Permeation Behavior of Dextrans by Charged Ultrafiltration Membranes of Polyacrylonitrile-Photografted with Ionic Monomers

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SYNOPSIS

Charged copolymers, polyacrylonitrile, having grafts of quaternized poly(N,N-dimethyl-aminoethylmethacrylate) and poly(sodium p-styrenesulfonate), were synthesized by photograft-polymerization. The charged ultrafiltration membranes, which had heterogeneous porous structures, were prepared by casting graft polymer solution containing poly(vinyl alcohol) into water.

The permeation of nonionic dextran and anionic dextransulfate through the charged membranes was studied by ultrafiltration in aqueous solution. The molecular weight cutoff characteristics of the charged membranes were obtained using dextrans of various molecular sizes. The permeation behavior was discussed in terms of the charge effect of the graft polymers and electrostatic interaction between the polymers and the solute molecules.

INTRODUCTION

Under normal circumstances the glomerular capillary wall of animal and man kidney markedly restricts the transmural passage of plasma protein and other macromolecules, while at the same time permitting high rates of fluid filtration. The basis of this selectivity was investigated by Brenner et al.,[1] who used charged dextran solute in vivo. It was suggested that the glomerular capillary wall, having fixed negative charges, provides an electrostatic barrier to filtration of the protein and other circulating polyanions.[2]

Few reports have been published on the subject of synthetic charged ultrafiltration membranes, from the standpoint of the model in vitro. These published studies have contributed to the understanding of the sieve effects of the glomerular capillary wall. In recent reports on the ultrafiltration of proteins, Miyama et al. prepared positively and negatively charged polyacrylonitrile membranes having an analogous ultrafiltration relationship to the glomerular capillary wall.[3,4] Results on the permeation of albumin and γ-globulin through the membranes indicated the possibility of protein separation. Kimura et al. have made cationic and anionic polysulfone membranes and controlled permeation of a protein mixture by the charge effects of the charged membranes.[5,6]

Accordingly, an ultrafiltration membrane having fixed charges is more interesting than an uncharged membrane for practical applications, since it has three variables, such as sign, density of the charges, and pore size. Therefore, in various industrial fields, such as food, medical, and biochemical industries, it is useful to apply the charged ultrafiltration mem-

brane to the separation of the constituents of proteins, enzymes, antibodies, hormones, and blood proteins.

In our previous reports,[3,4] cutoff characteristics of the membranes were not examined. Adsorption of proteins on the membranes also must be considered. Therefore, in the present work, cutoff characteristics of the charged polyacrylonitrile graft-copolymer membranes were examined by the permeation of dextrans of various molecular sizes. In addition, electrostatic interaction between the charged membrane and the solute was confirmed by the permeation of dextran and dextransulfate.

EXPERIMENTAL

Materials

All reagents used in the experiment were of reagent grade, unless otherwise described. Acrylonitrile and N,N-dimethylaminoethylmethacrylate (DAEM) were distilled before use. Other reagents were used without further purification.

Dextran of various molecular weights, that is, Dextran T-10, T-40, T-70, T-500, and T-2000, with molecular weights of 1×10^4, 4×10^4, 7×10^4, 5×10^5, and 2×10^6 (Pharmacia), was employed.

Water was purified by using ion-exchange resin, and was distilled.

Charged graft copolymers were synthesized photochemically. As trunk polymer, polyacrylonitrile (PAN-Br), containing about 15 bromine atoms per macromolecule, was used. The details of the preparation methods were described previously.[3,4]

Preparation of Membranes

The preparation of the charged ultrafiltration membranes proceeded as follows. The graft-copolymer, poly(acrylonitrile-graft-N,N-dimethylaminoethylmethacrylate) (abbreviated as PAN-g-DAEM) was dissolved in dimethylformamide (DMF) and poly-(acrylonitrile-graft-(sodium p-styrenesulfonate)) (abbreviated as PAN-g-SSS) in dimethylsulfoxide (DMSO). The solution was mixed with DMSO solution of poly(vinyl alcohol) (PVA) (PVA 203 of Kurare Co. Ltd. Degree of polymerization and sponification are 300 and 88%, respectively). The membrane was made by spreading the mixture solution on a ferrotype plate using a pair of 100 μm-thick spacer, coagulating in water, and washing with water to remove the solvent and PVA.

The quaternized reaction of the dimethylamino

groups of PAN-g-DAEM was accomplished by dipping the membrane into ethylbromide saturated aqueous methanol solution for 8 h at room temperature. Then, the membrane was washed with a large quantity of water and was kept in water in the dark. The positively charged membranes obtained by the quaternization are abbreviated as PAN-g-DAEM⁺.

The amount of fixed charges (meq/g polymer) in the charged ultrafiltration membranes was measured by an acid–base titration.[7] The water content of the membranes was calculated with wet base.

Apparatus and Measurements

The ultrafiltration experiments were carried out with an apparatus (Type 8050 cell) manufactured by Amicon Co. Ltd., 50 mL capacity and 43 mm effective diameter at 25°C, and under the pressure of 760 mm H_2O.

For the measurement of dextran permeation, 0.2 wt % of dextran aqueous solution was used. The ionic strength of the eluent solution was made 0.077 or 0.154 by adding NaCl.

The volume flux of the solution was calculated from the flow rate after the elution of 5 mL of solution, and was expressed by volume flux per unit area and unit time (L/m² h).

The amounts of the permeated dextran were measured by colorimetric reaction with acid and phenol for dextransulfate[8] and by GPC analysis for dextran. The experimentally obtained rejection, R, of charged membrane is defined as
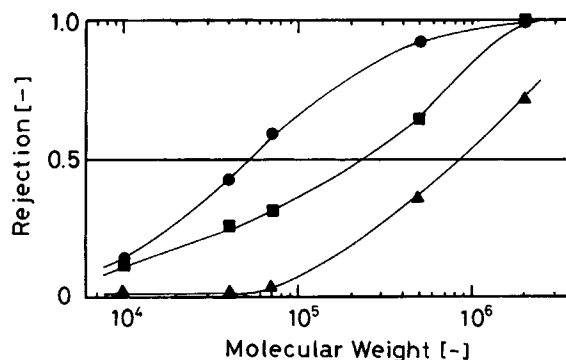
$$R = (C_b - C_p)/C_b$$

Figure 1 Molecular cutoff curves of PAN-g-DAEM⁺ membranes prepared from charged copolymer solution containing various amounts of PVA. (●) 1 wt % of PVA additive, (■) 3 wt % of PVA additive, (▲) 4 wt % of PVA additive. The PAN-g-DAEM⁺ membrane is sample no. C-4.

Table I   Properties of Uncharged and Charged Ultrafiltration Membranes[a]

Sample	Polymer	Fixed Charges (meq/g polymer)	Water Content[b] (%)	Volume Flux of Water (L/m^2 h)
N-1	PAN-Br	—	12.6	588
N-2	PAN-g-DAEM	—	65.3	139
C-1	PAN-g-DAEM$^+$	0.12	81.6	69
C-2[c]	PAN-g-DAEM$^+$	0.40	85.2	59
C-3	PAN-g-DAEM$^+$	0.59	90.6	33
C-4	PAN-g-DAEM$^+$	0.75	90.8	30
A-1	PAN-g-SSS	0.22	88.2	51
A-2	PAN-g-SSS	0.62	91.8	11

[a] Membranes were prepared from 8 wt % of polymer solution containing 1 wt % of PVA additives.
[b] Water content calculated with wet base by using weights of wet and dry membranes.
[c] The positively charged copolymer of C-2 was prepared by quaternization of N-2 with ethylbromide.

where C_b and C_p denote the concentrations of bulk and permeated dextran, respectively. Using this relationship, permeability (%), P, is given as

$$P = (1 - R) \times 100$$

RESULTS AND DISCUSSION

Miyama et al. found by taking scanning electron micrographs that the positively charged PAN-g-DAEM$^+$ membrane has a heterogeneous porous structure.[3,4] The size of membrane pores was controlled by conditions of the membrane preparation, especially by the amount of PVA added. So the effect of PVA additions on the permeation of proteins was examined. The increase of the permeation was observed with increasingly added PVA. Figure 1 shows the effects of PVA on the molecular weight cut off curves for the PAN-g-DAEM$^+$ membranes, which are prepared from DMF solution containing 1, 3, and 4 wt % of PVA, respectively. It is obvious that the addition of PVA increases permeability of dextran. On the other hand, the mechanical strength of the PAN-g-DAEM$^+$ membrane decreased with the increase of PVA and the membrane prepared with more than 3 wt % PVA was not strong enough to be used practically. Therefore, the charged membranes prepared from casting the solution containing 1 wt % of PVA were used in the following experiments.

Table I shows the values of fixed charges, water content, and volume flux of water of various membranes prepared from the casting solution containing 1 wt % PVA. The water content of PAN-Br membrane is of a very low value and that of the uncharged

PAN-g-DAEM membrane is significantly larger than that of the PAN-Br membrane. But, water contents of PAN-g-DAEM$^+$ membranes are much larger than those of PAN-Br and PAN-g-DAEM membranes, and water contents gradually increase with an increase in fixed charges. These results suggest that the hydrophilicity of the PAN-Br membrane is enhanced by the grafting of DAEM, and that the hydrophilicity of the PAN-g-DAEM membrane is further enhanced by quaternization of dimethylamino groups.

The PAN-Br membrane, which is of low hydrophilicity relative to the PAN-g-DAEM membrane, permeates water well. However, the volume flux of the charged membrane decreases with an increase in the fixed charges.

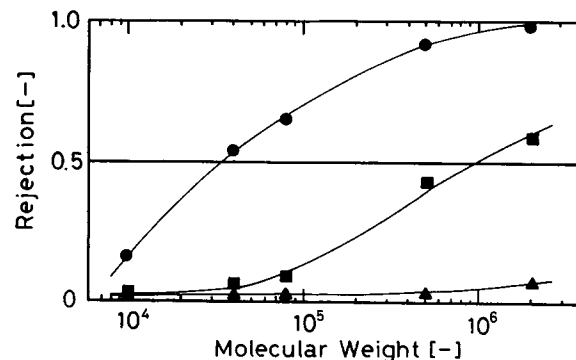The molecular cutoff curves of the PAN-Br, PAN-g-DAEM, and PAN-g-DAEM$^+$ membranes,

Figure 2 Molecular cutoff curves of membranes of PAN-Br, PAN-g-DAEM, and PAN-g-DAEM$^+$. (■) PAN-Br (sample no. N-1), (▲) PAN-g-DAEM (sample no. N-2), (●) PAN-g-DAEM$^+$ (sample no. C-2). The membranes were prepared from 8 wt % of polymer solution containing 1 wt % PVA.
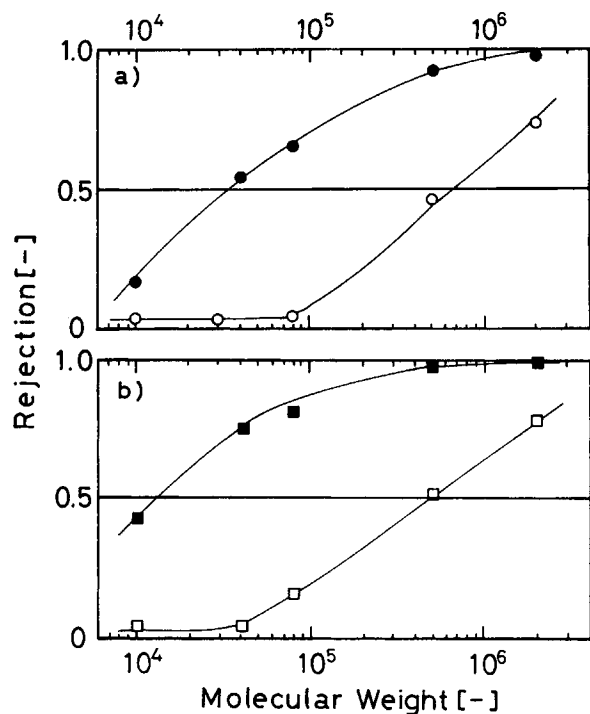
Figure 3 Molecular cutoff curves of PAN-g-DAEM$^+$ and PAN-g-SSS membranes prepared from charged graft copolymers having various amount of fixed charges: (a) (O) PAN-g-DAEM$^+$ (sample no. C-1); (●) PAN-g-DAEM$^+$ (sample no. C-4). (b) (□) PAN-g-SSS (sample no. A-1); (■) PAN-g-SSS (sample no. A-2). The membranes were prepared from 8 wt % of polymer solution containing 1 wt % PVA.

measured by permeation of solution containing various molecular size dextrans, are shown in Figure 2. The rejection of dextran by the PAN–Br membrane is higher than that by the PAN-g-DAEM membrane. This suggests that the grafting of DAEM increases the size of membrane pores and enhances the permeation of dextran, which may be due to the increase of hydrophilicity by the grafting of hydrophilic monomers, as reported by Vigo et al.[9]

As shown in Table I, in the positively charged PAN-g-DAEM$^+$ membranes prepared by quaternization, the volume flux of water decreases with an increase in the fixed charges. The cutoff curves of the PAN-g-DAEM$^+$ membranes markedly shift toward a low molecular weight side as compared with those of PAN-g-DAEM membranes.

Here, decrease of pore size of the PAN-g-DAEM$^+$ membrane may be explained by the fact that the coil conformation of the ionic residues extends in aqueous solution as a result of electrostatic repulsion between the charged neighboring groups.[10] Accord-

ingly, the extended conformation of the charged grafts seems to decrease the membrane pore size and the permeation of water and dextran.

Effects of fixed charges of graft-copolymers on the molecular cutoff curves of PAN-g-DAEM$^+$ and PAN-g-SSS membranes are shown in Figures 3 (a) and (b). When the amount of fixed charges of PAN-g-DAEM$^+$ increases from 0.12 to 0.75, the curve of PAN-g-DAEM$^+$ shifts towards a small molecular weight side. In addition, water volume flux decreases with increasing fixed charges as shown in Table I.

The dependence of cutoff curves of PAN-g-SSS membranes on the fixed charges shows the similar tendency as that of PAN-g-DAEM$^+$ membranes. The shift of the curves and the decrease of volume flux may be due to the decrease of pore size of the membranes.

According to a sieve slit model,[11,12] the solute rejection R relates to the solute size and the pore size as follows:

$$R = \gamma_s/\gamma \qquad (1)$$

where γ_s is the solute radius and γ is the pore radius of the membrane. Since the model is adopted to an asymmetric ultrafiltration membrane at the high rejection 0.8,[11] the average pore radius in the present work is calculated from 0.8 rejection in the cut-off curves of Figures 2 and 3. Here, the solute radius γ_s of the dextran is calculated by the Stokes-Einstein equation,[13]

$$\gamma_s = \kappa T/6\pi\eta D \qquad (2)$$

Table II Average Pore Radius and Practical Length for Charged Ultrafiltration Membranes

Sample No.	Polymer	Average Pore Radius[a] γ (nm)	Length[b] L (μm)
N-2	PAN-g-DAEM	42.8	11.0
C-1	PAN-g-DAEM$^+$	23.6	1.5
C-2	PAN-g-DAEM$^+$	12.8	2.9
C-3	PAN-g-DAEM$^+$	12.2	5.7
C-4	PAN-g-DAEM$^+$	11.9	2.8
A-1	PAN-g-SSS	38.7	2.6
A-2	PAN-g-SSS	16.5	2.6

[a] γ is estimated from eq. (1) by using 0.8 rejection and stokes radius of dextran.
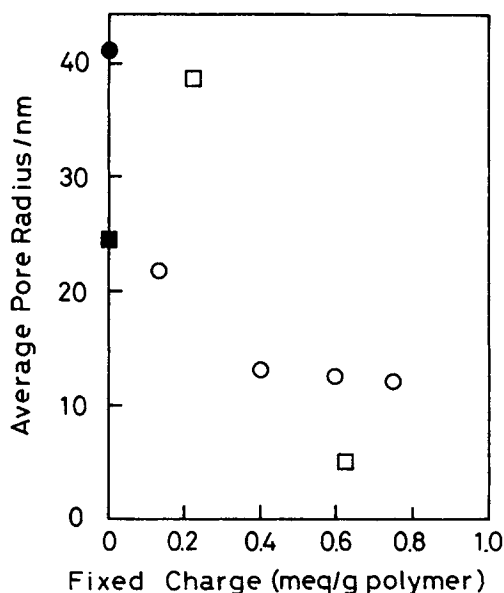[b] L is calculated from eq. (5).

Figure 4   Relationship between fixed charges of charged membrane and average pore radius γ for ultrafiltration membranes: (O) PAN-g-DAEM$^+$ membranes; ($\square$) PAN-g-SSS membranes; ($\bullet$) PAN-g-DAEM membrane; ($\blacksquare$) PAN-Br membrane.

where κ is the Bolzmann constant, T is absolute temperature, η is viscosity, and D is diffusivity (cm^2/s), which is expressed by the molecular weight of dextran Mw as follows.[13-15]

$$D = 8.76 \times 10^{-9}(Mw)^{-0.48} \qquad (3)$$

The linear relationship of equation (3) between log D and Mw is obviously limited to a very special case in the infinitely diluted concentration of a water soluble polymer such as dextran, polyethylene glycol, and poly(vinyl pyrrolidone).[14] As the dilute dextran

solutions were used in the experiment, the viscosity of water 8.9×10^{-4} Pa s at 298 K was used in the above calculations.[16]

Assuming that the pores in a dense layer of the present heterogeneous membrane are cylindrical and perpendicular to the membrane plane and that the rate of flow of water is governed by Poiseuille's law, the average pore radius of membranes is expressed as[12,17-19]:

$$\gamma^2 = 8VL\eta/tA(\Delta P)S \qquad (4)$$

where V is permeated volume of water (mL) in given time t (s), A is the effective membrane area (cm^2), ΔP is the pressure difference across the membrane (dyn/cm^2), S is the specific water content expressed by water absorption per unit weight of the membrane, and L is the practical length (cm), which corresponds to the membrane thickness when the solute permeates.

Using the average pore radius γ, estimated from the relationship of the equation (1), the length L can be calculated from equation (5) as derived from equation (4):

$$L = tA(\Delta P)S\gamma^2/8\eta V \qquad (5)$$

In Table II the calculated values of γ and L for the charged membranes are listed. Figure 4 shows the relationship between fixed charges of the charged membranes and the average pore radius γ. The pore radius of the membranes decreases with an increase in the fixed charges. The obtained values of the length L for the PAN-g-DAEM membrane is about 11 μm. This value, agrees well with the length of a dense layer of a few micrometer's thickness on the substrate side in the PAN-g-DAEM membrane ob-

Table III   Influence of Added Salts (NaCl) on Permeability of Dextran and Volume Flux of Water[a]

Ionic Strength[b]	Volume Flux of Water (L/m² h)		Permeability of Dextran T-10 (%)	
	PAN-g-DAEM$^+$	PAN-g-SSS	PAN-g-DAEM$^+$	PAN-g-SSS
0	59	11	85	57
0.15	65	42	93	83

[a] Membranes were prepared from 8 wt % of polymer solution containing 1% of PVA additives. Fixed charges for PAN-g-DAEM$^+$ and PAN-g-SSS membranes is 0.40 (sample no. C-2) and 0.62 meq/g polymer (sample no. A-2), respectively.
[b] Ionic strength of solution was prepared at 0.154 by adding NaCl.

Table IV   Volume Flux of Eluent and Permeability of Uncharged and Charged Dextran Through Charged Membranes[a]

	Volume Flux of Eluent (L/m^2 h)		Permeability (%)	
Solute	PAN-g-DAEM$^+$	PAN-g-SSS	PAN-g-DAEM$^+$	PAN-g-SSS
Dextransulfate	83	39	95	79
Dextran T-10	16	60	69	85

[a] The charged contents of PAN-g-DAEM$^+$ membrane are 0.59 (sample no. C-3) and PAN-g-SSS membrane are 0.22 (sample no. A-1).

[b] The eluent solution contains 0.2 wt % of dextran or dextransulfate. The molecular weight of dextransulfate is 8000 and dextran T-10 10,000. The ionic strength is prepared at 0.077 by adding NaCl.

served photographically in our previous study.[3] In addition, the results indicate that the length of the charged membrane is shorter than that of the PAN-g-DAEM membrane.

Table III shows the effect of ionic strength on volume flux of water and permeability of dextran T-10 for the samples of C-2 and A-2 membranes. The values of volume flux and permeability increase with the addition of salts. This increase suggests that the diameter of membrane pores is extended by adding NaCl because of the electrostatic shielding of ionic residues.

In order to examine the electrostatic effect of charged membranes in charged dextran, anionic charged dextransulfate was selected as the solute. The volume flux of eluent and the permeability of dextran and dextransulfate through the charged membrane are shown in Table IV. The volume flux of dextran solution in the PAN-g-DAEM$^+$ (sample no. C-3) membrane is smaller than that in the PAN-g-SSS (sample no. A-1) membrane. Furthermore, the permeability of dextran through the PAN-g-SSS membrane is larger than the permeability through the PAN-g-DAEM$^+$ membrane. As uncharged dextran does not interact with the membranes electrostatically; these results indicate differences of the pore diameter of those membranes. That is, the pore size of the PAN-g-DAEM$^+$ membrane is less than that of the PAN-g-SSS membrane.

In the case of the PAN-g-DAEM$^+$ membranes, the volume flux of the dextransulfate solution and the permeability of dextransulfate are higher than those of dextran as shown in Table IV.

Also, in the PAN-g-SSS membranes, the volume flux and the permeability of dextransulfate decrease in comparison with those of dextran. These results obviously support the conclusion in the previous reports[3,4] that electrostatic interaction between

charged membranes and charged polymer solutes controls permeation of charged polymer solutes.

CONCLUSION

Charged ultrafiltration membranes were prepared from charged graft-copolymers of polyacrylonitrile with quaternized N,N-dimethylaminoethylmethacrylate (PAN-g-DAEM$^+$) and sodium p-styrenesulfonate (PAN-g-SSS), and were used for the permeation of dextran and dextransulfate.

When increasing the number of fixed charges in the membranes, the volume flux of water decreased and the rejection of dextran increased despite the higher hydrophilicity of the charged membrane. Further, the addition of NaCl into the solution decreased the rejection of dextran and increased the volume flux of water. The results were explained by the change of pore size of the membranes. Also, the permeation of dextransulfate through the negatively charged membranes was smaller, but the permeation through the positively charged membranes was larger than the permeation through the neutral membrane. The results support the existence of an electrostatic barrier between the charged membranes and the charged polymer solutes.

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