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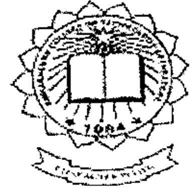

CHARACTERIZATION OF ULTRAFILTRATION MEMBRANE AND EFFECT OF PRESSURE ON FOULING

by

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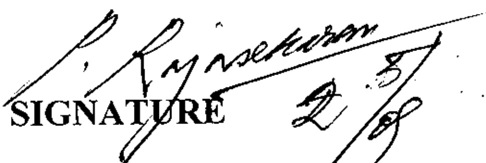
BONAFIDE CERTIFICATE

Certified that this project report **"CHARACTERIZATION OF ULTRAFILTRATION MEMBRANE AND EFFECT OF PRESSURE ON FOULING"** is the bonafide work of **"LUKKA THUYAVAN.Y."** who carried out the project work under my supervision.



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To Whom It May Concern

This is to certify that Mr. Y. Lukka Thuyavan, M.Tech., student of Kumaraguru College of Technology, Coimbatore worked in my laboratory on project titled '**CHARACTERIZATION OF UF MEMBRANE AND EFFECT OF PRESSURE ON FOULING**' for five months during December 2008 to April 2009. His conduct in the laboratory was exemplary.

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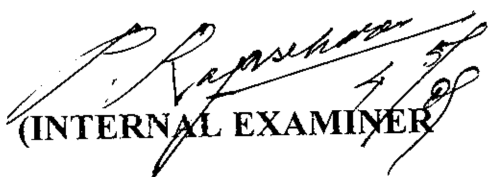

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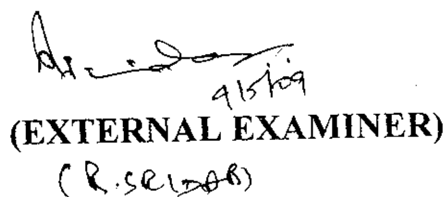

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LIST OF ABBREVIATIONS

CA	Cellulose acetate
Cp	Concentration in the permeate
Cr	Concentration in the retentate
DF	Diafiltration
ED	Electrodialysis
HPTFF	High performance tangential flow filtration
IEP	Isoelectic point
kDa	Kilodalton
kPa	Kilopascal
MF	Microfiltration
MW	Molecular weight
MWCO	Molecular weight cut off
NMWCO	Nominal Molecular weight cut off
NF	Nanofiltration
nm	Nano meter
PA	Polyamide
PAN	Polyacrylonitrile
PES	Polyethersulfone
PI	Polyimide
PS	polysulfone
PVA	Poly vinylalchol
RO	Reverse osmosis
RPM	Rotation per minute
SDS	Sodium dodecyl sulphate
SPS	Sulfonated polysulfone
TMP	Transmembrane pressure
TSS	Total suspended solids
UF	Ultrafiltration
μm	Micrometer

ABSTRACT

ABSTRACT

Membrane filtration is an attractive separation process, as it is usually performed under gentle conditions. Membrane processes have been widely used for bioseparations since well before the start of the modern membrane industry. During the last two decades, new membranes and modules have been developed specifically to meet the requirements of the biotechnology industry. This includes application of membranes for sterile purification, clarification, initial harvest; virus removal protein concentration and purification. There are four major pressures driven membrane process such as MF, UF, NF and RO. UF processes are widely used for protein purification and concentration. The main reason for underutilization of ultrafiltration membranes are fouling, concentration polarization and poor transmission of proteins. In view of this present study, systematic characterization of PAN E113 and PES- 30 were characterized in terms of permeability, transmission of proteins and fouling. BSA, ovalbumin, myoglobin and lysozyme were used as model proteins. The factors affecting the volumetric flux, transmission of each protein were evaluated using stirred cell (Module Amicon 8200) holding cell of 200 ml capacity at various TMP ranges from 20 to 106 kPa. Experimental data shows that low permeability E113 PAN reveals that higher transmission of protein at low pressure of 26 kPa. On the other hand, the high permeability PES-30 showed higher transmission at high pressure of 93 kPa. In both PES-30 and PAN E113 membranes showed higher fouling at their high applied pressure. Interactions between these operating parameters and the significance effects on UF operation were also discussed.

INTRODUCTION

1.0 INTRODUCTION

Membrane processes play a critical role in the purification of biotechnology products. Early membrane systems were adopted from technology originally developed for other industrial applications. During the last two decades, new membranes and modules have been developed specifically to meet the requirements of the biotechnology industry. This includes applications of membranes for sterile filtration, clarification, initial harvest, virus removal, protein concentration, buffer exchange, and protein purification. Membrane separation technology is a novel and highly innovative process engineering operation. Membrane processing is a technique that permits concentration and separation without the use of heat. The application of membrane separation processes is one of the most significant recent developments in chemical and biological process engineering. Such processes are used in a wide range of industrial operations to pharmaceuticals, biological macromolecules, separate organic molecules, colloids, ions and solvents. Membrane separation processes are often more capital and energy efficient when compared with conventional separation process (Syed Ali *et al.*, 1997).

1.1 Membrane and its Classification

Membranes based filtration extends this application further to include the separation of dissolved solutes in liquid streams and for separation of gas mixture. The major membrane separation process are reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF), microfiltration (MF), dialysis, electrodialysis (ED), pervaporation (PV). The primary role of a membrane is to act as a selective barrier. It should permit passage of certain

components and retain certain other components of a mixture. Membrane could be defined as a region of discontinuity interposed between two phases (Hwang and Kammermeyer, 1975), or as a phase that act as a barrier to prevent mass movement, but allows restricted or regulated passage of one or more species through it (Lakshminarayanaih, 1984). The most common pressure driven membrane processes are microfiltration, ultrafiltration, nanofiltration and reverse osmosis is the application of hydraulic pressure to speed up the transport process. The nature of the membrane itself controls which components permeate and which are retained. Particles are separated on the basis of their molecular size and shape with the use of pressure and specially designed semi-permeable membranes. In ultrafiltration the driving force is the difference in solute concentration at the membrane surface and at some arbitrarily defined point in the bulk fluid. Membranes are usually classified according to the size of the separated components, and thus particle sizes in MF applications are specified in microns (μm). However with UF membranes, it is customary to refer to the molecular weight cut-off (MWCO) instead of particle size. In the early days of membrane technology, UF membranes were characterized by studying the relative permeability of proteins and polyethylene glycols, which were characterized in terms of their molecular weights. Even though it is known that molecular weight alone does not determine the size of protein and indeed, many manufacturers use dextrans rather than proteins to characterize UF membranes, this terminology is still used, sometimes prefixed with word nominal as in NMWCO. Thus UF covers particles and molecules that range from about 1000 in molecular weight to about 500,000 Daltons. Ultrafiltration membranes can also be used for protein purification using a process known as high performance tangential flow filtration (HPTFF). High performance

tangential flow filtration is an emerging technology that uses semipermeable membranes for the separation of proteins without limit to their relative size HPTFF has been used to separate monomers from oligomers based on their difference in size, protein variants differing at only a single amino acid residue, and an antigen binding fragment from a similar size purity (Ebersold and Zydney, 2004).

1.2 Membrane Separation Processes

Reverse osmosis retains all components other than the solvents, while ultra filtration retains only macromolecules or particles larger than about 10-200 Å (about 0.001 -0.02 µm). microfiltration is designed to retain particles in the micron range that is suspended particles in the range of 0.10 µm to about 5 µm. RO is essentially considered to be a dewatering technique, while UF is essentially can be looked at as a method for simultaneous purifying, concentrating and fractionating macromolecules or fine colloidal suspensions. Microfiltration is used mainly as a clarification technique, separating suspended particles from dissolved substances, provided the particles meet the size requirements for microfiltration membranes.

Nanofiltration is relatively new processes that use charged membrane with pores that are larger than RO membranes, but too small to allow permeation of many organic compounds such as sugars. They also have a useful property in that they can separate dissociated forms of a compound from the undissociated form i.e. organic acids such as lactic acid and acetic acid pass through easily at low pH, but are rejected at higher pH when in their salt forms (Raman *et al.,* 1994). Membranes have four different modules. They are plate and frame, tubular, spiral wound, hollow fiber and thin channel flow. Generally, flow across the membrane surface is classified

as five categories. They are Co-current flow, completely mixed flow, Counter current flow, Cross flow, Dead-end flow. Ultrafiltration membranes have pore sizes between 1 and 20 nm and are designed to provide high retention of proteins and other macromolecules. Ultrafiltration membranes can also be used for protein purification using a process known as high performance tangential flow filtration. Microfiltration membranes have pore size between 0.05 and 10 nm and are designed to retain cells and cell debris while allowing proteins and smaller solutes to pass into the filtrate. Membranes designed specifically for virus filtration fall between these limits and have pore size between 20 and 70 nm. Nanofiltration is properly defined as a process that separates solvent, monovalent salts, and small organics from divalent ions and larger species. Depth filters are not typically considered as membranes since they retain key components throughout the porous structure. Removal rates are determined by both adsorptive and size-based retention mechanisms.

	Microfiltration	Virus filtration	Ultrafiltration	Nanofiltration	Reverse Osmosis
Components retained by membrane	Intact cells Cell debris Bacteria	Viruses	Proteins	Divalent ions Amino acids Antibiotics	Amino acids Sugars Salts
	Membrane				
Components passed through membrane	Colloids Viruses Proteins Salts	Proteins Buffer components	Amino acids Antifoam Buffer components	Salts Water	Water

Figure 1.2.1 Comparison of removal characteristics of different pressure-driven membrane processes

Membrane separation technology is a novel and highly innovative process engineering operation. Membrane processing is a technique that permits concentration and separation without the use of heat. The application of membrane separation processes is one of the most significant recent developments in chemical and biological process engineering. Such processes are used in a wide range of industrial operations to pharmaceuticals, biological macromolecules, separate organic molecules, colloids, ions and solvents.

1.3 ULTRAFILTRATION

Principle

Ultrafiltration is used for protein concentration and buffer exchange, largely replacing size exclusion chromatography for buffer exchange at industrial scale (Kurnik *et al.,* 1995). High protein retention is achieved by using a small pore size membrane, although recent studies have demonstrated the potential of exploiting both size and electrostatic interactions for enhanced ultrafiltration processes.

Ultrafiltration (UF) is a separation technique, which is now widely employed in the biotechnology industry for separating proteins and peptide drugs from fermentation broths. However, its use has been confined mainly to concentration and diafiltration of dilute protein solutions. Nevertheless, ultrafiltration may be a cost-efficient and easy to scale-up protein separation method for industrial use. The possibility of carrying out large-scale protein separations justifies further research in this direction. Proteins have special structures and properties, enabling them to fulfill many different biological functions. Ultrafiltration designates a membrane separation process, driven by a pressure gradient, in which the membrane fractionates components of a

liquid as a function of their solvated size and structure. Bioseparation can be defined as purification and isolation of functionally important Bio-molecules, which can be clubbed as, bioprocess industries (Keller *et al.*, 2001). Ultrafiltration is used for protein concentration and buffer exchange, largely replacing size exclusion chromatography for buffer exchange at industrial scale. High protein retention is achieved by using a small pore size membrane, although recent studies have demonstrated the potential of exploiting both size and electrostatic interactions for enhanced ultrafiltration processes (Mehta and Zydney, 2005).

Ultrafiltration (UF) is a pressure driven process used to separate or concentrate macrosolutes, from micro solutes and solvent. It is evident that resolving protein mixtures by UF cannot be regarded as a mere sieving process. The extent of separation is determined by a complex interplay of the boundary layer and mass transfer effects as well as the membrane surface characteristics. In ultrafiltration of macromolecules such as proteins, the solute rejection leads to concentration polarization near the membrane surface. As the transmembrane pressure increases, the permeate flux in case of protein solutions will increase nonlinearly and will be less than that of pure water flux due to this concentration polarization. After reaching the plateau, the flux is independent of the applied pressure which shows the formation of gel layer i.e. Gel Polarization. This polarized layer is a gel layer rather than a viscous liquid is open to debate, but experimental evidences indicated that it approaches a "close packed" configuration of low hydraulic permeability. As such, for designing effective fractionations, it is imperative to investigate the fundamental factors governing protein transport through UF membranes as it can be easily altered by hydrodynamic conditions.

Studies on single protein transmission through partially retentive membranes can provide valuable insights into this aspect.

Ultrafiltration process is most widely used membrane process and can be thought of performing one or more following function:

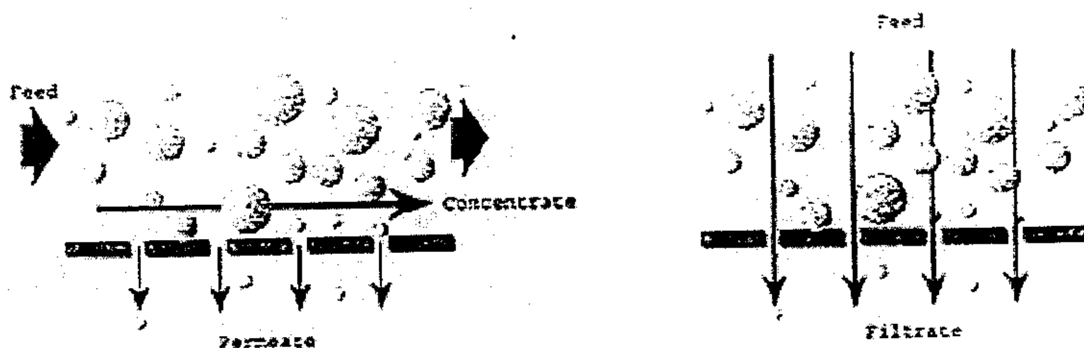
- Feed clarification
- Concentration of rejected solutes
- Fractionation of solutes

Separation is efficient when there is at least tenfold difference in the sizes of the species.

Ultrafiltration (UF) is increasingly used as a technology for surface water purification. UF membranes have a high selectivity and became economically attractive during the last 15 years. However, membrane performance is influenced by fouling. For this reason frequent cleaning of the membrane is required. In the short term the membrane is cleaned by means of backwashing and in the long term the membrane is cleaned with cleaning chemicals. Although back washing and chemical cleaning are useful methods to remove fouling, the execution of such procedures is often based on rules of thumb and/or pilot plant studies. UF is different from conventional filtration, also called normal or dead-end filtration, in that it operates in the cross flow mode; that is, the feed stream flows parallel to the filtration media (membrane). The difference between cross flow and dead-end filtration is illustrated in figure. Cross flow acts as a sweep stream to continuously cleanse the surface of the membrane from accumulated retentate. There are two products of UF; the permeate, containing components small enough to pass through the membrane, and the concentrate, containing the retentate. UF can improve both the economics

and the quality UF can reduce costs by reducing energy-intensive drying steps, and improve profitability

Types of Filtration



CROSSFLOW **NORMAL FLOW**

DEAD END MODE vs. CROSSFLOW MODE

Dead end mode **Cross flow mode**

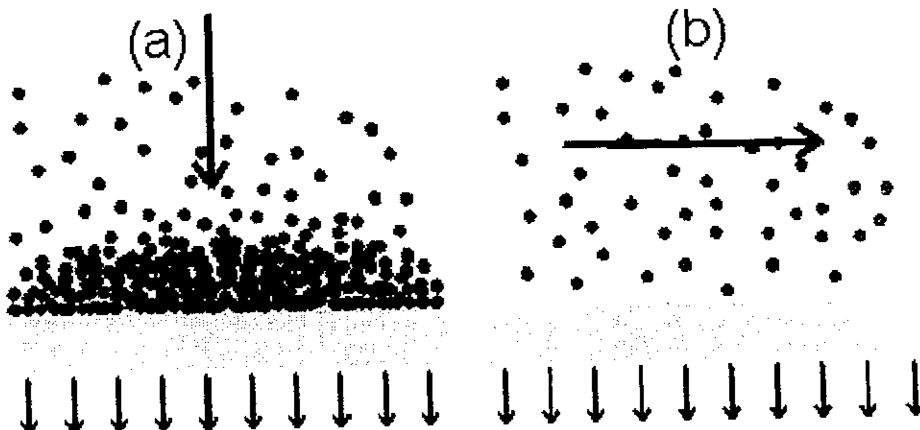


Figure 1.4.1 Cross flow mode vs. Dead end mode

The major advantage of ultrafiltration processes over conventional bioseparation processes is high throughput of product. However, in spite of wide spread use of ultrafiltration in processes such as diafiltration and concentration, the potential for its use in protein fractionation has not been exploited in the biotech industry (Ghosh and Cui, 2000).

1.4 Dead End Stirred Cell Ultrafiltration

Normal flow filtration, also referred to as direct flow or dead-end filtration, is used primarily for systems in which the retained components are present at very low concentration. Normal flow is also used in depth filtration and membrane chromatography where the removal occurs throughout the porous structure.The major use for these dead-end units is for rapid ultrafiltration (UF) or microfiltration (MF) of small volumes that may be used in clinical and analytical applications, for *invitro* diagnostic use and for determining ligand-macromolecular binding parameters. However, being dead-end devices, there is no way to control concentration polarization, and thus they are best used with relatively clean and dilute samples. These are also dead-end cells but have a means of controlling polarization by agitation of the fluid. The applications of UF processes are generally limited to the systems where the solutes to be separated have more than 10-fold difference in molecular weight (MW).Molecular size becomes the sole criteria for separation purposes in such cases. However, it is possible to separate proteins and enzymes with comparable MW by adequately manipulating the parameters such as solution pH, ionic strength, and transmembrane pressure (TMP) (Feins and Sirkar, 2005). Due to the very compressible filter cakes high pressures and long filtration times are often necessary, a problem which arise in biomass separation if the micro-organisms form a slimy capsule. In

this case the biopolymers of the capsule determine the filtration kinetics, and the filtration problem of these types of micro-organisms can be abstracted roughly to the filtration of the biopolymers of the slime capsule (Ralph Hofmann and Clemens Posten, 2001).

In contrast, membrane adsorber based chromatography offers several advantages: there are no long diffusion paths in the membrane; mass transfer takes place through convection rather than through diffusion. Due to this fact, membrane adsorbers enable a time-effective performance with high flow rates without high back pressure (Zou *et al.*, 2001). Besides, some methods like chromatography and electrophoresis require complex instrumentation support to run efficiently, and usually yield low throughput of the products at an extremely high process cost. Hence, the separation techniques that are able to yield high throughput of the products at a low cost are highly desired in biotechnological industries. Of these potential candidates, ultrafiltration (UF) has ever attracted a considerable amount of attention in recent years for the separation of proteins due to comparatively gentler towards the proteins than separation process on phase changes and more economical than gel chromatography (Rios *et al.*, 2007) .

Downstream processing refers to the recovery and purification of biosynthetic products, particularly pharmaceuticals, from natural sources such as animal or plant tissue or fermentation broth, including the recycling of salvageable components and the proper treatment and disposal of waste. It is an essential step in the manufacture of pharmaceuticals such as antibiotics, hormones (e.g. insulin and human growth hormone), antibodies (e.g. infliximab and abciximab) and vaccines; antibodies and enzymes used in diagnostics; industrial enzymes; and natural fragrance and flavor compounds. Downstream processing is usually considered a specialized field

in biochemical engineering, itself a specialization within chemical engineering, though many of the key technologies were developed by chemists and biologists for laboratory-scale separation of biological products.

The increasing number of applications of ultrafiltration for processing of proteins in dairy, food and biotechnology industries calls for better understanding of the transmission and flux behavior of protein solution. Therefore, in summary, the objectives of this study are

- To study the membrane characteristic, the transmission, rejection and volumetric flux are analyzed with various operating parameters such as pH, temp, pressure using Dead End stirred cell flow Module.

- To estimate the protein transmission percentage separately for the characterized UF membrane.

- To analyze the effect of pressure on fouling.

LITERATURE REVIEW

2.0 LITERATURE REVIEW

Membrane filtration has proved its potential in the field of Down Stream Processing of the fermented broth. It very efficiently can separate cell matter from the broth (Micro filtration) and then purification and concentration of the desired product by processes utilizing membranes (ultra filtration) having pore size specific for the product. Ultrafiltration (UF) is primarily a size exclusion based pressure driven membrane separation process. In ultrafiltration, the accumulation of retained molecules may form a concentrated gel layer. The impact of gel-layer formation is that it can significantly alter the performance characteristics of the membrane. This is commonly called concentration polarization. Fundamentally, the gel layer will limit filtrate flow rate and any increase in pressure will have no beneficial effect. The major technological barriers in the way of the membrane processes are due to the constraints imposed by one or a combination of the following factors: broad membrane pore size distribution, concentration polarization and membrane fouling.

Ultrafiltration (UF) is a pressure-driven membrane-based separation process in which particles and dissolved macromolecules smaller than 0.1μm and larger than about 2nm are rejected. Ultrafiltration is a selective fractionation process utilizing pressures up to 145 psi (2-10 bars). Ultrafiltration membranes typically have pore size in the range from 10 to 1000 Å. It concentrates suspended solids and solutes of molecular weight greater than 1,000. i.e., membranes Nominal Molecular Weight Cutoff (NMWCO) in the range of 1 kDa to 1000 kDa are used. The permeate contains low molecular weight organic solutes and salts. Most of the ultrafiltration membranes are described by the nominal molecular weight

cutoff (MWCO), which is usually defined as the smallest molecular weight species for which the membrane has more than 90% rejection. The MWCO of any given membrane can vary with changing feed chemistries as well as with factors such as molecular orientation, molecular configuration, operating conditions, etc. From the viewpoint of transport fundamentals, the distinction between reverse osmosis (RO) and UF is purely artificial (Loeb and Sourirajan, 1981). However the nature of the larger molecules that are usually separated by ultrafiltration leads to significant practical difference between UF and RO processes. The liquid phase diffusivity of these species is lower; hence membrane fouling and concentration polarization problems are more significant in the UF. In most ultrafiltration processes, the permeate flux becomes independent of the applied pressure for high enough values of the applied pressure (Karode, 2000).

The relationship between the applied ultrafiltration pressure and the rate of permeation (flux) for a pure solvent feed flowing under laminar conditions to tortuous membrane channels may be described by the Carman-Kozeny equation

Where

$$J = \frac{|\Delta p|}{\mu R_m}$$

J - Flux (Volumetric rate per unit area),

Δp- Transmembrane pressure difference

μ - Solvent Viscosity

R_m - Membrane resistance.

Table 2.01 Pressure driven membrane process

Process	Applied Pressure	Retentate	Permeate
Microfiltration	Up to 1.5 bar	Suspended particles	Dissolved solutes
Ultrafiltration	Up to 10 bar	Macromolecules such as proteins, viruses, colloids	Small molecules
Nanofiltration	10-30 bar	Small molecules large peptides, divalent salts, dissociated acids	Small peptides Monovalent ions, undissociated acids, amino acids
Reverse osmosis	35-100 bar	All solutes	Water

Feed phase mass transfer resistance and resistance due to gel layer formation on the membrane surface are extremely important effects in ultrafiltration processing. Consequently system design and operating protocol are also important. Membrane selection is aimed at decreasing the fouling tendencies of the membrane surface. The base polymer surface chemistry can be modified in order to increase hydrophilicity, which increases flux and reduce fouling in most. In commercial modules, concentration polarization is decreased by increasing the fluid shear at the membrane surface or by the turbulence inducers such as channel spacers.

Table 2.02 Membrane Separation Process

Process	Membrane Type and Pore Radius	Membrane Material	Process Diving Force	Applications
Microfiltration	Symmetric microporous, 0.1-10 microns	Cellulose nitrate or acetate, Polyvinylidene difluoride (PVDF), Polyamides, Polysulfone, PTFE, Metal Oxides etc	Hydro-static pressure difference at approx. 10-500 kPa	Sterile filtration, Clarification
Ultrafiltration	Asymmetric microporous, 1-10 nm	Polysulfone, Polypropylene, Nylon 6, PTFE, PVC,PAN	Hydrostatic pressure difference at approx. 0.1-1.0 Mpa	Separation of macromolecular solutions
Reverse Osmosis	Asymmetric skin-type, 0.5-1.5 nm	Polymers, Cellulosic acetate	Hydrostatic pressure difference at approx. 2-10 Mpa	Separation of salts and microsolutes from solutions
Electrodialysis	Cation and anion exchange membrane	Sulfonated cross-linked polystyrene	Electrical potential gradient	Desalting of ionic solutions
Gas Separation	Asymmetric homogeneous polymer	Polymers & copolymers	Hydrostatic pressure and concentration gradients	Separation of gas mixtures
Pervaporation	Asymmetric homogenous polymer	Polyacrylonitrile, Polymers	Vapour pressure gradient	Separation of azeotropic mixtures
Nanofiltration	Thin-film membranes	Cellulosic Acetate and Aromatic	9.3-15.9 bar	Removal of hardness and desalting

2.01 Membrane Material

Common requirements for membranes, regardless of the separation process, are high flux and selectivity, chemical resistance, and high durability (longest possible life), and of course material cost. The type of membrane material and the fabrication method used in its production determines these properties. Membranes are selective barriers that permit the passage of certain components and reject certain other components within a mixture. Membranes in ultrafiltration allow small molecule to pass through the membrane and they reject or do not allow macromolecules to pass through(Loeb, 1981).

Different membrane materials with the same nominal MWCO will appear to give different solute rejection. In addition to pore size distribution, the chemical nature of the membrane as it affects solute-membrane interaction (i.e. fouling) is important. Compared to polysulfone membranes, membranes made of cellulose acetate or regenerated cellulose had broader pore size distribution and higher rejections and showed less deviation between observed and true rejections and less effect of transmembrane pressure on rejection. These phenomena are probably related to fouling effects, which in turn are related to hydrophobicity, charge and surface roughness. In general, higher fluxes and lower adsorption effects have been observed with hydrophilic materials than hydrophobic membrane, for aqueous-based feeds. Materials used for the manufacture of ultrafiltrations are regenerated cellulose, ceramic composites (zirconia on alumina), polyacrylonitrile (PAN), polyvinyl alcohol (PVA), polysulfone (PS), polyethersulfone (PES), cellulose acetate (CA), polyimide (PI) and polyamide (PA).

The families of polysulfone membranes are widely used in MF and UF. Polysulfone itself is characterized by having in its structure diphenylene sulfone repeating units. The $-SO_2$ group in the polymeric membrane is quite stable, because of electronic attraction of resonating electrons between adjacent aromatic groups. The oxygen molecules projecting from this group each have two pairs of unshared electrons to donate to strong hydrogen bonding of solute or solvent molecules. Repeating phenylene rings create both steric hindrance to rotation within the molecule and electronic attraction of resonating electron system between adjacent molecules; both contribute to a high degree of molecular immobility, producing high rigidity, strength, creep resistance, dimensional stability and heat deflection temperature(Bhardwaj et al., 1996).

The first commercial UF membranes were introduced in the 1960s by Millipore and Amicon. Amicon introduced the first cellulose membranes in the 1970s.

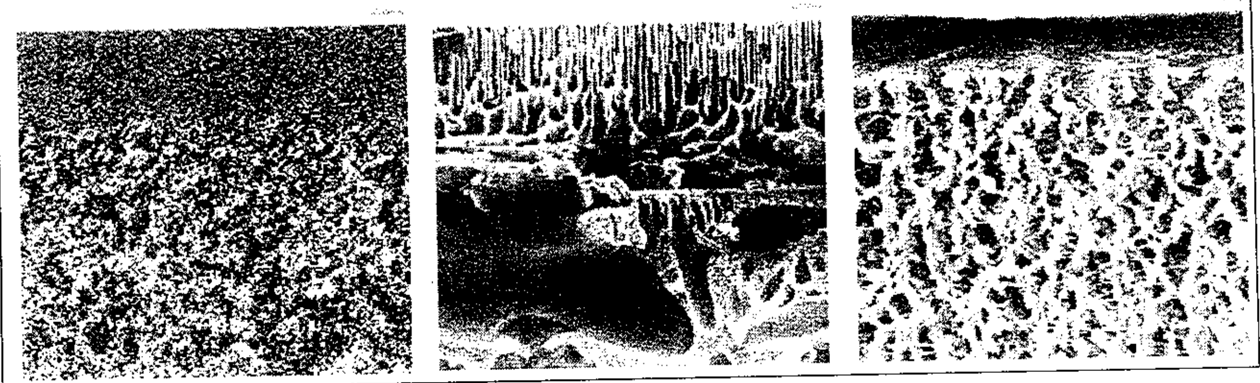


Figure 2.01. Different cross section of UF membranes (Millipore®) Electron micrograph (650 xs)

In the ultrafiltration process, there are two main types of membranes used. The first of the membranes are asymmetric skinned membranes. These membranes consist of a wide variety of synthetic polymers, copolymers, and

blends. The second main types of membranes used in ultrafiltration are inorganic membranes. These membranes consist of inorganic materials such as Zirconium Ozide and Alumina.

The most common membranes used in ultrafiltration are polysulfone (PS), polyethersulfone (PES), sulfonated polysulfone (SPS), polyamide (PA), cellulose acetate (CA), Zirconium Oxide (inorganic), and alumina (inorganic). All of these membranes are different and have various properties. There are a handful of important properties for a membrane in the ultrafiltration process. These properties include: pore-size, porosity, hydrophilic/hydrophobic nature, pH tolerances, temperature tolerances, strength, durability, and cleanability.

The membranes used in ultrafiltration are asymmetric porous and the pore sizes range from 0.05 microns to 1 nanometer. The separation principle for these membranes is a sieving mechanism and the driving force is a pressure range of 1 - 10 bars. The majority of these membranes (modified or unmodified) are able to withstand temperature greater than 100°C and pH ranges of 1 – 14.

2.1 UF Membrane Modules

Membranes have four different modules. They are plate and frame, tubular, spiral wound, hollow fiber and thin channel flow. Each configuration model has their inherent strengths and weaknesses and varies in their industrial and commercial applications. Choosing the right module configuration is important to the overall effectiveness of the ultrafiltration process. Membrane module designs have been in continual development is driven by four primary factors (Robert Hesketh, 2008).

They are

i. To obtain a high surface area in a small volume.
ii. To minimize the mass transfer limitation from fluid flow at the membrane interfaces.
iii. To maximize the ease and minimize the cost of module production an
iv. To minimize the modules contribution to the application operating cost.

The modules can be constructed in a number of ways including leafs extending perpendicular from a central shaft or disks stacked on a central core, either of which would then be put in a pressure housing(Agarwal et al., 1994).

2.1.1 Plate and Frame Module

Plate and Frame module was extensively discussed by Mehta and Zydney (2005). Plate modules use multiple flat sheet membranes in a sandwich arrangement consisting of a support plate, membrane and channel separator. The membranes are sealed to the plates using gaskets and hydraulically clamped to form a tight fit. Several of these membranes are stacked together and clamped to form a complete module. The feed channel can be a clear path with channel heights from 0.3 to 0.75 mm. The higher channel heights are necessary for high-viscosity feeds; reduction in power consumption of 20 to 40% can be achieved by using a 0.6mm channel compared to a 0.3 mm channel.

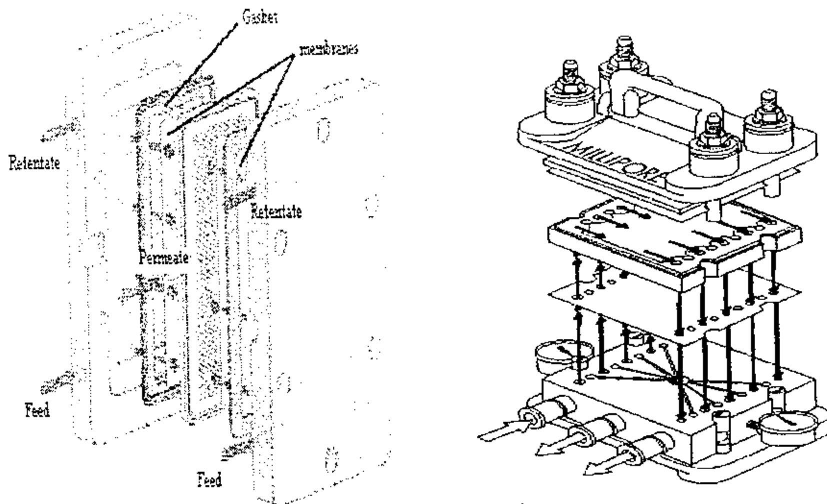


Figure 2.1.1 Diagram of Plate and Frame Ultrafiltration Module

This element incorporates sheet membrane stretched over a frame to separate the layers and facilitate collection of the permeate which is directed to a center tube. From the perspective of cost and convenience, it is beneficial to pack as much membrane area into as small a volume as possible. This is known as packing density. The greater the packing density, the greater the membrane area enclosed in certain sized device and generally the lower the cost of the membrane element. The downside of the high packing density membrane elements is their greater propensity for fouling.

Ultrafiltration in a plate and frame system occurs by pumping a feed solution with smaller MW undesired material contaminating the solution over a membrane under pressure. Usually, two membranes and a spacer are used but for our laboratory design, only one membrane and a spacer will be used. The permeate or undesired low MW contaminate is forced through the membrane to a holding tank and the retentate or concentrated high MW desired product can either be recirculated to the feed tank or passed to another holding tank.

These systems use a cross flow (tangential) filtration system. i.e. the solution flow parallel to the membrane. At the same time the feed are pushed by pressure through the membrane.

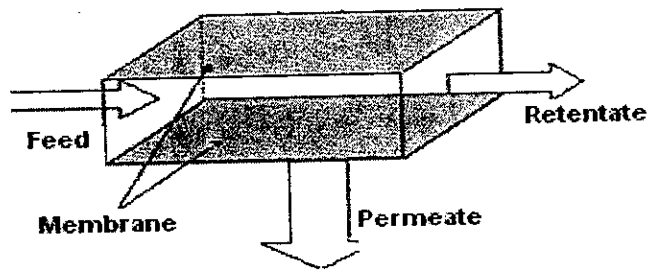


Figure 2.1.2 Flow Diagram of Plate and Frame Ultrafiltration Module (Cross flow module)

The feed solution flows radially over the membranes, alternating between an inward and outward flow as it moves through the stack of membranes. Permeate is collected around the periphery of the unit. The heart of the plate-and-frame module is the plastic support plate that is sandwiched between two flat-sheet membranes. The membranes are sealed to the plate by either gasket with locking devices, glue, or are directly bonded. The plate is internally porous and provides a flow channel for permeate, which is collected from a tube on the side of the plate.

The main advantages of the flat-plate module design are that they have high membrane packing densities and low hold-up volumes. This is due to the small channel height in the flat-plate modules. The main disadvantage of the flat-plate design is that they are susceptible to particulate plugging and the modules are difficult to clean .The main application for the plate and frame module is in recovering biological products.

2.1.2 Tubular Membrane Module

Tubular membrane devices are similar to their HF module counterparts, but employ much larger internal diameter tubes typically in the range of 0.3 to 2.5 cm. In this design, the semi-permeable membrane is cast in place within a porous support tube made of fiberglass or other appropriate materials. The individual tubes are then packed in small bundles, which are kept in place by two end plates. This is then encapsulated into a plastic or stainless steel sleeve to form a single tube cartridge. This module gives turbulent flow usually several types of tubular are in parallel.

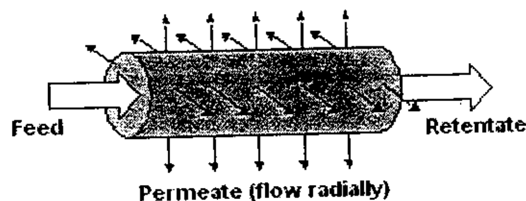


Figure 2.1.3 Flow Diagram of Tubular Membrane Module

During the filtration process the feed or "unfiltered" liquid flows into one end plate and the resulting permeate or "filtered" liquid flows radially through the membrane tube and towards the sleeve collection area and out the permeate outlet ports. The primary advantage of the tubular membrane module design is the large internal diameter of its tubes, which prevent the problem of particulate plugging. Generally speaking, the internal diameters of the tubes are 10 times the size of the largest expected particulate in the feed liquid. The primary disadvantages of tubular modules are their very low membrane packing density and high-energy costs. The tubular membrane is generally used in small flow, high solids loading applications. Its design is

inherently easy to clean; therefore it is the membrane of choice when severe fouling is expected.

2.1.3 Hollow-Fiber Module

The HF modules consist of an array of narrow-bore fibers with a dense skin layer, which is bound into bundles of fibers (e.g. 50–10,000 fibers) and potted at the ends with an epoxy or polyurethane resin to form a tube sheet. The tube sheet is machined to expose the open bores of the fibers and then encapsulated into plastic cylinder housing. These modules work in a similar fashion to the tubular modules. The feed liquid flows into the fibers through the end plates of the cylinder and the resulting permeate flows radially outward through the fiber walls into a collection area and then out through the permeate outlet ports.

Hollow fiber membranes provide extremely high mass transfer areas in a cost-effective module size. The small diameter of the fibers makes them more prone to plugging and fouling when the process feed is through the inside diameter (lumen), therefore pretreatment/filtration requirements are usually more stringent in this case.

The primary advantages of the HF modules are their high mass transfer rates, low energy costs and high packing densities. All of these are the result of the small internal diameter of the packed fibers. The main disadvantage of the HF module design is that it is susceptible to particulate plugging. This requires a pre-filtration of the feed liquid to remove large particulate matter prior to loading into the ultrafiltration process. The HF module is generally used in high flow, low solids loading applications. Its

design is inherently easy to clean; therefore it is also the design of choice in applications, which requires cleaning.

2.1.4 Spiral Wound Module

Spiral modules are constructed using flat sheet membranes in the form of a "pocket" consisting of two membrane sheets separated by a porous support plate and a permeable mesh. The membranes are sealed along the edges to form a pocket using an adhesive. Several of these pockets are spirally wound around a single collecting tube using a feed-side mesh as a spacer to establish the desired feed channel thickness. The entire spiral is then wrapped with fiberglass tape and fitted into a tubular steel or plastic pressure vessel with an anti telescoping device placed at both ends of each element to prevent distortion of the spiral during operation. The pressurized feed solution is fed into one end of the vessel and flows through the plastic screens along the surface of the membranes. The permeate flows into the closed membrane pockets and spirals radially inward where it is collected and through the central tube. The main advantages of these modules are their high membrane packing density, effective mass transfer characteristics and low energy costs. This is due to the spacing between the membrane sheets and the low flow rates of the feed solutions. The main disadvantages of the spiral modules are that they are highly susceptible to fouling and are generally difficult to clean. This eliminates them from consideration for applications where Total Suspended Solids (TSS) loading is high. The comparison of different Membrane Module in the forms with respect to flux, hold up volume, and mass transfer coefficient.

Table 2.1.1 **Comparison** of Different module

Type	Fluid flow regime	Membrane area/module volume	Mass transfer coefficient	Hold-up Volume	Special remarks
Plate and Frame	Laminar	Low	Low to moderate	Moderate	Easily dismantled and cleaned
Spiral wound	Laminar	Moderate	Low	Low	Low TMP only.
Hollow fibre	Laminar-turbulent	High	Low to moderate	Low	Susceptible to fibre blocking
Tubular	Turbulent	Low	Moderate to high	Moderate to high	Flow pattern easy to characterise.

2.2 Factors Affecting UF System

There are several factors that can affect the performance of an ultrafiltration system. A brief discussion of these is given here.

2.2.1 Operating Pressure

Permeate rate is directly proportional to the applied pressure across the membrane surface. However, due to increased fouling and compaction, the operating pressures rarely exceed 100 psig and are generally around 50 psig. The operating pressure for a given run was taken to be the average of the inlet and the outlet pressure readings. In some of the capillary-type ultrafiltration membrane modules the operating pressures are even lower due to the physical strength limitation imposed by the membrane module.

2.2.2 Operating Temperature

Increasing the temperature generally increases the permeate flux due to the dual effect of lowering the permeate viscosity, which assists flow rate, and of increasing diffusivity, which assists dispersion of the polarized layer in both UF.

2.2.3. Flow across the Membrane Surface

The permeate rate increases with the flow velocity of the liquid across the membrane surface. Flow velocity if especially critical for liquids containing emulsions or suspensions. Generally, flow across the membrane surface is classified as five categories. They are Co-current flow, completely mixed flow, Counter current flow, Cross flow and Dead-end flow.

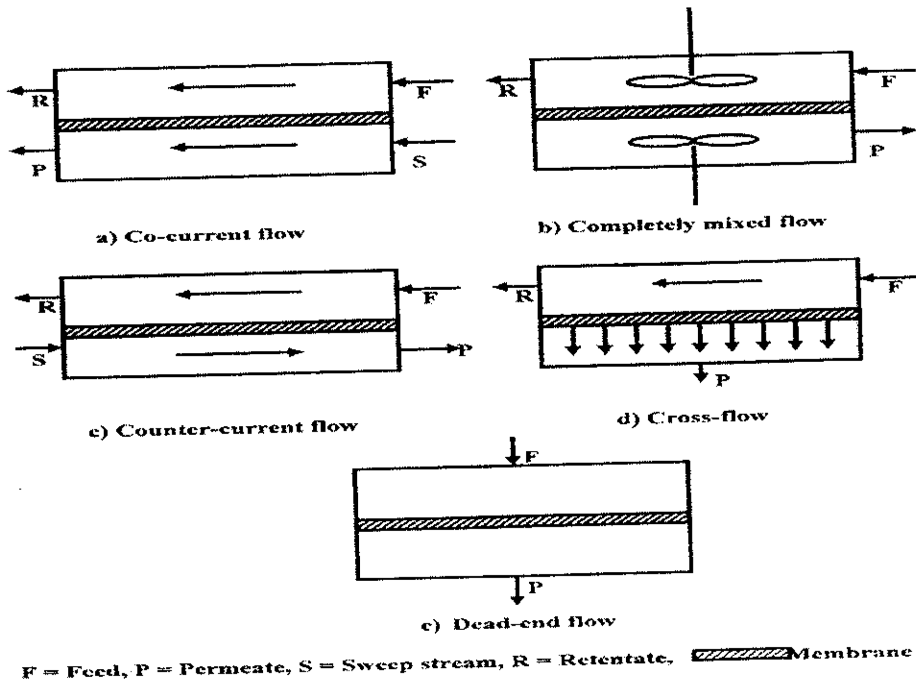


Figure 2.2.1 Classification of Membrane Process based on flow type

2.2.3.1 Co-current Flow

Co-current flow through a membrane module in which the fluids on the upstream and the downstream sides of the membrane move parallel to the membrane surface and in the some.

2.2.3.2 Completely-Mixed (Perfectly-Mixed)

This flow through a membrane module in which fluids on both the upstream and downstream sides of the membrane are individually well-mixed.

2.2.3.3 Counter-current

It is flow through a membrane module in which the fluids on the upstream and downstream sides of the membrane move parallel to the membrane surface but in opposite directions.

2.2.3.4 Dead-End

Dead end flow through a membrane module in which the only outlet for upstream fluid is through the membrane.

2.2.3.5 Cross Flow Pattern

UF is one of the most widely used forms of Cross-flow or tangential flow filtration (TFF).Cross flow through a membrane module in which the fluid on the upstream side of the membrane moves parallel to the membrane surface and the fluid on the downstream side of the membrane moves away from the membrane in the direction normal to the membrane surface. The cross-flow configuration helps control concentration-polarization and cake buildup. It is used to separate proteins from buffer components for buffer exchange, desalting, or concentration. Most ultrafiltration processes operate in cross-flow mode, and most ultrafiltration membranes are made from polymers or ceramic materials. Cross flow ultrafiltration is now used in a

wide range of industrial applications. The application of cross flow filtration in the biochemical and food industries is often limited by the rapid decline in flux of permeate due to fouling of the membrane and to the formation of concentration polarization(Palacio et al., 2003).

Higher flow also means higher energy consumption and larger pumps. Increasing the flow velocity also reduces the fouling of the membrane surface. Generally, an optimum flow velocity is arrived at by a compromise between the pump horsepower and increase in permeate rate.

2.3 Factors Affecting Transmission of Protein in UF

Model proteins were separated against their size gradient, so that the bigger protein is obtained in the permeate and transmission by manipulating the physico chemical properties of proteins. The following factors affect the transmission of a protein. Properties of protein

- Molecular size of protein
- Charge of protein

1. Membrane properties

- Pore size distribution
- Morphology
- Surface Properties
- Mechanical Strength
- Chemical Properties
- Hydrophilic and Hydrophobic of membrane
- Fouling

2. System hydrodynamics

- Transmembrane pressure (TMP)/ TMP pulsing
- Fluid system management
  - Cross flow velocity
  - Pump Speed
  - Laminar / Turbulent flow
  - Dean and Taylor vortices
- Stirrer speed
- Concentration polarization

3. Solution Environment

- Temperature
- Feed concentration
- pH
- Ionic strength

2.3.1 Temperature

Increase in temperature generally results in beneficial effects of lower viscosity and higher diffusion from transport point of view. However, for biological feed streams, too high a temperature will lead to protein denaturation and enzyme inactivation or other heat damages. This could result in poor product quality as well as lowering of flux. Hence thermal stability of feed stream dictates the operating temperature.

2.3.2 Feed Concentration

Transmission decreased with increase in the protein concentration. Higher protein concentration causes significant increase in viscosity and

decrease in diffusivity of protein. Protein retention with an ultrafiltration membrane is based on the steric hindrance, i.e. on the ratio between the protein size ad the membrane pore size. However the retention also depends on the physico-chemical environment of the solute and on the chemical nature of the membrane (Nilsson, 2008). The protein- protein interaction appeared to dominate the protein membrane interaction as concentration increased. Increasing the feed concentration during UF generally results in a decrease in the permeate flux.

2.3.3 pH and Ionic Strength

Several recent studies have demonstrated that it is possible to obtain high-resolution protein separations by adjusting solution pH and salt concentration to exploit electrostatic interactions between the proteins and membrane. However, in order to identify the optimum conditions for selective filtration, it is essential to understand the UF characteristics of single proteins. Based on these results, optimum conditions can be identified for the fractionation of mixtures (Attia *et al.*, 1991).

Changing the solution pH alters the electrical charge on both the protein and the membrane due to the ionization or deionization of various acidic/basic groups on the protein and membrane surface. This can cause either attractive or repulsive interactions depending upon the specific number and equilibrium constants (pKa values) of these charged ligands. Solution pH can alter the conformation of some proteins, and it can affect the protein-diffusion coefficient (which will alter the protein mass-transfer coefficient in the specific module). Finally, membrane fouling can be a strong function of solution pH due to the influence of protein–surface and

protein–protein interactions on both the rate and extent of fouling (Velasco *et al.*, 2003).

2.3.4 Effect of Stirring

Since stirring and cross flow increases shear and drag forces along a membrane surface it is expected that greater back diffusion of solutes take place thus reducing concentration polarization. Greater protein rejection is the result, and at the same time an increase in the permeation rate takes place. However, evidence shows that such observations may only be valid for ultrafiltration where the pore size is more comparable to that of the protein molecules

2.3.5 Effect of Solution Conditions

Depending on the prevailing solution conditions protein fouling can be minimized or exacerbated. Adjusting the solution pH and/or the ionic strength is a means by which intermolecular protein-protein and membrane-protein electrostatic forces can be manipulated.

At pH values below the isoelectric point (IEP) protein molecules acquire net positive charges while above the IEP they acquire negative charges. These charges increase in magnitude with increasing distance away from the IEP. At or close to the IEP protein molecules acquire a zero net charge. Furthermore away from the IEP protein molecules enlarge due to intramolecular electrostatic charge repulsion, resulting in relatively permeable protein deposits on membranes. When the protein molecules in solution have the same charge sign as of the membrane surface, electrostatic

repulsion ensues, thus having the effect of reducing fouling and protein adsorption. Alternately when they are oppositely charged, the likelihood of deposition is increased due to electrostatic attraction.

The electrostatic effects of pH can be offset when electrolytes are present in a feed solution. Salt ions bind to ionized groups on protein molecules and produce a charge-screening effect, dampening out any electrostatic attractive or repulsive forces and effectively compressing the electrostatic double layer. Therefore, as ionic strength increases, the range of electrostatic double layer attractive or repulsive forces decreases.

2.3.6 Effect of Solution Conditions for Single Protein Solutions

At pH of the IEP it has been shown that the absence of electrostatic forces results in increased protein adsorption to membrane surfaces and tightly packed protein deposits. (Swaminathan and Sirkar, 1981) remarked that the solubility of protein molecules goes through a minimum at the IEP where there also maximum tendency for protein agglomeration. Since membrane fouling by proteins is known to occur via the deposition of aggregates, consideration must be given to the effects that salts have on the solubility of proteins.

2.3.7 Protein Solubility and Precipitation

Since membrane fouling by proteins is known to occur via the deposition of aggregates, consideration must be given to the effects that salts have on the solubility of proteins. In aqueous solution the solubility of a protein is affected by polar interactions with the solvent, ionic interactions with the salts present and repulsive electrostatic forces between like-charged

molecules or small aggregates of molecules (Scopes, 1994). For a given pH and temperature, the variation of protein solubility with salt concentration can be illustrated schematically by Figure 2.3.7.1 Over the ionic strength range from zero to about 0.5 M, protein solubility tends to increase with salt concentration (Scopes, 1994). This progressive increase in solubility with salt concentration is known as "salting in". For salting-out electrolytes, protein solubility reaches a maximum before decreasing with increasing salt concentration at high salt concentrations. This decrease in solubility with increasing salt concentration is known as "salting out". The opposite effect occurs for salting-in electrolytes at high salt concentrations. In terms of pH, most proteins exhibit a minimum in solubility at their IEP that may be low enough to effect their precipitation out of solution. For moderate salt concentrations (< 0.5 M), this minimum increases with salt concentration. Figure 2.3.7.2 shows the solubility behavior of proteins with pH and increasing (moderate) salt concentration.

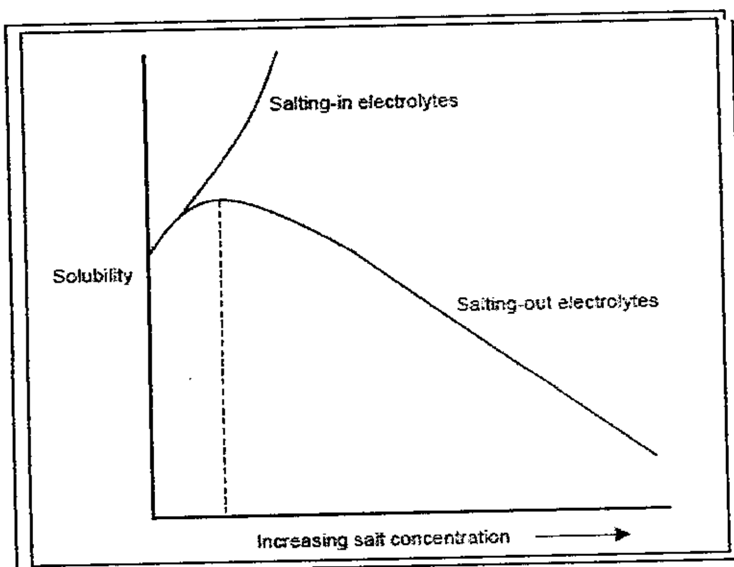


Figure 2.3.7.1: Protein solubility as a function of salt concentration

It is pertinent to note here that pH and ionic strength are interrelated. If the pH of solution is farther away from isoelectric point of protein, the greater is the net charge on protein and electrostatic interactions are thus increased. By increasing the salt concentration, these excess charges can be shielded and the extent of electrostatic interactions is decreased.

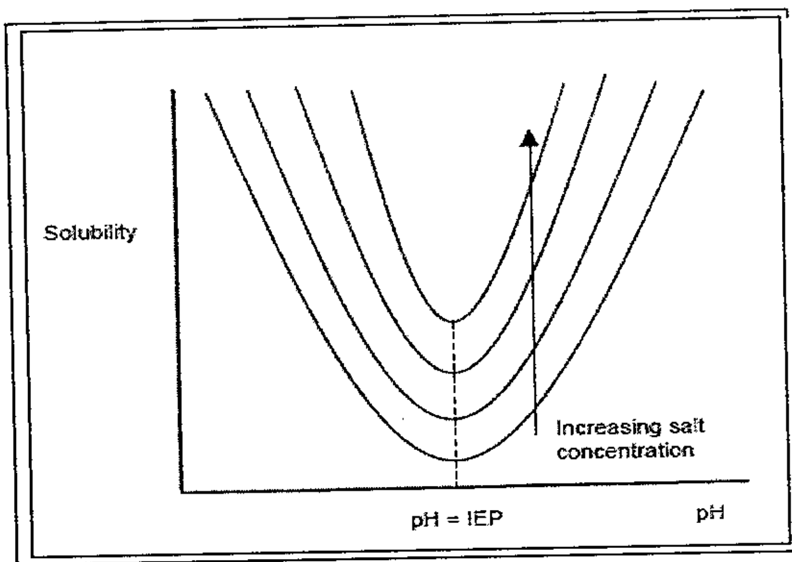


Figure 2.3.7.2 Solubility behavior of proteins with pH and salt concentration

Balakrishnan and Agarwal, (1996) evaluated the effect of solution pH on the transmission of ovalbumin, myoglobin, and lysozyme in a rotating (Taylor vortex) module. Ovalbumin and myoglobin transmission were greatest at the respective protein isoelectric points. The very large reduction in protein transmission at pH < pI was attributed to significant adsorption of the positively charged proteins on the negatively charged membrane under these conditions. The reduction in protein transmission at pH > pI was attributed to an expansion of the protein associated with intramolecular electrostatic interactions in combination with a reduction in the extent of concentration polarization due to the back mass transport caused by the

electrostatic repulsion between the proteins and membrane surface. Lysozyme transmission was actually greatest at pH 6.8, which is well below the protein isoelectric point (pH 10.8). This high transmission at pH 6.8 was thought to be due to an increase in the extent of concentration polarization associated with the attractive interaction between the positively charged lysozyme and the negatively charged membrane.

Zydney and Burns, (2008) demonstrated the importance of solution pH in determining protein sieving through semipermeable ultrafiltration membranes. The protein-sieving coefficient attained its maximum value near the protein isoelectric point and decreased at pH both above and below the pI. These pH effects could be quite dramatic. For example, the BSA sieving coefficient decreased by more than two orders of magnitude (from So = 0.22 to 0.002) as the pH was reduced from 4.7 to 3.5 due to the strong electrostatic exclusion of the positively charged protein from the membrane pores.

Nakao *et al.,* (2008) studied the ultrafiltration of BSA at the isoelectric point and another pH by the use of charged membrane. At the isoelectric point, the rejection of the protein was low, while it was high at the pH level, which gave the protein the same sign of charge as that of the membrane due to charge repulsions.

Yang and Tong, (1997) obtained data for the transport of myoglobin and cytochrome C through hydrolyzed PAN hollow- fiber membranes. Protein transmission was greatest at pH near the protein isoelectric point. The reduction in protein transmission at $pH < pI$ was attributed to an increase in protein adsorption, while the reduction at $pH > pI$ was assumed to be due to electrostatic repulsion between the positively charged membrane and the positively charged protein.

Saksena and Zydney, (1994) showed a dramatic increase in selectivity for the separation of bovine serum albumin (BSA) and immunoglobulins (IgG) by operating at pH 4.8 and low-salt concentrations. This increase in selectivity was attributed to the electrostatic exclusion of the positively charged IgG, with the uncharged BSA passing through the membrane.

Millesime *et al.*, (1994) has investigated the effect of ionic strength on the fractionation of BSA and Lysozyme using unmodified and modified inorganic membrane. At low ionic strength, a selectivity of 10 was observed irrespective of the type of membrane used. However, at moderate and high salt concentration, selectivity with modified membrane went down and was even lower with the unmodified membrane.

Sema Salgın, (2007) studied the influence of electrostatic interactions on membrane fouling during the separation of bovine serum albumin (BSA) from solution in a crossflow ultrafiltration system. The changes in permeate flux, cake layer resistance, zeta potentials of BSA and polyether sulfone (PES) membranes, and electrostatic interaction energies, were evaluated. At all of the ionic conditions studied, PES membranes are negatively charged. However, BSA molecules are either negatively or positively charged depending on the ionic environment. Whereas the cake layer resistance decreased with increasing pH and ionic strength, the permeate fluxes increased. The calculated electrostatic energy was a minimum at the isoelectric point (IEP) of BSA. However, at this point, the cake resistances corresponding to fouling at each ionic strength were not minimized. Below the IEP of BSA, the electrostatic forces were attractive, while above the IEP, repulsive electrostatic forces were dominant.

2.3.8 Bulk Concentration

Nystrom *et al.*, (2001) determined critical fluxes by constant flux ultrafiltration (UF) experiments under laminar flow conditions using hydrophilic C30G and hydrophobic GR51 ultrafiltration membranes and dilute myoglobin solutions and baker's yeast suspensions as model colloids. Solution concentration, pH and cross-flow were investigated. The critical flux increased with increasing flow velocity and decreasing solute concentration. The regenerated cellulose C30G membrane exhibited higher critical fluxes than the polysulphone GR51 membrane. The highest critical flux was obtained at pH 8 in the presence of repulsive electrostatic forces between the molecules and the surface of the membrane and the lowest at the isoelectric points of the colloids. In the case of baker's yeast below the critical flux, the flux was about the same as the pure buffer solution flux showing a strong form of the critical flux. This also occurred with the C30G membrane at low concentrations of myoglobin except at pH 6 when a weak form of the critical flux was measured. With the GR51 membrane, the permeate flux deviated from the pure buffer solution flux even at the lowest fluxes.

Zhang and Spencer, (2003) studied BSA and globulin separation and it was found that the total protein concentration was less than 1g/ L and no added salt, the separations was good. The separation went down with increase in both the salt concentrations and protein concentration

Balakrishnan and Agarwal, (1996) studied transmission of Lysozyme and Myoglobin and it was found that transmission decreased with increase in the protein concentration. Higher protein concentration causes significant increase in viscosity and decrease in diffusivity of proteins. The protein

concentration causes significant increase in viscosity and decrease in diffusivity of proteins. The protein- protein interaction appeared to dominate the protein membrane interaction as concentration increased.

Ghosh and Cui, (1997) examined the effect of pH on fractionation of BSA and Lysozyme by ultrafiltration through 50kDa MWCO polysulfone membrane. It was found that the selectivity of solute separation of dilute mixtures of BSA. However, at a higher feed concentration, the transmission of lysozyme through polysulfone membrane decreases quite dramatically resulting in lower throughput of product. The transmission of lysozyme was enhanced through the polysulfone ultrafiltration membrane by pretreating the surface of the membrane by adsorption of another protein, myoglobin. An increase in lysozyme transmission of up to 63% with respect to native membrane was observed.

Iritani *et al.*, (2005) have reported the fractionation of lysozyme and BSA using a 30 kDa polysulfone membrane. The membrane was assumed to be almost completely retentive for BSA but permeable for lysozyme. The study was meant to demonstrate that proteins electrostatic interactions between dissimilar molecules may control the solute rejection and the filtration rate in upward dead-end ultrafiltration of binary protein mixtures. The concentration of BSA was found to have a very strong influence on the transmission of lysozyme. At higher concentrations of BSA, greater rejection of lysozyme was observed.

2.4 Process Configurations and Diafiltration

A considerable purification of the protein can be done by direct ultrafiltration, but the flux drops to uneconomically low values, and the

pumping power required rises due to increase in viscosity of the retentate. Thus, in order to efficiently effect the maximum purification of a retained solute, diafiltration (DF) can be done. DF refers to the process of adding water to the retentate and continuing the elimination of membrane permeating species along with the water. DF can be conducted under either one of the two modes: discontinuous or continuous diafiltration. Discontinuous diafiltration refers to the operations where permeable solutes are cleared from the retentate by volume reduction, followed by redilution with water and reultrafiltration in repetitive steps. This generally results in the loss of soluble sugar molecules like lactose, non protein nitrogen and the smaller protein molecules making the retentate rich in larger size protein molecules.

Cleaning is the removal of foreign material from the surface and body of the membrane and associated equipment. The vast majority of the literature over the past two decades has focussed on fouling rather than cleaning, even though what appears to be a fouling problem may really be a cleaning problem. The frequency of cleaning is a critical economic factor, since it has a profound effect on the operating life of a membrane. Cleaning and sanitizing membranes is desirable for several reasons

- Laws and regulations may demand it in certain application
- Reduction of microorganism to prevent contamination of the product streams
- Process optimization – it may be better to take time off for cleaning and restoring the flux, rather than continuing with a fouled membrane with a low flux.

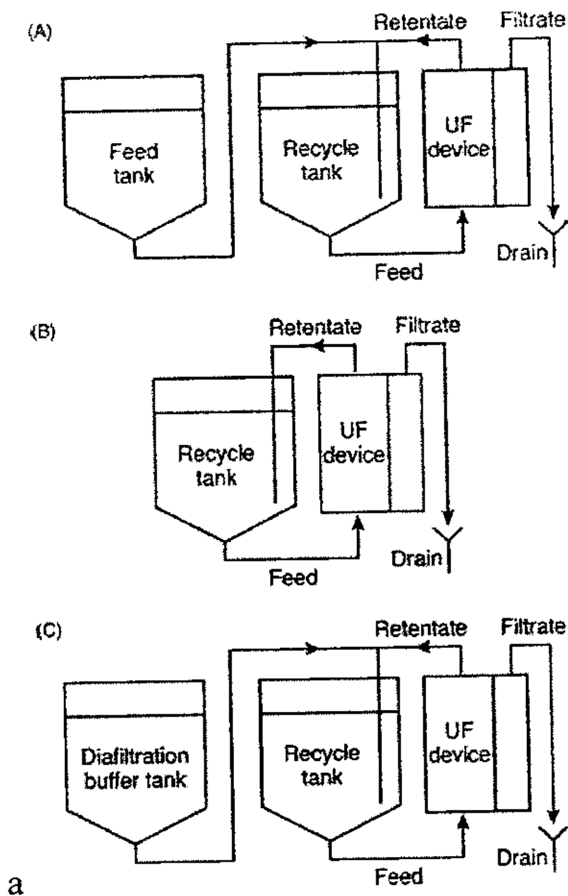


Figure 2.4.1 Process Configurations And Diafiltration

A) Fed Batch ultrafiltration operation for a retained product. (B) Batch ultrafiltration operation for a retained product. (C) Diafiltration operation for a retained product.

2.5 UF Membrane Properties

Mostly, the purity of the given feed depends on the differences in the properties used for the separation. If charge properties and adjustment of solution conditions as well as flow conditions are optimized, model proteins can be separated against their size gradient, so that the bigger protein is obtained in the permeate. Membrane properties in conjunction with operating conditions lead to fouling.

In the following subsections, the membrane properties are discussed first and then fouling effects are described.

- Pore size- 10 to 1000 °A
- High hydraulic permeability to solvent
- Sharp "retention cut-off" properties.
- Good mechanical durability
- Hydrophilic/hydrophobic nature,
- pH tolerances, Temperature tolerance
- Strength, durability
- The driving force is a pressure range of 1-10 bars.

These characteristics depend on membrane material as well as the fabrication technique. The properties are interrelated; a highly porous membrane structure can be maintained only if the polymer has adequate mechanical strength. The physiochemical properties governing the filtration models principally describe the effect of the concentration polarization at the membrane surface (Richard Bowen *et al.*, 2005). Properties such as resistance to compaction under pressure, cleaning chemicals, bacterial degradation, and temperature are important for industrial use. Surface properties and pore morphology have a bearing on fouling properties, and solute separation. The hydrodynamics of the system, i.e. the applied pressure and the membrane configuration have a strong bearing on the extent of concentration polarization on the membrane surface. The most important membrane properties are obviously the membrane productivity (flux) and the extent of separation (rejection of various feed components). Because of

the relatively large size of molecules rejected by the membrane as well as the high fluxes of most ultrafiltration membranes, the phenomena of concentration polarization and fouling are significant.

2.5.1 Flux

The amount of fluid passing through the membrane, the volumetric rate of flow of the permeate through the membrane. It is usually given in terms of rate of permeate flow per unit area per unit time.

$$\textbf{Flux} = \frac{Permeate\ \ volume}{Membrane\ \ area\ \ X\ \ Time}$$

Unit – $liter\,/\,m^2\,/\,hr.$

Flux is directly proportional to applied pressure and inversely proportional to viscosity, which depends on temperature. The flux in UF processes does not increase linearly with pressure after critical pressure has been reached.

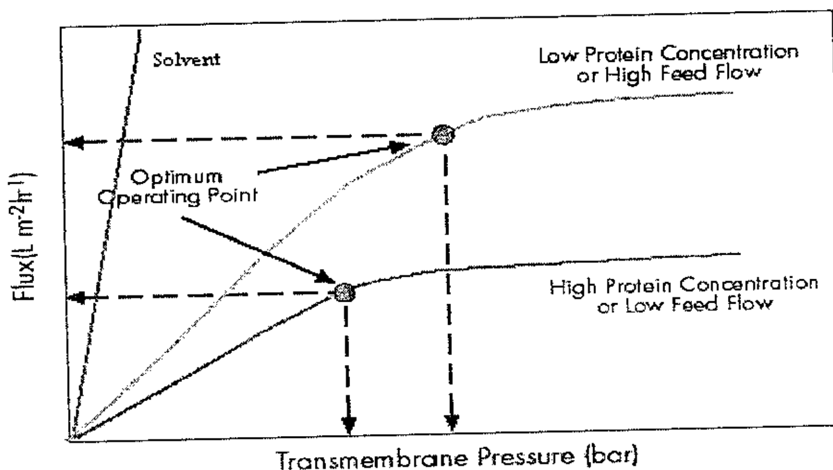


Figure 2.5.1 A typical trend of flux versus pressure for a UF process

These observations indicated the importance of fluid phase mass transfer effects. Some of the methods to increasing the flux. They are

1. Reduce concentration polarization
2. Reduce pressure
3. Reduce solids in feed
4. Reduce concentration at membrane surface

This process indicates the Mixing perpendicular to membrane, Low concentration factor, Prevent concentration at membrane surface, Increase back transfer of solids, High velocity gradient, increase temperature and short flow length.

2.5.2 Rejection and Transmission

The measure of how well a membrane retains or allows the passage of a solute. When based on the concentration in the bulk of the permeate and retentate streams, it is the apparent rejection. The intrinsic rejection is based on the concentration at the membrane surface. Transmission (*Tr*) Ratio depends on both of concentration in retentate (*Cr*) and permeate (*Cp*).

$$\textbf{Rejection} = 1 - \frac{C_p}{C_r}$$

$$\textbf{Transmission} = \frac{C_p}{C_r} \; X \; 100$$

Where,

C_p - Concentration in the permeate

C_r - concentration in the retentate

2.5.3 Molecular Weight Cutoff Profile

The rejection characteristics of ultrafiltration membranes are usually expressed as a nominal molecular weight (MWCO). Otherwise the membrane must be capable of retaining completely nearly all the solute above some specified value, Known as Molecular weight cut off (MWCO). The MWCO of membrane measures the sharpness of the separation possible. This number refers to the molecular weight in Daltons of a protein.

From mechanistic point of view, if the membranes are used only as sieving devices, the proteins to be separated should have at least a ten-fold difference in their molecular weights. Model proteins were separated against their size gradient, so that the bigger protein is obtained in the permeate by manipulating the physico chemical properties of proteins such as molecular size, charge, system hydrodynamics and other operating conditions of ultrafiltration such as pH, ionic strength, temperature, pressure and membrane pre-treatment. Membrane manufacturers are continuously striving to produce isoporous membranes with sharp molecular weight cut off values. The strategies to minimize the effects of concentration polarization and membrane fouling include operating in laminar flow, corrugated membrane surface, diafiltration mode, pulsatile and reverse flow, vortex mixing, and gas sparged membrane filtration. With recent developments in these directions, the ultrafiltration is well poised to play a major role in rapidly developing biotechnology for fraction of high valued proteins.

2.5.4 Concentration Polarization

A comprehensive study of effects of concentration polarization and gel formation on flux and retention of macro solutes showed the importance

of increasing back transport of rejected solutes from membrane surface in order to increase the flux and the rejection of smaller solutes, which would normally permeate in un-polarized conditions. Concentration polarization arises from accumulation of solute molecules adjacent to the membrane surface. When macromolecules and colloids are ultrafiltered, the accumulated solute can form a fairly viscous and gelatinous layer called a "gel layer" on the membrane surface. Concentration polarization is a common feature of all pressure driven membrane processes and it's a drastic effect on membrane performance. Concentration polarisation is the development of a concentration gradient of the retained components near the membrane (Gekas, 2008). It is a function of the hydrodynamic conditions in the membrane system and is independent of the physical properties of the membrane. The membrane pore size and porosity are not directly affected by concentration polarization (Marshall *et al.*, 2003).

Solute rejected by the membrane builds up at its surface to a concentration C_w. The value of C_w is determined by the balance between solute brought to the membrane surface by convenience flow of the solvent and that, which back-diffuses to the bulk. At times, however, C_w reaches its solubility limit, which is lower than what the hydrodynamics would predict. i.e. the applied pressure and the membrane configuration have a strong bearing on the extent of concentration polarization on the membrane surface. Operating conditions can lessen the severity of concentration polarization. It is particularly significant with the high-flux membranes used in ultrafiltration. Concentration polarization leads to smaller incremental increases in flux as pressure is increased until a gel layer is formed, at which point the flux shows no further increase with pressure. The flux at this point is called the *limiting flux*.

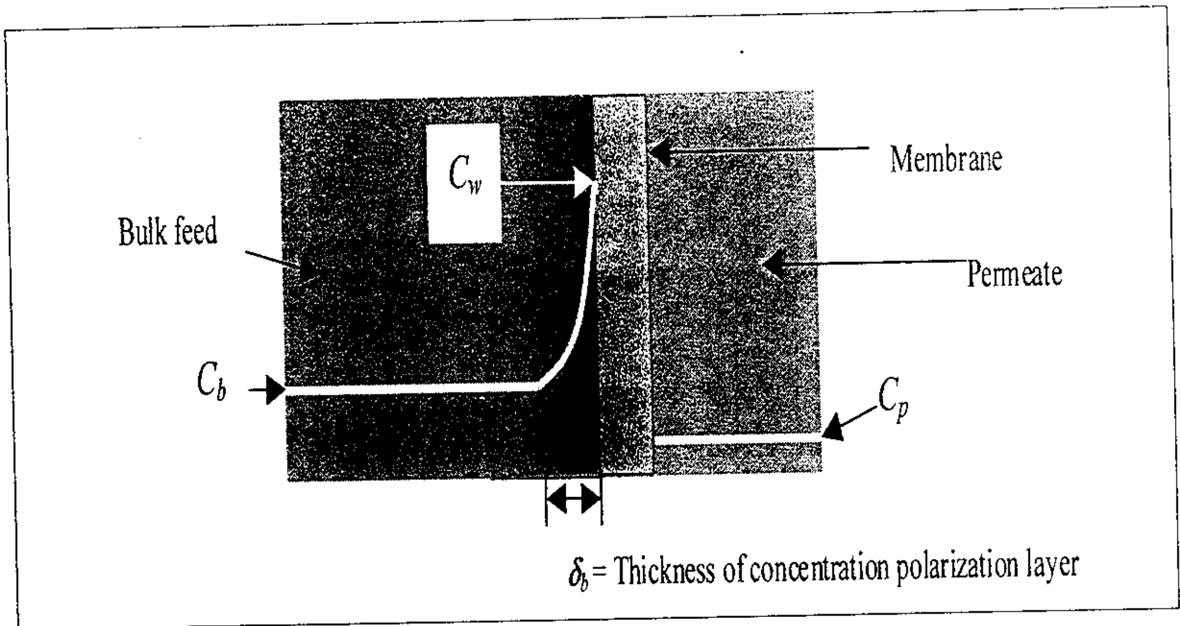


Figure 2.5.4.1 Concentration polarization

Flux-depressing effects due to membrane fouling are frequently confused with flux lowering phenomena associated with concentration polarization. In theory concentration polarization effects should be reversible by decreasing the transmembrane pressure, lowering the feed feed concentration, or increasing cross-flow velocity or turbulence. If this can be done, the cause of lower flux is polarization and not fouling.

2.5.5 Fouling

Fouling is a major limitation to the widespread use of membrane filtration. Fouling is a boundary layer phenomenon, caused or aggravated by concentration polarization, in which solutes deposit on the membrane surface and reduce membrane flux and selectivity Ultrafiltration is used for protein concentration, buffer exchange, and clarification of solutions containing low molecular weight products. Protein fouling in ultrafiltration generally occurs on the external membrane surface since most proteins are

too large to pass through the pores of the ultrafiltration membranes (Palacio *et al.,* 2003). Microfiltration is a pressure-driven membrane process. Membrane fouling during microfiltration can lead to more than an order of magnitude reduction in the filtrate flux, even during the filtration of relatively clean protein solutions. It severe pore plugging by protein occurs, in spite of the pores being an order of magnitude larger than the protein. Fouling within the membrane structure (pore plugging or pore narrowing) results in a change in the apparent pore size, pore size distribution and pore density of the membrane.

Fouling may be reversed by membrane cleaning; however, some irreversible fouling may also occur, which over time necessitates membrane replacement. The effect of fouling species on ht module also needs to be considered. While both concentration polarization and fouling reduce flux, they have opposing effects on the observed rejection. Another way to distinguish the two phenomena is through their time dependence. Concentration polarization is dependent on operating parameters such as pressure, temperature, feed concentration, and velocity but is not a function of time. Fouling s partially feed concentration, but is also time dependent.

The process resulting in loss of performance of a membrane due to the deposition of suspended or dissolved substances on its external surfaces, at its pore openings, or within its pores. The term "fouling" is often used indiscriminately in reference to any phenomenon that results in reduced product rates.

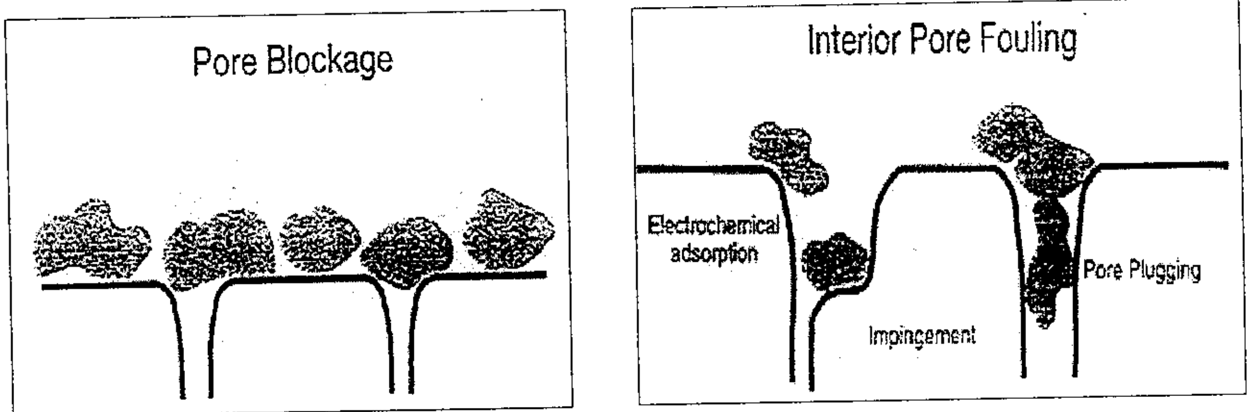


Figure 2.5.5.1 Fouling

Ever since the inception of membrane filtration as a mainstream separation process in the 1970's, one of the most widely studied facets of its operation is that of fouling. Whether reversible or irreversible in nature, fouling can affect the filtration throughput of the membrane, or flux, causing it to decline to low and impractical levels. This in turn reduces separation efficiency, resulting in adverse economic effects for the filtration process involved. Irreversible fouling refers the phenomenon whereby adsorbed solutes cause pore blockage and cake-layer formation on the membrane surface.

Membrane fouling can be affected by numerous factors, both physical and physico-chemical. Physical factors include those relating to the system hydrodynamics while physico-chemical factors involve the properties of the feed solution and the membrane itself. In any membrane filtration process, system hydrodynamic adjustments arising from adjustments to parameters such as the transmembrane pressure (TMP), flux, stirring rate and crossflow velocity can affect the balance of these forces and ultimately affect the likelihood of membrane fouling.

2.6 Properties of Protein

2.6.1 Model Proteins

Crystalline powders of ovalbumin, BSA, lysozyme from chicken egg white lot and Myoglobin from horse heart skeletal muscle, were procured from Sigma Chemical Co. (USA).

Proteins are commonly characterized by their isoelectric points (p*I*), the pH at which they have zero net charge. This net charge has commonly been used to predict the behaviour of proteins on ion exchange resins based on the assumptions that proteins will not be retained at their p*I*, and that they will be retained by anion exchangers at pHs above their p*I* or by cation exchange resins below their p*I*. In addition to p*I*, proteins also have different molecular weights (MW) which are usually used to predict the behaviour of proteins in molecular-sieve separations such as membrane technology and gel filtration chromatography.

2.6.1.1 Bovine Serum Albumin, BSA

Bovine serum albumin, bovine albumin, BSA, also known as "Fraction V", is a serum albumin protein that has numerous biochemical applications including ELISAs (Enzyme-Linked Immunosorbent Assay), immunoblots, and immunohistochemistry. It is also used as a nutrient in cell and microbial culture. In restriction digests, BSA is used to stabilize some enzymes during digestion of DNA and to prevent adhesion of the enzyme to reaction tubes and other vessels. This protein does not affect other enzymes that do not need it for stabilization. BSA is used because of its stability, its lack of effect in many biochemical reactions, and its low cost since large

quantities of it can be readily purified from bovine blood, a byproduct of the cattle industry. By manipulating solvent concentrations, pH, salt levels, and temperature, Cohn was able to pull out successive "fractions" of blood plasma. The process was first commercialized with human albumin for medical use and later adopted for production of BSA.

2.6.1.2 Ovalbumin

Ovalbumin is the main protein found in egg white, making up 60-65% of the total protein. While Ovalbumin displays sequence and three-dimensional homology to the serpin superfamily, it is a noninhibitory serpin. While most serpins control such processes as fibrinolysis and coagulation by inhibiting serine proteases, the function of ovalbumin is unknown, although it is presumed to be a storage protein.

Ovalbumin is an important protein in several different areas of research, including:

- General studies of protein structure and properties (because it is available in large quantities).
- Studies of serpin structure and function (the fact that ovalbumin does not inhibit proteases means that by comparing its structure with that of inhibitory serpins, the structural characteristics required for inhibition can be determined).
- Proteomics (chicken egg ovalbumin is commonly used as a molecular weight marker for calibrating electrophoresis gels).
- Immunology (commonly used to stimulate an allergic reaction in test subjects).

2.6.1.3 Myoglobin

Myoglobin is a single-chain globular protein of 153 amino acids, containing a heme (iron-containing prophyrin) prosthetic group in the center around which the remaining apoprotein folds. It has eight alpha helices and a hydrophobic core. It has a molecular weight of 16,700 daltons, and is the primary 0xygen-carrying pigment of muscle tissues. Unlike the blood-borne hemoglobin, to which it is structurally related, this protein does not exhibit cooperative binding of oxygen, since positive cooperativity is a property of multimeric or oligomeric proteins only. Instead, the binding of oxygen by myoglobin is unaffected by the oxygen pressure in the surrounding tissue.

Myoglobin is released from damaged muscle tissue (rhabdomyolysis), which has very high concentrations of myoglobin. The released myoglobin is filtered by the kidneys but is toxic to the renal tubular epithelium and so may cause acute renal failure.

Myoglobin is a sensitive marker for muscle injury, making it a potential marker for heart attack in patients with chest pain.

Myoglobin contains a prophyrin ring with an iron center. There is a proximal histidine group attached directly to the iron center, and a distal histidine group on the opposite face, not bonded to the iron.

Many functional models of myoglobin have been studied. This was used to show the importance of the distal prosthetic group. It serves three functions:

- To form hydrogen bonds with the dioxygen moiety, increasing the O_2 binding constant
- To prevent the binding of carbon monoxide, whether from within or without the body. Carbon monoxide binds to iron in an end-on

fashion, and is hindered by the presence of the distal histidine, which forces it into a bent conformation. CO binds to heme 23,000 times better than O_2, but only 200 times better in hemoglobin and myoglobin. Oxygen binds in a bent fashion, which can fit with the distal histidine.

- To prevent irreversible dimerization of the oxymyoglobin with another deoxymyoglobin species.

2.6.1.4 Lysozyme

Lysozymes, also known as muramidase or N-acetylmuramide glycanhydrolase, are a family of enzymes (EC3.2.1.17) which damage bacterial cell walls by catalyzing hydrolysis of 1, 4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in a pepidoglycan and between N-acetyl-D-glucosamine residues in chitodextrins. It is abundant in a number of secretions, such as tears, saliva, and mucus. Lysozyme is also present in cytoplasmic granules of the polymorphonuclear neutrophils (PMN). N-acetylmuramic acid and N-acetyl-D-glucosamineThe enzyme functions by attacking peptidoglycans (found in the cells walls of bacteria, especially Gram-positive bacteria) and hydrolyzing the glycosidic bond that connects N-acetylmuramic acid with the fourth carbon atom of N-acetylglucosamine. It does this by binding to the Peptidoglycan molecule in the binding site within the prominent cleft between its two domains. This causes the substrate molecule to adopt a strained conformation similar to that of the transition state. According to Phillips-Mechanism the lysozyme binds to a hexasaccharide. The lysozyme then distorts the 4th sugar in hexasaccharide (the D ring) into a half-chair conformation. In this stressed state the glycosidic bond is easily broken.

The amino acid side chains glutamic acid 35 (Glu35) and aspartate 52 (Asp52) have been found to be critical to the activity of this enzyme. Glu35 acts as a proton donor to the glycosidic bond, cleaving the C-O bond in the substrate, whilst Asp52 acts as a nucleophile to generate a glycosyl enzyme intermediate. The glycosyl enzyme intermediate then reacts with a water molecule, to give the product of hydrolysis and leaving the enzyme unchanged.

Lysozyme is part of the innate immune system. Children fed infant formula lack lysozyme in their diet and have three times the rate of diarrheal disease. Since lysozyme is a natural form of protection from pathogens like *Salmonella*, *E.coli* and *Pseudomonas*, when it is deficient due to infant formula feeding, can lead to increased incidence of disease. Whereas the skin is a protective barrier due to its dryness and acidity, the conjunctiva (membrane covering the eye) is instead protected by secreted enzymes, mainly lysozyme and defensin. However, when these protective barriers fail, conjunctivitis results.

2.6.2 Effect of Size of Protein on Transmission

The primary mechanism of protein transmission through membrane is based on size. When the size of protein is smaller than that of pore, it will be transmitted. But the protein is not a rigid molecule. If membranes are used only as sieving devices, the differences in protein molar mass have to be at least a decade. If charge properties and adjustment of solution conditions as well as flow conditions are optimized, model proteins can be separated against their size gradient, so that the bigger protein is obtained in the permeate(Marianne Nystro *et al.* , 2008).

It is a biopolymer consisting of basic building blocks called amino acids. Nature has evolved proteins to carry out different functions by varying their conformation due to changes in their surrounding solution environment. These conformational changes, consequently the size of protein, are governed by four types of non-covalent forces between the side chain groups of amino acids of the protein. These are electrostatic, hydrogen bond, Vander Waals interactions and hydrophobic interactions. Delicate balance of these forces determines the protein folding and the three dimensional conformation of protein and thus its size and shape. The extent of these interactions can be varied by the adjusting the pH and ionic strength of solution to manipulate the size as well as its charge.

The other contribution to protein transmission is due to electrostatic interactions of charges on protein and the surface charge of membrane. Their isoelectric points (pI) varied between 4.6 and 11 (Marianne Nystro *et al.*, 2008). In folded protein, most of the amino acids with charged groups occur on the surface with core of protein rich with hydrophobic amino acids. The relative amount of positive and a negatives charge is a function of the pH of the solution. The pH value where the surface of protein carries equal number of positive and negative charges is called isoelectric point (pI). Electrostatic interactions were dominated by the distortion of the electrical double layer surrounding the protein, leading to a distinct maximum in protein transmission at the protein isoelectric point. Attractive electrostatic interactions did occur when the protein and membrane had a large opposite charge, causing a second maximum in transmission at a pH between the isoelectric points of protein and membrane (Burns and Zydney, 2007). Proteins are commonly characterized by their isoelectric points (p*I*), the pH

at which they have zero net charge. If the pH of the solution is above the pI, the net charge on the protein will be negative and if it is below pI, the net charge of protein will be positive. Thus charge of protein can be varied to some extent by adjusting the pH. This net charge has commonly been used to predict the behavior of proteins on ion exchange resins based on the assumptions that proteins will not be retained at their p*I*, and that they will be retained by anion exchangers at pHs above their p*I* or by cation exchange resins below their p*I* (Yue Xu *et al., 2000*).

In addition to p*I*, proteins also have different molecular weights (MW), which are usually used to predict the behavior of proteins in molecular-sieve separations such as membrane technology and gel filtration chromatography.

MATERIALS AND METHOD

3.0 MATERIALS AND METHOD

3.1 STIRRED CELL MODULE:

This module was used during the course of experiments conducted to characterize the given membrane and also to check the transmission of proteins through the membrane. It requires small volume of feed and is useful only at the laboratory level.

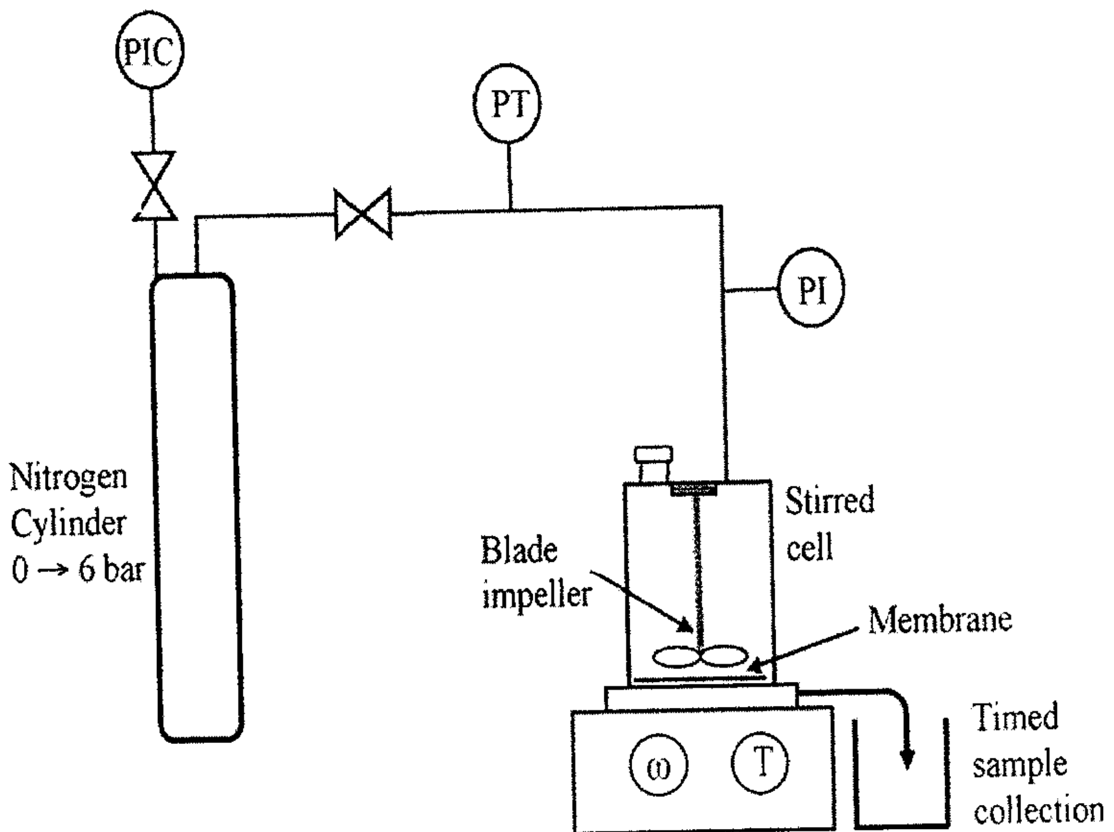
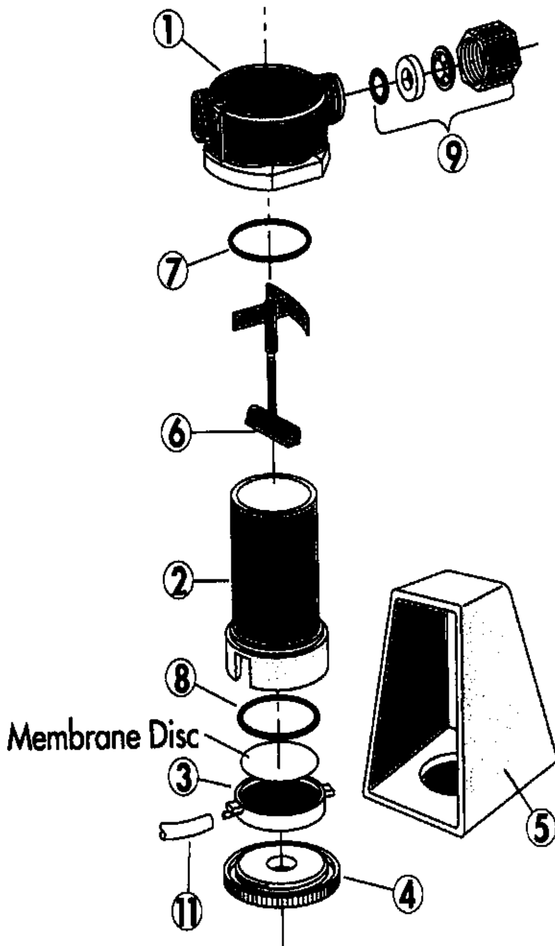


Figure 3.1 Schematic Diagrams of the Stirred Cell Apparatus

ADVANTAGES OF STIRRED CELL MODULE:

- Useful for small scale and research applications.
- Used for UF and MF.
- Provide uniform conditions near the membrane surface.
- Useful for small-scale process development work.

VIEW OF A STIRRED CELL MODULE:



1.	CAP ASSEMBLY
2.	BODY
3.	MEMBRANE HOLDER
4.	BASE
5.	RETAINING STAND ASSEMBLY
6.	STIRRER ASSEMBLY
7.	O-RING FOR CAP ASSEMBLY
8.	O-RING
9.	TUBING FITTING ASSEMBLY
10.	PRESSURE
11.	TUBING

Figure 3.2 Dead end stirred cell module

3.2 Apparatus and Materials

- Ultrafiltration Dead end Stirred cell Module (AMICON, USA), 2000 ml capacity
- Effective membrane area: $0.003166 m^2$
- Thickness of the liquid Channel 0.5mm.
- Maximum Surface temperature 40°C

MAGNETIC STIRRER

- RET Control Visc, IKA Labortechnik
- 0-1100 RPM

pH METER

- Cyberscan pH 510
- Resolution: 0.01 pH
- Accuracy: $\pm$ 0.01 pH
- Automatic temperature compensation

Weighing Balance

UV Spectrophotometer

Membrane

- E113 Unmodified Membrane (Supplied by NCL , Pune)
- PES -30 (Supplied by Millipore).

REAGENTS REQUIRED:

Table 3.2.1 Chemicals

CHEMICALS	DISTRIBUTORS
Sodium Dihydrogen Phosphate (extra pure)	Merck, Mumbai
Di sodium Hydrogen Phosphate	Merck, Mumbai
Sodium Chloride	SRL, Mumbai
Sodium Dodecyl Sulphate (SDS)	SRL,Mumbai

Protein

Crystalline powders of ovalbumin, BSA, lysozyme from chicken egg white Lot and Myoglobin from horse heart skeletal muscle, were procured from Sigma Chemical Co. (USA).

Table 3.2.2 Properties of protein

Protein	pI	MW
Lysozyme	10.6	13,900
Myoglobin	6.8	17,000
Ovalbumin	4.6	44,000
BSA	4.8	69,000

]Protein solution

The desired proteins were prepared by dissolving weighed amount (concentration of 0.3g/l) of protein in 0.1 M phosphate buffer at pH -7.

3.3 Protocol

To predict the flux data and obtain values for permeability, the following steps were followed (Narsaiah and Agarwal, 2007):

- The stirred tank was filled to its capacity with water.
- The transmembrane pressure was set by pressurized nitrogen gas and monitored using U-tube mercury manometer.
- The flow rate of permeate was measured by timing the collection of one ml of permeate at a set pressure, using a stop watch.
- The pressure was increased for a particular stirrer speed.
- After one set of data the stirrer speed is changed and the similar procedure is followed.
- Similarly for buffer and protein solution, the above procedure was repeated.
- Retentate and permeate samples were collected after ultrafiltration at each pressure.
- Permeability was measured before and after ultrafiltration using the buffer (in which the protein solution was prepared) to know the extent of fouling.
- A curve was plotted with transmembrane pressure (kPa) on X - axis and volumetric flux (m/s) on Y axis.
- The slope obtained gave permeability of the membrane for respective solutions.
- The permeate and retentate for protein solution were analyzed in the spectrophotometer to compute the protein content in each of the streams.
- Rejection data was then calculated from the concentrations of permeate and retentate streams.

Membrane Washing:

To use the membrane for subsequent runs, the membrane was cleaned with SDS (sodium dodecyl sulphate). This denatures any proteins present on the surface of the membrane or trapped in its pore channels. To free the membrane from any SDS, it was flushed with water at gradually increasing pressures.

After washing the membrane was stored into 1% Sodium Azide solution to prevent any microbial growth on the membrane surface and for the reuse of the membrane.

- Fouling (%) = 100 X [(Initial Buffer permeability- Final Buffer permeability)/ Initial Buffer permeability]

RESULTS AND DISCUSSION

4.0 RESULTS AND DISCUSSION

4.1 Characterization of membrane

E113 unmodified PAN and PES 30 membranes were used in this study. Characterization of above membranes were carried out by using model proteins such as BSA (69 kDA), ovalbumin (43.5 kDa), Myoglobin (17 kDA) and Lysozyme (13.9 kDa). The membranes were characterized in terms of permeability and transmission of the above model proteins. Transmission of protein and fouling characteristic of E113 unmodified PAN and PES 30 membranes were experimentally determined.

4.1.1 Flux Analysis

E113 unmodified PAN and PES-30 membranes were used for membrane characterization. The flux is usually given in terms of rate of permeate flow per unit area per unit time. All the experiments in the present study were performed using dead end stirred cell (Amicon 8200) supplied by Millpore. The pressure was varied from 20 kPa to 106 kPa. The stirrer speed was maintained from 150 to 250 of maximum RPM.

At the end of the experiment, the membrane was washed with demineralized water and the buffer flux was measured. If the membrane permeability was reduced due to membrane fouling, the membrane was washed with 0.2 % SDS. In Stirred cell ultrafiltration module, the cross flow velocity with respect to pressure. It is usually given in terms of rate of permeate flow per unit area per unit time. In most ultrafiltration processes, the permeate flux becomes independent of the applied pressure for high enough values of the applied pressure. The results are shown regarding the stirring speed (RPM) reading and various pressure.

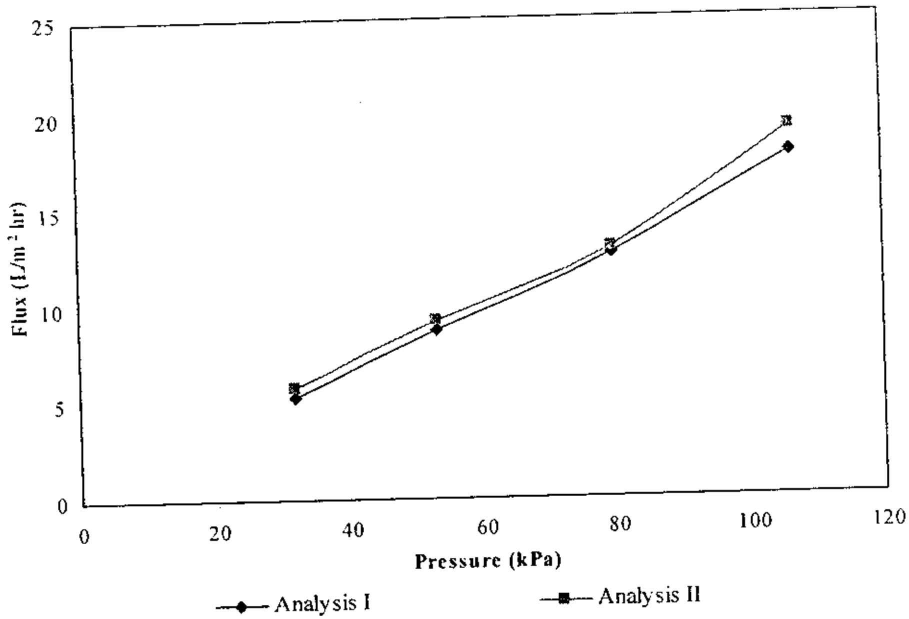


Figure 4.1.1 Water Flux data analysis for E113 unmodified PAN membrane

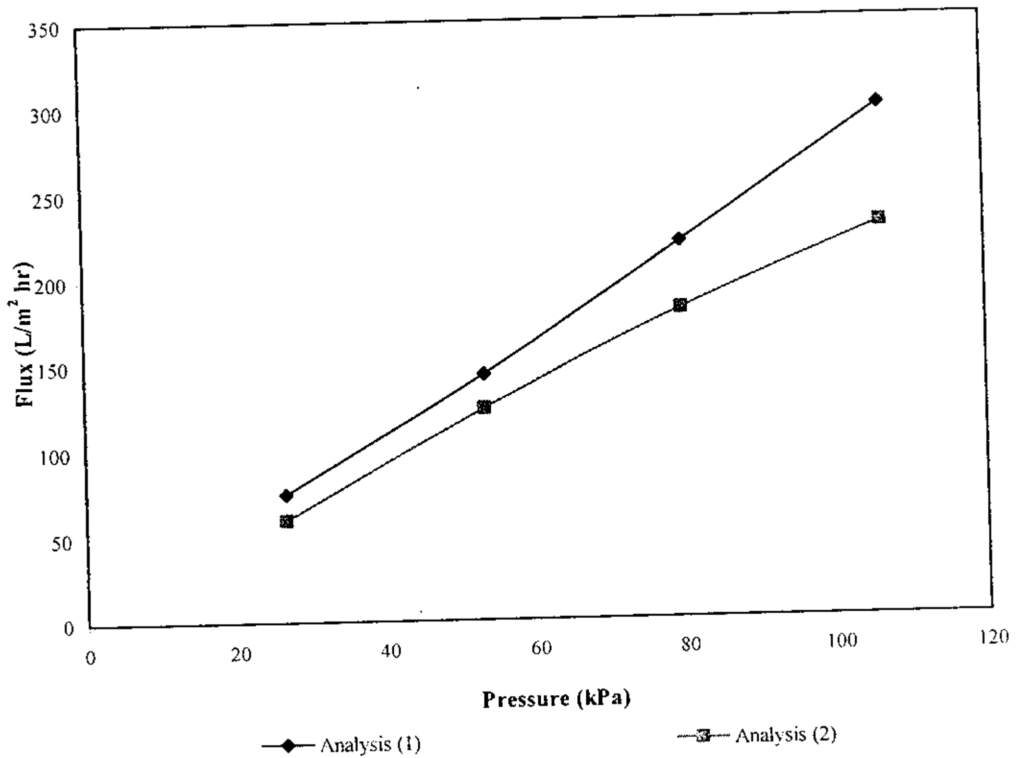


Figure 4.1.2 Water Flux data analysis for PES-30 membrane

Figure 4.1.1 and 4.1.2 showed water flux behavior of E113 unmodified PAN membrane and PES-30 membrane respectively. The water

permeability for the E113 unmodified PAN membrane and PES -30 membrane were 4.67 x 10^{-8} m/s kPa and 6.9 x 10^{-7} m/s kPa respectively.

4.2 Transmission analysis of protein solution

The primary mechanism of protein transmission through membrane is based on size. Transmission of protein through ultrafiltration membrane is influenced by surface characteristics of the membrane and good way to describe membrane characteristic. Membranes are a biopolymer consisting of basic building blocks called amino acids. Nature has evolved proteins to carry out different functions by varying their conformation due to changes in their surrounding solution environment.

Transmission of proteins through ultrafiltration membrane is influenced by surface characteristics of the membrane. If the membrane shows negligible fouling tendency, the steric and electrostatic contributions dictate the transmission of protein. When the fouling tendency of membrane is significantly high, both steric and electrostatic contributions change with time during ultrafiltration. Fouling alters the pore size distribution and reduces the pore size by narrowing down the pore. This results in reduced steric contribution to transmission. Fouling also changes the surface characteristics of membrane leading to variation of electrostatic interactions. Depending on the type of interaction, the protein transmission could increase or decrease.

4.2.1 E113 unmodified PAN membrane

4.2.1.1 BSA Analysis (0.3 g/l)

Table 4.2.1.1 E113 Membrane with BSA analysis

Stirrer Speed (RPM)	Initial Buffer Permeability (x 10^{-8}m/s.kPa)	Final Buffer Permeability (x 10^{-8}m/s.kPa)	Fouling (%)	BSA Permeability (x 10^{-8}m/s.kPa)	Transmission (%)
150	5.87	5.33	9	4.95	0.4
200	5.53	5.44	2	5.11	0.8
250	5.69	5.33	5	5.13	0.8

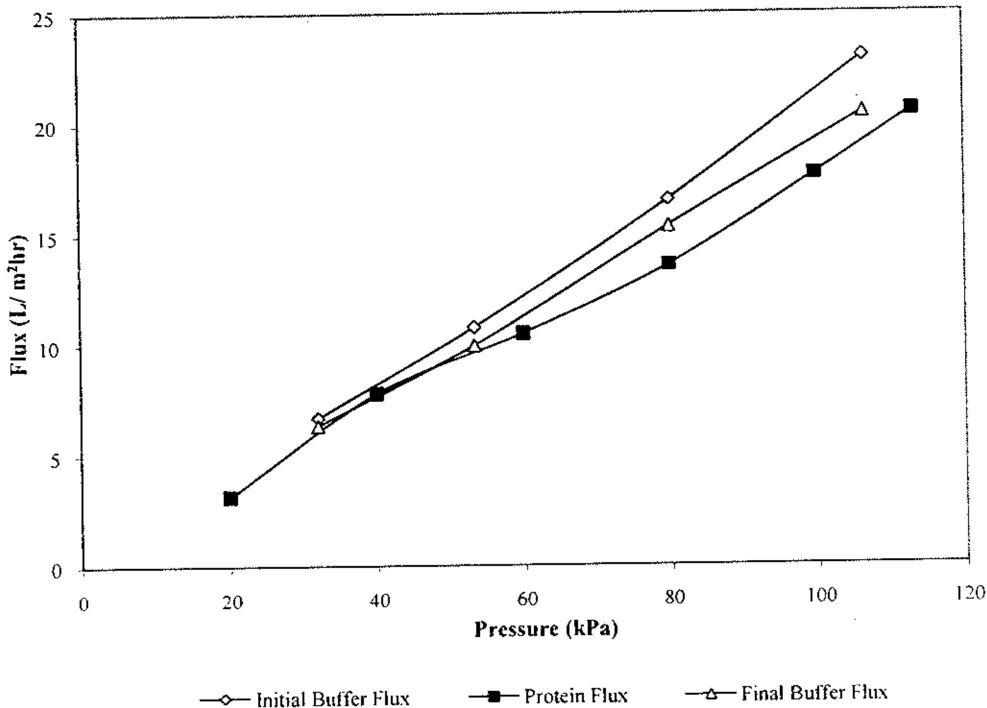


Figure 4.2.1.1(a) Flux data for BSA with respect to Stirrer speed 150 RPM for E113 unmodified PAN membrane

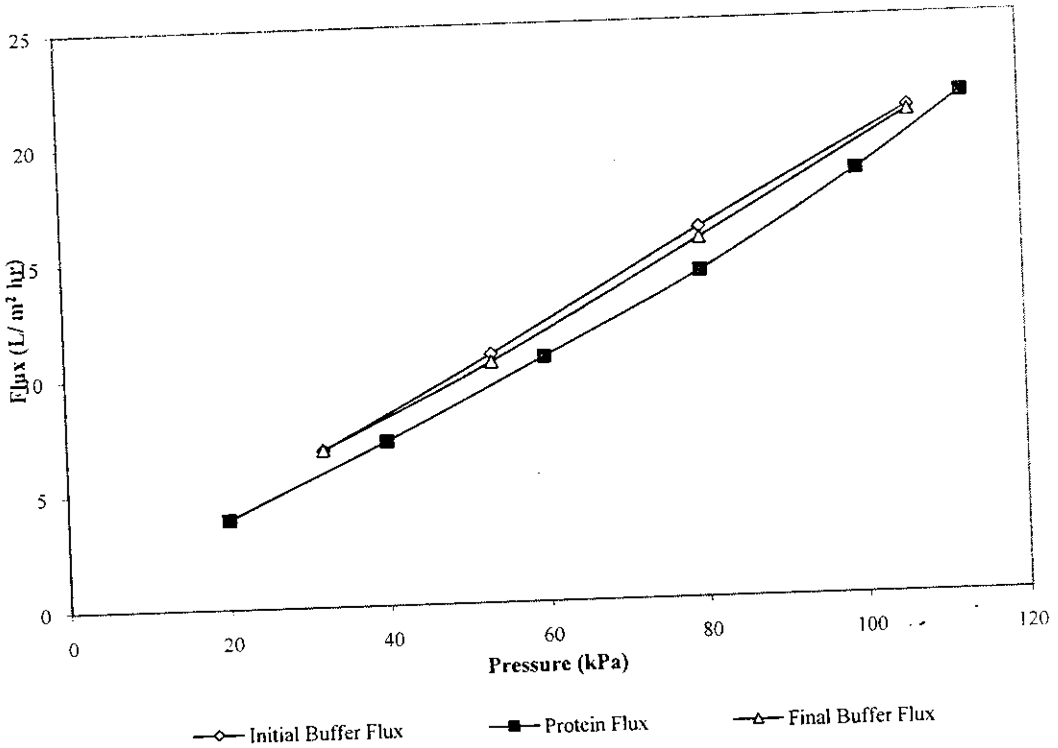


Figure 4.2.1.1(b) Flux data for BSA with respect to Stirrer speed 200 RPM for E113 unmodified PAN membrane

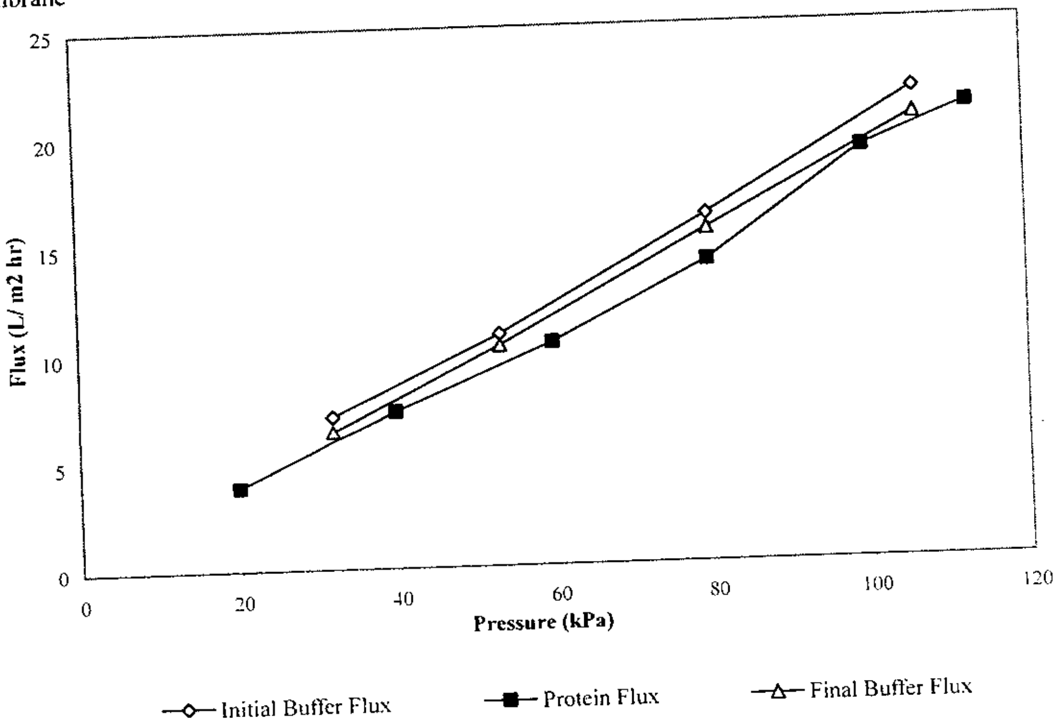


Figure 4.2.1.1(c) Flux data for BSA with respect to Stirrer speed 250 RPM for E113 unmodified PAN membrane

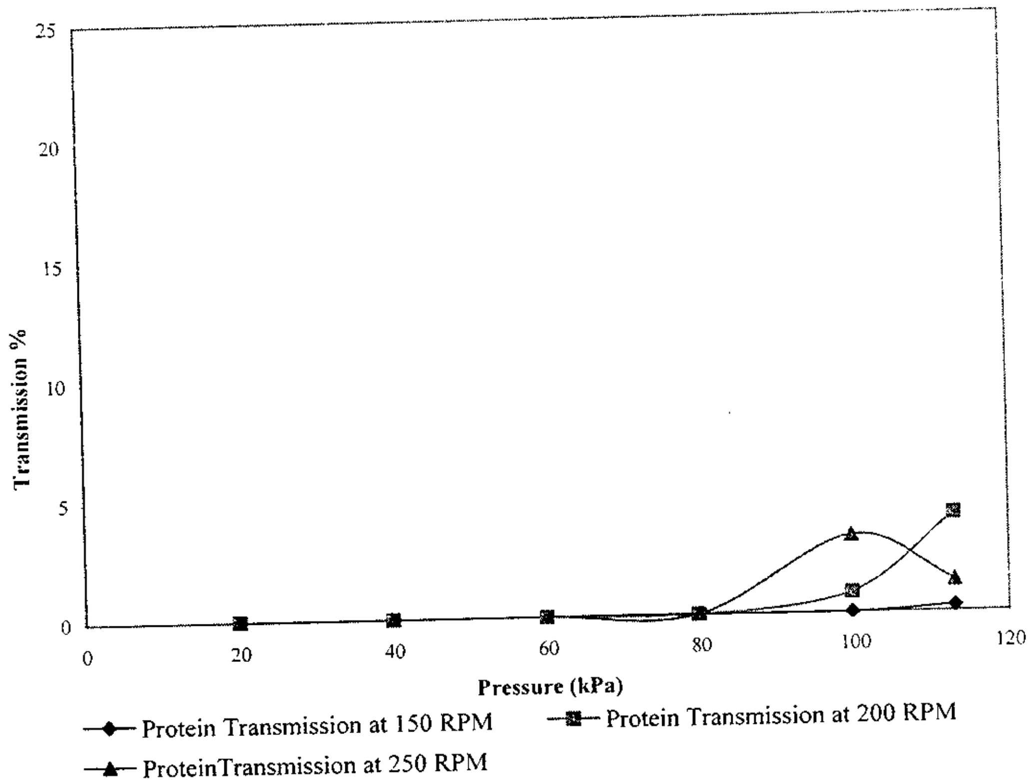


Figure 4.2.1.1(d) Comparison of Transmission data for BSA with different Stirrer speed (150-250) RPM for E113 unmodified PAN membrane

For BSA the permeability of the E 113 unmodified membrane was ranged between 4.95- 5.13 x 10^{-8} (m/s.kPa) for TMP range of 20 -106 kPa (Figure 4.2.1.1(a) to 4.2.1.1 (c)). Figure 4.2.1.1 (d) showed that for E113 unmodified membrane, the transmission of BSA (69 kDa) was negligible and the fouling % lied from 2% to 9% (Table 4.2.1.1). All the experiments were carried out at room temperature with 0.3 g/L concentration of BSA in feed solution.

4.2.1.2 Ovalbumin Analysis (Concentration: 0.3 g / L)

Table 4.2.1.2 E113 Membrane with Ovalbumin Analysis

Stirrer Speed (RPM)	Initial Buffer Permeability (x 10^{-8}m/s.kPa)	Final Buffer Permeability (x 10^{-8}m/s.kPa)	Fouling (%)	Ovalbumin Permeability (x 10^{-8}m/s.kPa)	Transmission (%)
150	5.58	5.37	4	5.19	0.9
200	5.54	5.15	7	5.27	0.5
250	5.79	5.14	11	5.05	0.2

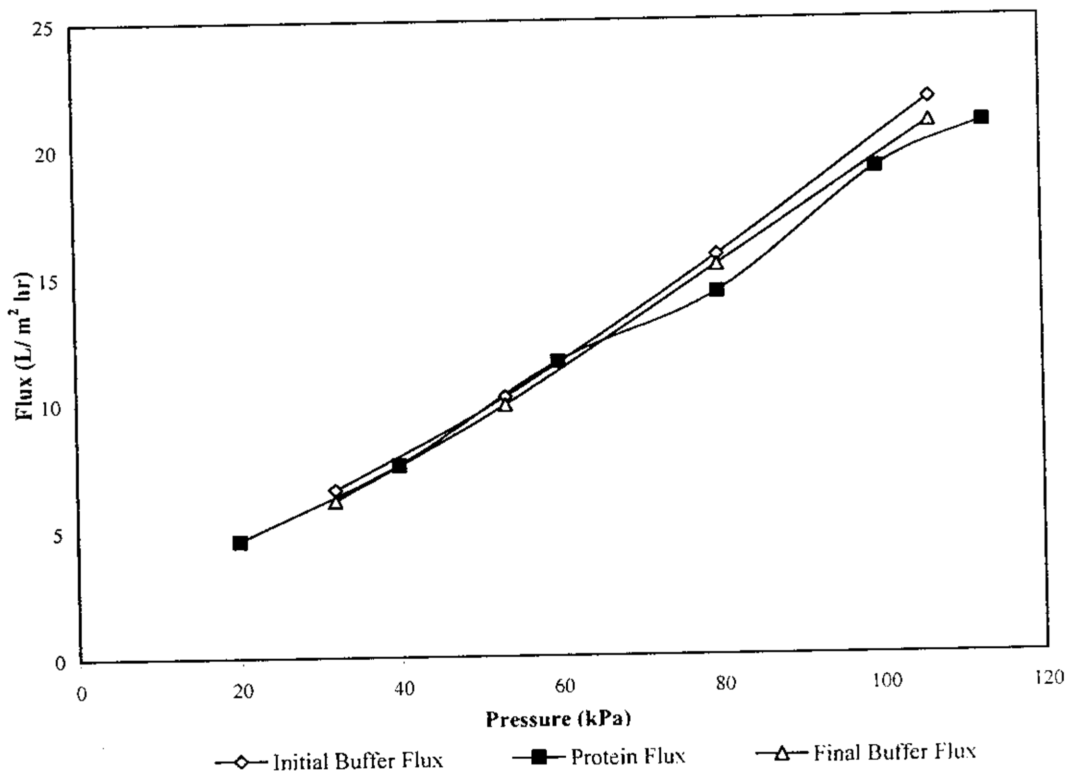


Figure 4.2.1.2(a) Flux data for Ovalbumin with respect to Stirrer speed 150 RPM for E113 unmodified PAN membrane

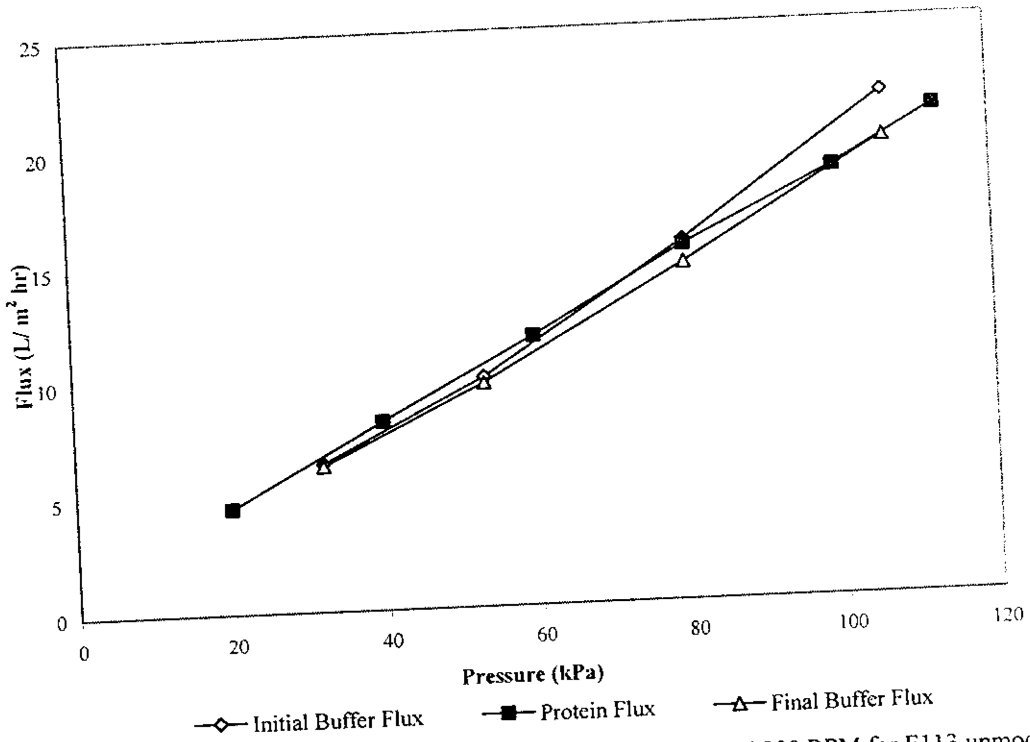


Figure 4.2.1.2 (b) Flux data for Ovalbumin with respect to Stirrer speed 200 RPM for E113 unmodified PAN membrane

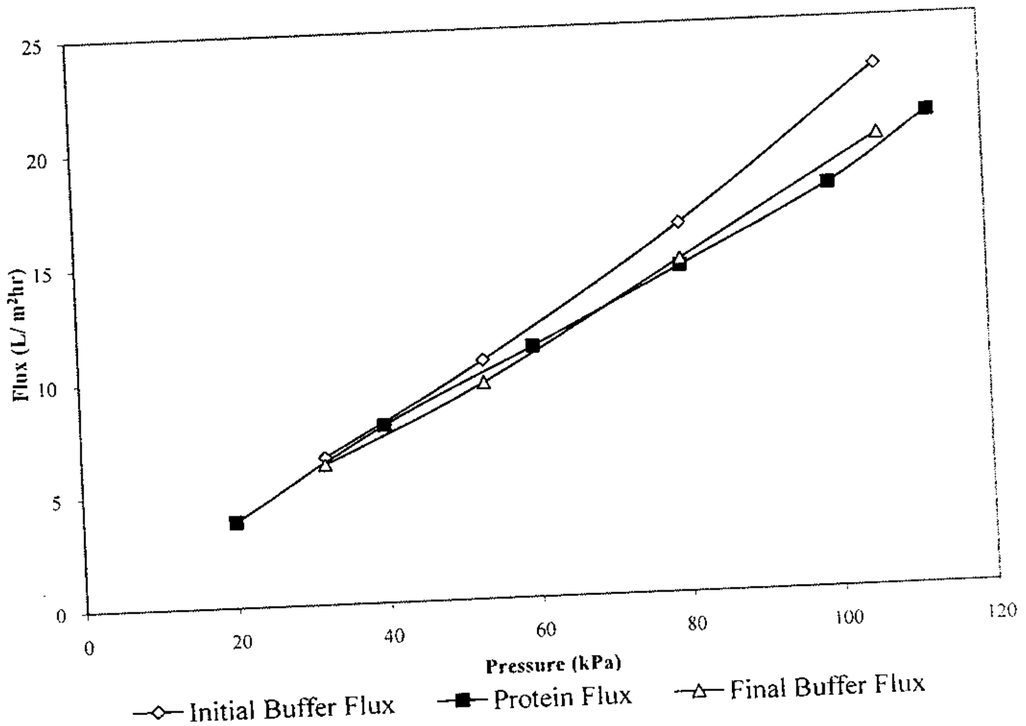


Figure 4.2.1.2(c) Flux data for Ovalbumin with respect to Stirrer speed 250 RPM for E113 unmodified PAN membrane

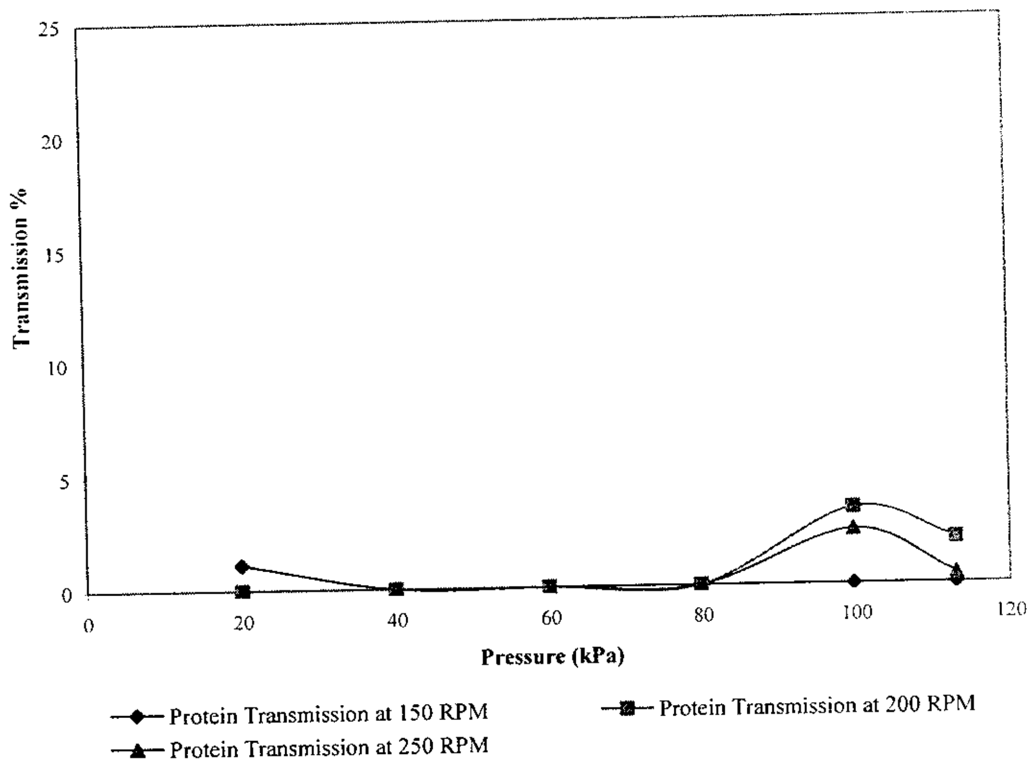


Figure 4.2.1.2 (d) Comparison of Transmission data for Ovalbumin with different Stirrer speed (150-250) RPM for E113 unmodified PAN membrane

4.2.1.3 Myoglobin Analysis (Concentration: 0.3 g /L)

Table 4.2.1.3 E113 Membrane with Myoglobin Analysis

Stirrer Speed (RPM)	Initial Buffer Permeability (x 10^{-8}m/s.kPa)	Final Buffer Permeability (x 10^{-8}m/s.kPa)	Fouling (%)	Myoglobin Permeability (x 10^{-8}m/s.kPa)	Transmission (%)
150	5.21	4.84	7	4.92	27
200	4.96	4.74	4	4.58	28
250	4.96	4.74	8	4.9	26

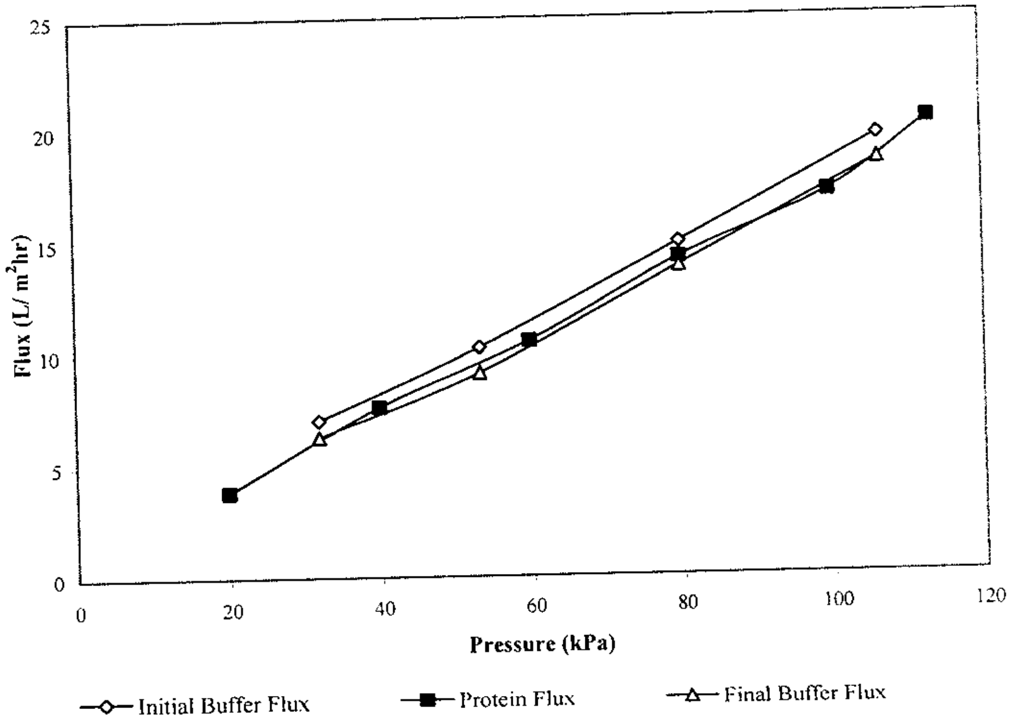


Figure 4.2.1.3(a) Flux data for Myoglobin with respect to Stirrer speed 150 RPM for E113 unmodified PAN membrane

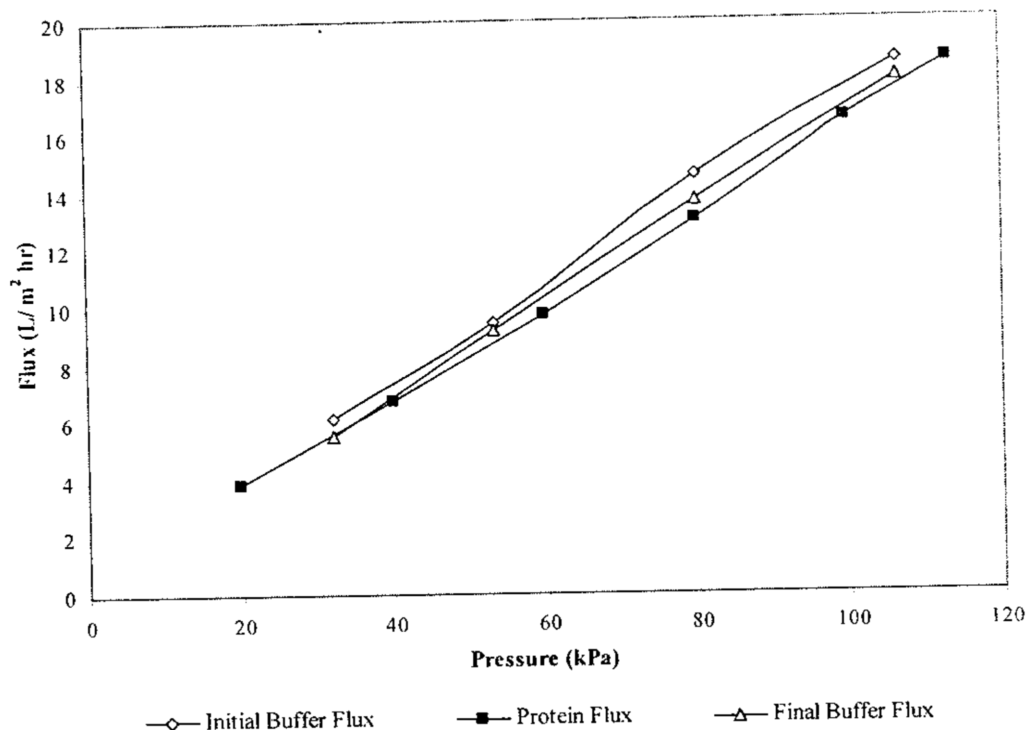


Figure 4.2.1.3(b) Flux data for Myoglobin with respect to Stirrer speed 200 RPM for E113 unmodified PAN membrane

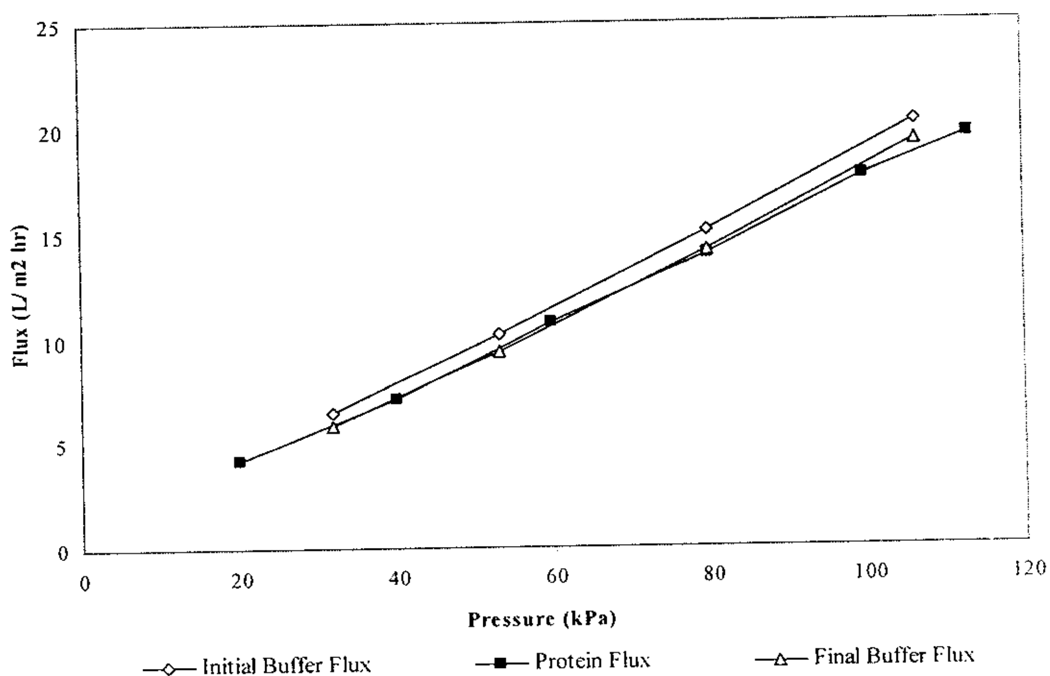


Figure 4.2.1.3(c) Flux data for Myoglobin with respect to Stirrer speed 250 RPM for E113 unmodified PAN membrane

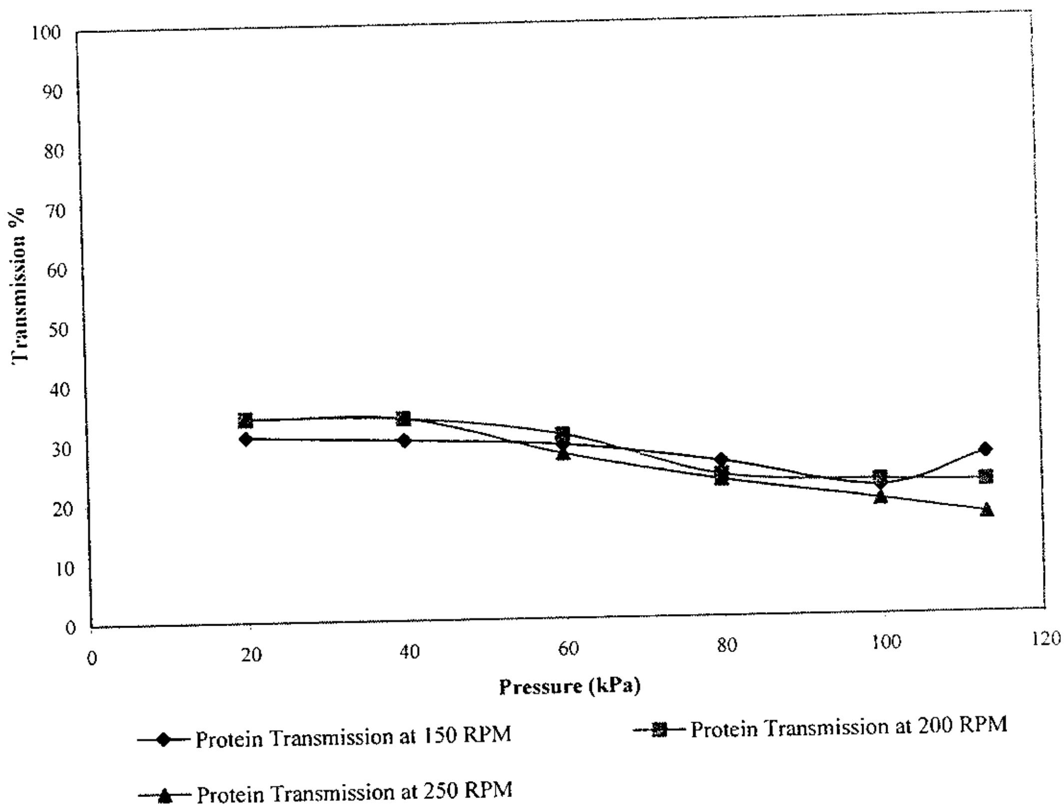


Figure 4.2.1.3(d) Comparison of Transmission data for Myoglobin with different Stirrer speed (150-250) RPM for E113 unmodified PAN membrane

The transmission of ovalbumin and myoglobin for E113 unmodified PAN membrane at 150 to 250 RPM stirred speed were 0.2 to 0.9 % and 26 to 28 % respectively for the pressure range of 20 kPa to 106 kPa. The permeability of the ovalbumin and myoglobin for E113 membrane were 5.0 to 5.3 x 10^{-8} (m/ s kPa) and 4.5 to 4.92 x 10^{-8} (m/ s kPa) at 150 to 200 RPM stirred speed as shown in figure (4.2.1.2 (a) to 4.2.1.2 (c) & 4.2.1.3(a) to 4.2.1.3(c)). The fouling percentage for these set of experiments was very low and ranged between 4 to 11 % for ovalbumin and 4 to 8 % for myoglobin.

4.2.1.4 Lysozyme Analysis (Concentration: 0.3 g/ L)

Table 4.2.1.4 E113 Membrane with Lysozyme Analysis

Stirrer Speed (RPM)	Initial Buffer Permeability (x 10^{-8}m/s.kPa)	Final Buffer Permeability (x 10^{-8}m/s.kPa)	Fouling (%)	Lysozyme Permeability (x 10^{-8}m/s.kPa)	Transmission (%)
150	5.04	4.53	10	4.14	0.0
200	4.56	4.31	5	3.83	0.2
250	4.77	4.5	5	4.02	0.0

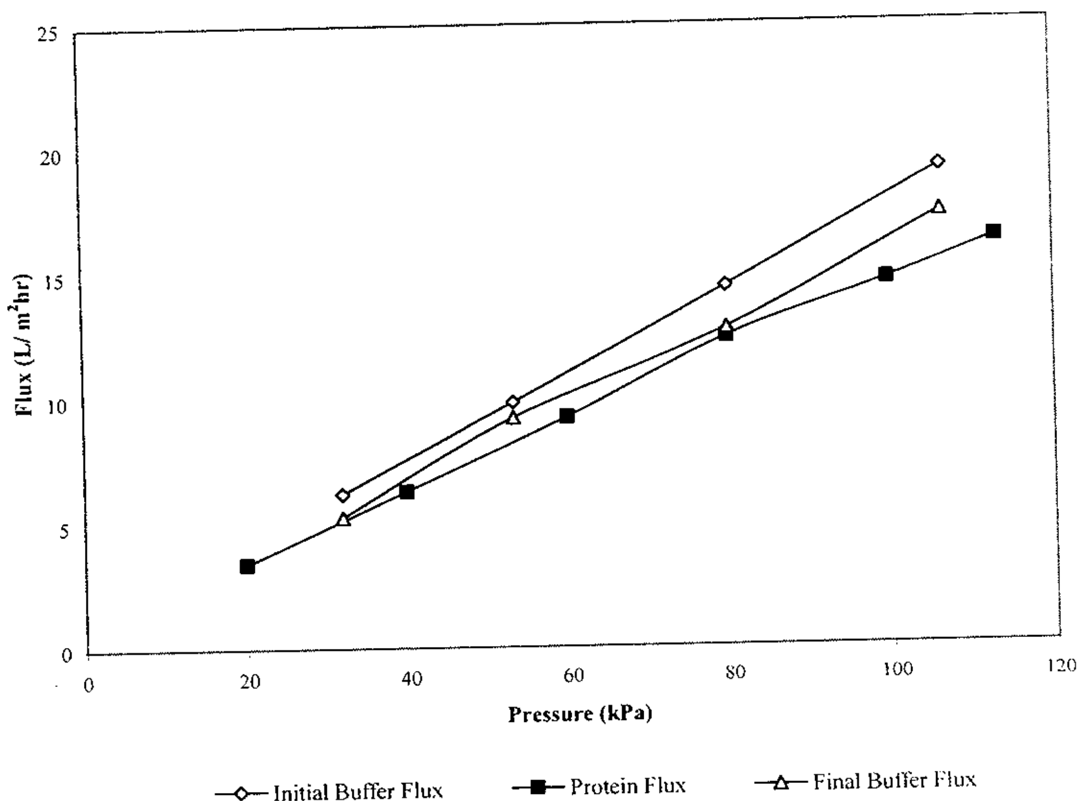


Figure 4.2.1.4(a) Flux data for Lysozyme with respect to Stirrer speed 150 RPM for E113 unmodified PAN membrane

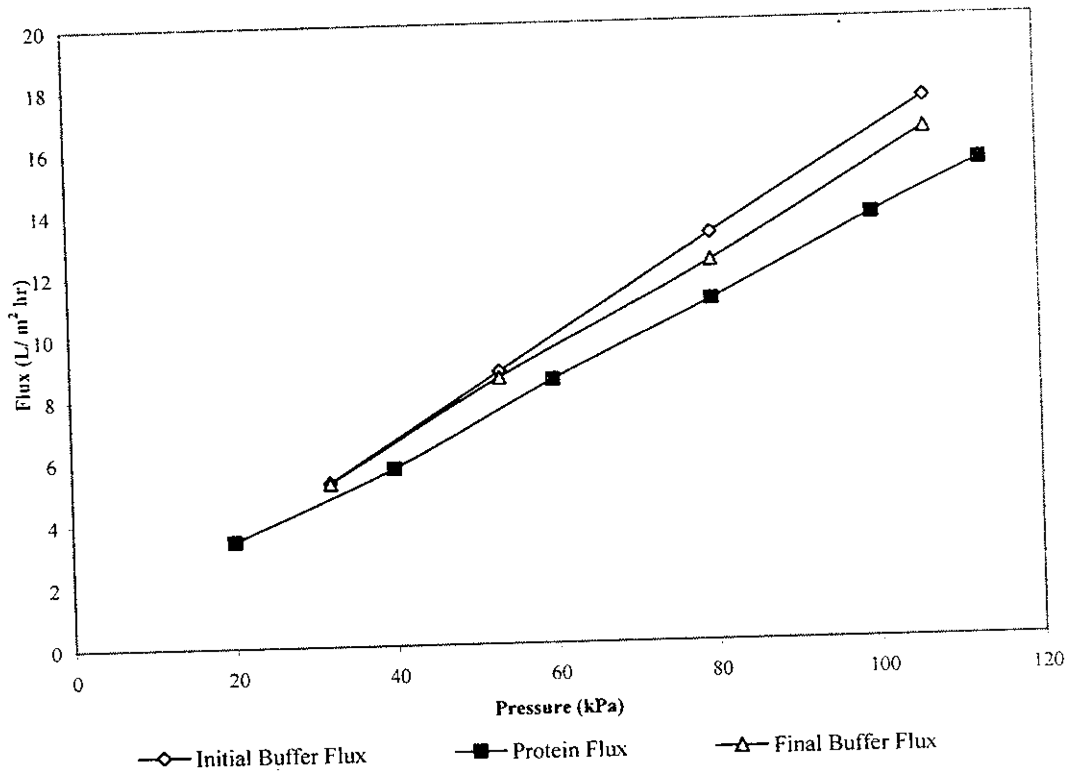


Figure 4.2.1.4 (b) Flux data for Lysozyme with respect to Stirrer speed 200 RPM for E113 unmodified PAN membrane

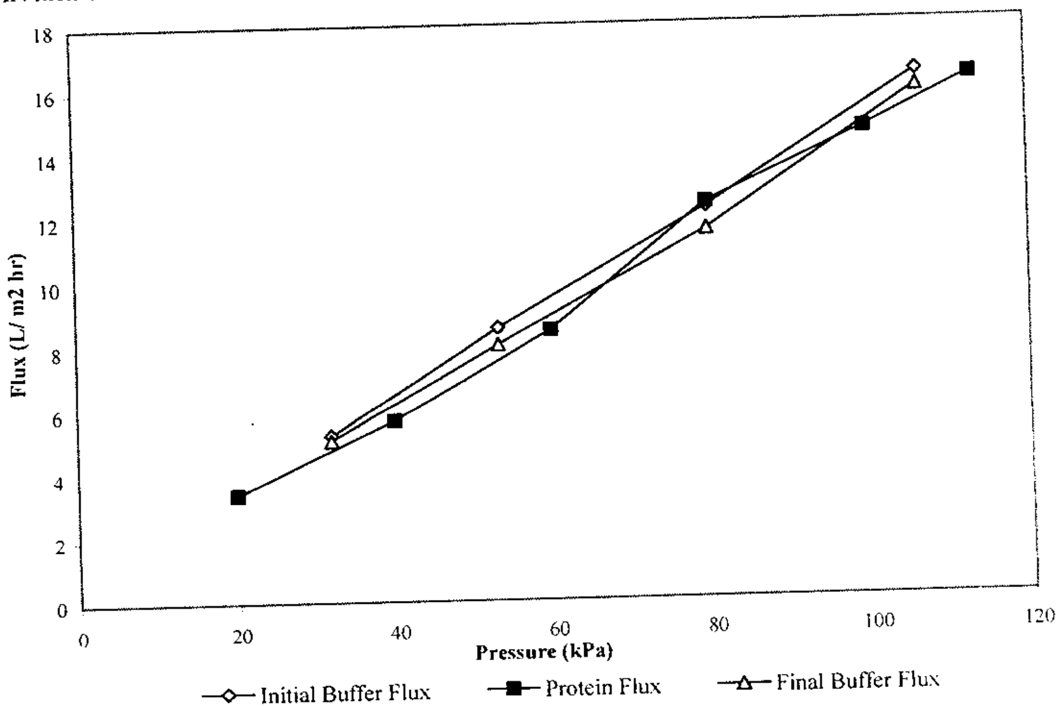


Figure 4.2.1.4(c) Flux data for Lysozyme with respect to Stirrer speed 250 RPM for E113 unmodified PAN membrane

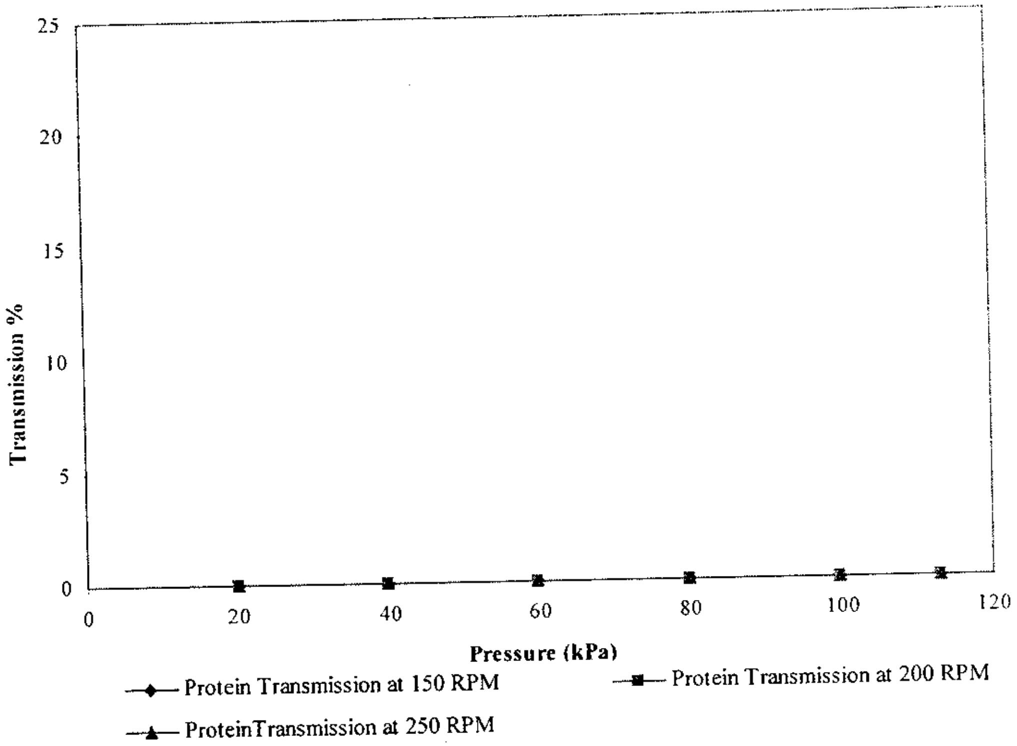


Figure 4.2.1.4(d) Comparison of Transmission data for Lysozyme at pH 7.0 with different Stirrer speed (150-250) RPM for E113 unmodified PAN membrane

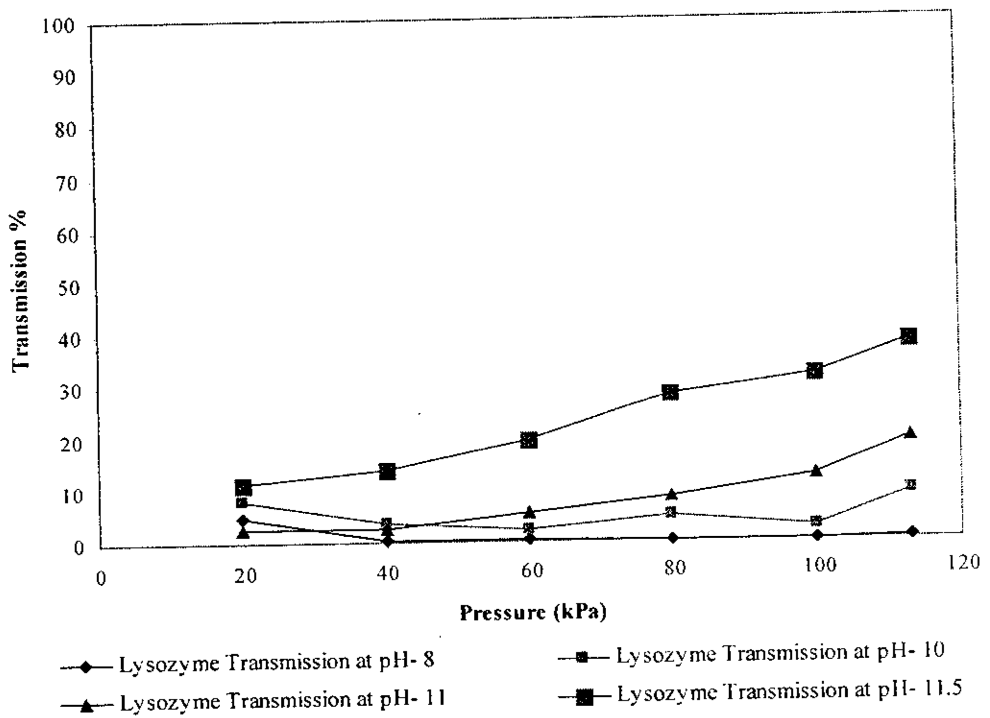


Figure 4.2.1.4(e) Comparison of Transmission data for Lysozyme with different pH (8-11.5) for E113 unmodified PAN membrane

E113 unmodified PAN membrane were also tested for lysozyme model protein which is of low molecular weight protein (13.9 kDa) and the permeability was ranged from 3.8 to 4.1 x 10^{-8} (m/s. kPa). Figure 4.2.1.4(d) showed that for E113 unmodified membrane, the transmission of lysozyme was negligible and the fouling % was observed from 5 to 10 % with respect to different stir speed. At pH 7 for E113 membrane myoglobin transmission was higher than lysozyme, while the molecular weight of lysozyme is less than myoglobin. To test this behavior of membrane, the lysozyme with different pH solution were passed through the membrane. The figure 4.2.1.4(e) showed that the transmission of lysozyme was maximum i.e. 23% for the pH 11.5, which is nearer to its Isoelectric Point (pI) value. For the pH values of 11 and 10, lysozyme showed the transmission around 15 and 8, which approached almost zero at pH 8.the reason of higher transmission at pH 11.5, was due to its pI value.

4.2.2 PES - 30 Membrane

4.2.2.1 BSA Analysis (Concentration: 0.3 g /L)

Table 4.2.2.1 PES - 30 membrane with BSA Analysis

Stirrer Speed (RPM)	Initial Buffer Permeability (x 10^{-7}m/s.kPa)	Final Buffer Permeability (x 10^{-7}m/s.kPa)	Fouling (%)	BSA Permeability (x 10^{-7}m/s.kPa)	Transmission (%)
150	6.57	3.24	51	3.17	0.0
200	6.78	3.4 1	50	3.33	0.0

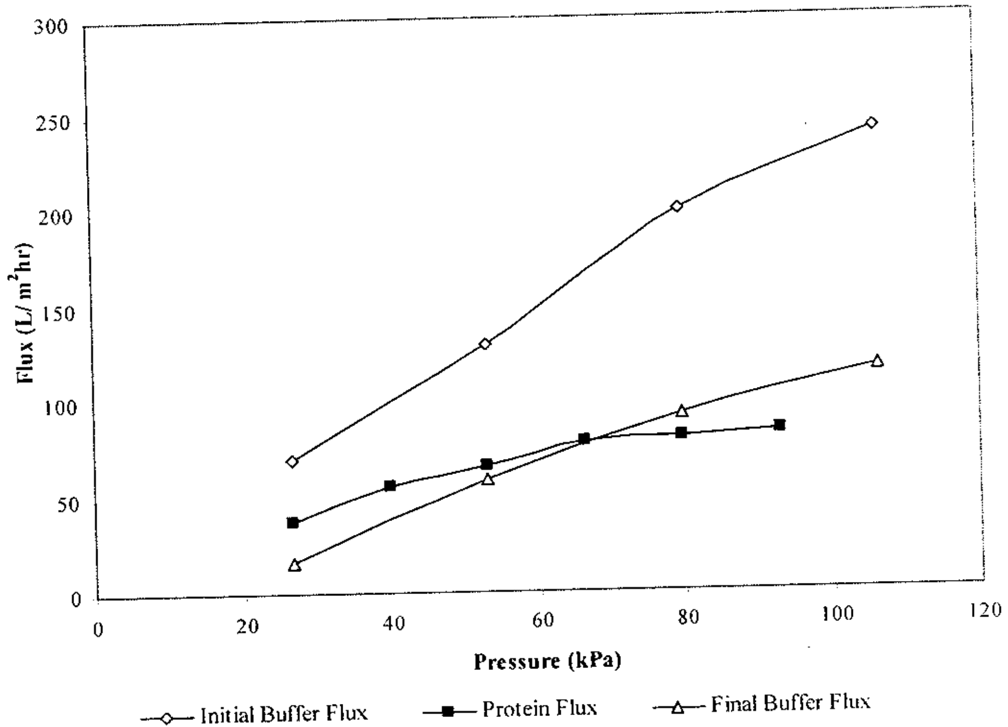


Figure 4.2.2.1(a) Flux data for BSA with respect to Stirrer speed 150 RPM for PES-30 membrane

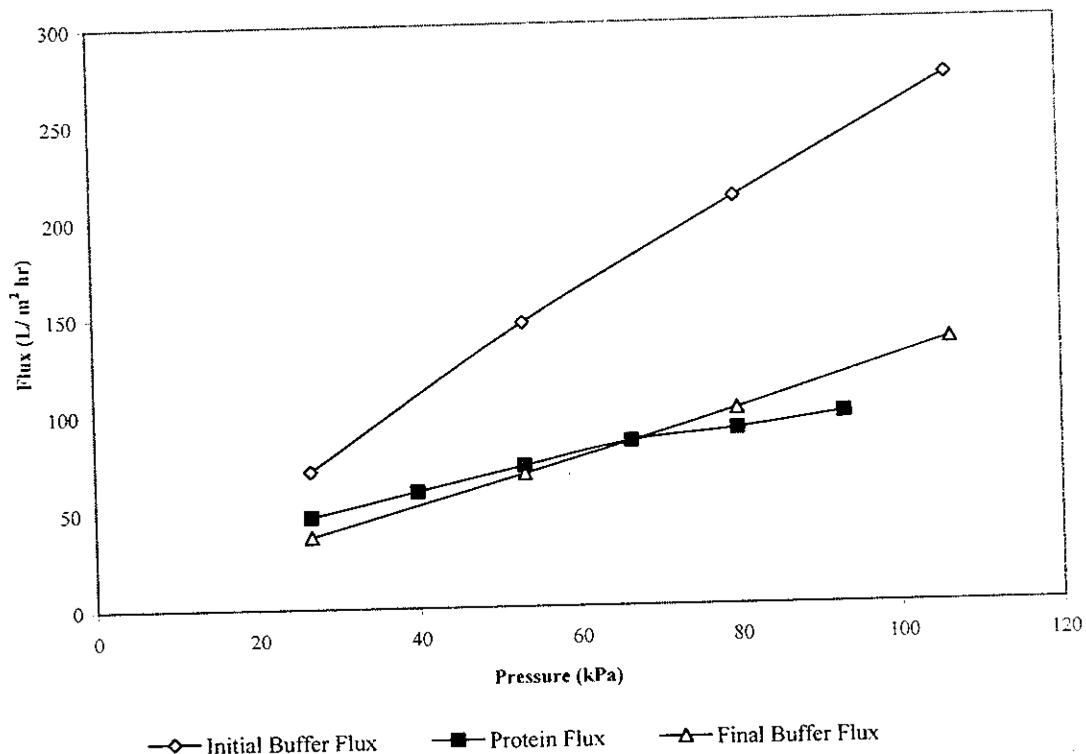


Figure 4.2.2.1(b) Flux data for BSA with respect to Stirrer speed 200 RPM for PES-30 membrane

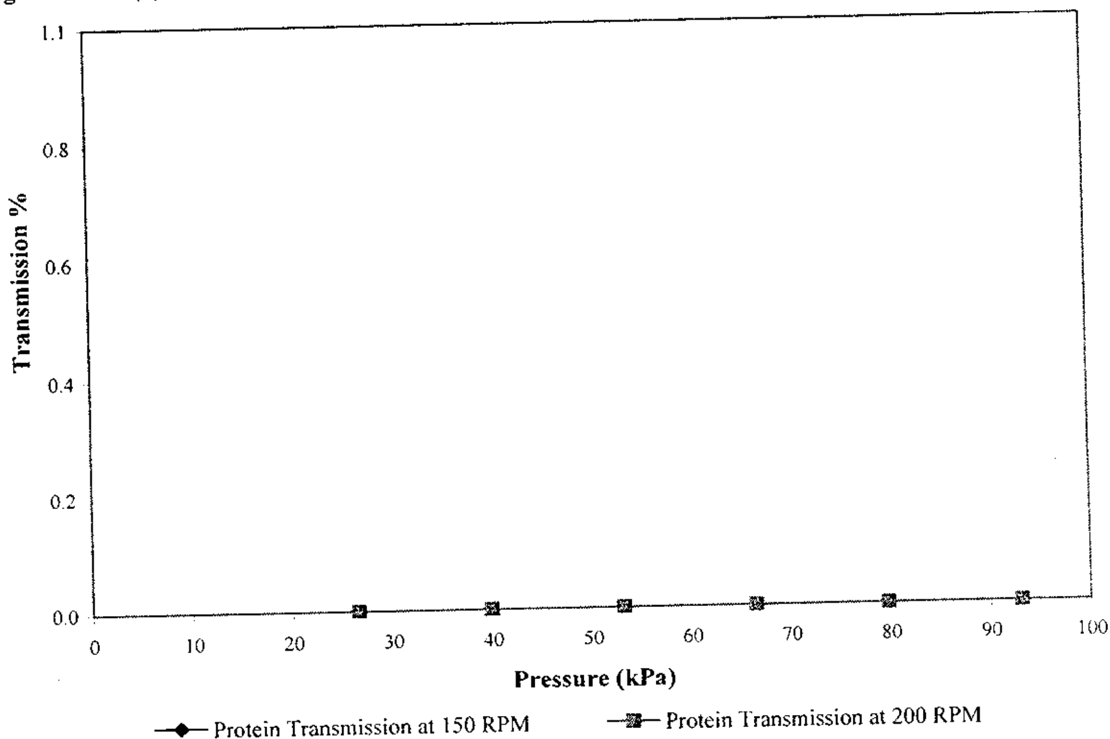


Figure 4.2.2.1(C) Comparison of Transmission data for BSA with different Stirrer speed (150-200) RPM for PES-30 membrane

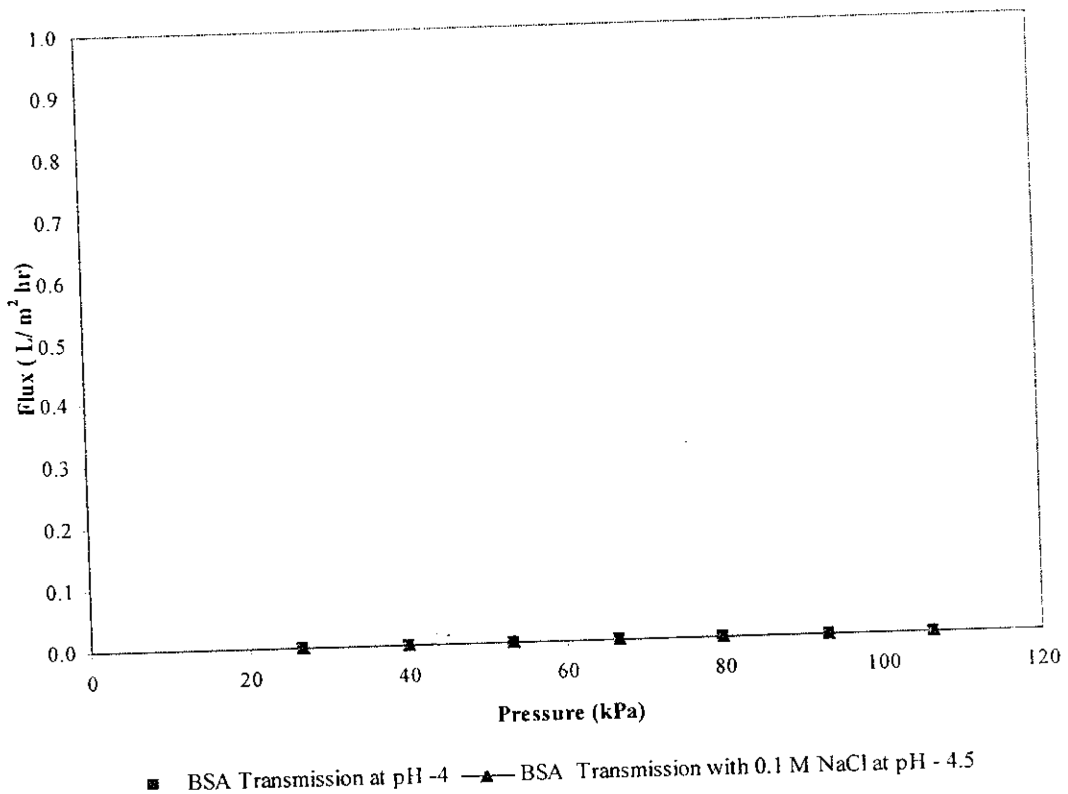


Figure 4.2.2.1(d) Transmission data for BSA with respect to different pH for PES-30 membrane

PES 30 membrane supplied by Millipore was also characterized using model proteins. All the parameters like (pH, Pressure, temperature and rpm) kept same as worked with E113 unmodified PAN membrane. The permeability of PES-30 membrane (MWCO-30kDa) for BSA was 3.25 ± 0.8 x 10^{-7} m/s kPa (Figure 4.2.2.1(a) to 4.2.2.1 (c)). The model protein BSA (69 kDa) showed 100 % rejection for PES 30 membrane at pH 7 and fouling was ranged from 50 to 51 %. Nakao *et al.*, (2008) studied the ultrafiltration of BSA at the isoelectric point and another pH by the use of charged membrane. At the isoelectric point, the rejection of the protein was low, while it was high at the pH level, which gave the protein the same sign of charge as that of the membrane due to charge repulsions

4.2.2.2 Ovalbumin Analysis (Concentration: 0.3 g/L)

Table 4.2.2.2 PES - 30 Membrane with Ovalbumin Analysis

Stirrer Speed (RPM)	Initial Buffer Permeability (x 10^{-7}m/s.kPa)	Final Buffer Permeability (x 10^{-7}m/s.kPa)	Fouling (%)	Ovalbumin Permeability (x 10^{-7}m/s.kPa)	Transmission (%)
200	5.63	3.24	42	3.15	24
250	5.12	3.43	33	3.45	32
200+ (0.1MNaCl)	4.72	3.03	35	3.88	43

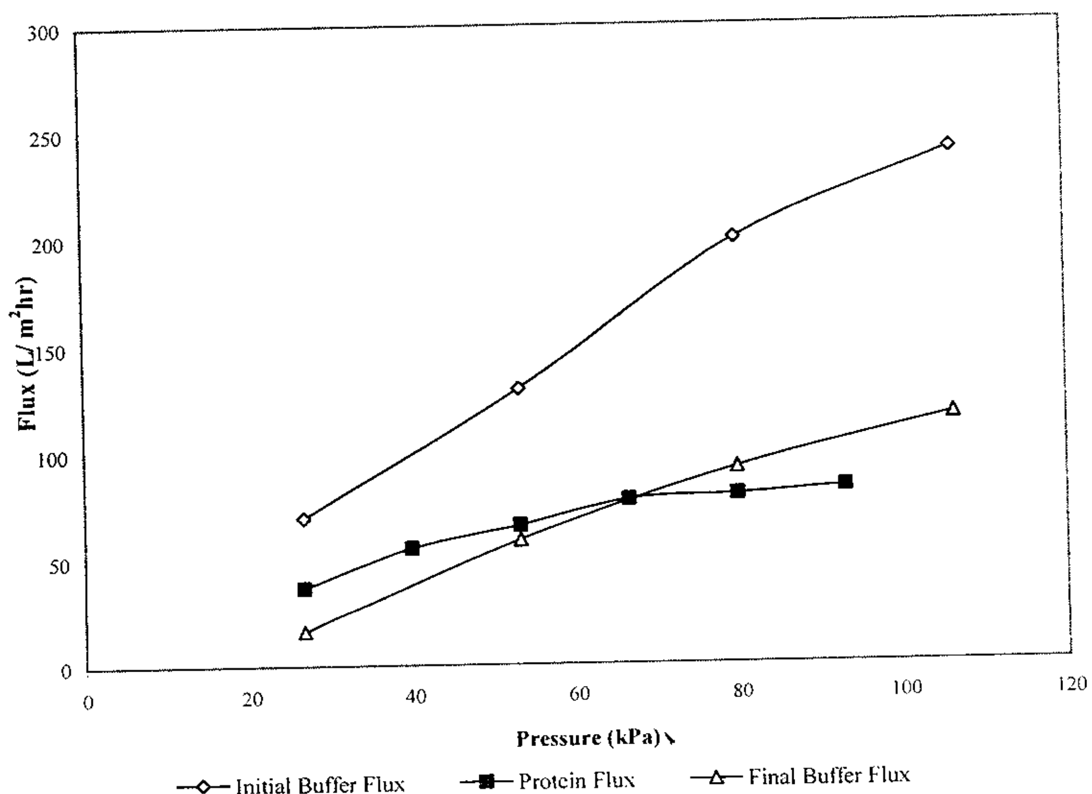


Figure 4.2.2.2(a) Flux data for Ovalbumin with respect to Stirrer speed 200 RPM for PES-30 membrane

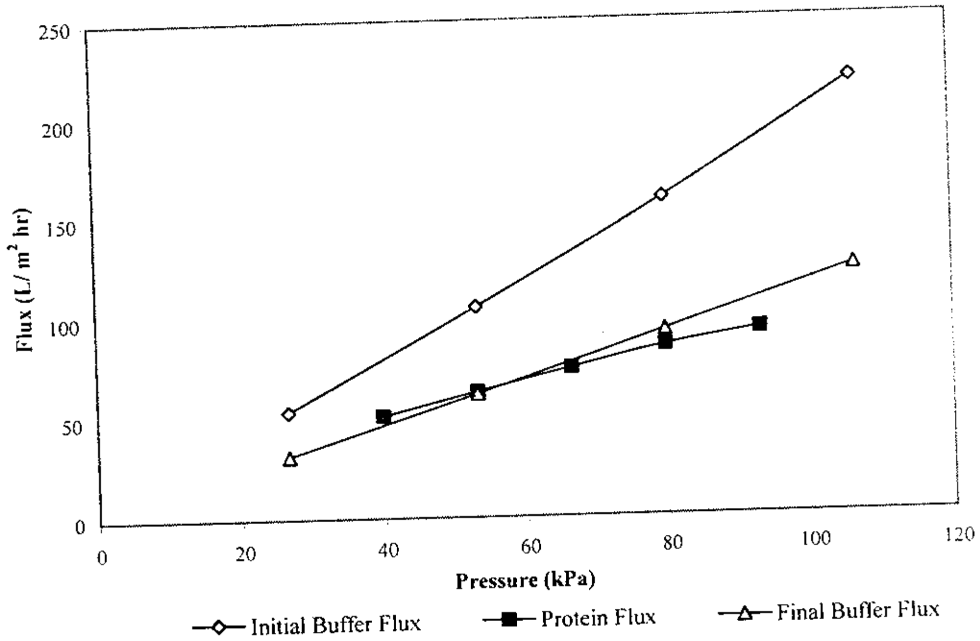


Figure 4.2.2.2(b) Flux data for Ovalbumin with respect to Stirrer speed 250 RPM for PES-30 membrane

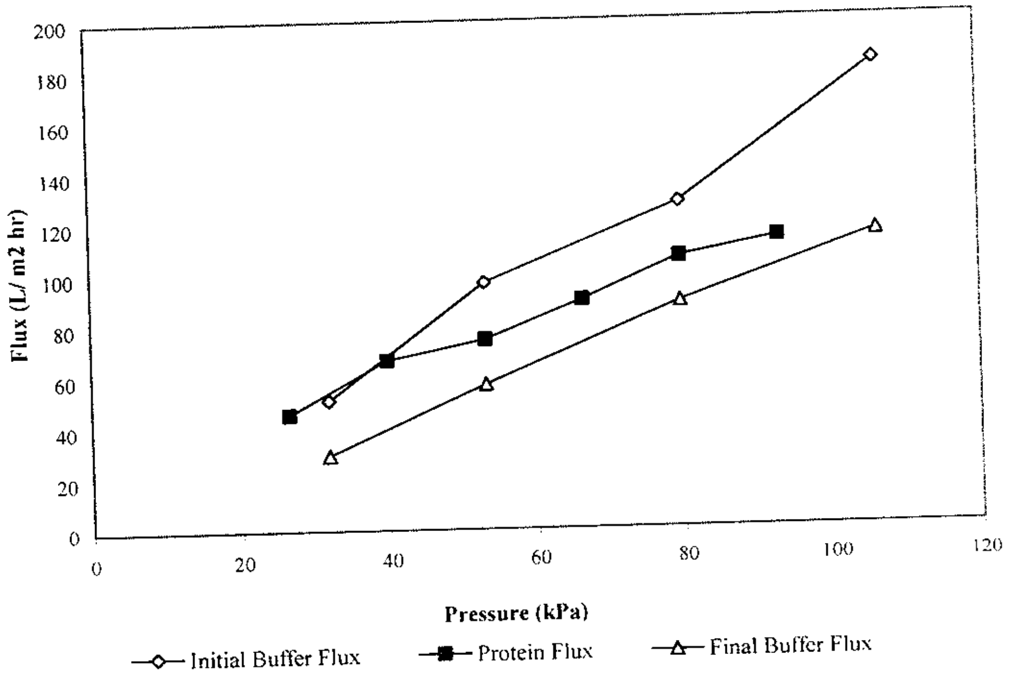


Figure 4.2.2.2(c) Flux data for Ovalbumin with NaCl respect to Stirrer speed 200 RPM for PES-30 membrane

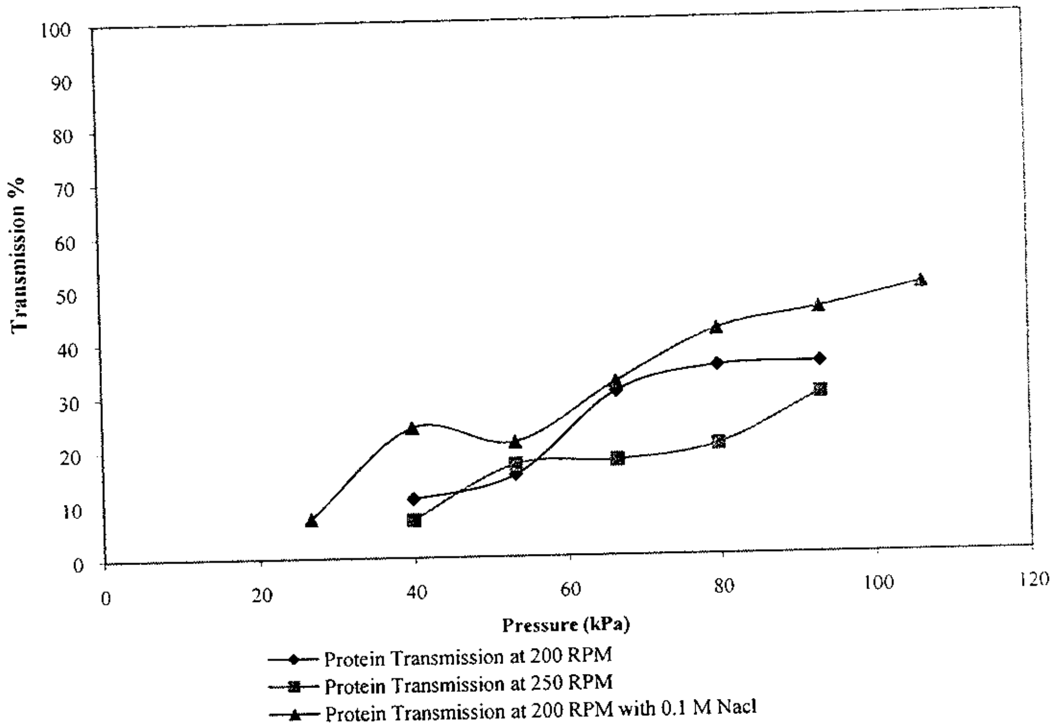


Figure 4.2.2.2(d) Comparison of Transmission data for Ovalbumin with different Stirrer speed (200-250) RPM for PES-30 membrane

Zydney and Burns, (2008) demonstrated the importance of solution pH in determining protein sieving through semipermeable ultrafiltration membranes. The protein-sieving coefficient attained its maximum value near the protein isoelectric point and decreased at pH both above and below the pI.

4.2.2.3 Myoglobin Analysis (Concentration: 0.3 g/L)

Table 4.2.2.3 PES - 30 Membrane with Myoglobin Analysis

Stirrer Speed (RPM)	Initial Buffer Permeability (x 10^{-7}m/s.kPa)	Final Buffer Permeability (x 10^{-7}m/s.kPa)	Fouling (%)	Myoglobin Permeability (x 10^{-7}m/s.kPa)	Transmission (%)
200	5.67	2.118	63	2.39	51
250	6.35	2.464	61	2.81	51
200 + (0.1 M NaCl)	5.56	2.65	52	3.15	54

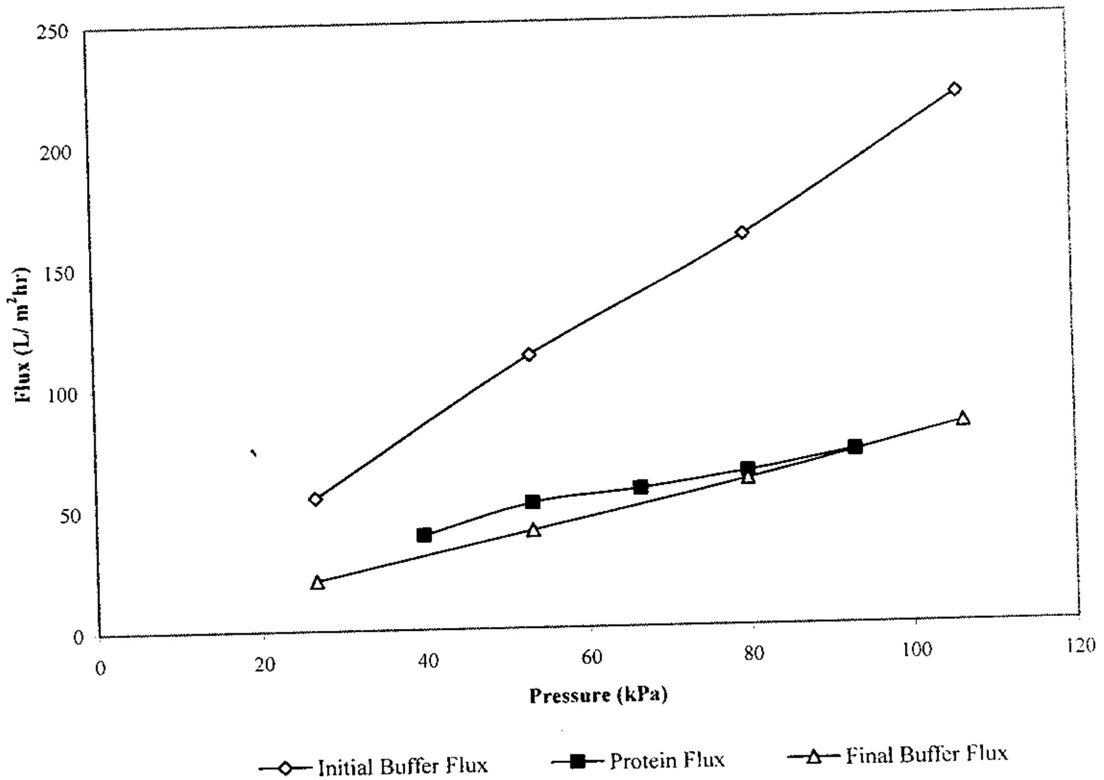


Figure 4.2.2.3(a) Flux data for Myoglobin with respect to Stirrer speed 200 RPM for PES-30 membrane

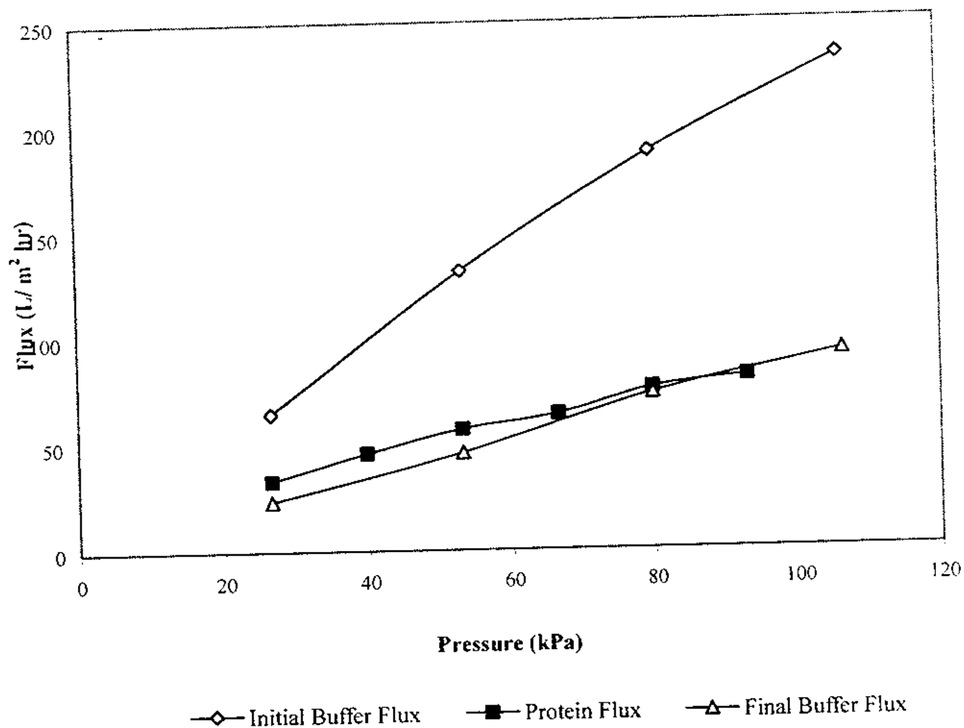


Figure 4.2.2.3(b) Flux data for Myoglobin with respect to Stirrer speed 250 RPM for PES-30 membrane

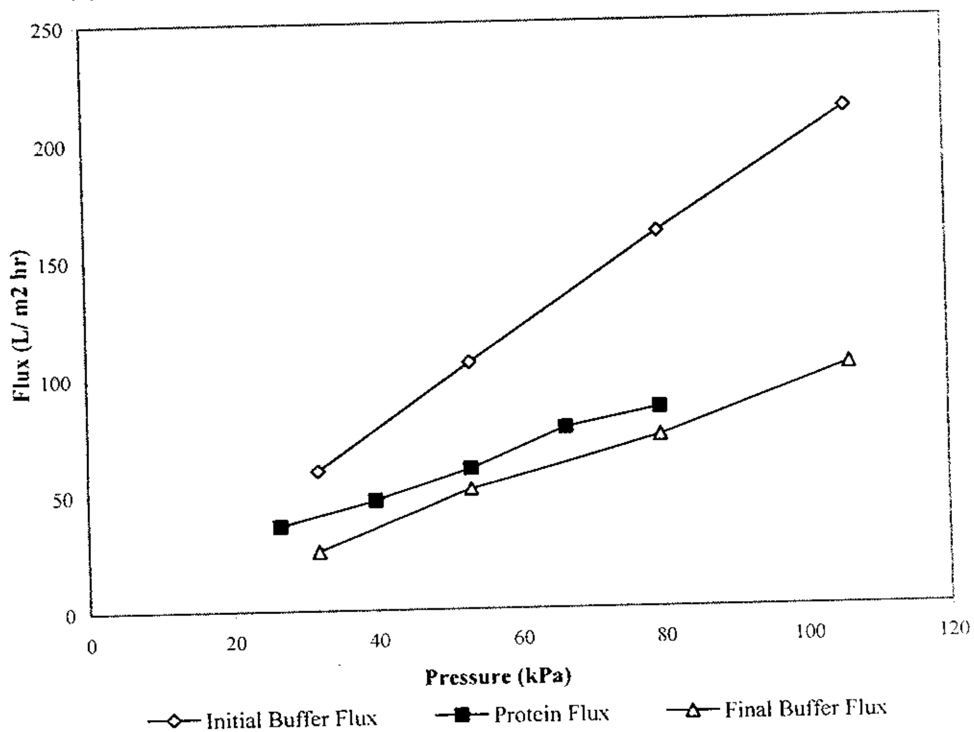


Figure 4.2.2.3(c) Flux data for Myoglobin with NaCl respect to Stirrer speed 200 RPM for PES-30 membrane

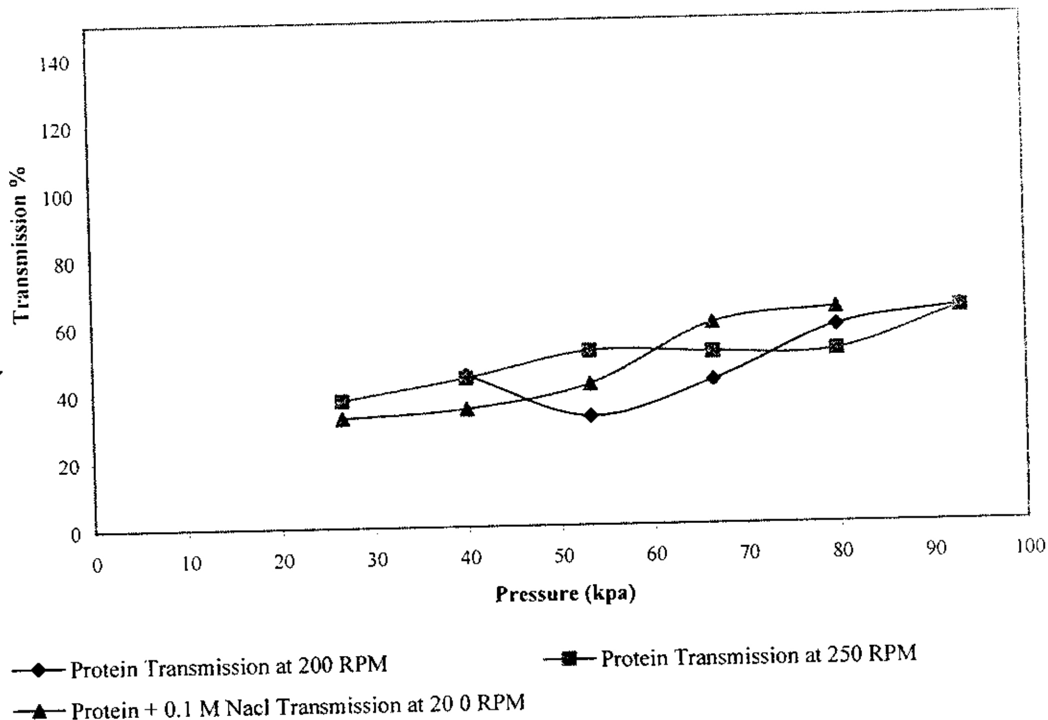


Figure 4.2.2.3(d) Comparison of Transmission data for Myoglobin with different Stirrer speed (200-250) RPM for PES-30 membrane

In PES-30 membrane, the transmission analysis of ovalbumin and myoglobin with stirrer speed of 200 and 250 RPM at pH 7 revealed that better transmission were observed at 200 rpm with the addition of 0.1 M NaCl. The percentage of ovalbumin (43.5 kDA) and myoglobin (17 kDa) transmission was 24 to 43 % and 51 to 54% respectively. The permeability range for ovalbumin and myoglobin were 3.0 to 3.4 x 10^{-7} m/s kPa and 2.3 to 3.15 x 10^{-7} m/s kPa (figure 4.2.2.2 (a) to 4.2.2.2 (c) and 4.2.2.3 (a) to 4.2.2.3 (c)). With addition of 0.1 M NaCl the transmission and protein permeability, for both ovalbumin and myoglobin were increased.

4.2.2.4 Lysozyme Analysis (Concentration : 0.3 g/L)

Table 4.2.2.4 PES - 30 membrane with Lysozyme Analysis

Stirrer Speed (RPM)	Initial Buffer Permeability (x 10^{-7}m/s.kPa)	Final Buffer Permeability (x 10^{-7}m/s.kPa)	Fouling (%)	Lysozyme Permeability (x 10^{-7}m/s.kPa)	Transmission (%)
150	6.14	4.56	26	4.38	92
200	6.16	4.66	23	4.44	94
250	6.21	4.45	28	4.39	93

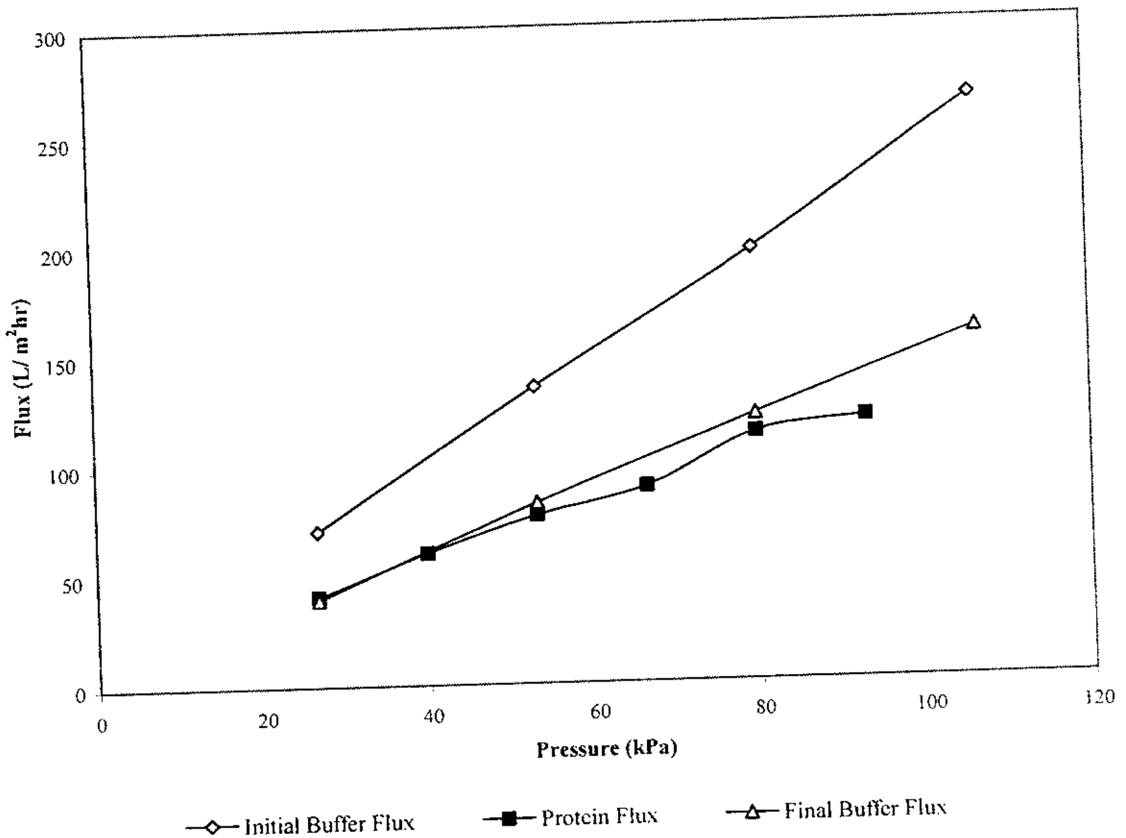


Figure 4.2.2.4(a) Flux data for Lysozyme with respect to Stirrer speed 150 RPM for PES-30 membrane

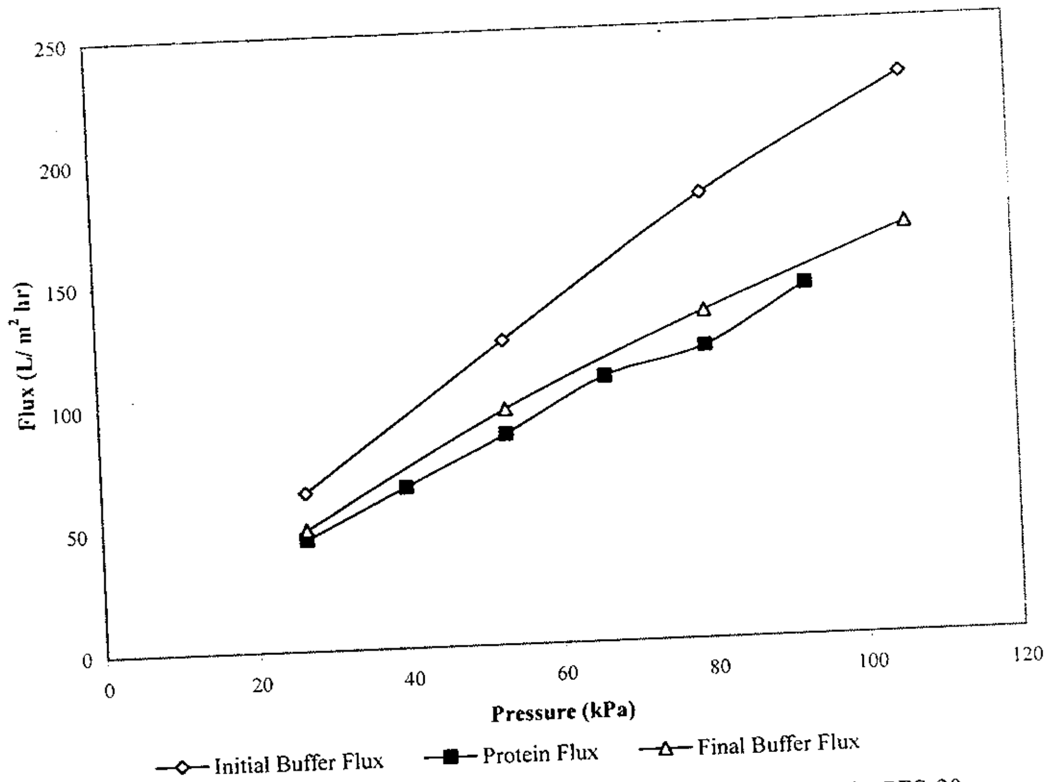


Figure 4.2.2.4(b) Flux data for Lysozyme with respect to Stirrer speed 200 RPM for PES-30 membrane

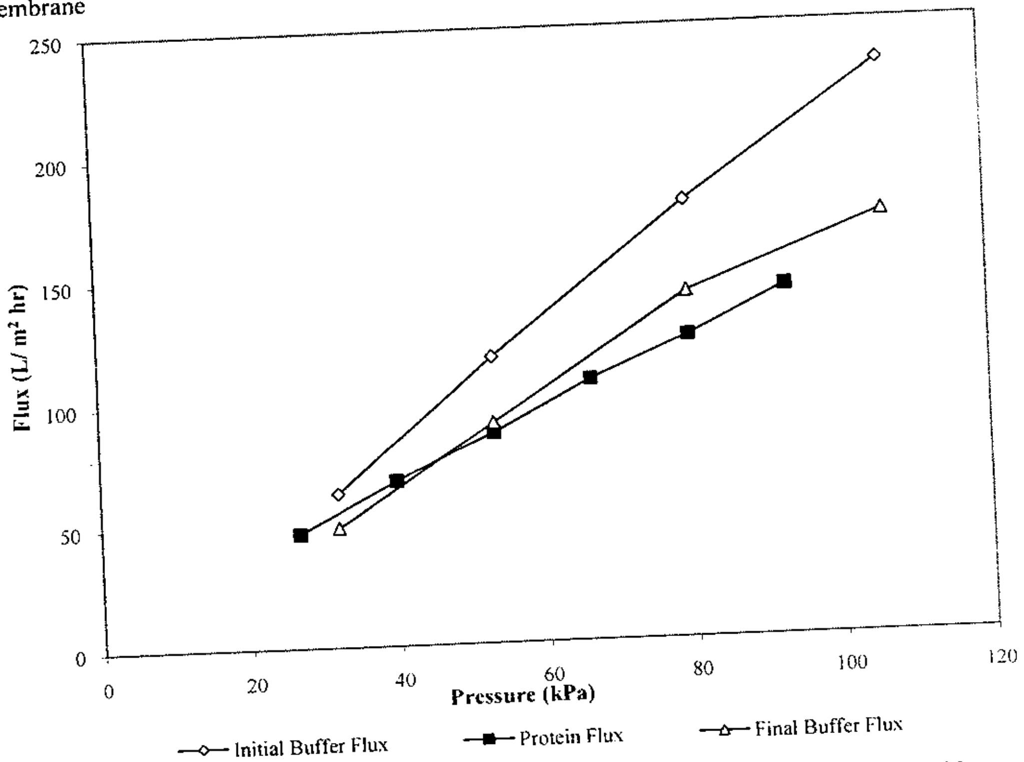


Figure 4.2.2.4(c) Flux data for Lysozyme with respect to Stirrer speed 250 RPM for PES-30 membrane

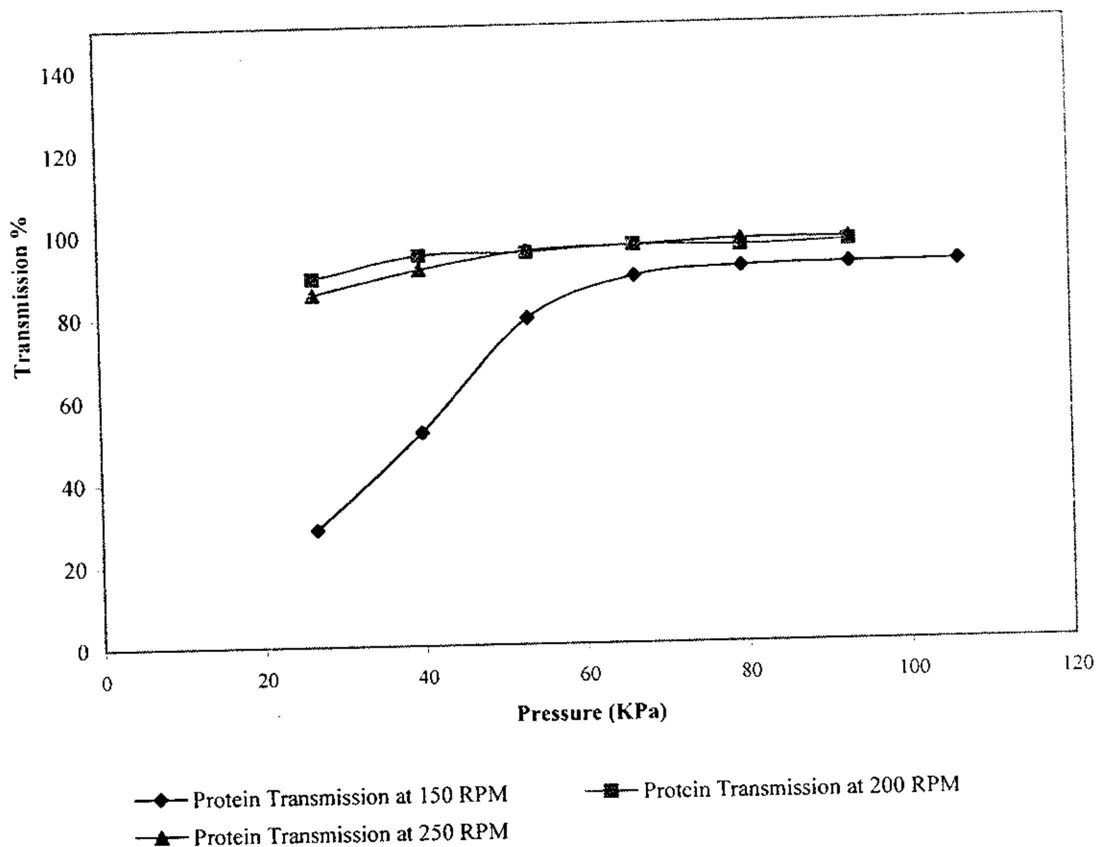


Figure 4.2.2.4(d) Comparison of Transmission data for Lysozyme with different Stirrer speed (150-250) RPM for PES-30 membrane

Transmission analysis of low molecular weight protein lysozyme (13.9 kDa) was also checked and ranged from 92 % to 94 %. Fouling percentage for lysozyme was lesser than other protein with PES-30 Membrane. The permeability of lysozyme for this high flux membrane was ranged from 4.38 to 4.44 x 10^{-7} m/s kPa (Figure 4.2.2.4(a) to 4.2.2.4(c)).

4.3 Effect of pressure:

The effect of pressure was tested for E 113 unmodified and PES 30 membranes. The transmission percentage of different model proteins and membrane fouling were measured at different operating pressure ranged from 26 kPa to 106 kPa. Different stirred speeds were also used to check effect of stirring on transmission and membrane fouling.

4.3.1 E113 unmodified PAN mebrane

4.3.1.1 Transmission and fouling analysis of Myoglobin at 100 RPM

Table 4.3.1.1 Analysis of Myoglobin for E113 unmodified PANmembrane for different pressure at 100 RPM

Pressure kPa	Initial Buffer Permeability (x 10^{-8} m/s.kPa)	Final Buffer Permeability (x 10^{-8} m/s.kPa)	Fouling (%)	Transmission (%)
26	4.63	4.23	9	20
106	5.14	3.93	23	12

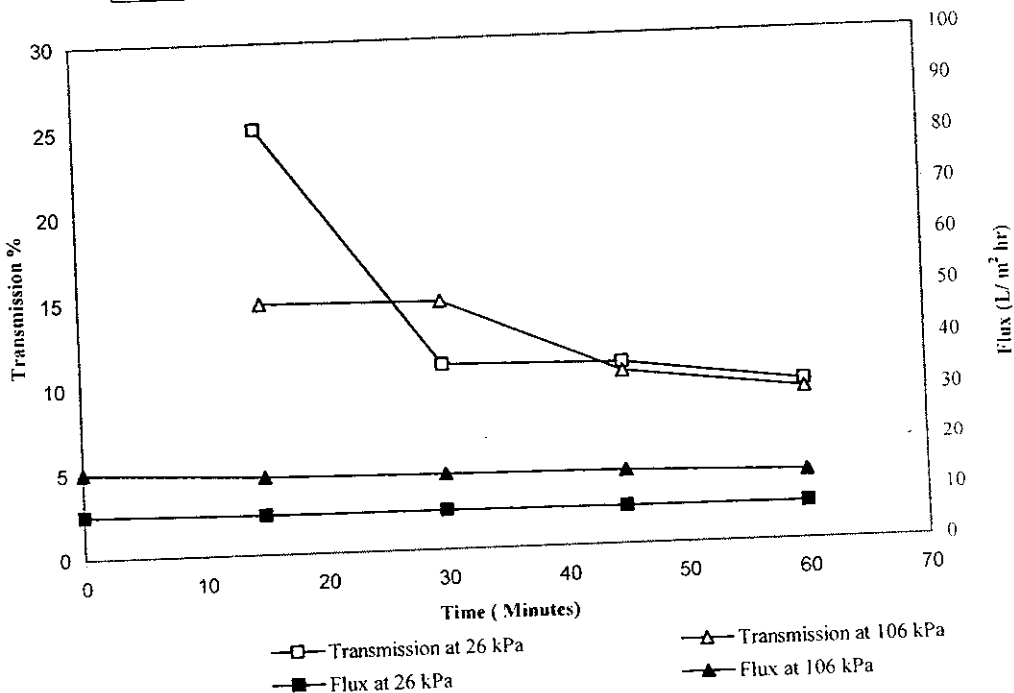


Figure 4.3.1.1(a) Transmission and Flux analysis of Myoglobin at 100 RPM with respect to time for E113 unmodified PANmembrane

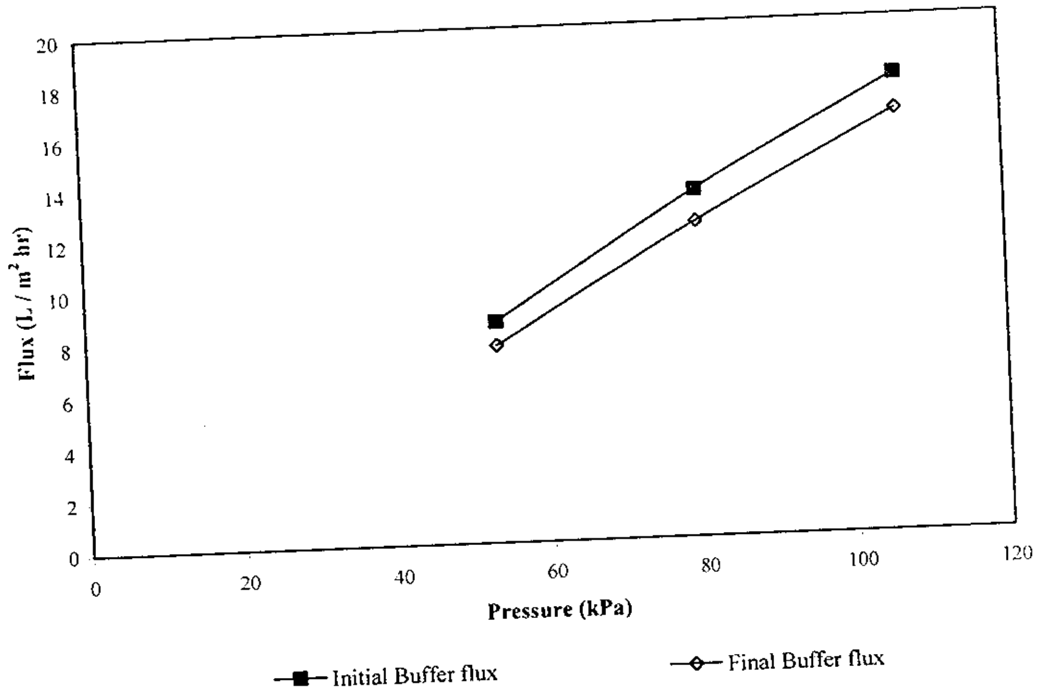


Figure 4.3.1.1(b) Fouling analysis of Myoglobin at 100 RPM and 26 kPa for E113 unmodified PAN membrane

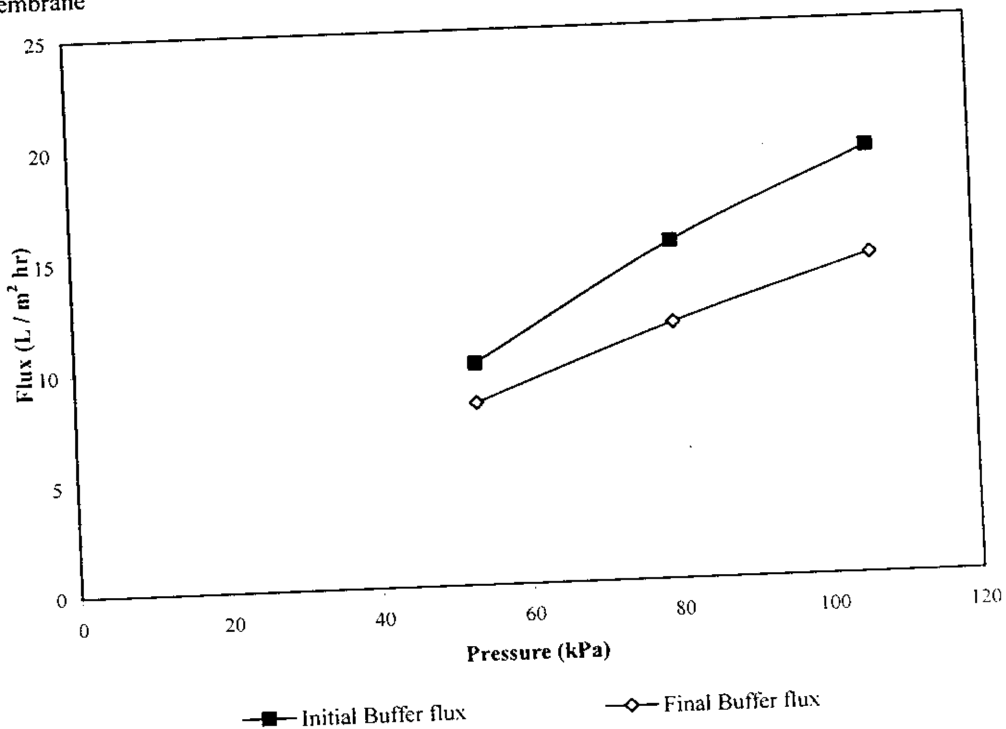


Figure4.3.1.1(c) Fouling analysis of Myoglobin at 100 RPM and 106 kPa for E113 unmodified PAN membrane

In these set of experiments the effect of pressure on protein transmission and fouling were tested. For E113 unmodified PAN membrane at 100 RPM, the transmission percentage of myoglobin were better in low pressure of 26 kPa (20 %) than 106 kpa (12 %) (Figure (4.3.1.1 (a)). Flux analysis with respect to time revealed that for both 26kPa and 106 kPa pressures had lesser deviation in their range.

Similarly effect of pressure on protein transmission and fouling were also tested for myglobin at 400 rpm using E 113 unmodified membrane. The figure (4.3.1.2 (b) and 4.3.1.2 (c). showed that the percentage of fouling at pressure 106 kPa (24 %) was twice than 26 kPa (12 %). Better transmissions were observed for dead end stirred cell, (Amicon 8200) in the time range of 0 to 30 minutes for E113 unmodified PAN membrane. At 400 RPM, lesser transmission was observed compared to myoglobin transmission at 100 RPM.

4.3.1.2 Transmission and fouling analysis of Myoglobin at 400 RPM

Table 4.3.1.2 Analysis of Myoglobin for E113unmodified PAN membrane for different pressure at 400 RPM

Pressure kPa	Initial Buffer Permeability (x 10^{-8} m/s.kPa)	Final Buffer Permeability (x 10^{-8} m/s.kPa)	Fouling (%)	Transmission (%)
26	4.67	4.09	12	14
106	5.24	3.99	24	11

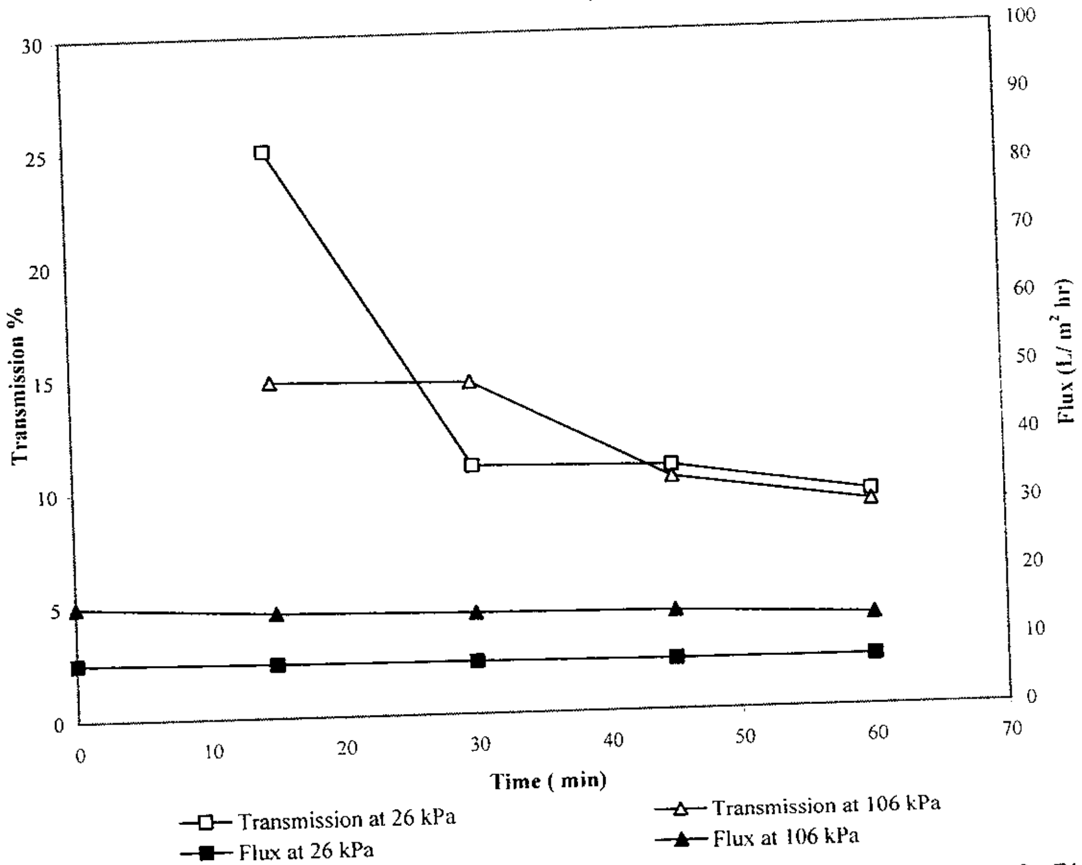


Figure 4.3.1.2(a) Transmission and Flux analysis of Myoglobin at 400 RPM with respect to time for E113 unmodified PANmembrane

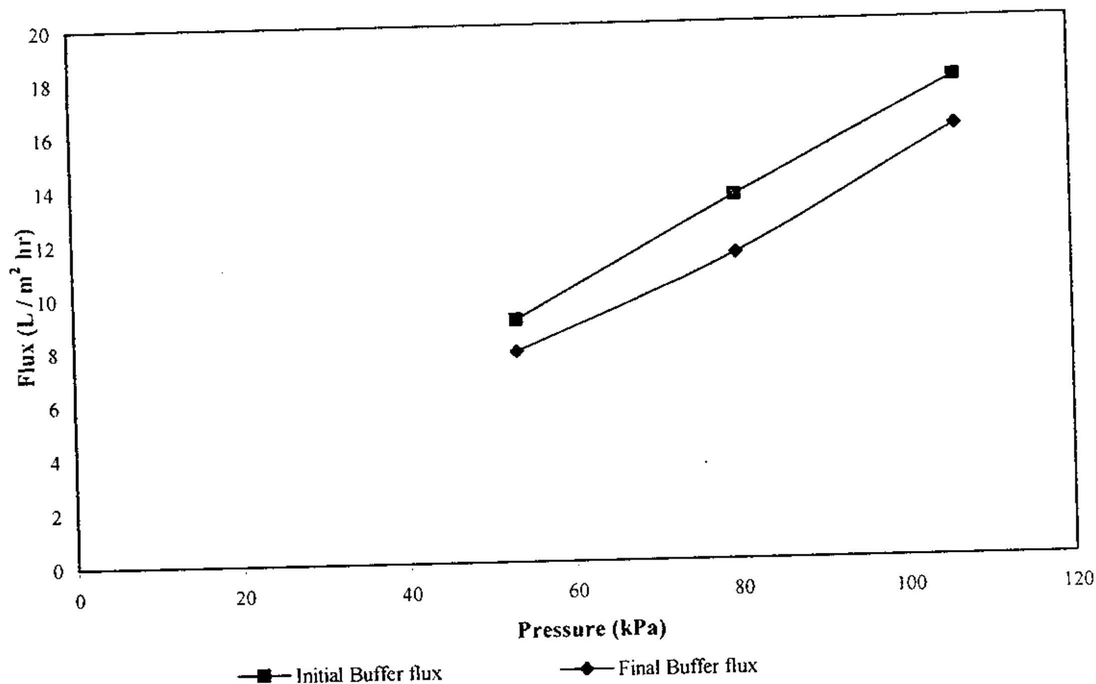


Figure 4.3.1.2(b) Fouling analysis of Myoglobin at 400 RPM and 26 kPa for E113 unmodified PAN membrane

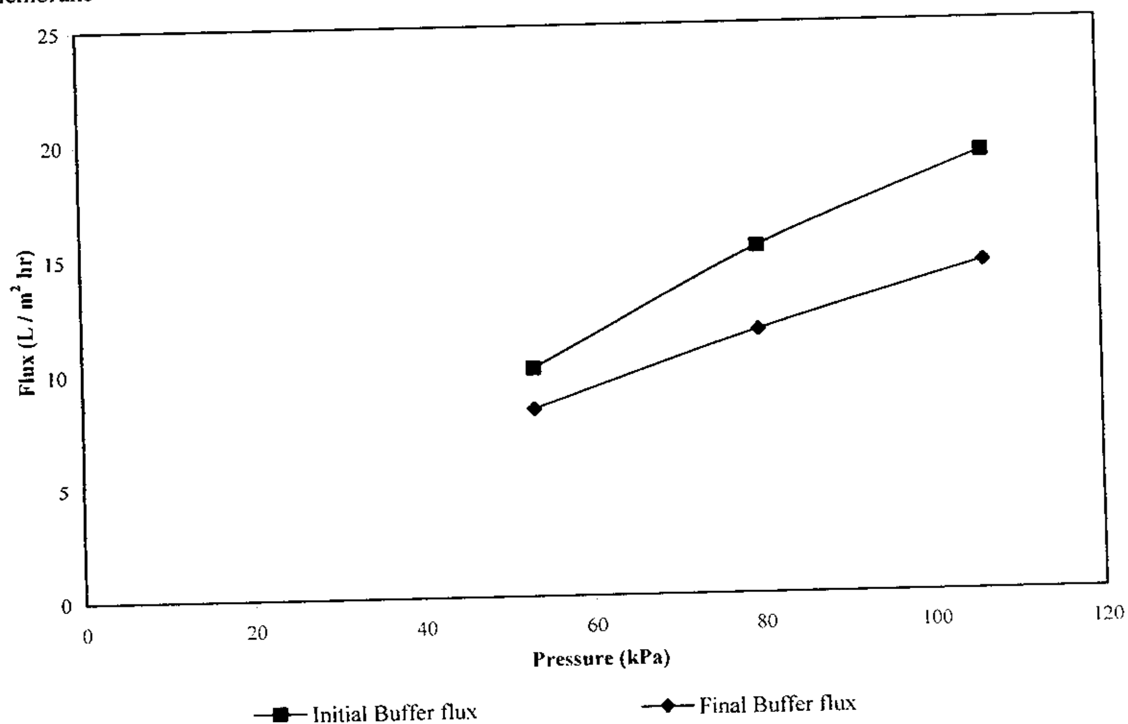


Figure4.3.1.2(c) Fouling analysis of Myoglobin at 400 RPM and 106 kPa for E113 unmodified PAN membrane

4.3.1.3 Transmission and fouling analysis of Lysozyme at 200 RPM

Table 4.3.1.3 Analysis of Lysozyme for E113 Unmodified PAN membrane for different pressure at 200 RPM

Pressure (kPa)	Initial Buffer Permeability (x 10^{-8} m/s.kPa)	Final Buffer Permeability (x 10^{-8} m/s.kPa)	Fouling (%)	Transmission (%)
40	2.90	2.71	7	10
73.5	3.16	2.86	9	7
106	3.93	3.17	19	1

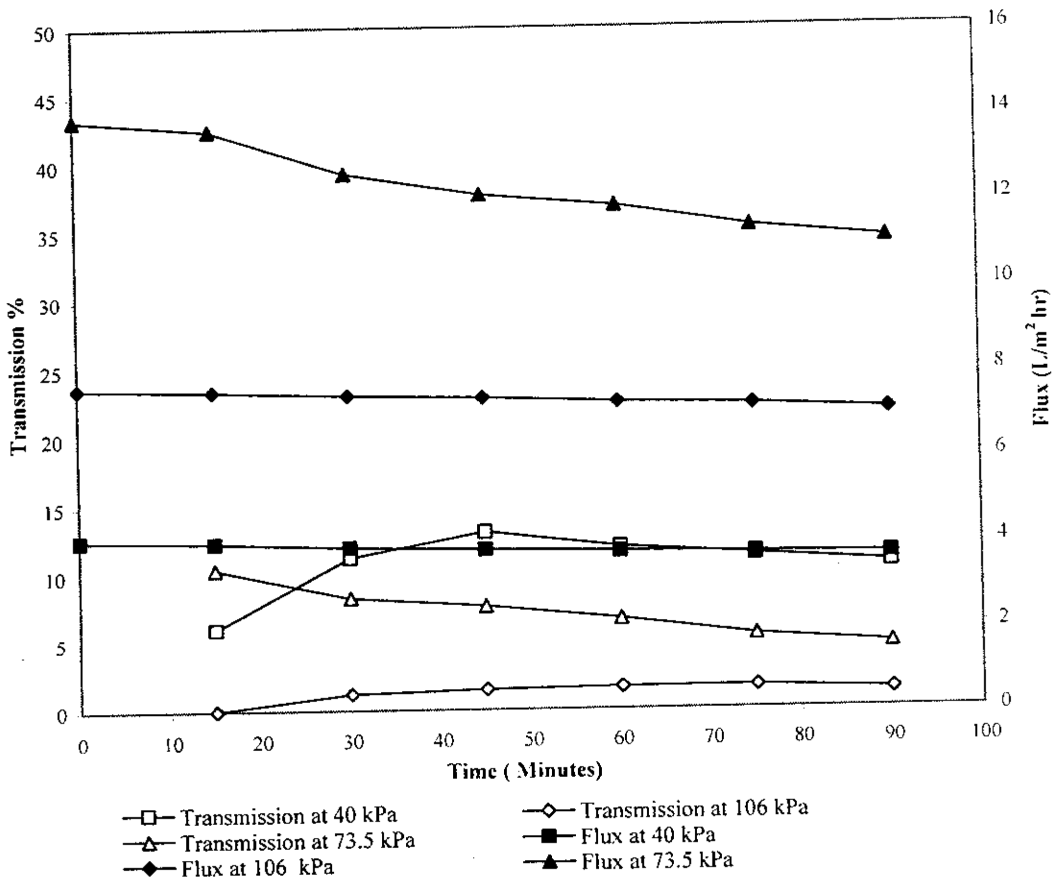


Figure 4.3.1.3 (a) Transmission and Flux analysis of Lysozyme at 200 RPM with respect to time for E113 unmodified PAN membrane

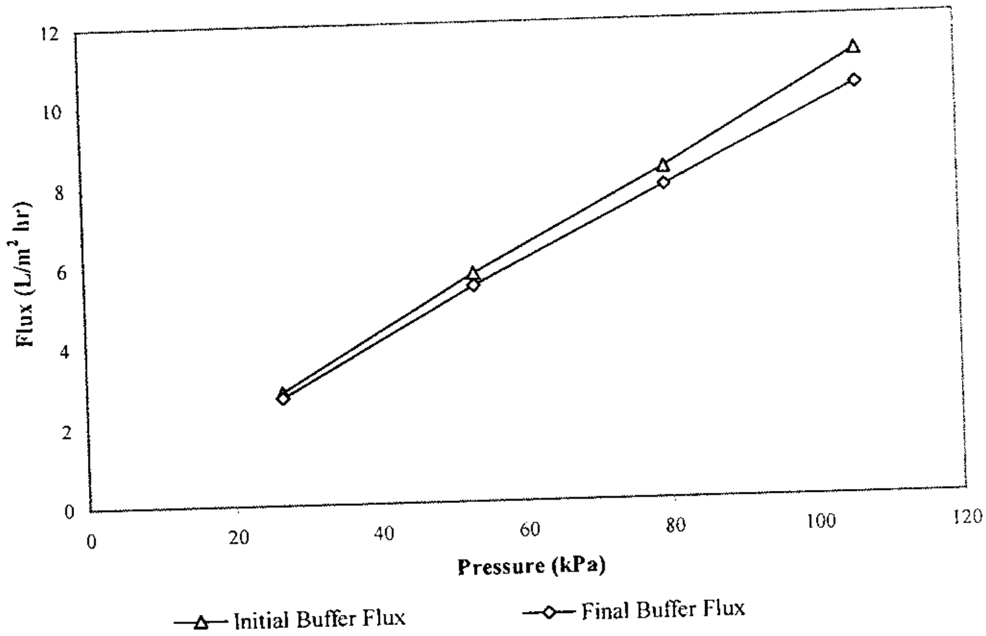


Figure 4.3.1.2 (b) Fouling analysis of Lysozyme at 200 RPM and 40 kPa for E113 unmodified PAN membrane

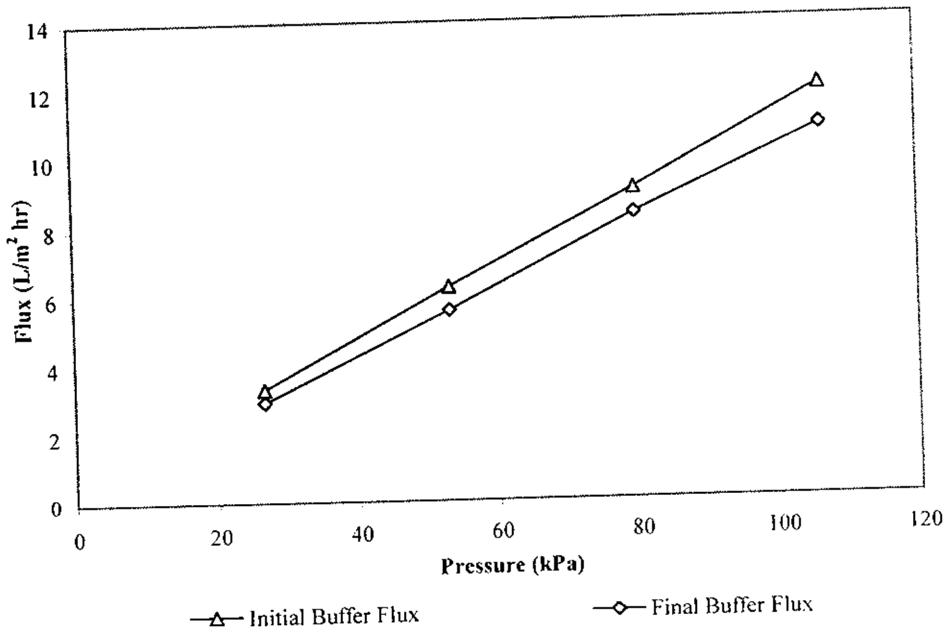


Figure4.3.1.2(c) Fouling analysis of Lysozyme at 200 RPM and 73.5 kPa for E113 unmodified PAN membrane

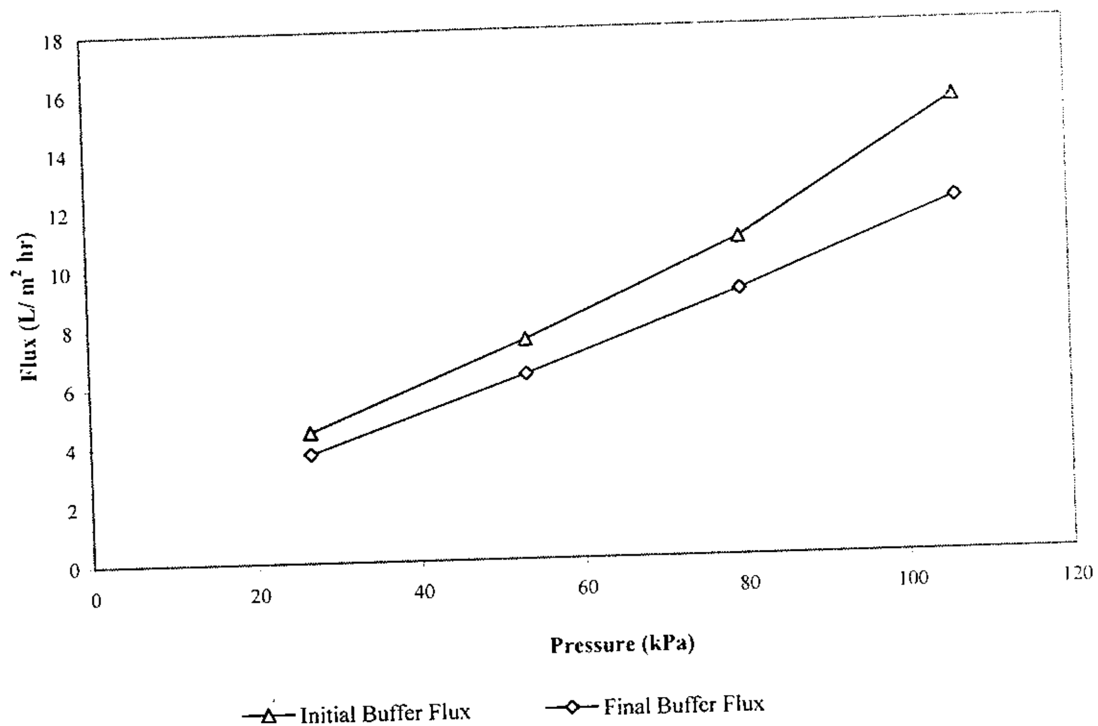


Figure 4.3.1.2(d) Fouling analysis of Lysozyme at 200 RPM and 106 kPa for E113 unmodified PAN membrane

4.3.1.4 Transmission and fouling analysis of Lysozyme at 350 RPM

Table 4.3.1.4(a) Analysis of Lysozyme for E113 Unmodified PAN membrane for different pressure at 350 RPM

Pressure KPa	Initial Buffer Permeability (x 10^{-8} m/s.kPa)	Final Buffer Permeability (x 10^{-8} m/s.kPa)	Fouling (%)	Transmission (%)
40	2.90	2.75	5	0.7
73.5	3.06	2.87	6	0.5
106	2.90	2.71	7	0.6

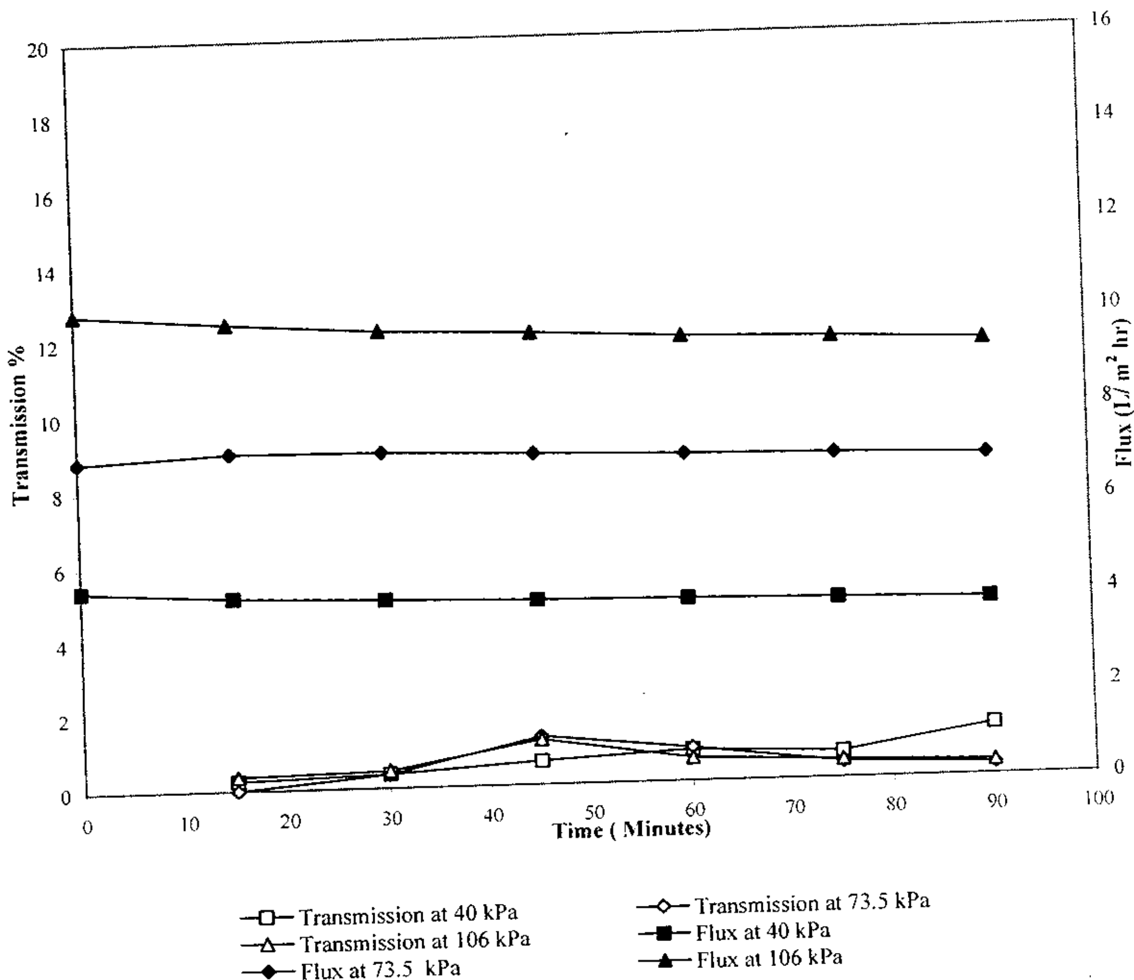


Figure 4.3.1.4(a) Transmission and Flux analysis of Lysozyme at 350 RPM with respect to time for E113 unmodified PANmembrane

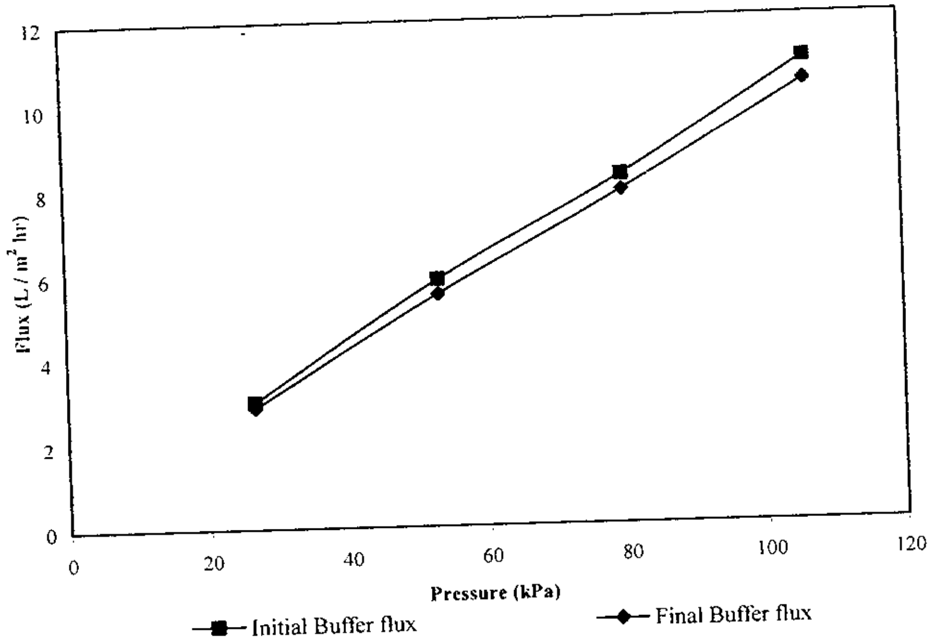


Figure 4.3.1.4(b) Fouling analysis of Lysozyme at 350 RPM and 40 kPa for E113 unmodified PAN membrane

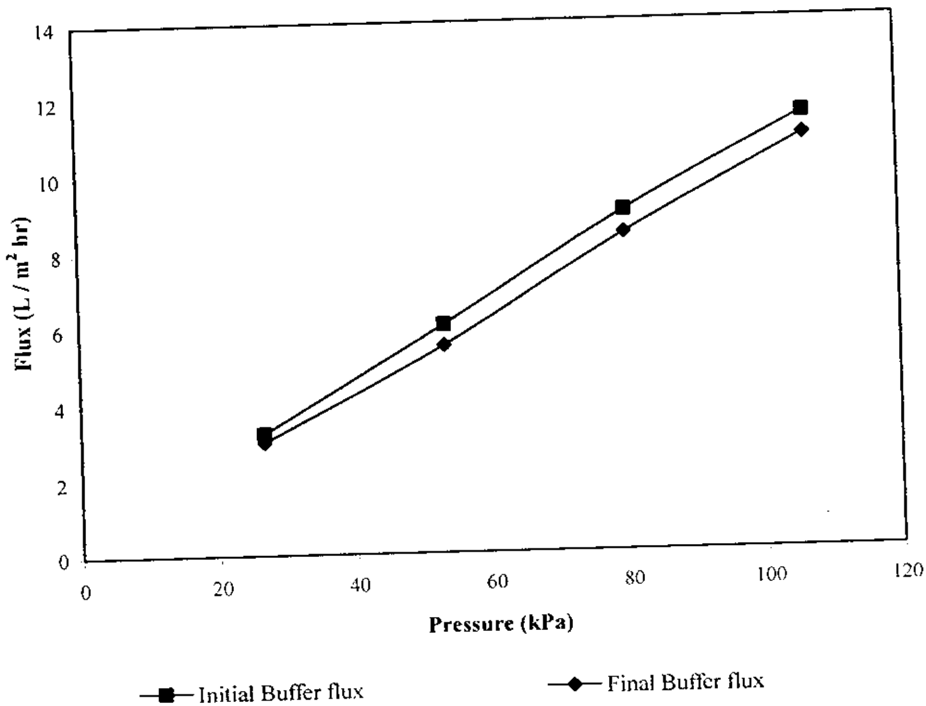


Figure 4.3.1.4(c) Fouling analysis of Lysozyme at 350 RPM and 73.5 kPa for E113 unmodified PAN membrane

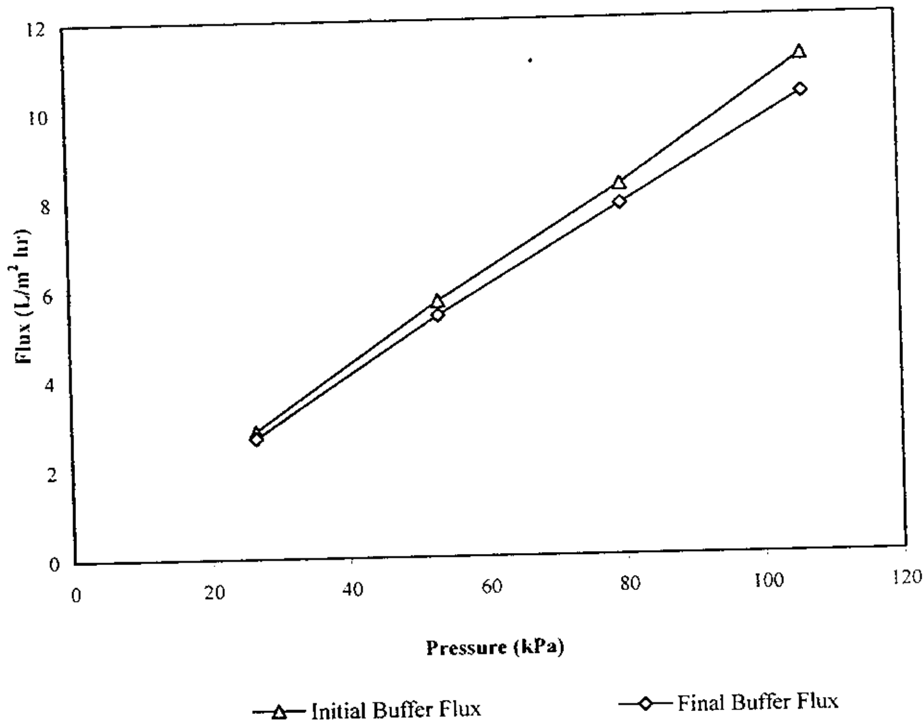


Figure 4.3.1.4(d) Fouling analysis of Lysozyme at 350 RPM and 106 kPa for E113 unmodified PAN membrane

At pH 11.0 with stirrer speed of 200 and 350 rpm the percentage of lysozyme transmission was higher in similar to the case of myoglobin. The transmission of lysozyme was 10% at low pressure which was approached to 1% for higher pressure of 106 kPa with 200 RPM (Table 4.3.1.3 and 4.3.1.4). Similarly the experiments were carried out at 350 RPM for the pressure range of 40 kPa to 106 kPa. The transmission percentages were observed very low at 350 RPM stirred speed as shown in figure 4.3.1.4 (a) In E113 unmodified PAN membrane, flux analysis with respect to time shows better for longer run. The fouling percentage was also higher at both RPM (200 and 350) for high pressure 106 kPa as shown in Table (4.3.1.4).

4.3.2. PES-30 Membrane

4.3.2.1 Transmission and fouling analysis of Ovalbumin at 100 RPM

Table 4.3.2.1 Analysis of Ovalbumin for PES-30 membrane for different pressures at 100 RPM

Pressure kPa	Initial Buffer Permeability (x 10^{-7}m/s.kPa)	Final Buffer Permeability (x 10^{-7}m/s.kPa)	Fouling (%)	Transmission (%)
26	5.51	4.16	32	18
93	5.19	3.49	33	35

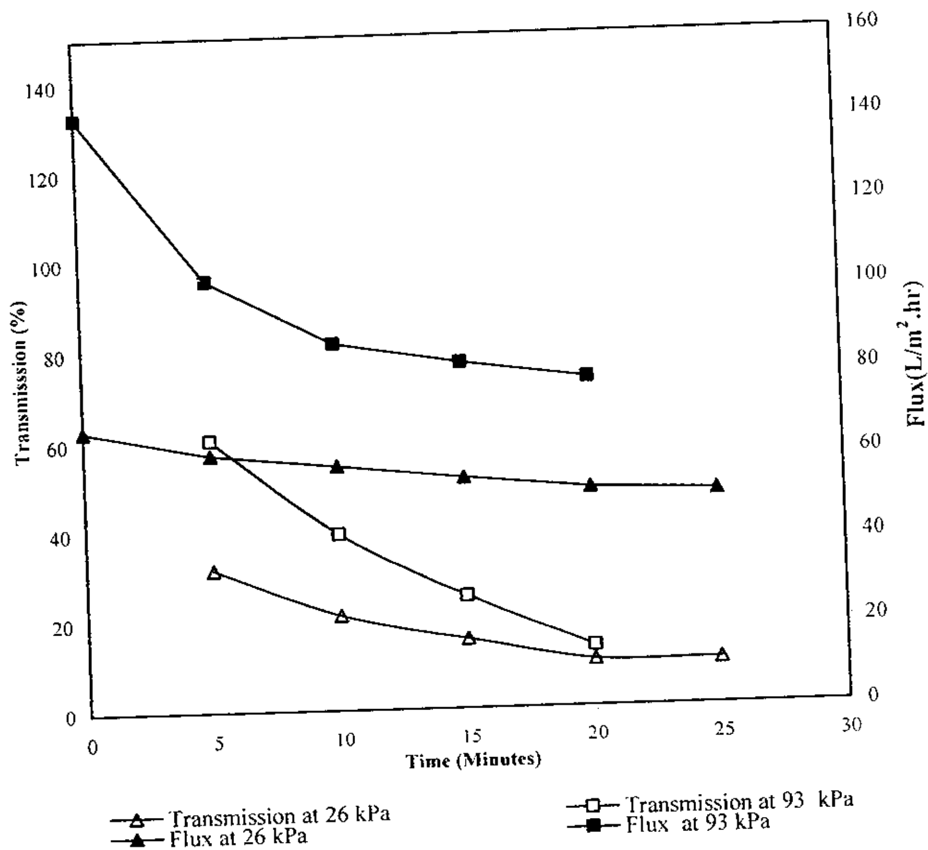


Figure 4.3.2.1(a) Transmission and Flux analysis of Ovalbumin at 100 RPM with respect to time for PES -30 membrane

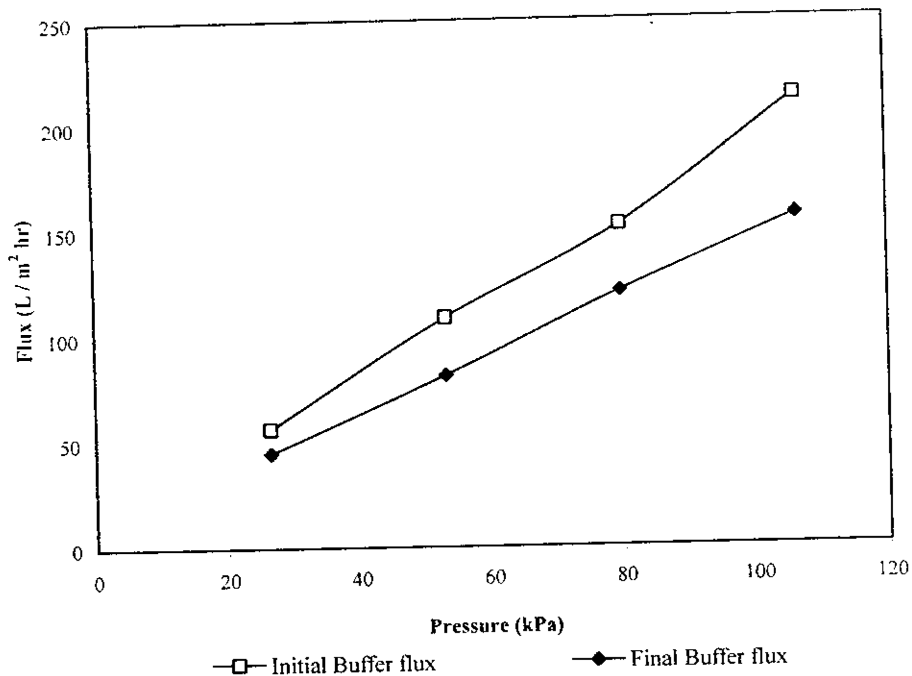


Figure 4.3.2.1(b) Fouling analysis of Ovalbumin at 100 RPM and 26 kPa for PES -30 membrane

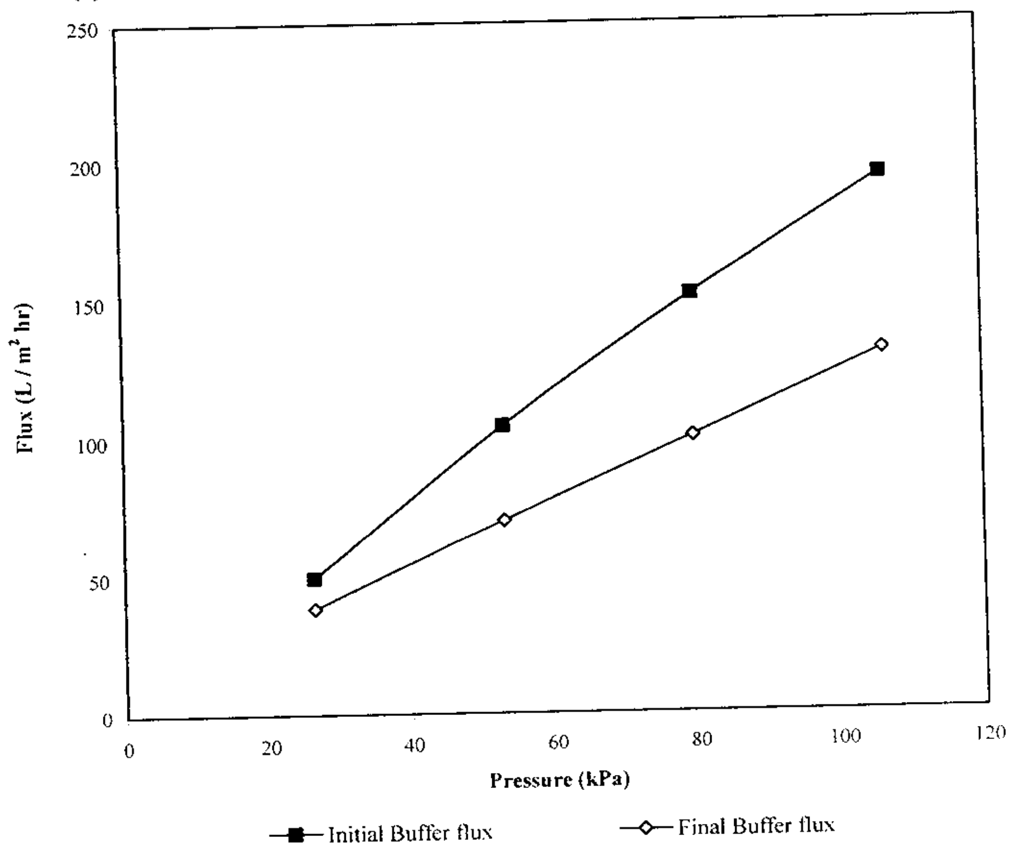


Figure 4.3.2.1(c) Fouling analysis of Ovalbumin at 100 RPM and 93 kPa for PES -30 membrane

Observed transmission data from Table 4.3.2.1 showed that percentage of ovalbumin transmission at 93 had two fold (35%) the amount than 26 kPa (18%) at 100 RPM. In PES-30 membrane ovalbumin flux analysis with respect to time reveals that flux was decreased from 133 to 76 L/m^2hr whereas, at pressure 26 kPa, flux profile deviation was lesser than 96 kPa pressure(Figue 4.3.2.1(a)). Fouling percentage was 33 for 96 kPa and 32 for 26 kPa pressure, Figure (4.3.2.1(b) and 4.3.2.1(c)).

4.3.2.2 Transmission and fouling analysis of Ovalbumin at 400 RPM

Table 4.3.2.2 Analysis of Ovalbumin for PES-30 membrane for different pressures at 400 RPM

Pressure kPa	Initial Buffer Permeability (x 10^{-7}m/s.kPa)	Final Buffer Permeability (x 10^{-7}m/s.kPa)	Fouling (%)	Transmission (%)
26	5.838	4.37	25	9
93	5.97	3.99	33	14

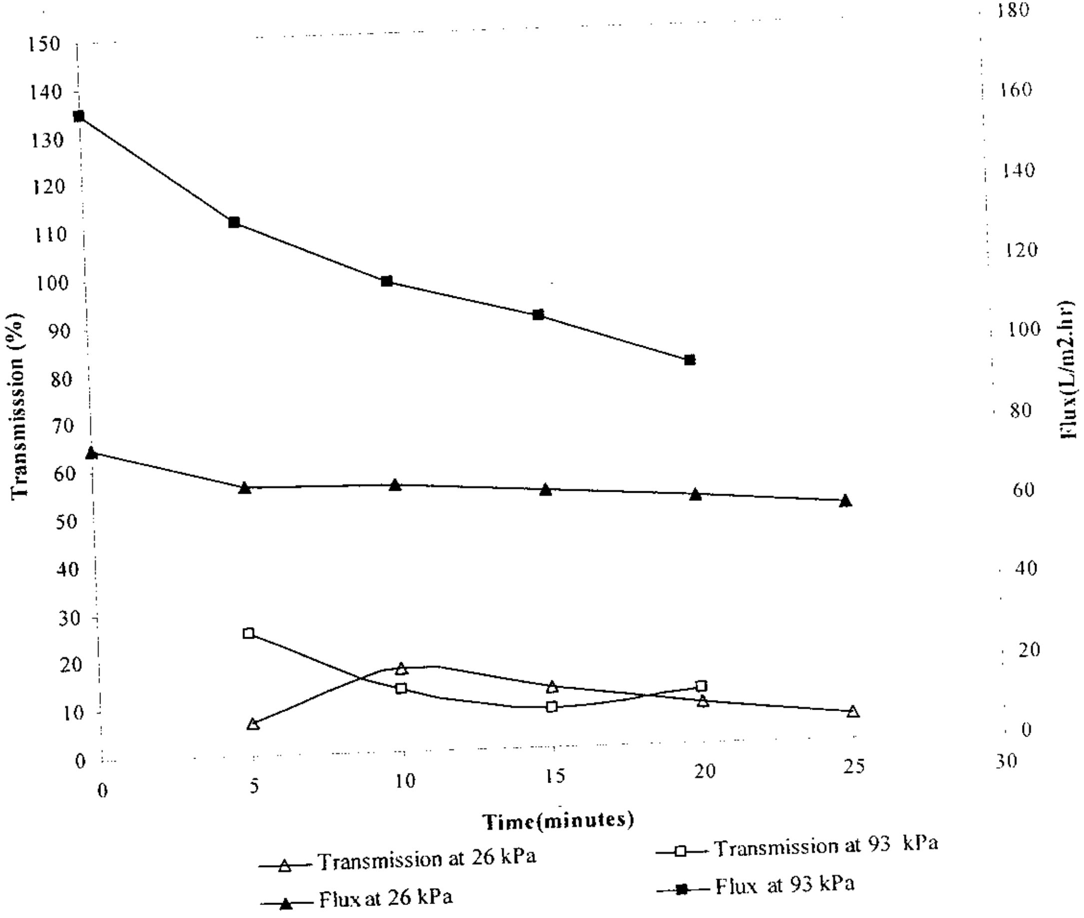


Figure 4.3.2.2(a) Transmission and Flux analysis of Ovalbumin at 400 RPM with respect to time for PES - 30 membrane

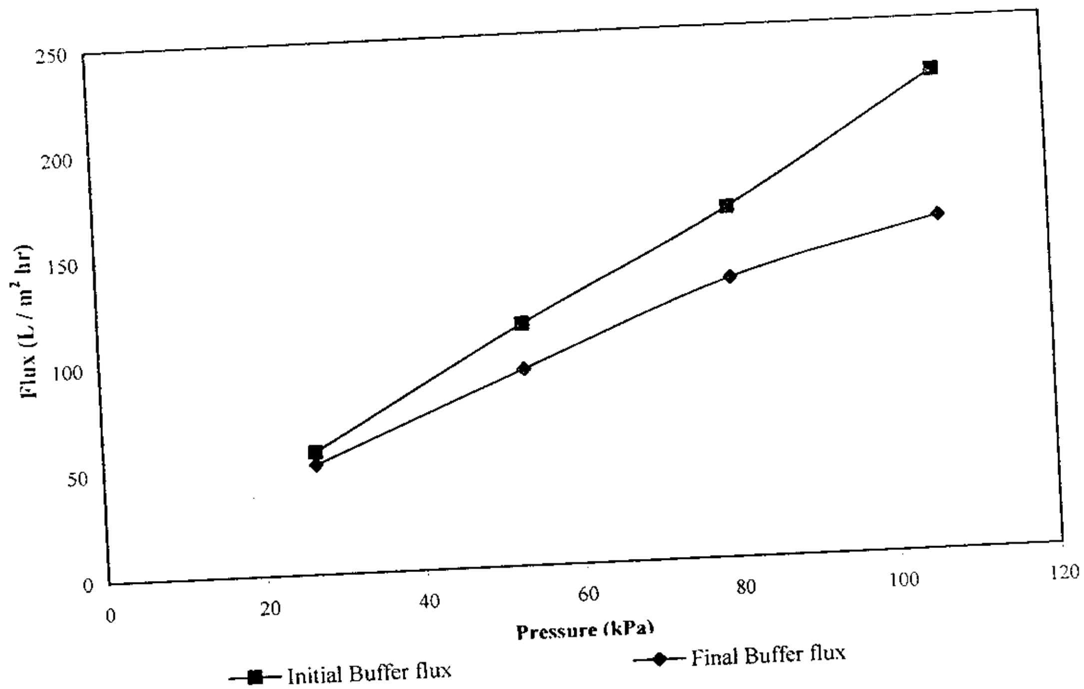


Figure 4.3.2.2(b) Fouling analysis of Ovalbumin at 400 RPM and 26 kPa for PES -30 membrane

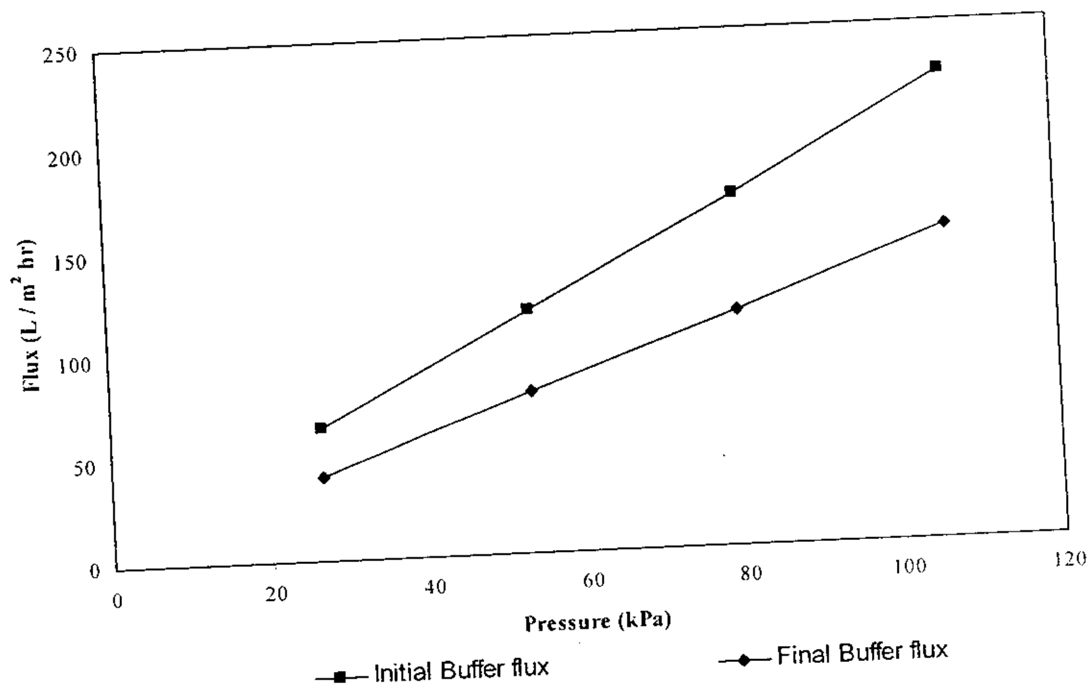


Figure 4.3.2.2(c) Fouling analysis of Ovalbumin at 400 RPM and 93 kPa for PES -30 membrane

In PES-30 membrane higher fouling were occurred at high pressure of 93 kPa with respect to stirrer speed of 100 and 400 rpm (Table 4.3.2.1 and 4.3.2.2). Figure 4.3.1.1 (a) and 4.3.1.2 (a) showed that percentage of Ovalbumin transmission was higher at 93 kPa (i.e. 35 %) and 100 rpm than 400 rpm (14 %). Better flux was observed in the low pressure of 26 kPa with respect to time at 400 RPM.

4.3.2.3 Transmission and fouling analysis of Ovalbumin at 200 RPM

Table 4.3.2.3 Analysis of Ovalbumin for PES-30 membrane for different pressures at 200 RPM

Pressure kPa	Initial Buffer Permeability (x 10^{-7}m/s.kPa)	Final Buffer Permeability (x 10^{-7}m/s.kPa)	Fouling (%)	Transmission (%)
40	6.43	4.8	25	10
66.5	6.0	4.7	22	16
93	6.38	4.0	37	18

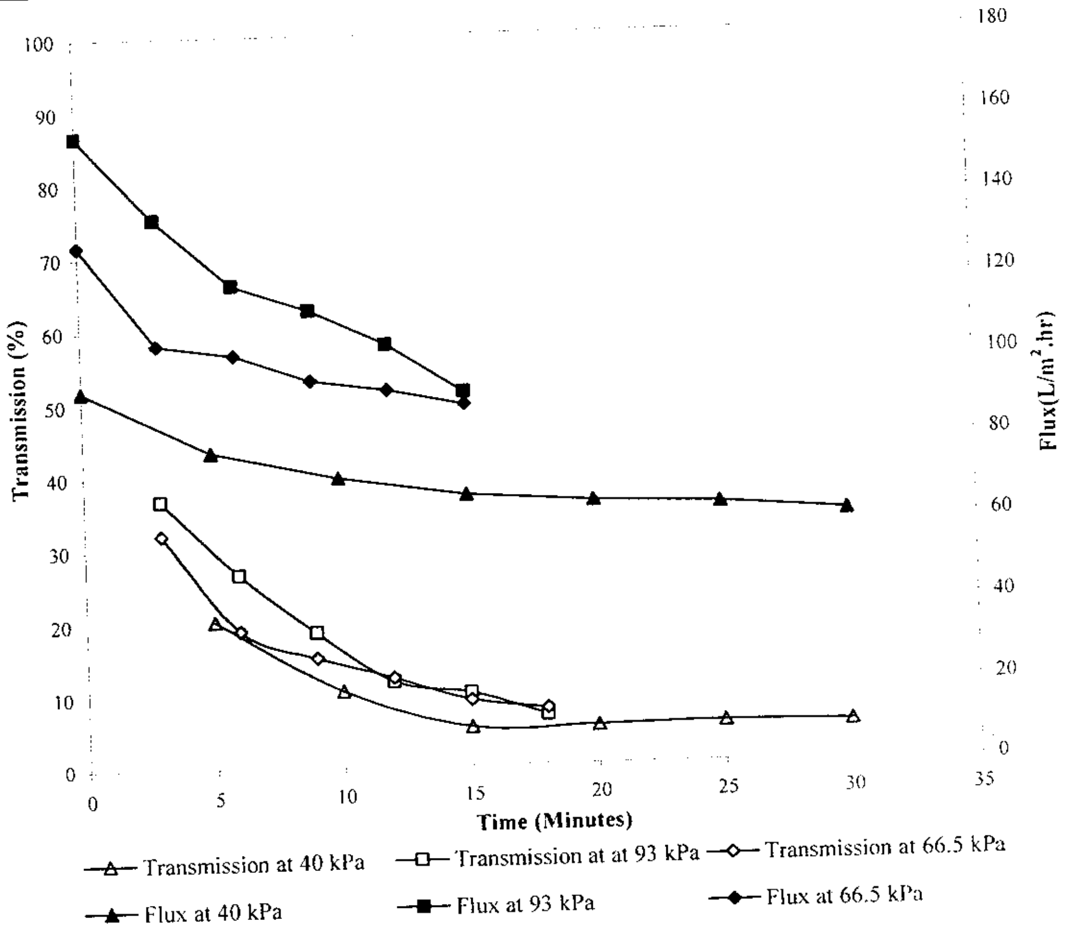


Figure 4.3.2.3(a) Transmission and Flux analysis of Ovalbumin at 200 RPM with respect to time for PES - 30 membrane

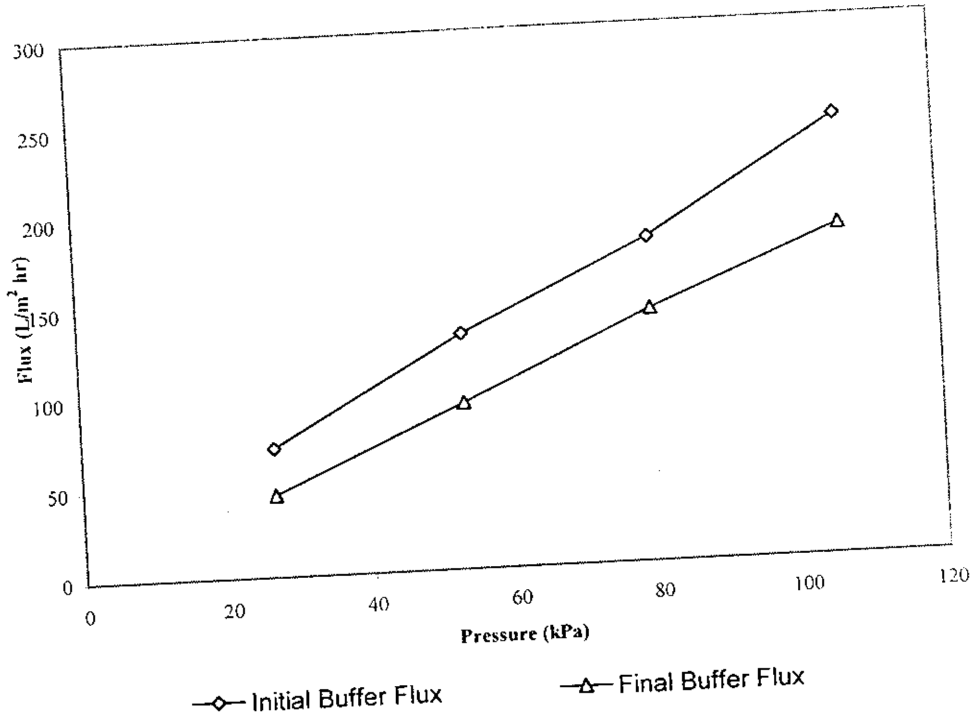


Figure 4.3.2.3(b) Fouling analysis of Ovalbumin at 200 RPM and 40 kPa for PES -30 membrane

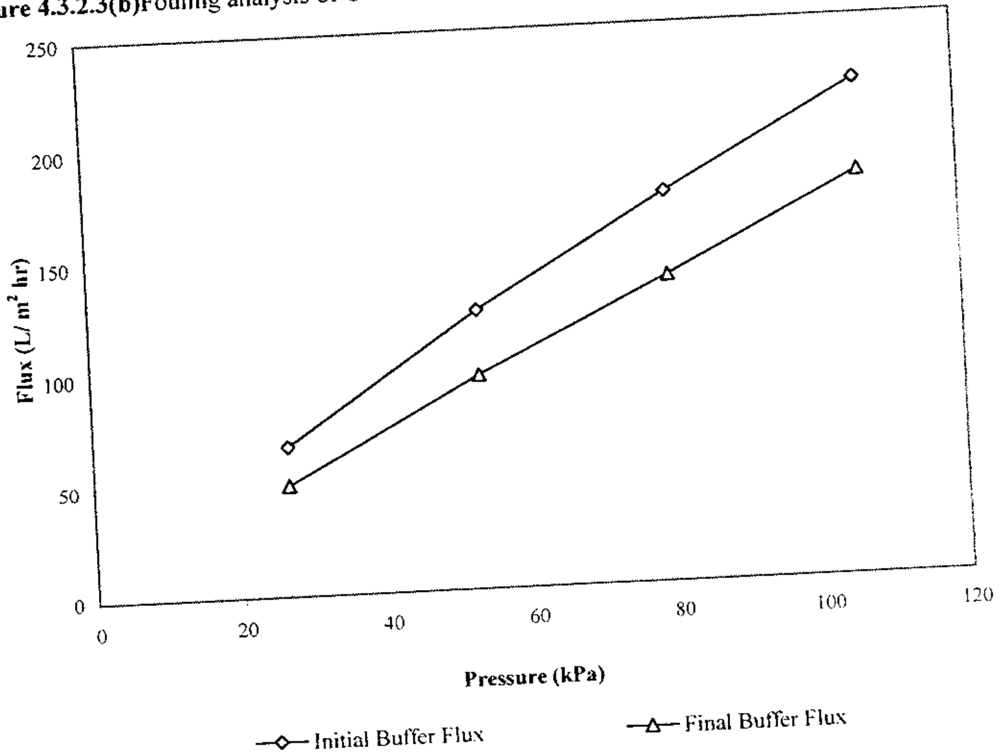


Figure 4.3.2.3(c) Fouling analysis of Ovalbumin at 200 RPM and 66.5 kPa for PES -30 membrane

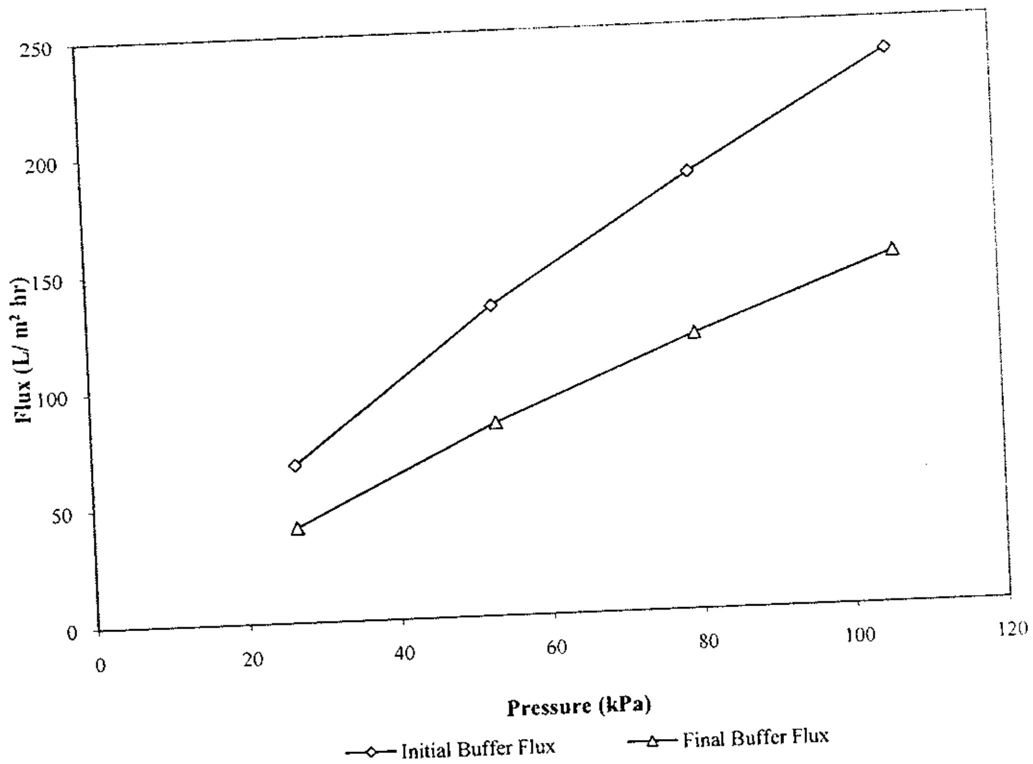


Figure 4.3.2.3(d) Fouling analysis of Ovalbumin at 200 RPM and 93 kPa for PES -30 membrane

4.3.2.4 Transmission and fouling analysis of Ovalbumin at 350 RPM

Table 4.3.2.4 Analysis of Ovalbumin for PES-30 membrane for different pressures at 350 RPM.

Pressure kPa	Initial Buffer Permeability (x 10^{-7}m/s.kPa)	Final Buffer Permeability (x 10^{-7}m/s.kPa)	Fouling (%)	Transmission (%)
40	6.17	4.5	27	5
66.5	6.0	4.7	21	10
93	6.29	4.38	30	8

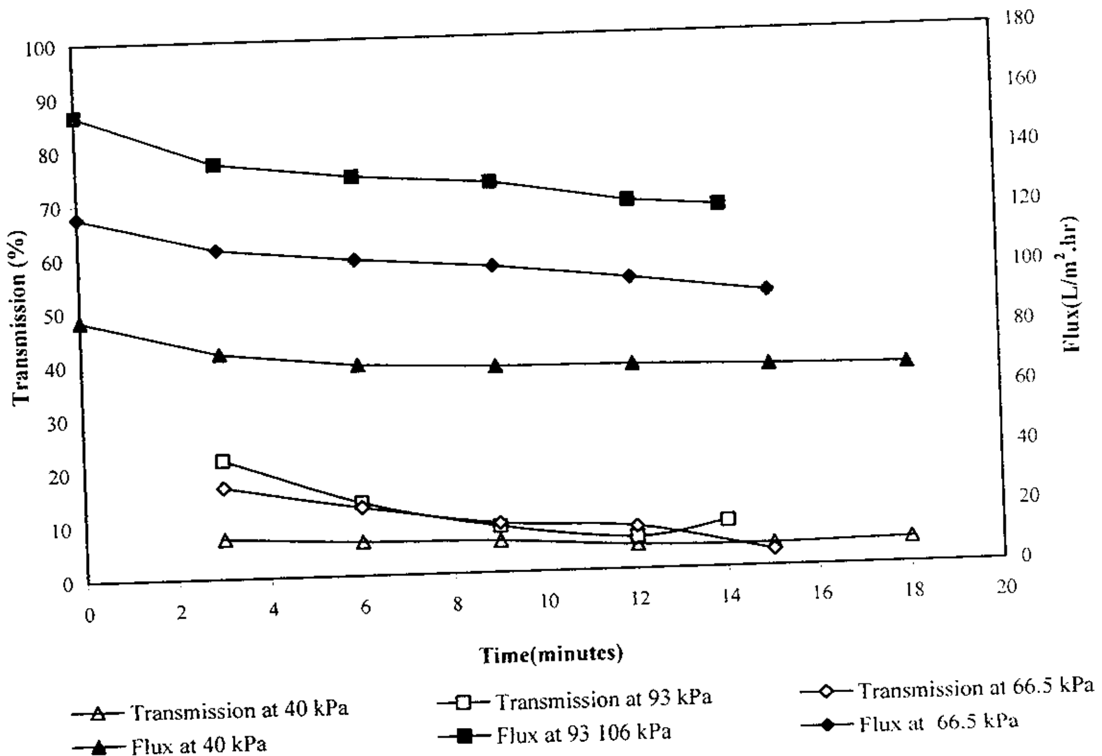


Figure 4.3.2.4(a) Transmission and Flux analysis of Ovalbumin at 350 RPM with respect to time for PES - 30 membrane

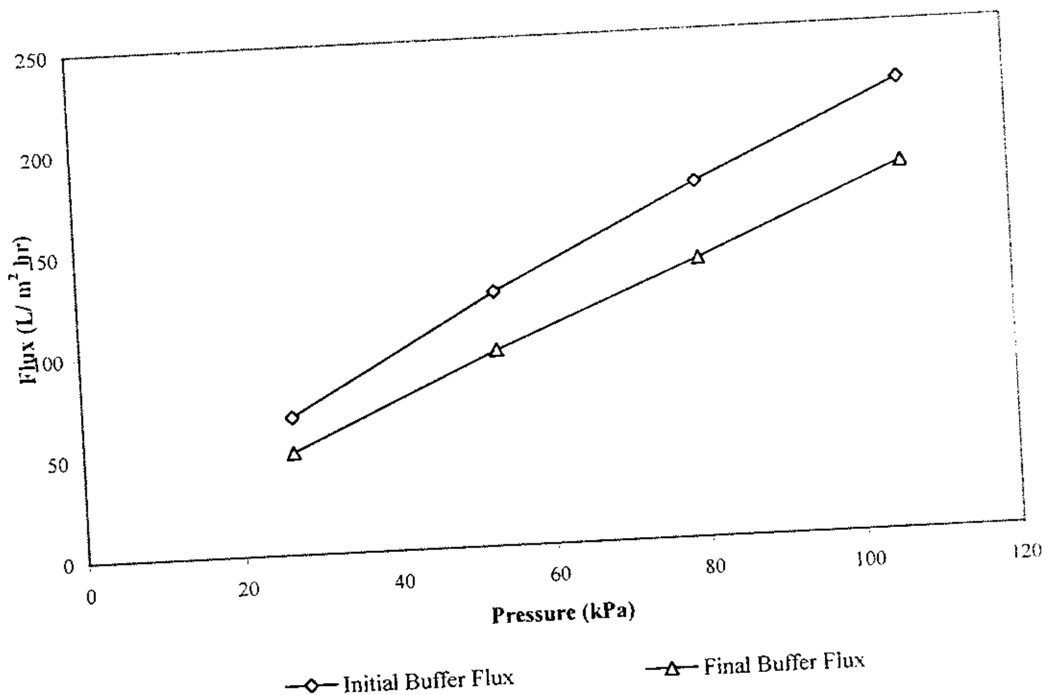


Figure 4.3.2.4(b) Fouling analysis of Ovalbumin at 350 RPM and 40 kPa for PES -30 membrane

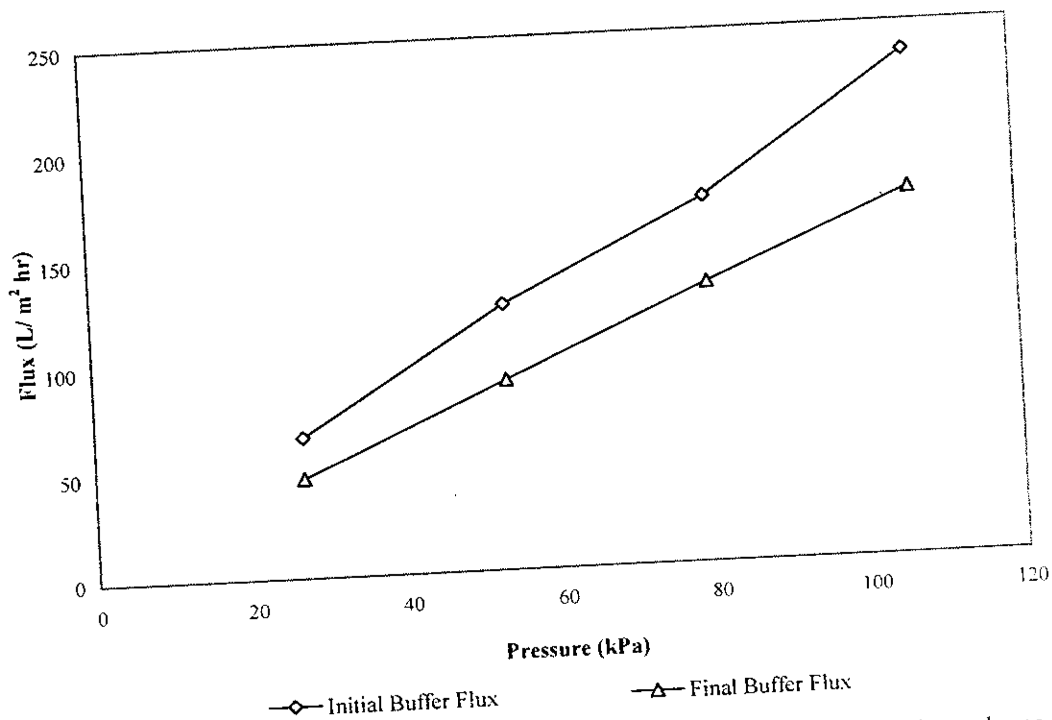


Figure 4.3.2.4(c) Fouling analysis of Ovalbumin at 350 RPM and 66.5 kPa for PES -30 membrane

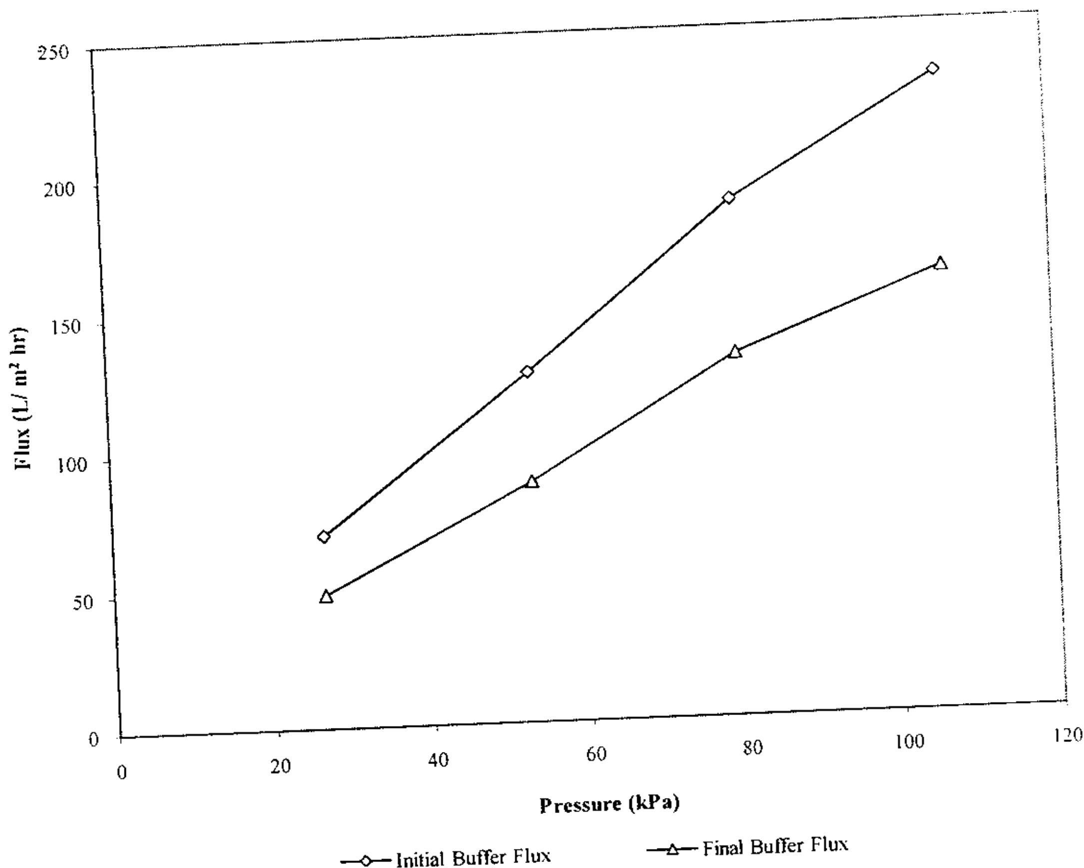


Figure 4.3.2.4(d) Fouling analysis of Ovalbumin at 350 RPM and 93 kPa for PES -30 membrane

Figure 4.3.2.3(a) and 4.3.2.4(b) showed that at 200 and 350 RPM, the ovalbumin transmission had linear relationship with pressure and increasing from lower pressure 40 kPa to higher pressure 106 kPa (Table 4.3.3 and 4.3.4). The figure (4.3.2.3(a) and 4.3.2.4(b)) showed at higher pressure, the fouling occurred after some time and reduced the transmission percentage of ovalbumin. At low pressure, transmission of protein was constant during the time interval up 20 minutes. High permeability PES-30 membrane with respect to stirrer speed (200 and 350 RPM) and time, the ovalbumin transmission was higher in the case of 200 rpm than 400 RPM.

4.4.1 Low Permeability E113 unmodified PAN membrane (4.67×10^{-8} m/s kPa)

The E 113 unmodified membrane is very low flux membrane and showed water permeability of ~ 4.67×10^{-8} m/s kPa. Results obtained from the experiments of various model proteins with E113 unmodified PAN membrane showed that the membrane was low molecular weight cut off membrane. E113 membrane showed negligible transmission for BSA and Ovalbumin at pH 7. Transmission for myoglobin was ~25 % at pH 7. Transmission analysis of lysozyme at pH 11.5 which was near to lysozyme pI value results that E113 membrane hold net negative charge on the surface. At pH lower than lysozyme pI value, the protein behave as positive charge and thus transmission was very less with E113 membrane. MWCO of the E113 membrane was expected as 6 to 10 kDa. For E113 membrane the fouling was very low and ranged from 1% to 15%.

In case of E113 low permeability membrane, the transmission of lysozyme was maximum at low pressure i.e. about 10 %. The transmission and flux with respect to time was constant for 350 RPM and all pressure ranges (40 to 106 kPa). From the results it was concluded that E113 PAN based membrane had low MWCO but it showed good reproducibility in results with low fouling.

4.4.2 High Permeability PES- 30 membrane (6.9×10^{-7} m/s kPa)

The flux of PES 30 membrane was higher than the E 113 unmodified membrane. This membrane showed better transmission for lysozyme, ovalbumin and myoglobin. In case of BSA it resulted in 100 % rejection. Fouling was too high compared to PAN membrane.

Generally PES membrane shows high fouling % with respect to hydrophilic membrane, but the flux was high and the water permeability range was tenfold higher than E113 membrane. In figure (4.3.2.4 (a)) at low pressures, the transmission and flux profile was almost same during whole experimental time that suggest that higher the rpm and lower the pressure gives better transmission of proteins and low fouling in compared to high pressure and higher RPM. By providing higher rpm, the solute particles have less chance to deposit on the membrane surface and better separation has occurred. Where as at low RPM, there was no good correlation between the transmission and flux with respect to time (Figure (4.3.2.3 (a)).

4.4.3 Fouling Minimization

Fouling is found to be maximum at isoelectric point due to least solubility at this pH and due to the absence of electrostatic forces, which resulted in increased protein adsorption to membrane surfaces. As pH increases from isoelectric point, electrostatic interaction between membrane and model protein becomes more and more repulsive thus reducing effect of fouling. Fouling was found to be increasing with protein concentration due to greater concentration polarization and therefore increased hydraulic resistance.

The presence of excess ion at higher salt concentration shield the surface charges of both protein and membrane and thus reducing the repulsive interactions between protein and membrane which might have increased the fouling on increasing salt concentration.

4.4.4 Effect of System Hydrodynamics and Solution Environment on protein solution

Effect of pH was studied on flux and transmission on model proteins. Protein transmission was greatest at pH near its isoelectric point. The reduction in protein transmission at pH < pI was attributed to an increase in protein adsorption, while the reduction at pH > pI was assumed to be due to electrostatic repulsion between the negatively charged membrane and the negatively charged protein. Balakrishnan and Agarwal, (1996) evaluated the effect of solution pH on the transmission of ovalbumin, myoglobin, and lysozyme in a rotating (Taylor vortex) module. Ovalbumin and myoglobin transmission were greatest at the respective protein isoelectric points. The very large reduction in protein transmission at pH < pI was attributed to significant adsorption of the positively charged proteins on the negatively charged membrane under these conditions. The reduction in protein transmission at pH > pI was attributed to an expansion of the protein associated with intramolecular electrostatic interactions in combination with a reduction in the extent of concentration polarization due to the back mass transport caused by the electrostatic repulsion between the proteins and membrane surface.

Average limiting flux was found to be decreasing with increasing bulk concentration as increased protein concentration can increase the viscosity of solution and can decrease the diffusivity of proteins through membrane. Similar kind of observation was seen with changing the temperature. Decrease in temperature increased viscosity therefore decreasing average limiting flux and transmission. Yang and Tong, (1997) obtained data for the transport of myoglobin and cytochrome c through hydrolyzed PAN hollow-fiber membranes. Protein transmission was greatest at pH near the protein

isoelectric point. The reduction in protein transmission at $pH < pI$ was attributed to an increase in protein adsorption, while the reduction at $pH > pI$ was assumed to be due to electrostatic repulsion between the positively charged membrane and the positively charged protein. Increase in stirrer speed reduced the concentration polarization, which leads to higher flux and reduced wall concentration of model proteins. Increase in salt concentration increased the concentration polarization, which leads to decreased flux and transmission.

CONCLUSION

5.0 CONCLUSION

The effects of operating parameters on the flux and separation factor in dead- end UF of model protein using PES-30 and PAN E113 membranes were systematically investigated. The following results were obtained.

- The UF flux and protein transmission were strongly affected by the applied pressure, solution pH and ionic strength, particularly mainly by the applied pressure and feed concentration.
- Under comparable condition, PES-30 displayed higher flux and higher fouling % than that of PAN E113 membrane. In both PES-30 and PAN E113 membranes showed higher fouling at their high applied pressure.
- E113 membrane displayed higher transmission at their low applied pressure but in PES -30 membrane showed better transmission at their higher pressure.

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6.0 REFERENCES

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