Concentration and Purification of Collagen Proteins by Ultrafiltration

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The objective of the present study was to evaluate the performance of the ultrafiltration process on the recovery of collagen solution constituents at laboratory scale, using a tangential flow filtration, with flat regenerated cellulose membrane (5000 Da). Also permeate flows were evaluated along with the physicochemical characteristics of the concentrates, the permeates and feed solutions. The regenerated cellulose membranes were morphological studied by electron microscopy and characterized by water solutions permeation at different pressures.

Keywords: collagen, concentration, protein, purification, ultrafiltration

Pressure-driven membrane separation processes such as reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF) and microfiltration (MF) are used either separately or as a combination of membrane techniques to achieve a high quality product by efficiently removing bacteria, viruses, dissolved solids, colloidal particles, biomolecules, organic micropollutants, proteins, polymers, sugars or inorganic ions [1-3].

Ultrafiltration is a very attractive membrane separation process (MSP) [4], as it does use heat and as a consequence does not involve a phase change, which makes the concentration process more economical [2, 5, 6].

UF processes are widely used in biotechnology for separation and purification of proteins and bioactive substances with molecular weights ranging from 50 to 50.000 Da which corresponds to a medium diameter of the pores between 0.1 - 1 nm (10 - 1000 Å) [2, 7], in the food industry for the improvement of taste and stability of beverages, in wastewater treatment and in production of drinking water mainly due to their excellent properties, such as their ability to remove various viruses and much of the dissolved organic matter [8-10].

Ultrafiltration membranes are frequently obtained from polymeric materials by the phase inversion process [11, 12].

Collagen is the most abundant component of the extracellular matrix and many types of soft tissues, accounting for almost 25 to 30% of the total protein in the animal body [13, 14] and it exercises various functions, depending on its location [15]. It can be extracted from various animal species and it is generally derived from slaughter by-products. The main sources of collagen are the skin, tendons, cartilage and bones.

Collagen is considered to be one of the most useful biomaterials because it has a wide range of industrial applications: in the food industry for collagen and gelatin, in the pharmaceutical fields, in the medicine has been used in cardiovascular surgery, plastic surgery, orthopedics, neurology [16].

Experimental part

Material and products

The chemical reagents used in the present study were collagens with the molecular weight of 2000 Da (S1), 5600 Da (S2), 7000 Da (S3) and 10000 Da (S4). The collagen was provided from bovine skin [15, 17] and the solutions were prepared by dissolving protein in ultrapure water, with 1% final concentration. The solutions were gently stirred for 1 h to ensure homogeneity at 25ºC. The water used was high-quality deionized water (>15 $M\Omega \cdot cm^{-1}$) produced by an Elix Technology Inside (Milli-Q, France) equipment.

During experiments, a regenerated cellulose UF membrane with the molecular weight of 5000 Da was purchased from Merck Millipore. In Table 1 the characteristics of the membrane was presented.

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Table 1
MEMBRANE SPECIFICATION AND PROPERTIES

Type	Ultrafiltration
Material	Regenerated cellulose
Molecular weight cutoff (MWCO)	5000 Da
Operation pressure	4.83 bar
pH in continuous operation	3-13
Maximum temperature	50°C

Membrane and filtration apparatus

The filtrations experiments were performed with the laboratory installations Koch membrane system LABCELL CF-1 type, ensuring a cross-flow mode (feed stream flowing tangentially to the membrane surface) that is schematically presented in Figure 1.

The experimental setup, exclusively made from stainless steel comprises the following equipments: feed tank (1) with a volume of 500 mL, pneumatic pump with a flow capacity of 1.8 L/min (2), manometers (3), a housing for membrane with diameter of membrane 76 mm and a membrane effective area of 28 cm^2 (4), finally a scale from 0 to 60 bar. The maximum working pressure was 35 bar and the optimal pressure was 8 bar. The maximum optimal temperature was 70ºC and the liquid velocity at tangential liquid flow was 2 m/s. A manual valve was used to control the transmembrane pressure.

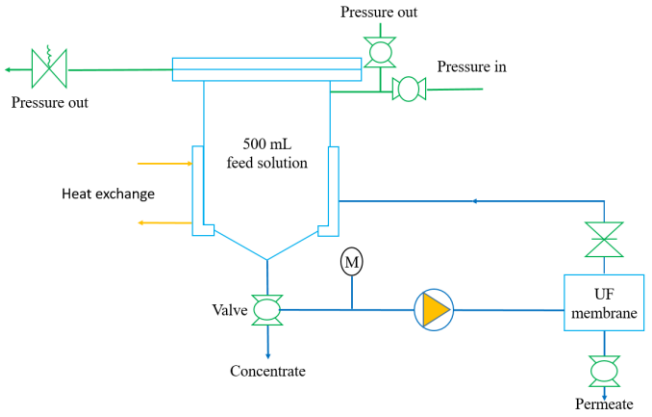

Fig. 1. KOCH LABCELL-CF1 ultrafiltration installation type

Experimental procedure

The experimental setup that has been used ensured a tangential flow mode in the separating process. The permeate was continuously collected outside the membrane module, while the retentate was recirculated to the feed tank.

First the characterization of the membrane's transport properties was made by determining the dependence of ultrapure water volumetric flux on a transmembrane pressure (TMP) in the range of 2 to 6 bar and the readings of the flow rate were made at several volumes (50 mL, 100 mL, 150 mL, 200 mL, 250 mL). From the data obtained results that the optimum pressure for the concentration of collagen is 5 bar.

The experiments consisted of four steps: in the first step the permeate flux (Jw) for a new membrane at 5 bar pressure with ultrapure water was measured. The ratio between this Jw and the TMP of the experiment provided the pure water permeability (PWP), which represents a main characteristic of a membrane. In the second step, the collagen filtration process was determined: the storage tank was filled with the solution (500 mL) containing the collagen at the desired initial concentrations (1%); 250 mL permeate and 250 mL concentrate were collected. In the three step, once the collagen filtration experiment was finished, the water permeate flux was measured again in order to determine the irreversible membrane fouling and thus, the different resistances of the filtration process. At the final of experiments, samples of the feed, retentate and permeate were analyzed.

Analytical methods

The concentration and purification characteristics of the initial solution, concentrate and permeate samples were determined by analysis of the following parameters: *temperature*, *pH*, *electrical conductivity, nitrogen content and protein concentration*.

The *temperatures, pH* and *conductivities* analysis were measured by a Multi 340i WTW multiparameter equipment with electrode model TetraCon 325 WTW for electrical conductivity and temperature and electrode model SenTix 41 for pH. A Shimadzu analyzer, model TOC-L CPH/CPN was used to determine the *nitrogen content*.

The *protein concentration* was determined by the Lowry method. These methods are spectrophotometric and the absorbance readings were performed in a UV visible spectrophotometer (Specord 210 PLUS from Analytic Jena). The method is based on the formation of a cupric complex when protein reacts with alkaline copper reagent (biuret reaction) and the reduction of phosphomolybdate and phosphotungstate from Folin-Ciocalteu reagent by phenolic compounds in protein. Membrane surface morphology was visualized by using a SEM Quanta FEG 250 equipament.

Theoretical calculations

Membrane fluxes were calculated as permeate function using measured volumes in a determined time interval with the equation (1):

$$J = \frac{1}{A} \cdot \frac{(\Delta V_{permeat})}{(\Delta t)} \qquad (1)$$

where:

J = volumetric permeate flux $\left(\frac{L}{m^2 \cdot h}\right)$;

A = membrane effective separation area (m^2);

ΔV = permeate volume collected during the time interval Δt;

Δt = time of permeation and sample collection (h).

The membrane rejection of regenerated cellulose membrane was calculated by a rejection coefficient determined by equation (2) as follows:

$$R = \left(\frac{C_f - C_p}{C_f}\right) \times 100 \qquad (2)$$

where:

R - removal rate of contaminant (%);

C_f and C_p are the concentrations of each contaminant in the feed and permeate solutions respectively (mg/L).

Results and discussions

Membrane characteristics

Transport properties of the regenerated cellulose RC membranes were evaluated on the basis of the TMP dependency on ultrapure water volumetric flux in the range of 2-6 bar and was presented in Figure 2.

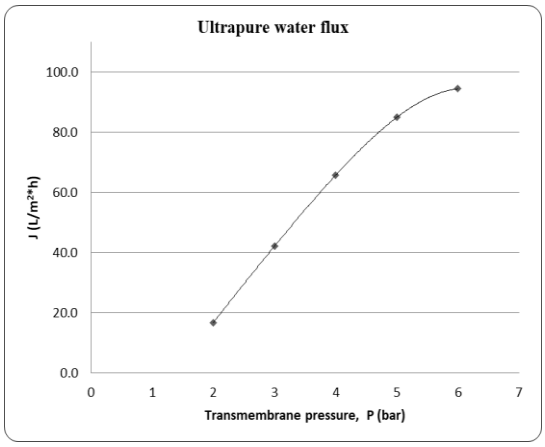

Fig. 2. Ultrapure water flux determined for tested RC membrane as a function of transmembrane pressure in the range of 2-6 bar

As shown in the curve in Figure 2, the permeation flux of ultrapure water increases in a linear manner (between a 2 to 4 bar) with increasing transmembrane pressure following a decrease in flux between a 4 to 6 bar. The phenomenon is

attributed to the pore closing effect by compressing the active layer as a result of the pressure effect. In Figure 2 the optimum pressure of 5 bar was observed.

Membrane permeate flux has been used to characterize the productivity of a membrane filtration system. The permeate flux, J was calculated by using the Darcy equation. The flows (L/m^2*h) obtained in the UF process were: 78.3 for S1, 75.2 for S2, 70.4 for S3 and 60.6 for S4. The permeate flows recorded for the four samples was shown in Figure 3.

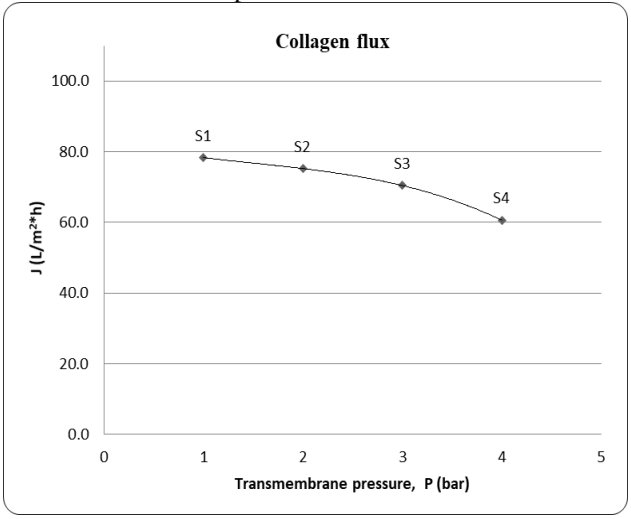

Fig. 3. Collagen solutions flux determined for tested RC membrane as a function of transmembrane pressure of 5 bar

It was found that as the molecular mass of collagen increases, the flux decreases, which is explained by the intensification of the polarization phenomenon.

The results obtained in the process of collagen ultrafiltration with different molecular weights by the same type of membrane are shown in Table 2.

Table 2
CHEMICAL AND PHYSICAL PROPERTIES OF COLLAGEN SOLUTIONS

Chemical-physical properties	Solution type											
	S1			S2			S3			S4		
	1	2	3	1	2	3	1	2	3	1	2	3
Temperature (ºC)	20.4	21.6	21.3	24.0	24.3	24.6	16.8	19.1	20.1	20.2	20.6	21.2
pH	8.28	8.07	8.26	7.08	6.95	7.02	8.07	7.90	8.0	8.46	8.28	8.27
Conductivity (µS/cm)	3420	3070	3370	1421	1246	1477	1656	1433	1680	2160	1850	2180
Nitrogen content (mg/L)	1218	615	1812	1251	148	2343	1779	117	3422	1895	70	3701
Protein concentration (mg/L)	6802	3322	10264	7486	790	14162	9486	490	18470	10096	340	19841
Ration protein: nitrogen	5.59	5.40	5.67	5.98	5.35	6.04	5.33	4.17	5.40	5.33	4.85	5.36

These data highlights the fact that the rejection is 49.5%, calculated according to the nitrogen content and 51.2% depending on the protein content for S1. For the collagen with molecular weight 5600 Da (S2) was obtained a rejection of 88.2% for nitrogen content and 89.4% for the protein. For S3 collagen solution a 93.4% rejection coefficient was obtained for nitrogen content and a 94.8% for protein content. For S4 was obtained a rejection of 96.3% calculated according to the nitrogen content and 96.6% depending on the protein content for S4.

The values obtained are quite close to the two indicators (nitrogen, protein), demonstrating a good correlation between the type of collagen and their nitrogen content. An average protein to nitrogen ratio of 5.37 is obtained which is close to the value of 5.62 mentioned in the literature [18].

The measurements of pH and electrical conductivity (µS/cm) showed that the solution was not degraded during the tests.

After each experimental test, the hydraulic performance of the membrane was investigated by filtration of the ultrapure water. In Figure 4 was presented ultrapure water flux determinate after collagen ultrafiltration.

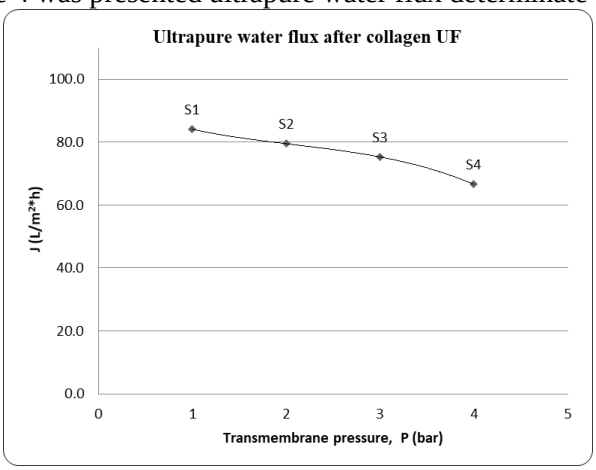

Fig. 4. Ultrapure water flux determinate after collagen ultrafiltration

Clogging aspects are also highlighted in Figure 5 in which membrane views are reproduced prior to collagen ultrafiltration and ultrafiltration of the S4 solution.

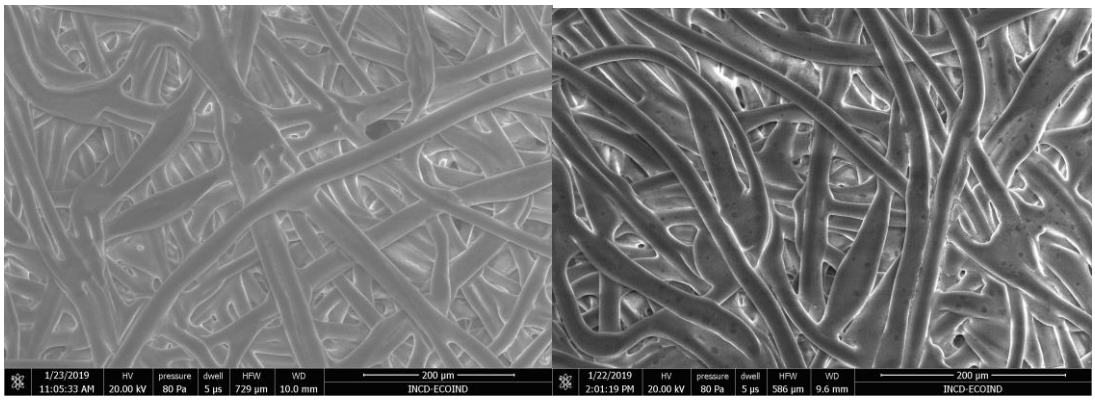

Fig. 5. SEM images of regenerated cellulose membranes (a - control membrane, b - membrane after ultrafiltration S4)

Figure 5 shows that the images that comprise aspects of the macroporous structure of the membranes before and after ultrafiltration indicate in the case of the corresponding S4 ultrafiltration the appearance of some points on the regenerated cellulose filaments which are not found in the control membrane images. These may be associated with collagen conglomerates retained in the membrane structure that contribute to the membrane clogging over time.

Conclusions

Ultrafiltration is an economic and attractive membrane separation process used to concentration and purification collagen proteins.

Ultrafiltration tests of collagen solutions were performed with a regenerated cellulose UF membrane with the molecular weight of 5000 Da. For the same operating conditions (5 bar), there was a significant difference in the permeate flux of the collagen solutions and of the ultrapure water; the flux of ultrapure water is greater than the flux of collagen permeate.

Protein ultrafiltration with collagen concentrations of 1.0 g/L were obtained the results regarding specific permeate flow ranging from 60.6 to 78.3 $L/m^2 \cdot h$. The experiments show a retention between 49.5% and 96.3% function of nitrogen content and 51.2% and 96.6% for protein concentration. The measurements of pH and electrical conductivity showed that the solution was not degraded during the experiments.

The structure and morphology of the regenerated cellulose membranes were scanning with electron microscopy SEM and the results show that the polarization layer and membrane fouling are formed.

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