Charged Ultrafiltration Membrane for Permeation of Proteins

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Synopsis

Polyacrylonitrile-*g*-poly(*N*,*N*-dimethylaminoethyl methacrylate) was synthesized photochemically and quaternized. The positively charged membranes made from the quaternized graftcopolymer showed high ultrafiltration rate for water by adding poly(vinyl alcohol) to casting solution and washing it out after the casting. In buffered saline solution, the permeability of the membranes was very small at pH below isoelectric point of albumin but increased markedly at higher pH. On the other hand, the permeability for γ-globulin was very small and did not show any pH dependence.

INTRODUCTION

Under normal conditions, the glomerular capillary wall markedly restricts the transmural passage of plasma proteins and other macromolecules, while at the same time it permits high rate of fluid filtration. Brenner et al.[1] suggested that the glomerular capillary wall contains fixed negative charges which provide an electrostatic barrier to filtration of serum albumin and other circulating polyanions. This suggestion was supported by a theoretical model of Deen et al.[2] for glomerular filtration of charged solutions. However, no report has been published on the synthetic charged ultrafiltration membrane for the permeation of proteins. Therefore, it is a challenging problem to prepare a charged ultrafiltration membrane from a synthetic polymer and to evaluate it for the permeation of plasma proteins. For this purpose, polyacrylonitrile-*g*-poly(*N*,*N*-dimethylaminoethyl methacrylate) (abbreviated as PAN-*g*-DAEM) was synthesized by the photograftpolymerization of DAEM, $CH_2{=}C(CH_3)COOC_2H_4N(CH_3)_2$, to polyacrylonitrile containing bromine atoms.[3] Then, membranes were prepared from the graftcopolymer and tested to permeation of albumin and γ-globulin.

EXPERIMENTAL

Synthesis of PAN-*g*-DAEM

Synthesis of the graftcopolymer was carried out by the photochemical method described previously.[3] In a reaction vessel of 1000-mL capacity, 200 mL of purified polyacrylonitrile, 400 mL of dimethylsulfoxide (DMSO), and 3.3 g of carbontetrabromide of the reagent grade were introduced. The

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mixture was irradiated from outside with a 100-W high-pressure mercury lamp in argon flow. After the photopolymerization for 4 h at 25°C, the mixture was poured into large quantity of aqueous methyl alcohol. The precipitated polymer was dried in vacuum, washed with hot distilled water and with methyl alcohol, and dried for more than 20 h at 50°C. The obtained photosensitive polymer contains bromine atoms and will be abbreviated as PANBr. Molecular weight of PANBr was measured by gel permeation chromatography (Waters, GPC-244) for *N,N*-dimethylforamide (DMF) solution, where calibration was made by using a standard polystyrene.

At various feeding ratios, PANBr and DAEM of the reagent grade were dissolved into DMSO. The mixture was photoirradiated from the outside of the vessel at 25°C for 6 h in argon with a 100-W high-pressure mercury lamp. The mixture was concentrated at reduced pressure and 50–60°C, and poured into large quantity of aqueous methyl alcohol. The precipitated polymer was washed thoroughly with methyl alcohol and with hot distilled water, dried, and purified by the reprecipitation. The chemical structure of the PAN-*g*-DAEM was identified by IR and ^{1}H-NMR. Especially, the DAEM content of the copolymer was determined from an area ratio of an NMR peak at about 4 ppm due to $-CH_2-CH_2-$ of DAEM to that at about 3.3 ppm due to

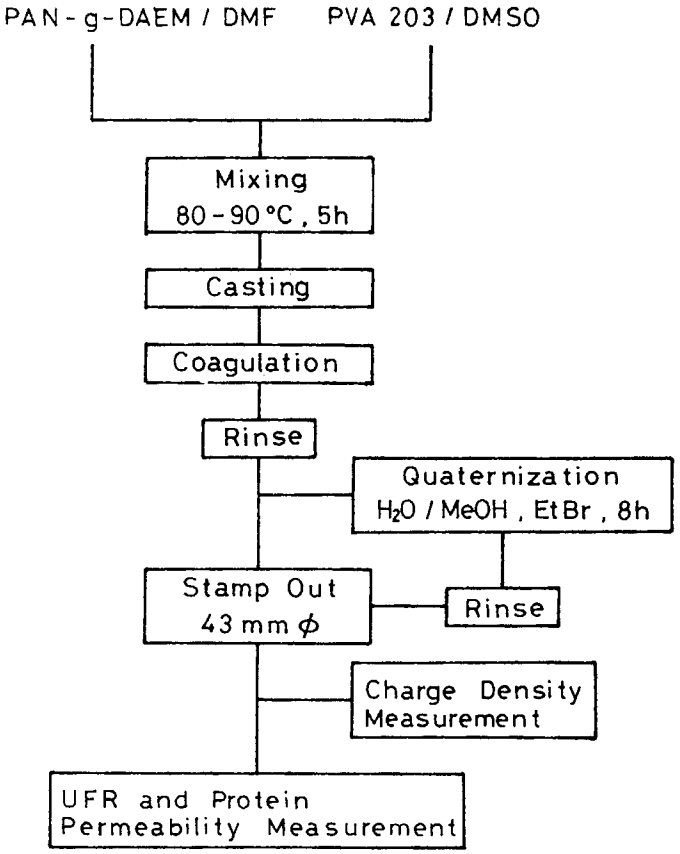


Fig. 1. Schematic diagram for preparation of charged membranes.

—CH— of PAN, where ^{1}H-NMR spectra were obtained by dissolving 10 mg of the PAN-*g*-DAEM into 0.6 mL DMSO-d_6 and using a 270-MHz FT-NMR spectrometer (JEOL, JNM-GX270).

Preparation of Membrane

Preparation of charged membranes is shown schematically in Figure 1. The membrane was made by spreading DMF solution of the PAN-*g*-DAEM with the addition of DMSO solution of poly(vinyl alcohol) (PVA 203 of Kurare Co. Ltd., degree of polymerization 300 and degree of saponification 88 mol %) on a ferrotype plate, coagulating it in water, and washing it with water to remove PVA. To convert the membrane into charged one, the membrane was quaternized as follows: It was dipped into aqueous methyl alcohol saturated with ethylbromide for 8 h at room temperature, washed with large quantity of distilled water, and kept in the dark in distilled water. Concentration of N^+ of the quaternized membrane was measured by the ion-exchange method.[4]

Evaluation of Membrane

The membranes of 100 ± 20 μm thickness and 43 mm effective diameter (13.4 cm^2 effective area) were used for measurements of ultrafiltration rate and permeation of albumin and γ-globulin in a Type 8050 cell (50 mL capacity) of Amicon Co. Ltd., where temperature and pressure were kept at 20°C and 76 cm H_2O (by using argon gas pressure), respectively. The ultrafiltration rate (UFR) was calculated from the flow rate of the water (purified by ion-exchange resin) after the elution of 5 mL water and expressed by water flux per unit area, unit time, and unit pressure (mL/m^2 h cm H_2O).

For the measurement of the protein permeation, a buffer solution containing 0.2 wt % of bovine serum albumin (Cohn Fraction V of Miles Co., isoelectric point 4.7–4.9, molecular weight 66,000, and purity 99%) or bovine serum γ-globulin (Cohn Fraction II of Povite Co., isoelectric point 6.5–7.0, molecular weight 156,000, and purity 99%) was used. Here, aqueous CH_3COOH—CH_3COONa buffer solution was used in the range of pH 3.6–5.6 and aqueous NaH_2PO_4—Na_2HPO_4 in the range of pH 5.3–8.0. Ionic strength of the solution was kept at 0.15 by adding NaCl. The membrane was set in the cell after dipping it for 2 h in the buffer solution. The cell solution and the

TABLE I
Composition of PAN-*g*-DAEM

Sample no.	M_n of PANBr	Composition (wt %)	
		PANBr	DAEM
1	2.4×10^5	95.6	4.4
2	2.8×10^5	96.0	4.0
3	2.1×10^5	89.7	10.3
4	2.5×10^5	91.6	8.4
5	2.5×10^5	91.0	9.0

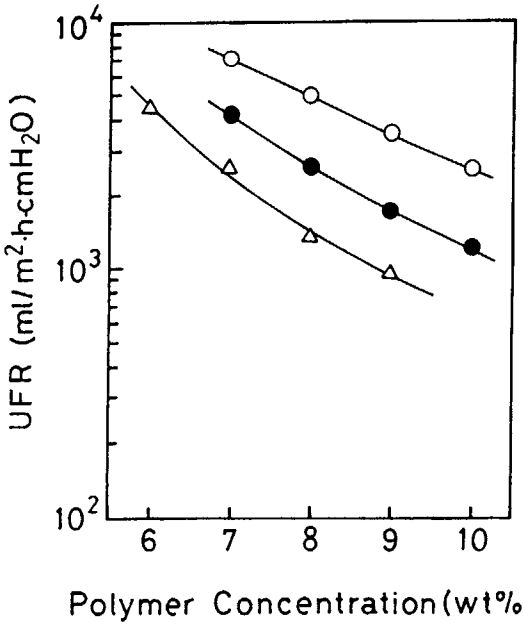


Fig. 2. Effect of polymer concentration and molecular weight (M_n) on UFR of PANBr membranes: M_n: (○) 1.7×10^5; (●) 2.8×10^5; (□) 4.1×10^5.

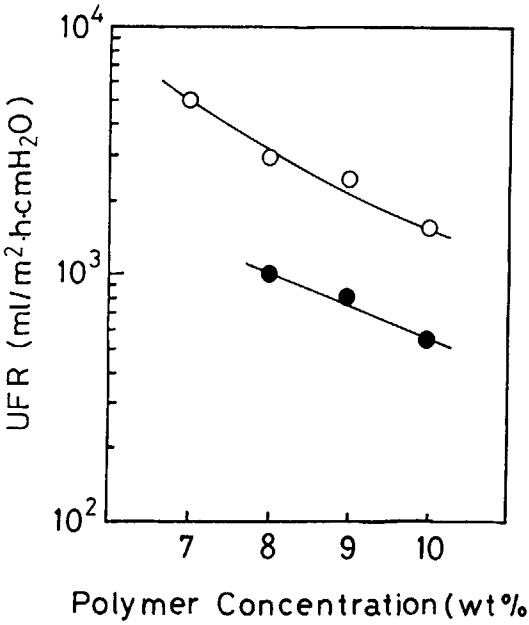


Fig. 3. Effect of polymer concentration on UFR of PANBr and PAN-*g*-DAEM membranes: (○) PANBr ($M_n = 2.4 \times 10^5$); (●) PAN-*g*-DAEM (sample no. 1).

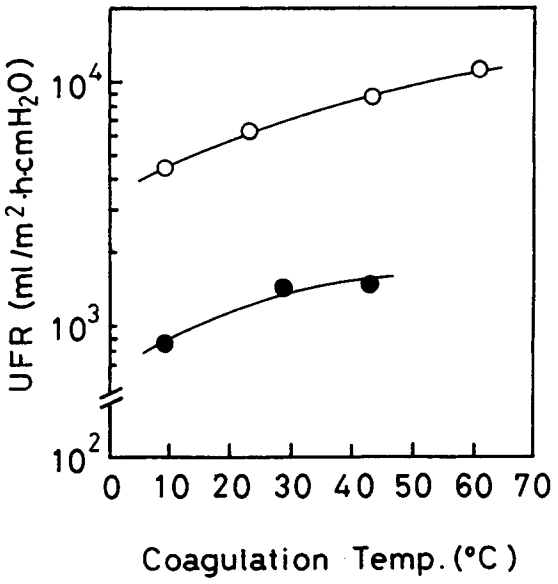


Fig. 4. Effect of coagulation temperature on UFR of PANBr and PAN-*g*-DAEM membranes: (○) PANBr (M_n = 1.7 × 10^5 and polymer concentration 8 wt %); (●) PAN-*g*-DAEM (sample no. 2 and polymer concentration 9 wt %).

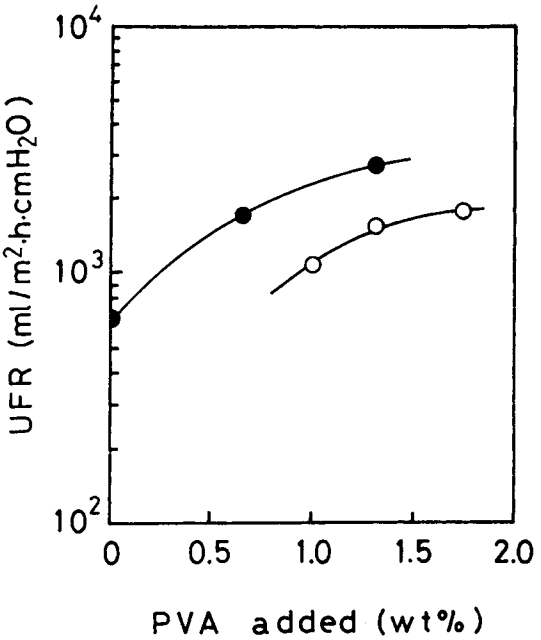


Fig. 5. Relationship between UFR of PAN-*g*-DAEM membranes and amount of PVA added: (●) sample no. 1 (polymer concentration 7 wt % and coagulation temperature 20°C); (○) sample no. 3 (polymer concentration 8 wt % and coagulation temperature 20°C).

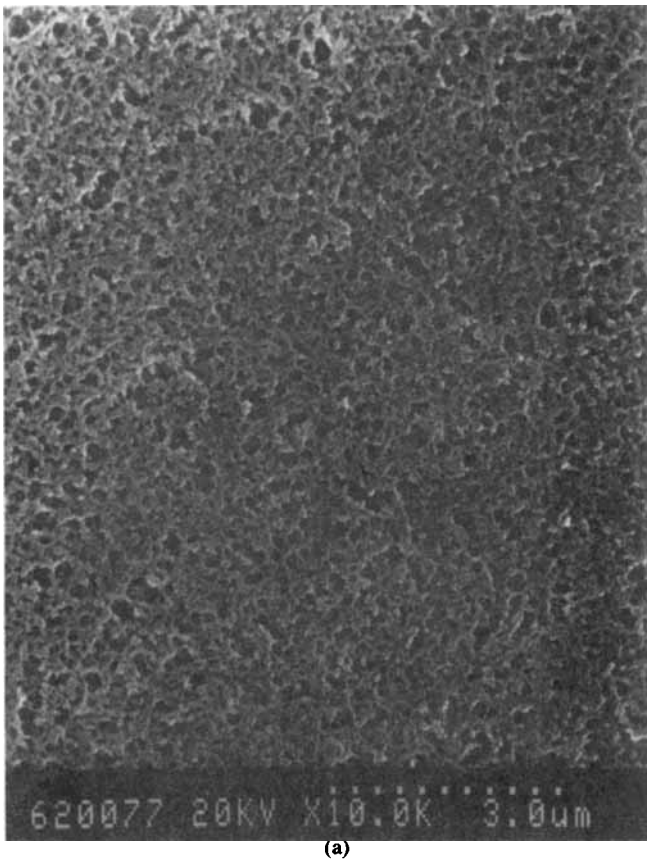
effluent were taken out for analysis after 5 min of elution. The permeability was obtained by adding Coomassie Brilliant Blue G-250 to the effluent and measuring the absorbance at 595 nm.

Morphology of the membrane was observed by a scanning electron x-ray microanalyzer (Hitachi, X-650).

RESULTS AND DISCUSSION

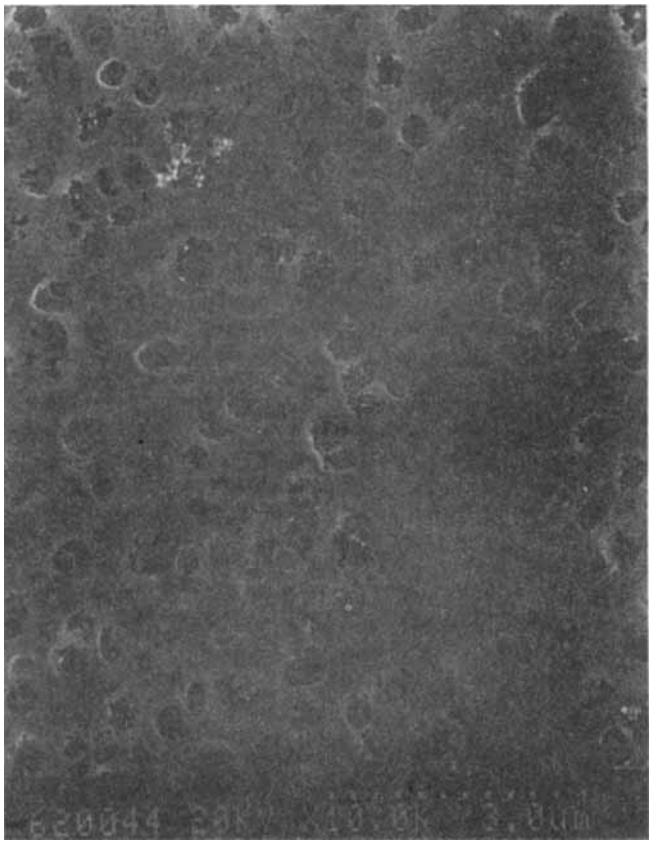
In Table I, composition of PAN-*g*-DAEM used in the present study are shown together with number average molecular weight (M_n) of the trunk polymer.

To find the optimum conditions for the membrane casting, UFR was measured by changing polymer concentration in the casting solution, molecular weight of the polymer, and coagulation temperature. As shown in Figure 2, the higher the polymer concentration and/or the higher the molecular weight of the polymer, the lower the UFR when the trunk polymer, PANBr, was used



(a)

Fig. 6. Scanning electron micrographs of PAN-*g*-DAEM membrane made from sample no. 5: (a) air-side surface of membrane (without addition of PVA); (b) air side surface of membrane (with addition of PVA); (c) cross section of membrane (with addition of PVA). Right is air-side surface and left is substrate-side.

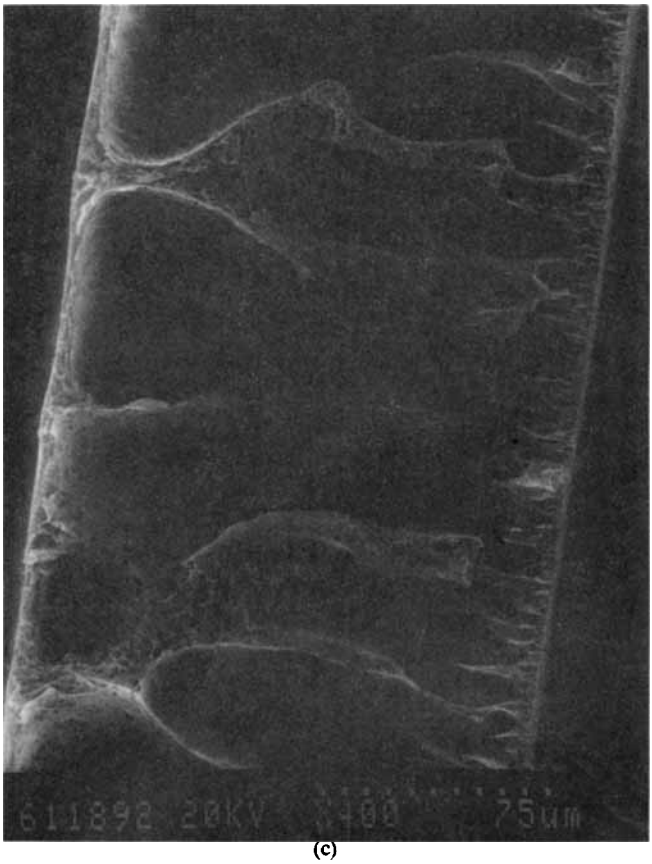


(b)

Fig. 6. (*Continued from the previous page.*)

for casting the membrane. Effect of polymer concentration on the PAN-*g*-DAEM membrane is shown in Figure 3. The tendency is similar to that of the PAN membrane. Also, grafting of DAEM reduces UFR of the membrane. Here, PVA was not added. Figure 4 shows that the higher the coagulation temperature, the higher the UFR for both the PANBr and PAN-*g*-DAEM membranes. On the other hand, decrease of the polymer concentration and increase of coagulation temperature reduced the mechanical strength of the membrane, which made difficult to peel off the membrane from the substrate. Thus, polymer concentration of 8 wt % and coagulation temperature of 20°C were chosen unless otherwise described. The quaternization reduced UFR of the membrane to about 25% of the original value. Thus, to increase UFR of the membrane, PVA 203 was added to the casting solution and washed away from the membrane after the casting. As shown in Figure 5, the addition of PVA increases UFR of both the PANBr and PAN-*g*-DAEM membranes.

In Figure 6, scanning electron micrographs of the membrane are shown. By the addition of PVA, pore size increases to 0.1–0.5 μm. From the cross section of Figure 5(c), it is found that the membrane is heterogeneous porous membrane having a dense layer of few micrometers thickness on the substrate side.



(c)

Fig. 6. (*Continued from the previous page.*)

The membrane made from PANBr having UFR in the range of 1000–2000 mL/m^2 h cm H_2O showed only few percent of albumin permeation. On the other hand, the membranes made from PAN-*g*-DAEM and PAN-*g*-$DAEM^+$ (quaternized membrane) show appreciable permeation at higher pH as shown in Figure 7. The permeability of membrane increases at pH 4.5–5.0 (near isoelectric point of albumin) and higher pH, while it is very small at pH below the point. This tendency is more marked for higher N^+ concentration than for lower N^+ concentration. The membranes before the quaternization shows the similar but less marked tendency. Since pKa of DAEM homopolymer measured preliminary was 7.1, more than half of DAEM of the PAN-*g*-DAEM is considered to be protonated in the pH range used in the experiment. Therefore, it is reasonable that the PAN-*g*-DAEM membrane, especially of higher DAEM content, shows similar pH dependence similar to that of the PAN-*g*-$DAEM^+$ membrane.

In Table II, permeability for γ-globulin is shown. Both the PAN-*g*-DAEM and the PAN-*g*-$DAEM^+$ membranes show very small permeability and no dependence on the permeability on pH.

The above-described result indicates that positive charges of the membrane attract negative charges of the albumin at pH higher than the isolectric point of the albumin, which increases the permeation of the albumin at higher pH.

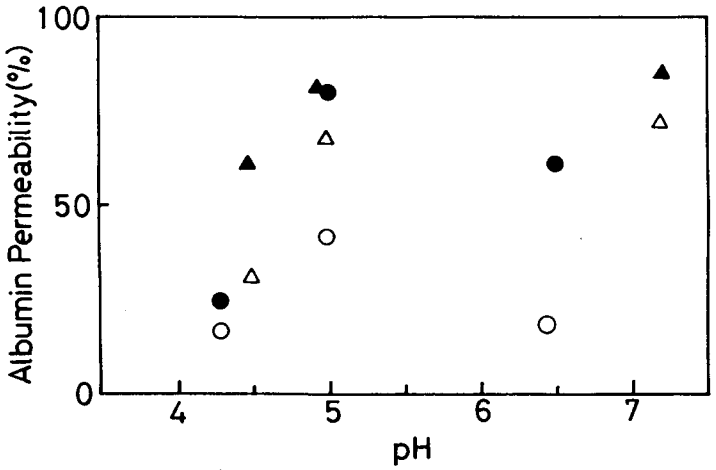


Fig. 7. Dependence of albumin permeability of pH of solution: (○) PAN-*g*-DAEM (sample no. 2 and UFR = 1400–1600 mL/m^2 h cm H_2O); (●) PAN-*g*-DAEM$^+$ (sample no. 2 and [N^+] = 0.16 meq/g); (△) PAN-*g*-DAEM (sample no. 4 and UFR = 1400–1800 mL/N^2 h cm H_2O); (▲) PAN-*g*-DAEM$^+$ (sample no. 4 and [N^+] = 0.43 meq/g). Here, UFR of PAN-*g*-DAEM membrane is about 25% of the corresponding PAN-*g*-DAEM membrane.

TABLE II
Permeability of γ-Globulin and Its pH Dependence

	pH				
Sample	4.87	6.30	7.33	7.45	7.90
5	3.0%	5.2%	3.2%	3.4%	2.8%
5^+	5.1%	4.6%	2.2%	4.3%	3.1%

[a] Here, UFR of sample no. 5 is 1800–2200 mL/m^2 h cm H_2O and that of sample no. 5^+ 450–550 mL/m^2 h cm H_2O. N^+ concentration of the latter is 015 meq/g.

From Figure 7 and Table II, it seems promising to separate albumin and globulin by using the positively charged ultrafiltration membrane. Further, we are examining permeation and separation of proteins by using the negatively charged membrane.

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