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Review

Large-scale ion-exchange column chromatography of proteins Comparison of different formats

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Abstract

This review describes the performance of various column designs available to process-scale users of low-pressure chromatography for protein purification. By carrying out a range of ion-exchange separations using Whatman microgranular ion-exchange celluloses we are able to compare and contrast the practical performance issues associated with several designs of axial and radial flow columns.

Keywords: Reviews; Stationary phases, LC; Ion-exchange chromatography; Axial flow; Radial flow; Preparative chromatography; Proteins

Contents

1. Introduction

Ion-exchange chromatography is an established technique used in the separation of charged molecules across a breadth of applications and industries.

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Chemically ion-exchange involves the exchange of solutes of like charge from a solid support bearing the opposite charge (adsorbent). The principles of ion-exchange chromatography and an introduction to market segments for its application are summarised elsewhere [1]. Ion exchange is a widely used technique in bioseparations since peptides, proteins, nucleic acids and related biopolymers have ionisable chemical moieties which render them susceptible to charge enhancement or reversion as a function of pH. Consequently under a set of defined mobile phase conditions a biopolymer mixture may be chromatographed using an ion-exchange medium in a suitable contactor. Dependent on the relative ionic charge of the components, some biopolymers will adsorb (adsorbates) and others will remain in solution. Desorption of bound material can then be effected resulting in a degree of purification of the target biomolecule. A generic process flow for ion-exchange chromatography may be regarded as:

(1) Adjust the feedstock composition to provide a mobile phase appropriate for adsorption of the desired component.

(2) Contact feedstock with ion exchanger to facilitate adsorption

(3) Wash unretained contaminants from the ion exchanger.

(4) Desorb and elute bound material from the ion exchanger.

(5) Regenerate the ion exchanger (clean-in-place, CIP).

When assessing opportunities for process-scale ion-exchange chromatography there are four generic approaches that the chromatographer might consider for large-scale use:

(1) Batch
(2) Column
(3) Expanded/fluidised bed
(4) Suspended bed

1.1. Adsorbent–adsorbate contacting

Batch contacting is a simple equilibrium system whereby the adsorbent is dispersed in a tank containing feedstock, which includes the adsorbate, and simply simply mixing the components effects adsorption. The depleted feed is removed from the adsorbent using a suitable solid–liquid separation technique such as continuous flow basket centrifugation, dead-end filtration or even decantation. Washing and desorption are typically carried out in a similar manner. This technique has been described in detail elsewhere and its performance relative with other modes of contacting discussed [2,3].

Column contacting systems, on the other hand, require that the ion exchanger is contained within a hollow device and retained there using bed supports that are porous to the feedstock and other mobile phases. Column chromatography is widely used in large-scale ion-exchange processes for protein separations and a variety of column formats are commercially available. This approach to process-scale protein purification forms the basis of this review. Column contactors can also be used to contain adsorbents in a loosely packed fashion. One example is their application in an expanded/fluidised mode of operation and this is an area that has been well reviewed in the literature [4]. Another example is the hybrid batch/column technique referred to as suspended bed chromatography which we have described previously [5,6].

2. Column chromatography

Large-scale chromatography columns are available from several manufacturers each with their own proprietary designs. These systems are designed to meet different process specifications and accordingly use a variety of appropriate materials of construction. The aim of this review is to compare and contrast the various approaches to large-scale column-based ion-exchange processes and not to discuss the specifications of the hardware or the engineering capabilities of the equipment suppliers.

Because ion exchange is an adsorption technique it can be used in either positive or negative capture modes. In either mode feedstock is applied to the ion exchanger packed in a column. Dependent on the pH and/or conductivity of the feedstock the target may adsorb while the contaminants remain unretained. This is referred to as positive chromatography. On the other hand where the target is unretained and the contaminants adsorb, this is referred to as negative chromatography. Intuitively, one might consider positive capture to be the preferred approach to

chromatography since the target is adsorbed to the ion exchanger and then selectively desorbed. However, this is not necessarily the optimal approach to chromatography. There are numerous cases of both approaches being used for protein purification and commercial manufacturing processes could use either technique. To demonstrate each approach we have reported the separation of immunoglobulins from goat serum by anion exchange [7] as an example of negative capture and the separation of monoclonal antibodies from tissue culture supernatant by cation exchange [8] as an example of positive capture. These studies demonstrate both principles of adsorption chromatography.

The selection of positive or negative capture steps is very much process dependent and a number of factors need to be evaluated in making the selection. For example, positive chromatography often concentrates while negative does not; if impurities are a minor component of the feedstream then negative chromatography would be preferred to maximise the capacity of the adsorbent; if the feedstock mobile phase is unsuitable for direct adsorption then a cost benefit analysis might suggest negative chromatography as the preferred approach so as to avoid costly buffer adjustments, etc.; the subsequent downstream process might dictate the mobile phase composition from the preceding ion-exchange process which affects selection of mode; validation is simpler for negative chromatography; desorption and CIP can be a single step in negative capture since only unwanted contaminants adsorb so their stability during elution is of low importance, whereas in positive capture each step will be discreet.

The outcome of this analysis should help facilitate the design of the process and hence its scale and economics. In this review the focus will be on positive capture using anion exchange but it should be emphasised that many of the comparative issues and scale-up challenges will be similar both for negative capture and also for cation-exchange separations.

3. Model feedstock

The model feedstock that will be used to compare and contrast the various column approaches is fresh hen egg-white. Egg-white contains a complex mixture of proteins that have been chromatographed using a variety of different ion-exchange systems [9–12]. This protein system has commercial significance in the food industry as well as for biochemical reagents. More recently though the hen is being used as a host for transgenic protein systems and the egg-white will be the major source of the expressed transgenic protein.

Egg-white is a viscous, translucent material containing 50–100 mg protein/ml which in its natural form would not be suitable for column chromatography. In our work, we typically dilute egg-white to a 10% (v/v) suspension using 0.025 *M* Tris–HCl buffer (pH 7.5), and then clarify it using the fibrous weak anion-exchange cellulose CDR (Whatman, Maidstone, UK) in batch mode. The clear feedstock containing 5–10 mg/ml total protein is suitable for column chromatography. Protocols for preparation of the feedstock and assaying the components using fast protein liquid chromatography (FPLC) are described elsewhere [13].

4. Anion-exchange chromatography of hen egg-white proteins

The mobile phase composition of the hen egg-white feedstock has been shown to influence its chromatography on anion-exchange celluloses [14] and the composition described above reflects a suitable system for comparative column performance testing. The process flow that has been followed for comparative column testing is summarised below:

Feedstock (8–20 column volumes)
↓
Anion-exchange column
(0.025 *M* Tris–HCl buffer, pH 7.5)
↓
Wash (6 column volumes)
(0.025 *M* Tris–HCl buffer, pH 7.5)
↓
Gradient elute (16 column volumes)
(0–0.5 *M* NaCl in 0.025 *M* Tris–HCl buffer, pH 7.5)
↓
CIP

The CIP process involves sequential washes with 0.5 *M* NaOH (2 column volumes), water (2 column volumes), 0.1 *M* Tris–HCl buffer, pH 7.5 (2 column volumes) and 0.025 *M* Tris–HCl buffer, pH 7.5.

Various anion-exchange celluloses (Whatman) were used according to manufacturer's instructions and recommended flow-rates. Chromatography columns were obtained from the manufacturer and used according to their supplied instructions.

A typical process-scale separation of hen egg-white proteins by anion exchange following the process flow summarised above is represented in Fig. 1. It is evident that the chromatogram does not provide a great deal of definition, giving broad peaks with no apparent resolution between protein species. The reason for this is that the protein concentrations throughout much of the separation are so high that the ultraviolet absorbance detector response is poor. This is a frequent observation in process-scale chromatography where at high protein concentrations the absorbance of the solution at a given wavelength is so high that Beer's law is disobeyed and large changes in protein concentration generate small detector response [15]. Beer's law [16] states that:

$$A = \epsilon lc$$

where A is the absorbance of the solution at a defined wavelength, ϵ is the molar absorbance coefficient of the solute, l is the path length through the sample and c is the solute concentration.

On this basis, as the concentration of the protein solution increases so there should be a proportional increase in absorbance, typically measured at 280 nm (A_{280}). A plot of A_{280} versus ovalbumin concentration for a 2.5 mm path length flow cell is presented in Fig. 2. It is evident from the data that linearity is observed at low protein concentrations of up to ~10 mg/ml. This may be typical of many biological feedstocks. However non-linearity is observed at higher protein concentrations which may often be representative of the levels obtained during desorption and peak elution following a process loading of feedstock. This explains the differences in peak shape that are observed when a process column

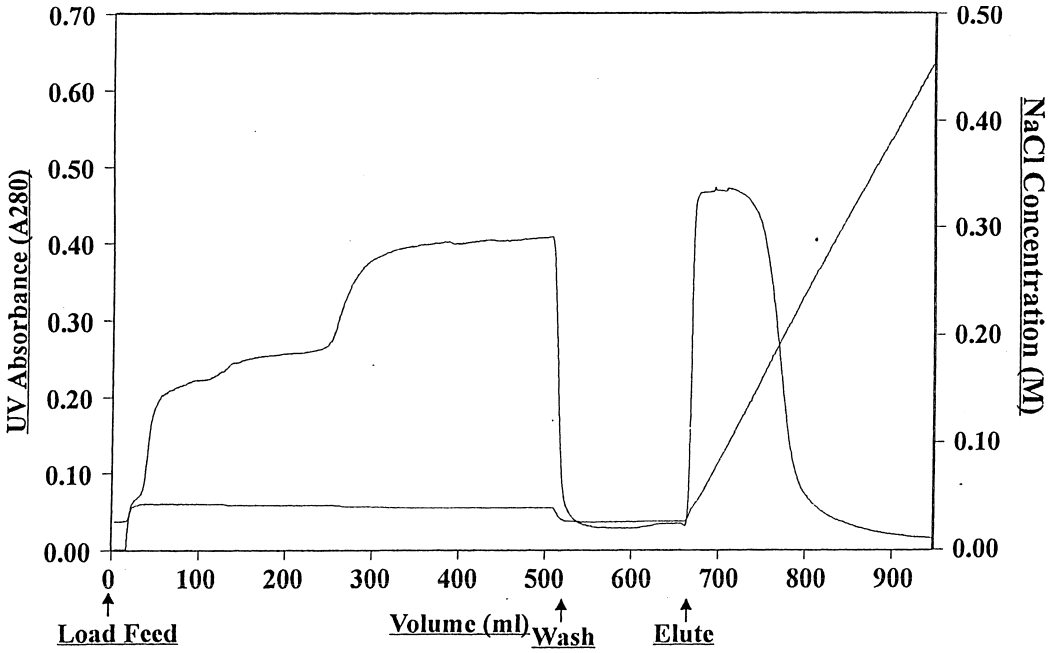

Fig. 1. Chromatography of 4.2 kg hen egg-white proteins on Express-Ion Q in a Moduline column (15.5 cm×45 cm I.D.) in 0.025 *M* Tris–HCl buffer, pH 7.5 at a flow-rate of 150 cm/h.

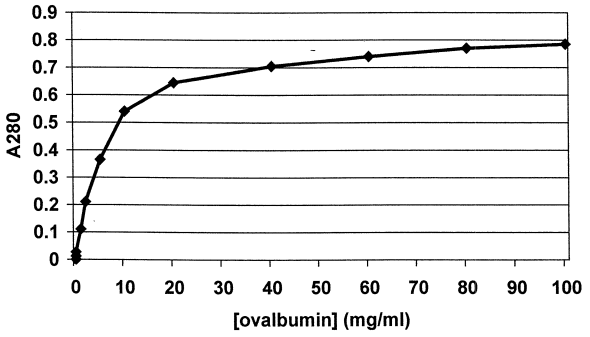

Fig. 2. Absorbance of ovalbumin solutions prepared in 0.025 *M* Tris–HCl buffer, pH 7.5 at 280 nm using a 2.5 mm path length flow cell.

containing an ion exchanger is used for separation of a low protein load (Fig. 3) compared with a high protein load (Fig. 1). This non-linearity of detector response will reduce the amount of information that can be deduced from a chromatogram in terms of product purity.

For this reason, an independent analytical chromatography technique is recommended for examination of the composition of these major peaks. One such technique that we have used routinely is FPLC. Examples of the analysis of fractions throughout the separation using FPLC and protocols for such analyses have been reported previously [13].

When one loads a small quantity of egg-white feedstock (0.3–0.4 column volumes), referred to as an ''analytical loading'', then one generates a reasonably well defined separation as shown in Fig. 3. The separation identifies a peak of lysozyme eluting in the non-bound fraction, which could be the regarded as a product of negative capture, and two major eluted peaks. Conalbumin elutes first followed by ovalbumin. In a process loading (Fig. 1) the large non-bound peak comprises lysozyme plus displaced conalbumin and the salt-eluted peak is essentially pure ovalbumin, as confirmed by FPLC [13].

5. Comparison of various column formats

A chromatography column is a pressurised system that may simply be considered as a hollow vessel into which is placed the chromatography medium and that is retained in place with porous bed supports at the inlet and outlet. The bed supports, often referred to as frits, have a porosity less than the size of the adsorbent particle such that the adsorbent is retained within the column unit, yet porous enough to allow free passage of feedstock and all mobile phases. Porosities in the range 5–50 μm would be typically used in large-scale protein separations. In order to ensure good chromatographic performance,

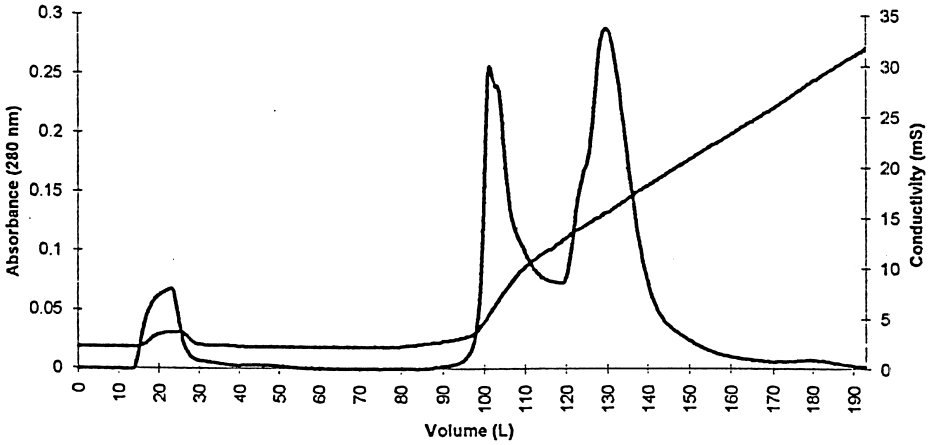

Fig. 3. Chromatography of ~100 g of hen egg-white proteins on Express-Ion Q in a Moduline column (15.5 cm×45 cm I.D.) in 0.025 *M* Tris–HCl buffer, pH 7.5 at a flow-rate of 150 cm/h.

a robust and reproducible column packing protocol must be developed and validated. This will usually be based on operating instructions supplied by the column manufacturer, recommendations of the chromatography medium vendor and experience of the process chromatographer. However, several general observations apply. For packed bed operations, it is necessary to avoid physical movement of the adsorbent bed during use due to physico–chemical interactions between the ion exchanger and the mobile phase. Accordingly, the packing pressure of the bed should be greater than the maximum operating pressure for the process. If the ion exchanger is prone to shrinkage during use then added mechanical compression of the bed or packing in the mobile phase that gives maximum shrinkage would be suggested. Clearly for expanded or fluidised bed techniques [4] such considerations are of less significance.

Having packed the bed a number of simple tests may be performed to demonstrate both its utility and confirm the reproducibility of the packing operation, as is often a pre-requisite for validated processes. These tests can include an estimate of the column packing density expressed as dry grams adsorbent per unit column volume, a measure of linear flow-rate at defined pressures and determination of the height equivalent to one theoretical plate (HETP) using a tracer spike [17]. As a final qualification test, we have found that an analytical loading of feedstock run under defined process chromatography conditions (Fig. 3) gives useful information on bed integrity and chromatographic performance of the packed column.

There are several column formats commercially available to the process chromatographer. These may be segregated into two paired groups. Firstly, fixed bed volume and adjustable bed volume. In the former case the length of the column barrel is fixed whereas in the latter case it may be adjustable using a variety of design options. Secondly, the flow characteristics through the bed may be axial or radial. In the former case the column is a tube with bed supports top and bottom. The fluid flow is axial and typically is top to bottom. In the latter case the bed supports are two concentric tubes with the adsorbent sandwiched between. The top and bottom of the column unit are sealed and consequently fluid flow is radial typically from the outer bed support to the inner one [18].

In order to scale-up an axial flow ion-exchange process, the column diameter is increased while maintaining chromatographic bed height and linear flow-rate. In the case of radial flow, the bed height is effectively the distance between the two concentric bed support tubes and this should be maintained. Linear velocity cannot be readily determined in this geometric configuration so a measure of column volumes per hour is suggested as a determinant of flow-rate. Scale-up is achieved simply by increasing the length of the radial flow unit, whilst maintaining flow-rates in terms of column volumes per hour.

In the following sections we compare several different column designs and formats for the anion-exchange chromatography of hen egg-white proteins. The list of columns used is not exhaustive due to availability of units in our laboratory nor is it intended to be prescriptive as to which make and model is recommended. For more detailed description of the experiments and their results the reader is referred to the original publications. Column designs used in these studies were:

(1) Fixed volume axial flow.
(2) Fixed volume radial flow.
(3) Fixed volume Side Pack axial flow.
(4) Adjustable volume slurry pack axial flow.
(5) Adjustable volume pump pack axial flow.

5.1. Fixed volume axial flow

In these studies we used a 16 cm×45 cm I.D. unit (PREP-25, Whatman) that is pump-packed using a slurry of anion-exchange cellulose. The column was packed by pumping a slurry of DE52 (Whatman) into the PREP-25 column at 15 p.s.i. and operated at ~10 p.s.i., to give a flow-rate of ~30 cm/h (1 p.s.i. = 6894.76 Pa). Chromatography of an analytical loading of egg-white proteins is shown in Fig. 4b. This study also demonstrates a 1000-fold scale-up from a laboratory-scale column (15.5 cm×1.5 cm I.D.) when operated at a similar linear flow-rate (Fig. 4a). Under these conditions we reported five consecutive process chromatograms each using 200 l of feedstock (10 mg/ml) [15]. As a result of these multiple runs we observed media fouling and re-

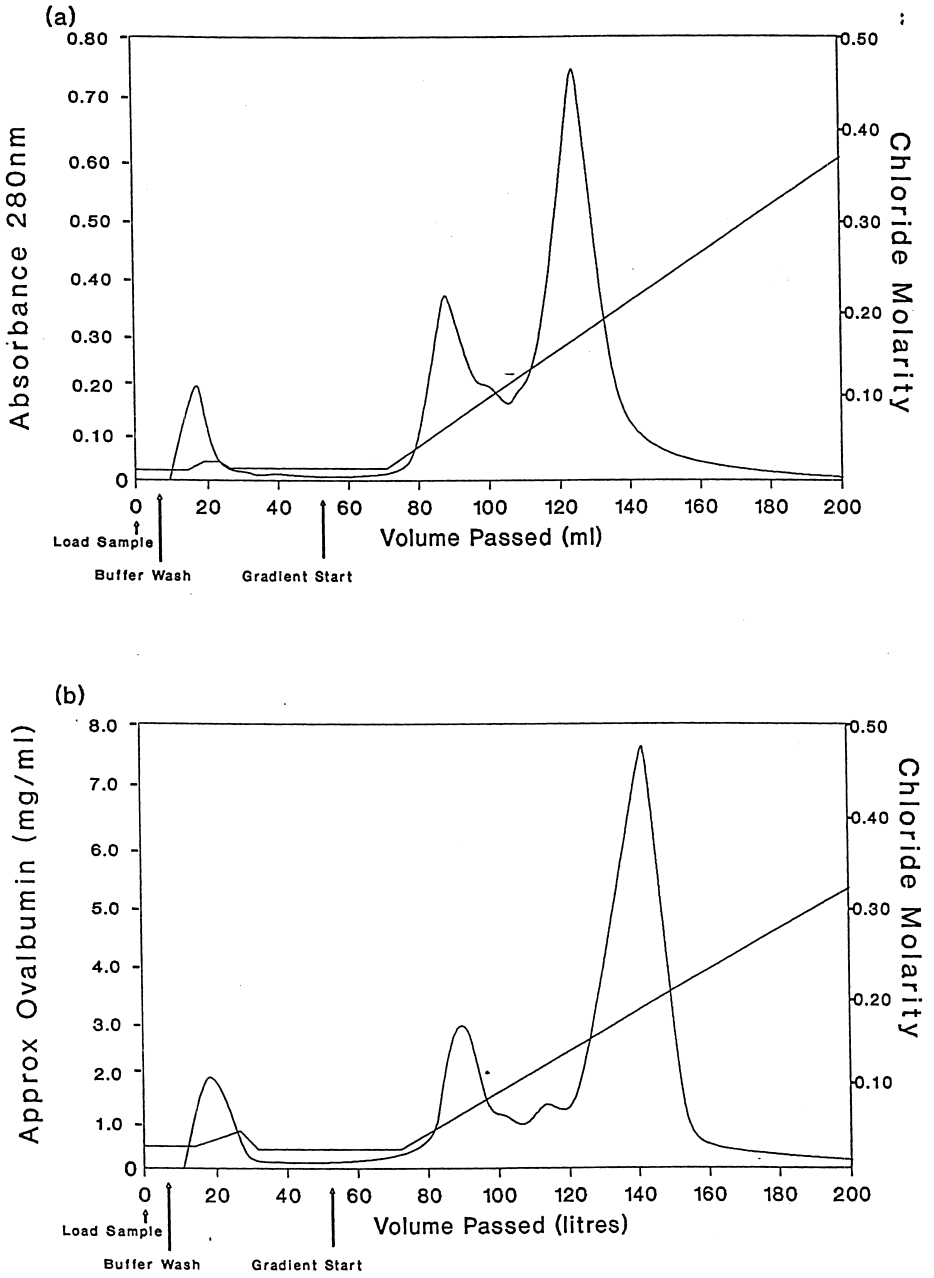

Fig. 4. Chromatography of hen egg-white proteins on DE52 using 0.025 *M* Tris–HCl, pH 7.5 at a flow-rate of ~30 cm/h at (a) laboratory scale (15.5 cm×1.5 cm I.D., 100 mg load) and (b) in a PREP-25 column (16 cm×45 cm I.D., 100 g load).

ported that chromatographic performance could be restored following overnight CIP using 0.5 *M* NaOH according to the process flow summarised in Section 4. Full experimental details are reported elsewhere [15].

Although this column unit was effective for large-

scale use its major limitation was inflexibility in volume, i.e., fixed at 25 l. No other production columns were readily available in this range and so it was discontinued. A key feature of this column was the ability to pump-pack the adsorbent that facilitated rapid and consistent bed formation in 15–30 min. This mode of operation will be examined in more detail below. Column unpacking, however, required column disassembly and manual excavation of the spent chromatography medium. In this case this was not problematic since the column barrel unit is only 16 cm deep allowing easy access. However, there are both health and safety and containment issues associated with this type of operation and this could well be a significant concern in a process environment.

5.2. Fixed volume radial flow

In this work we used a Superflo-100 and a Superflo-10L radial flow column (Sepragen, Hayward, CA, USA) with volumes of 100 ml and 10 l and ''effective bed heights'' of ~3 and ~10 cm, respectively, based on the distance between the concentric bed supports. In a comparative study we carried out analytical loadings of hen egg-white proteins using DE52 in each of these columns and compared the performance with a 100 ml axial flow column. These data are presented in Fig. 5. While the chromatographic profiles are as expected for such a separation, it is apparent that the resolution between conalbumin and ovalbumin is influenced by the ''effective bed height''. This is anticipated since an increase in bed height would provide more theoretical plates and enhanced resolution between eluting peaks as is illustrated in Fig. 5. In a subsequent study we demonstrated effective scale-ups between a Superflo-100, 100 ml column and a Superflo-500, 500 ml column and also between a Superflo-10L, 10 l column and a Superflo-20L, 20 l column, each pairing having similar ''effective bed heights'' [19]. A design feature of radial flow columns is the ability to pump-pack the slurry of ion exchanger directly into the column unit via the axial packing ports [18,20]. In a similar manner the design enables the user to pump-unpack the column thereby reducing the hazards associated with manual unpacking mentioned above. It should be noted that if in the event

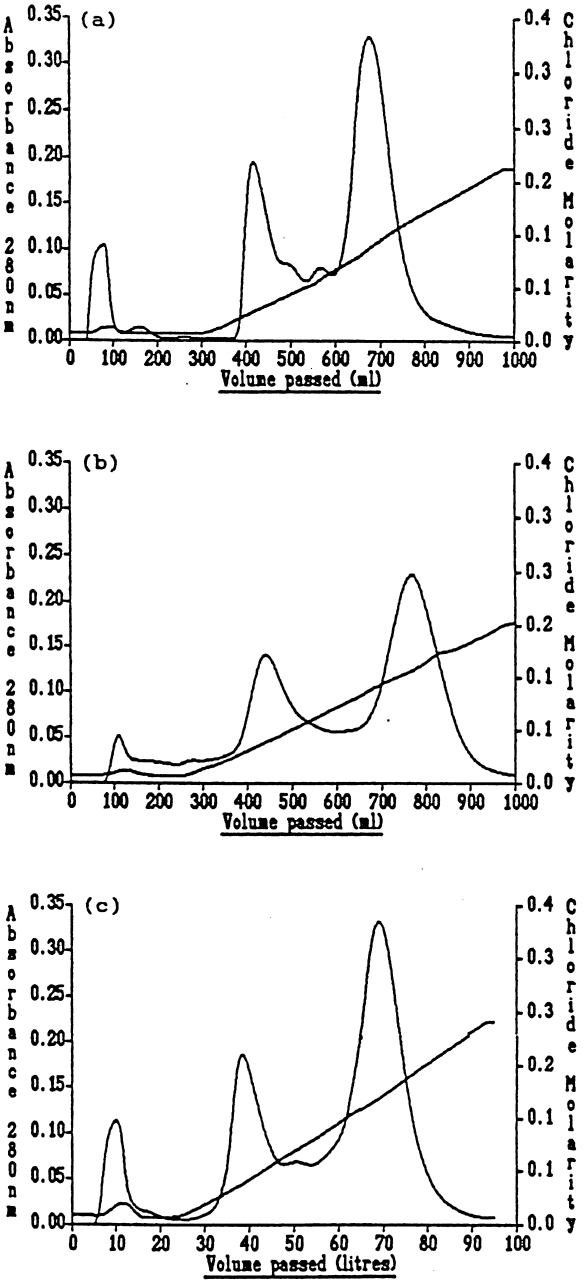

Fig. 5. Chromatography of hen egg-white proteins on DE52 in 0.025 *M* Tris–HCl buffer, pH 7.5 using (a) 100 ml axial flow column (6.6 cm×4.4 cm I.D.) and (b) 100 ml Superflo-100 radial flow column at flow-rates of 15 ml/min and (c) 10 l Superflow-10L radial flow column at a flow-rate of 1 l/min.

that fouling of the inlet bed supports were to occur due to components of the feedstock interacting with the support matrix itself then the column unit may require unpacking and disassembly. By comparison in axial flow systems it is possible to carefully replace the upper bed support, which typically contacts with the feedstock during loading, with minimal disruption to the integrity of the adsorbent bed.

A recent introduction for radial flow method scouting and process optimisation is the WEDGE column. This can be described as a segment of the radial flow column, which retains the ''effective bed height'' of the complete unit, so lends itself to scale-down studies [20].

One major feature of radial flow columns is their ability to support higher volumetric flow-rates than similar volume axial flow columns at the same pressure drop. We compared the chromatographic performance of DE52 and QA52 (Whatman) in a Superflo-100 column with a 100 ml axial flow column (6.6 cm×4.4 cm I.D.; Millipore, Stonehouse, UK). The pressure flow data generated in this study are summarised in Table 1. It is evident that under similar operating conditions we were able to achieve flow-rates ~fivefold greater using the radial flow system compared to the axial flow system. It was observed that under these operating conditions the chromatographic resolution of hen egg-white proteins using gradient elution was superior using axial flow compared to radial flow. A series of comparative chromatograms using QA52 is presented in Fig. 6. This effect on chromatographic resolution is anticipated since the ''effective bed height'' is less for the radial flow column than it is for the identical volume axial flow column. It should be emphasised that we used gradient elution, and had step elution been employed then such an effect would be minimised. Full experimental details for these studies are reported elsewhere [19,21].

Table 1
Pressure/flow-rate data for chromatography of hen egg-white proteins on DE52 and QA52 under axial or radial flow conditions

Flow-rate (ml/min)	Pressure (p.s.i.)			
	DE52 column		QA52 column	
	Axial	Radial	Axial	Radial
5	1	–	1	1
15	5	1	5	4
25	14	1	17	4
50	>45	1	>45	4
100	–	6	–	18
150	–	6	–	20

5.3. Fixed volume side pack axial flow

A recent innovation in column design is the Side Pack column [22]. This device is an axial flow unit which is packed by pumping a slurry through a side port, such that the bed formation is bi-directional with packing buffer emerging through both upper and lower bed supports [22]. We evaluated a 16 cm×35 cm I.D. Side Pack column (ProMetic BioSciences, Burtonsville, MD, USA) using Express-Ion Q (Whatman). The column was readily packed in ~20 min and had a packing density of 0.303 kg dry mass/l bed volume. This is higher than values of 0.218 and 0.227 kg/l that we have reported for Express-Ion Q in other process scale axial flow column designs [23,24], presumably a result of the bi-directional mode of packing and bed consolidation. This higher packing density may be of benefit for the isolation of small molecules as discussed below. Following packing we could operate the Side Pack unit at 280 cm/h and have reported the process-scale separation of egg-white proteins following the process flow summarised in Section 4 without difficulty [24]. An analytical loading and process loading of hen egg-white feedstock on Express-Ion Q in the Side Pack column operating at 280 cm/h are presented in Fig. 7.

Following use the Side Pack column required disassembly to facilitate unpacking which for the reasons discussed above may be undesirable. In keeping with the other fixed volume designs described above there is inflexibility in column volume and so the process engineer must design the process flow around these fixed adsorbent volumes.

5.4. Adjustable volume slurry pack axial flow

In each of the three column formats described above the column volume was fixed, such that the feedstock volumes, etc., would be tailored to meet the chromatographic performance of a fixed volume

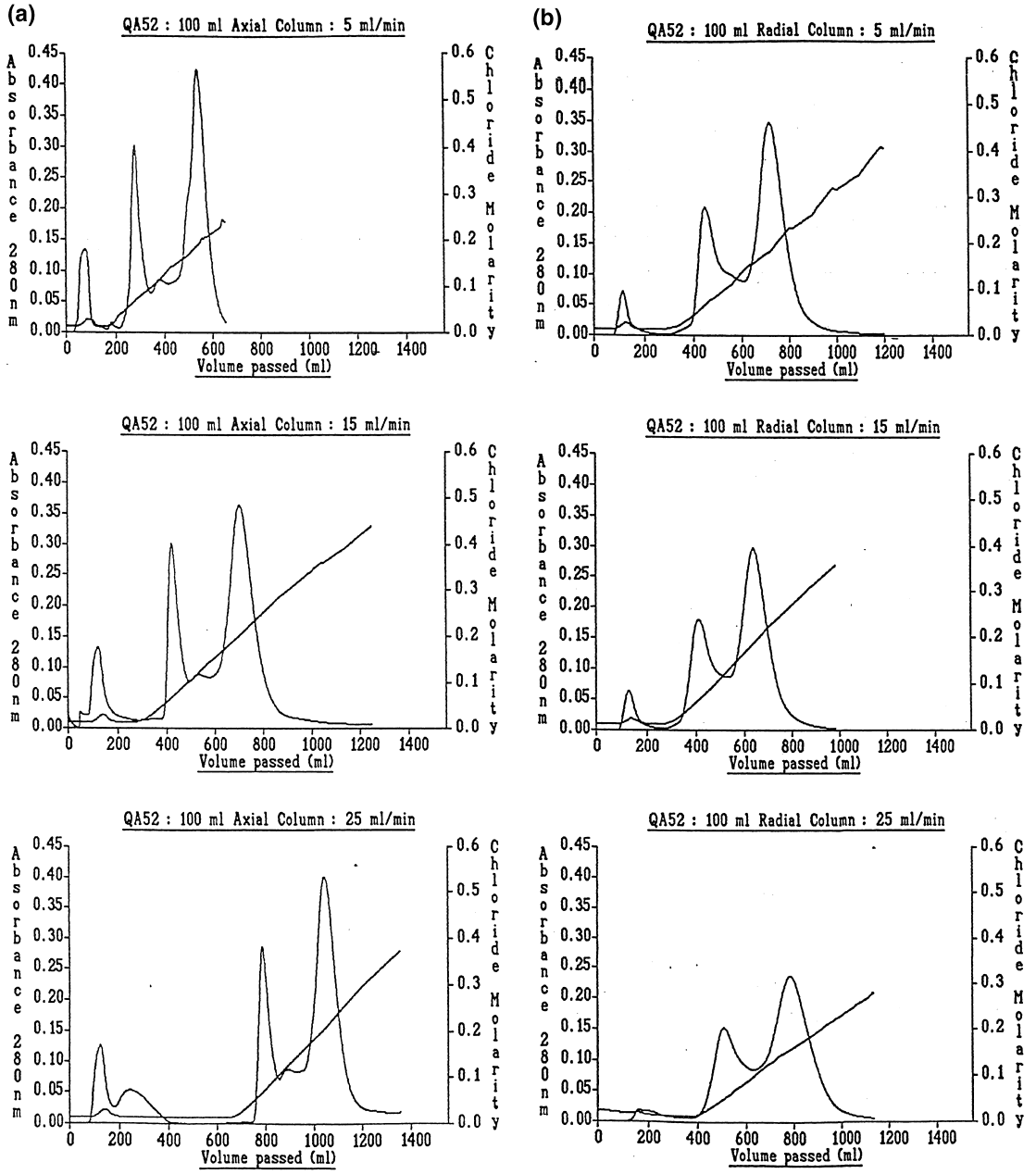

Fig. 6. Chromatography of hen egg-white proteins on QA52 in 0.025 *M* Tris–HCl buffer, pH 7.5 at flow-rates of 5–25 ml/min in (a) 100 ml axial flow column (6.6 cm×4.4 cm I.D.) and (b) 100 ml Superflo-100 radial flow column.

of ion exchanger. This may be unattractive to the user since using too much feedstock may lead to column overload and early breakthrough whereas too large a column makes inefficient use of costly adsorbent. In order to overcome these issues and add flexibility to the application adjustable volume axial

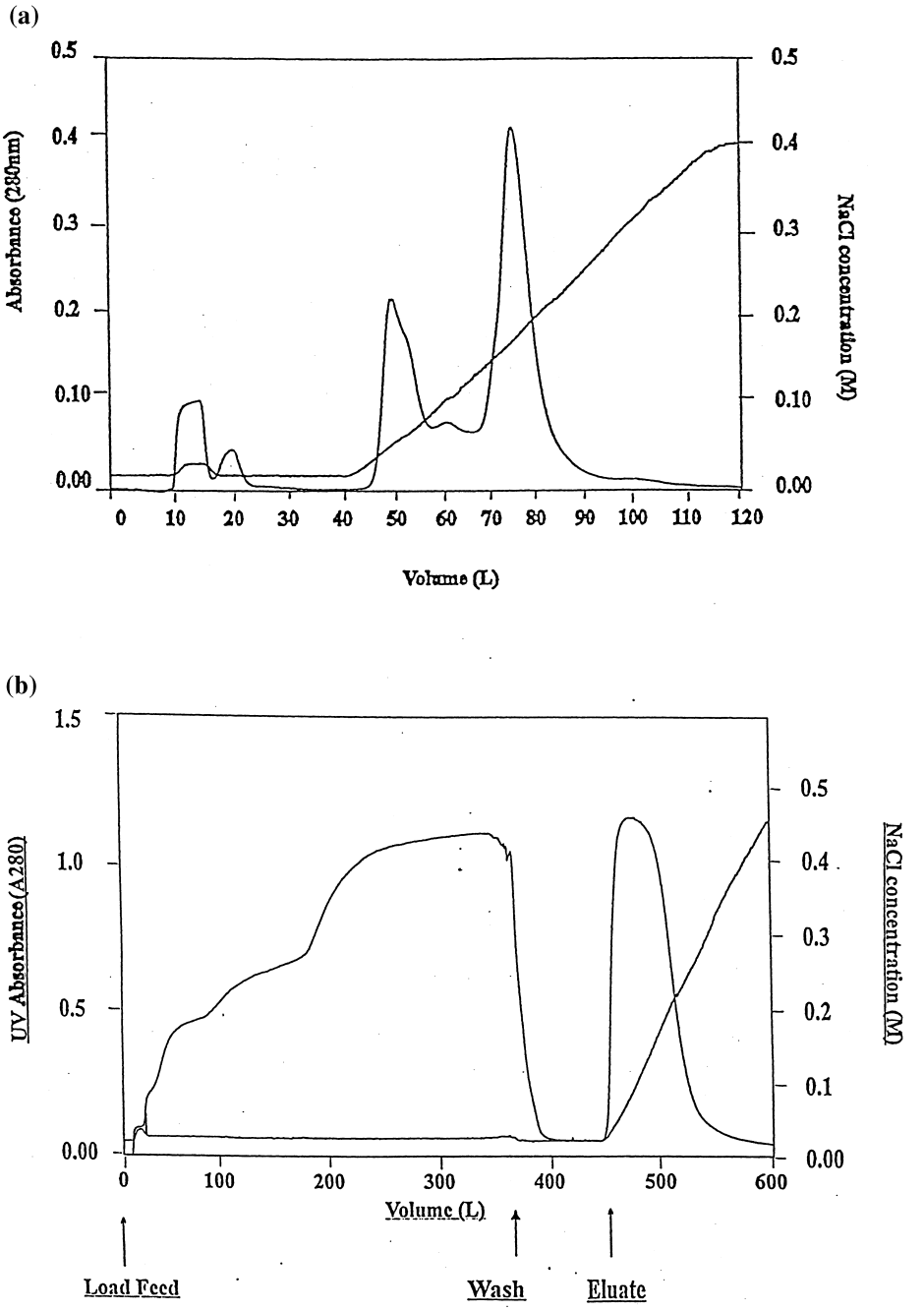

Fig. 7. Chromatography of hen egg-white proteins on Express-Ion Q using 0.025 *M* Tris–HCl buffer, pH 7.5 at a flow-rate of 280 cm/h using the Side Pack column (16 cm×35 cm I.D.) with loadings of (a) 44 g and (b) 2.2 kg total protein.

flow columns were designed. While a number of designs are available from several manufacturers the principle remains the same, namely bed height can be altered by vertical adjustment of a moveable upper flow adapter that houses the top bed support. One system that we have worked with is the Moduline range of columns from Millipore. Our system is the G450 x 500 unit, comprising a 45 cm I.D. column barrel that is 50 cm in length. In order to pack this unit, the slurry of ion exchanger is poured or pumped into the open column barrel assembly, which may be fitted with an extension tube if the volume of slurry exceeds ~75 l, with the outlet port closed. The upper flow adapter is rapidly fitted to the column assembly to minimise gravity settling of the adsorbent. The outlet port is opened and the adsorbent bed consolidated by pumping packing buffer through the system in downflow, typically at constant pressure.

Following bed consolidation the column is depressurised, headspace buffer removed, the upper flow adapter positioned on top of the bed. The bed is then physically compressed using the upper flow adapter 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. Furthermore, as the column diameter increases, the upper flow adapter becomes heavy and is cumbersome to manoeuvre, often requiring a mechanical lowering assembly. Additionally, its positioning can be a labour intensive operation, in order to ensure that no air is trapped under the flow adapter that could affect column performance. Using a 45 cm I.D. column of this design, we found the reproducibility of this operation to be highly operator dependent. We observed that small differences of ±1 cm in bed over or under compression would result in significant changes in the pressure/flow performance of the packed column [19].

In that study DE52 (20 kg) was packed into the column at a pressure of 10 p.s.i. Following depressurisation this gave a relaxed bed height of 17.6 cm and packing density of 0.198 kg dry mass/l bed volume. The bed was then manually compressed using the flow adaptor by up to 3 cm, and flow-rate measured at 10 p.s.i. These data are summarised in Table 2 with a linear relationship of the form:

$$y = 14.74x - 190 \quad (r = 0.9997; n = 6)$$

Table 2
Influence of bed height compression on packing density of a 45 cm diameter column containing DE52 and flow-rate at 10 p.s.i. in 0.025 *M* Tris–HCl buffer, pH 7.5

Bed height (cm)	Packing density (dry kg DE52/l column volume)	Flow-rate at 10 p.s.i. (cm/h)
17.6	0.198	69.6
17.2	0.203	63.6
16.0	0.218	46.2
15.5	0.225	38.0
15.0	0.232	30.7
14.5	0.240	24.4

It is evident that small variability in packing techniques could have major process implications and without adequate control this effect would have an impact on process economics and likely process validation.

We have carried out a number of process-scale evaluations using this column system, including Express-Ion D [13] and Express-Ion Q [23], at flow-rates of up to 225 cm/h following the process flow outlined in Section 4. The chromatography of egg-white proteins using Express-Ion Q with both analytical loading and process loading are presented in Figs. 3 and 1, respectively.

In a comparative study using DE52 we ran analytical loadings of hen egg-white feedstock at ~37 cm/h in both the fixed bed volume PREP-25 column (Section 5.1) and the adjustable bed volume Moduline G450 column. In each case bed heights were 16 cm. These chromatograms are presented in Fig. 8 and are essentially identical. This demonstrated the consistency in performance of these two axial flow systems with the added flexibility of adjustable volume associated with the Moduline system.

In the slurry pack format unpacking of the column following use is often a manual process. Typically, the upper flow adapter or column top section is removed and then the user manually excavates 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 section can prove a physical challenge! Our experiences have shown column unpacking to take at

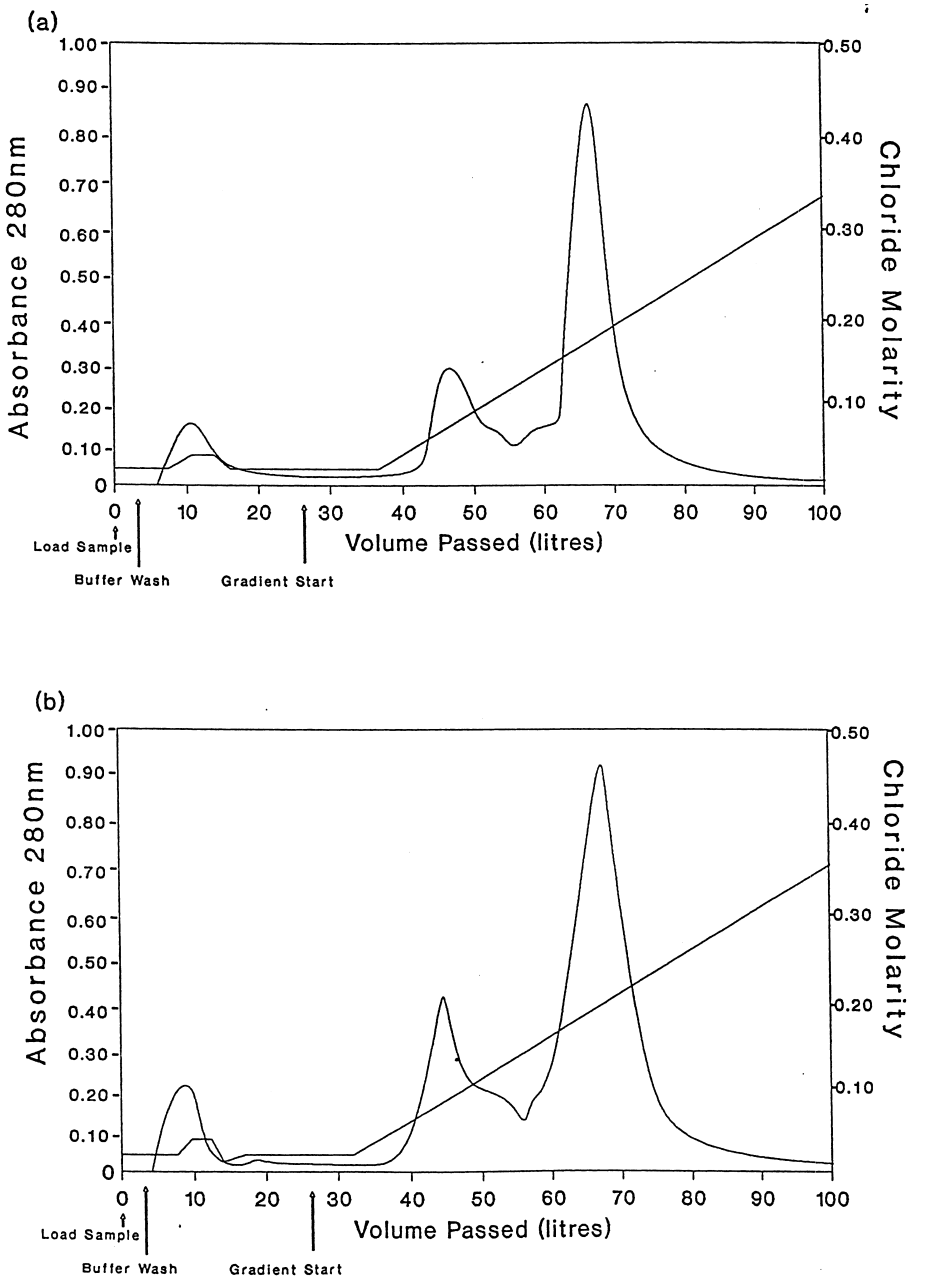

Fig. 8. Chromatography of ~100 g hen egg-white proteins on DE52 in 0.025 *M* Tris–HCl buffer, pH 7.5 at a flow-rate of ~30 cm/h in (a) the PREP-25 column (16 cm×45 cm I.D.) or (b) the Moduline column (16 cm×45 cm I.D.).

least 60 min an observation in keeping with the Side Pack column and PREP-25 which both took two operators 60 min to disassemble, unpack, clean and reassemble.

5.5. Adjustable volume pump pack axial flow

Based on the discussion in the previous sections on axial flow columns a system offering the advan-

tages of adjustable volume with the benefits of pump packing would appear highly desirable. Such systems have recently become available and their design and function is described in the literature [25,26]. These columns fulfil the criteria stated above but they are also configured for in situ pump unpacking. This facility addresses many of the concerns expressed in the previous sections. We evaluated a 50 cm×44 cm I.D. IsoPak column (Millipore) which had been adjusted to give a 16 cm bed. The column was packed with Express-Ion Q and a series of process-scale separations carried out at flow-rates of up to 300 cm/h following the process flow summarised in Section 4 [24]. An analytical loading and process loading of hen egg-white feedstock on Express-Ion Q in the IsoPak column operating at 300 cm/h are shown in Fig. 9.

The column packing process was a simple procedure taking one operator ~10 min to complete. Furthermore, column unpacking using the pump was a simple procedure again taking one operator ~10 min to complete. Using this design of column, the packing process is carried out in up-flow. This enables air to be displaced from the column during packing.

This design of column is suitable for the suspended bed technique mentioned above (Section 1.1) [5,6]. This has been demonstrated not only with a clarified hen egg-white feedstock [5] but also recently with an unclarified feed [27]. The process under evaluation was the anion-exchange chromatography of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) from milled Bakers' yeast. Method scouting demonstrated the optimal conditions to be using Express-Ion Exchanger Q at pH 7.5 and a biomass concentration of 3.5% (w/v). Under these conditions we could isolate partially purified G3PDH directly from the yeast disruptate. A small-scale system was developed at 1/756 scale and this was used to scale-up the process using a 44 cm I.D. IsoPak column, 18 kg of Express-Ion Q and 275 l of 3.5% (w/v) Bakers yeast disruptate. The results demonstrated the utility of suspended bed chromatography with the IsoPak column for direct adsorption from an unclarified feed. Aside from protein purity and mass balance criteria, this study demonstrated significant concentration of the G3PDH, reduction in turbidity of the preparation and removal of double-stranded DNA-like material. The elution data are presented in Fig. 10.

6. Comparative performance of different column formats

The studies which have been described in the preceding sections generate several items of comparable information. It should be noted that this work has been ongoing since the late 1980s and it has not been possible to compare one ion-exchange system across all column types primarily due to the development and introduction of new ion-exchange media throughout this period. However we are able to compare the performance of a DE52 system using the PREP-25, Superflo-10L and Moduline G450 and the performance of an Express-Ion Q system using the Moduline G450, Side Pack—16 l and IsoPak—44 cm systems. From these studies a number of general observations can be made:

(i) Pump packing is a simpler process in comparison with slurry packing and we consider it to be the more reproducible technique.

(ii) Pump unpacking is far simpler, faster and potentially less hazardous than manual unpacking.

(iii) If column inlet bed supports become fouled by components of the feedstock, and requires cleaning and/or replacement, then this may be easier and less disruptive in axial flow systems.

(iv) Where less rigid adsorbents are used, that may compress at higher pressures, radial flow columns offer superior flow performance to axial columns when used at similar pressure drops.

(v) The hardware design can influence the pressure/flow performance of the packed column. For example at similar pressures the IsoPak system containing Express-Ion Q supported flow-rates at least 50% greater than the Moduline system.

(vi) The column design and approach to packing influences the column packing density. Data generated in the various systems are summarised in Table 3. The packing densities obtained between the PREP-25 and Moduline columns are very similar. This may be anticipated since both units are depressurised immediately following packing and prior to final use, even though one column is pump packed whilst the other is slurry packed. On the other hand,

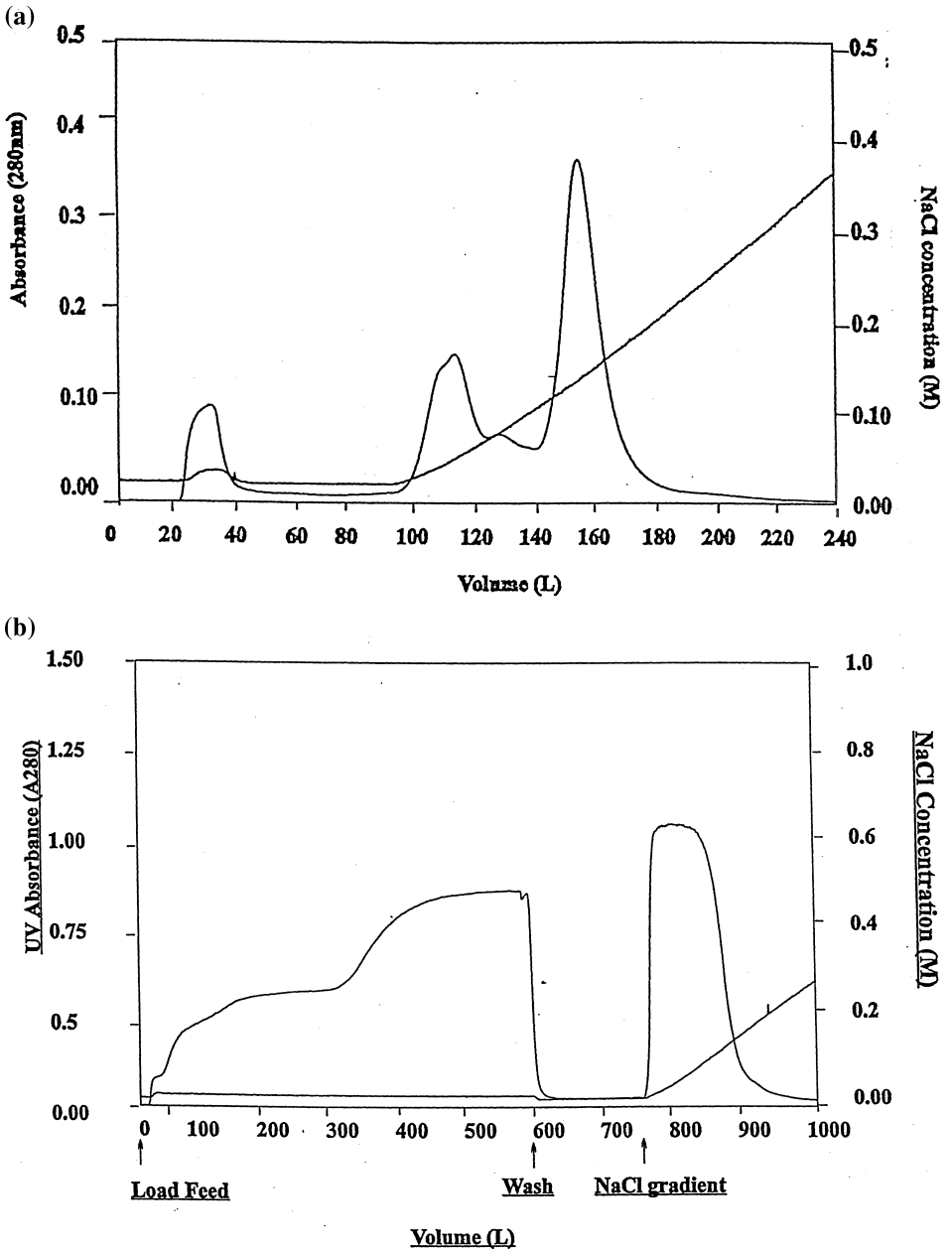

Fig. 9. Chromatography of hen egg-white proteins on Express-Ion Q using 0.025 *M* Tris–HCl buffer, pH 7.5 at a flow-rate of 300 cm/h using the IsoPak column (16 cm×44 cm I.D.) with loadings of (a) 70 g and (b) 3.4 kg total protein.

the IsoPak column appears to have a slightly higher packing density compared to the Moduline system presumably due to the fact that pressure is maintained across the bed throughout all stages of packing and equilibration. The radial flow columns have a lower packing density than the axial flow columns and the Side Pack column has a higher packing density than the other axial flow columns. We attribute these differences to the specifics of the packing processes which are unique to these column

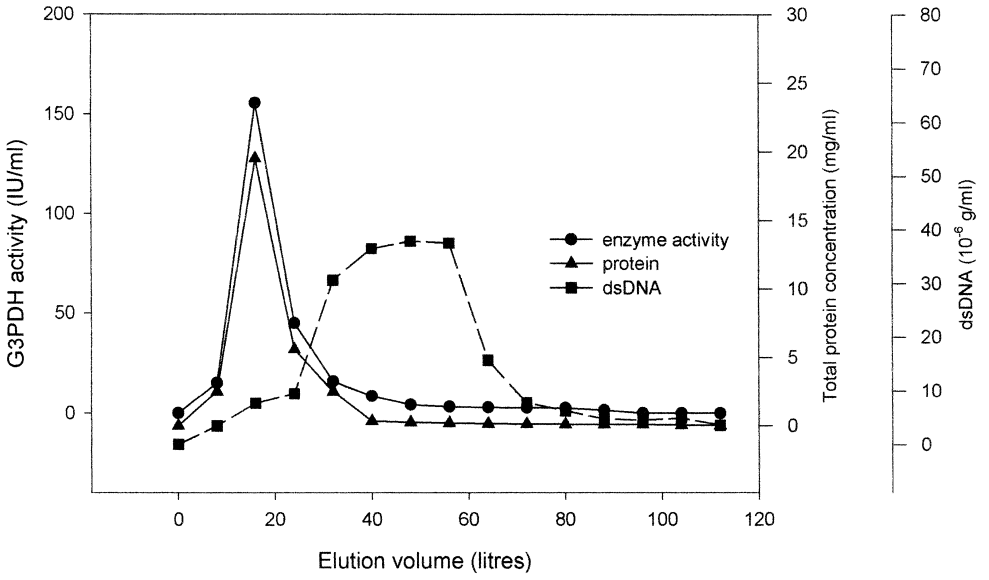

Fig. 10. Chromatography of unclarified 3.5% (wet w/v) Bakers' yeast disruptate on Express-Ion Q using 0.01 *M* Tris–HCl buffer, pH 7.5, containing 0.5 *M* NaCl and 0.001 *M* EDTA at a flow-rate of 158 cm/h using the IsoPak column (13 cm×44 cm I.D.) following suspended bed adsorption.

designs. Although the masses of adsorbent packed in these sets of columns varied we did not see any significant differences in protein capacity for the adsorbents tested. This is not unexpected since issues of pore diffusion and location of functional groups affect the dynamic binding capacities for large molecules and it is assumed that similar accessibility to target protein, namely ovalbumin, is consistent regardless of these differences in packing density. However this may not be the case in small molecule purification. We have shown that when purifying a small bioactive hexapeptide by cation exchange [28], the binding capacity correlates stoichiometrically to the number of functional groups present in the column. In this case the higher packing density obtained in a column such as Side Pack may directly offer capacity benefits. It should be noted that these capacity benefits might be offset by the increased packing cost since there is a need to pack more ion exchanger into the column.

(vii) Scale-up from small units to larger units was predictable and straightforward in all systems evaluated as described above.

(viii) Clean-in-place protocols were effective in all systems tested and these are reported in more detail in the relevant publications.

Table 3
Packing densities of ion-exchange celluloses in various column formats

Adsorbent	Column	Packing density (dry kg/l)	Ref.
DE52	PREP-25	0.194	[15]
DE52	Moduline G450	0.198	[15]
DE52	Superflo-10L	0.117	–
Express-Ion D	PREP-25	0.210	[13]
Express-Ion Q	Moduline G450	0.218	[22]
Express-Ion Q	Side Pack, 16 l	0.303	[23]
Express-Ion Q	IsoPak, 44 cm	0.227	[23]

7. Conclusions

The process chromatographer is faced with the dilemma of media selection and column hardware section during process development. In our opinion, and based on studies reported here, while column designs are different their comparative performance in large-scale ion-exchange processes is less variable. Clearly there are differences in their mode of

operation and ease of use and these may be the decision-making factors in column selection. It has been outside of the scope of this article to consider other protein systems or other chromatographic media, but in both our own work and that disseminated through the technical literature many of the observations and conclusions reported here are confirmed. Scaling up a process from laboratory bench, through pilot scale to manufacturing has often been viewed with some trepidation and this to some extent was manifested by the limited availability of column formats and designs.

For axial flow systems it is accepted that increasing the column diameter whilst maintaining bed height and linear velocity (Fig. 8) is the approach for scaling-up a process. However this stage of scale-up is often regarded as a time-consuming, labour-intensive challenge. With the recent innovations in column technology that we have evaluated above we consider that scaling-up to a large column may now be regarded as a simple, rapid and routine stage of a chromatographic separation.

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