Proteins Ultrafiltration using Polysulfone-polyaniline and Polysulfone-magnetite Nanoparticles Composite Membranes

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In this paper are presented the results of bovine serum albumin (BSA) aqueous solutions ultrafiltration using composite membranes based on polysulfone (PSf), polyaniline (PANI) or magnetite nanoparticles (NM). Operating the protein solutions ultrafiltration at 5 bars, pH 4.9 and 7.4, at BSA concentrations of 1 g/L and 1.5 g / L were obtained the results regarding specific permeate flow ranging from 80 to 110 L/m^2 x h and retentions between 85 and 99%. Flow-retention results are the best for PSf-PANI composite membrane, and regarding the retention PSf-NM composite membrane is superior to PSf membrane at the same permeate flow. Along with increasing protein concentration in the feed, flow slightly decreases, while retention increases with 1to 3 units reaching ideal retention threshold (98%).

Keywords: proteins ultrafiltration, composite membranes, polysulfone-magnetite, polysulfone-polyaniline

Some aqueous effluents are sources of valuable compounds that can, but it is also imposed in terms of environmental protection, to be recovered. Among these, proteins or enzymes represents a target group of great interest [1].

By conventional filtration technologies it can not be achieved a recovery degree economically advantageous, while membrane technology allows the concentration of interest component, which can be reused in the process [2,3].

Two branches of food industry that produce large volumes of wastewater whose organic load is given by high protein content are: milk processing industry and respectively brewing industry, but many fluids that occur in biomedicine are also protein sources [4,5].

Among the membrane methods used to separate proteins, ultrafiltration has attracted the attention of researchers due to the application of a methodology more convenient in terms of phase changes, and economic considerations compared to gel chromatography.

Development of Loeb - Sourirajan type polymer membranes [5,6] determined a moose impressive research on membrane separation of aqueous solutions [3-5]. However, although there are over four decades since then, we are still looking for innovative ways to increase the process performances (flow and retention) for this type of membranes [6-10].

Recent applications in the field of environmental protection and food industry [11-13], but especially the implications of membranes in biotechnology and biomedicine [14-17] have imposed researches for physico-chemical performance improvement in order to achive thermal or radiation sterilization and for providing opportunities in washing and clogging area [18-23]:

- temperature resistance;
- resistance on the entire range of *p*H;
- resistance to oxidizing environments (sodium hypochlorite, hydrogen peroxide);
- resistance to medium and large energy radiation (UV or with accelerated particles).

Making lately composite membranes [24-28] has reopened the researches on effective ultrafiltration of proteins aqueous solutions that occur in food industry, biotechnology and biomedicine.

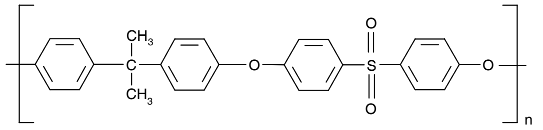
Proteins separation using membrane techniques was increasingly studied due to its multiple and potential applications: purification and fractionation of biological enzymes from varoius biological media; reducing water pollution by reducing the organic load; recovery of valuable compounds in the food industry from by-products [29,30].

In this paper are presented the results of bovine serum albumin (BSA) aqueous solutions ultrafiltration using composite membranes based on polysulfone (PSf), polyaniline (PANI) or magnetite nanoparticles (NM).

Experimental part

Materials and methods

For composite membrane preparation as base polymer polysulfone (Aldrich - M = 22000 Da) was used having the structural formula shown in the following diagram:



In order to prepare different composite membrane materials and to study their performances, aniline (Merck) and magnetite naoparticule prepared in the laboratory were used [29-31].

PANI was generated in-situ by polycondensation of aniline existing in the pores of the preformed membrane, using an ammonium peroxydisulfate extra purified (Merck)

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solution, in a mixture with fuming hydrochloric acid 37%, reagent for analysis (Merck).

For base polymer dissolution, two dipolar aprotic solvents 1-methyl-2-pyrrolidone (N-methylpyrrolidone) (Merck), N, N-dimethylformamide (Scharlau) were used.

In these solutions, aniline (composite membranes PSf-PANI) was dissolved, respectively, magnetic nanoparticles (PSf composite membranes, NM) were dispersed.

The solvents were selected from the polymer solubility at room temperature point of view in order to obtain membranes whose pore size are in the range nedeed for ultrafiltration.

In order to adjust the polymer solution viscosity, as adjuvant, synthesis reagents polyvinyl - pyrrolidone K90, K30 (Fluka); polyethylene glycol 8000, 9000 (Scharlau) were used.

As non-solvents for phase inversion, distilled water and a mixture of distilled water with or without aniline addition were used (Merck).

Prepared membranes conditioning was performed in glycerol solution (Merck).

In order to characterize the membranes, aqueous solutions covering a *p*H range from 1 to 13 were used, made using analytical pure reagents: 95-97% concentrated sulphuric acid (Merck) and sodium hydroxide pellets (Merck).

In the experiments were also used: bovine serum albumin (BSA) (Biochemika) buffers (Radeikis) in which it was prepared: citric acid - sodium citrate buffer solution (*p*H 4.9) and Tris - HCl buffer (*p*H = 7.4).

Solutions prepair procedures

a) polymer solution preparation

The amount of base polymer determined (depending on the concentration desired to be prepared) was introduced slowly, under stirring (magnetic stirrer) in the vessel provided with cover in which solvent mixture NMP - aniline (in the proportions set) are palced. The stirring was continued until complete dissolution of the polymer was achived.

After polymer dissolution step, polysulfone, in the solvents, in the resulting solutions adjuvants necessary to adjust the viscosity were added, continuing the stirring until complete dissolution, in the following order: polyethylene glycol (2%) and then after its dissolution, polyvinyl - pyrrolidone (2%).

Obtaining the desired polymer solution, depending on concentration, was achieved within 4 h.

The solution obtained was filtered through a device equipped with a metal mesh made of stainless steel wires with a mesh size of 30μm.

Through this process, solid impurities (undissolved) which adversely affect skinning process (appearance of continuous lines in the length membrane) and thus the structural characteristics of the membrane, removal is aimed.

Finally, basically before use, the solution was subject to deaerating in order to remove air embedded during dissolution process. Air bubbles from polymer solution can lead to discontinuities and defects in membrane structure during skinning process.

Deaeration was carried out by passing the solution into a vessel connected to a vacuum source (vacuum tube or vacuum pump) and empting it until full air removal from the polymer solution was achived.

Deaerated solutions were then stored in sealed vessels for further use in composite membrane making process.

b) chemical reaction solution preparation

For aniline existing in the preformed membrane pores polycondensation, was used, in all experimental variants, an oxidizing solution of 0.1 M ammonium peroxydisulfate and 0.1 M HCl. After preparation, the solution was introduced in the reaction tank, being used for the set of experiments from that day.

c) magnetite nanoparticles dispersion preparation

Polysulfone solution (100mL) is inserted in a Retsch^R PM100 colloid mill teflon pan along with the proper amount of magnetite nanoparticles [32-35] and 50g ceramic grinding. After 4 h of mixing by stirring at 300rpm brown dispersion is subject to deaeration by staying in dry place for 24 h.

Membranes preparation

In the conducted experiments, by phase inversion process, immersion-precipitation technique accompanied by *in-situ* polycondensation chemical reaction PSf-PANI composite membranes were obtained, while PSf-NM composite membranes were prepared by phase inversion precipitation.

Skinning polymer solution was performed at ambient temperature and humidity, by directly applying on a smooth glass surface, using a manual device generically called “doctor blade”. Polymer film was coagulated according to the previously presented data [29-31].

After conditioning, repeated washing with distilled water and drying in a vacuum oven at 60° C, the membranes are cut to the desired size for the experiments and characterization through: scanning electron microscopy (FESEM Hitachi S 4500), thermal analysis (TGA Thermogravimetric Analyzer Q5000 - TA Instruments Inc.) and pure water permeation -CF1 KMS Laboratory Cell (Koch-Membrane Germany).

Results and discussions

Widespread use of polysulfones (amorphous thermoplastic polymers class) for microporous, dense and composite membranes preparation, is justified by the performance of these polymers [2]

- high solubility in polar aprotic solvents and therefore, great possibilities for obtaining membranes by phase inversion;
- thermal resistance up to about 200°C;
- chemical resistance throughout the *p*H range and in oxidative environments;
- tensile strength;
- polysulfone selectivity for gas mixtures.

Currently, polysulfone membranes do not meet all the flow and retention requirements, but are an excellent support matrix for liquid membranes, macromolecular compounds or enzymes [1,2].

In order to combine the advantages of polysulfones (PSf) as membrane materials, with polyaniline (PANI) electrochemical properties and magnetic properties of magnetite nanoparticles (NM), lately have been prepared polysulphone - polyaniline composite membranes (PANI-PSf), and polysulfone- magnetite nanoparticles (PSf-NM) by using immersion-precipitation and phase inversion technique, respectively [32-34].

Polyanilines (PANI) represents an important class of polymers used in the preparation of composite membranes due to high conductivity, chemical stability satisfactory, and not least of non-prohibitive cost [35,36].

Polyanilines shows, compared to other types of conductive polymers, an unique feature namely, that the

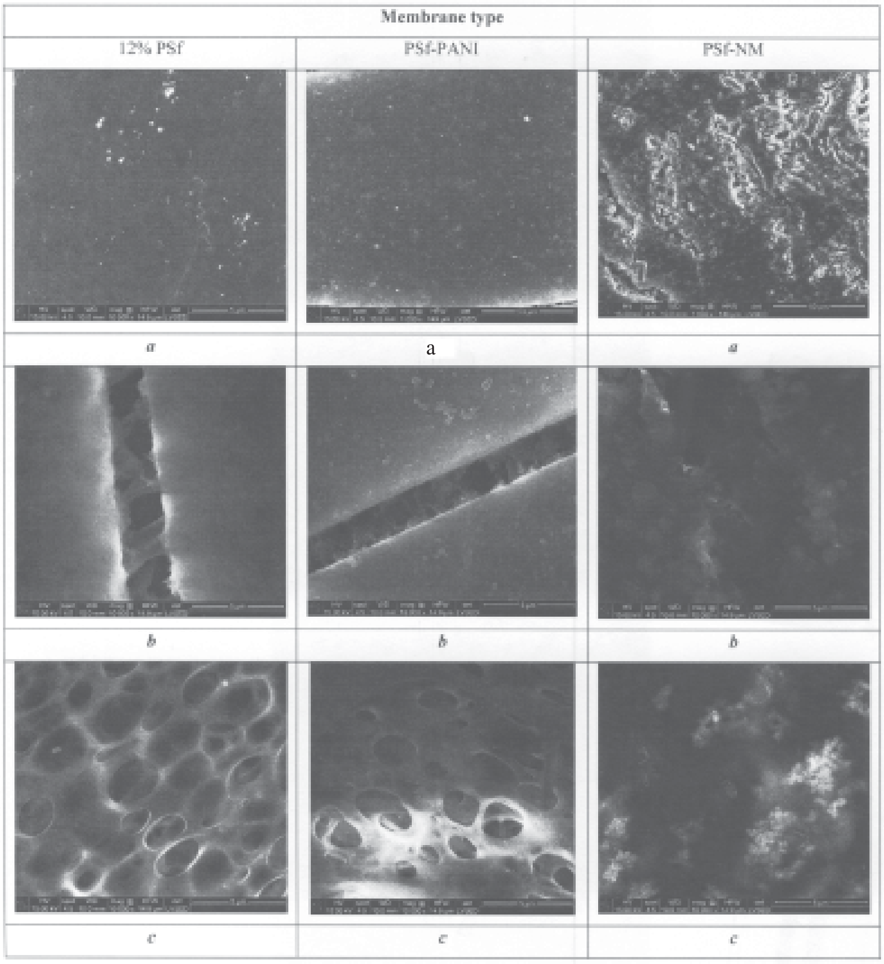


Table 1
SELECTED MEMBRANES CHARACTERISTICS:
a-TOP SUFACE, b-FRACTURE DETAIL,
c-BOTTOM

electrical properties can be modulated and reversibly controlled by doping [37].

On the other hand, magnetite nanoparticles shows adsorption properties for both inorganic and organic or biological (proteins, enzymes) compounds [38-40] being known in magneto-collection or magneto sedimentation separation applications [41,42].

Polysulfone 10-16% solutions (used to obtain membranes) are stable for a long time, have a favorable consistency for films formation, the films are adherent to various surfaces (glass, stainless steel, copper, aluminum, PVC, teflon) which has enabled the proposed composites [45].

Membranes characteristics

In this work, after assessment of general characteristics: overall appearance, colour, surface and roughness uniformity, defects absence (scratches, creases, cracks), membranes obtained from 12% PSf in N-methyl pyrrolidone and composites: PSf-PANI and PSf-NM which were obtained in 12% PSf solution in N-methyl pyrrolidone and polymer: add = 5: 1 mass ratio were selected for specific proteins retention tests (BSA).

In table 1 are presented: (a) active surface (top surface), (b) details of a fracture and (c) the back (bottom) part for the three membranes types.

Scanning electron microscopy analysis shows that polyaniline and magnetite nanoparticles additions lead to membrane compaction both at surface and in section, as evidenced in literature for other nanoscale additives (carbon nanotubes, graphene or fullerenes) [46-49].

Surprisingly, for porous media, thermal properties (fig. 1) correction of polysulfone membrane occurs, in the sense of thermal resistance increase for the composite with polyaniline.

Most likely, sulfone and polysulfone groups, interact strongly enough with aromatic amino groups from polyaniline. Interaction is also present in the case of oxide composite membranes, from magnetite, so that, thermal degradation of polysulfone membrane attenuates, but in a particular way (fig. 1), reflected by magnetite dehydration.

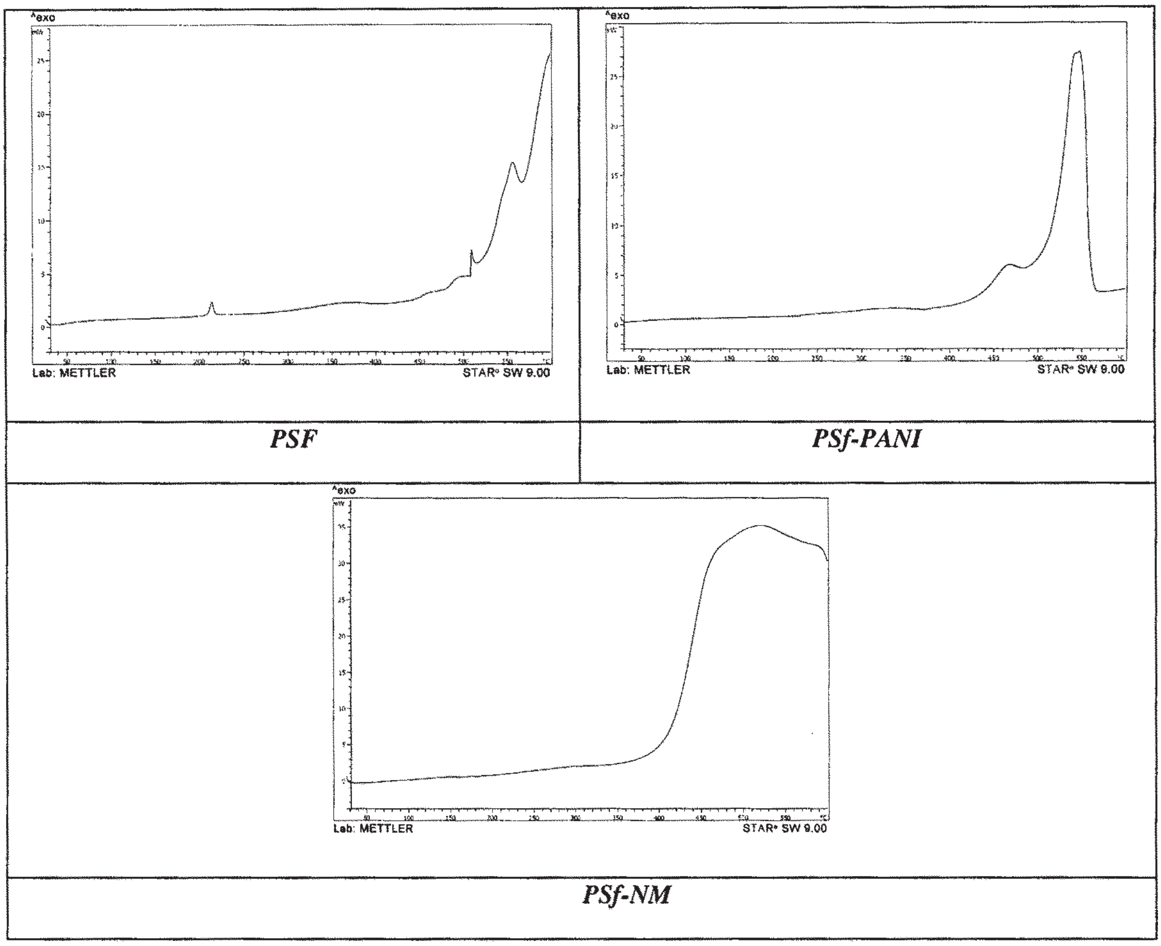
As highlighted properties are likely to improve the solvent flow and protein retention, composite membranes were subject to ultrafiltration at variable pressures and determination of proteins retention depending on the feed solution *p*H.

Influence of working pressure and pressure on pure water flow

Practical results obtained at the passage of aqueous solutions streams through prepared composite membranes were made in specific experimental conditions presented in table 2 [37-40]

-membrane diameter - 76 mm;
- effective membrane area - 28cm^2;
- feed tank volume - 500mL;
-liquid speed (at tangential flow) - 2m/s;
- maximum working pressure - 35 bar;
-working pressure – 2-6 bar;
-maximum working temperature - 70^{0}C;
-pump flow - 1.8 L/min;
-material –steel.

The study shows that the pure water flows are ranging from 80 L/m^2.h at a pressure of 1 bar and *p*H = 1, at about 500 L/m^2.h at a pressure of 6 bar and *p*H = 9 for PSf membrane, and from 25 L/m^2.h to about 85 L/m^2.h for PSf - NP composite membrane for the same working conditions.



PSF **PSf-PANI**

PSf-NM

Fig. 1. Thermal analysis charts for selected membranes: *PSf* (polysulfone), *PSf-PANI* (polysulfone -polyaniline), *PSf-NM* (polysulfone –magnetite nanoparticles)

Membrane type	Pressure (bar)	Water flow (L/m^{2}xh)					
		pH=1	pH=3	pH=5	pH=7	pH=9	pH=11
12%PSf	2	81.0	85.7	106.9	128.4	150.9	108.9
	4	136.0	173.1	233.2	287.6	290.1	264.2
	5	196.0	218.2	342.9	398.0	410.4	382.5
	6	231.2	268.0	376.7.	493.1	507.2	451.8
12%PSf-PANI	2	32.5	41.8	53.2	66.7	53.6	43.9
	4	55.5	59.7	69.4	98.0	80.9	78.5
	5	66.7	76.5	87.3	111.2	105.3	95.8
	6	87.9	98.0	118.7	134.2	123.9	112.6
12%PSf-NM	2	24.5	31.6	41.2	53.6	28.2	26.1
	4	39.4	47.1	51.2	64.0	50.5	46.7
	5	47.9	53.5	63.9	74.5	64.2	56.0
	6	54. 4	64.9	76.8	83.5	71.2	64.3

Table 2
WATER FLOWS AT DIFFERENT *p*H's AND PRESSURES, OBTAINED AT COMPOSITE MEMBRANES TESTING

For PSf-PANI composite membrane the values are intermediate, reaching values of 32 L/m^2.h at a pressure of 1 bar and *p*H = 1, up to 135 L/m^2.h at a pressure of 6 bar and *p*H = 9.

The data obtained recommend the use of membranes at pressures of 4-6 bar and neutral or slightly alkaline *p*H, because at *p*H increase transmembrane flow decreases, reducing technico - economical efficiency.

The behaviour of membranes at protein solution passing, depending on *p*H and transmembrane pressure (table 3) is different.

Thus, PSf membrane shows a decrease of almost an order of magnitude of flow in relation to that obtained with pure water, while the composite membrane PSf-NM, but especially, PSf-PANI composite membrane showed a decrease at most 20 -25%.

This observation is in agreement with the literature, which shows that, for hydrophobic (PSf) and weakly hydrophilic (PSf-NM) membranes, concentration polarization or even clogging strongly occurs, while in the case of hydrophilic membranes (PSf-PANI) concentration polarization is lower, and clogging occurs only in extreme conditions (i.e. dead end filtration).

At the same time, it may be noted that, the maximum flow at constant pressure, is obtained at *p*H 5 and then decreases, sometimes even pronounced (PSf-NM at *p*H 11).

Table 3
EXPERIMENTAL DATA OBTAINED AT COMPOSITE MEMBRANES CHARACTERIZATION FOR 1 g/L BSA SOLUTION

Membrane type	Pressure (bar)	Water flow (L/m^{2}xh)						
		pH=1	pH=3	pH=5	pH=7	pH=9	pH=11	pH=13
12%PSf	2	18.4	20.2	33.6	26. 6	17.2	14.9	13.3
	3	23.0	39.5	64.5	58.9	30.0	26,5	23.1
	4	29.1	44.7	70.4	79.8	42.2	37.0	31.1
	5	31.4	54.7	88.8	86.7	56. 4	48.0	41.4
12%PSf-PANI	2	23.3	27.0	43.4	49.2	39.9	30.1	26.4
	3	33.4	40.9	70.6	87.4	82.0	67.7	54.3
	4	46.8	67.3	104.2	96.5	84. 8	75. 8	59.7
	5	80.7	99.6	117.4	124.4	101.0	92.7	70.8
12%PSf-NM	2	21.4	24.7	40.0	28.2	19.8	16.54	13.4
	3	33.2	39.5	51.8	41.8	38.5	26.9	23.2
	4	37.8	51.7	61.1	52.0	41.3	28.4	21.3
	5	45.8	55.5	62.3	54.2	43.4	30.9	22.3

The composite membranes obtained performances, in terms of the proteins separation were highlighted by:

Protein separation ability or retention, evidenced by determining the rejection degree (R) of a protein taken as standard (BSA) was determined using the equation (1):

$$R = \frac{C_{in} - C_p}{C_{in}} \cdot 100 \qquad (1)$$

where:

C_{in} = solute concentration in feed (mg/L)
C_p = solute concentration in permeate (mg/L

Protein concentrations from permeate and concentrate solution were determined using the Lowry method by UV-Vis spectrophotometry [40-49].

Flow-retention results are the best for PSf-PANI composite membrane, and regarding the retention PSf-NM composite membrane is superior to PSf membrane at the same permeate flow. Operating the protein solutions ultrafiltration at 5 bars, *p*H 4.9 and 7.4, at BSA concentrations of 1 g/L and 1.5 g / L were obtained the results regarding specific permeate flow ranging from 80 to 110 L/m^2. h and retentions between 85 and 99%.

Along with increasing protein concentration in the feed, flow slightly decreases, while retention increases with 1to 3 units reaching ideal retention threshold (98%).

As observed in the case of pure water flows, the best permeate values are at weakly basic -neutral *p*H. In the same time, at the optimum pH the retention are higher, suggesting a BSA aggregation, resulting in a faster and more efficient filtration.

In optimal working conditions (P = 5bars, *p*H = 7.4 and concentration = 1 g/L) permeate flux decreases during operation (fig. 2) for the two composite membranes, more pronounced in the first 4 h.

Flow decrease is present for both composite membranes, with a slight accent at PSf -NM membrane.

The slight increase of pressure at 6 bars, the membrane flow slowly increase, and its behaviour in time is better for composite membranes, especially, PSf-PANI type (fig.3).

Table 4
RESULTS REGARDING COMPOSITE MEMBRANES PERFORMANCES OBTAINED IN THE SEPARATION PROCESS OF 1 g/L BSA SOLUTION

Membrane	Feed solution pH	Permeate flow (L/m^2·h)	Retention (R) (%)
12% PSf	4.9	90.9	85.7
	7.4	108.2	91.0
12% PSf-PANI	4.9	104.0	94.7
	7.4	116.4	98.2
12% PSf-NM	4.9	84.2	88.8
	7.4	96.2	93.6

Table 5
RESULTS REGARDING COMPOSITE MEMBRANES PERFORMANCES OBTAINED IN THE SEPARATION PROCESS OF 1.5 g/L BSA SOLUTION

Membrane	Solution pH	Permeate flow (L/m^2·h)	Retention (R) (%)
12% PSf	4,9	88.2	88.1
	7,4	106.0	91.4
12% PSf-PANI	4,9	99.6	95.0
	7,4	104.8	98.9
12% PSf-NM	4,9	87.9	90.5
	7,4	93.1	94.3

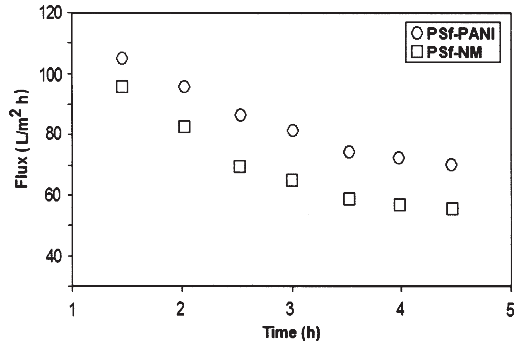


Fig. 2. Permeate flow for selected membranes: *PSf-PANI* (polysulfone-polyaniline), *PSf-NM* (polysulfone-magnetite nanoparticles), at 5 bars, pH 7.4 and 1.0 g/L BSA concentration

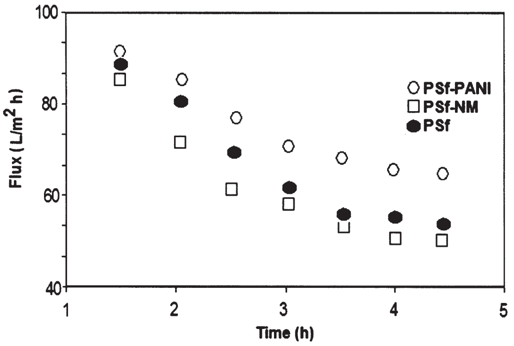


Fig. 3 Permeate flow for selected membranes: *PSf* (polysulfone), *PSf-PANI* (polysulfone-polyaniline), *PSf-NM* (polysulfone- magnetite nanoparticles) at 5 bars, pH 7.4 and 1.5 g/L BSA concentration

Conclusions

This paper presents the results of bovine serum albumin (BSA) ultrafiltration from aqueous solutions using composite membranes based on polysulfone (PSf), polyaniline (PANI) or magnetite nanoparticles (NM).

After assessment of general characteristics: overall appearance, colour, surface and roughness uniformity, defects absence (scratches, creases, cracks), membranes obtained from 12% PSf in N-methyl pyrrolidone and composites: PSf-PANI and PSf-NM which were obtained in 12% PSf solution in N-methyl pyrrolidone and polymer: add = 5: 1 mass ratio were selected for specific proteins retention tests (BSA).

From scanning electron microscopy analysis it was revealed that polyaniline and magnetite nanoparticles additions lead to membrane compaction both at surface and in section, as evidenced in literature for other nanoscale additives (carbon nanotubes, graphene or fullerenes).

Operating the protein solutions ultrafiltration at 5 bars, *p*H 4.9 and 7.4, at BSA concentrations of 1 g/L and 1.5 g / L were obtained the results regarding specific permeate flow ranging from 80 to 110 $L/m^2.h$ and retentions between 85 and 99%.

Flow-retention results are the best for PSf-PANI composite membrane, and regarding the retention PSf-NM composite membrane is superior to PSf membrane at the same permeate flow.

Along with increasing protein concentration in the feed, flow slightly decreases, while retention increases with 1to 3 units reaching ideal retention threshold (98%).

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