially at increased temperatures) and variable ionic strength (in both senses).

Flow characteristics of a Spheron derivative in a column are given in Fig. 13. Unlike the hydrophilic derivatives of cellulose and polydextran, Spheron shows a linear dependence of the over-pressure on the flow-rate up to a value of 500 ml/cm^2·h. The column height remains constant over the entire range. The flow characteristics

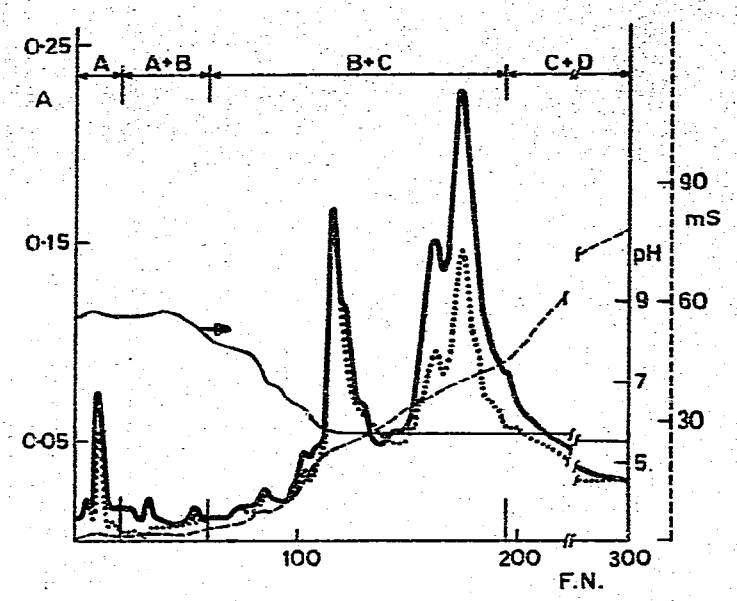

Fig. 10. Chromatography of a preparation of calf-thymus deoxyribonucleic acid on DEAE-Spheron Type 2. 1 ml of a solution containing 1.8 mg of the preparation was applied to a column (24 × 0.8 cm) of DEAE-Spheron Type 2 (bead size, 60–100 μm). Fractions, 2.2 ml per 4 min. Buffers: A, 0.005 *M* phosphoric acid–Tris (pH 8.57); B, 0.1 *M* phosphoric acid–Tris (pH 5.52); C, buffer B 1 *M* in NaCl; D, buffer B 2 *M* in NaCl. Elution: 50 ml of buffer A followed by linear gradients of 50 ml of A + 50 ml of B, 150 ml of B + 150 ml of C and 150 ml of C + 150 ml of D. No peak appeared on elution with the last gradient. Thick line, absorbance at 254 nm; dotted line, absorbance at 280 nm.

of the Spheron derivatives are practically independent of the ionic strength of the solution.

From Figs. 2–12 and from studies of the use of Spheron in the field of affinity[13–15] and covalent chromatography[17], it is evident that the surface area of these materials is sufficiently hydrophilic (although probably not as hydrophilic as the surfaces of cellulose or polydextran) and does not denature biopolymers by hydrophobic adsorptions. The elution profiles given in Figs. 3–12 suggest that there are no essential differences between cellulose (polydextran) derivatives and hydroxyalkyl methacrylate gels as regards their functional groups. The examples given in the figures include high-molecular-weight proteins (Figs. 3–5, 8 and 9) and nucleic acids (Fig. 10), and their fragments of medium molecular weight (Figs. 6 and 11) and of low molecular weight (Figs. 7 and 12). In these cases the derivatives of Spheron possess a resolving power similar to that of hydrophilic ion exchangers based on a cellulose or polydextran matrix. The Spheron derivatives can be applied in the most important fields of ion-exchange chromatography in biochemistry. The separation is based essentially on ionic interactions. The use of macroporous ion exchangers with a high exclusion limit is not restricted to high-molecular-weight substances. When such materials are used for the chromatography of low-molecular-weight compounds, they offer, compared to the classical ion exchangers, the advantage of higher diffusion rates within the beads and also the accessibility of all of the ionic groups on the inner surface of the pores.

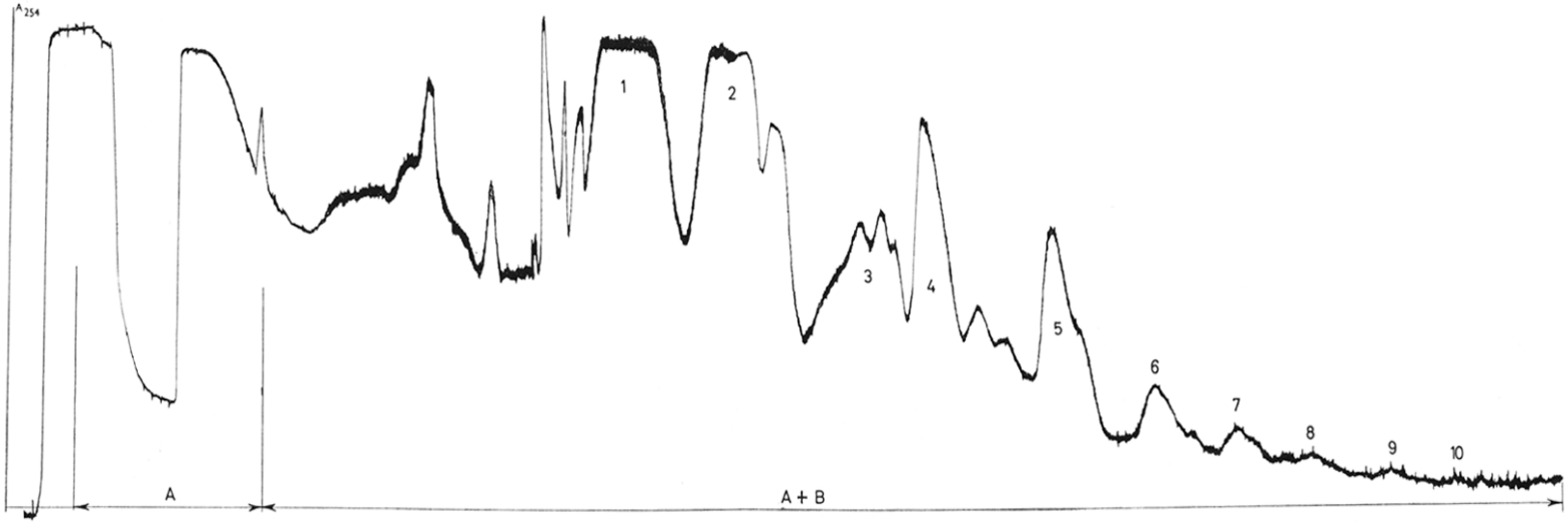

Fig. 11. Separation of pyrimidine oligodeoxyribonucleotides from specific diphenylamine-acid hydrolyzate of *Bacillus subtilis* DNA on DEAE-Spheron Type 2. The graph is a continuous record of the absorption at 254 nm from an UV absorption meter, carried out by courtesy of Dr. J. Šatava, Institute of Experimental Botanics, Czechoslovak Academy of Sciences, Prague. 50 mg of the digest were applied to a column (30 × 1 cm) of DEAE-Spheron Type 2 (bead size, 60–100 μm). Elution: 300 ml of buffer A followed by a linear gradient of 1000 ml of A + 1000 ml of B. Flow-rate, 50 ml/h. Buffers: A, 0.01 *M* sodium acetate (pH 5.3); B, buffer A 0.4 *M* in NaCl. The first peaks (not numbered) are due to products of non-specific cleavage and to reaction components of the initial solution; peaks 1 – 10 are mono- to deca-deoxyribonucleotides.

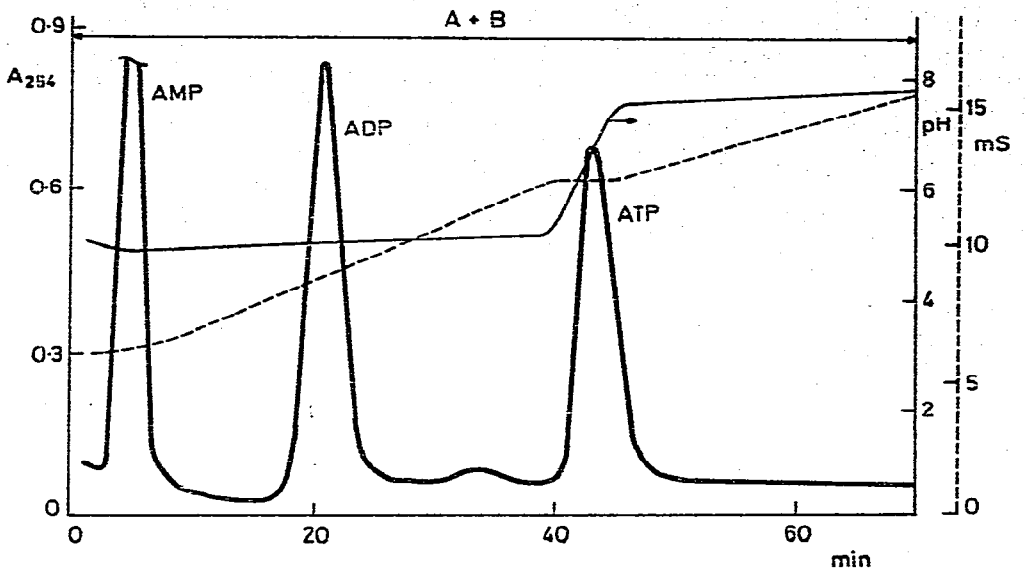

Fig. 12. Separation of a synthetic mixture of commercial adenosine phosphates on a column (18 × 0.6 cm) of DEAE-Spheron Type 2, (bead size, 25–32 μm). 2 mg of a mixture of adenosine monophosphate (AMP), diphosphate (ADP) and triphosphate (ATP) dissolved in 200 μl of water were applied to the column and eluted by a linear gradient developed with 150 ml of 0.1 *M* HCl–Tris buffer (pH 5) + 150 ml of 0.1 *M* HCl–Tris buffer (pH 8) (0.15 *M* in NaCl). Temperature, 25°. Flow-rate, 240 ml/h. Pressure, 8 atm. Fractions were collected at 1-min intervals.

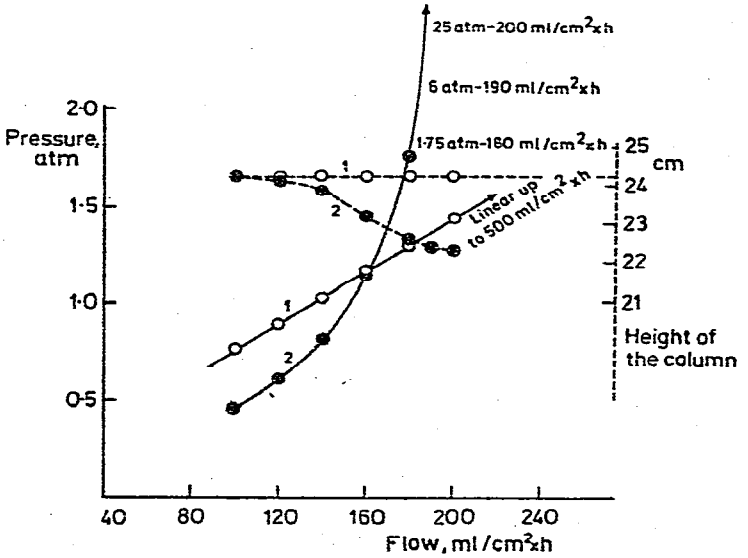

Fig. 13. Relations between the flow-rate and the counter pressure on chromatographic columns packed with hydrophilic ion-exchangers. 1 = Column (24 × 0.8 cm) of P-Spheron (bead size, 20–40 μm; nominal exclusion limit, $3 \cdot 10^5$ daltons); 2 = column of phosphonyl-cellulose. The experiments were made with 0.1 *M* sodium acetate (pH 5).

Fig. 14. Microphotograph of Spheron P-300.

Spheron derivatives also show a high chemical stability. Although they are based on an ester bond, they are resistant to hydrolysis and resemble the very stable esters of trimethylacetic acid. These ion exchangers can therefore be treated with 2 *M* solutions of acids and alkali. The gel can be sterilized in autoclaves. It is also resistant to mild oxidizing agents and to the action of organic solvents. The synthetic matrix does not undergo enzymatic cleavage and is not attacked by microorganisms.

The fact that the macroscopic beads of Spheron are composed of aggregates with a very high density of cross-linking (Fig. 1) means that the macroscopic particles do not swell much and hardly change their volume with ionic strength; for instance, there is no volume change when 2 *M* NaCl is replaced by water during the washing of a column.

Spheron is manufactured in the form of regular, rigid, spherical particles (Fig. 14). The flow characteristics of the Spheron derivatives in the column are good and can be defined by a column-flow resistance factor[28], φ, of *ca.* 850.

CONCLUSIONS

The derivatives of Spheron beads with controllable macropores permit the penetration of biopolymers into the beads, are sufficiently hydrophilic, almost do not swell and are practically undeformable in the chromatographic process; their volume is independent of pH and ionic strength, they can be prepared with the desired capacity and they show excellent flow characteristics. They are very resistant, both chemically and mechanically. They have been found to be of advantage in preliminary experiments on the chromatography of proteins, nucleic acids and their fragments.

We believe that their future development directed to smaller particles will pave the way to studies on the use of high-performance liquid chromatography in the separation of biopolymers.

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