Journal of Chromatography A, 658 (1994) 419–428
Elsevier Science B.V., Amsterdam

CHROM. 25 498

Process-scale evaluation of a fast-flowing anion-exchange cellulose

Peter R. Levison*, Stephen E. Badger, David W. Toome, Michael Streater and Jayne A. Cox

Whatman International Ltd., Springfield Mill, Maidstone, Kent ME14 2LE (UK)

ABSTRACT

The scale-up of the separation of hen egg-white proteins was investigated using Whatman DEAE-cellulose/D856, a fast-flowing microgranular anion-exchange cellulose. Under suitable conditions the maximum binding capacity for ovalbumin was determined to be 84 mg protein/ml packed DEAE-cellulose/D856 in a 25-ml column. The process was scaled-up 1000-fold and using a sub-maximum loading a working capacity of 56 mg protein/ml bed was obtained at a linear flow-rate of 150 cm/h. This reflects 100% recovery of applied ovalbumin with utilization of 66% of the theoretical maximum capacity of the medium. It is estimated that the productivity of this system would be up to 20 kg ovalbumin/$m^3 \cdot h$ based on these data.

INTRODUCTION

Ion-exchange chromatography is a widely used technique in the downstream processing of commercially important biopolymers. Low-pressure ion-exchange media are traditionally based on polysaccharide supports including cellulose, agarose and dextran [1,2]. Low-pressure ion-exchange chromatography is typically carried out on a large scale using either column or batch techniques depending on the nature and requirements of the process step [3]. In order for the process to be economically viable, it is important to optimize throughput at the required degree of product purity. Throughput is affected by capacity, flow-rate and resolution, all of which are interrelated.

Cellulose is a polysaccharide with a macroporous structure and in its non-regenerated form it offers a very high protein capacity [4,5] and fast adsorption–desorption kinetics [5,6], features which directly influence dynamic binding capacity and chromatographic resolution. While this satisfies two of the above three throughput criteria, cellulosics typically operate at low flow-rates in axial-flow columns [7,8], a factor which can adversely affect process time.

In order to address this aspect, we have shown that Whatman DE52 and QA52 microgranular anion-exchange celluloses can support flow-rates in radial-flow columns up to six times faster than in an axial-flow column of identical volume whilst maintaining good chromatographic resolution [7–9]. Further, the use of Whatman DE92, a fibrous anion-exchange cellulose, permits a 2–3-fold increased flow-rate in an axial-flow column compared with DE52, albeit at a reduced capacity [10].

In order to address the flow-rate limitations of microgranular ion-exchange celluloses without resorting to the use of either radial-flow columns or an alternative physical form of cellulose, we have developed a new range of fast-flowing microgranular ion-exchange celluloses. The products DEAE-cellulose/D856, QA-cellulose/D856, CM-cellulose/D856 and SE-cellulose/D856 were designed to operate at flow-rates

* Corresponding author.

SSDI 0021-9673(93)E0924-J

TABLE I

TYPICAL PROPERTIES OF DEAE-CELLULOSE/D856

Property	Value
Moisture content (%)	66.0
Regains (g/dry g):	
Free base form	1.88
Hydrochloride form	1.92
Small-ion capacity (mequiv./dry g)	0.98
Column packing density (dry g/ml)	0.21
Protein capacity[a] (mg/dry g)	290
Protein capacity[a] (mg/ml)	61
Flow-rate (cm/h)	
50 cmH_2O/cm[b]	425
75 cmH_2O/cm	614

[a] Bovine serum albumin, 0.01 *M* sodium phosphate buffer (pH 8.5).
[b] 1 $cmH_2O = 98.0665$ Pa.

>150 cm/h in process-scale axial-flow columns *ca.* 4–5 times faster than the traditional microgranular products DE52, CM52, etc. Typical properties of DEAE-cellulose/D856 are summarized in Table I.

In this study, we examined several scale-up parameters in the separation of hen egg-white proteins using DEAE-cellulose/D856. First, the chromatographic conditions were developed using analytical loadings of egg-white at the laboratory scale and shown to scale-up 1000-fold to a process column. Second, the maximum dynamic capacity of DEAE-cellulose/D856 was determined for the ovalbumin component of hen egg-white. Third, the process-scale separation of hen egg-white proteins was carried out using a 25-l column of DEAE-cellulose/D856 operating at 150 cm/h throughout. The effect of a clean-in-place procedure on the chromatographic performance of the bed after process loadings was examined. This latter stage complements our previous work on DE52 [11,12] and QA52 [13].

EXPERIMENTAL

Materials

Cell debris remover (CDR), DEAE-cellulose/D856 and a PREP-25 column were obtained from Whatman International (Maidstone, UK). Mono Q in a 5 cm × 0.5 cm I.D. column and Sephadex G-25 medium were obtained from Pharmacia (Milton Keynes, UK). Ovalbumin was obtained from Sigma (Poole, UK), bovine serum albumin (BSA) from ICN Biomedicals (High Wycombe, UK) and tris(hydroxymethyl)-aminomethane (Tris) from Merck (Poole, UK). All other chemicals were of analytical-reagent grade. Fresh size 2 hen eggs were obtained from Barradale Farms (Headcorn, UK).

Feedstock Preparation

Egg-whites were separated from 600 fresh hen eggs and diluted to 14% (v/v) with 0.025 *M* Tris–HCl buffer (pH 7.5). The egg-white suspension was clarified using a total of 22 kg of pre-equilibrated CDR in a batch mode. Spent CDR was removed by centrifugation through a 1.6 × 0.06 mm slotted screen (EHR 500 basket centrifuge; Robatel and Mulatier, Lyons, France) and the sample was clarified by filtration through a grade 541 filter-paper (Whatman International, Maidstone, UK). The clear solution (200 l) containing 9.28 mg/ml of total protein was used for chromatography on DEAE-cellulose/D856.

Batch kinetics

DEAE-cellulose/D856 was equilibrated with 0.01 *M* sodium phosphate buffer (pH 7.5). The adsorption of 1.075 mg/ml BSA (M_r 67 000) carried out in a batch stirred tank (50 ml) using DEAE-cellulose/D856 (105 mg dry mass equivalent) over a 60-min period, with continuous monitoring of the adsorbate solution at 280 nm.

Chromatographic development

Egg-white feedstock (7 ml) was applied to a DEAE-cellulose/D856 column (15.5 cm × 1.5 cm I.D.) pre-equilibrated with 0.025 *M* Tris–HCl buffer (pH 7.5) and non-bound material was removed by washing with 0.025 *M* Tris–HCl buffer (pH 7.5) (50 ml). Bound material was eluted with a linear gradient of 0–0.5 *M* NaCl in 0.025 *M* Tris–HCl buffer (pH 7.5) (200 ml). The chromatography was carried out at a flow-rate of 4 ml/min.

A parallel experiment was carried out using DEAE-cellulose/D856 packed in a PREP-25

column (16 cm × 45 cm I.D.) at a flow-rate of 4 l/min with a 1000-fold scale-up throughout.

Dynamic capacity determination

Egg-white feedstock was applied to a DEAE-cellulose/D856 column (15.5 cm × 1.5 cm I.D.) previously equilibrated with 0.025 *M* Tris–HCl buffer (pH 7.5) at a flow-rate of 4 ml/min over a period of 180 min, by which time the absorbance of the eluate at 280 nm was similar to that of the feedstock. Non-bound material was removed by washing with 0.025 *M* Tris–HCl buffer (pH 7.5) (100 ml). Bound material was eluted with 0.025 *M* Tris–HCl buffer (pH 7.5) containing 0.5 *M* NaCl (200 ml). The flow-rate was maintained at 4 ml/min throughout.

Process-scale chromatography

DEAE-cellulose/D856 (25 kg) was equilibrated with 0.025 *M* Tris–HCl buffer (pH 7.5) to give a final slurry concentration of 30% (w/v). The slurry of DEAE-cellulose/D856 was pump-packed into a PREP-25 column at a pressure of *ca.* 10 p.s.i. (1 p.s.i. = 6894.76 Pa). The ion-exchanger was used with the egg-white feedstock accordingly: (i) analytical loading (ii) preparative loading (iii) analytical loading (iv) clean-in-place (v) analytical loading. All procedures were carried out at room temperature (15–20°C).

(i) Analytical loading. Egg-white feedstock (7 l) was loaded on to the column and non-bound material removed by washing with 0.025 *M* Tris–HCl buffer (pH 7.5) (50 l). Bound material was eluted using a linear gradient of 0–0.5 *M* NaCl in 0.025 *M* Tris–HCl buffer (pH 7.5) (200 l). The DEAE-cellulose/D856 was re-equilibrated with 0.025 *M* Tris–HCl buffer (pH 7.5) (150 l). A flow-rate of 4 l/min was maintained throughout.

(ii) Preparative loading. Egg-white feedstock (200 l) was loaded on to the column and non-bound material removed by washing with 0.025 *M* Tris–HCl buffer (pH 7.5) (100 l). Bound material was eluted using a linear gradient of 0–0.5 *M* NaCl in 0.025 *M* Tris–HCl buffer (pH 7.5) (200 l). The DEAE-cellulose/D856 was re-equilibrated with 0.025 *M* Tris–HCl buffer (pH 7.5) (150 l). A flow-rate of 4 l/min was maintained throughout.

(iii) Analytical loading. Egg-white feedstock (7 l) was chromatographed on DEAE-cellulose/D856 as described in (i).

(iv) Clean-in-place procedure. The column of DEAE-cellulose/D856 was washed with 0.5 *M* NaOH (50 l), depressurized and allowed to stand at room temperature for 16 h. The column was repressurized and washed successively with water (50 l), 0.1 *M* Tris–HCl buffer (pH 7.5) (50 l) and 0.025 *M* Tris–HCl buffer (pH 7.5) (225 l). A flow-rate of 4 l/min was maintained throughout.

(v) Analytical loading. Egg-white feedstock (7 l) was chromatographed on DEAE-cellulose/D856 as described in (i).

Assays

Pooled fractions at various stages of chromatography were assayed for protein content by measuring the absorbance at 280 nm against standard solutions of ovalbumin. Throughout the column procedures the effluent was monitored for absorbance at 280 nm and by conductivity.

FPLC analysis

For the dynamic capacity study and the preparative loading run, fractions were collected at various stages of the procedure and analysed by fast protein liquid chromatography (FPLC) using Mono Q. For salt-eluted material, a sample was desalted by gel filtration through a column (12.5 cm × 1.0 cm I.D.) containing Sephadex G-25 medium previously equilibrated with 0.025 *M* Tris-HCl buffer (pH 7.5).

The fractions were chromatographed on a column of Mono Q using a Dionex Model 4500 ion chromatograph equipped with a Model VDM II UV–Vis detector and Model AI 450 data handling and processing system. FPLC was carried out using a linear gradient of 0–0.25 *M* NaCl in 0.025 *M* Tris–HCl buffer (pH 7.5) at a flow-rate of 3 ml/min and a 250-μl injection volume. The absorbance of the effluent was monitored at 280 nm.

RESULTS AND DISCUSSION

It is well established that Whatman micro-granular ion-exchange celluloses exhibit very fast kinetics of adsorption–desorption [5,6], features

which facilitate high throughput. For low-pressure polysaccharide-based adsorbents, the process of adsorbing the target molecule to the ionized functional group is generally limited by pore diffusion within the internal regions of the matrix. The rapid kinetic uptake rates observed for microgranular ion-exchange celluloses demonstrate that diffusion within the pore is relatively fast, thereby inferring that the adsorbate can diffuse to the binding site via a relatively untortuous path. If an ion exchanger has rapid binding kinetics, then it follows that the contact time required to effect protein adsorption will be low and accordingly a high-flow rate should be possible while still maintaining good capture efficiency. The limitation, as described previously, with traditional microgranular cellulosics is that they cannot sustain high flow-rates and DE52, for example, would typically be used at a flow-rate of *ca.* 35 cm/h on a process scale [3,5]. The aim of this product development was to produce a microgranular cellulose with the pore diffusion benefits offered by the structure of the matrix but with enhanced physical properties enabling it to sustain higher flow-rates, *i.e.*, make effective use of the rapid kinetics.

The uptake of BSA by DEAE-cellulose/D856 in a batch stirred tank is represented in Fig. 1 with data expressed in terms of mg BSA/ml

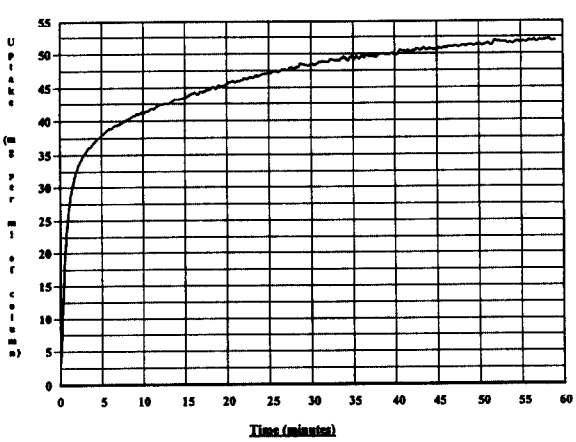

Fig. 1. Kinetic uptake of bovine serum albumin by DEAE-cellulose/D856 in 0.01 *M* sodium phosphate buffer (pH 7.5) in a batch stirred tank. Data are expressed as column volume equivalents based on a packing density of 0.21 dry g/ml column volume.

column volume for ease of interpretation (packing density 0.21 dry g/ml). The data demonstrate that DEAE-cellulose/D856 has rapid binding kinetics similar to those previously reported for DE52 and QA52 [5,6], with 50% uptake of BSA complete within 1 min and 90% complete within 23 min. In a packed column, of dimensions 16 cm × 45 cm I.D. assuming 75% (v/v) voidage, then a flow-rate of 1.0 l/min (37 cm/h) equates to a maximum residence time of *ca.* 22 min, a flow-rate of 2.7 l/min (100 cm/h) equates to a maximum residence time of *ca.* 8 min and a flow-rate of 4.0 l/min (150 cm/h) equates to a maximum residence time of *ca.* 5 min. The kinetic data therefore suggest that the matrix structure of DEAE-cellulose/D856 should lend itself to operation at high flow-rates with good capture efficiency. In our earlier studies on egg-white chromatography using DE52 [3,5] and DE92 [10], we used a feedstock containing *ca.* 10 mg/ml total protein as we considered this to be a typical and suitable working protein solution for preparative chromatography. Under preparative conditions we have previously seen displacement occurring for DE52 [14], DE92 [10] and CDR [13], whereby the more acidic components displace the less acidic material during sample loading.

In order to assess the chromatographic performance of the ion exchanger and optimize the mobile phase conditions, it is desirable to carry out preliminary investigations on an analytical scale where the total protein loaded is <5% of the total capacity of the medium. A typical separation of hen egg-white proteins on a laboratory column of DEAE-cellulose/D856 is represented in Fig. 2a. The non-bound fraction comprises the basic protein lysozyme (10–20 ml) and traces of the acidic protein conalbumin (40–55 ml). The adsorbed material contained conalbumin (80–90 ml), ovomucoid and ovoglobulin (90–110 ml), with the major component being ovalbumin (110–130 ml).

This chromatographic profile is similar to those seen for DE52 [14] and DE92 [10]. Having developed a suitable analytical test system on a laboratory scale, the separation was ready for scale-up. Scale-up of ion-exchange processes is generally achieved by simply increasing the col-

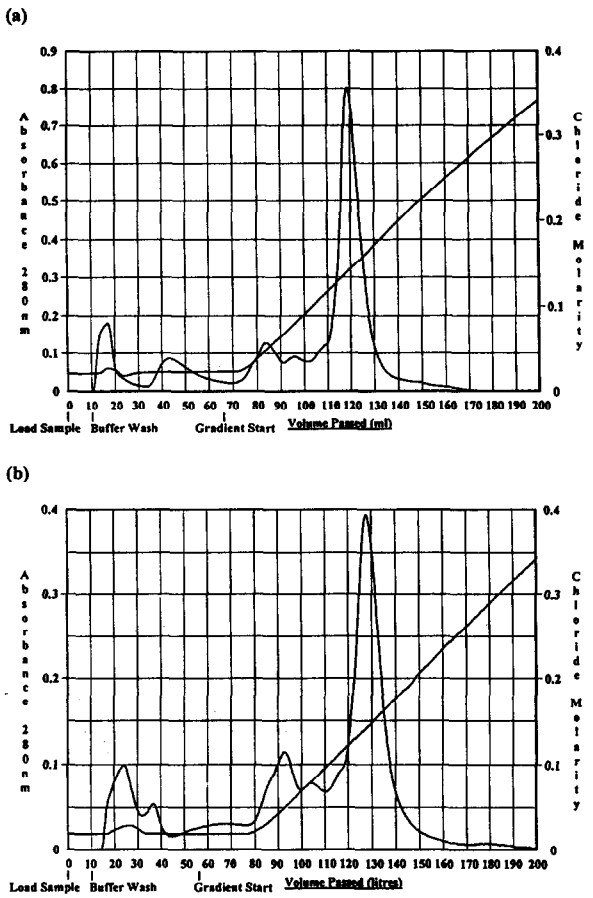

Fig. 2. Column chromatography of hen egg-white proteins on DEAE-cellulose/D856 using 0.025 *M* Tris–HCl buffer (pH 7.5) (a) on a laboratory scale (15.5 cm × 1.5 cm I.D.) and (b) on a process scale (16 cm × 45 cm I.D.).

umn diameter while maintaining the column bed height and linear flow-rate. This process was scaled-up 1000-fold by increasing the column diameter 30-fold at a flow-rate of 150 cm/h. The separation is represented in Fig. 2b and clearly the DEAE-cellulose/D856 has scaled-up in the expected manner.

Protein capacity is a key parameter influencing throughput and by its very nature is entirely application dependent, and will be influenced by several factors including pH, conductivity, p*I*, molecular mass, nature of the contaminants and temperature. In order to determine the dynamic protein binding capacity of an ion exchanger, a breakthrough curve would be obtained under conditions representative of those to be used for preparative chromatography. The breakthrough curve for DEAE-cellulose/D856 using the egg-white feedstock (9.28 mg/ml) is shown in Fig. 3. Following a loading of 750 ml of feedstock on to the column, the absorbance at 280 nm of the column effluent was similar to that of the feedstock, indicating that a saturation loading had been reached. FPLC analysis of the feedstock (Fig. 4a) and the fraction collected between 700 and 750 ml (Fig. 4e) confirm that both samples have identical composition. During the production of the breakthrough curve a typical frontal elution was observed (Fig. 3) and it can be seen from FPLC analysis that as loading starts (Fig. 4b; 50–100 ml) the least acidic conalbumin components (peaks b and c) are displaced by other acidic proteins. As loading proceeds (Fig. 4c; 300–350 ml), the ovoglobulin and ovomucoid components (peaks d and e) are also displaced. Finally, the most acidic protein ovalbumin begins to break through (Fig. 4d; 450–500 ml, peak f). Following a buffer wash and salt elution (Fig. 3), the eluted peak was a relatively pure fraction of ovalbumin (Fig. 4f). These data are similar to those reported for DE52 [14], DE92 [11] and CDR [12].

Following the column loading and buffer wash, a total of 2.310 g of protein was bound to the DEAE-cellulose/D856, which reflects a

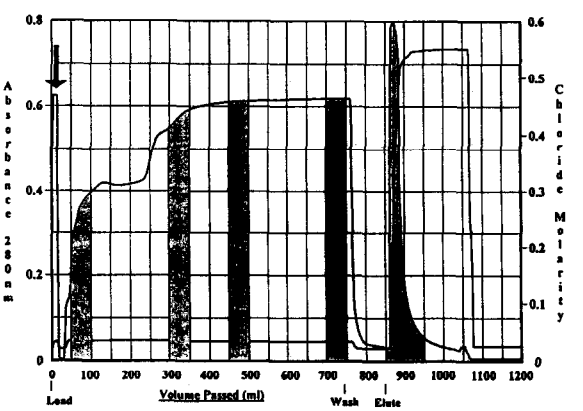

Fig. 3. Absorbance profile of column eluate during a saturation loading of DEAE-cellulose/D856 with 9.28 mg/ml hen egg-white proteins using 0.025 *M* Tris–HCl buffer (pH 7.5). The absorbance of the feedstock is identified by the arrow. Fractions were collected as denoted by the shaded areas.

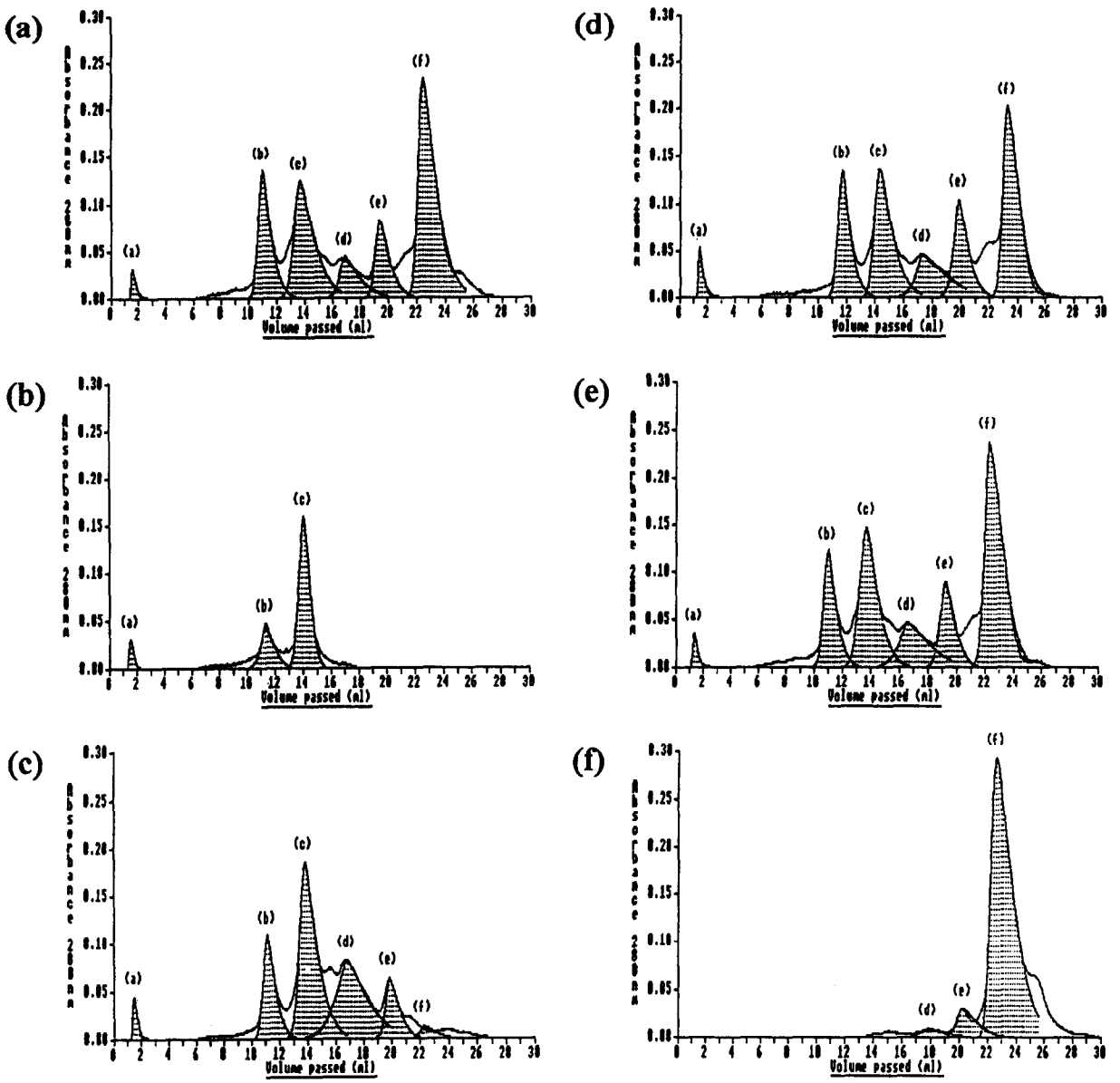

Fig. 4. FPLC analysis using Mono Q of individual fractions eluting during a saturation loading of DEAE-cellulose/D856 with hen egg-white proteins. (a) Egg-white feedstock; (b) non-bound fraction, 50–100 ml; (c) non-bound fraction, 300–350 ml; (d) non-bound fraction, 450–500 ml; (e) non-bound fraction, 700–750 ml; (f) salt-eluted material.

capacity of 84 mg protein/ml column volume when operated at a flow-rate of *ca.* 140 cm/h. In similar experiments using DE52 and DE92, capacities of 158 and 98 mg protein/ml were obtained for DE52 [14] and DE92 [10], respectively, but in these studies linear flow-rates of *ca.* 70 cm/h were used.

In the breakthrough study, a total of 9.690 g of protein was loaded on to the column, after which time breakthrough was complete and the medium was saturated with protein (Figs. 3 and 4). Hen egg-white is a multi-component mixture [15] with the ovalbumin component accounting for 63.8% (w/w) of total egg-white protein [16].

The ovalbumin bound to the DEAE-cellulose/D856 therefore accounts for only 52% (w/w) of the total ovalbumin present in the feedstock. Although the recovery of bound ovalbumin was good (*ca.* 95%), this type of overall process would only be efficient where the feedstock was freely available and *ca.* 50% yield losses were acceptable. In many cases it would not be commercially viable to run a column at its theoretical maximum capacity owing to the value of this non-bound target component. Consequently, the dynamic binding capacity gives an indication of the scale of the process but in a preparative separation the mass of feedstock loaded would be reduced such that loading stopped when the target begins to breakthrough.

This has been demonstrated in the process-scale chromatography of hen egg-white proteins on DEAE-cellulose/D856 using the PREP-25 column. Following column packing, an analytical loading of egg-white feedstock was carried out to check that the bed integrity was satisfactory and to provide final reassurance of chromatographic performance on a process scale (Fig. 5a). For the preparative run, a total of 1.856 kg of protein was loaded on to the column, which reflects a sub-maximum loading of *ca.* 73 mg protein/ml packed column volume. The protein mass balance data for this study are summarized in Table II. In order to assess the capture efficiency of the adsorptive process with respect to the ovalbumin component of the feedstock, a binding efficiency is calculated according to

$$\text{binding efficiency (\%)} = \frac{\text{mass of protein adsorbed}}{\text{mass of protein loaded}} \cdot 100 \qquad (1)$$

The data in Table II demonstrate that 1.413 kg of protein bound to DEAE-cellulose/D856 during the loading stage, which is equivalent to a protein capacity of 55.6 mg protein/ml column volume. The binding efficiency of *ca.* 76% (w/w) suggests that 100% adsorption of applied ovalbumin should have occurred based on an ovalbumin content of 63.8% (w/w) together with some acidic ovoglobulin and ovomucoid contaminants. This is supported by FPLC analysis of the non-bound and salt-eluted material collected

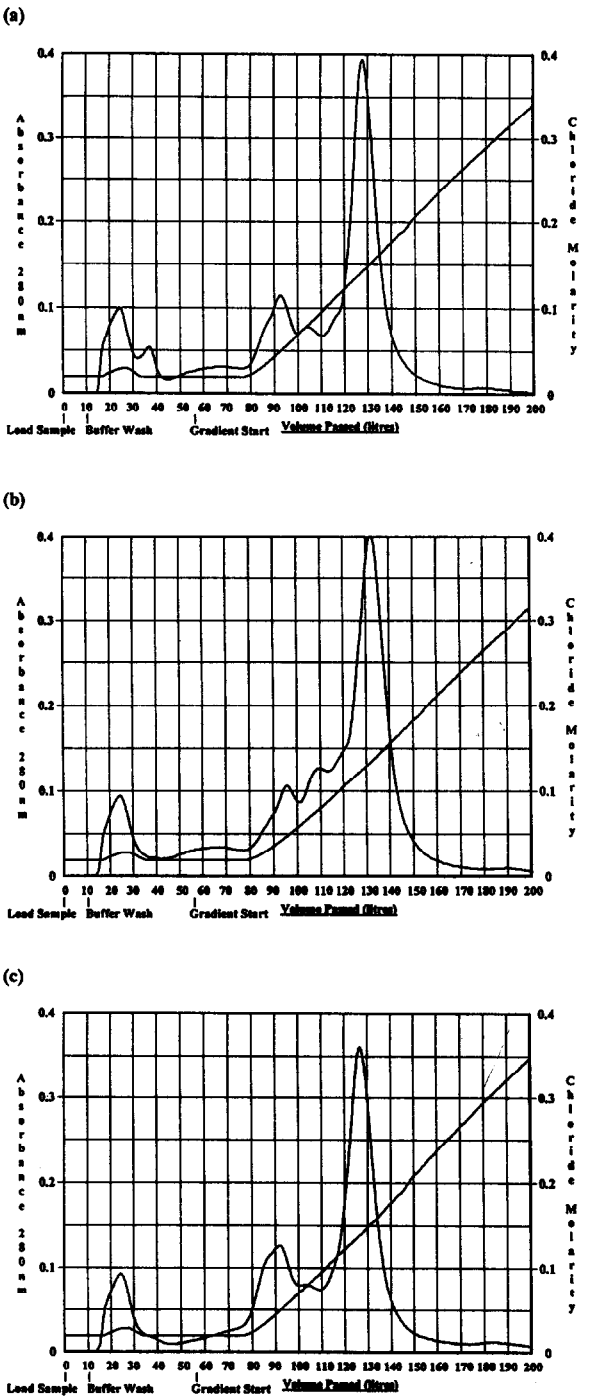

Fig. 5. Column chromatography of hen egg-white proteins on DEAE-cellulose/D856 using 0.025 *M* Tris–HCl buffer (pH 7.5) on a process scale (16 cm × 45 cm I.D.). (a) Analytical loading before preparative run; (b) analytical loading after preparative run; (c) analytical loading after CIP.

TABLE II

PROTEIN MASS BALANCE DURING PREPARATIVE CHROMATOGRAPHY OF EGG-WHITE PROTEINS

Stage of chromatography	Feedstock total protein (g)	Total protein (g)		Binding efficiency (%)
		In mobile phase	Adsorbed on DEAE-cellulose/D856	
Loading	1856	350	1506	
Wash		93	1413	76.1
Elution		1313	100	

during the preparative run (Fig. 6). The non-bound material (Fig. 6b) contained the lysozyme and conalbumin components of the egg-white feedstock (peaks a, b and c) while the salt-eluted fraction (Fig. 6c) contained the ovomucoid, ovoglobulin and ovalbumin components (peaks d, e and f).

In terms of overall throughput, the process was very efficient, giving a capacity of 55.6 mg protein/ml column volume at a flow-rate of 150 cm/h, and this reflects the utilization of *ca.* 66% of the maximum theoretical capacity of the DEAE-cellulose/D856. This degree of efficiency of binding is a reflection of the rapid binding kinetics which are a feature of DEAE-cellulose/D856 (Fig. 1), and clearly other media with inferior kinetics could not achieve such a high capture efficiency on a process scale with similar flow-rates.

The data in Table II demonstrate that the recovery of bound protein was *ca.* 93%, which, although acceptable, does indicate that some strongly acidic material was retained by the ion-exchanger under strong ionic strength desorption conditions. The problems of incomplete desorption, often referred to as "fouling", have been reviewed elsewhere [17] and we have studied the effect of fouling of hen egg-white proteins on DE52 [12] and goat serum proteins on QA52 [13]. In this study, we carried out an analytical loading on to the column of DEAE-cellulose/D856 immediately following the preparative run. The separation is shown in Fig. 5b and, in comparison with the starting conditions (Fig. 5a), it is evident that the resolution between the conalbumin component (*ca.* 90 ml) and the ovalbumin component (*ca* 125 ml) has been impaired. The peak of ovalbumin has broadened and is less well resolved from the conalbumin component. This suggests that an element of media fouling has occurred following preparative chromatography and the final elution condition of 0.5 *M* NaCl was insufficient to regenerate the bed fully. While a higher concentration of NaCl may effect improved regeneration, we have previously demonstrated that storage of DE52 [12] or QA52 [13] in 0.5 *M* NaOH for at least 12 h is an effective clean-in-place (CIP) regime. In the present study such an approach was taken and following re-equilibration of the DEAE-cellulose/D856 column, an analytical loading of egg-white feedstock was carried out.

This separation is represented in Fig. 5c and, by comparison with Fig 5a and b, it is apparent that the CIP protocol has restored chromatographic performance of the medium back to its initial state. These conditions for CIP have previously been demonstrated to be efficacious for the simultaneous sterilization and depyrogenation of heavily contaminated columns of DE52 [11,12], CDR [11,13] and QA52 [11,13], features which are of major importance in many biopharmaceutical processes.

Whatman microgranular ion-exchange celluloses have a high protein capacity and exhibit fast kinetics of adsorption [4–6] but are perceived as being incapable of sustaining the high flow-rates which in certain circumstances are desirable. To some extent this can be mitigated by using either batch techniques [3], radial-flow columns [7,9] or fibrous cellulosic media [10]. However any of these three options may be impractical owing to external factors, including process constraints, hardware constraints and

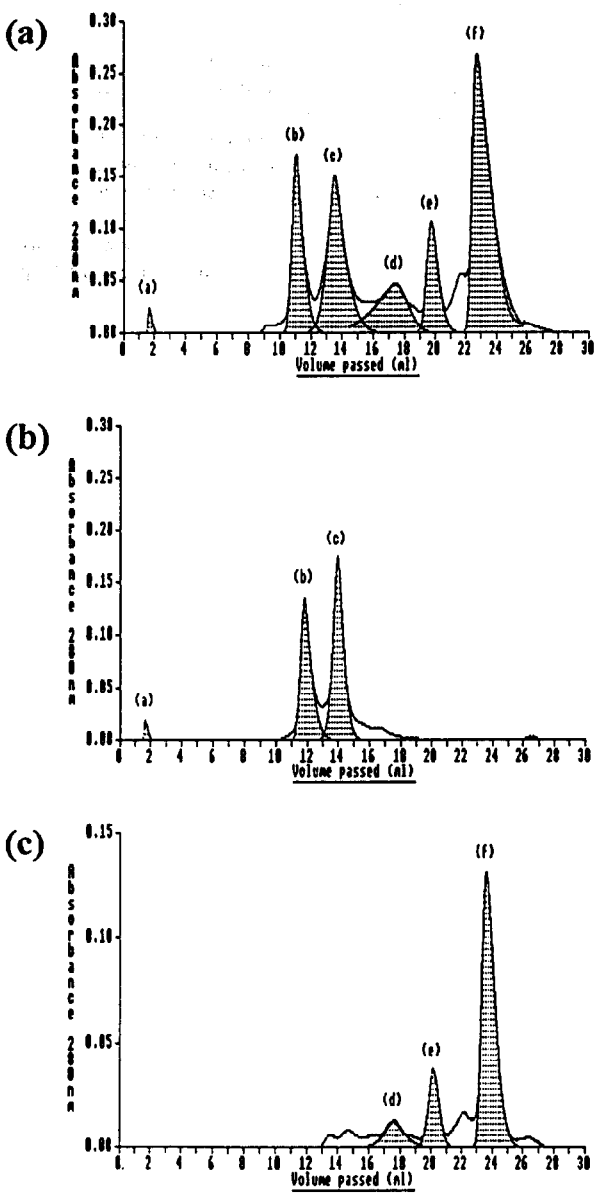

Fig. 6. FPLC analysis using Mono Q of individual fractions eluted during preparative chromatography of hen egg-white proteins on DEAE-cellulose/D856 in the PREP-25 column. (a) Egg-white feedstock; (b) non-bound material; (c) salt-eluted material.

regulatory issues. In the light of these constraints, we undertook the development of fast-flowing, high-capacity, microgranular ion-exchange celluloses with the key prerequisite of rapid kinetics to allow the effective use of high flow-rates in axial columns. In this study we have shown that DEAE-cellulose/D856, as an example of such a product, can sustain a linear flow-rate of 150 cm/h in a 25-l axial-flow column at a pressure of *ca.* 7 p.s.i. throughout a complete process cycle including adsorption, washing, desorption, CIP, etc., with no loss of performance. During this process a working capacity of *ca.* 56 mg/ml was obtained, reflecting *ca.* 66% of the total theoretical capacity of the DEAE-cellulose/D856 with 100% capture of target during a single pass through the column. Under the experimental conditions followed in this study it can be estimated that the productivity of this system would be *ca.* 19.4 kg ovalbumin/$m^3 \cdot h$ for a single cycle and this would reduce to *ca.* 3.3 kg/$m^3 \cdot h$ if a 12-h CIP were carried out after each run.

However, it should be emphasized that this separation was not optimized fully and if it were to be used as a commercial process productivity values would either increase or decrease, depending on the purity requirement of the ovalbumin component and the frequency of regeneration using either NaCl or NaOH treatments.

The data in this study demonstrate that DEAE-cellulose/D856 can be effectively used for process-scale ion-exchange chromatography in axial-flow columns at high flow-rates, which addresses the high productivity requirements of the biopharmaceutical processing industries.

REFERENCES

1 D. Friefelder, *Physical Biochemistry*, Freeman, San Francisco, 2nd ed., 1982, p. 249.
2 E.F. Rossomando, *Methods Enzymol.*, 182 (1990) 309.
3 P.R. Levison, in G. Ganetsos and P.E. Barker (Editors), *Preparative and Production Scale Chromatography*, Marcel Dekker, New York, 1993, p. 617.
4 P.R. Levison and F.M. Clark, *Pittsburgh Conference 1989, Atlanta, Abstracts*, p. 752.
5 P.R. Levison, S.E. Badger, D.W. Toome, D Carcary and E.T. Butts, in D.L. Pyle (Editor), *Separations for Biotechnology 2*, Elsevier Applied Science, London, 1990, p. 381.
6 P.R. Levison, D.W. Toome, S.E. Badger, D. Carcary and E.T. Butts, *Pittsburgh Conference 1990, New York, Abstracts*, p. 1079.
7 L. Lane, M.L. Koscielny, P.R. Levison, D.W. Toome and E.T. Butts, *Bioseparation*, 1 (1990) 141.

8 P.R. Levison, E.T. Butts, M.L. Koscielny and L. Lane, in Y. Briand, C. Doinel and A. Faure (Editors), *Protein Purification Technologies*, Vol. 4, GRBP, Clermont-Ferrand, 1990, p. 137.
9 P.R. Levison, S.E. Badger, D.W. Toome, E.T. Butts, M.L. Koscielny and L. Lane, in A. Huyghebaert and E. Vandamme (Editors), *Upstream and Downstream Processing in Biotechnology III*, Royal Flemish Society of Engineers, Antwerp, 1991, p. 3.21.
10 P.R. Levison, S.E. Badger, D.W. Toome, M.L. Koscielny, L. Lane and E.T. Butts, *J. Chromatogr.*, 590 (1992) 49.
11 P.R. Levison and F.M. Clark, *Pittsburgh Conference, 1989, Atlanta, Abstracts*, p. 754.
12 P.R. Levison, S.E. Badger, D.W. Toome, D. Carcary and E.T. Butts, in R. de Bruyne and A. Huyghebaert (Editors), *Downstream Processing in Biotechnology II*, Royal Flemish Society of Engineers, Antwerp, 1989, p. 2.11.
13 P.R. Levison, M.L. Koscielny and E.T. Butts, *Bioseparation*, 1 (1990) 59.
14 P.R. Levison, in G. Subramanian (Editor), *Preparative and Process-Scale Liquid Chromatography*, Ellis Horwood, Chichester, 1991, p. 146.
15 L. Stevens, *Comp. Biochem. Physiol.*, 100B (1991) 1.
16 W. Bolton, in C. Long, E.J. King and W.M. Sperry (Editors), *Biochemists Handbook*, Spon, London, 1971, p. 764.
17 G.K. Sofer and L.E. Nystrom, *Process Chromatography: a practical Guide*, Academic Press, San Diego, 1989, p. 93.