Effect of Pore Size Distribution on Enzyme Immobilization in Porous Supports

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Clark et al. (1985) recently formulated a kinetic model for coupling restricted diffusion and immobilized reaction of enzyme molecules in a cylindrical pore using the concept of "pore central core restricted diffusion." This model takes into consideration the increase of diffusion resistance by the reduction of available cross-sectional area during the enzyme immobilization process. It successfully predicts the results of nonuniform enzyme distribution in porous supports as observed in some experiments.

Clark's model assumes that supports have uniform pore size. This is in contrast to the fact that pore size distribution exists in almost all porous solids. Treating pores of various diameters as uniform in size (e.g., average pore diameter) tends to underestimate the plugging effect of small pores by enzyme molecules during the immobilization process, especially for porous solids with broad or bimodal pore size distributions. This might result in significant deviations of the predicted values of the amount of the enzyme loaded.

The objective of this study is to improve Clark's model by incorporating a pore size distribution into the pore central core restricted diffusion model. By using a refined equation for the void cross-sectional area of pore, we recalculate the amount of enzyme immobilized vs. time on stream. In addition, a real pore size distribution of silica supports is measured to investigate the deviation of the loaded amount of enzyme predicted by Clark's model.

Mathematical Model

Clark et al. (1985) proposed a quasisteady-state model to simulate the enzyme immobilization process within a cylindrical pore. By using the dimensionless concentrations, the total amount of immobilized enzyme within a cylindrical pore can be determined by evaluating the following integral:

$$E_s^*(t) = \int_0^1 E_s(x, t)\, dx \tag{1}$$

Here we consider a spherical particle with a pore volume density function, $P_v(r_p)$. In order to incorporate the effect of pore size distribution into Clark's model, some modifications have been made. The first one is to consider the hydrodynamic drag effects. According to Clark's model, the restricted diffusion coefficient is related to the bulk diffusion coefficient of enzyme by a hydrodynamic drag factor, $K_r(\lambda)$, i.e.,

$$D(z) = D_\infty K_r[\lambda(z)] \tag{2}$$

$$K_r[\lambda(z)] = 1 - 2.1044\lambda(z) + 2.089\lambda^3(z) - 0.948\lambda^5(z) \tag{3}$$

where λ is the ratio of enzyme to effective pore diameter, which is a function of the axial coordinate, z. Equation 3, proposed by Pappenheimer et al. (1951), is suitable only for $\lambda < 0.4$. Because a batch of commercial support preparation might contain a significant proportion of smaller pores, λ larger than 0.4 should be considered for the realistic application of Clark's model. Hence, Eq. 3 no longer applies, and a table of $K_r(\lambda)$ with a wider range of λ(0–0.9), developed by Paine and Scherr (1975), is used to calculate $K_r(\lambda)$ in the present work.

On the other hand, the void cross-sectional area at position z, with local immobilized enzyme concentration, $e_s(z)$, is given by

$$A(z) = A_0\left[1 - 4\lambda_0(1 - \lambda_0)\frac{e_s(z)}{e_{sm}}\right] \tag{4}$$

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where e_{sm} is the immobilized enzyme concentration at monolayer coverage, and λ_0 is the ratio of enzyme to initial pore diameter. Since the enzyme molecule is considered a rigid sphere, dead space between immobilized enzyme molecules and pore wall (shaded area in Figure 1) is not accessible for the passage of incoming enzyme molecules. When $\lambda_0 > 1/3$, the excluded volume arisen from the immobilized enzymes would be larger than that represented by the second term of Eq. 4. Hence a correction factor, α, is incorporated into Eq. 6 to compute the "real" void cross-sectional area, i.e.,

$$A(z) = A_0[1 - \alpha \cdot 4 \cdot \lambda_0(1 - \lambda_0)E_s(z)] \tag{5}$$

The values of α are determined from the geometric area and the actual maximum enzyme loading. For instance, when $\lambda_0 = 1/2$, the available cross-sectional area for further enzyme molecule diffusion will be zero when E_s equals the maximum loading, i.e., 0.5; hence α, calculated from Eq. 5, equals 2. Similarly, for $\lambda_0 = 1/3$, α equals 1.125. Thus, from several values of α corresponding to different values of λ_0, the following correlation may be obtained by the least square method:

$$\alpha = 2.4839 - 11.1953\lambda_0 + 23.9317\lambda_0^2 - 7.2398\lambda_0^3, \quad 1/3 \le \lambda_0 \le 1/2 \tag{6}$$

$$\alpha = 1, \quad 0 < \lambda_0 < 1/3 \tag{7}$$

Two key parameters, ϕ and ψ, defined in Clark's model, are modified as

$$\phi = \sqrt{\tau}\left(\frac{R_p}{3}\right)\sqrt{\frac{2k_{imm}}{D_\infty r_p}} \tag{8}$$

$$\psi = \frac{e_{bo}r_p}{2e_{sm}} \tag{9}$$

Here the tortuosity factor, τ, is included for the completeness of mathematical model. Since ϕ and ψ change with pore size, r_p, the following dimensionless terms, which are independent of the pore size, will be used, i.e.,

$$\phi' = \frac{\sqrt{R_p}}{3}\sqrt{\frac{2k_{imm}}{D_\infty}} \tag{10}$$

$$\psi' = \frac{e_{sm}}{e_{bo}R_p} \tag{11}$$

Rajagopalan and Luss (1979) proved that two particles, one having uniform pores and the other with a nonuniform pore size distribution, would have the same surface area and pore volume if the radius of the uniform pores is equal to the harmonic pore radius of the pore size distribution. The harmonic pore radius of a pore size distribution is defined as:

$$\frac{1}{r_{ph}} = \int_0^{r_{pm}} \frac{P_v(r_p)\,dr_p}{r_p} \tag{12}$$

In order to compare the total amount of enzyme loaded within a spherical particle predicted by Clark's and our models, r_{ph} is used as the uniform pore size in the calculation of Clark's model. Thus the comparison is based on the same surface area and pore volume.

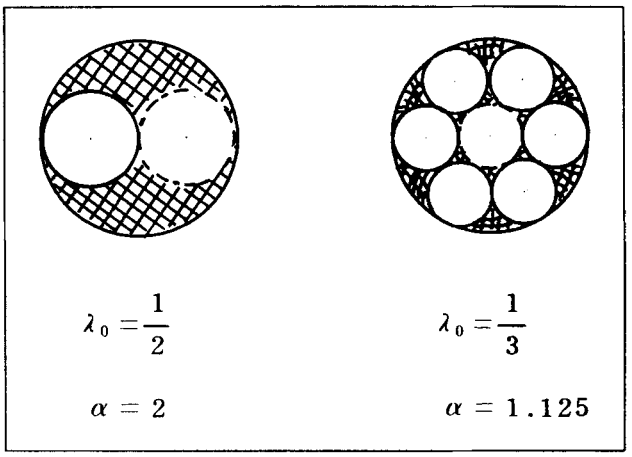

Figure 1. Effective pore cross-sectional area.

When Clark's model is used, it can be easily shown that the total amount of enzyme loaded per spherical particle, E_s^*, is the same as that expressed by Eq. 1. On the other hand, the total amount of the immobilized enzyme predicted by the present model can be obtained by evaluating the following integral (see Appendix for derivation):

$$E_s^*(t) = r'_{ph}\int_0^1 \frac{P'_v(r'_p)}{r'_p}\int_0^1 E_s(x,t)\,dx\,dr'_p \tag{13}$$

where r'_p and r'_{ph} are the dimensionless pore radius, and $P'_v(r'_p)$ is the dimensionless pore volume distribution. In calculating Eq. 13, ten or fifteen points of the Gaussian-Legendre quadrature formula were used to preserve the accuracy of integration. For

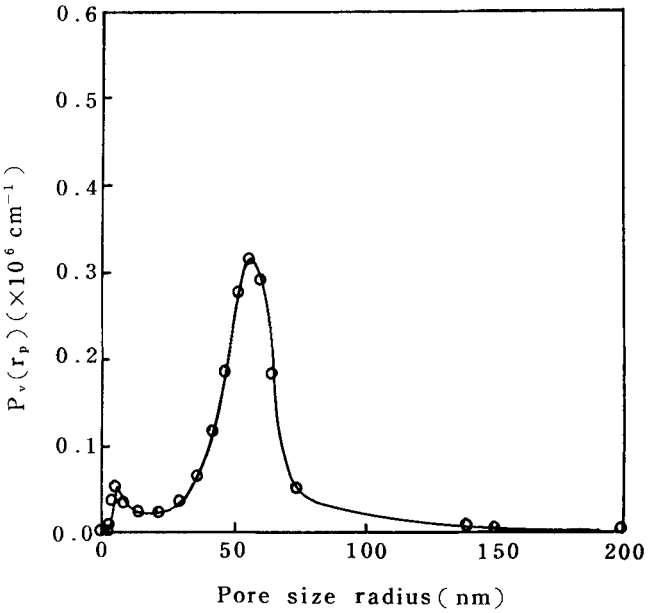

Figure 2. Pore volume distribution of silica sample, XOBO 15.

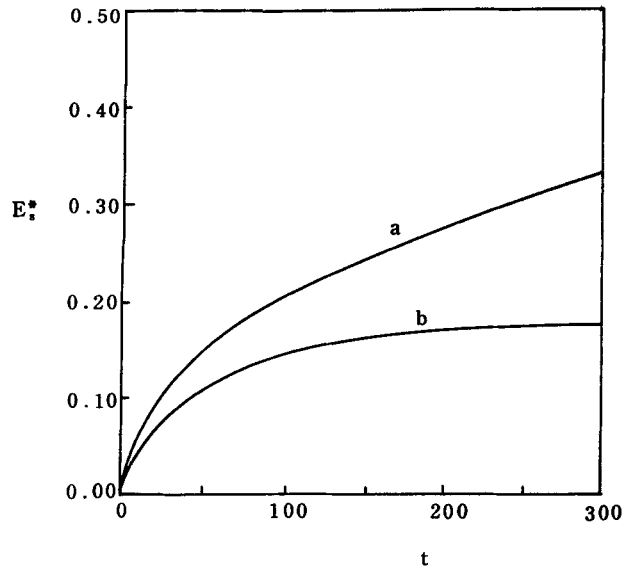

Figure 3. E_s^* predicted by (a) the present model and (b) Clark's model.

$a = 1 \times 10^{-6}$ cm, $D_e = 8.27 \times 10^{-7}$ cm^2/s, $k_{imm} = 3.4 \times 10^{-2}$ cm^3/m^2 − s, $R_p = 2.25 \times 10^{-2}$ cm, $e_{bo} = 1 \times 10^{-4}$ g/cm^3, $e_{sm} = 5 \times 10^{-8}$ g/cm^2, $\tau = 2$, $\epsilon_0 = 0.69$, $\phi' = 0.133$, $\psi' = 0.0222$

each pore size, r_p, the calculation of E_s will be the same as the original Clark's model.

Results and Discussion

A silica sample, XOBO15, manufactured by IBF Co., France, is adopted to investigate the effect of pore size distribution on the restricted diffusion of enzyme immobilization. Figure 2 shows that the pore volume distribution of this silica sample is a standard type of bimodal distribution, as that for most other catalysts. In addition, typical parameters for glucose-oxidase immobilization used in the calculations are described in the legend of Figure 3. According to Eq. 21, the harmonic pore radius is about five times the radius of the enzyme molecule, a ratio close to the values of industrial practice in enzyme immobilization.

Figure 3 illustrates a comparison of E_s^* predicted by Clark's model and the present model. It is worth pointing out that this comparison is made under the condition that the surface area and porosity are the same in both cases. The results from Figure 3 indicate that the effect of pore size distribution on E_s^* is quite significant. In this case, some larger pores within the particle have smaller plugging effect than that predicted by Clark's model; thus Clark's model underestimates E_s^*, and its deviation cannot be neglected. Although only one case is examined in this study, it can be easily seen from the mathematical model that the deviations between these two models will depend on the values of ϕ', ψ', and types of pore size distribution. Therefore, Clark's model may also overestimate E_s^*, depending on the operating conditions.

Appendix

Proof of Eq. 13. We denote by N, the total number of pores within a spherical particle, and by $P_n(r_p)dr_p$, the number fraction of pores with pore radius between r_p and $r_p + dr_p$. The pore structure within the spherical particle is considered homogeneous, thus each pore has a pore length of $(\sqrt{\tau}/3)R_p$. In order for a clear description, the following derivation is based on the dimensional quantities.

For each cylindrical pore with pore radius r_p, the total amount of enzyme loaded may be obtained from Eq. 1, i.e.,

$$e_{sp}^*(t') = 2\pi r_p \int_0^{\sqrt{\tau}R_p/3} e_s(z, t')\, dz \tag{14}$$

where e_s is the immobilized enzyme concentration based on the surface area, and t' is a dimensional time. For a spherical particle with a pore size distribution, the total amount of immobilized enzyme can be written as

$$e_s^*(t') = \int_0^{r_{pm}} NP_n(r_p) e_{sp}^*(t')\, dr_p \tag{15}$$

$$= \int_0^{r_{pm}} 2\pi N P_n(r_p) r_p \int_0^{(\sqrt{\tau}/3)R_p} e_s(z, t')\, dz\, dr_p \tag{16}$$

Since only the pore volume density function, $P_v(r_p)$, can be measured experimentally either by mercury penetration or nitrogen desorption methods, $P_n(r_p)$ in Eq. 16 is replaced by $P_v(r_p)$. For a spherical catalyst,

$$N\pi r_p^2 \frac{\sqrt{\tau}}{3} R_p P_n(r_p) dr_p = \frac{4}{3}\pi R_p^3 \epsilon_0 P_v(r_p) dr_p \tag{17}$$

so that Eq. 16 may be rewritten as

$$e_s^*(t') = \frac{8\pi R_p^2 \epsilon_0}{\sqrt{\tau}} \int_0^{r_{pm}} \frac{P_v(r_p)}{r} \int_0^{(\sqrt{\tau}/3)R_p} e_s(z, t')\, dz\, dr_p \tag{18}$$

The maximum amount of immobilized enzyme within a spherical particle is given as

$$e_{sm}^* = 4/3\pi R_p^3 \rho_b S_g e_{sm} \tag{19}$$

In addition, for a spherical particle, it can be easily proven that (Rajagopalan and Luss, 1979)

$$\frac{\rho_b S_g}{\epsilon_0} = \frac{2}{r_{ph}} \tag{20}$$

where r_{ph} is defined by Eq. 12. Dividing Eq. 18 by Eq. 19 and substituting $\rho_b S_g$ from Eq. 20 yields

$$\frac{e_s^*(t')}{e_{sm}^*} = \frac{r_{ph}}{\frac{\sqrt{\tau}}{3} R_p} \int_0^{r_{pm}} \frac{P_v(r_p)}{r_p} \int_0^{(\sqrt{\tau}/3)R_p} \frac{e_s(z, t')}{e_{sm}} dz\, dr_p \tag{21}$$

We denote by r_p', the dimensionless pore radius (r_p/r_{pm}) and by $P_v'(r_p')$, the dimensionless pore volume density function. Using the principle of statistics, we find that

$$\int_0^{r_{pm}} P_v(r_p)\, dr_p = \int_0^1 P_v'(r_p')\, dr_p' \tag{22}$$

Therefore,

$$P_v'(r_p') = r_{pm} P_v(r_p) \tag{23}$$

Finally, substitution of Eq. 22 into Eq. 21, and using the definitions of dimensionless terms, Eq. 13 is obtained.

Notation

a = enzyme radius
A = defined by Eq. 4
A_0 = initial cross-sectional pore area
D = restricted diffusion coefficient
D_∞ = bulk diffusivity of enzyme in liquid solution
e_{bo} = immobilizing solution enzyme conc. at particle's exterior surface
e_s = local immobilized enzyme conc.
e_{sm} = immobilized enzyme conc. at monolayer coverage
e_s^* = total immobilized enzyme in particle
e_{sp}^* = total immobilized enzyme in cylindrical pore
E_s = local immobilized enzyme dimensionless conc., e_s/e_{sm}
E_s^* = total immobilized enzyme in particle, dimensionless
k_{imm} = rate constant for enzyme immobilization
K_r = hydrodynamic drag factor
N = total number of pores in particle
$P_n(r_p)$ = pore number density function
$P_v(r_p)$ = pore volume density function
$P_v'(r_p')$ = dimensionless pore volume density function
r_p = pore radius
r_{pm} = maximum pore radius
r_{ph} = harmonic pore radius defined in Eq. 12
r_p' = dimensionless pore radius, r_p/r_{pm}
r_{ph}' = dimensionless harmonic pore radius, r_{ph}/r_{pm}
R_p = radius of spherical particle
S_g = surface area of particle per unit mass
t = dimensionless time $(= t'/(\tau \cdot R_p/3)^2/D_\infty)$
t' = dimension time
x = dimensionless axial coordinate $(= z/(\sqrt{\tau}/3)R_p)$
z = axial coordinate

Greek letters

λ = ratio of enzyme to effective pore dia.
λ_0 = ratio of enzyme to initial pore dia.
ϕ = defined in Eq. 8
ϕ' = defined in Eq. 10
ψ = defined in Eq. 9
ψ' = defined in Eq. 11
α = correction factor defined in Eq. 5
τ = tortuosity factor
ϵ_0 = particle porosity
ρ_b = bulk density of particle

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