

# Ascitic Fluid Ultrafiltration through Polysulfone–Polyaniline Composite Membranes

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*The paper presents the ascitic fluid ultrafiltration using asymmetric and composite membranes. For this reason, two variants of polysulfone - polyaniline composite membranes were used. Permeate flow was within the values known for this type of membranes. The retention of components is favourable, being characteristic for ultrafiltration using membranes with low hydrophilicity. The process was conducted at pH 6.5 (natural value of ascitic fluid). The ascites fluid used was preserved only through freezing and not chemically. In addition to albumin, bilirubin and uric acid were concentrated. Globulins are retained on the surface and inside the membranes. Ultrafiltration of ascites fluid through 12% polysulfone-polyaniline composite membranes in both coagulation variants provided good results that offer real outlooks for albumin recovery.*

**Keywords:** albumin recovery, ascitic fluid, composite membranes, ultrafiltration

Ascitic fluid is a biological fluid which accumulates in the abdominal cavity in the case of some serious diseases like: liver cirrhosis, alcoholic hepatitis, toxic or viral infection, abdominal cancer (stomach, pancreas, ovary, uterus, and kidney), lung cancer, acute pancreatitis and contains about 3 g/dL protein [1-3]. Usually, the removal of ascitic fluid from the body is done by puncture [4].

From the qualitative point of view, this undesirable fluid for the human body has the same content as the blood serum, but the amount of analytes is different [2, 4].

Albumin is the most abundant plasma protein (55 ÷ 65% of total protein) from 20 weeks of pregnancy and then for the whole life.

It is synthesized by the liver and has multiple roles, the most important being: colloid osmotic intravascular pressure maintenance and transport of hormones, degradation products which has to be eliminated from the body, vitamins, drugs, ions, water, etc. [4,5].

The amount of albumin dramatically decreases in some severe diseases like: liver cirrhosis, lupus erythematosus, glomerulonephritis, mellitus diabetes, requiring regular treatment with albumin injection [6,7].

The amount of albumin reported to the total amount of protein in the ascitic fluid is a differential diagnosis element for the cause of ascites [4,5]. Some of the physico-chemical properties of the albumin are presented in table 1 [8,9].

Globulin fractions detected by electrophoresis in alpha1, alpha2, beta and gamma globulin fulfill various functions in the body: source of nutrition, buffer systems components, immunological agents (immunoglobulins), conveyor of certain ions and molecules (haptoglobin), various proteolytic enzymes regulating activity role (antiproteases: alpha1-antitrypsin, alpha2-macroglobulin) [10].

In addition to protein (albumin and globulin), ascites fluid contains substances resulting from protein catabolism (table 1) [5-8].

Also, glucose plays a significant role from quantitative point of view in ascitic fluid composition in which may

occur in small quantities lipids, ions and enzymes [11].

The idea of albumin recovery from ascitic fluid was born as a result of fields multiplication in which membrane techniques have found applications and due to their continue improvement, due to the existence of practical applications of membrane separations in the medical field (the dialysis process, contraceptive membrane, implants, sterilization processes for biological materials) and, not at last, due to the high cost of injectable albumin on the pharmaceutical market: about 100 € / g [12-17].

The main objectives of usage the ultrafiltration process with polymeric membranes in the case of ascitic fluid were:

- the study of total proteins and their fractions separation;
- the study of albumin separation;
- the study of important analytes separation, mainly those resulting from protein catabolism.

## Experimental part

### Materials, methods and equipment


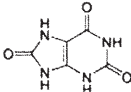
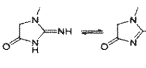

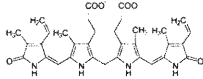
#### Membrane

Analyzing the results obtained in the previous experiments in the case of proteins separation from aqueous solutions using laboratory prepared polymeric membranes [18-20], for this process were chosen composite membranes obtained from polymeric solutions with 12% polysulfone – 1% polyaniline (PSf-PANI) in two variants of preparation: coagulation agent-water (variant 1) respectively, water - aniline (variant 2) which were tested in the separation of bovine serum albumin synthetic solutions. The morphology of used membrane samples prepared by immersion precipitation method [21-23], phase inversion with chemical reaction techniques [24-26] relieved asymmetrical structure and 100-120µm thickness (fig. 1).

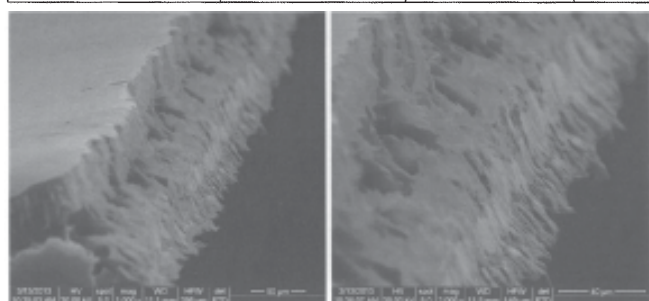
#### Instrumentation

The materials used for the experiments were: graded beakers, 500 mL, and hermetically closed, heat-resistant glass, sterile.

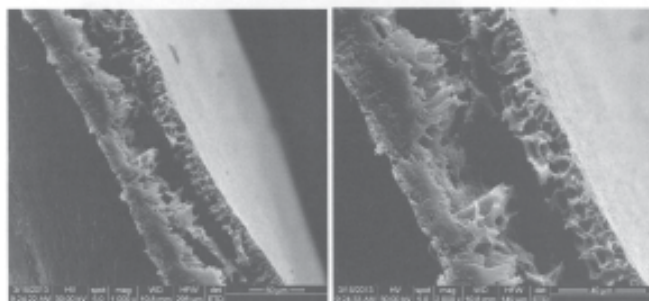
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Characteristics of chemical species from ascitic fluid				
albumin	uric acid	creatinine	urea	bilirubin
molecular weight 65÷67 kDa; isoelectric point: 4,9; it is composed of 609 amino acid; total number of negative ionic charge: 98; total number of positive ionic charge: 87	molar mass 168.11 g, resulting from nucleic acid degradation, is the final product of purine catabolism, constituent of deoxyribonucleic acid	molar mass 113.12 g, is the catabolic product of muscle phosphocreatine and is excreted exclusively by the kidney	molar mass 60.057 g, is the final product of the catabolism in the liver	molar mass 584.66 g, is a biliary pigment, yellow, pyrrole derivative, resulted from the degradation of hemoglobin
$C_{3076}H_{4833}N_{821}O_{919}S_4$	$C_5H_4N_4O_3$	$C_4H_7N_3O$	$CH_4N_2O$	$C_{33}H_{36}N_4O$
				

**Table 1**  
CHARACTERISTICS OF CHEMICAL  
SPECIES FROM ASCITIC FLUID



a. variant 1



b. variant 2

Fig. 1. Morphology of PSf-PANI membrane at two magnitudes: a-variant 1 (x1000 and x2000) and b-variant 1 (x1000 and x2000)

## Reagents

The reagents used were:

- Ethyl alcohol, p.a. Merck®, used for membranes activation;
- Biosystems® used for *in vitro* biochemical assays, initial analysis of ascitic fluid, concentrates and permeates resulting from the ultrafiltration process ;
- Kit for serum protein electrophoresis, DDS Diagnostic;
- Laboquick immunochromatography rapid tests;
- Biolab molded plaques culture media;
- Merck® pH indicator strips.

## Equipment

- Automatic Biochemistry Analyzer A25 - automated high-performance spectrometer, used for *in vitro* diagnosis in clinical laboratories.

**Performances:** the possibility to program operating parameters for 450 different tests organized in several

workshops throughout the clinical laboratory methodology means: absorbance, linear and non linear concentration, kinetics 1, kinetics 2, serial dilutions, with execution in duplicate of each sample, working speed: 240 tests/h, results archiving capacity, the possibility of performing emergency tests as first priority without disrupting routine work list, friendly in communication through an attached computer, and due its own high performance software, quality control subprogram archiving calibration and internal controls, preparation and archiving of Levey-Jennings charts.

**Main components:** power supply with voltage regulator incorporated, transfer system that performs pipetting operations and transport of reagents and samples, includes pump, fluid routes, robotic arm acting on the xyz coordinates, micro syringe with piston, needle pipetting reagents and samples, engines, the analyzer body including analysis chamber with rotor in which is installed a single plastic element with 120 cells where are occurring chemical reactions or enzymatic determination (also called rotor), optical system composed of: photometer, 9 interference filters, a halogen lamp (12V/20W) providing absorbance reading in the 340-670 nm domain, data processing system: measures the absorbances, calculates concentrations, generates results. The software includes self-test programs to highlight any mistakes or malfunction setting, samples to be analyzed dilution program, heating reagents and both ambient and own analysis camera temperature monitoring.

**Accessories:** stands for samples and reagents, plastic bowls for samples, two sizes reagent containers (20/50 mL), high quality plastic rotors that held chemical and biochemical reactions and through which is passed the light beam for absorbance measurement, distilled water containers, liquid system, and wash solution and for wastes.

**Operation principle:** Based on the installed program and with the use of movable arm performs pipetting of samples and reagents which are transferred into rotor tanks.

Here, the samples are mixed, heated at 37°C and after the scheduled time, according to the reaction type, the absorbance are measured through the optical system. The measurement result is processed in a very short time and the result is passed to the data base.

Printing the analysis report is achieved at manual command when all tests required for that sample were

performed. For each test the calibration is made towards the reference material. If errors occur, they are printed and displayed in order to be remedied by the operator. It is produced by Biosystems – Spain, calibrated by INM - Bucharest with maintenance checks done.

- Electrophoresis system ELF200 - apparatus used for carrying out agarose gel electrophoresis from biological fluids. The apparatus has a unitary construction and consists of a power supply and dryer provided with delay, the command and control block is at the top of the device, and on the front of the panel are the display, keyboard and signaling leds, the parameters that can be programmed are: voltage, migration and drying time. The apparatus uses visual and audible signaling if the load current exceeds 100 mA and working time is out.

*Accessories:* the migration cell with graphite electrode having a migration capacity of 10 samples, fixing/staining/discoloring plastic vats, scanner, computer provided with software application for scanning and interpretation of total protein fractions, hemoglobin and lipids separations. It is produced by Electronic April – Romania.

- Labsystems semi automatic micropipettes 0,1 ÷ 10 µL, 50 µL and 20 ÷ 200 µL, calibrated by INM - Bucharest with maintenance checks done and used in protein electrophoresis.

- Reference materials with traceability: calibrators and internal controls on two - value levels produced by Biosystems ® (biochemistry) and BioRad ® (electrophoresis).

#### Analytical methods

During the spectrometric analysis were used the methods recommended by the reagent manufacturer (Biosystems), generally based on the principle of enzymatic reactions (with or without chromophore group) which are carried out at 37°C, the analyte concentrations being automatically determined on the basis of the calibration curve from the memory of the analyzer.

The methods are standardized by the International Federation of Clinical Chemistry (IFCC).

The experiments were performed on automatic biochemistry analyzer A25 where daily are used internal control samples with traceability on two- value level. The analytical methods are validated according to I-12-RENAR - analytical methods validation instruction. Protein electrophoresis was carried out on agarose gel (manufacturer DDS Diagnosis - Romania). Working steps are: pipetting the analyzed samples on the celluloid support of the agarose gel (film), migration (separation of the fractions) is carried out in barbital TRIS buffer solution pH = 8.4, at 100 mV for 20 min, fixing the separation of the protein fractions in a solution of ethyl alcohol / acetic acid / distilled water followed by an intermediate drying, staining (revealing the separation of protein fractions) with amido black dye, discoloration, again with solution of ethyl alcohol / acetic acid / distilled water, but this time in a different ratio, final drying of the film.

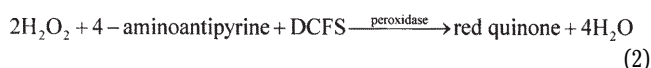
The separation of the protein fractions was interpreted in the software application provided by the device manufacturer (Electronic April - Romania). As in the case of the spectrometric analyzes, on each film are pipetted two internal control samples with traceability on two- value levels. The analytical method is validated according to I-12-RENAR - analytical methods validation instruction. Immunochromatography and microbiology assays for preliminary testing (possible infection) of the ascites fluid were carried out on the basis of the standard methods [27, 28]. The reaction principles of the main components of the ascitic fluid are [29]:

- Albumin - reacts at pH = 4.1 with bromocresol green forming a colored compound (emerald green) which can be determined by spectrometry. The optimum acidic environment for developing the reaction is created with the acetate buffer solution. The absorbance of the sample toward the blank is read at the wavelength  $\lambda = 630$  nm.

- Total proteins - reacts in an alkaline medium with  $\text{Cu}^{2+}$  ions forming a colored compound (purple) dosable by spectrometry (biuret reaction). The optimum acidic environment for developing the reaction is created with potassium hydroxide. The absorbance of the sample toward the blank is read at the wavelength  $\lambda = 545$  nm.

- Total bilirubin - in the presence of cetimides (needed to break the bonds of bilirubin-albumin conjugation) forms a colored compound diazosulfanilic acid dosable by spectrometry. The absorbance of the sample toward the blank is read at the wavelength  $\lambda = 540$  nm.

- Uric acid – forms according to the coupled reactions described below, a colored complex colorimetrically dosable:



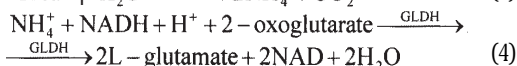
where:

DCFS = dichloro phenyl phosphate.

The absorbance of the sample toward the blank is read at the wavelength  $\lambda = 520$  nm.

- Creatinine - it is dosed on the basis of the reaction rate of the formation of a colored compound in the alkaline medium with picrate (kinetic reaction). The optimum alkaline environment for developing the reaction is created with sodium hydroxide (Jaffe reaction). The reaction is monitored at wavelength  $\lambda = 500$  nm.

- Urea – present in the sample is consumed according to the coupled reactions described below, being measured by spectrometry the nicotinamide dinucleotide dehydrogenase formed and consumed in the second reaction (kinetic reaction):



where:

GLDH = glutamate dehydrogenase.

The reaction is monitored at wavelength  $\lambda = 340$  nm.

- Calcium - reacts with methyl thymol blue in an alkaline medium, forming a colored complex that can be measured by spectrometry. In order to avoid the interference with magnesium, the reaction takes place in the presence of hydroxyquinoline. The absorbance of the sample toward the blank is read at the wavelength  $\lambda = 610$  nm.

- Magnesium - in an alkaline medium forms with xylidyl blue a colored complex, dosable by spectrometry. In order to avoid the interferences with calcium, the reaction is done in the presence of tetra-amino ethylene glycol. The absorbance of the sample toward the blank is read at the wavelength  $\lambda = 520$  nm.

- Iron – The  $\text{Fe}^{3+}$  ions bound to transferrin are released by guanidine and reduced to  $\text{Fe}^{2+}$  with hydroxylamine.  $\text{Fe}^{2+}$  ions are reacting with ferozzine, forming a colored complex colorimetrically dosable. The absorbance of the sample toward the blank is read at the wavelength  $\lambda = 630$  nm.

Protein electrophoresis - is based on the property of proteins to migrate in an electric field based on the electric charge and the isoelectric point of each-one.



**Table 2**  
PROTEIN FRACTIONS DISTRIBUTION IN ASCITIC FLUID  
(ALB = ALBUMIN, GLOB = GLOBULIN)

Proteins fractions	Fraction	
	%	g/dl
Albumin	45.20	1.14
Alfa-1 Glob	2.00	0.05
Alfa-2 Glob	10.30	0.26
Beta Glob	20.50	0.52
Gamma Glob	22.00	0.55
Alb/ Glob Ratio	0.83	

#### Personal protective measures and biohazardous waste management

In order to protect the employees handling biological samples and chemical reagents the standard precautions and instructions on occupational safety have to be respected [30-34].

It has to be avoided the ingestion or skin and mucous contact with reagents. In case of skin contact, rinse the affected area with water. In case of eye contact or ingestion is required emergency medical attention. After use, the bottles should be washed with water. The exposed metal surfaces should be cleaned with 10% sodium hydroxide solution and the worktables with Terallin 5% disinfectant solution.

All products of human origin should be considered as potentially infectious. It is recommended to handle the reagents and biological samples according to regulations such as: wearing gloves when handling reagents and samples, mouth pipetting is prohibited, eating, smoking or drinking while working are prohibited, performing daily decontamination and disposal of samples and reagents. Reagents and other used materials are labeled with warnings about the safety and environment, according to the European Community directives.

Biological liquid waste resulting from performed analyzes are treated with 5% sodium hypochlorite solution and then discharged to the toilet.

Biohazardous wastes as: soft materials, sample containers, supplies, are stored in the properly labeled yellow box. Stinging wastes are stored separately, in strong yellow boxes that close tightly, marked in accordance with European Community directives and the Romanian legislation are kept in special places, closed and are raised and transported for destruction at every 48 h by a specialized company under contract.

#### Working procedure

##### Preparation of biological material

We obtained the ascitic fluid from the Gastroenterology Department of Pitesti County Emergency Hospital from a patient diagnosed with nonviral liver cirrhosis:

- the total quantity was partitioned in 500 mL flasks, which have been preserved at - 20°C

Seeded environment	Selenite broth	Blood agar	CLED	AABTL	ADCL	Sabouraud
Obtained result	no increase	no increase	no increase	no increase	no increase	no increase
Conclusion: ascitic fluid was sterile						

Test	AgHBs	AntiHCV
Result	Negative	Negative
Conclusion: ascitic fluid was not infected with hepatitis		

**Table 3**  
SPECIFIC ANALYTE CONCENTRATION IN ASCITIC FLUID.

Analyte	U.M. IFCC	Value
Total proteins	g/dl	2.52
Albumin	g/dl	1.15
Total bilirubin	mg/dl	2.67
Urea	mg/dl	17.00
Creatinine	mg/dl	0.82
Uric acid	mg/dl	5.53
Glucose	mg/dl	170.00
Cholesterol	mg/dl	38.00
Triglycerides	mg/dl	35.00
Total calcium	mg/dl	7.50
Magnesium	mg/dl	1.39
Iron	µg/dl	56.00

- the composition of the ascitic fluid was physico-chemically, spectrophotometrically, electrophoretically, immuno chromatographically and bacteriologically analyzed, achieving the results set out below.

Physico-chemical properties: clear liquid, yellow fluorescent, specific odor, pH = 6.5.

Principal characteristics of used ascetic fluid were brief presented in tables 2-5.

After thawing, each sample was again analyzed, pointing out that glucose and lipid values were not preserved which means that the study of separation of these components is not the subject of this paper. In the case of proteins, substances resulting from protein catabolism and ions the differences were not significant.

#### Preparation of ultrafiltration plant

Tangential flow ultrafiltration plant CELFA System was used [35,36]. The plant preparation consisted in flushing with distilled water and pressure tested.

#### Membranes preparation

Activation of polyaniline - polysulfone composite membranes was made by keeping them prior to use during an hour in ethanol a.p./distilled water solution at 2:1 ratio [21-26].

#### Working parameters

In each of the two experimental variants it was started from a constant volume of 0.5 L ascitic liquid, collecting 0.25 L of permeate, respectively 0.25 L of concentrate, at a working pressure of 6 bar.

**Table 4**  
RESULTS OF ASCITIC FLUID  
MICROBIOLOGICAL TESTING

**Table 5**  
RESULTS OBTAINED FOR ASCITIC FLUID  
IMMUNOLOGICAL TESTING

Analyte	U.M.	C1	P1	C2	P2
Total proteins	g/dl mg/l	2.47 24700	0.24 2400	2.77 27700	0.18 1800
Albumin	g/dl mg/l	1.61 16100	0.32 3200	1.95 19500	0.29 2900
Total bilirubin	mg/dl mg/l	0.53 5.30	0.70 7	0.59 5.90	0.53 5.30
Urea	mg/dl mg/l	15 150	16 160	16 160	17 170
Creatinine	mg/dl mg/l	0.11 1.10	0.62 6.20	0.12 1.20	0.74 7.40
Uric acid	mg/dl mg/l	1.22 12.20	3.81 38.10	1.20 12.00	3.84 38.40
Total calcium	mg/dl mg/l	7.61 76.10	7.39 73.9	7.37 73.70	7.51 75.10
Magnesium	mg/dl mg/l	1.14 11.40	1.32 13.20	1.45 14.50	1.32 13.20
Iron	µg /dl mg/l	75.00 0.75	30.00 0.30	84.00 0.84	23.00 0.23

Fraction	C1		C2		Observations
	[%]	[g/dl]	[%]	[g/dl]	
Albumin	65.20	1.60	70.40	1.95	Globulin concentrations in the permeate were below the quantification limit.
Alfa-1 globulin	1.00	0.02	0.80	0.02	
Alfa-1 globulin	3.10	0.07	2.00	0.06	
Beta globulin	18.8	0.46	16.00	0.44	
Gamma globulin	11.90	0.29	10.80	0.30	
A/ G ratio	1.87		2.38		

Analyte	12%PSf+PANI membrane variant 1 permeate flux = 69,4 (l/m <sup>2</sup> ·h)		12%PSf+PANI membrane variant 2 permeate flux = 78 (l/m <sup>2</sup> ·h)	
	R (%)	I (mg/cm <sup>2</sup> )	R (%)	I (mg/cm <sup>2</sup> )
Total proteins	90.48	364.06	92.86	326.56
Albumin	72.17	57.81	74.78	9.38
Total bilirubin	73.78	0.64	80.15	0.66
Urea	5.88	0.47	0.00	0.16
Creatinine	24.39	0.14	9.76	0.12
Uric acid	31.10	0.94	30.56	0.94
Total calcium	1.47	0.00	-0.13	0.02
Magnesium	5.04	0.05	5.04	0.00
Iron	46.43	0.00	58.93	0.00

Separation process duration and the emergence of the first drops were different depending on the membrane used as follows:

- the series 1 of separation reached C1/P1 = 1:1 after about 4 1/2 h;
- the series 2 of separation reached C2/P2 = 1:1 after about 4 h.

## Results and discussions

The mean results for the analysis of separation fractions obtained during the two series of processing, used at results interpretation are reported in (table 6 and 7).

The ability to separate the components present in the fluid subjected to the ultrafiltration process is evidenced by determining the degree of rejection, R:

$$R = \frac{C_f - C_p}{C_f} \cdot 100 \quad (\%) \quad (5)$$

Where:

$C_f$  – feed solute concentration (mg/L)

$C_p$  = permeate solute concentration (mg/L)

The retention capacity (immobilization I) on the membrane surface and in its microporous structure by adsorption and ionic bonds formation between polysulfone reactive groups with reactive residues from the ascitic fluid components structure is presented in (table 7).

**Table 6**  
SPECTROMETRIC ANALYZES RESULTS  
OF THE CONCENTRATES AND  
PERMEATES OBTAINED

**Table 7**  
PROTEIN ELECTROPHORESIS RESULTS  
FROM CONCENTRATES AND PERMEATES  
OBTAINED  
(A = ALBUMIN, G = GLOBULIN)

**Table 8**  
HIGHLIGHTING THE REJECTION DEGREE -  
R AND IMMOBILIZATION CAPACITY - I OF  
KEY ANALYTES IN THE CONCENTRATES  
AND PERMEATES OBTAINED

$$I = \frac{m}{S} \quad (\text{mg/cm}^2) \quad (6)$$

were:

m = total protein retaining (mg)

S = active membrane surface (cm<sup>2</sup>)

The results are presented in the diagrams from table 6-8. Although the retention degree (R) of the total protein is greater than that of albumin, total protein retention capacity is less than that of albumin ( $R_{PT} > R_{Alb}$ ,  $I_{PT} < I_{Alb}$ ), in both variants of the membranes, which could be explained by the fact that globulins have more active functions available for over and intramembrane bond formation.

At the electrophoresis of permeates obtained on the globulin migration domain could not be detected anything, not even when the dilution of samples decreased, their concentration is also very low in the concentrates obtained, (fig. 2).

The fact that lipids were not stable in time in the ascitic fluid leads to the idea of lipoproteins formation. This second hypothesis is supported by the values of separation concentrations for proteic fractions (table 6).

Bilirubin, although having reactive acid groups, has a smaller retention capacity, but a rejection degree that should be taken into account for a possible attempt of albumin recovery from the ascitic fluid. The explanation is that it still remains bounded to the albumin (in order to be



Fig. 2. Proteins electrophoresis: C = Concentrate, P = Permeate, 1 = result at ultrafiltration through membrane variant 1, 2 = result at ultrafiltration through membrane variant 2, -1, -2 = number of the experiment; permeate fractions were below the limit of detection by this method

eliminated from the body, bilirubin is transported by the albumin).

The rejection level of nitrogen compounds (urea, creatinine, uric acid) is considerably different. Uric acid concentrates (higher molecular weight than the other compounds), while creatinine and urea pass in the permeate.

The fact that uric acid separates hardly could be an impediment to any attempt of concentrated albumin purification by membrane process.

Calcium is best separated through membrane variant 2 and fully past in the permeate.

## Conclusions

At ascitic fluid ultrafiltration was used asymmetric and composite membranes. Permeate flow was within the values known for this type of membranes. The retention of components is favourable, being characteristic for ultrafiltration using membranes with low hydrophilicity. The process was conducted at pH 6.5 (natural value of ascitic fluid). The ascites fluid used was preserved only through freezing and not chemically. In addition to albumin, bilirubin and uric acid were concentrated. Globulins are retained on the surface and inside the membranes.

Through variant 2 (12% polysulfone - polyaniline composite membranes coagulated in water and aniline), urea and calcium passed fully in permeate, while magnesium is present in the concentrate in a very small amount ( $R = 5.04\%$ ). Iron, in both variants, is concentrated at a rate of approx. 50%. Ultrafiltration of ascites fluid through 12% polysulfone-polyaniline composite membranes in both coagulation variants provided good results that offer real outlooks for albumin recovery.

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