Composite PSF-PANI Membranes for Proteins Recovery

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Abstract: - This work reports the performances of the polysulfone-polyaniline (PSf-PANI) porous composite membranes for proteins recovery. Polysulfone-polyaniline porous composite membranes are obtained by phase inversion with chemical reaction. PANI is obtained *in-situ* by oxidative aniline polymerization. Composite membranes are morphological studied by electron microscopy and characterized by water solutions permeation at different pH and pressures. Polysulfone-polyaniline porous composite membranes UF processes performances to BSA separation are also reported.

Key-Words: - PSf-PANI membranes, phase inversion, hydrodynamic characteristics, rejection, BSA retention.

1 Introduction

The membrane techniques that use composite polysulfone membranes are applied in various domains, replacing in many instances the technologies that use simple membranes [1, 2].

Polysulfone (PSf) are a thermoplastic rigid polymer, durable and transparent that maintains its characteristics in a temperature range from -100 degrees to over 160°C [3]. Chemical and physical properties of polysulfone including good chemical stability, mechanical strength and resistance to oxidation make it the preferred material for use as membrane substrate [4, 5]. Polysulfone are widely used at the manufacture of membranes for ultrafiltration and gas separation [6-8]. In particular, polysulfone is used as ion exchange membrane for processes such electrodialysis and electrolysis with polymer electrolyte-membrane [9].

Polysulfone-polyaniline porous composite membranes (PSf-PANI) exhibit an interesting advantage because they use PSf as the base polymer (PSf has good mechanical and chemical properties, good stability in acid and basic aqueous solutions, good solubility in the usually solvents in the process of membrane production) and specially performances in bioanalysis and bioseparations [11-14].

The paper presents the composite PSf-PANI membranes performances obtained by phase inversion, new immersion-precipitation technique with chemical reaction. The morphology and hydrodynamic characteristics are studied, as well as the capacity of separating albumin from bovine serum (BSA) – the reference protein.

2 Experimental

2.1 Materials

- Polysulfone material used in the present study was obtained from BASF (Ultrason S3010)
- Solvents: N methyl pyrrolidone (Merck) and aniline (Merck), p.a. reagent were used as solvents without advanced purification and methanol, ethanol and isopropanol (Merck) and bidistillate water were used as non solvents.
- Oxidizers: nitric acid (Merck), ammonium peroxodisulfate (Fluka), potassium persulfate (Merck) and hydrochloric acid p.a. (Merck) were used as 0.1-0.5 M solution.
- BSA – Biochemika, fraction V, ≥92%, M=67.000 g/mol, free protease

2.2 Working procedure:

- Polysulfone was dissolved in a solvent mixture from above solvents to form a solution at a preset temperature (25^0C)
- Degassed solution was cast to a glass plate using a Gardner preheated knife.
- The thickness filters polysulfone membrane type was 0.3-0.5 mm.

- *Polysulfone membranes* formed *were maintained for at least one day in nonsolvents mixture.*
- Composite membranes (**$M_{PSf/PANI}$ I-III**) were achieved by remanent aniline oxidation in membranes pores using three solutions with different concentrations (0.10M, 0.30M and 0.50M persulfate in hydrochloric acid). These were used to produce membrane type filters from polysulfone with polyaniline by oxidation for 48 hours.

2.3 Membrane characterization

Characterization of membranes was achieved by three complementary methods previously presented [15]. The main features of the membranes tested necessary for determination of lead and powders aerosols are illustrated as following.

Total porosity: Membranes porosity was describes by Eq. 1:

$$\text{Porosity} = \frac{V_m - V_p}{V_m} \times 100 \tag{1}$$

where:

- *Vm* - the volume of membrane mass (is determinate multiplying the membrane areas by thickness of membrane
- *Vp* - polymer volume
- *Vm* - membrane weight /density

Hydrodynamic characterizations were realized with a laboratory installations type **KOCH LABCELL-CF1** presented in Fig. 1 with the following functional characteristics:

- membrane diameter = 76 mm;
- membrane effective area = 28cm^2;
- volume of feeding tank = 500ml;
- liquid velocity at tangential liquid flow = 2m/s;
- maximum working pressure = 35 bar;
- optimal pressure = 8 bar;
- maximum optimal temperature = 70^{0}C;
- pump flow capacity = 1.8 l/min;
- material = steel.

Membrane fluxes were calculated as permeate function using measured volumes in a determined time interval, with Eq. (2):

$$J = \frac{1}{A} \cdot \frac{\left(\Delta V_{permeat}\right)}{\left(\Delta t\right)}, \quad \left(\frac{L}{m^2 \cdot h}\right) \tag{2}$$

where:

A = effective membrane area (0.0028 m^2)

ΔV = permeate volume collected during the time interval Δt

Pressure drop along tested membrane was measured using a pressure meter during the test period.

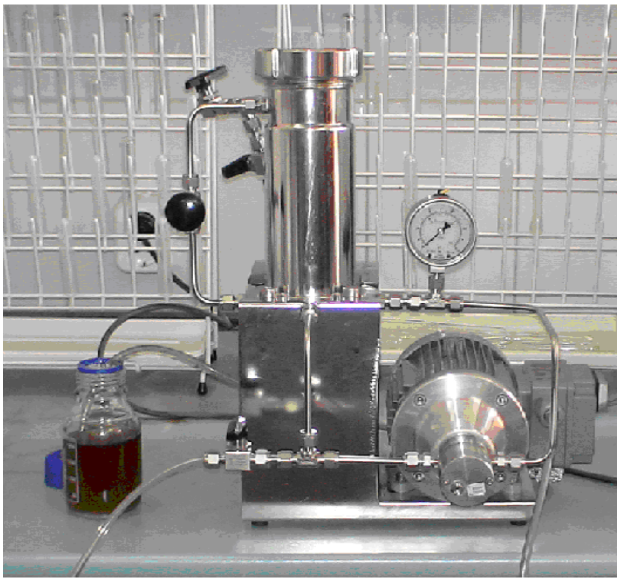


Fig. 1. KOCH LABCELL-CF1 Laboratory installation type

Membrane rejection (selectivity) of polysulfone and nanocompozite based on polysulfone and polyaniline was expressed by retention degree R of bovine serum albumin protein determined by Eq. (3):

$$R = \frac{c_f - c_p}{c_f} = 1 - \frac{c_p}{c_f}, \tag{3}$$

where:

c_f = BSA concentration in feeding fluid (g/L, moles/L);

c_p = BSA concentration in permeat (g/L, moles/L).

Protein analysis was done by Lowry method. 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 (especially tyrosine) [16, 17].

Optical density (absorbance) of protein solutions samples whose concentration should be measured in terms of concentration are processed using the standard curve.

Scanning electron microscopy analysis (SEM): The obtained membranes are kept in water: methanol solution (1:1). For characterization, the membranes are dried in vacuum at 60^{o}C for 4 hours. Membrane samples (4.8, 5.2 and 9.0 cm diameter disks) were characterized by scanning electron microscopy – SEM (using a FESEM Hitachi S 4500 microscope). To examine the membrane they were fractured in liquid nitrogen for obtaining of 1cm x 3cm samples and were cover with gold. The membranes were examined in cross-section (with x1.000 magnification) and on the surface (with x 2000 magnification).

3 Results and Discussion

Polysulfone-polyaniline porous composite membranes are prepared by phase inversion, immersion-precipitation techniques in a stepwise procedure [5, 6], as follows:

- polysulfone solubilization in a solvent mixture;
- filtration of polymer solution to gels removal;
- air removal into a desiccator;
- polymer film formation from polymer solution;
- coagulation of polymer film;
- chemical reaction.

Polysulfone-polyaniline porous composite membranes are obtained by polysulfone coagulation followed by oxidative polymerization of aniline from polymeric film._The polymeric base materials were PSf and PANI (obtained *in-situ*). Three solutions, with different concentration level of the PSf (10%PSf, 12%PSf, 14%PSf) were used. The solvents were N-methyl pyrrolidone (NMP) and aniline. The proportion between the base polymer PSf and PANI was: 100:1, 100:2 and 100:3. For the polycondensation of the adsorbed aniline in membrane pores, a mixture of HCl 0,1M and ammonium peroxodisulphate 0.05M in ratio 2:1 was used.

The paper presents the results of three experimental versions ($\mathbf{M_{PSf/PANI}}$ **I-III**) in the ultrafiltration of protein, particularly BSA. Three composite membranes with different characteristics were obtained. Polymer film was formed in a matter of seconds, at room temperature and humidity, using a “*doctor blade”* device type made from stainless steel, with 200 μm slot, to a smooth glass support. The coagulation time for each membrane was 3 minutes. The average time for the reaction to obtain each composite membrane was 3 hours. Visually, the process was controlled by monitoring the colour from white, to violet and finally to blue. In the end, the composite membranes were washed using fresh distilled water for eliminating the residual reaction compounds, followed by conditioning in glycerine solution 20%.

The performances of the obtained composite membrane were determined by evaluating the hydrodynamic characteristics and the retention of reference compounds. The experimental setup that has been used to determine the hydrodynamic characteristics of the composite membranes was the KMS Laboratory Cell CF-1 (Koch-Membrane - Germany) ensuring a tangential flow mode in separated process. In the first experiment a 1 g/L BSA solution dissolved in citric acid-sodium citrate buffer at pH = 4.9 was used. In the second experimental version a 1 g/L BSA solution dissolved in Tris-HCl buffer at pH = 7.4 was used.

The experimental setup that has been used in these experiments was the same KMS Laboratory Cell CF-1 (Koch-Membrane - Germany) ensuring a tangential flow mode in separated process. The experiments were conducted at 4 bar pressure. In each of the two experimental versions the volume feed solution BSA was initially 0.5 L, with 0.25 L collected permeate and 0.25 L concentrate. The protein concentration in permeate and concentrate were determined by the Lowry method, on spectrophotometry UV-VIS [15, 16]. It was used the molecular absorption spectrometer Specord 205 (Analytic Jena, Germany) quartz containers 1 cm thickness. The absorbance was measured at $\lambda = 751$ nm. The experiments realized along the described methodologies try to evaluate the influence of changing the PSf-PANI ratio upon the hydrodynamic and separation performances of the composite membrane, at different pH and pressure values. The results obtained by testing the composite membranes, measuring the permeate flux solutions and BSA retention, at different time period and pressures values, in all experimental versions, are shown in figures 2 - 5.

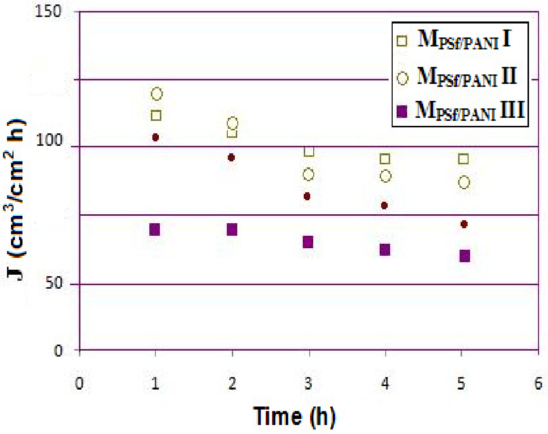


Fig.2. Evolution of water flux for PSf/PANI composite membranes

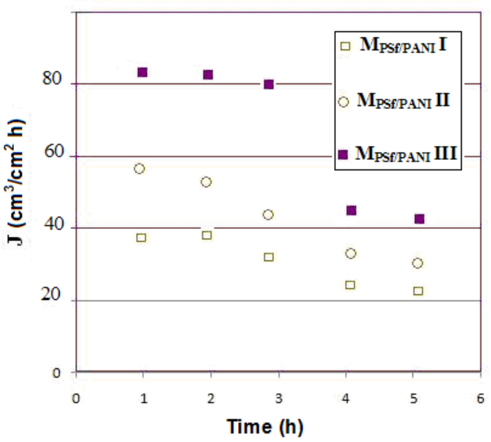


Fig.3. Evolution of BSA solution flux for PSf/PANI composite membranes

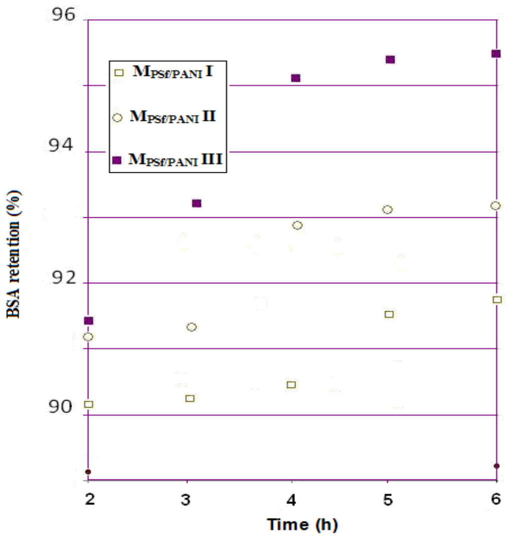


Fig.4. Evolution of BSA retention on PSf/PANI composite membranes

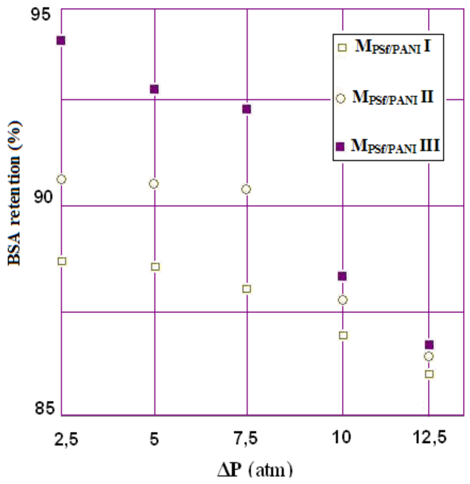


Fig.5. BSA retention on PSf/PANI composite membranes versus transmembrane pressure

a) Determination of the transmembrane

The performed experiments for trans-membrane pressure influence evaluation, on the obtained membranes by three different polymer concentration solutions, reveal the following:

- the water flux decreases with increased the time, regardless of the type of the composite membrane prepared and the pressure values which are tested;
- the maximal values of the water flux correspond to one our, for each of composite membranes, at all pressures tested;
- increases of the PSf polymer concentration from the base solution used to prepare the composite membrane leads to improved hydrodynamic characteristics of the composite membrane (increase flux of the permeate); this phenomenon may be explained by a looser structure of the PANI-PSf composite membrane due to the modification of the macromolecules conformation and orientation

The effect was observed especially in the case of the composite membrane obtained from the highest concentration of the polymer solution (14% PSf), when the difference between permeate flux determined in both experimental versions was the largest.

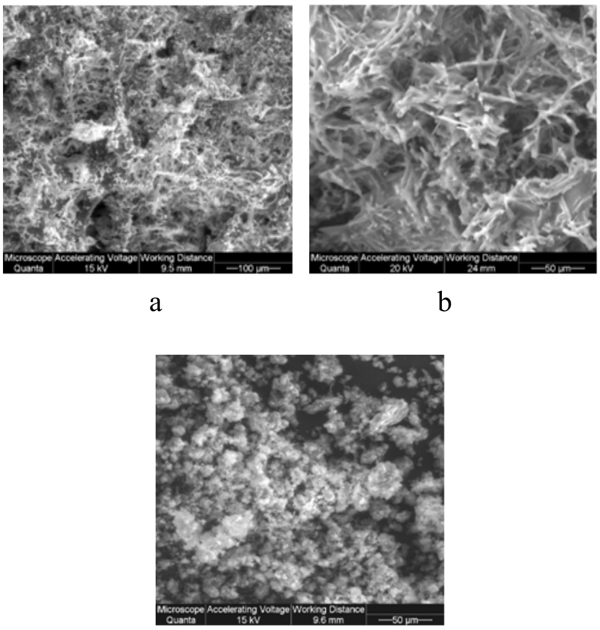
There is no effect of aniline addition in composite membranes prepared from 10%PSf solution; the permeate flux is similar in the two experimental versions. A possible explanation is that at lower PSf concentrations (10%), the membrane structure exhibits higher porosity. Increasing the PANI quantity does not lead to a significant increase of the permeate flux.

b) Determination of retention and flow of standard protein solution (BSA)

The permeate flux, the BSA concentration in permeate and concentrate, the percent of rejection (*R*) and the immobilization capacity (*I*) of the composite membranes obtained in all experimental were presented in figures 4 and 5..

The membranes prepared from polysulfone-polyaniline nanocomposites presents flows and retention levels of the protein bovine serum albumin (BSA) much higher than polysulfone membrane know alone.

The performed experiments for BSA retention, for the obtained membranes by those three membranes versions and three different polymer solutions concentration, can be discussed as following: the rejection values (*R*) determined for the three composite membranes tested are higher at pH = 7.4 as compared to the same parameters determined at pH = 4.9. The explanation is that the pH = 4.9 value is very near the iso-electrical pH level of the BSA. In these conditions, the BSA protein does not exhibit electrical charge and does not interact with the PANI reactive functional groups of the composite membranes; at pH=7.4 the PANI polymer has the best hydrodynamic characteristics, effect confirmed in the case of the two functional characteristics (R); the percent rejection of the $\mathbf{M_{PSf/PANI}}$ membranes type is higher than the percent rejection of the $\mathbf{M_{PSf/PANI}}$ **II**, $\mathbf{M_{PSf/PANI}}$ **III** membranes type corresponding to the higher polymer PSf and PANI concentrations from composite membrane ; this is because from solution with higher concentration the obtained composite have lower cut-off values; increasing the PANI content in composite membrane leads to an increase of the immobilization capacity (I) ; the immobilization capacity (I) for composite membrane, $\mathbf{M_{PSf/PANI}}$ **III** is higher (cca 95%) than composite membrane $\mathbf{M_{PSf/PANI}}$ **II** and for composite membrane $\mathbf{M_{PSf/PANI}}$ **II**, $\mathbf{M_{PSf/PANI}}$ **II** is higher (cca93%) than composite membrane $\mathbf{M_{PSf/PANI}}$ **I**, (cca 91%).



a b

c

Fig. 6 SEM images of PSf-PANI composite membranes from 10% polymer concentration (a – surface), 12% polymer concentration (b – surface) and 14% polymer concentration (c – surface)

c) The scanning electron microscopy - SEM of the PSF- PANI composite membranes reveals a very compact structure for the membranes obtained from 12% PSf solution, (potential uses being in ultraflitration and nanofiltration) and a more relaxed structure for the 14% PSf membrane, but still more compact than the one of the entirely 10% PSf membranes, for membranes obtained on 10% PSf solution.

The membrane morphology is in complete accord with BSA retention, respectively the increasing of compact grade of membranes in order 10% PSf, 12% PSf and 14% PSf (Table 1).

4 Conclusion

The influence of an increased polyaniline / polysulphone ratio in the composite membrane structures led to the following experimental conclusions:

- improvement of the hydrodynamic performances of the composite membranes
- concomitant increase in the separation capacity (R) and immobilization capacity (I) for the composite membrane with a higher PANI contents in relation to PSf
- the membranes morphology obtained by Scanning Electron Microscopy is in complete accord with BSA retention and hydrodynamic tests.

The best composite membrane obtained in this study from the point of view of BSA retention and separation is the $\mathbf{M_{PSf/PANI}}$ **III** type, corresponding to a pH level of 7.4.

Mechanical and chemical resistance of prepared membranes from PSF, and PSF/PANI is very good, matching all operations in the field and laboratory required by analytical technique.

Gas-dynamic resistance of membranes prepared is good, comparable to the literature type, which requires correction to normal volumes of air sampled. Water flows through PSF and PSF/PANI membranes are higher than those obtained by literature membranes.

Permeability PSF and PSF/PANI membranes is superior to those obtained by know membranes, regardless of the applied transverse pressure difference.

Standard protein retention (BSA) for membranes and PSF/PANI is superior to those obtained by PSF membranes.

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