Concentration of Biological Liquids by Dialysis with Modified Acrylonitrile Copolymer Membranes

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SYNOPSIS

Acrylonitrile copolymer membranes were subjected to partial hydrolysis in order to improve their dialysis characteristics. The partial hydrolysis time and temperature, as well as the concentration of the hydrolyzing agent, were the variable parameters. The changes in the chemical and physical characteristics of the membrane were proved by infrared spectroscopy, elemental analysis, differential scanning calorimetry, and Mercury intrusion porometry. After each degree of modification, the permeability of the membrane with respect to a solution of vitamin B_{12} was determined under static conditions and at temperature of 298 K. The improvement in the permeability at a definite degree of hydrolysis is explained by the conversion of nitrile groups to amide and carboxyl groups, as well as increase of the volume of the pores with average radius. The efficiency of the modified PAN membranes for concentration of biological liquids (diluted serum) was studied.

INTRODUCTION

The partial hydrolysis is one of the most frequently used methods for hydrophilization of polymeric membranes.[1] The hydrolysis of acrylonitrile (AN) copolymer is comparatively well studied.[2,3] The purpose of the present investigation is to modify AN copolymer membranes (PAN-10)[4] with KOH and to study their behavior during dialysis. The efficiency of acrylonitrile copolymer membranes (PAN-10,25) for concentration of biological liquids is reported in the literature.[5] In this work, the efficiency of the modified PAN membranes for concentrating diluted serum is studied and compared with that of unmodified ones.

EXPERIMENTAL

Materials and Methods

The following membranes were used in our experiments: poly(acrylonitrile-methylmethacrylate-sodium vinylsulphonate) membrane (PAN 10), mol. wt. cutoff 10000, supplied by Spartak Co., Bulgaria; polysulphon membrane (Diaflo 15), mol. wt. cutoff 15000, supplied by Amicon, USA.

Aqueous solutions of vitamin B_{12} (p.a., Fluka, Switzerland) were used for studying the membrane permeability. The efficiency of the modified PAN membranes for concentrating biological liquids was studied with diluted human serum (p.a., Versatol, USA).

Modification of PAN Membranes with KOH

The membranes were partially hydrolyzed with an aqueous solution of KOH with a concentration of 0–20 mass % at a temperature of 313, 333, 343, and 363 K for 0–180 min.

Determination of Permeability Coefficient (P)

The permeability coefficient was determined by using a laboratory dialysis cell[6] with respect to 0.01% vitamin B_{12} at a temperature of 298 K. The dialysis cell has two compartments. The volume of each chamber is 50 cm^3. The effective membrane area was 50 cm^2. Samples with a volume of 1 mL were

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taken from both the chambers simultaneously at a predetermined period of time and after the determination of the concentrations, the solutions were returned quickly to the chambers. The concentration of vitamin B_{12} solution was determined spectrophotometrically (Spekol, Carl Zeiss Jena, Germany) at 360 nm. The permeability coefficient was calculated by the following relationship:[7]

$$P = \frac{2.303 V_1 V_2}{At(V_1 + V_2)} \lg \frac{(C_1 - C_2)_0}{(C_1 - C_2)_t}, \text{cm/min}$$

where V_1 and V_2 are the volumes of donor and receptor chambers, respectively, cm^3; A = the effective membrane area, cm^3; C_1, C_2 = concentrations of the solutions in the donor and receptor compartments, respectively; t = dialysis time.

Concentration of Biological Liquids (Diluted Serum)

The diluted human serum was concentrated in a microconcentrator.[5] One milliliter of each of six normal serums is diluted 100 times with physiological solution and then is reconcentrated to the initial concentration. The total protein content was determined by ultraviolet absorbance at 278 nm.[8] The fractional separation of the proteins was done by electrophoresis[9] upon cellulose-acetate membranes (product of Sartorius). The reading of the phoregrams was performed by a densitometer "Boehringer," Austria.

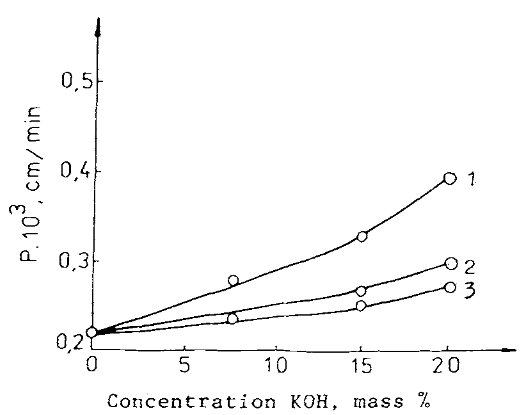

Figure 1 Permeability coefficient (with respect to 0.01 mass % aqueous solution of vitamin B_{12}) of modified membrane as a function of the hydrolyzing agent concentration (KOH) at different temperatures (K): (1)—343; (2)—333; (3)—313.

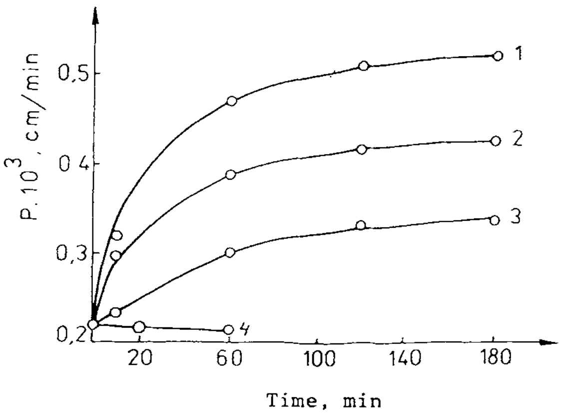

Figure 2 Permeability coefficient (with respect to 0.01 mass % aqueous solution of vitamin B_{12}) of modified membrane as a function of hydrolysis time at different temperatures. Concentration of KOH 20 mass%.: (1)—343 K; (2)—333 K; (3)—313 K; (4)—363 K.

Determination of Chemical and Physical Characteristics of the Modified Membranes

The appearance of the new hydrophilic groups after modification was proved by IR spectroscopy (Specord M80, Carl Zeiss Jena).[10] Nitrogen percentage was determined by elemental analysis (Carlo Erba).[11] The degree of hydrophilicity was determined by differential scanning calorimetry (DSC-111, Setaram, France).[12] The pore characteristics of the membranes were determined by mercury intrusion porometry (P-1500, Carlo Erba).[13]

RESULTS AND DISCUSSION

The influence of hydrolyzing agent concentration on the permeability of solution of vitamin B_{12} is shown in Figure 1. The suitable concentration of the hydrolyzing agent was found to be 20 mass %, which stipulates high permeability coefficient and the mechanical properties of the initial membranes are not deteriorated. At concentration over 20 mass % KOH the physicomechanical properties of membranes significantly decrease.

The change in the permeability of vitamin B_{12} with the hydrolysis time and temperature is represented in Figure 2. Obviously, the permeability coefficient of modified membranes at temperatures of 313, 333, and 343 K considerably increases up to a hydrolysis time of 60 min. Beyond that time, the increase is less marked.

The permeability coefficients, depending on the reaction temperature, follow the order $P_{343} > P_{333} > P_{313} > P_{363K}$ (Fig. 2). At modification temperature of 363 K, the permeability decreases abruptly. In order to clarify this fact, the pore distribution curves of membranes modified at 343 and 363 K were compared to those of unmodified ones (Fig. 3). The membranes modified at a temperature of 343 K were found to possess significantly larger volume of pores with average size (Fig. 3a). The range of the average pore size did not change. This is probably due to opening of some closed pores and increase of the volume of the pores with average radius within the same range under the influence of modifying agent and temperature.

It has been reported in the literature[14] that when PAN membranes are treated at temperature higher than 363 K the pores get contracted. The pore characteristics of the membranes modified at a temperature of 363 K support this fact (Fig. 3b).

The increase of the permeability coefficient with the increase of the degree of modification was attributed to the appearance of new hydrophilic groups in the polymer membranes.[15] With the increase in the degree of modification, more and more new hydrophilic groups appeared in the polymer chains. IR spectra of an initial membrane and those of modified one at 343 K are shown in Figure 4. The appearance of new adsorption peaks at wave numbers 1640 and 1670 cm^{-1} corresponds to the deformational vibrations of the N—H and C=O bonds, respectively, in the new amide and carboxyl groups. The changes in the peaks at wave numbers 1720 and 2220 cm^{-1} are attributed to the increase of the carboxyl content and decrease of the quantity of nitrile groups. The transformation of the nitrile groups into carbonyl

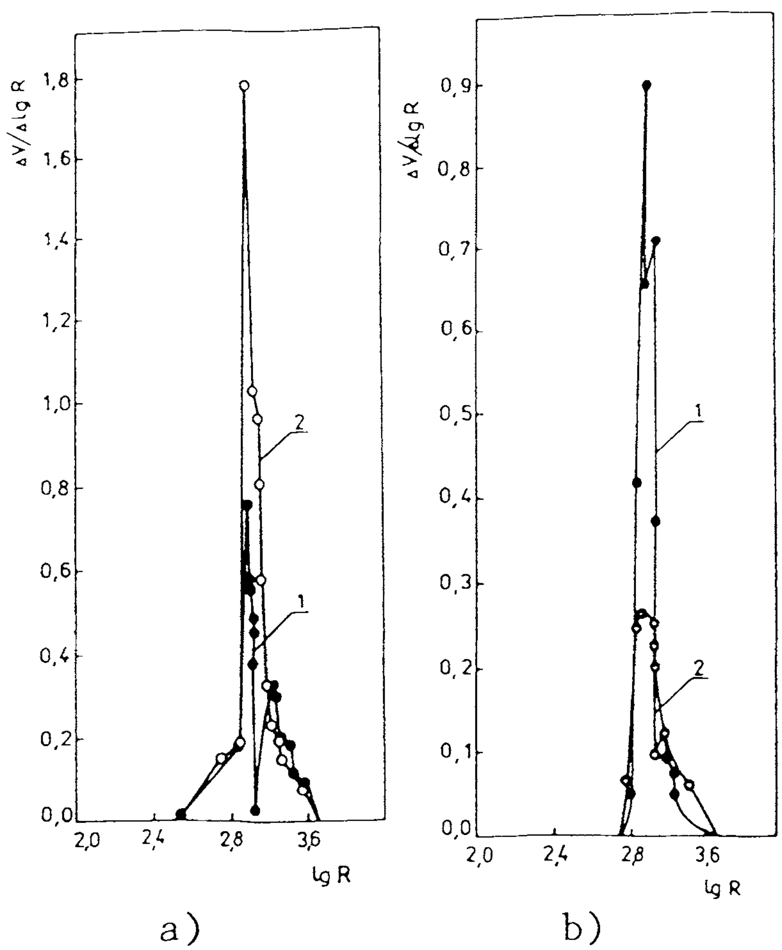

Figure 3 Different pore size distribution of the initial (1) and modified PAN membrane (2) treated with 20 mass % KOH for 120 min at (a) 343 and (b) 363 K.

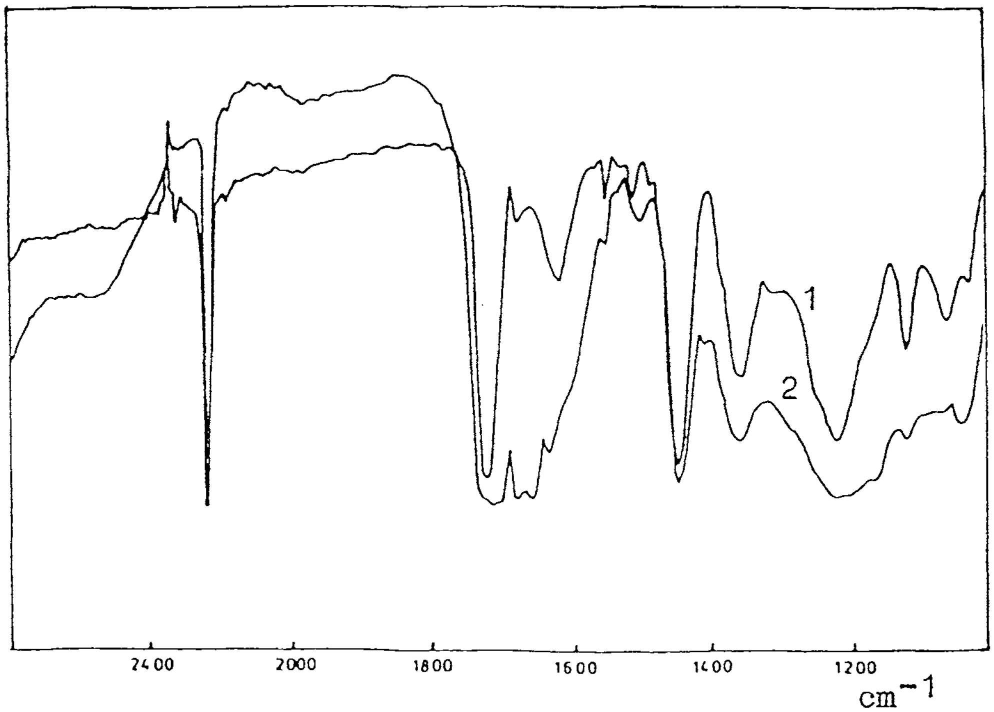

Figure 4 Infrared spectra of the initial membrane (1) and modified PAN membrane (2) treated with 20 mass % KOH for 120 min at 343 K.

ones in the modified membranes is confirmed by the reduced nitrogen percentage (from 22.05 to 18.05) determined by elemental analysis.

The increase of the hydrophility is illustrated by data obtained from DSC (Table I). The permeability of B_{12} increases with the increase of the amount of the total and free water adsorbed in the membrane.

The maximum permeability at modification temperature of 343 K was attributed not only to the appearance of new hydrophilic groups, but also to the increase of the pore volume.

At modification temperature of 363 K, the permeability decreases despite the increase of the hydrophilic groups' content, because the pore volume decreases (Fig. 3b).

The efficiency of the modified AN copolymer membranes for concentration of biological liquids with low content of proteins (diluted serum) was studied. The total protein content (percentage from the initial concentration) after concentration of diluted human serum, for different membranes, is as follows: 94.1 (Diaflo-15), 94.8 (PAN-10), and 94.2 (modified PAN-10).

The results of protein fractions separation by electrophoresis are shown in Table II. Obviously, the concentrating factor through three different types of dialysis membranes is of the same order. Due to the great number of clinical analyses, the important criterion for the effectiveness of the dialysis membrane is the time of concentrating. For

Table I Water Content and Permeability Coefficient of the Initial and Modified Membranes

		Water Content, gH_2O/g Dry Membrane						Permeability Coeff. With Respect to 0.01 Mass % Vitamin B_{12}
No.	Membrane	Total	S**	Free	S**	Bound	S**	$P \cdot 10^3$, cm/min
1.	Initial	0.45	0.05	0.43	0.01	0.02	0.01	0.20
2.	Modified	0.58	0.04	0.50	0.02	0.08	0.02	0.48

(Temperature of modification 343 K, reaction time 1 h, concentration of KOH 20 mass %, $n = 6$*)
* n = Number of experiments.
** S = Standard deviation.

Table II Mean Statistical Difference between Native and Reconcentrated Serum ($n = 6$)*

		Mean Statistical Difference Between Native and Reconcentrated Serum by Microconcentrator With Different Membranes, %					
Proteins	Native Serum %	Diaflo-15	S**	PAN-10	S**	Modified*** PAN-10	S**
Albumin	67.0	−2.1	0.1	−1.8	0.2	−1.9	0.1
α_1 Globulin	0.5	+2.5	0.2	+2.3	0.2	+2.1	0.2
α_2 Globulin	13.9	+1.2	0.1	+0.7	0.2	+1.1	0.1
β Globulin	7.6	−0.9	0.2	−2.8	0.2	−2.4	0.1
γ Globulin	11.0	+1.2	0.2	+1.7	0.1	+1.3	0.2

* n = Number of experiments.
** S = Standard deviation.
*** = Temperature of modification 343 K, reaction time 1 h, concentration of KOH − 20%.

Diaflo-15 and PAN-10, it is 2.5–3 h, and for the modified PAN-10 membrane-2 h.

The membrane modified by partial hydrolysis adsorbs smaller amount of proteins (0.7 mg/m^2) than the initial one (0.9 mg/m^2).

CONCLUSIONS

Poly(acrylonitrile-methylmethacrylate-sodium vinylsulphonate) membranes modified by partial hydrolysis show better permeability coefficient. The improvement of the permeability at a definite degree of hydrolysis was attributed to the appearance of the new functional amide and carboxyl groups, which are more hydrophilic than the nitrile groups present in the initial membranes and to the increase of the volume of pores with average radius within the same range. The latter is probably due to opening of some closed pores under the influence of modifying agent.

The modified PAN membranes are more suitable for concentration of biological liquids than initial ones.

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