

Original article

Parameters affecting nanofiltration rate of specific immunoglobulins at the National Blood Centre, Thai Red Cross Society

Teeraporn Chonsilapawit, Thongyip Nakdaeng and Wadee Rungpradubvong

National Blood Centre, Thai Red Cross Society

Abstract:

Introduction: Nanofiltration is one of the most important viral removal techniques to increase product safety in plasma-derived medicinal products (PDMPs). This filtration step in the production process of human rabies immunoglobulin (HRIG) was studied by Blood Product Production Department of National Blood Centre (NBC), Thai Red Cross Society. To optimize the filtration rate, there are many parameters that affect the filtration rate.

Objective: This study aimed to study the parameters that affected the nanofiltration process of NBC's specific immunoglobulins. **Materials and Methods:** Apheresis plasma for HRIG received from rabies-immunized donors was fractionated into precipitate IIC (PIIC). Dissolved PIIC with 1,500 mL water for injection (WFI) and filtrated were filtered through 20 nm nanofilter. Four batches (eight nanofiltration processes) were conducted from April 2019 to April 2020. The studies were separated into 2 periods. The first period was increasing pH-adjust reagent concentrations combined with temperature control of the solution. Then the filter-wetting process was added in the second period by pressure holding to eliminate the bubbles that were clogged within the filter pore. Three consecutive batches (six nanofiltration processes) were operated.

Results: In the first period, the results were unsatisfactory where the filtration rates were 4.83 and 2.11 kg/m²/10 hr. The results of the second period were more satisfactory where the filtration rates were 4.79, 4.70, 4.44, 4.15, 6.22 and 7.16 kg/m²/10 hr, respectively.

Conclusion: There were three parameters that influenced the nanofiltration rate, increased concentration of pH-adjusted reagents, controlled solution temperature, and the filter-wetting process by pressure holding. Combining all three conditions together helped the nanofiltration rate to be satisfactory.

Keywords : ● Immunoglobulin ● Nanofiltration ● Filtration rate

J Hematol Transfus Med. 2024;34:9-17.

Received 19 September 2023 Corrected 21 November 2023 Accepted 8 December 2023

Correspondence should be addressed to Teeraporn Chonsilapawit, National Blood Centre, Thai Red Cross Society, Rama 4 Rd., Pathumwan, Bangkok 10330 E-mail: teeraporn.c@redcross.or.th

นิพนธ์ต้นฉบับ

ปัจจัยที่มีผลต่ออัตราการกรองนาโนในผลิตภัณฑ์ Specific immunoglobulins ของศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย

ธีรพร ชลศิลป์วิทย์ ทองหยิบ นาคแดง และ วดี รุ่งประดับวงศ์

ศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย

บทคัดย่อ

บทนำ การกรองนาโน (nanofiltration) เป็นหนึ่งในเทคนิคสำคัญในการกำจัดไวรัสเพื่อเพิ่มความปลอดภัยในผลิตภัณฑ์จากพลาสมา (plasma derived medicinal products; PDMPs) ฝ่ายผลิตผลิตภัณฑ์โลหิต ศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย ได้ทำการศึกษาวิจัยในเรื่องการกรองไวรัสเพื่อลดความเสี่ยงในการปนเปื้อนของไวรัสในผลิตภัณฑ์จากพลาสมา คือ อิมมูโนโกลบูลินป้องกันโรคพิษสุนัขบ้า (human rabies immunoglobulin; HRIG) ซึ่งมีหลายปัจจัยที่มีผลต่ออัตราการกรอง **วัตถุประสงค์** เพื่อศึกษาปัจจัยที่มีผลต่อความสม่ำเสมอของอัตราการกรองด้วยตัวกรองนาโน (nanofilter) ในผลิตภัณฑ์ specific immunoglobulin ของศูนย์บริการโลหิตแห่งชาติ **วัสดุและวิธีการ** ใช้พลาสมาที่มาจากกระบวนการรับบริจาคเฉพาะส่วนจากผู้บริจาคที่มีภูมิคุ้มกันสูงต่อโรคพิษสุนัขบ้า (apheresis HRIG) เฉพาะที่ศูนย์บริการโลหิตแห่งชาติ แล้วนำไปผ่านกระบวนการตกตะกอนโปรตีนจนได้เป็นตะกอนชั้น IIC (precipitate IIC; PIIC) นำตะกอน PIIC HRIG ไปละลายน้ำ และนำไปกรองผ่านตัวกรองนาโนขนาด 20 นาโนเมตรโดยใช้ปริมาณครั้งละ 1,500 มิลลิลิตร ทำการศึกษาตั้งแต่เดือนเมษายน พ.ศ. 2562 ถึงเดือนเมษายน พ.ศ. 2563 จำนวนทั้งสิ้น 8 ครั้ง (4 รุ่นการผลิต) โดยแบ่งการศึกษาเป็น 2 ช่วง ช่วงที่ (1) การปรับเปลี่ยนความเข้มข้นของสารที่ใช้ในการปรับค่าความเป็น กรด-ด่าง ควบคุมกับ การควบคุมอุณหภูมิของสารละลายเริ่มต้นก่อนกรอง ช่วงที่ (2) การปรับปรุงวิธีการในการล้างตัวกรอง (filter-wetting process) ด้วยวิธี pressure hold เพื่อไล่ฟองอากาศที่ตกค้างในตัวกรองออกให้หมด โดยทำการทดลองใน 3 รุ่นการผลิต (3 consecutive batches) รวม 6 ครั้ง **ผลการศึกษา** ผลการศึกษาในช่วงที่ (1) พบว่าอัตราการกรองได้เท่ากับ 4.83 และ 2.11 kg/m²/10 hr ซึ่งค่าที่ได้ไม่เป็นที่พึงพอใจ ผลการศึกษาในช่วงที่ (2) พบว่า อัตราการกรองเป็น 4.79, 4.70, 4.44, 4.15, 6.22 และ 7.16 kg/m²/10 hr ซึ่งพบว่าผลที่ได้เป็นที่พึงพอใจ **สรุป** ปัจจัยที่มีผลต่ออัตราการกรองนาโน ได้แก่ การเพิ่มความเข้มข้นสารเคมีที่ใช้ในการปรับค่าความเป็นกรด-ด่าง การควบคุมอุณหภูมิของสารละลาย และเทคนิคการล้างตัวกรองให้เบี่ยงด้วยการทำ pressure hold การใช้ทุกสภาวะร่วมกันมีผลช่วยให้อัตราการกรองดีขึ้น

คำสำคัญ : ● อิมมูโนโกลบูลิน ● การกรองนาโน ● อัตราการกรอง

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2567;34:9-17.

Introduction

Nanofiltration is a viral removal technique commonly used in many medicinal products for several decades. It is used to increase safety in plasma-derived medicinal products (PDMPs) according to size exclusion principle. The filter has larger pore size than target protein but smaller than viruses hence the viruses would be detained on the upstream of filter membrane while the target protein passed through. However, if protein aggregation was formed until the size of the protein was equal to or larger than the viruses, because of many variations such as temperature change or pH, this kind of method could be ineffective.^{1,2}

Unlike other kinds of viral inactivation methods such as solvent-detergent (SD) treatment and pasteurization (heat treatment), which have limitations on non-enveloped virus, nanofiltration used the size exclusion technique to separate the protein and the viruses. Taking advantages over the sizes, it can be used to remove either enveloped or non-enveloped virus from the target protein. Combining nanofiltration together with SD treatment or heat treatment in the manufacturing process helps to increase viral inactivation effectiveness for both enveloped and non-enveloped virus.^{3,4}

Nanofilter sizes of 35 and 70 nanometers were first developed by Asahi Kasei, Japan. Thereafter, other filter manufacturers worldwide continued to produce nanofilters to serve the increasing demands. The most popular nanofilter sizes are 15, 20 and 50 nanometers. Nanofilters were widely used in pharmaceutical industry as well as for production of PDMPs. Many pharmaceutical companies have chosen nanofiltration combined with other methods to remove viruses from their products. At present, nanofiltration is the standard method for viral removal in many PDMPs such as coagulation factors and immunoglobulins.^{5,6}

Blood Product Production Department of National Blood Centre, Thai Red Cross Society (NBC, TRCS) is the sole manufacturer of specific intramuscular immu-

noglobulins (IMIG) in Thailand. The products consist of human rabies immunoglobulins (HRIG) 150 IU/mL and hepatitis B immunoglobulin (HBIG) 200 IU/mL. Both products have been produced for more than 30 years by using the process of cold ethanol protein precipitation and purification. Quality and safety of the products have been improved continually. However, the process of viral inactivation for IMIG was unknown in the past.⁷ To reduce risk of viral contamination and improve safety of the products, NBC began to find the way to implement viral inactivation process. In 2015, the research of viral removal process using 20-nm nanofilter was initiated by cooperation between Japan Blood Product Organization (JB) and NBC. The research started with many studies focused on finding the most appropriate nanofiltration condition for NBC's IMIG. After the best condition was acquired, many lab-scale and scale-up studies were performed.

According to self-study in the past, NBC's IMIG could not pass through nanofilter due to the fact that protein concentration and pH of products were not appropriate. To find the best nanofiltration condition, optimal protein concentration and pH were studied. After 22 attempts, the best nanofiltration condition for NBC's IMIG was found to be 2 percent protein and pH 4 using nanofilter from Pall Corporation.⁸ After that, five lab-scale (120 mL) and four scale-up (1,500 mL) studies were conducted. The results from those studies showed that filtration rates among each study were inconsistent. Filtration rate was one of the most important factors to determine filter area. Inconsistent filtration rate caused inappropriate filter area determination. Too large filter area led to higher expense while too small filter area led to the filter being clogged. To determine the filter area appropriately, a study that focused on the parameters that affected filtration rate which is specific to the products was necessary. This study aimed to investigate the parameters that affected the filtration rate of NBC's IMIG nanofiltration process.

Materials and Methods

Nanofiltration

Apheresis plasma for HRIG was fractionated into Precipitate II C (PIIC) by Cohn cold ethanol fractionation method.⁹ PIIC was dissolved with 1,500 mL of water for injection (WFI) to get approximately 4 percent protein concentration and was adjusted pH to 4. Dissolved PIIC (DIS PIIC) was purified by diafiltration using Ultrafiltration Pall OS050T12 50K OMEGA CENTRAMATE 0.1 m² (New York, USA). Protein concentration and pH of DIS PIIC were adjusted to 2 percent protein and pH 4 again to obtain appropriate nanofiltration conditions.

DIS PIIC was filtered through pre-filter 0.1 µm (KA02D-JLP2G, Pall, New York, USA) followed by nanofilter 20-nm (CLM05DV20P1G, Pall, New York, USA). Four batches of HRIG were studied, each of which was separated into half volume for nanofiltration process. Filter volume was 1,500 mL approximately. The study was divided into 2 periods.

Period 1 Concentrations of pH-adjust reagents were changed, combined with temperature controlled of the solution before filtration. One batch of HRIG (2 nanofiltration processes) was performed in this period. There were improvement steps from previous studies as shown below. Details were shown in Figure 1.

Step 1.1 Change from 0.1 N HCl to 0.5 N HCl

Step 1.2 Change from 0.1 N NaOH to 1.0 N NaOH

Step 1.3 Control temperature of solution before filtration to 20-25 C (from 4 C)

Period 2 Filter-wetting technique was changed by holding pressure at 2.1 bar for 20 minutes during wetting process (step 2 in Figure 1), all bubbles clogged in nanofilter would be totally removed. This technique was combined together with all improvements in period 1. Three consecutive batches were operated in this period, each of which was separated into 2 nanofiltration processes.

Filter-wetting process by pressure hold (Figure 2)

WFI was poured into the pressure tank and then the gas valve was opened to allow air into the pressure

tank, starting with 0.4 bar of air pressure. The air vent valve was gradually opened to remove all air-clogged in the filter. After removing air-clogged, the air vent valve was closed and then the air pressure was slowly increased to 2.1 bar. The solution outlet valve was opened until more than 1-liter of the solution were filtered. After 1-liter solution was reached, the outlet valve was closed to hold pressure inside the filter for 20 minutes. After that, the outlet valve was opened until 0.5-liters of the solution were filtered. The gas valve was closed and then the depressurization valve of the pressure tank was opened to release air pressure that remained in the pressure tank. After there was no air pressure in the pressure tank, tank's lid was opened to remove residual WFI and then protein solution was poured into the pressure tank to start filtration process.

Results

Period 1

The pH-adjust reagents were adjusted from 0.1 to 0.5 N HCl and from 0.1 to 1.0 N NaOH. Temperature of the solution before filtration was controlled at 20-25 C. The study was performed on HRIG batch no. 190430. The solution was separated into half volume, each of which was filtered through nanofilter (study no. 1.1 and 1.2). Filtration rate were 4.83 and 2.11 kg/m²/10 hr, respectively. The average filtration rate was 3.47 kg/m²/10 hr (SD = 1.92). The results were shown in Table 1.

Period 2

The pH-adjust reagents were adjusted from 0.1 to 0.5 N HCl and from 0.1 to 1.0 N NaOH. Temperature of the solution before filtration was controlled at 20-25 C. Wetting process by pressure hold was done at 2.1 bar for 20 minutes during filter wetting process. The studies were conducted in 3 batches of HRIG: 191106, 200129 and 200401. The solution was separated into half volume, each of which was filtered through nanofilter (study no. 2.1-2.6). Filtration rate were 4.79, 4.70, 4.44, 4.15, 6.22 and 7.16 kg/m²/10 hr, respectively. The average filtration rate was 5.24 kg/m²/10 hr (SD = 1.18). The results were shown in Table 1.

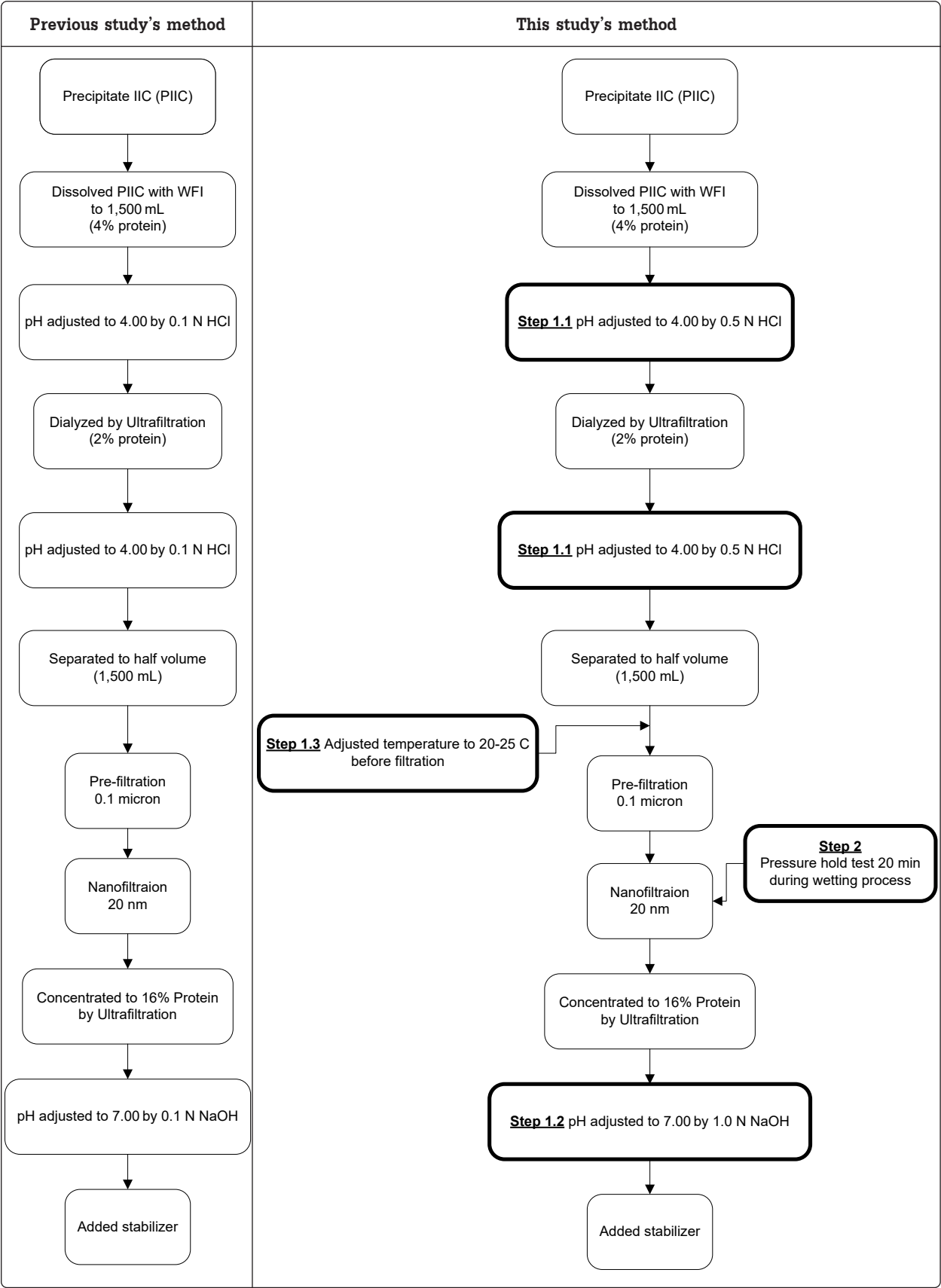


Figure 1 Comparison of previous study's method with this study's method

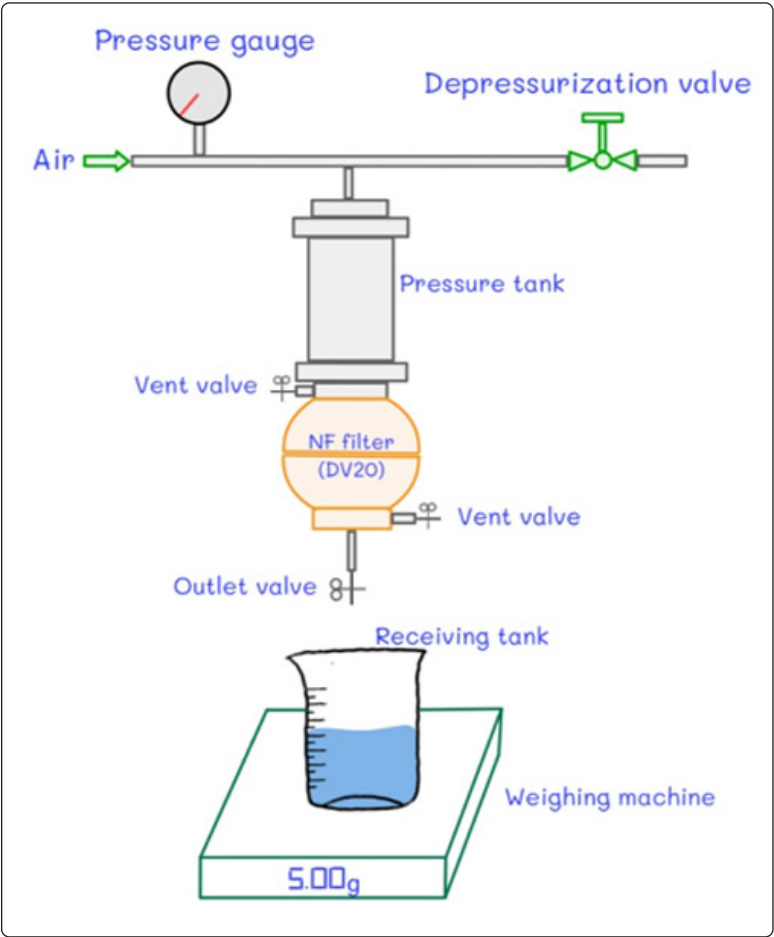


Figure 2 Filter-wetting process by pressure hold

Table 1 Results of nanofiltration rate from period 1 and period 2

Study period	Period 1		Period 2					
Test parameters	Increased pH-adjust reagent concentration + temperature control		Increased pH-adjust reagent concentration + temperature control + filter wetting process change					
Lot no.	190430		191106		200129		200401	
Study no.	1.1	1.2	2.1	2.2	2.3	2.4	2.5	2.6
Nanofiltration rate (kg/m ² /10 hr.)	4.83	2.11	4.79	4.70	4.44	4.15	6.22	7.16

From the results, after period 1 study, filtration rates remained unsatisfactory. In period 2, after combining filter wetting process by pressure hold together with period 1 conditions, filtration rates were found to be satisfactory in the first batch (batch no. 191106). The two nanofiltration rates were 4.79 and 4.70 kg/m²/10 hr, respectively (> 4 kg/m²/10 hr). Two batches of HRIG were studied afterward (batch no. 200129 and 200401). The filtration rates were also found to be satisfactory in those two batches. The filtration rates were 4.44, 4.15,

6.22 and 7.16 kg/m²/10 hr, respectively. In conclusion, the filtration rates were satisfactory in the three consecutive batches.

Discussion

From preliminary study, after finding the best condition for nanofiltration, five lab scale (120 mL) and four scale-up (1,500 mL) studies were performed. In scale-up studies, using 1,500 milliliters of HRIG plasma, the filtration rates were found to be unsatisfactory. To

analyze the problem, many parameters were considered. For example, low concentration of pH-adjust reagents led to high amount of reagent added, low temperature of the protein solution tended to change viscosity of the solution and made it difficult to be filtered. As a result, additional processes were performed in this study.

The studies were separated into 2 periods. According to the result from period 1, after increasing the concentrations of pH-adjust reagents together with controlling the temperature of the solution before filtration, the filtration rates were found to be 4.83 and 2.11 kg/m²/10 hr, respectively. These filtration rates results were unsatisfactory. After thoroughly analyzing the cause of the failure, filter-wetting technique was found to be unsuitable. Bubble elimination during filter-wetting process was not adequate which lead to decreased filtration efficiency. Period 2 study was planned in order to solve the problem. In period 2, filter-wetting process was improved by applying pressure hold at 2.1 bar for 20 minutes combined together with the conditions in period 1. Six studies were done in period 2. The filtration rates in period 2 were found to be 4.79, 4.70, 4.44, 4.15, 6.22 and 7.16 kg/m²/10 hr, respectively. The average filtration rate was 5.24 kg/m²/10 hr (SD = 1.18).

From the results, increasing the concentrations of pH-adjust reagents together with controlling the temperature of solution before filtration and improvement of filter-wetting technique helped to maintain the consistency of filtration rate. Increasing the concentrations of pH-adjust reagents led to decreased volumes of the reagents added. As a result, the total volume of nanofiltration solution was decreased. The total volume of solution was the important parameter that affected the filtration efficiency. The nanofilter used in this study had only 0.07 m² total area. Hence, it was necessary to limit the total solution volume. If the total solution volume exceeded filter capability, the filtration rate would be decreased.

Increasing the temperature would lower the solution viscosity, which greatly affected the filtration efficiency. Many studies stated that the solution temperature and viscosity were related. Low temperature solution would have high viscosity and vice versa. Unless temperature exceeded the product limit and affected product quality, increasing solution temperature would lead to lower solution viscosity which resulted in increased filtration efficiency.^{10,11}

Pressure hold was commonly used in filter integrity testing. The principle of pressure hold was wetting filter thoroughly then input pressure into the filter. The volume of input pressure depended on the type and size of the filter. The method was holding the pressure within time limit. If pressure drop not more than the maximum value allowed, the filter was determined to have integrity.¹²

In this study, pressure hold was applied in filter-wetting process by holding pressure at 2.1 bar for 20 minutes. After the time limit was reached, by opening the outlet valve, the pressure detained inside would completely push out the bubbles that were clogged in the filter pore. This process helped to increase the filtration efficiency since the bubbles hindered the solution to pass through the filter. Using this technique helped the solution to replace air in the filter more effectively. Hence the filtration rates were increased.

Other than pH, temperature and wetting process that affected filtration rate, pore size of the filter was one of important parameters. In this study, 20-nm nanofilter was used as it had been proven that immunoglobulins could pass through the filter and viruses were removed effectively.^{5,13} There was a study that compare between using 20-nm and 35-nm nanofilter in immunoglobulins manufacturing process. The result found that the 20-nm nanofilter demonstrated viral reduction from 4.1 to ≥ 7.3 while 35-nm nanofilter showed > 2.72 prion reduction factor.¹⁴ The result from this study indicated that 20-nm nanofilter had high viral removal capacity. Another

study stated that viruses of the size range from 25 nm to 40 nm (picornaviruses, calciviruses and SV40) were effectively removed by 15 to 20 nm pore size filters while the virus retention of parvoviruses which size range from 18 to 24 nm was $4 \log_{10}$ or greater which indicated high viral removal capacity of filters.⁵ Increasing pore size of the filter could get higher filtration rate but high risk of small-size viral contamination. Decreasing pore size could lead to clogged filter. Thus, filtration optimization process that was specific to each product was required.

By optimizing the parameters in this study, nanofiltration for NBC's IMIG could be efficiently operated. The average filtration rate was $5.24 \text{ kg/m}^2/10 \text{ hr}$ (5 L). As NBC commercial scale was 7 L, at least 20 percent of filtration margin was required to calculate optimal filter area. Based on calculation, 2 m^2 nanofilter was enough for $10.48 \text{ kg/m}^2/10 \text{ hr}$ (10 L). Therefore, 7 liters commercial-scale solution could be filtered within 10 hours by 2 m^2 nanofilter.

Limitations

In this study, we had to declare limitation in 3 aspects. Firstly, time constraint, as this study was one of the continuous development projects of NBC to enhance product quality and safety, the project timeline was separated into 3 phases. Phase I was finding the best filtration condition, phase II was lab-scale and up-scale production and phase III was commercial scale production. Since each phase had limited timeline, this study had to be finished within time limit in order to deliver high quality products to the patient as soon as possible. Secondly, plasma constraint, because the plasma was used as one of the life-saving drugs for the patients so the sample size of the study was limited. The plasma used in this study were rarely obtained because donor immunization process was required before collecting the plasma. Even the donors were immunized, not all of them could donate the plasma. Only donors who had high titer after immunization could donate the plasma. From NBC internal data, the qualified donors

were only 3.5 percent of all donors. Moreover, the process of plasma fractionation to obtain the precipitate IIC (PIIC) required many units of plasma. One batch of precipitate IIC (PIIC) required at least 180 units (90 L) and took a minimum of 3 weeks to get one PIIC. The PIIC used in this study was obtained and shared from routine production resulted in decreased amount of PIIC for routine immunoglobulin production. As a result, the immunoglobulins were not enough to provide to the patients. Lastly, financial constraint, nanofilters and reagents used in this study were very expensive and limited.

From the three constraints stated, after the result of filtration rate did not meet expectation in period 1, adding wetting process was implemented in period 2 to solve the problem immediately and the results were shown to meet the criteria.

Following good manufacturing practice (GMP) standard, at least three consecutive batches of the production were enough to prove consistency of the process.¹⁵ Hence, three batches of the production with six nanofiltration results were conducted as preliminary process validation prior to commercial batch.

As the production process was trade secret, the process optimization that specific to the products should be done by each manufacturer and was not widely published. As a result, only limited references which focus on finding the appropriate nanofiltration condition could be available.

Conclusion

Filtration rate was one of the most important factors that helped to determine the appropriate filter area. Using the same filter in different kind of products would give different results. In order to filter NBC's IMIG (HBIG and HRIG) through nanofilter, which was the key viral removal process, the study of the parameters that affected the nanofiltration which were specific to the products was necessary.

In this study, many parameters influencing the filtration rate were studied, such as increasing the concentrations of pH-adjust reagents together with controlling the temperature of the solution before filtration and improving filter-wetting technique leading to process improvement. Using all conditions together could increase the filtration rate. As a result, Blood Product Production Department could manufacture the nanofiltration products with high quality and safety for commercial scale in the near future.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We would like to acknowledge technical supports from Japan Blood Product Organization (JB) for dispatching the professional staff and providing useful advices to assist us to fulfil this study. We are very grateful to Quality Control and Quality Assurance Department of National Blood Centre, Thai Red Cross Society for their assistance and support in sample testing and data analyzing. This study would not be accomplished without their contributions.

References

1. Burnouf T, Radosevich M. Nanofiltration of plasma-derived biopharmaceutical products. *Haemophilia*. 2003;9:24-37.
2. World Health Organization. Guidelines on viral inactivation and removal procedures intended to assure the viral safety of human blood plasma products. Geneva: World Health Organization; 2004.
3. Burnouf T, Radosevich M, Goubran H, Wilkommen H. Place of nanofiltration for assuring viral safety of biologicals. *Curr Nanosci*. 2005;1:189-201.
4. Burnouf T. Modern plasma fractionation. *Transfus Med Rev*. 2007;21:101-17.
5. Roth NJ, Dichtelmüller HO, Fabbrizzi F, Flechsig E, Gröner A, Gustafson M, et al. Nanofiltration as a robust method contribution to viral safety of plasma-derived therapeutics: 20 years' experience of the plasma protein manufacturers. *Transfusion*. 2020;60:2661-74.
6. Burnouf T. An overview of plasma fractionation. *Ann Blood*. 2018;3:33.
7. Chantanakajornfung A. Methods of plasma fractionation. *J Hematol Transfus Med*. 2009;19:193-202.
8. Chonsilapawit T, Nakdaeng T, Moriguchi M, Rungpradubvong W. The study of filter selection and finding appropriate nanofiltration condition to increase National Blood Centre's immunoglobulin safety. *J Hematol Transfus Med*. 2018;28:347. (abstract).
9. Cohn EJ. Preparation and properties of serum and plasma proteins; a system for the separation into fractions of the protein and lipoprotein components of biological tissues and fluids. *J Am Chem Soc*. 1946;68:459-75.
10. Barukčić I, Božanić R, Kulozik U. Effect of pore size and process temperature on flux, microbial reduction and fouling mechanisms during sweet whey cross-flow microfiltration by ceramic membranes. *Int Dairy J*. 2014;39:8-15.
11. Caenn R, Darley HCH, Gray GR. The filtration properties of drilling fluids. In: Caenn R, Darley HCH, Gray GR, editors. *Composition and properties of drilling and completion fluids*. Texas: Gulf Professional; 2011. p. 271-306.
12. Trotter AM, Meltzer TH. The pressure hold/drop integrity test; its correlation to diffusive flow. *PDA J Pharm Sci Technol*. 1998;52:182-5.
13. Caballero S, Nieto S, Gajardo R, Jorquera JI. Viral safety characteristics of Flebogamma® DIF, a new pasteurized, solvent-detergent treated and Planova 20 nm nanofiltered intravenous immunoglobulin. *Biologicals*. 2010;38:486-93.
14. Soluk L, Price H, Sinclair C, Atalla-Mikhail D, Genereux M. Pathogen safety of intravenous Rh immunoglobulin liquid and other immune globulin products: enhanced nanofiltration and manufacturing process overview. *Am J Ther*. 2008;15:435-43.
15. Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme. Guide to good manufacturing practice for medicinal products. Geneva: PIC/S; 2015 [cited 2023 Oct 8] Available from: https://picscheme.org/users_uploads/news_news_documents/ps_inf_11_2015_pics_gmp_revised_annex_15.pdf

