Research Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2022.43.006892

Production of Equine Anti-Daboia Siamensis Immunoglobulin F(ab')$_2$ Fragments with Ammonium Sulphate and Caprylic Acid Precipitation and by Multimode Chromatography

Xin Li, Jiahui Shao and Xin Zhang*

Shanghai Serum Biotechnology CO.LTD, China

***Corresponding author:** Xin Zhang, Shanghai Serum Biotechnology CO.LTD, NO 1288 HuaQin Road, Shanghai (200000), China

ARTICLE INFO

Received: April 09, 2022

Published: April 21, 2022

Citation: Xin Li, Jiahui Shao, Xin Zhang. Production of Equine Anti-*Daboia Siamensis* Immunoglobulin F(ab')$_2$ Fragments with Ammonium Sulphate and Caprylic Acid Precipitation and by Multimode Chromatography. Biomed J Sci & Tech Res 43(3)-2022. BJSTR. MS.ID.006892.

Keywords: MEP Hyper Cel; DEAE; ELIS; F(ab')$_2$; Precipitation

ABSTRACT

Objective: To develop a novel procedure for preparation of equine anti-*Daboia siamensis* immunoglobulin F (ab')$_2$ fragments.

Methods: First, after plasma were diluted by 2 volume pure water, adjusted to pH3.2±0.1, the mixture was digested by 1.5% v/v pepsin for 70min, precipitated by combined with 0.5M ammonium sulphate and 1.75% (v/v) caprylic acid and centrifuged. Second, the supernatant adjusted to pH9.0±0.2 and conductive<40 was loaded into MEP Hypercel resin and eluted with 20 mM sodium dihydrogen phosphate citric acid buffer (pH 3.8±0.2). Last, the unbounded F(ab')$_2$ in the sodium dihydrogen phosphate citric acid buffer (pH5.8±0.2) flowed through the DEAE resin, and flow through fluid was diafiltrated into 0.02M PB buffer (pH 7.4±0.2) on a membrane with a MWCO of 50 kDa.

Results: After the whole process, the purity of final production was over 90% and the overall yield of F (ab')$_2$ was about 68%.

Conclusion: A novel procedure for preparation of equine anti-*Daboia siamensis* immunoglobulin F (ab')$_2$ fragments was developed. What's more, the optimized procedure is short-time, easy to operate and also promised for large-scale production.

Introduction

Published estimates of global burden suggested a range from a minimum of 420 000 snake envenoming and 20 000 deaths up to as many as 2.5 million cases and more than 100 000 deaths each year [1]. Snake antivenom immunoglobulins (antivenoms) from equine plasma are the specific therapeutic products for the treatment of snakebite envenoming. The production of the antivenom began in 1894 and nowadays all antivenom producers are based on salting out to precipitate specific proteins without or with enzyme digestion. The methods originated by pope [2,3] and later modified by other workers for the isolation, purification and concentration of the specific antibody. There are several protocols for whole (IgG) or fragments (F (ab')$_2$ and Fab) of immunoglobulins production by ammonium sulfate (AS) precipitation combined with other strategies. Two precipitation steps recommended by WHO are included employing two different AS concentrations in addition to the elimination of "euglobulins" by precipitation in a diluted acidic solution, which lead to the formation of protein aggregates and to a recovery of IgG of between 40 and 50% [1]. In fact, the recovery of F (ab')$_2$

reach to over 90% after serum was digested by pepsin and reach to over 80% after first AS precipitate. The lower yield may be caused by some aspects:

a) Heating may markedly increase the rate of destruction of antitoxin especially when the temperature is above 57°C.

b) The serum containing phenolic preservatives increase loss of greatly accelerated by rise in temperature.

c) There is still over 10% F (ab')$_2$ kept in the supernatant of second AS precipitate and the the supernatant is discarded [2,4]. After comparted the purification effect of two ion exchange adsorbents (Streamline TM SP and paramagnetic CM beads), thiophilic beads and guanidine beads, Kumpalume etc found that Streamline TM SP had better dynamic binding capacity and that the thiophilic and hydrophobic matrices showed better selectivity than the cation exchange matrices [5]. Zhang etc use two steps salting out with AS followed ultrafiltration concentration liquid purified by one successive protein G affinity chromatography [6]. In conclusion, the protocol of two precipitation steps maybe combined with other methods leading to the lower purify and the lower recovery of F (ab')$_2$.

There are also several protocols for F (ab')$_2$ production by caprylic acid (CA) precipitation combined with other strategies. Plasma was precipitated by 2% CA, digested by pepsin and then infiltrated with a 50 kDa molecular weight cut-off (MWCO) polyethersulfone membrane [7]. Plasma was precipitated by 2% CA, digested by pepsin and then based on anion exchange column in flowthrough mode [8], cationic ion-exchange column in bind-elute mode [9], or protein An affinity chromatography [10]. Plasma was digested by pepsin and further precipitated by 1.5% CA [8,9,10], or combination with AS [11,12]. Plasma was added with pepsin and 2% CA and then infiltrated with a 50 kDa MWCO polyethersulfone membrane [8]. CA precipitation approach has some advantages: First, since CA precipitates most plasma proteins-mainly albumin-except the immunoglobulin fraction, the production of antivenoms has relatively high purity and with a low aggregate protein and reach over to 97% of the IgG/F (ab')$_2$ yields [7]. Second, the administration of large volumes of albumin containing 4–20 mM CA for over 50 years suggests that CA at the concentration showed safe. Last, CA clear viruses' infectivity in a single step at 0.02M CA at pH 4-5 for 30min [13]. In this study, we provide an alternative method to obtain F (ab')$_2$ equine antivenom, and employed MEP Hyper Cel chromatography combined with DEAE chromatography to deal with the supernatant of AS precipitation combined with CA. The overall yield of F (ab')$_2$ was about 70% and 90% pure F (ab') 2 after finishing the whole process including digestion, precipitation, chromatography, and ultrafiltration.

Materials and Methods

Materials and Reagents

Our company purchased the antivenins production technologies in 1999 from Shanghai Institute Biological Technology Co., Ltd, which was one of antivenins manufactures. (https://apps.who.int/bloodproducts/snakeantivenoms/database/). The equine anti-*Daboia siamensis* antiserum was separated in our company from blood with adding phenol and stored at 4°C until purification. Pepsin from porcine stomach (Merck, Germany) was purchased. The DEAE Sepharose fast flow (name DEAE) were obtained from GE Healthcare (Uppsala, Sweden) and the resin MEP Hyper Cel was purchased from Pall life science (East Hills, NY, USA). Every resin was loaded into a 16x20mm column (XK 16/20, GE Healthcare) and the purifications were performed using an AKTA™ avant pure 150 system. TSK-Gel G3000SWXL column (7.8 × 300 mm) was purchased from Tosoh (Tokyo, Japan). Rabbit anti-horse IgG (Fc specific)-HRP (category: SAB3700145-2mg) was purchased from Sigma. Other reagents were bought directly from local markets.

Protein Concentration Determination

As described previously [7], total protein concentration was estimated spectrophotometrically during the isolation procedure.

Size-Exclusion Chromatography (SEC)

SEC, which was used to monitor the purity in all purification steps, was run on TSK-Gel G3000SWXL column (7.8 × 300 mm) with 0.1M phosphate-sulphate running buffer (pH 6.6) at a flow rate of 0.5 mL min-1 on Agilent 1260 Infinity II HPLC system (Agilent Technologies, USA). Standard molecular weights used to calibrate the column were γ-globulin (150 kDa), ribonuclease A (13.7 kDa), thyroglobulin (665 kDa) and ovalbumin (44.3 kDa). The effluent was monitored at 280 nm [7].

ELISA

The whole IgG in 100ml supernatant precipitated by CA (crude IgG) was further purified to obtain the purity of 98% (named pure IgG) by protein G chromatography [7], of which 20mg was labelled with horseradish peroxidase (HRP). As described [14], pure IgG labelled with HRP (HRP-IgG) and Rabbit anti-horse IgG (Fc specific)-HRP (HRP -Fc-IgG) were determined by direct ELISA. Briefly, a microliter plate was coated with 1ug/well of *Daboia siamensis* venom in 100ul 0.05 M sodium carbonate bicarbonate buffer (pH 9.6) and left overnight at 4°C. HRP- IgG (1mg/ml) with different dilutions (1/100-1/100000) were tested and the optimal HRP-IgG dilution was obtained when the titrate HRP-IgG was arrived at 1-1.5 OD units. Similarly, HRP -Fc-IgG dilution of 1/4000 was used to determine the pure IgG (1mg/ml) with different dilutions (1/100-

1/100000) in order to obtain the optimal pure IgG dilution. The indirect ELISA was applied to identify the degree during pepsin digestion. The microtiter plate was coated with 1ug/well venom and HRP -Fc-IgG dilution of 1/4000 was used as second antibody. The OD450 average value of the wells added with 100ul pure IgG (total 5*10-6mg) as primary antibody were defined as reference, and 100ul sample (1mg/ml) from different digestion time with appropriate dilutions (1/100-1/10000) were employed to be measured primary antibody. It was easy to determinate the relevant concentration of sample from different digestion time. For example, if the mean OD450 values of wells loaded with the sample after 60 min digestion with dilution 1/ 400 , plasma with dilution 1/10000 and pure IgG (1mg/ml) were 1.15, 1.20 and 1.25, the relevant concentration was 2%: (5*10-6mg/100ul x dilution factor 1200)/ (5*10-6mg/100ul x dilution factor 60000)=0.02 (this is 2%), which showed there was about 5% undigested intact IgG in the mixture.

As described previously [14], the competitive ELISA was used to quantify the antibody titer of IgG and F $(ab')_2$. In brief, the microliter plate was coated as same as above direct ELISA. Sample from each purification step with serial dilutions (1/5000-1/100000) were mixed with HRP-IgG dilution 1/20000 (named standard HRP-IgG dilution) in 100ul PBST-BSA (0.15 M PBS, pH7.4 containing 0.5% BSA and 0.05% Tween-20). The standard HRP-IgG dilution in PBST-BSA was used as the reference. The titer of each sample was defined as the sample dilution that had an absorbance equal to half the absorbance shown by the standard HRP-IgG dilution alone (50% competition).

Pepsin Digestion and Precipitation

Pepsin digestion and precipitation were performed in 10 L rector under automatic control. After diluted by 4L pure water to aid mixing, 2L plasma was mixed by stirring (200 rpm/min), heated to 37°C and then adjusted to pH3.2±0.1 with 0.5 mol/L HCL and pepsin at a ratio of 1.5% w/w of total antiserum volumes (1.5 g of pepsin for each liter of plasma) was added and then incubated at 37 °C. After the digestion had lasted for 30, 50, 60, 70, 80 and 90 minutes, 100ml mixture was drawn from the reactor, adjusted to pH 7.4±0.2 and detected by SDS-PAGE and the indirect ELISA, respectively. After the digestion had continued for 70min and the pH of the mixture was then raised to 5.2±0.1 by addition of 0.5 mol/L NaOH, the mixture was cooled down and five copies of 100 ml mixture were taken out. Each was added by a final concentration of 1.75% v/v CA and 5%, 7.5%, 10% or 12.5% AS, respectively and mixed by stirring (200rpm/min) for one hour at 25 °C, followed by centrifugation for 20 min at an rpm of 12000. The supernatant was collected and detected by HPLC, and competitive ELISA analysis and the precipitation was redissolved in PBS buffer in order to detect by HPLC. The supernatant after precipitated by 1.75% v/v CA and 7.5% AS was used to subsequent purifications.

MEP Hyper Cel Resin Multimode Chromatography (MMC) in Binding Model

After MEP Hyper Cel resin was equilibrated in 20mM Glycine-NaOH buffer (pH 9.0±0.2) containing different concentration AS, the supernatant was adjusted to pH 9.0±0.2, diluted with different volume 20mM Glycine-NaOH buffer (pH 9.0±0.2) and then loaded into the column. Proteins bound were washed with 20mM Glycine-NaOH buffer (pH 9.0±0.2) and eluted with 20 mM sodium dihydrogen phosphate citric acid buffer (pH 3.8±0.2). According to the manual, cleaning-In-Place (CIP) was conducted with 0.5M NaOH containing 1M NaCl and the buffer immediately was adjusted to pH7.4±0.2 for HPLC.

Anion Ion-Exchange Chromatography in Flow-Through Model

After DEAE resin was equilibrated in 20 mM sodium dihydrogen phosphate citric acid buffer (pH 5.8±0.2), the solution eluted from MEP chromatography was adjusted to pH 5.8±0.2, diluted by 20mM sodium dihydrogen phosphate citric acid buffer (pH 5.8±0.2) in order that its conductive was less than 40 and its protein concentration was less than 5mg/ml, loaded into the column. The target F $(ab')_2$ flowed through the resin and was collected. CIP was carried out by 0.5-1M NaOH followed by pure water to elute tightly bound molecules.

Diafiltration Step

F$(ab')_2$ sample eluted from DEAE chromatography was diafiltrated into 0.02M PB buffer (pH 7.4±0.2) on a membrane with a MWCO of 30 kDa. In each diafiltration step the buffer was exchanged by a factor of 8,000.

Result and Discussion

HRP -Fc-IgG and HRP- IgG

When the microtiter plate was coated with 1ug /well of *Daboia siamensis* venom and HRP- IgG (1mg/ml) was added with dilution 1/20000, the OD450 read value show around 1-1.2 OD units and so HRP- IgG (10-5 mg/ml) was named standard HRP-IgG dilution. Similarly, when the 100ul HRP -Fc-IgG dilution of 1/4000 and 100ul pure IgG (total 5*10-6 mg) were combined, the OD450 read arrive at about 1.2.

Digestion

It is necessary to be demonstrated that the manufacturing process is involved in inactivation and/or removal of potential viral contamination. Because CA can clear viruses' infectivity in a single step at 50g CA/kg solution at pH 5.5-5.8 for 1h [1], CA precipitation scheme was adopted in our study. There are two routes including:

a) Precipitation by CA and then digestion [7] or

b) Digestion and then precipitation by CA.

After compared the two routes in practice, we found they were practicable and therefor chose (b), the simpler one. As pepsin activity is highest at 37°C and below pH 4 and the digestion should be completed in 2h, we added pepsin 1.5 g/L into plasma at 37°Cand pH 3.2±0.1 and further identified the optimum digestion time.Digestion resulted in the complete elimination of albumin with 30min and of intact IgG by 70min (Figure 1). In addition, with conventional SDS-PAGE analysis, the indirect ELISA by means of HRP-Fc-IgG was first used to quantify the undigested IgG. When the plasma digested for 60 min was diluted with dilution factor 400 and carried out the indirect ELISA, which indicted there was about 2% undigested intact IgG at 60 min. The HPLC profiles showed there were four main peaks: at 10 min, 14.1 min, 15.4 min and 27 min, which represent unknown large protein, intact IgG, albumin, and phenol, respectively (Figure 2A). After the plasma digested for 70 min, there were three new peaks: at 15.2 min, 17.1 min and 18.4min, of which the peak at 15.2 min represents F $(ab')_2$ (Figure 2B). Based on ELISA results, the recovery of F $(ab')_2$ was 90±3% after the plasma was digested at pH 3.2±0.1 and 37°C by 1.5 g/L pepsin for 70min.

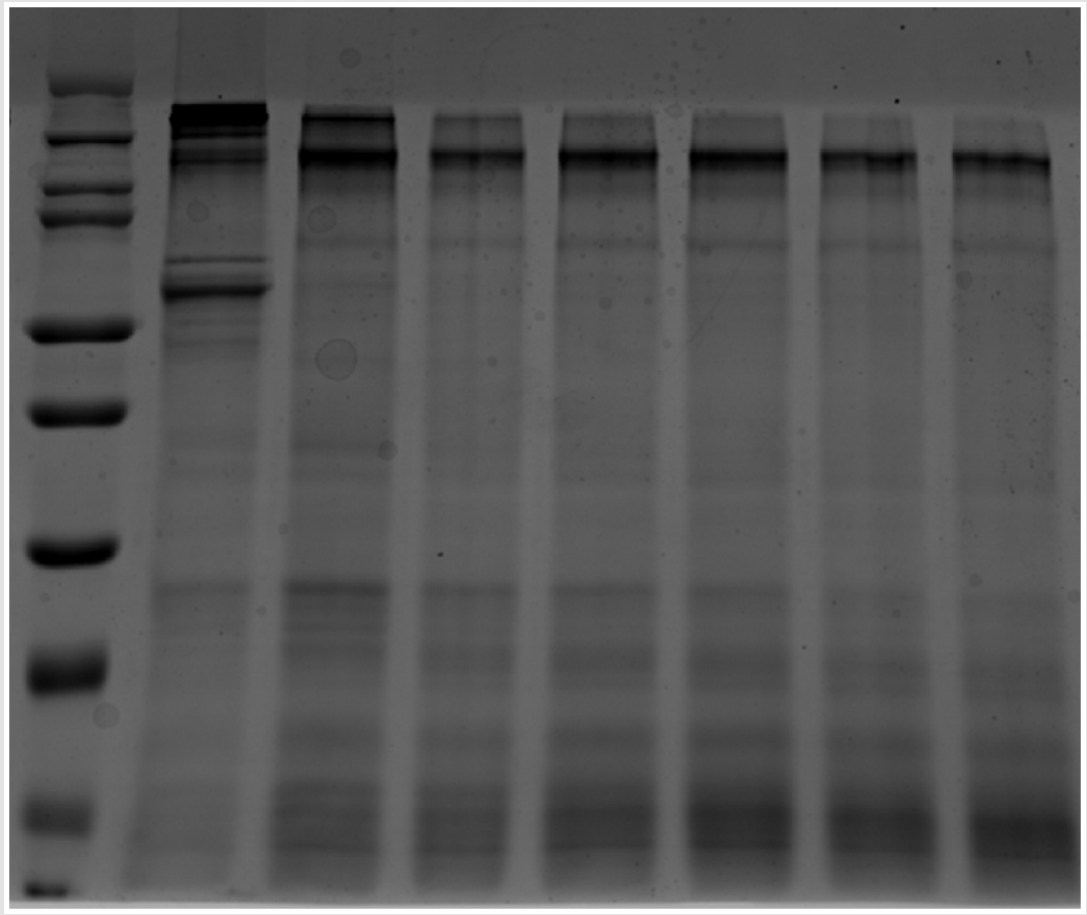

Figure 1: 12% of non-reducing SDS-PAGE analysis of digestion process. digestion samples at 0min (lane 2), 30min (lane 3), 50min (lane 4), 60min (lane 5), 70min (lane 6), 80min (lane 7), 90min (lane 8), and marker (Lane 1) (Bio-Rad catalog #161-0377), 250, 150, 100, 75, 50, 37 and 25 kDa From top to bottom.

Precipitation

Because AS precipitate can precipitate fibrin [3] and CA precipitates most plasma proteins mainly albumin except immunoglobulin, precipitation combined with CA and AS is necessary and economic. As the recovery of F $(ab')_2$ produced by two different AS concentration was between 40 and 50% [1], our study was to test the different AS concentrations' effect on the loss of F $(ab')_2$ by precipitation re-dissolution. It's clear that over 10% or higher concentration of AS lead to the loss of F $(ab')_2$ (Figures 2E-2F) and large molecular weight than F $(ab')_2$ including two peaks at 9.4 min and 10.1min were removed when compare Figure 2B with Figure 3B. we used 1.75% v/v CA and 0.5M AS (about 6.6%) to deal with the digested serum, which was apparent lower than 12% AS recommended by WHO [1] .Based on competitive ELISA results, the recovery of F $(ab')_2$ was 90±3 %, which suggested that lower AS lead to less loss of F $(ab')_2$.

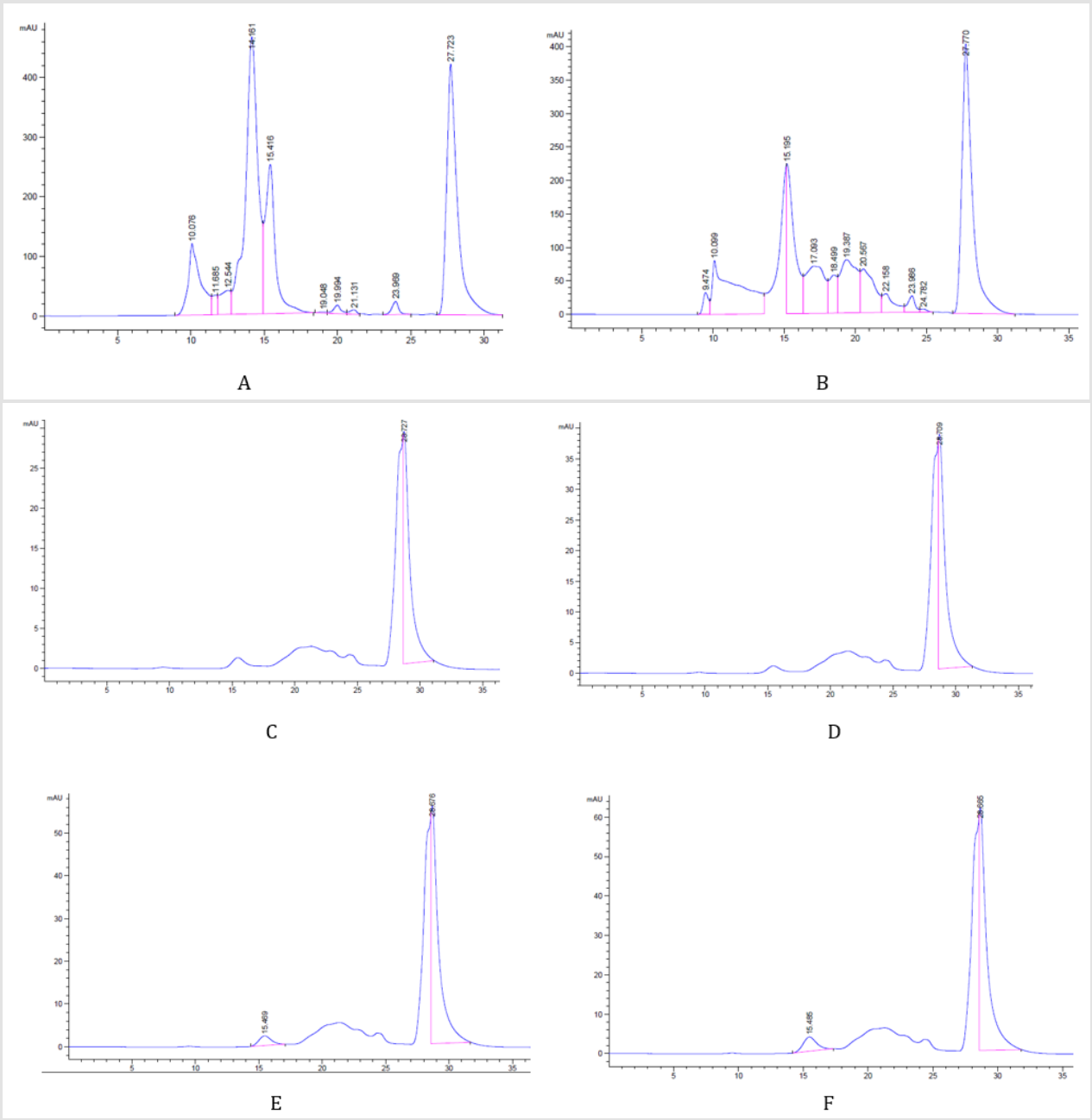

Figure 2: Different AS concentrations' effect on the loss of F (ab') 2. (A) plasma diluted by 2 volume pure water (pH7.8±0.3), (B) plasma digested by 1.5 g/L pepsin for 70 min, (C-F) the substances precipitated by 1.75 v/v CA and 5%、7.5%、10% or 12.5% AS and redissolved in PBS buffer, respectively.

Removal of Much Impurity by MEP MMC

Because this process that remove the small peptide fragments and/or reagents (AS or CA) by dialysis in cellulose (MWCO 30kDa) bags is time-consuming, costs much pyrogen-free water and also risks contamination with endotoxins. Although chromatography based on hydrophobic and/or ionicity of F (ab') 2 was widely tested for many years, the purity effect was unsatisfactory and not replace the salting out. We believe that the isoelectric point of F (ab ')$_2$ is widely distributed between 6-9 [5], which leads to the larger pH range of eluent and the poor effect of removing protein impurity when ion-exchange chromatography was used. What's more, the effect of removing macromolecular proteins by ion-exchange chromatography is poor; On the other hand, equine f (ab ')$_2$ has many subtypes [5] and the hydrophobicity of each subtype is very different, which results the poor separation effect when hydrophobic chromatography was used.

MMC provides more than one type of interactions including traditional ion and/or hydrophobic between the resin ligand and the sample component. As MEP resin owns immunoglobulin-selective merit in addition to weak anion and hydrophobicity the same as other mixed-mode resins, we applied MEP resin to purify F $(ab')_2$ from the supernatant in the study. F $(ab')_2$ could be bond the MEP resin in 20mM Gly-NaOH (pH 9.0±0.2) containing 0.2 AS (conductive<40), washed with 20 mM Gly-NaOH (pH 9.0±0.2), and eluted with 20 mM sodium acetate (pH 3.8±0.2). Much impurity made of small molecular weight proteins and phenol was flew through and there was almost no F $(ab')_2$ in CIP buffer. At the same time, the purity of eluted F (ab') 2 was about 85% by HLPC analysis and the yield was up 93±2% based on competitive ELISA (Figures 3B-3E).

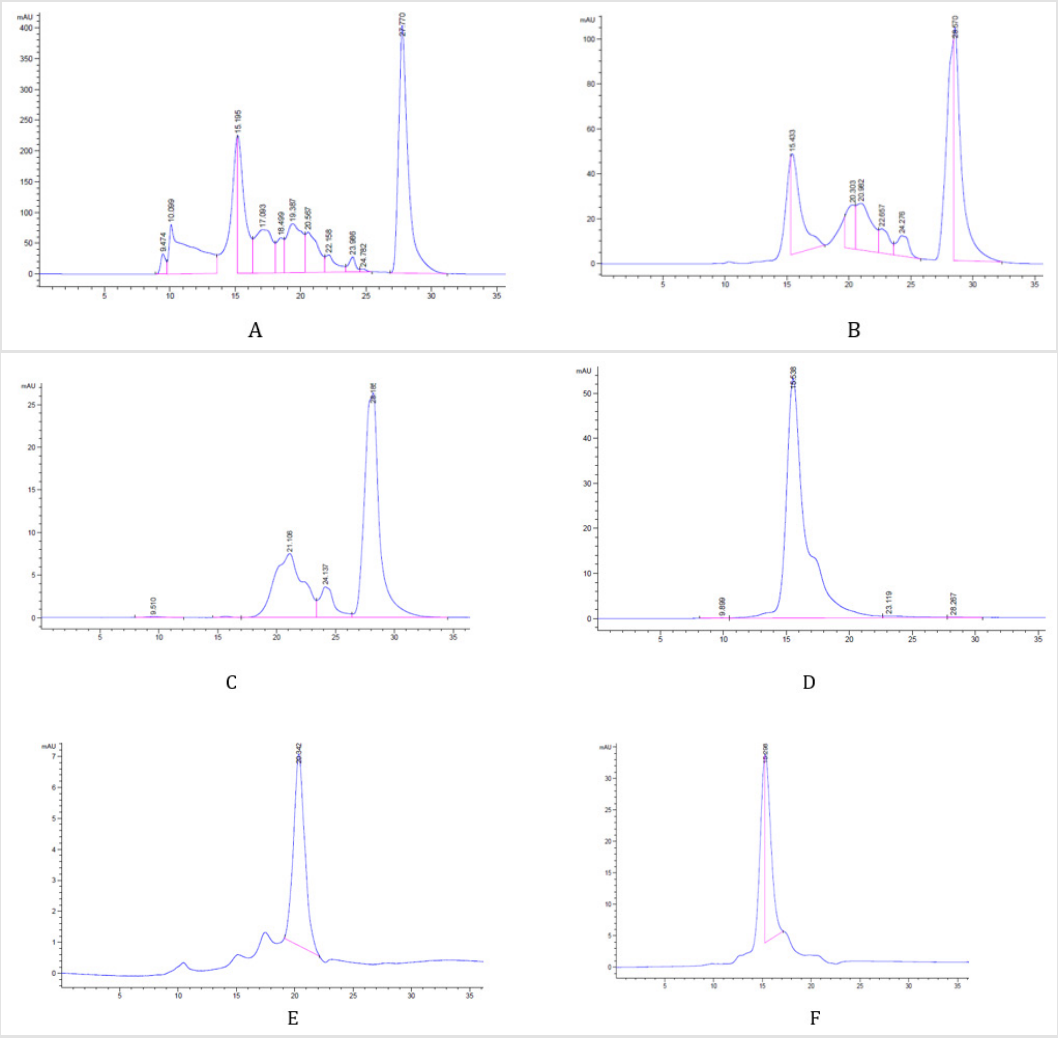

Figure 3: MEP MMC. (A) plasma digested by 1.5 g/L pepsin for 70 min (same as figure 2B), (B) loaded sample (the supernatant precipitated by 1.75 v/v CA and 7.5% AS), (C-E) the flow through fluid, elution, CIP solution of MEP chromatography, respectively; (F) the flow through fluid of DEAE chromatography.

The ideal purification effect may be based on the merits of MEP resin. First, protein A/G and MEP can bind immunoglobulin in a significant different mechanism [15]. Protein A/G mainly combines with Fc by means of antigen antibody binding, but MEP mainly combines with antibodies by the aid of the hydrophobicity of its ligand (4-mercapto-ethyl-pyridine, 4-MEP). The pH of the elution from MEP MMC is about pH 4, which is milder than protein A /G chromatography and reduced the loss of F$(ab')_2$ activity. Moreover, the subtype binding ability of protein A /G is different, which may be unfavorable to the purification of equine polyclonal antibody; Second, the absorption of F$(ab')_2$ is largely independent of ionic strength (e.g., in NaCl concentrations ranging from 50 mM to 1 M) and IgG concentrations ranging from 50 μg/mL to 5 mg/mL, which is suitable to deal with the supernatant containing about 0.2M AS and about 4mg/ml F $(ab')_2$. Near the pH 9.0, F$(ab')_2$ has higher dynamic binding capacity (DBC) of MEP and Fc has very low

absorption ability [15]. The binding of MEP ligands to F (ab')$_2$ is dominated mainly by hydrophobic interaction and most of hydrophilic and weaker hydrophobic molecules were washed with 20 mM Gly-NaOH (pH9.0±0.2). The hydrophobic interaction could be destroyed at pH4.0-5.8 so that the buffer (pH 3.8 ±0.2) can fully elute F (ab')$_2$.

F (ab')$_2$ refined by DEAE Chromatography and Diafiltration

As the ion exchange (IEX) chromatography is effective at removing pepsin (7, 10), DEAE chromatography was utilized to purify F (ab')$_2$ from the elution from MEP MMC in order to remove pepsin (isoelectric point about 2.2) and other proteins/peptides. HLPC analysis shows that the CIP contains some impurities and almost no F (ab ') $_2$, and that the purity of F (ab')$_2$ in the flow through solution exceeds 90% (Figure 3F). F (ab')$_2$ refined by ultrafiltration with membrane package (MWCO 50 kDa) from flow through solution and concentrated into 0.02M PB buffer (pH 7.4 ± 0.2). After completing the whole process including enzyme digestion, AS and CA co-precipitation, MEP and DEAE chromatography and ultrafiltration, HPLC analysis showed that the purity of F (ab')$_2$ was more than 90%, and the competitive ELISA results showed that the total yield of F (ab')$_2$ was about 68% (68 + 3%).

Conclusion

In conclusion, this study improved the traditional process:

1) HRP-FC-IgG was used for competitive ELISA detection, which can quantify the process of pepsin digestion more accurately than traditional SDS-PAGE and other methods, and effectively prevent over or insufficient digestion.

2) We confirmed that the recovery of F (ab ')$_2$ can improve as reducing the concentration of AS by redissolution.

3) Depending on the excellent properties of MEP resin, the method could effectively remove protein impurity that sample was loaded with "high salt and high pH" buffer and MEP resin was washed with "low salt and high pH" buffer and eluted with "low salt and low pH" buffer. In the whole process IgGs or F(ab')$_2$ fragments were kept in solution, ensuring quality and, therefore, safety of the final product. In addition, the process has the advantages of short operation time, simple operation and low cost. However, although this protocol has independently studied four batches of plasma on a laboratory scale, further research is needed, including the suitability for larger scale production and preclinical and clinical efficacy of the prepared final product.

Conflict of Interests

None.

References

1. (2017) WHO Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins.
2. Pope CG, Stevens MF (1951) The action of proteolytic enzymes on the antitoxins and proteins in immune sera. II. Heat denaturation after partial enzyme action 32(4): 314-324.
3. (1939) The action of proteolytic enzymes on the antitoxins and proteins in immune sera. I.-true digestion of the proteins.
4. Harms AJ (1948) The Purification of Antitoxic Plasmas by Enzyme Treatment and Heat Denaturation. 42(3): 390-397.
5. Kumpalume P, Boushaba R, Jones RGA, Slater NKH (2002) Facile F(ab´)$_2$ manufacturing: strategies for the production of snake antivenoms. Food and Bioproducts Processing 80: 88-97.
6. Xue Jun Zhang, Hai Ling Li, Da Yi Deng, Chong Ji, Xiao Dong Yao, et al. (2018) Functional and proteomic comparison of different techniques to produce equine anti-tetanus immunoglobulin F(ab')$_2$ fragments. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences 1092.
7. Tihana Kurtović, Maja Lang Balija, Marija Brgles, Dora Sviben, Monika Tunjić, et al. (2019) Refinement strategy for antivenom preparation of high yield and quality. PLoS Negl Trop Dis 13(6): e0007431.
8. Ibrahim Al Abdulla, Nicholas R Casewell, John Landon (2014) Single-reagent one-step procedures for the purification of ovine IgG, F(ab')$_2$ and Fab antivenoms by caprylic acid. J Immunol Methods 402(1-2): 15-22.
9. Sunil Kumar Arora, Partha Rakshit, Dilip Singh Rawat, Ravi Kumar Gupta (2016) Development of Modified Caprylic Acid Fractionation Method for the Enhanced Yield and Purity of Equine Polyvalent Antisnake Venom Immunoglobulins. J Bioprocess Biotech 6: 8.
10. Aldon Fernandes, John Oswald Kaundinya, Gautam Daftary, Lalit Saxena, Subhasis Banerjee (2008) Chromatographic purification of equine immunoglobulin G F(ab´)$_2$ from plasma, J Chromatogr B Analyt Technol Biomed Life Sci 876(1): 109-115.
11. Azadeh Taherian, Mohammad Fazilati, Ahmad Taghavi Moghadam, Hamid Tebyanian (2018) Optimization of purification procedure for horse F(ab´)$_2$ antivenom against Androctonus crassicauda (Scorpion) venom. Tropical Journal of Pharmaceutical Research 17(3).
12. A study on the use of caprylic acid and ammonium sulfate in combination for the fractionation of equine antivenom F(ab')$_2$ arapepsins Two Proteolytic Enzymes Associated with Porcine Pepsin.
13. (2009) The ELISA Guidebook (2nd Edn.)., humana press.
14. Ying-Di Luo, Qi-Lei Zhang, Xiao-Ming Yuan, Wei Shi, Shan-Jing Yao, et al. (2017) Selectivity evaluation and separation of human immunoglobulin G, Fab and Fc fragments with mixed-mode resins. J Chromatogr B Analyt Technol Biomed Life Sci 1040: 105-111.
15. Lewis M, Wagner B, Woof J (2008) The different effector function capabilities of the seven equine IgG subclasses have implications for vaccine strategies. Mol Immunol 45 (3): 818-827.

ISSN: 2574-1241

DOI: 10.26717/BJSTR.2022.43.006892

Xin Zhang. Biomed J Sci & Tech Res