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Downstream processing of MDCK cell-derived equine influenza virus

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Abstract

A microcarrier-based process was used to produce equine influenza virus (A/Equi 2 (H3N8), Newmarket 1/93) in Madin Darby Canine kidney (MDCK) cells. The virus was purified in a sequence of downstream processing steps comprising of depth filtration, inactivation, ultrafiltration (UF) and gel filtration. In the ultrafiltration step, the hemagglutinin (HA) was recovered to 100%. A high increase of neuraminidase (NA) activity indicated the removal of some inhibitory compounds during this step. At the same time, the level of contaminating proteins and DNA was reduced by more than 88%. In the subsequent size exclusion chromatography (Sepharose CL 2B), the recovery of HA and NA in the "virus peak" was 37.8 and 59.8%, respectively compared to the concentrated feed material. Inconsistencies in the overall mass balance for HA and NA (70.0 and 69.2%) during gel filtration indicated non-specific interactions of the inactivated virus to the gel matrix which is supported by a HA recovery of about 50% in shake flask experiments performed as a control. Overall 35.8% of HA and 291.6% of NA were recovered. More than 95.7% of the host cell proteins and 98.7% of the host cell DNA were removed during downstream processing.

Keywords: Chromatography; Downstream processing; Influenza; Virus; MDCK cell and vaccine

1. Introduction

Vaccines play an important role in the prevention, control and eradication of infectious and contagious diseases. Vaccination is the principal means of prophylaxis for human and veterinary use and there is no therapy in view after manifestation of the disease except for passive immunization and few chemotherapeutic successes e.g. against influenza or herpes virus. Though influenza vaccines are still produced in eggs, the cultivation of cells, which are grown in suspension or monolayer culture and are finally infected with virus, is the most important production system today. With increasing safety demands by the Food and Drug Administration (FDA) and the European Agency for Evaluation of Medicinal Products (EMEA) to reduce the levels of possible side effects such as allergic and autoimmune reactions, continuous efforts to improve downstream processing methods are required.

Influenza virus is a lipid-enveloped RNA virus that belongs to the *Orthomyxoviridae* family and causes respiratory infections that result in severe human and animal suffering and high economic losses. For decades, vaccine supply relied on embryonated chicken eggs as a substrate for influenza propagation [1]. However, to cope with a potential shortage of eggs in a pandemic situation [2], to increase the flexibility of production campaigns and to avoid problems related to egg-derived vaccines, i.e. the risk of allergies against egg albumin and the selection of egg adapted virus subtypes, large-scale mammalian cell culture systems were developed for human and veterinary influenza vaccines [3–11]. So far, most publications have focused on upstream

Abbreviations: BEI, binary ethyleneimine; DNA, deoxyribonucleic acid; EDTA, ethylene diamine tetra-acetic acid; FCS, foetal calf serum; GF, gel filtration; KDa, kilodalton; HA, hemagglutinin; MDCK, Madin Darby Canine kidney; MALS, multiangle laser light scattering; MW, molecular weight; $NaHCO_3$, sodium bicarbonate; NA, neuraminidase; PBS, phosphate buffer saline; SDS–PAGE, sodium dodecylsulphate–polyacrylamide gel electrophoresis; UF, ultrafiltration

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processing and virus yields from bioreactors as well as efficacy and safety of final vaccines while comparatively little is reported on downstream processing of cell culture derived viruses [3,6,10,12].

Downstream processing of influenza virus from allantoic fluids (egg-derived influenza virus) usually consists of clarification by centrifugation followed by concentration by ultrafiltration and purification by ultracentrifugation [13]. Earlier, Polson et al. [14] and Polson [15] demonstrated purification by polyethylene glycol precipitation. In addition, several authors describe the use of continuous zonal centrifugation using sucrose [6], potassium tartrate [10] and caesium chloride [16] to purify viruses from cultivation broths. However, while results for final virus yields and host cell DNA per dose load are given, there are no reports on HA recovery or reduction of contaminating DNA and host cell proteins for individual processing steps.

Downstream processing of cell culture derived influenza viruses also necessitates a multi step approach to fulfil pharmaceutical requirements. While it is possible to adapt inactivation and solubilisation procedures originally developed for egg-derived vaccines [4] the differences in starting materials, i.e. allontoic fluids versus cell culture media containing microcarriers and cell debris, usually require additional methods for the efficient purification of viral antigens. One option is the clarification of the cultivation broth by depth filters or separators followed by the concentration of the antigen by crossflow filtration and inactivation [7]. In a next step, virus is purified by one or more chromatography methods, e.g. a combination of size exclusion or anion exchange chromatography, to fulfill (BE versus AE) all pharmaceutical requirements concerning purity, efficacy and safety. Until now, no results have been published that critically evaluate such a process. In the present paper, we report experimental data on the recovery of HA and NA activity of inactivated influenza virus harvests as well as the removal of DNA and contaminating proteins. Typical results obtained for the downstream processing of MDCK cell-derived equine influenza virus (A/Equi 2 (H3N8), Newmarket 1/93) from large-scale microcarrier culture are discussed.

2. Materials and methods

2.1. Preculture in roller bottle and large-scale microcarrier culture

Madin Darby Canine kidney (MDCK) cells (no. 841211903, ECACC, UK) were grown on cytodex 1 microcarriers (Amersham Bioscience, Freiburg, Germany) at 37 °C in a 5 L fermenter (B. Braun Biotech., Melsungen, Germany) containing cell growth medium based on GMEM (Invitrogen/Gibco, Karlsruhe, Germany) supplemented with glucose (final concentration 5.5 g/L; Sigma-Aldrich, Germany), 10% (v/v) fetal calf serum (Invitrogen/Gibco, Karlsruhe, Germany), 2.0 g/L peptone (autoclaved 20%, v/v; International Diagnostic Group, Lancashire, UK) and 4.0 g/L $NaHCO_3$ (Sigma-Aldrich, Germany). Cells were infected at 37 °C with equine influenza virus (A/Equi 2 (H3N8) Newmarket 1/93, NIBSC, UK) in cell growth medium without serum containing low levels (5 mL, 10 mg/mL) of porcine trypsin (Invitrogen/Gibco, Cat No. 27250-018, Karlsruhe, Germany) and 4.5 g/L of glucose. This medium is called virus maintenance medium. MDCK cells were grown in roller bottles (Greiner, Esslingen, Germany, 850 cm^2) containing 250 mL cell growth medium (Sigma-Aldrich, Germany) for inoculum preparation. The detailed procedure is described elsewhere [17].

Virus culture broth was harvested after passing through two depth filters (Polyfil II, 5 μm and 1 μm, with 0.22 and 0.3 m^2 filteration area, respectively; Vokes Filtration Technology, Germany) under positive pressure and inactivated before downstream processing using a final concentration of 1.5 mM binary ethyleneimine (BEI) [18].

2.2. Downstream processing

The partially clarified virus culture was concentrated on a plate type flat sheet 100 kDa MW cut-off ultrafiltration (UF) polyethersulfone membrane (Sartocon 3021466907E-SG, Sartorius, Göttingen, Germany) through a Watson Marlow 505S peristaltic pump equiped with 6.4 mm silicon tubing and the concentrated virus material (UF retentate) was harvested. To collect virus particles adsorbed onto the membranes 500 mL PBS (pH 7.2) was added and circulated without throttling the outlet valve. The inlet and outlet pressures of the UF system were at atmospheric pressure at a flow rate of 18 L/h. The virus washed from the membrane is termed in the following as UF wash. The concentrated virus was fractionated on gel filtration XK 100 columns (100 cm × 1.6 cm; Amersham Bioscience, Freiburg, Germany) packed with Sepharose CL 2B (70–40,000 kDa; Amersham Bioscience, Freiburg, Germany) at a flow rate of 1 mL/min (30 cm/h) at room temperature. The height of the gel bed was 95 cm and the loading volume of the UF retentate 10 and 20 mL, which corresponded to 5 and 10% of the column volume. The pressure of the system increased about 0.3 bar during loading the column whereas the pressure during packing was about 0.2 bar at these operating conditions. Four fractions were collected either in smaller aliquots or as a whole from the column and analysed for HA, NA activities, DNA and protein concentrations (Tables 1 and 2): GF Fraction 1 (the fraction collected before the first peak), GF Fraction 2 (the fraction of the first peak, called virus peak), GF Fraction 3 (the fraction collected after the virus peak), and GF Fraction 4 (the fraction of the second peak containing phenol red and other low molecular weight components of the culture broth). A process flow sheet for upstream and downstream processing is shown in Fig. 1.

Table 1
HA, NA activities, protein and DNA concentrations in various steps of equine influenza downstream processing

Steps	Volume (mL)	log HA U/0.1 mL	NA (mU/mL)	Protein (mg/mL)	DNA (ng/mL)
Culture broth	2000	1.8	2.7	0.029	1444
Depth filtration	1454	ND	ND	ND	ND
Inactivation	1454	1.95	3.8	0.038	1112
UF, permeate	1367	0.00	0.5	0.030	468
UF, retentate	87	3.15	308.9	0.079	1363
UF, washings	76	1.95	24.5	0.014	53
GF Fraction 1	55	0.00	0.00	BQL	0.00
GF Fraction 2 (first peak)	30	2.25	61.6	0.009	81
GF Fraction 3	95	1.65	2.3	BQL	38
GF Fraction 4 (second peak)	110	0.45	0.6	0.009	7
GF (pooled fractions)	290	1.53	7.4	[a]	23.5

ND: not determined, BQL: below quantification limit (0.008 mg/mL).
[a] Not calculated.

Table 2
Recovery and mass balances during downstream processing of equine influenza virus

Steps	Concentration factor			% recovery		% protein reduction	% DNA reduction
	HA	NA	Volume	HA	NA		
Inactivation	1.00	1.0	1.0	100.0	100.0	0.0	0.0
UF (retentate)	22.4	81.3	16.7	94.8	490.3	87.6	92.7
UF (retentate + wash)	8.92	46.8	8.92	100.1	524.3	85.6	92.4
[a]GF Fraction 2 (first peak)	0.13	0.20	0.33	37.8	59.8	65.8	82.2
[b]GF (pooled fractions)	0.024	0.024	0.034	70.0	69.2	–	50.0
[c]Overall	2.82	22.8	5.6	35.8	291.6	95.7	98.7

Ten milliliter (5%, v/v) of UF concentrate was loaded to a GF, Sepharose CL 2B (95 cm × 1.6 cm) column. Flow rate 1 mL/min, eluting buffer PBS, pH 7.4. –, not calculated due to protein concentrations below detection limit for some fractions.
[a] Peak fraction of gel filtration (GF).
[b] Pooled fractions of all four fractions of GF.
[c] Based on UF retentate and GF peak fraction.

2.3. Assays

2.3.1. Hemagglutination assay

Hemagglutinin (HA) was quantified as described by Mahy and Kangro [19]. HA activities are presented as log HA U/0.1 mL of the sample.

2.3.2. Neuraminidase (NA) assays

Neuraminidase assays were carried out as described elsewhere [20].

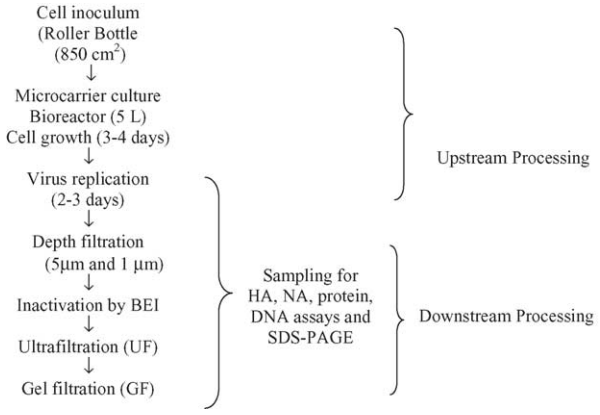

Fig. 1. Upstream and downstream processing of equine influenza virus A.

2.3.3. Protein assay

Protein contents of various samples were estimated by Bradford method [21] in a microtitre plate.

2.3.4. DNA estimation

DNA content of all the virus samples was measured using PicoGreen (Molecular Probes, The Netherlands). The detailed procedure is described in the data sheet of Molecular Probes (http://www.probes.com/media/pis/mp07581.pdf). The culture broth samples taken before depth filtration were centrifuged at 5000 × *g* for 30 min before carrying out the DNA assay.

2.3.5. Extraction of host cell protein from MDCK cells

MDCK cells cultivated in a roller bottle were trypsinized with the addition of 15 mL trypsin/EDTA solution (0.5 mg/mL, porcine trypsin, Cat No. 27250-018, Invitrogen/Gibco, Karlsruhe, Germany; 0.2 mg/mL of EDTA, Sigma-Aldrich, Taufkirchen, Germany) in PBS after washing with PBS. The settled cells were separated after decanting the liquid. The cells were then resuspended in a 250 mM homogenisation buffer containing 250 mM sucrose (Sigma-Aldrich, Taufkirchen, Germany), 3 M imidazole (Sigma-Aldrich, Taufkirchen, Germany) in Milli Q water, pH 7.4 and containing 10 μg/mL of aprotinin (Fluka, Taufkirchen, Germany), 1 μg/mL pepstatin (Sigma-Aldrich, Taufkirchen,

Germany) and 10 μg/mL leupeptin (Fluka, Taufkirchen, Germany) and 0.8 mM Pefabloc SC PLUS (Roche, Mannheim, Germany). The cells were centrifuged at 5000 × *g* and 4 °C for 30 min, and the buffer was decanted. The pellet was resuspended in homogenisation buffer with 1 mM EDTA (Sigma-Aldrich, Taufkirchen, Germany), protease inhibitors as described above and 30 μL of cycloheximide (Sigma-Aldrich, Taufkirchen, Germany) per 10 mL buffer.

2.3.6. Dissociation of viral proteins and electrophoresis

The dissociation and electrophoresis conditions were as described by Maizel et al. [22]. The virus culture broth, samples after depth filtration and inactivation; samples from ultrafiltation (retentate) and the GF virus peak were concentrated 10, 10, 10, 2 and 5-fold, respectively by VIVA spin filtration cartridges (Vivascience AG, Hannover, Germany, MW cut-off 5 kDa) followed by two to three washing steps with PBS and were applied onto sodium dodecylsulphate–polyacrylamide gel electrophoresis (SDS–PAGE) (Power PAC 200, BioRad Laboratories GmbH, Germany). Two times diluted, 25 μL MDCK cell extracts were loaded into a well.

3. Results and discussion

3.1. Characteristics of the virus culture broth

The protein and DNA concentration of our virus maintenance medium was 8.2 μg/mL and 790 ng/mL, respectively. With an initial cell number of about 1.0–1.2 × 10^6 cells/mL at the time of infection a typical virus culture broth harvested from a microcarrier based cell culture after 3 days cultivation had a DNA content in the range 1.10–4.96 μg/mL, a protein concentration from 0.03 to 0.18 mg/mL, HA from 1.8 to 2.7 log HA/100 μL and NA activities from 2.7 to 4.3 mU/mL, respectively. High HA titres seemed to be correlated with high protein and DNA concentrations of the bioreactor harvest. In contrast, NA activities varied and did not have any obvious correlation with other components. This could be explained by the influence of unspecific inhibitors released during virus replication and cell death followed by cell lysis (see discussion on NA recovery below). During clarification and subsequent inactivation, HA activities fluctuated within ±0.15 log HA U/0.1 mL, which is within the typical error range of such an assay. In contrast, NA activities increased slightly in the subsequent depth filtration and inactivation steps. Protein concentrations also sometimes slightly increased in the above-mentioned step, which was not clearly understood.

3.2. Recovery and material balances

Hemagglutinin and neuraminidase activities, protein and DNA concentrations including a summary of the recovery and the material balances for all process steps of a typical batch are presented in Tables 1 and 2, respectively. As mentioned above, hemagglutinin activity varied slightly after depth filtration and in subsequent inactivation steps, whereas the increase of NA activity and protein concentration was not clearly understood. DNA concentration decreased 23% (1444–1112 ng/mL) in this step. This was probably due to unspecific adsorption of host cell DNA to the polypropylene based depth filters similar to the binding of DNA to eppendorf tubes made of poplypropylene where centrifugation of λ phage DNA resulted in a 29% loss of DNA [23,24].

During ultrafiltration, a total of 41 mg protein (87.6%) and 0.64 mg DNA (92.7%) were removed in the permeate, indicating the membrane partially rejected these compounds (Table 1). While no HA activity was observed in the permeate, a low level of NA activity (0.5 mU/mL) was detectable indicating a loss of NA molecules. With a diameter of about 80–120 nm intact influenza virus particles can not pass through a 100 kDa cut-off membrane. NA is a homotetramer comprising of 50 kDa monomers [25]. Therefore, the NA activity in the permeate indicated either the release of NA molecules from the viral membrane due to shear damage during ultrafiltration or previous downstream processing steps or the presence of a low level of NA molecules in the virus harvest of the bioreactor, e.g. from the disintegration of the membrane of infected cells.

During the ultrafiltration process the initial flux decreased steadily from 17 to 7 L m^{-2} h^{-1} within about 10 min (Fig. 2). In the retentate (87 mL), protein and DNA accumulated to 0.079 mg/mL and 1363 ng/mL, respectively (Table 1). After an additional wash with 500 mL PBS through the system followed by a further concentration step, an additional 76 mL containing 0.014 mg/mL protein and 53 ng/mL DNA was harvested. Most of the reduction in flux is probably related to a polarization of proteins and an unspecific adsorption of DNA onto the membrane during the concentration. However, overall recovery of HA, DNA and protein of the ultrafiltration step are 100.1, 107.7 and 93.4%. The membrane could be easily regenerated by flushing using a standard protocol as per the manufacturer's instruction.

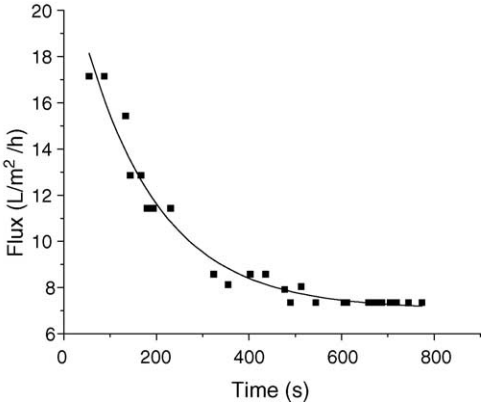

Fig. 2. Flux decline in ultrafiltration.

The UF retentate, which was the starting material for subsequent size exclusion chromatography, had a HA of 3.15 log HA U/0.1 mL and a NA activity of 308.9 mU/mL. Based on the antigen content of the inactivated virus harvest, this corresponded to a recovery of 94.8 and 490.3%, respectively. The unusual high recovery of NA activity is probably due to the removal of unspecific enzyme inhibitors. Other batches have shown similar tendencies (data not shown).

Using Sepharose CL 2B as a gel filtration media and a loading volume of 5% (v/v) of the column volume, three fractions (2, 3 and 4) of the outlet sample containing HA, NA, protein and DNA (Table 1) were obtained. A substantial amount of HA (37.8%) and NA (59.8%) was eluted in "GF Fraction 2", which corresponds to the "virus peak" (see Section 2.2, downstream processing) and is usually collected for further downstream processing steps in commercially available vaccines [7]. In addition, 1.65 log HA U/0.1 mL together with a low NA activity (2.3 mU/mL) were found in "GF Fraction 3" whereas "GF Fraction 4" attributed only a very low quantity of HA and minute NA activities. Most of the protein was eluted in "GF Fraction 2" and "4" while DNA coeluted with HA in "GF Fractions 2" and "3". The mismatch of the HA and volume concentration factor (GF First Peak, Table 2) and the comparatively high HA content of "GF Fractions 3" and "4" clearly indicated that the HA activity of the retentate was based on several virus fractions. Intact virions eluted within the void volume of the column due to their higher molecular size (GF Fraction 2) while smaller membrane based particles or micelles containing HA molecules in GF Fraction 3 and more or less freely dispersed HA molecules in GF Fraction 4. At the low shear conditions prevailing during gel filtration, virus disintegration is not very likely. Therefore, the presence of HA activity in most fractions collected during gel chromatography either reflects the initial properties of the starting material harvested from the bioreactor or is due to the disintegration of virions during inactivation and ultrafiltration. Which factor is more important could not be determined as it is extremely difficult to monitor the relevant properties of the culture broth, e.g. virus particle size distribution, during these unit operations. The low NA activity of fractions 3 and 4 is related to the different detection limit of HA and NA assays. In total, 70.0% of HA and 69.2% of NA were recovered during gel filtration while 50.0% of the genomic DNA was removed (Table 2).

In a gel filtration experiment from a different batch of virus culture with a UF retentate concentration of 3.68 log HA U/0.1 mL, 52 mU/mL NA and 0.07 mg/mL protein, 20 mL sample (10% column volume) was loaded onto the same gel matrix and the eluent of the first peak "GF Fraction 2" collected in 1 mL aliquots. Fig. 3 shows the profiles for HA, NA, protein and UV against the normalised volume. As expected, the UV signal showed two peaks, typically for influenza downstream processing with a Sepharose CL 2B gel i.e. a "virus peak" (corresponding to "GF Fraction 2" in Table 1) and a "second peak" (corresponding to "GF Fraction 4" in Table 1). Most of the NA activity as well as a high protein concentration can be found in the first peak. In contrast, HA showed a maximum of 3.15 log HA U/0.1 mL at about 0.5 column volume and remained comparatively high in between both UV peaks. On the average 1.14 log HA U/0.1 mL were found in the second peak in which small molecules such as phenol red (present in the culture medium) elute. Similar to the results obtained for a loading volume of 5% (v/v), this indicated a disintegration of virus particles.

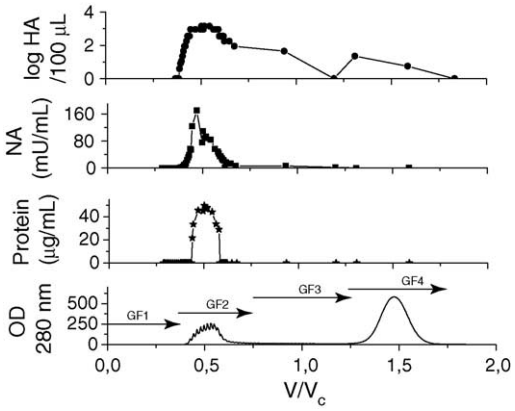

Fig. 3. HA, NA, protein and optical density profiles of influenza virus on a gel filtration column. Ten percent column volume of UF concentrate was fed into the gel filtration column from a different batch of culture broth.

Typically, total virus recovery from the pooled fractions collected during gel chromatography is less than 100% (about 70% for HA and NA, Table 2). Part of this mismatch could be due to the accuracy of the assays and corresponding errors in the mass balance. In a control to investigate non-specific interactions of virus particles with the gel matrix, 1 mL of gel was incubated with 1.5 mL of active and inactivated virus broths for 6 h at room temperature. While the HA activity of the supernatant was not reduced for the infectious virus it decreased by a factor of about 2 for the inactivated material suggesting 50% of the virus binding tightly to the gel filtration medium. A possible reason for such an unspecific interaction of inactivated influenza virus to the gel filtration media is the presence of free aziridine groups of the BEI, which might interact with the –OH group of the Sepharose gel matrix. These aziridine groups in BEI are electrophiles, which modify proteins during virus inactivation and have a tendency to bind various nucleophilic groups [26]. Presumably, these groups are very reactive towards the –OH groups of Sepharose. In other words, as BEI contains two aziridine groups with some of them available even after inactivation some unspecific binding of viral proteins is highly plausible and a factor to be further investigated with respect to optimizing virus recovery during gel filtration.

3.3. Characterisation by SDS–PAGE

Samples from various downstream processing steps were analysed by SDS–PAGE (Fig. 4). Several bands of proteins are found in the virus culture broth at the end of

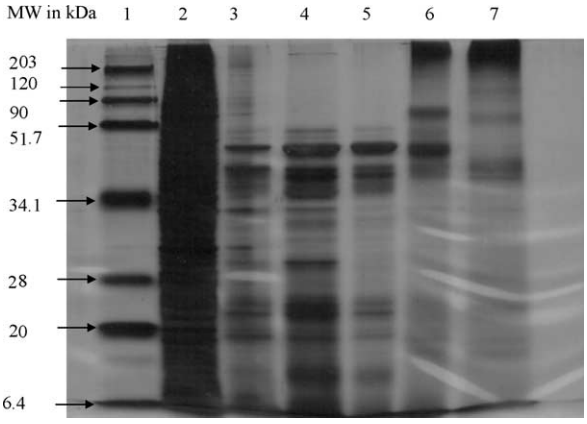

Fig. 4. SDS–PAGE of downstream processed samples. Lane 1: molecular weight markers; lane 2: MDCK cell extracts; lane 3: culture broth before depth filtration; lane 4: culture broth after depth filtration; lane 5: culture broth after inactivation; lane 6: ultrafiltration; and lane 7: gel filtration virus peak.

virus replication (lane 3, after centrifugation), most of them are probably MDCK cell-derived as low abundant viral proteins can hardly be detected by silver staining. The major antigenic component (HA) was visible at 66 kDa in between 51.7 and 90 kDa MW markers in lanes 6 and 7. After depth filtration (lane 4) some of the larger molecular weight compounds greater than 51.7 kDa disappear probably due to an unspecific adsorption to the depth filters. The reduction of bands with intermediate molecular weight within the range of 20–51.7 kDa (some protein bands in the lane) after inactivation is probably due to precipitation influenced by crosslinking of proteins with the aziridine groups of the BEI. These precipitates are always found after inactivation and storage at 4 °C. The presence of two larger molecular weight compounds greater than 51.7 and 90 kDa and a strong increase in molecules greater than 203 kDa in the retentate after ultrafiltration is clearly attributed to the cut-off of 100 kDa membrane while smaller molecules are withdrawn with the permeate. However, the appearance of some of the smaller molecules (<90 kDa) in the gel filtration peak clearly indicated that these molecules are bound to virus particles as otherwise a higher retention time due to the large pore size of the gel filtration matrix (70–40,000 kDa) would be expected.

4. Conclusion

Downstream processing of MDCK cell-derived equine influenza virus from a microcarrier culture on a sequence of steps comprising depth filtration, inactivation, ultrafiltration and gel filtration was carried out. During depth filtration and subsequent inactivation steps HA activity varied within a range of +/−0.15 log HA U/0.1 mL, DNA content was reduced whereas NA activity was increased. Ultrafiltration of the inactivated sample resulted in a sharp decline of the flux with a recovery of hemagglutinin of about 95%. NA activities strongly increased during ultrafiltration and gel filtration suggesting the removal of some inhibitory compounds. Gel filtration of the concentrated sample resulted in three different fractions with HA and NA activity. A “virus peak” with intact virus particles, a second fraction containing smaller membrane aggregates or micelles with HA and some lower NA activity and a third fraction mainly consisting of medium compounds but also with a very low HA and NA level. Lower recoveries of HA, NA and DNA after gel filtration indicated unspecific interactions of these molecules to the gel matrix. At present, further investigations are being performed to elucidate the influence of inactivation procedures on HA yields, to better characterize the composition of virus harvests during the various downstream processing steps and to finally improve overall recovery and purity of inactivated influenza vaccines. In addition, experiments have been extended to the downstream processing of inactivated human influenza viruses derived from static as well as microcarrier cultures of MDCK cells cultivated in a serum free medium.

References

[1] C. Gerdil, Vaccine 21 (2003) 1776.
[2] World Health Organization, Bulletin OMS 73(4) (1995) 431.
[3] I.V. Alymova, S. Kodihalli, E.A. Govorkova, B. Fanget, C. Gerdil, R.G. Webster, J. Virol. 72 (1998) 4472.
[4] R. Brands, J. Visser, J. Medema, A.M. Palache, G.J.M. van Scharrenburg, in: F. Brown, J.S. Robertson, G.C. Schild, J.M. Wood (Eds.), Inactivated Influenza Vaccine Prepared in Cell Culture, Karger, 1999, p. 18.
[5] T. Mabrouk, R.W. Ellis, Dev. Biol. Stand. 110 (2002) 125.
[6] O. Kistner, P.N. Barrett, W. Mundt, M. Reiter, S. Schober-Bendixen, F. Dorner, Vaccine 16 (1998) 960.
[7] U. Reichl, in: H. Brunner (Ed.), Proceedings of the Fourth International Congress of Biochemical Engineering, Fraunhofer IRB Verlag, 2000, p. 314.
[8] E.A. Govorkova, S. Kodihalli, I.V. Alymova, B. Fanget, R.G. Webster, Dev. Biol. Stand. 98 (1999) 39.
[9] N. Kessler, G. Thomas-Roche, L. Gerentes, M. Aymad, in: F. Brown, J.S. Robertson, G.C. Schild, J.M. Wood (Eds.), Inactivated Influenza Vaccine Prepared in Cell Culture, Karger, 1999, p. 18.
[10] O.-W. Merten, C. Hannoun, J.-C. Manuguerra, F. Ventre, S. Petres, in: S. Cohen, A. Shafferman (Eds.), Novel Strategies in Design and Production of Vaccines, Plenum Press, New York, 1996, p. 141.
[11] J.A. Tree, C. Richardson, A.R. Fooks, J.C. Clegg, D. Looby, Vaccine 19 (2001) 3444.
[12] I.G.S. Furminger, in: K.G. Nicholson, R.G. Webster, A.J. Hay (Eds.), Textbook of Influenza, Blackwell Science Ltd., London, 1988, p. 324.
[13] C. Liu, M.C. Eichelberger, R.W. Compans, G.M. Air, J. Virol. 69 (1995) 1099.
[14] A. Polson, A. Keen, C. Sinclair-Smith, I.G.S. Furminger, J. Hyg. Camb. 70 (1972) 255.
[15] A. Polson, Prep. Biochem. 23 (1993) 207.
[16] J.J. Skehel, G.C. Schild, Virology 44 (1971) 396.
[17] Y. Genzel, I. Behrendt, S. König, H. Sann, U. Reichl, Vaccine 22 (17–18) (2004) 2202.
[18] H.G. Bahnemann, Vaccine 8 (1990) 299.
[19] B.W.J. Mahy, H.O. Kangro, Virology Method Manual, Academic Press, London, 1996, p. 41.

[20] D.P. Nayak, U. Reichl, J. Virol. Methods 2004(1 December) 122(1) 9–15.
[21] M.M. Bradford, Anal. Biochem. 72 (1976) 248.
[22] J.V. Maizel, D.O. White, M.D. Scharff, Virology 36 (1968) 115.
[23] B.P. Belotserkovskii, B.H. Johnston, Science 271 (1996) 222.
[24] C. Gaillard, F. Strauss, Science 271 (1996) 223.
[25] R.W.H. Ruigrok, in: K.G. Nicholson, R.G. Webster, A.J. Hay (Eds.), Textbook of Influenza, Blackwell Science Ltd., London, 1988, p. 29.
[26] F. Käsermann, K. Wyss, C. Kempf, Antiviral Res. 52 (2001) 33.