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DIFFUSION THROUGH LAMINATED GEL

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The diffusion coefficients of probe molecules having different molecular weights ranging from 4.0 $\times 10^1$ to 1.6$\times 10^5$ within poly(acrylamide) gel, agarose gel and the laminated gel are determined by the time-lag method. It is found that the diffusion coefficients of these probe molecules in agarose gel are much larger than that in poly(acrylamide) gel. The diffusion coefficients of probe molecule becomes smaller with molecular weight in both gels. In the case of bovine serum albumin (M.W. = 6.6 $\times 10^4$), the diffusion coefficient in poly(acrylamide) gel (tickness of the gel: 0.14 cm) could not be determined because of a large time-lag of more than 40 days. Similar results are obtained for the laminated gel. These results suggest that the transport properties of laminated gel of poly(acrylamide) and agarose are mainly determined by that of the poly (acrylamide) gel layer. The poly(acrylamide) gel layer behaves as a cut-off filter in the laminated gel.

Key Words: Diffusion coefficient, Poly (acrylamide) gel, Agarose gel, Laminated gel, Time-lag method

INTRODUCTION

Hydrogels consist of a polymer network and water. Since the total amount of the polymer network is much less than that of water, the substances can diffuse through the gel. The rate of diffusion through the gel is mainly determined by the sizes of the polymer network and the diffusing substances. Thus, the gels having various mesh sizes are necessary to control the transport of substance through gels.

Recently, we reported the time-lag method is useful to determine the transport properties of porous materials such as gel [1]. In this paper, we report about the transport properties of several gels consist of poly(acrylamide) and agarose. The sample gels used here are poly(acrylamide) gel, agarose gel, and the laminated gel of them. In the case of laminated gel, the interpenetrated network is formed at the interface of poly(acrylamide) gel and agarose gel.

MATERIALS AND METHODS

Preparation of gels

The acrylamide monomer, ammonium peroxide, N,N'-methylenebisacrylamide and N,N,N',N'-tetramethylethylenediamine were obtained from Bio Rad Ltd., Japan. The agarose (M.W. = 1.0$\times 10^5$) was supplied from Shimizu Shokuhin Ltd., Japan.

Three types of gel samples were prepared: poly (acrylamide) gel, agarose gel and laminated gel of them. The procedur sample of preparation is schematically illustrated in Fig 1.

Poly(acrylamide) gels were prepared by the standard method. The acrylamide monomer and N,N,N',N'-tetramethylethylenediamine were dissolved into the deionized water. Then, the gel holder was immersed into the pre-gel solution and degassed for 30 min. The polymerization reaction was initiated by adding 1 ml of 4 % (w/w) ammonium peroxide solution. The fraction of N,N'-methylenebisacrylamide was fixed at 1 mol% of the total monomer concentration.

Agarose were dissolved in water at 98°C for 1 hour. The gel holder was immersed into the agarose solution. The solution was quenched to 0°C for 10 min. Them the gel was maintained at 4°C for one day in order to complete the gelation.

The laminated gels consist of 10% poly(acrylamide) gel and 1 % agarose gel was prepared as follows. The gel bond film having circular opening was stuck on the acryl plate. This "half gel mold" was immersed in the pre-gel solution of acrylamide. Then the reaction was made. The gel mold, which contains poly(acrylamide), was taken out of the gel. The second "half gel mold" was stuck on the first one, which consist of agarose gel bonding film and acryl plate , and immersed into agarose solution at 98°C. The temperature was lowered to 0°C to form agarose gel.

The cross section of sample was 0.76 cm^2, and the thickness of the gel was 0.14 cm. All gel samples were washed using Tris-HCl buffer solution for 24 hours before measurement. The surface of gel was protected by stainless steel mesh that also prevent the swelling of gel. The measurements of diffusion the swelling of gel.

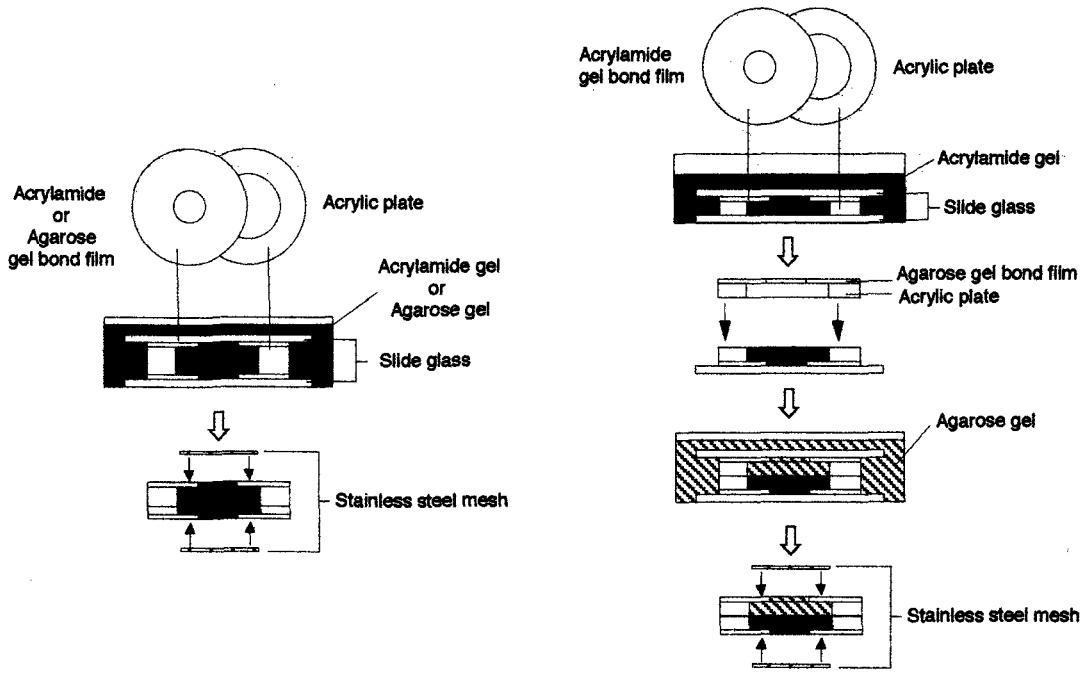

Fig. 1 Strchretic illustration of the sample preparation.
Left: agasose gel and poly(acrylamide) gel. Right: laminated gel of gasose gel and poly(acrylamide) gel

coefficients of probe molecules were, thus, made under constant-volume conditions.

Probe substances

Following five molecules (ion) were used as probe substances: calcium ion (Ca^{2+}, M.W. = 4.0×10^{1}), glucose (Glc, M.W. = 1.8×10^{2}), vitamin B12 (VB12, M.W=1.36×10^{3}), immunoglobulin G (IgG, M.W. = 1.6×10^{5}) (those were obtained from Wako Pure Chemical Industries Ltd., Japan), and Bovine serum albumin (BSA, M.W. = 6.6×10^{4}) (this was obtained from Sigma, USA). These probe molecules were disolved in a buffer solution of tris-hydroxy aminomethane (Tris)-HCl at pH=7.2, and used in the following experiments.

Apparatus

Figure 2 shows the schematic illustration of the apparatus used for diffusion measurement. The probe molecules were added into compartment A. Then, the concentration of probe molecule in the compartment B is measured as a function of time. The initial concentrations of probe molecules in the compartment A were follows: 4.40×10^{2} mM, Ca^{2+}; 4.44×10^{2} mM, Glc; 7.38×10^{-1}, VB12; 1.52×10^{-1} mM, BSA; 7.6×10^{-2}, IgG mM. The detectors used to determine the concentration in the compartment B for each probe molecule were follows: Ca^{2+}, the ion meter (SR23, HORIBA Ltd., Japan); Glc, the refractive index meter (RI-70, Showa Denko, Japan); VB12, BSA and IgG, the spectrometer (UV-150, Shimazu Ltd., Japan) operated at wavelengths of 360 nm (VB12) and 280 nm (BSA and IgG).

In the time-lag method, the diffusion coefficient is determined as follows [1]. Compartment A and B, which are filled with solvent, are separated by the gel. Then, the probe molecule is added into compartment A. The probe molecule diffuses into the gel. Finally, the probe molecule diffuses out of the gel. The concentration gradient of probe molecule is established in the gel in the initial stage. During measurements, the steady state is realized and hence the permeation rate is constant. Under the steady state conditions, the concentration of probe molecule in compartment B is given as follows,

$$C(t)=\frac{A D C_o}{V_2 d}\left(t-\frac{d^2}{6D}\right)$$

where A is the cross section of the gel (m^2), V_2 is the volume of compartment B (m^3), D is the diffusion

coefficient in gel (m^2/sec), d is the thickness of the gel (m), Co is the initial concentration in compartment A (M) and t is the time. Hence, the diffusion coefficient of the probe molecule can be determined from the time-lag τ that is determined by extrapolation of elution curve at the steady state to $C(\tau)=0$. Diffusion coefficient is calculated as follows,

$$D=\frac{d^2}{6\tau}$$

RESULTS AND DISCUSSION

In Figuer 3, the diffusion coefficient of probe molecules in the gels and the molecular weight of the probe molecules are plotted in a double logarithmic form. It is clear from these results that the diffusion coefficient of various probe molecules in agarose gel can be expressed by a straight line. The slope of the straight line in the double logarithmic plot becomes -1/3. It indicates that the diffusion coefficient of probe molecule in the agarose gel can be written as follows.

$$D \propto M^{-1/3}$$

Since the probe molecules used here are rather compact and are soluble in water, the molecular weight of probe molecules can be expressed as

$$M \propto R^3$$

where R denotes the radius of the probe molecule.

Thus, the diffusion coefficient of the probe molecule in agarose gel can be written as follows.

$$D \propto R^{-1}$$

These results indicate that the diffusion in agarose gel can be simply explained by Stokes-Einstein relationship for the diffusion coefficient of substances [2].

$$D=\frac{kT}{6\pi\eta R}$$

It has been reported that the aggregated domains of double helices of polysaccharide chain construct the network structure of agarose gel. Besides, the network structure of agarose gel may be tenuous since the concentration of agarose in the gel is 1%. Under these conditions, the mesh size of the agarose gel is larger than that of synthetic polymer gels such as poly(acrylamide) gel. The probe molecules, therefore, can easily diffuse through the large mesh. The polymer network of the gel does not effect the diffusion process of probe molecule in the case of agarose gel. The diffusion coefficient of probe molecules, therefore, can be simply explained by Stokes-Einstein relationship.

In contrast, the presence of poly(acrylamide) gel network affects the diffusion process of probe molecules. In the present case, the protein molecules, BSA and IgG, can not permeate through the poly (acrylamide) gel. In the case of BSA, the diffusion coefficient in poly(acrylamide) gel could not be determined because of a large time-lag of more than 40

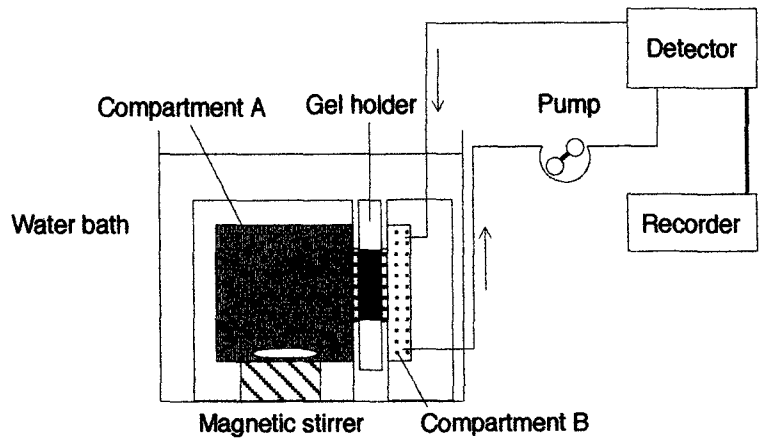

Fig. 2 Schematic illustration of the apparatus. The apparatus consist og two compartments A and B. The probe molecules are added in compartment A. The probe molecules diffuse to compartment B through the gel fixed with the gel holder. The concentration of probe molecule in compartment B is measured as afunction of time. The temperature was maintained at 30°C by a water bath. The compartments A and B were closed to avoid the evaporation of solvent.

days. The polymer network is, therefore, practically behaves as a cut-off membrane for the diffusion of these protein molecules.

It is obvious from these results that the smaller probe molecules can diffuse through the laminated gel. It is, however, also clear that the probe protein, BSA. can permeate through the laminated gel but IgG can not. The transport properties of laminated gel are, therefore, intermediate between the transport properties of agarose gel and that of poly(acrylamide) gel. These results indicate that the laminated gel prepared here can separate these proteins.

By changing the features of polymer chains, the thickness of each gel, and the concentrations, various kinds of laminated gels having various transportproperties can be prepared. Such laminated gels can be widely used in separation technology, implanting and culturing of cells.

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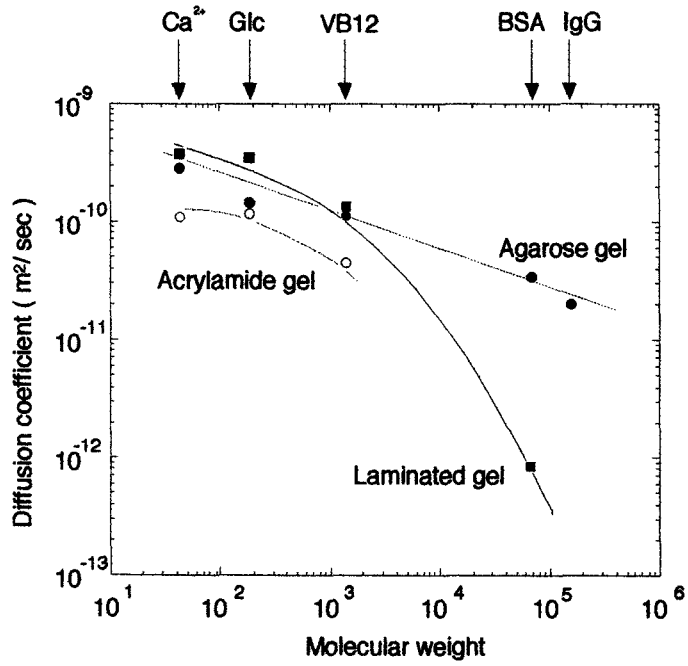

Fig. 3 Diffusion coefficients of the probe molecules in the gel, ●: 1 % ararose gel, ○: 10 % poly (acrylamide) gel, and ■: laminated gel (1 % agarose - 10 % acrylamide).

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