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CHARACTERIZATION OF TSK-GEL DEAE-TOYOPEARL 650 ION EXCHANGER

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SUMMARY

Basic properties and applications to chromatographic separations of the weak anion exchanger TSK-GEL DEAE-Toyopearl 650 were investigated. DEAE-Toyopearl 650 was found to be an ideal support for the medium-performance ion-exchange chromatography of biological substances such as proteins.

INTRODUCTION

Although high-performance liquid chromatography is widely used in many fields, high performance preparative columns are very expensive and the scale-up of analytical separations is seriously inhibited. For this reason, supports for medium-performance liquid chromatography, which are of large particle size, inexpensive and easy to pack, have been required[1].

TSK-GEL Toyopearl (Toyo Soda, Tokyo, Japan), introduced a few years ago, is a support of this kind and has been demonstrated to be an excellent support for medium-performance gel filtration[2]. In addition, a derivative of Toyopearl, a weak anion exchanger, has become commercially available recently. According to the manufacturer, the weak anion exchanger was derived by introducing diethylaminoethyl groups into Toyopearl HW65 with a large pore size and was named DEAE-Toyopearl 650. The basic properties and the applications to chromatographic separations of this ion exchanger are described in this paper.

EXPERIMENTAL

All measurements were performed on DEAE-Toyopearl 650S (superfine grade) and 650M (medium grade) of lot No. DE65103. The same materials are available from E. Merck (Darmstadt, G.F.R.).

The titration curve was measured with a Model RAT-11 autotitrator (Hiranuma, Ibaragi, Japan). DEAE-Toyopearl 650M (10 ml in the swollen state) was titrated in 100 ml of 0.5 *M* sodium chloride solution with 0.5 *N* hydrochloric acid.

The adsorption capacity for proteins was determined by the static method. DEAE-Toyopearl 650M (3 ml) was mixed with 10 ml of a 1% solution of thyroglo-

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bulin, ferritin or bovine serum albumin and 0.05 *M* Tris–HCl buffer (pH 8.30), which is referred to later as adsorption buffer, was added to make the total volume 50 ml. After the mixture had been left at 25°C for 30 min with occasional swirling, it was filtered through filter-paper, washed with adsorption buffer and then washed repeatedly with *ca.* 15 ml of 0.05 *M* Tris–HCl buffer (pH 8.30) containing 0.5 *M* sodium chloride, which is referred to later as desorption buffer. The filtrate containing desorption buffer was pooled until it became 100 ml in total and the protein in the filtrate was determined spectrophotometrically at 280 nm.

The particle size distribution was observed with a microscope and determined with a Model TA II Coulter Counter (Coulter Electronics, Hialeah, FL, U.S.A.).

The microstructure was observed with a Model JSM-50A scanning electron microscope (Japan Electron Optics Lab., Tokyo, Japan).

The pore size was evaluated by measuring the relationship between molecular weight and elution volume, *i.e.*, the molecular weight calibration graph in gel filtration, for protein and polyethylene glycol. Proteins were measured on a DEAE-Toyopearl 650M column (15 × 1.6 cm I.D.) in 0.1 *M* phosphate buffer (pH 5.5) containing 0.5 *M* sodium chloride with UV detection at 280 nm. Polyethylene glycols with narrow molecular weight distributions were measured on the same column in 0.1 *M* sodium chloride solution with refractive index detection. The flow-rate was 1 ml/min.

The mechanical stability was evaluated by measuring the relationship between flow-rate and pressure drop on DEAE-Toyopearl 650S and 650M columns (15 × 1.6 cm I.D.) in 0.1 *M* sodium chloride solution at 25°C.

The swelling properties were evaluated by measuring the bed volume in 0.05 *M* phosphate buffers of pH 5–10 and in 0.02 *M* phosphate buffers of pH 7.50 containing 0–0.5 *M* sodium chloride. DEAE-Toyopearl 650M was filled in a 60 × 1.6 cm I.D. column to a height of *ca.* 40 cm. The exact bed height in a buffer was determined after elution of the buffer had been continued for 1 day at a flow-rate of *ca.* 1 ml/min. This was repeated for all buffers with a single column.

The chemical stability was evaluated by measuring the change in ion-exchange capacity on treatment of DEAE-Toyopearl 650M with 0.5 *M* sodium hydroxide solution and 0.5 *M* hydrochloric acid at 25°C. The ion-exchange capacity was determined by measuring the titration curve.

The thermal stability was evaluated by measuring the change in ion-exchange capacity on treatment of DEAE-Toyopearl 650M with 0.1 *M* phosphate buffer (pH 7.0) at 120°C.

Re-equilibration of columns in ion-exchange chromatography with gradient elution was investigated on a DEAE-Toyopearl 650M column (15 × 1.6 cm I.D.). The column was first equilibrated with the final buffer, then the eluent was changed to the initial buffer and the conductance and pH of the column effluent were monitored as a function of elution volume. The conductance was monitored continuously with a flow cell type conductivity detector and the pH was measured for fractions of 30 ml (one column volume). The flow-rate was 1.5 ml/min. Combinations of initial and final buffers were (1) 0.05 *M* Tris–HCl buffer (pH 8.60) and 0.05 *M* Tris–HCl buffer (pH 8.60) containing 0.5 *M* sodium chloride, (2) 0.05 *M* Tris–HCl buffer (pH 8.94) and 0.05 *M* Tris–HCl buffer (pH 7.10) containing 0.5 *M* sodium chloride and (3) 0.05 *M* Tris–HCl buffer (pH 8.44) and 0.05 *M* phosphate buffer (pH 5.90) containing 0.5 *M* sodium chloride.

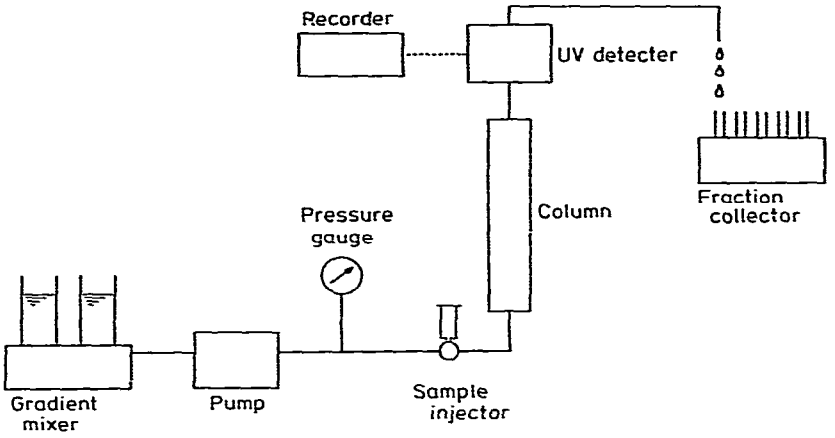

Fig. 1. Schematic diagram of the set-up for ion-exchange chromatography on DEAE-Toyopearl 650.

The application to chromatographic separations was investigated with the set-up shown in Fig. 1. Proteins and nucleic acid related compounds were separated by linear gradient elution of two buffers at a flow-rate of 1.5 ml/min at 25°C. A glass column (15 × 1.6 cm I.D.) with end-fittings at both ends was employed. The packing of the column was carried out by the semi-constant pressure method[3] with a peristaltic pump. The column effluent was monitored continuously with a UV detector at 280 or 254 nm. Then the portion of interest was collected manually and subjected to analytical gel filtration and ion-exchange chromatography to examine the degree of purification.

In the separation of commercial urease, all of the column effluent was collected in 3 ml with a fraction collector and 1-ml aliquots of the fractions were examined for urease activity by the colorimetric timing method[4]. The analytical gel filtration was performed on a Toyo Soda TSK-GEL G3000SW (60 × 0.75 cm I.D.) two-column system in 0.1 *M* phosphate buffer (pH 6.8) containing 0.1 *M* sodium sulphate at a flow-rate of 1 ml/min at 25°C. Under these conditions, proteins in the molecular weight range 10,000–500,000 could be separated in *ca.* 40 min, as can be seen from the molecular weight calibration graph in Fig. 2.

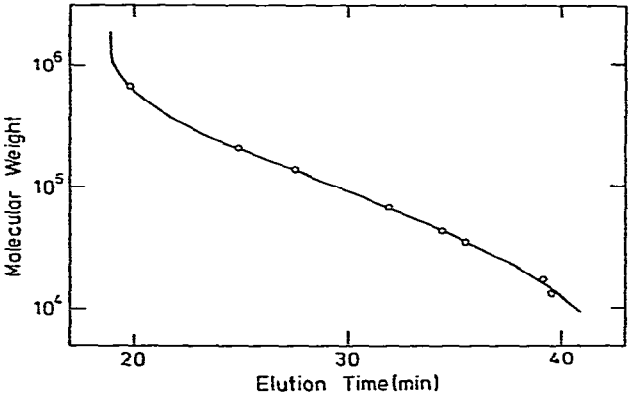

Fig. 2. Calibration graph of TSK-GEL G3000SW two-column system for globular protein.

The analytical ion-exchange chromatography was performed either on TSK-GEL IEX-545 DEAE SIL (15 × 0.6 cm I.D.) packed with weak anion exchanger of 10 μm diameter or on TSK-GEL IEX-535 CM SIL (15 × 0.6 cm I.D.) packed with weak cation exchanger of 10 μm diameter with linear gradient elution of two buffers at a flow-rate of 1 ml/min at 25°C. A UV detector at 280 nm was also used in analytical separations.

Commercial urease (from jack bean; P-L Biochemicals, Milwaukee, WI, U.S.A.) (30 mg in 1 ml of initial buffer) was separated on DEAE-Toyopearl 650S and 650M with linear gradient elution from 0.05 *M* phosphate buffer of pH 6.70 (150 ml) to 0.05 *M* phosphate buffer of pH 6.70 containing 0.5 *M* sodium chloride (150 ml). Egg white (0.25 ml, containing *ca.* 30 mg of proteins) diluted to 1 ml with initial buffer was separated on DEAE-Toyopearl 650S with linear gradient elution from 0.05 *M* Tris–HCl buffer of pH 8.60 (200 ml) to 0.05 *M* Tris–HCl buffer of pH 8.60 containing 0.5 *M* sodium chloride (200 ml). Standard human serum (Boehringer, Mannheim, G.F.R.) (0.5 ml, containing *ca.* 40 mg of proteins) diluted to 1 ml with initial buffer was separated on DEAE-Toyopearl 650S and 650M with linear gradient elution from 0.05 *M* Tris–HCl buffer of pH 8.60 (200 ml) to 0.05 *M* Tris–HCl buffer of pH 8.60 containing 0.5 *M* sodium chloride (200 ml). Commercial bovine β-lactoglobulin (ICN Pharmaceuticals, Cleveland, OH, U.S.A.) (60 mg in 2 ml of initial buffer) was separated on DEAE-Toyopearl 650M with linear gradient elution from 0.05 *M* phosphate buffer of pH 5.80 (150 ml) to 0.05 *M* phosphate buffer of pH 5.80 containing 0.25 *M* sodium chloride (150 ml). Commercial bovine pancreas ribonuclease (Sigma, St. Louis, MO, U.S.A.) (30 mg in 1 ml of initial buffer) was separated on DEAE-Toyopearl 650S with linear gradient elution from 0.01 *M* diethanolamine–HCl buffer of pH 9.70 (200 ml) to 0.01 *M* diethanolamine–HCl buffer of pH 9.70 containing 0.15 *M* sodium chloride (200 ml). A mixture of four ribonucleotides (0.2 mg of each in 1 ml of initial buffer) was separated on DEAE-Toyopearl 650S and 650M with linear gradient elution from 0.05 *M* Tris–HCl buffer of pH 8.60 containing 0.05 *M* sodium chloride (200 ml) to 0.05 *M* Tris–HCl buffer of pH 8.60 containing 0.5 *M* sodium chloride (200 ml). A mixture of adenosine mono-, di and triphosphates (0.2 mg of each in 1 ml of initial buffer) was separated on DEAE-Toyopearl 650S and 650M with linear gradient elution from 0.05 *M* Tris–HCl buffer of pH 8.60 containing 0.05 *M* sodium chloride (150 ml) to 0.05 *M* Tris–HCl buffer of pH 8.60 containing 0.5 *M* sodium chloride (150 ml).

In the above separations, elution conditions typical of anion-exchange chromatography were employed and no attempt was made to achieve the best results.

RESULTS AND DISCUSSION

Basic properties

Fig. 3 shows the titration curve, indicating that DEAE-Toyopearl 650 contains only one type of ionic group with $pK_a \approx 11.5$ and shows almost no buffering activity at pH < 10. Therefore, little pH fluctuation of the eluent in the column, little change in adsorption strength of the ion exchanger and rapid re-equilibration of the column can be expected in ion-exchange chromatography with pH gradient elution on DEAE-Toyopearl 650. Furthermore, DEAE-Toyopearl 650 may be applicable to the separation of some basic proteins owing to the high pK_a value of the ionic group. The

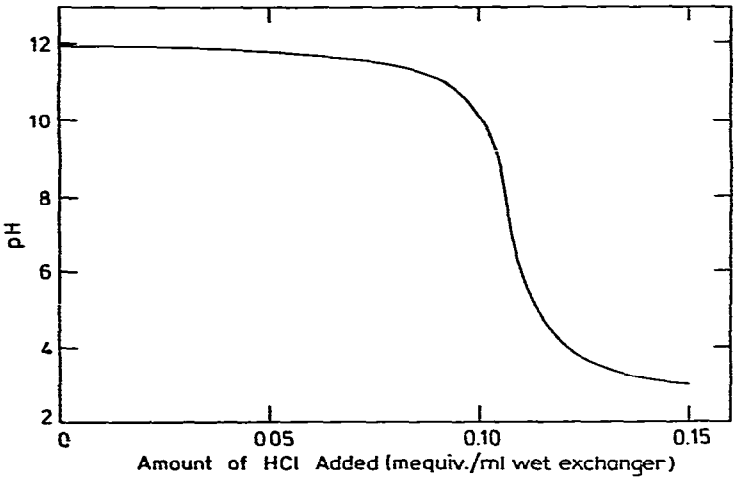

Fig. 3. Titration curve of DEAE-Toyopearl 650M.

ion-exchange capacity is *ca.* 0.1 mequiv./ml, which is comparable to those of many commercial DEAE-type ion exchangers.

The adsorption capacity for proteins is summarized in Table I. DEAE-Toyopearl 650 had a fairly high adsorption capacity even for ferritin and thyroglobulin, with molecular weights of 440,000 and 660,000, respectively. Therefore, DEAE-Toyopearl 650 should be applicable to proteins with very high molecular weights.

The recovery of proteins is summarized in Table II. The proteins were recovered almost quantitatively except for haemoglobin, for which a low recovery was observed. Haemoglobin seems to be easily adsorbed on many types of supports.

TABLE I

ADSORPTION CAPACITY OF DEAE-TOYOPEARL 650M FOR PROTEINS IN 0.05 *M* TRIS–HCl BUFFER OF pH 8.30 AT 25°C

Protein	*Adsorption capacity (mg/ml)*
Thyroglobulin	12
Ferritin	15
Bovine serum albumin	26

TABLE II

RECOVERY OF PROTEINS FROM DEAE-TOYOPEARL 650M IN 0.05 *M* TRIS–HCl BUFFER OF pH 8.30 AT 25°C

Protein	*Recovery (%)*	*Protein*	*Recovery (%)*
Thyroglobulin	95	Ovalbumin	95
Ferritin	101	β-Lactoglobulin	105
γ-Globulin	103	Trypsin inhibitor	100
Bovine serum albumin	103	Myoglobin	100
Haemoglobin	81		

TABLE III

PARTICLE SIZE DISTRIBUTION OF DEAE-TOYOPEARL 650S AND 650M

Particle size range (μm)	*Frequency (%)*	
	650S	*650M*
16–20	2	0
20–25	14	0
25–32	35	1
32–40	41	6
40–50	8	29
50–64	0	40
64–80	0	24

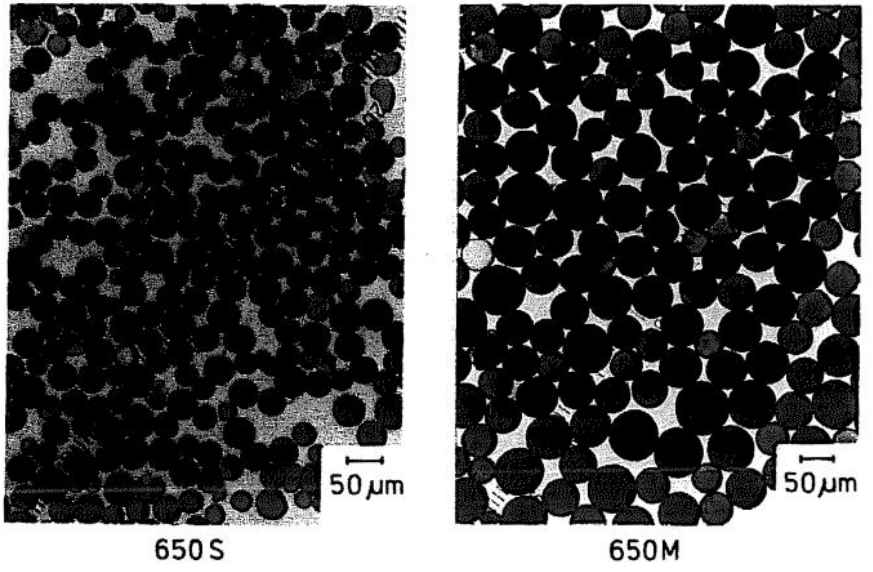

Fig. 4. Photomicrographs of DEAE-Toyopearl 650S and 650M.

Fig. 4 shows photomicrographs of DEAE-Toyopearl 650S and 650M. Table III shows the particle size distribution determined with the Coulter Counter. These results indicate that both grades have narrow particle size distributions. More than 90% by volume of superfine and medium-grade particles are within the ranges 20–40 and 40–80 μm, respectively.

Fig. 5 shows the microstructure of DEAE-Toyopearl 650, illustrating that DEAE-Toyopearl 650 is macroreticular and has pores of several thousand ånstroms into which even very-high-molecular-weight substances can easily penetrate.

Fig. 6 shows the molecular weight calibration graphs in the gel filtration of proteins and polyethylene glycols. Proteins and polyethylene glycols were separated according to their molecular size without ionic interactions because a buffer of low

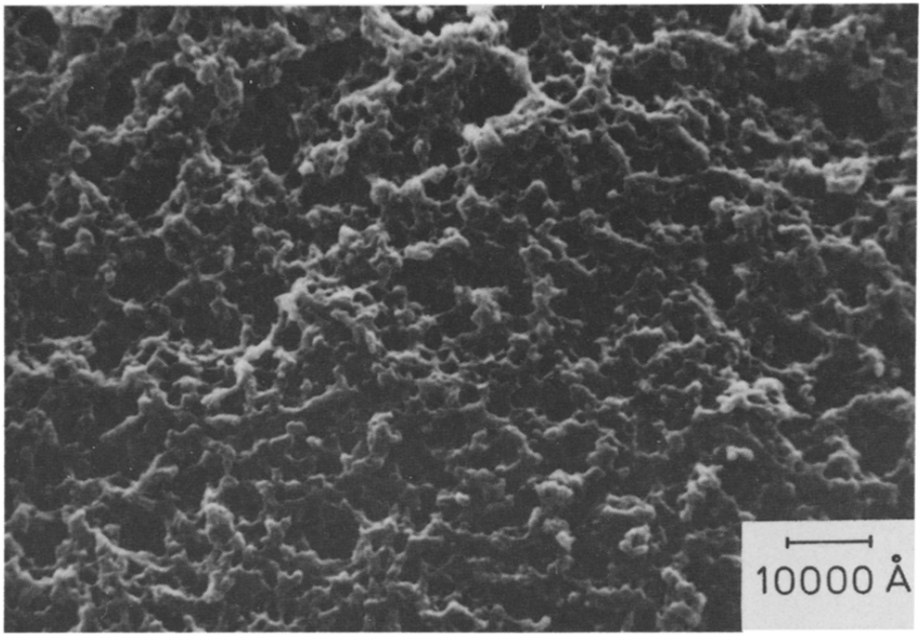

Fig. 5. Scanning electron photomicrograph of DEAE-Toyopearl 650M.

pH with a high salt concentration was employed as the eluent. As the total exclusion volume was *ca.* 13 ml, the exclusion limit for proteins is presumed to be tens of millions of daltons, again suggesting that DEAE-Toyopearl 650 is applicable to proteins with very high molecular weights. The exclusion limit for polyethylene glycol is *ca.* 1,000,000.

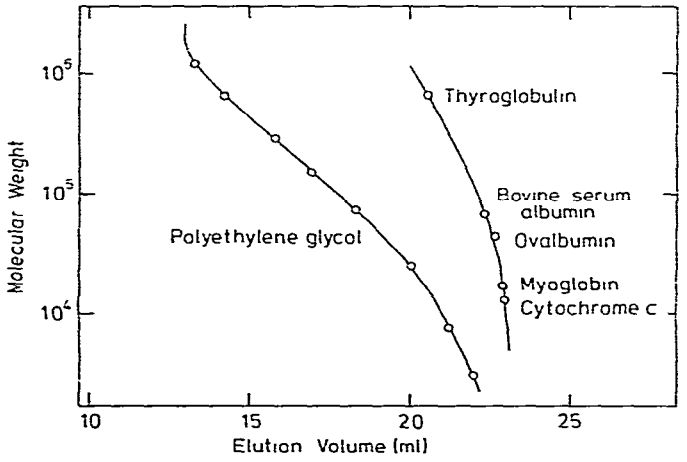

Fig. 6. Plots of molecular weights against elution volumes for proteins and polyethylene glycols on DEAE-Toyopearl 650M.

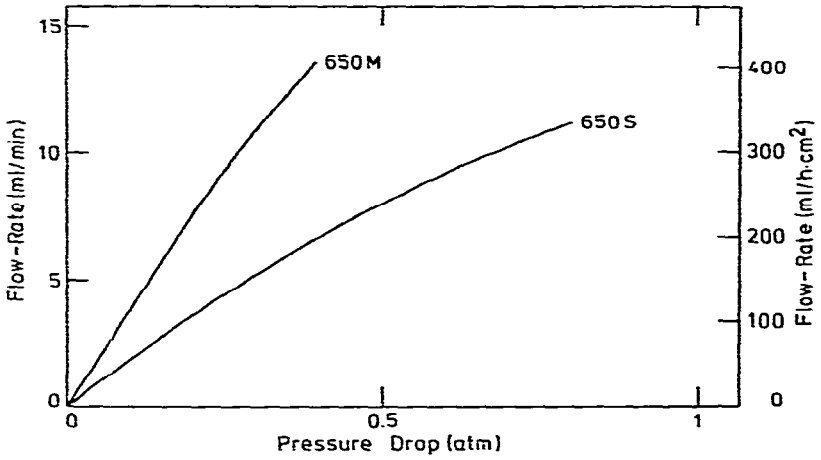

Fig. 7. Flow-rate as a function of pressure drop on DEAE-Toyopearl 650S and 650M columns (15 × 1.6 cm I.D.).

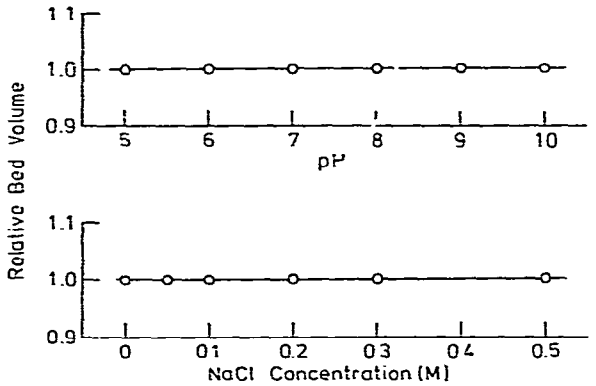

Fig. 8. Bed volume of DEAE-Toyopearl 650M in 0.05 *M* phosphate buffers of pH 5–10 and in 0.02 *M* phosphate buffers of pH 7.5 containing 0–0.5 *M* sodium chloride.

Fig. 7 shows the relationship between flow-rate and pressure drop. DEAE-Toyopearl 650 was found to be mechanically so stable that flow-rates higher than 300 ml/h · cm^2 were attainable on a 15 × 1.6 cm I.D. column. The upper limit of flow-rate or pressure drop was not established.

Fig. 8 shows the dependence of the swollen volume of DEAE-Toyopearl 650M on the pH and salt concentration of the buffer. No change in the swollen volume was observed in the pH range 5–10 and at sodium chloride concentrations in the range 0–0.5 *M*. Therefore, it should be possible to repeat separations and re-equilibrations on a column without re-packing.

Fig. 9 shows the change in the ion-exchange capacity of DEAE-Toyopearl 650M on treatment with 0.5 *M* sodium hydroxide solution and 0.5 *M* hydrochloric acid at 25°C. The ion-exchange capacity remained unchanged for the first 10 days and subsequently decreased gradually at a very slow rate of less than 0.001 mequiv./ml per

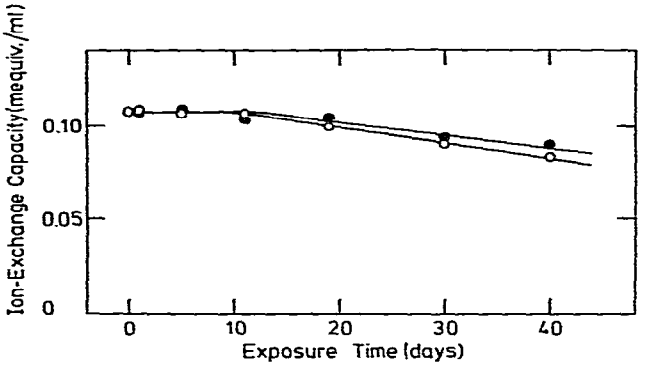

Fig. 9. Chemical stability of DEAE-Toyopearl 650M in alkaline and acidic aqueous solutions at 25°C. ○, 0.5 *M* sodium hydroxide solution; ●, 0.5 *M* hydrochloric acid.

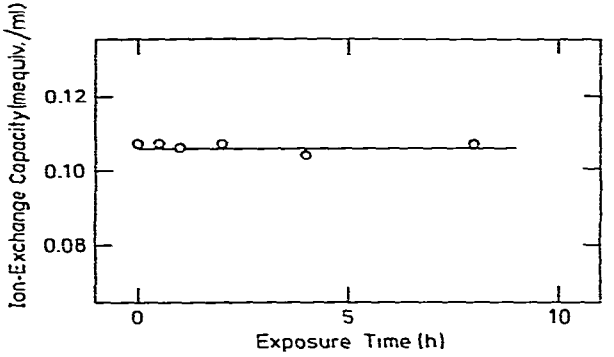

Fig. 10. Thermal stability of DEAE-Toyopearl 650M in 0.1 *M* phosphate buffer (pH 7.0) at 120°C.

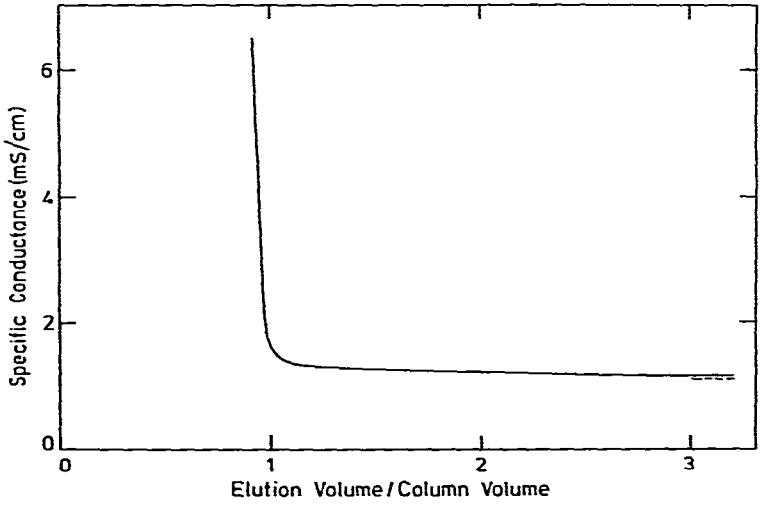

Fig. 11. Conductance of column effluent as a function of elution volume during re-equilibration of a DEAE-Toyopearl 650M column (15 × 1.6 cm I.D.) at a flow-rate of 1.5 ml/min. Initial buffer, 0.05 *M* Tris–HCl buffer (pH 8.60); final buffer, 0.05 *M* Tris–HCl buffer (pH 8.60) containing 0.5 *M* sodium chloride. Broken line indicates the conductance of the initial buffer.

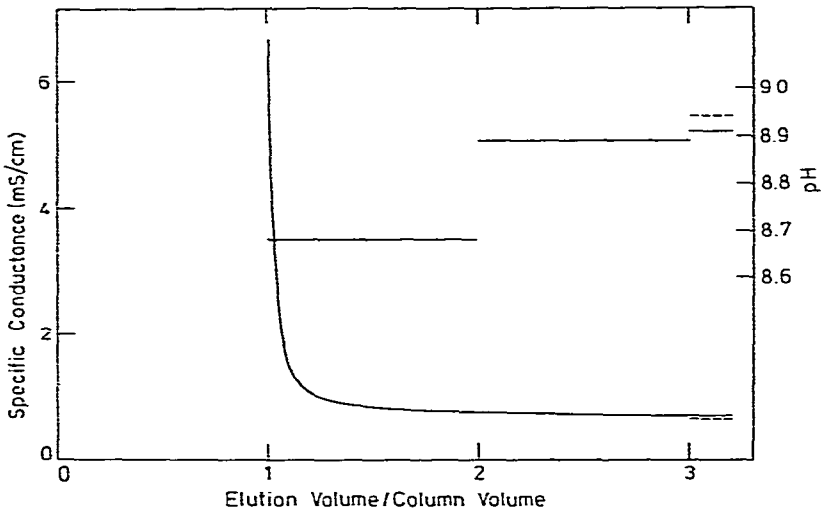

Fig. 12. Conductance and pH of column effluent as a function of elution volume during re-equilibration of a DEAE-Toyopearl 650M column (15 × 1.6 cm I.D.) at a flow-rate of 1.5 ml/min. Initial buffer, 0.05 *M* Tris–HCl buffer (pH 8.94); final buffer, 0.05 *M* Tris–HCl buffer (pH 7.10) containing 0.5 *M* sodium chloride. Broken lines indicate the conductance and pH of the initial buffer.

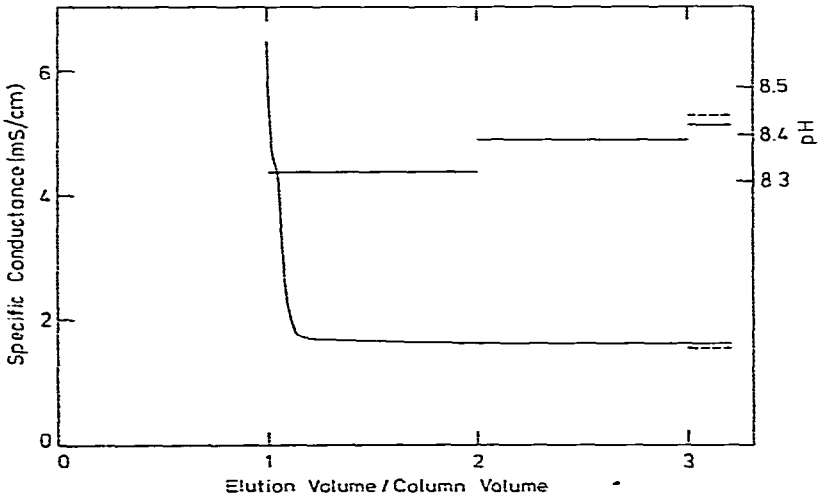

Fig. 13. Conductance and pH of column effluent as a function of elution volume during re-equilibration of a DEAE-Toyopearl 650M column (15 × 1.6 cm I.D.) at a flow-rate of 1.5 ml/min. Initial buffer, 0.05 *M* Tris–HCl buffer (pH 8.44); final buffer, 0.05 *M* phosphate buffer (pH 5.90) containing 0.5 *M* sodium chloride. Broken lines indicate the conductance and pH of the initial buffer.

day. Consequently, there should be no problem in washing DEAE-Toyopearl 650 with alkaline and acidic aqueous solutions for regeneration.

Fig. 10 shows the change in ion-exchange capacity of DEAE-Toyopearl 650M on treatment with 0.1 *M* phosphate buffer (pH 7.0) at 120°C. No change in ion-exchange capacity was observed during 8 h. Accordingly, DEAE-Toyopearl can be sterilized repeatedly by autoclaving at 120°C.

Figs. 11–13 show the profiles of pH and conductance of the column effluent during re-equilibration of DEAE-Toyopearl 650M column with initial buffers in ion-exchange chromatography with gradient elutions of three different types. The conductance of the column effluent was around 35–40 mS up to the elution volume of *ca.* 0.9 column volumes, then decreased very rapidly and became identical with those of initial buffers at about 0.1 mS (equivalent to *ca.* 1 m*M* sodium chloride concentration) at an elution volume of 3 column volumes. At this time, the difference in pH between the column effluent and the initial buffers was 0.02–0.03 in the latter two cases. Therefore, it can be said that 3 column volumes of initial buffer of 0.05 *M* concentration is almost enough to re-equilibrate DEAE-Toyopearl 650 columns. The elution of 3 column volumes of initial buffer was completed in 1 h in the above experiments.

Applications to chromatographic separations

Fig. 14 shows the results of the separation of commercial urease. The total recovery of enzymatic activity was approximately 85%. Of the applied urease activity, 66 and 74% were found in seven fractions containing most urease activity on DEAE-Toyopearl 650S and 650M, respectively. Aliquots of 2 ml of the seven fractions were pooled and the absorbance at 280 nm of the pooled fractions was measured to calculate degrees of purification. The degrees of purification based on specific activity were 30- and 28-fold for the pooled fractions obtained on DEAE-Toyopearl 650S and 650M, respectively. The pooled fractions were also subjected to analytical gel filtration. Fig. 15 shows chromatograms of the pooled fractions and original commercial urease obtained by analytical gel filtration. Both of the peaks appearing at elution times of *ca.* 19 and 23 min are assumed to be urease peaks because urease activity was found in two peaks eluted first in the previous separation of the same

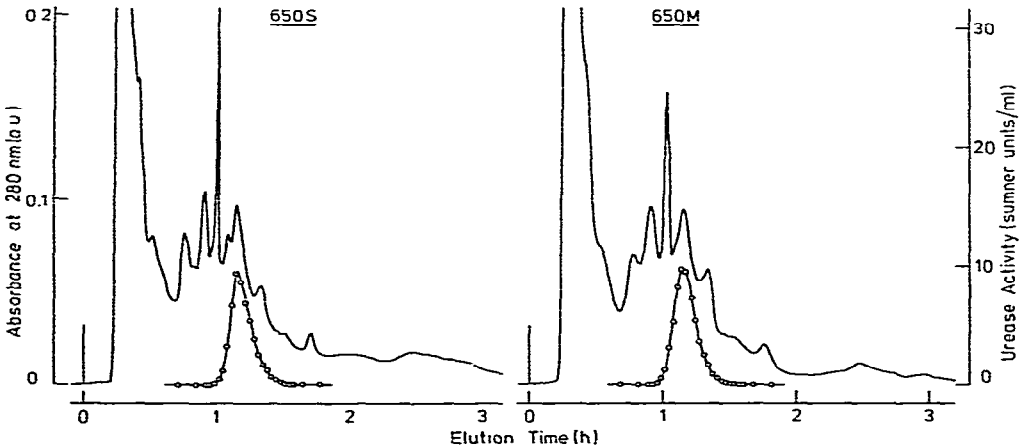

Fig. 14. Separation of commerical crude urease by ion-exchange chromatography on DEAE-Toyopearl 650S and 650M. Sample load, 30 mg in 1 ml of initial buffer; column size, 15 × 1.6 cm I.D.; elution, linear gradient from 0.05 *M* phosphate buffer of pH 6.70 (150 ml) to 0.05 *M* phosphate buffer of pH 6.70 containing 0.5 *M* sodium chloride (150 ml); flow-rate, 1.5 ml/min; temperature, 25°C; detection, UV absorbance at 280 nm (———) and enzymatic activity (O—O).

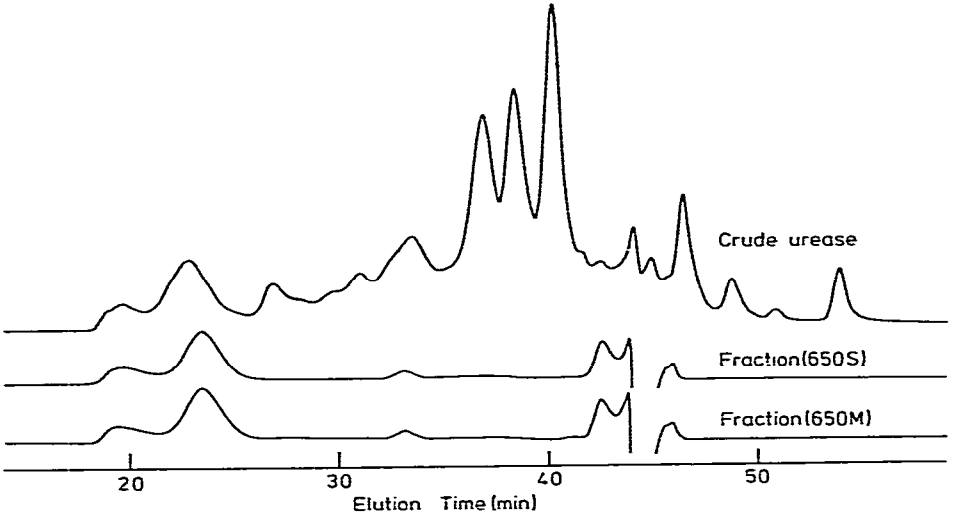

Fig. 15. Analytical gel filtration chromatograms of crude urease and urease fractions on TSK-GEL G3000SW two-column system.

commercial urease sample on preparative G3000SWG columns[5]. Therefore, Fig. 15 suggests that fairly pure urease was obtained. Only a small peak of impurity was found at an elution time of *ca.* 33 min in each chromatogram of the fractions.

Fig. 16 shows the results of the separation of egg white. The peak corresponding to albumin between the two vertical lines was fractionated and subjected to

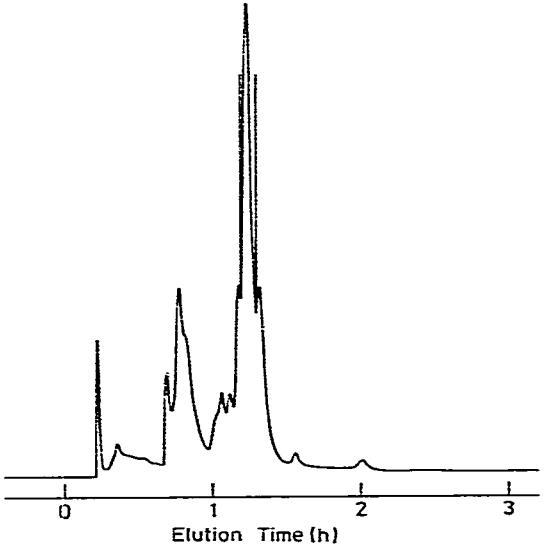

Fig. 16. Separation of egg white proteins by ion-exchange chromatography on DEAE-Toyopearl 650S. Sample load, 1 ml of 1:4 diluted solution; column size, 15 × 1.6 cm I.D.; elution, linear gradient from 0.05 *M* Tris–HCl buffer of pH 8.60 (200 ml) to 0.05 *M* Tris–HCl buffer of pH 8.60 containing 0.5 *M* sodium chloride (200 ml); flow-rate, 1.5 ml/min; temperature, 25°C; detection, UV absorbance at 280 nm.

analytical gel filtration and ion-exchange chromatography. The results are shown in Figs. 17 and 18, and indicate that the fraction contains only very small amounts of impurities in addition to albumin. The peaks at elution times of *ca.* 42–45 min in the gel filtration chromatogram of the fraction and the peaks just after the sample injection in the ion-exchange chromatogram of the fraction are solvent peaks.

Fig. 19 shows the results of the separation of standard human serum. The peaks corresponding to albumin between the two vertical lines were fractionated and subjected to analytical gel filtration and ion-exchange chromatography. The results are shown in Figs. 20 and 21. According to the gel filtration chromatograms, the

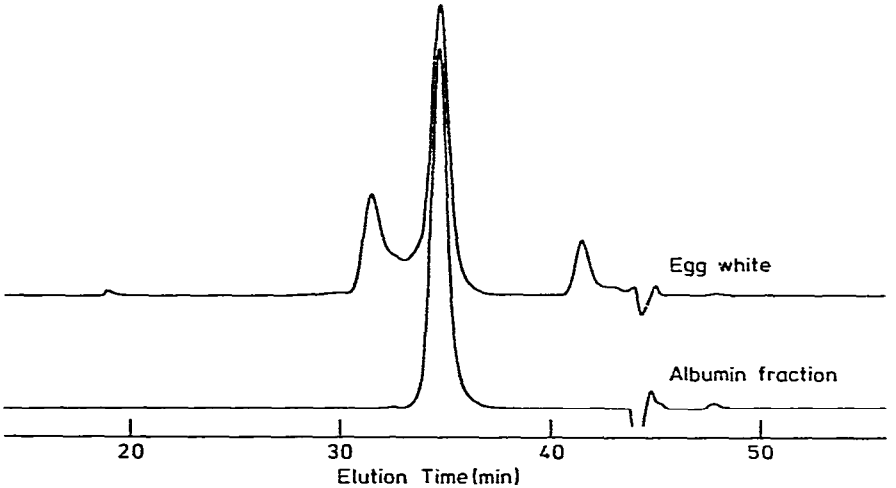

Fig. 17. Analytical gel filtration chromatograms of egg white and albumin fraction on TSK-GEL G3000SW two-column system.

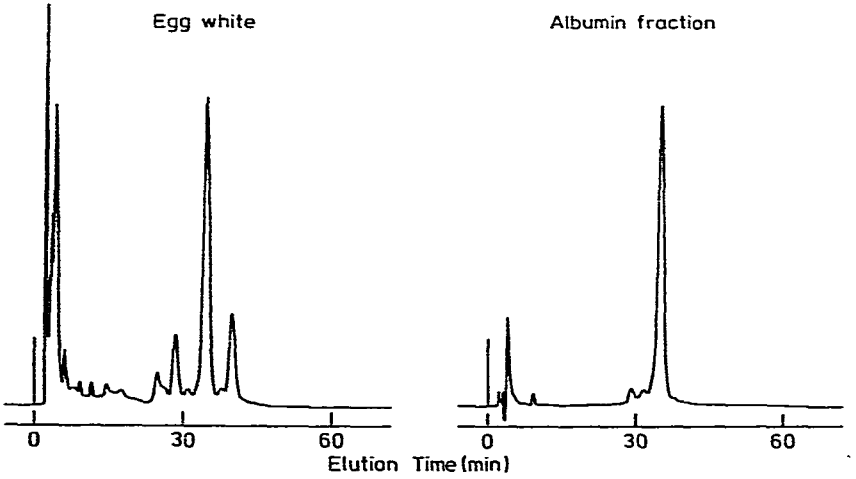

Fig. 18. Analytical ion-exchange chromatograms of egg white and albumin fraction on TSK-GEL IEX-545 DEAE SIL with 180-min linear gradient elution from 0.1 *M* Tris–HCl buffer of pH 7.50 to 0.1 *M* Tris–HCl buffer of pH 7.50 containing 0.2 *M* sodium chloride.

fractions are contaminated with small amounts of high-molecular-weight components, presumed to be α-globulin. However, the whole main peaks including shoulders in ion-exchange chromatograms are assumed to be albumin peaks because the contents of the shoulders are so high compared with that of the main peak that they cannot be ascribed to any other proteins.

Fig. 22 shows the results of the separation of commercial β-lactoglobulin. β-Lactoglobulins A and B were completely separated. The two fractions collected between the two vertical lines contain pure β-lactoglobulins A and B, respectively, as can be seen from the analytical ion-exchange chromatograms in Fig. 23.

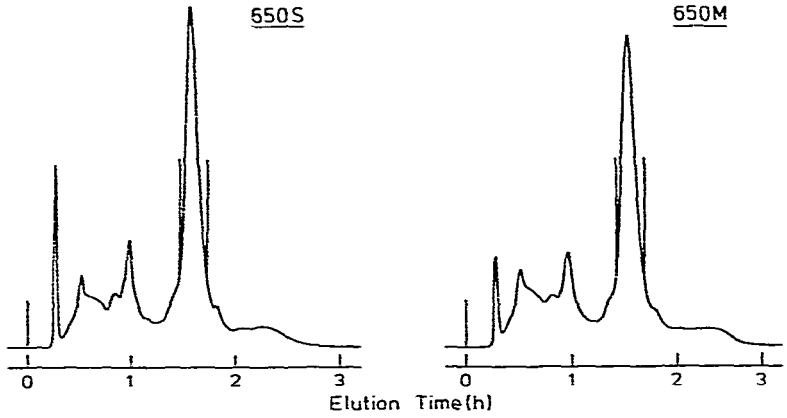

Fig. 19. Separation of human serum proteins by ion-exchange chromatography on DEAE-Toyopearl 650S and 650M. Sample load, 1 ml of 1:2 diluted solution; column size, 15 × 1.6 cm I.D.; elution, linear gradient from 0.05 *M* Tris–HCl buffer of pH 8.60 (200 ml) to 0.05 *M* Tris–HCl buffer of pH 8.60 containing 0.5 *M* sodium chloride (200 ml); flow-rate, 1.5 ml/min; temperature, 25°C; detection, UV absorbance at 280 nm.

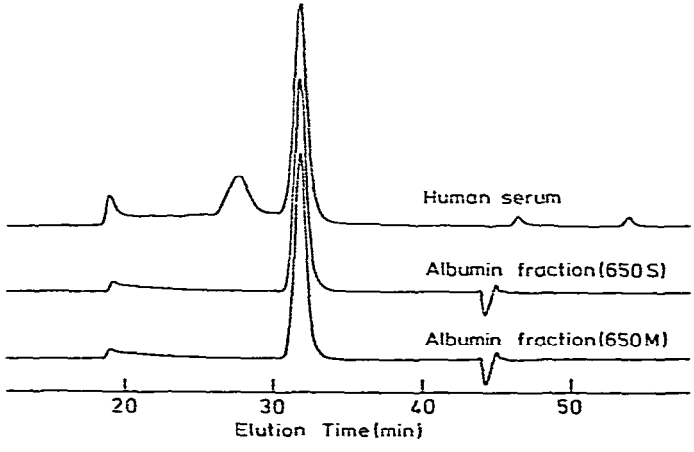

Fig. 20. Analytical gel filtration chromatograms of human serum and albumin fractions on TSK-GEl G3000SW two-column system.

Fig. 24 shows the results of the separation of commercial ribonuclease. The peak corresponding to ribonuclease between the two vertical lines was fractionated and subjected to analytical gel filtration and ion-exchange chromatography. The results are shown in Figs. 25 and 26. Although ribonuclease is a basic protein with an isoelectric point of *ca.* 9, it could be adsorbed and purified to great extent on DEAE-Toyopearl 650 by using buffers of high pH.

Fig. 27 shows the separation of ribonucleotides. Baseline separation of four ribonucleotides was achieved in 50 min on superfine-grade material. Fig. 28 shows the separation of adenosine phosphates. Three components were separated completely in 50 min on both grades of material and evidently the separation time can further be decreased on the superfine grade.

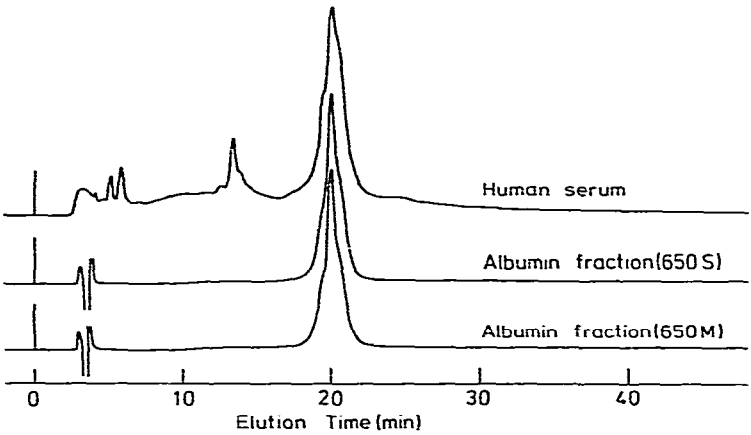

Fig. 21. Analytical ion-exchange chromatograms of human serum and albumin fractions on TSK-GEL IEX-545 DEAE SIL with 60 min linear gradient elution from 0.05 *M* Tris–HCl buffer of pH 8.00 to 0.05 *M* Tris–HCl buffer of pH 8.00 containing 0.5 *M* sodium chloride.

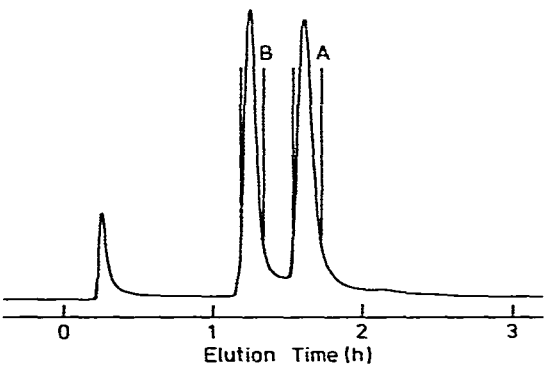

Fig. 22. Separation of commercial β-lactoglobulin by ion-exchange chromatography on DEAE-Toyopearl 650M. Sample load, 60 mg in 2 ml of initial buffer; column size, 15 × 1.6 cm I.D.; elution, linear gradient from 0.05 *M* phosphate buffer of pH 5.80 (150 ml) to 0.05 *M* phosphate buffer of pH 5.80 containing 0.25 *M* sodium chloride (150 ml); flow-rate, 1.5 ml/min; temperature, 25°C; detection, UV absorbance at 280 nm.

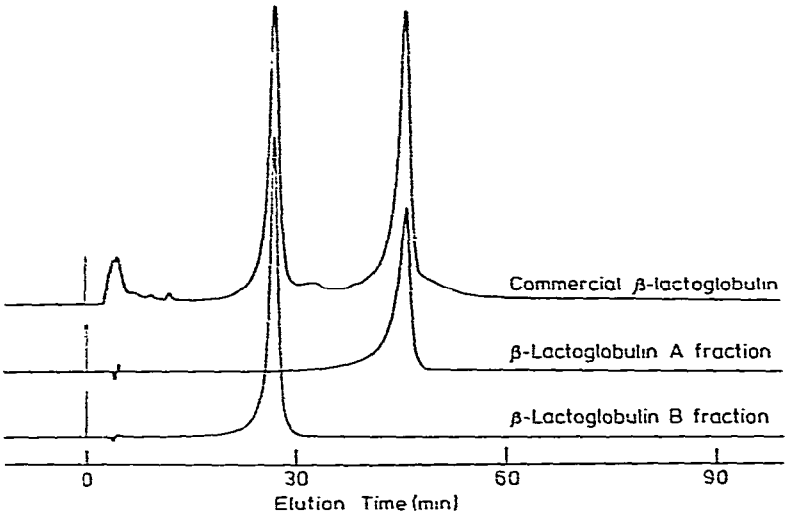

Fig. 23. Analytical ion-exchange chromatograms of commercial β-lactoglobulin and β-lactoglobulin A and B fractions on TSK-GEL IEX-545 DEAE SIL with 180-min linear gradient elution from 0.05 *M* phosphate buffer of pH 5.80 containing 0.05 *M* sodium chloride to 0.05 *M* phosphate buffer of pH 5.80 containing 0.25 *M* sodium chloride.

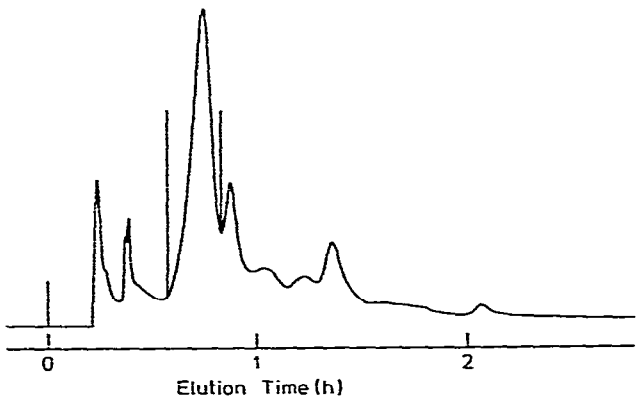

Fig. 24. Separation of commercial ribonuclease by ion-exchange chromatography on DEAE-Toyopearl 650S. Sample load, 30 mg in 1 ml of initial buffer; column size, 15 × 1.6 cm I.D.; elution, linear gradient from 0.01 *M* diethanolamine–HCl buffer of pH 9.70 (200 ml) to 0.01 *M* diethanolamine–HCl buffer of pH 9.70 containing 0.15 *M* sodium chloride (200 ml); flow-rate, 1.5 ml/min; temperature, 25°C; detection, UV absorbance at 280 nm.

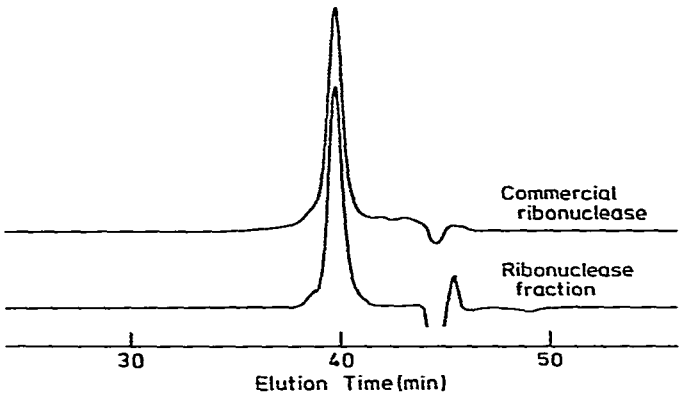

Fig. 25. Analytical gel filtration chromatograms of commercial ribonuclease and ribonuclease fraction on TSK-GEL G3000SW two-column system.

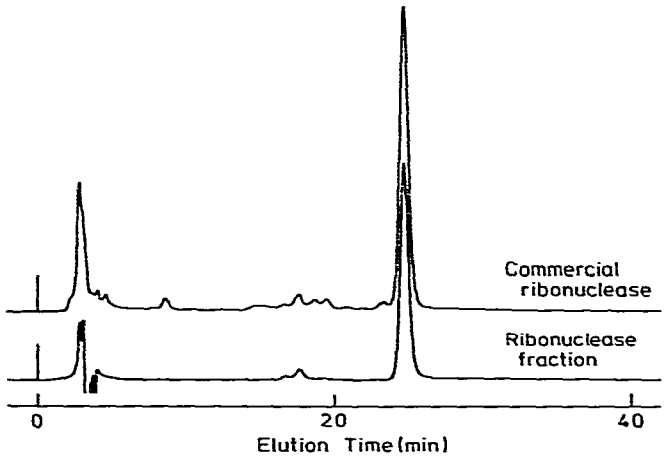

Fig. 26. Analytical ion-exchange chromatograms of commercial ribonuclease and ribonuclease fraction on TSK-GEL IEX-535 CM SIL with 120-min linear gradient elution from 0.05 *M* Tris–HCl buffer of pH 7.50 to 0.05 *M* Tris–HCl buffer of pH 7.50 containing 0.2 *M* sodium chloride.

CONCLUSION

DEAE-Toyopearl 650 provides high resolutions, especially in the separation of proteins at medium speed, and also is applicable to a wide range of samples including urease with a high molecular weight of *ca.* 400,000, ribonuclease of a basic protein and low-molecular-weight nucleic acid related compounds. In addition, DEAE-

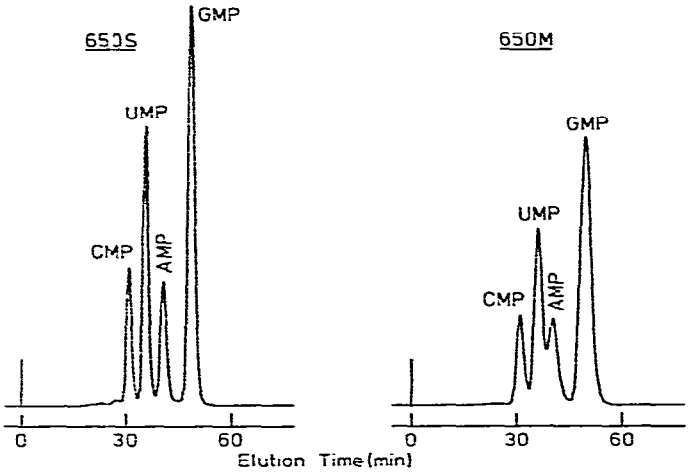

Fig. 27. Separation of ribonucleotides, adenylic acid (AMP), cytidylic acid (CMP), guanylic acid (GMP) and uridylic acid (UMP) by ion-exchange chromatography on DEAE-Toyopearl 650S and 650M. Sample load, 0.2 mg of each nucleotide in 1 ml of initial buffer; column size, 15 × 1.6 cm I.D.; elution, linear gradient from 0.05 *M* Tris–HCl buffer of pH 8.60 containing 0.05 *M* sodium chlroide (200 ml) to 0.05 *M* Tris–HCl buffer of pH 8.60 containing 0.5 *M* sodium chloride (200 ml); flow-rate, 1.5 ml/min; temperature, 25°C; detection, UV absorbance at 254 nm.

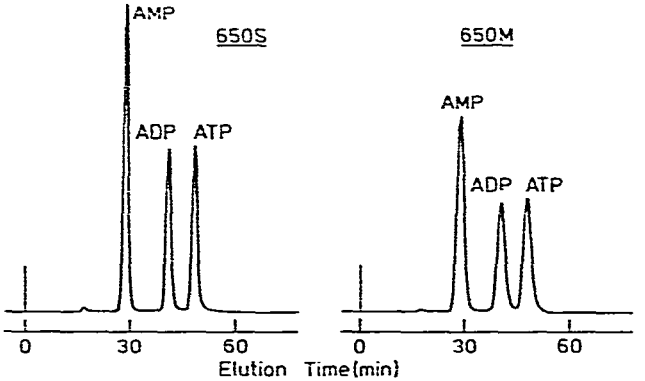

Fig. 28. Separation of adenosine 5'-mono-, di-, and triphosphate by ion-exchange chromatography on DEAE-Toyopearl 650S and 650M. Sample load, 0.2 mg of each in 1 ml of initial buffer; column size, 15 × 1.6 cm I.D.; elution, linear gradient from 0.05 *M* Tris–HCl buffer of pH 8.60 containing 0.05 *M* sodium chloride (150 ml) to 0.05 *M* Tris–HCl buffer of pH 8.60 containing 0.5 *M* sodium chloride (150 ml); flow-rate, 1.5 ml/min; temperature, 25°C; detection, UV absorbance at 254 nm.

Toyopearl 650 should be very suitable for scale-up to industrial-scale separations owing to its high mechanical stability, stable swollen volume independent of pH and salt concentration, high chemical stability and easy re-equilibration.

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