The permeation of protein solution at ultrafiltration through indigenous polyurethane membranes

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Abstract

The results of the current investigation confirm that both, the pore size distribution and interaction forces working between solute and membrane material govern solute separation in ultrafiltration. Negatively charged macromolecules (ionically charged casein) were sieved more effectively, while positively charged ones less effectively, than neutral macromolecules which result is consistent with the existence of negative charges on the membrane surface.

The mass transfer through the MB_1 and MB_2 polyurethane membranes highlights the correlation between permeability and the value of the rH parameter of the insulin solutions.

Keywords: polyurethane membrane (PU), ultra and microfiltration (UF-MF), rH parameter, electrostatic charge.

Introduction

Ferry has reviewed much of the early work in his 1936 review of ultrafiltration-microfiltration (UF-MF) [1]. Principled concerns were expressed with the filtration of proteins [2], blood sera [3], enzymes [4], toxins [5], bacteriophages [6].

The last two decades have witnessed the renaissance of interest in membrane separation processes to the extent that their large-scale application in the water treatment, food and medical industries appears imminent.

The increasing demand of protein concentrates and the need of economic treatment in order to reduce pollution explains the increasing interest in protein recovery, especially of other useful compounds as well, from the residual waters and their possible reuse in animal feed, in food products and industrial-related fields.

The protein and other useful industrial compounds recovery, for instance, the lactose, can be advantageous for glucose production by the enzymatic hydrolysis of the recovered lactose, having a benefit in the balance of payments as main consequence.

The casein recovery from the milk-based products (separated milk, milk whey) within a plant producing 25000 liters of whey ensure a reduction of the pollution similar to that released in a city of 500000 inhabitants [7].

The chemical, the chemico-physical methods and the conventional biological treatment render this recovery possible, but destroy the important compounds present in the residual waters. A special method such as flocculation with ligninsulphonates renders this recovery from casein water (0,6-0,7% casein) [8], and from the waters used in the amide-producing industry [9] (1,3-1,6%), difficult and non-economic.

The ultrafiltration-microfiltration (UF-MF) is likely to be used besides heat clotting, with less energy consumption (lower by 40% than in case of heat clotting) [10].

This technique (UF-MF) used cellulose and polysulphone acetate [11], at pressures of 10 atm. For the casein separation (M ≅ 20000) from the carbohydrates, for instance, the lactose (M ≅ 360) and from the mineral salts and amino acids.

The permeability efficiency was not significantly influenced by a pressure increase, which can, in return, cause an increase of the concentration polarization. A flow (3-7 m^3/h) and temperature increase determine, instead, a linear enhancement of permeability [12].

The temperature was maintained between such values which allow process economy, avoiding the degeneracy of the recovered protein material and operating in such conditions as to avoid a possible microbial contamination. Such last objective can be achieved either by reducing the critical range or operating at temperatures of 10 or 50^0C [8].

The membrane, a region of imperfect discontinuity between two phases, allows certain particles to pass through it and to stop the passing of others; the membrane permeability is generally correlated to the sizes of the substance particles and of the membrane pores. The membrane becomes an active or passive element of separating the two phases, among which a mass transfer manifests, the electrical charge playing an important role.

In order to highlight certain correlation possible between the polyurethane membranes permeability studied in this work and the value of the rH parameter of the insulin solution used in the measuring system, determinations were performed regarding the mass transfer [13].

The objective of this work was to determine the proper operating conditions, the efficiency for ultrafiltration-microfiltration (UF-MF) of casein solutions and of separated milk.

Material and method

Materials

We used the polyurethane membranes of MABEDAN: MB_1 with the dimension of pores of 3-4 μm and MB_2 with the dimension of pores of 1-2 μm and PR_2S with the dimension of pores of 0,5-1μm.

The experiments have been carried out with solutions of casein (0,5%) and separated milk with 3% protein; 0,8% fat; 3,85% lactose, N% total 0,46% at 25^0C and 60 mmHg, respectively 2 bar [14].

Method

The membranes of polyurethane (PU) type, MABEDAN, were prepared by casting and the development of membrane structure while the polymer processing was achieved [15].

The permselectivity of membranes for the casein solutions was tested in a laboratory installation of UF-MF (vacuum: 60 mmHg, $S_{membrane}$ = 0,003m^2).

The permselectivity of membranes for the separated milk was tested in a laboratory installation of UF-MF (2 bars; 0,03m^2).

In order to highlight certain possible correlation between polyurethane membranes permeability and the value of the rH parameter ($rH = \frac{E_h + 0{,}058\,pH}{0{,}029}$; E_h: the redox potential), for the protein solutions, the insulin transfer was monitored (the first protein to be synthetically achieved) by the MB_2 membranes. An original device (redoxtron) was used, which allows for the rH linking at the desired values and highlighting the substance transfer by the membrane separating two compartments. In a redoxtron compartment, 100 cm^3 of

$K_2SO_4.10^{-4}$M solution and the researched substance in the known concentration (donor phase) was added and in the other compartment, only 500 cm^3 of $K_2SO_4.10^{-4}$M solution (acceptor phase). In the donor phase, a stirring applied and in the acceptor phase, continuous current applied at a 30V voltage, no stirring, with a certain position of the electrodes to the membrane, so that, at the membrane level, certain values of the rH were created (between 2,75 reducer and 42,16 oxidant). The insulin transfer through the PU membrane was highlighted conductometrically, by measuring the current intensity (mA) in the solution of the acceptor phase. The value of the redox E_h potential was determined for the insulin solution, potentiometrically, using a SFIBOLD device equipped with a platinum electrode and a reference electrode of saturated calomel, in an air-tight pan and placed on ice. Concomitantly, the pH values were determined, by the potentiometric method, using a glass electrode and a calomel electrode.

The electrokinetic measurements on the membranes were performed using an Electrokinetic Analyser (EKA) (Anton Paar KG,Graz, Austria), by measuring the streaming potential between two electrodes.

A 10^{-3}M aqueous solution of KCl was used as a test solution. The zeta potential was calculated from the streaming potential determined in dependence on the pressure difference and from the specific conductivity of the solution according to the Smoluchowski equation.

The miscibility of the polyurethane system has been characterized by scanning electronic microscopy (SEM). All scanning electron microscopy observations were made with the aid of a Phillips 400 electron microscope.

The presence of casein was tested through quantitative analytical determination. The membrane performance is given by the capacity of retaining $R\% = (1 - C_1/C_2)100$ where C_1 and C_2 represent the permeate concentration, the initial solution concentration respectively.

Results and discussions

The ultra-microfiltration system schematic design is shown in Figure nr. 1 and Figure nr. 2. All experiments were performed in the batch mode at room temperature.

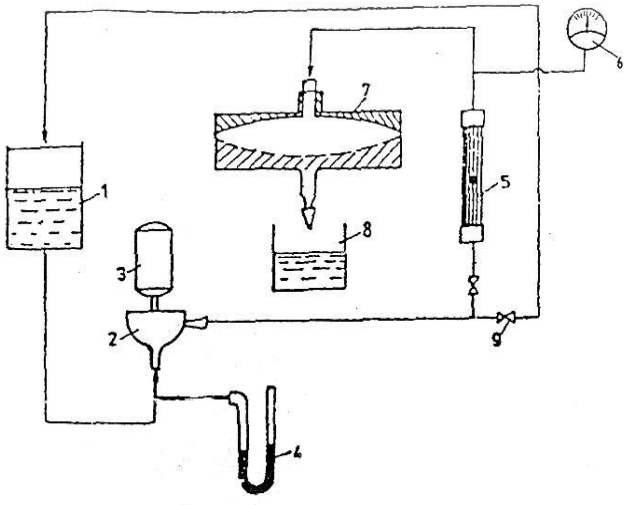
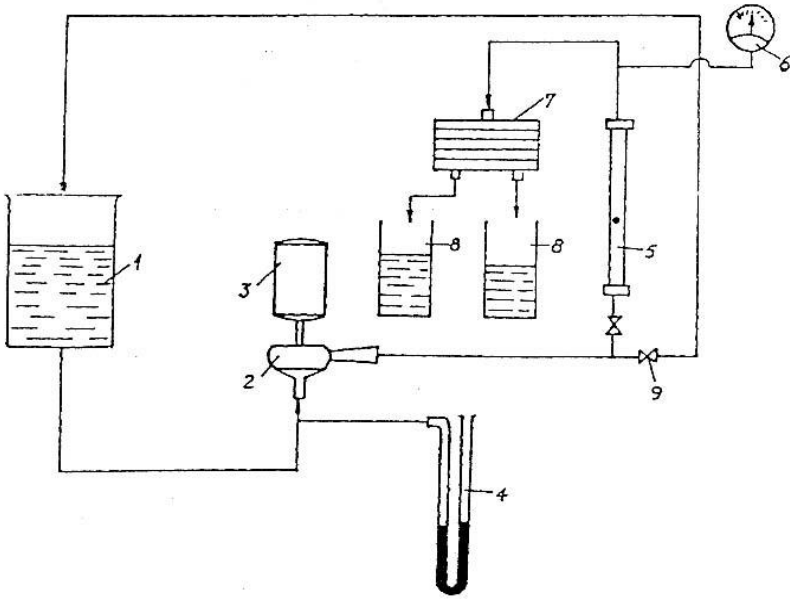


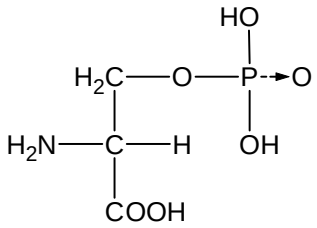
Figure 1. The UF-MF laboratory installation
(vacuum: 60 mmHg, $S_{membrane}$= 0,003 m^2): 1) tank, 2) centrifugal pump,
3) electric motor, 4), 6) manometers, 5) rotameter, 7) monolayer ultrafiltration module, 8) permeate tank



1) loading ta) multilayer ultrafiltration module, 8) tanks for concentrate and diluting milk-based product

The main milk protein is a phosphoproteid called casein. The casein is not a unitary substance; it was separated by electrophoresis and chromatography in fractions $\alpha_{S1,}$ α_{S2}, β and γ.

The molecular weight varies between 6500 and 27000. The casein contains approximately 0,9% bonded phosphor, in the form of phosphoric ester of the serine CH_2OH – groups (phosphoserine):



phosphoserine

The phosphoric acid residues (phosphoserine) gives the casein acid character (isoelectric point 4,6). The milk caseins probably have, in native state, globular conformation as the milk viscosity is not much higher than water viscosity. γ-casein (molecular weight 19000), unlike other caseins, is soluble and forms soluble complexes with other caseins. γ-casein contains 165 ± 5 amino-acids and it is the only one among the caseins to contain sugars (glyco-proteid).

In general, the membranes used for ultrafiltration are characterized by their molecular weight cut-off (MWCO).

The MWCO is the molecular weight of a globular molecule that is 90% rejected by the membrane. The MWCO is used to predict the ability of a membrane to filter out globular macromolecules. The MWCO is not precise because it depends on the solute rejection.

The electrostatic charge is a determining structural characteristic of the considered polyurethane membranes, the substances transmission or retention respond to a double mechanism based on the molecular weight and the peptides charge. Various strategies are offered in order to maximize this double selectivity. The said strategies are linked to the selection of the physical-chemical conditions: pH, the solutions' ionic force. The peptides and membranes electrostatic charge, according to the pH and the solutions' ionic force can determine the direction (attraction or repulsion) but also the amount of peptides-membrane interactions.

Figure 3 schematically shows such differences in transport property between the usual semi-permeable membranes and a charge membrane. In the former case, the volume flow occurs from the diluted side to the concentrated side, while in the latter case, the direction of volume flow is reversed due to the coupling of volume and salt flow (negative osmosis occurs).

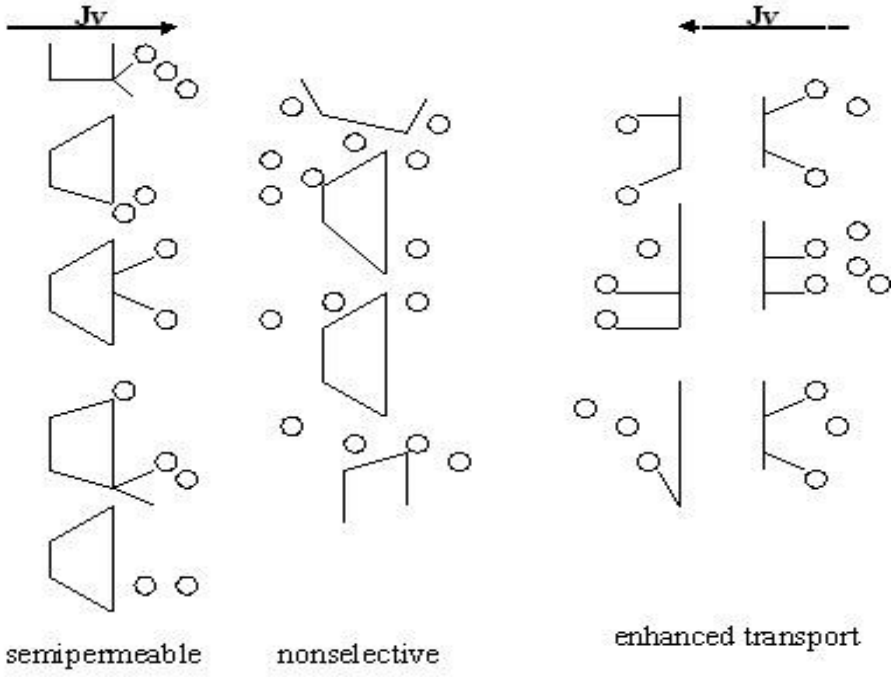


Figure 3. Differences in transport properties between usual semi-permeable membranes and a charge membrane

It is now possible to predict membrane performance in ultrafiltration as a function of data on average pore size, pore size distribution, and the interfacial forces, in addition to the operating conditions of the experiment.

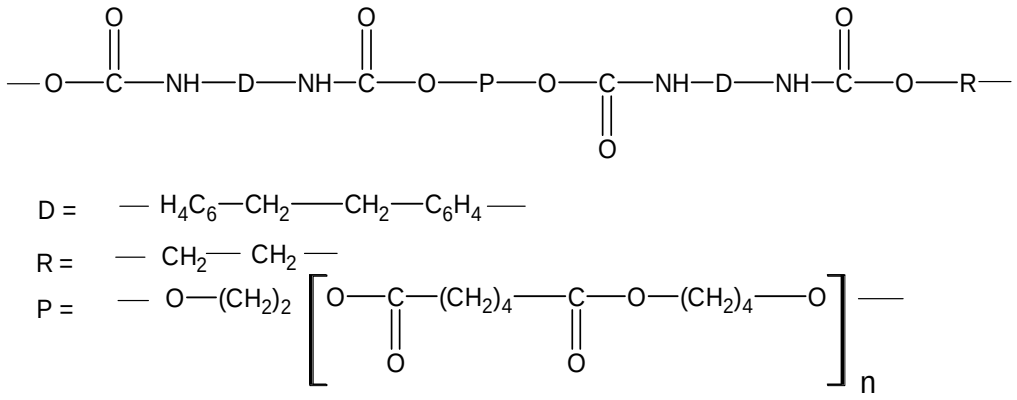
The experimental data regarding the PU membranes PU by UF-MF (void: 60mmHg, T = 25^0C) of the casein-based solutions 0,5% are presented in Table 1. The transformation efficiencies are of 95%

Table 1. The experimental data regarding the PU membranes valorization by UF-MF (void: 60mmHg, T: 25^0C) of the casein-based solution 0,5%

Membranes	Φ μm	Permeability speed l/m^2h	Transformation efficiency %	Protein %
MB_1	4	5	95	0,025
MB_2	2	3	90	0,050

The experimental results regarding UF-MF of the separated milk (3% protein; 0,46% N total; 0,8% fat; 3,85% lactose), at: 2 bar; T = 25^0C are presented in Table 2.

The PU membranes were used PU: MB_1 and MB_2 in which the base polymer is the PR 100 polyurethane PR 100 with structure:



And the PRS_2 membrane in which the basic polymer is PR 200 with structure:

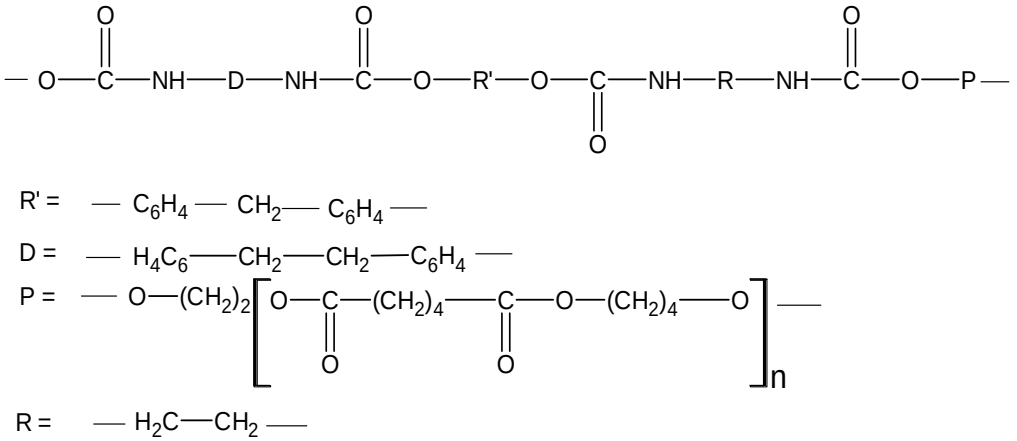


Table 2. The experimental results regarding UF-MF with the polyurethane membrane at 2 bar; 25^0C of the separated milk: 3% protein; 0,46 $ N total;0,8% fat; 3,85% lactose

Membrane	Protein %	Transformation efficiency %	N total %	Lactose %	Permeability speed l/m^2h
MB_1	1,1	63,33	0,19	3,56	10
MB_2	1,5	50,00	0,23	3,56	5
PR_2S	1,0	66,66	0,17	3,70	70

Spectacular results were found at the UF-MF of the separated speed, with 50-70% casein retention degree. The casein release is executed in this case from systems in the form of emulsions (separated milk), highlighting, at the same time, a lactose selective separation (in the permeate, the lactose is found in proportion of 3,56- 3,70%, while in the initially separated milk, it is of 3,85%)[14].

The different permeability speed for the three membranes, recorded at the milk UF-MF verifies the different morphologies achieved by scanning electronic microscopy (Figure 4). The lowest value is recorded by the MB_2 membrane (5 l/m2h).

a)

Figure 4. SEM micrograph of the polyurethane membrane: a) MB_1 b) MB_2 c) PR_2S (x 3000)

The electronic microscopic images (x 3000) highlight higher globular microphases in the MB_2 membrane and lower in the MB_1 and PR_2S membranes. The image is the result of the electrons' impacting with the atoms in the membrane structure and their reflection in a distribution, more or less deviated.

Thus, the structural indicators influence the samples functionality. If we take a look at the PR 100 and PR 200 basic polymers chemical structure, we notice a different separation degree of the soft and hard phases (higher in PR100 than in PR200):

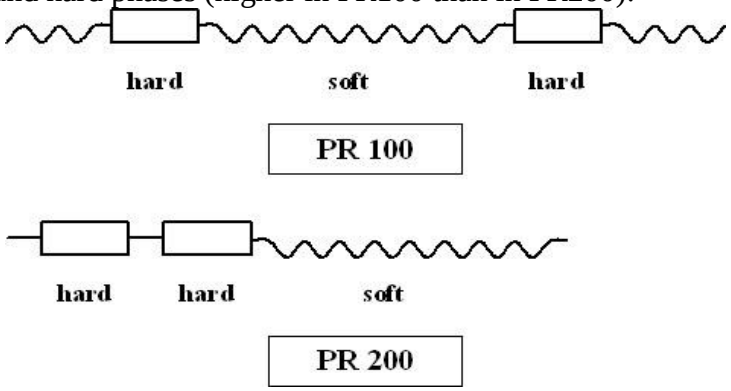
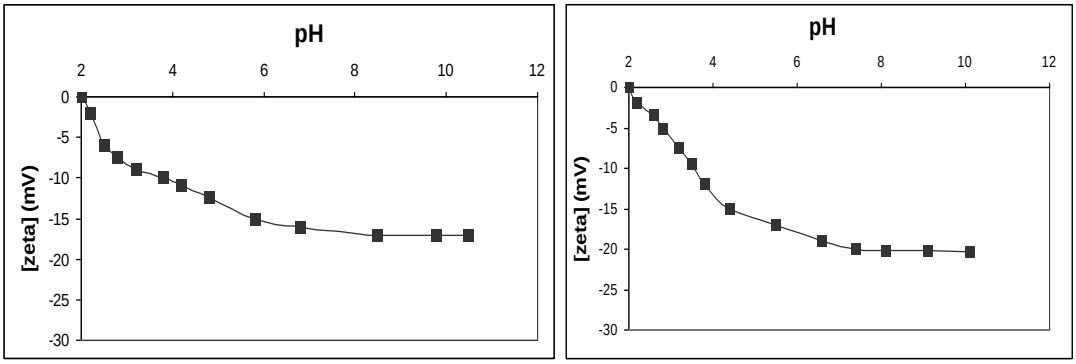


Chart1

In context, the supra-molecular laying of the polymeric chains will be different as well. A higher supra-molecular uniformity is noticed for the MB_1 and PR_2S membranes due to the technical conditions of the membrane preparation as well.

The negative electrostatic charge is a determinant structural characteristic of the studied polyurethane membranes.

Thus, the electro-kinetic measurements performed on the MB_1 and MB_2 membranes showed a zeta potential of -25 V for the MB_1 membranes while for the MB_2 membranes, a lower value of -20 V (figure 5).



(a) (b)

Figure 5. [zeta] potential/pH profile for (a) MB_2 polyurethane membrane and (b) MB_1 polyurethane membrane

In addition, for highlighting certain correlations between the MB_1 and MB_2 asymmetric polyurethane membranes permeability (with a preponderance of the negative electric charges on one side and positive on the other side) and the value of the rH parameter of the insulin solutions (a polypeptide with molecular mass of 5750-6000, formed by the condensation of 51 molecules of amino-acids) the mass transfer was monitored depending on the rH.

The initial value of the a rH parameter of the insulin solution is of 27,38. Considering that the rH values between 0 and 28,2 is the reducing field of the rH and those between 28,2 and 42,5 represent the oxidant field, we find that the insulin subject to dialysis through the polyurethane membranes has an rH optimum to the membrane transfer in the reducing field, while the procaine subject to dialysis through the same polyurethane membranes shows a rH

optimum to the membrane transfer in the oxidant field. The nistatine subject to the same treatment shows amphoteric character.

This outcome results in the molecular volume to be in direct linking with the molecular mass and which seems not to significantly influence the dialysis process under the conditions of the imposed rH (Table 3)

Table 3. The molecular mass (M) and the optimum rH to the substances used to highlight the polyurethane membrane in various rH values

Substance	M	Optimum rH
Nistatine	986,08	Extreme Red ; extreme Ox
Insulin	6000,00	25,00
Procaine. HCl	272,78	29,50

Highlighting the membrane selectivity dependence upon the phases' redox characteristics allows us to propose the described procedure as parameter and the membranes characterization method, along with the other criteria as well as for the analysis and separation of the membrane separation processes.

Conclusions

The PU membrane becomes an active element of two phases' separation, between which a mass transfer manifests and in which the electrical charge plays an important role.

The peptides and the membranes' electrostatic charge according to the pH and to the solutions' ionic force can determine the direction (attraction or repulsion) but also the amount of the peptide-membrane interactions.

The structural indicators influence the samples functionality. The supra-molecular uniformity depends upon the soft and hard phases distribution on the macromolecular chain.

Highlighting the membrane selectivity dependency upon the redox characteristics allows us to propose the determination procedure of the rH optimum to transfer, as method of characterizing the asymmetric feature for the studied polyurethane membranes.

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