EFFECT OF IONIC STRENGTH ON WHEY PROTEIN SEPARATION IN HIGH PERFORMANCE TANGENTIAL FLOW FILTRATION

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JUDUL : EFFECT OF IONIC STRENGTH ON WHEY PROTEIN SEPARATION IN HIGH PERFORMANCE TANGENTIAL FLOW FILTRATION

SESI PENGAJIAN: 2010/2011

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EFFECT OF IONIC STRENGTH ON WHEY PROTEIN SEPARATION IN HIGH PERFORMANCE TANGENTIAL FLOW FILTRATION

MUHAMMAD FAKHRULLAH BIN MOHD NOR

A thesis submitted to the Faculty of Chemical and Natural Resource Engineering in partial fulfilment of the requirement for the Degree of Bachelor of Engineering in Chemical Engineering

Faculty of Chemical and Natural Resource Engineering
Universiti Malaysia Pahang

DECEMBER 2010

I declare that this thesis entitled "*Effect of Ionic Strength on Whey Protein Separation in High Performance Tangential Flow Filtration*" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and it is not concurrently submitted in candidature of any degree.

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Dedicated to my beloved families and friends
for their love and encouragement

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ABSTRACT

High performance tangential flow filtration (HPTFF) is an emerging technology developed from the conventional cross flow filtration (CFF). HPTFF could separate protein components that differ less than three fold in size compared to the CFF, which only applicable to separate the protein components that differ more than tenfold in size. In the current study, HPTFF was operated using PES membrane with MWCO of 30kDa to separate whey protein components into two fractions which is enriched in α-lactalbumin (α-lac) on the permeate side and β-lactoglobulin (β-lg) in the retentate side. In order to get higher permeate value of α-lac, ionic strength of the solution from 0M to 0.5M in HPTFF was studied in this study. The best separation factor was observed at intermediate salt concentration of 0.125M concentration.

ABSTRAK

Prestasi tinggi aliran filtrasi tangensial (HPTFF) adalah sebuah teknologi baru yang dibangunkan dari penapisan aliran konvensional silang (CFF). HPTFF dapat memisahkan bahagian-bahagian protein yang berbeza kurang daripada tiga kali ganda dalam saiz berbanding dengan CFF, yang hanya berlaku untuk memisahkan bahagian-bahagian protein yang berbeza lebih dari sepuluh kali ganda dalam saiz. Dalam kajian ini, HPTFF diterapkan menggunakan PES membrane yang mempunyai MWCO 30kDa untuk memisahkan bahagian-bahagian whey protein menjadi dua fraksi yang diperkaya dengan α-lactalbumin (α-lac) di sebelah permeat manakala β-lactoglobulin (β-lg) di sebelah retentat. Dalam rangka untuk mendapatkan nilai α-lac yang lebih tinggi di sebelah permeat, kekuatan ion larutan dari 0M untuk 0.5M di dalam HPTFF dipelajari dalam kajian ini. Faktor pemisahan terbaik didapati berlaku pada kepekatan garam yang berkepekatan sederhanaiaitu 0.125M konsentrasi.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

HPTFF	-	High Performance Tangential Flow Filtration
BSA	-	Bovine Serum Albumin
α-lac	-	α-lactalbumin
β-lg	-	β-lactoglobulin
CFF	-	Conventional Cross Flow Filtration
PES	-	Polyethersulfone
IgG	-	Immunoglobulin
MF	-	Microfiltration
UF	-	Ultrafiltration
NF	-	Nanofiltration
RO	-	Reverse Osmosis
PVDF	-	Polyvinylidene Difluoride
MWCO	-	Molecular Weight Cut-Off
SEC	-	Size Exclusion Chromatography
NaCl	-	Natrium Chloride
HCl	-	Hydrochloride Acid
TMP	-	Transmembrane Pressure
NaOH	-	Sodium Hydroxide
IP	-	Isoelectric Point
RPC	-	Reverse Phase Chromatography
TFA	-	Trifluoroacetic Acid

LIST OF SYMBOLS

A	-	Peak area (mAu*mL)
C	-	Concentration (mg/ml)
M	-	Molarity (mol/L)
m	-	Mass of samples (g)
MW	-	Molecular weight (mass/number of moles)
V	-	Volume (mL)
%	-	Percent
°C	-	Degree Celsius
mg	-	milligram
mL	-	milliliter
L	-	Liter
psig	-	Pound per square inch gauge
mg/mL	-	milligram / milliliter
nm	-	nanometer
μm	-	micrometer
g/L	-	gram / Liter
kDa	-	kilo Dalton
mS/cm	-	milliSiemens / centimeter

LIST OF APPENDIX

CHAPTER 1

INTRODUCTION

1.1 Research Background

In the recent year, there is an increased of the product developed using biotechnology. Protein separation has become more important in order to fulfill the final product requirement in this biotechnology industrial. In bioseparation, the protein of interest is purified and recovered from crude products to produce a pure and single protein product.

Among the separation methods that widely been used to separate the protein mixture to individual protein component is membrane filtration. Compared to other methods, membrane filtration has been extensively used in large-scale process to concentrate milk, whey and fermentation broths, and to clarify a fruit juices. Membrane separation is energy efficient and easily scaled up, but the process is limited by the fouling and lack of durability of membrane materials. Membranes are more effective for bulk than for precise separations, and seem best suited to the commercial production of low-value products. The emergence of high-performance materials and modules should expand the use of membranes in product recovery (Singh and Singh, 1996).

There are two types of membrane operation that commonly being used, which is dead end filtration and cross flow filtration. Figure 1.1 illustrated these two types of operation. Cross flow also referred as tangential flow filtration.

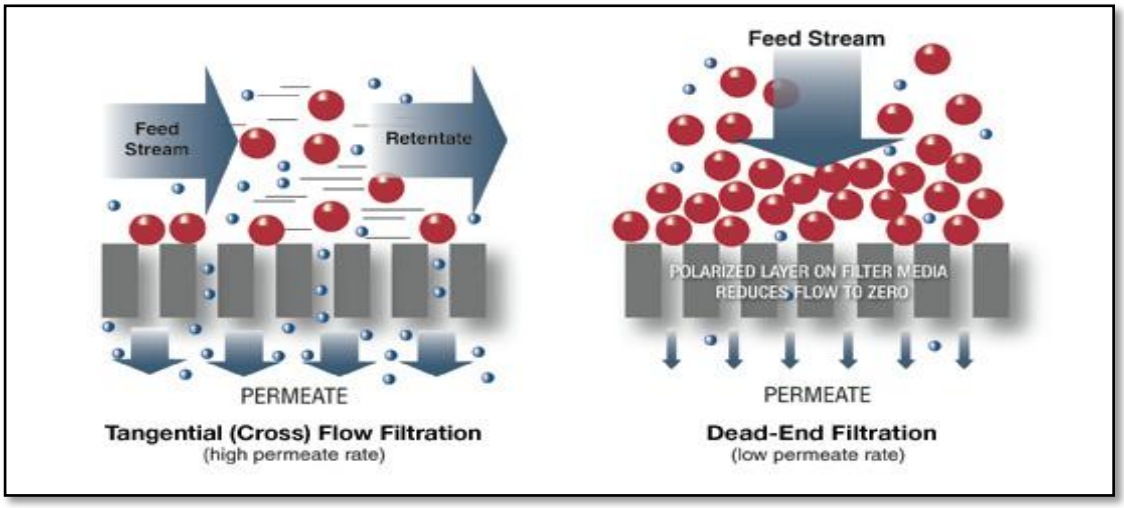


Figure 1.1: Tangential flow filtration and dead-end filtration.

In dead end filtration, the feed will be forced perpendicularly through the pore of the membrane. Depending on the membrane pore size, the components that have bigger size will stay behind while the smaller components will flow out as permeate. However the membrane fouling is increase over time in dead end filtration due to the cake layer formation occurred on the surface of the membrane. This layer will become thicker over a time and will reduced the filtration rate and pressure need to push the feed through the membrane (Vogel and Todaro, 1997).

For tangential flow filtration, the feed will flow across the membrane tangentially rather than flow into the membrane. This is better than dead end filtration as the flow will wash away the cake layer formed on the surface of membrane. It will reduce the clogging at the inner side of the membrane and will increase the capability of the membrane to separate the mixtures.

Whey contains a lot of proteins such as β-lactoglobulin (β-lg), α-lactalbumin (α-lac), immunoglobulin (Ig), bovine serum albumin (BSA), lactoferrin and lactoperoxidase. These single and pure proteins actually can be purified for the benefit of their own value in certain application. In the current study, whey will be separated into two fractions which is enriched in α-lac on the permeate side and β-lg in the retentate side by using high performance tangential flow filtration (HPTFF). In order to get higher permeate value of α-lac, the ionic strength during the operation of HPTFF will be optimized.

1.2 Problem Statement

Whey protein normally regarded as a by-product in cheese manufacturing industry. It is a remaining liquid after casein was precipitated from milk to produce a cheese. It contains different types of protein which had its specific value and application. Fractionation of whey protein into single pure protein could add the value of these whey proteins and can be used specifically in certain application.

Whey protein fractionation using conventional membrane cross-flow system is not possible due to the similar size of whey protein component, especially between α-lac and β-lg. In conventional cross-flow filtration, the protein component should be differ 10 fold in size to be effectively separate. However, with the new concept of HPTFF, it is now possible to separate α-lac and β-lg from whey protein.

1.3 Objective of Research

The objective of this research is to separate α-lac and β-lg from whey protein using HPTFF technique. In order to get the good separation factor, the ionic strength of the whey and buffer solution during HPTFF will be studied.

1.4 Scope of Research

Several research scopes have been outlined in order to fulfill the objective of this research. These research scopes are:

- Preparation of whey from fresh milk
- Setup and operate HPTFF using 30 kDa polyethersulfone (PES) membrane in Kvick-lab filtration system
- Study the effect of ionic strength of whey and buffer solution from 0M to 0.5M on whey protein separation in HPTFF
- Characterize the whey protein component in permeate and retentate side

CHAPTER 2

LITERATURE REVIEW

2.1 Membrane Separation

Membrane separation is operates mainly based on the size different between the components to be separated and the pore size of the membrane. Components that have smaller size than the membrane pores will pass through it and known as permeate while the larger components will be blocked and retained as retentate. Either permeates or retentate could be collected as a product depends on the purpose of separation. The classification of membrane separations based on the pore size is shows in Figure 2.1.

2.1.1 Microfiltration

Microfiltration (MF) is a pressure-driven membrane process for the separation of particles in the size range of 0.1 to 10 μm. MF is commonly used to recover macromolecules and retain suspended colloidal particles A large range of MF applications are pretreatment steps, removal of small molecules from bigger protein molecules, clarify suspensions for cell harvesting, and sterilize liquids to remove viruses and bacteria. Module configuration of MF include hollow-fiber, tubular, flat plate, spiral-wound and rotating devices. The MF membranes are made from natural or synthetic polymers such as cellulose nitrate or acetate, polyvinylidene difluoride (PVDF), polyamides, polysulfone, polycarbonate, polypropylene, etc. The inorganic materials such as metal oxides (alumina), glass, zirconia coated carbon etc. are also used for manufacturing the MF membranes (Saxena et al., 2009).

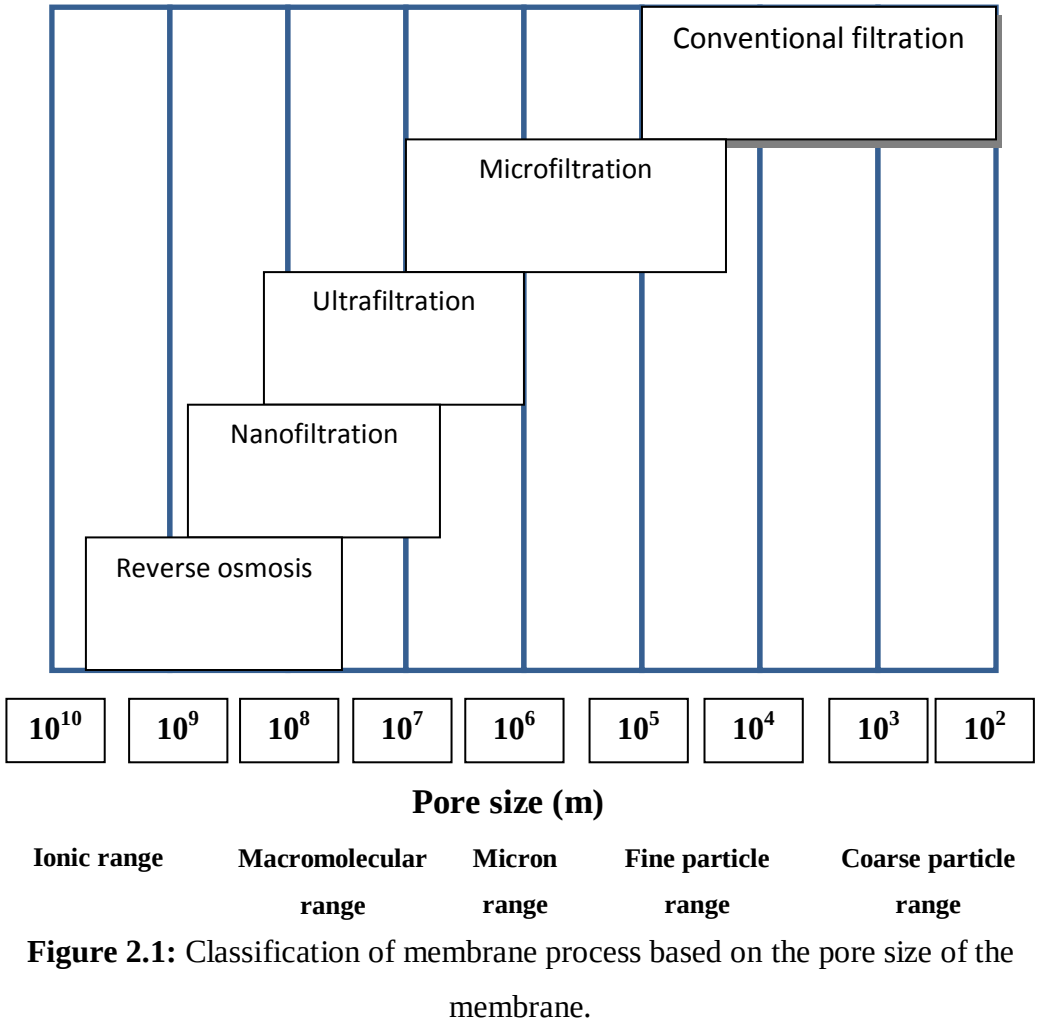


Figure 2.1: Classification of membrane process based on the pore size of the membrane.

2.1.2 Ultrafiltration

Ultrafiltration (UF) is a separation process in which the membranes having pore size around 10-1000Å. UF has been used widely in variety of applications ranging from biological macromolecules to waste water treatment. Major applications of UF are fractionation of macromolecules like proteins and nucleic acids, removal of cells and cell from fermentation broth, virus removal from therapeutic products etc. Nearly 40 percent of total usage of UF membranes comes from food and biotechnological applications. Having high throughput of product, relative ease of scale-up and ease of equipment cleaning and sanitization are the major advantages of UF. UF membranes are made from organic polymer such as polysulfone, polyethersulfone etc. or inorganic polymer such as glass, metals and ceramics (Ghosh, 2003).

2.1.3 Nanofiltration

Nanofiltration (NF) is typically referred to as "loose" reverse osmosis (RO) due to its larger membrane pore structure as compared to the membranes used in RO, and allows more salt passage through the membrane. Because it can operate at much lower pressures, and passes some of the inorganic salts, NF is used in applications where high organic removal and moderate inorganic removals are desired. Besides that, NF is capable of concentrating sugars, divalent salts, bacteria, proteins, particles, dyes and other constituents that have a molecular weight greater than 1000 daltons.

Membranes used for NF are of cellulosic acetate and aromatic polyamide type having characteristics as salt rejections from 95% for divalent salts to 40% for monovalent salts and an approximate 300 molecular weight cut-off (MWCO) for organics. An advantage of NF over RO is that NF can typically operate at higher recoveries.

2.1.4 Reverse Osmosis

Reverse osmosis (RO) is a similar separation technique to ultrafiltration except that membranes of a much smaller pore diameter are employed and the operating pressure is much higher. RO could separate particles ranging from 0.2-0.5 nm. The operating pressure must exceed the natural osmotic pressure for the system, resulting in the movement of solvent, usually water, from the solution of high analyte concentration to that of low analyte concentration. Reverse osmosis is therefore suitable for preconcentrating relatively large volumes of dilute solutions such as river or drinking water. The rate of permeation of organic solutes through the membrane depends on the chemical compatibility of the membrane and analytes, not sieving, as was the case for ultrafiltration. Reverse osmosis can therefore be used to separate solutes of similar size.

2.2 Membrane Configuration

2.2.1 Hollow Fiber

Hollow fiber had a typical 0.25 to 2.5 mm in diameter. Figure 2.2 show example of hollow fiber membrane modules. Hollow fiber module usually consists of a bundle of several hundred fibers. These are spun separately, bundled and potted into tube headers using epoxy resin. The fiber bundles are housed inside pressure vessels and the feed material normally flows through the inside (the lumen) of the fiber. Hollow fiber membranes are made from a variety of polymers including polyethersulfone, polysulfone, polypropylene, polyvinylidene fluoride, and mixed cellulose esters. Pre-sterilized disposable hollow fiber modules have also been developed, eliminating the need for cleaning and regeneration (Ghosh, 2003).

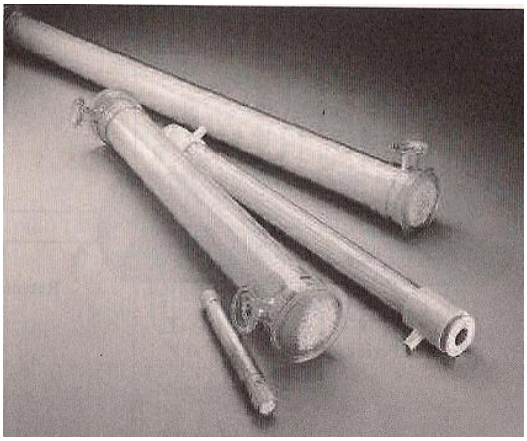


Figure 2.2: Hollow fiber membrane modules.

2.2.2 Flat Sheet

The basic flat sheet membrane module consists of a shallow rectangular flat sheet membrane on one or both sides of the channel as shown in Figure 2.3. The feed that enters this type of configuration will be separated into a permeate side at top and bottom side, while producing retentate at the end of flow. Intermediate and large scale flat sheet modules resemble a plate and frame filter press. These devices can be easily disassembled for cleaning and for replacement of defective membrane elements. The material to build these types of membranes comes from a wide range of polymer including polysulfone, polyethersulfone, cellulose, and hydrophilized polyvinylidene fluoride. In order to increase hydrophilicity and to reduce fouling, the surfaces of these materials are always modified with certain functional group (Ghosh, 2003; van Reis and Zydney, 2007).

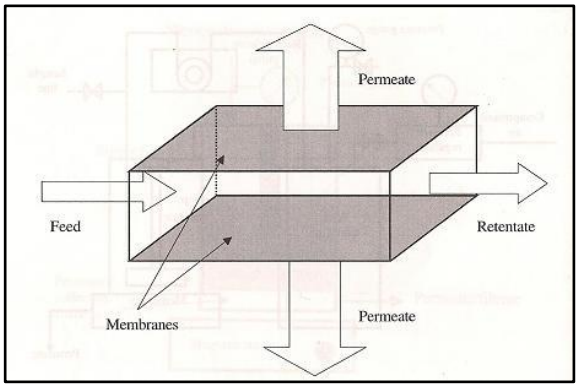


Figure 2.3: Basic flat sheet tangential flow membrane module.

2.2.3 Spiral Wound

This module is prepared from flat sheet membranes wound in the form of a spiral envelope using a feed spacer as indicated in Figure 2.4. The feed will flow on the outside of the envelope at high pressure and permeate is collected on the inside. The collected permeate runs out of the end of the module. The advantages of the spiral wound configuration include high membrane packing density and relatively low cost. A major limitation is that these devices cannot be operated at high transmembrane pressure (Ghosh, 2003).

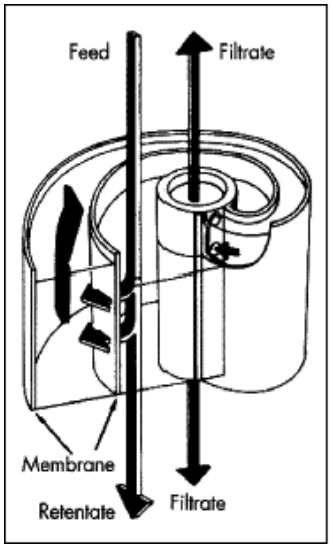


Figure 2.4: Internal arrangement of a spiral wound membrane module

2.2.4 Tubular

Tubular membrane element is shows in Figure 2.5 which is generally had a diameter size more than 3 mm. Normally, a tubular membrane module is made up of several tubes arranged as in a shell and tube exchanger. The feed stream enters the lumen of the tubes and the retentate exits at the other hand of the tubes. The permeate passes through the wall (membrane) and is collected on the shell side. The advantages of a tubular membrane module include turbulent flow (leading to low solute/particulate matter build-up), relatively easy cleaning, easy to handle the feed containing suspended particulate matter and viscous fluid, and the ability to replace or plug a failed membrane element (Ghosh, 2003).

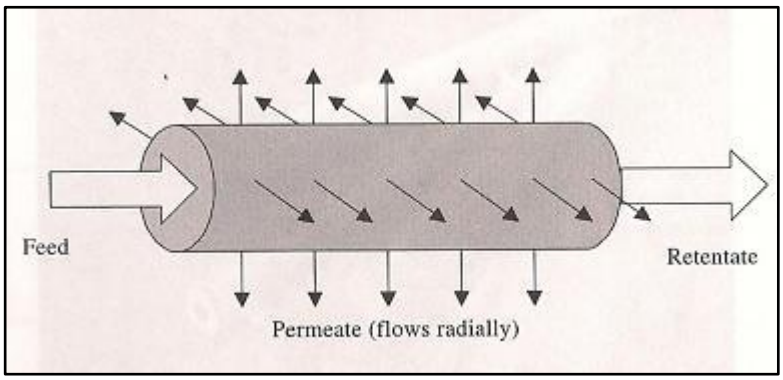


Figure 2.5: Tubular membrane

2.3 High Performance Tangential Flow Filtration

High performance tangential flow filtration (HPTFF) is an emerging technology that uses semi permeable membranes for the separation of proteins without limit to their relative size. This is in sharp contrast to conventional ultrafiltration processes that are generally thought to require a 10-fold difference in size for effective separation. HPTFF is a two-dimensional purification method that exploits differences in both size and charge characteristics of biomolecules. Molecules that differ less than threefold in size can be separated by using highly selective charged membranes and careful optimization of buffer and fluid dynamics.

Current protein separation processes often use ion-exchange chromatography, ultrafiltration (UF) and size exclusion chromatography (SEC) for concentration, purification and buffer exchange. HPTFF makes it possible to perform all of these steps in a single unit operation, thereby reducing production costs. HPTFF can provide high-resolution purification while maintaining the inherent high throughput and high yield characteristics of conventional UF. HPTFF can therefore be used in initial, intermediate and final purification stages.

HPTFF has been used to separate monomers from oligomers based on their difference in size, protein variants differing at only a single amino acid residue, and an antigen binding fragment from a similar size impurity. HPTFF also can potentially be used throughout the purification process to remove specific impurities (e.g., proteins, DNA, or endotoxins) and/or eliminate protein oligomers or degradation products. In addition, HPTFF can effect simultaneous purification, concentration, and buffer exchange, providing an opportunity to combine several different separation steps into a single scalable unit operation (Christy et al., 2002 : van Reis and Zydney, 2007).

HPTFF obtained high selectivity by control of filtrate flux and device fluid mechanics in order to minimize fouling and exploit the effects of concentration polarization. Increasing the concentration of a solute at the membrane wall increases the effective sieving of the solute in the absence of fouling. At higher wall concentrations fouling will occur resulting in a reduction in the effective pore size.

This will result in decreased sieving of the solute, despite the higher wall concentration. Therefore, an optimum flux for separation of solutes using ultrafiltration membranes has been studied. This involves operating the membrane device in the pressure dependent, rather than the pressure-independent, flux regime. In addition, concurrent flow on the filtrate side of the membrane can be used to maintain the optimal flux, and thus the maximum selectivity, throughout the module.

Significant improvements in performance could be obtained by controlling buffer pH and ionic strength to maximize differences in the effective hydrodynamic volume of the different proteins in HPTFF operation (van Reis et al., 1999). For example, Saksena and Zydney (1994) showed that the selectivity (defined as the ratio of the protein sieving coefficients) for the filtration of bovine serum albumin (BSA) and immunoglobulin G (IgG) could be increased from a value of only two, at pH 7 and high salt concentrations, to more than 30 simply by adjusting the pH to 4.7 and lowering the solution ionic strength. The dramatic improvement in performance was due to the strong electrostatic exclusion of the positively charged IgG at pH 4.7, with the transmission of the (uncharged) BSA remaining fairly high. Similar improvements in performance by controlling pH and salt concentration have been reported for laboratory-scale filtration of BSA and hemoglobin (Zydney et al., 1995), BSA and lysozyme (Iritani et al.,1995), and myoglobin and cytochrome C (Yang and Tong,1997).

2.4 Whey Protein

Normal milk contains 30-35 g/L proteins, approximately 78% of it is a caseins protein with the remainder being the whey proteins. The major milk component is showed in Figure 2.6. The caseins are used primarily in the manufacture of cheese, although they can also be added to baked goods, sausages, etc. Whey itself is not a balance source of nutrients because of the high concentration of water (93.5%) and lactose (4.5%). Whey protein concentrates and isolates are used as food additives in the production of a variety of bakes goods, dairy products, meats and beverages. However, the lack of consistency in the gross composition and functionality of these products has limited their acceptance by the food processing

industry. In addition of their nutritional value, whey proteins have good water-holding capacity, are effective emulsifying and foaming agents, and can improve rheological properties.

The unique nutritional, therapeutic and functional characteristics of the individual whey protein are largely unrealized in these whey products due to interactions between component and degradation during processing. This has thus been considerable commercial interest in the production of individual (purified) whey proteins with well-characterized functional and biological properties.

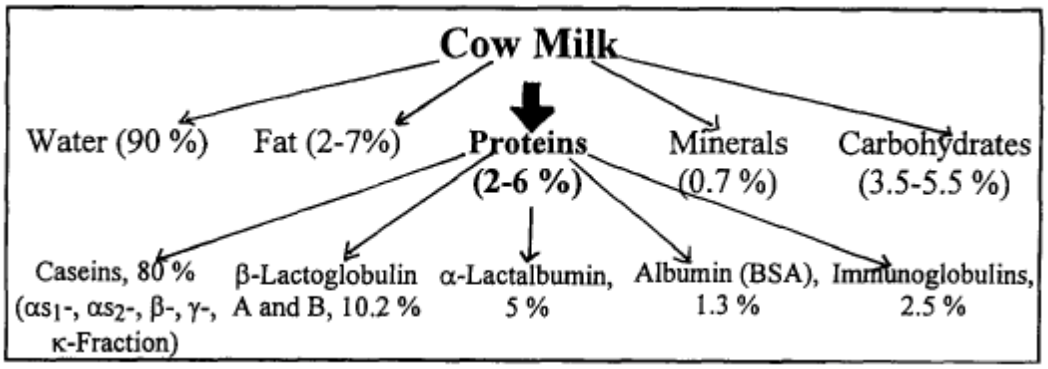


Figure 2.6: Major component in milk (Splittet al., 1996).

However, the fractionation of this complex mixture of protein is very difficult, as the proteins tend to foul the membranes and the protein sizes are close to each other. But, there were several studies done in order to separate this whey protein. Lucas et al. (1998) for example extracted α-lac from whey protein concentrate with modified inorganic membranes. Muller et al. (1999) used a combination of UF and diafiltration for the separation of α-lac from acid casein whey. Xu et al. (2000) used an anion exchanger and UF for the separation of IgG and glycomacropeptide from dairy whey. Cheang and Zydney (2004) combined two different pore size UF membranes in the purification of α-lac and β-lg from whey protein isolate.

Table 2.1: Physical Characteristics of Major Whey Protein (Andersson and Mattiasson, 2006)

Protein	Concentration [g/L]	Molecular weight [kDa]	Isoelectric Point
β-Lactoglobulin (β-lg)	2.7	18.362	5.2
α-Lactalbumin (α-lac)	1.2	14.147	4.5–4.8
Immunoglobulin (Ig)	0.65	150 –1,000	5.5–8.3
BSA	0.4	69	4.7–4.9
Lactoferrin	0.1	78	9.0
Lactoperoxidase	0.002	89	9.5
Glycomacropeptide	1–1.2	7	<3.8

Whey protein contains a wide array of proteins as summarized in the Table 2.1. Each individual whey protein components have their own unique nutritional, functional and biological characteristics. β-lg is commonly used to stabilize food emulsions because of its surface-active properties. Besides that, β-lg also is a better foam stabilizer than the other whey protein components, and can be used in the production of confectionary.

α-lac has been claimed as a neutraceutical and a food additive in infant formula owing to its high content in tryptophan and as a protective against ethanol and stress-induced gastric mucosal injury. It also provides enhanced whippability in meringue-like formulations. In addition, α-lac has strong affinity for glycosylated receptors on the surface of oocytes and spermatozoids and may thus have potential as a contraceptive agent.

Bovine immunoglobulins can enhance the immunological properties of infant formula and they can be used therapeutically in the treatment of animal neonates and, in the form of special supplements, they can offer, in many situations, an important reduction of risk to acquire diarrhoea causing infections and other illnesses. BSA has gelation properties and it is of interest in a number of food and therapeutic applications, for instance, because of its antioxidant properties.

CHAPTER 3

METHODOLOGY

3.1 Whey Protein Preparation

Whey protein solution was prepared according to the method by Han et al. (1996) by using fresh milk as raw material. Firstly, milk was centrifuged at 4420 rpm at room temperature for 30 min for delipidation. Then, the pH of the skimmed milk was adjusted to 4.7 by the slow addition of 1M HCl. After casein precipitation, the solution was stirred for a further 30 min to complete precipitation. Casein was removed by centrifugation at 10 000 rpm and 25^{o}C for 30 min. The obtained whey was diluted with distilled water until a conductivity of 2.7 mS/cm was obtained. The pH of whey then was the adjusted to pH 4.3. The ionic strength of the whey solution was adjusted by adding certain amount of NaCl according to the targeted molarity from 0M to the 0.5M. Finally, the prepared whey solution was filtered through vacuum filter with the pore of filter was 0.45 µm. The simplified steps are shown in Figure 3.1.

3.2 Buffer Preparation

Throughout the experiment, acetate buffer at pH 4.3 was used. This buffer was prepared by adding 410 mL 0.2M glacial acetic acid and 90 mL 0.2M sodium acetate. Then, ultrapure water was top up into this mixture to make up to 1L buffer solution. The ionic strength of acetate buffer was adjusted by adding appropriate amount of NaCl according to the targeted molarities. Finally, the buffer was vacuum filtered through 0.45µm membrane filter.

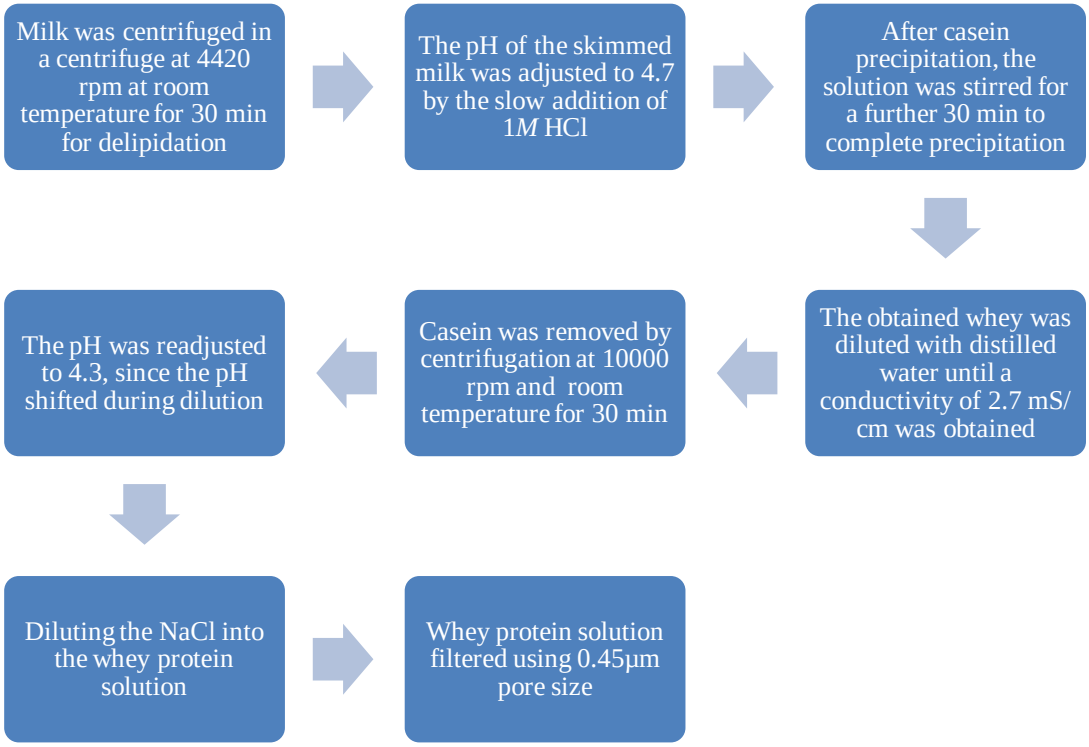


Figure 3.1: Preparation of whey from fresh milk (Hahn et al., 1996).

3.3 High Performance Tangential Flow Filtration

Cross-flow filtration experiments were performed using Kvick Lab Cross Flow System (Dimensions, approximate (Width = 38cm, length = 61cm, height = 53cm) as showed in Figure 3.2. The membrane used in HPTFF experiment was purchased from GE Healthcare which made from polyethersulfone with 30 kDa MWCO and 0.11 m^2 membrane area. The steps involved in HPTFF experiment was summarized in Figure 3.3.

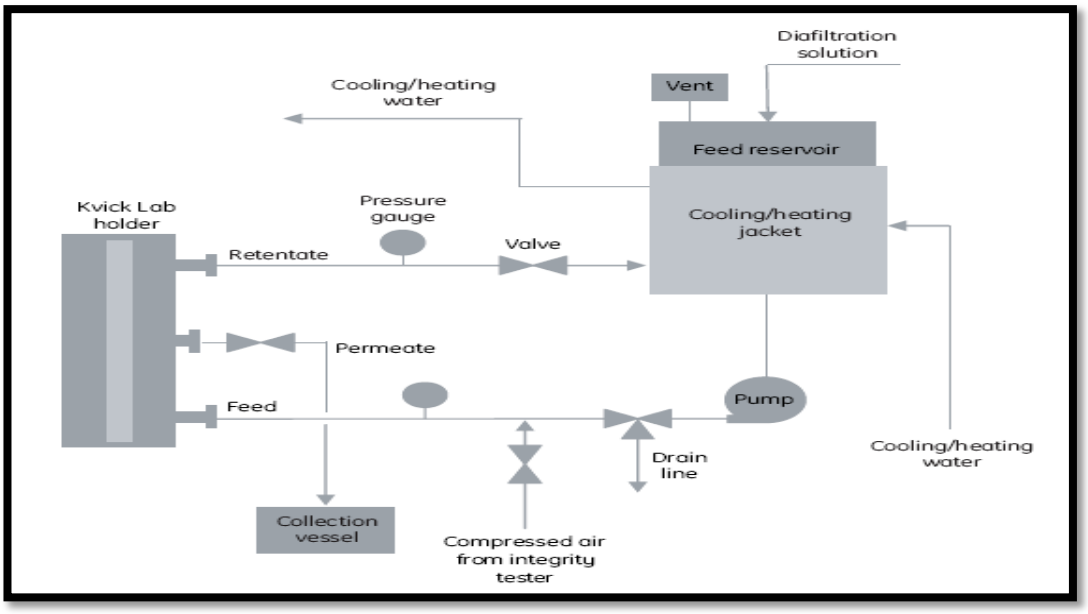


Figure 3.2: Kvick lab cross-flow system diagram.

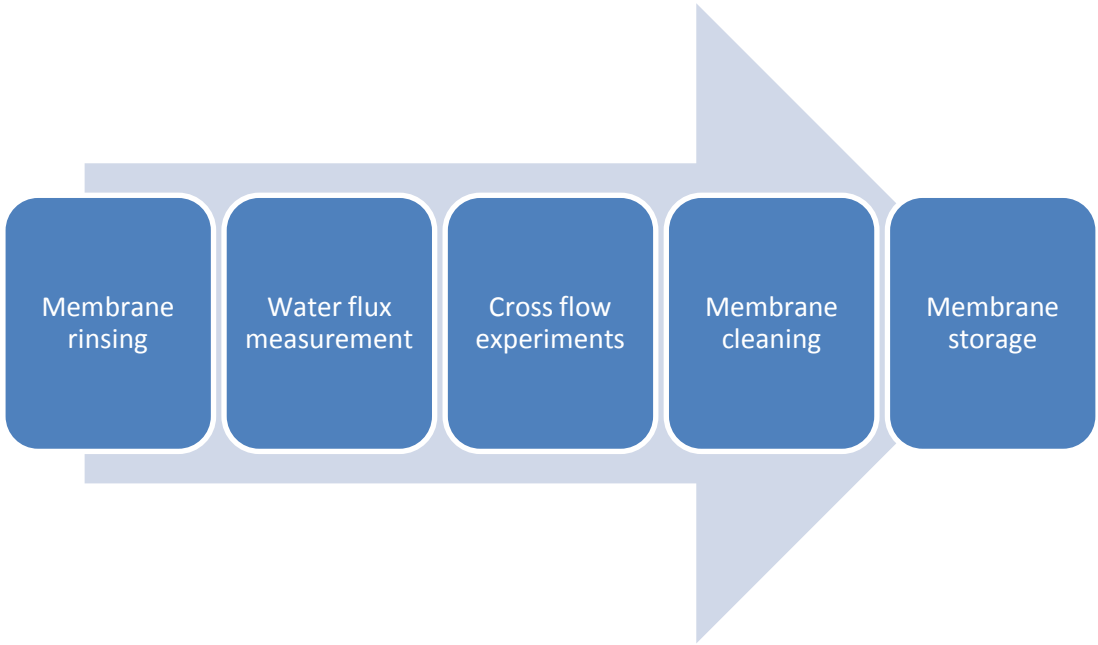


Figure 3.3: Simplified steps for Kvick Lab cross-flow filtation

Upon receiving a new membrane, it is essential to rinse the storage solution (i.e. 0.2 N NaOH and 21% glycerin) from the cassette. Rinsing was done using ultrapure water at TMP 0.3 bar until the permeate fraction reach pH 7.

3.4 Water Flux Measurement

The water flux (WF) of original or initially used membrane was measured in order to calculate the effectiveness of membrane cleaning process and as a guideline on the lifetime of the membrane cassette. Average water flux was taken at TMP 0.3 bar and 1 bar. Calculated water flux was normalized to 20°C and 1 psig. This flux was multiplied with an appropriate viscosity correction factor as shown in Appendix A1.

3.5 Cross-Flow Experiments

One liter of buffer solution was added in the feed tank. This buffer was circulated for about 20 minutes through the membrane by return both retentate and permeate line to the feed tank to equilibrate the system at the desired operating pH and ionic strength. The permeate line was taken out from the feed tank and 700 mL of whey was added into the tank and the retentate line was returned to the feed reservoir with permeate. The pump speed was set at 200 rpm with feed pressure of 7psig and retentate pressure of 3psig. In HPTFF operation, retentate will be recirculated to the feed tank for 3 times. Permeate was collected in the cumulated permeate tank and the volume of permeate was measured. Samples of retentate and permeate were taken at the end of the experiment for quantification of individual proteins.

3.6 Membrane Cleaning

Membrane cleaning is important to remove all the leftover products that have a potential to contaminate the membrane material and the subsequent product applied onto the membrane. Besides that, cleaning will remove fouling material so that it can maintain or recover the membrane filtration efficiency.

The cleaning solution used during this study is 0.5 M NaOH. The retentate side was circulated and flushed two times with water after cross flow experiment to discard the remaining solution in the system. Next, the system was drained and 2L of cleaning solution was added into the feed tank. Firstly, the feed and retentate valve was opened and about 10% of the cleaning solution was pumped through the retentate line to waste. Then, same step was followed but this time another 10% of the cleaning solution was pumped through the permeate line to waste. Then, all the feed, retentate and permeate line was circulated to the feed tank and the cleaning process was run for about 1 hour at TMP 0.3 bar. After cleaning the whole system was flushed with water until the permeate line reach a water pH. The effectiveness of a cleaning protocol was examined by measuring the water flux recovery (%) compared to its original flux. Water flux recovery of 90% was selected as the satisfaction of the cleaning process.

3.7 Membrane Storage

Cleaned casettes should be stored properly to prevent membranes from drying out or developing microbial growth. Typical storage solutions and conditions are listed in Table 3.1. Membrane cassettes was stored in air-tight containers and a small volume of storage solution was added into the containers.

Table 3.1: Recommended membrane storage conditions

Duration	**Temperature**	**Chemical**
Up to 3 days	Ambient (20-25°C)	Water or 1-3% NaOCl
Up to 1 month	Ambient (20-25°C)	0.1 N NaOH
Up to 12 months	Refrigerated (4°C)	0.05 N NaOH

3.8 Protein Assays

RPC was used to analyze the protein composition from the fractions collected during cross-flow run. The RPC procedure was referred to Elgar et al. (2000) using 1-mL Resource RPC column (Pharmacia Biotech) and was operated at room temperature and at a flow-rate of 2 mL/min. The column was equilibrated in 80% solvent A (0.1%, v/v, TFA in ultra pure water) and after sample injection a 1-min isocratic period was applied followed by a series of linear gradients to 100% solvent B (0.09%, v/v, TFA, 90%, v/v, MeCN in ultra pure water) as follows: 1-6 min, 20-40% B; 6-16 min, 40-45% B; 16-19 min, 45-50% B; 19-20min, 50% B; 20-23 min, 50-70% B; 23-24 min, 70-100% B. the column was re-equilibrated after a 1-min hold at 100% B by a 2-min linear gradient to 20% B followed by an isocratic period of 3 min. Detection was by absorbance at 214 nm and total run time was 30 min.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Chromatogram Results

The protein content in feed, retentate and permeate was analyzed using RPC protocol. The typical RPC chromatogram for whey protein component is shown Figure 4.1. The concentrations of single whey protein can be calculated using the standard curve explained detail in the next section.

4.2 Standard Curve Graph

Figure 4.2 show the standard curve for the calculation the concentration of β-lg. The concentration was unknown sample was calculated from the peak area of particular protein in RPC chromatogram.

β-lg concentration could be determined using the Equation 4.1:

$$C = 0.0010600 \times \text{Peak Area} \times \text{Sample Dilution Factor} \quad \text{(Eq. 4.1)}$$

Where C is the concentration of β-lg in mg/ml, A is the peak area in mAu*ml. Dilution factor is the amount of sample diluted during the RPC measurement.

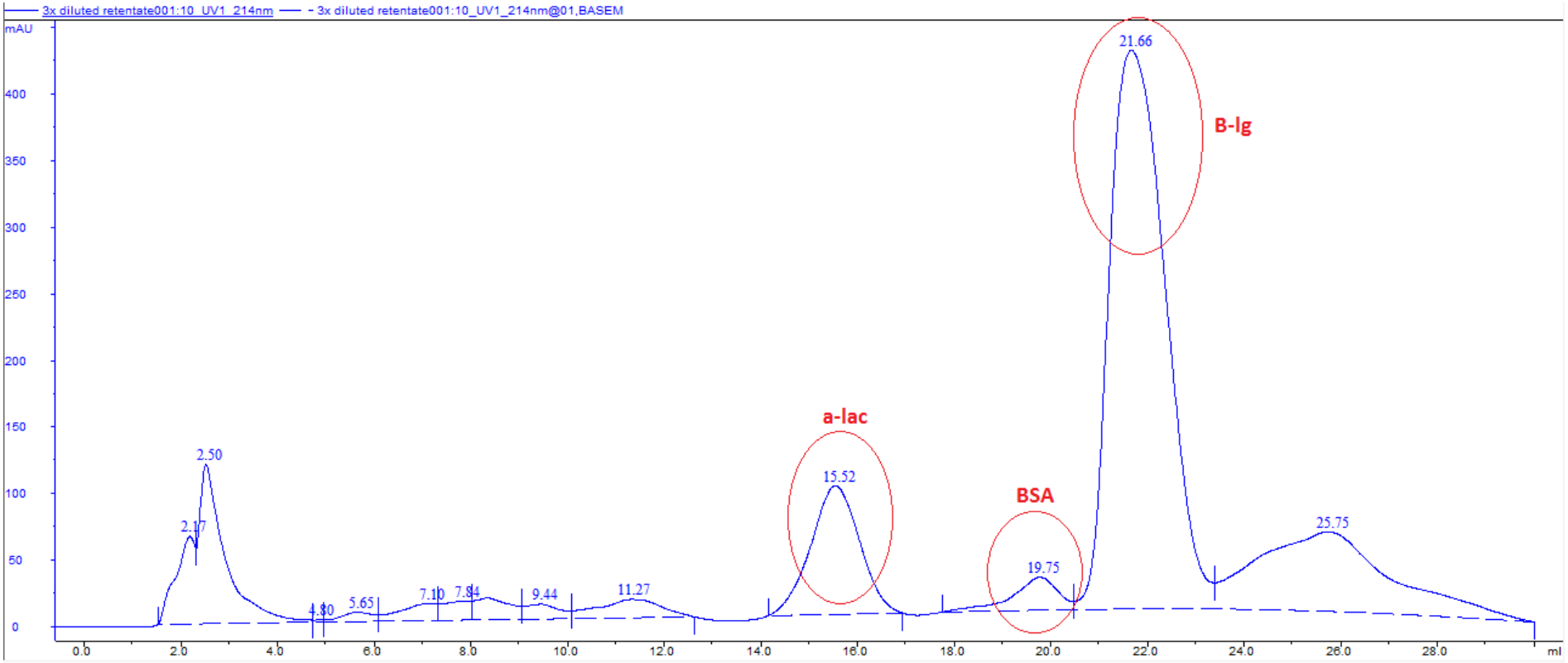


Figure 4.1: Chromatogram Result

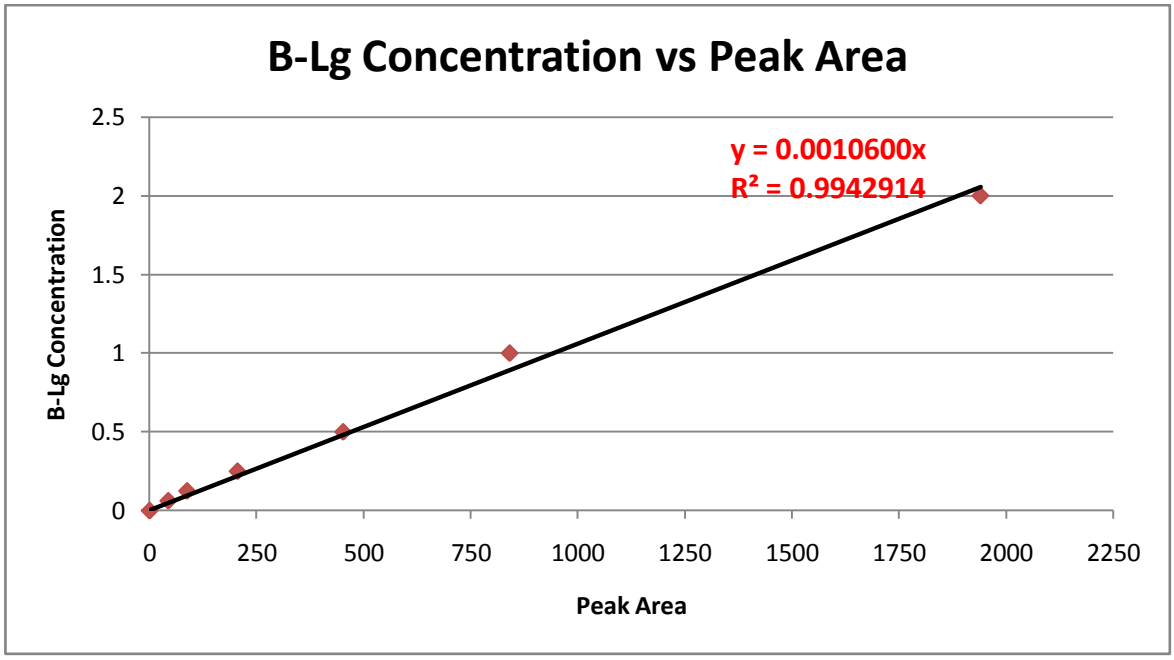


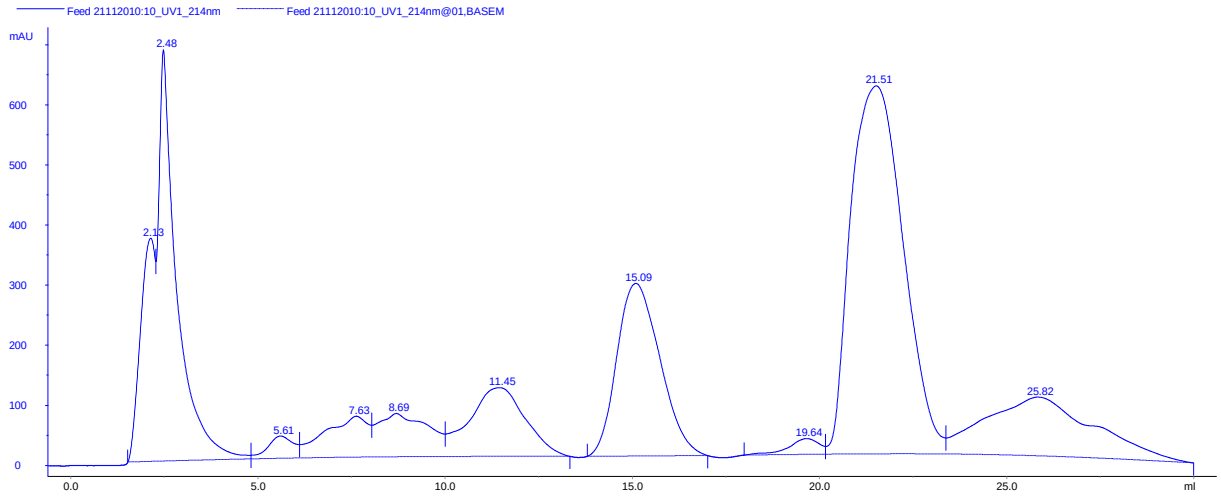
Figure 4.2: Standard curve graph for β-lg

Standard curve for α-lac could not be developed due to the unavailable standard protein of α-lac. However, the percentage of the α-lac retained and permeated still could be determined by comparing the peak areas of α-lac available in the feed, retentate and also in permeate.

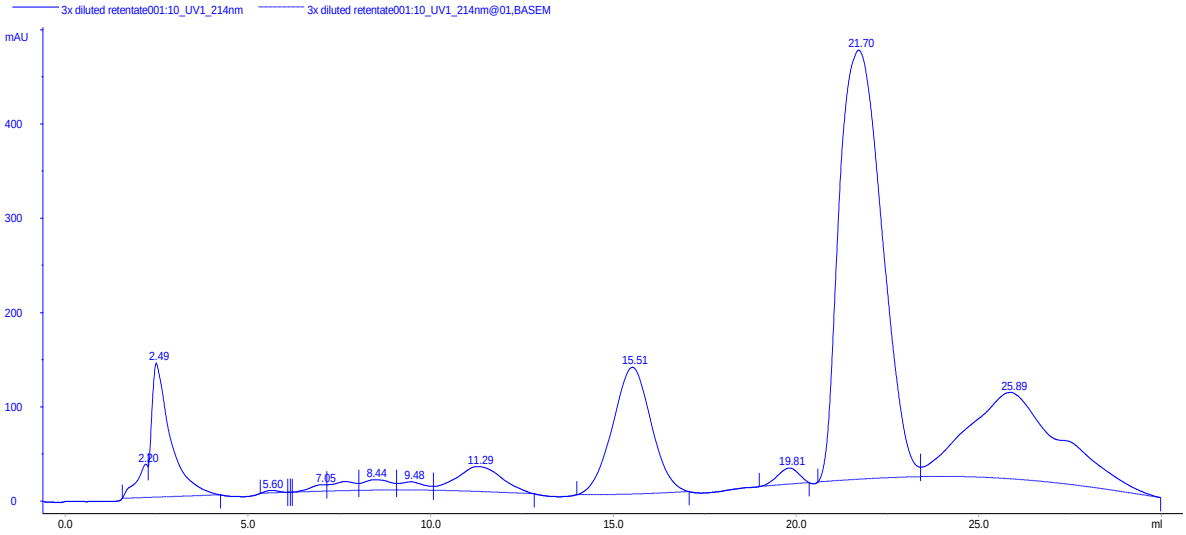
4.3 Effect of Ionic Strength

Four HPTFF experiments at different ionic strengths of 0M, 0.125M, 0.25M and 0.5M were conducted during this study. The RPC chromatograms of feed, retentate and permeate for each ionic strength was showed in Figure 4.3 to Figure 4.6.

(a)



(b)



(c)

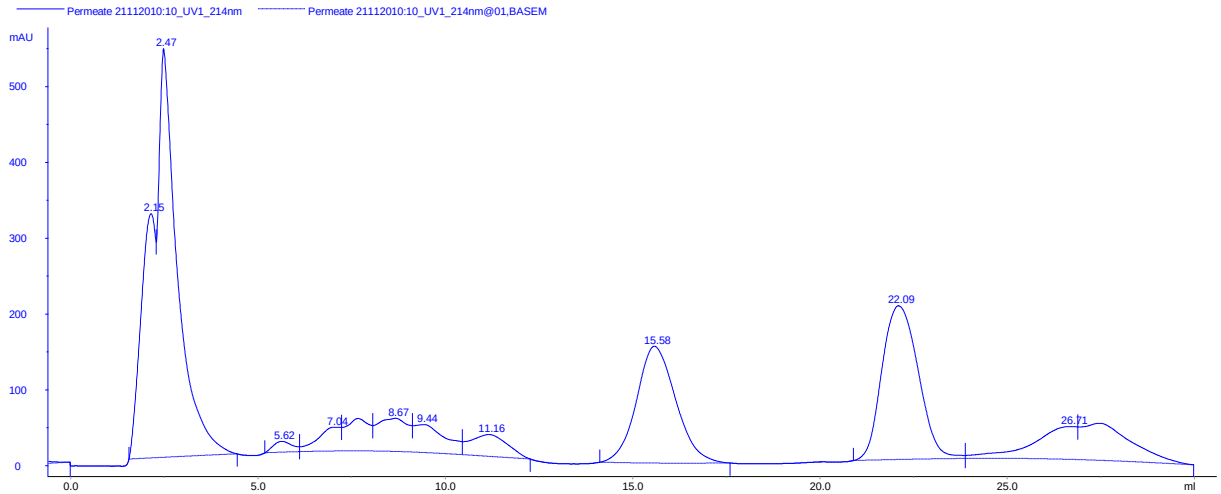
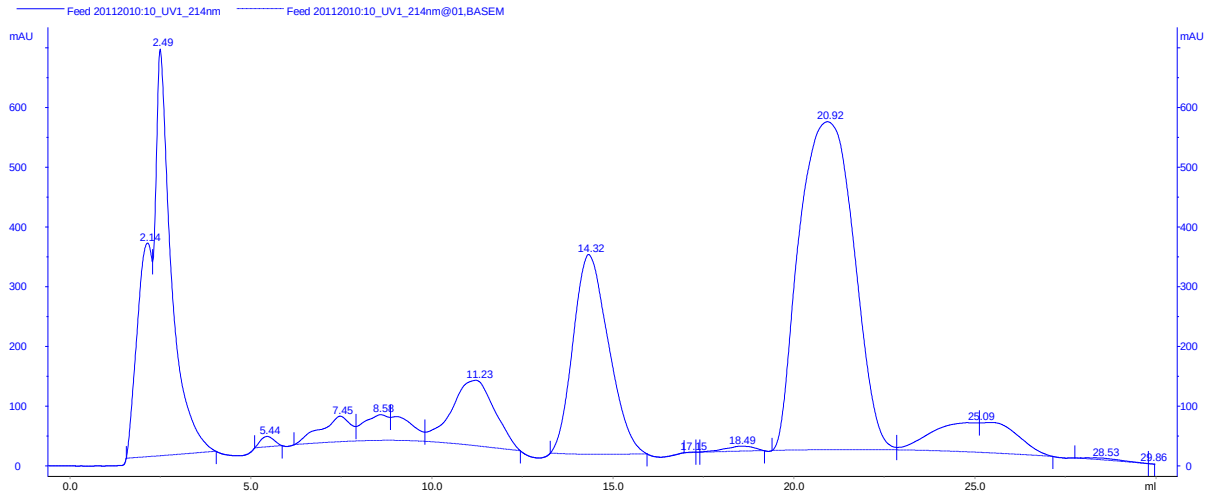
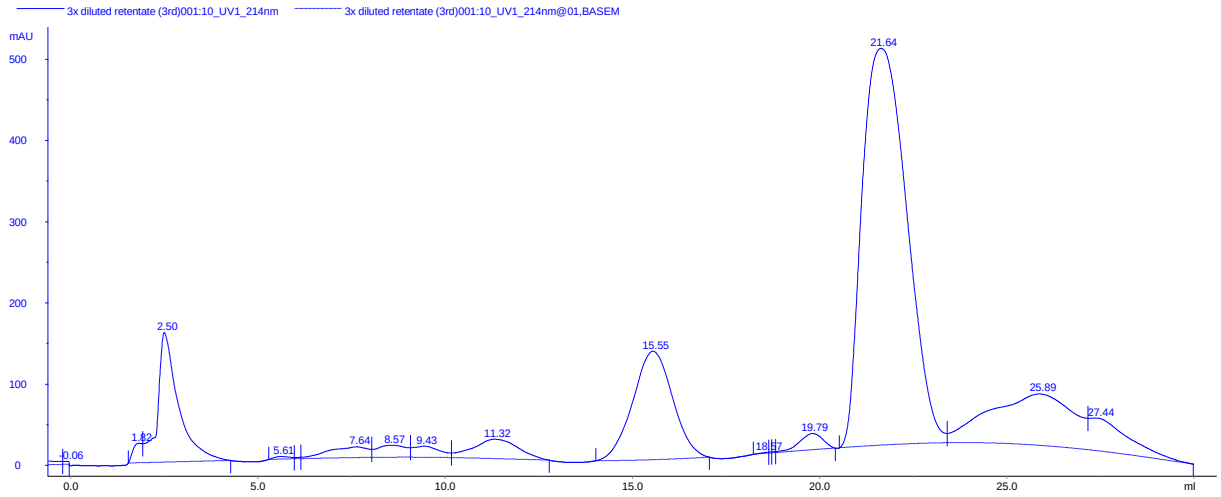


Figure 4.3: Chromatogram results for 0M NaCl: (a) feed, (b) retentate and (c) permeate

(a)



(b)



(c)

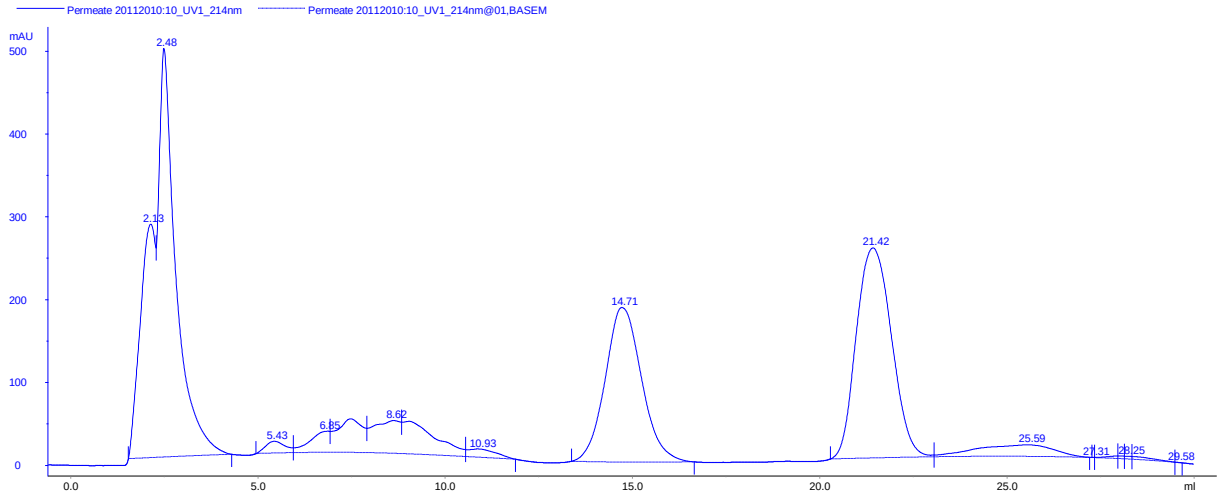
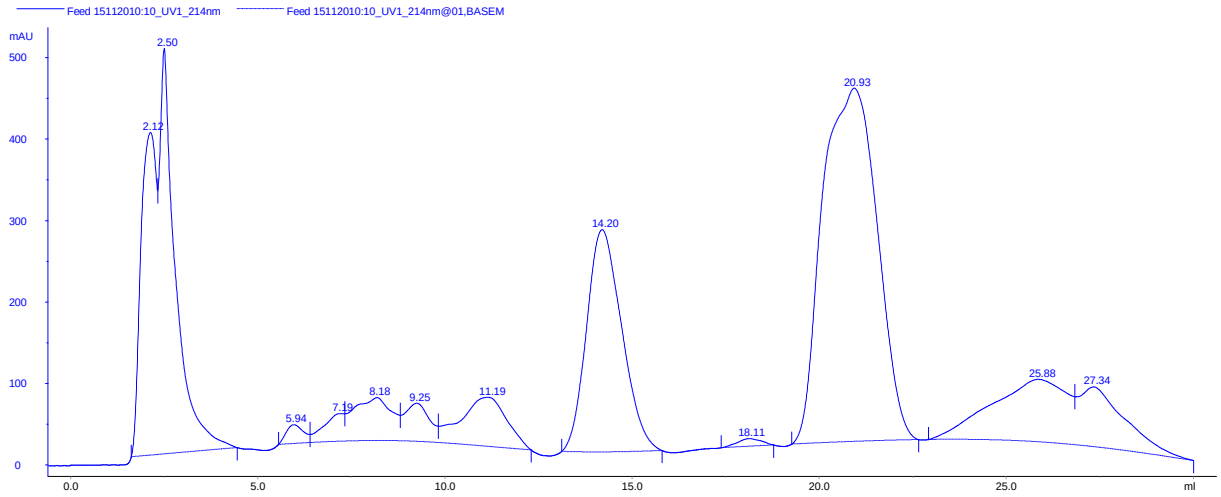
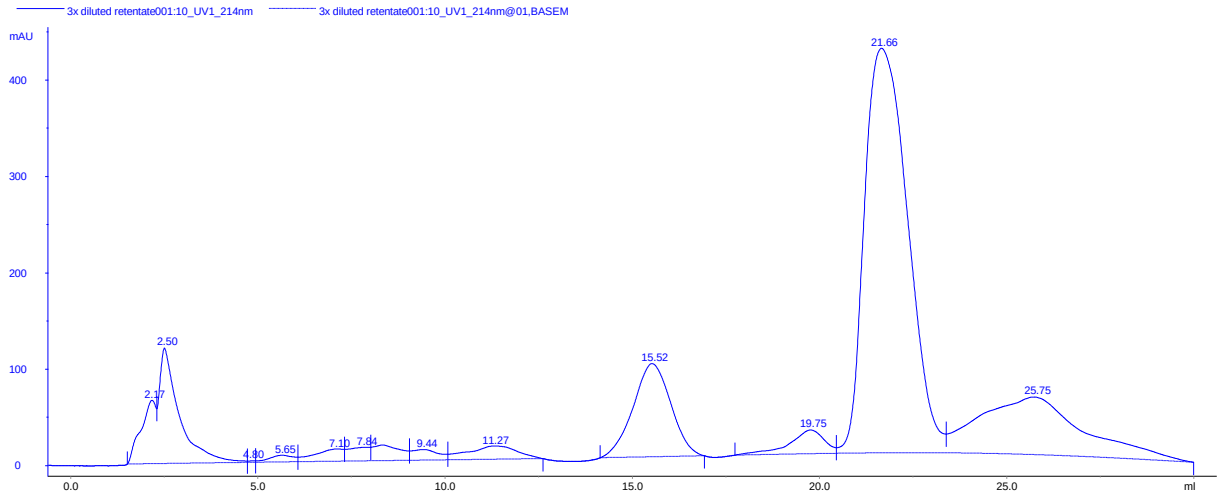


Figure 4.4: Chromatogram results for 0.125M NaCl: (a) feed, (b) 3x diluted retentate and (c) permeate

(a)



(b)



(c)

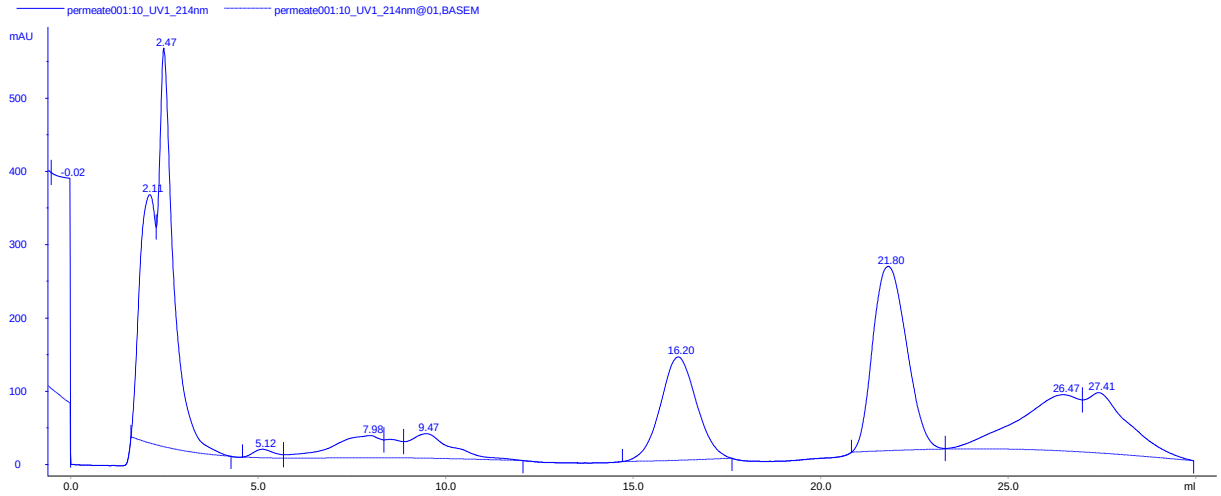
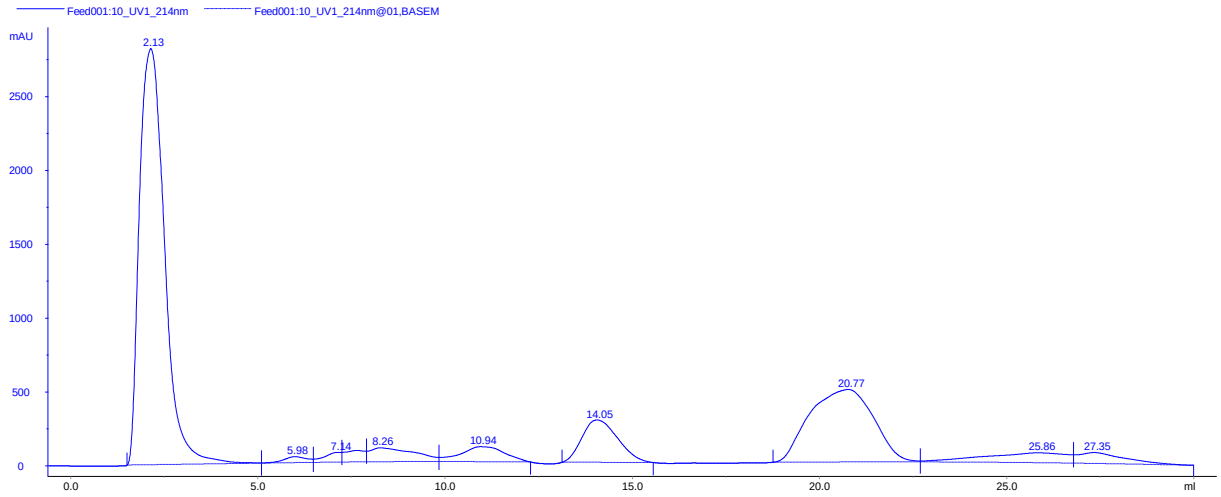
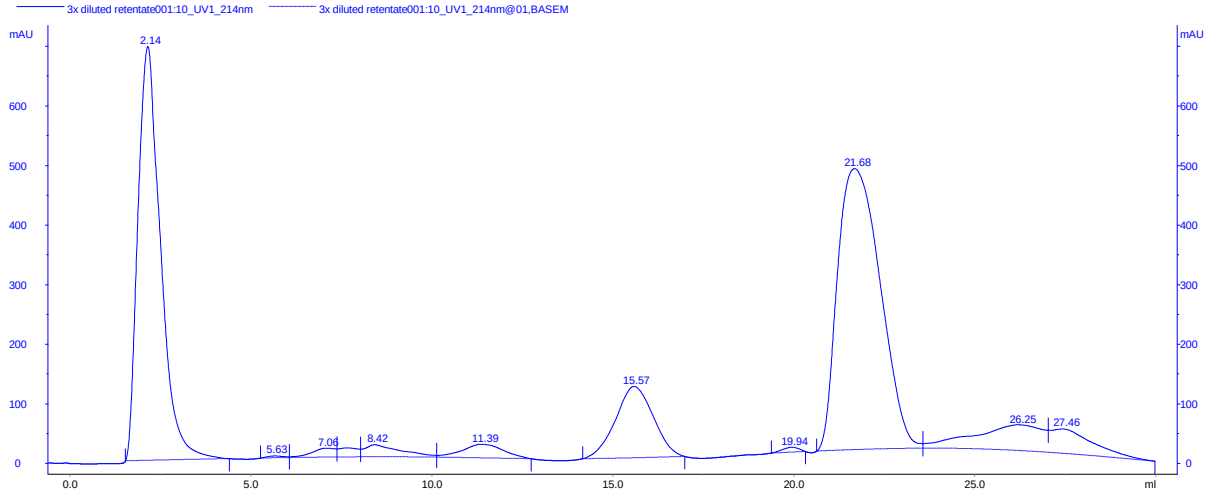


Figure 4.5: Chromatogram results for 0.25M NaCl: (a) feed, (b) retentate and (c) permeate

(a)



(b)



(c)

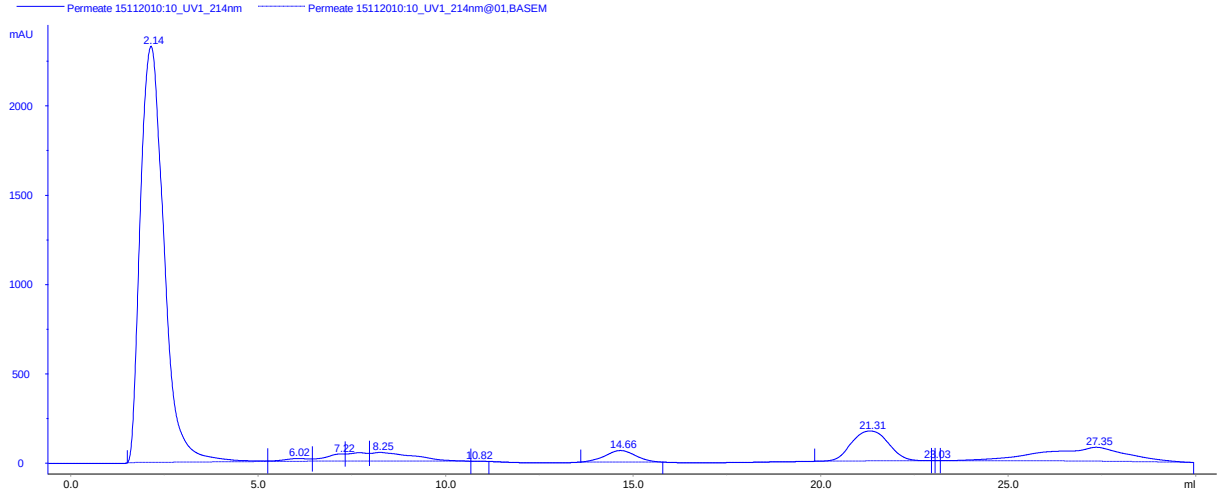


Figure 4.6: Chromatogram results for 0.5M NaCl: (a) feed, (b) retentate and (c) permeate

The percentage of α-lac and β-lg retained and permeated at different ionic strength were summarized in Table 4.1 and Figure 4.7.

Table 4.1: Percentage of α-lac and β-lg retained and permeated at different ionic strength

Salt concentration, M	Components	% Permeated	% Retained	% loss
0	α-lac	46%	26%	28%
	β-lg	19%	44%	36%
0.125	α-lac	59%	30%	11%
	β-lg	24%	52%	23%
0.25	α-lac	55%	25%	20%
	β-lg	30%	52%	18%
0.5	α-lac	21%	32%	47%
	β-lg	17%	28%	56%

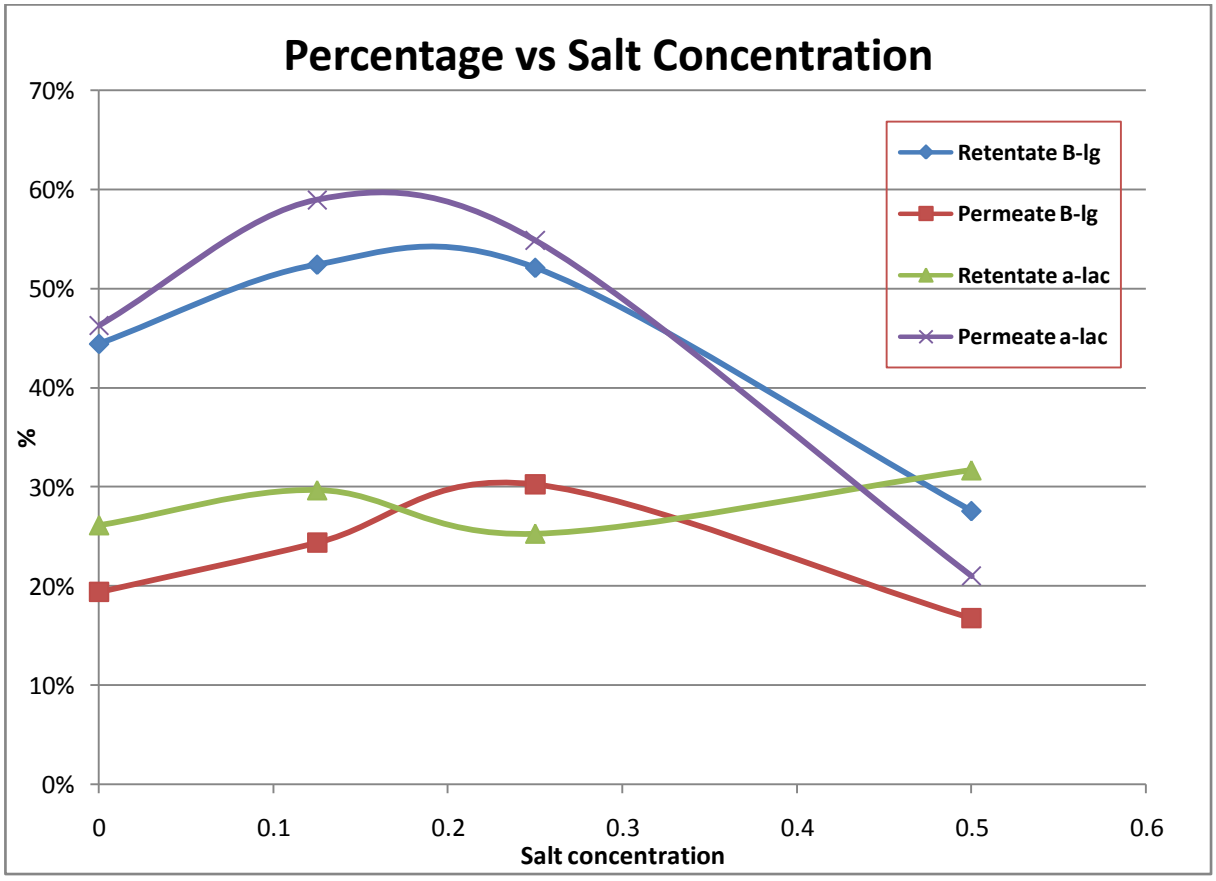


Figure 4.7: Percentage of the components at different salt concentration

Based on the results, the best separation of α-lac and β-lg is observed at 0.125M. At this salt concentration, most of 59% of α-lac was permeated and 52% of β-lg was retained. It is believed that at optimum salt, maximum electrostatic interactions occurred between the proteins. The presence of electrical double layer has increased the size of the charged protein, which will increase the effective hydrodynamic volume of charged protein, thereby reducing the β-lg transmission through the membrane (Zydney, 1998).

As the salt concentration increase to 0.5M, the permeation of α-lac decreased heavily while the retention of β-lg decreased slightly. The selectivity of protein components was low at higher conductivity level demonstrating the importance of electrostatic interaction in providing effective protein separation. Based on this statement, it is understand that high ionic strength tends to reduce electrostatic repulsion between proteins due to the shielding of ionizable groups by mobile ions (Cheang and Zydney, 2004).

Table 4.2. Retentate and permeate table at all salt concentration

Salt concentration	Components	Retentate			Permeate		
		Total Area	**Mass**	**% Retained**	**Total Area**	**Mass**	**% Permeated**
0	α-lac	62913.08	-	26%	111369.31	-	46%
	β-lg	234941.14	294.14	44%	135603.15	294.14	19%
0.125	α-lac	62913.08	-	30%	125001.26	-	59%
	β-lg	234941.14	249.04	52%	164233.43	249.04	24%
0.25	α-lac	43140.1	-	25%	93598.88	-	55%
	β-lg	214143.87	226.99	52%	155880.37	226.99	30%
0.5	α-lac	55370.27	-	32%	36732.85	-	21%
	β-lg	246176.18	260.95	28%	114345.94	260.95	17%

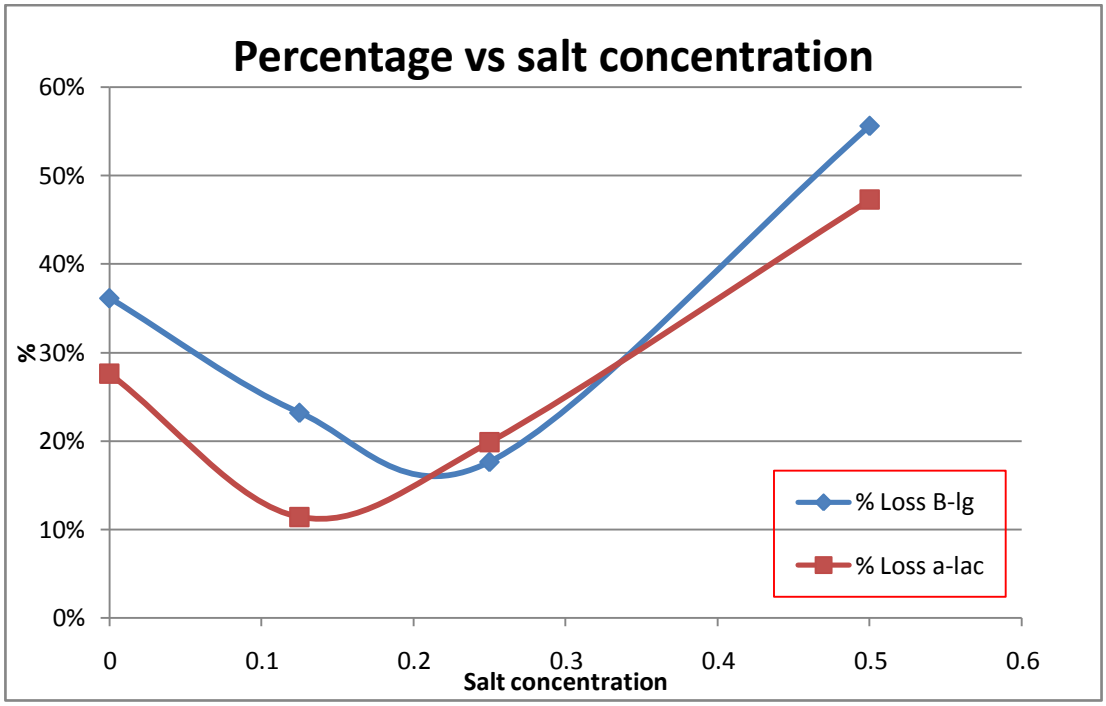


Figure 4.8: Percentage of protein lost at different salt concentration

The lost of the protein also significant at high salt concentration as shown in Figure 4.8. This is because the protein components tend to precipitate at high salt concentration. The protein lost at low salt concentration can be caused by protein adsorption to the membrane and protein denaturation by shear stress caused by circulation of the retentate stream at high velocities as explained by Almejica *et al*, (2007).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The ionic strength plays a role in the selectivity of α-lac and β-lg through the UF membrane. The best separation of α-lac and β-lg was achieved at 0.125M salt concentrations, which 59% of α-lac was permeated and 52% of β-lg was retained.
At this optimum ionic strength, the electrostatic interaction plays an important role which alters the hydrodynamic volume of the individual whey protein component. Individual proteins separated from whey have their own value and can be used for specific application.

5.2 Recommendation

There are a lot of parameters that can be further investigated in HPTFF experiment. Among them are transmembrane pressure, pH and flow velocity. The temperature of the whey protein to be separated also can be study as suggested by Cheang and Zydney (2003).

Besides that, separation of α-lac and β-lg in HPTFF could be enhanced in two-stage operation using 30 and 100 kDa membranes in series. This two-stage operationcould result in purification of α-lac and β-lg will be greater than 10-fold at 90% yield. (Cheang and Zydney, 2003)

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APPENDICES

APPENDIX A

WATER FLUX MEASUREMENTS

A1: Viscosity Correction Factor

Temperature in °C when permeate flow was measured	**Viscosity correction factor**	**Temperature in °C when permeate flow was measured**	**Viscosity correction factor**
4	1.567	25	0.89
5	1.519	26	0.871
6	1.472	27	0.851
7	1.428	28	0.833
8	1.386	29	0.815
9	1.346	30	0.798
10	1.307	31	0.781
11	1.271	32	0.765
12	1.235	33	0.749
13	1.202	34	0.734
14	1.169	35	0.719
15	1.139	36	0.705
16	1.109	37	0.692
17	1.081	38	0.678
18	1.053	39	0.665
19	1.027	40	0.653
20	1.000	41	0.641
21	0.978	42	0.629
22	0.955	43	0.618
23	0.933	44	0.607
24	0.911	45	0.597

A2: Water Flux Recovery for Each Run

TMP	Before Run			0M			0.125M		
	Original WF	**After**	**WF Recovery**	**Original WF**	**After**	**WF Recovery**	**Original WF**	**After**	**WF Recovery**
5	86.54	78.72	91%	86.54	78.719	91%	86.54	78.392	90%
15	252.73	228.46	90%	252.73	78.392	91%	252.73	246.93	97%
TMP	**0.25M**			**0.5M**					
	Original WF	**After**	**WF Recovery**	**Original WF**	**After**	**WF Recovery**			
5	86.54	83.285	96%	86.54	84.221	97%			
15	252.73	235.33	93%	252.73	227.3	90%			

APPENDIX B

RPC CHROMATOGRAPHY RESULTS

B1: Salt Concentration: 0M

a) Feed

No	Retention (ml)	Area (mAU*ml)	Height (mAU)
1	-1.97	2.0789	5.051
2	2.13	166.0483	371.529
3	2.48	386.1133	684.741
4	5.61	29.6972	37.366
5	7.63	90.9495	68.375
6	8.69	112.2736	72.160
7	11.45	198.3543	114.522
8	15.09	384.0648	287.311
9	19.64	25.4003	26.453
10	21.51	997.2175	612.531
11	25.82	327.8371	97.891
12			
13	Total number of detected peaks		11
14	Total area (mAU*ml)		2720.0360
15	Area in evaluated peaks (mAU*ml)		2720.0346
16	Ratio peak area / total area		0.999999
17	Total peak width (ml)		27.92
18	Column height (cm)		3.00
19	Column Vt (ml)		0.77
20	Calculated from		Feed 21112010:1(
21	Baseline		Feed 21112010:1(
22	Peak rejection on		
23	Maximum number of peaks ()		20
24			

Peaks table for feed of 0M NaCl

b) Retentate

A: 3x diluted retentate001:10_UV1_214nm@01,PEAK

No	Retention (ml)	Area (mAU*ml)	Height (mAU)
1	-1.98	2.5486	5.440
2	-0.94	0.0316	0.264
3	2.20	12.9063	35.596
4	2.49	84.1176	142.576
5	5.60	1.1771	2.762
6	6.13	0.0125	0.191
7	6.18	0.0105	0.190
8	7.05	3.6463	7.132
9	7.64	7.3123	9.926
10	8.44	9.7626	11.236
11	9.48	6.9623	8.901
12	11.29	38.0971	26.574
13	15.51	165.1262	134.585
14	19.81	11.5638	17.153
15	21.70	616.6434	455.137
16	25.89	269.8301	91.744
17			
18	Total number of detected peaks		16
19	Total area (mAU*ml)		1229.7562
20	Area in evaluated peaks (mAU*ml)		1229.7483
21	Ratio peak area / total area		0.999994
22	Total peak width (ml)		25.13
23	Column height (cm)		3.00
24	Column Vt (ml)		0.77
25	Calculated from		3x diluted rete
26	Baseline		3x diluted rete
27	Peak rejection on		
28	Maximum number of peaks ()		20

Peaks table for retentate of 0M NaCl

c) Permeate

A: Permeate 21112010:10_UV1_214nm@01,PEAK

No	Retention (ml)	Area (mAU*ml)	Height (mAU)
1	-1.97	2.3141	9.770
2	-1.62	1.3216	8.535
3	-1.51	2.9349	8.760
4	-0.87	3.7120	7.858
5	2.15	143.3037	322.043
6	2.47	315.6243	538.870
7	5.62	8.6394	14.435
8	7.04	22.3217	31.558
9	7.66	30.9971	42.857
10	8.67	41.7450	43.916
11	9.44	36.3705	37.063
12	11.16	33.1625	28.928
13	15.58	187.8066	154.287
14	22.09	228.6731	202.912
15	26.71	59.2393	43.382
16	27.47	76.3024	49.064
17			
18	Total number of detected peaks		16
19	Total area (mAU*ml)		1194.4740
20	Area in evaluated peaks (mAU*ml)		1194.4681
21	Ratio peak area / total area		0.999995
22	Total peak width (ml)		24.50
23	Column height (cm)		3.00
24	Column Vt (ml)		0.77
25	Calculated from		Permeate 211120
26	Baseline		Permeate 211120
27	Peak rejection on		
28	Maximum number of peaks ()		20

Peaks table for permeate of 0M NaCl

B2: Salt Concentration: 0.125M

a) Feed

A: 3x diluted retentate (3rd)001:10_UV1_214nm@01,PEAK

No	Retention (ml)	Area (mAU*ml)	Height (mAU)
1	-1.98	5.1735	11.839
2	-1.35	2.4485	8.093
3	-0.86	4.9095	8.108
4	-0.06	0.6469	3.868
5	1.82	6.8930	24.176
6	2.50	102.3477	159.809
7	5.61	1.6269	3.448
8	6.12	0.2943	1.854
9	7.64	16.8713	13.403
10	8.57	13.4760	14.827
11	9.43	11.7012	13.898
12	11.32	33.8386	24.058
13	15.55	165.6617	133.860
14	18.57	0.3051	1.273
15	18.69	0.1019	1.274
16	18.77	0.1395	1.345
17	19.79	14.6378	20.199
18	21.64	676.2252	488.661
19	25.89	163.6422	63.606
20	27.44	49.5744	40.389
21			
22	Total number of detected peaks		20
23	Total area (mAU*ml)		1270.5217
24	Area in evaluated peaks (mAU*ml)		1270.5155
25	Ratio peak area / total area		0.999995
26	Total peak width (ml)		26.86
27	Column height (cm)		3.00
28	Column Vt (ml)		0.77

29	Calculated from		3x diluted rete
30	Baseline		3x diluted rete
31	Peak rejection on		
32	Maximum number of peaks ()		20

Peaks table for feed of 0.125M NaCl

b) Retentate

A: Feed 20112010:10_UV1_214nm@01.PEAK

No	Retention (ml)	Area (mAU*ml)	Height (mAU)
1	-1.98	0.1756	3.057
2	-1.83	0.8516	2.199
3	2.14	164.2209	358.097
4	2.49	353.3138	681.978
5	5.44	7.6472	17.660
6	7.45	41.4362	43.021
7	8.58	34.4568	43.187
8	9.04	28.8994	40.000
9	11.23	150.4083	109.794
10	14.32	378.9463	334.773
11	17.15	0.2192	0.918
12	17.36	0.0769	0.758
13	18.49	8.1191	8.526
14	20.92	961.7532	549.149
15	25.09	72.6151	49.278
16	25.49	61.3995	51.253
17	28.53	3.4048	3.194
18	29.86	0.0323	0.275
19			
20	Total number of detected peaks		18
21	Total area (mAU*ml)		2267.9799
22	Area in evaluated peaks (mAU*ml)		2267.9762
23	Ratio peak area / total area		0.999998
24	Total peak width (ml)		25.02
25	Column height (cm)		3.00
26	Column Vt (ml)		0.77
27	Calculated from		Feed 20112010:10
28	Baseline		Feed 20112010:10
29	Peak rejection on		
30	Maximum number of peaks ()		20

Peaks table for 3x diluted retentate 0.125M NaCl

c) Permeate

No	Retention (ml)	Area (mAU*ml)	Height (mAU)
1	-1.97	0.2500	3.904
2	-1.76	0.5618	2.835
3	-1.67	0.8258	2.818
4	2.13	129.2977	281.960
5	2.48	265.9374	493.655
6	5.43	8.8003	14.338
7	6.85	15.5762	25.612
8	7.46	32.5110	40.652
9	8.62	33.0742	39.641
10	9.05	40.6011	39.450
11	10.93	8.8415	10.168
12	14.71	210.7947	186.856
13	21.42	276.9535	253.829
14	25.59	34.1371	14.245
15	27.31	0.0395	0.575
16	27.92	1.0780	3.134
17	28.05	0.5847	3.396
18	28.25	0.6743	3.509
19	28.38	2.3347	3.480
20	29.58	0.0411	0.288
21			
22	Total number of detected peaks		22
23	Total area (mAU*ml)		1062.9639
24	Area in evaluated peaks (mAU*ml)		1062.9148
25	Ratio peak area / total area		0.999954
26	Total peak width (ml)		23.14
27	Column height (cm)		3.00
28	Column Vt (ml)		0.77
29	Calculated from		Permeate 201120
30	Baseline		Permeate 201120
31	Peak rejection on		
32	Maximum number of peaks ()		20
33			

Peaks table for permeate of 0.125MNaCl

B3: Salt Concentration: 0.25M

a) Feed

No	Retention (ml)	Area (mAU*ml)	Height (mAU)
1	-4.87	5.2018	7.734
2	-3.17	0.0106	0.101
3	2.12	186.7390	396.062
4	2.50	251.0079	497.544
5	5.94	12.5320	23.123
6	7.19	21.6563	34.026
7	8.18	62.3439	52.688
8	9.25	35.9500	46.876
9	11.19	84.9625	60.308
10	14.20	297.8653	273.014
11	18.11	7.0091	9.545
12	20.93	735.7176	433.756
13	25.88	176.9263	76.687
14	27.34	105.1287	73.480
15			
16	Total number of detected peaks		14
17	Total area (mAU*ml)		1983.0527
18	Area in evaluated peaks (mAU*ml)		1983.0510
19	Ratio peak area / total area		0.999999
20	Total peak width (ml)		25.95
21	Column height (cm)		3.00
22	Column Vt (ml)		0.77
23	Calculated from		Feed 15112010:1(
24	Baseline		Feed 15112010:1(
25	Peak rejection on		
26	Maximum number of peaks ()		20
27			

Peaks table for feed of 0.25M NaCl

b) Retentate

A: 3x diluted retentate001:10_UV1_214nm@01,PEAK

No	Retention (ml)	Area (mAU*ml)	Height (mAU)
1	-1.97	0.1996	3.485
2	-1.78	0.4928	3.173
3	-1.68	0.7524	3.298
4	-1.39	0.6212	1.730
5	-0.81	0.0498	0.372
6	2.17	30.5045	65.922
7	2.50	73.8491	119.940
8	4.80	0.3451	1.588
9	5.65	5.3089	7.010
10	7.10	11.4342	12.814
11	7.84	9.4439	14.279
12	8.32	13.9499	16.354
13	9.44	9.2084	11.150
14	11.27	21.2377	13.809
15	15.52	113.2286	96.870
16	19.75	26.8817	24.739
17	21.66	562.0574	419.885
18	25.75	191.5817	59.977
19			

Peaks table for retentate of 0.25M NaCl

c) Permeate

A: permeate001:10_UV1_214nm@01,PEAK

No	Retention (ml)	Area (mAU*ml)	Height (mAU)
1	-1.25	382.5583	293.604
2	-0.92	202.6436	304.839
3	-0.02	152.7013	307.220
4	2.11	159.4629	339.367
5	2.47	251.8397	544.439
6	5.12	7.7934	11.871
7	7.98	46.3990	30.450
8	8.52	12.8065	25.296
9	9.47	44.3667	33.751
10	16.20	157.8396	141.338
11	21.80	262.8674	251.957
12	26.47	144.6360	77.055
13	27.41	112.0860	82.626
14			

Peak table for permeate of 0.25M NaCl

B4: Salt Concentration: 0.5M

a) Feed

No	Retention (ml)	Area (mAU*ml)	Height (mAU)
1	-4.88	1.4171	6.662
2	-4.45	4.9507	6.542
3	-3.24	0.1545	1.377
4	-3.13	0.1735	1.227
5	-2.94	0.1147	1.173
6	-2.85	0.1677	1.217
7	-2.66	0.3313	1.153
8	-2.30	0.3985	0.957
9	-1.83	0.1254	0.578
10	-1.44	0.0226	0.208
11	2.13	2192.9594	2819.028
12	5.98	29.9458	41.303
13	7.14	35.7747	67.423
14	7.63	47.6867	78.399
15	8.26	133.2164	95.335
16	10.94	141.2597	102.614
17	14.05	318.3337	287.709
18	20.77	973.7615	491.931
19	25.86	173.7290	67.954
20	27.35	109.6129	74.794
21			
22	Total number of detected peaks		22
23	Total area (mAU*ml)		4164.1595
24	Area in evaluated peaks (mAU*ml)		4164.1357
25	Ratio peak area / total area		0.999994
26	Total peak width (ml)		27.93
27	Column height (cm)		3.00
28	Column Vt (ml)		0.77
29	Calculated from		Feed001:10_UV1_
30	Baseline		Feed001:10_UV1_
31	Peak rejection on		
32	Maximum number of peaks ()		20
33			

Peaks table for feed of 0.5M NaCl

b) Retentate

No	Retention (ml)	Area (mAU*ml)	Height (mAU)
1	-1.97	0.3148	5.477
2	-1.78	0.7728	4.384
3	-1.68	0.9961	4.496
4	-1.37	0.9307	2.488
5	2.14	502.8738	694.613
6	5.63	1.7364	3.263
7	7.06	11.5194	15.252
8	7.62	9.3969	15.299
9	8.42	23.8322	20.373
10	11.39	30.8570	23.113
11	15.57	145.3288	119.927
12	19.94	4.4250	8.432
13	21.68	646.1317	471.584
14	26.25	95.0048	43.435
15	27.46	56.4361	41.050
16			
17	Total number of detected peaks		15
18	Total area (mAU*ml)		1530.5572
19	Area in evaluated peaks (mAU*ml)		1530.5565
20	Ratio peak area / total area		1.000000
21	Total peak width (ml)		24.67
22	Column height (cm)		3.00
23	Column Vt (ml)		0.77
24	Calculated from		3x diluted rete
25	Baseline		3x diluted rete
26	Peak rejection on		
27	Maximum number of peaks ()		20
28			

Peaks table for retentate of0.5M NaCl

c) Permeate

No	Retention (ml)	Area (mAU*ml)	Height (mAU)
1	-4.88	1.5966	6.369
2	-4.48	2.3543	5.263
3	-3.88	0.9300	2.148
4	-3.08	0.0020	0.057
5	2.14	1700.4840	2329.610
6	6.02	13.0266	16.112
7	7.22	24.2933	40.754
8	7.67	28.6863	47.526
9	8.25	64.8226	49.112
10	10.82	0.5941	1.793
11	14.66	61.9441	65.091
12	21.31	192.8262	168.243
13	23.03	0.0577	0.634
14	23.14	0.0938	0.697
15	27.35	205.9711	78.708
16			
17	Total number of detected peaks		15
18	Total area (mAU*ml)		2297.6920
19	Area in evaluated peaks (mAU*ml)		2297.6826
20	Ratio peak area / total area		0.999996
21	Total peak width (ml)		23.77
22	Column height (cm)		3.00
23	Column Vt (ml)		0.77
24	Calculated from		Permeate 151120
25	Baseline		Permeate 151120
26	Peak rejection on		
27	Maximum number of peaks ()		20
28			

Peaks table for permeate of 0.5M NaCl