

Recovery of Virus Producing NDV Vaccine By Tangential Flow Filtration (TFF)

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Abstract: Currently, virus separation process is mainly conducted by using ultracentrifugation or sucrose gradient electrophoresis methods. Both methods however have several drawbacks wherein the ultracentrifugation method requires very high rotational speed to separate the virus while the sucrose gradient electrophoresis method is very time consuming. Alternative to both methods are by using Tangential Flow Filtration (TFF). In this study, we have separated and concentrated the Newcastle Disease Virus (NDV) harvested from embryonic specific pathogen free (SPF) eggs using TFF method by utilizing microfilter membrane with pore size of 0.45 μ m. Hollow fiber membrane was selected as filter because of their chemical and thermal stability. The study was conducted according to the design developed using Taguchi method which consists of selected three parameters and two levels of factors. The results revealed that higher pressure input (P_{in}), lower pressure output (P_{out}), and higher virus concentration led to higher virus titer. Optimum Trans-Membrane-Pressure (TMP) value of 15 psi and virus concentration of 28% had given the maximum titer of the virus which was 512 haemagglutination assay (HA) unit.

Keywords: Separation, Tangential Flow Filtration (TFF), Newcastle Disease virus (NDV), hollow fiber membrane

INTRODUCTION

Newcastle disease virus (NDV) is a member of the Paramyxoviridae family (Alexander, 1980; Alexander, 1988; Alexander, 1990; Lamb and Kolakofsky, 1996; Stevens et al., 1976). NDV infection, the Newcastle Disease (ND) has been a devastating disease of poultry, and in many countries the disease remains one of the major problems affecting existing or developing poultry industries. Newcastle disease attacks chickens and turkeys and various other birds. Certain mammals, including man, may also be infected with the virus. In poultry the disease can cause high mortality (up to 100%). Surviving birds show impaired growth, poor food utilization, reduced egg production, and impairment of eggshell formation (Berg et al., 1947; Lorenz et al, 1944). Fertility and hatchability of eggs are also reduced. Humans exposed to NDV develop granular conjunctivitis (Lippmann, 1952) which leads to some lasting impairment of vision, as well as lymphadenitis, headache, malaise and chills.

Production of NDV particles for vaccine production requires the development of robust separation operations. Commonly, ultracentrifugation and sucrose gradient electrophoresis have been used for virus separation and concentration (Polson, 1993). However, the introduction of tangential flow filtration (TFF), a filtration technique that uses different types of membrane, to separate small micro particles may offer the alternative for high yield vaccine production. The ability of TFF method to separate the virus from other particles is due to the size difference between them. TFF has many advantages over other separation methods. It does not involve the use of toxic reagents; rapid separation time; it makes use of tangential flow across the membrane filter with nominal cutoff under proper conditions (Wang, 2001).

The aim of this study was to obtain the ideal conditions for separating and concentrating Newcastle Disease virus which was harvested from embryonic specific pathogen free (SPF)

chicken eggs by using tangential flow filtration (TFF) method. The conditions of the filtration process which are the inlet pressure P_{in} , outlet pressure P_{out} and virus concentration were optimized in this study which was conducted according to the Taguchi Design developed using STATISTICA 8 to achieve the maximum virus titre.

APPROACH AND METHODS

Virus Sample

Newcastle disease virus which has been utilized during this study is the lentogenic F strain. The selection of this strain is due to its characteristics which are low virulence and low mortalities compared to the other strains and the vaccine produced from it is suitable for chicken of all ages. The virus stock has been acquired from the Virology Lab, Faculty of Veterinary, Universiti Putra Malaysia.

Virus Dilution

The samples were centrifuged using 4000 rpm at 4°C for 10 minutes. The supernatant is taken and diluted using 2 liters 1X PBS solution. Two different concentrations were made; low concentration (8.5%) and high concentration (25%), the stocks were then kept in the freezer at -80°C.

Virus Filtration

The method for tangential flow filtration using microfilter hollow fiber membrane is adopted from GE Healthcare Biosciences.

Measuring Water Flux

Cartridges can be reused if they are properly clean and stored. However, after a number of uses/cleaning cycles, the permeability performance of the cartridge may decrease to

an undesirable level. The cartridge's permeability was determined while the cartridge is new, because by comparing the permeability of the cartridge over time, it is easier to determine when the cartridge should be replaced. To monitor the performance of the cartridge and the effectiveness of each cleaning, the permeability of the cartridge was measured after each cleaning and compared that figure with the cartridge's performance when new. The water flow was measured using stopwatch and graduated cylinder from the permeate port at a particular transmembrane pressures (TMP). The water flux was calculated in L/m²/hr (LMH). Water temperature was measured at the point of flux determination. The flux value was recorded as a benchmark for future comparison. The formula to calculate the transmembrane pressure (TMP) and water flux is as follows:

$$\text{Flux} = V * 0.06 / t * A \quad (1)$$

where V is volume (L)
t is hour (hr)
A is area (m²)

$$\text{TMP} = P_{\text{in}} + P_{\text{out}} / 2 \quad (2)$$

where TMP is transmembrane pressure (psi)
P_{in} is inlet pressure (psi)
P_{out} is outlet pressure (psi)

Sanitizing the Cartridge

Distilled water and 0.1M to 1M NaOH solutions were used. The solutions were re-circulated at 5 to 10 psi inlet / 1 to 5 psi outlet pressure for 30 to 60 minutes. Distilled water was flushed to drain out the NaOH until pHs on both permeate and retentate is neutral in order to re-use the cartridge.

Operating and Preparing the System

Sampling/drain valve was closed, the cartridge was secured in upper and lower manifolds and the pump tubing was correctly positioned and tensioned within the pump head. Flexible tubing must be firmly connected from the retentate outlet on the upper manifold to one of the tubing barbs on the reservoir cap. If the process solution tends to foam, ensure there is a retentate down comer pressed into the reservoir cap for the retentate line. The flexible tubing was directed from the upper permeate line to a collection flask. The sanitary clamp was removed from the reservoir cap and the reservoir cap was slide to one side. The sample solution was added to the reservoir. The reservoir cap was repositioned on the silicone gasket and it was clamped in place using the sanitary clamp. The backpressure-tubing valve was opened several times. The pump was started at slow speed and waits about 30 seconds for the pressure to build up. The pressure gauges are mechanically dampened and respond slowly. The pump speed was increased slowly. The inlet pressure will build up while the outlet pressure gauge may still read zero. Backpressure was applied by slowly closing the tubing valve. The inlet pressure gauge was observed. If the pressure rises too high, the pump speed was lowered. Upon completion the product was recovered from reservoir via the drain/recovery valve. The filter membrane was rinsed using 1M NaOH

followed by distilled water.

The Taguchi experimental design was used in this project. Table 1 shows Taguchi experimental design for three parameters with two levels; 1 and 2.

Table 1: Taguchi experimental design

Run	F1	F2	F3	P _{in} (psi)	P _{out} (psi)	Virus concentration (%)
1	1	1	1	30	5	28
2	1	2	2	30	10	8.5
3	2	2	1	20	10	28
4	2	1	2	20	5	8.5

Processing 0.5% Red Blood Cell (RBC)

Whole blood was collected in an EDTA bottle. The blood was transferred into 50ml centrifuge tube. 32ml of 1X PBS was added into the tube and the volume was adjusted to make up to 50ml. Then it was centrifuged at 1000rpm for 10 minutes at 4°C. The supernatant was discarded. The steps were repeated until clear supernatant was observed. 0.0625ml of the concentrated RBC was added to 12.5ml PBS into 15ml centrifuge tube. Then it was stored in the chiller.

Haemagglutination Assay (HA) Test

This test was done to obtain the titer of the virus. Before applying the test, spot test was done to confirm whether good result may be attained and whether the virus is still active. This test is done by adding small amount of washed RBC with the virus sample on the plate. Then the agglutination was observed.

For HA test, 50µl PBS was added into the well 2 to well 12 in a V-shape microtiter plate. 100µl of virus was then added in well 1, 50µl of it was transferred in well 2, 50µl of well 2 was transferred to well 3, these dilution steps were repeated until it reaches well 11. In well 11, 50µl of it was discarded. 50µl of RBC was added in each well (well 1-12). The addition of RBC may starts from well 12 to well 1 if the same tip was to be used. After 15-20 minutes, the results were observed. Note that the results must be observed immediately since the agglutination will vanish eventually.

RESULTS

There are four runs of experiment in total and they are conducted according to Taguchi design which has been developed using statistical software, STATISTICA 8. The design consists of three independent factors which are the inlet pressure P_{in}, outlet pressure P_{out}, and the virus concentration and there are two levels for each factors. The results for change of virus titer before and after filtration are summarized in Table 2.

Table 2: Virus titer before and after microfiltration

Run	P _{in} (psi)	P _{out} (psi)	Virus conc. (%)	Before filtration (HA)	After Filtration (HA)
1	30	5	28	32	512
2	30	10	8.5	8	16
3	20	10	28	32	128
4	20	5	8.5	8	16

Table 2 shows that in all four runs of experiment, the virus titer increased after went through the tangential flow filtration process. Run 1 initially has the virus titer of 32 HA unit. After the completion of the filtration process, the virus titer has increased to 512 HA unit at the retentate. Meanwhile, the initial virus titer for Run 2 is 16 HA unit and it was doubled to 32 HA unit at the retentate after finished. Run 3 has the same initial virus titer as Run 1 which was 32 HA unit and the final virus titer achieved were 128 HA at the retentate. For Run 4, the initial virus titer is 8 HA unit and the final virus titer was doubled to 16 HA at the retentate.

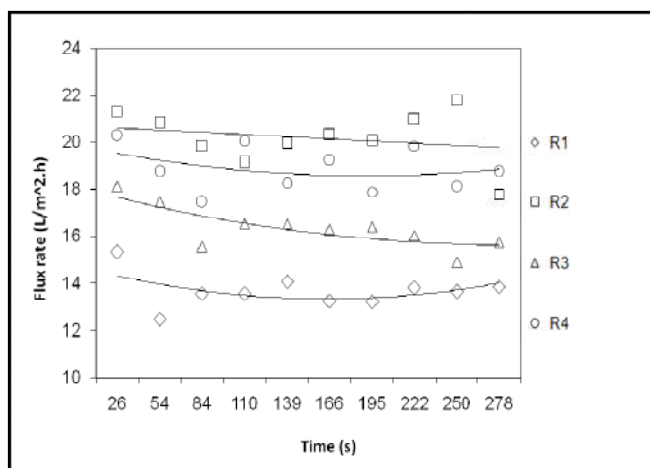


Figure 1: Flux rate at different run

Figure 1 illustrates the plot of flux rate versus time for each run. The flux rates differed for each run. The flux rates of Run 1 and Run 3 are much lower compared to the rates of Run 2 and Run 4. This may due to the higher virus concentration in the sample of Run 1 and Run 3 compared to Run 2 and Run 4. So, the probability of solid formation on the membrane surface is higher since it has higher number of viruses in the sample. The highest flux rate is obtained during Run 2. The reasons maybe lie on its operating conditions where it has used the highest P_{in} and P_{out} leading to the highest trans-membrane pressure (TMP) exerted on the filtration system. Overall, the flux rate is consistent for each runs and it did not drop drastically throughout the experiment. Meanwhile, Figure 2 shows the plot of time versus volume. By comparing Figure 1 and Figure 2, it can be assumed that, as the volume is increased throughout time, the flux rate will be decreased.

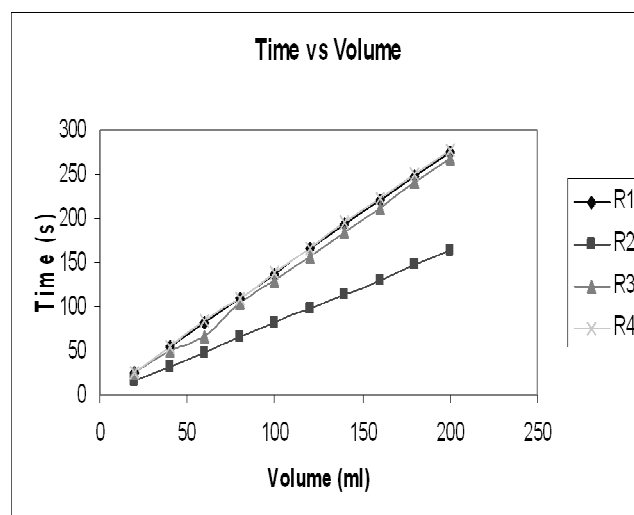


Figure 2: Time versus Volume

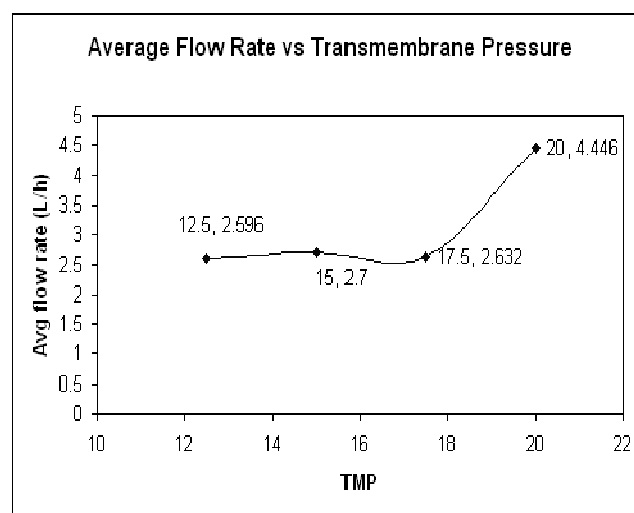


Figure 3: Average Flow Rate versus TMP

Figure 3 describes the relationship between average flow rate and trans-membrane pressure, TMP. The graph depicts the highest average flow rate exerted on the highest trans-membrane pressure applied and the lowest average flow rate exerted on the lowest trans-membrane pressure applied. This simply follows the theoretical nature of the filtration process by which the higher the trans-membrane pressure, the higher the flow rate it can run. However, the average flow rate at trans-membrane pressure of 17.5 psi is decreased which was 2.632 L/h. This maybe because the virus concentration used at 17.5 psi is higher than that of 15 psi. So, the rate is slower since it is operated using higher number of viruses which leads to higher tendency of buildup of the solids on the filter membrane.

Taguchi Analysis

Figure 4 shows the means plot of virus titer at the retentate of microfiltration. The plots indicate the effect of each parameter on the virus titer. From the figure shown, the virus concentration gives the most significant effect on the virus

titer. Meanwhile the effect of both pressure in and pressure out is almost the same since the plot was not much different. In general, it can be considered that high pressure in, low pressure out and high virus concentration led to higher final virus titer.

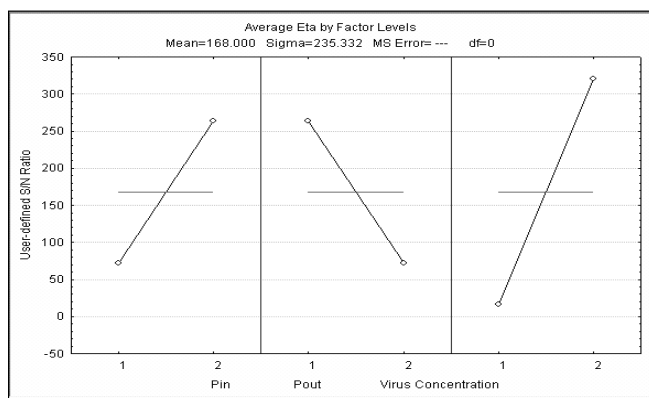


Figure 4: Means plot for virus titer at microfiltration

From Table 3, it can be observed that the interaction between pressure in and pressure out is probability of 0.254. In the mean time, the interaction between both pressure in and pressure out give to the virus concentration has same probability of 0.529. Here we can see that virus concentration in the sample has the biggest role in affecting the final virus titer.

Table 3: Interaction between parameters and their effect on final virus titer

Interaction	P_{in}	P_{out}	Virus Concentration
Inlet Pressure (Pin)	0	0.254	0.529
Outlet Pressure (Pout)	0.254	0	0.529
Virus Concentration	0.529	0.529	0

DISCUSSIONS

Theoretically, the virus titer should have not been obtained at the retentate side of the membrane since the process has been designed so that the virus should passed through the membrane and to be collected at the permeate side. However, it was observed that most viruses did not pass through the membrane during the filtration process and they were collected at the retentate side of the microfiltration. This was because the pore size of the membrane used was small enough for most of the viruses. Several reviews (Alexander, 1980; Alexander, 1988) gave the size range of the Newcastle Disease virus, NDV as 150 to 350 nm. However after completing the project, another review gave the size range of NDV to be between 100 to 500 nm. NDV size range based on the initial reviews influenced

the selection of membranes with the suitable pore size. Therefore, with the latest review, it could explain why most viruses were concentrated at the retentate. More so, it might

be that the viruses could be in the different stages of growth; hence they have differences in size.

During the project, there are some possible errors may have occurred such as drastically increase the pumping speed during the filtration process and the filter membrane is not washed properly according to the recommended cleansing steps. Firstly, the pump should be slowly increased at some interval of seconds to adjust the transmembrane pressure, TMP. This is because drastic change in the pump speed may lead to the sudden change of the pressure as well as the flow of the filtration. This can result blockage in the filter membrane (fouling). The filter membrane should be washed properly to ensure good performance of the filter and to maintain the reliability and reusability of the filter membrane in the future. Lastly, the system should be connected and clamped properly to guarantee the smooth flow of the operation. After completion of the process, the pump tube has to be loosened to allow the release pressure and to maintain the performance of the pump.

CONCLUSION

In conclusion, we have achieved the aims of this study which were to separate and concentrate NDV using the tangential flow filtration and also to obtain the ideal operating condition using the system which is by applying trans-membrane pressure of 15 psi and by using virus concentration of 28% that has given us final virus titer of 512 HA unit. We are currently using this technique to prepare concentrate NDV for vaccine production. It is considered that the tangential flow filtration process described in this report will expedite research studies related to Newcastle Disease virus or NDV.

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