Diffusion of Saccharides and Amino Acids in Cross-linked Polymers

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Diffusion coefficients of saccharides and amino acids were measured in cross-linked polymers such as dextran gels, polyacrylamide gels and photo-crosslinkable resins of different gel concentrations or various degrees of crosslinkage. Comparison of diffusion coefficients in gels with those in polymer solutions indicated that the diffusional velocity in gels was restricted not only by the interaction between diffusing substances and gel components but also by the steric hindrance of gel matrix. The diffusion coefficients in dextran gels and polyacrylamide gels were appreciably lower than those in polymer solutions, and the degree of lowering depended on both the gel concentration and the size of diffusing substances. The photo-crosslinkable resins basically composed of polyethylene glycol showed a higher permeability than dextran and polyacrylamide gels on account of a weak interaction between diffusing substances and resin component. Furthermore, an attempt was made to correlate the decrease of diffusion coefficients in gels with the diffusion coefficients in polymer solutions and the distribution coefficient of the diffusing substances.

In recent years cross-linked polymers such as dextran gels and polyacrylamide gels have been extensively utilized as support for immobilized enzymes and in gel chromatography. The reaction rate of an immobilized enzyme is frequently found restricted by the diffusional velocity of substrates within particles. Similarly, the column efficiency in gel chromatography is appreciably influenced by the gel phase diffusion. Information on diffusion coefficient in gels is therefore very important.

Diffusivity in gels is considered to be lower than that in solution because of the interaction between diffusing substances and gel components and of the steric hindrance. However, only a few works have been reported on diffusion in gels in comparison with those on gaseous diffusion in solid catalysts. Nelson compared the diffusion coefficient in an ion-exchange resin with that in a model electrolyte solution. Horowitz and Fenichel measured diffusion coefficients of molecules of a molecular weight less than 100 in a dextran gel (Sephadex G-34). They pointed out that the ratio of the diffusion coefficient in the gel to that in aqueous solution was in the 0.6~0.7 range regardless of the size, chemical grouping and temperature of solutes. Ackers and Steere studied diffusion of proteins through agar-gel membranes and explained the decrease of the diffusion coefficients by a theoretical equation of Renkin.

In a previous paper on diffusion of amino acids and saccharides in polymer solutions such as dextran and its derivatives, we reported our observation that the diffusion coefficient decreased with the increase in polymer concentration. The lowering degree of the diffusion coefficient could be well explained by a model in which a direct interaction (hydrogen bond) between diffusing substances and polymer was taken into consideration.

In this study, we measured the diffusional velocity of relatively low molecular weight substances such as saccharides and amino acids in dextran gels, polyacrylamide...
gels and photo-crosslinkable resins\textsuperscript{10,11} of different gel concentrations or various degrees of crosslinkage. For this purpose, spherical beads of dextran gels and polyacrylamide gels or a plane sheet of the photo-crosslinkable resins were prepared. Comparison of the diffusion coefficients in the gels with those in the polymer solutions indicated that diffusion in gels was restricted not only by the interaction between gel components and diffusing substances but also by the steric hindrance. An attempt was made to correlate the decrease of diffusion coefficients in gels with the diffusion coefficients in polymer solutions and the distribution coefficient which represents the partition of solutes between the gel phase and the space outside the gel particle.

MATERIALS AND METHODS

Materials. All chemicals used in this study were of analytical grade. Dextran T-40 (mol. wt. \(4 \times 10^4\)) was purchased from Pharmacia Fine Chemicals. Acrylamide, \(N, N'\)-methylenebisacrylamide (BIS), \(N,N,N',N'\)-tetramethylethylenediamine (TEMED), ammonium persulfate, epichlorohydrin, \(d\)-glucose, maltose, glycine, \(L\)-\(\beta\)-alanine, \(L\)-valine and \(L\)-arginine were obtained from Wako Pure Chemical Industries, maltotriose from Hayashibara Biochemical Inc., glucoamylase (pure grade) from Seikagaku Kogyo Co., and Sorbitan sesquioleate from Tokyo Kasei Co.

Preparation of dextran gel beads. Dextran gel beads were prepared by a modification of the pearl polymerization method.\textsuperscript{12} Ten grams of dextran was suspended in 4 ml of distilled water and dissolved by adding 6 ml of 5 N sodium hydroxide solution, and the solution was allowed to stand overnight in a vacuum to eliminate air bubbles. To this alkaline dextran solution, 1\textendash}6 ml of epichlorohydrin was added in order to vary the degree of crosslinkage of the gel. The mixture became viscous after 10\textendash}20 min of slow stirring at 40\(\degree\)C with a glass stick. At this stage, this solution was immediately poured into liquid parafin which had been incubated at 50\(\degree\)C in a 250 ml beaker under vigorous stirring, accomplished with an agitator.

The polymerization reaction was allowed to proceed for 20 hr at 50\(\degree\)C. The gel produced was washed with \(n\)-hexane and acetone, and then with water till it became neutral. The shape of the gel was found to be quite spherical by photographic observation, and the water regain was in the range of 3.8\textendash}24.0 g/dry gel. The gel beads having a diameter of 2\textendash}4 mm were used in the experiment.

Preparation of polyacrylamide gel beads. Acrylamide monomer and a cross-linking agent, BIS, were dissolved in distilled water to the prescribed concentration. The final volume was adjusted to 5 ml. To this solution was added a catalyst system consisting of 1 ml of ammonium persulfate solution (5\%) and 0.1 ml of TEMED. The final concentration of the total monomer (acrylamide plus BIS) was varied from 10 to 40\% (w/v), with the BIS content, in terms of percentage (w/w) of the total amount of monomer, kept at 9.1\%. The BIS content was varied from 5 to 25\% at a fixed total monomer concentration of 22\%.

This mixture was poured with an injector into a measuring cylinder containing 600 ml of an organic phase (toluene-chloroform; 435/165) and 0.5 ml of an emulsion stabilizing agent, Sorbitan sesquioleate. During polymerization, the organic phase was kept under nitrogen atmosphere at room temperature. The polymerization was completed before the bead reached the bottom of the cylinder. The gel bead was washed with toluene to remove chloroform and then with a large amount of distilled water.

Preparation of photo-crosslinkable resins. A 1-mm thick plane sheet of photo-crosslinkable resins was prepared by the same method as previously reported,\textsuperscript{10,12} and a piece of the original sheet of 3\textendash}4 cm was used in the experiment. The photo-crosslinkable resin named ENT 100\textsuperscript{11} is basically composed of polyethylene glycol.

Measurement of diffusion coefficient. Diffusion coefficients in dextran or polyacrylamide gels were measured by a method similar to that by Horowitz and Fenichel.\textsuperscript{5} A single swollen gel bead was transferred to an excess of the sample solution at the prescribed concentration (\(d\)-glucose 1 M, maltose 0.5 M, maltotriose 0.33 M, glycine 1.4 M, \(L\)-\(\beta\)-alanine 1.0 M, \(L\)-valine 0.3 M, \(L\)-arginine 0.5 M), and allowed to be equilibrated for 24 hr at room temperature under shaking.

Then, the bead was removed from the solution, blotted with a piece of filter paper to eliminate excess liquid on the surface, and put in a holder made of stainless steel wire. This holder was immersed successively at appropriate time intervals (1\textendash}10 min) in a test tube (I.D. 22 mm), in which 5 ml of distilled water or 1.0 M acetate buffer (pH 4.3) in case of \(L\)-arginine as a diffusing substance had been pipetted beforehand. Mixing of solution in the tube was accomplished with a magnetically rotated bar at the bottom. The temperature was controlled at 25\textpm\ 0.1\(\degree\)C.

In most runs, eight tubes were used. Each transfer
was accomplished in 3–5 seconds, and this loss of
time could be ignored compared with the experimental
time. The bead was allowed to remain in the final
test tube for 2–6 hr to recover all remaining solute.
The concentration of glucose in a wash-out solution
in each tube was determined by the glucose oxidase
method, and that of amino acids by the ninhydrin
reaction. Maltose and maltotriose were completely
hydrolyzed into glucose with glucoamylase, and the
amount of glucose produced was determined by the
above described method.

To photo-crosslinkable resins was applied the
method. A swollen resin sheet equilibrated
with a diffusing substance (glucose) was put into a
beaker filled with 150 ml of distilled water which was
kept well stirred with a magnetically rotated bar. At
appropriate time intervals, 0.5 ml of the sample
solution was taken, and the concentration of glucose
was determined by the glucose oxidase method.

Analysis of the experimental data. In case of
dextran and polyacrylamide gels, the relative average
concentration of solute in the bead at any time,
\[ \frac{C_g(t_j)}{C_0} \]
was given by

\[ \frac{C_g(t_j)}{C_0} = \frac{1}{N} \sum_{i=1}^{N} \frac{C_s(t_j)}{C_s(t_i)} \]

(1)

where \( C_0 \) represents the initial concentration of solute
in the bead, \( C_g(t_j) \) the concentration of each wash-out
solution, \( N \) the total number of test tubes and \( t \) the
time. In the experiment with the photo-crosslinkable
resin, \( C_g(t_j) \) was calculated from the concentration
of the sample solution as

\[ \frac{C_g(t_j)}{C_0} = 1 - \frac{C_g(t_j)}{C_s(t_j)} \]

where \( C_g(t_j) \) represents the concentration of the sample
solution at time \( t_j \) and \( C_s(t_j) \) the concentration of the
sample solution after all the solute in the resin sheet
has been eluted.

The relations of \( \frac{C_g(t_j)}{C_0} \) with \( \frac{t_j}{r^2} \) for a spherical
bead and a plane sheet are easily derived by Eqs. (2)
and (3), respectively, on the basis of the assumptions:
(1) the concentration of solute in a bulk solution out-
side a bead is negligibly low and (2) the dependency of
diffusion coefficient on the concentration is negligible.

\[ \frac{C_g(t_j)}{C_0} = \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp \left( -D_e \pi^2 r^2 t_j / r^3 \right) \]

for spherical bead

(2)

\[ \frac{C_g(t_j)}{C_0} = \frac{8}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp \left( -D_e \pi^2 r^2 t_j / l^3 \right) \]

for plane sheet

(3)

where \( D_e \) is the diffusion coefficient in the gel, \( r \) the
radius of a bead and \( l \) the half thickness of a sheet.
The diffusion coefficient was so determined as to
achieve the maximum consistency between the
experimental data and the calculated results by Eq. (2)
or (3). In most experimental runs, \( \frac{C_g(t_j)}{C_0} \) was in
the range of 0.2 to 1.0. The radius of a bead and
the thickness of a sheet were measured with a profile

The distribution coefficient for poly-
acrylamide gels and photo-crosslinkable resins were
determined experimentally, since no theory for these
gels has yet been established. A single gel bead or
sheet was equilibrated in the saccharide solutions of
the same concentration as used in the diffusion ex-
periment. Then, the gel was removed from the
solution, blotted to eliminate excess liquid on the surface
and placed in a test tube until all the solute was eluted.

The test tube was filled with 25 ml of distilled water.

\[ K = \exp \left( -\pi L (r_s + r_r)^2 \right) \]

(4)

where \( L \) is the concentration of rods (dextran molecules),
expressed in cm rod/cm³, \( r_s \) the equivalent radius of a
solute molecule and \( r_r \) the radius of the rod. Laurent
and Killander experimentally determined \( r_r \) to be
7 Å. They also gave the relationship between \( L \) and
dextran concentration; i.e., in a dextran gel \( L \) had a
value of \( 4 \times 10^{12} \) cm/cm³ for the concentration
of 0.1 g/ml. The values of \( r_r \) were estimated from the
diffusion coefficient in a dilute solution \( (D) \) by Stoke’s
law or the radius of a sphere of equal weight and
density \( (\phi) \) as shown in Table I.

The distribution coefficients of saccharides for poly-
acrylamide gels and photo-crosslinkable resins were
determined experimentally, since no theory for these
gels has yet been established. A single gel bead or
sheet was equilibrated in the saccharide solutions of
the same concentration as used in the diffusion ex-
periment. Then, the gel was removed from the
solution, blotted to eliminate excess liquid on the surface
and placed in a test tube until all the solute was eluted.

Figure 1 shows a comparison between the experi-
mental and calculated results obtained for a dextran
gel, in which \( \frac{C_g(t_j)}{C_0} \) was plotted against \( \frac{t_j}{r^3} \). A
fairly good agreement was observed between them.
Thus, a reasonable diffusion coefficient might be
obtained by the method described above.

Distribution coefficient. For evaluation of distribu-
tion coefficients of the solutes for dextran gels, the
correlation derived by Ogston was available. He
proposed the following formula:

\[ K = \exp \left( -\pi L (r_s + r_r)^2 \right) \]

(4)

FIG. 1. Theoretical and Experimental Relations
between \( \frac{C_g(t_j)}{C_0} \) and \( \frac{t_j}{r^3} \) for Dextran Gels.

Diffusing substances: ○, glucose; □, maltotriose.
The gel concentration was evaluated at 20.6% (w/v).
The solid curves show the calculated results by Eq. (2),
with \( 0.23 \times 10^{-4} \) and \( 0.10 \times 10^{-4} \) cm²/sec used as \( D_e \)
for glucose and maltotriose, respectively.

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for glucose and maltotriose, respectively.

\[ \frac{D_e}{D_e} \]
TABLE I. ESTIMATED MOLECULAR DIMENSIONS

<table>
<thead>
<tr>
<th>Substances</th>
<th>$D$ at 25°C (cm²/sec × 10⁶)</th>
<th>Molecular radius (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.68</td>
<td>3.6</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.50</td>
<td>4.9</td>
</tr>
<tr>
<td>Maltotriose</td>
<td>0.43</td>
<td>6.4</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.06</td>
<td>2.3</td>
</tr>
<tr>
<td>L-β-Alanine</td>
<td>0.93</td>
<td>2.6</td>
</tr>
<tr>
<td>L-Valine</td>
<td></td>
<td>3.4</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>0.52</td>
<td>4.1</td>
</tr>
</tbody>
</table>

a) The value for raffinose was used.¹⁹
b) The radius of a sphere of equal weight and density was calculated.¹⁹ As the value of $\rho$, 1.23 was used.¹⁹

c) Measured for 0.5 M L-arginine in 1 M acetate buffer pH 4.5.¹⁹

Diffusion in solution $K=25C_0(C_0V_g)$, where $C_0$ represents the initial concentration of the saccharide solution, $C_s$ the final concentration of a solution in the test tube and $V_g$ the volume of a gel bead or a sheet.

**Gel concentration.** Since the cross-linked polymers used in this study swelled in water or some aqueous solutions, the gel concentration was calculated on the basis of the water regain and the partial specific volume of the gel component, i.e., 0.61 for dextran gels,²⁰ 0.89 for polyacrylamide gels²¹ and 0.91 for the photo-crosslinkable resin (polyethylene glycol).²¹

**Results**

**Distribution coefficient**

Figure 2 shows a plot of the calculated values of the distribution coefficients of saccharides and amino acids versus gel concentrations for dextran gels. Figure 3-a shows a plot of the experimental data of the distribution coefficients of saccharides versus gel concentrations for polyacrylamide gels at a constant BIS content (9.1%). In Fig. 3-b, $K$ is plotted against BIS content at a constant monomer concentration (22%).

**Diffusion coefficient of saccharides and amino acids in dextran gels**

The observed values of relative diffusion

![Fig. 2. Theoretical Relations between $K$ and Gel Concentrations for Various Saccharides and Amino Acids in Dextran Gels.](image)

![Fig. 3-a. Experimental Relations between $K$ and Gel Concentrations for Various Saccharides in Polyacrylamide Gels.](image)

![Fig. 3-b. Relations between $K$ and BIS Content for Various Saccharides in Polyacrylamide Gels.](image)
coefficients $D_e/D_o$ of saccharides and amino acids in dextran gels against gel concentrations are shown in Figs. 4-a and 4-b, respectively. In a previous paper, the diffusion of saccharides and amino acids in dextran solution, we pointed out that the decrease of diffusion coefficient was ascribable to a direct interaction (hydrogen bond) between diffusing substances and dextran molecules. The solid curves in Figs. 4-a and 4-b represent the theoretical results of the ratios of diffusion coefficient in dextran solution $D_e$ to $D_o$, which were in good agreement with the experimental ones.

As seen in Figs. 4-a and 4-b, $D_e/D_o$ was appreciably lower than $D_p/D_o$ and the degree of the lowering depended on both the gel concentration and the size of diffusing substances. This implies that diffusion in gels is restricted not only by the interaction between diffusing substances and the gel component (dextran) but also by the steric hindrance of gel matrix.

**Diffusion coefficient of saccharides in polyacrylamide gels**

The diffusion coefficients of saccharides in polyacrylamide gels are shown in Figs. 5-a and 5-b. Figure 5-a shows a plot of $D_e/D_o$ versus gel concentrations at a constant BIS content (9.1%), in which the theoretical results of $D_p/D_o$ in acrylamide polymer solution are also drawn in solid lines. Details of the calculation are described in the Appendix. The values of $D_e/D_o$ in polyacrylamide gels were much lower than $D_p/D_o$ in the same
FIG. 5-b. Effect of BIS Content on $D_e/D_0$ for Various Saccharides in Polyacrylamide Gels.

Total monomer concentration was kept at 22%. The symbols are the same as shown in Figs. 4-a and 5-a.

way as observed in dextran gels.

In Fig. 5-b, $D_e/D_0$ is plotted against BIS content at a total monomer concentration of 22%. The minimum $D_e/D_0$ was observed at a BIS content of about 15%. This striking and unexpected feature of the permeability of polyacrylamide gels was also found in the studies so far reported. Hjerten et al. observed a marked increase in gel permeability for proteins at a higher BIS content above 15%. Degani and Miron observed in the study of polyacrylamide-entrapped cholinesterase that the entrapping efficiency strongly depended on BIS content and reached its maximum at 5%.

**Diffusion coefficient of glucose in photo-crosslinkable resins**

The diffusion coefficient of glucose was also measured in the photo-crosslinkable resins to be compared with that in dextran and polyacrylamide gels. As shown in Table II, the values of $D_e/D_0$ were larger than those for dextran and polyacrylamide gels at the same gel concentrations. On the other hand, the distribution coefficients of glucose were much smaller than those for dextran and polyacrylamide gels, and were nearly equal to $D_e/D_0$. This might mean that the diffusion in the photo-crosslinkable resin was restricted only by the steric hindrance of gel matrix and that the interaction between glucose and the resin was negligible. As a matter of fact, the possibility of hydrogen bond formation with polyethylene glycol is considered to be very small, since it contains only ether-like oxygen.

**DISCUSSION**

Various models have been proposed for establishment of relations between the diffusion coefficient in porous matrix such as ion-exchange resins and solid catalysts and that in the absence of such matrix. In most of the previous approaches, the ratio $D_e/D_0$ was expressed as a function of a void fraction in porous materials $\varepsilon$ and tortuosity factor $\tau$ which accounts for both tortuosity and varying pore cross section, i.e., $D_e/D_0 = \varepsilon/\tau$. In these treatments, however, neither the interaction between solutes and pore wall (gel component) nor the effect of solute size was taken into consideration.

The experimental results obtained in the present study showed that diffusion in gels might be restricted not only by the interaction between solute and gel component but also...
Diffusion in Cross-linked Polymers

Fig. 6. Experimental Relations between $D_e$ and $KD_p$ for Various Saccharides and Amino Acids in Dextran Gels.

The solid line shows $D_e = D_p(K/\Delta)$, in which $\Delta$ is specified as 1.1. Diffusing substances: ○, glucose; △, maltose; □, maltotriose; ●, glycine; ⊙, L-β-alanine; ▲, L-valine; ■, L-arginine.

Fig. 7. Experimental Relations between $D_e$ and $KD_p$ for Various Saccharides in Polyacrylamide Gels.

The experimental data shown in Fig. 5-a were employed. The solid line had a slope larger than unity, and the application of $D_e = D_p(K/\tau)$ to the experimental data failed. See the text for details. Diffusing substances: ○, glucose; △, maltose; □, maltotriose.

by the steric hindrance of gel matrix. Thus, $D_e$ should be expressed as $D_e = D_p(K/\tau)$. $K$ was employed in place of $\varepsilon$ to account for the effect of the size of diffusing substances. In Fig. 6, $D_e$ measured in dextran gels are plotted against $KD_p$ in a log-log coordinate. As seen in the figure, the plot of $D_e$ versus $KD_p$ gave a straight line with a slope of unity and $\tau$ was evaluated as 1.1.

Figure 7 shows a plot of $D_e$ versus $KD_p$ for polyacrylamide gels. The experimental data gave a nearly straight line, but the slope was higher than unity. This means that diffusion coefficient in polyacrylamide gels cannot be correlated with $D_e = D_p(K/\tau)$, probably owing to the heterogeneous structure of polyacrylamide gels. According to recent studies with a scanning electron microscope, polyacrylamide gels have a cellular structure, that is, the gel is composed of a closed space (a small cell) with a diameter of a few microns and a cell wall, and is extremely different from a homogeneous structure presumed for dextran gels. In such a heterogeneous structure of the polyacrylamide gel, the structure of the cell wall may be strongly responsible for the overall permeability. The distribution coefficient in the wall may be much smaller than the observed value. Similarly, an unusual dependency of $D_e$ on BIS content shown in Fig. 5-b might be explained by the change of the cell wall structure with BIS content.

On the other hand, in the photo-crosslinkable resin, $D_e/D_o$ was nearly equal to $K$ as described previously.

The findings obtained above presented the following information on the permeability of cross-linked polymers: The diffusional velocity in the gel was restricted not only by the interaction between diffusing substances and gel components but also by the steric hindrance of gel matrix. The photo-crosslinkable resin showed the highest permeability, where the interaction between the diffusing substance and the resin component was considered negligible. The diffusion coefficients in dextran gels were well correlated with $D_e = D_p(K/\tau)$, in which both the interaction and the obstruction by the gel matrix were considered. However, with polyacrylamide gels, an attempt to correlate the experimental data with $D_e = D_p(K/\tau)$
failed. It was pointed out as the reason for this failure that the structure of the polyacrylamide gels was heterogeneous and different from that of dextran gels. Further studies on the structure of pore matrix must lead to a better understanding on the permeability in gels.

APPENDIX

Since the polyacrylamide gel has two atoms per one monomer molecule which can form hydrogen bond, a model proposed in the previous paper9) might be applied to the evaluation of $D_p/D_o$. Thus, $D_p/D_o$ is expressed as

$$D_p/D_o = \frac{1}{2} \left[ 1 + \frac{K_H - nC_{dt} + nC_{At}}{\sqrt{B^2 + 4nC_{At}K_H}} \right]$$

(A-1)

where

$$B = K_H + n(C_{dt} - C_{At})$$

(A-2)

In these equations, $C_{dt}$ represents the polymer concentration based on monomer unit, $C_{At}$ the concentration of a diffusing substance, $K_H$ the dissociation constant of hydrogen bonding ($=29 \text{ m}^3$)9) and $n$ the number of atoms which can form hydrogen bond in the diffusing substance.

REFERENCES