Purification of Vaccines by the UF-3000PS Ultrafiltration Membrane

Kenji KOYAMA
Yoshiyuki MIYAKI
Michihiro AKAZAWA

Japanese encephalitis (JE) and rabies viruses propagated in mouse brain and tissue culture, respectively, were purified by the use of a newly-developed ultrafiltration membrane UF-3000PS, which has an asymmetric pore structure of middle pore sizes between ultrafiltration and microfiltration. Host proteins were separated from the starting viral suspensions with the infectivity recovery close to 100%. It was found that our ultrafiltration method using the UF-3000PS membrane can be applied to a large-scale purification of viruses in place of the conventional centrifugation or precipitation methods because of its rapidity, simplicity and inexpensiveness.

1. Introduction

Viruses for preparation of vaccines were propagated by infectious animal's organs or tissue culture. Thus the starting materials contain a significant amouint of proteins and other impurities come from the host organs or cells, which cause allergic reactions as the side effect when the vaccine is injected into an animal's body.

Many studies have been made in order to purify such viruses,[1-11] where centrifugation and precipitation methods were mainly investigated. However, the following problems are found in the previous methods for large-scale purification:

1) low yield of virus or appreciable contamination of host proteins because of the closeness of the specific gravities of virus and host proteins,
2) time and labor consumable,
3) contamination of chemicals such as polyethylene glycol, and
4) high maintenance cost.

A membrane separation seems to be promising method for purification of virus. However, conventional microfiltration (MF) membranes allow proteins to permeate but cannot completely retain virus and conventional ultrafiltration (UF) membranes retain both proteins and virus. In this work, we developed a new type of UF membrane, UF-3000PS, which have a middle pore size between UF and MF. A part of characteristics of the membrane was reported previously.[12-13] We applied the membrane to purification of Japanese encephalitis (JE) and rabies viruses.

2. Experimental Section

[1] Preparation of Virus.

Japanese encephalitis (JE) virus, Nakayama NIH strain, was inoculated into brains of mice, twenty-one to twenty-eight-day-old ddY strain mice, and cultured for four days. A 20% suspension of JE virus-infected mouse brain was prepared with phosphate-buffered saline (PBS) and centrifuged for

30 min at 18,000×g. The supernatant fluid was treated with protamine sulfate at a final concentration of 0.15% and centrifuged again for 15 min at 18,000×g. The supernatant fluid was used as the starting virus solution in this work.

Rabies virus, Nishigahara strain, was inoculated into BHK-21 cells and cultured for five days with Eagle's minimum essential medium containing 1% of fetal bovine serum. The virus was centrifuged for 30 min at 5,000×g. The supernatant fluid was used as the starting virus solution in this work.

〔2〕 Assay of virus infectivity.

The virus solutions was diluted with PBS to prepare $10^{-3}-10^{-8}$ units solutions. Each diluted virus solution (0.03 mℓ) was injected into brains of twenty-eight-day-old mice. Mice which showed symptoms peculiar to the JE were regarded as positive. The value of log LD_{50} was caluculated by the Behrens-Karber's method.

〔3〕 Ultrafiltration.

The large-pore UF membrane, UF-3000PS was prepared by a phase conversion method: polysulfone dissolved in an organic solvent was uniformly spread on a non-woven sheet of polyethyrene and then dipped into the precipitation bath. The pore size could be controlled by adjusting concentration of polysulfone, solvent composition, and the temperature of the precipitation bath.

The membrane was throughly washed with pure water, dipped into a glycerin-water solution, and air-dried at room temperature.

The UF-3000PS membrane was attached to the plate-and-frame-type UF module, UF-LMS II, (Tosoh, Corp.) which has an effective membrane area of 200 cm^2 and can be conveniently used for the cross-flow filtration in a laboratory, but easily scaled up. In Fig. 1, is shown the flow diagram of the UF system, which consists of the UF-LMS II module, a peristaltic recirculation pump, two pressure gauges, a feed reservoir, and a permeate reservoir.

The virus solution was recirculated through the module at 700 mℓ/min. The permeation rate was adjusted to 5 mℓ/min. This filtration mode ensures that the concentration of solids on the membrane surface

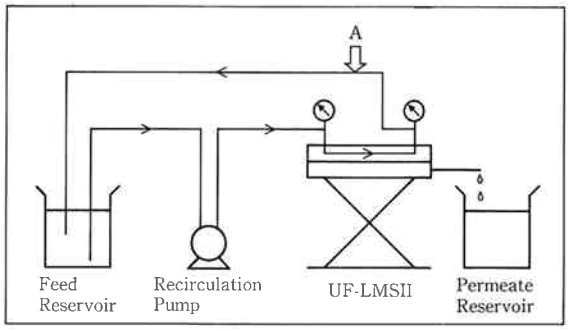

Fig. 1 Flow diagram of the UF system.
A, a screw-stopper used to pressurize the circulating feed solution by narrowing the tube at the outlet.

will not increased significantly above that in the feed reservoir. A 600 mℓ of the virus solution was concentrated to 100 mℓ, dilluted to the initial volume with PBS, and concentrated again to 100 mℓ. This operation was repeated four and three times for the JE and rabies virus solutions, respectively. The temperature of the test virus solutions was kept at 8°C all through the UF experiment.

3. Results and Discussion

Fig. 2 shows the cross section and top surface of the obtained membrane, which were observed by a scanning electron microscope. Prior to the observation, the non-woven support material had been removed from the membrane. It can be seen that the membrane has an asymmetric pore structure consisting of a very thin active dense layer (top surface) and sponge-like support layer and has homogeneous pores larger than 10 nm diameter on the top surface.

The molecular weight cutoff (MWCO) curve of the UF-3000PS membrane was observed by use of a 0.1% solution of pure standard proteins. The curve is shown in Fig. 3. The MWCO of this membrane estimated from the curve is higher than 10^6 (about 3×10^6). The flux of pure water oberved at 1 kgf/cm^2 was 1050±200 ℓ/m^2hr.

Fig. 4 shows show the relationships between the permeate volume and the operation time for the JE and rabies virus solutions. The permeate volume is proportional to the operation time, i. e., the flux is constant, for either solution, indicating that the separation was well performed without suffering significant

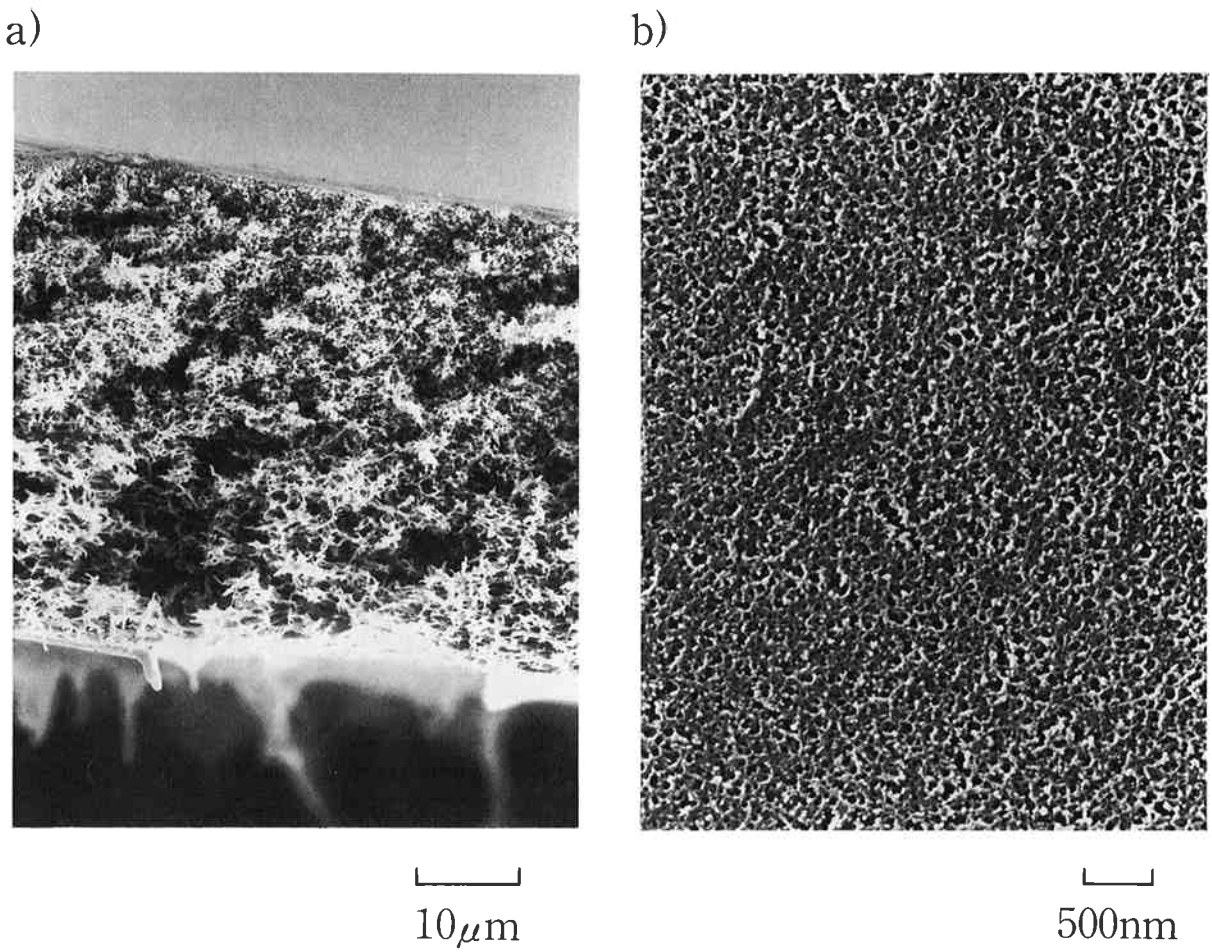

Fig. 2　The scanning electron micrographs of the UF-3000PS membrane.
(a) the cross section; (b) the top surface.

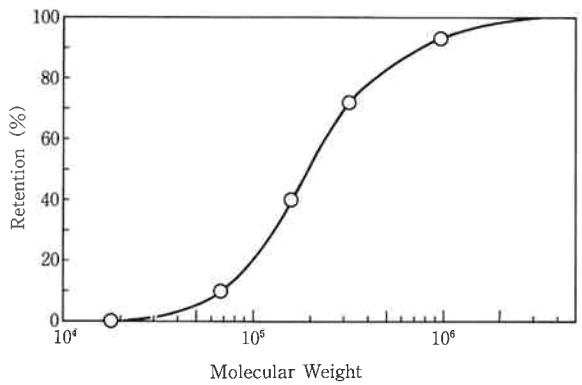

Fig. 3　The molecular weight cutoff curve of the UF-3000PS membrane, which was observed with an aqueous solution of pure standard proteins.

concentration polarization effect or membrane fouling. This result may be due to the asymmetric structure of the membrane and the cross-flow filtration mode.

In Fig. 5, is shown the protein content in 600 mℓ of

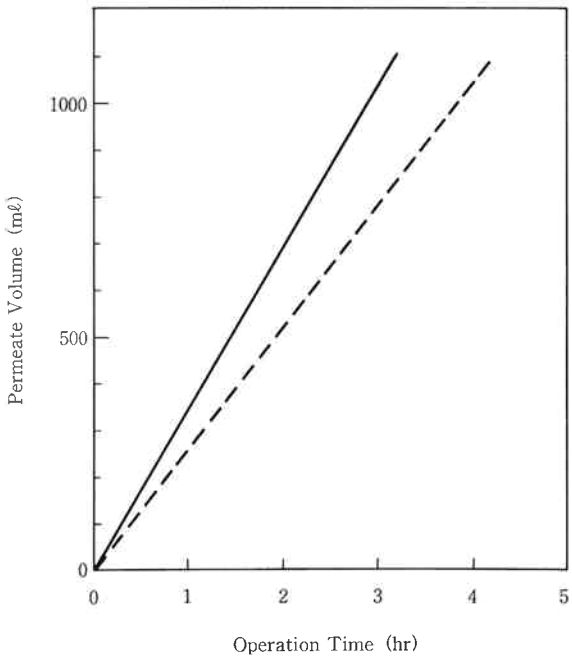

Fig. 4　The relationships between the permeate volume and the operation time for the JE and rabies virus solutions.
Solid line, rabies virus; broken line, JE virus.

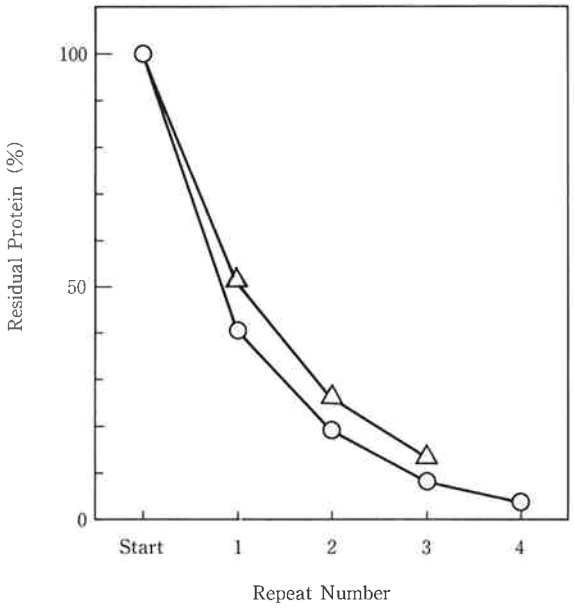

Fig. 5 The protein content in 600 mℓ of the virus solutions at each step of ultrafiltration. ○, JE virus solution; △, rabies virus solution.

Table 1 Results of purification of JE virus by use of the UF-3000PS membrane.

	volume (mℓ)	protein content (μgN/mℓ)	infectivity	
			log LD_{50}	recovery (%)
Initial soltn.	600	540	7.5	100
Purified soltn.	600	17	7.4	80
Filtrate	2,000	150	5.6	1

the virus solutions at each step of ultrafiltration. In the case of JE virus, the protein content in the starting virus solution was 0.54 mgN/mℓ. It was reduced to about 40% in every UF steps and finally became 0.0172 mgN/mℓ. This means that 97% of the host protein was removed from the virus solution. Moreover, the purified virus solution was almost colorless, although the starting solution assumed red color. This fact indicates that hemoglobin could be separated from the virus solution by the ultrafiltration with UF-3000PS. In the case of rabies virus, the protein content in the starting virus solution was 0.13 mgN/mℓ. It was reduced to about 51% in every UF steps and finally became 0.017 mg/mℓ. This means that 87% of the host protein was removed from the virus solution.

Table 2 Results of purification of rabies virus by use of the UF-3000PS membrane.

	volume (mℓ)	protein content (μgN/mℓ)	infectivity	
			log LD_{50}	recovery (%)
Initial soltn.	600	130	6.8	100
Purified soltn.	600	17	6.8	100
Filtrate	1,500	45	—	—

The results of the purification of JE and rabies viruses by ultrafiltraion are summarized in Table 1 and 2, respectively. The recoveries of infectivity were about 80% and 100% for the JE and rabies virus solutions, respectively.

4. Conclusion

The pore size of the UF-3000PS membrane prepared in this work is large enough to allow most proteins to permeated. Therefore, the virus propagated by infectious animal's organs or tissue culture can be easily purified by filtering out the host proteins through the membrane. The filtration is little influenced by the concentration polarization and membrane fouling because of the asymmetric pore structure of the membrane and the cross-flow filtration mode. Such UF purification offers high yield of virus and much saving in time and labor compared with conventional precipitation and centrifugation methods,[7] and can be easily scaled up.

5. Acknowledgment

The authors are grateful to Mr. S. Ohno, Tosoh Corporation, Shin-Nanyo, for his support and useful suggestion.

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著　者
氏名　小　山　憲　治
Kenji KOYAMA
入社　昭和44年3月4日
所属　科学計測事業部
機器製造部
生産技術課
副主任研究員

著　者
氏名　宮　木　義　行
Yoshiyuki MIYAKI
入社　昭和55年4月1日
所属　科学計測事業部
機器製造部
生産技術課
主任研究員

著　者
氏名　赤　沢　道　博
Michihiro AKAZAWA
入社　昭和47年4月16日
所属　科学計測事業部
ゲル製造部
技術課
技術課長