# Cellulose acetate membranes with controlled porosity and their use for the separation of aminoacids and proteins

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The properties of a polymer membrane can be managed via synthesis of filter materials with controlled porosity. This paper surveys the effect of three cationic surfactants (dimethyl-dioctodecil ammonium bromide, alkyl-benzyl-dimethyl ammonium chloride, N-dodecyl-pyridinium chloride) on the geometry and pore size of cellulose acetate membranes. Porosity control is carried out by adding surfactant to the polymer solution (10 % wt cellulose acetate in N, N-dimethylformamide), followed by membrane coagulation. Synthesized membranes were characterized by scanning electron microscopy to study the surfactant's influence on porosity. Also the membranes were characterized by measuring the hydrodynamic flow of water and alcohol. The retention capacity was measured for the separation of the two proteins - bovine serum albumin and hemoglobin and for the separation of four amino acids - alanine, phenylalanine, tryptophan and lysine. For the bovine serum albumin retention, the higher rejection degree was shown by the dimethyl-dioctodecil ammonium bromide membrane (91%) and in the case of hemoglobin retention, the same membrane showed a rejection rate of 84%.

(Received February 24, 2014; accepted July 10, 2014)

*Keywords:* Cellulose acetate membrane, Surfactant, Proteins, Aminoacids

## 1. Introduction

The research regarding synthesis, characterization and applications of polymeric membranes progresses fast because of the many practical applications of these materials in key areas of interest such as water purification [1], artificial organs [2], fuel cells [3], membrane reactors [4], composite materials [5]. Among the polymers used to prepare membranes are polysulfone, polyphenilen oxide, polyphenilen sulphate, polyether ketone, cellulose and cellulose derivatives.

Cellulose is a syndiotactic homopolymer composed by D-glucopyranose units connected through β-(1-4)-glycosidic bonds. This polymer is the most common organic material in nature, about $5 \times 10^{11}$ tons of cellulose are generated annually in the biosphere. It has no color or smell and it has some excellent properties, such as good mechanical strength, good bicompatibility and hydrophilicity, high sorption capacity and relatively good thermal resistance [6]. Due to the fact that cellulose is sparingly soluble in usual solvents, dissolving in particular highly toxic or difficult to remove mixtures, such as $N_2O_4$ / N,N - dimethylformamide (DMF) or N,N – dimethyl acetamide / LiCl, the use of cellulose derivatives is generally preferred in practice. The most important cellulose derivatives are carboxymethyl cellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, nitrocellulose and cellulose acetates, soluble in a wide range of common organic solvents or water-soluble [6-8].

The importance of cellulose in membranologie lies also in the fact that the first synthetic membranes were synthesized from nitrocellulose by Bechold 1907 [9]. Although it is the oldest polymer used in this area, in the last two decades one can observe a steady increase in the number of articles published on cellulose membranes and cellulose derivatives, from 255 documents in 1995 to 576 documents in 2013, according to Scopus Database (Fig. 1).

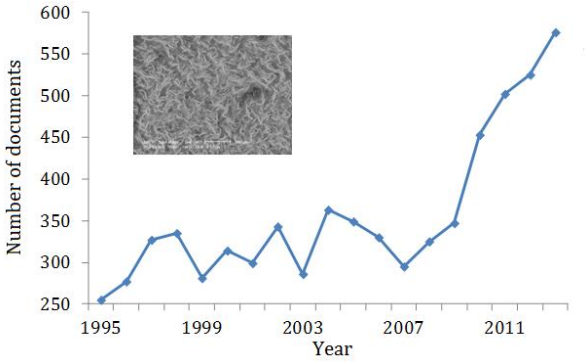


*Fig. 1. Evolution of the number of documents published during 1995-2013 highlighting membranes of cellulose and cellulose derivatives*

Cellulose acetate is an acetylated derivative with different DS (Degree of Substitution). Typically the DS is 2.5 due to the molecular weight, solvation properties in a wide range of polar organic solvents and melt flow

properties [10]. It is used to prepare membranes for all types of membrane separation processes (ultrafiltration, microfiltration, reverse osmosis, nanofiltration) due to polymer solution versatility and possibility of polymer membranes synthesis by phase inversion.

The properties of a polymer membrane can be managed via synthesis of filter materials with controlled porosity. The use of surfactants as additives in polymer solutions is a good strategy for membrane materials geometry and pore size modeling and control [11].

Madaeni et al. [12] have designed pores of a cellulose acetate membrane using as surfactants: cetyl trimethyl ammonium bromide, Triton X-100 and 3,5 – dinitrosalicylic acid. The membranes were synthesized by dissolving cellulose acetate in an acetone:formamide mixture, followed by surfactant mechanical stirring and polymer film precipitation. Membranes with different geometries and pore sizes were used to remove nitrophenols from aqueous solutions. The same group of researchers [13] has synthesized cellulose acetate membranes from the same polymer solution, using this time sodium dodecyl sulfate as additive. Synthesized membranes were used to purify potable water contaminated with pesticides.

This paper surveys the effect of three cationic surfactants (dimethyl-dioctodecil ammonium bromide, alkyl-benzyl-dimethyl ammonium chloride, N-dodecyl-pyridinium chloride) on the geometry and pore size of cellulose acetate membranes. Synthesized membranes were characterized by scanning electron microscopy to study the surfactant's influence on porosity. Also the membranes were hydrodynamic characterized through water and alcohol permeation and aminoacids and proteins retention.

## 2. Experimental

The membranes were synthesized via phase inversion, starting from a 10% wt cellulose acetate solution (Merck) in N, N-dimethylformamide (DMF, Fluka), through coagulation in deionized water. To prepare the polymer solution, the polymer is added to the solvent in small portions to avoid sparingly soluble blocks formation and continuously stirred. When the polymer was completely dissolved, the solution was deaerated for 48 hours before use.

Three surfactants were used for the design of the membrane pores (Fig.2): dimethyl-dioctodecil ammonium bromide, alkyl-dimethyl-benzyl ammonium chloride, N-dodecyl-pyridinium chloride (all from Fluka, of analytical purity). The surfactants were added to the polymer solution at a $10^{-5}$ M concentration and dispersed in the solution mass by ultrasonic for 30 minutes.

The membranes were synthesized by film deposition onto a glass substrate with a film thickness of 300 µm, immersing the substrate into a deionized water coagulation bath. After synthesis, the membrane was washed with water and held in deionized water for at least 24 hours to remove traces of surfactant remaining in the pores.

For the retention of proteins, Bovine Serum Albumin (BSA, Merck) and Hemoglobin (Merck) were used, both of analytical purity. The amino acids used are alanine, phenylalanine, tryptophan and lysine, all of analytical purity, purchased from Merck. For the preparation of the solutions (250 mL, $10^{-5}$ M concentration) deionized water was used.

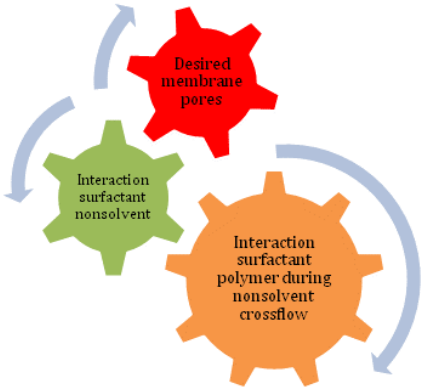


*Fig. 2. The interaction implications between the molecules of surfactant, polymer and nonsolvent*

Scanning electron microscopy was performed with a FEI VX35 Microscope after the samples have been pre-coated with gold [14-16], BSA retention analysis with a UV-viz Specrophotometer Camspec [17, 18], permeation tests and protein and amino acids retention analysis were performed on a Sartorius vacuum system under 0.1 atm using membrane discs with a 45 mm diameter.

## 3. Results and discussion

The surfactants belong to the detergent category. The surfactants molecules consist of a hydrophobic moiety attached to a hydrophilic moiety.

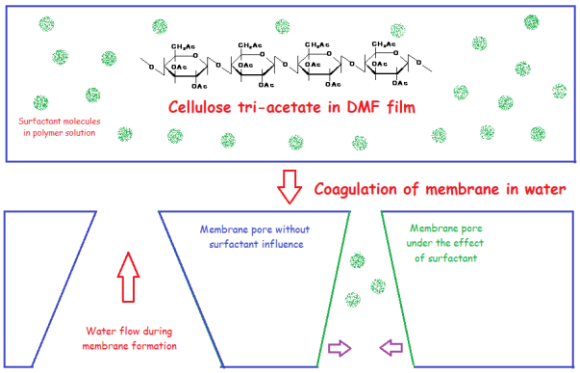


*Fig. 3. The surfactant molecules influence on polymer film in membrane formation*

The advantages of using surfactants in membrane synthesis consisted of the removal of polymer microspheres formed on the membranes surfaces [12, 13].

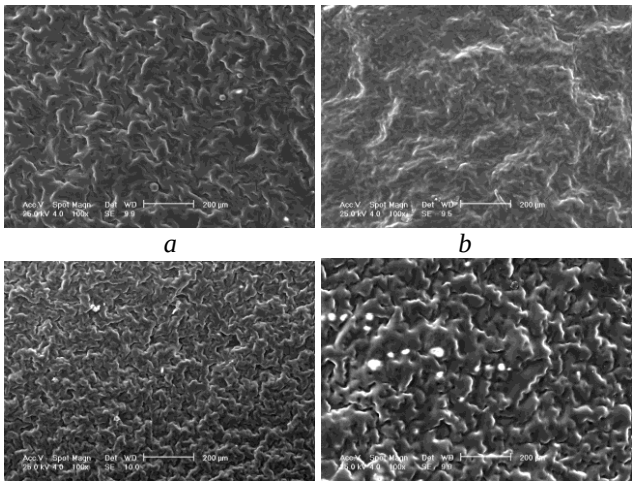

*Fig. 4. Scanning electron microscopy of active surfaces for cellulose acetate membranes (a) and synthesized membranes using as additives dimethyl-dioctodecil ammonium bromide (b), alkyl-benzyl-dimethyl ammonium chloride (c), N-dodecyl –pyridinium chloride (d) - × 100*

The porosity design process of polymer membranes using surfactants can be explained first of all via surfactant molecules interaction with non-solvent molecules resulting in a modified surface tension of the non-solvent (in this case, water). Modified surface tension changes the non-solvent flow properties and therefore the speed at which it crosses the polymer film.

Synthesis of polymer membranes by phase inversion depends on several process parameters. The most influential factors are related to polymer solution and non-solvent physical properties. Temperature plays an important role here. If polymer film precipitation occurs in a warm nonsolvent, membrane pore size decreases with membrane cooling due to material contraction effect. Another important factor is the non-solvent flow speed through the polymer film, excessive speed resulting in large pores formation, while a very slow speed leads to small pores formation with an extensive distribution on the membrane surface (such as membranes obtained through solvent evaporation).

Initially, the surfactant molecules are found only in the polymer solution and do not affect membrane formation because no dynamic process takes place. When non-solvent molecules appear, the dynamic regime in which molecules travel through the polymer film is modified due to surface tension changes. The nonsolvent 'slips' in different ways through polymer macromolecules, depending on the surfactant nature and character (Figure 3). The three surfactants (dimethyl-dioctodecil ammonium bromide, alkyl-dimethyl-benzyl ammonium chloride, N-dodecyl pyridinium chloride) were chosen because in a previous paper [11] the surfactants were used to study the effect on polysulfone membranes formation.

Changes in the membranes morphology were studied by SEM microscopy. Active surface and porous surface have been studied at the same magnification for a cellulose acetate membrane obtained from a polymer solution without surfactants, as well as for membranes obtained with the three cationic surfactants used as additives. Microscopy was performed after a prior sample drying, therefore the active surface morphology reveals more information about surface structure and not about pore size or shape (Fig. 4). The surface organization structures observation still provides information about the differences between membrane pores. The polymer is able to organize during drying only depending on the membrane channels initial form.

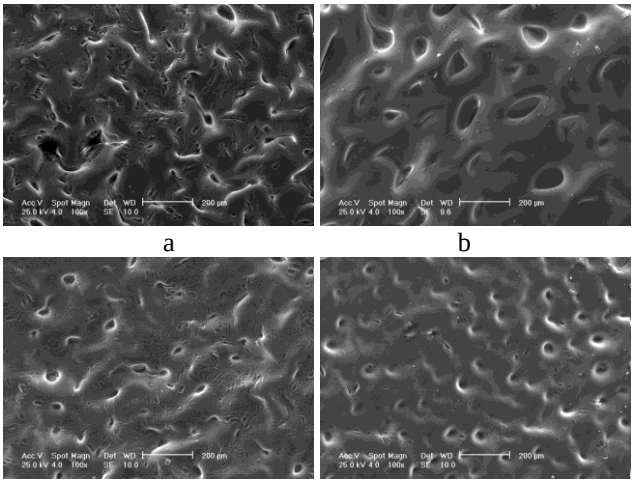

*Fig. 5. Scanning electron microscopy of porous surfaces for cellulose acetate membranes (a) and synthesized membranes using as additives dimethyl-dioctodecil ammonium bromide (b), alkyl-benzyl-dimethyl ammonium chloride (c), N-dodecyl –pyridinium chloride (d) - × 100*

The cellulose acetate membrane with no additive in the polymer solution (Fig. 4a) has a polymer structure defining grooves with a length of between 50 and 200 μm on the active surface. The membrane surface reveals polymer microspheres from place to place, resulting from the phase-inversion process (macromolecular tangles are driven from the polymer solution volume into the membrane surface). The surface structure of the membrane which used dimethyl-dioctodecil ammonium bromide is more compact (Fig. 4b). The dimensional characterization of polymer self-organization forms is much more difficult. Membranes obtained by using alkyl-benzyl-dimethyl ammonium chloride (Fig. 4c) and N-dodecyl pyridinium chloride (Fig. 4d) have pores becoming larger. In the last membrane case it can be observed surface grooves forming length up to 500 μm. Also this membrane surface reveals microspheres, more even than the membrane without any addition. As pore size and pore distribution, cellulose acetate membranes without additives are somewhere between alkyl-benzyl-dimethyl ammonium chloride membranes and N-dodecyl-pyridinium chloride membranes.

Membrane porous surface analysis reveals several important aspects, in contrast with the active surface. Membranes using alkyl-benzyl-dimethyl ammonium chloride and N-dodecyl-pyridinium chloride as surfactants have the same pore size of about 20 μm. However, the second membrane (Fig. 5d) has a larger surface pore distribution. Due to this pore distribution, on the alkyl-benzyl-dimethyl ammonium chloride membrane surface (Fig. 5c) polymer structure ribs can be seen after the material drying process. The cellulose acetate membrane without additives (Fig. 5) has pores with diameters in a large range between 10 and 100 μm. Also in this case, a dry polymer folding can be seen, most likely around the pores. Dimethyl-dioctodecil ammonium bromide membranes reveals larger pores, with a diameter of about 180 μm, a uniform surface distribution, without dry polymer folding. This indicates that the pore structure is more stable than the other membranes.

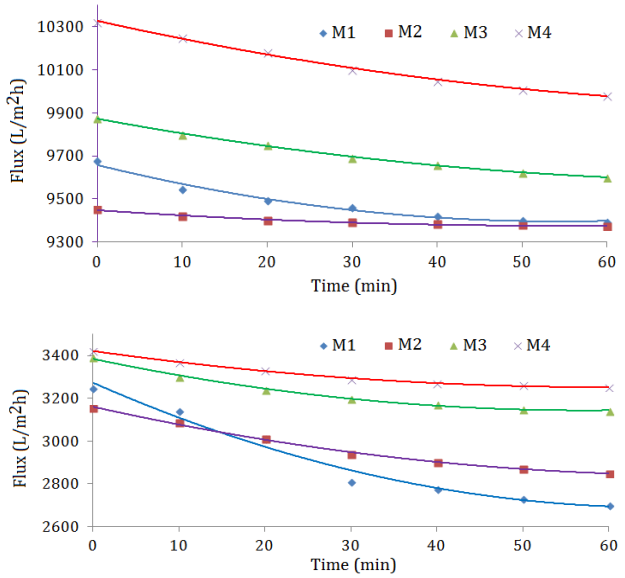


*Fig. 6. Water flow (top) and ethanol flow (bottom) for cellulose acetate membranes - M1, dimethyl-dioctodecil ammonium bromide polymer - M2, alkyl-benzyl-dimethyl ammonium chloride polymer - M3, N-dodecyl-pyridinium chloride polymer - M4*

In order to assess the synthesized membranes hydrodynamic proprieties, water flow and ethanol flow were studied. The results are shown in Figure 6. The results are in agreement and can be correlated with the electronic microscopy micrographs observations. The water flow value of cellulose acetate membrane without surfactant started from 9675 L/m$^2$h. After one hour the water flow value decreased to 9391 L/m$^2$h. For the dimethyl-dioctodecil ammonium bromide membrane, the water flow value started from 9450 L/m$^2$h, and after one hour it decreased to 9373 L/m$^2$h. For the alkyl-benzyl-dimethyl ammonium chloride membrane, the water flow started from 9874 L/m$^2$h and after one hour it decreased to 9600 L/m$^2$h . For the N-dodecyl-pyridinium chloride membrane, the water flow value started from 10321 and after one hour it decreased to 9980 L/m$^2$h.

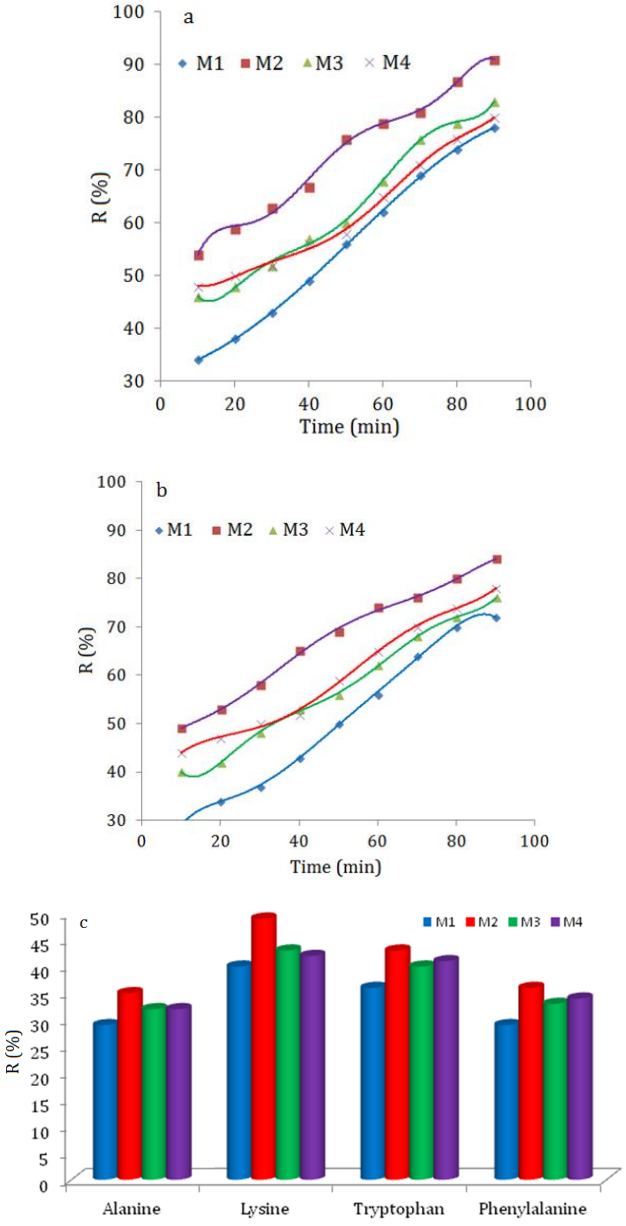


*Fig. 7. Bovine Serum Albumin (a), hemoglobin (b), amino acids - alanine, lysine, tryptophan, phenylalanine (c) retention for cellulose acetate membranes - M1, dimethyl-dioctodecil ammonium bromide polymer - M2, alkyl-benzyl-dimethyl ammonium chloride polymer - M3, N-dodecyl-pyridinium chloride polymer - M4*

The decreasing water flow values can be explained via compactness and stability of the membrane internal structure, resulting in increasing the porous layer resistance against the advancing solvent. For dimethyl-dioctodecil ammonium bromide membrane the flux decrease was the smallest, due to a very low initial porosity and a greater interlayer stability in macroporous structure. The flow decrease had the same trend for both cellulose acetate membrane and membranes with surfactant.

The ethanol flow value of cellulose acetate membrane without surfactant started from 3246 L/m$^2$h. After one hour the ethanol flow value decreased to 2700 L/m$^2$h. For the dimethyl-dioctodecil ammonium bromide membrane, the ethanol flow value started from 3154 L/m$^2$h, and after one hour it decreased to 2850 L/m$^2$h. For the alkyl-benzyl-dimethyl ammonium chloride membrane, the ethanol flow started from 3390 L/m$^2$h and after one hour it decreased to 3140 L/m$^2$h . For the N-dodecyl-pyridinium chloride membrane, the ethanol flow value started from 3420 and after one hour it decreased to 3250 L/m$^2$h.

The decreasing ethanol flow values can be explained via cellulose and cellulose derivatives hydrophilicity. Ethanol is an organic solvent and do not wet the pore walls. The ethanol flow is slower than water flow. The largest flow decrease was recorded for the cellulose acetate membrane without any surfactant. This may be explained by interaction between the hydrophobic alcohol and a surfactant residue in the membrane structure. Also it should be noted the fact that ethanol molecules are heavier and larger than water molecules, hence more pronounced influence on the internal structure of the membrane.

In order to evaluate the membrane retention capacity, a bovine serum albumin and hemoglobin solution ($10^{-5}$M concentration) was filtered. The solution was refluxed for 90 minutes. Retention is calculated using formula 1:

$$R=(1-C_p/C_f) \times 100 \quad (1)$$

where Cp is the concentration in permeate (the resulting solution after passing through the membrane) and Cf is the concentration of the feed solution. The results are shown in Figure 7. Separation efficiency is lower than in the case of a polysulfone membrane, but it reveals good values for proteins. In the case of bovine serum albumin, separation efficiency was 78% for cellulose acetate membranes, 91% for dimethyl-dioctodecil ammonium bromide membranes, 83% for alkyl-benzyl-dimethyl ammonium chloride membranes and 80% for N-dodecyl-pyridinium chloride membranes. The optimum value for dimethyl-dioctodecil ammonium bromide membrane can be explained by its porosity (diameter distribution). Other membranes do not allow a very efficient separation process due to poor pore distribution on active surface and unevenness. Also, large pore diameter in conjunction with poor pore distribution, favors clogging. The separation process becomes difficult in time and it requires a higher operating pressure.

The separation efficiency of hemoglobin is lower than in case of bovine serum albumin, 78% for cellulose acetate membranes, 91% for dimethyl-dioctodecil ammonium bromide membranes, 83% for alkyl-benzyl-dimethyl ammonium chloride membranes and 80% for N-dodecyl-pyridinium chloride membranes. The difference between the two separation is explained by the different sizes of the two proteins and their shape. Also in the case of hemoglobin, the dimethyl-dioctodecil ammonium bromide membrane has the best separation efficiency, due to its porosity.

Another interesting observation would be that for polysulfone using the same surfactants [11], dimethyl-dioctodecil ammonium bromide membranes did not show the best performance for solvent permeation or protein filtration and retention tests . A possible explanation could be the different nature of the two polymers: polysulfone is a hydrophobic technopolymer, while cellulose acetate is a hydrophilic natural polymer derivative. The different chemical nature results in different behavior of the two films during the polymer membrane synthesis due to the interactions between the molecules of surfactant, solvent, nesolovent and the macromolecular polymer chain.

Separation of amino acids was not performed with spectacular results. The synthesized membranes are not suitable for this type of filtration. The interpretation of the data flows of water, alcohol and protein filtration reveals that this type of membranes are suitable for microfiltration and limited for ultrafiltration. The higher rejection degree was for lysine, in the case of dimethyl-dioctodecil ammonium bromide membrane with a value of 49%, and the lowest was for alanine, in the case of cellulose acetate membrane without surfactant. Values for the separation of amino acids are due to the molecular size alone. The membranes cannot be used for full separation from an aqueous solution, but can be used for amino acid solutions dilution or for partial removal.

## 4. Conclusions

In order to design the pores of cellulose acetate membranes and to study the possibility of inducing filter properties, to the polymer solutions were added three different surfactants - dimethyl-dioctodecil ammonium bromide, alkyl-benzyl-dimethyl ammonium chloride, N-dodecyl-pyridinium chloride. Membranes were characterized by scanning electron microscopy to observe morphological changes. Dimethyl-dioctodecil ammonium bromide membranes have smaller and denser pores than pure polymer membranes, while the other two membranes have larger pores and a smaller distribution area. Results of the microscopy study were correlated with water and ethanol permeation. For water, the lowest flow value after 60 minutes was shown by the dimethyl-dioctodecil ammonium bromide membrane (9373 L/m$^2$h), and the highest value was shown by the N-dodecyl-pyridinium chloride membrane (9980 L/m$^2$h). However, for ethanol permeation, the lowest flow value was shown by the membrane without additives (2700 L/m$^2$h) and the highest flow value was shown by the N-dodecyl-pyridinium chloride membrane (3250 L/m$^2$h). For the bovine serum albumin retention, the higher rejection degree was shown by the dimethyl-dioctodecil ammonium bromide membrane (91%) and in the case of hemoglobin retention, the same membrane showed a rejection rate of 84%. Synthesized membranes did not show a good performance in amino acids separation. The rejection degree did not exceed 49%.

**Acknowledgements**

The work has been funded by the Sectoral Operational Programme Human Resources Development 2007-2013 of the Ministry of European Funds through the Financial Agreement POSDRU/159/1.5/S/ID134398.

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