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EVALUATION OF CONVENTIONAL AND MEDIUM-PERFORMANCE ANION EXCHANGERS FOR THE SEPARATION OF PROTEINS

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SUMMARY

Conventional and medium-performance anion exchangers for the separation of proteins were compared with respect to titration curve, protein adsorption capacity, pore size, mechanical stability, swelling property and resolution in column chromatography. Each anion exchanger had different characteristics; under the conditions used, the properties of DEAE-Toyopearl 650 seemed to be advantageous over other anion exchangers.

INTRODUCTION

Ion-exchange chromatography has been extensively employed for the separation and purification of proteins since Peterson and Sober[1] developed cellulose ion-exchangers in 1956. Furthermore, high-performance ion-exchange chromatography and medium-performance ion-exchange chromatography are becoming popular mainly in analytical separation and in preparative separation, respectively. This paper reports the results of an evaluation of the conventional and medium-performance anion exchangers which have been employed in the preparative separation of proteins.

EXPERIMENTAL

Eleven commercial anion-exchangers (Table I) were evaluated with respect to various characteristics. Since DEAE-Toyopearl 650S and 650M differ only in particle size[2], DEAE-Toyopearl 650S was evaluated only in the studies of mechanical stability and chromatographic separation. Titration curves were measured in 0.5 *M* sodium chloride with 0.5 *M* hydrochloric acid by using an autotitrator. Protein adsorption capacities were determined for bovine serum albumin, ferritin and thyroglobulin in 0.05 *M* Tris–HCl buffer of pH 8.30 by the static method. Pore sizes were evaluated by measuring the relationship between molecular weight and elution volume, *i.e.* molecular weight calibration curves in gel filtration, for poly(ethylene glycol) in 0.1 *M* aqueous sodium chloride solution. Mechanical stability was evaluated by measuring the relationship between flow-rate and pressure drop on 15 × 1.6 cm I.D. columns in

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TABLE I

EVALUATED COMMERCIAL ANION EXCHANGERS

Anion exchanger	*Manufacturer*	*Anion exchanger*	*Manufacturer*
DEAE-Sephadex A-25	Pharmacia	DEAE-Cellulose	Serva Feinbiochemica
DEAE-Sephadex A-50	Pharmacia	DEAE-Trisacryl M	LKB
DEAE-Sepharose CL-6B	Pharmacia	DEAE-Bio-Gel A	Bio-Rad Labs.
DEAE-Sephacel	Pharmacia	DEAE-Toyopearl 650S	Toyo Soda
DEAE-Cellulose DE-52	Whatman	DEAE-Toyopearl 650M	Toyo Soda
DEAE-Cellulose DE-23	Whatman		

0.1 *M* aqueous sodium chloride solution. 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. Calf serum and bovine haemoglobin (Wako, Osaka, Japan) were measured on 15 × 1.6 cm I.D. columns with linear gradient elution of salt concentration to evaluate the resolution in column chromatography. All experiments were performed at 25°C. The experimental procedures have been described in detail elsewhere[2].

RESULTS AND DISCUSSION

Fig. 1 shows titration curves, indicating that many anion exchangers contain three types of ionic group with pK_a values of *ca.* 11, 9 and 6. However, DEAE-Trisacryl M contains two types of ionic group with pK_a values of *ca.* 11 and 6, and DEAE-Bio-Gel A and DEAE-Toyopearl 650 each contain only one type of ionic group with pK_a values of *ca.* 9.5 and 11.5, respectively. It may be advantageous that these three anion exchangers show almost no buffering activity in the pH range 7–9

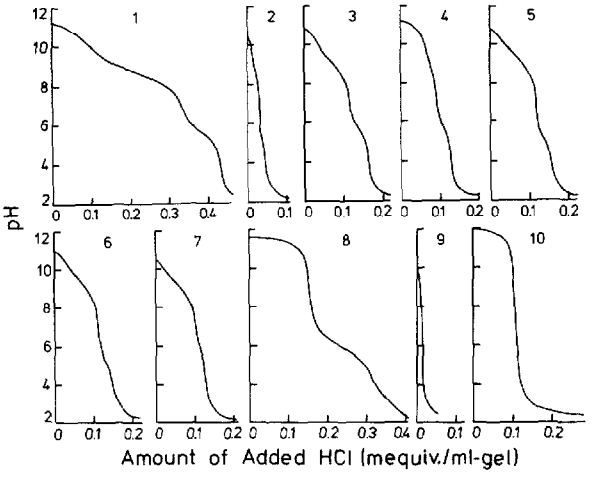

Fig. 1. Titration curves of commercial anion exchangers measured in 0.5 *M* sodium chloride with 0.5 *M* hydrochloric acid. Curves: 1 = DEAE-Sephadex A-25; 2 = DEAE-Sephadex A-50; 3 = DEAE-Sepharose CL-6B; 4 = DEAE-Sephacel; 5 = DEAE-Cellulose DE-52; 6 = DEAE-Cellulose DE-23; 7 = DEAE-Cellulose; 8 = DEAE-Trisacryl M; 9 = DEAE-Bio-Gel A; 10 = DEAE-Toyopearl 650M.

TABLE II

ION-EXCHANGE CAPACITY OF COMMERCIAL ANION EXCHANGERS

Anion-exchanger	*Ion-exchange capacity* (mequiv./ml)*	*Anion-exchanger*	*Ion-exchange capacity* (mequiv./ml)*
DEAE-Sephadex A-25	0.354	DEAE-Cellulose DE-23	0.108
DEAE-Sephadex A-50	0.030	DEAE-Cellulose (Serva)	0.097
DEAE-Sepharose CL-6B	0.114	DEAE-Trisacryl M	0.175
DEAE-Sephacel	0.093	DEAE-Bio-Gel A	0.013
DEAE-Cellulose DE-52	0.118	DEAE-Toyopearl 650M	0.108

* Evaluated at pH 7.0.

where anion-exchange chromatography is usually performed. Furthermore, DEAE-Trysacryl M and DEAE-Toyopearl 650 may be applicable to the separation of some basic proteins, owing to the high pK_a values of their ionic groups. Many anion-exchangers had ion-exchange capacities of *ca.* 0.1 mequiv. per millilitre of swollen exchangers (Table II).

Protein adsorption capacities are summarized in Table III. Values for some anion exchangers were as high as *ca.* 100 mg/ml for bovine serum albumin. However, even those anion exchangers had adsorption capacities of less than 10 mg/ml for ferritin and thyroglobulin of high molecular weight. DEAE-Bio-Gel A and DEAE-Toyopearl 650 had comparatively high adsorption capacities for ferritin and thyroglobulin, although they had fairly low adsorption capacities for bovine serum albumin compared with other anion exchangers.

Fig. 2 shows molecular weight calibration curves for poly(ethylene glycol). Although all anion exchangers employed here, except DEAE-Sephadex A-25, are for the separation of proteins, they differ considerably in pore size. This difference should greatly affect protein adsorption capacities. DEAE-Toyopearl 650 had the largest pore size.

TABLE III

PROTEIN ADSORPTION CAPACITY OF COMMERCIAL ANION-EXCHANGERS

Anion-exchanger	*Protein adsorption capacity (mg/ml)*		
	Bovine serum albumin	*Ferritin*	*Thyroglobulin*
DEAE-Sephadex A-25	22	–*	–*
DEAE-Sephadex A-50	78	2.1	2.9
DEAE-Sepharose CL-6B	97	4.3	3.5
DEAE-Sephacel	89	9.7	6.5
DEAE-Cellulose DE-52	116	9.9	6.6
DEAE-Cellulose DE-23	58	5.9	3.9
DEAE-Cellulose	18	–*	–*
DEAE-Trisacryl M	73	10.7	8.6
DEAE-Bio-Gel A	28	17.2	10.1
DEAE-Toyopearl 650M	26	15.0	11.5

* Not determined.

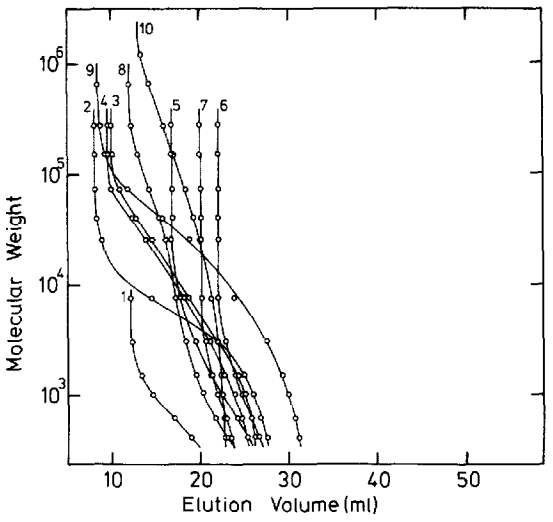

Fig. 2. Molecular weight calibration curves of commercial anion exchangers for poly(ethylene glycols) obtained on 15 × 1.6 cm I.D. columns in 0.1 *M* aqueous sodium chloride solution at flow-rates of 0.5–1 ml/min. Curves as in Fig. 1.

Fig. 3 shows the relationship between flow-rate and pressure drop. The mechanical stabilities of DEAE-Sephadex A-50, DEAE-Bio-Gel A, DEAE-Sephacel and DEAE-Sepharose CL-6B were low, and the upper limits of applicable flow-rate or pressure drop were observed for these four anion exchangers even on columns as small as 15 × 1.6 cm I.D.

Figs. 4 and 5 show the dependences of bed volume on pH and ionic strength of the buffer, respectively. The rise of pH 5 to 10 or the increase of sodium chloride concentration from 0 to 0.5 *M* resulted in a decrease of several percent in the bed

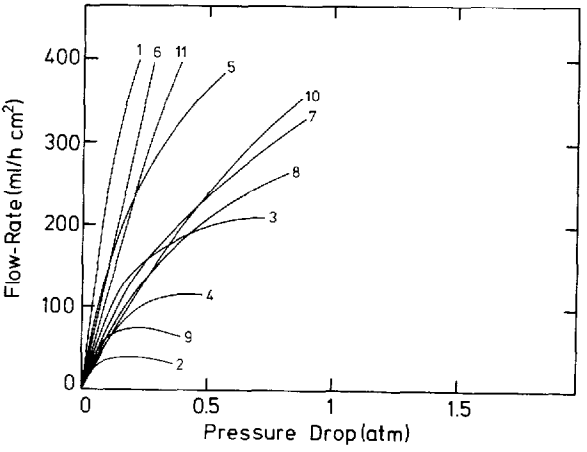

Fig. 3. Relationship between flow-rate and pressure drop for commercial anion exchangers obtained on 15 × 1.6 cm I.D. columns in 0.1 *M* aqueous sodium chloride solution. Curves: 1–9 as in Fig. 1; 10 = DEAE-Toyopearl 650S; 11 = DEAE-Toyopearl 650M.

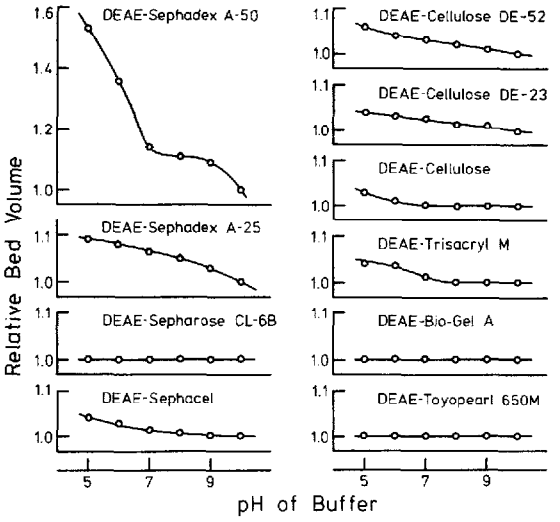

Fig. 4. Dependence of bed volume on buffer pH in 0.05 *M* phosphate buffers of pH 5–10 with bed sizes of 35–45 cm in height and 1.6 cm in diameter.

volume of many anion exchangers. However, no change in bed volume was observed for DEAE-Sepharose CL-6B, DEAE-Bio-Gel A and DEAE-Toyopearl 650 over the range of pH and sodium chloride concentration investigated. Accordingly, it should be possible to repeat separations and re-equilibrations without re-packing on columns of these three anion-exchangers.

Figs. 6 and 7 show chromatograms of calf serum and bovine haemoglobin, respectively. Since DEAE-Sephadex A-50 could not be operated at flow-rates greater than 1 ml/min, it was omitted here. Although similar patterns were obtained on all

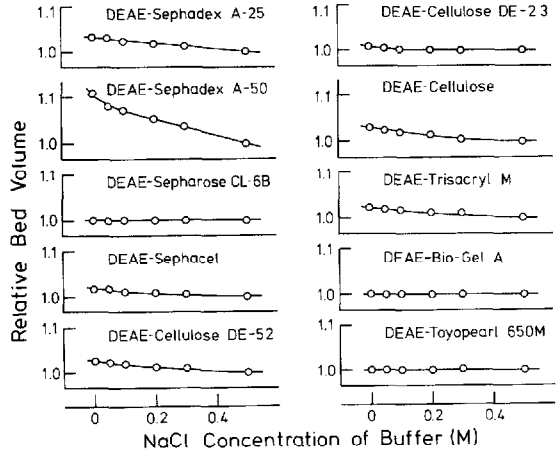

Fig. 5. Dependence of bed volume on ionic strength of the buffer in 0.02 *M* phosphate buffers of pH 7.50 containing 0–0.5 *M* sodium chloride with bed sizes of 35–45 cm in height and 1.6 cm in diameter.

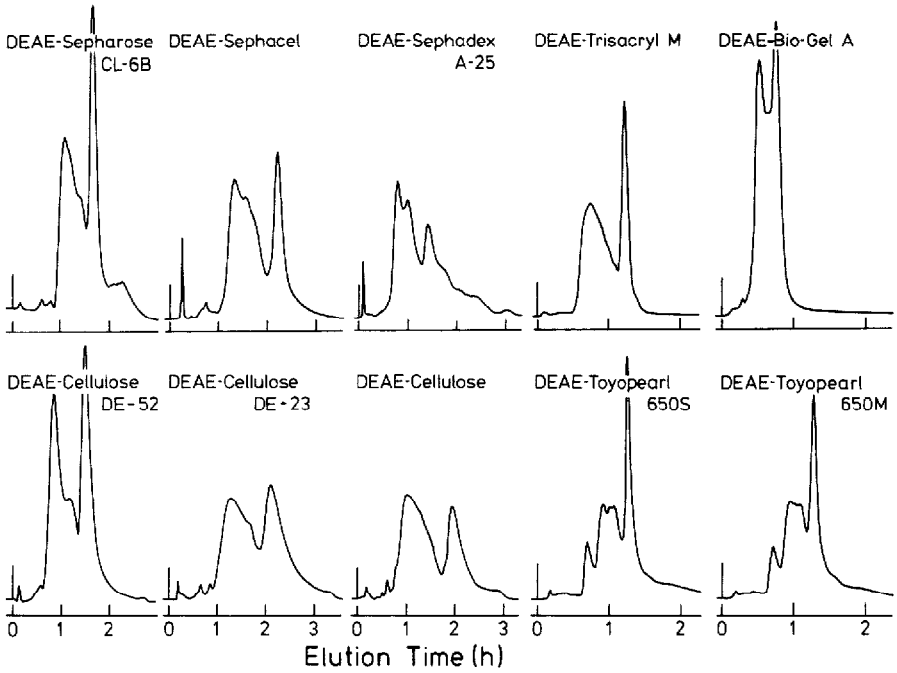

Fig. 6. Chromatograms of calf serum (0.5 ml of 50% diluted calf serum) obtained by ion-exchange chromatography on 15 × 1.6 cm I.D. columns with linear gradient elution from 0.05 *M* Tris–HCl buffer of pH 8.60 (200 ml) to 0.05 *M* Tris–HCl buffer of pH 8.60 containing 0.5 *M* sodium chloride (200 ml) at a flow-rate of 2 ml/min.

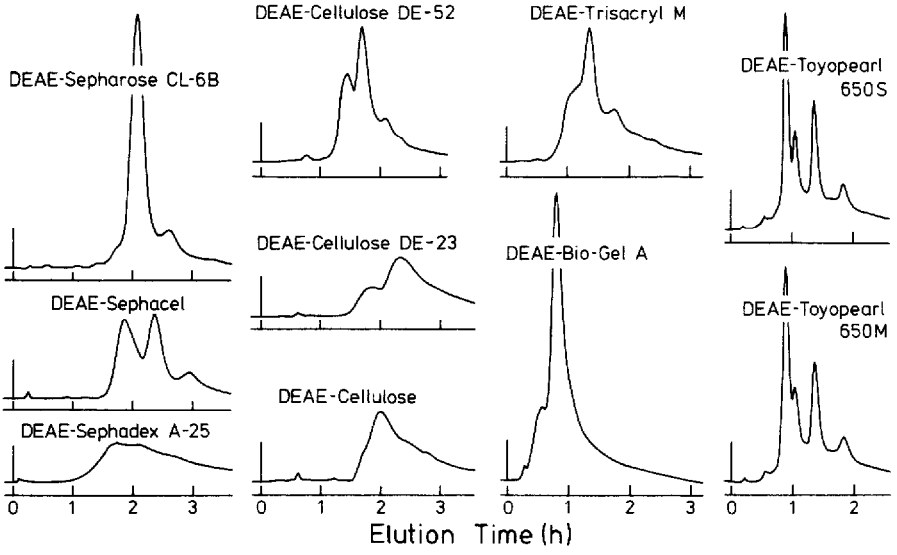

Fig. 7. Chromatograms of bovine haemoglobin (0.5 ml of 5% solution) obtained by ion-exchange chromatography on 15 × 1.6 cm I.D. columns with linear gradient elution from 0.05 *M* Tris–HCl buffer of pH 8.60 (250 ml) to 0.05 *M* Tris–HCl buffer of pH 8.60 containing 0.15 *M* sodium chloride (250 ml) at a flow-rate of 2 ml/min.

anion exchangers, both resolution and elution position considerably differed. Both samples were best separated under these conditions on DEAE-Toyopearl 650S.

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