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Recovery of biomolecules by Nanofiltration membranes

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GRATEFULNESS

First I thank God for illuminating my path.

I thank my parents and siblings for each of their advice and words of encouragement that every day have helped me grow as a person and fulfill each of my goals.

I also thank my director for the time, dedication, patience and trust placed in me.

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ABSTRACT

The separation, purification and recovery of amino acids plays an important role in many pharmaceutical, food and cosmetic chemical processes. Nanofiltration (NF) is a pressuredriven membrane process with intermediate characteristics of ultrafiltration and Reverse Osmosis, which gives Nanofiltration a unique performance.

The separation of biomolecules by nanofiltration membranes is governed not only by the pore size of the membrane, but also by the ion charge itself, which is a function of the pH of the medium and the solution of the solute. If you have a neutral pH, the recovery of the biomolecules is basically given by the pore size of the membrane and when they have an acidic or basic pH, the ionic charge of the membrane and the solute for its recovery play a very important role. It is because the nanofiltration membranes are generally charged due to their terminal functional groups and this electrical charge may depend on the composition of the feed and the pH. NF membranes are usually negatively charged under neutral or alkaline conditions and positively charged under highly acidic conditions.

In this work, the recovery of biomolecules through nanofiltration membranes will be studied, since biomolecules are complex organic molecules. The work will consist of two main parts, the first part consists of the bibliographic review of scientific studies in which the application of nanofiltration membrane technology for the separation of amino acids and peptides, published in high impact journals and the second part consists of an experimental work, which was performed with a peptide that is triglycine, said peptide was worked both in a solution of deionized water, and in a solution in which a concentration of sodium chloride was added. The experimental results obtained confirmed the experimental results of other scientific articles in which the same behavior of the nanofiltration membrane is observed with respect to the retention of said peptide because the experimental results are adjusted with good correlation with the Spiegler-Kedem model allows to determine σ , *Lp* and *Ls* for each experiment.

It is important to mention that the rejection of triglycine (MPF 36) depends very much on the solution in which it is found and on the pH, since according to the experimental results obtained, the maximum rejection of triglycine in a solution of deionized water was 80 % - 90 % with an acidic pH, with a neutral pH has a rejection of 40 % (close to its isoelectric point) and 80 % - 90 % at basic pH.

Triglycine 0.001 M solution with a concentration of NaCl 0.1 M presents a behavior in which rejection is reduced even when it has an acidic, neutral and basic pH, since NaCl produces an effect that protects the surface charge of the membrane , changing its electrical structure, decreasing the repulsion of triglycine molecules and, therefore, its rejection. It should also be mentioned that an increase in the ionic strength of the feed solution decreased the maximum rejection of the amino acid and the peptide, due to an alteration in the electrical charge of the membrane MPF 36.

This work is one more step for the application of the NF, since it plays an important role in many areas; water treatment, dairy industry, biomedical processes, among others. It is a call to improve, investigate and develop the ability of NF to separate monovalent, polyvalent and amphoteric biomolecules, which will be the key to the development of processes that imply clean production and make this tool, not an alternative, an application.

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1. INTRODUCTION

Membrane filtration is a pressure-driven process in which the membrane acts as selective barriers to restrict the passage of contaminants such as organics, nutrients, turbidity, microorganisms, inorganic metal ions and other contaminants that deplete oxygen, and allows the passage of relatively transparent water. In addition, it is important to mention that the performance or efficiency of a membrane is determined by the parameters of selectivity and the flow through the membrane.

The membranes could be a selective barrier between two phases, since the processes of the membrane are characterized by the fact that the supply current is divided into two currents, which are the permeate current and the retained current, as is observed in *Figure 1.1.*

Figure 1.1 Schematic representation of membranes process where the feed stream has been separated into a retentate and a permeate stream.

One of the classifications of the most important membranes is based on their nature. The biological membranes have many drawbacks such as a limited operating temperature and pH range, however, the synthetic membranes work well and these can be both organic (polymeric) and inorganic (ceramic, metallic) membranes, for which in the present study will work with the type of organic membrane for its high efficiency.

The driving force in the processes based on membrane technology that allows the passage of substances through a membrane is the difference of electrochemical potential that can be differentiated 4 types of processes according to the motive power that can be by Microfiltration, Ultrafiltration, Nanofiltration and Reverse Osmosis. For the present work, it will be carried out with the Nanofiltration process for the recovery of biomolecules.

Nanofiltration is a pressure-driven membrane process that lies between ultrafiltration and reverse osmosis, has a working pressure range of 5-20 bar, has a molecular weight limit of 200 to 1000 Da and the size of its pores is approximately 1nm; The membranes can be tubular, spiral or flat.

Nanofiltration membranes are usually negatively charged under neutral or alkaline conditions and positively charged under very acidic conditions, likewise, the electrical charge of the functional groups of the membrane can affect the composition of the solute and the pH; before this behavior of the membrane, it is known that it has a minimal rejection of the salts when the pH is near the isoelectric point of the membrane, therefore, the rejection of the discharged molecules is dominated by the exclusion of the size, while The species with charge is influenced both by the exclusion of the size and by the electrostatic interactions between the membrane and the solute.

It is important to relate the properties of the material with the performance of the membrane, which makes the characterization of the membrane very important. Most of the available commercial NF membranes are of polymeric or organic nature and are applied to perform works in aqueous solution, with which it is sought to swell and the pores are open, that is why in the present work an experimental test with an organic nanofiltration membrane which is adapted to the methodology that will be used and will be described later. It is also important to mention that at present nanofiltration membranes are applied in the industrial sector mainly for the processes of demineralization, color removal and desalination.

A part of the mechanism of transfer of matter in the NF process is based on the dissolution-diffusion process, which is related to the phenomenon of osmotic pressure. When a driving force is applied to the input current of a membrane module, two currents are produced, permeate and rejection. The rejection is that current that does not cross the semipermeable membrane, this current has a higher concentration of solute than food. The rejection factor is used as an index of effectiveness for the use of membranes that define the measure of the concentration change of the retained solute and the solute that it goes through the membrane:

$$
R_{obs} = \frac{C_b - C_p}{C_b} \tag{1}
$$

where Robs is the rejection observed experimentally, C_b is the concentration of solute in the Reject current and C_p is the concentration of solute in the permeate stream. A solute will be very rejected by a membrane when the rejection factor tends to 1, and the solute will permeate completely the membrane when the rejection tends to 0. The transport of a binary solution (consisting of salt and water) through NF membranes can be described by the phenomenological model of Kedem and Katchalsky, which is based on the non-equilibrium thermodynamics. The flow of water through a membrane, *Jv*, depending on the Pressure difference between the two faces of the membrane, ΔP, is given by the following expression:

$$
Jv = Lp.(\Delta P - \sigma. \Delta \pi) \tag{2}
$$

where L_p is the permeability of solvent in the membrane, $\Delta \pi$ is the difference the osmotic pressure, σ is the reflection coefficient, which is a measure of the degree of solute penetration through the membrane and corresponds to the rejection of maximum solute. Spiegler-Kendem modified the model of Kendem and Katchalsky by a differential form of equation (2) and takes into account the Concentration profile inside the membrane. The rejection of solute for a solution ideal was deduced by Spiegler-Kendem by integrating the differential equation of the solute transport and was expressed as a function of the reflection coefficient in the following equations:

$$
R = \frac{(1 - F) \cdot \sigma}{1 - \sigma \cdot F} \tag{3}
$$

$$
F = \exp\left(-\frac{(1-\sigma)J_v}{R_e \cdot T \cdot L_s}\right) \tag{4}
$$

where Rg is the constant of the ideal gases and T the temperature of permeation. So he NF separation process is characterized by the determination of *Lp, Ls* and σ. *(Spiegler, K.S. et al. 1966).*

It is also important to mention that the Biomolecules are complex organic molecules. These molecules form the basic structural constituent of a living cell. Among the most studied biomolecules we have the amino acids, amines, peptides and proteins.

- Amino acids: amino acids are the most versatile small biomolecules. An amino acid is a molecule that contains amino (NH₂) and carboxyl (COOH) functional groups.
- Amines: amines are organic compounds that contain nitrogen as a key element in the amine functional group. They are derived from ammonia and are the result of the hydrogen substitution of the molecule by the alkyl radicals.
- Peptides: the peptides are formed by the union of amino acids through peptide bonds. When the number of amino acids that make up the molecule is not greater than ten, it is called an oligopeptide, and if it is greater than ten, it is called a polypeptide.
- Proteins: proteins are polypeptides formed by the combination of a large number of amino acid units. Protein molecules are polymers of different sizes and shapes with different physical and chemical properties.

Over time, different studies have been conducted on the behavior of nanofiltration membranes with respect to the recovery of biomolecules such as amino acids, peptides, amines and proteins, and also the membranes used can be both organic and inorganic. study that used the inorganic Nanofiltration membrane for the selective separation of Amino Acids, in which it was possible to see that these membranes, with their high selectivity, are based on both the size of the solute and the charge and also the pH has

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to be It is a dominant parameter for the recovery of amino acids due to the rejection presented, since in this type of inorganic membranes it is necessary to have a higher percentage of recovery at neutral $pH - Tr > 85$ % -in contrast to acidic or basic pH 's a much lower retention percentage, Tr < 25 % and Tr < 30 %, respectively *(Garem et al., 1996).*

When working with inorganic membranes, regardless of the type of solution in which the membrane is located, the electrical neutrality of the membrane will always be present, as well as the solute and membrane loads to explain the amino acid and peptide transmissions *(Martin-Orue et al., 1997).*

Now, although working with inorganic membranes, there was a higher prevalence of pH, when working with organic membranes there was greater influence of the ionic strength in the electrostatic interactions between the membrane and the amino acids *(Civit et al., 2012).* Also, electrostatic repulsion is the dominant mechanism that governs the separation process; since the pH produces in the electrical charge of the membrane and the amino acids as well as the corresponding repulsions, and consequently in the membrane-solute interactions, a clear example is in the retention of the biogenic amines since they present higher retentions at pH acids 95 %, at alkaline pH have a retention of 20-40 % and neutral pH retentions of 5-10 %, since when you have neutral pH is at its isoelectric point of the membrane and the passage of the solutes will be done only Because of the pore size of the membrane *(Sabaté et al., 2007)*, the same can be observed when working with diprotic amino acids such as L-glutamine and glycine, since the pH dependence of the osmotic pressure is identified as the main parameter that affects the separation behavior in a higher concentration regime *(Kovacs et al., 2007)*, the same behavior can be observed when working with genes such as glycine and triglycine that have neutral pH (pH 6-7), with minimal solute retention *(Civit et al., 2012).*

Similarly, the behavior of organic membranes has also been studied through modeling, in which the main advantage of the models is that it requires very little input data, it can be easily obtained from a simple experimental design, and the Glycine test was carried out on what retentions were obtained according to the pH variation; as pH 6.0 $(Tr = 74\%)$, pH 6.6 (Tr = 75 %), pH 7.9 (Tr = 75 %), pH 8.9 (Tr = 76 %), pH 9.9 (Tr = 83 %), pH 10.8 (Tr = 92 %), in which we can also confirm that the pH has an important contribution to the osmotic pressure for amino acid permeation *(Kovács et al., 2009).*

Another important factor to take into account for solute retention is also the temperature with which one works, since one can have variations with respect to this factor. With respect to solute rejections and permeates, a qualitative theoretical discussion shows that a possible change in the pore radius distribution with temperature may play a role, in addition to the diffusivity, the chemical structure and the concentration of individual compounds. *(Pruksasri et al., 2015),* a serious example with respect to the experimentation that was had for the recovery of monosaccharides in which it was obtained that even working at neutral pH, that is to say between pH 6-7 at lower temperature they had higher percentages of rejection that we have higher temperatures (25 ° C more retention, 30 ° C regular retention and 45 ° C negligible retention). *(Benkun Qi et al., 2011).*

In the same way, we have to work with other types of membranes such as polyelectrolyte multilayers for the retention of neutral amino acids such as glycine, L-alanine, L-serine, L-glutamine and basic amino acids such as L-lysine. Neutral amino acids can be easily separated according to the size of the solute using nanofiltration membranes with multilayer polyelectrolyte membranes, as well as mentioning that L-glutamine is the best neutral amino acid for its retention in this type of membranes, since it obtained a greater percentage of rejection of 96% approximately, and the one that lower percentage of rejection would be the Glycine with 25% approximately *(Honga et al., 2006).*

At the present time, the use of NF membranes has been developed and commercialized from the last decade, they were first used in the treatment of effluents with high calcium and magnesium sulphate and bicarbonate concentrations, for the softening of waters now with more than 10 years of research are used for separations gas-gas, gas-liquid, liquid-liquid or solid-liquid. Among the main applications of the Nanofiltration in the industry are:

- Water softening: (García, 2002).
- Desalination and purification: (Yu, et al., 2001) (Wang, et al, 2002).
- Drinking water: (García et al, 2006) (Réguillon et al., 2008) (Radjenović et al, 2008).
- Wastewater treatment: (Ducom et al, 1999) (Jiraratananon et al, 2000).
- Food industry: in the production of fruit and vegetables, clarifications of fruit juices, wine clarification (Koltuniewicz, et al., 2008) (Butylina et al, 2006)

2. OBJETIVES

- **2.1.** General Objective
	- The aim of this work is to review the key characteristics to recover biomolecules by nanofiltration membranes.
- **2.2.** Specific Objectives
	- Compile and analyze bibliographic information of studies carried out with nanofiltration membranes for the recovery of biomolecules.
	- Determine the behavior of the triglycine permeation in the nanofiltration membrane by means of an experimental test at different concentrations, determining the influence of pressure, pH, conductivity and concentration of NaCl in the flow of permeate and rejection.

3. EXPERIMENTAL WORK

- **3.1.** Materials
	- Deionized water

Deionized water is water that has eliminated almost all of its mineral ions, such as cations such as sodium, calcium, iron and copper and anions such as chloride and sulfate. Deionization is a chemical process that uses specially manufactured ion exchange resins, which exchange hydroxide and hydroxide ions for dissolved minerals, and then recombine to form water. Because most non-particulate water impurities are dissolved salts, deionization produces highly pure water that is generally similar to distilled water, with the advantage that the process is faster and does not increase the scale. However, deionization does not significantly eliminate organic molecules, viruses or discharged bacteria, except by incidental capture in the resin.

• Sodium chloride (NaCl)

The chemical formula of sodium chloride is NaCl and its molar mass is 58.44 g/mol . It is an ionic compound consisting of a sodium cation (Na⁺) and a chloride anion (Cl⁻). Solid NaCl has a crystalline structure, in which each $Na⁺$ ion is surrounded by six chloride ions in an octahedral geometry.

Sodium chloride is a white crystalline solid with a density of 2.16 g/mL, and a melting point of 801 °C. It is also available as aqueous solutions of different concentrations, called saline solutions.

• Triglycine (GGG)

It is the simplest tripeptide, composed of three units of glycine. It has a molecular weight of 189.17 g / mol, has a pKa = 3.2 and pKb = 7.89. It is the tripeptide with greater structural flexibility because it does not have a voluminous side chain that causes steric hindrance as seen in Figure 3.1, it also has a melting point of around 240-250 \degree C and it is also important to mention that triglycine has the three charges electrics as well as nanofiltration membranes.

Figure 3.1 Chemical structure of triglycine

In the nanofiltration test, 99% triglycine was used as the supplier of the commercial house ALFA AESAR, product: A13778 / L11565, 5g.

Description	Triglycine
Symbology	GGG
Molecular formula	$H_2NCH_2(CONHCH_2)_2CO_2H$
	$(C_6H_{11}N_3O_4)$
Molecular weight (Da)	189.17
pK α-COOH	3.2
$pK \alpha$ -NH ₃ ⁺	7.89
Isoelectric point	5.5
Solubility in water at 25 °C (g / L)	64.9

Table 3.I Characteristics of triglycine

• Nanofiltration commercial membrane MPF 36

Table 3.II Characteristics of commercial membrane MPF-36

Description	MPF-36
Made	KOCH Membrane SelRO®
Type of membrane	Acid / Stable base NF
Cutting molecular weight (Da)	1000
Glucose retention	$0.13 - 0.50$
PH conditions	$0-13$
Maximum pressure (bar)	35
Maximum temperature (C)	70
$Lp(Lm-2 h-1 bar-1)$	5.76
Isoelectric point	$5 - 7$

- Osmonics module (SEPA CF II Membrane Element Cell).
- Thermostatic bath (Huber Polystat cc2) and cooling (Haake CK20).
- Piston pump (CAT M / 3CP 1121 pump).
- Speed controller (Yaskawa, VS mini J7).
- Manometers installed at the entrance and exit of the membrane module.
- Pressure regulating valve.
- pH solutions: hydrochloric acid 1 N and sodium hydroxide 1 M.

Figure 3.2 Photograph of the nanofiltration system with membrane MPF 36

Figure 3.3 Commercial membrane MPF 36 Figure 3.4 Food Bottle

Figure 3.5 Thermostatic bath Figure 3.6 Osmonics module

Figure 3.7 Manometers installed at the entrance and exit of the membrane module

Figure 3.8 Piston pump

3.2. Methodology

The peptide used will be triglycine which is a peptide formed by three amino acids of glycine and is the tripeptide with greater structural flexibility because it does not have a voluminous side chain that causes a steric hindrance, also the membrane used will be the commercial membrane of nanofiltration MPF 36, which will be placed in the Osmonics Module (Membrane Element Cell SEPA CF II) which will be connected to a piston pump. The feeding solution of the feed tank will pass through a pump positive displacement (PD pump) and the supply temperature will be set at 20ºC by means of a thermostatic water bath, and manometers will be installed at the entrance and exit of the module and a pressure regulating valve, as shown in *figure 3.9.*

The experimental work was carried out with a discontinuous circulation where the concentrate and permeate streams are returned to the feed tank to reach the stable state and the transmembrane pressure will be measured from the difference between the pressures in the concentrate and the feed streams. The flow rates of the permeate, the rejection and the infiltration were determined from a volume collected at the same time that it was 10 ml.

Two synthetic feed solutions were prepared, first 0.01 M Triglycine was prepared with demineralized water and, as a second solution, triglycine and NaCl were prepared in demineralized water at concentrations of 0.001 M and 0.1 M, respectively. Likewise, we work with pH averages of 2,4,6,8 10, approximately; for which the solutions of hydrochloric acid and sodium hydroxide will be used to reach the desired pH, and also the tests were carried out at pressures of 10, 7 and 3 bar.

Analysis system: TOC (total organic carbon)

For the measurement of triglycine the technique of total organic carbon was used.

The equipment used is a SHIMADZU TOC-VCSH / CSN, with Pt catalyst (in alumina capsules). The TOC technique is used to identify carbon in a sample. This determines the total carbon (TC), but also must measure the inorganic carbon (IC), to obtain the TOC by difference. Determining under acidic conditions all the IC is converted to $CO₂$, which is measured in the infrared analyzer. Under these conditions the organic carbon is not oxidized, so only the IC is determined, the sample is injected into a reaction chamber, at 680 ° C, filled with the oxidizing catalyst. The water vaporizes and the carbon (organic and inorganic) is oxidized to $CO₂$. This $CO₂$ is transported, in the air stream, and measured in a non-dispersive infrared analyzer.

- **3.3.** Experimental Procedure
	- \checkmark The membrane is washed with distilled water lasting approximately one hour.
	- \checkmark The *Lw* (permeability of distilled water) is determined in a range of 2 to 10 bar in increasing order, by means of the regression of the data of Lw and ΔP.
	- \checkmark First the Triglycine solution of 0.01 M is prepared with 4.7286 gr. in an initial volume of 2.5 L, which is placed in the thermostatic basin for temperature monitoring, in which the filtration equilibrium is established, the diagram of the same is shown in figure 3.9.
	- \checkmark The temperature was maintained at 20 °C and the pH of the food constant for each determination.
	- \checkmark The filtration was started with the Triglycine solution at 20 \degree C, at the pH of the solution which was 5.18 without pressure, and after 60 minutes of stabilization of the permeate flow, then it was started by determining the

permeate flow and the sample collection (permeate and rejection). At the same time the parameters of pH and electrical conductivity are measured.

- \checkmark The collected samples are prepared to analyze the total organic carbon (TOC) concentration.
- \checkmark The measurements are made in this way allowing the medium to equilibrate for the next 60 minutes, after each increase in pressure.
- \checkmark Again the whole procedure of pressure variation is performed, but now the pH is varied, firstly in an acid range of 6 to 2 in descending order, using hydrochloric acid to adjust the pH and then in the range of 6 - 10, in ascending order using NaOH to increase the pH.
- \checkmark An additional evaluation is made with respect to the synthetic feed composed of Triglycine 0.001 M and 0.1 M NaCl, 0.4728 g and 14.6179 g respectively, were used for its elaboration and the same procedure was followed.

Figure 3.9 Schematic diagram of experimental device with commercial membranes MPF 36

4. RESULTS AND DISCUSSION

The analysis was made in five trends observed in the present experimental work, the first analysis was made based on the circulation of deionized water through the membrane, the second analysis was performed with the NaCl 0.1 M solution to analyze its behavior with a neutral pH, the third and fourth analysis observed the behavior of triglycine 0.01M and triglycine 0.001 M with NaCl 0.1 M as a function of the variation of pH and pressure and finally the comparative analysis of the behavior of Triglycine 0.01 M and triglycine 0.001 M with NaCl 0.1 M in rejection, permeability, pressure and pH.

4.1. Nanofiltration of Deionized water to 20 °C

The filtration of the deionized water through the membrane at different transmembrane pressures (TMP) was done mainly to densify its support. It is also important to mention that this stage is known as initial pressurization.

In Figure 4.1 we can see that a permeability of 10.17 L/m²h was obtained; likewise, it is observed that there is an optimum slope that crosses the origin, thus obtaining the pressurization that was desired for the stabilization of the membrane which was worked with 4 different pressures of 10, 8 and 5 bar, respectively.

Figure 4.1 Correlation between the pressure and the density of flow of deionized water with commercial membranes MPF 36¹

 \overline{a} ¹ *The line have been added to facilitate the view of the trend of the experimental points.*

4.2. Nanofiltration of Sodium chloride 0.1 M to 20 °C

In this test, the experiment was carried out with saline water at a concentration of 0.1 M, in which the characteristics of the membrane module were observed with respect to the NaCl used.

In the figure 4.2 shows the trend of the flow density (*Jv*) as a function of the variation of the pressure applied to a neutral pH of 6.3, which is observed to be directly proportional, being within the desired values and the expected behavior; also, in figure 4.3 it can be seen that the variation of the rejection as a function of the temperature increase to a pH of 6.3 is directly proportional, reaching a maximum rejection of 10% at an applied pressure of 10 bar, which would comply with the behavior of the studies carried out in the nanofiltration membrane, mainly with what is mentioned in the scientific article about the influence of pH and the operation of variables in biogenic nanofiltration amines mentioned in the introduction of this work, in which it is mentioned that in the neutral pH there will be lower percentages of solute rejection since the passage of the same will be only by the size of it with respect to the pore of the membrane, and as the size of the NaCl ions are small, these would pass without difficulty.

Figure 4.2 Correlation between permeate flow and applied pressure of NaCl 0.1 M

Figure 4.3 Correlation between the rejection of NaCl 0.1 M and the applied pressure at a neutral pH²

 \overline{a} ² *The line have been added to facilitate the view of the trend of the experimental points.*

4.3. Nanofiltration of Triglycine 0.01 M to 20 °C

With respect to the filtration process in the membrane of the triglycine solution in deionized water at a concentration of 0.01 M, there was a directly proportional relation between the applied pressure (bar) and the permeate flow density (Jv).

As can be seen in Figure 4.4, it can be observed that the flow of permeate is directly proportional to the variation of the transmembrane pressure and the variation of pH, since in the 5 samples made at different pH, both acidic and neutral and basic , the permeabilities obtained show that there is a greater permeability to the basic pH with respect to the acid pH, since at $pH = 7.7$ there is a permeability of 6.02 L/m²h and at pH = 2.7 there was a permeability of 2.87 L/m²h as shown in Figure 4.5.

In Figure 4.5 it can be seen that the permeate trend line indicates that even if there is no pressure if there can be a minimum of permeate, this is because the pore size of the nanofiltration membrane is large, so if there is a small flow although there is no pressure, it can still permeate; It is also important to mention that other studies have also analyzed the behavior of the type of organic membrane and have the same behavior. *(Moreno, Carmen; 2006).*

Figure 4.4 Correlation between applied pressure and permeate flow of Triglycine 0.01 M

Figure 4.5 Evolution of the permeate flow (Jv) in function of the applied pressure (bar) at different pH of Triglycine 0.01 M

Figure 4.6 shows the variation of the rejection percentage of Triglycine as a function of the pressure applied to the pH solutions. It is observed that there is a directly proportional relation with respect to the increase of the rejection in relation to the increase of the applied pressure, until reaching in each solution the coefficient of reflection in which a maximum rejection of approximately 80 % generally takes place, presenting then a stability of rejection itself. Likewise, it can be observed that the highest rejections observed were with the most acidic pH solutions and the most basic pH, which have the same behavior, with maximum rejections of 80 % in both solutions. In the same way, the solution that presents a lower rejection would be the neutral pH of 5.1 that would be in agreement with the isoelectric point of the membrane making the passage of the solutes only by the action of the pore size and not by the electric charge.

Figure 4.6 Correlation between the rejection of Triglycine 0.01 M and the applied pressure at acid, neutral and basic pH³

Figure 4.7 shows the relation between rejection in function of pH, in which the variation of the maximum rejection of triglycine as a function of pH is U-shaped. The maximum rejection is high in the solution of the most acidic pH with 81 %, decreases when the pH increases reaching a minimum value of approximately $40%$ at pH = 5.1, and the rejection increases again as the pH increases, acquiring the maximum rejection of 80 % at $pH = 9.2$, this behavior is mainly due to the electrostatic interaction between triglycine molecules and the surface of the membrane.

It must be borne in mind that the surface charge of the membrane also changes with the pH of the solution and how the organic membrane used (MPF 36) has an isoelectric point of about 5 and 7, that is, at an acidic pH the load of the

 \overline{a}

³ *The lines have been added to facilitate the view of the trend of the experimental points.*

membrane is positive and superior pH values the membrane presents a negative charge. At acid pH, the triglycine molecule is positively charged by the protonation of the carboxylic group and the surface charge of the membrane is also positive. Therefore, the repulsion between charges of the same sign minimizes the permeation of the triglycine molecule and, consequently, the maximum rejection is very high. At basic pH, the effect is very similar, with very high rejection values being observed. In this case, both the charge of the triglycine molecule and the surface charge of the membrane are negative and, therefore, exclusion by electrostatic repulsion occurs. At intermediate pH, the triglycine molecule increases its penetration through the membrane due to the decrease in electrical repulsion. In the pH range of 5 to 7, the isoelectric points of triglycine and membrane are located. Therefore, the compensation of the positive and negative charges reduces the electrostatic repulsion, obtaining the value of the smallest maximum rejection at a pH of approximately 5, which will be solely due to the pore size of the membrane, which will corroborate the scientific studies previously mentioned in the present work, since pH plays a very important role at the time of solute retention.

Figure 4.7 Correlation between the pH and the rejection obtained in the different solutions of the triglycine 0.01 M

In the Figure 4.8 shows that at higher rejections, there are lower permeated, so it is verified that the relation between rejection and permeate is inversely proportional and also can be seen in Figure 4.9 said inverse behavior, since it presents the form of an inverted U, which is the opposite of what is observed in Figure 4.7 which has the shape of a U.

Figure 4.8 Correlation between the rejection and the water permeability of the triglycine 0.01 M

Figure 4.9 Correlation between the pH and the water permeability of the triglycine 0.01 M

4.4. Nanofiltration of Triglycine 0.001 M and Sodium chloride 0.1 M to 20 °C

With respect to the filtration process in the membrane of the 0.001 M triglycine solution with 0.1 M NaCl, there was also a directly proportional behavior between the applied pressure (bar) and the flow density of the permeate (*Jv*).

As can be seen in Figure 4.10, it can be observed that the flow of permeate is directly proportional to the variation of the transmembrane pressure and the variation of pH, since in the 5 samples made at different pH, both acidic and neutral and basic , the permeabilities obtained show that there is a greater permeability to the basic pH with respect to the acidic pH, since at $pH = 10$ there is a permeability of 5.97 L / m²h and at pH = 3.5 there was a permeability of 5.22 L / m²h as shown in the Figure 4.11.

In Figure 4.11 it can be seen that the permeate trend line indicates that even if there is no pressure if there can be a minimum of permeate, this is because the pore size of the nanofiltration membrane is large, so if there is a small flow although there is no pressure, it can still permeate; It is also important to mention that other studies have also analyzed the behavior of the type of organic membrane and have the same behavior. *(Moreno, Carmen; 2006).* Then, comparing with the experimental data of figure 4.5, it is observed that regardless of the concentration of the solute in the solution, although there is no pressure in the membrane module, there can also be a minimum permeate.

Figure 4.10 Correlation between permeate flow and applied pressure of Triglycine 0.001 M and Sodium chloride 0.1 M

Figure 4.11 Evolution of the permeate flow (Jv) in function of the applied pressure (bar) at different pH of Triglycine 0.001 M and Sodium chloride 0.1 M

The effect of ionic strength on the nanofiltration of triglycine was analyzed by adding a concentration of NaCl 0.1 M. Figure 4.12 shows the maximum rejection of triglycine depending on the pH, in which a clear tendency is observed of the maximum rejections which would have the acidic pH of 2.4 and the more basic pH of 10, it could also be say that they are around 60 - 70 %. This value is relatively close to that obtained in absence of NaCl as shown in Figure 4.6, where the maximum rejections of triglycine were in 80 %. In addition, a decrease in rejection at neutral pH of approximately 40 % to 30 % in the presence of NaCl. This decrease observed in the rejection of triglycine in presence of NaCl, may be due to the decrease of the electrostatic interaction between the molecules of triglycine and the surface of the membrane. Thus, it can be considered that NaCl produces an effect shielding the surface charge of the membrane, changing its electrical structure, decreasing the repulsion of the triglycine molecules and, therefore, their rejection. This effect is difficult to specify its mechanism of interaction, in fact in the bibliography no articles have been found to specify the causes of the decrease in electrical interaction. Some authors point that the increase of the ionic strength can cause a variation of the dielectric constant within the pores of the membrane that could facilitate the permeation of triglycine molecules.

Figure 4.12 Correlation between the rejection of Triglycine 0.001 M with NaCl 0.1 M and the applied pressure at acid, neutral and basic pH⁴

In Figure 4.13 it was observed that the variation of the maximum rejection of the triglycine molecule of 0.001 M in the presence of NaCl 0.1 M at different pH, has the V-shaped, which in comparison with figure 4.7 in which the solution of triglycine 0.01 M had no concentration of NaCl presents minor rejections. Also, it is important to mention that the maximum rejection is found both in the acid pH of 2.4 and in the most basic pH of 10. In this case, the maximum rejection is between 60 to 70% and the minimum rejection is between 30 to 40 % at a

 \overline{a}

⁴ *The lines have been added to facilitate the view of the trend of the experimental points.*

neutral pH of 6.5. Therefore, the presence of NaCl reduces the maximum rejection of triglycine, since NaCl produces a screening effect of the membrane surface charge increasing the permeation of the triglycine molecule with positive charge, being able to conclude that when increasing the force ion of the feed solution, the MPF 36 membrane changes its electrical condition, observing a change of the reflection coefficient (σ) .

Figure 4.13 Correlation between the pH and the rejection obtained in the solution of triglycine 0.001M with NaCl 0.1 M⁵

Figure 4.14 shows that at higher rejections, there is greater permeability having a directly proportional behavior observing the opposite with respect to Figure 4.8, this is due to the precise NaCl since the effect of screening on the membrane makes that the permeability is much greater, it is also observed that figure 4.15 that the relationship between pH and permeability does not have a definite shape.

Figure 4.14 Correlation between the rejection and the water permeability with a solution of triglycine 0.001M with NaCl 0.1 M

 \overline{a}

⁵ *The line have been added to facilitate the view of the trend of the experimental points.*

4.5. Comparison between the solutions of triglycine 0.01 M and the triglycine 0.001M with NaCl 0.1 M

Figure 4.16 shows the comparison of the rejection behavior as a function of the applied pressure of both the triglycine solution at 0.01 M and the 0.001 M triglycine solution with NaCl 0.1 M, in which we can see that in both behaviors they have the same tendency to reach a maximum rejection and then follow a horizontal trend, but it is also observed that in the presence of NaCl there is a reduction of approximately 10 % of the rejections obtained in relation to the rejections of the single triglycine solution, because the NaCl produces an effect that protects the surface charge of the membrane , changing its electrical structure, decreasing the repulsion of triglycine molecules and, therefore, its rejection.

Figure 4.17 shows the comparison of the rejection behavior in function to different pH, in which it can be observed that the action of the pH plays a very important role in all solutions, since in spite of the solution of triglycine 0.001 M with NaCl 0.1 M presents minor rejections, the tendency of the permeation behavior of the solute is similar, because it has greater rejections when you have the acid and basic pH of both solutions due to the protonation of the carboxyl group and the de-protonation of the amine group that triglycine possesses.

In figure 4.18 it can be seen that depending on the triglycine solution 0.01 M the relation between rejection and permeability is inversely proportional, however the opposite happens in the solution of triglycine 0.001 M with NaCl 0.1 M since it presents a relationship directly proportional, this may be due to the action of the screening that produces NaCl on the surface of the membrane.

It is observed in Figure 4.19 that both solutions show similar behaviors, since it is observed that at higher pH, basic pH, the tendency is to have higher permeabilities, and when there are lower pH, acid pH, the permeability decreases, said action is due to the proper behavior of the charge of triglycine with the charge of the surface of the membrane, mainly by the protonation of the carboxyl group and deprotonation of the amine that it has in its chain.

Figure 4.16 Correlation between the rejection and the pressure applied in the solution of triglycine 0.01 M and triglycine 0.001 M with NaCl 0.1 M ⁶

Figure 4.17 Correlation between the rejection and the pH in the solution of triglycine 0.01 M and triglycine 0.001 M with NaCl 0.1 M ⁷

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Figure 4.18 Correlation between the Lp and the rejection in the solution of triglycine 0.01 M and triglycine 0.001 M with NaCl 0.1 M

Figure 4.19 Correlation between the Lp and the pH in the solution of triglycine 0.01 M and triglycine 0.001 M with NaCl 0.1 M

⁶ *The lines have been added to facilitate the view of the trend of the experimental points.*

⁷ *The lines have been added to facilitate the view of the trend of the experimental points.*

5. CONCLUSIONS

A nanofiltration membrane has been characterized by the filtration of deionized water and a solution of NaCl. The membrane presented a high water permeate flux and very low NaCl retention. This is due to the fact that this membrane has a molecular weight cut off of 1000 Da. On the other hand, the main conclusions arising from the results obtained with the triglycine are as follows:

The rejection of triglycine (MPF 36) depends very much on the solution pH, since the maximum rejection of triglycine in a solution only of deionized water is 80 % - 90 % with an acidic pH, with a pH neutral has a rejection of 40 % (near its point isoelectric) and 80 % - 90 % at basic pH.

When it has triglycine in a solution of deionized solution with NaCl, the rejection is reduced even when it has an acid, neutral and basic pH, since NaCl produces an effect that protects the surface charge of the membrane, changing its electrical structure, decreasing the repulsion of the triglycine molecules and, therefore, their rejection.

It was observed that an increase in the ionic strength of the feed solution decreased the maximum rejection of the amino acid and the peptide, the initial premise is an alteration in the electrical charge of the MPF 36 membrane.

6. RECOMMENDATIONS

It is recommended to carry out studies to identify the phenomenon that occurred when increasing the ionic strength in the MPF 36 membrane and its repercussion with the maximum rejection of solute.

It is recommended to study other peptides with the application of the Spiegler-Kedem model, in order to analyze the variation of the ionic strength of feeding, the selectivity and the variation of the pH with the type of organic membrane MPF 36.

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8. ANNEX

Membranes	◡ Benefits	Shortcomings
Organic (Polymeric)	Easy to process	Limited selectivity
	Good mechanical properties	Limited resistance to high
		temperature
Inorganic	Relatively inexpensive	Limited chemical durability
	High selectivity	Difficult to process
	High temperature capability	Brittle
	Chemically resistant	Expensive

Table 8.I Summary of benefits and shortcoming of organic and inorganic membrane

Figure 8.1 General classification of membranes, based on their nature, separation regime and geometry

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Table 8.II Case studies of the application of nanofiltration membrane technology for the separation of amino acids and peptides, published in high impact journals. (full study)

* There is no information

^b The feed solution contained a mixture of glycine, l-alanine, l-serine, and l-glutamine (0.001M of each amino acid).

c The feed solution contained a mixture of glycine, l-serine, and l-glutamine (0.001M of each amino acid).

^d The feed solution contained a mixture of glycine, l-alanine, and l-glutamine (0.001M of each amino acid).

CalibrationReport

Calibration with fixed sample volume: 500,0µl

CalibrationReport

Replicate area units

