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Significant Improvements in the Performance of an Established Affinity Chromatography Procedure Employed to Purify a Monoclonal Antibody in 100 Purification Cycles

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Abstract

Protein A-Sepharose affinity chromatography is a very successful method for the purification of immunoglobulins for pharmaceutical use. However, the chromatography efficiency and lifetime of this method have to be always adjusted to specific chromatography conditions (biological source, buffers, flow rates, antibody properties, temperature, protein concentration, cleaning protocol, etc). This study sought to demonstrate improvements in the performance of an established affinity chromatography procedure employed to purify the CB.Hep-1 monoclonal antibody (mAb) used in the purification of the active pharmaceutical ingredient of a Hepatitis B vaccine. In conclusion, the relative poor mAb recovery observed in 150 mM PBS; pH 8.0/100 mM citric acid; pH 3.0 buffer system conditions was attributed to the inefficacy of the elution buffer to disrupt completely interactions between the matrix and mAb. In this regard, retention of the CB.Hep-1 mAb into the matrix was helped by the ligand coupled to the matrix and not by unspecific interactions. The 1.5M glycine-NaOH/3M NaCl; pH 9.0/200 mM glycine-HCl; pH 2.5 buffer system significantly improved the affinity chromatography recovery without affecting mAb purity, molecular homogeneity, ligand leakage and mouse DNA content in 100 purification cycles. Thus, application of 1.5M glycine-NaOH/3M NaCl; pH 9.0/200 mM glycine-HCl; pH 2.5 as buffer system allowed the reduction of the CB.Hep-1 mAb and Hepatitis B vaccine costs, respectively.

Keywords: Affinity chromatography; CB.Hep-1 monoclonal antibody; Protein A-Sepharose

Introduction

Monoclonal antibodies (mAb) play an important role in therapy, immunodiagnostic and purification of molecules by immunoaffinity chromatography [1,2]. Bottlenecks of mAb manufacturing processes currently have moved to downstream processes; thus purification costs are now outbalancing upstream costs as a consequence of fermentation capacity increase protein expression level of the cell lines [3,4].

Generally, the platform of mAb downstream processes includes particle removal by centrifugation or filtration followed by an affinity chromatography and some viral inactivation steps [5]. The most important step of this platform is the affinity chromatography, because of its high selectivity, which leads to a very high mAb purity. Within affinity chromatography techniques used to purify mAb staphylococcal Protein A-Sepharose affinity chromatography is perhaps the most widely applied. It is a very robust and universal platform to purify almost any kind of immunoglobulins from a diverse range of starting biological sources. Nevertheless, this matrix is usually a significant component of the whole purification cost with respect to other properties. Therefore, an optimal usage of expensive Protein A-Sepharose affinity chromatography matrices would significantly reduce purification process cost. In this sense, there are several parameters, which can affect the yield, purity of eluted mAb and Protein A-affinity chromatography matrices lifetime; and consequently the cost of the purification process. Within them, adsorption and elution buffers play a significant role [6,7].

On the other hand, the decreasing trend in prices of the Hepatitis B vaccines, due to a declining incidence of this disease as a consequence of a successfully worldwide vaccination program makes mandatory improvements in the CB.Hep-1 mAb [8], downstream process to increase the recovery and thus reduce the mAb and vaccine production cost.

For that reason, the subjects of the present research were (I) to detect principal drawbacks in an established Protein A-Sepharose Fast Flow (PASFF) affinity chromatography used at industrial scale to purify the CB.Hep-1 mAb, which employs a phosphate/citric acid buffer system and (II) to test a new buffer system to demonstrate significant improvements in the CB.Hep-1 mAb recovery in 100 purification cycles for reducing the mAb and vaccine purification process cost, respectively.

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Materials and Methods

Chromatography matrices

PASFF, Sepharose 4 Fast Flow and Sephadex G-25 were from Amersham-Biosciencies (Uppsala Sweden).

Loading study of the CB.Hep-1 mAb using the established PASFF affinity chromatography procedure

Ascites contained the CB.Hep-1 mAb (30 mg) was applied to each PD-10 column (Amersham-Biosciences, Uppsala, Sweden) packed with 2 mL of PASFF using both buffer systems (I, 150 mM M PBS; pH 8.0/100 mM citric acid; pH 3.0 and II, 1.5M glycine-NaOH/3M NaCl; pH 9.0/200 mM glycine-HCl; pH 2.5). All eluted fractions were neutralized with 2M Tris to allow buffer exchange to 20 mM Tris/150 mM NaCl; pH 7.6 by gel filtration chromatography in Sephadex G-25 and protein characterization. Affinity columns were operated at 48 cm/h and the concentration of mAb applied to the columns was always 15 mg/mL.

Regeneration and in-situ cleaning of PASFF matrix

The PASFF column was regenerated after each purification cycle with 100 mM citric acid; pH 3 and the in-situ cleaning was performed after each purification cycle with five times column volume of 70% ethanol (Merck) at 1 mL/min.

Quantification of proteins

Proteins were quantified by the procedure described by Lowry using Bovine serum albumin (BSA) as standard [9]. Calibration curve ranged 100-500 µg/mL and sample absorbance was measured at 730 nm in a UV-Visible Ultrospec 2000 Spectrophotometer (Pharmacia Biotech, Cambridge, UK).

Estimation of the CB.Hep-1 mAb Concentration by Enzyme-Linked Immunosorbent Assay (ELISA)

A validated ELISA was employed to estimate mAb concentration. Detection of antigen-antibody reaction was achieved using an anti-mouse IgG horseradish peroxidase conjugated, revealing the enzyme activity with o-phenylenediamine (OPD) (Sigma, St Louis, USA) substrate. Reaction was stopped with 2.5 M H_2SO_4 and absorbance was measured at 492 nm using a Multiskan ELISA reader (Labsystem, Helsinki, Finland) [10].

Detection of the CB.Hep-1 mAb Retained in the PASFF by SDS-PAGE

Samples (20 µg) of the previously purified CB.Hep-1 mAb were analyzed under reducing conditions by electrophoresis on 12.5% SDS-PAGE [11]. Bands of proteins were stained with Coomassie blue R-250 (Bio-Rad, California, USA). The purity was analyzed by densitometry using the Molecular Analyst, version 1.4.1 software. Before sample application in SDS-PAGE, PASFF (0.1 g) was heated at 100°C under reducing conditions for 5 min. The sepharose 4 FF, without Protein A, used in only one the CB.Hep-1 mAb purification cycle served as negative control.

Estimation of the mouse DNA content by hybridization method

A sample volume containing 1 mg of mAb was mixed with 1 mL of phenol. Then, a centrifugation at 1300 g for 15 min was done. Top phase was blended with similar phenol/chloroform volume (v/v) and centrifuged 140 at 1300 xg for 15 min. Supernatant was mixed with 2-butanol (v/v) and centrifuged again, top phase was discarded. In parallel, different dilutions of mouse chromosomal DNA (1 ng-3.9 pg) were prepared. The BSA (100 µg) free of DNA was used as negative control. The technique quantification limit was 7.5 pg/mg of protein [12].

Estimation of the CB.Hep-1 mAb purity by SDS-PAGE in the lifetime study

Samples (20 µg) of the purified CB.Hep-1 mAb were analyzed under reducing conditions by electrophoresis on 12.5% SDS-PAGE [11]. Bands of proteins were stained with Coomassie blue R-250 (Bio-Rad, California, USA). Samples were analyzed from purification cycles 1, 10, 30, 60, 100, respectively.

Estimation of the CB.hep-1 mAb purity and molecular homogeneity in the lifetime study

The molecular distribution and purity of the CB.Hep-1 mAb were also estimated by using a HPLC-GF column TSK G3000 PW (600 mm/57.5 mm I.D., TosoHaas, Japan). The chromatographic mobile phase employed was 150 mM PBS; pH 7.0 and 100 µg of the samples dissolved in 150 mM PBS; pH 7.0 were directly applied into the system. The volumetric flow rate employed was 200 µL/min and absorbance was measured at 226 nm. Samples were analyzed from purification cycles 1, 10, 30, 60, 100, respectively.

Estimation of the protein a concentration by ELISA

A high binding plate (COSTAR, USA) was coated with 10 µg/mL of specific sheep polyclonal antibodies against Protein A (SpAc1). Concentrations of standard Protein A ranged 0.19-3.13 ng/mL previously diluted in 0.5% skim milk/0.15 M PBS/0.1% Tween 20; pH 7.2. Standard and samples were boiled for 3 min and centrifuged at 10000 xg in a Universal 16 centrifuge, (Hettich, Germany). Then, both were applied to the plate and incubated for 2 h at 37°C. Reaction between SpAc1 and Protein A was detected through the SpAc1-peroxidase conjugate. Reaction was revealed adding 0.015% H_2O_2 and OPD (Sigma, St Louis, USA) and stopped with 2.5M H_2SO_4. Absorbance was measured at 492 nm using a Multiskan ELISA reader (Labsystem, Helsinki, Finland) [13].

Estimation of the CB.hep-1 mAb "Specific Activity"

The antibody specific activity was calculated as the ratio between the CB.Hep-1 mAb concentration measured by ELISA [10] and total protein concentration measured by the Lowry method [9].

Preliminary proof-of - concept studies

Preliminary study I. A PD-10 column (Amersham-Biosciences, Uppsala, Sweden) packed with 2 mL of PASFF was loaded with the purified CB.Hep-1 mAb (15.2 mg/mL) using a buffer system based on 1.5M glycine- NaOH/3M NaCl; pH 9.0/200 mM glycine-HCl; pH 2.5.The eluted fractions were also neutralized with 2M Tris to allow buffer exchange to 20 mM Tris/150 mM NaCl; pH 7.6 by gel filtration chromatography in Sephadex G-25. The affinity column was operated at 48 cm/h.

Preliminary study II. Ascites (12 mL) contained the CB.Hep-1 mAb was applied to each PD-10 column (Amersham-Biosciences, Uppsala, Sweden) packed with 2 mL of PASFF using both buffer systems (I. 150 mM M PBS; pH 8.0/100 mM citric acid; pH 3.0 and II, 1.5M glycine- NaOH/3M NaCl; pH 9.0/200 mM glycine-HCl;

pH 2.5). All eluted fractions were neutralized with 2M Tris to allow buffer exchange to 20 mM Tris/150 mM NaCl; pH 7.6 by gel filtration chromatography in Sephadex G-25 and protein characterization. Both affinity columns were operated at 48 cm/h. Concentration of mAb applied to the columns was always 16.4 mg/mL. Regeneration of the Protein A-Sepharose FF matrix was done after each purification cycle with 100 mM citric acid; pH , while the in-situ cleaning of the Protein A-Sepharose column was performed after each purification cycle but with five time column volume of 70% ethanol (Merck) at 1 mL/min. This experiment was done in triplicate.

Lifetime study

Each PD-10 column (Amersham-Biosciences, Uppsala, Sweden) packed with 2 mL of PASFF were loaded with ascites (12 mL) contained the CB.Hep-1 mAb (load 15.5 mg/mL of matriz) using both buffer systems (I, 150 mM M PBS; pH 8.0/100 mM citric acid; pH 3.0 and II, 1.5M glycine-NaOH/3M NaCl; pH 9.0/ 200 mM glycine-HCl; pH 2.5). Elution fractions were also neutralized with 2M Tris to allow buffer exchange to 20 mM Tris/150 mM NaCl; pH 7.6 by gel filtration chromatography in Sephadex G-25. Both assessed affinity columns were operated at 48 cm/h. This procedure was repeated 100 purification cycles. The protocols used for regeneration and in-situ cleaning of the PASFF matrix were similar to those describe above.

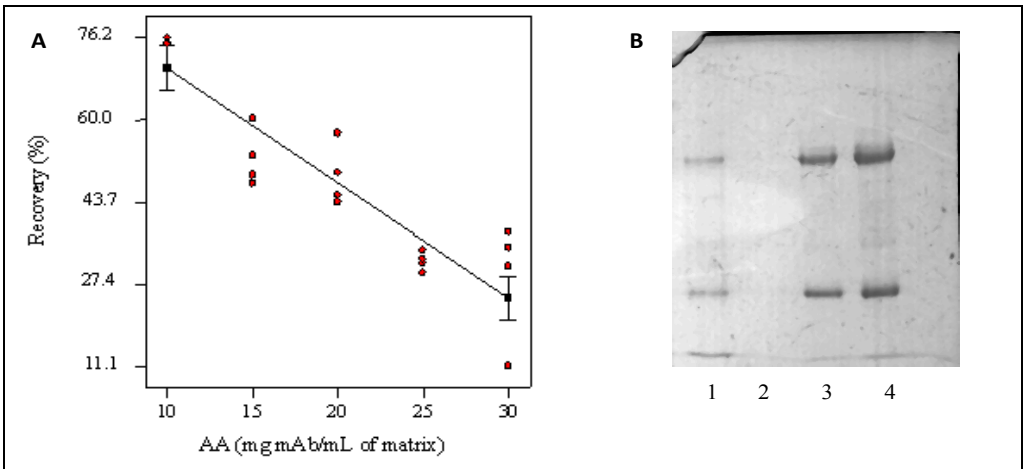
Statistical analysis

Statgraphics Plus version 5.0 (2000) from Statistical Graphics, Design Expert version 6.0.1 and Microsoft Excel softwares were used in this study. One factor experimental design with 5 levels was performed to study the load influence; four repetitions were carried out in each level. A student t's test with α=0.05 was used to compare chromatographic parameters among assessed buffer systems in the preliminary study. Adsorption and elution capacity were also statistically analyzed by the Mann and Whitney W test in the lifetime study.

Results and Discussion

The purification of mAb used for vaccine production with low aggregated values needs the design of very efficient downstream processes to reduce vaccine cost. In order to improve an industrial scale CB.Hep-1 mAb purification protocol based on the PASFF affinity chromatography, two buffer systems were assessed in repetitive 100 purification cycles at analytical scale. To start this study, matrix adsorption capacity, matrix elution capacity and recovery of mAb were assessed as a function of the applied amount of the CB.Hep-1 mAb (10, 15, 20, 25 and 30; (mg/mL of matrix)). In this sense, the best statistical models for each chromatographic parameter were illustrated in Figure 1A.

Adsorption capacity increased from 9.7 to 14.1 mg mAb/mL of matrix within the assessed range of the applied amount of mAb. While the elution capacity maintained statistically invariable (8.4 mg mAb/mL of matrix). Difference between adsorption and elution capacity could be explained by inefficiency in the elution procedure based on 10 mM citric acid; pH 3 buffer. It is well known that responsiveness of the Protein A to pH is a function of hydrophobicity. At low pH, the hystidyl residues, highly conserved residues, in the Protein A-binding site of IgG are fully charged minimally hydrophobic and mutually



Parameters	Goodness of Fit Test	Lack of fit test	Statistical Model*
Adsorption Capacity	0.02	0.35	AC = 7.94 + 0.21 AA
Recovery (%)	<0.0 01	0.06	R = 93.03-2.28AA
Elution capacity	-	0.43	EC = 8.34

AA represents the applied amount of CB.Hep-1 mAb (mg mAb/mL of matrix)

AC adsorption capacity expressed in mg mAb/mL of matrix

EC elution capacity expressed in mg mAb/mL of matrix

Figure 1: (A) Recovery plot for different applied amounts (AA) of the CB.Hep-1 mAb (phosphate/citric acid buffer system). (B) SDS-PAGE to demonstrate retention of the CB.Hep-1 mAb into the PASFF. Line 1, CB.Hep-1 mAb released from the PASFF after elution step. Line 2, CB.Hep-1 mAb released from the Sepharose 4 FF with the purified CB.Hep-1 mAb. Line 3, CB.Hep-1 mAb (15 µg) (SDS-PAGE control). Line 4, CB.Hep-1 mAb (20 µg) (SDS-PAGE control).

repellent [14]. Perhaps, this elution buffer was unable to charge fully of the hystidyl residues. As consequence, the mAb recovery decreased by 47.8% through studied interval of applied amount of mAb. Even the highest recovery (73.9% at 10 mg mAb/mL of matrix) observed in this study would not be suitable for Protein A-Sepharose FF affinity chromatography because the recovery of this chromatography usually ranged 80-90% [14].

Due to the ELISA applied to the samples of the unbound fractions did not revealed presence of the CB.Hep-1 mAb, we hypothesize that about 30% of the CB.Hep-1 mAb was retained into the affinity chromatography matrix. To confirm the CB.Hep-1 mAb retention into the PASFF, a SDS-PAGE experiment using this matrix after elution step was done (Figure 1B). As results, retention of mAb was confirmed when heavy and light chains of IgG were detected in heated PASFF after the elution step. Similar results were noted by us when the purification of the antigen was performed by immunoaffinity chromatography (data no shown).

To discard unspecific interactions of the CB.Hep-1 mAb with the PASFF, a size-exclusion chromatography using the same inert matrix but without the ligand (Protein A) was carried out. In this case, no IgG bands were observed in SDS-PAGE analysis, which did not reveal unspecific retention of mAb into the matrix. Therefore, retention of the CB.Hep-1 mAb into the matrix was helped by specific interactions with the Protein A.

Taken into consideration the relative poor recovery (52.3±5.7%) caused by the reduced adsorption capacity and elution capacity manifested when phosphate/citric acid buffer system was used; both, adsorption and elution buffers were redefined. The new adsorption buffer assessed was 1.5 M glycine-NaOH/3M NaCl; pH 9, which has been recommended for this purpose because it enhances IgG binding capacity of Protein A [14]. However, sometimes it is not recommended for large scale mAb purification because adsorption improvements are not always justified in comparison with the cost increase. Regarding to this, sometimes glycine has same effectivity as other compounds such as sodium sulphate, polyethylene glycol (PEG), sodium chloride, ammonium sulphate and potassium phosphate. However, we choose glycine because this mAb is quite stable in glycine and it does not have PEG disadvantages in terms of viscosity and of ammonium sulphate, which is volatile creating instability in buffer pH, toxicity and protein denaturalization.

Results of a preliminary proof-of-concept experiment applying the previously purified CB.Hep-1 mAb and the glycine buffer system to the affinity matrix showed an increase by 67% in the adsorption capacity (P=1x10^{-4}) and consequently in productivity, when 15 mg of mAb/mL of matrix was applied (data no shown). The adsorption capacity enhancement detected in the PASFF could be explained by the high molar concentration of buffer because of the majority of binding energy comes from hydrophobic interactions. Similar results were observed in the elution capacity analysis (P=3x10^{-5}) and recovery (P=2.5x10^{-5}). Summarizing, recovery increased up to 96%, which corroborated results reported by other authors [14].

After the first preliminary proof-of concept experiment using the previously purified CB.Hep-1 mAb, another preliminary experiment using the CB.Hep-1 mAb and glycine buffer system but with ascitic fluid, a complex biological source was performed. In this demonstration, the recovery observed with phosphate/citric acid buffer system (control) was 52.3±5.7%, whereas 96.0±1.9% was detected in the case of buffer system based on glycine (P=2.4 x 10^{-5}). Therefore, the use of glycine buffer system increased the CB.Hep-1 mAb recovery by 1.4 fold in this assessment (Table 1). In this case, both parameters (adsorption (9.0±1.0-15.0±0.2 and elution 7.8±0.8-14.5±0.2) showed significant improvements.

The mAb purity measured by SDS-PAGE and HPLC-GF showed no differences between the CB.Hep-1 mAb purified using both buffer systems (Table 1). Bands of 50-55 kDa and 25 kDa representing classical profile of heavy and light chains of IgG were observed and evidences of mAb fragmentation were not detected. The new buffer system also improved substantially purification yield (2.3 mg mAb/mL of ascites) with respect to phosphate/citric acid buffer system (1.6 mg mAb/ mL of ascites).

Several toxic effects such as alteration of the tissue uptake of immunocomplex, and toxic shock syndrome are associated with the presence of the staphylococcal Protein A [14]. In these mAb CB.Hep-1 elution fractions, the Protein A leakage was determined by an specific ELISA validated in presence of mouse inmunoblobulins, which permitted quantification without non-specific reactions the content of Protein A in mAb samples [13]. The content of this contaminant was less than 10 ppm for all CB.Hep-1 samples (Table 1). It represents a very low amount of Protein A because other authors have reported values of Protein A leakage up to 64 ppm using ELISA in ng range of sensitivity [15-17]. This might be explained by the fact that the interaction between Protein A and IgG_{2bk} is not to high as to provoke a relative high release of the covalently-linked Protein A from the sorbent.

Due to the malignant transformation properties of the mammalian cellular DNA, the quantification of mouse DNA content in the mAb preparation is another point to consider. In such sense, regulatory agencies are very strict. Nowadays, the acceptable level ranged 100 pg-10 ng of residual cellular DNA per human dose [18]. The dot-blot analysis performed to estimate the mouse DNA content in the mAb CB.Hep-1 samples evidenced the high purification capacity of this chromatography because the mouse DNA presented in the purified mAb CB.Hep-1 did not exceed 7.5 pg/mg of IgG (Table 1). These values did not exhibited significant differences between both experimental conditions.

Parameters	A	B
Adsorption capacity (mg/mL of matrix)	9.0 ± 1.0	15.0 ± 0.2
Adsorption capacity (%)	67.8 ± 2.7	98.7 ± 8.3
Elution capacity (mg/mL of matrix)	7.8 ± 0.8	14.5 ± 0.2
Elution capacity (%)	86.6 ± 0.3	96.6 ± 0.4
Recovery (%)	52.3 ± 5.7	96.0 ± 1.9
Purity measured by SDS-PAGE (%)	99.2 ± 1.0	99.6 ± 0.5
Purity measured by size-exclusion-HPLC (%)	96.5 ± 0.8	97.0 ± 2.82
Mouse DNA content (mAb pg/mg total protein)	< 7.5	< 7.5
Specific activity of purified CB.Hep-1 mAb (%)	98.3 ± 22.1	97.9 ± 10.2
mAb concentration (mg/mL)	5.6 ± 0.7	3.3 ± 0.6
Protein A concentration (ng/mL)	16.4 ± 1.2	4.2 ± 1.7
Protein A content (ppm)	2.9 ± 1.7	1.2 ± 1.2
Buffer system cost ($/g of purified mAb)	1.0	4.4
Productivity (mg mAb/L of matrix per hour)	50.9	96.1
Productivity increase (%)	100.0	188.6

Table 1: Results of the preliminary experiment II to purify the CB.Hep-1 mAb using ascites as complex biological source and both buffer systems (n=3). Buffer System A: 150 mM PBS, pH 8.0/100 mM citric acid, pH 3.0. Buffer System B: 500 mM glycine-NaOH/3M NaCl, pH 9.0/200 mM glycine-HCl, pH 2.5.

It is usual that matrix manufactures recommend the use of pH values in the elution buffer lower than 3 (1.5-2) to increase the elution capacity of matrixes, although these pH conditions would considerably affect the antigen binding capacity of mAb. In this study, the influence of the pH value 2.5 on the antigen recognition capacity of the CB.Hep-1 mAb was indirectly measured by the determination of the mAb "specific activity". Results demonstrated that this pH condition did not modify the HBsAg recognition capacity of the CB.Hep-1 mAb (Table 1).

Finally, the stability of the PASFF purifying the CB.Hep-1 mAb with the glycine buffer system was assessed in 100 purification cycles using ascites rich in the CB.Hep-1 mAb. The affinity chromatography columns were loaded at a capacity equivalent to 15 mg IgG/mL of matrix in every purification cycle and mAb were then harvested in one elution fraction as indicated above. Figure 2 illustrates an overlapping representation of representative chromatograms (purification cycles 1, 10, 30, 69 and 100) registered in the lifetime study. As it can be seen individual chromatograms were almost distinguishable in both buffer conditions demonstrating the high repeatability or consistence of the improved procedure.

The recovery trend of the CB.Hep-1 mAb was illustrated in the Figure 3. This study resulted in an average mAb recovery = 29.4±19.79% for phosphate/citric acid buffer system and 47.18± 26.67% for glycine buffer system (P=1.9 x 10^{-5}), which allow to corroborate results of the preliminary experiments but in 100 purification cycles. Again statistical differences were detected in the adsorption (P=1.94x10^{-11}) and elution capacity (P =8.79x10^{-5}) between both experimental conditions.

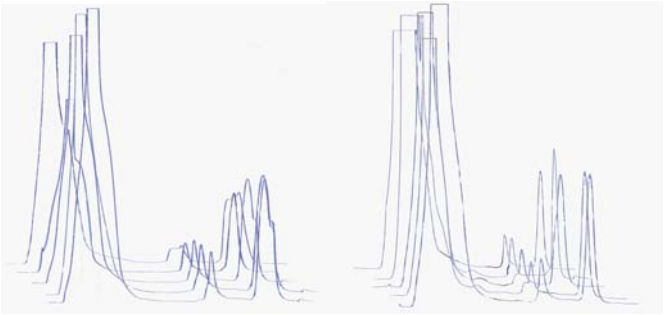


Figure 2: Partial overlay of the elution profiles of representative purification cycles (1, 10, 30, 60 and 100) of the life time study. (Left) 150 mM PBS, pH 8.0/100 mM citric acid, pH 3.0 buffer system. (Right). 1.5M glycine-NaOH/3M NaCl; pH 9.0/200 mM glycine-HCl; pH 2.5.

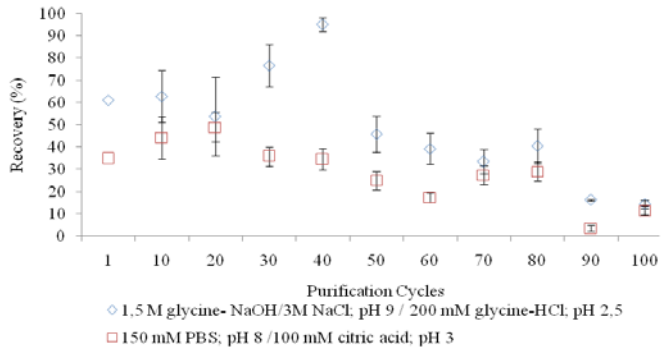


Figure 3: Results of the CB.Hep-1 mAb recovery in the PASFF affinity chromatography of the life time study.

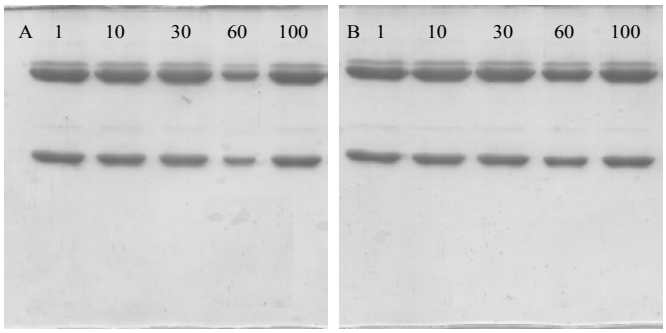


Figure 4: Results of the SDS-PAGE analysis to measure the purity of the CB.Hep-1 mAb purified by the PASFF affinity chromatography in the lifetime study (purification cycles: (1, 10, 30, 60 and 100). (Left) 150 mM PBS; pH 8.0/100 mM citric acid; pH 3.0 buffer system. (Right) 1.5M glycine-NaOH/3M NaCl; pH 9.0/200 mM glycine-HCl; pH 2.5 buffer system.

The antibody SDS-PAGE profiles obtained under reducing conditions (Figure 4) reveals no substantial differences among the purity measured in samples of the different purification cycles. Bands of approximately 50-55 kDa and 25 kDa represent the typical profile of the heavy and light chains of IgG. These profiles showed no evidence of fragmentation in the CB.Hep-1 molecule during the whole life time study. Results of the analysis of the purity and molecular homogeneity of the CB.Hep-1 performed by HPLC-GF did not evidenced differences in this parameter in samples of the mAb purified using both purification buffer systems (Figure 5). The second small fraction observed corresponded always with residues of the acid PASFF elution buffer and nit with fragment of the proteins. Thus, the number of the purification cycles had no significant effect on the purity of mAb CB.Hep-1.

Summarizing, taking into account the application of this mAb in vaccine manufacturing, our findings (mAb yield and process productivity increase) would make possible the purification 40% approximately more Hepatitis B surface antigen and consequently of vaccine doses using the same starting biological source volume for mAb purification. Beside the positive economical impact provoked by this vaccine production increase, the decrease in the CB.Hep-1 mAb unitary cost would additionally decrease drastically vaccine production cost. Therefore, mAb purification cost increase by glycine buffer system price (four-fold) is not significant with respect to benefits of its application, which is contradiction with previous reports [14].

Conclusions

The relative poor mAb recovery observed in 150 mM PBS; pH 8.0/100 mM citric acid; pH 3.0 buffer system conditions can be attributed to the elution buffer was not able to disrupt completely interactions between the PASFF and mAb. Retention of the CB.Hep-1 mAb into the matrix was helped by the ligand (Protein A) coupled to the matrix and not by unspecific interactions. The buffer system based on 1.5M glycine-NaOH/3M NaCl; pH 9.0/200 mM glycine-HCl; pH 2.5 significantly improved the affinity chromatography recovery without affecting the CB.Hep-1 mAb purity, molecular homogeneity, Protein A leakage and mouse DNA content in 100 purification cycles. Application of 1.5M glycine-NaOH/3M NaCl; pH 9.0/ 200 mM glycine-HCl; pH 2.5 as buffer system allowing to reduce the CB.Hep-1 mAb and vaccine cost. As general recommendation, it is necessary to remark that special cares should be taken during preparation and storage of glycine buffers

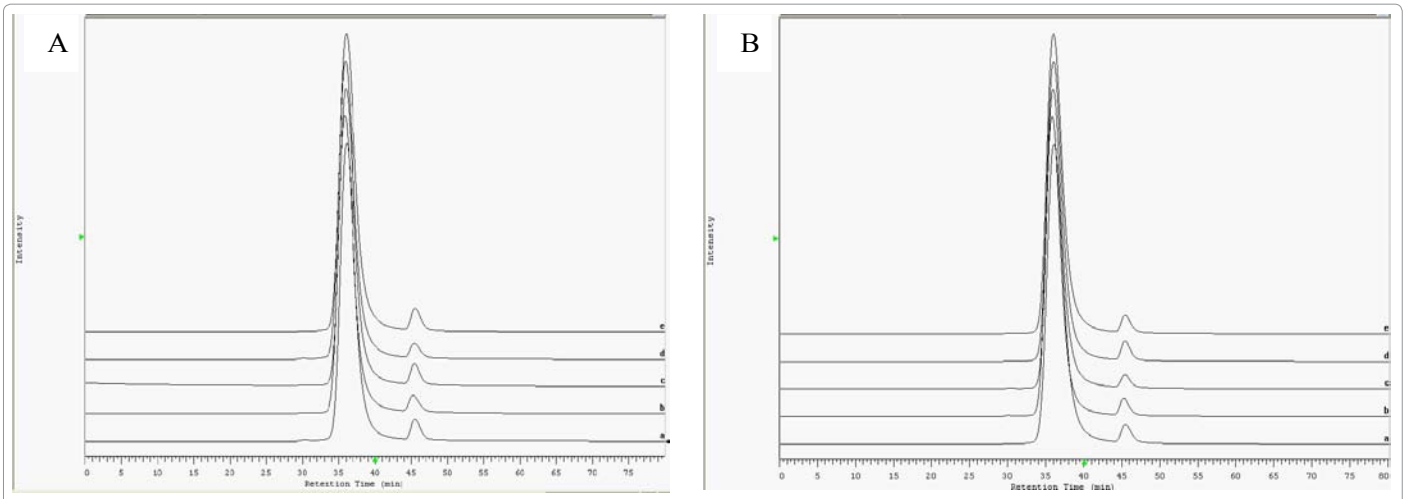


Figure 5: Results of the HPLC-GF analysis to measure the purity of the CB.Hep-1 mAb purified by the PASFF affinity chromatography in the life time study (purification cycles: 91, 10, 30, 60 and 100). (Left) 150 mM PBS; pH 8.0/100 mM citric acid; pH 3.0 buffer system. (Right) 1.5M glycine-NaOH/3M NaCl; pH 9.0/200 mM glycine-HCl; pH 2.5 buffer system.

for large scale processing because they obviously are more susceptible to microbial contamination.

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