ROLE OF ELECTROKINETIC PARAMETERS ON ASYMMETRIC ULTRAFILTRATION MEMBRANE FLUX AND REJECTION DURING SEPARATION OF BOVINE SERUM ALBUMIN (BSA)

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JUDUL **ROLE OF ELECTRO KINETIC PARAMETERS ON ASYMMETRIC ULTRA FILTRATION MEMBRANE FLUX AND REJECTION DURING SEPARATION OF BOVINE SERUM ALBUMIN (BSA)**

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SITI MARNI AZIZ

A thesis submitted in fulfillment of the
requirement for the award of the degree of
Bachelor in Chemical Engineering

Faculty of Chemical Engineering & Natural Resources Engineering
Universiti Malaysia Pahang

MAY 2008

I declare that this thesis entitled "*Role of Electro kinetic Parameters on Asymmetric Ultrafiltration Membrane Flux and Rejection during Separation of Bovine Serum Albumin (BSA)"* is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Special Dedication of This Grateful Feeling to My…

Beloved parent;

Mr. Aziz Yusuf & Mrs. Sati Marayam Rahaman

Loving brother and sister;

Mohd Asrul and Siti Mastura

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ABSTRACT

The protein concentration involves the removal of solvent (usually water) from protein solution. There will be various methods that can be used for this protein concentration process. One of the methods is by using ultra filtration method. During ultra filtration, the macromolecules such as protein are retained on the membrane. The main objective in this experiment is to determine the optimum pH condition and ionic strength concentration which is give maximum permeate flux which is through the membrane. The molecular weight cut off for the membrane is 50 kDa. This study is carried out in order to know the effect of pH protein solution to the permeate flux and the percent rejection of protein molecules with the various value of pH. Besides, the effect of ionic strength concentration to the permeate flux and rejection protein molecule also had been highlight in this study. From the result acquire, optimum flux for BSA protein filtration is been observed in pH 8 solution and in 0.5M NaCl ionic strength concentration.

ABSTRAK

Pemekatan protein melibatkan penyingkiran pelarut (kebiasaan adalah air) daripada larutan protein. Terdapat pelbagai kaedah yang boleh digunakan untuk proses pemekatan protein ini. Salah satu caranya ialah dengan menggunakan kaedah penurasan ultra. Semasa penurasan ultra, makromolekul seperti protein akan tertahan pada membran. Dalam eksperimen ini, objektif utama ialah untuk menentukan keadaan pH dan kekuatan ionic yang optima yang dapat memberikan peresapan fluks yang tinggi melalui membran. Membran yang digunakan di dalam mempunyai liang keporosan 50 kDa. Kajian ini dilakukan bertujuan untuk mengetahui kesan larutan pH keatas peresapan fluks serta peratusan protein yang tertahan dengan menggunakan pelbagai nilai pH. Selain itu, kesan kepekatan ion ke atas peresapan fluks juga diberi perhatian di dalam kajian ini. Daripada keputusan yang diperolehi, fluks yang optima untuk penurasan protein BSA didapati pada larutan pH 8 dan pada kepekatan kekuatan ion 0.5M.

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Ultrafiltration is a pressure –driven, a separation process using membrane having pore sizes that range from 10-1000Å and widely used for concentration, diafiltration, clarification and fractionation of macromolecules such as proteins, nucleic acids, and synthetic polymer (Ghosh, 2003) . Basically, ultra filtration will remove high molecular-weight substances, colloidal materials, and organic and inorganic polymeric molecules. Low molecular-weight organics and ions such as sodium, calcium, magnesium chloride, and sulfate are not removed. Low applied pressures are therefore sufficient to achieve high flux rates from an ultrafiltration membrane (Zator *et al*., 2003). The major advantages of this type of filtration are high throughput of product, relative ease of scale up and ease of equipment cleaning and sanitization.

A membrane can be defines as a thin barrier or film through which solvents and solutes are selectively transported (Ghosh, 2003). An ideal ultrafiltration membrane should have a few characteristic such as high hydraulic permeability towards solvent, good mechanical durability, good chemical and thermal stability, ease of manufacture and others. Ultra filtration membranes can have extremely high fluxes but in most practical applications the flux varies between 50 and 200 GFD (gallons per square foot per day) at an operating pressure of about 50 psig in contrast, reverse osmosis membranes only produce between 10 to 30 GFD at 200 to 400 psig.

1.2 Problem Statement

The ultra filtration process can be run whether in batch or continuous mode. Batch mode filtration is efficient due to the lowest possible material exposure to the membrane. However, in a constant pressure operation, the permeate flux would decline with time. The main limitation of the batch concentration is large dedicated tank is required. The limitation can be overcome by using fed-batch operation. In the fed-batch mode of operation, the exposure of the membrane to material is greater when compared with the batch mode. Therefore, the permeate flux and efficiency is lower (Ghosh, 2006).

1.3 Objective

The purpose of doing this research is to identify the optimum value of pH which is can give high flux to the filtration of bovine serum albumin protein. Besides, the effect of ionic strength to the protein also will be highlighted in this research.

1.4 Scope of Research Work

There are few purposes that lead up to this research. The purposes are:

- To study the effect of electro kinetic parameter (pH and ionic strength) to the flux and rejection during separation of BSA
- To identify the optimum pH and ionic strength for BSA during the protein separation. The range of pH that being study is pH 5 to pH 8. Meanwhile, the range of ionic strength that being study is 0.5M to 2.0M.

CHAPTER 2

LITERATURE REVIEW

2.1 Definition of membrane

Membrane can be define as a thin barrier which is allow passage of particle with a certain size, particular physical or chemical properties (Ghosh, 2003). A membrane can be dividing into types which are cell membrane and synthetic membrane. The cell membrane is a semi permeable lipid bilayer which can be found in all cells (Ghosh, 2003). Meanwhile, the synthetic membrane is a membrane that being prepared for separation task in laboratory and industry. Their active part, which permits selective transport of material, usually consists of polymer or ceramics, seldom glass or material (Ghosh, 2003). Membrane can be prepare in variety forms like flat sheets, tubes, capillary and hollow fibres. Membrane is built in membrane modules like plate and frame, spiral-wound module, hollow fibre module or tube-in-shell module (Ghosh, 2006).

2.1.1 Driving force in membrane separation process

Different driving force does include in membrane separation process. Some of this are being applied when to transport solute and solvent molecules through.

membranes. The forces include transmembrane pressure, concentration or electrochemical gradient, osmotic pressure and electric field (Ghosh, 2003)

2.1.1 Transmembrane pressure

The transmembrane pressure is the main applied driving force (Ghosh, 2003). Due to this applied driving force, the bulk liquid medium which is the solvent is forced through the pores. The solvent molecules carry the solute molecules towards the membrane and in certain case through membrane. Solute molecules might be fully transmitted, partially transmitted or totally retained (or rejected) by membrane (Ghosh, 2003).

2.1.2 Concentration or Electrochemical Gradient

When a solute is partially retained, there is a build up of rejected solute molecules near the membrane surface. This leads to the solute concentration of upstream side is higher than that on the downstream side.

2.1.3 Osmotic pressure

The concentration difference across the membrane also leads to the development of a transmembrane osmotic pressure difference, which is can cause the flow of solvent from the downstream side back to the upstream side. This is referred to as osmotic-back pressure and acts against the applied transmembrane pressure (Ghosh, 2003).

2.1.4 Electric field

Externally applied electrical fields are sometimes used to increase the efficiency of ultra filtration processes. An electric field is usually used to encourage the back-transport of accumulated solute molecules from the membrane surface to the bulk feed. Besides, an electric field could also be applied to encourage the transport of specific solute molecules to a membrane (Ghosh, 2003).

2.2 Ultra filtration Membrane

Ultra filtration membrane can be used to purify material passing through the filter and also to collect material retained by the filter (Ghosh, 2003). For the ultra filtration membranes, pore diameters usually range from 1 to 20 nm. These pores are sufficiently small in order to retain proteins of low molecular mass. (Walsh, 2002).The type of membrane that being used during this research is polyethersulfone membrane. This type of membrane is highly mechanical, thermal and chemical resistance (Rahimpour *et al*., 2008).

2.2.1 Characteristic of Ultrafiltration Membrane

2.2.1.1 Asymmetric Membrane

An asymmetric membrane is composed of two or more structural planes of non-identical composition or morphology. From a morphological point of view, membranes can be classified into two categories which are porous or dense (Ghosh, 2006).

2.2.1.2 Porous membrane

Porous membrane has tiny pores or pores network (Ghosh, 2006).A porous membrane with pores (permeable to the solvent) of identical sizes is called the homogeneous porous membrane. A membrane with pores of different sizes is non homogeneous porous membrane (Kargol and Kargol, 2003).

2.2.2 Types of flow in Ultra filtration Process

In ultra filtration membrane process, most are carried out in cross flow mode. This cross flow is effective in controlling of cake build-up and allowing relatively high fluxes to be maintained.

In a cross-flow separation process, liquid stream that to be treated which is feed, flows tangentially along the membrane surface, hence producing two streams. The stream where the liquid that comes through the membrane is called permeates. The type and amount of species left from permeate will depend on the few factors such as the characteristics of the membrane, the operating conditions, and the quality of feed. Meanwhile, the other liquid stream is called concentrate and gets progressively

concentrated in those species removed by the membrane. In cross-flow separation, therefore, the membrane only acts as a barrier to the ions, molecules, or colloids and does not act as a collector to these species (Cheryan, 1998).

Meanwhile, for dead end flow or normal flow, the feed flow perpendicularly to the membrane surface. So, when the process flows continuously, it cans causes of filter cake on membrane (Cheryan, 1998). This phenomena will lead to the reducing of permeate flux filtration.

2.2.3 Protein Separation Mechanism in Ultra filtration Membrane

In general, low molecular weight solutes, whose molecular size is much smaller than the smallest pore on the membrane, will be freely permeable, they will have zero rejection, unless they interact with or bind to the impermeable compound in the feed. The permeability of individual components in a mixture depends on the relative sizes of those components and the pores. If a large pore membrane is used with a feed containing large solutes, which are of the same order of magnitude in size as pores, then the large solute may be only partially rejected.

The smaller solutes such as salt will not usually affect the permeability of the large molecules and cause molecular changes. However, changes in operating conditions such as pressure may force more of larger solute through the pores, resulting in a decrease in rejection of the large solute (Cheryan, 1998).

2.2.4 Factors affecting the Ultra filtration Process

2.2.4.1 Temperature

Temperature gives effect to the filtration process. By increasing the temperature of the process, this will reduce the fluid viscosity and will increase the permeate flux.

2.2.4.2 Ratio of Concentration

The concentration of macromolecule in retentate phase increase with the ratio of feed concentration by the time increase. Consequently, this phenomena will increase the membrane fouling and cause of permeate flux decline in ultra filtration processes. Besides, this will lead to the difficulty during the membrane cleaning.

2.2.4.3 Viscosity and Volume Flow rate

Volume flow rate can be defined as volume of fluid which passes through a given surface per unit time (Zator *et al*., 2007). The volume flow rate through surface is proportional to the pressure difference and inversely proportional to the flow of resistance. Meanwhile, the fluid viscosity is proportionally to the resistance. As the viscosity increased, the resistance also increased.

2.3 Proteins

Proteins were first described and named by the Swedish chemist Jon Jacob Berzelius in 1838 (Walsh, 2002). A protein is a biopolymer composed from basic building blocks which called amino acids. Naturally occurring proteins are made up from 20 different amino acids. Proteins are by far the most biopolymers in living cell which constitutes about 40 to 70 percent of dry cell weight and have diverse biological functions which are structural components, catalyst, transport molecules and others (Ghosh, 2003).

2.3.1 Classification of Protein

A protein molecule can be a single poly-(amino acid) chain or may comprise more than one poly-(amino acid), held together by covalent bonds or by non-covalent interactions. The structure of a protein can be defined at different levels, these being: primary, secondary, tertiary and quaternary (Ghosh, 2003).

2.3.2 Protein Composition based on Protein Structure

Protein's structure can be defined as four aspects which are:

- Primary structure: the primary structure of polypeptide can be refers to its exact amino acid sequence, along with the exact positioning of any disulphide bonds present. The twenty commonly occurring acid-amino can be divided into R group classifications which are non-polar, aromatic, polar but uncharged, positively charged and negatively charged. Nineteen of these amino acids contain a central (α) carbon atom, to which is attached a hydrogen atom (H), an amino

group(NH_2), a carboxyl group (COOH) and additional side chain (R) group which are can differentiate amino acid to amino acid (Walsh, 2002).

- Secondary structure: the secondary structure can be described as the local spatial conformation of a polypeptide's backbone, excluding the constituent amino acid's side chains. The major elements of secondary structure are the α-helix and ß-strands (3). The α-helix containing 3.6 amino acids residues in a full turn. This approximates to a length of 0.56nm long the exist of the helix. The participating amino acid side chains protrude outward from the helical backbone. The helical structure is stabilized by hydrogen bonding , with every backbone c=o group forming a hydrogen bond with the N-H group four residues ahead of it in the helix. ß-strands represent the other major recurring structural element of proteins. ß-strands usually are five to ten amino acid residues in length, with the residues adopting an almost fully extended zigzag conformation (Walsh, 2002).

- Tertiary structure: the formation of tertiary structure is usually driven by the burial of hydrophobic residues, but other interaction such as hydrogen bonding, ionic interactions and disulphide bonds can also stabilize the tertiary structure. The tertiary structure encompasses all the no covalent interactions that are not considered secondary structure (Walsh, 2002).

- Quaternary structure: The quaternary structure is the interaction between several chains of peptide bonds. The individual chains are called subunits. The individual subunits are not necessarily covalently connected, but might connect by disulphide bond. Not all proteins have quaternary structure since they might be functional as monomers. The quaternary structure is stabilized by the same range of interaction as the tertiary structure (Walsh, 2002).

2.4 Bovine Serum Albumin Protein

Albumin is generally referred to mean serum albumin or plasma albumin. The word albumin is also used to describe a protein or a group of proteins defined by solubility in water for example the albumin fraction of wheat (Musale and Kulkarni, 1997). Bovine serum albumin (BSA) is a large molecular weight protein that is 66,000 Dalton with a good essential amino acid profile. Bovine serum albumin has isoelectric point at pH 4.9 (Tung *et al*., 2007).

2.5 Effect of pH to the Permeate Flux and Rejection

The feed solution pH is give influenced to the membrane fouling in protein ultra fitration (Ghosh, 2003). Different solution pH values led to different filtration behaviors due to different protein isoelectric points (Tung *et al*., 2007). From the study show, that the steady-state flux increases when solution pH increases with BSA and with 50 kDa PES membranes. When the pH is 4, BSA and the membrane have opposite charge.

Thus, BSA is adsorbed onto the membrane surface and inside the pore wall at the beginning of the filtration period, leading to membrane fouling and flux decline. Though firmly deposited on the membrane, BSA easily passes through the membrane due to transmembrane pressure and vertical drag force during filtration flow. When the pH is within the range of BSAs pI, BSA forms a macromolecule and obstructs the membrane causing very low transmission (Tung *et al*., 2007).

2.6 Effect of Ionic Strength Solution to the Permeate Flux and Rejection

In addition to solution pH, ionic strength plays an important role in protein separation due to electrostatic interaction forces. From study, higher rate of protein fouling is observed at higher ionic strength and pH near to the isoelectric point of bovine serum albumin (BSA) (Mo *et al.*, 2008).

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CHAPTER 3

METHODOLOGY

3.1 Apparatus

During the research, a few chemical apparatus had been used. The apparatus would be:

- Ultra filtration system (AMERSHAM BIOSCIENCES-cross flow filtration)
- Ultra filtration membrane with MWCO 50kDA
- Beaker
- Stop watch
- Magnetic stirrer
- Uv- Vis Spectrophotometer Hitachi U-1800
- pH meter
- Measuring cylinder 100ml

3.1.1 Ultrafiltration System

An important component of a membrane separation system is the actual equipment, within which the membrane element is housed. This equipment can also be referred to as the membrane module. Membrane modules can be classified into different.

types which are stirred cell module, flat sheet tangential flow module, tubular membrane module, spiral wound membrane module and hollow fiber membrane module (Ghosh, 2003).

In this ultra filtration processes, the system that is choose is polyethersulfone. The unit membrane have cross-section area which is $0.11m^2$ .These devices can easily be gathered for cleaning and for replacement of defective membrane elements. Other advantages include the ability to handle reasonably high levels of suspended particulate matter and viscous fluids (Ghosh, 2006).

Figure 3.1: Ultra filtration System

3.1.1 Ultra filtration Plate

The membrane filtration used in this research is Polyethersulfone membrane plate. The membrane plate has cross-section area which is 0.11 m^2 with molecular weight cut off 50 kDA. A membrane plate has two holes beside the plate in order to install the plate to the filtration holder.

Figure 3.2: Ultra filtration Membrane

3.1 List of Chemicals

There are chemicals used during this research. The chemicals would be:

- Phosphate buffer solution
- BSA protein solution
- Deionized water
- Modified Lowry reagent
- Folin-Ciocalteu reagent
- 0.2M KH_2PO_4
- 0.2M K_2HPO_4

3.1.1 Preparation of Phosphate Buffer Solution

The phosphate buffer solution is prepared to dissolve the bovine serum albumin protein powder. The phosphate buffer is prepared by adding 0.2 M Potassium Dihydrogen Phosphate, KH_2PO_4 solution and 0.2M Potassium Hydrogen Phosphate, K_2HPO_4. Phosphate buffer solutions are prepared in pH 5.0, 6.0, 7.0 and 8.0. The mass need for prepare 1 liter potassium dihydrogen phosphate, KH_2PO_4 solution is 27.2 g while 45.6g is need to prepare 1 liter potassium hydrogen phosphate , K_2HPO_4.

In order to prepare the phosphate buffer solution in the desire pH, the solution of potassium dihydrogen phosphate and potassium hydrogen phosphate are mixes in a certain volume which can be conclude in the Table 3.1

Table 3.1: Phosphate Buffer Solution

Desired pH	potassium dihydrogen phosphate solution(ml)	potassium hydrogen phosphate (ml)
5	87.7	20.3
6	50.0	61.0
7	35.3	23.0
8	2.0	94.0

3.1.2 Preparation of Bovine Serum Albumin Protein Solution

The concentration of protein solution which needs to prepare is 1g bovine serum albumin protein powder for 4 liter solution. The solution is prepared by mixing 1g of protein with phosphate buffer solution at desired pH. Then, deionized water will be added to the mixture until getting the 4 liter of solution. The solution is then will be stirred on the magnetic stirrer to get the homogenous solution.

In order to observed the effect of the ionic strength to the permeate, the NaCl solution was prepared in 0.5 M, 1.0 M, 1.5 M and 2.0 M concentration. Then, the NaCl solution will be added to the protein solution at desired pH.

3.2 Ultra filtration System

Before initiating the experiment, the membrane must be installed and the system must be cleaned up first. To clean-up the ultra filtration system, the deionized water which is about 1 liter will be through to system. After that, in order to clean-up the remainder of protein molecule that still trapped in membrane pore, the NaOH 0.1 M solution will be through to the system. For the last step, the deionized water will be supply again to clean-up the membrane.

3.2.1 The Protein Filtration Process

The process starts with the start-up the ultra filtration system. The pressure system which is 0.85 bar and velocity which is 275 rpm is then set-up. The protein solution is 4 liter with the concentration 1g / 4 liter.

During the filtration process, the permeate that flow out is measure using measuring cylinder 50 ml in every half minute and volume of permeate is collected. All the experiment is being conducted in ambient temperature which is 25^0C. Experiment is conducted with the constant pressure, velocity and protein concentration but with the varies of protein solution pH.

3.2.2 Ultra filtration Plate Cleaning Process

Plate should be cleaned properly before reuse or storage. Hot water is being used by through the water by running the filtration process in backward. This can help to discharge the protein which retained on the membrane surface.

3.3 Standard Calibration Curve

A standard calibration curve is a method that can determine the unknown concentration protein in a protein solution sample by comparing the unknown to a set of standard sample of known protein concentration sample. This standard calibration curve is essential because it can determined the concentration of protein that present in permeate flux when the OD of this solution is observed.

To get the OD for the known concentration of protein sample, 0.2 ml of sample and 1.0ml Modified Lowry Reagent are mix and was incubated at room temperature for 10 minutes. After that, 0.1 ml of 1N Folin- Ciocalteu reagent was added into the mixture and left for room temperature at thirty minutes before the OD reading is observed at wavelength which is 750nm. The calibration curve was prepared in protein concentrations which are 0.1g/L,0.2g/L, 0.3g/L, 0.4g/L and 0.5g/L. The readings for OD are taken for each concentration and the graph of OD readings was plot against the protein concentration (Tung *et al*., 2007).

3.4 Flux Analysis

The permeate that flow out will be measure using measuring cylinder to measure the volume. The flux will be calculated using the following equation (3.1):

$$\text{Flux} = \frac{\text{Volume of Permeate}}{\text{Time x Total Cross Sectional Area}} \quad (3.1)$$

3.5 Protein Rejection Analysis

The protein rejection by using ultra filtration membrane is an essential parameter in order to detect the percentage of protein that go through the membrane. The rejection can be calculated by using following equation:

$$R = 1 - \frac{C_p}{C_f}, \quad (3.2)$$

Where, Cp = protein concentration in permeate flow

Cf = protein concentration in feed

In the experiment, the sample of permeate will be taken in every five minute. The sample then will be collected in a test tube and will be analyze for OD readings. In order to get the protein concentration in sample of permeate, OD readings will be refer with the standard calibration curve.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Standard Calibration Curve

The standard calibration curve that had been plotted is a linear line and its intercept is equal to zero. The standard curve had been prepared for each pH in this experiment.

Table 4.1: Data for Standard Curve with BSA Protein Solution at pH 5

Concentration (g/l)	OD (750 nm)
0	0.0000
0.1	2.5749
0.2	3.3767
0.3	3.7757
0.4	6.9992
0.5	9.9630

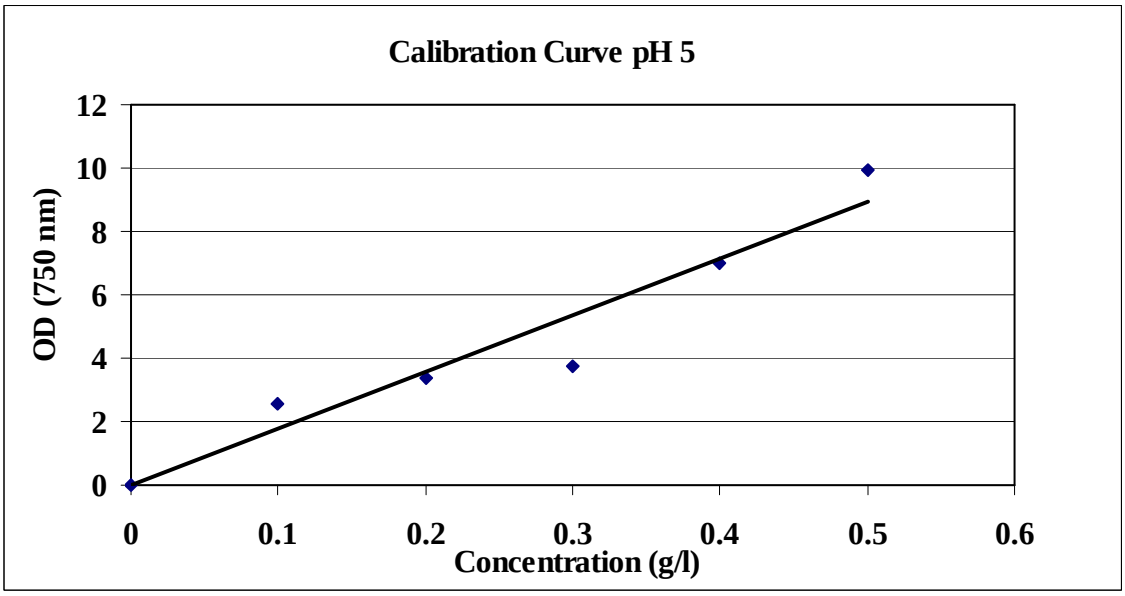

Figure 4.1 Standard Curve for BSA Protein Concentration at pH 5

Table 4.2: Data for Standard Curve with BSA Protein Solution at pH 6

Concentration (g/l)	OD (750 nm)
0	0.0000
0.1	0.5118
0.2	0.7219
0.3	0.8381
0.4	2.0656
0.5	3.3517

Table 4.3: Data for Standard Curve with BSA Protein Solution at pH 7

Concentration (g/l)	OD (750 nm)
0	0.0000
0.1	2.1825
0.2	2.9179
0.3	1.6403
0.4	2.9525
0.5	3.3544

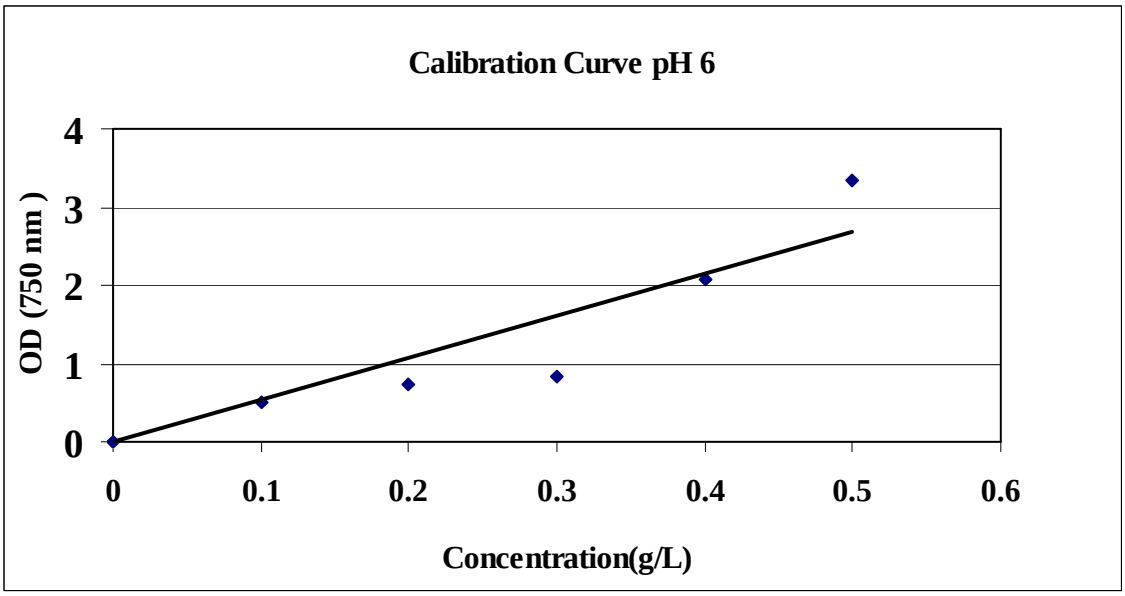

Figure 4.2: Standard Curve for BSA Protein Concentration at pH 6

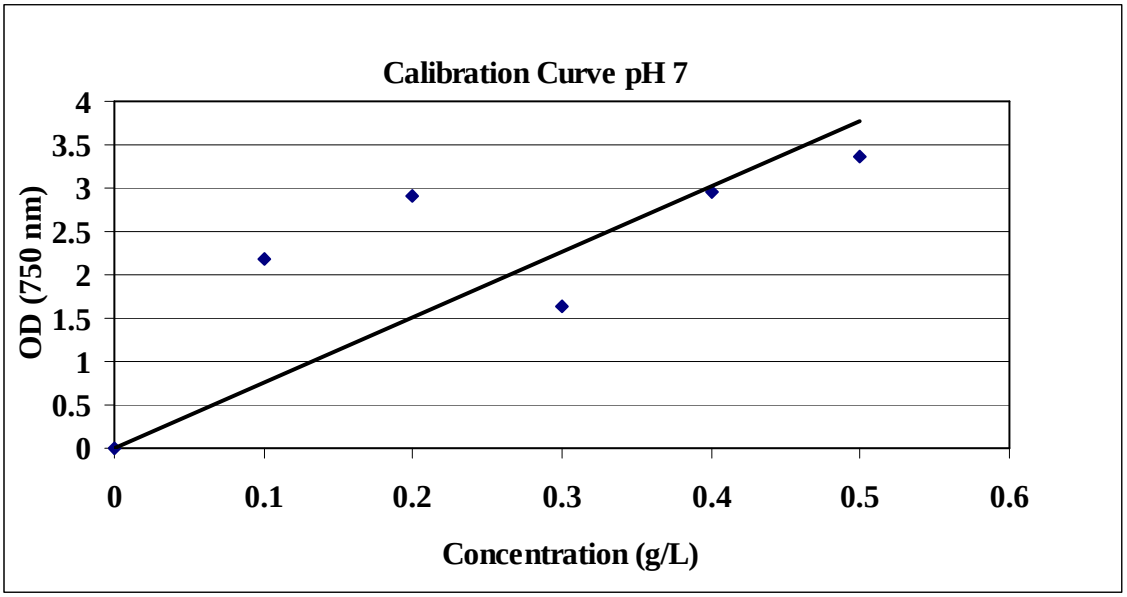

Figure 4.3: Standard Curve for BSA Protein Concentration at pH 7

Table 4.4: Data for Standard Curve with BSA Protein Solution at pH 8

Concentration (g/l)	OD (750 nm)
0	0.0000
0.1	0.2447
0.2	0.3443
0.3	1.8305
0.4	2.0795
0.5	2.3864

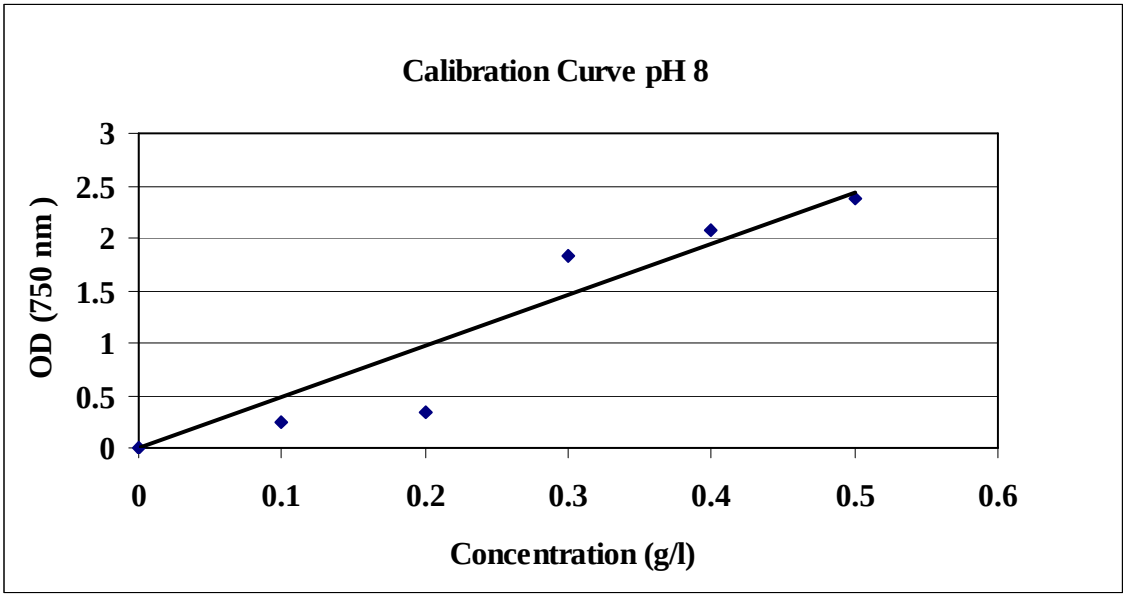

Figure 4.4: Standard Curve for BSA Protein Concentration at pH 8

4.2 Effect of pH on Membrane Flux During BSA Separation

Permeate flux that exit from filtration system are being calculated using formula:

$$\text{Flux} = \frac{\text{Volume of permeate}}{\text{Time x Total Cross Sectional Area}} \quad (4.1)$$

4.2.1 Flux Decline during BSA Separation at pH 5

Figure 4.5 shows the flux decline during the separation of BSA at pH 5. In the pH solution which is within BSAs pI, BSA protein tends to form macromolecule and obstruct the membrane. Hence it was causing low flux transmission. The percent of flux decline is 4%. The flux is slowly decreased and achieved the steady-state phase at 30 minute of filtration time.

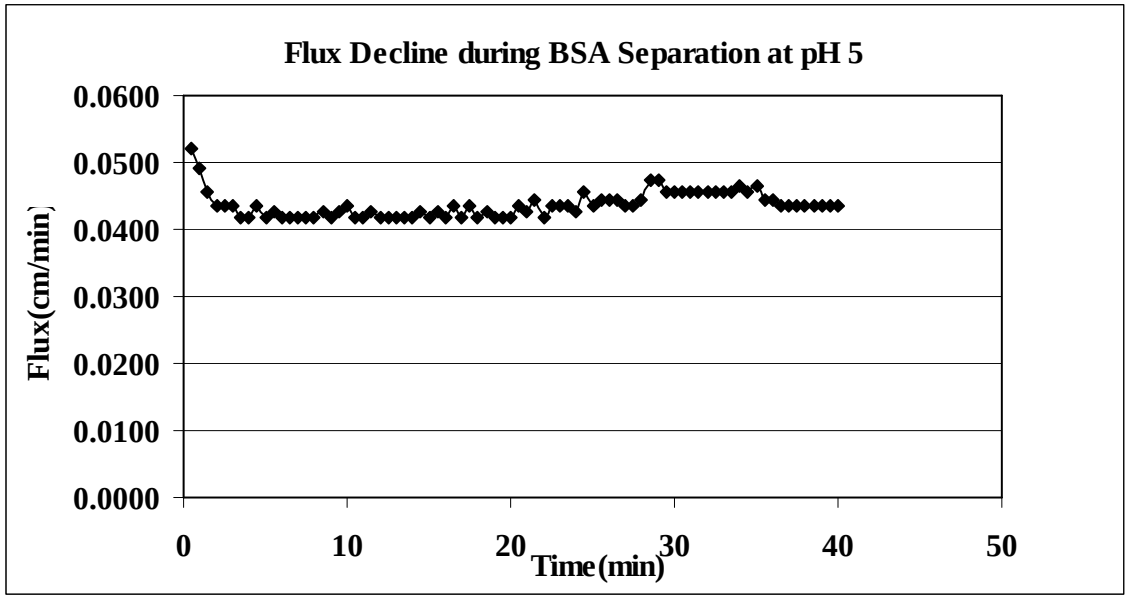

Figure 4.5: Flux Decline during BSA Separation at pH 5

4.2.2 Flux Decline during BSA Separation at pH 6

Figure 4.6 shows the flux decline during the separation of BSA at pH 6. In this pH solution, the BSA protein tends to aggregate and in the same time there is some of BSA protein is adsorbed onto the membrane surface. The percent of flux decline is 3.23%. The steady-state is achieved at 35 minute of filtration time.

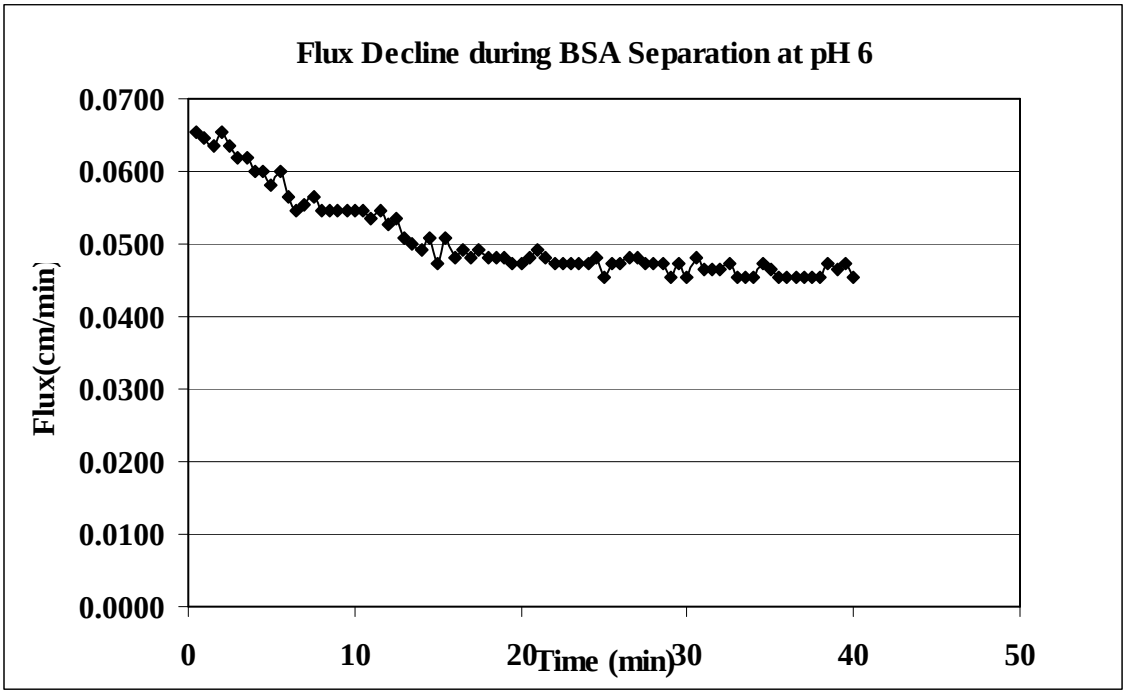

Figure 4.6: Flux Decline during BSA Separation at pH 6

4.2.3 Flux Decline during BSA Separation at pH 7

Figure 4.7 shows the flux decline during the separation of BSA at pH 7. Increasing the pH to 7 causes the negative charge to BSA protein and membrane, produce repulsion between both membrane and BSA protein molecule. The percent of flux decline is 4.48%. The steady-state phase then is achieved in the 36 minute of filtration time.

4.2.4 Flux Decline during BSA Separation at pH 8

Figure 4.8 shows the flux decline during the separation of BSA at pH 7. In this protein solution, the optimum flux is being observed. This occurred because the BSA protein tends to create strong electrostatic repulsion with the membrane and hence decreased the membrane fouling. The percent of flux decline is 2.63%. After 37 minutes of filtration, steady- state phase is achieved.

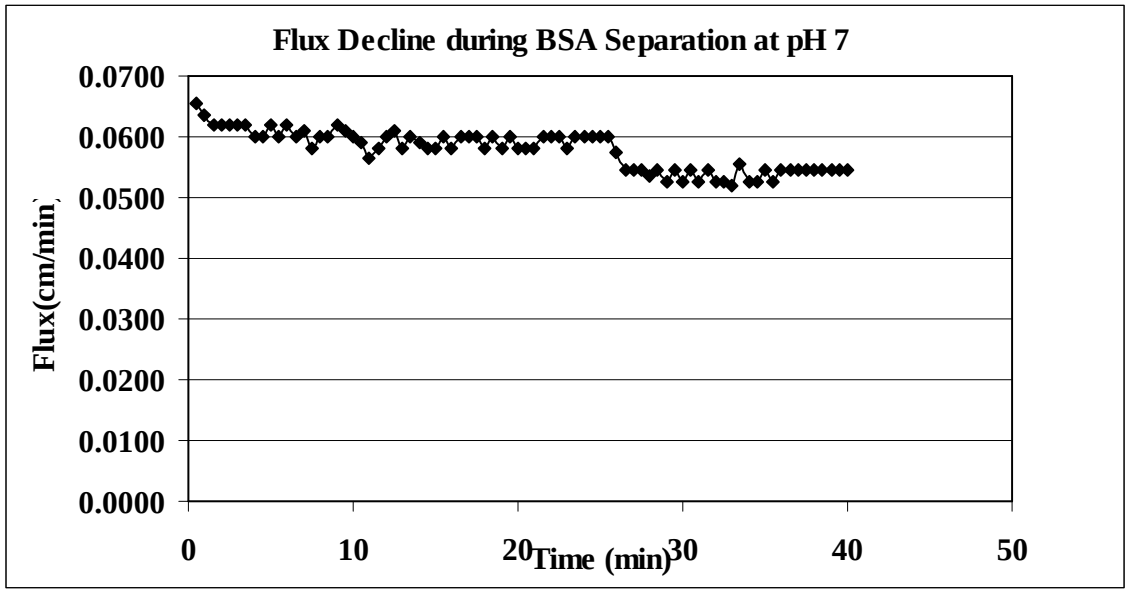

Figure 4.7: Flux Decline during BSA Separation at pH 7

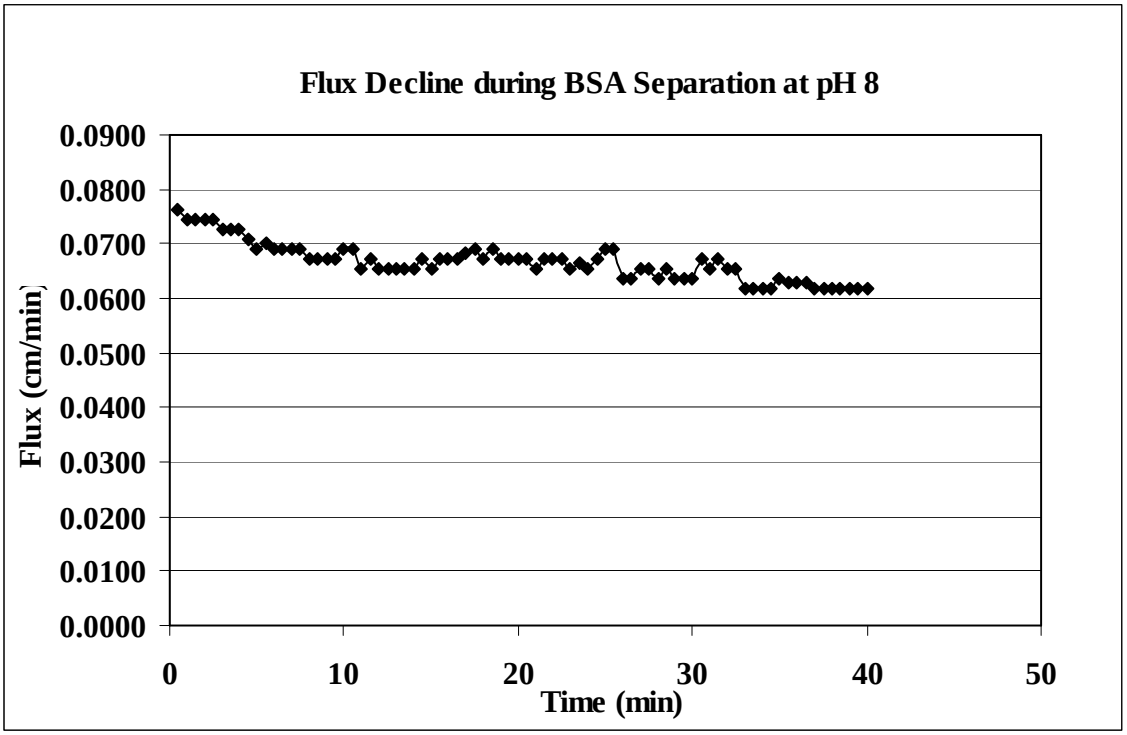

Figure 4.8: Flux Decline during BSA Separation at pH 8

4.2.5 Overall Flux Analysis at Different pH Solution During BSA Separation

Figure 4.9 shows the graph of flux decline for all the four pH protein solution that being study versus the time by using ultra filtration system. From the graph that been plotted, the flux is been decreased as the time increased. In the early experiment, the value of flux is high and became decreased in every five minute filtration process. However, there will be a condition when permeate flux is start to being constant. This condition had been observed when collect the volume of permeate in every one minute where the volume shows the value which is almost the same.

In the early of the experiment, the permeate give high volume because the protein retained on membrane pore which is causing membrane fouling is low. This condition provides low resistance for flux to pass through the membrane. But, as the time increased there will be more protein molecule would be trapped on the membrane

surface. Hence this phenomenon will lead to the reducing of volume flow rate and will decrease the flux. The graph also shows that optimum flux is at pH 8 solution because of the strong electrostatic repulsion between BSA protein molecule and membrane surface. Meanwhile, in the pH 5 solution, the minimum flux is observed. This occurred because when the BSA in the isoelectric point, BSA protein tends to forms a macmolecule and this causing low flux transmission (Tung *et al*., 2007).

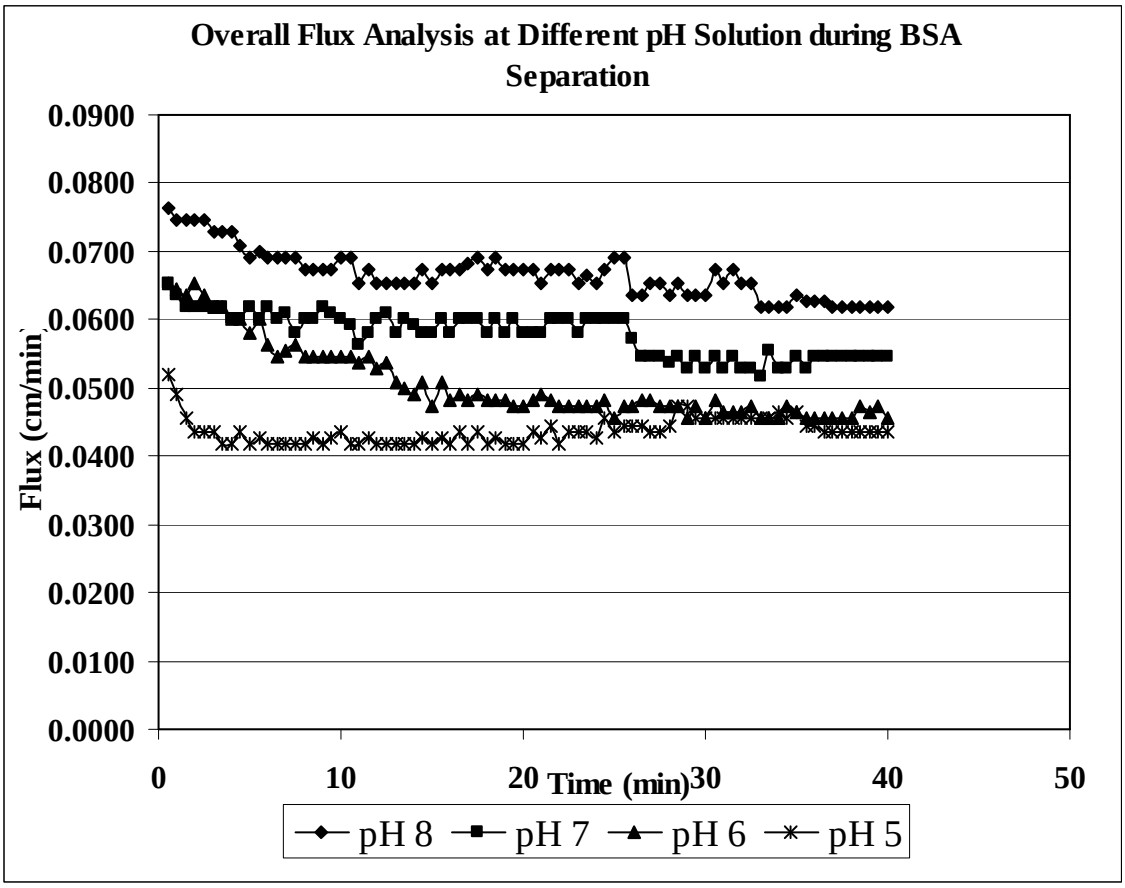

Figure 4.9: Overall Flux Analysis at Different pH Solution during BSA Separation

4.3 Effect of pH on Membrane Rejection

The percent of rejection protein on the membrane can calculate using formula:

$$R = 1 - \frac{C_p}{C_f},$$

Where, Cp = protein concentration in permeate flow

Cf = protein concentration in feed (4.1)

4.3.1 Rejection of BSA at pH 5 using UF membrane

At pH 5 solution, the rejection of BSA is decreased with the percent at 20.88% after 5 minutes filtration and 47.92 % at 40 minutes of filtration time. The steady state of rejection is achieved at 35 minutes of filtration time. The rejection was occurred due to the macromolecule of BSA proteins that adsorbed on the membrane surface because of electrostatic attraction.

Table 4.5: Rejection of BSA at pH 5 using UF Membrane

Time (min)	OD (750 nm)	Concentration (g/l)	Rejection (%)
0	-	0.2500	-
5	0.9593	0.1978	20.88
10	0.9242	0.1906	23.78
15	0.8153	0.1681	32.76
20	0.8055	0.1661	33.57
25	0.7879	0.1625	35.02
30	0.7061	0.1456	41.76
35	0.6340	0.1307	47.71
40	0.6315	0.1302	47.92

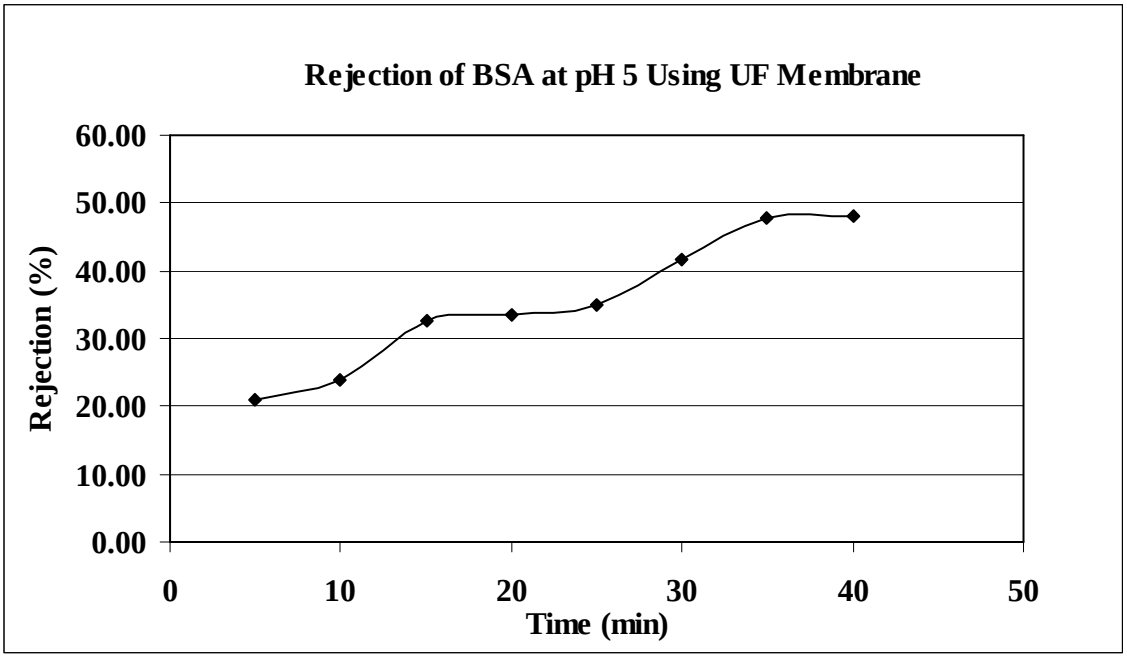

Figure 4.10: Rejection of BSA at pH 5 Using UF Membrane

4.3.2 Rejection of BSA at pH 6 using UF Membrane

In the pH 6, the rejection of BSA is about 35.05 % after 5 minutes of filtration time and 51.97 % at 40 minutes after filtration time. BSA protein is tend to aggregate on the membrane surface due to the electrostatic attraction occurred since the protein and membrane surface had opposite charges in the solution. The steady state is achieved at 35 minutes of filtration time.

Table 4.6: Rejection of BSA at pH 6 using UF Membrane

Time (min)	OD (750 nm)	Concentration (g/l)	Rejection (%)
0	-	0.2500	-
5	2.9069	0.1624	35.05
10	2.8539	0.1594	36.24
15	2.7881	0.1557	37.71
20	2.7580	0.1541	38.38
25	2.6065	0.1456	41.76
30	2.5063	0.1400	44.00
35	2.2153	0.1237	50.50
40	2.1497	0.1201	51.97

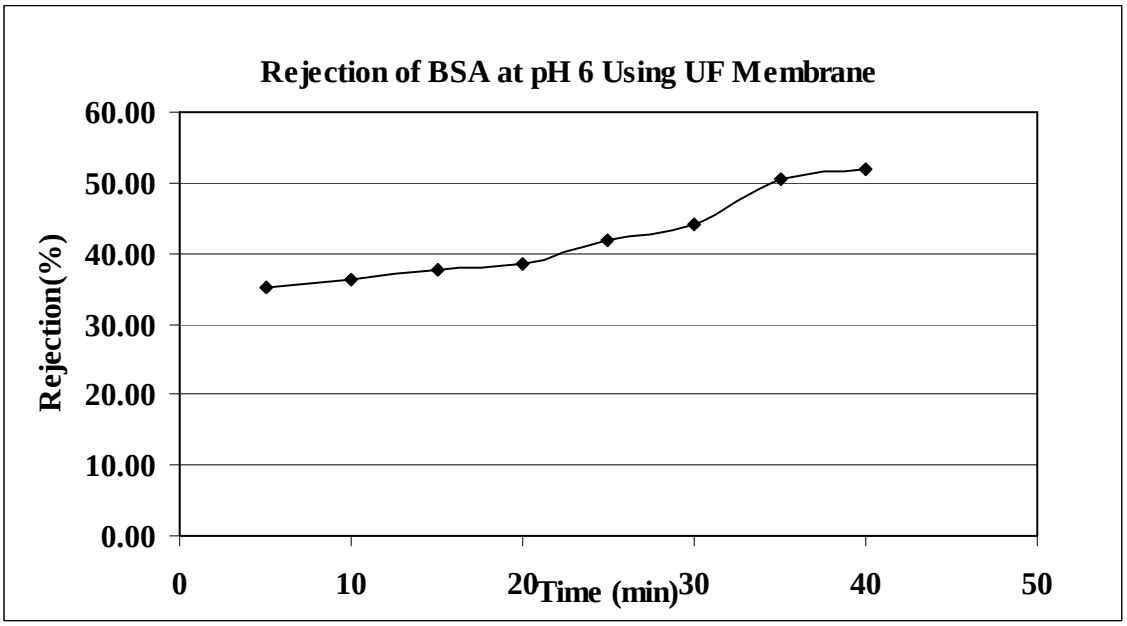

Figure 4.11: Rejection of BSA at pH 6 Using UF Membrane

4.3.3 Rejection of BSA at pH 7 using UF Membrane

In the pH 7, the percent rejection after 5 minutes of filtration is about 48.38 % and at 40 minutes of filtration time is about 64.76%. This occurred due the electrostatic repulsion between BSA protein and membrane which both have same negative charge. The steady state is achieved at 35 minutes of filtration time.

Table 4.7: Rejection of BSA at pH 7 using UF Membrane

Time (min)	OD (750 nm)	Concentration (g/l)	Rejection (%)
0	-	0.2500	-
5	0.9743	0.1291	48.38
10	0.9425	0.1248	50.06
15	0.9305	0.1233	50.70
20	0.918	0.1216	51.36
25	0.8732	0.1157	53.73
30	0.8145	0.1079	56.84
35	0.7129	0.0944	62.23
40	0.6650	0.0881	64.76

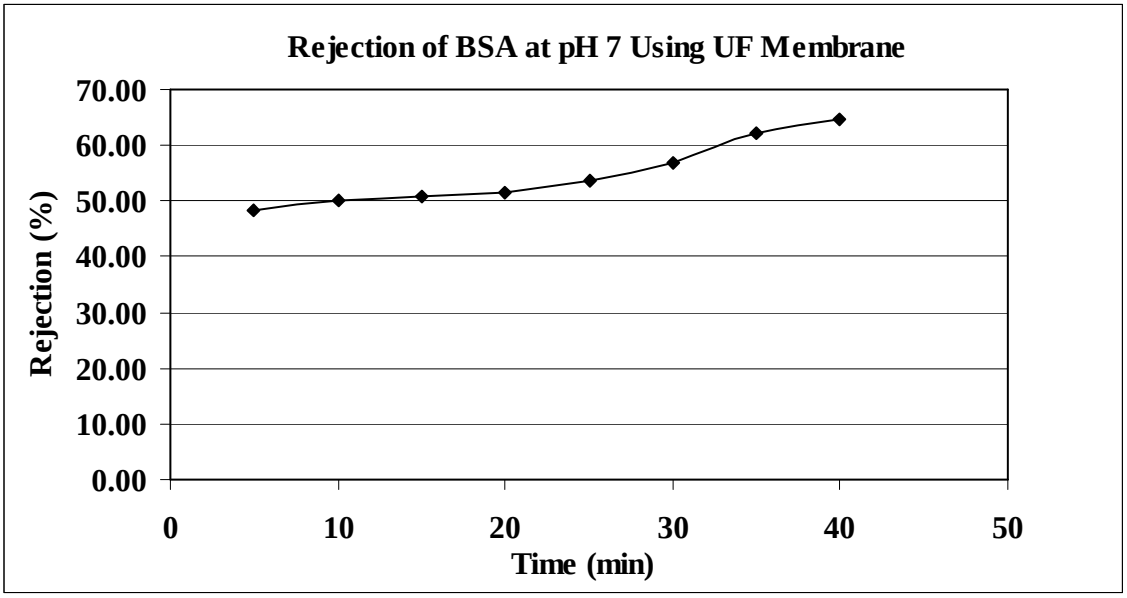

Figure 4.12: Rejection of BSA at pH 7 Using UF Membrane

4.3.4 Rejection of BSA at pH 8 using UF Membrane

In the pH 8 solution, the rejection is optimum. The rejection is about 56.29% at 5 minutes after filtration time and 83.41% after 40 minutes of filtration time. BSA protein and the membrane surface were both negatively charged, and electrostatic repulsion was the dominant interaction between the two materials. The steady state is achieved at 35 minutes after filtration time.

Table 4.8: Rejection of BSA at pH 8 using UF Membrane

Time (min)	OD (750 nm)	Concentration (g/l)	Rejection (%)
0	-	0.2500	-
5	0.5859	0.1093	56.29
10	0.5524	0.1030	58.79
15	0.5384	0.1004	59.84
20	0.5237	0.0977	60.93
25	0.3923	0.0732	70.73
30	0.3752	0.0700	72.01
35	0.2632	0.0491	80.37
40	0.2224	0.0415	83.41

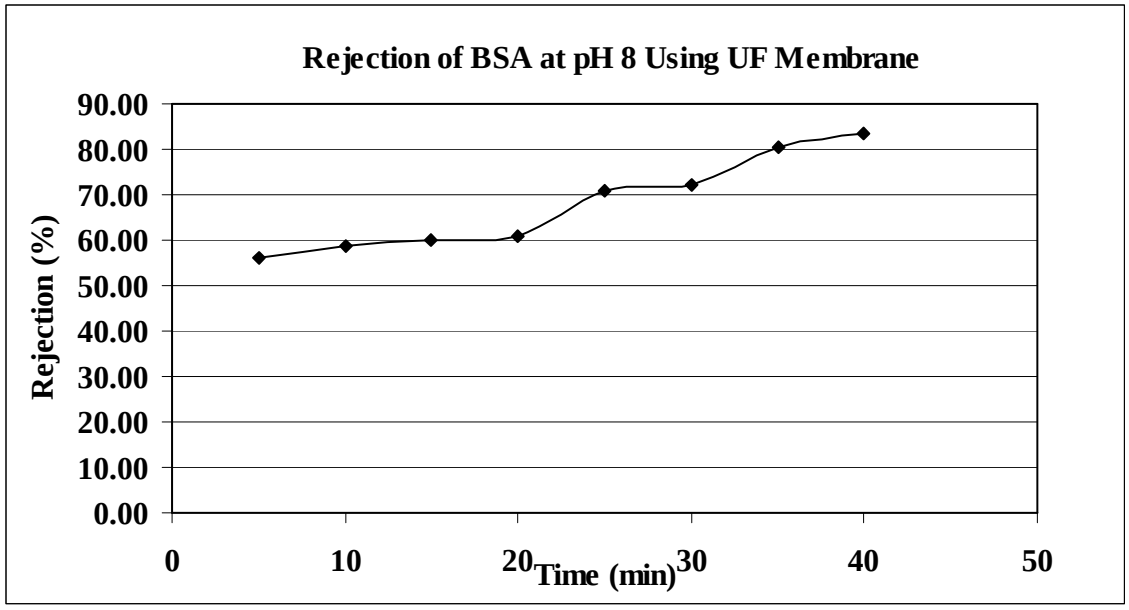

Figure 4.13: Rejection of BSA at pH 8 Using UF Membrane

4.3.5 Overall Rejection Analysis of BSA at Different pH Solution using UF Membrane

Figure 4.14 shows the rejection of BSA protein molecule for all pH values. In the pH 5, BSA protein shows the lowest rejection while the higher rejection can be seen in the pH 8 solution. At pH within the range of BSAs isoelectric point, BSA protein tends to form macromolecule and electrostatic attraction occurred since the protein and membrane surface had opposite charges in the aqueous solution. This encouraged the adsorption of BSA on the membrane surface.

When pH was above the isoelectric point of BSA, BSA and the membrane surface were both negatively charged, and electrostatic repulsion was the dominant interaction between the two materials. Any adsorption of BSA onto the membrane surface in this pH range was attributed to other interactions between BSA and membrane surface such as structural interaction (Mo *et al.*, 2008).

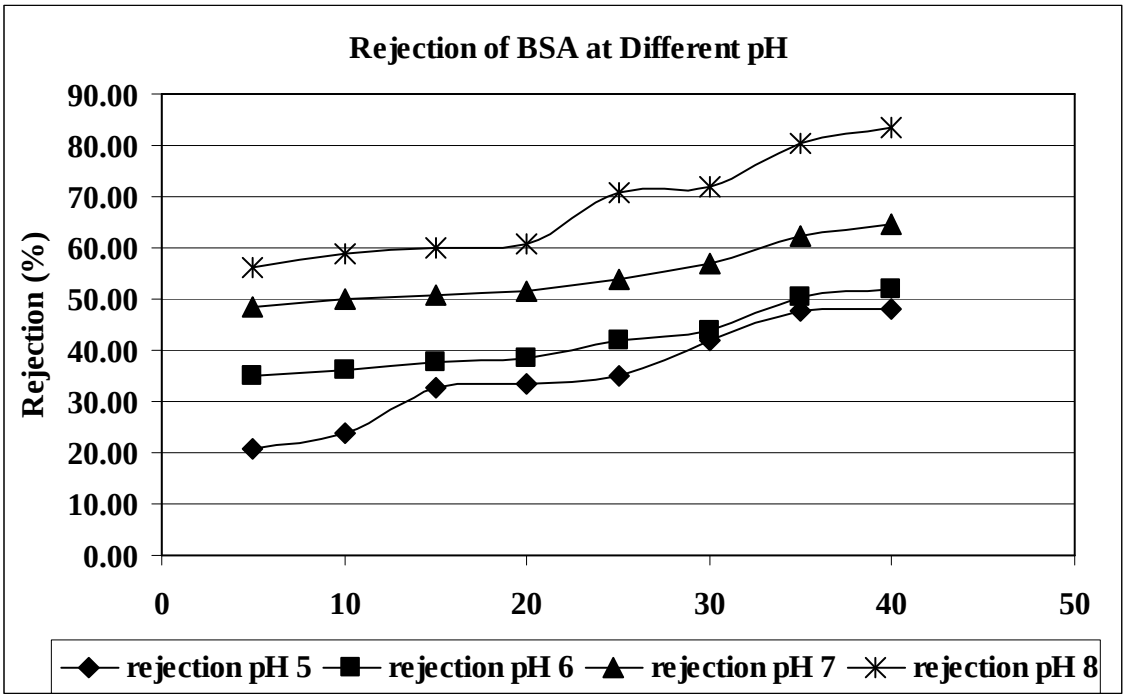

Figure 4.14: Rejection of BSA at Different pH

4.4 Effect of the Ionic Strength on the Membrane Flux

4.4.1 Flux Decline during BSA Separation at Ionic Strength 0.5 M NaCl

In the ionic strength solution, the BSA protein is tending to form aggregation due to the hydrophilic characteristic. In the 0.5M NaCl, initial flux decline show the high rate which is 0.06 cm/ min. as the time increase, the flux is decreased due to the BSA protein deposition on membrane surface. The percent of flux decline is 6.76%. From figure 4.15, the steady state is achieved after 37 minutes of filtration time.

4.4.2 Flux Decline during BSA Separation at Ionic Strength 1.0M NaCl

As the molarity of ionic solution is increase to 1.0M, there will be more of protein is retained on membrane pore makes the flux is relatively low and achieved steady-state after 35 minutes of filtration time. The percent of flux decline is 5.88%.

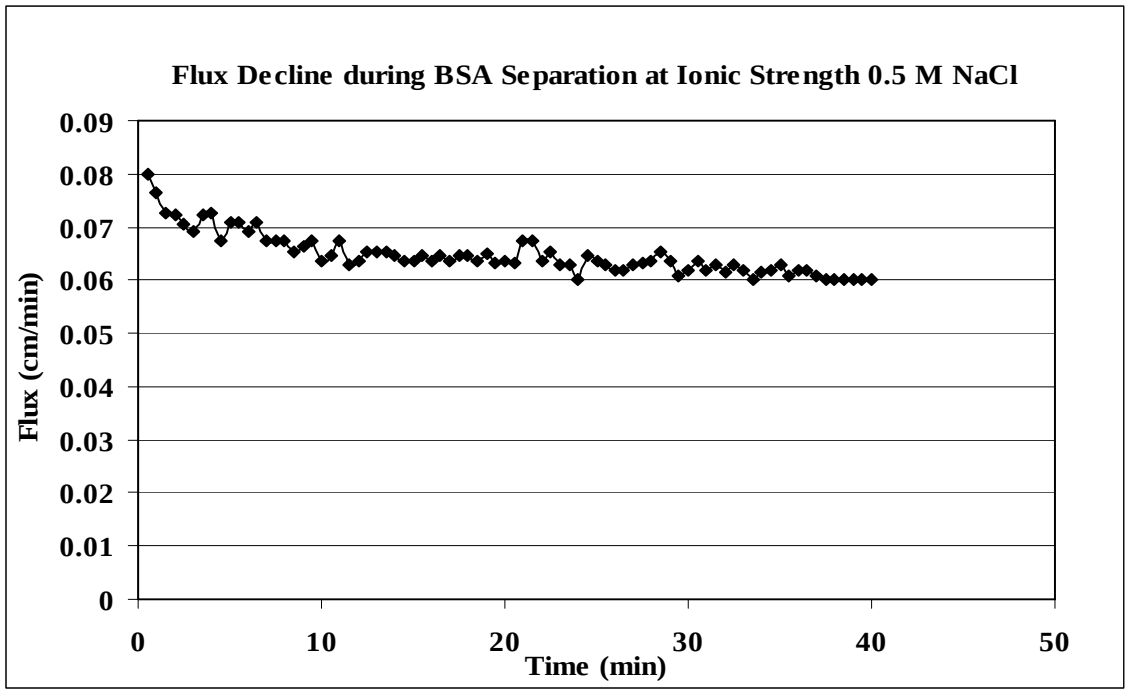

Figure 4.15: Flux Decline during BSA Separation at Ionic Strength 0.5 M NaCl

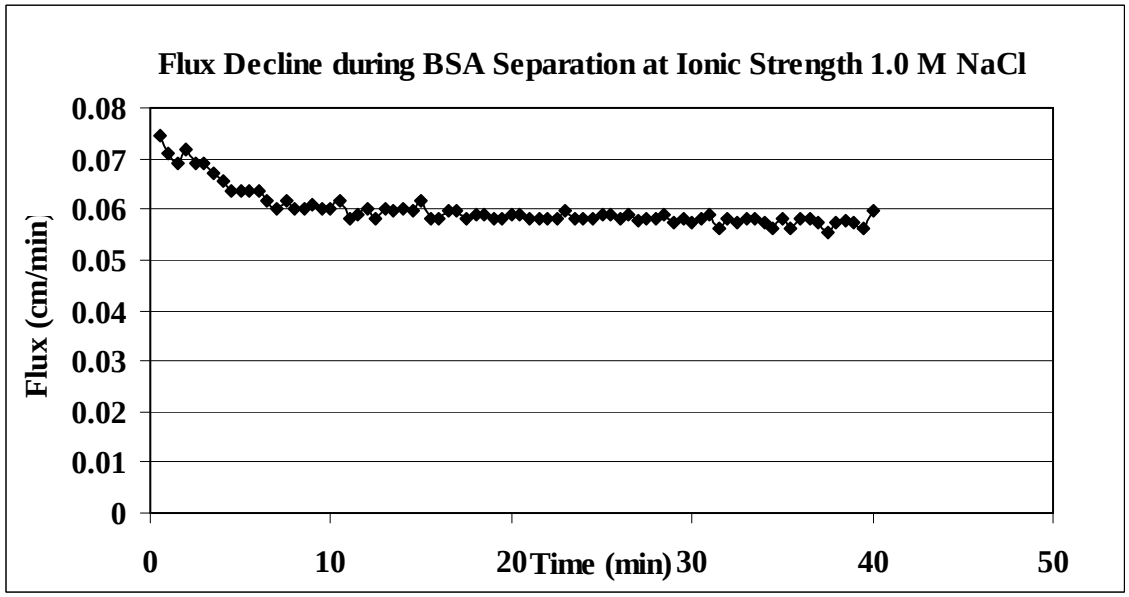

Figure 4.16: Flux Decline during BSA Separation at Ionic Strength 1.0 M NaCl

4.4.3 Flux Decline during BSA Separation at Ionic Strength 1.5 M NaCl

As the ionic strength increase, BSA protein tends to form macromolecule and absorbed onto membrane surface. This will lead to the decreasing of flux through the membrane. The steady-state is achieved at 37 minutes of filtration time. The percent of flux decline is 6.15%.

4.4.4 Flux Decline during BSA Separation at Ionic Strength 2.0 M NaCl

In the high concentration of the ionic solution, the steady- state of flux was become very low. This occurred due to the more macromolecule was formed and deposited on the membrane surface. The steady-state then achieved after 35 minutes of filtration time. The percent of flux decline is 4.92%.

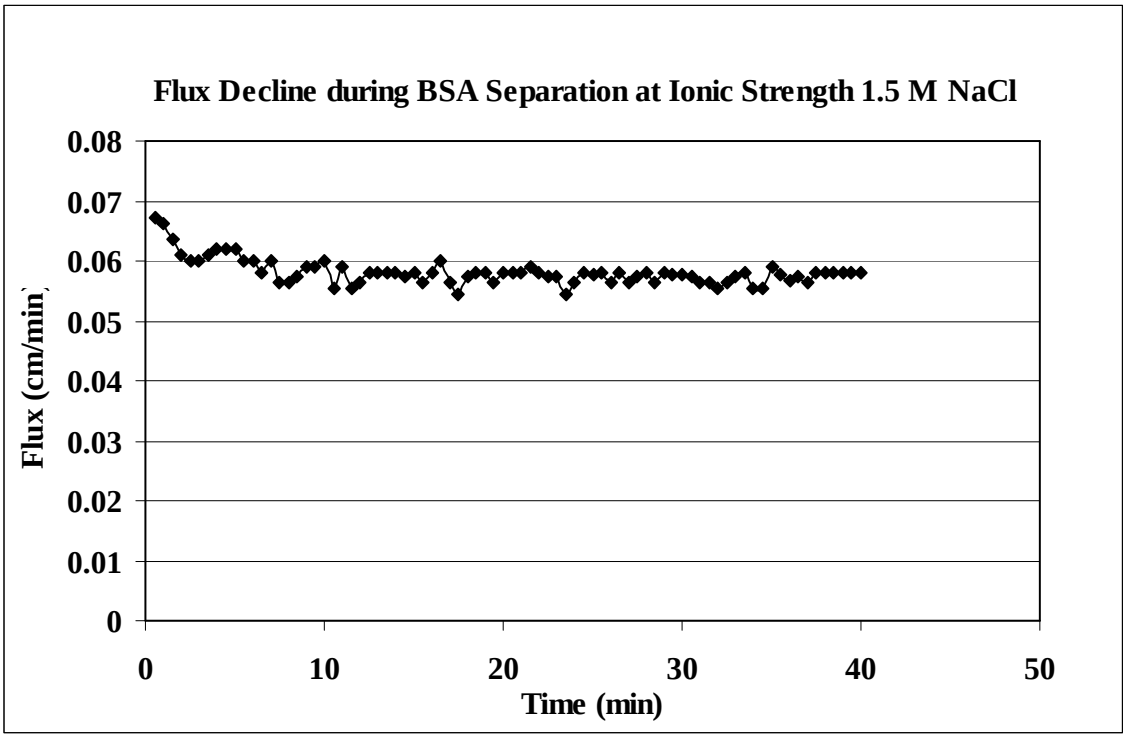

Figure 4.17: Flux Decline during BSA Separation at Ionic Strength 1.5 M NaCl

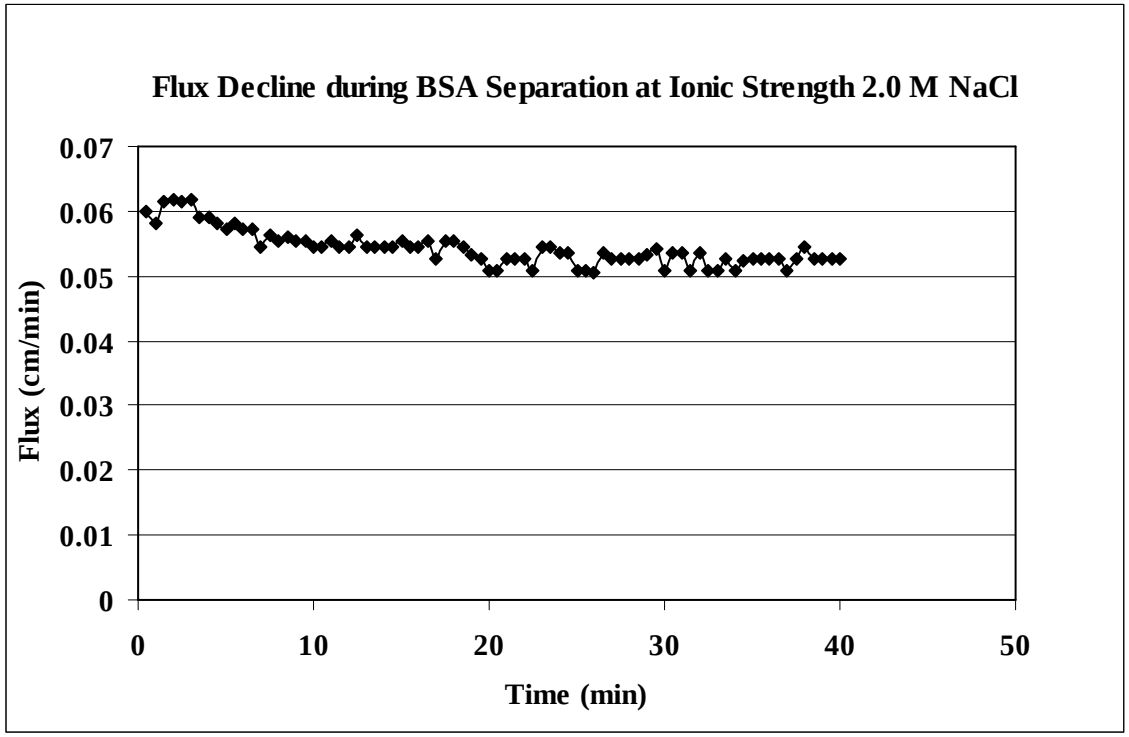

Figure 4.18: Flux Decline during BSA Separation at Ionic Strength 2.0 M NaCl

4.4.5 Overall Flux Analysis at Different Ionic Strength during BSA Separation

Figure 4.19 shows the overall flux analysis for type of ionic strength in pH 8 protein solution. From the graph that have been plotted, the flux is been decreased as the time increased.

Generally, at low concentration of NaCl, there will be more protein adsorbed on the membrane surface. It is also noticed that steady-state flux was decreases as ionic strength increases. In the early experiment, the value of flux is high and became decreased in every five minute filtration process. However, there will be a condition when permeate flux is start to show the steady-state phase. This condition had been

observed when collect the volume of permeate in every one minute where the volume shows the value which is almost the same.

From the result, it also shows that ionic strength 0.5M give the higher flux compare to the others ionic strength concentration. However, as the ionic strength increase, the BSA proteins have a tendency to aggregate and form a macromolecule. The electrostatic attraction between BSA and the membrane affected the initial flux decline rate at the initial fouling stage. After a lag-time, however, the macromolecule of BSA protein starts to deposit and adsorb onto the membrane surface and pore wall leading to enhanced fouling of the membrane and seriously lowering the steady-state flux (Tung *et al*., 2007).

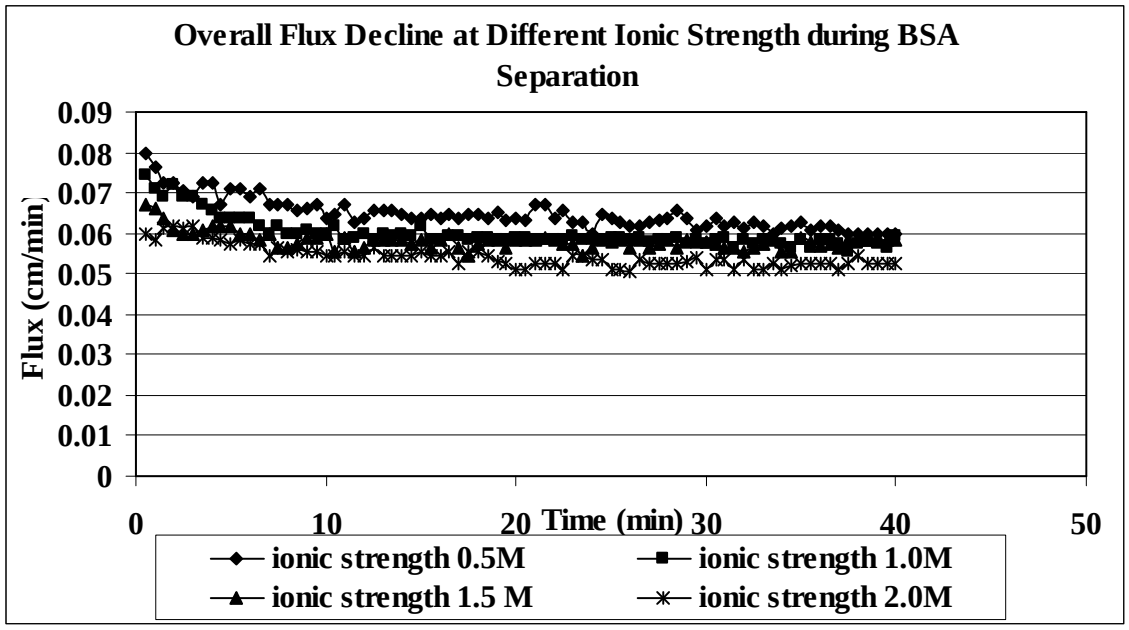

Figure 4.19: Overall Flux Decline at Different Ionic Strength during BSA Separation

4.5 Effect of Ionic Strength on Membrane Rejection during BSA Separation

4.5.1 Membrane Rejecting at 0.5M NaCl (pH 8) Using UF Membrane

From Table 4.9, the percent rejection is about 51.75 % for the early five minutes of experiment. In the low salt concentration and pH solution 8, BSA protein will have same charge with surface membrane. This will lead to electrostatics repulsion between the both and higher rejection had been achieved (Mo *et al*., 2008). The steady-state is achieved at 35 minutes of filtration time. After 40 minutes, the percent of rejection is about 64.42%.

Table 4.9: Membrane Rejecting at 0.5M NaCl (pH 8) Using UF Membrane

Time (min)	OD (750 nm)	Concentration(g/l)	Rejection (%)
0	-	0.2500	-
5	0.6468	0.1206	51.75
10	0.6191	0.1155	53.82
15	0.6025	0.1124	55.05
20	0.5905	0.1101	55.95
25	0.5611	0.1046	58.14
30	0.5411	0.1009	59.63
35	0.4800	0.0895	64.19
40	0.4770	0.0890	64.42

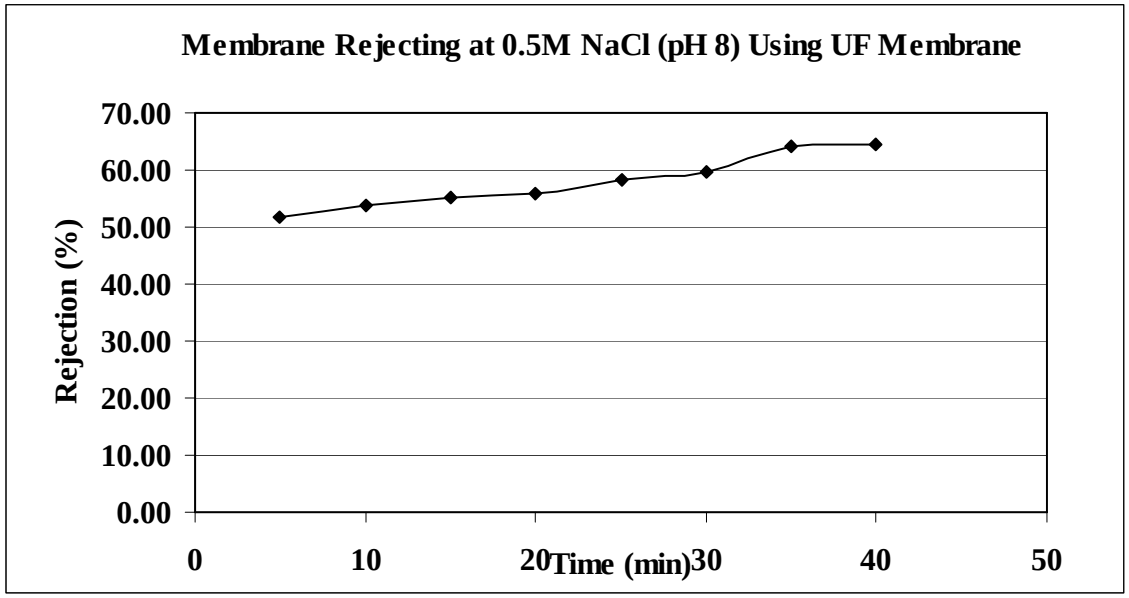

Figure 4.20: Membrane Rejecting at 0.5M NaCl (pH 8) Using UF Membrane

4.5.2 Membrane Rejecting at 1.0M NaCl (pH 8) Using UF Membrane

From Table 4.10, the percent rejection is 41.64% for the early five minutes of experiment. The rejection is decreased as the ionic strength increased. This occurred due to the BSA protein have a tendency to forms aggregation because ionic strength will reduced the electrostatic repulsion between the BSA and membrane surface (Mo *et al*., 2008). In the minute 35, the steady-state is achieved. After 40 minutes of experiment, the percent of rejection is increased to 51.80%.

Table 4.10: Membrane Rejecting at 1.0M NaCl (pH 8) Using UF Membrane

Time (min)	OD (750 nm)	Concentration (g/l)	Rejection (%)
0	-	0.2500	-
5	0.7823	0.1459	41.64
10	0.7551	0.1408	43.67
15	0.7436	0.1387	44.53
20	0.7246	0.1351	45.95
25	0.6778	0.1264	49.44
30	0.6635	0.1237	50.50
35	0.6506	0.1213	51.47
40	0.6461	0.1205	51.80

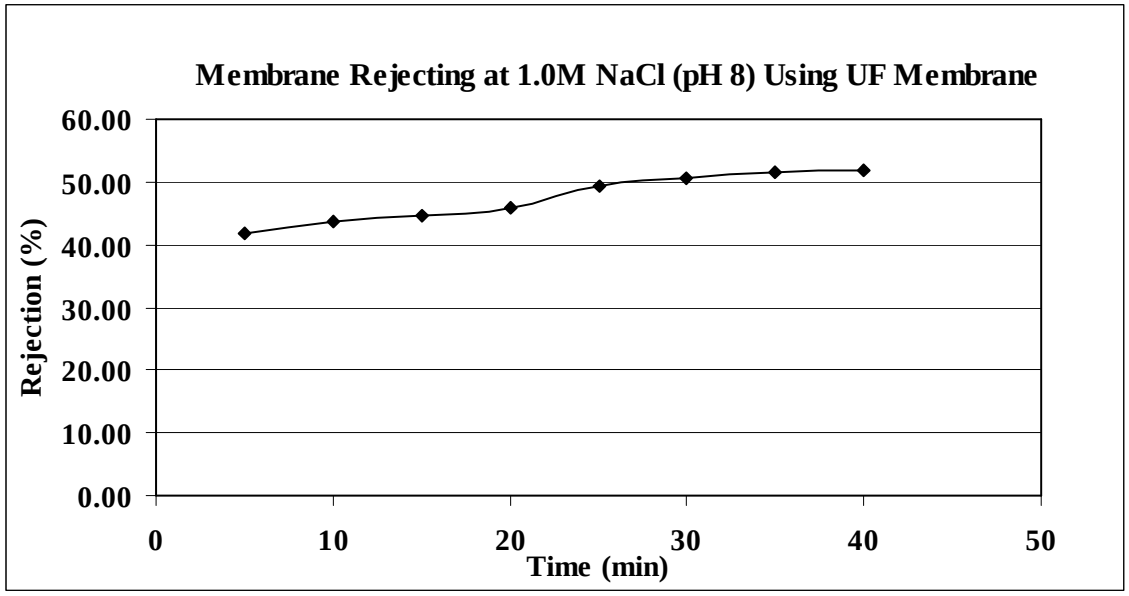

Figure 4.21: Membrane Rejecting at 1.0M NaCl (pH 8) Using UF Membrane

4.5.3 Membrane Rejecting at 1.5M NaCl (pH 8) Using UF Membrane

From Table 4.11, the percent rejection is about 33.56% for the early five minutes of experiment. In this ionic strength, the BSA proteins is start to form macromolecule which is restrain permeate flux to through the membrane. The steady-state is achieved after 35 minutes of filtration. After 40 minutes of experiment, the percent of rejection is increased to 51.32%.

Table 4.11: Membrane Rejecting at 1.5M NaCl (pH 8) Using UF Membrane

Time (min)	OD (750 nm)	Concentration(g/l)	Rejection (%)
0	-	0.2500	-
5	0.8906	0.1661	33.56
10	0.8842	0.1649	34.04
15	0.8678	0.1618	35.26
20	0.8556	0.1596	36.17
25	0.7800	0.1455	41.81
30	0.6690	0.1248	50.09
35	0.6537	0.1219	51.23
40	0.6526	0.1217	51.32

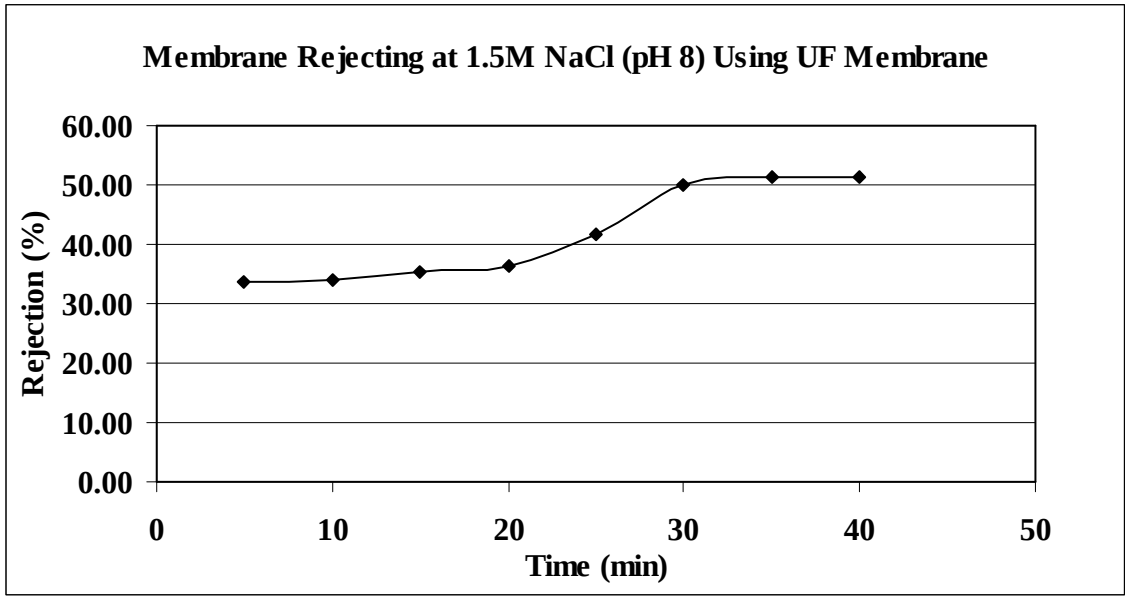

Figure 4.22: Membrane Rejecting at 1.5M NaCl (pH 8) Using UF Membrane

4.5.4 Membrane Rejecting at 2.0M NaCl (pH 8) Using UF Membrane

From Table 4.12, the percent rejection is about 28.15 % for the early five minutes of experiment. In 2.0M ionic strength, the BSA proteins tend to forms macromolecule and obstruct the membrane surface. The rejection is low due to the resulting in a decrease in electrostatic interactions (Salgin *et al*., 2005). The steady-state is achieved after 35 minutes of filtration. After 40 minutes of experiment, the percent of rejection is increased to 51.50%.

Table 4.12: Membrane Rejecting at 2.0M NaCl (pH 8) Using UF Membrane

Time (min)	OD (750 nm)	Concentration (g/l)	Rejection (%)
0	-	0.2500	-
5	0.9631	0.1796	28.15
10	0.9458	0.1764	29.44
15	0.9006	0.1680	32.82
20	0.8706	0.1624	35.05
25	0.7583	0.1414	43.43
30	0.6695	0.1249	50.06
35	0.6523	0.1217	51.34
40	0.6502	0.1213	51.50

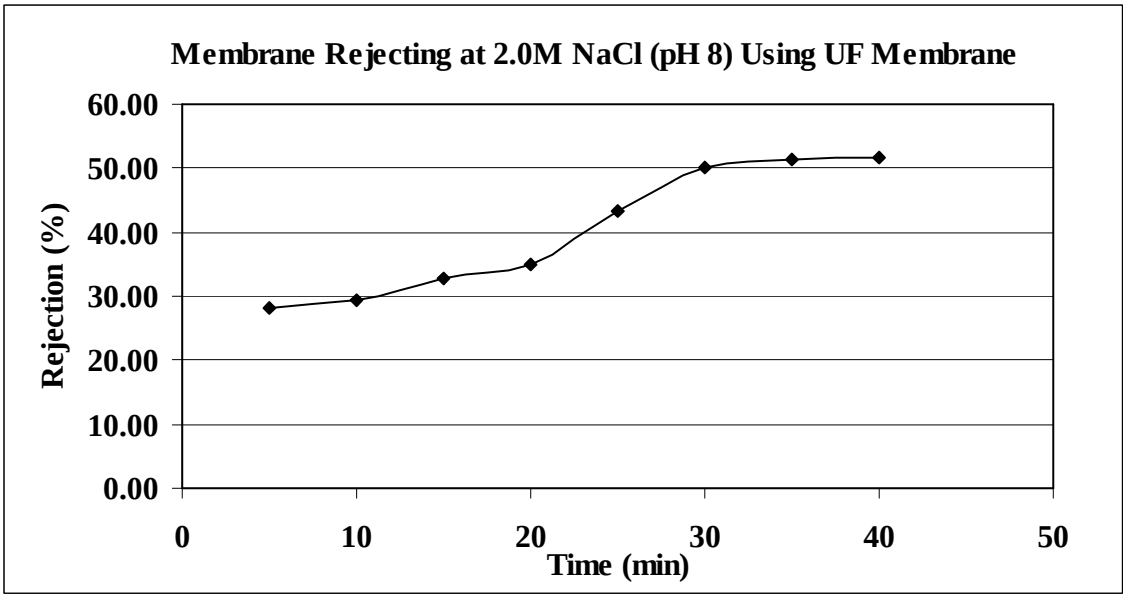

Figure 4.23: Membrane Rejecting at 2.0M NaCl (pH 8) Using UF Membrane

4.5.5 Overall Rejection of BSA at Different Ionic Strength Using UF Membrane

The figure 4.24 shows the overall rejection of BSA at different ionic strength using UF membrane. The rejection of protein molecule is increased by the time increased. The general trend of increasing protein rejection is observed for all ionic strength concentration (Salgin *et al*., 2005).

From the result also shows that, the ionic strength 2.0M have the minimum rejection protein and ionic strength 0.5M have the maximum protein rejection. So it is shows that rejection is decrease as ionic strength increases. Despite this high ionic strength the compressed electric double layers of protein and membrane result in less electrostatic interaction between the protein and the membrane. The protein is, therefore, easily deposited on the membrane forming a cake layer with serious fouling and making the steady-state flux very low (Tung *et al*., 2007).

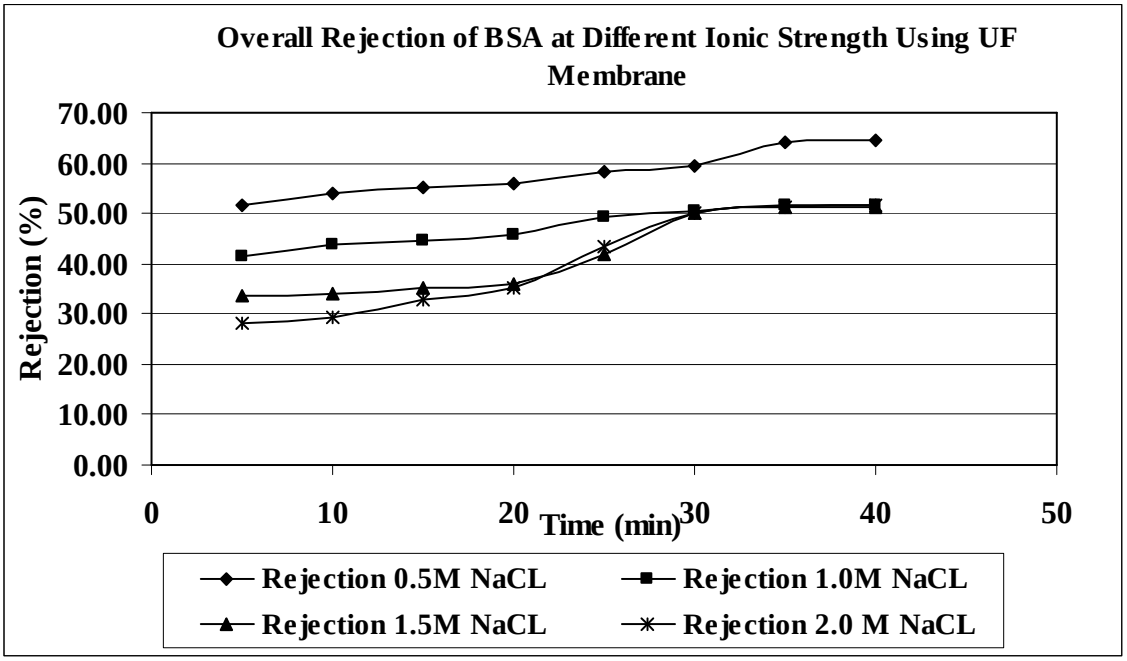

Figure 4.24: Overall Rejection of BSA at Different Ionic Strength Using UF Membrane

CHAPTER 5

CONCLUSION

5.1 Conclusion

From the result that being observed, in order to get optimum condition of filtration, the flux must be relatively maximum. So, the pH that fulfills this optimum condition is pH 8, which is give maximum flux for BSA protein filtration.

The percent of the rejection shows that the amount of protein retained on the membrane. To get the optimum flux which is optimum protein through the membrane, the rejection of the protein should be the least among all of the pH solution (Mo *et al*., 2008). So, from the result that being achieved shows that pH 8 show the least protein rejection.

The salt concentrations are found to strongly influence membrane fouling in protein ultra filtration. Generally, at low electrolyte, high rejection of protein is observed. As salt concentration increased, the protein rejection is decreases (Mo *et al*., 2008). From the result achieved, the 0.5 M NaCl in pH 8 solution give the optimum condition compare with others ionic strength protein solution , which is give high flux of protein filtration.

5.2 Recommendations and Future Work

There will be a few suggestions that might be used in order to improve this research in future. The suggestion would be:

1) This method can be applied for other type of complex sample such as BSA from egg.
2) The study for protein mechanism by using Scanning Electron Microscopic could be including in future.
3) For future work, by using the home made membrane, the comparison can be perform in order to know which of them is effective and give the better result.

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APPENDIX A

Appendix A1: Flux Decline during BSA Separation at pH 5

Time (minute)	Volume (cm3)	Flux
0.5	28.6	0.0520
1.0	27.0	0.0491
1.5	25.0	0.0455
2.0	24.0	0.0436
2.5	24.0	0.0436
3.0	24.0	0.0436
3.5	23.0	0.0418
4.0	23.0	0.0418
4.5	24.0	0.0436
5.0	23.0	0.0418
5.5	23.5	0.0427
6.0	23.0	0.0418
6.5	23.0	0.0418
7.0	23.0	0.0418
7.5	23.0	0.0418
8.0	23.0	0.0418
8.5	23.5	0.0427
9.0	23.0	0.0418
9.5	23.5	0.0427
10.0	24.0	0.0436

10.5	23.0	0.0418
11.0	23.0	0.0418
11.5	23.5	0.0427
12.0	23.0	0.0418
12.5	23.0	0.0418
13.0	23.0	0.0418
13.5	23.0	0.0418
14.0	23.0	0.0418
14.5	23.5	0.0427
15.0	24.0	0.0418
15.5	23.5	0.0427
16.0	23.0	0.0418
16.5	24.0	0.0436
17.0	23.0	0.0418
17.5	24.0	0.0436
18.0	23.0	0.0418
18.5	23.5	0.0427
19.0	23.0	0.0418
19.5	23.0	0.0418
20.0	23.0	0.0418
20.5	24.0	0.0436
21.0	23.5	0.0427
21.5	24.5	0.0445
22.0	23.0	0.0418
22.5	24.0	0.0436
23.0	24.0	0.0436
23.5	24.0	0.0436
24.0	23.5	0.0427
24.5	25.0	0.0455
25.0	24.0	0.0436
25.5	24.5	0.0445
26.0	24.5	0.0445

26.5	24.5	0.0445
27.0	24.0	0.0436
27.5	24.0	0.0436
28.0	24.5	0.0445
28.5	26.0	0.0473
29.0	26.0	0.0473
29.5	25.0	0.0455
30.0	25.0	0.0455
30.5	25.0	0.0455
31.0	25.0	0.0455
31.5	25.0	0.0455
32.0	25.0	0.0455
32.5	25.0	0.0455
33.0	25.0	0.0455
33.5	25.0	0.0455
34.0	25.5	0.0464
34.5	25.0	0.0455
35.0	25.5	0.0464
35.5	24.5	0.0445
36.0	24.5	0.0445
36.5	24.0	0.0436
37.0	24.0	0.0436
37.5	24.0	0.0436
38.0	24.0	0.0436
38.5	24.0	0.0436
39.0	24.0	0.0436
39.5	24.0	0.0436
40.0	24.0	0.0436

Appendix A2: Flux Decline during BSA Separation at pH 6

Time	Volume (cm3)	Flux
(minute)	36.0	(cm/min)
0.5	35.5	0.0655
1.0	35.0	0.0645
1.5	36.0	0.0636
2.0	35.0	0.0655
2.5	34.0	0.0636
3.0	34.0	0.0618
3.5	33.0	0.0618
4.0	33.0	0.0600
4.5	32.0	0.0600
5.0	33.0	0.0582
5.5	31.0	0.0600
6.0	30.0	0.0564
6.5	30.5	0.0545
7.0	31.0	0.0555
7.5	30.0	0.0564
8.0	30.0	0.0545
8.5	30.0	0.0545
9.0	30.0	0.0545
9.5	30.0	0.0545
10.0	30.0	0.0545
10.5	29.5	0.0545
11.0	30.0	0.0536
11.5	29.0	0.0545
12.0	29.5	0.0527
12.5	28.0	0.0536
13.0	27.5	0.0509

13.5	27.0	0.0500
14.0	28.0	0.0491
14.5	26.0	0.0509
15.0	28.0	0.0473
15.5	26.5	0.0509
16.0	27.0	0.0482
16.5	26.5	0.0491
17.0	27.0	0.0482
17.5	26.5	0.0491
18.0	26.5	0.0482
18.5	26.5	0.0482
19.0	26.0	0.0482
19.5	26.0	0.0473
20.0	26.5	0.0473
20.5	27.0	0.0482
21.0	26.5	0.0491
21.5	26.0	0.0482
22.0	26.0	0.0473
22.5	26.0	0.0473
23.0	26.0	0.0473
23.5	26.0	0.0473
24.0	26.5	0.0473
24.5	25.0	0.0482
25.0	26.0	0.0455
25.5	26.0	0.0473
26.0	26.5	0.0473
26.5	26.5	0.0482
27.0	26.0	0.0482
27.5	26.0	0.0473
28.0	26.0	0.0473
28.5	25.0	0.0473

29.0	26.0	0.0455
29.5	25.0	0.0473
30.0	26.5	0.0455
30.5	25.5	0.0482
31.0	25.5	0.0464
31.5	25.5	0.0464
32.0	26.0	0.0464
32.5	25.0	0.0473
33.0	25.0	0.0455
33.5	25.0	0.0455
34.0	26.0	0.0455
34.5	25.5	0.0473
35.0	25.0	0.0464
35.5	25.0	0.0455
36.0	25.0	0.0455
36.5	25.0	0.0455
37.0	25.0	0.0455
37.5	25.0	0.0455
38.0	26.0	0.0455
38.5	25.5	0.0473
39.0	26.0	0.0464
39.5	25.0	0.0473
40.0	36.0	0.0455

Appendix A3: Flux Decline during BSA Separation at pH 7

Time (minute)	Volume(cm3)	flux (cm/min)
0.5	36.0	0.0655
1.0	35.0	0.0636
1.5	34.0	0.0618
2.0	34.0	0.0618
2.5	34.0	0.0618
3.0	34.0	0.0618
3.5	34.0	0.0618
4.0	33.0	0.0600
4.5	33.0	0.0600
5.0	34.0	0.0618
5.5	33.0	0.0600
6.0	34.0	0.0618
6.5	33.0	0.0600
7.0	33.5	0.0609
7.5	32.0	0.0582
8.0	33.0	0.0600
8.5	33.0	0.0600
9.0	34.0	0.0618
9.5	33.5	0.0609
10.0	33.0	0.0600
10.5	32.5	0.0591
11.0	31.0	0.0564
11.5	32.0	0.0582
12.0	33.0	0.0600
12.5	33.5	0.0609
13.0	32.0	0.0582

13.5	33.0	0.0600
14.0	32.5	0.0591
14.5	32.0	0.0582
15.0	32.0	0.0582
15.5	33.0	0.0600
16.0	32.0	0.0582
16.5	33.0	0.0600
17.0	33.0	0.0600
17.5	33.0	0.0600
18.0	32.0	0.0582
18.5	33.0	0.0600
19.0	32.0	0.0582
19.5	33.0	0.0600
20.0	32.0	0.0582
20.5	32.0	0.0582
21.0	32.0	0.0582
21.5	33.0	0.0600
22.0	33.0	0.0600
22.5	33.0	0.0600
23.0	32.0	0.0582
23.5	33.0	0.0600
24.0	33.0	0.0600
24.5	33.0	0.0600
25.0	33.0	0.0600
25.5	33.0	0.0600
26.0	31.5	0.0573
26.5	30.0	0.0545
27.0	30.0	0.0545
27.5	30.0	0.0545
28.0	29.5	0.0536

28.5	30.0	0.0545
29.0	29.0	0.0527
29.5	30.0	0.0545
30.0	29.0	0.0527
30.5	30.0	0.0545
31.0	29.0	0.0527
31.5	30.0	0.0545
32.0	29.0	0.0527
32.5	29.0	0.0527
33.0	28.5	0.0518
33.5	30.5	0.0555
34.0	29.0	0.0527
34.5	29.0	0.0527
35.0	30.0	0.0545
35.5	29.0	0.0527
36.0	30.0	0.0545
36.5	30.0	0.0545
37.0	30.0	0.0545
37.5	30.0	0.0545
38.0	30.0	0.0545
38.5	30.0	0.0545
39.0	30.0	0.0545
39.5	30.0	0.0545
40.0	30.0	0.0545

Appendix A4: Flux Decline during BSA Separation at pH 8

Time (minute)	Volume(cm3)	Flux (cm/min)
0.5	42.0	0.0764
1.0	41.0	0.0745
1.5	41.0	0.0745
2.0	41.0	0.0745
2.5	41.0	0.0745
3.0	40.0	0.0727
3.5	40.0	0.0727
4.0	40.0	0.0727
4.5	39.0	0.0709
5.0	38.0	0.0691
5.5	38.5	0.0700
6.0	38.0	0.0691
6.5	38.0	0.0691
7.0	38.0	0.0691
7.5	38.0	0.0691
8.0	37.0	0.0673
8.5	37.0	0.0673
9.0	37.0	0.0673
9.5	37.0	0.0673
10.0	38.0	0.0691
10.5	38.0	0.0691
11.0	36.0	0.0655
11.5	37.0	0.0673
12.0	36.0	0.0655
12.5	36.0	0.0655
13.0	36.0	0.0655
13.5	36.0	0.0655
14.0	36.0	0.0655
14.5	37.0	0.0673

15.0	36.0	0.0655
15.5	37.0	0.0673
16.0	37.0	0.0673
16.5	37.0	0.0673
17.0	37.5	0.0682
17.5	38.0	0.0691
18.0	37.0	0.0673
18.5	38.0	0.0691
19.0	37.0	0.0673
19.5	37.0	0.0673
20.0	37.0	0.0673
20.5	37.0	0.0673
21.0	36.0	0.0655
21.5	37.0	0.0673
22.0	37.0	0.0673
22.5	37.0	0.0673
23.0	36.0	0.0655
23.5	36.5	0.0664
24.0	36.0	0.0655
24.5	37.0	0.0673
25.0	38.0	0.0691
25.5	38.0	0.0691
26.0	35.0	0.0636
26.5	35.0	0.0636
27.0	36.0	0.0655
27.5	36.0	0.0655
28.0	35.0	0.0636
28.5	36.0	0.0655
29.0	35.0	0.0636
29.5	35.0	0.0636
30.0	35.0	0.0636
30.5	37.0	0.0673
31.0	36.0	0.0655

31.5	37.0	0.0673
32.0	36.0	0.0655
32.5	36.0	0.0655
33.0	34.0	0.0618
33.5	34.0	0.0618
34.0	34.0	0.0618
34.5	34.0	0.0618
35.0	35.0	0.0636
35.5	34.5	0.0627
36.0	34.5	0.0627
36.5	34.5	0.0627
37.0	34.0	0.0618
37.5	34.0	0.0618
38.0	34.0	0.0618
38.5	34.0	0.0618
39.0	34.0	0.0618
39.5	34.0	0.0618
40.0	34.0	0.0618

APPENDIX B

Appendix B1: Flux Decline during BSA Separation at Ionic Strength 0.5 M NaCl

Time (min)	Volume (cm3)	flux (cm/min)
0.5	44.0	0.0800
1.0	42.0	0.0764
1.5	40.0	0.0727
2.0	39.8	0.0723
2.5	38.8	0.0705
3.0	38.0	0.0691
3.5	39.8	0.0723
4.0	40.0	0.0727
4.5	37.0	0.0673
5.0	39.0	0.0709
5.5	39.0	0.0709
6.0	38.0	0.0691
6.5	39.0	0.0709
7.0	37.0	0.0673
7.5	37.0	0.0673
8.0	37.0	0.0673
8.5	36.0	0.0655
9.0	36.5	0.0664
9.5	37.0	0.0673
10.0	35.0	0.0636

10.5	35.5	0.0645
11.0	37.0	0.0673
11.5	34.5	0.0627
12.0	35.0	0.0636
12.5	36.0	0.0655
13.0	36.0	0.0655
13.5	36.0	0.0655
14.0	35.5	0.0645
14.5	35.0	0.0636
15.0	35.0	0.0636
15.5	35.5	0.0645
16.0	35.0	0.0636
16.5	35.5	0.0645
17.0	35.0	0.0636
17.5	35.5	0.0645
18.0	35.5	0.0645
18.5	35.0	0.0636
19.0	35.8	0.0650
19.5	34.8	0.0632
20.0	35.0	0.0636
20.5	34.8	0.0632
21.0	37.0	0.0673
21.5	37.0	0.0673
22.0	35.0	0.0636
22.5	36.0	0.0655
23.0	34.5	0.0627
23.5	34.5	0.0627
24.0	33.0	0.0600
24.5	35.5	0.0645
25.0	35.0	0.0636
25.5	34.5	0.0627
26.0	34.0	0.0618

26.5	34.0	0.0618
27.0	34.5	0.0627
27.5	34.8	0.0632
28.0	35.0	0.0636
28.5	36.0	0.0655
29.0	35.0	0.0636
29.5	33.5	0.0609
30.0	34.0	0.0618
30.5	35.0	0.0636
31.0	34.0	0.0618
31.5	34.5	0.0627
32.0	33.8	0.0614
32.5	34.5	0.0627
33.0	34.0	0.0618
33.5	33.0	0.0600
34.0	33.8	0.0614
34.5	34.0	0.0618
35.0	34.5	0.0627
35.5	33.5	0.0609
36.0	34.0	0.0618
36.5	34.0	0.0618
37.0	33.5	0.0609
37.5	33.0	0.0600
38.0	33.0	0.0600
38.5	33.0	0.0600
39.0	33.0	0.0600
39.5	33.0	0.0600
40.0	33.0	0.0600

Appendix B2: Flux Decline during BSA Separation at Ionic Strength 1.0 M NaCl

Time (min)	Volume (cm3)	Flux (cm/min)
0.5	41.0	0.0745
1.0	39.0	0.0709
1.5	38.0	0.0691
2.0	39.5	0.0718
2.5	38.0	0.0691
3.0	38.0	0.0691
3.5	37.0	0.0673
4.0	36.0	0.0655
4.5	35.0	0.0636
5.0	35.0	0.0636
5.5	35.0	0.0636
6.0	35.0	0.0636
6.5	34.0	0.0618
7.0	33.0	0.0600
7.5	34.0	0.0618
8.0	33.0	0.0600
8.5	33.0	0.0600
9.0	33.5	0.0609
9.5	33.0	0.0600
10.0	33.0	0.0600
10.5	34.0	0.0618
11.0	32.0	0.0582
11.5	32.5	0.0591
12.0	33.0	0.0600
12.5	32.0	0.0582
13.0	33.0	0.0600
13.5	32.8	0.0595
14.0	33.0	0.0600

14.5	32.8	0.0595
15.0	34.0	0.0618
15.5	32.0	0.0582
16.0	32.0	0.0582
16.5	32.8	0.0595
17.0	32.8	0.0595
17.5	32.0	0.0582
18.0	32.5	0.0591
18.5	32.5	0.0591
19.0	32.0	0.0582
19.5	32.0	0.0582
20.0	32.5	0.0591
20.5	32.5	0.0591
21.0	32.0	0.0582
21.5	32.0	0.0582
22.0	32.0	0.0582
22.5	32.0	0.0582
23.0	32.8	0.0595
23.5	32.0	0.0582
24.0	32.0	0.0582
24.5	32.0	0.0582
25.0	32.5	0.0591
25.5	32.5	0.0591
26.0	32.0	0.0582
26.5	32.5	0.0591
27.0	31.8	0.0577
27.5	32.0	0.0582
28.0	32.0	0.0582
28.5	32.5	0.0591
29.0	31.5	0.0573
29.5	32.0	0.0582

30.0	31.5	0.0573
30.5	32.0	0.0582
31.0	32.5	0.0591
31.5	31.0	0.0564
32.0	32.0	0.0582
32.5	31.5	0.0573
33.0	32.0	0.0582
33.5	32.0	0.0582
34.0	31.5	0.0573
34.5	31.0	0.0564
35.0	32.0	0.0582
35.5	31.0	0.0564
36.0	32.0	0.0582
36.5	32.0	0.0582
37.0	31.5	0.0573
37.5	30.5	0.0555
38.0	31.5	0.0573
38.5	31.8	0.0577
39.0	31.5	0.0573
39.5	31.0	0.0564
40.0	32.8	0.0595

Appendix B3: Flux Decline during BSA Separation at Ionic Strength 1.5 M NaCl

Time (minute)	Volume (cm3)	flux (cm/min)
0.5	33.8	0.0673
1	39.0	0.0664
0.5	41.0	0.0636
1.0	39.5	0.0609
1.5	38.0	0.0600
2.0	38.0	0.0600
2.5	37.0	0.0609
3.0	36.0	0.0618
3.5	35.0	0.0618
4.0	35.0	0.0618
4.5	35.0	0.0600
5.0	35.0	0.0600
5.5	34.0	0.0582
6.0	33.0	0.0600
6.5	34.0	0.0564
7.0	33.0	0.0564
7.5	33.0	0.0573
8.0	33.5	0.0591
8.5	33.0	0.0591
9.0	33.0	0.0600
9.5	34.0	0.0555
10.0	32.0	0.0591
10.5	32.5	0.0555
11.0	33.0	0.0564
11.5	32.0	0.0582
12.0	33.0	0.0582
12.5	32.8	0.0582

13.0	33.0	0.0582
13.5	32.8	0.0573
14.0	34.0	0.0582
14.5	32.0	0.0564
15.0	32.0	0.0582
15.5	32.8	0.0600
16.0	32.8	0.0564
16.5	32.0	0.0545
17.0	32.5	0.0573
17.5	32.5	0.0582
18.0	32.0	0.0582
18.5	32.0	0.0564
19.0	32.5	0.0582
19.5	32.5	0.0582
20.0	32.0	0.0582
20.5	32.0	0.0591
21.0	32.0	0.0582
21.5	32.0	0.0573
22.0	32.8	0.0573
22.5	32.0	0.0545
23.0	32.0	0.0564
23.5	32.0	0.0582
24.0	32.5	0.0577
24.5	32.5	0.0582
25.0	32.0	0.0564
25.5	32.5	0.0582
26.0	31.8	0.0564
26.5	32.0	0.0573
27.0	32.0	0.0582
27.5	32.5	0.0564
28.0	31.5	0.0582

28.5	32.0	0.0577
29.0	31.5	0.0577
29.5	32.0	0.0573
30.0	32.5	0.0564
30.5	31.0	0.0564
31.0	32.0	0.0555
31.5	31.5	0.0564
32.0	32.0	0.0573
32.5	32.0	0.0582
33.0	31.5	0.0555
33.5	31.0	0.0555
34.0	32.0	0.0591
34.5	31.0	0.0577
35.0	32.0	0.0568
35.5	32.0	0.0573
36.0	31.5	0.0564
36.5	30.5	0.0582
37.0	31.5	0.0582
37.5	31.8	0.0582
38.0	31.5	0.0582
38.5	31.0	0.0582
39.0	32.8	0.0582
39.5	32.0	0.0582
40.0	32.0	0.0582

Appendix B4: : Flux Decline during BSA Separation at Ionic Strength 2.0 M NaCl

Time (minute)	Volume(cm3)	Flux (cm/min)
0.5	33.0	0.0600
1.0	32.0	0.0582
1.5	33.8	0.0614
2.0	34.0	0.0618
2.5	33.8	0.0614
3.0	34.0	0.0618
3.5	32.5	0.0591
4.0	32.5	0.0591
4.5	32.0	0.0582
5.0	31.5	0.0573
5.5	32.0	0.0582
6.0	31.5	0.0573
6.5	31.5	0.0573
7.0	30.0	0.0545
7.5	31.0	0.0564
8.0	30.5	0.0555
8.5	30.8	0.0559
9.0	30.5	0.0555
9.5	30.5	0.0555
10.0	30.0	0.0545
10.5	30.0	0.0545
11.0	30.5	0.0555
11.5	30.0	0.0545
12.0	30.0	0.0545
12.5	31.0	0.0564
13.0	30.0	0.0545

13.5	30.0	0.0545
14.0	30.0	0.0545
14.5	30.0	0.0545
15.0	30.5	0.0555
15.5	30.0	0.0545
16.0	30.0	0.0545
16.5	30.5	0.0555
17.0	29.0	0.0527
17.5	30.5	0.0555
18.0	30.5	0.0555
18.5	30.0	0.0545
19.0	29.3	0.0532
19.5	29.0	0.0527
20.0	28.0	0.0509
20.5	28.0	0.0509
21.0	29.0	0.0527
21.5	29.0	0.0527
22.0	29.0	0.0527
22.5	28.0	0.0509
23.0	30.0	0.0545
23.5	30.0	0.0545
24.0	29.5	0.0536
24.5	29.8	0.0536
25.0	28.0	0.0509
25.5	28.0	0.0509
26.0	27.8	0.0505
26.5	29.5	0.0536
27.0	29.0	0.0527
27.5	29.0	0.0527
28.0	29.0	0.0527
28.5	29.0	0.0527

29.0	29.3	0.0532
29.5	29.8	0.0541
30.0	28.0	0.0509
30.5	29.5	0.0536
31.0	29.5	0.0536
31.5	28.0	0.0509
32.0	29.5	0.0536
32.5	28.0	0.0509
33.0	28.0	0.0509
33.5	29.0	0.0527
34.0	28.0	0.0509
34.5	28.8	0.0523
35.0	29.0	0.0527
35.5	29.0	0.0527
36.0	29.0	0.0527
36.5	29.0	0.0527
37.0	28.0	0.0509
37.5	29.0	0.0527
38.0	30.0	0.0545
38.5	29.0	0.0527
39.0	29.0	0.0527
39.5	29.0	0.0527
40.0	29.0	0.0527