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CONCENTRATION OF COLLAGEN PROTEINS BY ULTRAFILTRATION COMPARED TO A STANDARD PROTEIN (BSA)

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Introduction

Membranes are used for the concentration and purification of proteins (ultrafiltration) and for small molecule clearance (ultrafiltration/diafiltration). The membrane separation techniques that use regenerated cellulose membranes are applied in various domains.

The aim of this study was to examine the ultrafiltration process on the rejection of the collagen protein using a tangential flow filtration, with flat regenerated cellulose membrane (5 kDa), evaluating permeate flows and physicochemical characteristics of the concentrates, permeates and feed solutions. Regenerated cellulose membranes UF processes performances to bovine serum albumin (BSA) separation are also reported.

Materials and methods

The UF process was conducted in laboratory with Koch membrane system Labcell CF-1, ensuring a tangential flow mode. The system was composed of a feed tank with a volume of 0.5 dm^3, a pneumatic pump, manometers, housing for membrane and a membrane with an effective area of 28 cm^2. During experiments, a regenerated cellulose UF membrane from Merck Millipore with a corresponding molecular weight cut-off (MWCO) equal to 5 kDa was used. The collagen was provided from bovine skin and BSA was purchased from Sigma-Aldrich. Three protein solutions were used to perform the experiments as follows: S_1 - collagen solution with molecular weight M=2000 Da, S_2 - collagen solution with molecular weight M=5600 Da and S_3 - BSA with molecular weight M=6600 Da. All the solutions were prepared in ultrapure water, with a concentration of 0.1%. The solution was gently stirred for 1 h to ensure homogeneity at 25ºC.

The characterization of membrane's transport properties was first made by determining a dependence of the ultrapure water volumetric flux on a transmembrane pressure (TMP) in the range of 2 to 6 bar. All tangential flow mode experiments were performed at a pressure of 5 bar.

The experiment consisted of three stages: in the first stage, the ultrapure water flux (Jw) was measured at a new membrane; in the second stage, the collagen solution flux was determinate and in the third stage, the ultrapure water was measured again for determining the flux of membrane after collagen filtration. In each experiment the

volume of the feed solution was initially 500 mL, with 250 mL collected permeate and 250 mL concentrate. Samples of feed, retentate and permeate were determined by analysis of the following parameters: temperature, pH, electrical conductivity, nitrogen content, protein concentration (Lowry method).

Results and conclusions

Tabel 1 shows the chemical and physical properties of the feed solution, permeate, and concentrate of collagen and BSA solutions.

Tabel 1. Chemical and physical properties of collagen solutions

Chemical-physical properties	Solution type								
	S_1			**S_2**			**S_3**		
	1	2	3	1	2	3	1	2	3
Temperature (ºC)	19.5	19.7	19.8	19.7	20.0	20.2	19.8	19.9	20.3
pH	7.4	7.1	7.0	6.9	6.6	6.6	7.3	8.0	6.9
Conductivity (µS/cm)	524	444	684	160	138	196	26	7	34
Nitrogen content (mg/L)	162	84.3	231	174	21.9	325	183	14.3	352
Protein concentration (mg/L)	908	472	1296	975	121	1834	1026	78.5	1978

*1-feed solution, 2-permeate, 3-concentrate.

Membrane permeate flux has been used to characterise the productivity of the membrane filtration system. The permeate flux, J was been calculated by using Darcy equation. The flows (L/m^2*h) obtained in the UF process were of 78.8 for S_1, 70.4 for S_2 and 67.0 for S_3.

The data shows it can be concluded that the regenerated cellulose membrane with molecular weight 5000 Da was able to perform a good rejection of 48% for 0.1% collagen M=2000, 87% for 0.1% collagen M=5600 and 92% for 0.1% BSA depending on the nitrogen content and depending on the protein concentration with small differences. It can be seen that although the molecular weight of the solution is higher than the exclusion value of the membrane, a good separation is achieved in case of S_2 and S_3. For S_1 a low separation was observed because of the molecular weight of the solution.

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