Direct isolation of monoclonal antibodies from tissue culture supernatant using the cation-exchange cellulose Express-Ion S

Graeme Denton[a], Andrea Murray[b], Michael R. Price[a], Peter R. Levison[c],*

[a]*Cancer Research Laboratories, School of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK*
[b]*Academic Unit of Medical Physics, Queen's Medical Centre, University Hospital, Nottingham NG7 2UH, UK*
[c]*Whatman International Ltd., Springfield Mill, James Whatman Way, Maidstone, Kent ME14 2LE, UK*

Abstract

The chromatography of the murine hybridoma cell C595/102 culture supernatant expressing the therapeutic monoclonal antibody C595, on the cation-exchange cellulose Whatman Express-Ion Exchanger S has been investigated. Initial method scouting studies using purified C595 in 1-ml mini columns demonstrated that binding capacity and binding efficiency were dependent not only on decreasing pH but also on the buffer salts used to prepare the mobile phase. Under optimised conditions of 0.1 M sodium acetate buffer, pH 5.0, we were able to separate purified C595 from BSA, the major contaminant in tissue culture fluid. Under these conditions immunoreactive C595 could be isolated directly from tissue culture supernatant. A scale-down study was carried out using a 25-ml column operated at a flow-rate of 150 cm/h which also yielded purified immunoreactive antibody. This procedure should now be suitable for scale-up. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Immunoglobulins may be isolated from their respective host expression systems by several chromatographic techniques. Affinity chromatography using, for example, immobilised antigens or group specific ligands such as Protein A, G or L is well recognised in the field. Protein A adsorbents are established in the purification of monoclonal antibodies [1], although attention has been drawn to potential validation issues associated with the leakage of the Protein A ligands from the adsorbent matrix during use [2]. Antibodies have been reported

to have isoelectric points within the range 6–8 [3] and, therefore, are ideal for purification by ion-exchange. Ion-exchange chromatography, whilst less selective than affinity, offers an opportunity for the isolation of immunoglobulins, either using anion-exchange at pH>pI or cation-exchange at pH<pI. Examples of both approaches are reported in the literature [3]. In traditional immunoglobulin purification from plasma, ion-exchange chromatography is widely used in a negative step [4] where the immunoglobulins pass unretained through an anion-exchange column and many contaminating proteins including albumin bind. This can be effected since immunoglobulins are relatively de-ionised around pH 7, whereas albumin is anionic due to its more acidic isoelectric point of ca. 4.7. We have reported such a separation in the isolation of immunoglobulins from

goat serum [4,5]. On the other hand immuno-globulins can also adsorb to various cation-ex-changers at pH<7 [3,4,6] and this can be exploited as a positive chromatographic step. We reported the separations of an IgG2a monoclonal antibody [7] from murine hybridoma L243 tissue culture fluid using the cation-exchange cellulose, Whatman SE53, at pH 5.5 [4]. More recently several other groups have described the use of various cation-exchange chromatography media for the purification of mono-clonal antibodies from various cell lines [8–10]

Mucus glycoproteins or mucins defined by the MUC1 gene are large, extensively glycosylated transmembrane macromolecules [11] that have had a considerable impact as markers of many human carcinomas. Measurement of these molecules in blood and serum samples gives an indication of patient tumour burden and may be used to provide a clinical guide, reflecting disease recurrence, progres-sion or response to therapy. The majority of clinically relevant anti-MUC1 antibodies recognise repeated epitopes within the hydrophilic region, APDTRPAPG, of the protein core, enabling these motifs to be exploited as tumour associated markers [12].

One such antibody is a murine monoclonal anti-body, C595 (IgG3, κ), also known as NCRC-48. C595 recognises the epitope RPAP within the MUC1 mucin protein core [13,14] and has a proven track record of clinical utility [15–20]. A therapeutic clinical trial using ^{67}Cu-labelled C595 for the treat-ment of superficial bladder cancer is due to com-mence in 2000. The ability to purify C595 from a biological sample in an efficient and cost-effective process is therefore essential for its development as a diagnostic or therapeutic modality. As a representa-tive of the vast number of monoclonal antibodies globally produced with potential use in therapeutic strategies, C595 serves as a suitable model to assess the potential use of single step chromatographic purification strategies in the development of such reagents.

In the present study we have examined the chro-matography of the monoclonal antibody C595 from murine hybridoma cell culture supernatants using the cation-exchange cellulose, Whatman Express-Ion S. The Express-Ion range of ion-exchange celluloses were designed for use in large-scale column based

processes. We have reported their chromatographic performance in several process column designs at volumes of up to 25 l [21–23] where flow-rates of over 300 cm/h were demonstrated. On the basis of our previous studies on the cation-exchange chroma-tography of immunoglobulins [4,5] we selected Express-Ion S as the preferred adsorbent. While the carboxymethyl derivatised Express-Ion C may have been suitable under higher pH conditions, as the pH approaches the pK_a of the carboxylic acid group (ca 4.5) so it would de-ionise which would not only affect the ionic adsorption capability of the adsorbent but could also give rise to unwanted secondary interactions [24]. On the other hand the sulphoxy-ethyl functional group on Express-Ion S will remain fully ionised to pH<0, so is better suited for this type of study.

In a typical process development, mobile phase optimisation may be carried out in a series of scouting experiments followed by several scale-down experiments to emulate a large-scale process sepa-ration. We have previously described such a scale-down study into the chromatography of a bioactive peptide by cation-exchange [25], and now we describe a number of approaches in the development of a purification of the C595 antibody.

2. Experimental

2.1. Materials

Express-Ion S and Express-Ion S Mini Columns were obtained from Whatman (Maidstone, UK). Phosphate buffered saline (PBS) tablets were from Oxoid (Basingstoke, UK). Purified C595 antibody (5.88 mg/ml in PBS) was obtained as described previously [26]. Bovine serum albumin (BSA) was obtained from Sigma (Poole, UK). Rabbit anti-mouse IgG HRP conjugate was obtained from Dako Ltd. (Ely, UK). All other chemicals were of a reagent grade or higher.

2.2. Tissue culture supernatants

Murine hybridoma cells C595/102 expressing the C595 antibody were grown in RPMI 1640 media (Sigma, Poole, UK) supplemented with 10% (v/v)

heat inactivated foetal calf serum (Sigma) and 2 m*M* glutamine (Sigma). The cells were grown beyond the log-phase of growth to allow for maximum antibody production which is up to ca. 70 µg of C595/ml culture fluid [26], in large (175 cm^3) tissue culture treated flasks (Nunc) at 37°C in an atmosphere containing 5% (v/v) CO_2. Supernatants were harvested by centrifugation at 48 000 *g* for 1 h, sterile filtered through a 0.2-µm membrane and NaN_3 [0.02% (w/v)] added. Supernatants were stored at 4°C for further use.

2.3. Mini column studies

2.3.1. Method scouting

An Express-Ion S mini column (1.9 cm×0.8 cm) was equilibrated with 0.1 *M* sodium phosphate buffer, pH 7.0 [27]. Purified C595 antibody [26] was diluted to a concentration of 200 µg/ml in 0.1 *M* sodium phosphate buffer, pH 7.0 and a 1-ml aliquot was loaded onto the column. The column was washed with 0.1 *M* sodium phosphate buffer, pH7.0 (10 ml) and bound material was eluted stepwise using 0.1 *M* NaCl, 0.2 *M* NaCl, 0.3 *M* NaCl, 0.4 *M* NaCl and 0.5 *M* NaCl in 0.1 *M* sodium phosphate buffer, pH 7.0 (1-ml steps). The flow-rate was maintained at 60 cm/h, collecting 1-ml fractions. All the procedures were carried out at room temperature (15–20°C). The complete study was repeated using 0.1 *M* sodium phosphate buffer, pH 6.4 and 5.8, and 0.1 *M* sodium acetate buffer, pH 5.8, 5.4 and 5.0.

2.3.2. BSA spiking study

An Express-Ion S mini column was equilibrated with 0.1 *M* sodium acetate buffer, pH 5.8. A sample of 20 mg/ml BSA in PBS (10 µl) was added to 200 µg/ml C595 in 0.1 *M* sodium acetate buffer, pH 5.8 (1 ml), and the sample was loaded on to the mini column. The column was washed with 0.1 *M* sodium acetate buffer, pH 5.8 (10 ml) and bound material was eluted stepwise using 0.1 *M* NaCl, 0.2 *M* NaCl, 0.3 *M* NaCl, 0.4 *M* NaCl and 0.5 *M* NaCl in 0.1 *M* sodium acetate buffer, pH 5.8 (1-ml steps). The flow-rate was maintained at 60 cm/h and was collected in 1-ml fractions. All the procedures were carried out at room temperature (15–20°C).

The complete study was repeated using 0.1 *M* sodium acetate buffer, pH 5.4 and 5.0.

2.3.3. Tissue culture supernatant

The C595 tissue culture supernatant (20 ml) was adjusted to pH 5.0 using 0.1 *M* acetic acid and loaded on to an Express-Ion S mini column, previously equilibrated with 0.1 *M* sodium acetate buffer, pH 5.0. The column was washed with 0.1 *M* sodium acetate, pH 5.0 (10 ml) and the bound material was eluted stepwise with 0.1 *M* NaCl, 0.2 *M* NaCl, 0.3 *M* NaCl, 0.4 *M* NaCl and 0.5 *M* NaCl in 0.1 *M* sodium acetate buffer, pH 5.0 (1-ml steps). The flow-rate was maintained at 60 cm/h, collecting 1-ml fractions. All procedures were carried out at room temperature (15–20°C).

2.3.4. Breakthrough study

The C595 tissue culture supernatant was adjusted to pH 5.0 using 0.1 *M* acetic acid and a sample (130 ml) was loaded on to an Express-Ion S mini column previously equilibrated with 0.1 *M* sodium acetate buffer, pH 5.0. The flow-rate was maintained at 60 cm/h, collecting 10-ml fractions. All the procedures were carried out at room temperature (15–20°C).

2.4. Scale-down study

The Express-Ion S was equilibrated with 0.1 *M* sodium acetate buffer, pH 5.0 and packed into a chromatography column (12.5 cm×1.6 cm I.D.) to give a volume of ca. 25 ml. The C595 tissue culture supernatant was adjusted to pH 5.0 using 0.1 *M* acetic acid and a sample (950 ml) was loaded on to the column. The column was washed with 0.1 *M* sodium acetate buffer, pH 5.0 (250 ml) and the bound material was eluted using a linear gradient of 0–0.5 *M* NaCl in 0.1 *M* sodium acetate buffer, pH 5.0 (250 ml). The flow-rate was maintained at 150 cm/h, collecting 10-ml fractions. All the procedures were carried out at room temperature (15–20°C).

2.5. Assays

Fractions obtained at various stages of chromatography were assayed for protein by absorbance at 280 nm and/or SDS–PAGE and for the presence of monoclonal antibody by enzyme linked immunosorbent assay (ELISA) and/or Western blotting.

2.5.1. ELISA

The immunoreactivity of isolated antibody fractions and material that had passed through the column was determined using a standard indirect ELISA procedure to determine antibody binding to MUC1 mucin dried to the wells of microtitre plates. Purified urinary mucin was adhered to the plate by overnight incubation at 37°C as previously described [26]. After blocking non-specific binding sites by incubation with PBS containing 1% (w/v) BSA, antibody preparations were diluted appropriately and added to the microtitre plate (50 µl per well) in triplicate. After incubation for 1 h, the plates were washed four times with PBS containing Tween 20 [0.05% (v/v)]. HRP-conjugated rabbit anti-mouse immunoglobulin (Dako), diluted to 0.1% (v/v) in PBS containing 1% (w/v) BSA (50 µl) was added to each well and the plates were incubated for 1 h. After a final wash, ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); Merck] chromogenic substrate was added (50 µl/well). ABTS was prepared as a 0.033% (w/v) solution in 0.1 M citrate phosphate buffer (pH 4) containing 33% (v/v) hydrogen peroxide (1 µl/ml). The presence of an immunoreactive anti-MUC1 mucin antibody in each well was determined by absorption at 405 nm using a Milenia Kinetic Analyser (Diagnostic Products Corporation, Llanberis, Wales, UK).

2.5.2. SDS–PAGE and western blotting

SDS–PAGE was performed using a PhastSystem Separation and Control Unit (Pharmacia). Chromatographic fractions were diluted to 50% (v/v) using 0.1 M EDTA, pH 8.0 containing 0.5% (w/v) SDS, 40% (w/v) sucrose and 0.05% (w/v) bromophenol blue and boiled for 5 min. The samples were applied to a 12.5% (w/v) homogeneous polyacrylamide PhastGel precast gel and electrophoresed according to the manufacturer's instructions. Protein bands were visualised by silver staining using the PhastGel silver staining kit according to the manufacturer's instructions. Phast Gels forwarded for Western blotting were processed using the Phast Gel semi-dry Western blot transfer unit (Pharmacia) onto nitrocellulose paper (Biorad, Hemel Hempstead, UK) using Western blot transfer buffer (25 mM Tris, 190 mM glycine in a 1:4 methanol–water mixture). After transfer, the membrane was incubated for 1 h in 0.1% (w/v) casein in PBS to block non-specific binding sites. After four washes with PBS–Tween, the membrane was incubated with 0.1% (v/v) rabbit anti-mouse–HRP conjugate (Dako) in PBS for 1 h. After four washes with PBS–Tween the blot was developed using 20 ml of a solution containing 0.4% (w/v) 3-amino-9-ethylcarbazole in DMF (0.4 ml), 50 mM sodium acetate buffer, pH 5.5 (19 ml), PBS–Tween (0.5 ml) and hydrogen peroxide (0.1 ml) (3-AEC substrate).

3. Results and discussion

When developing a separation mobile phase optimisation studies provide a useful starting point. We have demonstrated the significant influence of buffer composition on the separation of a bioactive peptide on Express-Ion C [25] and egg-white proteins on QA52 [28]. The influence of the pH of a 0.1 M sodium phosphate buffer over the range 5.8–7.0 on the protein mass-balance and relative binding capacity of Express-Ion S for purified C595 antibody under our experimental conditions is summarised in Table 1 and represented in Fig. 1. As pH is reduced so the antibody should become more cationic as it moves further away from its isoelectric pH. Our data support this since binding efficiency increases as the pH is lowered. In order to reduce pH further, we moved to 0.1 M sodium acetate buffers over the pH range 5.0–5.8. The adsorptive performance of Express-Ion S for purified C595 antibody under these conditions is summarised in Table 1 and represented in Fig. 2. These data demonstrate improved performance at lower pH. Our results reveal two interesting observations. Firstly, there is a major performance difference between the adsorption of C595 antibody to Express-Ion S in sodium acetate buffer compared to sodium phosphate buffer at the same pH (Table 1). Secondly, the influence of pH on the separation is very marked over the range 5–7. While this may be anticipated, it should be remembered that pH is a logarithmic measure. Accordingly as the pH moves away from the pI of a protein so its ionic properties would increase but following an exponential relationship. Consequently, one might have anticipated a greater influence of pH closer to the pI and less of an effect further away, since the

Table 1
Influence of pH on the protein mass balance during the adsorption–desorption of C595 antibody to Express-Ion S

Buffer	pH	Non-bound C595 (μg)	Eluted C595 (μg)	Effective C595 capacity (μg/ml)
Sodium phosphate	7.0	147	48	50
	6.4	175	61	64
	5.8	169	73	76
Sodium acetate	5.8	12	204	214
	5.4	34	195	204
	5.0	0	253	265

relative ionisation as a function of pH should be greater closer to the pI. These two effects of mobile phase composition on this adsorbent: adsorbate interaction could have a profound effect on subsequent process development yet neither may have been predicted. This clearly demonstrates the benefits of such a small scale and rapid scouting study. On the basis of these observations all subsequent studies utilised the sodium acetate buffer systems.

In tissue culture supernatant containing foetal calf serum, BSA will be a major contaminant. In order to demonstrate selectivity between C595 antibody and BSA using Express-Ion S, the BSA-spiking study was carried out. The separation of equal masses of C595 antibody and BSA in 0.1 M sodium acetate buffer over the pH range 5.0–5.8 is represented in Fig. 3. The data show that protein separates between retained and unretained material. While each fraction contains immunoreactive material, it should be noted that this ELISA assay is at best only semi-quantitative so detailed interpretation of the immunoreactive response is impractical. Nevertheless separation of immunoreactive material appears to be better at pH 5.0 (Fig. 3c) compared to pH 5.4 (Fig. 3b) or pH 5.8 (Fig. 3a) in agreement with the earlier scouting study (Fig. 2). The SDS–PAGE analysis of the various components of the separation using 0.1 M acetate buffer is presented in Fig. 4. At pH 5.0 (Fig. 4c), our data show that the protein eluting from the column after desorption (lanes 4 and 5) has a molecular weight similar to that of the C595 antibody (lane 6), whereas non-bound material (lanes 1 and 2) has a similar molecular weight to BSA (lane 3). There was no indication of BSA contamination of the bound material. Similar results were obtained for the sepa-

rations carried out at pH 5.4 (Fig. 4b) and pH 5.8 (Fig. 4a).

These initial sets of experiments demonstrated that the use of 0.1 M sodium acetate buffer, pH 5.0 gave the optimal separation. However, it must be emphasised that these conditions were developed using purified proteins and may not mirror the effects occurring in tissue culture supernatants where other components are present which may influence the separation. The separation of 20 ml of C595 cell culture supernatant on an Express-Ion S mini column is represented in Fig. 5, expressed in terms of immunoreactive material. The feedstock gave a background ELISA response of ca. 5 mOD/min, and the data clearly demonstrate elution of immunoreactive material following the desorption steps, and at a higher concentration than that present in the feed. The SDS–PAGE analysis of this separation is presented in Fig. 6. Lane 1 revealed protein bands corresponding to M_r ca. 50 kDa and ca. 25 kDa for reduced C595 and lane 2 revealed a protein band corresponding to M_r ca. 150 kDa for non-reduced C595, as previously described [26]. The tissue culture supernatant (lane 3) contained several protein bands, and following chromatography on Express-Ion S, desorbed material (lanes 4–6) had protein bands of a similar M_r to the C595 antibody. This eluted protein of M_r ca. 150 kDa was proven to be a murine antibody, following Western blotting with a rabbit anti-mouse IgG-HRP conjugate (Fig. 7).

When designing a process-scale separation it is desirable to use as much of the column capacity as possible in a single contacting operation. In order to assess the column volume required to handle the volume of feedstock available, a breakthrough study
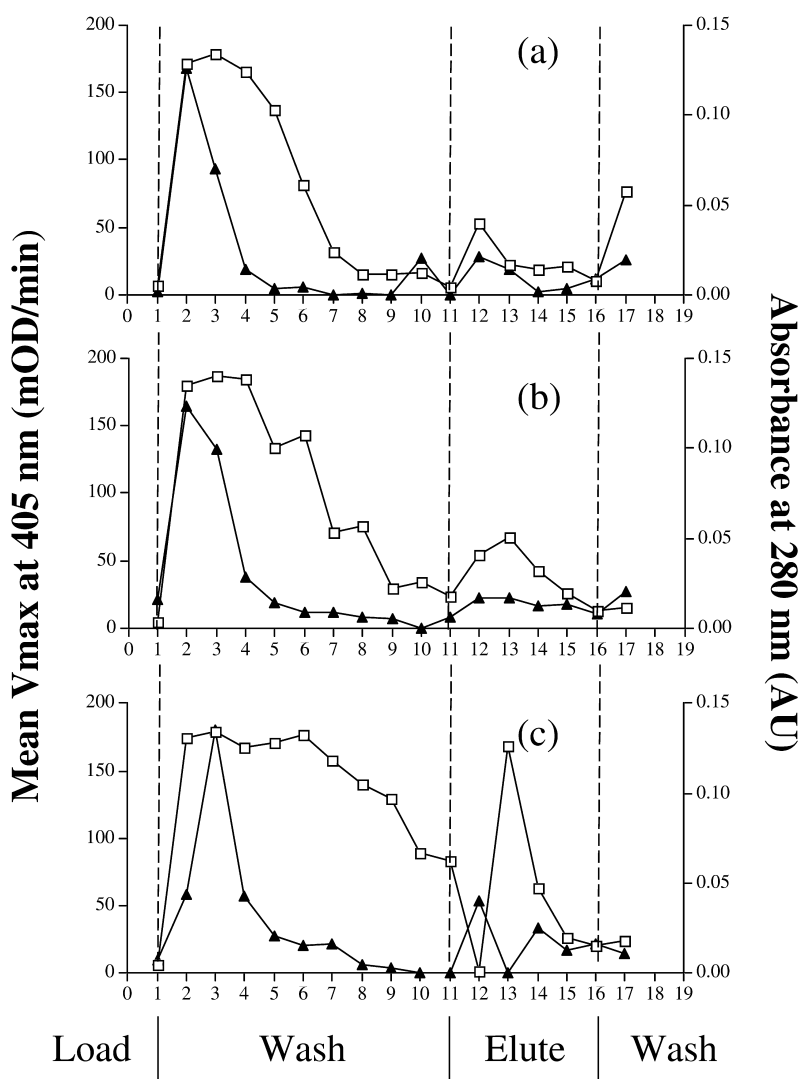
Fig. 1. Chromatography of 200 μg of purified C595 on an Express-Ion S mini column at a flow-rate of 60 cm/h using 0.1 *M* sodium phosphate buffer at (a) pH 7.0 (b), pH 6.4 and (c) pH 5.8, collecting 1-ml fractions. Fractions were analysed by (□) ELISA and (▲) absorbance at 280 nm.

would typically be carried under scale-down conditions. We have reported such studies previously [21]. In this work we applied C595 tissue culture supernatant to a mini column containing Express-Ion S to assess the breakthrough point. These data are summarised in Fig. 8. In this case we monitored an immunoreactive C595 antibody by ELISA using MUC1 mucin coated microtitre plates. In this assay the culture supernatant gave a background ELISA

response of ca. 24 mOD/min, which is higher than the baseline noise level of 12–25 mOD/min generated in our assay system. The data demonstrate adsorption of the monoclonal antibody from the culture supernatant over the first 60 ml loaded with breakthrough occurring after 70–80 ml feed had been loaded. Based on a typical expression level of 70 μg/ml in this tissue culture system [26], an Express-Ion S Mini Column has an apparent capacity
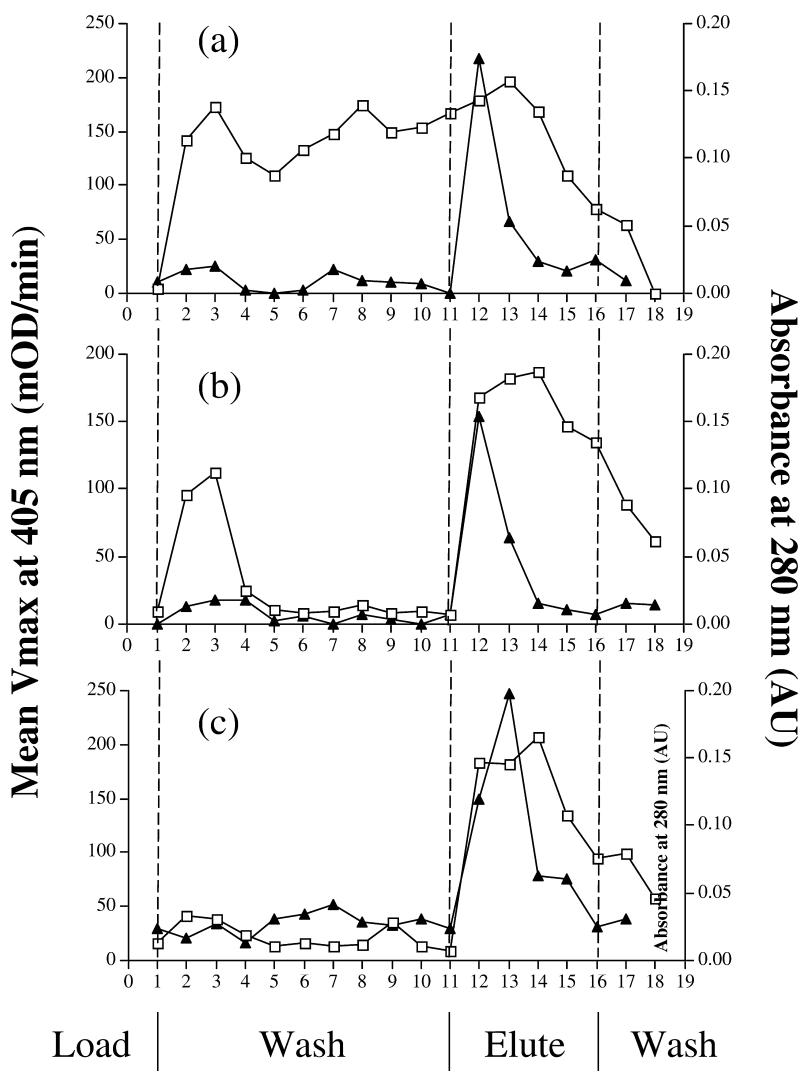
Fig. 2. Chromatography of 200 μg of purified C595 on an Express-Ion S mini column at a flow-rate of 60 cm/h using 0.1 *M* sodium acetate buffer at (a) pH 5.8 (b), pH 5.4 and (c) pH 5.0, collecting 1-ml fractions. Fractions were analysed by (□) ELISA and (▲) absorbance at 280 nm.

for C595 of 5–6 mg/ml under our mobile phase conditions which is of similar order to several Protein A adsorbents [1,2].

In our previous work using Express-Ion exchangers [21] we have carried out scale-down studies using 25-ml columns with bed heights of up to 20 cm and flow-rates of 150–300 cm/h. These have been found to scale-up linearly 1000-fold to 25-l columns. In this work we carried out a similar

scale-down study using a 25-ml column of Express-Ion S operating at a flow-rate of 150 cm/h. The results of this study are presented in Fig. 9. As observed in the previous study, the culture supernatant gave a background ELISA response of ca. 24 mOD/min, which is higher than the baseline noise level of 5–15 mOD/min generated in our assay system. The data show that antibody breakthrough had not occurred after ca 950 ml of tissue culture
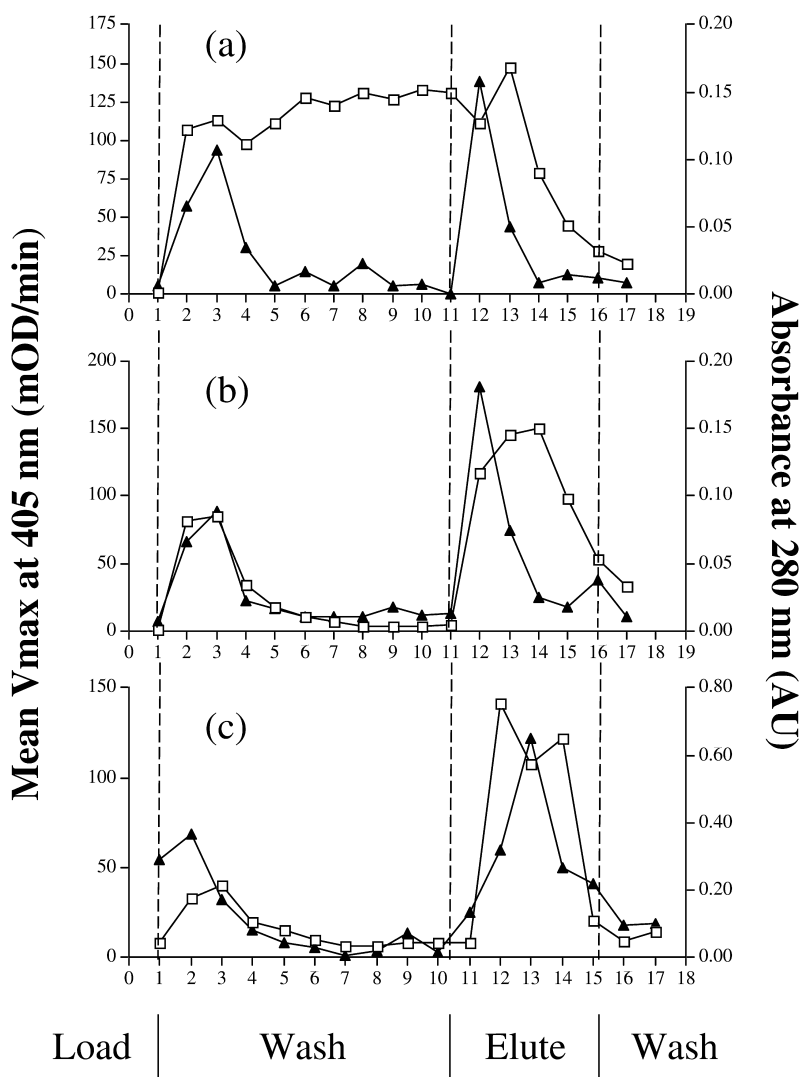
Fig. 3. Chromatography of 200 μg of purified C595 on an Express-Ion S mini column in the presence of 200 μg of BSA at a flow-rate of 60 cm/h using 0.1 M sodium acetate buffer at (a) pH 5.8 (b), pH 5.4 and (c) pH 5.0, collecting 1-ml fractions. Fractions were analysed by (□) ELISA and (▲) absorbance at 280 nm.

supernatant has passed through the column. This is to be expected based on the earlier breakthrough study and assuming lot-to-lot consistency between cell cultures, then breakthrough might have been anticipated after the passage of 2–3 l of culture supernatant through the column. After washing the column, immunoreactive C595 antibody was eluted from the Express-Ion S during the salt gradient. This material could be further purified by other chromatographic techniques as appropriate.

In the present study we have investigated some of the method scouting and mobile phase optimisation studies which can be carried out during the early stages of process development. We used Express-Ion S as our chosen ion-exchanger since this has been shown to be effective in the large-scale separation of proteins from 900 l of 10% (v/v) egg-white feed-stock using a 25-l column [29]. Furthermore it will withstand a cleaning and sanitisation regime appropriate to the type of validation requirements of the

Lane

(a)   (b)   (c)
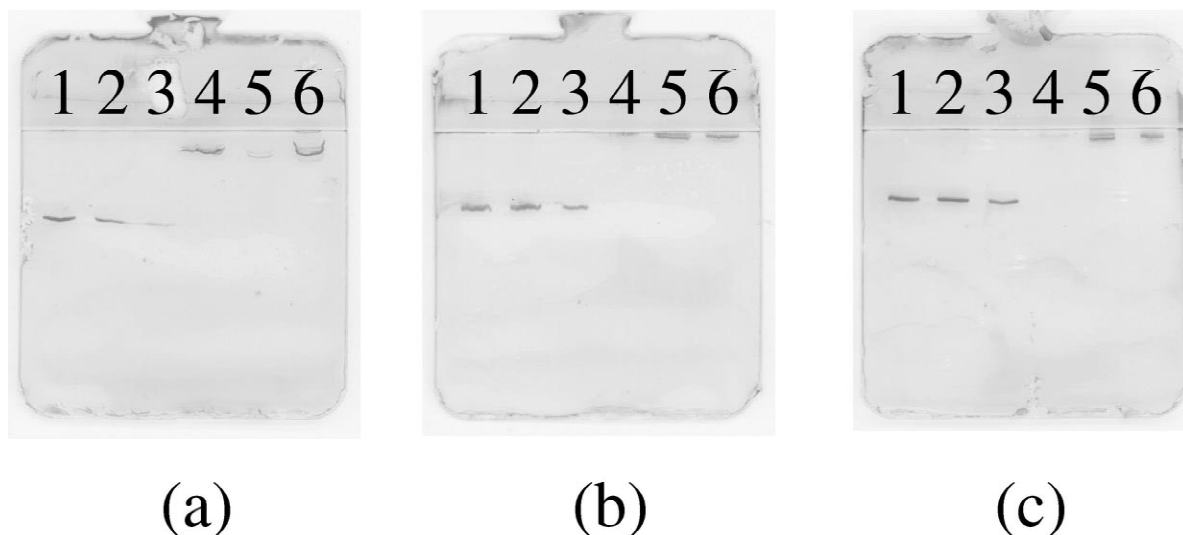
Fig. 4. SDS–PAGE analysis of fractions obtained following the chromatography of 200 μg of purified C595 on an Express-Ion S mini column in the presence of 200 μg of BSA at a flow-rate of 60 cm/h using 0.1 M sodium acetate buffer at (a) pH 5.8 (b), pH 5.4 and (c) pH 5.0, collecting 1-ml fractions (Fig. 3). In each case, lane 1 contains fraction 2; lane 2 contains fraction 3; lane 3 contains a BSA standard; lane 4 contains fraction 12; lane 5 contains fraction 13; lane 6 contains a C595 standard.

biopharmaceuticals industry [29,30]. We developed a protocol for the isolation of the IgG3 isotype monoclonal antibody C595 targeted against the product of
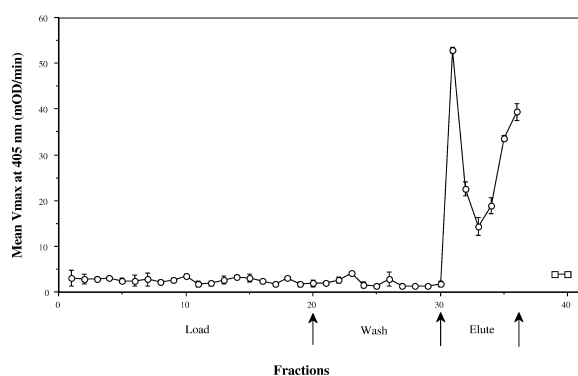

Fig. 5. Chromatography of 20 ml of C595 tissue culture supernatant on an Express-Ion S mini column at a flow-rate of 60 cm/h using 0.1 M sodium acetate buffer, pH 5.0, collecting 1-ml fractions. Fractions were analysed by (○) ELISA and are compared with the (□) ELISA response for the C595 tissue culture supernatant.

MUC1 gene from its hybridoma cell culture supernatant, using Express-Ion S. While the process has not been scaled-up, the studies reported here take us to a stage where scale-up should be both practical and predictable.

In previous work we have reported major selectivity differences from ion-exchanger to ion-exchanger for similar test systems [31] and there is no reason to expect this to be any different for monoclonal antibody expression systems. In a related study, we carried out a simple loading of tissue culture supernatant from a murine hybridoma expressing Ca2 an IgG1 anti-MUC1 antibody [32] on to an Express-Ion S mini column. In similar studies to C595, we isolated immunoreactive antibody from tissue culture supernatant using 0.1 M acetate buffer. While the method developed for C595 appears to be suitable for Ca2, further method developments of the type described in this report may improve the efficiency and selectivity of the separation.

With the introduction of automated chromatography systems with comprehensive method develop-

Lane

Fig. 6. SDS–PAGE analysis of fractions obtained following the chromatography of 20 ml of C595 tissue culture supernatant on an Express-Ion S mini column at a flow-rate of 60 cm/h using 0.1 *M* sodium acetate buffer, pH 5.0, collecting 1-ml fractions. (Fig. 5). Lane 1 contains a reduced affinity purified C595 standard; lane 2 contains a non-reduced affinity purified C595 standard; lane 3 contains a C595 tissue culture supernatant; lane 4 contains material eluted in 0.1 *M* NaCl; lane 5 contains material eluted in 0.2 *M* NaCl; lane 6 contains material eluted in 0.3 *M* NaCl. The two major bands visible in lane 1 are indicative of antibody heavy chains (upper, approximately 50kDa) and light chains (lower, approximately 25 kDa). The major band visible in lane 3 is BSA from the foetal calf serum present in the tissue culture media.

ment software, parametric studies of the type described here can be carried out rapidly using minicolumns and scale-down columns in order to assist with media selection and mobile phase optimisation.

We have described a simple, reliable and robust method for isolating potentially clinically relevant antibodies from heterogeneous feedstocks. The scouting methodology enables process optimisation that may simplify the final process routing. Whilst buffer conditions may vary from antibody to anti-
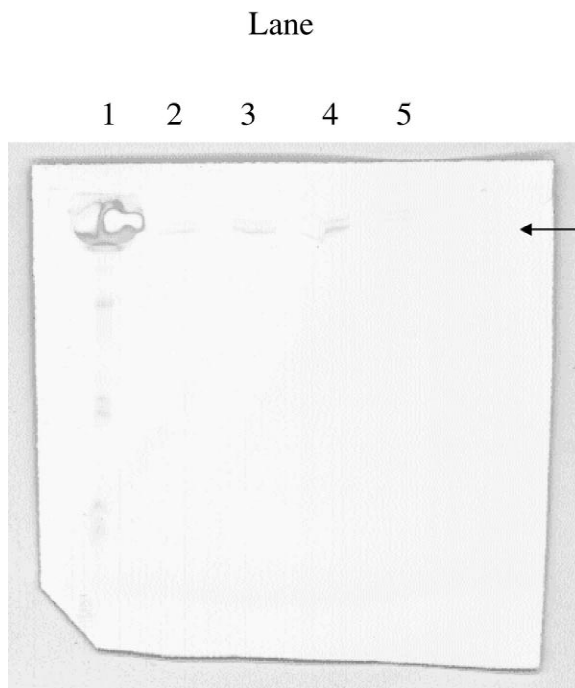
Lane

Fig. 7. Western blot analysis of fractions obtained following the chromatography of 20 ml of C595 tissue culture supernatant on an Express-Ion S mini column at a flow-rate of 60 cm/h using 0.1 *M* sodium acetate buffer, pH 5.0, collecting 1-ml fractions. (Fig. 5). Lane 1 contains a non-reduced affinity purified C595 standard; lane 2 contains material eluted in 0.1 *M* NaCl; lane 3 contains material eluted in 0.2 *M* NaCl; lane 4 contains material eluted in 0.3 *M* NaCl.
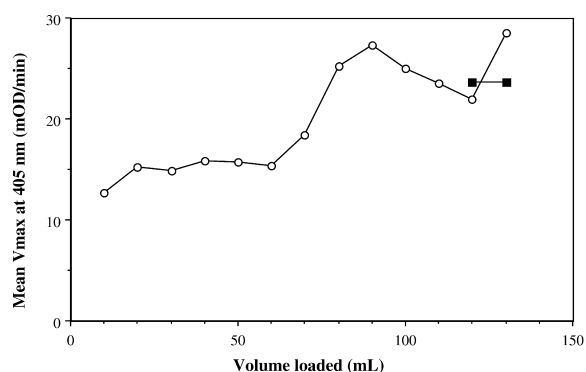

Fig. 8. Chromatography of 130 ml of C595 tissue culture supernatant on an Express-Ion S mini column at a flow-rate of 60 cm/h using 0.1 *M* sodium acetate buffer, pH 5.0, collecting 10-ml fractions. Fractions were analysed by (○) ELISA and are compared with the (■) ELISA response for the C595 tissue culture supernatant.
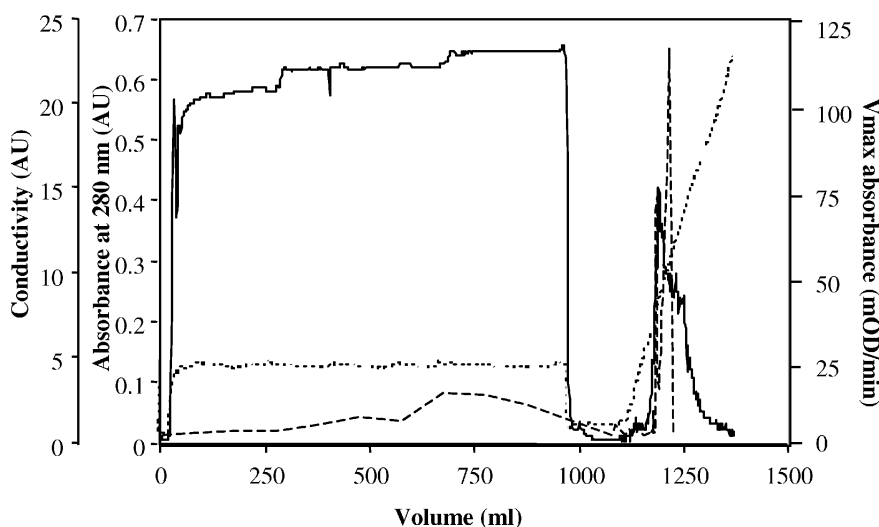
Fig. 9. Chromatography of 950 ml of C595 tissue culture supernatant on an Express-Ion S column (12.5 cm×1.6 cm I.D.) at a flow-rate of 150 cm/h using 0.1 M sodium acetate buffer, pH 5.0, collecting 10-ml fractions. Fractions were analysed by (------) ELISA. Absorbance at 280nm (—) and conductivity (`·····`) was measured throughout.

body, the general approach described here using Express-Ion S should lend itself to successful scale up for future large-scale bioprocessing.

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