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Influence of column design on process-scale ion-exchange chromatography

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Abstract

The performance of two new designs of pump-packed axial flow process chromatography columns have been evaluated for the preparative anion-exchange chromatography of hen egg-white proteins using Whatman Express-Ion Exchanger Q. A 16 l Side-Pack column and a 24 l IsoPak column containing Express-Ion Q were used in this study. In each case ca. 20 l feedstock containing 5–7 g protein/l, was applied per litre packed bed at flow-rates of ca. 150 and 300 cm/h. In each case the ovalbumin binding capacity was ca. 70 g/l packed bed with ca. 100% (w/w) recovery of applied protein. A clean-in-place procedure involving storage in 0.5 *M* NaOH was effective in maintaining chromatographic performance in all cases. These data were consistent with our previous work using the more traditionally configured slurry-packed axial flow columns. Each of these column designs were easy to use facilitating rapid packing with this adsorbent and in the case of IsoPak rapid pump unpacking. The introduction of these column designs significantly improves the task of column packing, hitherto a labour intensive, physically demanding and potentially unreproducible process.

Keywords: Preparative chromatography; Column design; Proteins

1. Introduction

The downstream processing of commercially important biopolymers has been gaining significance over the recent past. Traditionally proteins were isolated from various sources including animal tissue, microbial culture and plants. More recently with emerging techniques in biotechnology the isolation of recombinant proteins, peptides, carbohydrates and nucleic acids have gained importance. Ion-exchange chromatography is routinely carried out in these applications and for low-pressure separations widely used ion-exchange media are based on polysaccharide supports including cellulose, agarose and dextran [1,2].

Protein separations can be carried out in either a positive or negative ion-exchange step, where either the target or contaminants are retained, respectively [3] and in each case the separation could be carried out using a batch or column-based contacting system. We have compared process-scale ion-exchange separations using each of these techniques and reported them elsewhere [4,5]. Packed bed column chromatography is the ideal technique where a contained system approach is preferred, for example where the process requires automation or where regulatory and validatory concerns are critical [6]. Several pilot and production scale columns are

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available including two geometric configurations namely axial flow and radial flow [7]. We have evaluated each of these column configurations at different scales and compared their performance elsewhere [8,9]. Axial flow designs include systems for slurry and pump-packing and manual or pump-unpacking with either fixed or adjustable volume. For the avoidance of doubt, a slurry-packed column requires the introduction of a slurry of adsorbent in packing buffer (typically 30%, w/v) into the column barrel section fitted with an extension tube/packing reservoir. The bed is pressure consolidated, headspace buffer removed and a flow adaptor moved into contact with the packed bed. A pump-packed column enables the adsorbent slurry to be pumped directly into the column assembly through a packing port. This eliminates the need for an extension tube/packing reservoir and the subsequent operations associated with headspace buffer removal.

In previous studies we have reported ion-exchange separations using a 25 l pump-pack fixed volume column [10] and a 25 l slurry pack adjustable volume column [11]. In each of these studies the columns required manual unpacking, a labour intensive process typically leading to adsorbent loss. More recently however, adjustable volume columns have been designed for pump-packing a slurry of media to facilitate rapid bed consolidation and are also configured to permit rapid pump unpacking using in situ slurry preparation within the column barrel itself [12,13]. Another recent design is the side-packed chromatographic column (SPC) which include fixed volume axial flow columns into which the slurry is pump-packed through a side port [14].

We have previously reported the process-scale purification of hen egg-white proteins using Whatman Express-Ion Exchanger Q in a slurry-packed adjustable volume column (16 cm×45 cm I.D.) operated at flow-rates of up to 225 cm/h [11]. Hen egg-white is a complex protein mixture [15] and is an ideal feedstock for anion-exchange chromatography. In the present study we compare the chromatographic performance of Express-Ion Q for the process-scale separation of hen egg-white proteins using two new designs of pump-packed axial flow chromatography columns.

2. Experimental

2.1. Materials

Cell debris remover (CDR) and Express-Ion Q were obtained from Whatman (Maidstone, UK). A Side-Pack column (16 cm×35 cm I.D.) was obtained from ProMetic BioSciences (Burtonsville, MD, USA) and an IsoPak column (50 cm×44 cm I.D.) with associated recirculating slurry preparation station was obtained from Millipore (Stonehouse, UK). Tris(hydroxymethyl)aminomethane (Tris) was obtained from Merck (Poole, UK). All other chemicals were of analytical reagent grade. Fresh large hen eggs were obtained from Barradale Farms (Headcorn, UK).

2.2. Feedstock preparation

Egg-whites (55 l) were separated from 1440 fresh hen eggs and diluted to 10% (v/v) with 0.025 *M* Tris–HCl buffer (pH 7.5). The egg-white suspension was clarified using a total of 35 kg of pre-equilibrated CDR in a batch mode. Spent CDR was removed by centrifugation through a 1.6×0.6 mm slotted screen (EHR 500 basket centrifuge, Robatel and Mulatier, Lyons, France) and the sample clarified through a Grade 541 filter-paper (Whatman). The clear solution (550 l) containing 5–7 mg/ml of total protein was used for chromatography on Express-Ion Q.

2.3. Process-scale chromatography

2.3.1. Side-Pack column

Express-Ion Q (15 kg) was equilibrated with 0.025 *M* Tris–HCl buffer (pH 7.5) to give a final slurry concentration of ca. 30% (w/v). The slurry was transferred to the SPC (16 cm×35 cm I.D.) through the packing port and the bed consolidated at a pressure of ca. 12 p.s.i. (1 p.s.i.=6894.76 Pa) according to the column manufacturer's guidelines. The packed column of Express-Ion Q (16 cm×35 cm I.D.) had a volume of ca. 15.4 l and a packing density of 0.303 kg/l. All procedures were carried out at room temperature (15–20°C). The pressure/flow performance of the packed bed was measured

using 0.025 *M* Tris–HCl buffer (pH 7.5), prior to egg-white chromatography. The ion exchanger was used with the egg-white feedstock accordingly (i) analytical loading, (ii) preparative loading, (iii) analytical loading, (iv) clean-in-place (CIP), (v) analytical loading.

(i) Analytical loading. Egg-white feedstock (6 l) was loaded on to the column and non-bound material removed by washing with 0.025 *M* Tris–HCl buffer (pH 7.5) (30 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) (100 l). The Express-Ion Q was equilibrated with 0.025 *M* Tris–HCl buffer (pH 7.5) (50 l). Flow-rate was maintained at 150 cm/h throughout.

(ii) Preparative loading. Egg-white feedstock (300 l) was loaded on to the column and non-bound material removed by washing with 0.025 *M* Tris–HCl buffer (pH 7.5) (90 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) (220 l). The Express-Ion Q was re-equilibrated with 0.025 *M* Tris–HCl buffer (pH 7.5) (100 l). Flow-rate was maintained at 150 cm/h throughout.

(iii) Analytical loading. Egg-white feedstock (6 l) was chromatographed on Express-Ion Q as described in (i).

(iv) Clean-in-place procedure. The column of Express-Ion Q was washed with 0.5 *M* NaOH (30 l), depressurised and allowed to stand at room temperature for 72 h. The column was repressurised and washed successively with 0.1 *M* Tris–HCl buffer (pH 7.5) (75 l) and 0.025 *M* Tris–HCl buffer (pH 7.5) (50 l). Flow-rate of 150 cm/h was maintained throughout.

(v) Analytical loading. Egg-white feedstock (6 l) was chromatographed on Express-Ion Q as described in (i) above.

The complete study was repeated at a flow-rate of 280 cm/h.

2.3.2. IsoPak column

Express-Ion Q (20 kg) was equilibrated with 0.025 *M* Tris–HCl buffer (pH 7.5) to give a final slurry concentration of ca. 30% (w/v) using the recirculating slurry preparation station associated with the IsoPak column. The height of the 44 cm I.D. IsoPak column was adjusted to 16 cm, and the column was pump-packed in upflow from the slurry tank at a pressure of ca. 10 p.s.i. according to the column manufacturer's guidelines. The packed column of Express-Ion Q (16 cm×44 cm I.D.) had a volume of ca. 24.3 l and a packing density of 0.227 kg/l. All procedures were carried out in downflow at room temperature (15–20°C). The pressure/flow performance of the packed bed was measured using 0.025 *M* Tris–HCl buffer (pH 7.5), prior to egg-white chromatography. The ion exchanger was used with the egg-white feedstock accordingly (i) analytical loading, (ii) preparative loading, (iii) analytical loading, (iv) clean-in-place (CIP), (v) analytical loading.

(i) Analytical loading. Egg-white feedstock (10 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) (100 l). The Express-Ion Q was equilibrated with 0.025 *M* Tris–HCl buffer (pH 7.5) (150 l). Flow-rate was maintained at 150 cm/h throughout.

(ii) Preparative loading. Egg-white feedstock (500 l) was loaded on to the column and non-bound material removed by washing with 0.025 *M* Tris–HCl buffer (pH 7.5) (150 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) (400 l). The Express-Ion Q was re-equilibrated with 0.025 *M* Tris–HCl buffer (pH 7.5) (150 l). Flow-rate was maintained at 150 cm/h throughout.

(iii) Analytical loading. Egg-white feedstock (10 l) was chromatographed on Express-Ion Q as described in (i).

(iv) Clean-in-place procedure. The column of Express-Ion Q was washed with 0.5 *M* NaOH (50 l), depressurised and allowed to stand at room temperature for 72 h. The column was repressurised 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) (150 l). Flow-rate of 150 cm/h was maintained throughout.

(v) Analytical loading. Egg-white feedstock (10 l) was chromatographed on Express-Ion Q as described in (i) above.

The complete study was repeated at a flow-rate of 300 cm/h.

2.4. Assays

Pooled fractions at various stages of chromatography were assayed for total protein and ovalbumin content by fast protein liquid chromatography (FPLC) [10]. Throughout the column procedures the effluent was monitored for absorbance at 280 nm and by conductivity.

3. Results and discussion

The microgranular cellulose matrix upon which the Whatman ion-exchange product ranges are based is chemically and physically robust, lending itself to mechanical pumping and agitation under appropriate conditions [16]. This feature has enabled their application in batch processes where significant numbers of slurry transfers are required [4,5]. Furthermore we have shown their suitability for process-scale use in earlier designs of pump-packed fixed volume axial [10] and radial flow columns [9]. In the present study we have investigated the performance of Express-Ion Q in two new designs of pump-packed columns using a well-proven hen egg-white separation [10,11]. In each case a 30% (w/v) slurry of Express-Ion Q in 0.025 *M* Tris–HCl buffer (pH 7.5) formed a well packed bed following the pump-packing operations recommended by the manufacturer. The pressure-flow performance of the SPC and IsoPak columns is summarised in Fig. 1. In each case linear pressure/flow performance was observed over the flow-rate range 100–300 cm/h, with maximal flow-rates of 280 and 320 cm/h for the SPC and IsoPak, respectively using these system configurations. The pressure-flow performance of the Express-Ion Q throughout the process-scale separations remained constant, observations in keeping with our earlier studies on Express-Ion exchangers [10,11]. The column packing operations were relatively straightforward with the columns packed and operational

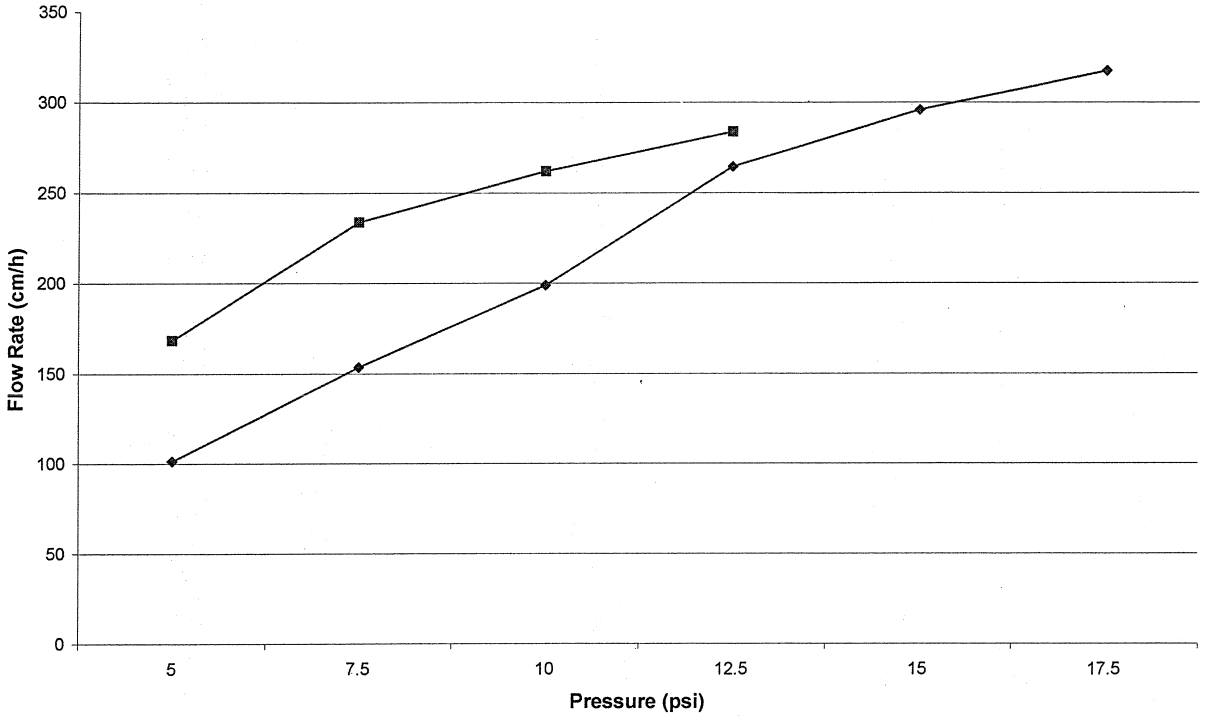


Fig. 1. Pressure flow performance of Express-Ion Q using 0.025 *M* Tris–HCl buffer (pH 7.5) in the Side-Pack column (■) and the IsoPak column (♦).

within 20 min for the SPC and 10 min for the IsoPak column. This compares with packing times of ca. 60 min for the more traditionally configured slurry-pack columns we have previously used [11]. It is noteworthy that the column packing density of 0.303 kg dry mass/l for the SPC is greater than the density of 0.227 kg dry mass/l for the IsoPak column. This is presumably attributed to the different column designs, and packing mechanism. What effect, if any, this may have on the column operation and chromatographic performance remains to be established. However, it has been suggested that adsorption of proteins to ionic groups on an ion exchanger results in a utilisation of ca. 5% (w/w) the number of charged groups attached to the ion-exchange matrix [17]. In this case a tighter packing in a chromatographic column may have little effect on protein capacity. However, for small molecules, this may not be the case. We have recently reported the binding of a hexapeptide (M_r 873) to the cation-exchange cellulose Express-Ion C [18], and showed that ca. 40% of the available binding sites were used for peptide adsorption. In this instance, a tighter packing density may give rise to a proportional increase in binding capacity and consequently throughput, but this remains to be established. However it should be noted that packing more adsorbent into a column will affect the overall economics of this unit operation, both in terms of adsorbent cost and also buffer costs since the equilibration stages will be chemically more demanding.

It has previously been reported that Express-Ion Q has an ovalbumin binding capacity of ca. 70 mg/ml [11,17]. Hen egg-white contains 63.8% (w/w) ovalbumin [19] and in the present study we loaded the Express-Ion Q columns with 0.11–0.14 kg total protein/l packed bed, i.e., levels of ovalbumin in excess of the anticipated maximum dynamic capacities of each column [11], and directly equivalent to our earlier studies using a 25 l slurry-packed adjustable volume column [11]. The protein capacity data for Express-Ion Q in the SPC and IsoPak column at flow-rates of 150 and ca. 300 cm/h are summarised in Table 1 and the chromatograms represented in Fig. 2. Since we are dealing with a natural feedstock and it would have been impractical for us to produce and store a pooled feedstock of 1700 l and use this for each of the four runs, there is a variability in the mass of protein loaded and this is reflected in the mass of protein bound. Notwithstanding, feedstock variability it is clear that the protein capacity of the Express-Ion Q is in the range 60–70 g/l packed bed. This is similar to the levels we have reported previously for preparative separations of egg-white proteins using Express-Ion D [10] and Express-Ion Q [11] at similar flow-rates. There does appear to be a slight reduction in protein capacity with increased flow-rates, observations in keeping with previous studies on Express-Ion Q and Q-Sepharose Fast Flow [11]. However it should be recognised that in these columns, assuming 75% (v/v) voidage of the adsorbent, the mobile phase turnover time is <3 min at a flow-rate of 300 cm/h so the maximum contact time between adsorbent and adsorbate will be of similar duration. We have reported very rapid diffusion kinetics of Express-Ion Q for ovalbumin [11], but nonetheless contact times of <5 min may give rise to a slightly reduced capture efficiency of proteins. Protein recovery was high in every case (Table 1) and FPLC analysis of the desorbed protein demonstrated it to be predominantly ovalbumin, with some ovomucoid and ovoglobulin content, observations in keeping with those previously reported for Express-Ion D [10] and Q [11].

The data in Table 1 indicates that some residual protein may be retained on the Express-Ion Q

Table 1
Protein capacities of Express-Ion Q during preparative chromatography of hen egg-white proteins

Flow-rate (cm/h)	Side-Pack column			IsoPak column		
	Feedstock total protein (kg)	Protein adsorbed (kg)	Protein desorbed (kg)	Feedstock total protein (kg)	Protein adsorbed (kg)	Protein desorbed (kg)
150	2.196	1.025	0.962	2.660	1.705	1.831
300[a]	2.188	0.939	1.118	3.355	1.622	1.602

[a] 280 cm/h for the SPC.

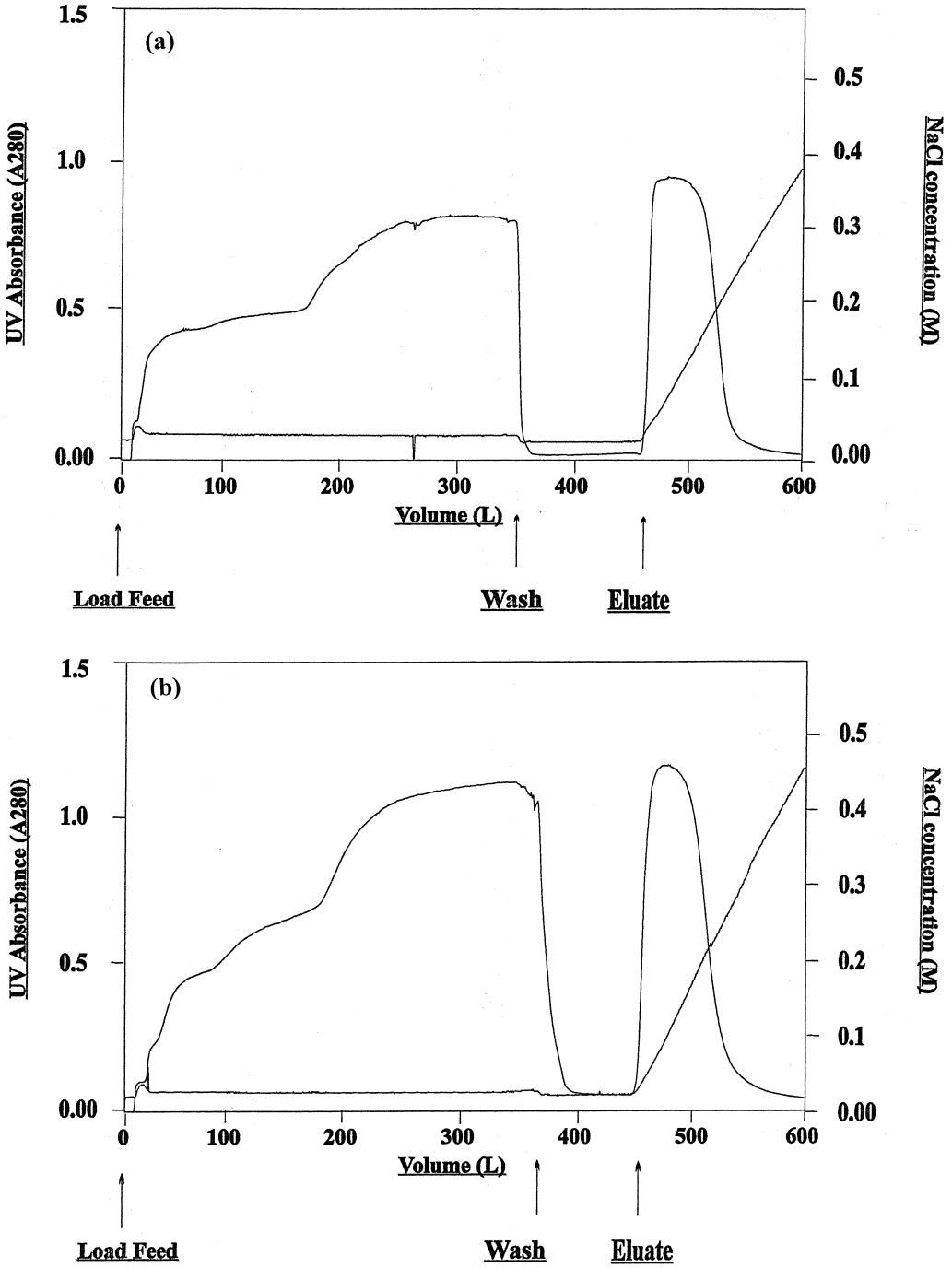
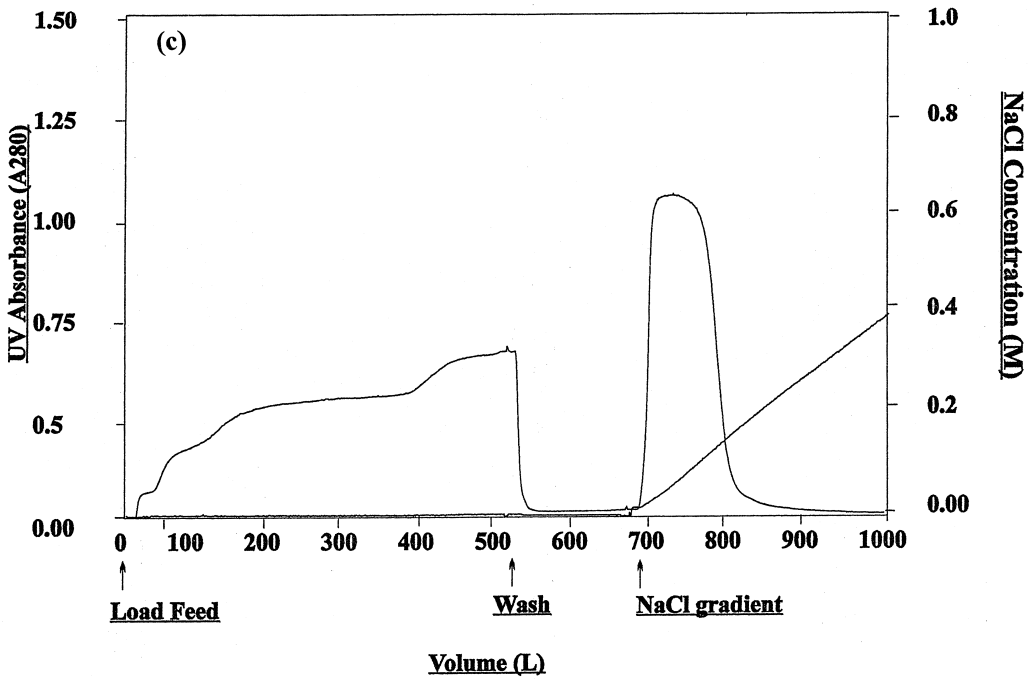


Fig. 2. Process scale chromatography of hen egg-white proteins on Express-Ion Q in the Side-Pack column at (a) 150 cm/h and (b) at 280 cm/h and in the IsoPak column at (c) 150 cm/h and (d) at 300 cm/h, using 0.025 *M* Tris–HCl buffer (pH 7.5).



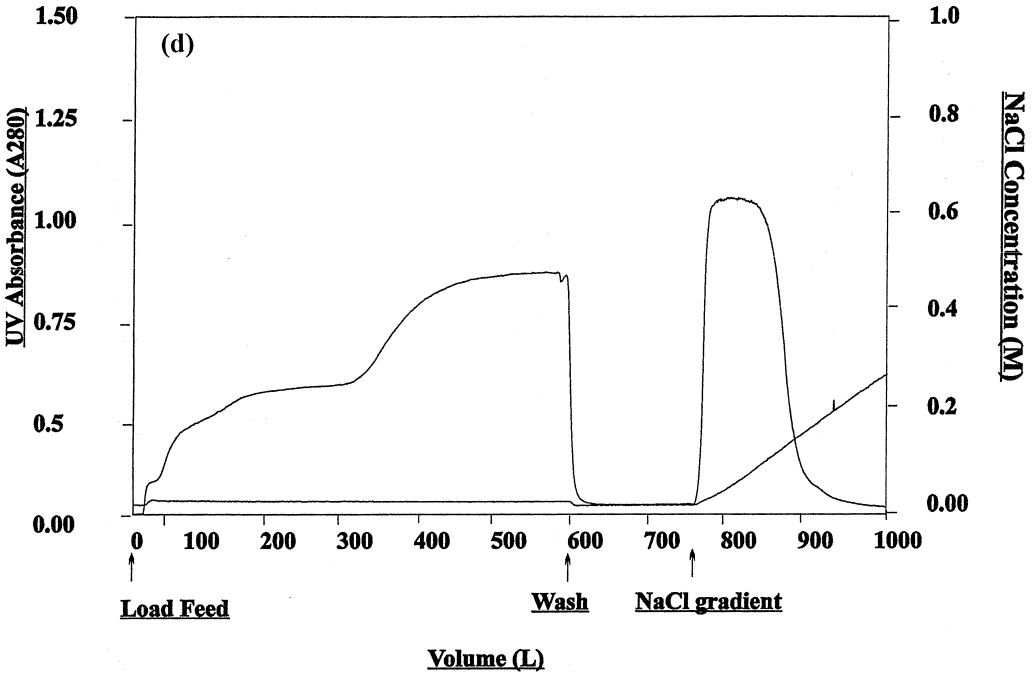


Fig. 2. (*continued*)

following the elution step. We carried out a CIP between preparative runs and there was no apparent reduction in performance of the Express-Ion Q following this operation (Fig. 2a–d). We have examined column fouling in more detail elsewhere [16,20] and consider that inclusion of analytical separations of feedstock are a useful monitor of media fouling. A representative series of analytical runs is presented in Fig. 3. Prior to the preparative run (Fig. 3a), a typical separation of egg-white proteins is observed [11] with the conalbumin component (ca. 50 l) eluting before the ovalbumin component (ca. 75 l). Following the preparative loading (Fig. 3b), it is evident that the resolution between these components is significantly impaired. 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. The CIP protocol using 0.5 *M* NaOH has restored chromatographic performance of the medium back to its initial state (Fig. 3c). These conditions for CIP have been demonstrated to be efficacious for simultaneous sanitisation of heavily contaminated columns of Express-Ion exchangers [20].

In the traditional design of slurry-pack columns, the slurry would be poured into the column barrel, possibly fitted with an extension tube and the bed would be consolidated at constant applied pressure. The next operation would involve depressurisation, removal of headspace buffer and positioning of the upper flow adaptor on top of the bed, followed by a physical bed compression to a height similar to that established during the initial packing step. This procedure is time-consuming, typically taking at least 60 min to complete. Additionally, as column diameter increases, the upper flow adaptor becomes heavy and is cumbersome to manoeuvre, often requiring a mechanical lowering assembly. Furthermore, its positioning can be a labour intensive operation in order to ensure that no air is trapped under the flow adaptor which could affect column performance. In our earlier studies using a 45 cm I.D. column of this design, we have found this operation to be highly operator dependent and small differences of ca. 1 cm in bed over or under compression can lead to significant changes in the pressure/flow performance of the packed column [9,16]. This would most likely affect the process economics and may have an impact on process validation.

With the IsoPak columns, packing pressure is maintained throughout all stages of bed consolidation and this problem is therefore eliminated. This should result in operator-independent consistent packing facilitating process validation. The packing process takes ca. 10 min to complete and can be readily achieved with one operative. Any mechanical issues associated with bed height adjustment may be carried out prior to column packing and in principle external to the chromatographic suite. In the case of the SPC, pump-packing using the dedicated side port enables the bed to be consolidated at a prescribed pressure and once this port is clamped shut following packing, the bed is retained within the upper and lower bed supports at its original packing density. In earlier fixed volume column designs, without dedicated packing ports, the column bed could relax following packing, thereby reducing packing density and potentially causing voids within the bed. Both designs of pump-packed column use upflow thereby displacing air from the column barrel during packing. This eliminates the difficulties often encountered with slurry-packed columns in removal of trapped air during flow adaptor replacement and bed re-compression.

Column unpacking has hitherto been an area where little attention has been directed in the literature. A widely adopted approach is to remove the upper flow adaptor/column top section and manually excavate the spent adsorbent. Not only is this labour intensive and gives rise to adsorbent loss, it is a potential health and safety hazard since the used adsorbent is now in an open, albeit contained, environment. Furthermore, reaching the base of a 50 cm high column barrel can prove a physical challenge!! Our experiences based on previous studies [8–11] have shown column unpacking to take at least 60 min an observation in keeping with the SPC which took two people 60 min to disassemble, unpack, clean and reassemble.

It has been reported that pump-packed adjustable volume columns have a capability for pump-unpacking [12,13]. The IsoPak column could be rapidly unpacked within 10 min by means of an in situ reslurrying of the bed and pump-unpacking of this

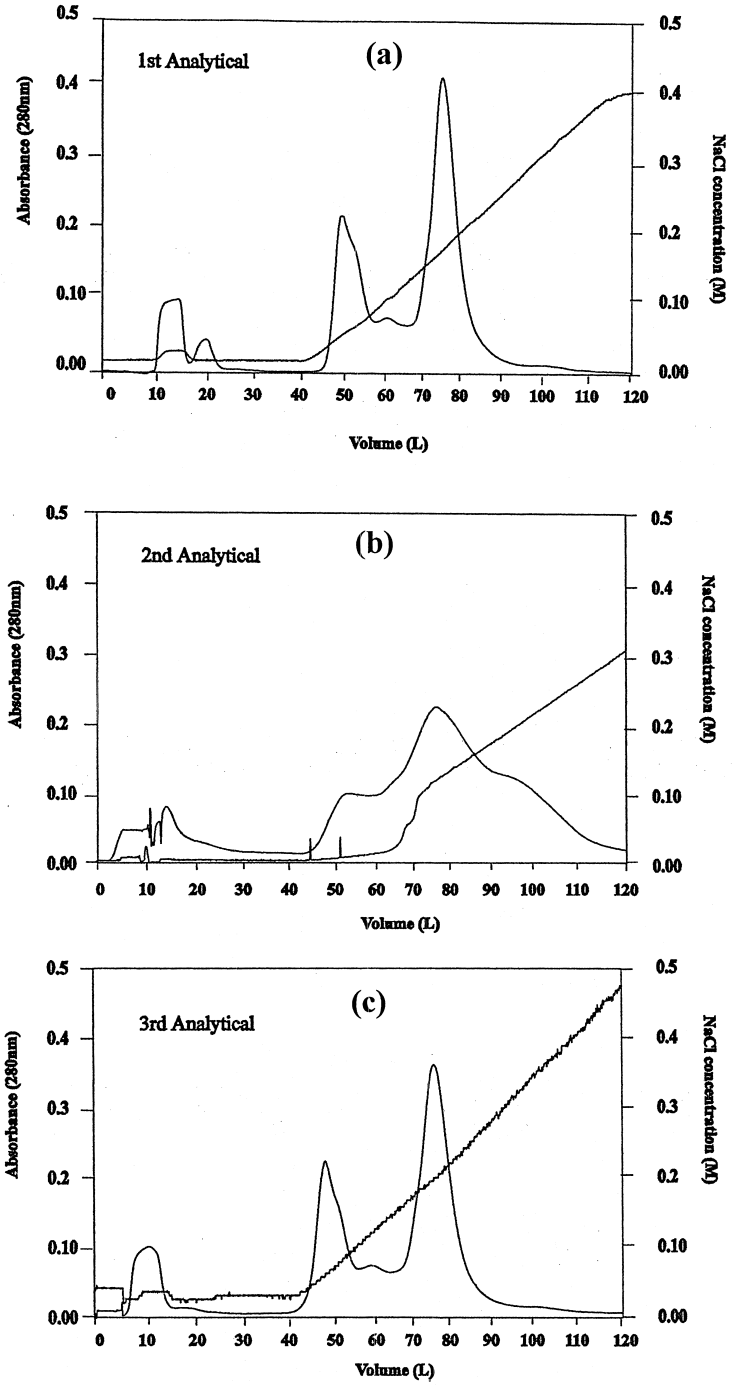


Fig. 3. Chromatography of hen egg-white proteins on Express-Ion Q in a Side-Pack column using 0.025 *M* Tris–HCl buffer (pH 7.5), (a) analytical loading before preparative run; (b) analytical loading after preparative run; (c) analytical loading after CIP, at a flow-rate of 280 cm/h.

slurry, using the recirculating slurry preparation station. We found this a very facile task requiring a single operative. Furthermore it reduces, if not eliminates, many of the labour, material loss and safety issues described above for manual unpacking.

In the present investigation we have carried out a process-scale separation of hen egg-white proteins using Express-Ion Q in two designs of axial flow column. The key difference between the SPC and IsoPak is their method of packing. We found that both column designs were highly effective in this separation, facilitating use of the absorbent at high flow-rates of up to 300 cm/h and supporting our earlier studies using the traditional slurry-packed columns. The ability to pump-pack a slurry of adsorbent directly into the column, which should minimise variability from run to run offers a major benefit to the user. The ease of unpacking the IsoPak column is particularly advantageous, especially in applications where single use of the ion exchanger is appropriate, for example negating the need for CIP and associated system validation. Our studies demonstrate that the packing and unpacking of process-scale chromatography columns, hitherto seen as a time-consuming, labour-intensive challenge can now be regarded as a simple, rapid and routine stage of a chromatographic separation.

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