Original Article

Comparison of two gel filtration chromatography resins for the purification of foot-and-mouth disease virus as a purified vaccine antigen

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ABSTRACT

Introduction: Foot-and-mouth disease (FMD) is a highly contagious and economically devastating viral disease of livestock that is categorized in list A of animal diseases by the World Organisation for Animal Health (OIE). Vaccination is effective against FMD and the vaccine production centers largely use the industrial ultra-filtration and chromatography in order to remove the cellular proteins as well as the non-structural viral proteins. The recommended method for the purification and quantification of the active ingredient of vaccines is 140S quantitative sucrose density gradient analysis. Despite many advantages, this method is highly operator-dependent and is not suitable for large-scale vaccine productions. The main objective of this study was to compare and evaluate two chromatography resins (i.e. Sephacryl S-300 and Sephacryl S-500) to separate FMD virus particles from the non-structural viral proteins. **Methods:** The resins were compared for gel filtration chromatography and the virus infectivity titration (CCID50% / ml) and real-time PCR amplification analyses were performed. **Results:** The results indicated that Sephacryl S-500 was not able to separate blue dextran from bovine serum albumin; therefore, it was not suitable for separation of the whole virus from the non-structural proteins while Sephacryl S-300 was suitable for this purpose. **Conclusion:** Sephacryl S-300 is a suitable resin for preparation of purified virus for large-scale FMD vaccine production.

KEYWORDS: Foot-and-mouth disease virus; Size exclusion chromatography (SEC); NSP-free FMD vaccine.

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious disease in cloven-hoofed ruminants such as sheep, goats, cattle and pigs [1, 2]. Seven serologic types and many subtypes of the virus have been identified, among them serotypes A and Asia are prevalent in Iran [3, 4]. FMD virus (FMDV) is a small, non-enveloped virus that belongs to the Aphthovirus genus of the Picornaviridae family. Like other picornaviruses, FMDV possesses a single-stranded positive-sense RNA genome of approximately 8'500 nucleotides, encapsidated in 25 nm icosahedral capsid made of 60 copies of four capsid proteins, namely VP1, VP2, VP3 and VP4 [5-8].

The molecular weight (Mw) of the whole virus and its RNA are 8'000 and 2'800 kDa, respectively [9, 10]. FMD is endemic in many parts of the world and the World Organisation for Animal Health (OIE) periodically reports its distribution and outbreak maps. FMD sanitary status has a profound economic impact on countries with meat-based economies [11, 12]. In FMD control and eradication programs in both enzootic and non-enzootic settings, the inactivated vaccines have been used which their effectiveness is highly based on the integrity of FMDV particles.

The need to further purify vaccine antigens was not only arisen to prevent the unwanted allergic reactions to the cell proteins by the animals after multiple vaccinations but also to allow the differentiation of the infected from the vaccinated animals during control campaigns[13-16].

In gel filtration or size-exclusion chromatography (SEC), the molecules in a solution are separated according to the differences in their sizes as they pass through a column packed with a chromatographic medium such as a gel. The pores in the gel matrix which are filled by the liquid phase are comparable in size to the molecules we may wish to separate. The relatively

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small molecules can diffuse into the gel from a surrounding solution whereas the relatively large molecules will be prevented by their size from diffusing into the gel to the same degree. Here, we attempted to compare and evaluate two chromatography resins used in such chromatography setups, namely Sephacryl S-500 and S-300 columns for the purpose of FMD virus purification.

MATERIALS and METHODS

Preparation of virus

FMDV strains O1 Iran 2010 vaccine strains were originally obtained from Razi Vaccine and Serum Research Institute (RVSRI; FMD Department). The virus was inoculated into 48-h BHK21 clone 13 cell monolayer culture incubated at 37° C for 20 h and then were stored in -70 °C. All materials (the virus and BHK21 cells) were obtained from RVSRI, Karaj, Iran.

Blue dextran and BSA solutions

The comparison of packed columns with two resins (Sephacryl S-300 and S-500) for separation of the virus from the cell proteins as well as the non-structural viral proteins was performed by mixing of blue dextran and bovine serum albumin (BSA) in 2 mg/ml and 5 mg/ml concentrations; respectively. BSA was served as a marker for the viral non-structural proteins (i.e. DNA polymerase and protease) and blue dextran was served as a marker for the intact viral particles.

Infectivity assay

The virus infectivity was determined as follows. Ten-fold serial dilutions of the virus sample made in EMEM cell culture medium (pH 7.3) were inoculated into 48-h IBRS2 (BA) monolayer cell culture (obtained from RVSRI) in 96-well micro plate and were incubated for 72 h after the inoculation. The 50% cell-culture-infective-dose (CCID50) was calculated by the method of Reed and Muench [17].

Gel chromatography

Knauer UV detector 2550 chromatographic system was used. The equipment consists of a 10 mm optical path flow cell with selectable 254/280 nm filters and a ChromGate 3.3.2 control and analysis software. The chromatograph was fitted with XK 16/40 column. Harvested virus suspension was clarified by centrifugation at 10’000 × g for 20 min. Blue dextran and BSA solutions and the clarified virus were applied separately to a Sephacryl S300 and S500 (Pharmacia Fine Chemicals, Uppsala, Sweden) column (XK 16/40, 1.6 by 40 cm) equilibrated with 0.01 M phosphate buffer (pH 7.2) containing 0.15 M NaC1. The column was eluted with the same buffer. The flow-rate was set at 0.65 ml/min. Fractions (4 ml) [18] were collected and analyzed by quantitative RT-PCR (SYBR Green) and the virus infectivity was measured using the CCID50 assay.

Quantitative RT-PCR

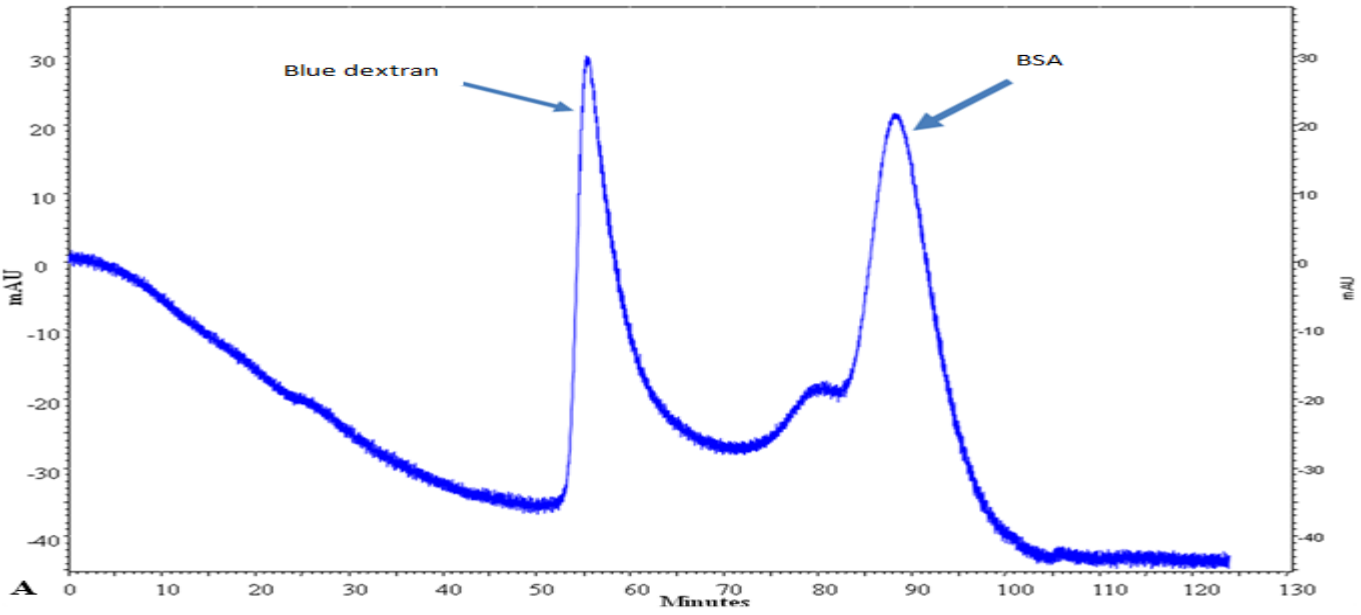
Viral RNA was extracted from the selected fractions by High Pure viral RNA extraction kit (Roche, Germany). RNA was subjected to one-step Quantitative RT-PCR using the universal primers for FMD virus detection, namely forward primer, Callahan 3DF (sequence: ACT GGG TTT TAC AAA CCT GTG A) and reverse primer, Callahan 3DR (sequence: GCG AGT CCT GCC ACG GA). RT-PCR mix containing 0.5 µl forward primer 0.3 pmol, 0.5 µl reverse primer 0.3 pmol, 1 µl Super Script One-Step RT-PCR with Platinum Taq Polymerase (Invitrogen, USA), 12.5 µl enzyme buffer and 10.5 µl DEPC water in 25 µl total volume reactions.

Amplification was carried out in a rotor-gene 6000 thermal cycler (Corbett Life Science) using the following program: 45°C for 5 min , 1 cycle ; 95° C for 5 s , 1 cycle ; 95° C for 5 s and 60 °C for 20 s , 40 cycles. Threshold cycle (Ct) was assigned to each PCR reaction [19].

RESULTS

Gel filtration chromatography

The blue dextran and BSA mixture for resin evaluation and virus preparations of O2010 strain were applied to Sephacryl S-300 and Sephacryl S-500, separately. The elution profile obtained is shown in Fig. 1. After applying blue dextran and BSA solution to Sephacryl S-300 HR column, two separate curves were seen where a retention time for blue dextran was 58 min and for BSA was 90 min (Fig. 1A). After applying the same solution to Sephacryl S-500 HR column, two overlapped curves were seen. The retention time for blue dextran was 60 min and for BSA was 58 min (Fig. 1B).



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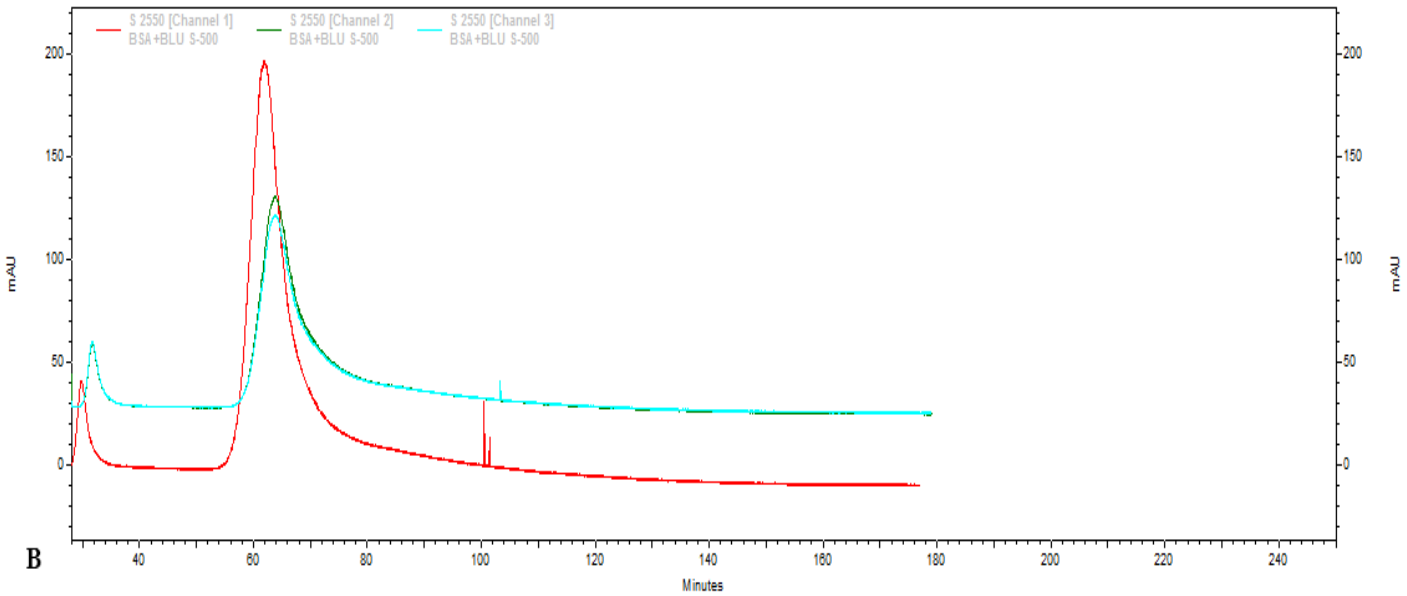
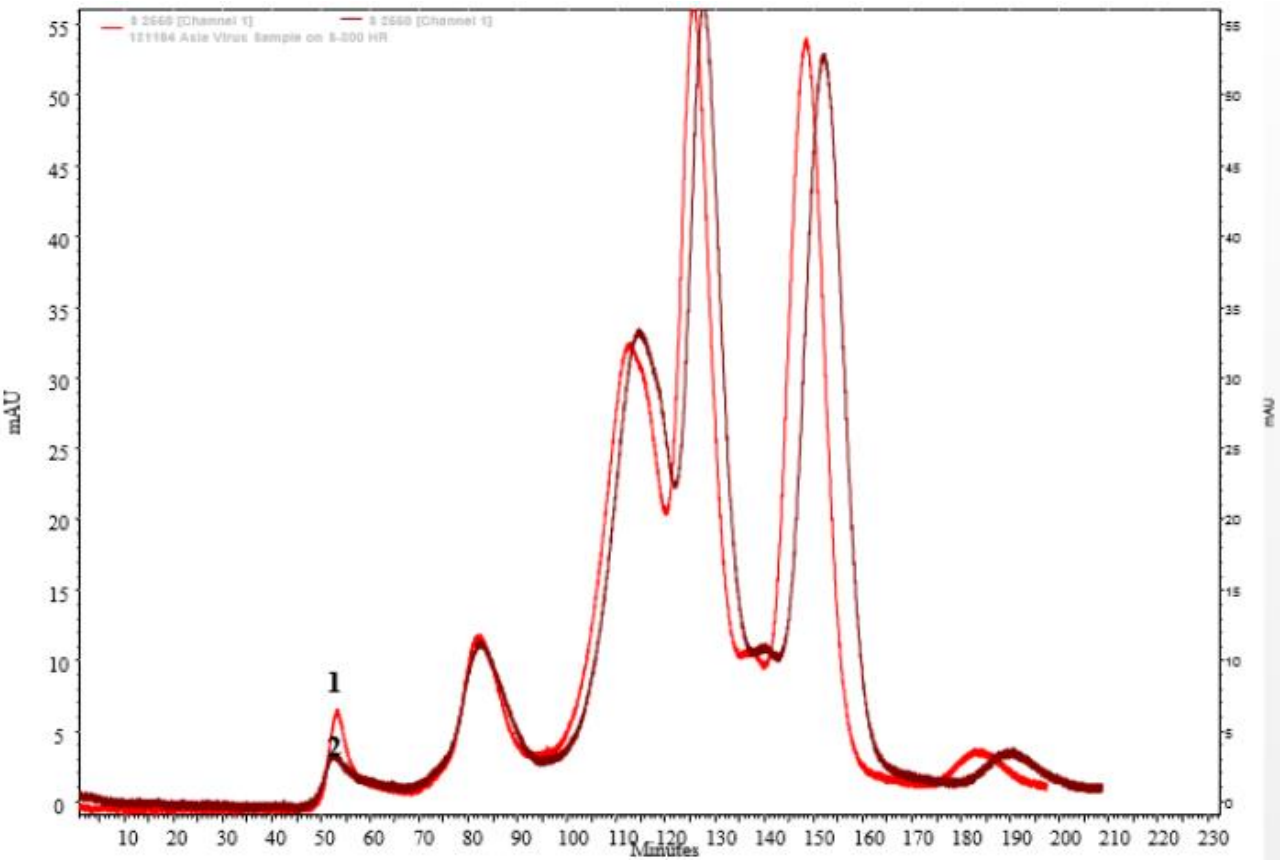


Fig. 1. Gel filtration chromatography (blue dextran and BSA). The peak lines represent the absorbance at 280 nm.
A) Samples were loaded onto a Sephacryl S-300 HR column, resolved at a flow rate of 0.65 ml/min and were collected as 4-ml fractions.
B) Samples were loaded onto a Sephacryl S-500 HR column, resolved at a same condition as A.

After applying the virus sample (FMD virus type O2010) to Sephacryl S-300 HR column, the purified FMDV was eluted by approximately 200 ml of the elution buffer and after 50 min in the first asymmetric peak. These peaks were collected respectively in 4-ml fractions (Fig. 2). Several peaks were seen, the first one represents the virus (Peaks 1 and 2) that were eluted from the column at 50 min. The other one represents the culture medium protein impurity in the virus suspension. This result indicated that Sephacryl S-300 was an efficient resin for FMDV virus separation from the other medium protein components of the culture.

On the other hand, after applying the virus sample to Sephacryl S-500 HR column, the first peak contained a mix of the virus and the culture medium proteins. This resin could not separate the virus from the other proteins, rendering Sephacryl S-500 not applicable for FMD virus purification.



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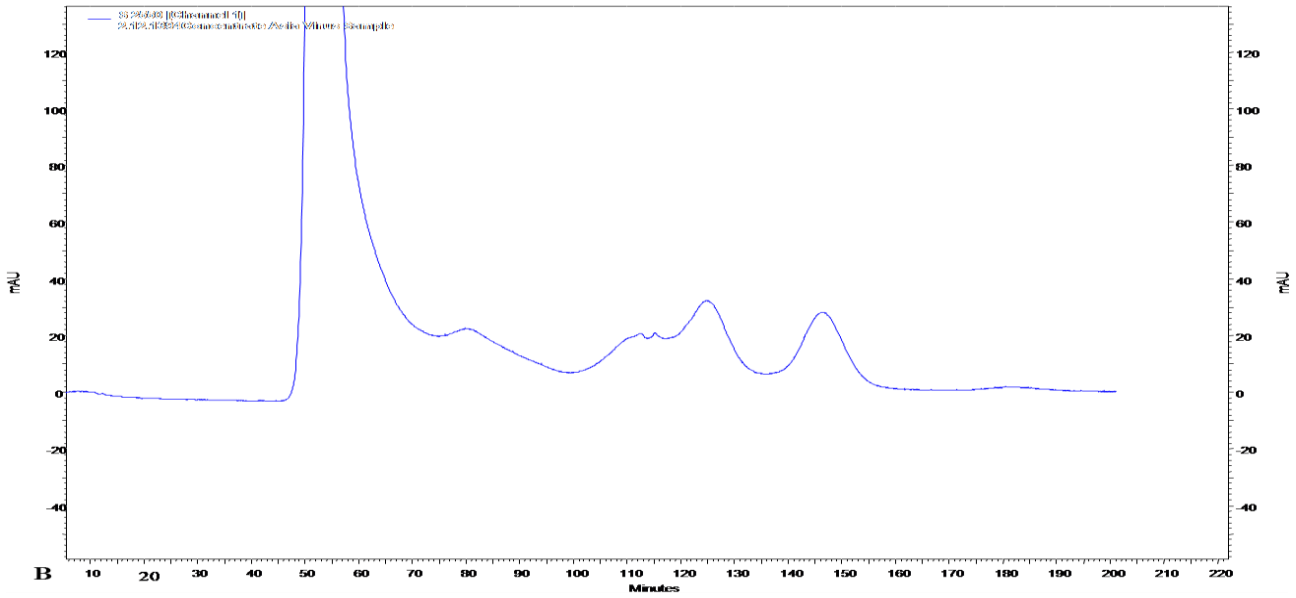

Fig. 2. Applying FMD virus to chromatography column.
A) Sephacryl S-300 column: the first asymmetric peak represents the eluted virus after 200 ml elution buffer running.
B) Sephacryl S-500 column: the first peak contains the virus and other protein impurities.

qRT- PCR reactions

The main objective of this set of experiments was to compare the results of the elution time of the virus with CCID50 of the eluted fractions during the chromatography with the Ct values obtain from real time RT-PCR, in order to find the optimum time for the virus isolation with the highest titer. As shown in Fig. 3, the fluorogenic reactions from the eluted fractions (shown in different colors on the plot) from Sephacryl S-300 indicated a stepwise amplification at earlier cycles reflecting a better separation in a stepwise manner and the presence of the virus in the earlier fractions (Fig. 3A).in compare with Sephacryl S500 amplification at later cycle and presense of the virus in the later fractions (Fig. 3B).

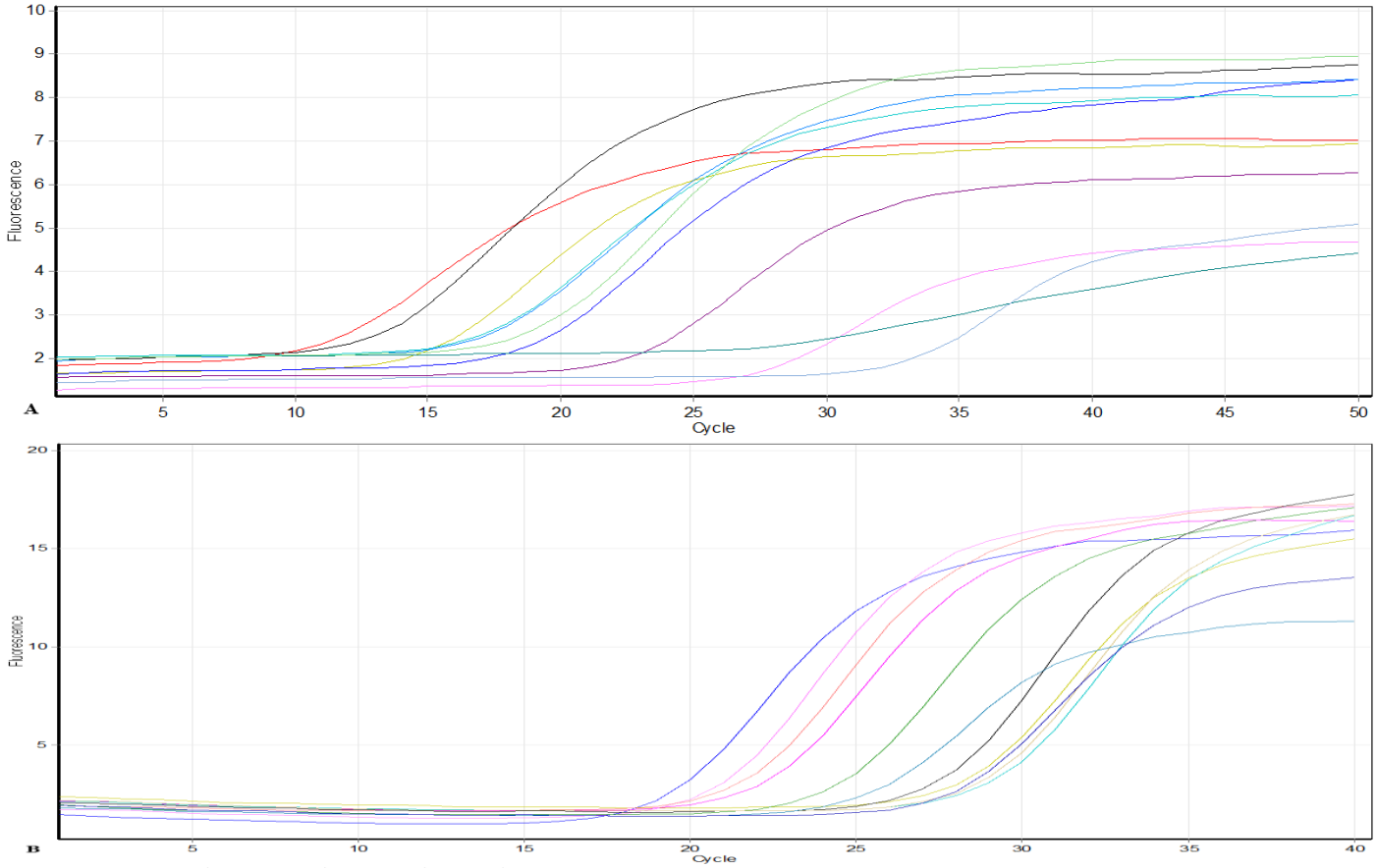

Fig. 3. Sigmoidal amplification plot of the eluted fractions from Sephacryl S-300HR (A) and Sephacryl S-500 HR (B) by real-time RT-PCR assay.

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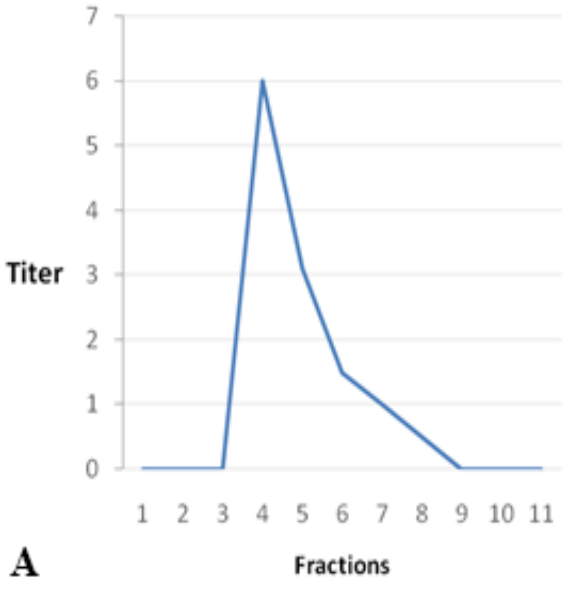
The virus titer

According to the results, the lower Ct values correspond to a higher virus assay. The Ct value for fraction 3 in Sephacryl S-300 and fraction 4 in Sephacryl S-500 is lower than the others and show higher concentrations of the virus according the virus titer (Table 1A, 1B). The titer of the virus before applying to the column was 6.25 CCID50/ml. The infectivity titer curve of the eluted virus sample fractions obtained from Sephacryl S-300 and Sephacryl S-500 column chromatography is shown in Fig. 4 which confirmed higher virus titers obtained at an earlier fraction (i.e. 3) by Sephacryl S-300 column.

Table 1. The Ct obtained from the quantitative RT-PCR in comparison with the virus infectivity titer (CCID50/ml).

Fraction (A)	Elution time	Titer CCID50/ml	Ct value
F1	45	0	31.17
F2	50	0	36.43
F3	55	6	14.61
F4	60	3.1	15.99
F5	65	1.5	17.79
F6	70	1	20.45
F7	75	0.5	20.42
F8	80	0	21.60
F9	85	0	25.70
F10	90	0	42.81
F11	95	0	32

Fraction (B)	Elution time	Titer CCID50/ml	Ct value
F1	45	0	26.15
F2	50	0	26.34
F3	55	0	25.86
F4	60	4.2	16.49
F5	65	3.4	17.96
F6	70	1.8	18.7
F7	75	1	19.75
F8	80	1	21.63
F9	85	0	24.70
F10	90	0	23.03
F11	95	0	25.54



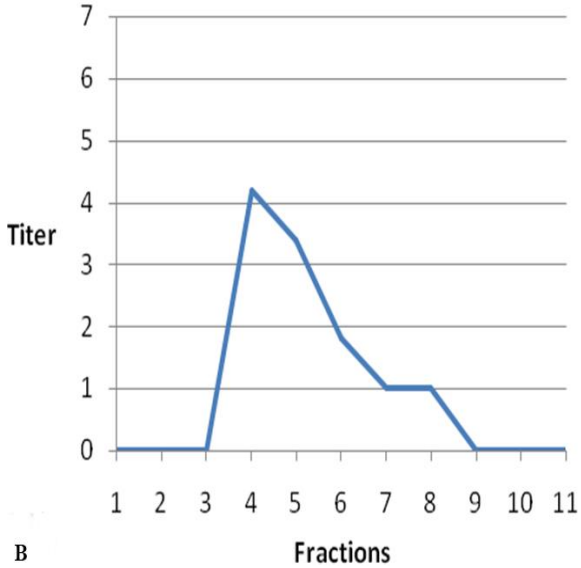


Fig. 4. The virus infectivity titer from the fractions eluted from Sephacryl S-300 (A) and Sephacryl S-500 (B).

DISCUSSION

SEC, also known as molecular exclusion or gel permeation chromatography (GPC), is a separation technique for molecules based on their size which has been utilized for the purification of different viruses in many species [20, 21]. To our knowledge, there are few reports of purification of FMD virus by SEC method. In the present study, the SEC method was used to compare two resins, namely Sephacryl S-300 and S-500 for the purpose of purification of FMD virus, designed for a large-scale production. The fractionation ranges in Mw for Sephacryl S-300 HR are from 10'000 to 1'000'000 Da while for Sephacryl S-500 are from 10'000 to 100'000'000 Da. Small molecules can diffuse into the gel from a surrounding solution whereas the relatively large-size molecules such as FMD virus will be prevented from diffusing into the gel at the same level.

FMD virus has a Mw of approximately 8,000,000 Da; therefore, it can pass through the column between the resin beads and can be rapidly eluted. However, the BHK cell and cell culture medium proteins and also the non-structural or disassembled viral capsid proteins with a low Mw are eluted between the void and the total volumes. In our practice, non-purified preparations of FMDV were still contaminated by the host cell (BHK) proteins passing through the column packed with Sephacryl S-500 HR. This is due to the very wide fractionation and large pore size of the beads where small molecules pass rapidly from the pores and can be detected in the void volume; hence, complete separation will not happen. On the contrary, when Sephacryl S-300 HR was used, the virus particles were completely separated from the protein impurity. This happens since the small molecule could be trapped in the gel pores and be completely separated from the large molecules. A purification procedure is used to remove the non-structural proteins (NSP) from the structural protein (SP) of the viral capsid components, in order to produce high-quality FMDV vaccines. However, during natural FMDV infection, the virus NSP may induce immune responses which are detectable using diagnostic measures.

SEC methods have been used in previous studies for the purification of the viruses. Spitteler and colleagues have used SEC method to quantify FMD virus particles during the

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production of the vaccine. In that study, they have used XK 16/70 column fitter Shephacryl S-400 with a flow rate of 1 or 1.3 [20]. Similar to our approach, Nagano and associates in 1989 had preferred to use column chromatography method instead of SDG, using Sephacryl S1000 resin to purify infectious bronchitis virus (IBV) [21]. Many years earlier in 1964, Giron and colleagues had used Sephadex G-25 resin for the purification of polio virus which like FMDV belongs to the Picornaviridae family [22]. According to the previous studies, chromatography methods have performed better for the purification of viruses in researches involved with viral vaccines such as influenza and recombinant hepatitis B [23, 24]. The compilation of the results in this study showed that Shephacryl S-300 is a more effective resin than Shephacryl S-500 for purification of FMDV by SEC method; hence, Shephacryl S-300 could be recommended for large scale vaccine production against this virus.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Muhammad K, Tariq MA, Altaf I, Rabbani M, Khawaja RM, Akram Q et al. Effect of Different Adjuvants Used in Foot and Mouth Disease Virus Vaccine on Antibody Response of Buffalo Calves. J Anim Plant Sci. 2013;23(3):736-9.
2. Chinsangaram J, Piccone ME, Grubman MJ. Ability of foot-and-mouth disease virus to form plaques in cell culture is associated with suppression of alpha/beta interferon. J Virol. 1999;73(12):9891-8.
3. Knowles NJ, Samuel AR. Molecular epidemiology of foot-and-mouth disease virus. Virus Res. 2003;91(1):65-80.
4. Mahravani H, Keyvanfar H, Izadi H, Salehizadeh M, Taghizadeh M, Sotudeh M et al. Genetic and antigenic analysis of type O and A FMD viruses isolated in Iran. Archives of Razi institute. 2007;62(2):63-8.
5. Bradish CJ, Henderson WM, Kirkham JB. Concentration and electron microscopy of the characteristic particle of foot-and-mouth disease. J Gen Microbiol. 1960;22:379-91. doi:10.1099/00221287-22-2-379.
6. Zhang Z, Alexandersen S. Detection of carrier cattle and sheep persistently infected with foot-and-mouth disease virus by a rapid real-time RT-PCR assay. J Virol Methods. 2003;111(2):95-100.
7. Mason PW, Grubman MJ, Baxt B. Molecular basis of pathogenesis of FMDV. Virus Res. 2003;91(1):9-32.
8. Gu C, Zheng C, Shi L, Zhang Q, Li Y, Lu B et al. Plus- and minus-stranded foot-and-mouth disease virus RNA quantified simultaneously using a novel real-time RT-PCR. Virus Genes. 2007;34(3):289-98. doi:10.1007/s11262-006-0019-2.
9. Cann AJ. Virology Labfax. Molecular Biotechnology. 1994;1(3):313-.
10. Brown F. The history of research in foot-and-mouth disease. Virus research. 2003;91(1):3-7.
11. Vallat B. Role of the international organisation for animal health (Office International des Epizooties: OIE) in the control of foot and mouth disease. Comp Immunol Microbiol Infect Dis. 2002;25(5-6):383-92.
12. Alexandersen S, Zhang Z, Donaldson AI, Garland AJ. The pathogenesis and diagnosis of foot-and-mouth disease. J Comp Pathol. 2003;129(1):1-36.
13. Doel TR. FMD vaccines. Virus Res. 2003;91(1):81-99.
14. Vannie P, Capua I, Le Potier MF, Mackay DK, Muylkens B, Parida S et al. Marker vaccines and the impact of their use on diagnosis and prophylactic measures. Rev Sci Tech. 2007;26(2):351-72.
15. Graves JH. Transfer of Neutralizing Antibody by Colostrum to Calves Born of Foot-and-Mouth Disease Vaccinated Dams. J Immunol. 1963;91:251-6.
16. Rodriguez LL, Grubman MJ. Foot and mouth disease virus vaccines. Vaccine. 2009;27 Suppl 4:D90-4. doi:10.1016/j.vaccine.2009.08.039.
17. Reed SC. Uniovular Twins in Mice. Science. 1938;88(2270):13. doi:10.1126/science.88.2270.13.
18. Wang R, Wang J, Li J, Wang Y, Xie Z, An L. Comparison of two gel filtration chromatographic methods for the purification of Lily symptomless virus. J Virol Methods. 2007;139(2):125-31. doi:10.1016/j.jviromet.2006.09.008.
19. Valasek MA, Repa JJ. The power of real-time PCR. Adv Physiol Educ. 2005;29(3):151-9. doi:10.1152/advan.00019.2005.
20. Spitteler MA, Fernandez I, Schabes E, Krimer A, Regulier EG, Guinzburg M et al. Foot and mouth disease (FMD) virus: quantification of whole virus particles during the vaccine manufacturing process by size exclusion chromatography. Vaccine. 2011;29(41):7182-7. doi:10.1016/j.vaccine.2011.05.078.
21. Nagano H, Yagyu K, Ohta S. Purification of infectious bronchitis coronavirus by Sephacryl S-1000 gel chromatography. Vet Microbiol. 1989;21(2):115-23.
22. Giron DJ, Heliman A. Purification of Poliovirus by Deae 'Sephadex a-25'. Nature. 1964;204:263-4.
23. Huang Y, Bi J, Zhang Y, Zhou W, Li Y, Zhao L et al. A highly efficient integrated chromatographic procedure for the purification of recombinant hepatitis B surface antigen from Hansenula polymorpha. Protein Expr Purif. 2007;56(2):301-10. doi:10.1016/j.pep.2007.08.009.
24. Kalbfuss B, Wolff M, Morenweiser R, Reichl U. Purification of cell culture-derived human influenza A virus by size-exclusion and anion-exchange chromatography. Biotechnol Bioeng. 2007;96(5):932-44. doi:10.1002/bit.21109.

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