백 영 빈 교수 지도

석사학위 청구논문

Effects of sucrose as an excipient on the performance of ultrafiltration/diafiltration for antibody-based therapeutic production

2021

성신여자대학교 대학원

생 물 학 과

이 지 은

Effects of sucrose as an excipient on the performance of ultrafiltration/diafiltration for antibody-based therapeutic production

Adviser: Youngbin Baek, Ph.D.

Submitted in partial fulfillment of the

requirements for the degree of master.

November, 2020

Department of Biology

The Graduate School of Sungshin University

Jieun Lee

Certificate of Committee Approval

Be accepted partial fulfillment of the requirements for the degree of:

Master of Science

Signatures:

Chairperson	Changsoo Kang, Ph.D
Committee member	Youngbin Baek, Ph.D
Committee member	Hyunsuk Oh, Ph.D

Sungshin University

Graduate School

Abstract

Jieun Lee
Department of Biology
Graduate School
Sungshin University

Ultrafiltration / diafiltration (UF/DF) processes are used for final formulation of biotherapeutics like antibody-based therapeutics. Therapeutic proteins are produced at high concentrations (>100 mg/mL) according to the limited volume (1—2 mL) for the subcutaneous administration. Thus, additives which induce changes in proteins' stability are used to preserve the colloidal stability and guarantee reliable processing and safe formulations. The objective of this study was to evaluate the effects of sucrose as an excipient on the performance of UF/DF. The normalized flux data corresponding to the permeate flux converted using measured viscosity values of each solutions were obtained using tangential flow filtration (TFF), normal flow filtration (NFF) system during UF/DF process. Analysis of membrane surface were performed to inspect surface morphologies of the fouled membranes. The normalized flux decreased during UF/DF process, which was larger as the concentration of sucrose increased. The flux decline was

similar regardless of the material of the membrane. On the other hand, it was greater as the permeate velocity increased. It was confirmed that the sucrose adsorbed on the membrane surface during filtration by analysis of membrane surface through SEM. The flux decline phenomenon caused by sucrose appears to be affected by both concentration polarization and adsorption. These results provide important insights into the factors governing the optimization of UF/DF process including excipients.

Contents

List of Figures

List of Tables

Chapter 1. Introduction

Biotherapeutics are pharmaceutical products that are manufactured from raw or material that originate from people or other living things. Antibody-based therapeutics are a type of biotherapeutics that are mainly used as a treatment for cancer and autoimmune diseases such as rheumatoid arthritis and Crohn's disease (Scott *et al*., 2012; Nelson *et al*., 2010; Weiner *et al*., 2010). These are included monoclonal antibody (mAb), antibody drug conjugate (ADC), and Fc-fusion protein. These are based on targeted therapy, so they generally cause less side effects. They also have the advantage of being combined with other products for efficient drug delivery (Wang *et al*., 2007). The biopharmaceutical market was estimated at $243 billion as of 2018, accounting for about 30 percent of the total drug market, which is expected to continue to grow and reach $388 billion by 2024. Among them, antibody-based therapeutics account for seven of the top 10 drugs in the biopharmaceutical market, and research is also actively underway to develop antibodies for commercial use.

Antibody-based therapeutics are produced by separation/purification through a several downstream processes, starting with the growing of cells capable of producing the desired pharmaceutical compounds in the bio-reactor (Zydney, 2016). The separation/purification process is carried out by clarification, primary

capture, polishing, virus filtration and formulation (Reis and Zydney, 2007). In the formulation process, which is the final stage of the separation process, ultrafiltration (UF) and diafiltration (DF) are performed. Ultrafiltration is used for concentration or diafiltration of protein solutions to remove low-molecular-weight (LMW) impurities or exchange buffers (Etzel, 2007). Figure 1 shows a schematic of ultrafiltration/diafiltration (UF/DF) process.

UF/DF process is performed by UF-DF or UF-DF-UF depending on the final concentration of protein. For example, in cancer patients, relatively low concentrations (<100 mg/mL) of protein are needed because they are administered through intravenous injections. Thus, in this case, products are produced through the UF-DF process. On the other hand, patients with rheumatoid arthritis need high concentrations (>100 mg/mL) of proteins because they are administered through subcutaneous injections rather than intravenous injections (Baek and Zydney, 2018). Thus, in this case, products are manufactured through the UF-DF-UF process. However, the highly concentrated proteins are tendency to form aggregates and have high viscosity (Shire *et al*., 2009). Thus, additives which either induce changes in proteins' conformational or colloidal stability in solution are used to preserve the colloidal stability and guarantee reliable processing and safe formulations (Shire, 2009).

Sucrose is a common excipient used to stabilize the protein at the formulation process. It is well known for improving the stability of the protein structure and

reducing denaturation by hydrating the protein. There are two well-known benefits of sucrose used as an excipient during liquid formulation or freeze-drying process (Wang *et al*., 2007; Shire *et al*., 2004). One is to ensure isotonicity and the other is to improve the stability of therapeutic products (Falconer, 2019; Le Basle *et al*., 2019). Ensuring isotonicity is important to reduce red blood cells damage during injection caused by osmotic pressure and to minimize tissue damage around the injection site. The osmotic pressure of therapeutic products to prevent their damage should be about 290 mOsm/L, for which many excipients such as NaCl, sucrose, trehalose, mannitol, glycerol, and sorbitol can be used. Sucrose, along with NaCl, has been widely used since 2010 while NaCl was mainly used until the 2000s (Falconer, 2019). Sucrose can also improve the stability of the therapeutic protein by preferential exclusion of water molecules from around the protein, which can be explained that sucrose surrounds the therapeutic proteins instead of water molecules (Kim *et al*., 2018).

However, several disadvantages of sucrose have also been reported. For example, it has been shown that the preferential sugar-sugar interaction has become more dominant due to phase separation, reducing the sucrose interacting with protein when the sucrose concentration was greater than 0.234 M (Tzannis and Prestrelski, 1999). In addition, sucrose also generates acute kidney injury. Since the kidneys do not express sucrase, it has been confirmed that sucrose, which is used as a stabilizer to prevent side effects, rather accumulates in the proximal tubules and

causes hyperosmolality which leads to renal injury (Dantal, 2013). In fact, 79 of 88 renal-damaged patients reported to the FDA were found to have been received sucrose-stabilized intravenous immunoglobulin (Orbach *et al*., 2004). Another side effect of sucrose is a protein glycation that can be caused by sucrose hydrolysis at low pH conditions (Gadgil *et al*., 2007; Banks *et al*., 2009). Protein glycation is known to cause chronic diseases such as diabetes, vascular disease, and immune response, and to decrease the stability and safety of therapeutic products (Fischer *et al*., 2008). Although the effects of sucrose on the therapeutic protein and patients were reported, studies on the effects of sucrose on the membrane process were minimal.

The objective of this study was to examine the effects of sucrose on the membrane during UF/DF process. Data were obtained by performing ultrafiltration at tangential flow filtration (TFF) and normal flow filtration (NFF) system using 20 mM histidine buffers with different concentrations of sucrose. Diafiltration was performed using 7 g/L of human immunoglobulin G (IgG). Date also obtained for the results of analysis of membrane surface.

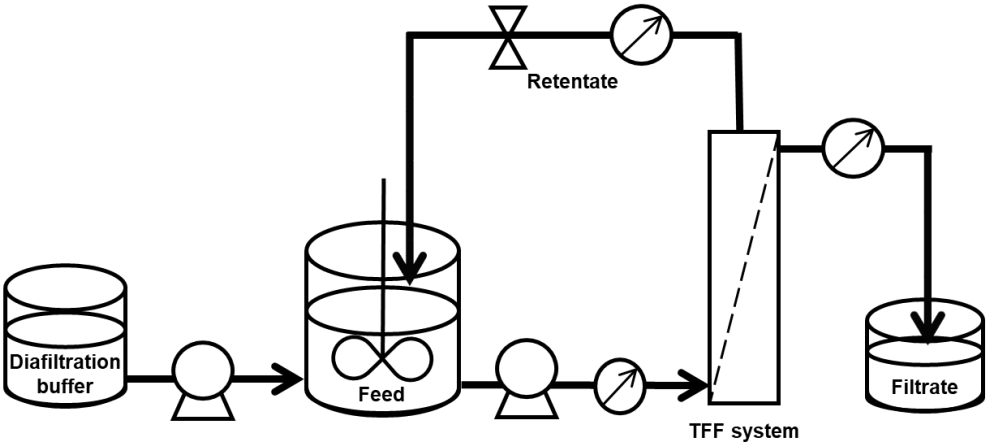


Figure 1. Schematics of ultrafiltration/diafiltration (UF/DF) process.

Chapter 2. Materials and Methods

2.1. Buffer formulations

The experiments were conducted using 20 mM histidine buffer solutions at pH 6. The value of [HA] and [A^-] was calculated using the Henderson-Hasselbalch equation corresponding to the following equation. The pK_a value of histidine is used as 6.

$$\mathrm{pH} = \mathrm{pKa} + \log \frac{[A^-]}{[HA]}$$

The buffer solutions were prepared by dissolving the calculated amount of L-histidine and L-histidine HCl (Sigma Aldrich) in deionized (DI) water (Merck Millipore). After mixing, they were checked for pH with an ORION VERSA STAR pro pH meter. It was measured at 24-25℃. If necessary, the pH was set using 0.2 N NaOH (Samchun) or HCl (Daejung CHEMICALS & METALS). The completed buffer solutions were added with sucrose (Daejung CHEMICALS & METALS) depending on the concentration (1, 5, 10, 25, 50, and 100 mM). Each buffer solution was used after performing vacuum filtration using a 0.2 ㎛ PTFE-Hydrophilic membrane filter (SciLab). After filtration, the pH was measured again to confirm that there was no change.

2.2. Viscosity measurements

The viscosity of the buffer solutions was measured using an AR-G2 stress-controlled rheometer (TA instrument). It was measured twice, and the average value was used. When analyzing the experimental data, a value of shear rates from 100 was used. The solution measured were 20 mM histidine buffer with 25, 50, 100, 200, and 300 mM sucrose at pH 6. The viscosity of buffer solutions that include 1, 5, and 10 mM sucrose was predicted by the viscosity values of 20 mM histidine and 20 mM histidine with 25 mM sucrose. The experimental conditions are 23℃ and a 60 mm parallel plate was used for geometry.

2.3. Filtration experiments

2.3.1 Ultrafiltration

Ultrafiltration experiments were performed at tangential flow filtration (TFF) and normal flow filtration (NFF) system. TFF systems are used for almost all large-scale commercial ultrafiltration devices. In tangential flow filtration, also referred to as cross flow filtration, the feed flow is parallel to the membrane and thus perpendicular to the filtrate flow. Therefore, the retentate flows along the surface of the membrane and out of the module, which controls the accumulation of suspended solids or colloids on the membrane. While in normal flow filtration, also referred to as dead-end filtration, the feed flow is the same as the filtrate flow. Thus, the retentate accumulates within the membrane module. NFF systems are used primarily when laboratory-scale separations and residual concentration are low.

The tangential flow ultrafiltration experiments were performed using Pellicon 3 TFF cassettes with D-screen and 30 kD Ultracel membranes with 88 cm^2 of surface area (Merk Millipore). The pellicon 3 cassette was mounted vertically in a cassette holder and the pressure gauge is located on the feed line and the retentate line. All cassettes were used after being flushed into DI water for more than 10 min at a feed flow rate of 45 mL/min using Masterflex roller pump. Before starting the experiment, the water permeability of the cassettes was measured

using DI water. Before ultrafiltration, membrane conditioning was performed with the 20 mM histidine buffer at pH 6 for 20 min. The permeate flux was calculated with the permeate weight for one minute at a feed flow rate of 45 mL/min. It was measured at 0.8 bar of the transmembrane pressure (TMP) using the pressure regulator on the retentate exit. During that one minute, feed pressure, retentate pressure and permeate weight were monitored. The experiments were conducted in total recycle mode (both retentate and permeate recycled back to the feed). The feed pressure, retentate pressure and the permeate weight were checked every 20 min. The flux was measured 3 times and the average value of those was obtained. During the experiment, the pressure was checked frequently and the pressure regulator in the retentate exit was used to adjust the TMP. The flux was measured for 5 h. After the experiment, the cassettes were cleaned using 0.2 NaOH for 30 min and then flushed with 3 liters of DI water.

The normal flow ultrafiltration experiments were performed using ultrafiltration disc type membranes with Ultracel 30 kD, Ultracel 100 kD, Biomax 30 kD (Merk Millipore). Ultracel 30 kD membranes were mainly used, and Ultracel 100 kD and Biomax 30 kD membranes were used to compare aspects according to the types of membrane. All the membranes were initially immersed in IPA for 15 min and the surface of them were washed with DI water. Then, flushing 50 mL of DI water at 1 bar, they were completely washed. After washing, membrane conditioning was performed with 15 mL of 20 mM histidine buffer at pH 6. During ultrafiltration,

the solutions were stirred at 800 rpm. The permeate flux was calculated by measuring the time taken to increase the weight of the permeate by 10 g. It was performed until the weight of the permeate was 280 g. During that time, the pressure gauge was continuously monitored to check if the pressure drop occurs at 1 bar. The permeate measured in weight units was converted to volume for each solution. Then, the normalized flux value, which was normalized as the pressure and viscosity value, was used for the data analysis.

Additional experiments with different experimental conditions of methods were conducted with NFF system. These include experiments under different pressure (5 bar) and pressure release experiments. Both experiments were performed with Ultracel 30 kD membranes. Pressure release experiments were performed by releasing the pressure to zero for 10 min at the point where the permeate volume became 100, 200 mL instead of constant pressure, and then applying the same pressure (1 bar) again. While releasing the pressure, the solutions were continued to stirred at 800 rpm.

The long-term experiments were conducted with TFF system. The cassette was flushed with 100 mM sucrose solution in total recycle mode for 1 h at 0.8 bar, and the permeate flux was checked every 20 min. After filtration, the cassette was cleaned sequentially using 0.2 N NaOH and DI water for 30 min, and then conditioned with 20 mM histidine buffer at pH 6 for 30 min. After conditioning,

the solution was replaced with a sucrose solution, and all this process was repeated.

2.3.2. Diafiltration

Diafiltration experiments were performed using Pellicon 3 cassettes with D-screen and 30 kD Ultracel membranes (Merk Millipore). The effective membrane area is 88 cm^2. The cassettes were initially flushed into DI water at least 500 mL to remove any storage solution. Diafiltration was conducted using a constant feed volume of 50 mL. The TMP was fixed at 1.3 bar by pressure regulator on the retentate exit. The feed flow rate was set at 51 mL/min using a Masterflex roller pump.

Before diafiltration, membrane conditioning was performed with the 20 mM histidine buffer at pH 6 for 20 min. The permeate flux was calculated with the time it took for the permeate to increase by 50 mL corresponding to 1 diavolume. It was checked while performing 10 diavolumes. During that time, feed pressure, retentate pressure and permeate weight were monitored continuously. After experiments, the cassettes were cleaned using 0.2 N NaOH for 30 min followed by flushing with 3 L of DI water. Feed solution was made with 7 g/L of IgG, purchased from Sigma Aldrich, in 20 mM histidine buffer at pH 6.

2.4. Analysis of membrane surface

Surface morphologies of Ultracel 30 kD membranes were inspected by scanning electron microscopy (SEM, JSM-6701F, JEOL) using a field emission scanning electron microscope (FESEM). The sucrose concentrations of the membrane surface were quantitatively analyzed through the phenol-sulfuric method. It was conducted in two ways, one by sampling permeate during filtration, and the other by desorbing sucrose from the membrane surface after filtration. Permeate was sampled by 1.5 mL each time the permeate increased by 10 g during filtration of 100 mM sucrose solution 280 mL using the Ultracel 30 kD membrane.

Desorption was performed after ultrafiltration experiment. First, filtration was carried out using DI water 280 mL to wash the remaining sucrose without adsorbing on the surface. Then, the sucrose was desorbed from the membrane contained in DI water 30 mL through 5 min of sonication and 3 min of vortexing. The sucrose concentrations of the samples were determined using a SpectraMax M5 microplate reader (Molecular Devices) at 490 nm of the absorbance. The experimental conditions were set at 24 degrees. The absorbed value of the sample was measured 3 times and the average value was used. Before the measurement, 1 mL of the samples diluted with 20 mM histidine at pH 6 were treated for 30 min at room temperature with 25 $\mu\ell$ of 8 % phenol (Sigma Aldrich) and 2.5 mL of 99.999% sulfuric-acid (Sigma Aldrich). The concentrations of sucrose were

analyzed through a standard curve using measured absorbed values obtained in advance.

Chapter 3. Results and Discussion

3.1. Effects of sucrose on ultrafiltration performance

3.1.1. Tangential flow filtration (TFF) system

Figure 2 shows the normalized flux obtained by performing tangential flow ultrafiltration experiments for 5 h using 20 mM histidine buffer at pH 6 with different concentration of sucrose. The normalized flux value (g/s^2), which is the multiplying of the viscosity (g/cm·s) of each solution by the flux value (LMH/bar), was used. The viscosity of each solution is shown in Table 1. The normalized flux decreased over time in the solution with sucrose of more than 25 mM. On the other hand, the flux of 1 mM, 5 mM sucrose solution was similar to that of DI water and 20 mM histidine solution used as control. The flux decreased more significantly as the concentration of sucrose increased, and it decreased significantly in the first 20 min.

Sucrose passes through the pore of the membrane when ultrafiltration is performed using the 30 kD membrane because the particles are small to 342 Da. Therefore, it was unexpected result that the normalized flux value, which ruled out the effects of viscosity, decreased. Also, it was interesting that the degree of decline in flux varies depending on the concentration of sucrose.

The flux decline is usually caused by concentration polarization and fouling (Matthiasson and Sivik, 1980). Firstly, concentration polarization is a natural consequence of the selectivity of a membrane (Bacchin *et al*., 2006). The filtrate flow causes an accumulation of partially (or completely) retained components at the upstream surface of the membrane. The accumulation of particles/solutes at the membrane surface increases the overall resistance to the filtrate flow through the formation of a particle cake or gel layer and it can reduce the effective pressure driving force through the osmotic pressure of the retained solutes (Zydney, 2000). Secondly, there is fouling which may take the forms of adsorption, pore blockage, deposit. Thus, several experiments were conducted to determine the cause of sucrose effect on the flux decline.

Table 1. Viscosity of 20 mM histidine buffer according to the concentration of sucrose.

Sucrose concentration (mM)	Viscosity (mPa·s)
0	1.138
25	1.223
50	1.26
100	1.31
200	1.515
300	1.811

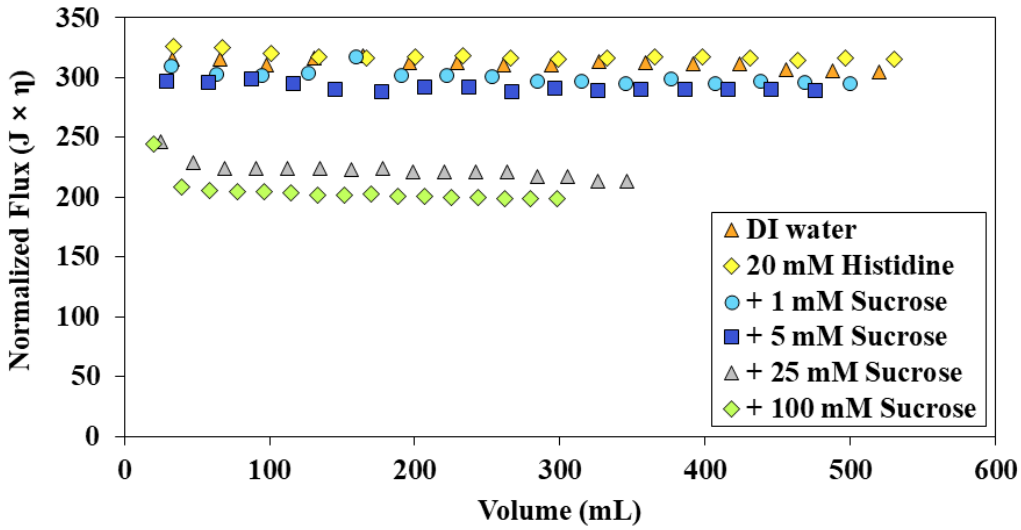


Figure 2. Normalized flux for tangential flow ultrafiltration (UF) of 20 mM histidine buffer at pH 6 with different concentration of sucrose. Data obtained using the Pellicon 3 cassette at a transmembrane pressure of 0.8 bar.

3.1.2. Normal flow filtration (NFF) system

Figure 3 displays the normalized flux obtained by conducting normal flow ultrafiltration experiments using the Ultracel 30 kD membrane. The normalized flux of solutions added with sucrose decreased over time. On the other hand, the flux of DI water and 20 mM histidine solution used as control remained constant. As with results of TFF system, the flux decline was larger as the concentration of sucrose increased. The 5 – 25 mM sucrose solution showed a gradual decrease in flux as the concentration of sucrose increased, but the trend of flux between 50 and 100 mM sucrose solution was almost the same.

Figure 4 shows the relative flux as a function of the sucrose concentration using TFF and NFF system. These values of the last flux divided by the initial flux in each solution were used to determine the degree of flux decline according to the concentration of sucrose. Comparing the trend of flux decline by each system, NFF system shows the flux decline from the lower concentration of sucrose than TFF system. The degree of flux decline was also greater. The reduction rate of flux was 19 and 70% for TFF and NFF system, respectively, based on 100 mM. It can be attributed to the difference in the shear stress with flow of fluid in each system, which generally results in more fouling in NFF system than in TFF system (Jackson and Landolt, 1973).

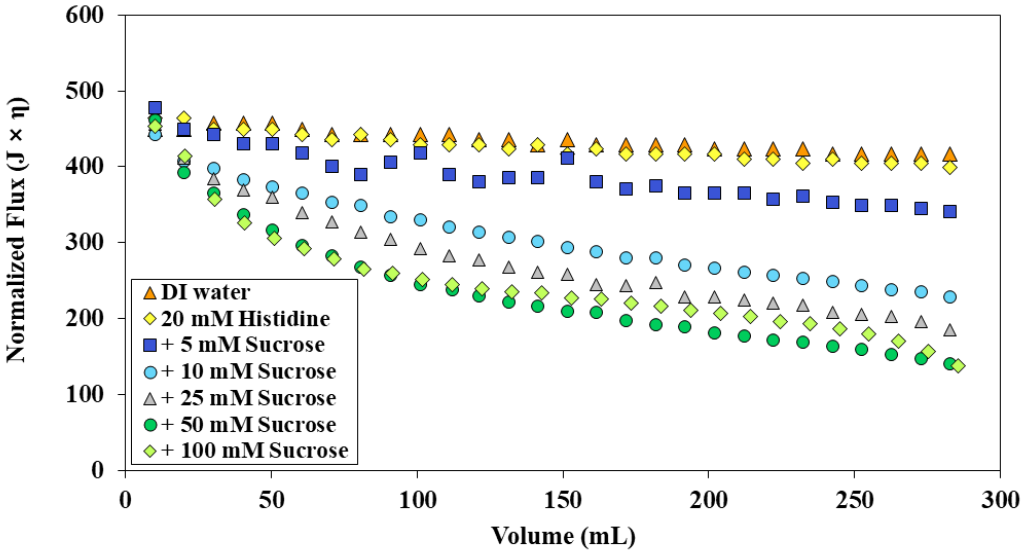


Figure 3. Normalized flux for normal flow ultrafiltration (UF) of 20 mM histidine buffer at pH 6 with different concentration of sucrose. Data obtained using the Ultracel 30 kD membrane at a transmembrane pressure of 1 bar. During filtration, the solutions were stirred at 800 rpm.

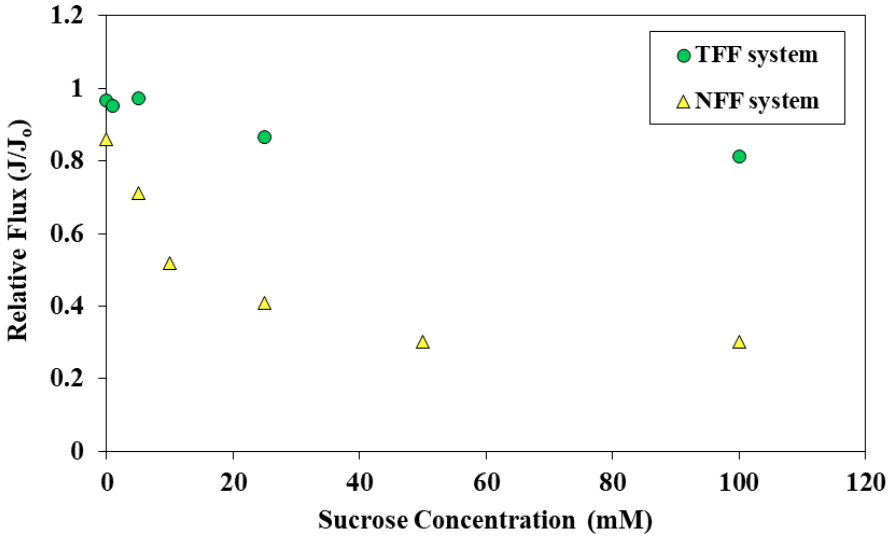


Figure 4. Relative flux (J/J_0) as a function of the sucrose concentration using TFF and NFF system.

3.2. Analysis of membrane surface

To confirm the effects of sucrose on the membrane in more detail, the surface morphology of the membrane was characterized by SEM. Figure 5 represents SEM images of the Ultracel 30 kD membrane before (a) and after (b) normal flow ultrafiltration using 20 mM histidine buffer at pH 6 with 100 mM sucrose. As shown in figure 5 (b), something appeared to be stacked on the surface of the membrane after filtration, which was observed the same in all parts of the membrane. On the other hand, nothing was observed in Figure 5 (a). Therefore, it was confirmed that the sucrose adsorbed on the membrane surface during filtration, causing fouling.

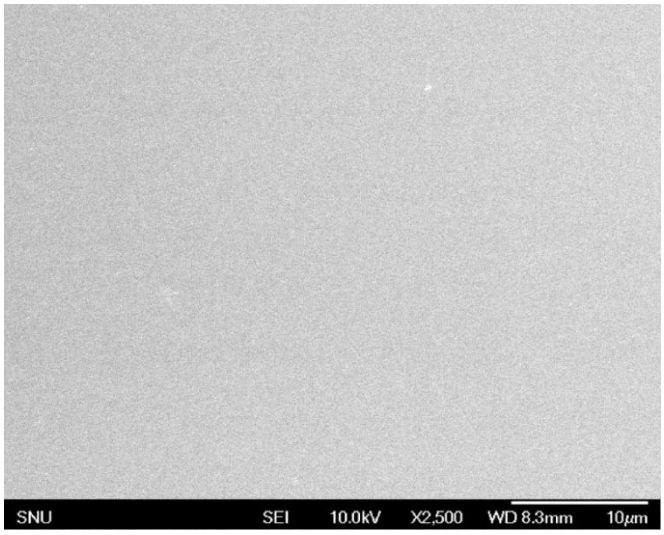
Adsorption is one of the forms of fouling, as described in section 3.1.1. It occurs when specific interaction between the membrane and the solute or particles exist. A monolayer of particles and solutes can grow even in the absence of permeation flux leading to an additional hydraulic resistance. If the degree of adsorption is concentration dependent then concentration polarization exacerbates the amount of adsorption (Bacchin *et al*., 2006). Therefore, it can be explained that sucrose, whose size is much smaller than the pore of the membrane, affects the fouling of the membrane due to adsorption.

Quantitative analysis of sucrose was performed with the Phenol-sulfuric method. Table 2 shows the concentration of sucrose confirmed through sampled permeate

during filtration. The decrease in the sucrose concentration of permeate shows that the sucrose was partially adsorbed by the membrane as mentioned earlier. The continuous decrease in the concentration of sucrose over time can explain the continued decrease in flux shown in the same previous experiment (Figure 3).

The phenomenon of sucrose adsorbing the surface of the membrane can explain the same flux decline trend seen in solutions of 50 and 100 mM (Figure 3). The reason seems to be that the surface of the membrane was so full of sucrose that there was no more site to be adsorbed (Hanemaaijer *et al*., 1989). Then, to determine the amount of sucrose adsorbed in the membrane surface after filtration, the sucrose was desorbed from the membrane and quantitatively analyzed. It was 55.1 mg/cm^2 after filtration using 100 mM sucrose solution.

(a)



(b)

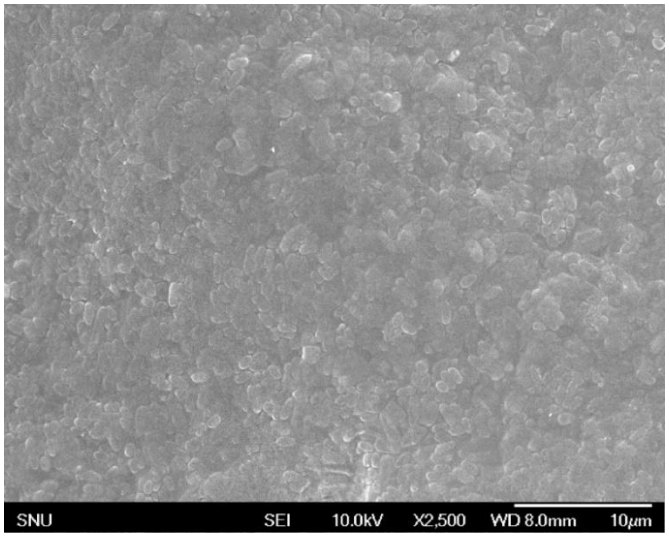


Figure 5. Scanning electron microscopy (SEM) image of the Ultracel 30 kD membrane before (a) and after (b) normal flow ultrafiltration (UF) using 20 mM histidine buffer at pH 6 with 100 mM sucrose.

Table 2. Sucrose concentration of permeate during normal flow ultrafiltration (UF) analyzed quantitatively with phenol-sulfuric method.

Initial sucrose concentration (mM)	**Permeate sucrose concentration (mM)**			
	30 g	**50 g**	**100 g**	**200 g**
100	91.8	91	85.3	79.3

3.3. Effects of concentration polarization

Figure 6 represents the normalized flux for normal flow ultrafiltration using the Ultracel 30 kD membrane obtained by temporarily releasing pressure at 100 and 200 mL of permeate volume. As shown at the point where the permeate volume is 100 and 200 mL, the decreased flux recovered to some extent and the degree was similar at both points. The reason for the recovery of the flux seems to be that the effects of the concentration polarization has been temporarily excluded due to the solution being mixed with the released pressure. However, since no complete recovery has been shown in both points, there are other factors affecting the decrease in flux in addition to the concentration polarization. This can be explained as the effect of adsorption as identified in section 3.2. Therefore, the decline in flux caused by sucrose appears to be affected by both concentration polarization and adsorption. The percentage calculated by the extent the flux decreased and recovered was 56% affected by the concentration polarization and 44% affected by the adsorption.

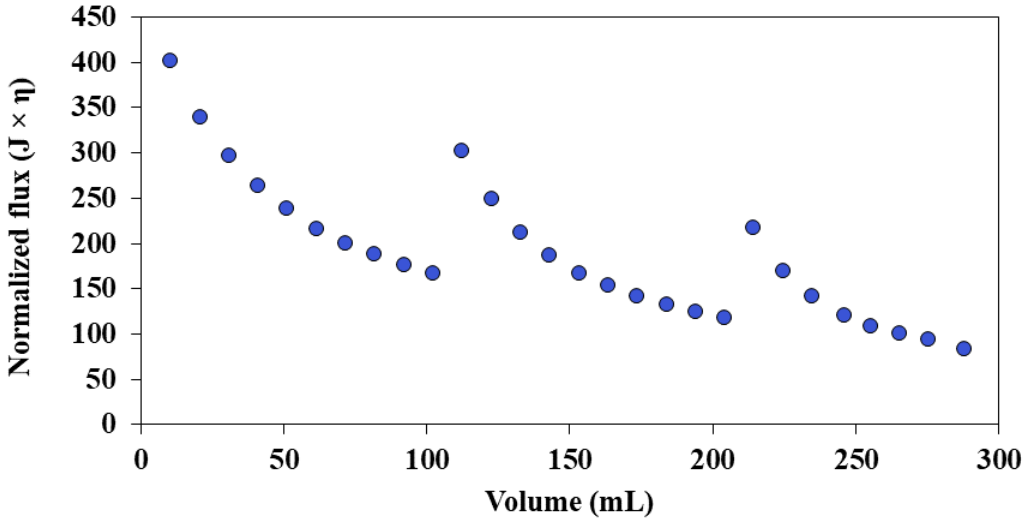


Figure 6. Normalized flux for normal flow ultrafiltration (UF) of 20 mM histidine at pH 6 with 100 mM sucrose obtained by temporarily releasing pressure at 100 and 200 mL of permeate volume. Data obtained using the Ultracel 30 kD membrane at a transmembrane pressure of 1 bar. During filtration, the solutions were stirred at 800 rpm.

3.4. Effects of other factors

3.4.1. Types of ultrafiltration membrane

To confirm if the pattern of flux decline due to sucrose varies depending on the pore size or material of the membrane, the normal flow ultrafiltration experiments were conducted using 100 mM sucrose solution. Ultracel 100 kD with greater pore size of the same material (regenerated cellulose, RC) and Biomax 30 kD with the same pore size of different material (polyethersulfone, PES) were used. These were compared because the membrane of RC, PES material is mainly used in UF/DF process due to its low protein adsorption.

As shown in Figure 7, the flux decline caused by sucrose were seen regardless of the type of membrane. Ultracel 30 kD and Biomax 30 kD showed the flux decline in almost the same form, whereas Ultracel 100 kD showed a faster rate of flux decline, which was larger at the beginning. It was noted that there was no material effect on the reduction of the flux because of the same trend between Ultracel 30 kD and Biomax 30 kD. The reason for the difference in the degree of fouling depending on the size of the pore can be attributed to the difference in permeate velocity. Since Ultracel 100 kD has a greater permeate velocity and is less affected by shear stress, it seems that the flux decline appears to be greater. This is the same as the general fouling phenomenon in which the larger the permeate velocity, the more serious the flux decreases (Matthiasson and Sivik, 1980).

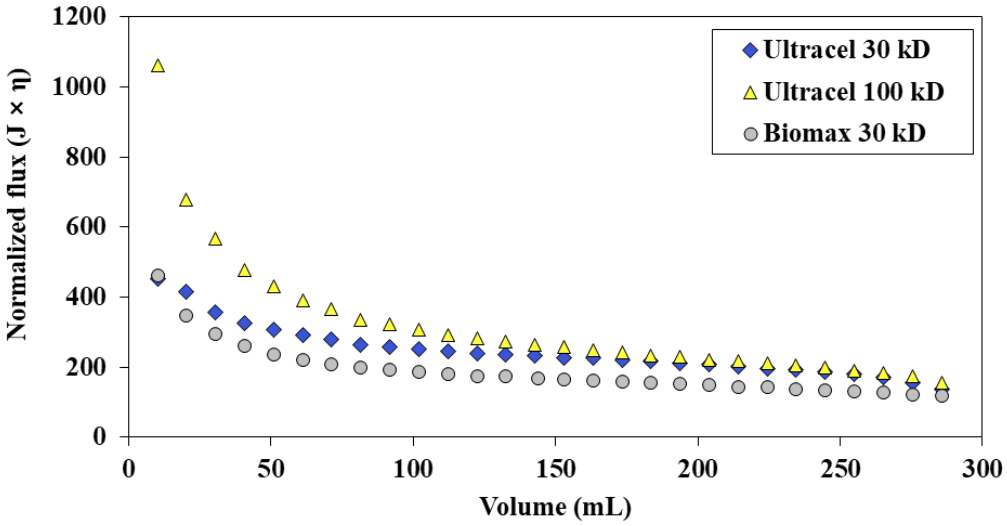


Figure 7. Normalized flux for normal flow ultrafiltration (UF) of 20 mM histidine at pH 6 with 100 mM sucrose according to the types of membrane. Data obtained at a transmembrane pressure of 1 bar. During filtration, the solutions were stirred at 800 rpm.

3.4.2. Transmembrane pressure (TMP)

To confirm the pattern of flux decline under different pressure condition, the normal flow ultrafiltration experiments were carried out at 5 bar using the Ultracel 30 kD membrane. Figure 8 shows the normalized flux during ultrafiltration using 100 mM sucrose solution according to the TMP. The normalized flux decreased faster at 5 bar than 1 bar. The flux in the two conditions became similar when the solution was passed around 200 mL, and the flux decline was shown with the same trend since then.

The reason why the higher pressure, the more fouling can be explained by the general fouling phenomenon caused by difference in permeate velocity as explained in the previous section (Jackson and Landolt, 1973). In addition, the reason why the flux, which had been rapidly decreasing due to higher pressure (5 bar), has decreased slowly since the permeate volume became 200 mL can be explained by the effect of the adsorption described earlier. As described in section 3.2., the adsorption sites of the membrane surface are limited (Hanemaaijer *et al*., 1989). Therefore, the degree of adsorption between the two conditions at that point appears to have become similar.

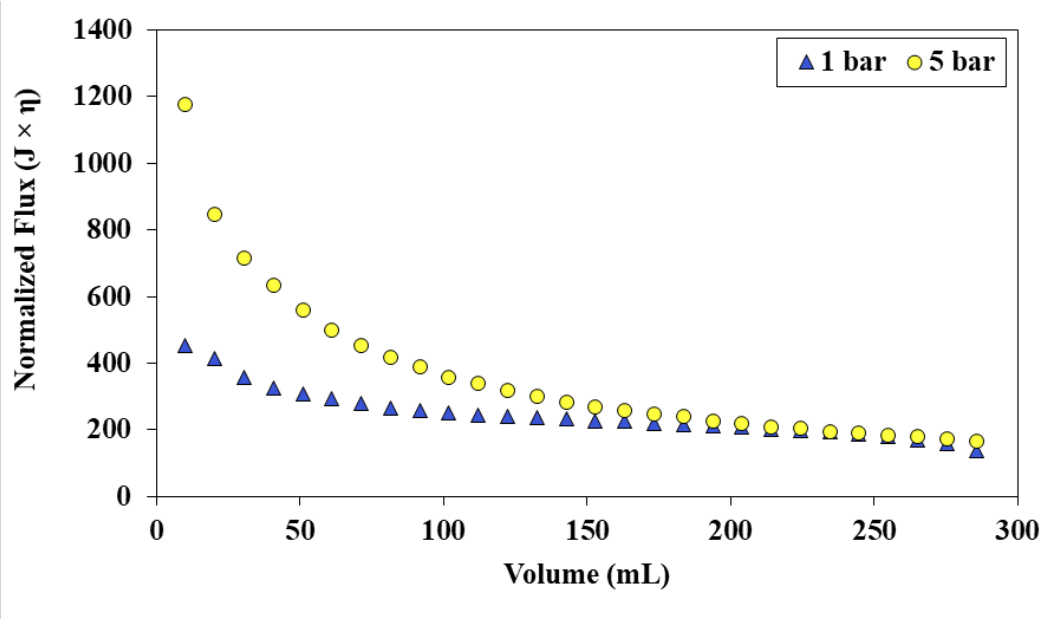


Figure 8. Normalized flux for normal flow ultrafiltration (UF) of 20 mM histidine at pH 6 with 100 mM sucrose according to the transmembrane pressure. Data obtained using the Ultracel 30 kD membrane at a transmembrane pressure of 1 bar. During filtration, the solutions were stirred at 800 rpm.

3.4.3. Operation time

Ultrafiltration membrane of the cassette type used in TFF system can be reused if it produces the same antibody-based therapeutics and has a normalized water permeability value of 80% or more of its initial value after being cleaned. Thus, long-term experiments were conducted to confirm how many times the cassette could be reused when washed with a common cleaning method after ultrafiltration using 100 mM sucrose solution.

Figure 9 displays the relative initial flux of 100 mM sucrose solution as a function of the number of ultrafiltration. The initial flux decreased according to the number of reuses. The initial flux of each cycle was divided into the initial flux of the first cycle and 14 cycles were performed. Since additional use of the cassette is possible when the J/J_0 value is 0.8 or higher, the graph shows that it can be reused approximately 7 times.

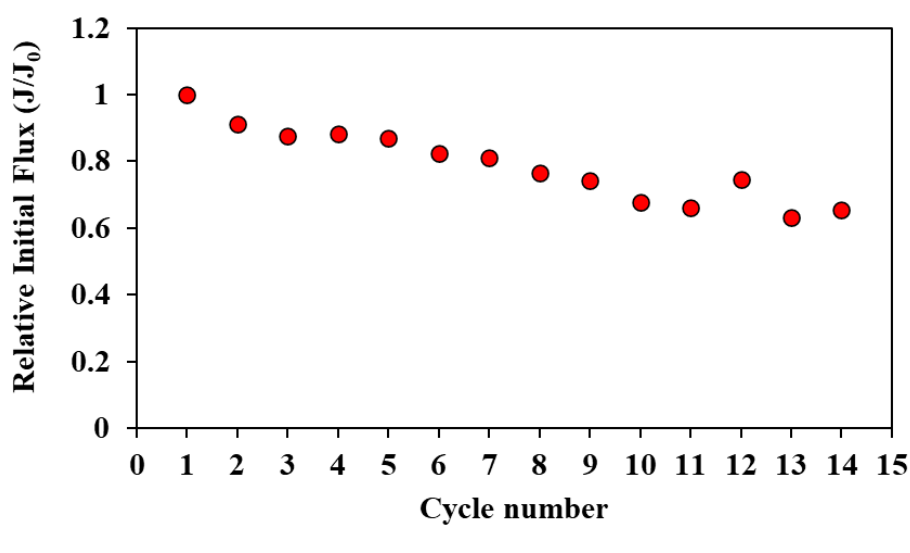


Figure 9. Relative initial flux (J/J_0) as a function of the number of tangential flow ultrafiltration (UF) using 20 mM histidine buffer at pH 6 with 100 mM sucrose. Data obtained using the Pellicon 3 cassette.

3.5. Effects of sucrose on diafiltration performance

Diafiltration experiments were conducted to confirm what aspects would be caused by sucrose, as sucrose was identified to affect the membrane fouling during ultrafiltration. Figure 10 represents the flux obtained by diafiltration from buffer to buffer with 100 mM sucrose under with and without IgG. During diafiltration, the flux decline was shown in both conditions. There was difference due to the viscosity of the IgG solution between the two values, but the trend of the decrease in flux was similar.

In general, as the sucrose concentration of the feed solution gradually increases from 0 to 100 during diafiltration, the flux decreases because of viscosity (Baek *et al*., 2017). Therefore, the roughly calculated viscosity value by considering the rate of change in the composition of the feed solution according to the number of diavolumes was used to exclude the effects of viscosity. However, the effect of viscosity on flux decline was minimal. Therefore, the sucrose affects the membrane fouling even during diafiltration.

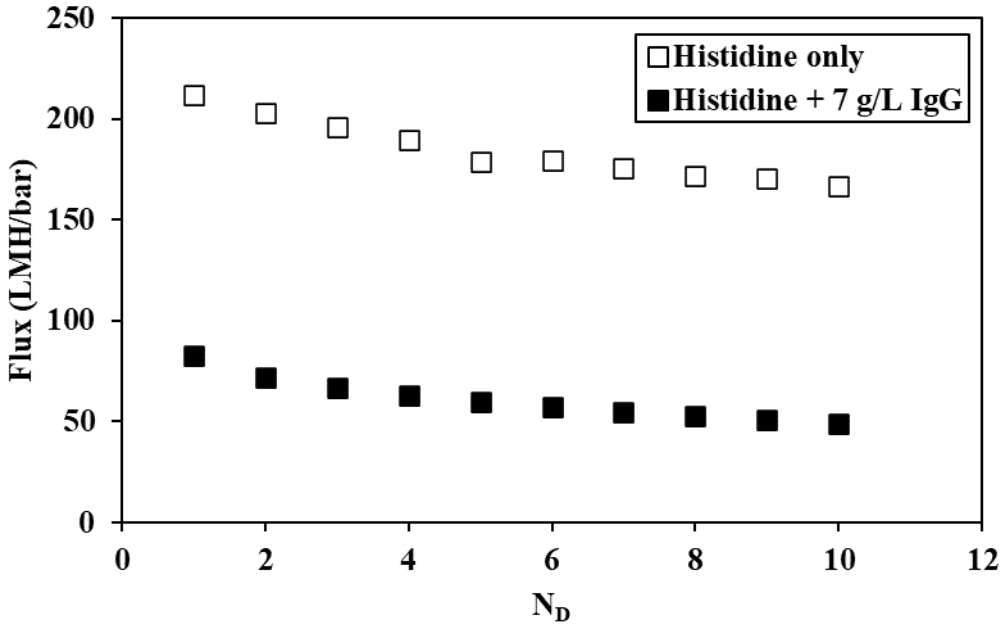


Figure 10. Flux during diafiltration (DF) from buffer to buffer with 100 mM sucrose under with and without IgG. Data obtained using the Pellicon 3 cassette at a transmembrane pressure of 1.3 bar.

Chapter 4. Conclusion

Although various characteristics of sucrose used as an excipient in UF/DF process for final formulation of biotherapeutics like antibody-based therapeutics have been reported, there has been minimal data on its impact on the performance of UF/DF. This paper presents data examining the effects of sucrose as an excipient on the performance of UF/DF. The normalized flux data corresponding to the permeate flux converted using measured viscosity values of each solutions decreased during ultrafiltration, which was larger as the concentration of sucrose increased. The flux decline was similar regardless of the material of the membrane. On the other hand, it was greater as the permeate velocity increased. During diafiltration, the flux also decreased due to the effects of sucrose.

It was confirmed that the sucrose absorbed on the membrane surface by analysis of fouled membrane surface through SEM. In addition, the concentration of sucrose continued to decrease as a result of quantitative analysis of sucrose by sampling per section during ultrafiltration. The decreased flux recovered to some extent in additional experiments that temporarily excluded the effect of concentration polarization by controlling process. However, no complete recovery has been shown, which can be seen as an effect of adsorption. Therefore, the flux

decline caused by sucrose appears to be affected by both concentration polarization and adsorption.

In general, TFF cassette can be reused if it produces the same antibody-based therapeutics and has a normalized water permeability value of 80% or more of its initial value after being cleaned. Thus, long-term experiments were conducted to cause fouling on the membrane for 1 h with a sucrose solution, and then clean it using a common cleaning method, and repeated this process. As a result, it can be reused approximately 7 times. These results provide important insights into the factors controlling the optimization of UF/DF process including excipients.

References

A. M. Scott, J. D. Wolchok, and L. J. Old, Antibody therapy of cancer, *Nat. Rev. Cancer*, 12, 278–287, 2012

A. L. Nelson, E. Dhimolea, and J. M. Reichert, Development trends for human monoclonal antibody therapeutics, *Nat. Rev. Drug Discov.*, 9, 767–774, 2010

L. M. Weiner, R. Surana, and S. Wang, Monoclonal antibodies: Versatile platforms for cancer immunotherapy, *Nat. Rev. Immunol.*, 10, 317–327, 2010

W. Wang, S. Singh, D. L. Zeng, K. King, and S. Nema, Antibody Structure, Instability, and Formulation, *J. Pharm. Sci.*, 96, 1–26, 2007

A. L. Zydney, Continuous downstream processing for high value biological products: A Review, *Biotechnology and Bioengineering*, 113, 465–475, 2016

R. van Reis and A. Zydney, Bioprocess membrane technology, *Journal of Membrane Science*, 297, 16–50, 2007

M. Etzel, Process Scale Bioseparations for the Biopharmaceutical Industry, 2007

Y. Baek and A. L. Zydney, Intermolecular interactions in highly concentrated formulations of recombinant therapeutic proteins, *Current Opinion in Biotechnology*, 53, 59–64, 2018

S. J. Shire, Z. Shahrokh, and J. Liu, Challenges in the development of high protein concentration formulations, *J. Pharm. Sci.*, 93, 1390–1402, 2004

S. J. Shire, Formulation and manufacturability of biologics, *Current Opinion in Biotechnology*, 20, 708–714, 2009

R. J. Falconer, Advances in liquid formulations of parenteral therapeutic proteins, *Biotechnology Advances*, 37, 2019

Y. Le Basle, P. Chennell, N. Tokhadze, A. Astier, and V. Sautou, Physicochemical Stability of Monoclonal Antibodies: A Review, *Journal of Pharmaceutical Sciences*, 109, 169–190, 2019

N. A. Kim, R. Thapa, and S. H. Jeong, Preferential exclusion mechanism by carbohydrates on protein stabilization using thermodynamic evaluation, *Int. J. Biol. Macromol.*, 109, 311–322, 2018

S. T. Tzannis and S. J. Prestrelski, Moisture effects on protein-excipient interactions in spray-dried powders. Nature of destabilizing effects of sucrose, *J. Pharm. Sci.*, 88, 360–370, 1999

J. Dantal, Intravenous Immunoglobulins: In-Depth Review of Excipients and Acute Kidney Injury Risk, *Am. J. Nephrol.*, 38, 275–284, 2013

H. Orbach, M. Tishler, and Y. Shoenfeld, Intravenous immunoglobulin and the kidney - A two-edged sword, *Semin. Arthritis Rheum.*, 34, 593–601, 2004

H. S. Gadgil, P. V. Bondarenko, G. Pipes, D. Rehder, A. Mcauley, N. Perico, T. Dillon, M. Ricci and M. Treuheit, The LC/MS analysis of glycation of IgG molecules in sucrose containing formulations, *J. Pharm. Sci.*, 96, 2607–2621, 2007

D. D. Banks, D. M. Hambly, J. L. Scavezze, C. C. Siska, N. L. Stackhouse, and H. S. Gadgil, The effect of sucrose hydrolysis on the stability of protein therapeutics during accelerated formulation studies, *J. Pharm. Sci.*, 98, 4501–4510, 2009

S. Fischer, J. Hoernschemeyer, and H. C. Mahler, Glycation during storage and administration of monoclonal antibody formulations, *Eur. J. Pharm. Biopharm.*, 70, 42–50, 2008

E. Matthiasson and B. Sivik, Concentration polarization and fouling, *Desalination*, 35, 59–103, 1980

P. Bacchin, P. Aimar, and R. W. Field, Critical and sustainable fluxes: Theory, experiments and applications, *Journal of Membrane Science*, 281, 42–69, 2006

A. L. Zydney, Membrane Bioseparations, *Encyclopedia of Separation Science*, 1728-55, 2000

J. M. Jackson and D. Landolt, About the mechanism of formation of iron hydroxide fouling layers on reverse osmosis membranes, *Desalination*, 12, 361–378, 1973

J. H. Hanemaaijer, T. Robbertsen, T. van den Boomgaard, and J. W. Gunnink, Fouling of ultrafiltration membranes. The role of protein adsorption and salt precipitation, *J. Memb. Sci.*, 40, 199–217, 1989

Y. Baek, N. Singh, A. Arunkumar, and A. L. Zydney, Effects of Histidine and Sucrose on the Biophysical Properties of a Monoclonal Antibody, *Pharm. Res.*, 34, 629–639, 2017

초록

한외여과/정용여과 공정은 항체의약품과 같은 바이오의약품의 제조공정에서 최종 제제화를 위해 사용한다. 단백질치료제는 피하주사를 위한 제한된 부피(1—2 mL)에 따라 고농도(>100 mg/mL)로 농축하여 생산한다. 따라서, 농축단백질 용액의 안정성을 보존하고, 처리과정의 신뢰성과 안전한 제형을 확보하기 위해 sucrose 와 같은 첨가제를 사용한다. 본 연구에서는 첨가제로 사용되는 sucrose 가 한외여과/정용여과 성능에 미치는 영향을 평가하고자 하였다. 한외여과/정용여과 공정동안 tangential flow filtration (TFF), normal flow filtration (NFF) 시스템을 사용하여 유량을 측정하였고, 이는 각 용액의 점도 값을 사용하여 Normalized flux 값으로 변환하였다. 공정 후 오염된 분리막의 표면분석 또한 수행하였다. 그 결과, UF/DF 공정동안 Normalized flux 값이 감소했고 이는 sucrose 의 농도에 따라 더 크게 나타났다. 유량감소는 분리막의 재질에 따라 차이가 없었으며, 유속이 빠를수록 더 크게 나타났다. 주사전자현미경(SEM)을 통한 분리막의 표면분석에서는 sucrose 가 여과(filtration)공정 동안 분리막의 표면에 흡착됨이 보였다. Sucrose 로 인해 나타난 유량감소현상은 농도 분극, 흡착 모두의 영향을 받은 것으로 보이며, 이러한 결과들은 첨가제가 들어간 UF/DF 공정의 최적화를 위한 요소들에 대한 중요한 통찰력을 제공한다.