SEPARATION OF LYSOZYME USING ULTRAFILTRATION MEMBRANE, EFFECT ON pH AND IONIC STRENGTH ON FLUX AND REJECTION

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NUR SHARLINAWATI BT. MD. SAID

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

Faculty of Chemical and Natural Resources Engineering
University Malaysia Pahang

APRIL 2008

“I hereby declare that I have read this thesis and in my opinion this thesis is sufficient in terms of scope and quality for the award of the degree of Bachelor Degree of Chemical Engineering”.

Signature : .....

Supervisor’s Name : Dr. Mimi Sakinah bt. Abd. Munaim

Date :

I declare that this thesis entitled “Separation of Lysozyme using Ultrafiltration Membrane, Effect on Ionic Strength on Flux and Rejection” 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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Name	:	Nur Sharlinawati Bt. Md. Said
Date	:	…….

To beloved Mom, Pn. Pudziah bt. Shariff, Siblings and in ever loving memory of my late Dad, Hj. Md. Said b. Hj. Abd. Manas.

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ABSTRACT

The separation of solid, macro molecule and non-dissolve particle results from the fermentation process which contains the desired product is a first step to get the product for the industry. One of the methods to separate this non-dissolve is by using ultrafiltration membrane. The main objective of this research is to investigate the effect of pH and ionic strength on membrane flux and rejection as well as to determine the optimum pH and ionic strength in order to obtain high flux and high lysozyme rejection by using ultrafiltration membrane. The range of pH that has been used is between 5 until 8. From the result, the alkaline conditions of the lysozyme solution resulted maximum membrane flux and rejection. This was due to the absorption of lysozyme onto the membrane and the charges between the membrane and the lysozyme solution itself. The optimum pH for high membrane flux and rejection and rejection is pH 8 while the optimum ionic strength for membrane flux is 0.5 M NaCl.

ABSTRAK

Pemisahan bahan pepejal, bahan makromolekul dan partikel tidak larut adalah langkah pertama bagi proses fermentasi yang akan memberikan produk yang diingini oleh industri. Salah satu kaedah untuk menjalankan proses pemisahan ini adalah dengan menggunakan membran penuras ultra. Ini adalah untuk memastikan proses fermentasi dapat dijalankan dengan optimum untuk mendapatkan penolakan protein yang maksimum dan juga fluks yang maksimum. Objektif utama kajian ini adalah untuk mengkaji larutan lysozyme pada pH yang optimum dan kekuatan ionik yang optimum untuk mendapatkan nilai fluks yang maksimum. Daripada hasil kajian, didapati bahawa keadaan larutan protein yang beralkali akan memberikan nilai fluks yang maksimum dan nilai penolakan lysozyme yang maksimum iaitu pada pH 8. Untuk kekuatan ionik pula, pada kemolaran larutan NaCl ialah 0.5 M ia akan memberikan nilai fluks yang maksimum dan nilai penolakan lysozyme yang minimum. Manakala pada kemolaran larutan 2.0 M NaCl ia memberikan nilai fluks yang minimum dan nilai penolakan lysozyme yang maksimum.

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NaCl	-	Sodium Chloride
NaOH	-	Sodium Hydroxide
KH_2PO_4	-	Sodium dihydrogen phosphate
K_2HPO_4	-	Sodium hydrogen phosphate
$CuSO_4.5H_20$	-	Cuprum Sulphate
Na_2CO_3	-	Sodium Citrate
OD	-	Optical Density

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CHAPTER 1

INTRODUCTION

This chapter will briefly describe the background of the study which contained the description of the application of the ultrafiltration membrane in the industry. The problem statement will discuss about why this research is done. It is also including the objective and scope of this research which will be achieved in this research and this experiment is done under certain scope.

1.1 Background of Study

Nowadays, membrane technology is widely use as a filtration medium. The advantages of using membrane are it can separate the solution base on the molecular size; it works at ambient temperature operation which also can avoid the phase change and extreme temperature. It use a modest requirement energy which is because no phase change thus there is no latent heat. Instead of the retentate which is recyclable there no other waste product and the closed module separating operation, avoiding formation of aerosols. Reducing the risk to operator with relatively low capital and running cost. Membrane filtration offers a direct separation, eliminating the use of additives, foam fractionation, filter aid filtration and its flexibility: “Tailor made” to meet individual requirement.

There are a few types of membrane process which have been develop commercially through the membrane technology which are, the microfiltration process, ultrafiltration process, hemodialysis process, electrodialysis process, hyperfiltration process, gas separation, membrane distillation process, reverse osmosis process and pervaporation process.

1.2 Application of the Ultrafiltration Membrane Technology in the Industry

The separation process for solid such as biomass solid, the unsolved particle and the macromolecule effect from the normal fermentation usually is the 1st step in the production of product in the industry. In certain process the separation of the particle, the fermentation products have to go through a pre-treatment process to make the process easier. The examples of the pre-treatment process are treatment with heat, changes in pH of the solution or addition of chemicals such as coagulation agent. For product which has miscible particles as enzyme, it has to be separated from the solution before the purity process of the product. The separation process can be done by using vacuum, filtration device, microfiltration or Ultrafiltration and also by using coagulation agent and flocculation agent (Anderson *et al*., 1981).

The filtration method is the most effective way from the cost aspects and its separation efficiency in separating large size particle or cell from the fermentation process. This technique is getting improvised and started to get attention to replace the conventional method of separation. The cross flow filtration is the most suitable membrane to be use to separate the large immiscible particle in the solution (Tung *et al.*, 2007).

However, while the filtration process is done, there is possibility of the product such as protein will be filtrate in a certain condition. It is important that to find an optimum condition for the filtration process to be complete at maximum value of flux of permeate.

1.3 Problem Statement

The chemical process industries are faced with an increasingly competitive environment, ever-changing market conditions, and government regulations. Yet, they still must increase productivity and profitability. Bottom line performance can be adversely affected by many factors, such as production economies and product quality. Many of these factors are extremely complex and subject to varying degrees of unpredictability. The concentrated lysozyme are mostly used in pharmaceutical industry to facilitate bioseparation steps such as salt and solvent induced precipitation, vaccine, monoclonal antibody, facilitate detection and for the analysis.

Thus, this research is done to investigate the effects of pH and ionic strength on membrane flux and rejection of lysozyme in the industry by using different approach and also to determine the optimum pH and ionic strength in order to obtain high flux and high protein rejection by using Ultrafiltration membrane. In the other hand, by using membrane, it will reduce the usage of chemicals which will contribute to pollution.

1.4 Objective

The objective of this research is to investigate:

(i) To investigate the effect of pH and ionic strength on membrane flux and rejection.

(ii) To determine the optimum pH and ionic strength in order to obtain high flux and high protein rejection.

1.5 Scope of Research

This experiment will be done under these scopes of experimental, which are:

(i) The lysozyme solution will be prepared in 4 samples of pH which are pH 5, pH 6, pH 7 and pH 8.

(ii) The protein which is used is Lysozyme protein which has 14.4 kDa number of molecular cut off.

(iii) The pressure as a driving force is 0.95 bar.

(iv) The wavelength that used in order to obtain the optical density (OD) is at 750 nm

(v) The speed of the rotary is 275 rpm.

(vi) The experiment is done in room temperature which is 27°C.

(vii) The membrane is made from polyethersulfone.

(viii) The method that will be used is cross flow filtration.

CHAPTER 2

LITERATURE REVIEW

This chapter will describe detail about the basic concepts of membrane separation technology, background of the membrane and type of membrane which will be use in the experiment (ultrafiltration membrane). It will cover it characteristic, the filtration mechanism and the factor which can affect it process. This chapter also will explain about the protein (lysozyme) background and it classification. Each of them is quoted from previous research which had been done. Even though the research is using different method, material and equipment but at some point it does give some useful information. The previous literature review is done base on 10 previous journals which has been extracted.

2.1 Membrane Definition

Membrane comes from the original word: “membrana “(Latin, which mean skin). The other definition of membrane are a selective barrier between two phases, a thin barrier that permits selective mass transport, a phase that acts as a barrier to prevent mass movement, but allows restricted and / or regulated passage of one or more species (Phillips, 1986).

Membrane is a thin layer which allows smaller molecule liquid or gas than its pore size to pass through it. These pores size normally measured in Armstrong scale or

micron (1 micron = 10 000 Armstrong). The thickness of the membrane usually is between 100 nm until a few centimetres over. The membrane layer is supported by a supported layer which is strong and thick. These limited routes of membrane only allow selected liquid or gas which means the other particle could not get into this membrane. The separation through membrane is effected by absorption, convection, concentration, pressure, the charge value of the solution and the temperature (Balasubramaniam, 2003).

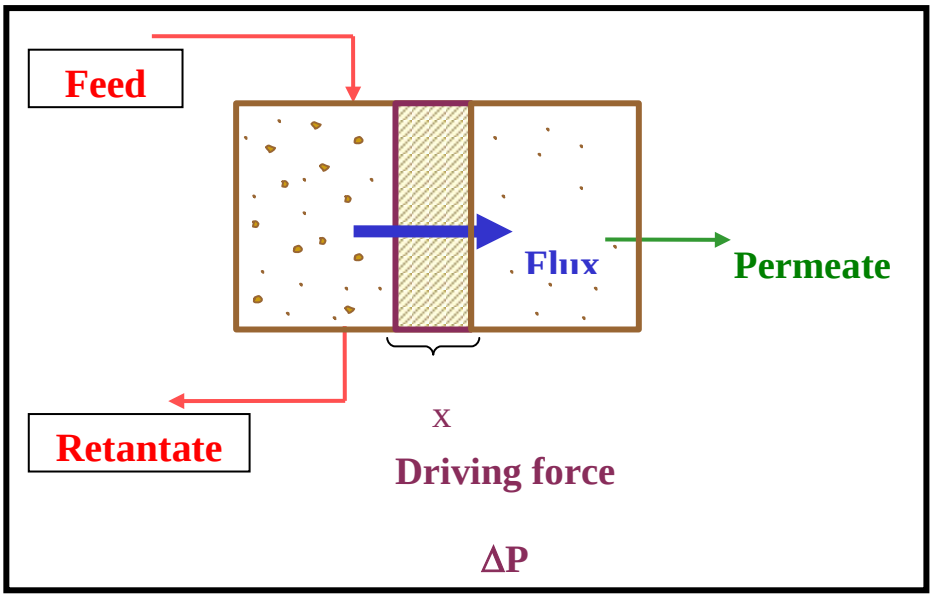

Figure 2.1: The Basic Membrane Separation Process

2.1.1 Driving Force Condition in Membrane Separation Process

In using membrane as a medium separation, it's only the pressure which will cause the flux. There is hydrostatic pressure, concentration and different ionic strength.

2.1.2 Hydrostatic Pressure

The difference of the hydrostatic pressure between 2 phases which is separated by the membrane will cause the flux base on the volume and will cause the separation of chemicals when the permeable hydrodynamic for membrane is different for each component (Stryer, 1981).

2.1.3 The Difference of Ionic Strength

The difference of ionic strength between 2 phases which is divided by membrane will cause the mass movements for most of chemicals when every each particle with different ionic strength will show different type of movements (Stryer, 1981).

2.1.4 The Concentration Difference

The difference of ionic strength between 2 phases which is divided by membrane will cause the mass movements for most of chemicals when every each component with different concentration will show different type of absorbance ability (Stryer, 1981).

2.2 Ultrafiltration Membrane

There are a few types of membrane in the industry and one of them is Ultrafiltration. This membrane has the range of 0.001 – 0.2 micron pore size. Its molecular weight cut off (MWCO) is only particles with molecular weight or less than MWCO pass through the membrane and emerge as permeate.

2.2.1 Characteristic of Ultrafiltration Membrane

2.2.1.1 The Symmetry

The Ultrafiltration membrane is a symmetry membrane. It will stop the miscible molecule at the surface and will trap the molecule which can pass through its pore. This membrane is different from the asymmetry membrane which in asymmetry membrane will not allow the bigger molecule to pass through the membrane and will stop at the surface. While the smaller molecule will pas through the membrane and out as permeate.

2.2.1.2 The Cross Flow Filtration

The transmembrane pressure was adjusted to 0.95 bar by a control valve with cross flow at specific velocity. All experiments were conducted at 27°C by maintaining protein solution temperature through a constant temperature water bath. The filtration continued for 50 minutes.

2.3 Definition of Protein

Protein was first found in 1838 which named fibrin, serum albumin, casein and crystalline from animal fat and 4 plant fat which are miscible albumin, lump of albumin, legumin and gluten. In 1871, 24 types of animal protein and 12 types of plant protein were found.

Around 1860’s, the development in chemical studies including the protein study had successfully produce first table of amino acid which is one of the composition in protein. The research is interminable by some researches who study about the characteristic of protein. Emil Fisher found that, the chain of amino acid is the basic structure of protein. While in the Harvard University, the researches found

the method how to separate the protein chain base on the difference and miscibility which cause by temperature change, pH value, ionic strength, dielectric constant and present of specific cation divalent.

2.3.1 The Characteristic of Protein

Protein is a natural polymer has high molecular weight in the range 6000 until a few hundred thousand Dalton. The protein structure consists of chain of amino acid which is bond by peptide. Protein is a 50% of the overall organic component in protoplasmic and it is the biggest component in live organism and also have the important usage.

Most of the protein is miscible in water, in the solution which has the medium ionic strength and the organic solvent. Half of them are immiscible at all. Protein can be denaturised by the heat and it will lose its miscibility in water. And the other half of protein does not react or lose anything even it being heated to 100°C. The miscibility of protein increases when the temperature increases.

2.3.2 The Classification of the Protein

Protein can be classified either into its chemical composition, shape or its function. The easiest and practical classification is base on either it is protein enzyme or not. Protein also can be classified into its premier structure, secondary structure, tertiary structure and the quarterly structure. Base on the chemical composition, protein is divided into simple protein and the conjugate protein.

2.3.2.1 The Classification Base on Chemical Composition

Simple proteins will results into acid amino when it's been dihydrolysis. 2 types of simple protein are:

(i) Globular protein: Miscible in liquid and dilute salt solution and has an ellipse shape. The examples are albumin, protamines, histones, prolamines, and glutelin.

(ii) Fibros protein : Immiscible in water or salt solution. It is much stabilized with acid or alkali and enzyme proteolytik.

Conjugate protein is a protein which is bond with carbohydrate, nucleic acid, lipid or phosphate. The non-protein part is known as prosthetic group and it is bond by covalent bond, heteropolar bond or coordinate bond.

2.3.2.2 The Classification Base on The Protein Structure

Protein has 3 dimensional structures which can be divided into 4 groups which are:

(i) Premier Structure: This structure consists of linear structure of amino acid chain. Its basic structure is one dimension protein which brings to the existence of the 3 structure dimension of protein and it will define the function of the protein (Walshaw, 1995).

(ii) Secondary Structure: This structure comes from the extension of polypeptide bond which happens because of the hydrogen bond between 2 residue is not separated. 2 types of this secondary structure are the helix structure and piece structure. The helix

structure consists of ά-helix or triple helix. Inside the structure of ά-helix, the hydrogen bond can occur between the carboxyl from one chain with the –NH group at another chain. In the other hand, the triple helix structure is more firm and more capable of strain (Walshaw, 1995).

(iii) Tertiary Structure: This structure is the cause of the interaction between functional group –R which separated along the chain. The folded part of the amino acid chain which is caused by this interaction formed a tertiary structure of protein. The functional group –R can be interacted by covalent bond, sulphide bond or hydrogen bond. It's also can cause the hydrophobic or hydrophilic bond interaction (Walshaw, 1995).

(iv) Quaternary Structure: This structure only found in the protein which has 1 or more polypeptide chain. The interaction between the polypeptide will affect the quaternary structure. The bonds are disulphate bond or other weak bond (Walshaw, 1995).

2.3.2.3 The Classification Base on the Usage of the Protein

Most of the protein is an enzyme which playing a role as a catalyst for the biochemical reaction to cater the metabolism to the living cell. This reaction is controlled by the modification of the activity or quantity such as the synthesis rate for the enzyme. Some of the regulator and transmitter in the reaction is also a protein such as hormone and activation molecule.

The membrane's protein is a protein which determines the intracellular concentration value for most enzyme substrate and product. While the structure of the protein give the contribution to the mechanical structure for organ and tissue such as elastin and collagen.

The remaining protein such as ovalbumin protein from white egg and casein from milk plays a role as an energy keeper. Some of the protein also acts as a carrier such as haemoglobin and serum albumin. Protein also involve in biochemical detain such as antibody and lots of protein in blood clotting.

2.3.3 Separation and Purity of the Protein

There are a few methods in the separation process and purifying protein. The characteristic of the protein can be exploiting with many separation methods. The characteristic are miscibility, molecule size and charge value (Tung *et al.*, 2007).

From the miscibility factor, one of the method that can be us is precipitation. In this method, the ionic strength, pH value, dielectric constant and temperature have to be changed. For the molecule sizing, the separation method such as dialysis, Ultrafiltration and gel filtration can be used. As for the charge value, the method which can be use such as electrophoresis and chromatography ionic separation (Tung *et al.*, 2007).

2.4 Lysozyme

Lysozyme is a protein discovered by Alexander Fleming in 1922.as early as 1921 Fleming announced that he had found a "remarkable bacteriolytic element" present in many tissues and secretions which was able to interfere with the growth of some specific bacterial colonies. This lysing element was called "lysozyme" by Fleming himself who went on studying its different characteristics and in 1922 isolated the enzyme from hen white egg, other tissues and biological secretions of living organisms. Some years later the bactericidal activity of lysozyme was widely confirmed and after 1930 many studies revealed how in nature every living organism both in the animal and plant kingdoms produces lysozyme. In order to give a proper

definition of lysozyme we have to take into consideration certain peculiar characteristics of the substance (Kuroki *et al.*, 1993).

The term “lysozyme" (or rather lysozyme considering their ubiquity and their various structural differences) refers to an enzyme with well-defined hydraulic activity. In nature are present different types of lysozyme with different characteristics according to their origin. Roughly we can distinguish between human lysozyme, which is contained in various secretions such as tears and saliva, and lysozyme present in products belonging to the animal and vegetal kingdom. Nature has provided hen egg-white with a high content of lysozyme, for the protection of yolk integrity, making albumen the preferred raw material for lysozyme industrial production. Lysozyme is extracted from hen egg-white consisting of 129 amino acid residues. The four disulfide bridges among the eight cysteine residues are essential for lysozyme activity (Kuroki *et al.*, 1993).

Lysozyme (1,4-β-N-acetylmuramidase) is an enzyme that plays an important role in the prevention of bacterial infections. It does this by attacking a specific component of certain bacterial cell walls, peptidoglycan. Peptidoglycan is composed of the repeating amino sugars, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), crosslinked by peptide bridges. Lysozyme acts by hydrolyzing the bond between NAG and NAM, increasing the bacteria's permeability and causing the bacteria to burst. Lysozyme is widely distributed in plants and animals (Biological Magnetic Resonance Data Bank, 2006).

The primary structure of lysozyme is a single polypeptide containing 129 amino acids. In physiological conditions, lysozyme is folded into a compact, globular structure with a long cleft in the protein surface. This cleft is the active site involved in binding to the bacterial carbohydrate chain and subsequently cleaving it (Biological Magnetic Resonance Data Bank, 2006).

2.5 Theory on Effects of pH Value on Filtration of Lysozyme Protein

There are two main chains in protein molecule which are the chain of carboxylic acid and amina chain. When the protein ionizes, the cis-carboxylic acid will formed carboxylate ion with negative charge. While the amina chain will formed the positive charge. Both of the charges will determine the pH value of the protein solution. If the values of both charges are equal it is known as isoelectric point (Cheetham *et al.*, 1992).

2.5.1 The Effects on Flux Product

By using the Ultrafiltration membrane to filtrate the protein, the sensitivity of flux is high towards the condition of the solution including its pH value. The flux value will change according to the pH value and it will reach its minimum value for the protein solution. The existence of the ionize component in the solution will reduce the flux rate even at the high value of pH. This means that the pH value of the flux will also increase (Kargol and Kargol, 2002).

2.5.2 The Effects on Protein Rejection

The pH value of the solution will effect the rejection for lysozyme in Ultrafiltration. The flux rejection will increase if the pH value increasing. For the lower value of pH, the protein solution and membrane are having a different charge which causes the electrostatic attraction. This will also cause the shrinking of the pore size when the protein absorb into the membrane. However, a small rejection of protein will also happen (Kargol and Kargol, 2002).

CHAPTER 3

METHODOLOGY

In this chapter, there will be an explanation about the detailed procedure that will be going through in the experiment to achieve the objective of this research.

3.1 Overall Methodology

The flowchart below shows the framework of this research.

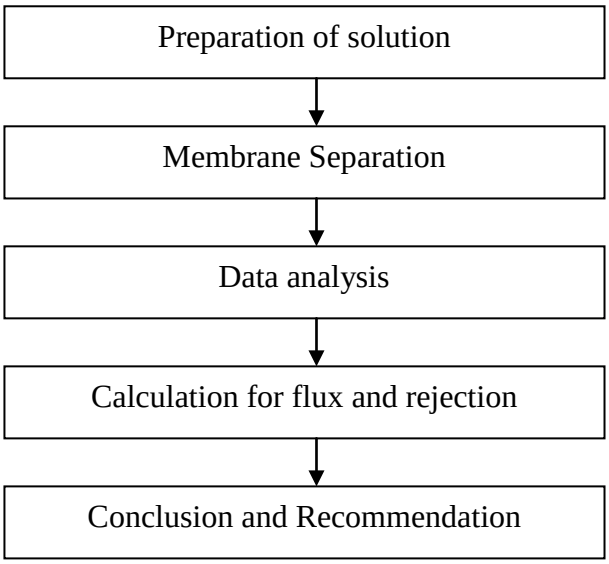

Figure 3.1: Overall Methodology

3.2 List of Apparatus

1) Cross flow Ultrafiltration with polyethersulfone membrane material.
2) UV-vis spectrometer
3) 5 beakers
4) 2 measuring cylinders
5) pH meter
6) Aluminum foil
7) 20 test tubes

3.3 List of Chemicals

1. Lysozyme
2. Sodium dihydrogen phosphate (KH_2PO_4)
3. Sodium hydrogen phosphate (K_2HPO_4)
4. Sodium hydroxide (NaOH)
5. $CuSO_4.5H_20$
6. Sodium citrate
7. Na_2CO_3

3.4 Preparation of Solution

There are few solutions need to be prepared for the experiment.

1. Potassium phosphate buffer solution
   Solution A: 27.2g KH_2PO_4 per liter
   Solution B: 45.6g K_2HPO_4 per liter

2. Preparation of pH solution

Table 3.1: Preparation of pH solution

Desired pH	Solution A (ml)	Solution B (ml)
5.0	87.7	20.3
6.0	50.0	61.0
7.0	35.3	23.0
8.0	2.0	94.0

3. Preparation of Lysozyme solution
   a. 1 g of Lysozyme + pH solution + DI water
   b. DI water was added until it reaches 4L of solution

   The solution must be kept in a refrigerator at 4C and must be used in 2 days to avoid contamination.

4. Preparation of Lowry Reagent
   a. Reagent A : 20g Na_2CO_3 + 4g NaOH to be dissolved in 1 liter DI water
   b. Reagent B : 2.5g $CuSO_4.5H_20$ + 5g sodium citrate to be dissolved in 1 liter DI water

3.5 Separation of Lysozyme using Ultrafiltration Membrane

1. The membrane must be cleaned properly before being used. Before cleaning membrane should be rinsed with buffer and water. It is highly recommended to optimize the membrane cleaning method as below;
   i. Direct the retentate and permeate lines to the feed tank. Open the feed and retentate valves. Close the permeate valve.
   ii. Circulate a minimal volume of buffer across the retentate side for 5-10 minutes at the process cross flow.

iii. Drain the system. Add a minimal volume of water and circulate water across the retentate side for 5-10 minutes at the process cross flow.

iv. Drain the system. Add cleaning solution to the feed tank at a ratio of 15-20 liters per m^2 of membrane area.

v. Open the feed and retentate valves and pump about 10% of the cleaning of the cleaning solution through permeate line to waste.

vi. Open the permeate valve, close the retentate valve and pump about 10% of the cleaning solution through permeate valves.

vii. Stop the pump and direct the retentate and permeate lines into the feed tank. Open the feed, retentate and permeate valves.

viii. Start the pump and adjust the cross the flow to the process cross flow. Circulate the cleaning solution for 30-60 minutes.

ix. Drain the cleaning solution from the system.

x. Flush the system using 15-20 liters of clean water per m3 of membrane area and the same process describe in steps 5 through 9 above. The circulation time may be reduced to 15 minutes.

Cleaning efficiency is commonly evaluated by comparing clean water fluxes pre- and post-use. It is good practice to record clean water flux after each cleaning. Commonly, users will discard membrane if the flux has reduced to 60-70 of original water flux. Note that reduced water flux often does not indicate that process fluid flux likewise be reduced.

Table 3.2 Recommended cleaning conditions

Cleaning Reagent	**Cleaning Conditions**
0.1-0.5 N sodium hydroxide	Contact time = 60 minutes Temperature = 40°C
0.5 N NaOH with 100-300 ppm	Contact time = 60 minutes
Sodium hypochlorite	Temperature = 20°C

2. pH 5 solution is added to the 10g of lysozyme and DI water is added until it reaches 4L.

3. The pressure is set to 0.95 bar.
4. The rotation speed is set to 275 rpm.
5. The solution is filtrate and the volume of permeate is recorded for every 30 seconds.
6. For every 5 minutes the sample is collected for the data analysis.
7. The data is analyzed by using UV-Vis at 750 nm.
8. The above step is repeated for pH 6, 7 and 8.

3.6 Data analysis

1. Determination of protein concentration
   i. Adding reagent A + reagent B in proportion 50:1.
   ii. Reaction mixture containing 1.0ml Lowry and 0.2ml sample was incubated at room temperature for 10 minutes
   iii. Folin-ciocalteu reagent is added about 0.1ml and left at room temperature for 30 minutes
   iv. Then the OD is measured using UV-vis
   v. Determine the concentration from calibration curve.

2. Determination of membrane flux and rejection
   i. Calculation of flux and rejection

CHAPTER 4

RESULT AND DISCUSSION

This chapter will describe about the result that have been obtained from the experiment.

4.1 Result for Calibration Curve

The calibration curve is plotted base on lysozyme concentration versus optic density (OD) at 750 nm.

Table 4.1: Calibration Curve

OD	Concentration (g/L)
0.0000	0.0000
0.0072	0.1000
0.0529	0.2000
0.1873	0.3000
1.2345	0.4000
1.2934	0.5000

4.2 Results for Lysozyme Flux at Various pH using Ultrafiltration Membrane

Flux rejection is calculated by using this equation;

$$\text{Flux} = \frac{\Delta \text{ Volume}}{\Delta \text{ t x area of membrane}}$$

4.2.1 Lysozyme Flux at pH 5 using Ultrafiltration Membrane

Table 4.2 and Figure 4.2 below shows the permeate flux for lysozyme solution at pH 5. The flux is recorded at every 30 seconds. The concentration of lysozyme solution at pH 5 is 0.25g/l. From the figure, it shows that the permeate flux will be decreasing in proportional to time. This is because the permeate flux depends on the ability of the lysozyme protein to get through the membrane pores. For pH 5, the charge of the protein will cause the membrane pores to reduce and it will lead to the low flux rate. From Figure 4.2 it shows that at 8 minutes of the flux time the permeate flux rate is constant which is from 2.59 (m/min) until 2.54 (m/min$^{)}$ and the percentage of declination is 1.93%. The value of flux achieve the steady state because of the certain static amount of lysozyme already clog the membrane pores in certain period of time and the value was not changing.

Table 4.2: Lysozyme at pH 5 using Ultrafiltration Membrane

Time (min)	V (ml)	Flux (m/min)	Permeability (m/kg.min)
0.5000	7.0000	1.2727	1.33971E-05
1.0000	15.5000	2.8182	2.96651E-05
1.5000	16.5000	3.0000	3.15789E-05
2.0000	17.0000	3.0909	3.25359E-05
2.5000	16.0000	2.9091	3.0622E-05
3.0000	16.0000	2.9091	3.0622E-05
3.5000	15.5000	2.8182	2.96651E-05
4.0000	15.5000	2.8182	2.96651E-05
4.5000	14.5000	2.6364	2.77512E-05
5.0000	15.0000	2.7273	2.87081E-05
5.5000	15.0000	2.7273	2.87081E-05
6.0000	14.7500	2.6818	2.82297E-05
6.5000	14.5000	2.6364	2.77512E-05
7.0000	14.7500	2.6818	2.82297E-05
7.5000	14.2500	2.5909	2.72727E-05
8.0000	14.2500	2.5909	2.72727E-05
8.5000	14.0000	2.5455	2.67943E-05
9.0000	14.0000	2.5455	2.67943E-05
9.5000	14.2500	2.5909	2.72727E-05
10.0000	14.0000	2.5455	2.67943E-05
10.5000	14.0000	2.5455	2.67943E-05
11.0000	14.0000	2.5455	2.67943E-05
11.5000	13.7500	2.5000	2.63158E-05
12.0000	14.0000	2.5455	2.67943E-05
12.5000	14.0000	2.5455	2.67943E-05
13.0000	14.0000	2.5455	2.67943E-05
13.5000	13.7500	2.5000	2.63158E-05
14.0000	14.0000	2.5455	2.67943E-05
14.5000	14.0000	2.5455	2.67943E-05
15.0000	13.7500	2.5000	2.63158E-05
15.5000	13.7500	2.5000	2.63158E-05
16.0000	11.5000	2.0909	2.20096E-05
16.5000	14.0000	2.5455	2.67943E-05
17.0000	14.0000	2.5455	2.67943E-05
17.5000	14.0000	2.5455	2.67943E-05
18.0000	13.5000	2.4545	2.58373E-05
18.5000	13.5000	2.4545	2.58373E-05
19.0000	13.7500	2.5000	2.63158E-05
19.5000	13.5000	2.4545	2.58373E-05
20.0000	14.0000	2.5455	2.67943E-05
20.5000	14.0000	2.5455	2.67943E-05
21.0000	13.5000	2.4545	2.58373E-05
21.5000	13.7500	2.5000	2.63158E-05
22.0000	13.5000	2.4545	2.58373E-05
22.5000	13.5000	2.4545	2.58373E-05
23.0000	13.7500	2.5000	2.63158E-05
23.5000	14.0000	2.5455	2.67943E-05
24.0000	13.0000	2.3636	2.48804E-05
24.5000	13.5000	2.4545	2.58373E-05

25.0000	13.2500	2.4091	2.53589E-05
25.5000	13.7500	2.5000	2.63158E-05
26.0000	13.5000	2.4545	2.58373E-05
26.5000	13.0000	2.3636	2.48804E-05
27.0000	13.2500	2.4091	2.53589E-05
27.5000	13.5000	2.4545	2.58373E-05
28.0000	13.2500	2.4091	2.53589E-05
28.5000	13.7500	2.5000	2.63158E-05
29.0000	13.2500	2.4091	2.53589E-05
29.5000	13.5000	2.4545	2.58373E-05
30.0000	13.5000	2.4545	2.58373E-05
30.5000	13.5000	2.4545	2.58373E-05
31.0000	13.5000	2.4545	2.58373E-05
31.5000	13.5000	2.4545	2.58373E-05
32.0000	13.5000	2.4545	2.58373E-05
32.5000	13.0000	2.3636	2.48804E-05
33.0000	13.5000	2.4545	2.58373E-05
33.5000	13.5000	2.4545	2.58373E-05
34.0000	13.5000	2.4545	2.58373E-05
34.5000	13.7500	2.5000	2.63158E-05
35.0000	13.0000	2.3636	2.48804E-05
35.5000	13.5000	2.4545	2.58373E-05
36.0000	13.5000	2.4545	2.58373E-05
36.5000	13.7500	2.5000	2.63158E-05
37.0000	13.2500	2.4091	2.53589E-05
37.5000	13.2500	2.4091	2.53589E-05
38.0000	13.5000	2.4545	2.58373E-05
38.5000	13.5000	2.4545	2.58373E-05
39.0000	13.5000	2.4545	2.58373E-05
39.5000	13.2500	2.4091	2.53589E-05
40.0000	13.7500	2.5000	2.63158E-05
40.5000	13.5000	2.4545	2.58373E-05
41.0000	13.5000	2.4545	2.58373E-05
41.5000	13.7500	2.5000	2.63158E-05
42.0000	13.0000	2.3636	2.48804E-05
42.5000	13.5000	2.4545	2.58373E-05
43.0000	13.7500	2.5000	2.63158E-05
43.5000	13.2500	2.4091	2.53589E-05
44.0000	13.0000	2.3636	2.48804E-05
44.5000	13.0000	2.3636	2.48804E-05
45.0000	13.0000	2.3636	2.48804E-05
45.5000	13.0000	2.3636	2.48804E-05
46.0000	13.0000	2.3636	2.48804E-05
46.5000	13.0000	2.3636	2.48804E-05
47.0000	13.5000	2.4545	2.58373E-05
47.5000	13.0000	2.3636	2.48804E-05
48.0000	13.0000	2.3636	2.48804E-05
48.5000	13.0000	2.3636	2.48804E-05
49.0000	13.0000	2.3636	2.48804E-05
49.5000	13.2500	2.4091	2.53589E-05
50.0000	13.0000	2.3636	2.48804E-05

4.2.2 Lysozyme Flux at pH 6 using Ultrafiltration Membrane

Table 4.3 and Figure 4.3 below shows the permeate flux for lysozyme solution at pH 6. The flux is recorded at every 30 seconds. The concentration of lysozyme solution at pH 6 is 0.25g/l. From the figure, it shows that the permeate flux will be decreasing in proportional to time. This is because the permeate flux depends on the ability of the lysozyme protein to get through the membrane pores. For pH 6, the charge of the protein will cause the membrane pores to reduce and it will lead to the low flux rate but it is a little bit higher than pH 5. From Figure 4.3 it shows the area of steady state which at 27.5 minutes of flux time the permeate flux rate is constant which is from 1.63 (m/min) until 1.54 (m/min) and the percentage of declination is about 1.63%. The value of flux achieve the steady state because of the certain static amount of lysozyme already clog the membrane pores in certain period of time and the value was not changing but it is a little less than pH 5 because of the presence of the positive charge in the solution itself.

Table 4.3: Lysozyme at pH 6 using Ultrafiltration Membrane

Time (min)	V (ml)	Flux (m/min)	Permeability (m/kg.min)
0.5000	18.0000	3.2727	3.44498E-05
1.0000	20.0000	3.6364	3.82775E-05
1.5000	12.0000	2.1818	2.29665E-05
2.0000	16.0000	2.9091	3.0622E-05
2.5000	15.0000	2.7273	2.87081E-05
3.0000	14.0000	2.5455	2.67943E-05
3.5000	14.0000	2.5455	2.67943E-05
4.0000	14.0000	2.5455	2.67943E-05
4.5000	14.0000	2.5455	2.67943E-05
5.0000	13.0000	2.3636	2.48804E-05
5.5000	13.0000	2.3636	2.48804E-05
6.0000	13.0000	2.3636	2.48804E-05
6.5000	13.0000	2.3636	2.48804E-05
7.0000	13.5000	2.4545	2.58373E-05
7.5000	12.0000	2.1818	2.29665E-05
8.0000	12.0000	2.1818	2.29665E-05
8.5000	11.0000	2.0000	2.10526E-05
9.0000	12.0000	2.1818	2.29665E-05
9.5000	11.0000	2.0000	2.10526E-05
10.0000	11.5000	2.0909	2.20096E-05
10.5000	11.0000	2.0000	2.10526E-05
11.0000	11.5000	2.0909	2.20096E-05
11.5000	11.0000	2.0000	2.10526E-05
12.0000	11.0000	2.0000	2.10526E-05
12.5000	11.0000	2.0000	2.10526E-05
13.0000	10.5000	1.9091	2.00957E-05
13.5000	10.5000	1.9091	2.00957E-05
14.0000	11.0000	2.0000	2.10526E-05
14.5000	10.0000	1.8182	1.91388E-05
15.0000	11.0000	2.0000	2.10526E-05
15.5000	10.0000	1.8182	1.91388E-05
16.0000	11.0000	2.0000	2.10526E-05
16.5000	10.5000	1.9091	2.00957E-05
17.0000	10.0000	1.8182	1.91388E-05
17.5000	10.5000	1.9091	2.00957E-05
18.0000	10.5000	1.9091	2.00957E-05
18.5000	10.0000	1.8182	1.91388E-05
19.0000	10.5000	1.9091	2.00957E-05
19.5000	10.5000	1.9091	2.00957E-05
20.0000	10.0000	1.8182	1.91388E-05
20.5000	10.0000	1.8182	1.91388E-05
21.0000	10.5000	1.9091	2.00957E-05
21.5000	10.5000	1.9091	2.00957E-05
22.0000	10.0000	1.8182	1.91388E-05
22.5000	10.0000	1.8182	1.91388E-05
23.0000	10.0000	1.8182	1.91388E-05
23.5000	10.5000	1.9091	2.00957E-05
24.0000	10.0000	1.8182	1.91388E-05
24.5000	10.0000	1.8182	1.91388E-05

25.0000	10.0000	1.8182	1.91388E-05
25.5000	6.5000	1.1818	1.24402E-05
26.0000	8.5000	1.5455	1.62679E-05
26.5000	9.5000	1.7273	1.81818E-05
27.0000	8.5000	1.5455	1.62679E-05
27.5000	9.0000	1.6364	1.72249E-05
28.0000	9.0000	1.6364	1.72249E-05
28.5000	9.0000	1.6364	1.72249E-05
29.0000	9.0000	1.6364	1.72249E-05
29.5000	9.0000	1.6364	1.72249E-05
30.0000	9.0000	1.6364	1.72249E-05
30.5000	9.0000	1.6364	1.72249E-05
31.0000	9.2500	1.6818	1.77033E-05
31.5000	9.0000	1.6364	1.72249E-05
32.0000	9.0000	1.6364	1.72249E-05
32.5000	9.0000	1.6364	1.72249E-05
33.0000	9.2500	1.6818	1.77033E-05
33.5000	9.0000	1.6364	1.72249E-05
34.0000	9.2500	1.6818	1.77033E-05
34.5000	9.2500	1.6818	1.77033E-05
35.0000	9.0000	1.6364	1.72249E-05
35.5000	9.0000	1.6364	1.72249E-05
36.0000	9.5000	1.7273	1.81818E-05
36.5000	8.5000	1.5455	1.62679E-05
37.0000	9.2500	1.6818	1.77033E-05
37.5000	9.0000	1.6364	1.72249E-05
38.0000	9.0000	1.6364	1.72249E-05
38.5000	9.0000	1.6364	1.72249E-05
39.0000	9.0000	1.6364	1.72249E-05
39.5000	9.0000	1.6364	1.72249E-05
40.0000	9.2500	1.6818	1.77033E-05
40.5000	9.0000	1.6364	1.72249E-05
41.0000	9.2500	1.6818	1.77033E-05
41.5000	9.0000	1.6364	1.72249E-05
42.0000	9.5000	1.7273	1.81818E-05
42.5000	8.5000	1.5455	1.62679E-05
43.0000	9.0000	1.6364	1.72249E-05
43.5000	10.0000	1.8182	1.91388E-05
44.0000	8.5000	1.5455	1.62679E-05
44.5000	9.0000	1.6364	1.72249E-05
45.0000	9.2500	1.6818	1.77033E-05
45.5000	9.0000	1.6364	1.72249E-05
46.0000	9.2500	1.6818	1.77033E-05
46.5000	9.0000	1.6364	1.72249E-05
47.0000	9.0000	1.6364	1.72249E-05
47.5000	9.5000	1.7273	1.81818E-05
48.0000	9.0000	1.6364	1.72249E-05
48.5000	8.7500	1.5909	1.67464E-05
49.0000	9.0000	1.6364	1.72249E-05
49.5000	9.0000	1.6364	1.72249E-05
50.0000	9.5000	1.7273	1.81818E-05

4.2.3 Lysozyme Flux at pH 7 using Ultrafiltration Membrane

Table 4.4 and Figure 4.4 below shows the permeate flux for lysozyme solution at pH 7. The flux is recorded at every 30 seconds. The concentration of lysozyme solution at pH 7 is 0.25g/l. From the figure, it shows that the permeate flux will be decreasing in proportional to time. At this point the protein solution is in neutral phase and there are no changes for buffer solution. From Figure 4.4 it shows that at 25 minutes of flux time the permeate flux rate is constant which is from 1.90 (m/min) until 1.72 (m/min) and the percentage of declination is 9.47%. The value of flux achieve the steady state because of the certain static amount of lysozyme already clog the membrane pores in certain period of time and the value was not changing.

Table 4.4: Lysozyme at pH 7 using Ultrafiltration Membrane

Time (min)	V (ml)	Flux (m/min)	Permeability (m/kg.min)
0.5000	10.0000	1.8182	1.91388E-05
1.0000	11.5000	2.0909	2.20096E-05
1.5000	12.5000	2.2727	2.39234E-05
2.0000	11.5000	2.0909	2.20096E-05
2.5000	12.0000	2.1818	2.29665E-05
3.0000	12.0000	2.1818	2.29665E-05
3.5000	12.5000	2.2727	2.39234E-05
4.0000	12.0000	2.1818	2.29665E-05
4.5000	12.0000	2.1818	2.29665E-05
5.0000	12.0000	2.1818	2.29665E-05
5.5000	12.0000	2.1818	2.29665E-05
6.0000	11.5000	2.0909	2.20096E-05
6.5000	11.0000	2.0000	2.10526E-05
7.0000	11.5000	2.0909	2.20096E-05
7.5000	11.5000	2.0909	2.20096E-05
8.0000	11.0000	2.0000	2.10526E-05
8.5000	11.5000	2.0909	2.20096E-05
9.0000	11.5000	2.0909	2.20096E-05
9.5000	11.7500	2.1364	2.2488E-05
10.0000	11.5000	2.0909	2.20096E-05
10.5000	11.2500	2.0455	2.15311E-05
11.0000	11.5000	2.0909	2.20096E-05
11.5000	11.2500	2.0455	2.15311E-05
12.0000	11.2500	2.0455	2.15311E-05
12.5000	11.0000	2.0000	2.10526E-05
13.0000	11.2500	2.0455	2.15311E-05
13.5000	11.2500	2.0455	2.15311E-05
14.0000	11.0000	2.0000	2.10526E-05
14.5000	11.0000	2.0000	2.10526E-05
15.0000	11.0000	2.0000	2.10526E-05
15.5000	11.2500	2.0455	2.15311E-05
16.0000	11.2500	2.0455	2.15311E-05
16.5000	11.5000	2.0909	2.20096E-05
17.0000	11.5000	2.0909	2.20096E-05
17.5000	11.2500	2.0455	2.15311E-05
18.0000	11.2500	2.0455	2.15311E-05
18.5000	11.0000	2.0000	2.10526E-05
19.0000	11.2500	2.0455	2.15311E-05
19.5000	11.5000	2.0909	2.20096E-05
20.0000	11.5000	2.0909	2.20096E-05
20.5000	11.2500	2.0455	2.15311E-05
21.0000	11.5000	2.0909	2.20096E-05
21.5000	11.0000	2.0000	2.10526E-05
22.0000	11.5000	2.0909	2.20096E-05
22.5000	11.0000	2.0000	2.10526E-05
23.0000	9.5000	1.7273	1.81818E-05
23.5000	11.5000	2.0909	2.20096E-05
24.0000	11.0000	2.0000	2.10526E-05
24.5000	10.5000	1.9091	2.00957E-05

25.0000	9.5000	1.7273	1.81818E-05
25.5000	9.5000	1.7273	1.81818E-05
26.0000	9.5000	1.7273	1.81818E-05
26.5000	9.5000	1.7273	1.81818E-05
27.0000	10.0000	1.8182	1.91388E-05
27.5000	9.5000	1.7273	1.81818E-05
28.0000	9.2500	1.6818	1.77033E-05
28.5000	9.5000	1.7273	1.81818E-05
29.0000	9.7500	1.7727	1.86603E-05
29.5000	10.0000	1.8182	1.91388E-05
30.0000	9.7500	1.7727	1.86603E-05
30.5000	10.0000	1.8182	1.91388E-05
31.0000	9.5000	1.7273	1.81818E-05
31.5000	9.5000	1.7273	1.81818E-05
32.0000	9.5000	1.7273	1.81818E-05
32.5000	10.0000	1.8182	1.91388E-05
33.0000	9.5000	1.7273	1.81818E-05
33.5000	9.7500	1.7727	1.86603E-05
34.0000	9.5000	1.7273	1.81818E-05
34.5000	9.2500	1.6818	1.77033E-05
35.0000	9.5000	1.7273	1.81818E-05
35.5000	10.0000	1.8182	1.91388E-05
36.0000	10.0000	1.8182	1.91388E-05
36.5000	9.5000	1.7273	1.81818E-05
37.0000	9.7500	1.7727	1.86603E-05
37.5000	9.7500	1.7727	1.86603E-05
38.0000	9.5000	1.7273	1.81818E-05
38.5000	9.7500	1.7727	1.86603E-05
39.0000	10.0000	1.8182	1.91388E-05
39.5000	9.5000	1.7273	1.81818E-05
40.0000	10.0000	1.8182	1.91388E-05
40.5000	10.5000	1.9091	2.00957E-05
41.0000	9.5000	1.7273	1.81818E-05
41.5000	10.0000	1.8182	1.91388E-05
42.0000	9.7500	1.7727	1.86603E-05
42.5000	9.7500	1.7727	1.86603E-05
43.0000	10.0000	1.8182	1.91388E-05
43.5000	9.7500	1.7727	1.86603E-05
44.0000	9.2500	1.6818	1.77033E-05
44.5000	10.0000	1.8182	1.91388E-05
45.0000	10.0000	1.8182	1.91388E-05
45.5000	10.2500	1.8636	1.96172E-05
46.0000	10.5000	1.9091	2.00957E-05
46.5000	10.0000	1.8182	1.91388E-05
47.0000	10.5000	1.9091	2.00957E-05
47.5000	10.0000	1.8182	1.91388E-05
48.0000	10.0000	1.8182	1.91388E-05
48.5000	10.0000	1.8182	1.91388E-05
49.0000	10.0000	1.8182	1.91388E-05
49.5000	10.2500	1.8636	1.96172E-05
50.0000	10.0000	1.8182	1.91388E-05

4.2.4 Lysozyme Flux at pH 8 using Ultrafiltration Membrane

Table 4.5 and Figure 4.5 below shows the permeate flux for lysozyme solution at pH 8. The flux is recorded at every 30 seconds. The concentration of lysozyme solution at pH 8 is 0.25g/l. From the figure, it shows that the permeate flux will be decreasing in proportional to time. This is because the permeate flux depends on the ability of the lysozyme protein to get through the membrane pores. For pH 8, it is found that it has the highest flux rate over all pH. From Figure 4.5 it shows that at 32 minutes of flux time, the permeate flux rate is constant which is from 3.90 (m/min) until 3.81 (m/min$^{)}$ and the percentage of declination is 2.31%. The value of flux achieve the steady state because of the certain static amount of lysozyme already clog the membrane pores in certain period of time and the value was not changing.

Table 4.5: Lysozyme at pH 8 using Ultrafiltration Membrane

Time (min)	V (ml)	Flux (m/min)	Permeability (m/kg.min)
0.50	19.00	3.45	3.63636E-05
1.00	23.50	4.27	4.49761E-05
1.50	24.50	4.45	4.689E-05
2.00	22.00	4.00	4.21053E-05
2.50	23.00	4.18	4.40191E-05
3.00	23.00	4.18	4.40191E-05
3.50	22.00	4.00	4.21053E-05
4.00	22.00	4.00	4.21053E-05
4.50	21.50	3.91	4.11483E-05
5.00	21.75	3.95	4.16268E-05
5.50	21.00	3.82	4.01914E-05
6.00	23.00	4.18	4.40191E-05
6.50	19.00	3.45	3.63636E-05
7.00	21.00	3.82	4.01914E-05
7.50	20.00	3.64	3.82775E-05
8.00	20.00	3.64	3.82775E-05
8.50	21.00	3.82	4.01914E-05
9.00	20.50	3.73	3.92344E-05
9.50	20.50	3.73	3.92344E-05
10.00	20.50	3.73	3.92344E-05
10.50	20.25	3.68	3.8756E-05
11.00	21.00	3.82	4.01914E-05
11.50	20.00	3.64	3.82775E-05
12.00	20.50	3.73	3.92344E-05
12.50	20.00	3.64	3.82775E-05
13.00	20.50	3.73	3.92344E-05
13.50	20.00	3.64	3.82775E-05
14.00	21.00	3.82	4.01914E-05
14.50	20.50	3.73	3.92344E-05
15.00	20.50	3.73	3.92344E-05
15.50	20.00	3.64	3.82775E-05
16.00	20.50	3.73	3.92344E-05
16.50	20.50	3.73	3.92344E-05
17.00	20.00	3.64	3.82775E-05
17.50	20.00	3.64	3.82775E-05
18.00	20.50	3.73	3.92344E-05
18.50	20.50	3.73	3.92344E-05
19.00	20.00	3.64	3.82775E-05
19.50	20.00	3.64	3.82775E-05
20.00	20.50	3.73	3.92344E-05
20.50	20.00	3.64	3.82775E-05
21.00	20.50	3.73	3.92344E-05
21.50	20.75	3.77	3.97129E-05
22.00	20.25	3.68	3.8756E-05
22.50	20.25	3.68	3.8756E-05
23.00	20.00	3.64	3.82775E-05
23.50	20.00	3.64	3.82775E-05
24.00	20.50	3.73	3.92344E-05
24.50	20.50	3.73	3.92344E-05

25.00	20.50	3.73	3.92344E-05
25.50	19.75	3.59	3.7799E-05
26.00	21.00	3.82	4.01914E-05
26.50	19.00	3.45	3.63636E-05
27.00	21.50	3.91	4.11483E-05
27.50	20.00	3.64	3.82775E-05
28.00	20.50	3.73	3.92344E-05
28.50	19.50	3.55	3.73206E-05
29.00	21.00	3.82	4.01914E-05
29.50	20.00	3.64	3.82775E-05
30.00	21.00	3.82	4.01914E-05
30.50	19.50	3.55	3.73206E-05
31.00	20.00	3.64	3.82775E-05
31.50	20.50	3.73	3.92344E-05
32.00	20.00	3.64	3.82775E-05
32.50	20.00	3.64	3.82775E-05
33.00	21.00	3.82	4.01914E-05
33.50	19.00	3.45	3.63636E-05
34.00	20.00	3.64	3.82775E-05
34.50	20.50	3.73	3.92344E-05
35.00	20.50	3.73	3.92344E-05
35.50	20.00	3.64	3.82775E-05
36.00	20.00	3.64	3.82775E-05
36.50	19.50	3.55	3.73206E-05
37.00	20.25	3.68	3.8756E-05
37.50	20.00	3.64	3.82775E-05
38.00	20.00	3.64	3.82775E-05
38.50	20.00	3.64	3.82775E-05
39.00	20.50	3.73	3.92344E-05
39.50	19.50	3.55	3.73206E-05
40.00	20.00	3.64	3.82775E-05
40.50	20.50	3.73	3.92344E-05
41.00	19.50	3.55	3.73206E-05
41.50	19.50	3.55	3.73206E-05
42.00	20.50	3.73	3.92344E-05
42.50	20.00	3.64	3.82775E-05
43.00	20.00	3.64	3.82775E-05
43.50	20.00	3.64	3.82775E-05
44.00	20.50	3.73	3.92344E-05
44.50	20.00	3.64	3.82775E-05
45.00	20.00	3.64	3.82775E-05
45.50	20.00	3.64	3.82775E-05
46.00	20.00	3.64	3.82775E-05
46.50	19.50	3.55	3.73206E-05
47.00	20.25	3.68	3.8756E-05
47.50	20.00	3.64	3.82775E-05
48.00	20.50	3.73	3.92344E-05
48.50	19.00	3.45	3.63636E-05
49.00	20.00	3.64	3.82775E-05
49.50	20.00	3.64	3.82775E-05
50.00	21.00	3.82	4.01914E-05

4.3 Overall Result Analysis for Lysozyme Flux at Various pH using Ultrafiltration Membrane

From the results, it shows that the steady-state flux increases when solution pH increases with lysozyme. At pH 5, lysozyme and the membrane have opposite charge. Thus, lysozyme 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, lysozyme easily passes through the membrane due to transmembrane pressure and vertical drag force during filtration flow. When the pH is within the range, lysozyme forms a macromolecule and obstructs the membrane causing very low transmission. Increasing the pH to 7 and 8 causes the same negative charge in both the protein and the membrane, creating a strong electrostatic repulsion between the two because membrane fouling is low and has a higher flux compared to other cases.

Table 4.6: Overall Result Analysis for Lysozyme Flux at Various pH using Ultrafiltration Membrane

Time (min)	Flux pH 5 (m/min)	Flux pH 6 (m/min)	Flux pH 7 (m/min)	Flux pH 8 (m/min)
0.5000	1.2727	3.2727	1.8182	3.4545
1.0000	2.8182	3.6364	2.0909	4.2727
1.5000	3.0000	2.1818	2.2727	4.4545
2.0000	3.0909	2.9091	2.0909	4.0000
2.5000	2.9091	2.7273	2.1818	4.1818
3.0000	2.9091	2.5455	2.1818	4.1818
3.5000	2.8182	2.5455	2.2727	4.0000
4.0000	2.8182	2.5455	2.1818	4.0000
4.5000	2.6364	2.5455	2.1818	3.9091
5.0000	2.7273	2.3636	2.1818	3.9545
5.5000	2.7273	2.3636	2.1818	3.8182
6.0000	2.6818	2.3636	2.0909	4.1818
6.5000	2.6364	2.3636	2.0000	3.4545
7.0000	2.6818	2.4545	2.0909	3.8182
7.5000	2.5909	2.1818	2.0909	3.6364
8.0000	2.5909	2.1818	2.0000	3.6364
8.5000	2.5455	2.0000	2.0909	3.8182
9.0000	2.5455	2.1818	2.0909	3.7273
9.5000	2.5909	2.0000	2.1364	3.7273
10.0000	2.5455	2.0909	2.0909	3.7273
10.5000	2.5455	2.0000	2.0455	3.6818
11.0000	2.5455	2.0909	2.0909	3.8182
11.5000	2.5000	2.0000	2.0455	3.6364
12.0000	2.5455	2.0000	2.0455	3.7273
12.5000	2.5455	2.0000	2.0000	3.6364
13.0000	2.5455	1.9091	2.0455	3.7273
13.5000	2.5000	1.9091	2.0455	3.6364
14.0000	2.5455	2.0000	2.0000	3.8182
14.5000	2.5455	1.8182	2.0000	3.7273
15.0000	2.5000	2.0000	2.0000	3.7273
15.5000	2.5000	1.8182	2.0455	3.6364
16.0000	2.0909	2.0000	2.0455	3.7273
16.5000	2.5455	1.9091	2.0909	3.7273
17.0000	2.5455	1.8182	2.0909	3.6364
17.5000	2.5455	1.9091	2.0455	3.6364
18.0000	2.4545	1.9091	2.0455	3.7273
18.5000	2.4545	1.8182	2.0000	3.7273
19.0000	2.5000	1.9091	2.0455	3.6364
19.5000	2.4545	1.9091	2.0909	3.6364
20.0000	2.5455	1.8182	2.0909	3.7273
20.5000	2.5455	1.8182	2.0455	3.6364
21.0000	2.4545	1.9091	2.0909	3.7273
21.5000	2.5000	1.9091	2.0000	3.7727
22.0000	2.4545	1.8182	2.0909	3.6818
22.5000	2.4545	1.8182	2.0000	3.6818
23.0000	2.5000	1.8182	1.7273	3.6364
23.5000	2.5455	1.9091	2.0909	3.6364

24.0000	2.3636	1.8182	2.0000	3.7273
24.5000	2.4545	1.8182	1.9091	3.7273
25.0000	2.4091	1.8182	1.7273	3.7273
25.5000	2.5000	1.1818	1.7273	3.5909
26.0000	2.4545	1.5455	1.7273	3.8182
26.5000	2.3636	1.7273	1.7273	3.4545
27.0000	2.4091	1.5455	1.8182	3.9091
27.5000	2.4545	1.6364	1.7273	3.6364
28.0000	2.4091	1.6364	1.6818	3.7273
28.5000	2.5000	1.6364	1.7273	3.5455
29.0000	2.4091	1.6364	1.7727	3.8182
29.5000	2.4545	1.6364	1.8182	3.6364
30.0000	2.4545	1.6364	1.7727	3.8182
30.5000	2.4545	1.6364	1.8182	3.5455
31.0000	2.4545	1.6818	1.7273	3.6364
31.5000	2.4545	1.6364	1.7273	3.7273
32.0000	2.4545	1.6364	1.7273	3.6364
32.5000	2.3636	1.6364	1.8182	3.6364
33.0000	2.4545	1.6818	1.7273	3.8182
33.5000	2.4545	1.6364	1.7727	3.4545
34.0000	2.4545	1.6818	1.7273	3.6364
34.5000	2.5000	1.6818	1.6818	3.7273
35.0000	2.3636	1.6364	1.7273	3.7273
35.5000	2.4545	1.6364	1.8182	3.6364
36.0000	2.4545	1.7273	1.8182	3.6364
36.5000	2.5000	1.5455	1.7273	3.5455
37.0000	2.4091	1.6818	1.7727	3.6818
37.5000	2.4091	1.6364	1.7727	3.6364
38.0000	2.4545	1.6364	1.7273	3.6364
38.5000	2.4545	1.6364	1.7727	3.6364
39.0000	2.4545	1.6364	1.8182	3.7273
39.5000	2.4091	1.6364	1.7273	3.5455
40.0000	2.5000	1.6818	1.8182	3.6364
40.5000	2.4545	1.6364	1.9091	3.7273
41.0000	2.4545	1.6818	1.7273	3.5455
41.5000	2.5000	1.6364	1.8182	3.5455
42.0000	2.3636	1.7273	1.7727	3.7273
42.5000	2.4545	1.5455	1.7727	3.6364
43.0000	2.5000	1.6364	1.8182	3.6364
43.5000	2.4091	1.8182	1.7727	3.6364
44.0000	2.3636	1.5455	1.6818	3.7273
44.5000	2.3636	1.6364	1.8182	3.6364
45.0000	2.3636	1.6818	1.8182	3.6364
45.5000	2.3636	1.6364	1.8636	3.6364
46.0000	2.3636	1.6818	1.9091	3.6364
46.5000	2.3636	1.6364	1.8182	3.5455
47.0000	2.4545	1.6364	1.9091	3.6818
47.5000	2.3636	1.7273	1.8182	3.6364
48.0000	2.3636	1.6364	1.8182	3.7273
48.5000	2.3636	1.5909	1.8182	3.4545
49.0000	2.3636	1.6364	1.8182	3.6364
49.5000	2.4091	1.6364	1.8636	3.6364
50.0000	2.3636	1.7273	1.8182	3.8182

4.4 Results for Rejection of Lysozyme at Various pH using Ultrafiltration Membrane

Rejection of protein is calculated by using this equation;

$$\text{Rejection} = \left(1 - \frac{Cp}{Cf}\right) \times 100\%$$

with; Cp = protein concentration in permeate

Cf = protein concentration in retentate

4.4.1 Rejection of Lysozyme at pH 5 using Ultrafiltration Membrane

Table 4.7 and Figure 4.7 below show the percentage of protein rejection for lysozyme solution at pH 5. It shows that the percentage of lysozyme rejection is increasing in proportional to the time. The lysozyme of rejection is between 19.6% until 54.7%. In average percentage of lysozyme rejection only 42.46% which means this amount of lysozyme will be clog at the membrane surface. This is because in acidic condition, the solution is in positive charges. These charges will attracted to the membrane which having the negative charges. The more acidic the solution, the more positive charges in it, the more it will attract to the membrane and it will cause clogging to the membrane surface. The OD values is recorded at 750 nm and the permeate concentration is determine from the calibration curve (Figure 4.1). The concentration of lysozyme solution at pH 5 is 0.25g/l.

Table 4.7: Lysozyme Rejection at pH 5 using Ultrafiltration Membrane

Time (min)	OD	Concentration (g/L)	Rejection (%)
0.00	0.00	0.00	0.00
5.00	0.08	0.22	11.50
10.00	0.07	0.21	14.30
15.00	0.05	0.19	23.50
20.00	0.04	0.18	27.40
25.00	0.04	0.18	29.10
30.00	0.04	0.17	31.70
35.00	0.04	0.17	33.90
40.00	0.04	0.16	34.70
45.00	0.03	0.15	38.30
50.00	0.03	0.15	39.50

4.4.2 Rejection of Lysozyme at pH 6 using Ultrafiltration Membrane

Table 4.8 and Figure 4.8 below show the percentage of protein rejection for lysozyme solution at pH 6. It shows that the percentage of lysozyme rejection is increasing in proportional to the time. The percentage of rejection is between 19.8% until 51.2%. In average the percentage of lysozyme rejection is 38.98% which means this amount of lysozyme will be clog at the membrane. This is because in acidic condition, the solution is in positive charges. These charges will attracted to the membrane which having the negative charges. The more acidic the solution, the more positive charges in it, the more it will attract to the membrane and it will cause clogging to the membrane surface. In this case, at pH 6, the solution has less positive charges than pH 5, thus the rejection is high but the amount of lysozyme clog at the membrane area is less. The OD values is recorded at 750 nm and the permeate concentration is determine from the calibration curve (Figure 4.1). The concentration of lysozyme solution at pH 6 is 0.25g/l.

Table 4.8: Lysozyme Rejection at pH 6 using Ultrafiltration Membrane

Time (min)	OD	Concentration (g/L)	Rejection (%)
0.00	0.00	0.25	0.00
5.00	0.07	0.21	15.90
10.00	0.06	0.21	16.80
15.00	0.06	0.21	17.30
20.00	0.05	0.19	22.80
25.00	0.05	0.19	25.20
30.00	0.05	0.18	28.70
35.00	0.04	0.17	30.20
40.00	0.04	0.15	38.60
45.00	0.03	0.14	44.50
50.00	0.02	0.13	46.90

4.4.3 Rejection of Lysozyme at pH 7 using Ultrafiltration Membrane

Table 4.9 and Figure 4.9 below show the percentage of lysozyme rejection for lysozyme solution at pH 7. It shows that the percentage of lysozyme rejection is increasing in proportional to the time. The percentage of rejection is between 15.9% until 46.9%. In average the percentage of lysozyme rejection is 28.69%. This is because in acidic condition, the solution is in positive charges. These charges will attracted to the membrane which having the negative charges. But in this case, pH 7 solution has more positive charges than the negative although it is in neutral condition because the value of isoelectric point of lysozyme is 11. The more acidic the solution, the more positive charges in it, the more it will attract to the membrane and it will cause clogging to the membrane surface. The presence of the negative charges will make the lysozyme to remain in the tank rather than clog at the membrane area. The OD values is recorded at 750 nm and the permeate concentration is determine from the calibration curve (Figure 4.1). The concentration of lysozyme solution at pH 7 is 0.25g/l.

Table 4.9: Lysozyme Rejection at pH 7 using Ultrafiltration Membrane

Time (min)	OD	Concentration (g/L)	Rejection (%)
0.00	0.00	0.00	0.00
5.00	0.05	0.20	19.80
10.00	0.05	0.19	24.30
15.00	0.04	0.17	32.50
20.00	0.03	0.15	38.90
25.00	0.03	0.15	41.40
30.00	0.03	0.14	42.50
35.00	0.02	0.14	44.90
40.00	0.02	0.14	45.20
45.00	0.02	0.13	49.10
50.00	0.02	0.12	51.20

4.4.4 Rejection of Lysozyme at pH 8 using Ultrafiltration Membrane

Table 4.10 and Figure 4.10 below show the percentage of lysozyme rejection for lysozyme solution at pH 8. It shows that the percentage of lysozyme rejection is increasing in proportional to the time. The percentage of rejection is between 11.5% until 39.5%. In average the percentage of lysozyme rejection is 28.39%. The amount of lysozyme rejected is the amount of the lysozyme remains in the tank due to the negative charges of the solution which will not lead to clog at the membrane surface. These charges will attract to the membrane which has the negative charges. But in this case, pH 8 solution has more negative charges. The more acidic the solution, the more positive charges in it, the more it will attract to the membrane and it will cause clogging to the membrane surface. The presence of the negative charges will make the lysozyme to remain in the tank rather than clog at the membrane area. The OD values is recorded at 750 nm and the permeate concentration is determine from the calibration curve (Figure 4.1). The concentration of lysozyme solution at pH 8 is 0.25g/l.

Table 4.10: Lysozyme Rejection at pH 8 using Ultrafiltration Membrane

Time (min)	OD	Concentration (g/L)	Rejection (%)
0.00	0.00	0.00	0.00
5.00	0.05	0.20	19.60
10.00	0.05	0.19	22.80
15.00	0.04	0.17	33.70
20.00	0.03	0.14	42.50
25.00	0.02	0.14	45.60
30.00	0.02	0.13	48.40
35.00	0.02	0.12	51.30
40.00	0.02	0.12	52.40
45.00	0.01	0.12	53.60
50.00	0.01	0.11	54.70

4.5 Overall Result Analysis for Lysozyme Rejection at Various pH

The value of permeate flux through the membrane depends on the rejection of the lysozyme molecule towards the membrane surface. When the rejection is low due to its same charges, it means that the lysozyme remains in the tank without clogging at the membrane and it will cause a high flux.

The lysozyme rejection also can be absorbance of the lysozyme onto the membrane because of the different charge between membrane and the Lysozyme itself. When the lysozyme absorbed in a high quantity it will reduce the pore size of the membrane and it will cause clogging and will decrease the flux flow as in pH 5 and pH 6 lysozyme solutions.

From Figure 4.11, it shows that percentage of rejection is increasing proportional to the time. The maximum value of Lysozyme rejection is at acidic, pH 8 and the minimum is at alkali, pH 5. This is because in acidic solution, the lysozyme will be in positive charge while polyethersulfone membrane is in negative charge and it will lead to a strong ionic bond between membrane and Lysozyme. However, in alkaline solution lysozyme will not attract to the membrane because both of them have the same charge. So lysozyme will remain in the tank without deposit onto the membrane surface due to repulsive mechanism.

Table 4.11: Overall Result Analysis for Lysozyme Rejection at Various pH

Time (min)	Rejection (%) at pH 5	Rejection (%) at pH 6	Rejection (%) at pH 7	Rejection (%) at pH 8
0	0	0	0	0
5	11.5	15.9	19.8	19.6
10	14.3	16.8	24.3	22.8
15	23.5	17.3	32.5	33.7
20	27.4	21.3	38.9	42.5
25	29.1	24.5	41.4	45.6
30	31.7	26.7	42.5	48.4
35	33.9	28.2	44.9	51.3
40	34.7	33.6	45.2	52.4
45	38.3	40.1	49.1	53.6
50	39.5	46.9	51.2	54.7

4.6 Effect of Ionic Strength on The Membrane Flux Using Ultrafiltration Membrane

The ionic strength test is done to the highest value of permeate flux which is pH 8 lysozyme solution. 4 sample of the solution is prepared and added with various molarities of Sodium Chloride which are 0.5 M, 1.0 M, 1.5 M and 2.0 M. This test is to determine the effect of the ionic strength towards membrane flux by using Ultrafiltration membrane.

4.6.1 Flux Decline of Lysozyme at 0.5 M NaCl using Ultrafiltration Membrane

Table 4.12 and Figure 4.12 below shows the permeate flux for Lysozyme solution at pH 8 with 0.5 M of NaCl. The flux is recorded at every 30 seconds. The concentration of lysozyme solution at pH 8 is 0.25g/l. From the figure, it shows that the permeate flux will be decreasing in proportional to time. This is because the permeate flux depends on the ability of the Lysozyme protein to get through the membrane pores. For ionic strength, the stronger the ionic strength, the more it will form bond between themselves. The flocculation will form and will lead to clogging on the membrane surface. From Figure 4.12 it shows at time t=32 minutes until t=49.50 minutes the permeate flux rate is constant and after that it drop from 3.90 (ml/min.m^2) until 3.81 (ml/min.m^2) and the percentage of declination is about 2.31%. They value of flux achieve the steady state because of the certain static amount of lysozyme already clog the membrane pores in certain period of time and the value was not changing.

Table 4.12: Table of Flux Decline of Lysozyme at 0.5 M NaCl using Ultrafiltration Membrane

Time (min)	V (ml)	Flux (m/min)	Permeability (m/kg.min)
0.5000	23.0000	4.1818	4.40191E-05
1.0000	24.0000	4.3636	4.5933E-05
1.5000	25.0000	4.5455	4.78469E-05
2.0000	26.0000	4.7273	4.97608E-05
2.5000	25.0000	4.5455	4.78469E-05
3.0000	24.0000	4.3636	4.5933E-05
3.5000	25.0000	4.5455	4.78469E-05
4.0000	24.0000	4.3636	4.5933E-05
4.5000	23.0000	4.1818	4.40191E-05
5.0000	23.0000	4.1818	4.40191E-05
5.5000	24.0000	4.3636	4.5933E-05
6.0000	23.0000	4.1818	4.40191E-05
6.5000	23.0000	4.1818	4.40191E-05
7.0000	23.0000	4.1818	4.40191E-05
7.5000	23.0000	4.1818	4.40191E-05
8.0000	22.5000	4.0909	4.30622E-05
8.5000	23.0000	4.1818	4.40191E-05
9.0000	22.5000	4.0909	4.30622E-05
9.5000	23.0000	4.1818	4.40191E-05
10.0000	22.0000	4.0000	4.21053E-05
10.5000	22.0000	4.0000	4.21053E-05
11.0000	22.0000	4.0000	4.21053E-05
11.5000	22.0000	4.0000	4.21053E-05
12.0000	21.0000	3.8182	4.01914E-05
12.5000	19.0000	3.4545	3.63636E-05
13.0000	20.0000	3.6364	3.82775E-05
13.5000	22.0000	4.0000	4.21053E-05
14.0000	21.5000	3.9091	4.11483E-05
14.5000	20.5000	3.7273	3.92344E-05
15.0000	20.5000	3.7273	3.92344E-05
15.5000	19.0000	3.4545	3.63636E-05
16.0000	21.0000	3.8182	4.01914E-05
16.5000	20.0000	3.6364	3.82775E-05
17.0000	20.2500	3.6818	3.8756E-05
17.5000	20.0000	3.6364	3.82775E-05
18.0000	20.0000	3.6364	3.82775E-05
18.5000	19.5000	3.5455	3.73206E-05
19.0000	20.0000	3.6364	3.82775E-05
19.5000	21.0000	3.8182	4.01914E-05
20.0000	20.5000	3.7273	3.92344E-05
20.5000	21.0000	3.8182	4.01914E-05
21.0000	19.5000	3.5455	3.73206E-05
21.5000	20.0000	3.6364	3.82775E-05
22.0000	20.0000	3.6364	3.82775E-05
22.5000	19.5000	3.5455	3.73206E-05
23.0000	20.0000	3.6364	3.82775E-05
23.5000	19.0000	3.4545	3.63636E-05

24.0000	20.0000	3.6364	3.82775E-05
24.5000	20.0000	3.6364	3.82775E-05
25.0000	18.5000	3.3636	3.54067E-05
25.5000	19.0000	3.4545	3.63636E-05
26.0000	19.5000	3.5455	3.73206E-05
26.5000	19.0000	3.4545	3.63636E-05
27.0000	20.5000	3.7273	3.92344E-05
27.5000	20.5000	3.7273	3.92344E-05
28.0000	20.0000	3.6364	3.82775E-05
28.5000	19.0000	3.4545	3.63636E-05
29.0000	21.0000	3.8182	4.01914E-05
29.5000	20.0000	3.6364	3.82775E-05
30.0000	19.0000	3.4545	3.63636E-05
30.5000	20.0000	3.6364	3.82775E-05
31.0000	21.0000	3.8182	4.01914E-05
31.5000	19.0000	3.4545	3.63636E-05
32.0000	21.5000	3.9091	4.11483E-05
32.5000	21.0000	3.8182	4.01914E-05
33.0000	20.0000	3.6364	3.82775E-05
33.5000	21.0000	3.8182	4.01914E-05
34.0000	21.0000	3.8182	4.01914E-05
34.5000	20.0000	3.6364	3.82775E-05
35.0000	20.5000	3.7273	3.92344E-05
35.5000	19.0000	3.4545	3.63636E-05
36.0000	21.0000	3.8182	4.01914E-05
36.5000	21.0000	3.8182	4.01914E-05
37.0000	21.0000	3.8182	4.01914E-05
37.5000	21.0000	3.8182	4.01914E-05
38.0000	21.0000	3.8182	4.01914E-05
38.5000	21.0000	3.8182	4.01914E-05
39.0000	20.0000	3.6364	3.82775E-05
39.5000	19.0000	3.4545	3.63636E-05
40.0000	20.0000	3.6364	3.82775E-05
40.5000	20.0000	3.6364	3.82775E-05
41.0000	21.0000	3.8182	4.01914E-05
41.5000	20.0000	3.6364	3.82775E-05
42.0000	18.5000	3.3636	3.54067E-05
42.5000	18.0000	3.2727	3.44498E-05
43.0000	18.0000	3.2727	3.44498E-05
43.5000	19.5000	3.5455	3.73206E-05
44.0000	21.0000	3.8182	4.01914E-05
44.5000	21.0000	3.8182	4.01914E-05
45.0000	21.0000	3.8182	4.01914E-05
45.5000	22.0000	4.0000	4.21053E-05
46.0000	18.0000	3.2727	3.44498E-05
46.5000	20.0000	3.6364	3.82775E-05
47.0000	21.0000	3.8182	4.01914E-05
47.5000	21.0000	3.8182	4.01914E-05
48.0000	21.0000	3.8182	4.01914E-05
48.5000	21.0000	3.8182	4.01914E-05
49.0000	21.0000	3.8182	4.01914E-05
49.5000	21.0000	3.8182	4.01914E-05

50.0000	20.5000	3.7273	3.92344E-05

4.6.2 Flux Decline of Lysozyme at 1.0 M NaCl using Ultrafiltration Membrane

Table 4.13 and Figure 4.13 below shows the permeate flux for Lysozyme solution at pH 8 with 1.0 M of NaCl. The flux is recorded at every 30 seconds. The concentration of lysozyme solution at pH 8 is 0.25g/l. From the figure, it shows that the permeate flux will be decreasing in proportional to time. This is because the permeate flux depends on the ability of the Lysozyme protein to get through the membrane pores. For ionic strength, the stronger the ionic strength, the more it will form bond between themselves. In this case, the flocculation will form more than 0.5 M NaCl and will lead to clogging on the membrane surface and the flux is less than 0.5 M NaCl. From Figure 4.13 it shows at time t=38 minutes until t=50 minutes the permeate flux rate is constant and after that it drop from 3.81 (ml/min.m^2) until 3.63 (ml/min.m^2) and the percentage of declination is about 4.72%. They value of flux achieve the steady state because of the certain static amount of lysozyme already clog the membrane pores in certain period of time and the value was not changing.

Table 4.13: Flux Decline of Lysozyme at 1.0 M NaCl using Ultrafiltration Membrane

Time (min)	V (ml)	Flux (m/min)	Permeability (m/kg.min)
0.5000	20.5000	3.7273	3.92344E-05
1.0000	21.0000	3.8182	4.01914E-05
1.5000	22.0000	4.0000	4.21053E-05
2.0000	22.0000	4.0000	4.21053E-05
2.5000	22.0000	4.0000	4.21053E-05
3.0000	22.0000	4.0000	4.21053E-05
3.5000	21.0000	3.8182	4.01914E-05
4.0000	21.0000	3.8182	4.01914E-05
4.5000	21.5000	3.9091	4.11483E-05
5.0000	19.5000	3.5455	3.73206E-05
5.5000	21.0000	3.8182	4.01914E-05
6.0000	19.5000	3.5455	3.73206E-05
6.5000	21.0000	3.8182	4.01914E-05
7.0000	20.0000	3.6364	3.82775E-05
7.5000	20.5000	3.7273	3.92344E-05
8.0000	20.0000	3.6364	3.82775E-05
8.5000	20.7500	3.7727	3.97129E-05
9.0000	21.0000	3.8182	4.01914E-05
9.5000	20.5000	3.7273	3.92344E-05
10.0000	20.0000	3.6364	3.82775E-05
10.5000	21.5000	3.9091	4.11483E-05
11.0000	20.0000	3.6364	3.82775E-05
11.5000	21.5000	3.9091	4.11483E-05
12.0000	18.0000	3.2727	3.44498E-05
12.5000	20.0000	3.6364	3.82775E-05
13.0000	20.5000	3.7273	3.92344E-05
13.5000	20.0000	3.6364	3.82775E-05
14.0000	20.0000	3.6364	3.82775E-05
14.5000	20.0000	3.6364	3.82775E-05
15.0000	20.0000	3.6364	3.82775E-05
15.5000	20.5000	3.7273	3.92344E-05
16.0000	20.0000	3.6364	3.82775E-05
16.5000	20.5000	3.7273	3.92344E-05
17.0000	20.0000	3.6364	3.82775E-05
17.5000	20.5000	3.7273	3.92344E-05
18.0000	20.0000	3.6364	3.82775E-05
18.5000	20.0000	3.6364	3.82775E-05
19.0000	20.0000	3.6364	3.82775E-05
19.5000	20.5000	3.7273	3.92344E-05
20.0000	20.0000	3.6364	3.82775E-05
20.5000	20.0000	3.6364	3.82775E-05
21.0000	20.0000	3.6364	3.82775E-05
21.5000	20.0000	3.6364	3.82775E-05
22.0000	20.5000	3.7273	3.92344E-05
22.5000	20.0000	3.6364	3.82775E-05
23.0000	20.0000	3.6364	3.82775E-05
23.5000	20.5000	3.7273	3.92344E-05

24.0000	20.0000	3.6364	3.82775E-05
24.5000	20.0000	3.6364	3.82775E-05
25.0000	20.0000	3.6364	3.82775E-05
25.5000	20.0000	3.6364	3.82775E-05
26.0000	20.0000	3.6364	3.82775E-05
26.5000	20.5000	3.7273	3.92344E-05
27.0000	20.0000	3.6364	3.82775E-05
27.5000	20.0000	3.6364	3.82775E-05
28.0000	19.5000	3.5455	3.73206E-05
28.5000	21.0000	3.8182	4.01914E-05
29.0000	20.0000	3.6364	3.82775E-05
29.5000	20.5000	3.7273	3.92344E-05
30.0000	19.5000	3.5455	3.73206E-05
30.5000	20.0000	3.6364	3.82775E-05
31.0000	20.0000	3.6364	3.82775E-05
31.5000	20.0000	3.6364	3.82775E-05
32.0000	18.0000	3.2727	3.44498E-05
32.5000	20.0000	3.6364	3.82775E-05
33.0000	19.0000	3.4545	3.63636E-05
33.5000	20.0000	3.6364	3.82775E-05
34.0000	20.0000	3.6364	3.82775E-05
34.5000	20.0000	3.6364	3.82775E-05
35.0000	20.0000	3.6364	3.82775E-05
35.5000	20.0000	3.6364	3.82775E-05
36.0000	20.0000	3.6364	3.82775E-05
36.5000	20.0000	3.6364	3.82775E-05
37.0000	19.5000	3.5455	3.73206E-05
37.5000	21.0000	3.8182	4.01914E-05
38.0000	20.0000	3.6364	3.82775E-05
38.5000	20.0000	3.6364	3.82775E-05
39.0000	20.0000	3.6364	3.82775E-05
39.5000	20.0000	3.6364	3.82775E-05
40.0000	20.0000	3.6364	3.82775E-05
40.5000	20.5000	3.7273	3.92344E-05
41.0000	20.0000	3.6364	3.82775E-05
41.5000	19.0000	3.4545	3.63636E-05
42.0000	20.0000	3.6364	3.82775E-05
42.5000	20.0000	3.6364	3.82775E-05
43.0000	20.0000	3.6364	3.82775E-05
43.5000	20.0000	3.6364	3.82775E-05
44.0000	20.0000	3.6364	3.82775E-05
44.5000	20.0000	3.6364	3.82775E-05
45.0000	20.0000	3.6364	3.82775E-05
45.5000	20.0000	3.6364	3.82775E-05
46.0000	20.0000	3.6364	3.82775E-05
46.5000	20.0000	3.6364	3.82775E-05
47.0000	20.0000	3.6364	3.82775E-05
47.5000	20.0000	3.6364	3.82775E-05
48.0000	20.0000	3.6364	3.82775E-05
48.5000	20.0000	3.6364	3.82775E-05
49.0000	20.0000	3.6364	3.82775E-05
49.5000	20.0000	3.6364	3.82775E-05

50.0000	20.0000	3.6364	3.82775E-05

4.6.3 Flux Decline of Lysozyme at 1.5 M NaCl using Ultrafiltration Membrane

Table 4.14 and Figure 4.14 below shows the permeate flux for Lysozyme solution at pH 8 with 1.5 M of NaCl. The flux is recorded at every 30 seconds. The concentration of lysozyme solution at pH 8 is 0.25g/l. From the figure, it shows that the permeate flux will be decreasing in proportional to time. This is because the permeate flux depends on the ability of the Lysozyme protein to get through the membrane pores. For ionic strength, the stronger the ionic strength, the more it will form bond between themselves. In this case, the flocculation will form more than 0.5 M NaCl and 1.0 M NaCl and will lead to more clogging on the membrane surface and the flux is also less than 0.5 M NaCl and 1.0 M NaCl. From Figure 4.14 it shows at time t=20.50 minutes until t=35 minutes the permeate flux rate is constant and after that it drop from 3.59 (ml/min.m^2) until 3.54 (ml/min.m^2) and the percentage of declination is about 1.39%. The value of flux achieve the steady state because of the certain static amount of lysozyme already clog the membrane pores in certain period of time and the value was not changing

Table 4.14: Flux Decline of Lysozyme at 1.5 M NaCl using Ultrafiltration Membrane

Time (min)	V (ml)	Flux (m/min)	Permeability (m/kg.min)
0.5000	11.5000	2.0909	2.20096E-05
1.0000	21.0000	3.8182	4.01914E-05
1.5000	19.0000	3.4545	3.63636E-05
2.0000	20.0000	3.6364	3.82775E-05
2.5000	20.0000	3.6364	3.82775E-05
3.0000	20.5000	3.7273	3.92344E-05
3.5000	19.7500	3.5909	3.7799E-05
4.0000	20.0000	3.6364	3.82775E-05
4.5000	19.5000	3.5455	3.73206E-05
5.0000	20.0000	3.6364	3.82775E-05
5.5000	19.7500	3.5909	3.7799E-05
6.0000	19.5000	3.5455	3.73206E-05
6.5000	19.5000	3.5455	3.73206E-05
7.0000	19.5000	3.5455	3.73206E-05
7.5000	19.2500	3.5000	3.68421E-05
8.0000	20.0000	3.6364	3.82775E-05
8.5000	20.0000	3.6364	3.82775E-05
9.0000	20.0000	3.6364	3.82775E-05
9.5000	19.7500	3.5909	3.7799E-05
10.0000	20.0000	3.6364	3.82775E-05
10.5000	19.5000	3.5455	3.73206E-05
11.0000	20.0000	3.6364	3.82775E-05
11.5000	20.0000	3.6364	3.82775E-05
12.0000	20.0000	3.6364	3.82775E-05
12.5000	19.7500	3.5909	3.7799E-05
13.0000	19.5000	3.5455	3.73206E-05
13.5000	19.0000	3.4545	3.63636E-05
14.0000	19.7500	3.5909	3.7799E-05
14.5000	19.0000	3.4545	3.63636E-05
15.0000	19.5000	3.5455	3.73206E-05
15.5000	19.0000	3.4545	3.63636E-05
16.0000	19.0000	3.4545	3.63636E-05
16.5000	19.2500	3.5000	3.68421E-05
17.0000	19.5000	3.5455	3.73206E-05
17.5000	19.0000	3.4545	3.63636E-05
18.0000	19.5000	3.5455	3.73206E-05
18.5000	19.7500	3.5909	3.7799E-05
19.0000	19.0000	3.4545	3.63636E-05
19.5000	19.0000	3.4545	3.63636E-05
20.0000	19.5000	3.5455	3.73206E-05
20.5000	19.5000	3.5455	3.73206E-05
21.0000	19.5000	3.5455	3.73206E-05
21.5000	18.0000	3.2727	3.44498E-05
22.0000	18.7500	3.4091	3.58852E-05
22.5000	19.0000	3.4545	3.63636E-05
23.0000	18.5000	3.3636	3.54067E-05
23.5000	19.0000	3.4545	3.63636E-05
24.0000	19.0000	3.4545	3.63636E-05

24.5000	19.0000	3.4545	3.63636E-05
25.0000	19.5000	3.5455	3.73206E-05
25.5000	19.5000	3.5455	3.73206E-05
26.0000	19.0000	3.4545	3.63636E-05
26.5000	19.7500	3.5909	3.7799E-05
27.0000	19.5000	3.5455	3.73206E-05
27.5000	19.0000	3.4545	3.63636E-05
28.0000	19.5000	3.5455	3.73206E-05
28.5000	19.0000	3.4545	3.63636E-05
29.0000	20.0000	3.6364	3.82775E-05
29.5000	19.2500	3.5000	3.68421E-05
30.0000	19.7500	3.5909	3.7799E-05
30.5000	19.0000	3.4545	3.63636E-05
31.0000	19.5000	3.5455	3.73206E-05
31.5000	19.0000	3.4545	3.63636E-05
32.0000	18.0000	3.2727	3.44498E-05
32.5000	19.5000	3.5455	3.73206E-05
33.0000	19.2500	3.5000	3.68421E-05
33.5000	19.2500	3.5000	3.68421E-05
34.0000	19.5000	3.5455	3.73206E-05
34.5000	20.0000	3.6364	3.82775E-05
35.0000	19.5000	3.5455	3.73206E-05
35.5000	18.0000	3.2727	3.44498E-05
36.0000	19.2500	3.5000	3.68421E-05
36.5000	19.0000	3.4545	3.63636E-05
37.0000	19.5000	3.5455	3.73206E-05
37.5000	18.5000	3.3636	3.54067E-05
38.0000	18.7500	3.4091	3.58852E-05
38.5000	19.5000	3.5455	3.73206E-05
39.0000	19.2500	3.5000	3.68421E-05
39.5000	20.0000	3.6364	3.82775E-05
40.0000	19.0000	3.4545	3.63636E-05
40.5000	19.7500	3.5909	3.7799E-05
41.0000	19.7500	3.5909	3.7799E-05
41.5000	19.0000	3.4545	3.63636E-05
42.0000	19.0000	3.4545	3.63636E-05
42.5000	19.7500	3.5909	3.7799E-05
43.0000	19.5000	3.5455	3.73206E-05
43.5000	19.7500	3.5909	3.7799E-05
44.0000	19.5000	3.5455	3.73206E-05
44.5000	19.7500	3.5909	3.7799E-05
45.0000	19.5000	3.5455	3.73206E-05
45.5000	19.7500	3.5909	3.7799E-05
46.0000	19.7500	3.5909	3.7799E-05
46.5000	19.0000	3.4545	3.63636E-05
47.0000	19.7500	3.5909	3.7799E-05
47.5000	19.7500	3.5909	3.7799E-05
48.0000	19.7500	3.5909	3.7799E-05
48.5000	19.7500	3.5909	3.7799E-05
49.0000	20.0000	3.6364	3.82775E-05
49.5000	19.5000	3.5455	3.73206E-05
50.0000	19.5000	3.5455	3.73206E-05

4.6.4 Flux Decline of Lysozyme at 2.0 M NaCl using Ultrafiltration Membrane

Table 4.15 and Figure 4.15 below shows the permeate flux for Lysozyme solution at pH 8 with 2.0 M of NaCl. The flux is recorded at every 30 seconds. The concentration of lysozyme solution at pH 8 is 0.25g/l. From the figure, it shows that the permeate flux will be decreasing in proportional to time. This is because the permeate flux depends on the ability of the Lysozyme protein to get through the membrane pores. For ionic strength, the stronger the ionic strength, the more it will form bond between themselves. In this case, the flocculation will form more than 0.5 M NaCl 1.0 M NaCl and 1.5 M NaCl and it will lead to more clogging on the membrane surface and the flux is also less than 0.5 M NaCl 1.0 M NaCl and 1.5 M NaCl. From Figure 4.15 it shows at time t=21 minutes until t=44 minutes the permeate flux rate is constant and after that it drop from 2.63 (ml/min.m^2) until 2.54 (ml/min.m^2) and the percentage of declination is about 3.42%. The value of flux achieve the steady state because of the certain static amount of lysozyme already clog the membrane pores in certain period of time and the value was not changing.

Table 4.15: Flux Decline of Lysozyme at 2.0 M NaCl using Ultrafiltration Membrane

Time (min)	V (ml)	Flux (m/min)	Permeability (m/kg.min)
0.5000	14.5000	2.6364	2.77512E-05
1.0000	14.0000	2.5455	2.67943E-05
1.5000	13.7500	2.5000	2.63158E-05
2.0000	13.0000	2.3636	2.48804E-05
2.5000	13.0000	2.3636	2.48804E-05
3.0000	12.7500	2.3182	2.44019E-05
3.5000	13.5000	2.4545	2.58373E-05
4.0000	13.0000	2.3636	2.48804E-05
4.5000	13.2500	2.4091	2.53589E-05
5.0000	13.0000	2.3636	2.48804E-05
5.5000	13.2500	2.4091	2.53589E-05
6.0000	13.0000	2.3636	2.48804E-05
6.5000	13.0000	2.3636	2.48804E-05
7.0000	12.7500	2.3182	2.44019E-05
7.5000	13.2500	2.4091	2.53589E-05
8.0000	12.5000	2.2727	2.39234E-05
8.5000	13.0000	2.3636	2.48804E-05
9.0000	13.0000	2.3636	2.48804E-05
9.5000	13.0000	2.3636	2.48804E-05
10.0000	13.0000	2.3636	2.48804E-05
10.5000	13.5000	2.4545	2.58373E-05
11.0000	12.5000	2.2727	2.39234E-05
11.5000	13.5000	2.4545	2.58373E-05
12.0000	13.0000	2.3636	2.48804E-05
12.5000	13.0000	2.3636	2.48804E-05
13.0000	13.0000	2.3636	2.48804E-05
13.5000	13.5000	2.4545	2.58373E-05
14.0000	13.0000	2.3636	2.48804E-05
14.5000	13.5000	2.4545	2.58373E-05
15.0000	13.0000	2.3636	2.48804E-05
15.5000	13.2500	2.4091	2.53589E-05
16.0000	13.5000	2.4545	2.58373E-05
16.5000	13.2500	2.4091	2.53589E-05
17.0000	13.0000	2.3636	2.48804E-05
17.5000	13.7500	2.5000	2.63158E-05
18.0000	13.0000	2.3636	2.48804E-05
18.5000	13.7500	2.5000	2.63158E-05
19.0000	13.5000	2.4545	2.58373E-05
19.5000	13.5000	2.4545	2.58373E-05
20.0000	13.0000	2.3636	2.48804E-05
20.5000	13.7500	2.5000	2.63158E-05
21.0000	14.5000	2.6364	2.77512E-05
21.5000	12.7500	2.3182	2.44019E-05
22.0000	13.5000	2.4545	2.58373E-05
22.5000	13.7500	2.5000	2.63158E-05
23.0000	13.5000	2.4545	2.58373E-05
23.5000	13.5000	2.4545	2.58373E-05

24.0000	13.7500	2.5000	2.63158E-05
24.5000	14.0000	2.5455	2.67943E-05
25.0000	13.5000	2.4545	2.58373E-05
25.5000	14.0000	2.5455	2.67943E-05
26.0000	14.0000	2.5455	2.67943E-05
26.5000	13.5000	2.4545	2.58373E-05
27.0000	13.0000	2.3636	2.48804E-05
27.5000	14.0000	2.5455	2.67943E-05
28.0000	13.7500	2.5000	2.63158E-05
28.5000	14.0000	2.5455	2.67943E-05
29.0000	13.5000	2.4545	2.58373E-05
29.5000	14.0000	2.5455	2.67943E-05
30.0000	14.0000	2.5455	2.67943E-05
30.5000	13.5000	2.4545	2.58373E-05
31.0000	14.5000	2.6364	2.77512E-05
31.5000	14.2500	2.5909	2.72727E-05
32.0000	13.5000	2.4545	2.58373E-05
32.5000	14.0000	2.5455	2.67943E-05
33.0000	13.7500	2.5000	2.63158E-05
33.5000	13.5000	2.4545	2.58373E-05
34.0000	13.7500	2.5000	2.63158E-05
34.5000	14.0000	2.5455	2.67943E-05
35.0000	13.7500	2.5000	2.63158E-05
35.5000	14.0000	2.5455	2.67943E-05
36.0000	13.7500	2.5000	2.63158E-05
36.5000	14.2500	2.5909	2.72727E-05
37.0000	13.7500	2.5000	2.63158E-05
37.5000	14.2500	2.5909	2.72727E-05
38.0000	13.7500	2.5000	2.63158E-05
38.5000	14.2500	2.5909	2.72727E-05
39.0000	14.0000	2.5455	2.67943E-05
39.5000	14.2500	2.5909	2.72727E-05
40.0000	13.7500	2.5000	2.63158E-05
40.5000	14.5000	2.6364	2.77512E-05
41.0000	14.0000	2.5455	2.67943E-05
41.5000	14.0000	2.5455	2.67943E-05
42.0000	14.0000	2.5455	2.67943E-05
42.5000	14.2500	2.5909	2.72727E-05
43.0000	14.0000	2.5455	2.67943E-05
43.5000	14.5000	2.6364	2.77512E-05
44.0000	14.0000	2.5455	2.67943E-05
44.5000	14.2500	2.5909	2.72727E-05
45.0000	14.0000	2.5455	2.67943E-05
45.5000	14.5000	2.6364	2.77512E-05
46.0000	14.5000	2.6364	2.77512E-05
46.5000	14.5000	2.6364	2.77512E-05
47.0000	13.7500	2.5000	2.63158E-05
47.5000	14.7500	2.6818	2.82297E-05
48.0000	14.0000	2.5455	2.67943E-05
48.5000	14.7500	2.6818	2.82297E-05
49.0000	14.2500	2.5909	2.72727E-05
49.5000	14.0000	2.5455	2.67943E-05

50.0000	14.0000	2.5455	2.67943E-05

4.7 Overall Result Analysis for Effect of Ionic Strength on The Membrane Flux Various Molarity of NaCl using Ultrafiltration Membrane

Ionic strength plays an important role in Lysozyme separation due to electrostatic interaction forces. Steady-state flux generally decreases and Lysozyme transmission increases 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. Transmembrane pressure and vertical drag force affect protein transmission during filtration flow as the protein desorbs from the membrane surface and pore wall and passes through the membrane into the filtrate. With a soft Lysozyme protein which has 14.4 kDa and 10 kDa for the membrane (which has a membrane pore size larger than molecular weight of Lysozyme) with high ionic strength cause the Lysozyme to collect on the membrane surface. Molecular conformation of the protein changes allowed the protein to enter the membrane and adsorb onto the pore wall thereby decreasing the membrane pore size. Results indicate that the steady-state flux and the transmission decrease rapidly at the same time at the beginning of the filtration period.

Increased ionic strength resulting from matching pore size and protein molecular weight reduces electrostatic interaction between them. Protein can then pass through the membrane easily, creating a higher protein recovery rate. Most proteins pass through the membrane into the filtrate during the initial filtration stage when the pore size is greater than the protein molecular weight. After a lag-time, however, the 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 even if the ionic strength is increased.

Table 4.16: Overall Result Analysis for Effect of Ionic Strength on The Membrane Flux Various Molarity of NaCl using Ultrafiltration Membrane

Time (min)	Flux at 0.5 M NaCl (m/min)	Flux at 1.0 M NaCl (m/min)	Flux at 1.5 M NaCl (m/min)	Flux at 2.0 M NaCl (m/min)
0.5000	4.1818	3.7273	2.0909	2.6364
1.0000	4.3636	3.8182	3.8182	2.5455
1.5000	4.5455	4.0000	3.4545	2.5000
2.0000	4.7273	4.0000	3.6364	2.3636
2.5000	4.5455	4.0000	3.6364	2.3636
3.0000	4.3636	4.0000	3.7273	2.3182
3.5000	4.5455	3.8182	3.5909	2.4545
4.0000	4.3636	3.8182	3.6364	2.3636
4.5000	4.1818	3.9091	3.5455	2.4091
5.0000	4.1818	3.5455	3.6364	2.3636
5.5000	4.3636	3.8182	3.5909	2.4091
6.0000	4.1818	3.5455	3.5455	2.3636
6.5000	4.1818	3.8182	3.5455	2.3636
7.0000	4.1818	3.6364	3.5455	2.3182
7.5000	4.1818	3.7273	3.5000	2.4091
8.0000	4.0909	3.6364	3.6364	2.2727
8.5000	4.1818	3.7727	3.6364	2.3636
9.0000	4.0909	3.8182	3.6364	2.3636
9.5000	4.1818	3.7273	3.5909	2.3636
10.0000	4.0000	3.6364	3.6364	2.3636
10.5000	4.0000	3.9091	3.5455	2.4545
11.0000	4.0000	3.6364	3.6364	2.2727
11.5000	4.0000	3.9091	3.6364	2.4545
12.0000	3.8182	3.2727	3.6364	2.3636
12.5000	3.4545	3.6364	3.5909	2.3636
13.0000	3.6364	3.7273	3.5455	2.3636
13.5000	4.0000	3.6364	3.4545	2.4545
14.0000	3.9091	3.6364	3.5909	2.3636
14.5000	3.7273	3.6364	3.4545	2.4545
15.0000	3.7273	3.6364	3.5455	2.3636
15.5000	3.4545	3.7273	3.4545	2.4091
16.0000	3.8182	3.6364	3.4545	2.4545
16.5000	3.6364	3.7273	3.5000	2.4091
17.0000	3.6818	3.6364	3.5455	2.3636
17.5000	3.6364	3.7273	3.4545	2.5000
18.0000	3.6364	3.6364	3.5455	2.3636
18.5000	3.5455	3.6364	3.5909	2.5000
19.0000	3.6364	3.6364	3.4545	2.4545
19.5000	3.8182	3.7273	3.4545	2.4545
20.0000	3.7273	3.6364	3.5455	2.3636
20.5000	3.8182	3.6364	3.5455	2.5000
21.0000	3.5455	3.6364	3.5455	2.6364
21.5000	3.6364	3.6364	3.2727	2.3182
22.0000	3.6364	3.7273	3.4091	2.4545
22.5000	3.5455	3.6364	3.4545	2.5000
23.0000	3.6364	3.6364	3.3636	2.4545
23.5000	3.4545	3.7273	3.4545	2.4545

24.0000	3.6364	3.6364	3.4545	2.5000
24.5000	3.6364	3.6364	3.4545	2.5455
25.0000	3.3636	3.6364	3.5455	2.4545
25.5000	3.4545	3.6364	3.5455	2.5455
26.0000	3.5455	3.6364	3.4545	2.5455
26.5000	3.4545	3.7273	3.5909	2.4545
27.0000	3.7273	3.6364	3.5455	2.3636
27.5000	3.7273	3.6364	3.4545	2.5455
28.0000	3.6364	3.5455	3.5455	2.5000
28.5000	3.4545	3.8182	3.4545	2.5455
29.0000	3.8182	3.6364	3.6364	2.4545
29.5000	3.6364	3.7273	3.5000	2.5455
30.0000	3.4545	3.5455	3.5909	2.5455
30.5000	3.6364	3.6364	3.4545	2.4545
31.0000	3.8182	3.6364	3.5455	2.6364
31.5000	3.4545	3.6364	3.4545	2.5909
32.0000	3.9091	3.2727	3.2727	2.4545
32.5000	3.8182	3.6364	3.5455	2.5455
33.0000	3.6364	3.4545	3.5000	2.5000
33.5000	3.8182	3.6364	3.5000	2.4545
34.0000	3.8182	3.6364	3.5455	2.5000
34.5000	3.6364	3.6364	3.6364	2.5455
35.0000	3.7273	3.6364	3.5455	2.5000
35.5000	3.4545	3.6364	3.2727	2.5455
36.0000	3.8182	3.6364	3.5000	2.5000
36.5000	3.8182	3.6364	3.4545	2.5909
37.0000	3.8182	3.5455	3.5455	2.5000
37.5000	3.8182	3.8182	3.3636	2.5909
38.0000	3.8182	3.6364	3.4091	2.5000
38.5000	3.8182	3.6364	3.5455	2.5909
39.0000	3.6364	3.6364	3.5000	2.5455
39.5000	3.4545	3.6364	3.6364	2.5909
40.0000	3.6364	3.6364	3.4545	2.5000
40.5000	3.6364	3.7273	3.5909	2.6364
41.0000	3.8182	3.6364	3.5909	2.5455
41.5000	3.6364	3.4545	3.4545	2.5455
42.0000	3.3636	3.6364	3.4545	2.5455
42.5000	3.2727	3.6364	3.5909	2.5909
43.0000	3.2727	3.6364	3.5455	2.5455
43.5000	3.5455	3.6364	3.5909	2.6364
44.0000	3.8182	3.6364	3.5455	2.5455
44.5000	3.8182	3.6364	3.5909	2.5909
45.0000	3.8182	3.6364	3.5455	2.5455
45.5000	4.0000	3.6364	3.5909	2.6364
46.0000	3.2727	3.6364	3.5909	2.6364
46.5000	3.6364	3.6364	3.4545	2.6364
47.0000	3.8182	3.6364	3.5909	2.5000
47.5000	3.8182	3.6364	3.5909	2.6818
48.0000	3.8182	3.6364	3.5909	2.5455
48.5000	3.8182	3.6364	3.5909	2.6818
49.0000	3.8182	3.6364	3.6364	2.5909
49.5000	3.8182	3.6364	3.5455	2.5455

50.0000	3.7273	3.6364	3.5455	2.5455

4.8 Results for Rejection of Lysozyme at Various Molarities of NaCl using Ultrafiltration Membrane

Rejection of protein is calculated by using this equation;

$$\text{Rejection} = \left(1 - \frac{Cp}{Cf}\right) \times 100\%$$

with; Cp = protein concentration in permeate

Cf = protein concentration in retentate

4.8.1 Rejection of Lysozyme at 0.5 M NaCl using Ultrafiltration Membrane

Table 4.17 and Figure 4.17 below show the percentage of protein rejection for lysozyme solution with 0.5 M of NaCl. The data is recorded every 5 minutes until it reaches 50 minutes. The value of lysozyme concentration is determined from the calibration curve. It shows that the percentage of lysozyme rejection is increasing in proportional to the time. The lysozyme of rejection is between 18.4% until 55.3%. In average percentage of lysozyme rejection only 39.85 % which means this amount of lysozyme will remain in the tank. This is because, the molarity of NaCl will cause the lysozyme to bond among them and it will lead to high rejection. The amount of the bonding lysozyme will cause fouling at the membrane surface due to the attraction of the different charges of polyethersulfone membrane with negative charge while the lysozyme with a positive charge. The fouling will prevent permeate from flowing over the membrane and the more it clogs over the membrane surface the more it will reduce the flux reading. The OD values are recorded at 750 nm and the concentration of lysozyme solution at pH 5 is 0.25g/l.

Table 4.17: Lysozyme Rejection at 0.5 M NaCl using Ultrafiltration Membrane Protein Rejection

Time (min)	OD	Concentration (g/L)	Rejection (%)
0.00	0.00	0.00	0.00
5.00	0.05	0.20	18.40
10.00	0.05	0.19	25.30
15.00	0.03	0.16	35.40
20.00	0.03	0.14	44.60
25.00	0.02	0.13	48.70
30.00	0.02	0.13	49.60
35.00	0.02	0.12	52.80
40.00	0.02	0.12	53.70
45.00	0.01	0.11	54.50
50.00	0.01	0.11	55.30

4.8.2 Rejection of Lysozyme at 1.0 M NaCl using Ultrafiltration Membrane

Table 4.18 and Figure 4.18 below show the percentage of protein rejection for lysozyme solution with 1.0 M of NaCl. The data is recorded every 5 minutes until it reaches 50 minutes. The value of lysozyme concentration is determined from the calibration curve from Figure 4.1. It shows that the percentage of lysozyme rejection is increasing in proportional to the time. The lysozyme of rejection is between 21.3 % until 56.60%. In average percentage of lysozyme rejection only 39.67 % which means this amount of lysozyme will remain in the tank. This is because, the molarity of NaCl will cause the lysozyme to bond among them and it will lead to high rejection. Although it will bond among them, the rejection values of 1.0 M of NaCl are higher than 0.5 M NaCl because of the stronger ionic bonding. The flux for 1.0 M NaCl is higher than 0.5 M NaCl because the stronger ionic bond the more fouling will happen at the membrane surface. The OD values are recorded at 750 nm and the concentration of lysozyme solution at pH 5 is 0.25g/l.

Table 4.18: Lysozyme Rejection at 1.0 M NaCl using Ultrafiltration Membrane Protein Rejection

Time (min)	OD	Concentration (g/L)	Rejection (%)
0.00	0.00	0.00	0.00
5.00	0.05	0.20	21.30
10.00	0.04	0.18	26.80
15.00	0.03	0.16	35.70
20.00	0.03	0.14	42.50
25.00	0.02	0.13	46.90
30.00	0.02	0.13	48.40
35.00	0.02	0.12	51.30
40.00	0.02	0.12	52.80
45.00	0.01	0.11	54.10
50.00	0.01	0.11	56.60

4.8.3 Rejection of Lysozyme at 1.5 M NaCl using Ultrafiltration Membrane

Table 4.19 and Figure 4.19 below show the percentage of protein rejection for lysozyme solution with 1.5 M of NaCl. The data is recorded every 5 minutes until it reaches 50 minutes. The value of lysozyme concentration is determined from the calibration curve. It shows that the percentage of lysozyme rejection is increasing in proportional to the time. The lysozyme of rejection is between 22.10 % until 58.40%. In average percentage of lysozyme rejection only 41.21 % which means this amount of lysozyme will remain in the tank. This is because, the molarity of NaCl will cause the lysozyme to bond among them and it will lead to high rejection. Although it will bond among them, the rejection values of 1.5 M of NaCl are higher than 0.5 M NaCl and 1.0 M NaCl because of the stronger ionic bonding. The higher rejection of lysozyme means that the lower the values of permeate flux. From the table, it is clear that the permeate flux for 1.5 M NaCl is lower than 1.0 M NaCl and 0.5 M NaCl. The OD values are recorded at 750 nm and the concentration of lysozyme solution at pH 5 is 0.25g/l.

Table 4.19: Lysozyme Rejection at 1.5 M NaCl using Ultrafiltration Membrane Protein Rejection

Time (min)	OD	Concentration (g/L)	Rejection (%)
0.00	0.00	0.00	0.00
5.00	0.05	0.19	22.10
10.00	0.05	0.19	25.90
15.00	0.03	0.16	35.50
20.00	0.03	0.14	44.10
25.00	0.02	0.13	46.90
30.00	0.02	0.13	48.40
35.00	0.02	0.12	53.60
40.00	0.01	0.11	56.90
45.00	0.01	0.10	58.40
50.00	0.01	0.10	61.50

4.8.4 Rejection of Lysozyme at 2.0 M NaCl using Ultrafiltration Membrane

Table 4.20 and Figure 4.20 below show the percentage of protein rejection for lysozyme solution with 2.0 M of NaCl. The data is recorded every 5 minutes until it reaches 50 minutes. The value of lysozyme concentration is determined from the calibration curve. It shows that the percentage of lysozyme rejection is increasing in proportional to the time. The lysozyme of rejection is between 22.80 % until 68.40%. In average percentage of lysozyme rejection only 42.68 % which means this amount of lysozyme will remain in the tank. This is because, the molarity of NaCl will cause the lysozyme to bond among them and it will lead to high rejection. Although it will bond among them, the rejection values of 2.0 M of NaCl are higher than 0.5 M NaCl, 1.0 M NaCl and also 1.5 M NaCl because of the stronger ionic bonding. The higher rejection of lysozyme means that the lower the values of permeate flux. From the table, it is clear that the permeate flux for 2.0 M NaCl is lower than 1.5 M NaCl, 1.0 M NaCl and 0.5 M NaCl. The OD values are recorded at 750 nm and the concentration of lysozyme solution at pH 5 is 0.25g/l

Table 4.20: Lysozyme Rejection at 2.0 M NaCl using Ultrafiltration Membrane Protein Rejection

Time (min)	OD	Concentration (g/L)	Rejection (%)
0.00	0.00	0.00	0.00
5.00	0.05	0.19	22.80
10.00	0.04	0.18	27.50
15.00	0.04	0.17	33.70
20.00	0.03	0.14	42.50
25.00	0.02	0.13	47.80
30.00	0.02	0.12	51.90
35.00	0.01	0.11	54.30
40.00	0.01	0.10	58.90
45.00	0.01	0.10	61.70
50.00	0.00	0.08	68.40

4.9 Overall Result Analysis for Lysozyme Rejection at Various Molarities of NaCl using Ultrafiltration Membrane

From Figure 4.21, it shows that percentage of rejection is increasing proportional to the time. The maximum value of lysozyme rejection is at 2.0 M of NaCl and the minimum is at 0.5 M NaCl. This is because, the stronger the ionic bond the more it will cause the lysozyme to bond among them and the more it will remain in the tank. The strongest ionic bond is in 2.0 M NaCl lysozyme solution, thus, the strongest bond among them will occur and it will lead to membrane fouling because it will prevent the flux permeate from flowing over the membrane. The weakest ionic bond among all molarities of NaCl is 0.5 M NaCl lysozyme solution because the bond is less strong from 2.0 M NaCl lysozyme solution and the lysozyme rejection is less compared to the others. The stronger the ionic bond, the more it will clog the membrane surface and also will cause low permeate flux.

Table 4.21: Overall Result Analysis for Lysozyme Rejection at Various Molarities of NaCl using Ultrafiltration Membrane

Time (min)	Rejection (%) at 0.5 M NaCl	Rejection (%) at 1.0 M NaCl	Rejection (%) at 1.5 M NaCl	Rejection (%) at 2.0 M NaCl
0.00	0.00	0.00	0.00	0.00
5.00	18.40	21.30	22.10	22.80
10.00	25.30	26.80	25.90	27.50
15.00	35.40	35.70	35.50	33.70
20.00	44.60	42.50	44.10	42.50
25.00	48.70	46.90	46.90	47.80
30.00	49.60	48.40	48.40	51.90
35.00	52.80	51.30	53.60	54.30
40.00	53.70	52.80	56.90	58.90
45.00	54.50	54.10	58.40	61.70
50	39.5	46.9	51.2	54.7

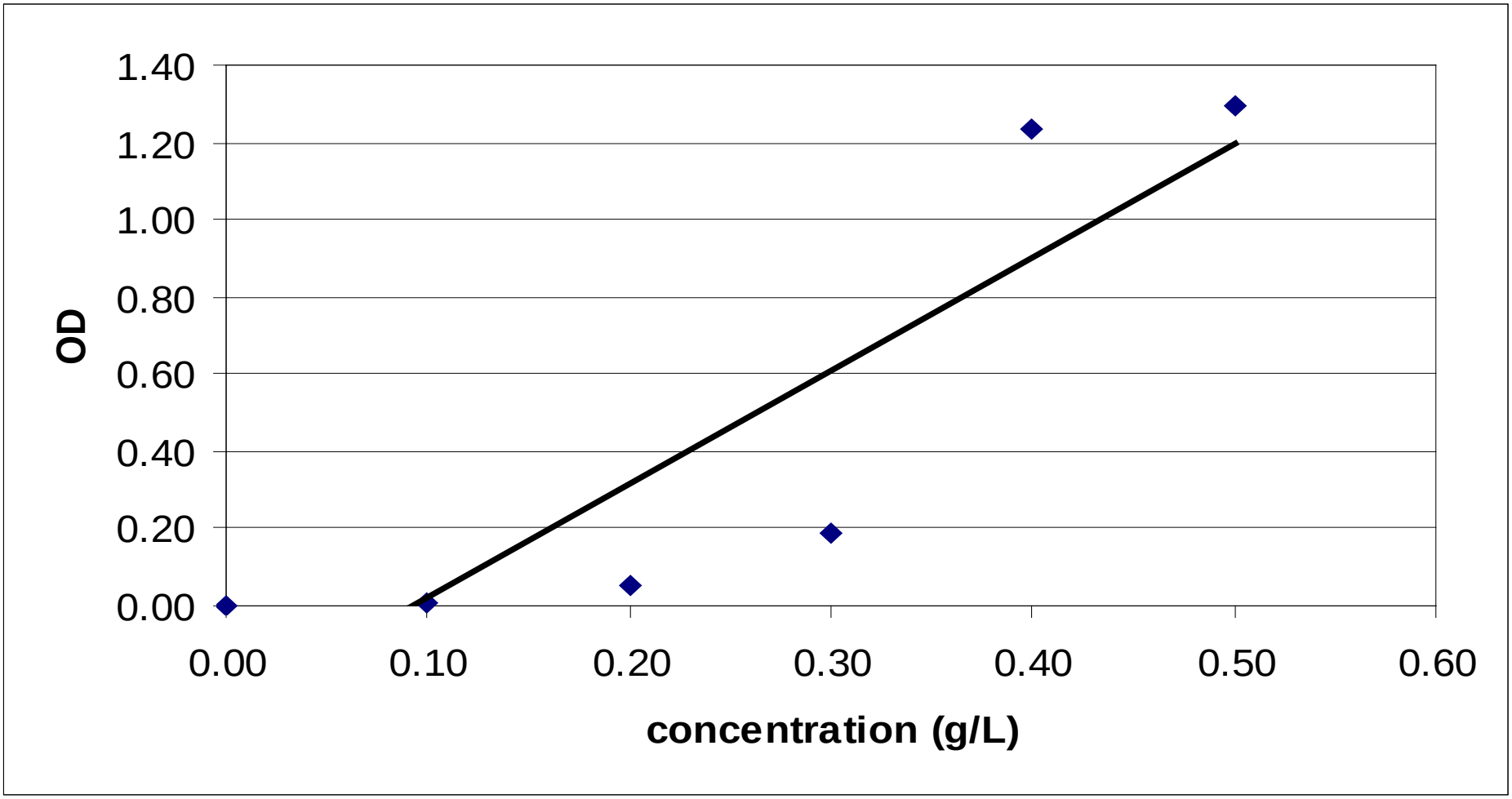

Figure 4.1: Calibration Curve

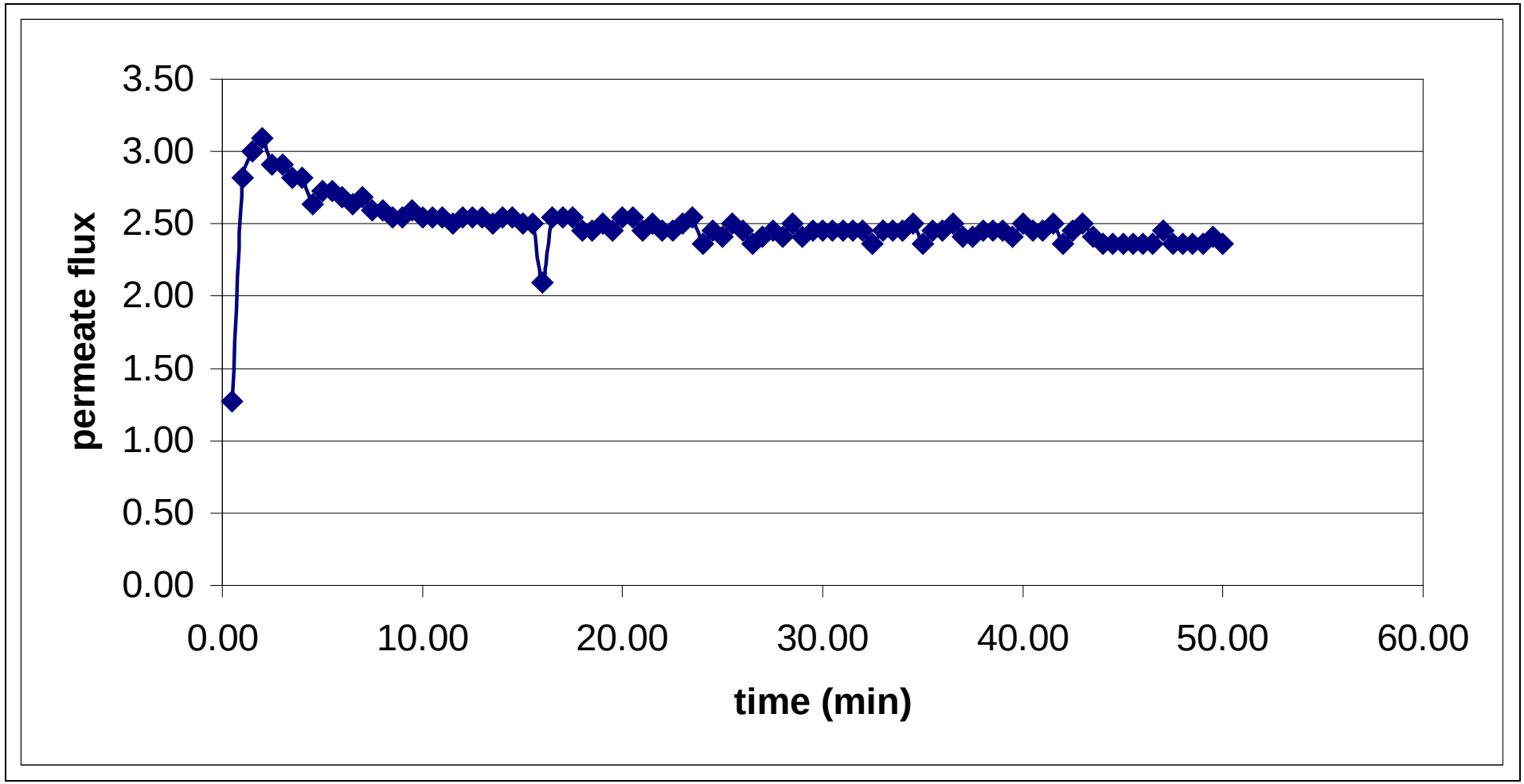

Figure 4.2: Flux Decline of Lysozyme Solution at pH 5 using Ultrafiltration Membrane

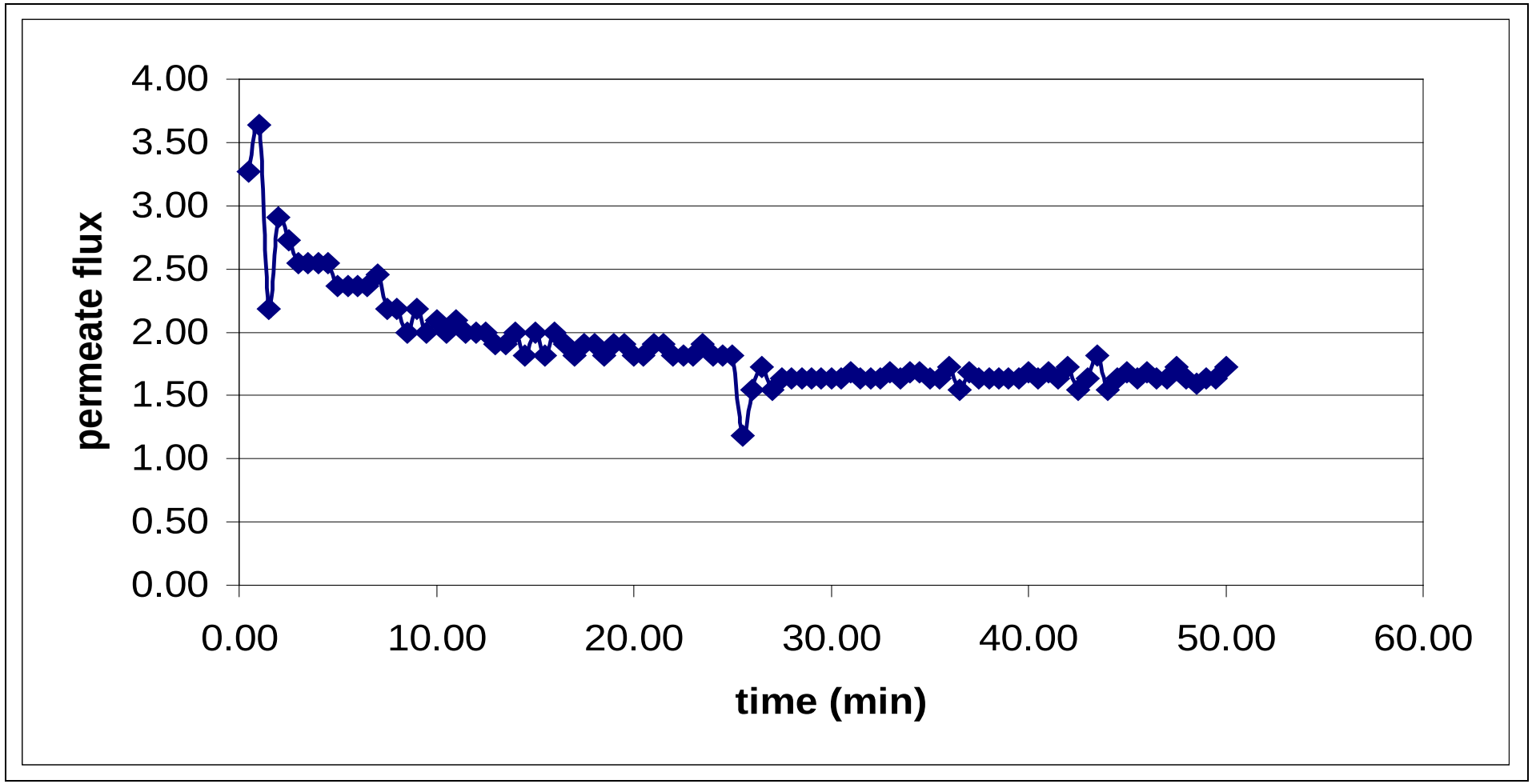

Figure 4.3: Flux Decline of Lysozyme Solution at pH 6 using Ultrafiltration Membrane

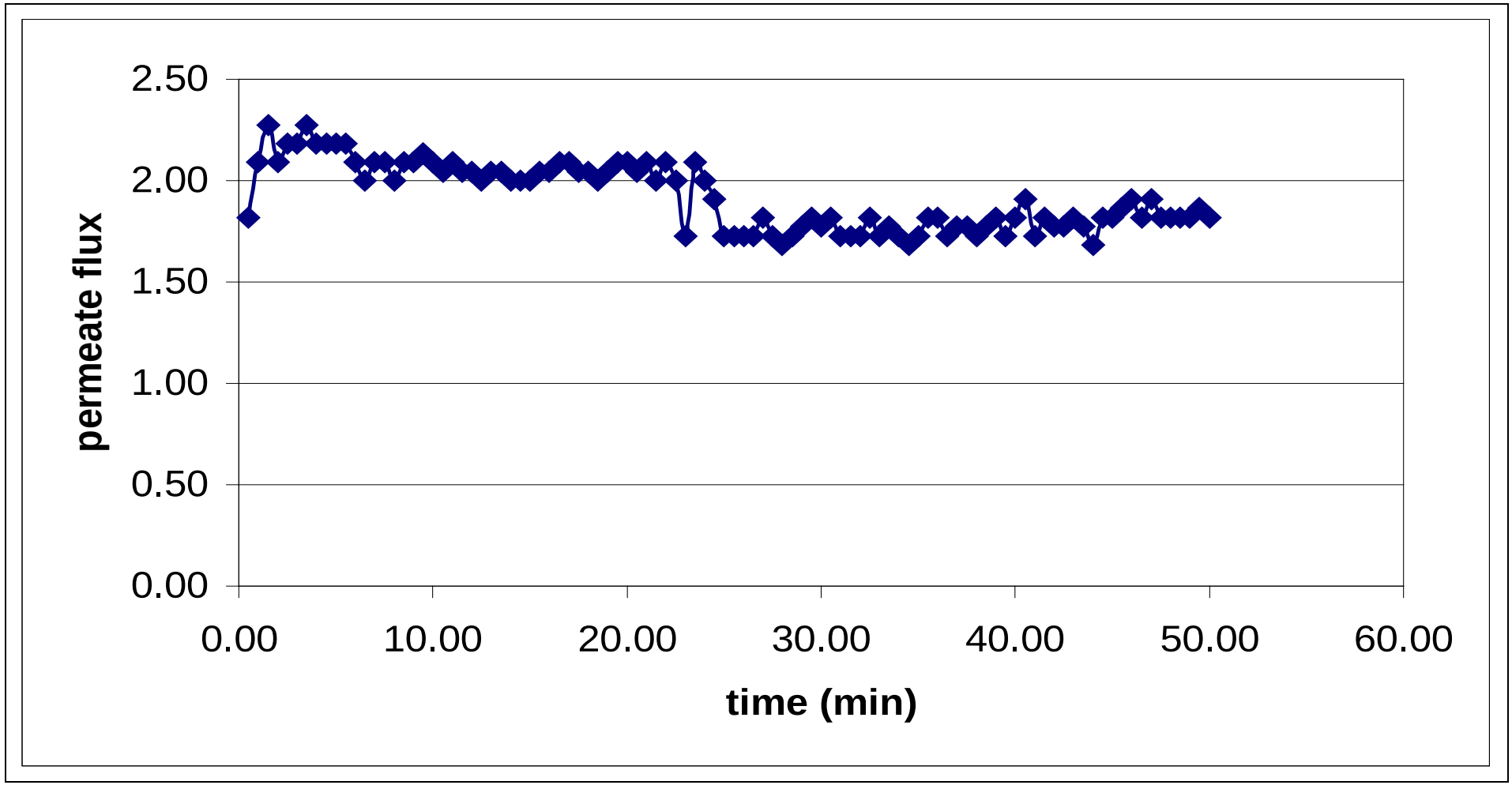

Figure 4.4: Flux Decline of Lysozyme Solution at pH 7 using Ultrafiltration Membrane

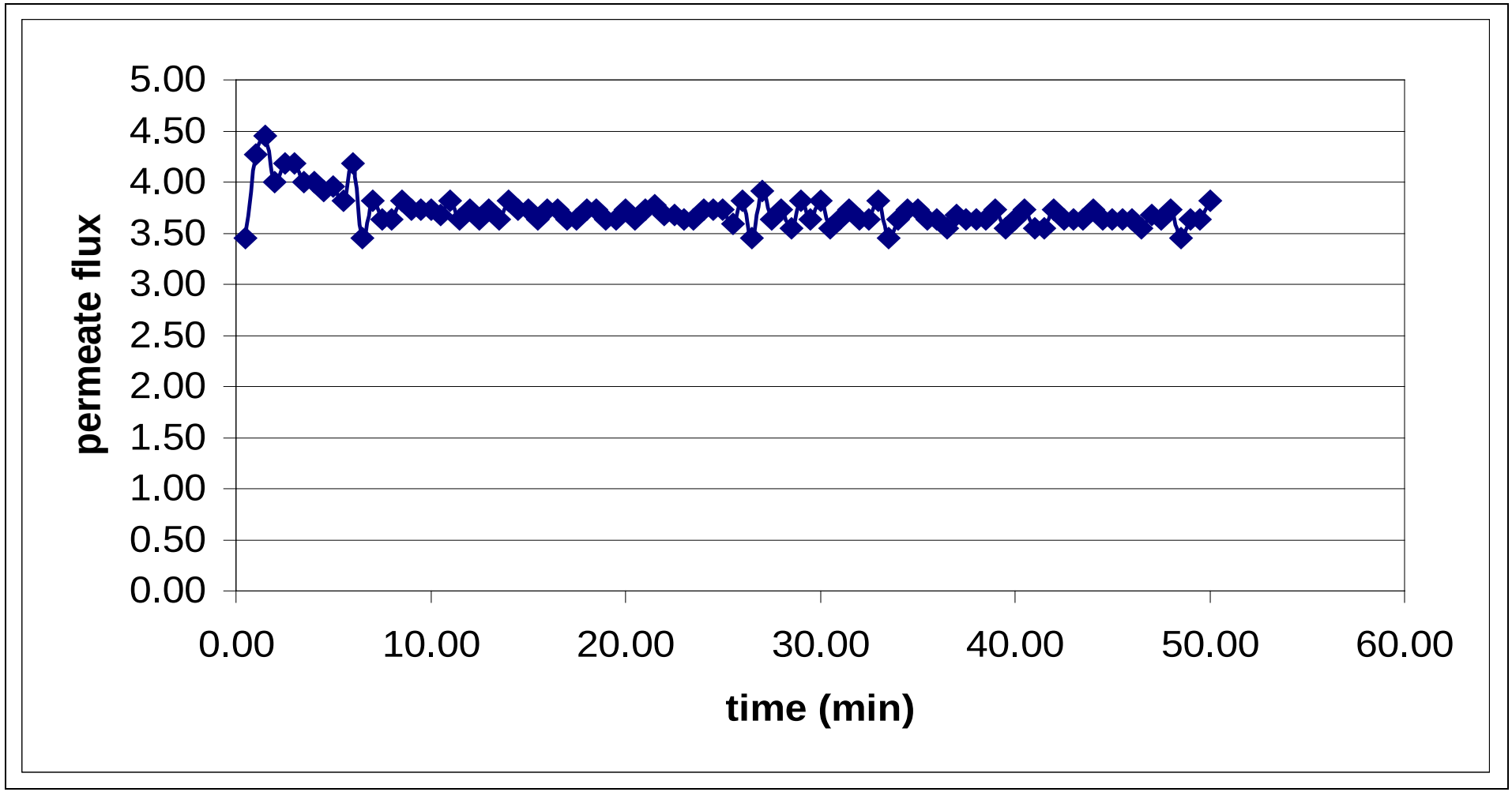

Figure 4.5: Flux Decline of Lysozyme Solution at pH 8 using Ultrafiltration Membrane

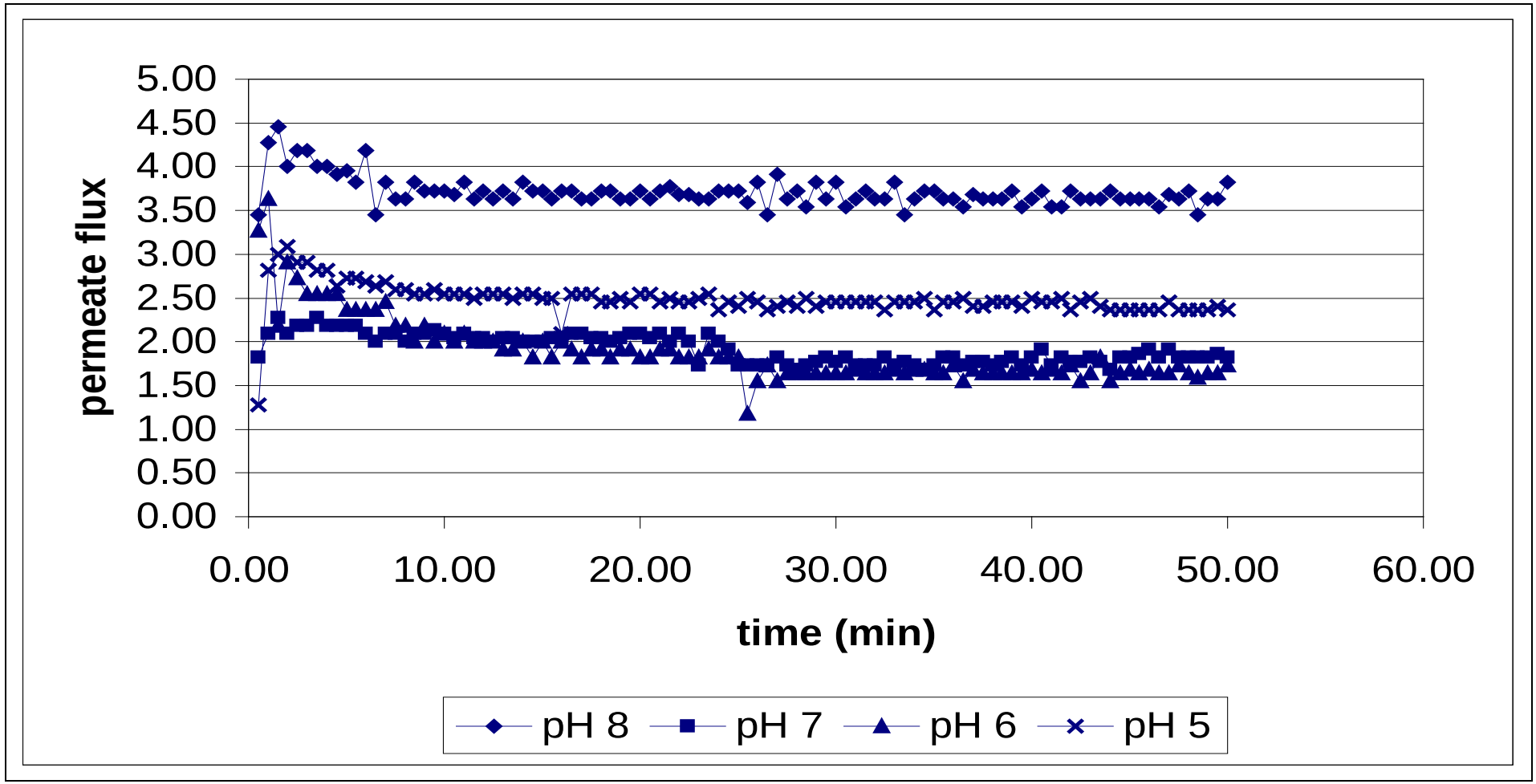

Figure 4.6: Overall Result Analysis for Flux Decline of Lysozyme Solution at Various pH using Ultrafiltration Membrane

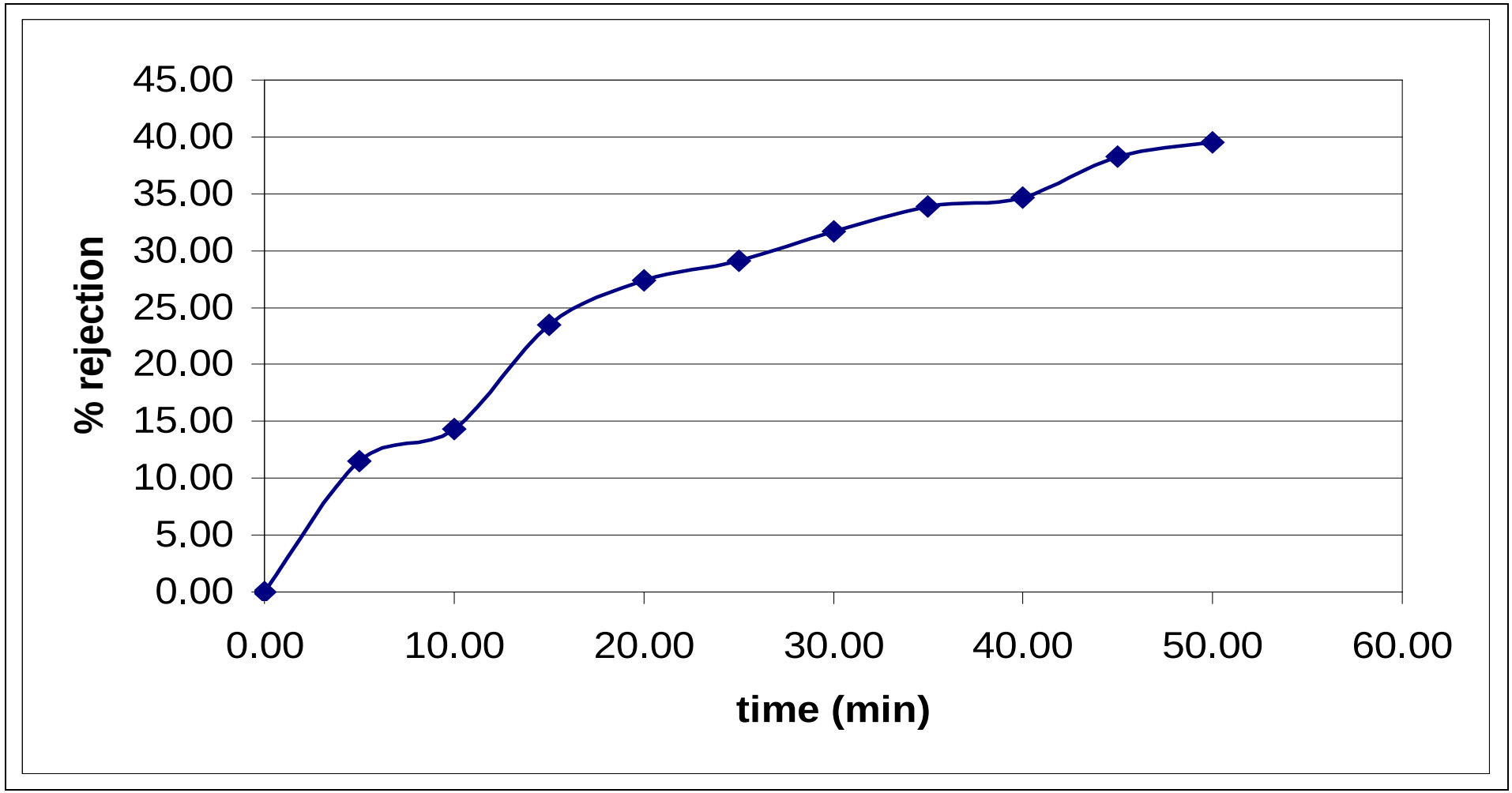

Figure 4.7: Lysozyme Rejection at pH 5 using Ultrafiltration Membrane Protein Rejection

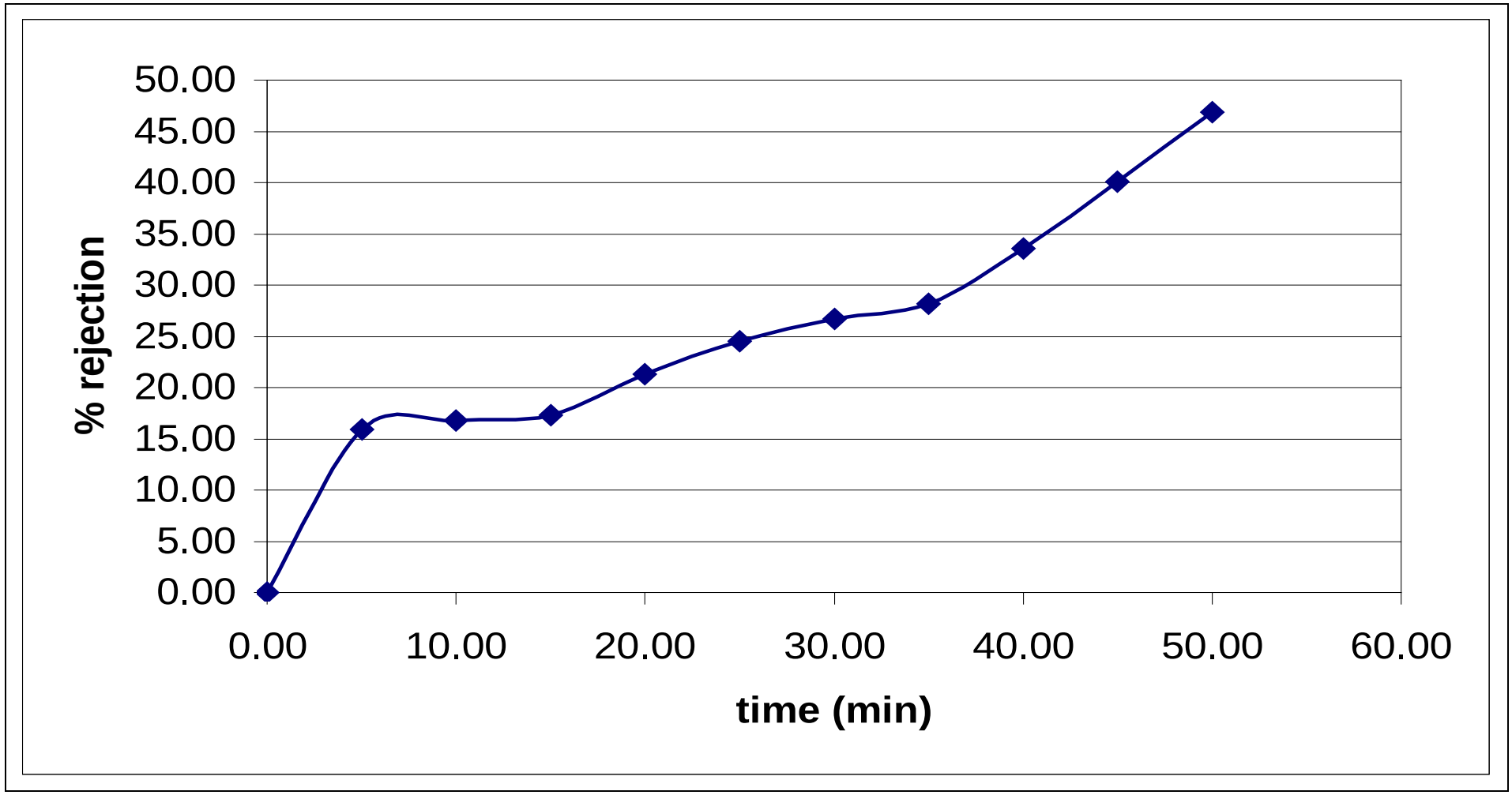

Figure 4.8: Lysozyme Rejection at pH 6 using Ultrafiltration Membrane Protein Rejection

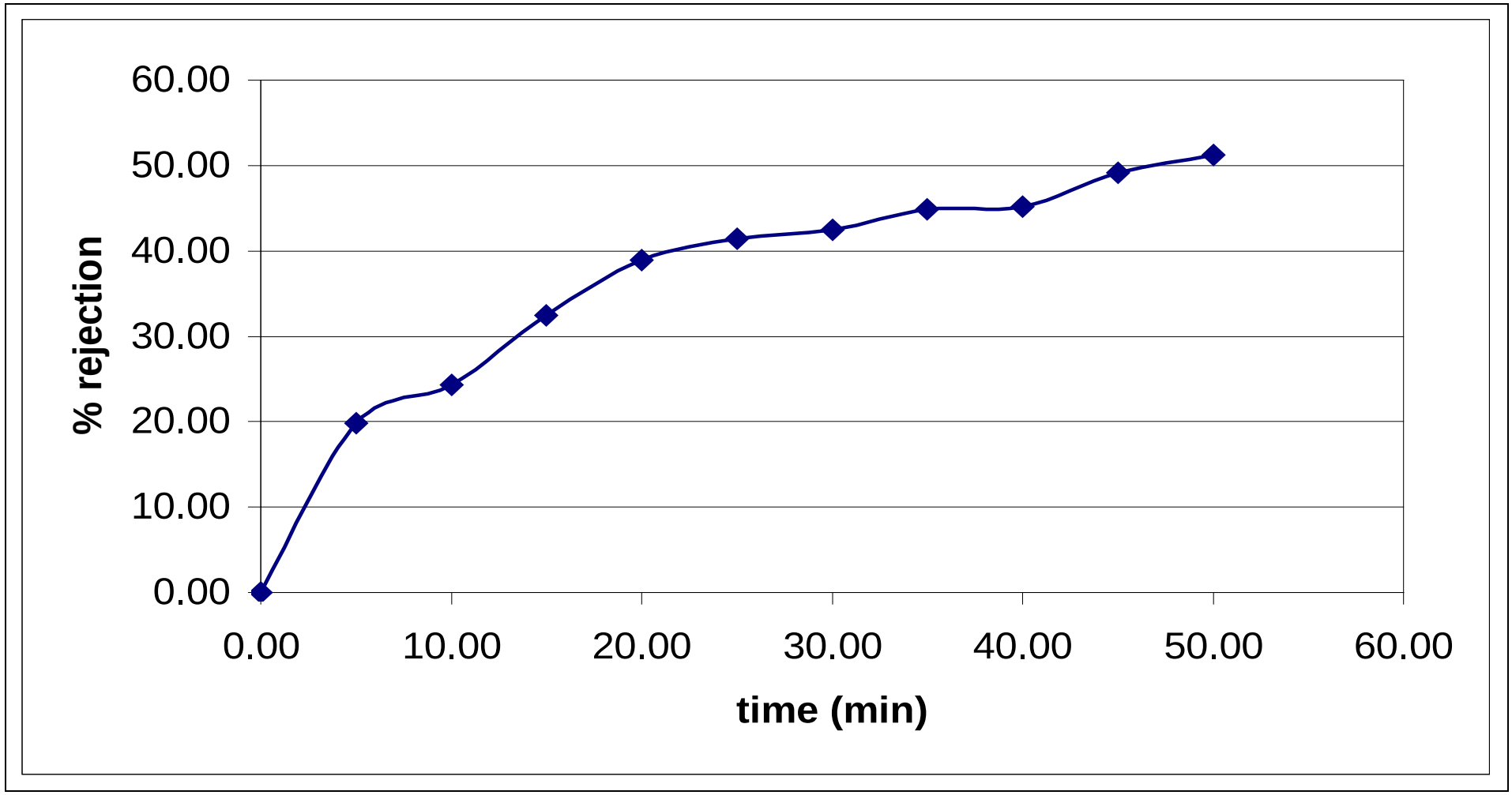

Figure 4.9: Lysozyme Rejection at pH 7 using Ultrafiltration Membrane Protein Rejection

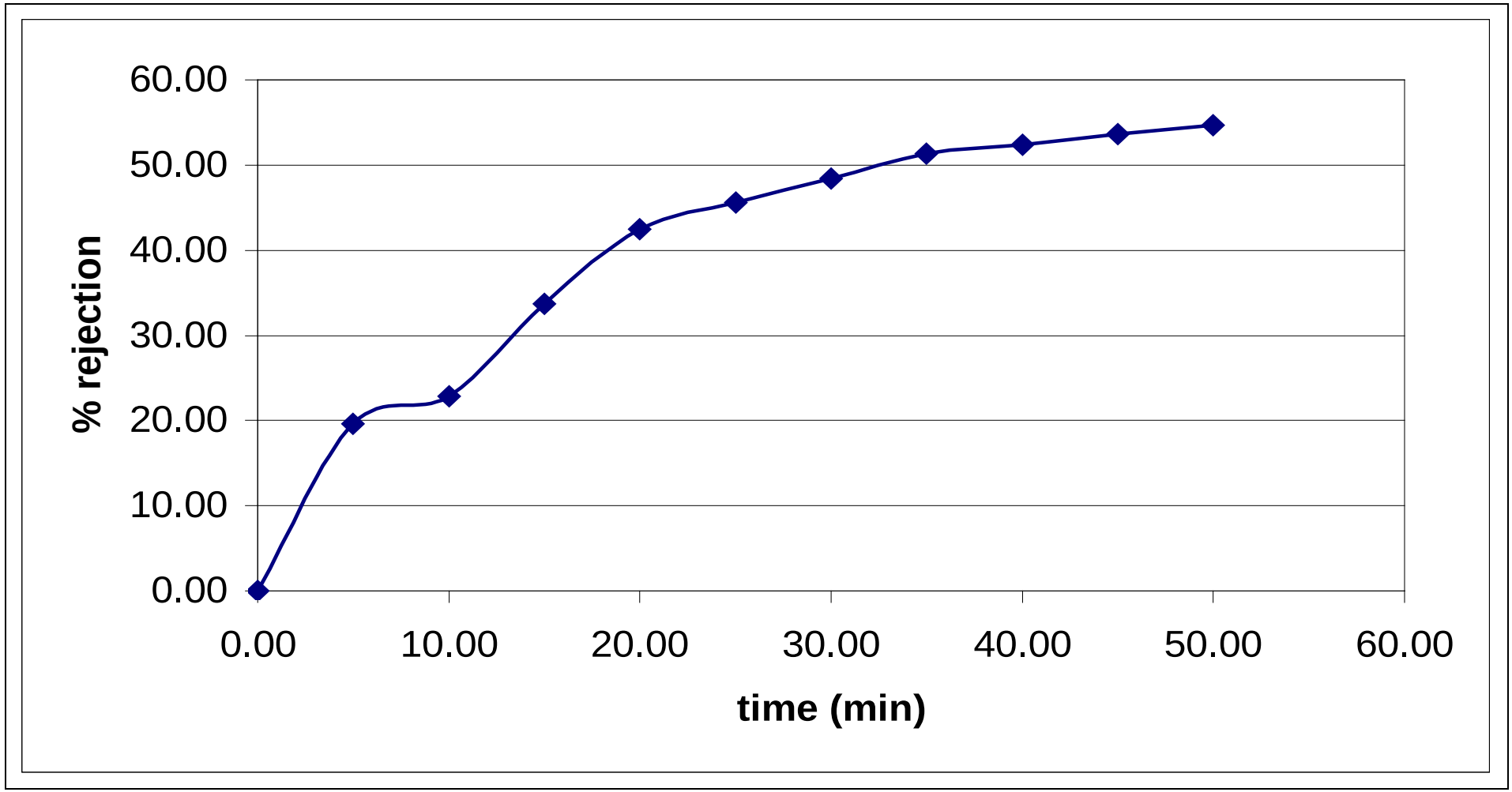

Figure 4.10: Lysozyme Rejection at pH 5 using Ultrafiltration Membrane Protein Rejection

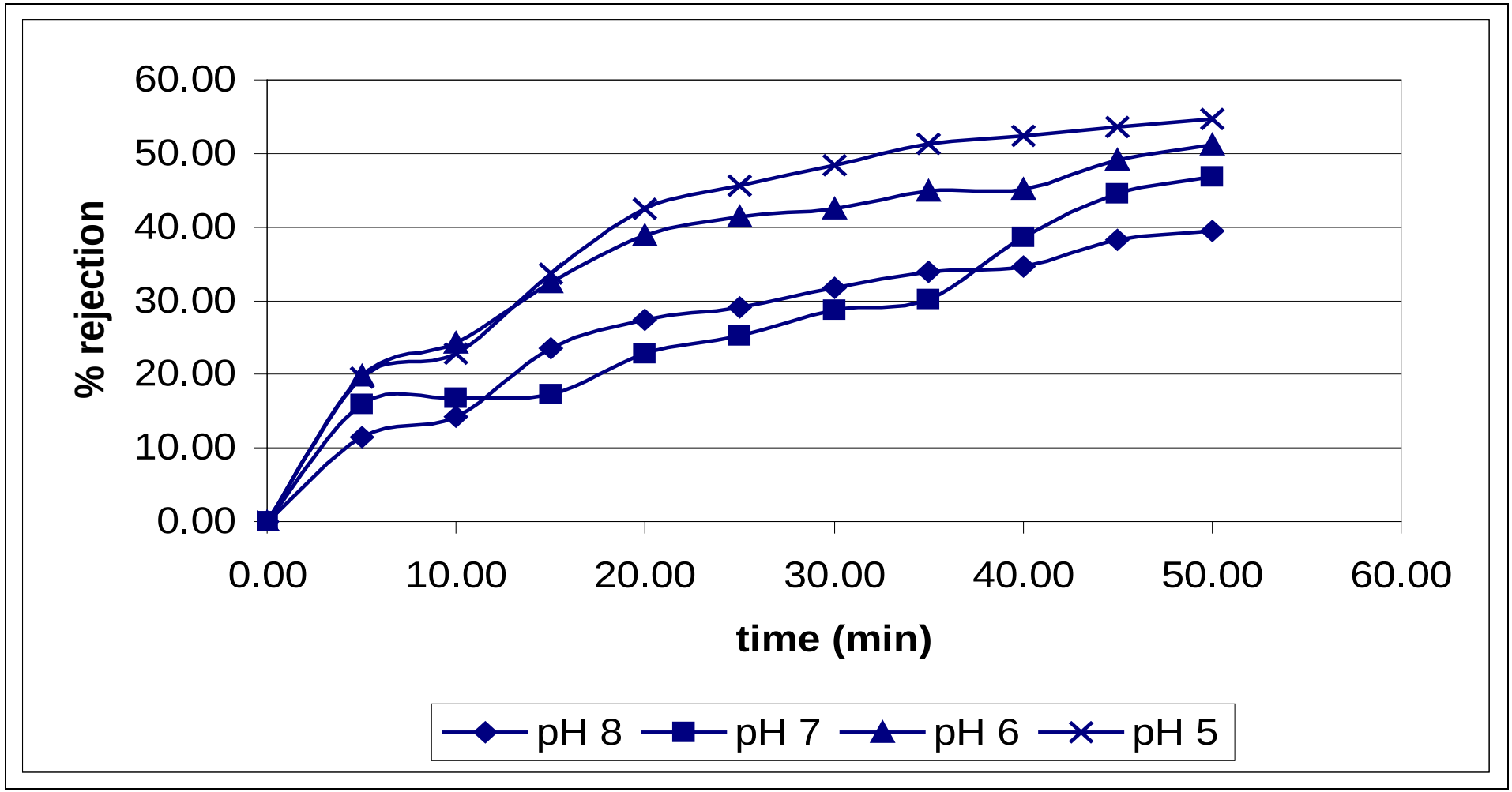

Figure 4.11: Overall Result Analysis for Lysozyme Rejection at Various pH

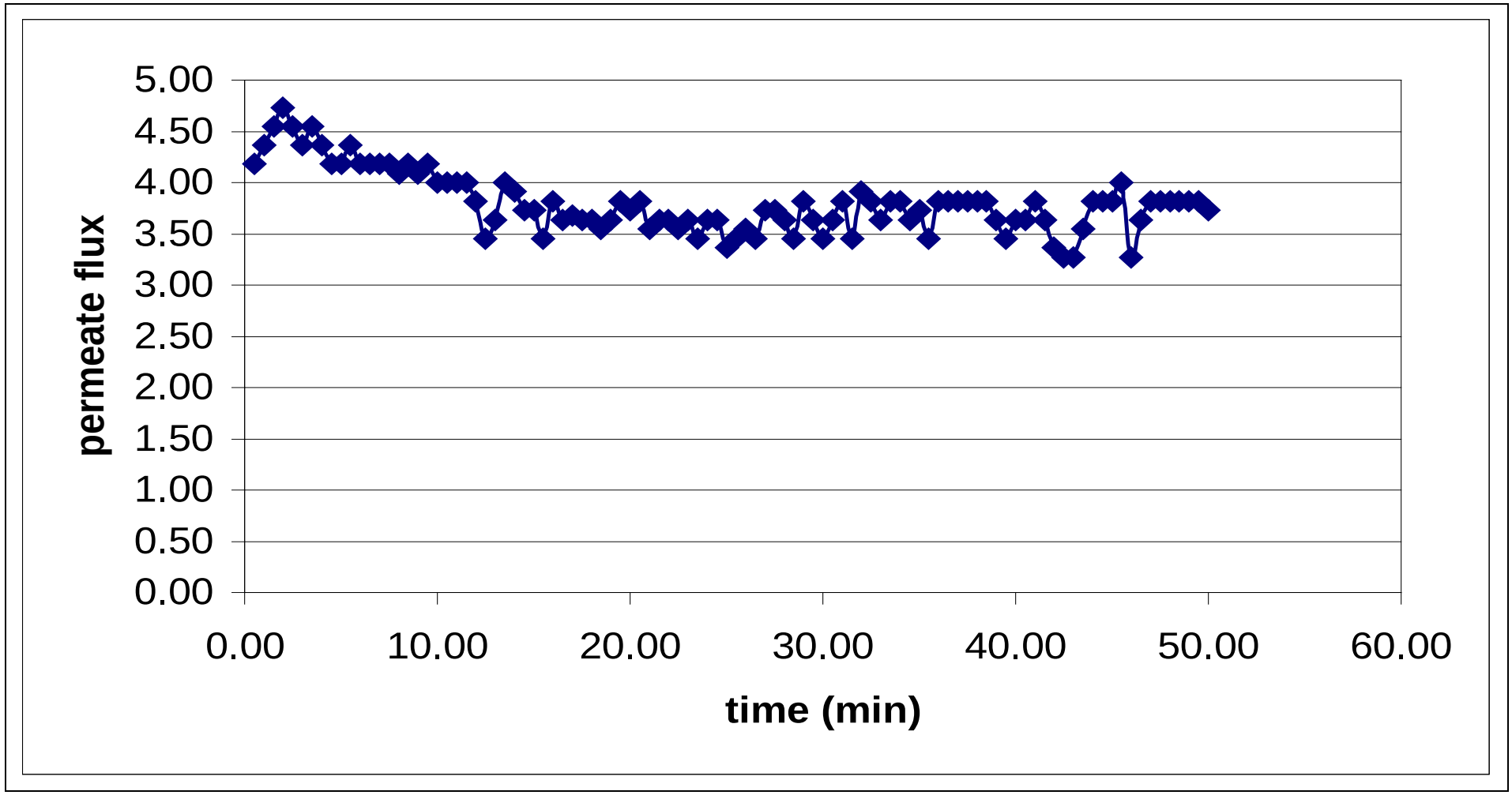

Figure 4.12: Flux Decline of Lysozyme Solution at 0.5 M NaCl using Ultrafiltration Membrane

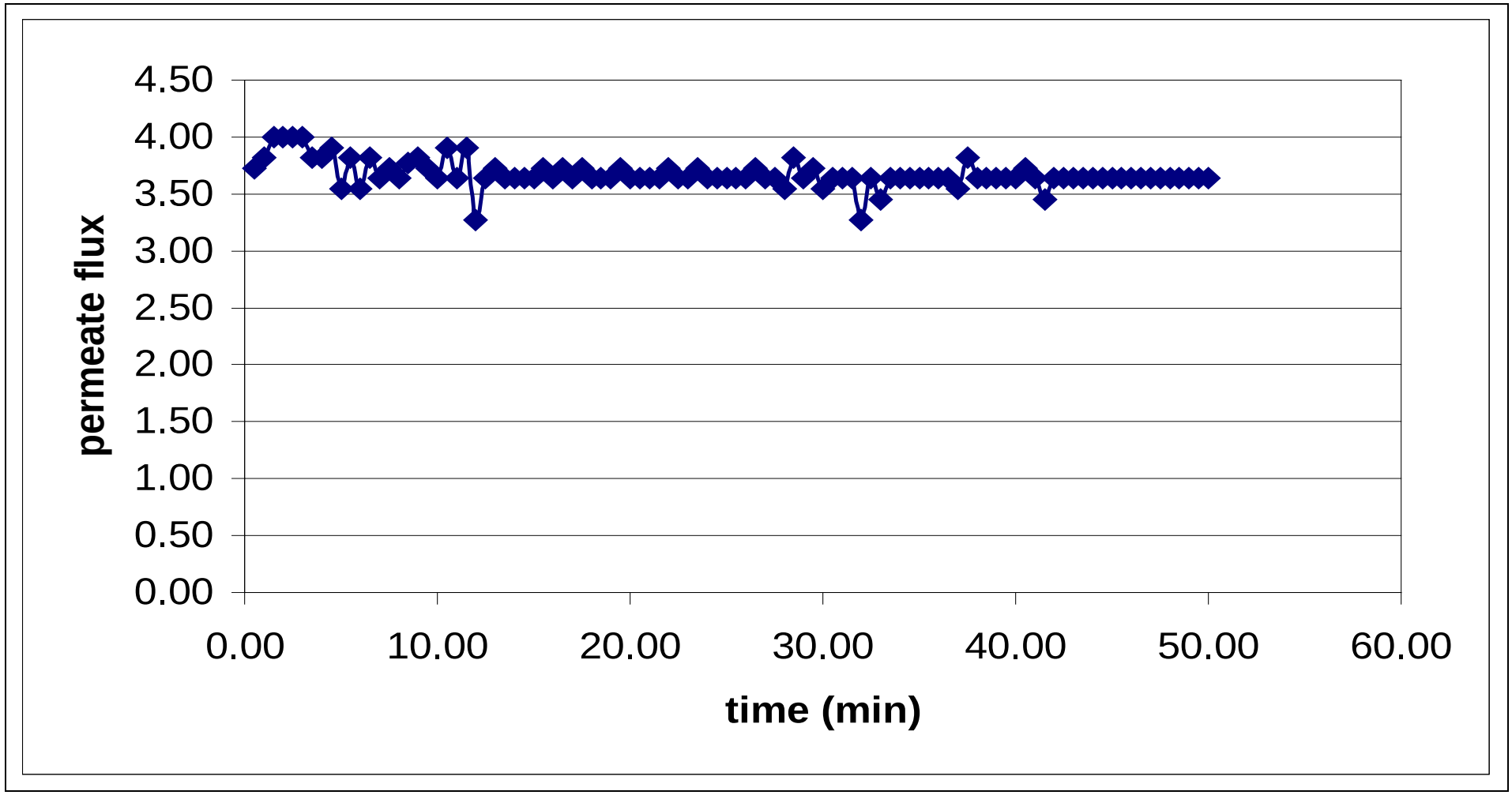

Figure 4.13: Flux Decline of Lysozyme Solution at 1.0 M NaCl using Ultrafiltration Membrane

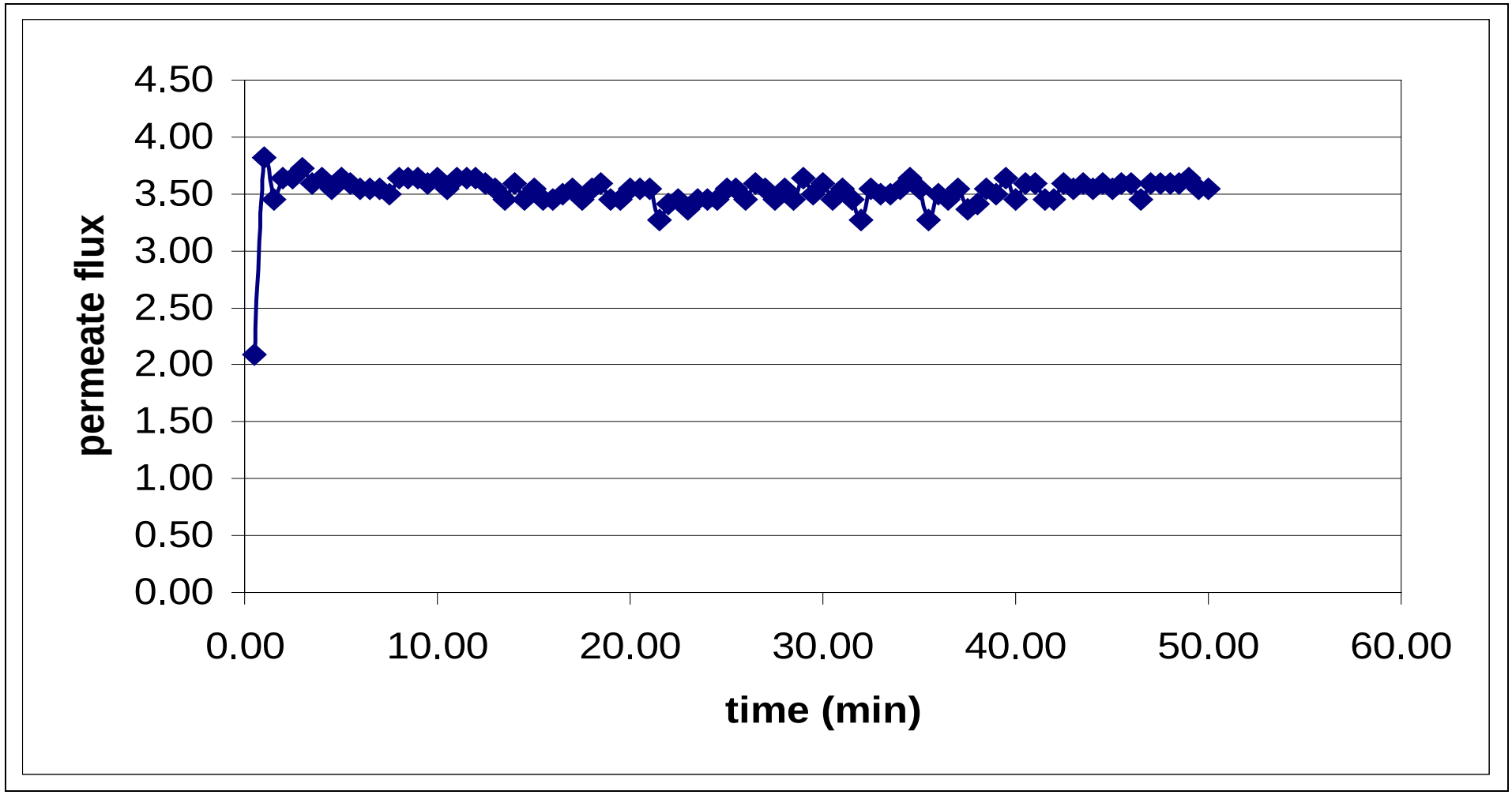

Figure 4.14: Flux Decline of Lysozyme Solution at 1.5 M NaCl using Ultrafiltration Membrane

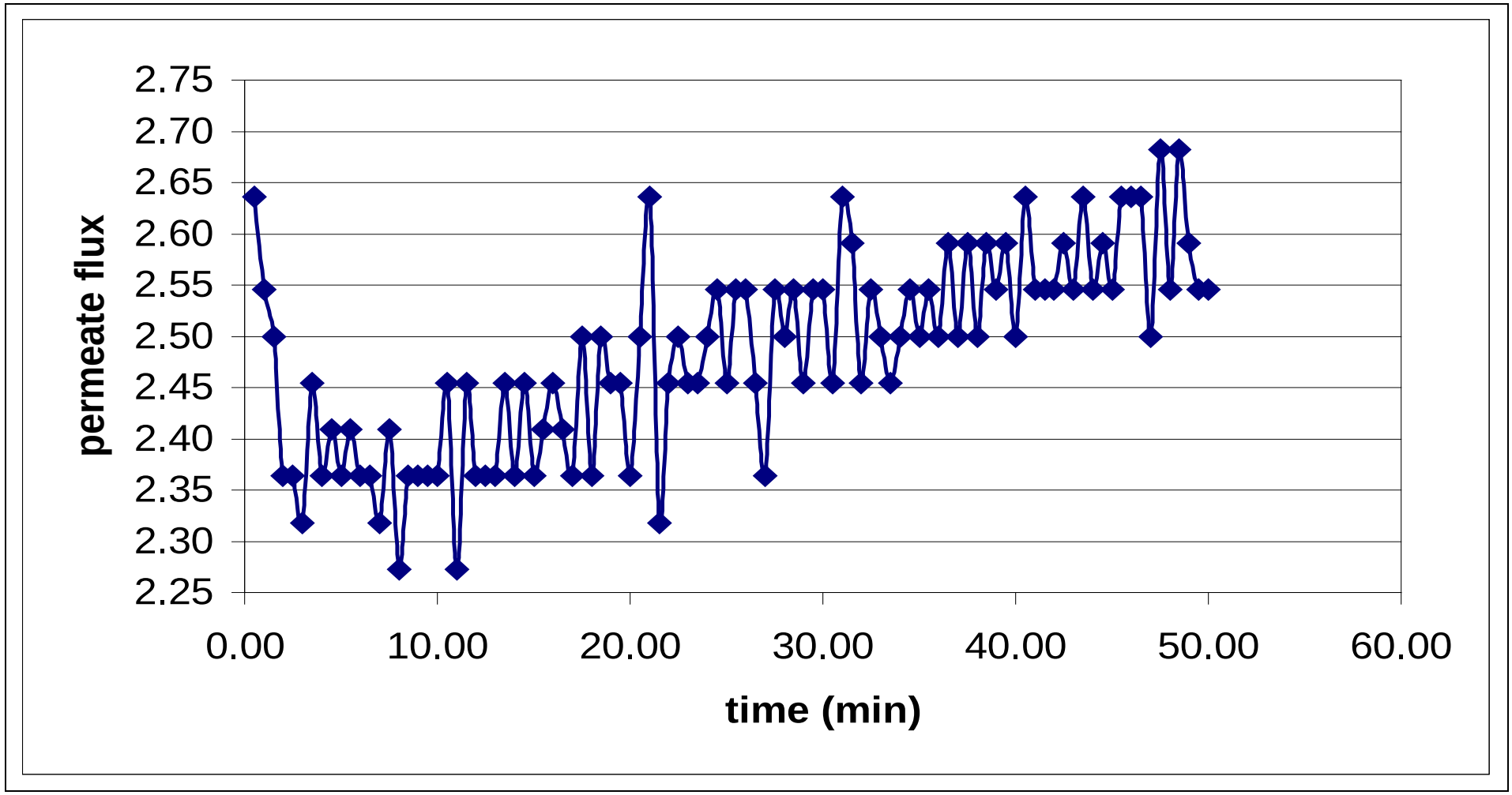

Figure 4.15: Flux Decline of Lysozyme Solution at 2.0 M NaCl using Ultrafiltration Membrane

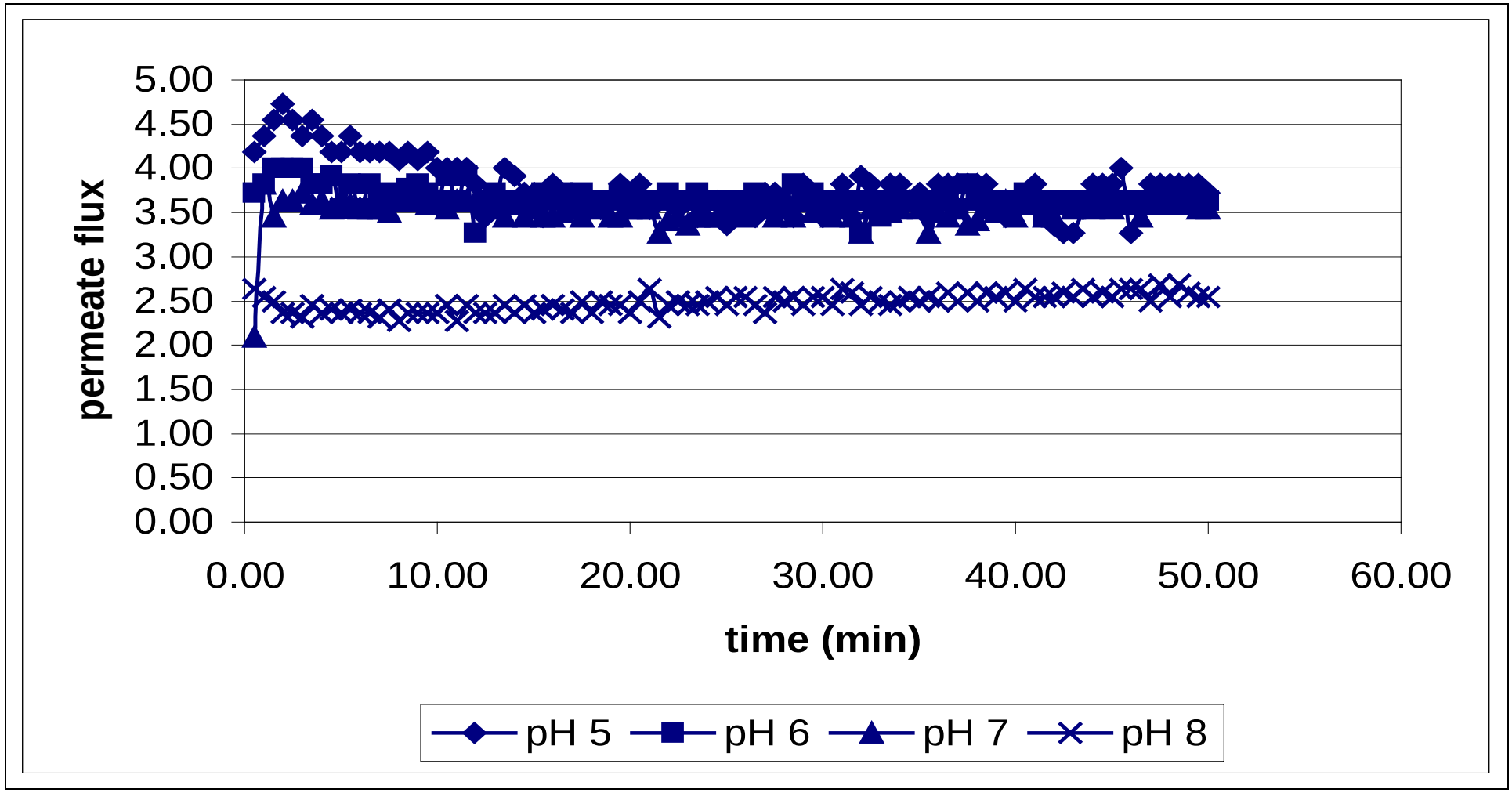

Figure 4.16: Overall Result Analysis for Effect of Ionic Strength on the Membrane Flux Various Concentration of NaCl using Ultrafiltration Membrane

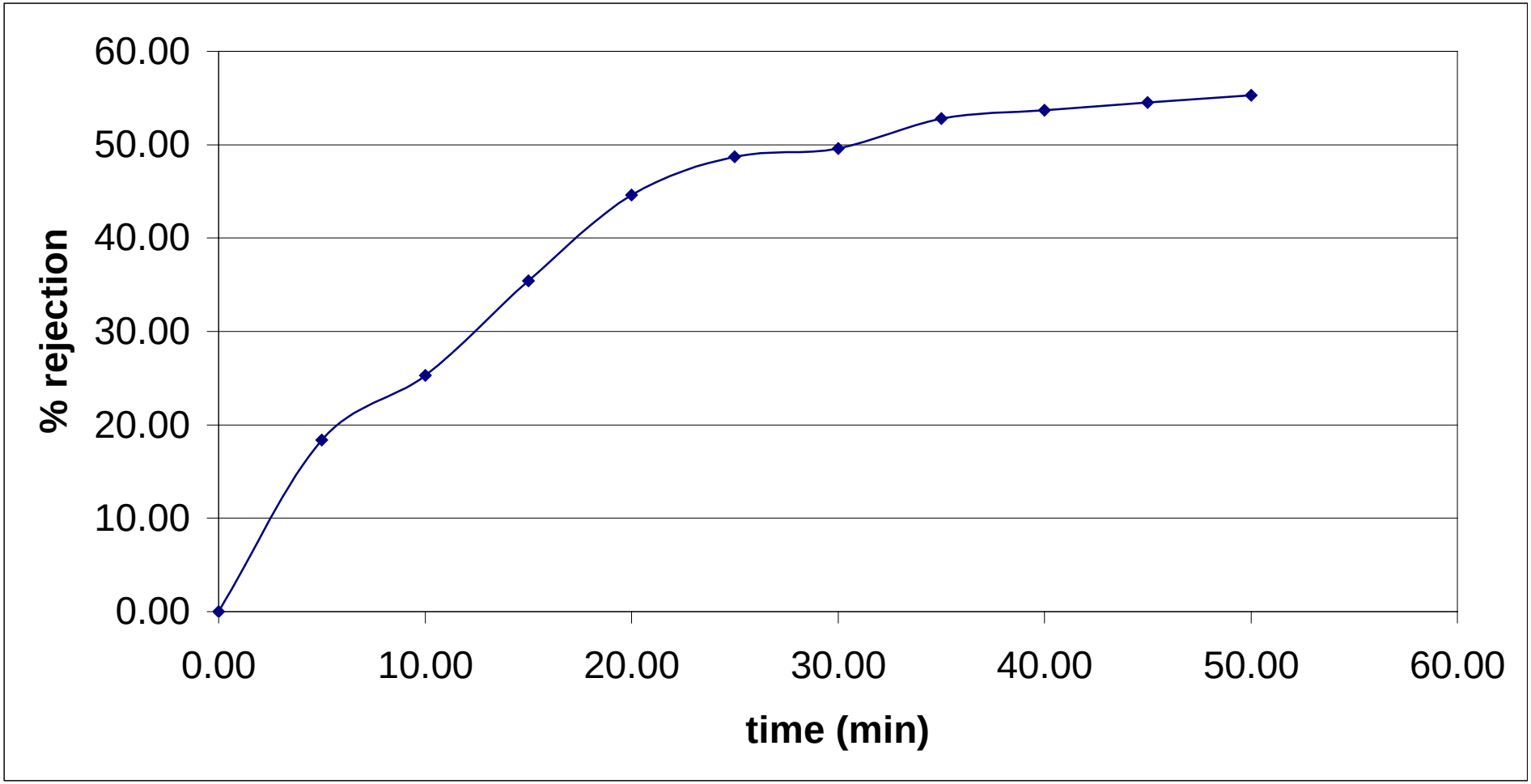

Figure 4.17: Lysozyme Rejection at 0.5 M NaCl using Ultrafiltration Membrane Protein Rejection

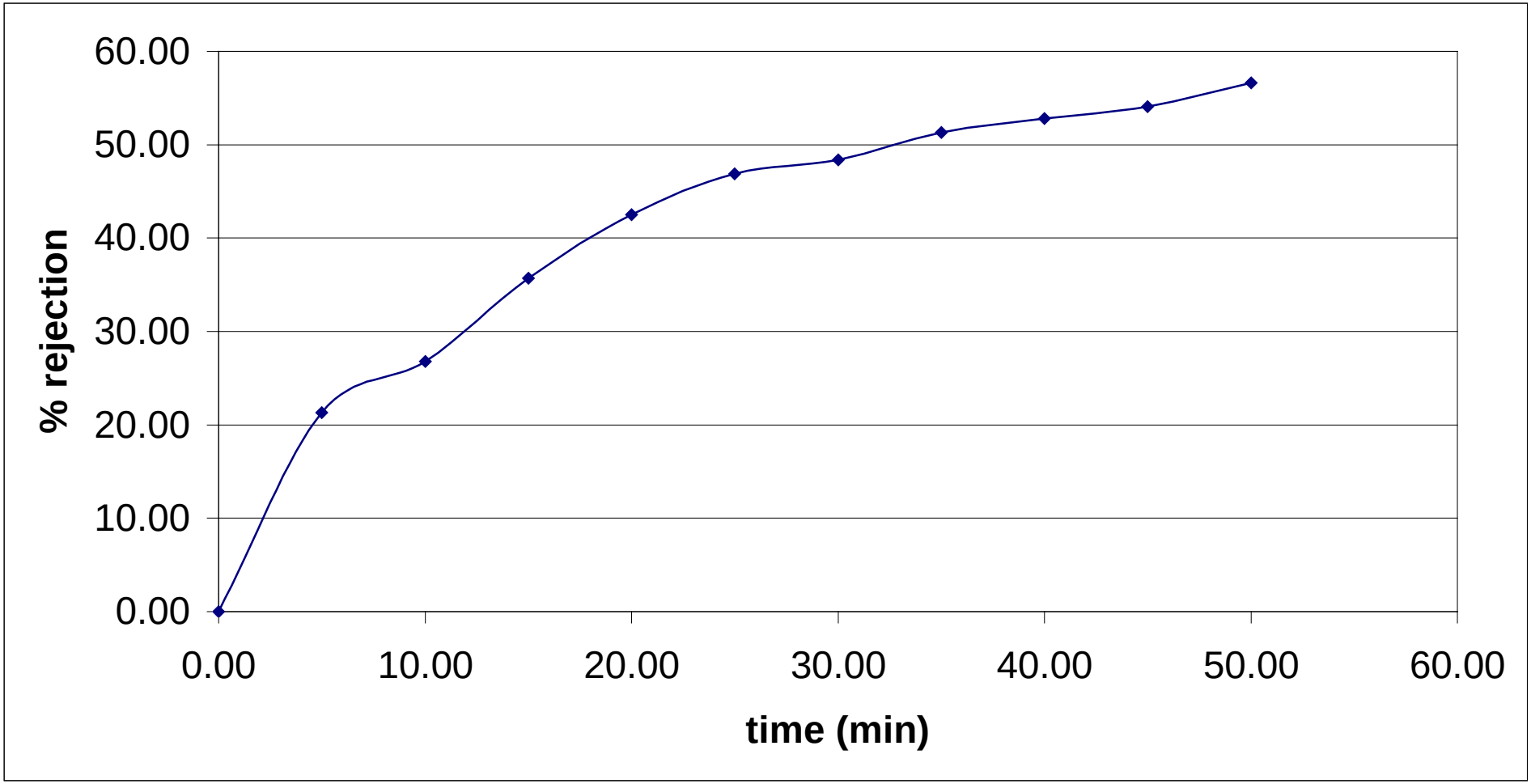

Figure 4.18: Lysozyme Rejection at 1.0 M NaCl using Ultrafiltration Membrane Protein Rejection

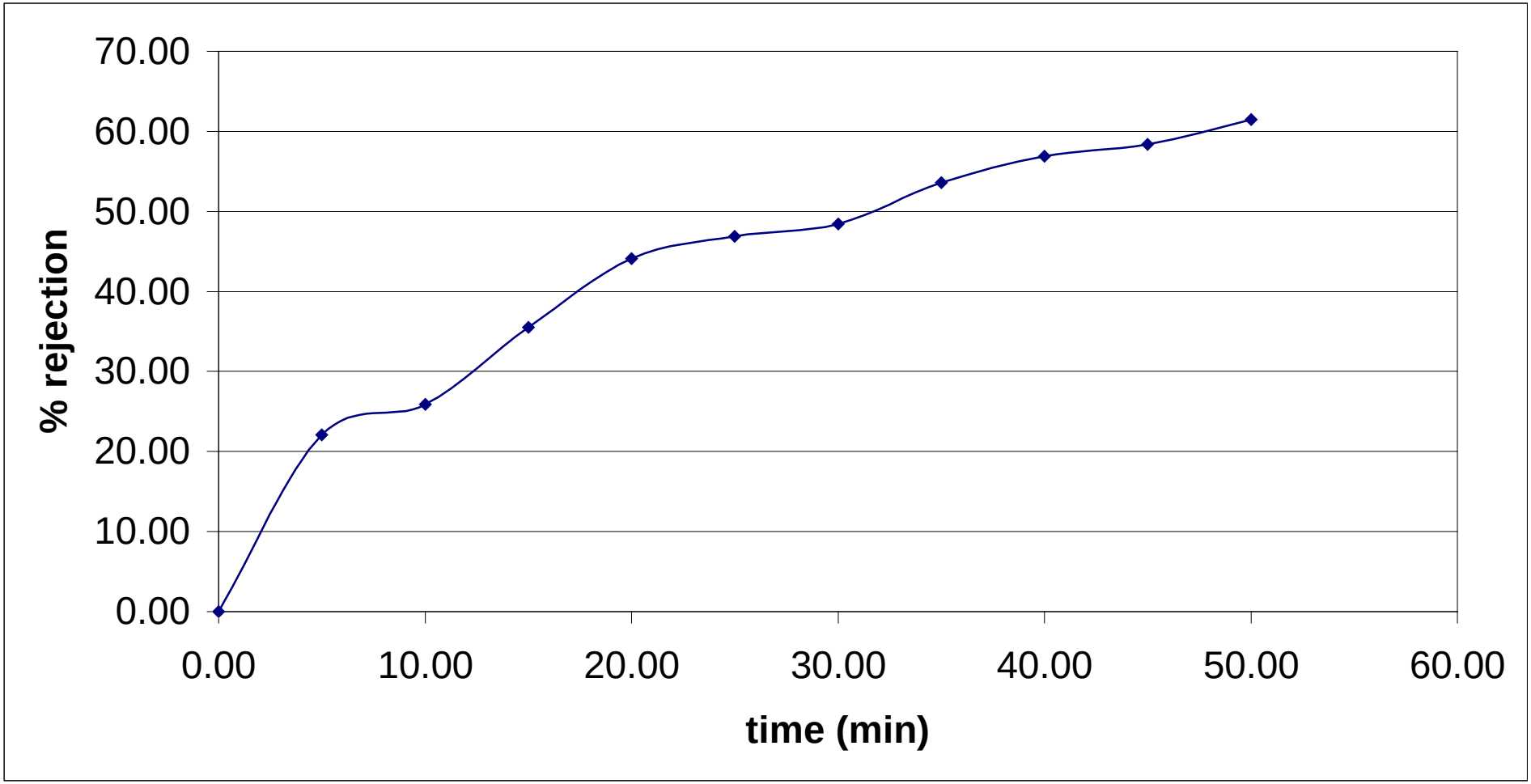

Figure 4.19: Lysozyme Rejection at 1.5 M NaCl using Ultrafiltration Membrane Protein Rejection

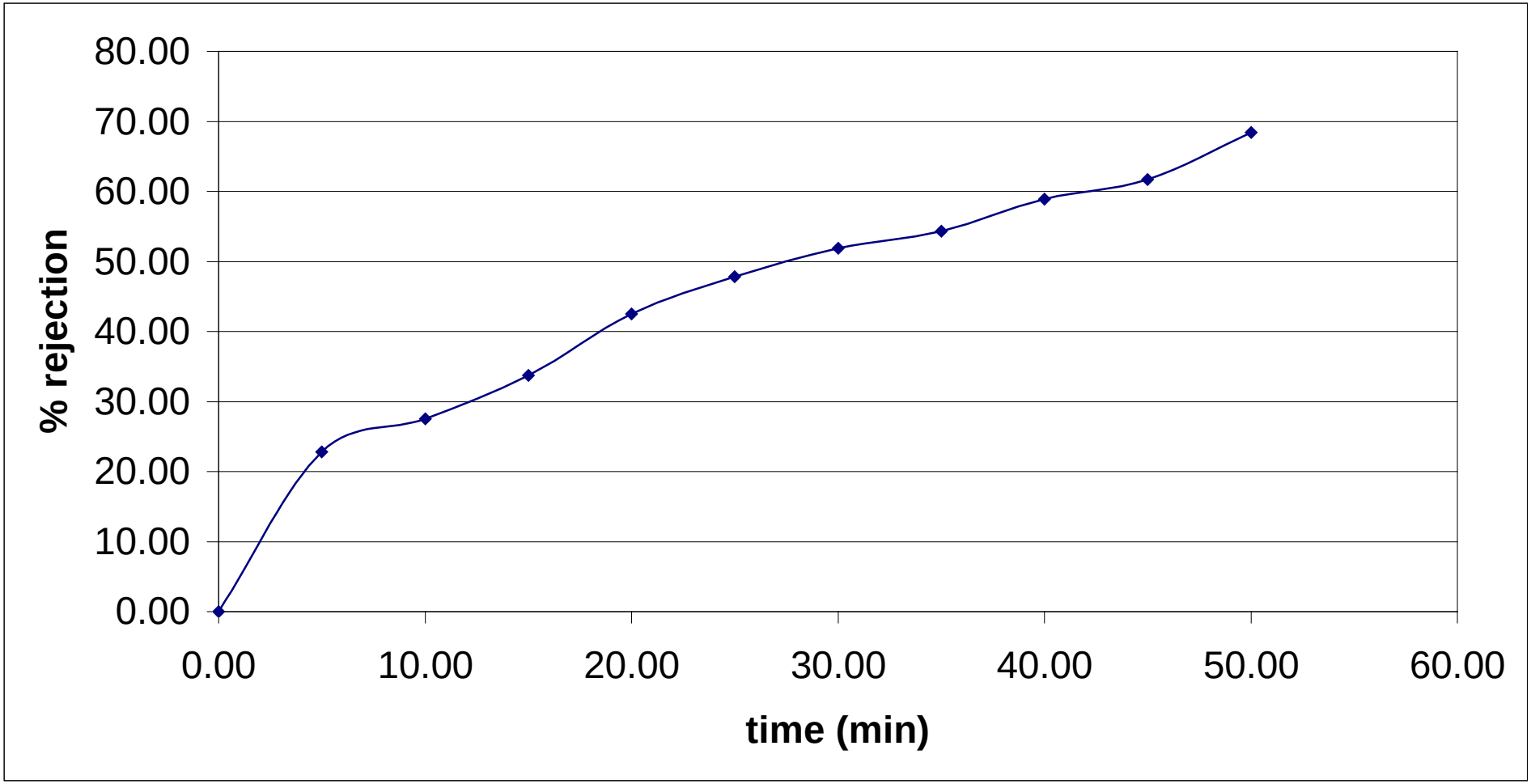

Figure 4.20: Lysozyme Rejection at 2.0 M NaCl using Ultrafiltration Membrane Protein Rejection

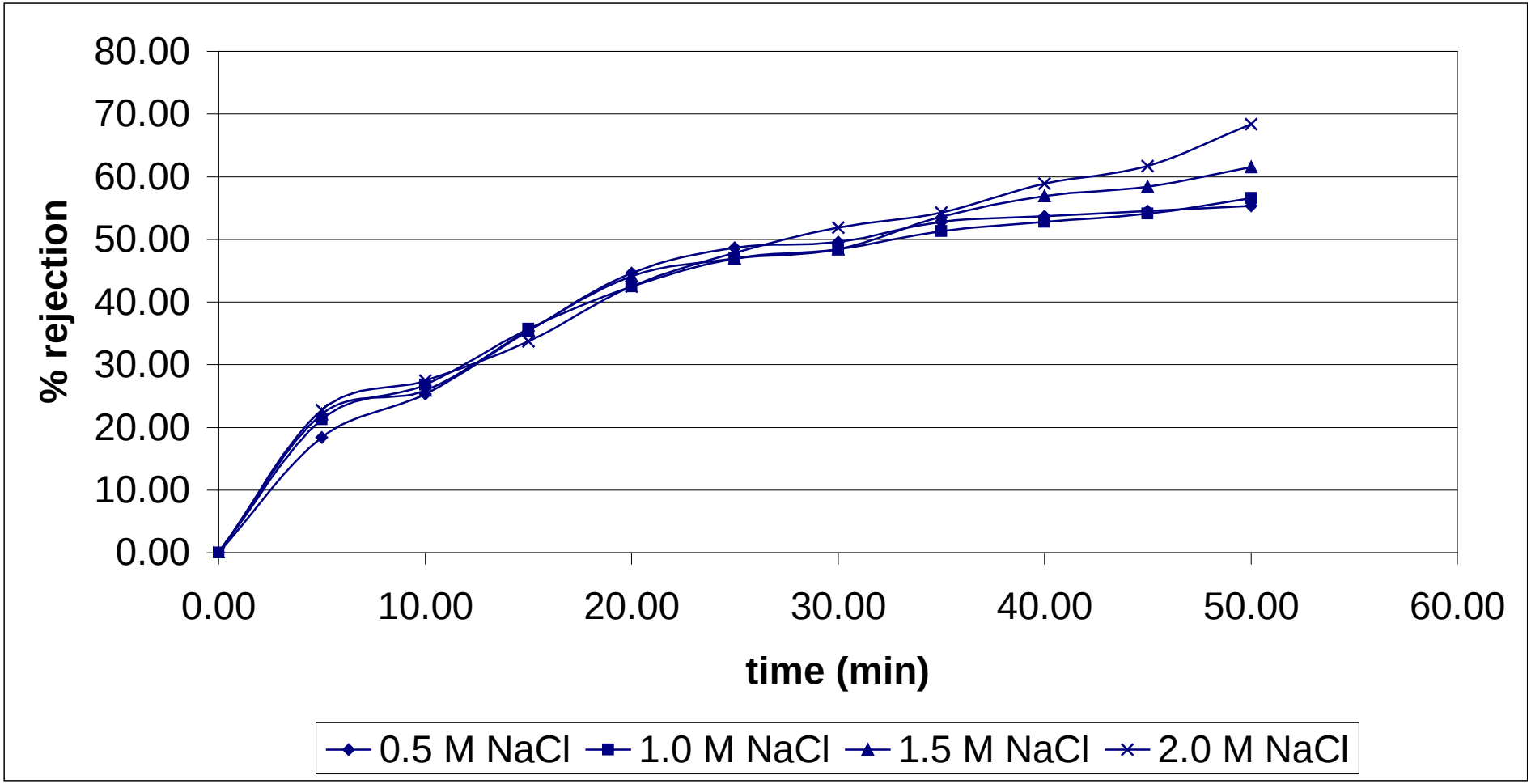

Figure 4.21: Overall Result Analysis for Lysozyme Rejection at Various Molarities of NaCl using Ultrafiltration Membrane

CHAPTER 5

CONCLUSION AND RECOMMENDATION

This chapter will discuss about the conclusion that could be made about the experiment and the recommendation for better results in the future.

5.1 Conclusion

The flux analysis is done to the various permeate with different pH to get high lysozyme rejection and maximum permeate flux the protein solution must be in alkali form. Which means that pH 8 is the most suitable to get those results.

The percentage of protein rejection analysis is done to various pH of protein solution to get an optimum condition for a low percentage rejection of protein. Which means the lowest percentage of protein rejection is at alkali form which is only 28.39%.

The ionic strength test in done to various concentration of NaCl which has been added to the protein solution to get an optimum condition for highest permeates flux. Which means the highest permeate flux is at lowest concentration of NaCl, 0.5M which will not lead to strong ionic bond.

5.2 Recommendation

Here is some recommendation that could be made for a better result in the future.

i) To obtain the concentration of the permeate flux, the OD reading should be taken 3 times for the average value.

ii) The membrane must be kept in the recommended conditions to avoid membrane clogging and to move out all the protein in the pores.

iii) The permeate volume must be measured carefully to avoid any split and will effect the reading.

iv) The protein sample must be analyzed in 2 days to avoid contamination.

v) All of the prepared solutions must be kept in a refrigerator at 4°C.

vi) The Lowry Reagent B must be wrapped to avoid the light.

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APPENDIX A

APPENDIX A1: Example of Flux and Rejection Calculation

Example of Flux Calculation for Protein Solution at pH 5 at t = 0.5 min;

$$\text{Flux} = \frac{\Delta \text{ Volume}}{\Delta \text{ t x area of membrane}}$$

$$= \frac{7}{(0.5)\ (11)}$$

$$= 1.272727273 \text{ ml/ min}$$

Example of percentage of Protein Rejection for Protein Solution at pH 5 at t = 5 minutes;

$$\text{Robs} = \left(1 - \frac{Cp}{Cf}\right) \times 100\%$$

$$= \left(1 - \frac{0.22125}{0.25}\right) \times 100\%$$

$$= 11.5\ \%$$

APPENDIX A2: Permeate Volume for Every pH

Time (min)	V (ml), at pH 5	V (ml), at pH 6	V (ml), at pH 7	V (ml), at pH 8
0.50	7.00	18.00	10.00	19.00
1.00	15.50	20.00	11.50	23.50
1.50	16.50	12.00	12.50	24.50
2.00	17.00	16.00	11.50	22.00
2.50	16.00	15.00	12.00	23.00
3.00	16.00	14.00	12.00	23.00
3.50	15.50	14.00	12.50	22.00
4.00	15.50	14.00	12.00	22.00
4.50	14.50	14.00	12.00	21.50
5.00	15.00	13.00	12.00	21.75
5.50	15.00	13.00	12.00	21.00
6.00	14.75	13.00	11.50	23.00
6.50	14.50	13.00	11.00	19.00
7.00	14.75	13.50	11.50	21.00
7.50	14.25	12.00	11.50	20.00
8.00	14.25	12.00	11.00	20.00
8.50	14.00	11.00	11.50	21.00
9.00	14.00	12.00	11.50	20.50
9.50	14.25	11.00	11.75	20.50
10.00	14.00	11.50	11.50	20.50
10.50	14.00	11.00	11.25	20.25
11.00	14.00	11.50	11.50	21.00
11.50	13.75	11.00	11.25	20.00
12.00	14.00	11.00	11.25	20.50
12.50	14.00	11.00	11.00	20.00
13.00	14.00	10.50	11.25	20.50
13.50	13.75	10.50	11.25	20.00
14.00	14.00	11.00	11.00	21.00
14.50	14.00	10.00	11.00	20.50
15.00	13.75	11.00	11.00	20.50
15.50	13.75	10.00	11.25	20.00
16.00	11.50	11.00	11.25	20.50
16.50	14.00	10.50	11.50	20.50
17.00	14.00	10.00	11.50	20.00
17.50	14.00	10.50	11.25	20.00
18.00	13.50	10.50	11.25	20.50
18.50	13.50	10.00	11.00	20.50
19.00	13.75	10.50	11.25	20.00
19.50	13.50	10.50	11.50	20.00
20.00	14.00	10.00	11.50	20.50
20.50	14.00	10.00	11.25	20.00
21.00	13.50	10.50	11.50	20.50
21.50	13.75	10.50	11.00	20.75
22.00	13.50	10.00	11.50	20.25
22.50	13.50	10.00	11.00	20.25

23.00	13.75	10.00	9.50	20.00
23.50	14.00	10.50	11.50	20.00
24.00	13.00	10.00	11.00	20.50
24.50	13.50	10.00	10.50	20.50
25.00	13.25	10.00	9.50	20.50
25.50	13.75	6.50	9.50	19.75
26.00	13.50	8.50	9.50	21.00
26.50	13.00	9.50	9.50	19.00
27.00	13.25	8.50	10.00	21.50
27.50	13.50	9.00	9.50	20.00
28.00	13.25	9.00	9.25	20.50
28.50	13.75	9.00	9.50	19.50
29.00	13.25	9.00	9.75	21.00
29.50	13.50	9.00	10.00	20.00
30.00	13.50	9.00	9.75	21.00
30.50	13.50	9.00	10.00	19.50
31.00	13.50	9.25	9.50	20.00
31.50	13.50	9.00	9.50	20.50
32.00	13.50	9.00	9.50	20.00
32.50	13.00	9.00	10.00	20.00
33.00	13.50	9.25	9.50	21.00
33.50	13.50	9.00	9.75	19.00
34.00	13.50	9.25	9.50	20.00
34.50	13.75	9.25	9.25	20.50
35.00	13.00	9.00	9.50	20.50
35.50	13.50	9.00	10.00	20.00
36.00	13.50	9.50	10.00	20.00
36.50	13.75	8.50	9.50	19.50
37.00	13.25	9.25	9.75	20.25
37.50	13.25	9.00	9.75	20.00
38.00	13.50	9.00	9.50	20.00
38.50	13.50	9.00	9.75	20.00
39.00	13.50	9.00	10.00	20.50
39.50	13.25	9.00	9.50	19.50
40.00	13.75	9.25	10.00	20.00
40.50	13.50	9.00	10.50	20.50
41.00	13.50	9.25	9.50	19.50
41.50	13.75	9.00	10.00	19.50
42.00	13.00	9.50	9.75	20.50
42.50	13.50	8.50	9.75	20.00
43.00	13.75	9.00	10.00	20.00
43.50	13.25	10.00	9.75	20.00
44.00	13.00	8.50	9.25	20.50
44.50	13.00	9.00	10.00	20.00
45.00	13.00	9.25	10.00	20.00
45.50	13.00	9.00	10.25	20.00
46.00	13.00	9.25	10.50	20.00
46.50	13.00	9.00	10.00	19.50
47.00	13.50	9.00	10.50	20.25
47.50	13.00	9.50	10.00	20.00
48.00	13.00	9.00	10.00	20.50
48.50	13.00	8.75	10.00	19.00

49.00	13.00	9.00	10.00	20.00
49.50	13.25	9.00	10.25	20.00
50.00	13.00	9.50	10.00	21.00

APPENDIX A3: Flux for Every pH

Time (min)	pH 8	pH 7	pH 6	pH 5
0.5000	3.4545	1.8182	3.2727	1.2727
1.0000	4.2727	2.0909	3.6364	2.8182
1.5000	4.4545	2.2727	2.1818	3.0000
2.0000	4.0000	2.0909	2.9091	3.0909
2.5000	4.1818	2.1818	2.7273	2.9091
3.0000	4.1818	2.1818	2.5455	2.9091
3.5000	4.0000	2.2727	2.5455	2.8182
4.0000	4.0000	2.1818	2.5455	2.8182
4.5000	3.9091	2.1818	2.5455	2.6364
5.0000	3.9545	2.1818	2.3636	2.7273
5.5000	3.8182	2.1818	2.3636	2.7273
6.0000	4.1818	2.0909	2.3636	2.6818
6.5000	3.4545	2.0000	2.3636	2.6364
7.0000	3.8182	2.0909	2.4545	2.6818
7.5000	3.6364	2.0909	2.1818	2.5909
8.0000	3.6364	2.0000	2.1818	2.5909
8.5000	3.8182	2.0909	2.0000	2.5455
9.0000	3.7273	2.0909	2.1818	2.5455
9.5000	3.7273	2.1364	2.0000	2.5909
10.0000	3.7273	2.0909	2.0909	2.5455
10.5000	3.6818	2.0455	2.0000	2.5455
11.0000	3.8182	2.0909	2.0909	2.5455
11.5000	3.6364	2.0455	2.0000	2.5000
12.0000	3.7273	2.0455	2.0000	2.5455
12.5000	3.6364	2.0000	2.0000	2.5455
13.0000	3.7273	2.0455	1.9091	2.5455
13.5000	3.6364	2.0455	1.9091	2.5000
14.0000	3.8182	2.0000	2.0000	2.5455
14.5000	3.7273	2.0000	1.8182	2.5455
15.0000	3.7273	2.0000	2.0000	2.5000
15.5000	3.6364	2.0455	1.8182	2.5000
16.0000	3.7273	2.0455	2.0000	2.0909
16.5000	3.7273	2.0909	1.9091	2.5455
17.0000	3.6364	2.0909	1.8182	2.5455

17.5000	3.6364	2.0455	1.9091	2.5455
18.0000	3.7273	2.0455	1.9091	2.4545
18.5000	3.7273	2.0000	1.8182	2.4545
19.0000	3.6364	2.0455	1.9091	2.5000
19.5000	3.6364	2.0909	1.9091	2.4545
20.0000	3.7273	2.0909	1.8182	2.5455
20.5000	3.6364	2.0455	1.8182	2.5455
21.0000	3.7273	2.0909	1.9091	2.4545
21.5000	3.7727	2.0000	1.9091	2.5000
22.0000	3.6818	2.0909	1.8182	2.4545
22.5000	3.6818	2.0000	1.8182	2.4545
23.0000	3.6364	1.7273	1.8182	2.5000
23.5000	3.6364	2.0909	1.9091	2.5455
24.0000	3.7273	2.0000	1.8182	2.3636
24.5000	3.7273	1.9091	1.8182	2.4545
25.0000	3.7273	1.7273	1.8182	2.4091
25.5000	3.5909	1.7273	1.1818	2.5000
26.0000	3.8182	1.7273	1.5455	2.4545
26.5000	3.4545	1.7273	1.7273	2.3636
27.0000	3.9091	1.8182	1.5455	2.4091
27.5000	3.6364	1.7273	1.6364	2.4545
28.0000	3.7273	1.6818	1.6364	2.4091
28.5000	3.5455	1.7273	1.6364	2.5000
29.0000	3.8182	1.7727	1.6364	2.4091
29.5000	3.6364	1.8182	1.6364	2.4545
30.0000	3.8182	1.7727	1.6364	2.4545
30.5000	3.5455	1.8182	1.6364	2.4545
31.0000	3.6364	1.7273	1.6818	2.4545
31.5000	3.7273	1.7273	1.6364	2.4545
32.0000	3.6364	1.7273	1.6364	2.4545
32.5000	3.6364	1.8182	1.6364	2.3636
33.0000	3.8182	1.7273	1.6818	2.4545
33.5000	3.4545	1.7727	1.6364	2.4545
34.0000	3.6364	1.7273	1.6818	2.4545
34.5000	3.7273	1.6818	1.6818	2.5000
35.0000	3.7273	1.7273	1.6364	2.3636
35.5000	3.6364	1.8182	1.6364	2.4545
36.0000	3.6364	1.8182	1.7273	2.4545
36.5000	3.5455	1.7273	1.5455	2.5000
37.0000	3.6818	1.7727	1.6818	2.4091
37.5000	3.6364	1.7727	1.6364	2.4091
38.0000	3.6364	1.7273	1.6364	2.4545
38.5000	3.6364	1.7727	1.6364	2.4545
39.0000	3.7273	1.8182	1.6364	2.4545
39.5000	3.5455	1.7273	1.6364	2.4091
40.0000	3.6364	1.8182	1.6818	2.5000
40.5000	3.7273	1.9091	1.6364	2.4545
41.0000	3.5455	1.7273	1.6818	2.4545

41.5000	3.5455	1.8182	1.6364	2.5000
42.0000	3.7273	1.7727	1.7273	2.3636
42.5000	3.6364	1.7727	1.5455	2.4545
43.0000	3.6364	1.8182	1.6364	2.5000
43.5000	3.6364	1.7727	1.8182	2.4091
44.0000	3.7273	1.6818	1.5455	2.3636
44.5000	3.6364	1.8182	1.6364	2.3636
45.0000	3.6364	1.8182	1.6818	2.3636
45.5000	3.6364	1.8636	1.6364	2.3636
46.0000	3.6364	1.9091	1.6818	2.3636
46.5000	3.5455	1.8182	1.6364	2.3636
47.0000	3.6818	1.9091	1.6364	2.4545
47.5000	3.6364	1.8182	1.7273	2.3636
48.0000	3.7273	1.8182	1.6364	2.3636
48.5000	3.4545	1.8182	1.5909	2.3636
49.0000	3.6364	1.8182	1.6364	2.3636
49.5000	3.6364	1.8636	1.6364	2.4091
50.0000	3.8182	1.8182	1.7273	2.3636

APPENDIX A4: Percentage of Rejection for Every pH

time (min)	pH 5	pH 6	pH 7	pH 8
0.00	0.00	0.00	0.00	0.00
5.00	11.50	15.90	19.80	19.60
10.00	14.30	16.80	24.30	22.80
15.00	23.50	17.30	32.50	33.70
20.00	27.40	22.80	38.90	42.50
25.00	29.10	25.20	41.40	45.60
30.00	31.70	28.70	42.50	48.40
35.00	33.90	30.20	44.90	51.30
40.00	34.70	38.60	45.20	52.40
45.00	38.30	44.50	49.10	53.60
50.00	39.50	46.90	51.20	54.70

APPENDIX A5: Permeate Volume for Every Molarities of NaCl

Time (min)	V (ml) for 0.5 M NaCl	V (ml) for 1.0 M NaCl	V (ml) for 1.5 M NaCl	V (ml) for 2.0 M NaCl
0.50	23.00	20.50	11.50	14.50
1.00	24.00	21.00	21.00	14.00
1.50	25.00	22.00	19.00	13.75
2.00	26.00	22.00	20.00	13.00
2.50	25.00	22.00	20.00	13.00
3.00	24.00	22.00	20.50	12.75
3.50	25.00	21.00	19.75	13.50
4.00	24.00	21.00	20.00	13.00
4.50	23.00	21.50	19.50	13.25
5.00	23.00	19.50	20.00	13.00
5.50	24.00	21.00	19.75	13.25
6.00	23.00	19.50	19.50	13.00
6.50	23.00	21.00	19.50	13.00
7.00	23.00	20.00	19.50	12.75
7.50	23.00	20.50	19.25	13.25
8.00	22.50	20.00	20.00	12.50
8.50	23.00	20.75	20.00	13.00
9.00	22.50	21.00	20.00	13.00
9.50	23.00	20.50	19.75	13.00
10.00	22.00	20.00	20.00	13.00
10.50	22.00	21.50	19.50	13.50
11.00	22.00	20.00	20.00	12.50
11.50	22.00	21.50	20.00	13.50
12.00	21.00	18.00	20.00	13.00
12.50	19.00	20.00	19.75	13.00
13.00	20.00	20.50	19.50	13.00
13.50	22.00	20.00	19.00	13.50
14.00	21.50	20.00	19.75	13.00
14.50	20.50	20.00	19.00	13.50
15.00	20.50	20.00	19.50	13.00
15.50	19.00	20.50	19.00	13.25
16.00	21.00	20.00	19.00	13.50
16.50	20.00	20.50	19.25	13.25
17.00	20.25	20.00	19.50	13.00
17.50	20.00	20.50	19.00	13.75
18.00	20.00	20.00	19.50	13.00
18.50	19.50	20.00	19.75	13.75
19.00	20.00	20.00	19.00	13.50
19.50	21.00	20.50	19.00	13.50
20.00	20.50	20.00	19.50	13.00
20.50	21.00	20.00	19.50	13.75
21.00	19.50	20.00	19.50	14.50
21.50	20.00	20.00	18.00	12.75
22.00	20.00	20.50	18.75	13.50
22.50	19.50	20.00	19.00	13.75

23.00	20.00	20.00	18.50	13.50
23.50	19.00	20.50	19.00	13.50
24.00	20.00	20.00	19.00	13.75
24.50	20.00	20.00	19.00	14.00
25.00	18.50	20.00	19.50	13.50
25.50	19.00	20.00	19.50	14.00
26.00	19.50	20.00	19.00	14.00
26.50	19.00	20.50	19.75	13.50
27.00	20.50	20.00	19.50	13.00
27.50	20.50	20.00	19.00	14.00
28.00	20.00	19.50	19.50	13.75
28.50	19.00	21.00	19.00	14.00
29.00	21.00	20.00	20.00	13.50
29.50	20.00	20.50	19.25	14.00
30.00	19.00	19.50	19.75	14.00
30.50	20.00	20.00	19.00	13.50
31.00	21.00	20.00	19.50	14.50
31.50	19.00	20.00	19.00	14.25
32.00	21.50	18.00	18.00	13.50
32.50	21.00	20.00	19.50	14.00
33.00	20.00	19.00	19.25	13.75
33.50	21.00	20.00	19.25	13.50
34.00	21.00	20.00	19.50	13.75
34.50	20.00	20.00	20.00	14.00
35.00	20.50	20.00	19.50	13.75
35.50	19.00	20.00	18.00	14.00
36.00	21.00	20.00	19.25	13.75
36.50	21.00	20.00	19.00	14.25
37.00	21.00	19.50	19.50	13.75
37.50	21.00	21.00	18.50	14.25
38.00	21.00	20.00	18.75	13.75
38.50	21.00	20.00	19.50	14.25
39.00	20.00	20.00	19.25	14.00
39.50	19.00	20.00	20.00	14.25
40.00	20.00	20.00	19.00	13.75
40.50	20.00	20.50	19.75	14.50
41.00	21.00	20.00	19.75	14.00
41.50	20.00	19.00	19.00	14.00
42.00	18.50	20.00	19.00	14.00
42.50	18.00	20.00	19.75	14.25
43.00	18.00	20.00	19.50	14.00
43.50	19.50	20.00	19.75	14.50
44.00	21.00	20.00	19.50	14.00
44.50	21.00	20.00	19.75	14.25
45.00	21.00	20.00	19.50	14.00
45.50	22.00	20.00	19.75	14.50
46.00	18.00	20.00	19.75	14.50
46.50	20.00	20.00	19.00	14.50
47.00	21.00	20.00	19.75	13.75
47.50	21.00	20.00	19.75	14.75
48.00	21.00	20.00	19.75	14.00
48.50	21.00	20.00	19.75	14.75

49.00	21.00	20.00	20.00	14.25
49.50	21.00	20.00	19.50	14.00
50.00	20.50	20.00	19.50	14.00

APPENDIX A6: Flux for Every Molarities of NaCl

time (min)	0.5 M	1.0 M	1.5 M	2.0 M
0.5000	4.1818	3.7273	2.0909	2.6364
1.0000	4.3636	3.8182	3.8182	2.5455
1.5000	4.5455	4.0000	3.4545	2.5000
2.0000	4.7273	4.0000	3.6364	2.3636
2.5000	4.5455	4.0000	3.6364	2.3636
3.0000	4.3636	4.0000	3.7273	2.3182
3.5000	4.5455	3.8182	3.5909	2.4545
4.0000	4.3636	3.8182	3.6364	2.3636
4.5000	4.1818	3.9091	3.5455	2.4091
5.0000	4.1818	3.5455	3.6364	2.3636
5.5000	4.3636	3.8182	3.5909	2.4091
6.0000	4.1818	3.5455	3.5455	2.3636
6.5000	4.1818	3.8182	3.5455	2.3636
7.0000	4.1818	3.6364	3.5455	2.3182
7.5000	4.1818	3.7273	3.5000	2.4091
8.0000	4.0909	3.6364	3.6364	2.2727
8.5000	4.1818	3.7727	3.6364	2.3636
9.0000	4.0909	3.8182	3.6364	2.3636
9.5000	4.1818	3.7273	3.5909	2.3636
10.0000	4.0000	3.6364	3.6364	2.3636
10.5000	4.0000	3.9091	3.5455	2.4545
11.0000	4.0000	3.6364	3.6364	2.2727
11.5000	4.0000	3.9091	3.6364	2.4545
12.0000	3.8182	3.2727	3.6364	2.3636
12.5000	3.4545	3.6364	3.5909	2.3636
13.0000	3.6364	3.7273	3.5455	2.3636
13.5000	4.0000	3.6364	3.4545	2.4545
14.0000	3.9091	3.6364	3.5909	2.3636
14.5000	3.7273	3.6364	3.4545	2.4545
15.0000	3.7273	3.6364	3.5455	2.3636
15.5000	3.4545	3.7273	3.4545	2.4091
16.0000	3.8182	3.6364	3.4545	2.4545
16.5000	3.6364	3.7273	3.5000	2.4091
17.0000	3.6818	3.6364	3.5455	2.3636

17.5000	3.6364	3.7273	3.4545	2.5000
18.0000	3.6364	3.6364	3.5455	2.3636
18.5000	3.5455	3.6364	3.5909	2.5000
19.0000	3.6364	3.6364	3.4545	2.4545
19.5000	3.8182	3.7273	3.4545	2.4545
20.0000	3.7273	3.6364	3.5455	2.3636
20.5000	3.8182	3.6364	3.5455	2.5000
21.0000	3.5455	3.6364	3.5455	2.6364
21.5000	3.6364	3.6364	3.2727	2.3182
22.0000	3.6364	3.7273	3.4091	2.4545
22.5000	3.5455	3.6364	3.4545	2.5000
23.0000	3.6364	3.6364	3.3636	2.4545
23.5000	3.4545	3.7273	3.4545	2.4545
24.0000	3.6364	3.6364	3.4545	2.5000
24.5000	3.6364	3.6364	3.4545	2.5455
25.0000	3.3636	3.6364	3.5455	2.4545
25.5000	3.4545	3.6364	3.5455	2.5455
26.0000	3.5455	3.6364	3.4545	2.5455
26.5000	3.4545	3.7273	3.5909	2.4545
27.0000	3.7273	3.6364	3.5455	2.3636
27.5000	3.7273	3.6364	3.4545	2.5455
28.0000	3.6364	3.5455	3.5455	2.5000
28.5000	3.4545	3.8182	3.4545	2.5455
29.0000	3.8182	3.6364	3.6364	2.4545
29.5000	3.6364	3.7273	3.5000	2.5455
30.0000	3.4545	3.5455	3.5909	2.5455
30.5000	3.6364	3.6364	3.4545	2.4545
31.0000	3.8182	3.6364	3.5455	2.6364
31.5000	3.4545	3.6364	3.4545	2.5909
32.0000	3.9091	3.2727	3.2727	2.4545
32.5000	3.8182	3.6364	3.5455	2.5455
33.0000	3.6364	3.4545	3.5000	2.5000
33.5000	3.8182	3.6364	3.5000	2.4545
34.0000	3.8182	3.6364	3.5455	2.5000
34.5000	3.6364	3.6364	3.6364	2.5455
35.0000	3.7273	3.6364	3.5455	2.5000
35.5000	3.4545	3.6364	3.2727	2.5455
36.0000	3.8182	3.6364	3.5000	2.5000
36.5000	3.8182	3.6364	3.4545	2.5909
37.0000	3.8182	3.5455	3.5455	2.5000
37.5000	3.8182	3.8182	3.3636	2.5909
38.0000	3.8182	3.6364	3.4091	2.5000
38.5000	3.8182	3.6364	3.5455	2.5909
39.0000	3.6364	3.6364	3.5000	2.5455
39.5000	3.4545	3.6364	3.6364	2.5909
40.0000	3.6364	3.6364	3.4545	2.5000
40.5000	3.6364	3.7273	3.5909	2.6364
41.0000	3.8182	3.6364	3.5909	2.5455

41.5000	3.6364	3.4545	3.4545	2.5455
42.0000	3.3636	3.6364	3.4545	2.5455
42.5000	3.2727	3.6364	3.5909	2.5909
43.0000	3.2727	3.6364	3.5455	2.5455
43.5000	3.5455	3.6364	3.5909	2.6364
44.0000	3.8182	3.6364	3.5455	2.5455
44.5000	3.8182	3.6364	3.5909	2.5909
45.0000	3.8182	3.6364	3.5455	2.5455
45.5000	4.0000	3.6364	3.5909	2.6364
46.0000	3.2727	3.6364	3.5909	2.6364
46.5000	3.6364	3.6364	3.4545	2.6364
47.0000	3.8182	3.6364	3.5909	2.5000
47.5000	3.8182	3.6364	3.5909	2.6818
48.0000	3.8182	3.6364	3.5909	2.5455
48.5000	3.8182	3.6364	3.5909	2.6818
49.0000	3.8182	3.6364	3.6364	2.5909
49.5000	3.8182	3.6364	3.5455	2.5455
50.0000	3.7273	3.6364	3.5455	2.5455

APPENDIX A7: Percentage of Rejection for Every Molarities of NaCl

Time (min)	Rejection (%) for 0.5 M NaCl	Rejection (%) for 1.0 M NaCl	Rejection (%) for 1.5 M NaCl	Rejection (%) for 2.0 M NaCl
0.00	0.00	0.00	0.00	0.00
5.00	18.40	21.30	22.10	22.80
10.00	25.30	26.80	25.90	27.50
15.00	35.40	35.70	35.50	33.70
20.00	44.60	42.50	44.10	42.50
25.00	48.70	46.90	46.90	47.80
30.00	49.60	48.40	48.40	51.90
35.00	52.80	51.30	53.60	54.30
40.00	53.70	52.80	56.90	58.90
45.00	54.50	54.10	58.40	61.70
50.00	55.30	56.60	61.50	68.40