Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1045 (2004) 93–98
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Integrated isolation of antibody fragments from microbial cell culture fluids using supermacroporous cryogels

Maria B. Dainiak[a,b], Ashok Kumar[a,b], Fatima M. Plieva[a,b], Igor Yu. Galaev[a], Bo Mattiasson[a,*]

[a] *Department of Biotechnology, Center for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, SE-22100 Lund, Sweden*
[b] *Protista Biotechnology AB, P.O. Box 86, SE-26722 Bjuv, Sweden*

Received 9 February 2004; received in revised form 15 June 2004; accepted 16 June 2004

Abstract

The present paper describes a chromatographic capture/purification step for the recovery of proteins directly from undiluted and unclarified cell culture broths using supermacroporous dimethylacrylamide (DMAA) cryogel. The interconnected character and the size (10–100 μm) of the pores of the adsorbent make it possible to process whole cell fermentation broths without blocking the column. Cu^{2+}–iminodiacetic acid (IDA) DMAA cryogel has been used for the isolation and purification of excreted $(His)_6$-tagged single chain (sc) Fv antibody fragments, $(His)_6$-scFv, from *E. coli* cell culture. Bound protein was recovered with 0.2 M imidazole or with 20 mM EDTA and was practically cell-free. Chromatographic capture using Cu^{2+}–IDA cryogel column was performed at flow rates of 300 and 600 cm/h, respectively and resulted in 84–96% recovery of $(His)_6$-scFv fragments with a purification factor of 13–15. The DMAA cryogel adsorbent is mechanically stable, can withstand harsh cleaning-in-place procedure and is relatively inexpensive. Chromatographic isolation of proteins using cryogels allows efficient removal of cells and can be operated at a flow rate as high as 600 cm/h. This novel technique has proven to be a scalable process, does not require special equipment and can be a good alternative to expanded bed adsorption and other integrated isolation techniques.

Keywords: Cryogels; Antibodies; Proteins; Dimethylacrylamide

1. Introduction

Along with progress in recombinant gene technology that offers almost unlimited possibilities for production of biomolecules with desired structure and function, there is an increased need for innovations in the bioseparation techniques, which can be a major bottleneck for the commercialization of the bioproduct. Indeed, conventional multi-step downstream processing is often time-consuming, labor-intensive and can account for up to 50–80% of the total process costs [1–3]. Each step, i.e. biomass separation, concentration, purification and polishing, contributes to the loss of the product yield and to the product cost.

One of the most promising strategies for achieving a better operational economy is the reduction of the number of steps by integrating the primary capture of the product from the cell suspension with the initial purification. Such integrated downstream processing implies the application of separation technologies capable of processing particle-containing solutions. Among developed integrated techniques which have received considerable attention are magnetic separations [4–6], aqueous two-phase systems [7–11] and expanded bed adsorption (EBA) [12–19]. These methods allow avoiding centrifugation and filtration steps but are limited either in scale, resolution or efficiency. Magnetic adsorption techniques where affinity ligands are immobilized on small magnetic particles require special equipment and costly magnetic adsorbents. Due to limitations in magnet capacity, this technique is mainly used in small-scale separations, although successful pilot-scale separations have also been reported [6]. In aqueous two-phase extraction, a batch-wise technique, both binding and elution are single equilibrium steps leading to poor separation efficiency. Moreover, there can be difficulties in collecting the target product due to

* Corresponding author. Tel.: +46 46 222 8264; fax: +46 46 222 4713.
E-mail address: bo.mattiasson@biotek.lu.se (B. Mattiasson).

0021-9673/$ – see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.chroma.2004.06.029

material gathering at the interface of two phases. Expanded bed chromatography, currently the most practical technology based on fluidization of chromatographic adsorbents of defined size and density, have several major limitations. These are the narrow range of flow velocities possible for desired expansion values [12], the need for the column to be absolutely vertical [20], the long equilibration time needed to obtain a stable steady state [21], the need to adjust the flow rate depending on the physical properties of the feed [12] and the necessity of feed dilution and/or pretreatment [22]. Further, conventional EBA adsorbents are prone to non-specific interactions with the biomass and to fouling [16,23,24] and therefore, can require special pretreatment to reduce cell binding [25]. As a result, the process costs for the integrated isolation/purification using EBA can be higher as compared to those of the conventional multi-step bioseparation [26].

It is clear from the above that there is still no universal direct capture procedure that could find wide application in commercial production of bioproducts.

In this work we introduce a robust and scaleable chromatographic technique for integrated product isolation from cell culture fluids using continuous supermacroporous dimethylacrylamide (DMAA) cryogel column. Cryogels, i.e. the polymeric gels formed in moderately frozen media, have a continuous system of interconnected macropores with a size of 10–100 μm. The large pore size and the interconnected morphology of pores allow unhindered mass transport of solutes of practically any size [27]. The DMAA cryogel columns consist only of 1/5 stationary phase (swollen gel) with the remaining 4/5 of the volume being pores. Due to the convective flow of the mobile phase through the interconnetced pores, the mass transfer resistance is practically negligible and the height equivalent to a theoretical plate (HETP) is practically independent either of flow rate or of the size of the marker (from acetone to *Escherichia coli* cells) [28]. Here we describe the preparation of small- and pilot-scale Cu^{2+}–iminodiacetic acid (IDA) DMAA cryogel adsorbents. The obtained immobilized metal affinity columns were used for direct capture of extracellularly expressed $(His)_6$-tagged single chain Fv fragments [$(His)_6$-scFv] from whole *E. coli* cell culture broth at flow rates up to 600 cm/h.

2. Experimental

2.1. Chemicals

N,*N*-Dimethylacrylamide (DMAA, 99%), aluminium oxide, ammonium persulfate (APS) and allyl glycidyl ether (AGE, 99%) were bought from Aldrich (Steinheim, Germany). *N*,*N*′-Methylenebis(acrylamide) (MBAAm) was from Acros (Geel, Belgium). Iminodiacetic acid (IDA) was from Fluka (Buchs, Switzerland). *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine (TEMED), carbonate–hydrogen carbonate buffer capsules, enzyme-linked immunosorbent assay (ELISA) buffer pouches (10 mM phosphate buffer pH 7.4, 0.15 M NaCl, 0.05% Tween 20), Sigma Fast *o*-phenylenediamine dihydrochloride tablet sets (OPD peroxidase substrate), rabbit anti-α ScFv, lysozyme, copper sulphate, EDTA-tetrasodium salt, imidazole and BCA (bicinchoninic acid) protein assay reagent were purchased from Sigma (St. Louis, MO, USA). Porcine anti-rabbit immunoglobulin/HRP was purchased from Dako (Glostrup, Denmark). Anti-6×-histidine [anti-$(His)_6$] antibody was obtained from R&D Systems Europe (Abingdon, UK). Low-salt LB-Broth, micro agar, sodium ampicillin and isopropyl-β-D-thiogalactopyranoside (IPTG) were of the brand Duchefa (Haarlem, The Netherlands). Terrific Broth (TB medium) was from Difco (Detroit, USA). The buffer salts were of the best quality available.

2.2. Production of supermacroporous monolithic Cu^{2+}–IDA cryogel

Monomers (6.3 ml of DMAA, 2.2 g of MBAAm and 0.85 ml of AGE) were dissolved in deionized water (final concentration 5%). Free radical polymerization was initiated by TEMED (115 μl) and APS (90 mg). The reaction mixture was poured into plastic syringes (5 ml) and was frozen at −12 °C for 16 h. After washing with water the columns were stored at 4 °C. Coupling of IDA ligand is presented in detail elsewhere [29,30].

2.3. Cell culture and production of $(His)_6$-scFv fragments

A recombinant strain of *E. coli* producing extracellular $(His)_6$-tagged single chain Fv antibody fragments [31] was provided by BioInvent International (Lund, Sweden) as a kind gift. The starter culture of the recombinant *E. coli* cells was grown in LB medium (trypton 10 g; yeast extract 5 g and sodium chloride 5 g in 1 l distilled water, pH 7.2) containing 0.1 mg/ml ampicillin. Expression of the target protein was carried out in TB medium (pancreatic digest of casein 12 g; yeast extract 24 g; dipotassium phosphate 9.4 g and monopotassium phosphate 2.2 g in 1 l distilled water, pH 7.2) supplemented with glycerol 4 ml/l and ampicillin 0.1 mg/ml and induced by 0.1 mM isopropyl β-D-thiogalactopyranoside at $A_{600} = 0.5$. The batch was cultivated at 37 °C for 24 h with shaking at 175 rpm. The obtained fermentation broth (turbidity 18–23 units A_{450}/ml; protein = 8–10 mg/ml) was used directly for the chromatographic analyses.

2.4. Chromatographic capture of $(His)_6$-scFv fragments from the E. coli cell culture fluid

The freshly obtained non-diluted cell culture fluid (0.25, 1, 10 or 200 ml) was applied to Cu^{2+}–IDA DMAA cryogel column (5 ml with 1.24 cm i.d. or 500 ml with 4.8 cm

i.d., respectively) followed by washing with the running buffer (20 mM HEPES, 200 mM NaCl, 2 mM imidazole, pH 7.0). Elution was performed using 0.2 M imidazole in 20 mM HEPES, 200 mM NaCl, pH 7.0 or 20 mM EDTA, 50 mM NaCl, pH 8.0 (which also stripped Cu^{2+} from the carrier). Flow velocities of 50, 300 or 600 cm/h were used. The cell content in the applied samples and in the fractions collected throughout chromatographic runs was analyzed spectrophotometrically by measuring absorbance at 450 nm. Cu^{2+}/EDTA solutions at the concentrations used do not absorb light at this wavelength. The chromatographic profile of $(His)_6$-scFv fragments was determined by running an ELISA on single fractions collected during feed application, washing and elution stages. Yield was determined as the percent of loaded antibody that eluted in the purified pool.

After removing Cu^{2+} ions with EDTA, regeneration of the adsorbent proceeded by washing the columns with distilled water (4 bed volumes) and application of 4 bed volumes of 0.25 M Cu^{2+} solution and finally followed by washing with another 4 bed volumes of distilled water. Cleaning-in-place with 0.1 M NaOH was not carried out between the runs.

2.5. Protein assay techniques

Protein concentration was determined using BCA protein assay reagent according to the established method [32]. Samples containing Cu^{2+} or imidazole were dialyzed against 20 mM HEPES, 200 mM NaCl, pH 7.0 prior to BCA assay.

The content of $(His)_6$-scFv fragments in the broth and in the chromatographic fractions was detected by indirect sandwich ELISA using peroxidase conjugated (HRP) secondary antibody. Monoclonal anti-$(His)_6$ antibody was adsorbed on ELISA plate wells (60 μl anti-$(His)_6$ 3 μg/ml in each well) followed by washing with phosphate-buffered saline with Tween 20, pH 7.4 (PBST). The plates were incubated with the analyzed samples applied at successive dilutions. Bound $(His)_6$-scFv fragments were detected with rabbit α ScFv antibody 1:2000 and swine anti-rabbit HRP antibody 1:1000. The wells were washed five times with PBST buffer after each step. Peroxidase activity was detected with *o*-phenylenediamine dihydrochloride (Sigma Fast™ OPD). The concentration of the target protein was determined using the ELISA titration curve obtained for pure standard solution of His-tagged scFv fragments.

The fermentation broth and the fractions from chromatographic runs were analyzed by sodium dodecyle sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) in 4–20% gradient polyacrylamide gels using a Bio-Rad Mini-protein II system according to the instructions of the manufacturer. The protein bands were stained with Coomasie Blue.

All cell containing samples were clarified by centrifugation at 14.000 × *g* for 3 min prior to protein analyses described above.

2.6. Preparation of cryogel samples for microscopy

The samples were fixed in 2.5% glutaraldehyde in 0.12 M sodium phosphate buffer pH 7.2 over night, postfixed in 1% osmium tetroxide for 1 h, dehydrated in ethanol and critical point dried. Dried samples were coated with gold–palladium (40:60) and examined using a JEOL-5600LV scanning electron microscope.

3. Results and discussion

3.1. Properties of continuous supermacroporous Cu^{2+}–IDA DMAA cryogels

Recently, we have developed new monolithic supermacroporous matrices with pore size up to 100 μm [29,30]. These matrices are produced by polymerization of the hydrophilic monomers or by cross-linking of hydrophilic polymers at subzero temperatures when most of the solvent, water, is frozen while the dissolved substances (monomers or polymers) are concentrated in small non-frozen regions. The reaction proceeds in these non-frozen regions while the crystals of frozen solvent perform like porogen. After melting the ice crystals, a system of large interconnected pores is formed. The pore size depends on the initial concentration of reagents in solution and the freezing conditions. In the IDA DMAA cryogel used in the present work the pore size was around 100 μm (Fig. 1) allowing direct application of cell suspension on a column. The produced DMAA cryogel columns were mechanically stable and did not compress at flow rates as high as 900 cm/h (Table 1). The back pressure increased linearly with increasing the flow rate through the column. The static binding capacity for a model protein, lysozyme, was practically independent of the flow rate used (Table 1).

Two sizes of IDA DMAA cryogel columns were produced, analytical columns of 5 ml volume (Fig. 2A) as well as preparative columns of 500 ml volume composed of 1.5 cm high disks with 4.8 cm diameter (Fig. 2B).

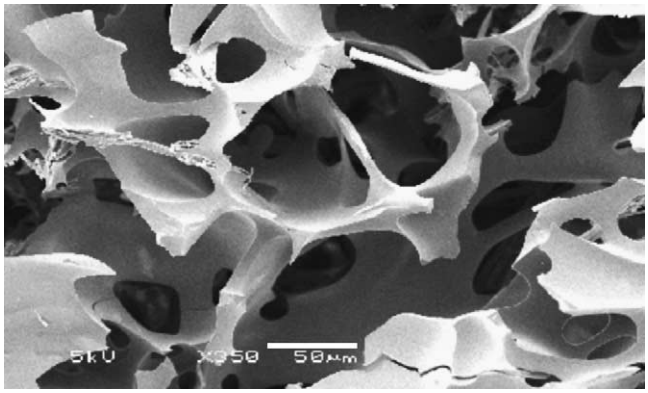

Fig. 1. Scanning electron microscope photograph of cryopolymerized DMAA matrix.

Table 1
Properties of the IDA-DMAA columns

IDA-DMAA column volume (ml)[a]	Applied flow rate (cm/h)	Pressure in the system (MPa)	Lysozyme static capacity (mg/ml)[b]
5	76	0.34	0.21
	917	0.92	0.19

[a] Monolithic cryogel was removed from 5 ml syringe packed into a chromatographic column (10 mM i.d.) equipped with upper and lower adapters and connected to a biologic HR chromatographic system (Bio-Rad, Hercules, USA).

[b] Chromatography was performed in 20 mM HEPES buffer with 0.2 M NaCl, pH 7.0 as a binding buffer. Elution was performed with 0.2 M imidazole solution in the binding buffer.

3.2. Direct capture of $(His)_6$-scFv fragments from E. coli cell culture fluid using Cu^{2+}–IDA DMAA cryogel

E. coli cell culture broth (OD_{600} = 9.5–12.1 U/ml) contained 8.4–10.0 mg/ml bulk protein and 24–32 μg/ml of the extracellularly expressed $(His)_6$-scFv fragments. A pulse of *E. coli* cell culture broth (0.25 ml) was passed through the IDA DMAA cryogel column (5 ml) not loaded with Cu^{2+}. Under the conditions used, the cryogel adsorbent did not bind any detectable amount of $(His)_6$-scFv fragments or other proteins present in the fermentation broth. *E. coli* cells passed freely through the interconnected network of macropores and were not mechanically entrapped within the cryogel matrix. When loaded with Cu^{2+}, the cryogel column bound quantitatively the target protein from the pulse [no $(His)_6$-scFv fragments were detected in the flow-through], while essentially all the cells and the bulk protein passed through without interacting with the affinity column. Bound $(His)_6$-scFv fragments were recovered with 20 mM EDTA or with 0.2 M imidazole with the yields of 96 and 84%, respectively. The eluted target protein was purified 15-fold. The addition of 2 mM imidazole in the running buffer was used to decrease the non-specific binding of bulk proteins to the Cu^{2+}–IDA cryogel column. Higher concentrations of imidazole in the running buffer decreased the adsorption of the target protein, i.e. 15–20% of $(His)_6$-scFv fragments was found in the breakthrough in the presence of 4 mM imidazole.

The ability of Cu^{2+}–IDA cryogel column to withstand high cell loads was challenged in a frontal adsorption experiment. Two bed volumes of *E. coli* cell culture broth (10 ml with turbidity of 23.4 units/ml) were applied to the 5 ml cryogel column at a flow rate of 300 cm/h (Fig. 3A). The particulate material passed through unhindered and was removed during the washing step. The feed load exceeded the binding capacity of the adsorbent and 65% of the applied target protein was in the breakthrough. The dynamic

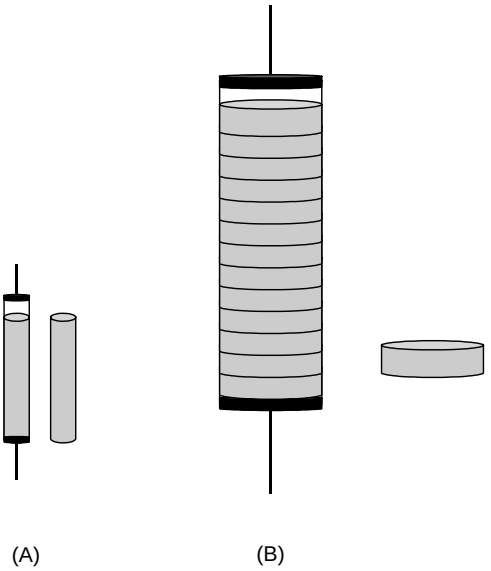

Fig. 2. Schematic presentation of (A) analytical Cu^{2+}–IDA cryogel column (5 ml with 1.24 cm i.d.) and monolithic Cu^{2+}–IDA cryogel removed from the column; (B) preparative Cu^{2+}–IDA cryogel column (200 ml with 4.8 cm i.d.) and a "building block", 1.5 cm high cryogel disk with 4.8 cm diameter.

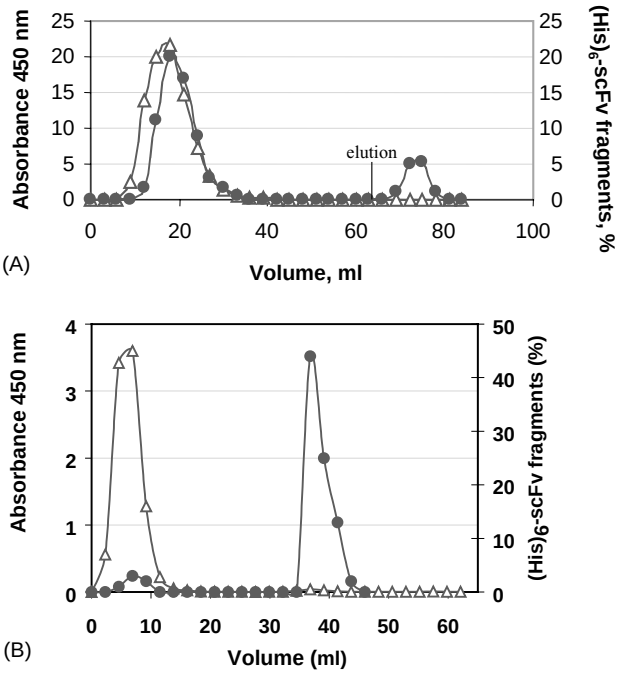

Fig. 3. The content of *E. coli* cells (open triangles) and $(His)_6$-scFv fragments (closed circles) in the breakthrough and eluate during chromatographic capture of $(His)_6$-scFv fragments from *E. coli* cell culture fluid on Cu^{2+}–IDA DMAA cryogel column. Experimental conditions: 10 ml (A) or 1 ml (B) of *E. coli* fermentation broth containing 24–32 μg/ml of the target His-tagged protein was applied to the column (5 ml with 1.24 cm i.d.) at a flow velocity of 300 cm/h. Running buffer: 20 mM HEPES, 200 mM NaCl, 2 mM imidazole, pH 7.0. Elution buffer: 20 mM HEPES, 200 mM NaCl, 0.2 M imidazole, pH 7.0.

binding capacity of the column with respect to $(His)_6$-scFv fragments was found to be 4.8 μg/ml (at break-through level of 5% of maximal) while the static binding capacity was 6.0 μg/ml.

Low capacity of the developed adsorbent is due to the relatively low surface area available for protein binding. Most of the column volume is occupied by large pores with rather small (as compared to microporous adsorbents) internal surface. However, when using cryogel columns the decrease in capacity can be compensated by very high flow rates. Besides, cryogel adsorbent with higher ligand density can be prepared. The small contribution of diffusion in the protein capture on monolithic macroporous adsorbent is indicated by the sharp breakthrough profile even at high flow rates and by the small difference between the static and dynamic binding capacities.

The optimized feed load (1 ml of the broth containing 30 μg of the target protein) was processed through 5 ml cryogel column at a flow rate of 300 cm/h (Fig. 3B). Quantitative adsorption of the product was achieved in a single pass through the column while most of the impurities were found in the effluent (Fig. 4). Elution with 0.2 M imidazole recovered 87% of $(His)_6$-scFv fragments with a purification factor of 13. The chromatographic run with the optimized feed load was repeated three times resulting in the product yields of 84, 80 and 84%, respectively.

In order to evaluate the limits of the cryogel column, the optimized purification cycle was carried out at flow rates up to 600 cm/h. The recovery and purity of the target product at flow rate of 600 cm/h were almost the same as in the runs carried out at flow rates of 50 and 300 cm/h, respectively (Table 2). The application of the undiluted feed at the maximal flow rate did not cause compression or any other kind of deformation of the cryogel matrix.

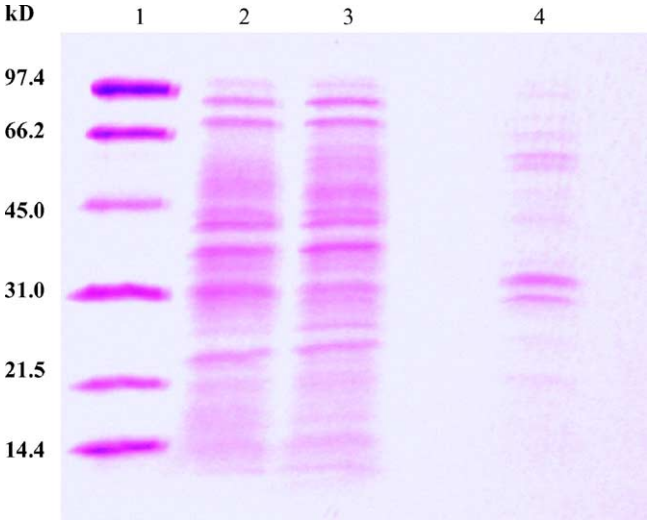

Fig. 4. SDS-PAGE (4–20% gradient polyacrylamide gel): lanes: 1 = low-molecular-mass standards); 2 = clarified fermentation broth; 3 = flow-through peak fraction from Fig. 2B; 4 = elution peak fraction from Fig. 2B. kD = kilodalton.

Table 2
The performance of the Cu^{2+}–IDA cryogel column at different flow rates

Flow velocity (cm/h)	Yield (%)	Purification factor
50	84	15
300	87	13
600	81	13

E. coli cell culture fluid (1 ml) was applied to the Cu^{2+}–IDA cryogel column (5 ml). After washing with 20 mM HEPES, 200 mM NaCl, 2 mM imidazole, pH 7.0 the bound proteins were eluted with 20 mM HEPES, 200 mM NaCl, 0.2 M imidazole, pH 7.0.

It is important to note that all purification cycles described above were carried out using one single column, which was regenerated between the runs. As 98–100% of the applied cells were in the flow-through in each purification cycle, particulate material was not accumulated in the column and there was no deterioration in the performance of the adsorbent from run to run. No build up of contaminants on the column over time was observed despite of the fact that cleaning-in-place procedure was not carried out throughout all analytical-scale experiments.

The separation process using Cu^{2+}–IDA DMAA cryogel column was scaled-up 100-fold to a 500 ml cryogel column (4.8 cm i.d.). The cryogel discs used in the pilot-scale column had an increased ligand density (approximately 22 μmol/ml) as compared with the 5 ml monolithic cryogel column (8.7 μmol/ml). Therefore, the feed load in the pilot-scale chromatographic runs was increased 200-fold up to 200 ml of the cell culture broth. The flow rate in the pilot-scale tests was 300 cm/h. $(His)_6$-scFv fragments were recovered with 20 mM EDTA with a yield of 94% and purification factor of 13. The elution volume in the pilot-scale chromatographic run was not increased as compared to the volume in which the bound product was eluted from the analytical column (Fig. 5). Thus, the performance properties of the scaled-up process were practically not altered as compared with the analytical scale tests (Table 3).

The reproducibility of analytical scale results to pilot scale of operation has demonstrated that the technique is easy and

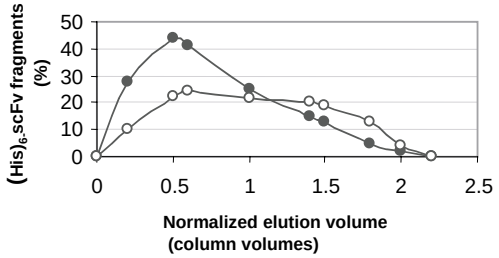

Fig. 5. Elution profiles of $(His)_6$-scFv fragments from an analytical (closed circles) and a preparative (open circles) Cu^{2+}–IDA cryogel columns. Experimental conditions: 1 ml or 200 ml of *E. coli* fermentation broth containing 24–32 μg/ml of $(His)_6$-scFv fragments was applied to the analytical (5 ml with 1.24 cm i.d.) and the preparative (200 ml with 4.8 cm i.d.) columns, respectively. The chromatographic runs were performed at a flow velocity of 300 cm/h. Running buffer: 20 mM HEPES, 200 mM NaCl, 2 mM imidazole, pH 7.0. Elution buffer: 20 mM EDTA, 50 mM NaCl, pH 8.0.

Table 3
Scaling up of $(His)_6$-scFv fragments capture directly from cell culture broth from 5 to 500 ml column

	Column volume 5 ml	Column volume 500 ml
Feed load (ml)	1	200
Linear velocity (cm/h)	300	300
Elution volume (ml) (column volumes)	11 (2.2)	1100 (2.2)
$(His)_6$-scFv fragments yield (%)[a]	87	97
Purification factor	15	13
Cell content in the eluted purified pool (%)[b]	0.5	7.2

See experimental protocol for conditions.
[a] Amount of applied $(His)_6$-scFv fragments was taken as 100%.
[b] Amount of applied cells was taken as 100%.

straightforward to scale up. The composed structure of the scaled-up multi-disc column did not cause entrapment of particulate material between the discs. There was no increase in flow resistance and the eluted $(His)_6$-scFv fragments were efficiently clarified. Thus, the proportional enlargement of the bed volume by increasing the number of discs of adsorbent can provide ample possibilities for scaling-up of chromatography using cryogels.

Acknowledgements

The authors are grateful to Cristina Glad at BioInvent International AB (Lund, Sweden) for providing recombinant *E. coli* strain. The supports of the Swedish Foundation for Strategic Research, The Swedish Foundation for International Cooperation in Research and Higher education (STINT, IG2003-2089), The Swedish Institute (Visby Program, project 2886/2002), Royal Swedish Academy of Sciences and INTAS (project 00-57) are gratefully acknowledged.

References

[1] A. Sadana, A.M. Beelaram, Bioseparation 4 (1994) 221.
[2] R.G. Harrison, Protein Purification Process Engineering, Marcel Dekker, New York, 1994.
[3] G. Sofer, L. Hagel, Handbook of Process Chromatography: A Guide to Optimization, Scale-Up and Validation, Academic Press, New York, 1997.
[4] S.M. O'Brien, R.P. Sloane, O.R.T. Thomas, P. Dunnill, J. Biotechnol. 54 (1997) 53.
[5] X.-D. Tong, B. Xue, Y. Sun, Biotechnol. Prog. 17 (2001) 134.
[6] J.J. Hubbuch, O.R.T. Thomas, Biotechnol. Bioeng. 79 (2002) 301.
[7] K.H. Kroner, H. Hustedt, M.-R. Kula, Process Biochem. 19 (1984) 170.
[8] H. Umakoshi, K. Yano, R. Kuboi, I. Komasawa, Biotechnol. Prog. 12 (1996) 51.
[9] S.G. Walker, C.J. Dale, A. Lyddiatt, J. Chromatogr. B 680 (1996) 91.
[10] J.W. Choi, G.H. Cho, S.Y. Byun, D.I. Kim, Adv. Biochem. Eng./Biotechnol. 72 (2001) 63.
[11] T. Cunha, A.-B. Raquel, Mol. Biotechnol. 20 (2002) 29.
[12] H.A. Chase, Trends Biotechnol. 12 (1994) 296.
[13] A.K.B. Frej, R. Hjorth, A. Hammarström, Biotechnol. Bioeng. 44 (1994) 922.
[14] C. Spence, C.A. Schaffer, S. Kessler, P. Bailon, Biomed. Chromatogr. 8 (1994) 236.
[15] M. Hansson, S. Staahl, R. Hjorth, T. Moks, Bio/Technology 12 (1994) 285.
[16] N. Ameskamp, C. Priesner, J. Lehmann, D. Lutkemeyer, Bioseparation 8 (1999) 169.
[17] J.T. Beck, B. Williamson, B. Tipton, Bioseparation 8 (1999) 201.
[18] B. Mattiasson, Expanded Bed Chromatography, Kluwer, Dordrecht, 1999.
[19] M. Dainiak, I.Yu. Galaev, B. Mattiasson, J. Chromatogr. A 942 (2002) 123.
[20] L.G. Bruce, S. Ghose, H.A. Chase, Bioseparation 8 (1999) 69.
[21] R.H. Clemmitt, L.G. Bruce, H.A. Chase, Bioseparation 8 (1999) 53.
[22] Y.K. Chang, H.A. Chase, Biotechnol. Bioeng. 49 (1996) 204.
[23] J.C. Erickson, J.D. Finch, D.C. Greene, in: B. Griffiths, R.E. Spier, W. Berthold, (Eds.), Animal Cell Technology: Products for Today, Prospects for Tomorrow, Butterworth and Heinemann, Oxford, 1994, p. 557.
[24] J. Feuser, J. Walter, M.-R. Kula, J. Thömmes, Bioseparation 8 (1999) 99.
[25] M.B. Dainiak, I.Yu. Galaev, B. Mattiasson, Biotechnol. Prog. 18 (2002) 815.
[26] G.S. Blank, G. Zapata, R. Fahrner, M. Milton, C. Yedinak, H. Knudsen, C. Schmelzer, Bioseparation 10 (2001) 65.
[27] V.I. Lozinsky, F.M. Plieva, I.Yu. Galaev, B. Mattiasson, Bioseparation 10 (2002) 163.
[28] F.M. Plieva, I.N. Savina, S. Deraz, J. Andersson, I.Yu. Galaev, B. Mattiasson, J. Chromatogr. B 807 (2004) 129.
[29] P. Arvidsson, F.M. Plieva, I.N. Savina, V.I. Lozinsky, S. Fexby, L. Bulow, I.Yu. Galaev, B. Mattiasson, J. Chromatogr. A 977 (2002) 27.
[30] P. Arvidsson, F.M. Plieva, V.I. Lozinsky, I.Yu. Galaev, B. Mattiasson, J. Chromatogr. A 986 (2003) 275.
[31] E. Söderlind, L. Strandberg, P. Jirholt, N. Kobayashi, V. Alexeiva, A.-M. Åberg, A. Nilson, B. Jansson, M. Ohlin, C. Wingren, L. Danielson, R. Carlsson, C.A.K. Borrebaeck, Nat. Biotechnol. 18 (2000) 852.
[32] P.K. Smith, R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson, D.C. Klenk, Anal. Biochem. 150 (1985) 76.