Morphology of Microporous Neosepta Ion-Exchange Membranes and Its Effect on Separation of Biological Mixtures

GALINA TISHCHENKO,[1,*] MIROSLAV BLEHA,[1] JIŘÍ ŠKVOR,[2] LADISLAV BUREŠ,[2] YUKIO MIZUTANI,[3] and NOBUHIKO OHMURA[3]

[1]Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, 162 06 Prague, Czech Republic; [2]SEVAC Co., 101 03 Prague, Czech Republic; [3]TOKUYAMA Corp., Tokuyama City 745, Japan

SYNOPSIS

Specially prepared microporous Neosepta ion-exchange membranes were investigated to establish a correlation between their structural characteristics (pore-size distribution, porosity) and permeability to components of immunoglobulin (Ig) fractions of mouse ascitic fluids. The solutions to be separated contained IgG_1 with specificity to horseradish peroxidase or to the heavy chain of human IgM, some other proteins, and a large amount of ammonium sulfate (0.22–0.35*M*). Analysis of the membrane morphology carried out by scanning electron microscopy and mercury porosimetry showed that the membranes possess a polymodal pore-size distribution. There are large open pores (400–600 and 200–300 nm in diameter) on the membrane surfaces, but the void volume of the membranes is a system of connected pores of smaller diameters (from 60–100 to 7–10 nm). The main part of the pores in the membranes displaying the best separation ability was 8–17 nm in diameter. It was found that highly porous charged membranes (relative porosity 58–60%) with low ion-exchange capacity (0.02–0.1 meq/g) made it possible to achieve the desired desalination degree of protein mixture (80–83%) within 5–7 h instead of 5 days needed in the traditional dialysis. Moreover, the amount of separated accompanying proteins reached 25–30% depending on membrane porosity and the quality of specific IgG_1 was considerably improved.

INTRODUCTION

As was reported earlier,[1,2] the microporous Neosepta ion-exchange membranes display an excellent retention ability toward some bacteria, colloidal particles (0.1 μ), and proteins (hemoglobin) due to their morphological features and the charged surface of pores. The morphology of membranes and volume concentration of ionogenic groups were adjusted by the conditions of synthesis and further chemical transformations. It was found that the Neosepta ion-exchange membranes, from which the microporous ion-exchange membranes were obtained, had the microheterogeneity of colloidal dimensions. This means that they consisted of a poly(vinyl chloride) (PVC) matrix and microglobules (approximately 10 nm in diameter) of styrene–divinylbenzene (St–DVB) copolymer (resin component), which formed its own finely dispersed and continuous phase within the PVC phase.[3,4] To make the membranes porous, the Fe^{3+} form of sulfonated or the $Fe(CN)_6^{3-}$ form of aminated resin components were treated[5,6] with H_2O_2. By controlling the conditions of synthesis and using selective decomposition of the resin components, it was possible to obtain microporous ion-exchange membranes with various pore-size distributions and volume concentrations of ionogenic groups.

We have already reported that the nontraditional dialysis desalination of Ig fractions of mouse ascitic fluids by using[7] the neutral microfiltration Synpore (0.1 μ) or Sartorius (0.05 μ) and microporous Neosepta ion-exchange membranes[8] resulted not only in a decrease in the dialysis time (more than 20

* To whom correspondence should be addressed.

Journal of Applied Polymer Science, Vol. 58, 1341–1348 (1995)
 CCC 0021-8995/95/081341-08

Table I Characteristics of Microporous Neosepta Ion-exchange Membranes

Membrane	Ion-Exchange Capacity (meq/g)	Water Content (mL H_2O/g)	Wet Polymer Content[a] (mL/g)	Relative Porosity (%)	Specific Volume (mL/g)	Specific Surface (m^2/g)
Cation-exchange membranes						
C-1	1.97	0.754	0.804	48	1.56	10.0
C-2	1.54	0.905	0.890	50	1.80	23.8
C-3	0.28	1.341	1.050	56	2.39	17.1
C-4	0.10	1.300	1.022	56	2.32	16.4
C-5	0.07	1.491	1.030	59	2.52	< 0.1
C-6	0.03	1.547	1.340	60	2.58	< 0.1
C-7	0.08	1.430	1.360	58	2.47	4.4
C-8	0.06	1.525	1.017	60	2.54	1.8
C-9	0.04	1.625	0.921	64	2.54	23.1
C-10	0.03	1.659	0.945	64	2.60	29.9
Anion-exchange membranes						
A-1	0.82	0.278	0.858	24	1.14	< 0.1
A-2	0.35	0.796	0.838	49	1.64	10.3
A-3	0.19	0.929	0.825	53	1.75	5.6
A-4	0.16	1.130	0.818	58	1.95	19.3
A-5	0.08	1.330	0.820	62	2.15	12.1
A-6	0.32	1.045	0.719	59	1.76	14.5
A-7	0.28	1.092	0.760	59	1.85	10.0
A-8	0.12	1.600	0.925	63	2.53	26.7
A-9	0.11	1.628	0.897	64	2.53	59.1
A-10	0.02	1.878	0.889	68	2.77	34.1

All the membrane characteristics were calculated for 1 g of dry membrane.
[a] The difference between specific volume of hydrated membrane and its water content.

times), but also in an improved purification of antibodies.

The aim of the present study was the analysis of the influence of structural features of microporous Neosepta ion-exchange membranes (porosity, pore-size distribution) and charged groups on their permeability to components of Ig fractions of mouse ascitic fluids containing immunoglobulins IgG_1 with different specificity and pI.

EXPERIMENTAL

Membranes

The microporous charged membranes were prepared from the ion-exchange Neosepta membranes which contained sulfonic acid or quaternary ammonium groups (Tokuyama Corp., Japan). The characteristics of membranes used are presented in Table I.

Table II Characteristics of Solutions of Ig Fractions

Specificity of Ig G_1 to	pI of Ig G_1	Diameter of Colloids (nm)	pH	Ammonium Sulfate (mg/mL[a])	Organic Substances (mg/mL)	Proteins and (Ig G_1) (mg/mL)
Human Ig M	5.45–5.85	149 ± 3	6.33	46.53 (0.35)	15.04	14.00 (1.39)
Horseradish peroxidase	5.95–6.30	114 ± 3	7.85	28.92 (0.22)	8.51	7.20 (1.00)

[a] Mol/L.

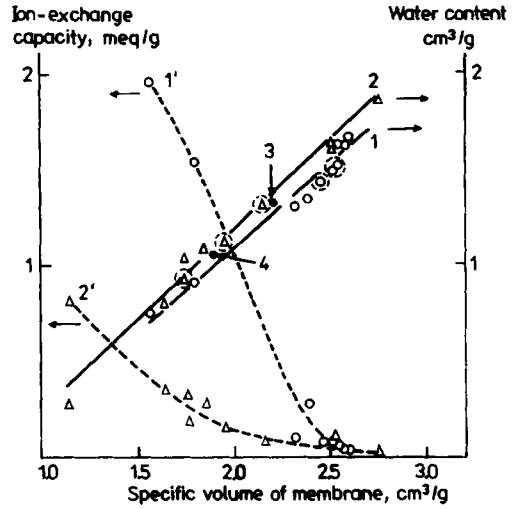


Figure 1 The hydration of microporous Neosepta (1) cation- and (2) anion-exchange membranes. (3) Synpore membrane (pore size 0.1 μ); (4) Sartorius membrane (0.05 μ). (1', 2') Ion-exchange capacity.

The membranes were regenerated with 0.5M HCl and 0.5M NH_4OH, conditioned with 0.5M NaCl, and thoroughly washed with deionized water. The amount of water within the pore space of the membranes was determined by drying at 110°C in vacuum to constant weight.

For scanning electron microscopy, the membranes were dried by the critical point method and then fractured in liquid nitrogen. Subsequently, a gold layer 10 nm thick was vacuum-deposited on the samples in the cell of a sputtering device (Balzers). The morphology of membrane surfaces and of the fracture was observed in a scanning electron microscope JSM-35 (JEOL, Japan) at 25 kV.

The specific surface area of the pores was determined by the gas-adsorption method using a Quantasorb (Quantachrome Corp., U.S.A.) apparatus. The total pore volume was measured by mercury porosimetry with the aid of a Porosimeter 225 (Carlo Erba Strumentazione, Italy).

Solutions of Immunoglobulin Fractions

The solutions of Ig fractions were manufactured by Sevac Co. (Czech Republic). They were obtained by ammonium sulfate precipitation of mouse ascitic fluids which contained the specific IgG_1 with specificity to horseradish peroxidase or to the heavy chain of human IgM. The characteristics of the solutions to be desalinated are presented in Table II.

Dialysis Permeation

The permeability of membranes was investigated in a three-compartment membrane cell[9] equipped with two membranes. The effective area of the membrane was 5.6 cm^2; the distance between the membranes was 3 mm. The volume of each diffusate and the dialysate compartments of the membrane cell were 0.5 and 1.5 cm^3, respectively. Water in the chambers collecting the diffusate (10 mL) and the solution to be desalinated (8 mL) were thermostatted at 25 ± 0.1°C. Circulation of the solutions in the membrane compartments was maintained by a peristaltic pump PP 1-05 (Zalimp, Poland). After 30 min, the experiment was stopped and the volume of solutions in the chambers was measured with a calibrated vessel. Their electric conductivity was measured by a digital conductivity meter (Philips, U.S.A.). Water in the diffusate compartments was replaced by new batches, and the process was continued. When the electric conductivity in the dialysate reached its maximum of 6 mS/cm corresponding to the 0.03–0.04M concentration of ammonium sulfate, the dialysis was stopped. The efficiency of purification of the solutions of Ig fractions in dialysis desalination was checked by ion-exchange chromatography of dialysates.

Analysis

The total concentration of proteins which passed into the diffusate during the dialysis was calculated using the relation[10]

$$C_{pr} = 1.55 \times A_{280} - 0.76 \times A_{260}\ (\text{mg/mL})$$

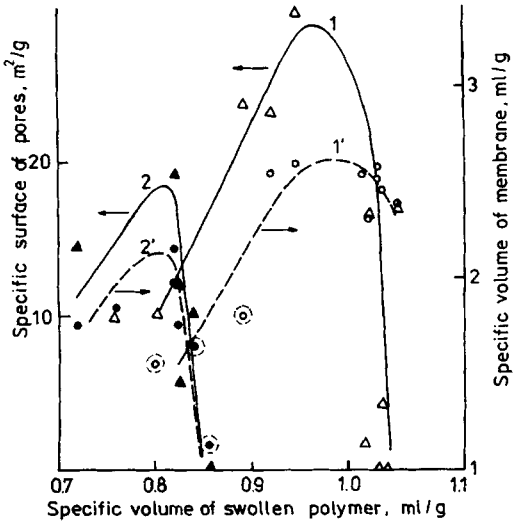
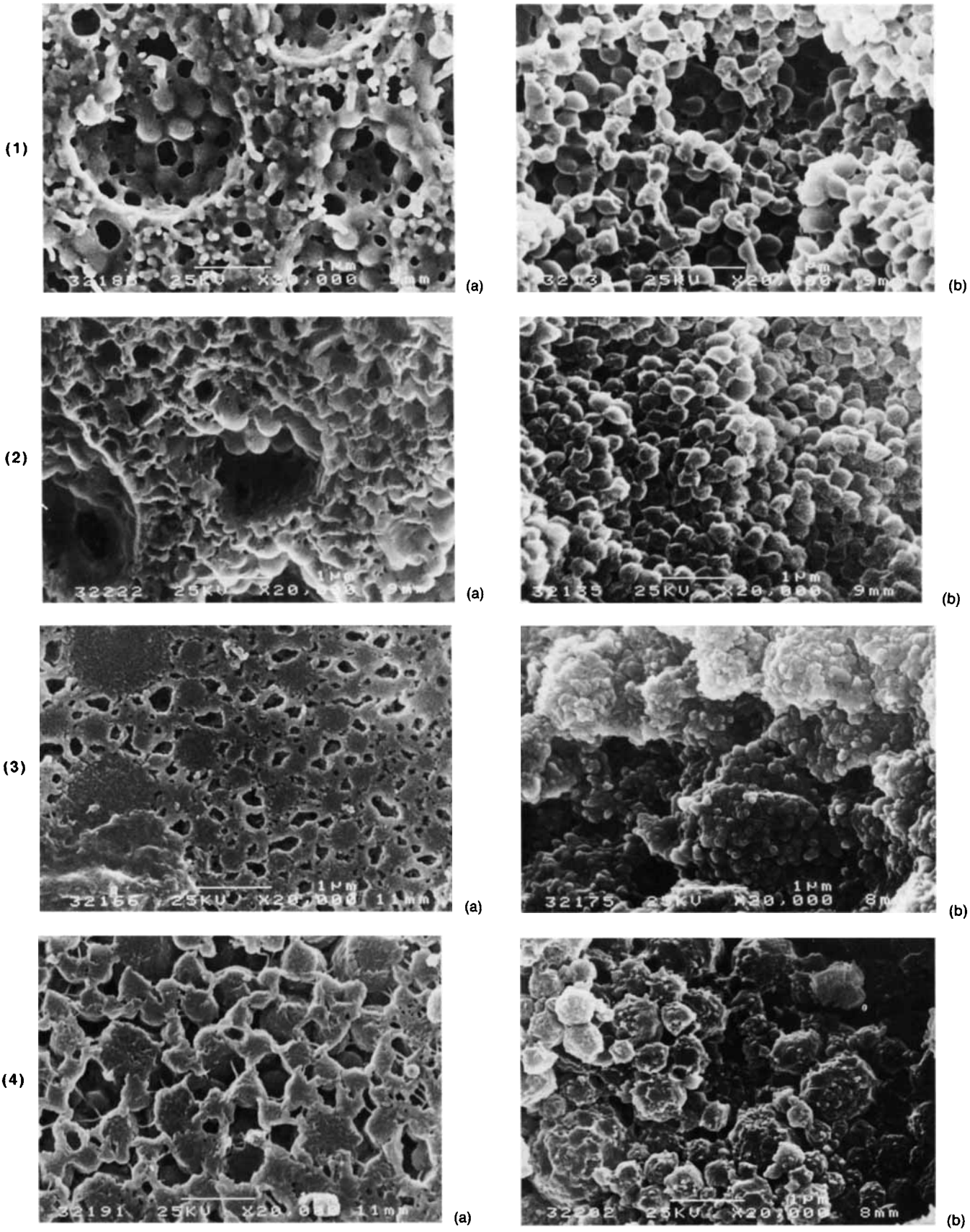


Figure 2 Dependence of (1, 2) specific surface of pores and (1', 2') specific volume of membrane on specific volume of swollen polymer in microporous Neosepta ion-exchange membranes. (1, 1') Cation-exchange and (2, 2') anion-exchange membranes.


(1)
(a)
(b)
(2)
(a)
(b)
(3)
(a)
(b)
(4)
(a)
(b)

where A_{260} and A_{280} are the absorbances at 260 and 280 nm, respectively, measured by a diode array spectrophotometer (Hewlett Packard, U.S.A.). The content of dry matter as well as of nitrogen and sulfate in lyophilized samples of solutions from the diffusate compartments was also determined.

Desalted solutions of the Ig fractions were chromatographed on a column packed with DEAE cellulose DE-52 (Whatman, U.S.A.) equilibrated with a 0.02*M* tris-HCl buffer solution (pH 7.8, buffer A). The ion-exchange chromatography was carried out at a rate 0.6 mL cm^{-2} min^{-1}, in a linear gradient of NaCl using buffer *G*, obtained by mixing buffer A and buffer B (0.4*M* NaCl in 0.02*M* tris-HCl buffer, pH 7.8). The concentration of specific immunoglobulins in the eluate fractions and in the permeate was determined by sandwich ELISA titration with standards.[11]

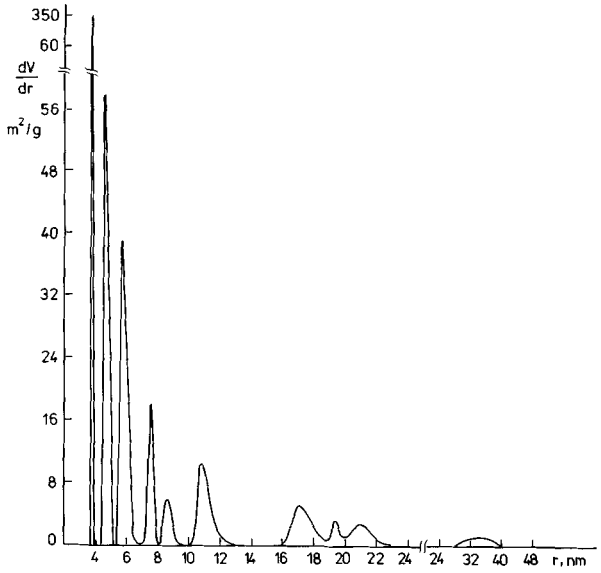


Figure 5 Pore-size distribution of cation-exchange membrane C-6.

RESULTS AND DISCUSSION

Membrane Morphology

As the water content within porous ion-exchange membranes is the sum of water bound by hydrophilic ionogenic groups of the polymer (water of hydration) and of free water filling the pores, it can be regarded as a measure of membrane porosity. As shown in Table I, water volume in wet membranes varies considerably (relative porosity 48–64% and 24–68% for C and A membranes, respectively). Decrease in the ion-exchange capacity of membranes with increasing water content is the result of chemical decomposition of ion-exchange St–DVB component (dashed lines in Fig. 1). The fact that the water contents of all the C membranes are always lower (approximately by 10%) than those of A membranes can be explained by different hydration degrees of sulfonic acid and quaternary ammonium groups. As shown in Figure 2, both the specific pore surfaces and specific volumes of the C membranes are higher than those of A membranes. It is noteworthy that both these dependencies possess maxima, which can be explained by different porosity levels of the membranes. The circled points define the region in the series of the C and A membranes (Fig. 2) which show the highest ion-exchange capacity and, as was reported previously,[8] the smallest permeability to ammonium sulfate. The reason for this phenomenon is the Donnan exclusion of coions which more hinders transfer of salt molecules through the membrane the less the salt concentration is.

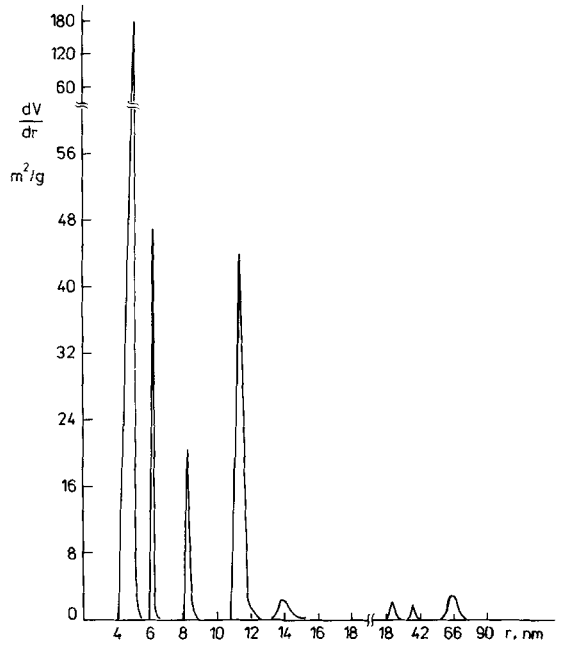


Figure 4 Pore-size distribution of cation-exchange membrane C-3.

Figure 3 Scanning electron micrographs of microporous cation-exchange membranes (1) C-3 and (2) C-6 and anion-exchange membranes (3) A-3 and (4) A-4. (a) Surface; (b) fracture.

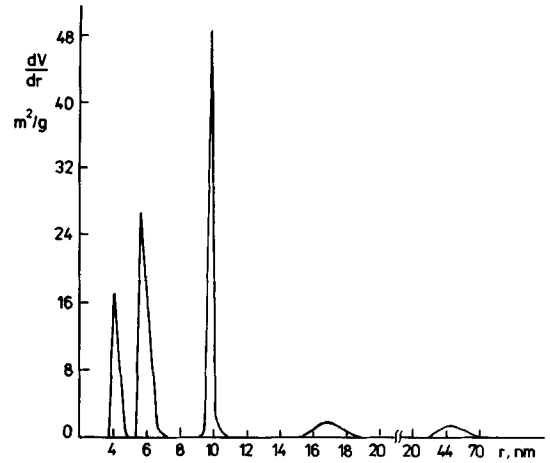


Figure 6 Pore-size distribution of anion-exchange membrane A-3.

It should be noted that both specific volumes of the swollen polymer (V_{sp}) and those of the membranes (V_m) are always larger for C membranes than for A membranes. Thus, these values V_{sp} (V_m) for C-3, C-6 and A-3, A-4 membranes are 1.05 (2.39), 1.035 (2.58) and 0.825 (1.75), 0.82 (1.95), respectively. The packing of PVC microglobules in C membranes can be assumed to be looser than in A membranes. Scanning electron micrographs showed that the surface of membranes [Fig. 3(a)] have open pores, the diameter of which varies with increasing porosity. The diameters of predominant pores on the surface of A-3 and C-3 membranes range between 200 and 300 nm. The more porous membrane (A-4) has some entrance pores with a larger diameter of 400–600 nm, in addition to the small ones. The surface of C membranes differs from that of A membranes in the existence of a denser polymer layer mentioned previously.[1]

Inner parts of the membranes have a fine cellular structure with a high void volume [Fig. 3(b)]. Each cell opens up to the neighboring cells via a number of pores in the cell walls. It should be mentioned that the micrographs do not show the substructure of the membranes and, therefore, the distribution of the St–DVB copolymer cannot be seen. As was observed,[4] the PVC microparticles cannot dissolve in the monomers. They are incompletely gelled and mostly retain their shape. The state of the residual ion-exchange resin in microporous membranes is still obscure.

The fractures of membranes showed the difference between the spatial packing of PVC microglobules. This can be distinctly seen in fractures of C and A membranes. The polydispersity of PVC microparticles is considerably larger in A membranes (200–1000 nm) than in C membranes (270–320 nm).

The investigation of membrane porosity by mercury porosimetry showed (Figs. 4–7) that the membranes have a polymodal pore-size distribution but that the part of the total volume of the pores belonging to the large ones (50–100 nm in diameter) is small. The diameter of the main pores within the C-3 membrane is in the range of 8–11 nm. It was found that large pores (32–46 nm in diameter) and small ones (7–8, 8–10, and 11–14 nm in diameter) occur within the C-6 membrane. The main part of the inner free volume of the A-3 membrane is occupied by pores 18–22, 8–10, and 11–14 nm in diameter, while the A-4 membrane at the same time has pores 25–38 nm in diameter. The total pore volume of these membranes varies as follows: 0.0462 (A-3), 0.0777 (A-4), 0.0954 (C-3), and 0.1629 cm^3/g (C-6). These values correspond approximately to 25–30% of the water content within wet membranes. This unexpected result can be explained by the influence of solvents (ethyl alcohol, diethyl ether) used in drying on the membrane structure. It seems certain that the proportion of large pores within the membranes was, in fact, larger.

Dialysis Desalination

The results of dialysis are presented in Tables III and IV. The dialysis time decreases with increasing porosity of the membranes and decreasing ion-exchange capacity. The desalination of the IgG_1 solution of the first type required more time (7.5–6.5 h) than that of second type (5.5–4 h), which con-

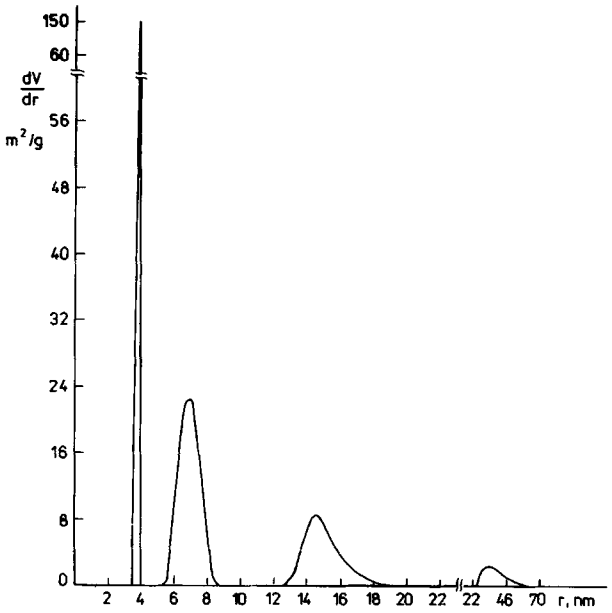


Figure 7 Pore-size distribution of anion-exchange membrane A-4.

Table III Parameters of Dialysis Desalination of Solutions of IgG_1 Specific to Horseradish Peroxidase, Using the Microporous Neosepta Ion-exchange Membranes

Membrane	ε (%)	Ion-exchange Capacity (meq/g)	Dialysis Time (min)	IgG_1 (Proteins)[a] Dialysate	Diffusate (%)	Membrane	Desalination (%)
A-3	53	0.19	450	92.9 (71.7)	0.7 (11.3)	6.4 (17.0)	69.5
A-4	58	0.16	330	98.5 (76.0)	0.2 (6.2)	1.3 (17.8)	76.8
A-5	62	0.08	240	97.5 (75.1)	0.1 (4.4)	2.4 (20.5)	79.3
C-3	56	0.28	390	83.5 (64.4)	2.5 (19.9)	14.1 (15.7)	62.3
C-4	56	0.10	360	89.1 (68.5)	0.8 (5.2)	10.1 (26.3)	72.7
C-6	60	0.03	330	97.2 (75.0)	0.8 (16.1)	2.0 (8.9)	82.0

ε = relative porosity of membrane.
[a] Relative to the respective amount of proteins and IgG_1 contained in 1 mL of the initial IgG_1 solution.

tained a lower amount of ammonium sulfate. The desalination degree thus obtained was only 70 and 62%, if membranes with high ion-exchange capacity (A-3 and C-3, Table III) were used, in spite of the long dialysis time. However, the amount of proteins transferred into the diffusate and sorbed within these membranes was higher in comparison with the more porous and less charged A-4 and C-6 mem-

Table IV Parameters of Dialysis Desalination of Solutions of IgG_1 Specific to Human IgM, Using the Microporous Neosepta Ion-exchange Membranes

Membrane	ε (%)	Ion-exchange Capacity (meq/g)	Dialysis Time (min)	IgG_1 (Proteins)[a] Dialysate	Diffusate (%)	Membrane	Desalination (%)
A-4	58	0.19	390	98.6 (93.8)	0.3×10^{-3} (1.6)	1.6 (4.6)	93.2
A-6	59	0.32	480	99.1 (96.1)	1×10^{-3} (0.9)	0.9 (3.0)	84.9
A-7	59	0.28	450	97.8 (92.5)	0.03 (0.7)	2.2 (6.8)	85.0
C-7	58	0.08	390	98.1 (87.7)	0.7×10^{-3} (6.3)	1.9 (6.0)	97.8
C-8	60	0.06	360	97.0 (80.6)	0.03 (10.0)	2.9 (9.4)	99.1
C-9	64	0.04	330	96.0 (77.1)	0.02 (10.3)	4.0 (12.6)	99.4
C-10	64	0.03	320	96.3 (77.5)	4×10^{-3} (10.8)	3.7 (11.7)	99.5

[a] See Table III.

branes (35.3%, 36.5% and 24%, 25%, respectively). This can be due to the influence of ionogenic groups on hindering the transport of salt molecules through those membranes in which the volume concentration of charged groups was higher. The influence of the nature of ionogenic groups on separation of the components can be seen in Table IV. The amount of proteins transferred through the cation-exchange membranes into the diffusate and sorbed within them was slightly higher than that observed for the anion-exchange ones. Another feature of the cation-exchange membranes observed with the membranes having the highest ion-exchange capacity was a higher water transport to the dialysate.

The IgG_1 losses were not high (approximately 1.5–3%) in the diffusate and within the membranes with the optimum morphology (circled points in Fig. 1). The purification degree of Ig fractions from accompanying proteins reached 25–30% in these cases. This made easier further separation of the components by ion-exchange chromatography. It was found that the serum albumin peak in chromatograms was very small, and it was completely absent when counterpressure was applied to the permeate compartments. This did not occur if the dialysis was carried out using neutral microfiltration Synpore (0.1 μ) or Sartorius (0.05 μ) membranes.[7]

CONCLUSIONS

The study of the morphology of microporous Neosepta ion-exchange membranes shows that the membranes have high porosity (relative pore volume 56–68%) with polymodal pore-size distribution. The large entrance pores (400–600 and 200–300 nm) ensure an easy penetration of the components to be separated into the membranes. The selectivity of isolation of the high molecular weight proteins from the low molecular weight ones and salt is provided by a system of connected pores of smaller diameters (60–100, 22–40, 12–16, 7–10 nm). Best results can be achieved using membranes of which the porosity and charge density are in the range 58–60% and 0.02–0.1 meq/g, respectively. The results obtained in the dialysis desalination of solutions of both IgG_1 types are close. However, there are two essential differences: First, the degree of desalination of solutions of IgG_1 possessing specificity to the heavy chain of human IgM is considerably higher. Second, the more porous membranes appear to be better suited for the dialysis of this IgG_1 than for the dialysis of IgG_1 possessing specificity to horseradish peroxidase. These data also demonstrate the role of the exclusion mechanism of protein aggregates differing in their size.

The authors wish to thank the Grant Agency of the Academy of Sciences of the Czech Republic for support by Grant No. 45056.

REFERENCES

1. Y. Mizutani and N. Nishimura, *J. Appl. Polym. Sci.*, **25,** 2273 (1980).
2. Y. Mizutani, K. Kusumoto, M. Nishimura, T. Nishimura, and E. Asada, *J. Appl. Polym. Sci.*, **39,** 1087 (1990).
3. Y. Mizutani, R. Yamane, H. Ihara, and M. Motomura, *Bull. Chem. Soc. Jpn.*, **36,** 361 (1963).
4. Y. Mizutani, *J. Membr. Sci.*, **49,** 121 (1990).
5. Y. Mizutani, *Bull. Chem. Soc. Jpn.*, **43,** 595 (1970).
6. Y. Mizutani and M. Nishimura, *J. Appl. Polym. Sci.*, **31,** 1147 (1986).
7. G. A. Tishchenko, M. Bleha, J. Škvor, and L. Bureš, *Bioseparation,* **5,** 19 (1995).
8. G. Tishchenko, M. Bleha, J. Škvor, L. Bureš, Y. Mizutani, and N. Ohmura, *J. Membr. Sci.*, to appear.
9. M. Bleha and G. Tishchenko, *J. Membr. Sci.*, **73,** 305 (1992).
10. C. Warburg and W. Christian, *Biochem. Z.*, **310,** 384 (1941).
11. R. H. Yolken, *J. Biol. Med.*, **53,** 85 (1980).

Received February 21, 1995
Accepted May 17, 1995