Comparison of Column Chromatography Techniques For The Purification of Influenza A/NWS/33 (H1N1) Virus

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ABSTRACT

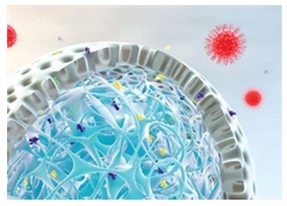
The gold standard in purification of influenza virus is by means of ultracentrifugation. Although effective, this process is very expensive and thus impractical for developing countries. We hypothesize that column chromatography can be a cost efficient alternative that is as effective as ultracentrifugation. If correct, this method of purification could revolutionize vaccine production in third world countries. We tested the purification ability of column chromatography by comparing two different chromatography resins. The Capto Q resin separates proteins on the basis of protein charge. The Capto 700 resin separates proteins on the basis of both size and charge. Samples following chromatography were collected, and evaluated for virus specific proteins as well as total protein content. After chromatography selected samples were evaluated by electrophoresis to determine protein separation. Although it is evident that some protein separation occurred, the results are inconclusive and suggest more testing.

INTRODUCTION

One vital step in vaccine production is the purification of the virus in the vaccine. Normally this purification is done through ultracentrifugation. While effective ultracentrifugation is extremely expensive making it unpractical for developing countries. Column Chromatography is a purification alternative that is extremely cost efficient. We hypothesize that column chromatography may be as effective as ultracentrifugation and could be utilized in developing countries for vaccine production. Influenza A/NWS/33 (H1N1) virus was used to test the purification ability of column chromatography. MDCK cells were infected with virus, the virus was then inactivated by means of Binary Ethyleneimine (BEI). To compare the purification ability of column chromatography two different chromatography resins were used: Capto Q and Capto 700.

Capto Q is a resin that separates proteins on the basis of charge. This is done by anion exchange. The Capto Q resin uses a quaternary Amine group that has a positive charge. This allows proteins with a negative charge to bind to the column allowing them to be separated from the rest of the solution flowing through the column.

Capto 700 is a resin that separates by size as well as charge. This is because the resin is composed of little beads. These beads have a ligand-activated core and an outer layer that is composed of little microscopic pores. The outer shell prevents large molecules from entering and binding to the hydrophobic and positively charged ligands.



Because both resins separate on the basis of charge, elution buffers with a step gradient of increasing salt concentrations were used for elution of bound proteins.

METHODS

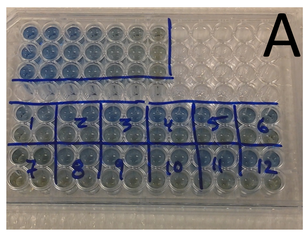
Sample Collection from Columns:

Samples were collected from both columns after buffer was added to the columns. The columns were initially washed with a phosphate buffer followed by 150ml of the BEI inactivated virus. After the virus solution had been added to the columns they were washed again with the phosphate buffer. 100ml increments of different KCl containing phosphate buffers were then added to each column starting with a 0.1M KCl buffer to a 0.5M KCl. The columns were washed a third time and final eluates were collected.

METHODS CONTINUED

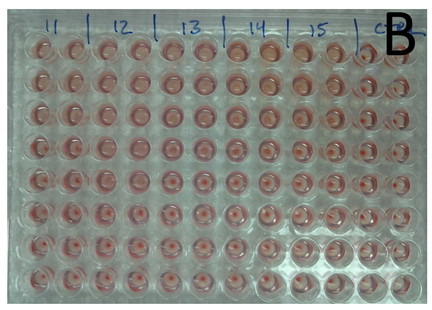
Protein Assay:

A Bradford Protein Assay was used to evaluate total protein concentration. The samples were compared to a standard containing Bovine Serum Albumin that ranged from 0 to 2000 µg of protein.



Hemagglutination Assay:

To determine viral protein content, samples were evaluated by hemagglutination assay. This was done by mixing sample dilutions in “u” bottom plates with turkey red blood cells.

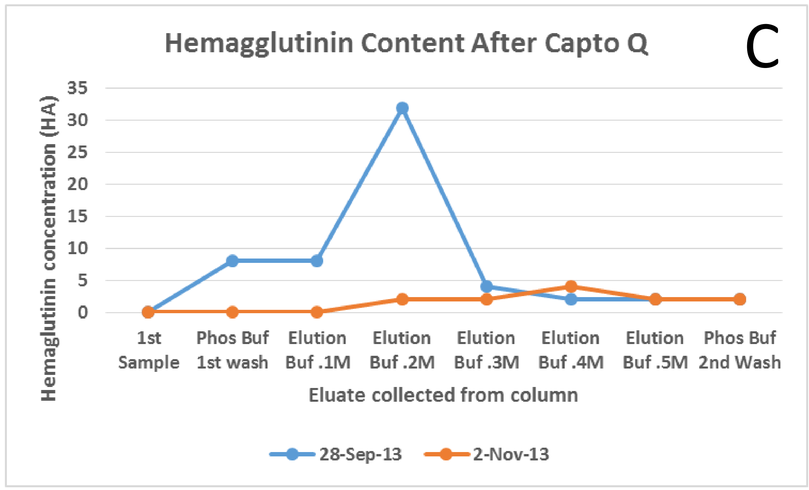


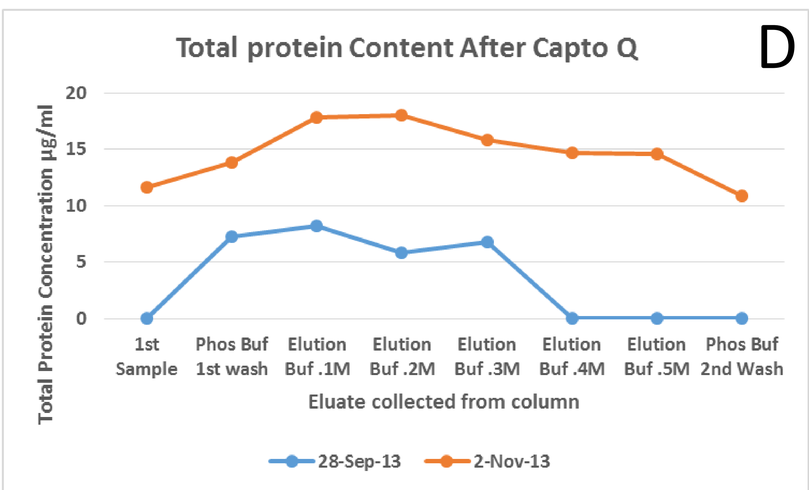
Sodium Dodecyl Sulfate (SDS)-Polyacrylamide Gel Electrophoresis (PAGE)

Once protein concentrations were determined, selected samples were passed through a 7.5% precast polyacrylamide gel. An electrical current of 200V was applied for 10 minutes and then reduced to 150V for 40 additional minutes. Total run time was 50 minutes.

RESULTS

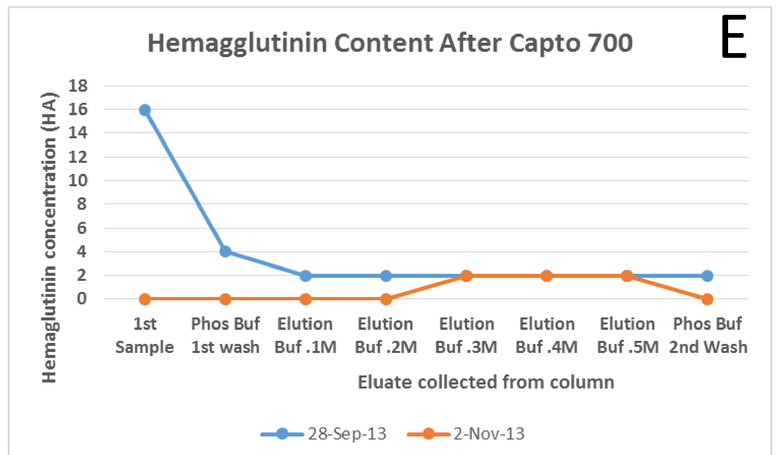
The following graphs (figures C and D) are from the column containing the Capto Q resin. The first graph shows the total hemagglutinin concentration detected by the hemagglutination assay. There was a peak of hemagglutinin in the 0.2M eluates collected on the 28 of September 2013. The second graph comes from the Bradford Protein Assay and shows the total protein concentration present in the eluates collected. This assay also showed a peak in protein concentration. When comparing the two graphs one can see that the protein peak corresponds to the hemagglutinin peak concentration.

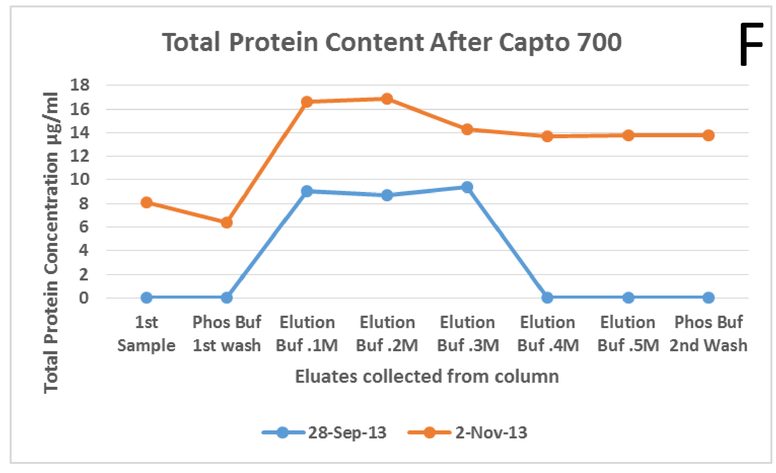




RESULTS CONTINUED

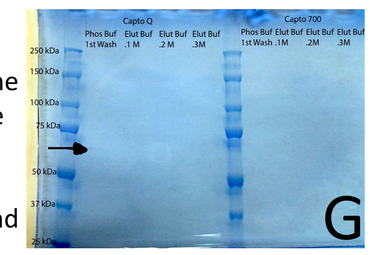
The following graphs (figures E and F) show the eluates collected from the column containing the Capto 700 resin. The first shows the concentration of the viral hemagglutinin protein. The data collected from the 28 of September 2013 suggests that the viral protein did not bind to the column, but rather flowed through the column. The second graph shows the total protein concentration present in the eluates. This graph indicates a similar peak of total protein concentration on both occasion in which the column was run. There does not seem to be a correlation between the total protein concentration and the hemagglutinin content in the eluates collected from the column containing the Capto 700 resin.





Results from SDS-PAGE:

In figure G, one can see a single band of protein that was identified at approximately 60kDa. The fact that the band was identified after the 1st phosphate buffer wash suggests that either the viral protein did not stick to the column, or that the protein visible on the gel was due to a contaminant (possibly bovine serum albumin.) As only one band of protein was visible, a more sensitive dye will be needed to detect lower concentrations of protein.



CONCLUSION

- A peak protein concentration appears to correlate with peak hemagglutinin content in both columns (Figures D and F) suggesting that little protein purification has occurred.
- A band of 60kDa protein was identified by the SDS-PAGE from the 1st phosphate buffer wash (Figure G). Because the hemaglutination assay identifies viral protein in later eluates (Figure C), the data suggests that the band of protein is a contaminant and not the desired viral protein.
- Sensitivity of the Simply Blue die was too low. A more sensitive die such as a silver stain will be required to identify remaining proteins in the sample.
- The overall results are inconclusive and suggest more testing is required to fully evaluate our hypothesis.

REFERENCES

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