The Study of Protein Recovery using Molecular Flotation and Ultrafiltration

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Two branches of the food industry producing large volumes of wastewater in which organic load is given by the high protein content, possibly to be separate and recovered by means of molecular flotation or ultrafiltration are dairy industry and brewing industry.In this work, will be presented our own results regrading the use of molecular flotation and composite membranes ultrafiltration in processing synthetic aqueous solutions simulating wastewater discharged from both dairy and brewing industries.

Keywords: protein separation, ultrafiltration, foam separation, molecular flotation, environmental protection

Proteins fall into the category of the compounds which have to be regenerative separated from organic effluents because they pollute the waters, but also have important economic and technical value [1-3].

Usually, the purpose of a separation process is to remove as much as possible the solute from the feed solution, in order to facilitate the re-use and recovery of the concentrated solution [4-8]. These considerations are significant in determining the process and the clean up system that has to be used [8-10].

By conventional remediation technologies cannot be achieved an economically advantageous recovery, while bubbles and foam flotation or ultrafiltration based technologies allows the concentration of the interest component and its reuse in the process [8-11].

Two branches of the food industry producing large volumes of wastewater in which organic load is given by the high protein content, possibly to be separate and recovered by means of molecular flotation or ultrafiltration are dairy industry and brewing industry [12 -15].

In this work will be presented our own results regrading the use of molecular flotation and composite membranes ultrafiltration in processing synthetic aqueous solutions simulating wastewater discharged from both dairy and brewing industries.

Experimental part

Materials and methods

Molecular flotation protein recovery

In figure 1 it is shown the laboratory set-up operating in discontinuous mode used to determine the proteins separation efficiency using foam bubbles, and in table 1 are illustrated the technical characteristics of the laboratory set-up [16,17].

Table 1

TEHNICAL CHARACTERISTICS OF THE LABORATORY FACILITY [16]

Components	*Characteristics*
Column	Standard length = 1200 mm Outer diameter = 60 mm Inner diameter = 50 mm
Porous plate	Diameter = 50 mm Pore diameter of the μm order
Compressor	Operating pressure = 2-3 atm

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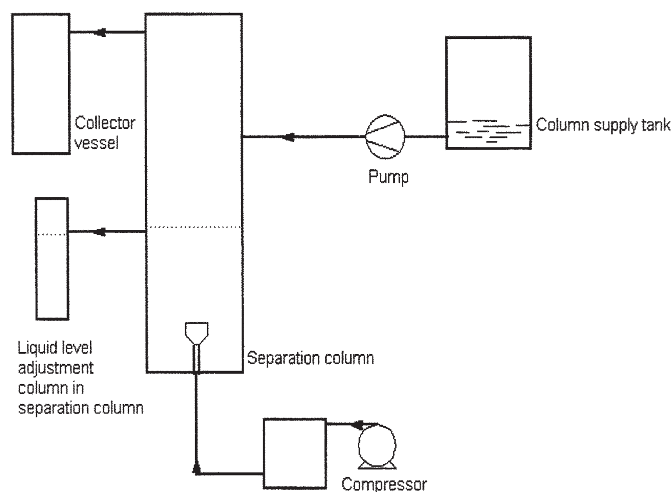

Fig. 1. Simplified digram of the flotation set-up

Working installation is relatively simple, versatile and does not require special precautions because the gas (air) introduction system consists of a compressor connected to the system lens [17].

Synthetic solutions containing standard protein, BSA (Merck) were introduced in the flotation column made of glass and having a maximum capacity of 1500 cm^3.

Air dispersion was achieved by porous plate and air flow setting was performed using a dosing system equipped with pressure and flow reducer.

In each of the two experimental variants: (1) without surfactant and (2) with dodecylpyridinium chloride, 10^{-6} M, the experiment started from a constant BSA solution volume, namely 0.5 L, collecting 0.25 L foam, respectively 0.25 L residue in the flotation column.

Molecular flotation performances in terms of proteins separation, has been highlighted by two criteria [18,19]:

- *The protein recovery*, as evidenced by determining the recovery degree (R) of a reference protein (BSA) was determined using the equation (1):

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

where:

C_{in} = protein concentration in feed solution (mg/L);

C_p = protein concentration remaininig in the column (mg/L).

- *Protein concentration factor,* shows how many times the protein concentration in the foam is higher than the initial protein concentration being determined using equation (2):

$$fc = \frac{C_p}{C_{in}} \quad (2)$$

Proteins concentration in permeate and concentrate solutions was determined using the Lowry method by UV-Vis spectrophotometry [20-23].

The principle of this method is based on forming a cupric complex when the protein is reacting with an alkaline copper reagent (biuret reaction) and reducing phosphomolybdates and phosphotungstate from Folin-Ciocâlteu reagent by phenolic residues from the protein. We used molecular absorption spectrometer Specord 205 (Analytik Jena, Germany) and quartz cuvettes with 1 cm thickness.

Sample absorbance was measured at λ = 751 nm compared to blank sample, namely distilled water. From the calibration curve were determined the protein concentrations in all the samples (foam and exhausted) obtained.

Proteic solutions ultrafiltration

In order to highlight the performances of composite membranes from BSA (Merck) separation/retention point of view by means of ultrafiltration were selected the membranes obtained in the following experimental conditions:

·Polymer based solutions containing 10% and 12% BASF polysulfone (PSf).

·Solvent – N-methyl pirolidone (Merck, NMP)+Aniline (Merck) mixture.

·In the two usage versions as coagulation solution - water (MI) and water with aniline addition (MII).

·Manual skinning of the polymer solution on the smooth glass surface, without textile support and mixed oxidant polycondensation with ammonium peroxodisulphate (Merck) and hydrochloric acid (Merck).

The retention of BSA protein through the obtained membranes was studied and the morphology of the PSf (10%)-PANI composite membrane was evidenced by scanning electron microscopy (fig. 2-4).

The performances of the composite membranes obtained in an ultrafiltration module (fig.5), from the point of view of proteins separation, have been revealed by two criteria:

- *Protein recovery capacity*, evidenced by determining the degree of rejection (R) for a protein taken as standard (BSA) was determined using equation (1).

- *The retention capacity (immobilization I)* at the membrane surface and in its microporous structure of the same standard protein (BSA) through adsorption and the formation of ionic bonds between the reactive groups of PANI and the reactive residues from the BSA structure; the retention capacity (I) is expressed in mass units per membrane area unit (mg/cm^2) and it is determined from the balance relationship (3) and formula (4):

$$V_{in} \bullet C_{in} = V_p \bullet C_p + V_c \bullet C_c + m \quad (3)$$

$$I = \frac{m}{S} \quad (mg / cm^2) \quad (4)$$

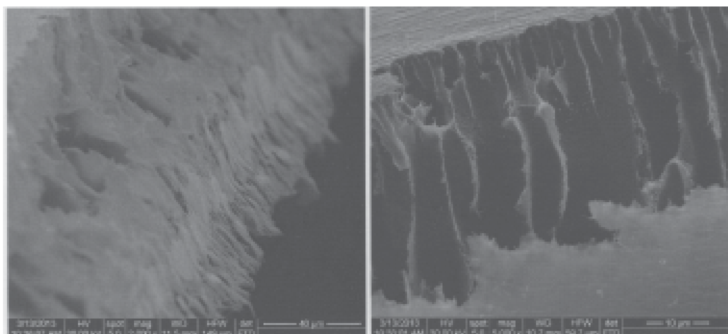

Fig. 2. SEM images of PSf (10%)-PANI composite membrane (cross - section) at different magnitudes (x2000, x5000)

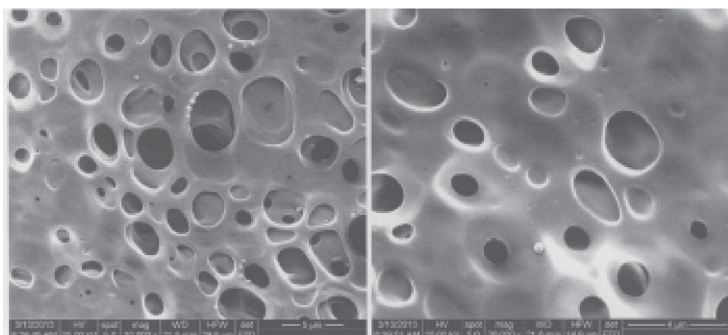

Fig. 3. SEM images of PSf (10%)-PANI composite membrane (porous surface) at different magnitudes (x100000, x20000)

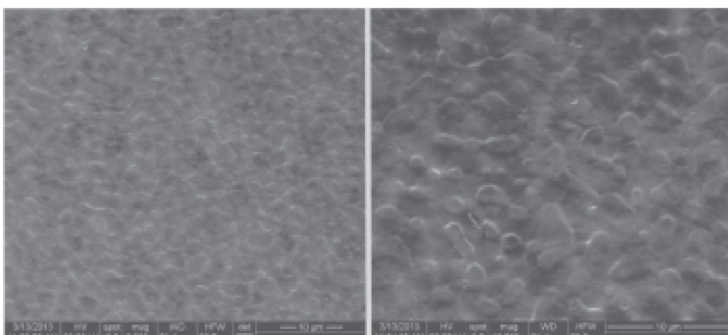

Fig. 4. SEM images of PSf (10%)–PANI composite membrane (active surface) at different magnitudes (x5000, x10000)

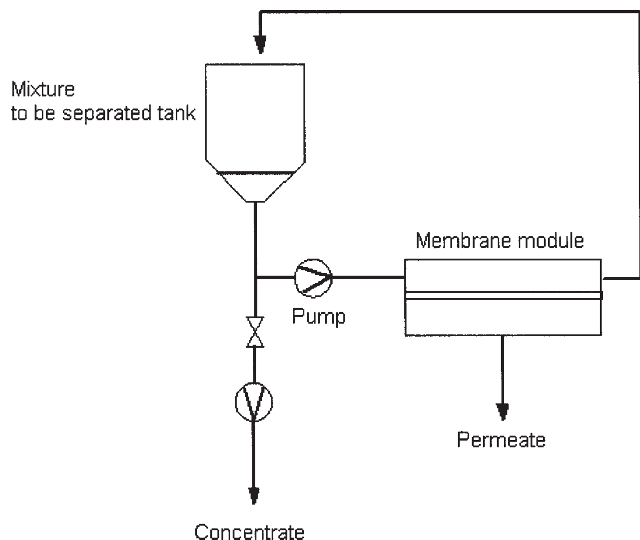

Fig. 5.Ultrafiltration system (UF) scheme

where :

V_{in} = feed solution volume (L)
C_{in} = solute concentration in feed (mg/L)
V_p = permeate volume (L)
C_p = solute concentration in permeate (mg/L)
V_c = concentrate volume (L)
C_c = solute concentration in concentrate (mg/L)
m = protein total mass retained by the membrane (mg)
S = membrane active surface (cm^2)

The membranes were tested using a solution having a concentration of 1g/L BSA dissolved in:

-citric acid-sodium citrate buffer solution with pH=4.9;

-Tris-HCl buffer solution with pH=7.4.

In order to achieve protein separation, the experimets were realised using a laboratory installation [18-20].

In each of the two experimental variants we started from a constant volume of BSA solution namely 0.5 L, collecting 0.25 L from permeate, respectively 0.25 L from concentrate.

The protein concentrations from permeate and concentrate solution were determined, as in the case of flotation, using Lowry method, by UV-Vis spectrophotometry [20-23].

Results and discussions

Molecular flotation protein separation

The study of BSA separation by means of molecular flotation using the flotation set-up (fig. 1) aimed to:

-establish the optimum air flow through the column containing 20-200 mg/L BSA solution;

-determine the BSA concentration factor and recovery efficiency;

-establish the surfactants influence on BSA regenerative separation performances.

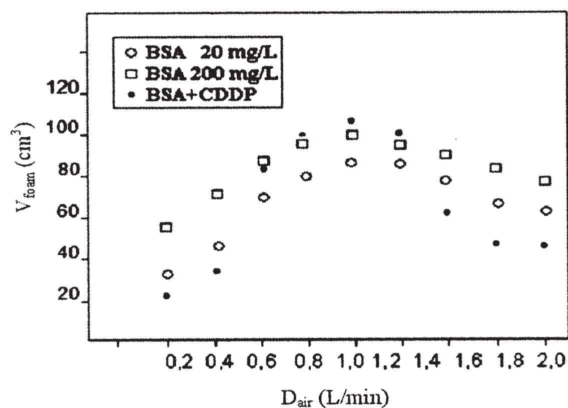

Fig.6. Foam production depending on air in the column

Establishing the optimum air flow through the column

The experiments aimed to establish the maximum volume of foam, which can be obtained in the molecular flotation column, for a 20 mg/L BSA minimum solution concentration and a 200 mg/L BSA maximum solution concentration, compared with a solution containing 100 mg/L BSA and 10^{-6} M dodecylpyridinium chloride.

From figure 6 it can be observed that the foam production increases in the range 0.2-1.0 L/min air, after which, flattening or even decrease in the foam volume collected for all three cases studied, takes place.

It is remarkable that the BSA foaming effect, although dependent on the concentration in the feed solution, is enough to achieve a limited foam production required for the separation.

Practically, working at 1 L/min ensures a smooth running of the process, in terms of the amount of foam folowed by the BSA concentration determination.

Increasing flow over 1 L/min is not recommended, both in terms of increased energy consumption and due to the effect of breaking the column layer solution, by creating bubbles approaching the column diameter.

Determination of recovery degree and concentration factor of BSA

In table 2 are presented the results obtained for the recovery degree and concentration factor of BSA achieved from one liter of solution at an air flow of 1 L/min and variable BSA concentrations in feeding solution.

The experiments showed a concentration factor comprised between 7.3 in variant A and 2.0 in variant E, indicating its decrease with increasing BSA concentration in the feed solution.

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In the same time, the recovery degree decreases, but more attenuated from 63.5% in varaint A, to 41.7% in variant E.

Table 2
RESULTS REGARDING THE PERFORMANCES OF FOAM SEPARATION IN THE BSA SEPARATION PROCESS

Working variant	Foam production (mL)	BSA concentration(mg/L)		Concentration factor	Recovery degree (%)
		feed	concentrate		
A	87	20	146	7.3	63.5
B	152	80	261	3.0	49.6
C	181	120	304	2.5	45.8
D	196	160	346	2.1	41.9
E	208	200	401	2.0	41.7

Table 3
RESULTS REGARDING THE PERFORMANCES OF FOAM SEPARATION IN THE BSA SEPARATION PROCESS

Working variant	Foam production (mL)	BSA concentration (mg/L)		Concentration factor	Recovery degree (%)
		feed	concentrate		
F	99	20	151	7.5	74.4
G	215	120	264	2.2	47.3
H	278	200	317	1.6	44.0

From an operational perspective it is found that is more appropriate to use molecular flotation for BSA separation and concentration from dilute solutions.

Another relevant technical aspect is to conduct the process in at least two steps.

Establishing the influence of surfactants on the performances of BSA regenerative separation

In table 3 are presented the results obtained for the recovery degree and concentration factor of BSA achieved from one liter of solution at an air flow of 1 L/min, variable BSA concentrations and constant dodecylpyridinium chloride concentration in feeding solution.

The use of a surfactant, dodecylpyridinium chloride, in feeding, is favorably influencing the concentration factor, but especially the BSA recovery degree at low concentrations in feeding (20 mg/L); for concentrations exceeding 100 mg/L BSA, the surfactant effect is insignificant.

The experiments show that BSA has sufficient foaming capacity in order to contribute to separation when the concentrations exceed 100 mg/L.

The use of surfactants, especially at low BSA concentrations, is justified when they do not contaminate the final product.

Molecular flotation is a regenerative separation technique techno-economic accessible which can be successfully applied for the separation and concentration of proteins in aqueous solutions.

The experiments indicate a concentration factor comprised between 7.3 at 20 mg/L BSA and 2.0 at 200 mg/L, indicating its decrease with increasing the BSA concentration in feed solution. In the same time, the recovery degree decreases, but more attenuated from 63.5% at 20 mg/L BSA, to 41.7% at 200 mg/L BSA. From the operational perspective it is found that is more appropriate to use molecular flotation for BSA separation and concentration from dilute solutions. Another relevant technical aspect is to conduct the process in at least two steps.

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Proteins recovery by ultrafiltration

Proteins separation using membrane techniques has been increasingly studied in recent years as a result of multiple and potential applications (purification and fractionation of enzymes from different biological environments, reduction of water pollution by reducing the organic load, recovery of valuable compounds from secondary products of food industry). Among membrane processes used for protein separation, ultrafiltration has attracted the attention of the researchers due to the application of a methodology more convenient in terms of phase changes and economic considerations versus gel

Table 4
RESULTS REGARDING THE PERFORMANCES OF COMPOSITE MEMBRANES OBTAINED BY THE VARIANT 1 IN THE BSA SEPARATION PROCESS

Membrane	Soil pH	Permeate flow ($l/m^2 \cdot h$)	BSA concentration (mg/l)		R (%)	I (mg/cm^2)
			permeate	concentrate		
10% PSf+PANI (variant 1)	4.9	138.5	241.1	1549.0	74.72	1.81
	7.4	190.3	183.9	1508.8	80.80	2.74
12% PSf+PANI (variant 1)	4.9	42.6	148.2	1520.2	85.05	3.08
	7.4	79.4	76.3	1389.9	92.32	4.67

Table 5
RESULTS REGARDING THE PERFORMANCES OF COMPOSITE MEMBRANES OBTAINED BY THE VARIANT 2 IN THE BSA SEPARATION PROCESS

Membrane	Soil pH	Permeate flow ($l/m^2 \cdot h$)	BSA concentration (mg/l)		R (%)	I (mg/cm^2)
			permeate	concentrate		
10% PSf+PANI (variant 2)	4.9	146.9	180.9	1575.4	82.25	2.21
	7.4	192.4	79.0	1544.3	92.04	3.42
12% PSf+PANI (variant 2)	4.9	73.9	84.3	1501.9	92.16	3.73
	7.4	115.1	13.9	1311.2	98.67	6.11

Table 6
COMPARISON BETWEEN ULTRAFILTRATION AND ION FLOTATION IN PROTEINS SEPARATION

Separation process	Investment (lei)	Raw material	Additional materials	Energy consumption (kWh)	Operating time (h)	Recovery degree (%)
Flotation	3.000	solution BSA 20-200 mg/L	surfactants	0,5	6	40-80
Ultrafiltration	10.000 (includes membrane)	solution BSA 1000 mg/L	pH adjustment agents	1,8	6	65-95

chromatography. In this part of the experimental section are presented the composite membrane performances obtained in terms of the separation / retention of some proteins in synthetic media.

The values for permeate flow, BSA concentration in the permeate and concentrate, degree of rejection (R) and retention capacity (I) are presented in table 4 for the membranes obtained by the variant 1 and in table 5 for the membranes obtained by the variant 1.

The results of the experiments carried out in order to track BSA retention by the obtained membranes through the two types of coagulation and the two polymer solutions of different concentrations shown that:

- for all the membranes tested, the values obtained for the retention degree (R) and the separation capacities (I) are higher at *p*H = 7.4 compared to the values measured at *p*H = 4.9; this is due to the fact that the *p*H value of 4.9 is very close to the isoelectric *p*H of the BSA protein and under these conditions, the protein, having no electrical charges does not interact with the reactive groups of the PANI composite membrane;
- at *p*H = 7.4, PANI polymer has the best performance from the hydrodynamic point of view (aqueous flows determined during membranes testing were maximum), fact confirmed also in the case of the two functional characteristics;
- comparing the rejection level (R) of the composite membranes from the 12% PSf solution with the one of the membranes from the 10% PSf solution, it is found that the values of this parameter are higher in the case of composite membranes from the 12% PSf solution corresponding to higher PSf and respectively PANI base polymer concentrations in the composite membrane; the explanation is that from the solution with higher concentration are generated membranes with lower cut-off
- the increase of PANI content toward PSf in the composite membrane has direct effect on increasing the retention degree (I). Thus, in the case of 12% PSF + PANI membrane (variant 1) was determined an increase of the retention degree (I) with cca. 27% toward to 12% PSF + PANI membrane (variant 2), and in the case of 10% PSf+PANI membrane (variant 1) the increase is about 24% toward 10% PSf+PANI (variant 2), both at pH=7.4.

Regenerative proteins separation by means of molecular flotation and ultrafiltration (table 6), shows that can be efficiently carried through both processes.

Molecular flotation requires investments and lower energy consumption but has a lower protein recovery degree, which decreases with increasing of the concentration of protein in the feed solution.

On the other side, although ultrafiltration requires greater investment and increased energy consumption, allows processing concentrated protein solutions for recovery means. What can be recommend as optimum operating would be protein concentration by molecular flotation to about 200 ppm and continuing with full recovery by ultrafiltration.

Conclusions

From the operational perspective it is found that is more appropriate to use molecular flotation for BSA separation and concentration from dilute solutions. Another relevant technical aspect is to conduct the process in at least two steps.

The use of a surfactant, dodecylpyridinium chloride, in feeding, is favorably influencing the concentration factor, but especially the BSA recovery degree at low concentrations in feeding (20 mg/L); for concentrations exceeding 100 mg/L BSA, the surfactant effect is insignificant.

The experiments show that BSA has sufficient foaming capacity in order to contribute to separation when the concentrations exceed 100 mg/L. The use of surfactants, especially at low BSA concentrations, is justified when they do not contaminate the final product.

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Acknowledgements: This research was supported by the Sectoral Operational Programme Human Resources Development 2007-2013 through the Financial Agreement POSDRU/107/1.5/S/76813.

References

1.JIANG, C.S., WU, Z.L., LI, R., LIU, Q., Biochemical Engineering Journal, **55**(1), 2011, p.43.
2.BRITES, A.M., PINHO, M.N., , J. Membr. Sci., **78**, 1993, p. 265.
3.NECHIFOR, G., VOICU, S.I., NECHIFOR, A.C., GAREA, S., Desalination, **241**, 2009, p. 342.
4.MEIRELES, M., AIMAR P., SANCHEZ, V., Biotechnol. Bioeng., **38**, 1991, p. 528.
5.JARUDILOKKUL, S., RUNGPHETCHARAT, K., BOONAMNUAYVITAYA, V., Journal of Chemical Engineering of Japan, **44(2),** 2011, p. 78.
6.VILLAMIEL M., DE JONG, P., J. Agric. Food Chem., **48**, 2000, p. 472.
7.VOICU, S. I., ALDEA, F., NECHIFOR, A. C., Rev. Chim. (Bucharest), **61**, no. 9, 2010, p. 817.
8.BARACKOV, I., MAUSE, A., KAPOOR, S., WINTER, R., SCHEMBECKER, G., BURGHOFF, B., Journal of Biotechnology, **161**(2), 2012, p. 138. 9.LINKE, D., BERGER, R. G.,Journal of Biotechnology, **152(4),** 2011, p. 125
10.CRACIUN, M. E., RUSE, E., NECHIFOR, G., Rev. Chim. (Bucharest), **57**, no. 9, 2006, p. 936.
11.TENG, M., LIN, S., JUANG, R., Desalination, **200**, 2006, p. 280.
12.NECHIFOR, G.. ALBU, B., RATA, D., POPESCU, G., Rev. Chim. (Bucharest), **47**, no. 3, 1996**,** p.260.
13.COROBEA, C., DONESCU, D., RADITOIU, S., VOICU, S. I., NECHIFOR**,** G., Rev. Chim. (Bucharest), **57**, no. 9, 2006, p. 981.
14.VOICU, S.I., STANCIU, N.D., NECHIFOR, A.C., VAIREANU, D.I., NECHIFOR, G., Romanian Journal of Information Science and Technology, **12**(3), 2009, p. 410.
15.NAKABAYASHI, T., TAKAKUSAGI, Y., IWABATA, K., SAKAGUCHI, K., Analytical Biochemistry, **419**(2), 2011, p. 173.
16.NECHIFOR, A. C., SAVA, S., TANCZOS, S.K., GALES, O., MIRON**,** A.R., Rev. Chim. (Bucharest), **64**, no. 1, 2013, p. 11.
17.LIU, Z.M., WU, Z.L., LI, R., FAN, X.J., Journal of Food Engineering, **114(2)**, 2013, p. 192.
18.LI, X., EVANS, G. M., STEVENSON, P., Chemical Engineering Research & Design, **11A,** 2011, p. 2298.
19.MATTHIASSON, E., J. Membr. Sci., **16**, 1983, p. 23.
20.CUCIUREANU, A., BATRINESCU, G., BADEA, N.N., RADU, D. A., NECHIFOR, G., Mat. Plast., **47**, no. 4, 2010, p.416.
21.POPESCU, G., NECHIFOR, G., ALBU, B. G., LUCA, N., Rev. Roum. Chim., **34**(2), 1989, p. 577.
22.GUELL, C., DAVIS, R.H., J. Membr. Sci., **119**, 1996, p. 269.
23.NECHIFOR A.C., D. E. PASCU, NEAGU (PASCU) M., TRAISTARU G.A., Rev. Chim. (Bucharest), **64**, no. 4, 2013, p. 343.
24.FAN, Z, WANG, Z., DUAN, M., WANG, J., WANG, S., J. Membr. Sci., **310**, 2008, p. 402.

Manuscript received: 19.07.2013