NON UNIFORM ENZYME DISTRIBUTION INSIDE CARRIERS

K. Buchholz

Dechema-Institut, D-6000 Frankfurt/M.97

Abstract: For catalyst optimization, enzyme immobilization may be controlled in such a way that most of the protein is fixed in the outer shells of a porous particle. Calculation of the profiles of fixed enzymes predicts efficiencies similar to those found experimentally.

INTRODUCTION

Immobilized enzymes in porous carriers are heterogeneous catalysts which may be subject to mass transfer limitations, resulting in low catalyst efficiencies. This is especially true for low substrate concentrations, high molecular weight substrates or hydrolysis reactions leading to a pH-shift. The most important general factor is limitation of the overall reaction rate by pore diffusion of substrates (1). Catalyst optimization should aim at high local activities in the outer shells of porous carriers with short diffusion paths, which may be achieved by pellicular carriers (2,3). We have demonstrated a different catalyst design (4) which takes advantage of diffusion control in the immobilization procedure. Rapid adsorption and covalent coupling leads to fixation of the enzyme in the outer shell of the catalyst particle. Such preparations show higher catalyst efficiencies as compared with equal amounts of enzyme immobilized without diffusion control (4). The experimental procedure (4) involved synthesis of the catalyst by rapid adsorption of enzyme (trypsin) into the outer shells of the porous carrier (porous glass) and fixation of the resulting gradient of adsorbed protein over the carrier radius by rapid covalent binding.

The total immobilized enzyme activity of the catalyst is determined from its activity *Vmax* at high substrate concentrations. The effectiveness of the catalyst is determined from its overall reaction rate, at low substrate concentration (N- α-Benzoyl-L-arginine ethyl ester, BAEE, 10^{-4} mol/1).

Model

A qualitative understanding of the underlying molecular processes can be achieved by a simple model. It takes into consideration the experimental procedure and results, experimental determination of adsorption and reaction constants, and simple basic assumptions for adsorption, diffusion and reaction steps.

The adsorption process is coupled with external transport of enzyme to the outer carrier surface; the mass transfer coefficient k_{se} is calculated from experimental determinations (5) (at high $Re > 10.000$) and the diffusion coefficient for trypsin from (6) (data summarised in table 1). The adsorption process is also coupled with diffusion of the protein inside the carrier, the effective diffusion coefficient being half the value of that in free solution (estimated from effusion experiments). The adsorption equilibrium is established very rapidly; the adsorption may be described approximately by a Langmuir isotherm, the constants being estimated from experiments (table 1; K_a can only be estimated as to the order of magnitude). The profile of adsorbed enzyme is immediately fixed by covalent coupling at a selected time during adsorption. Half of the bound enzyme remains active.

The catalysis (hydrolysis of BAEE) is a coupled process of external transport (k_s), pore diffusion (D_s, equal to the value for free solution, error $\pm$ 30%), and enzyme catalyzed reaction.

From these experimental conditions and model assumptions the following mathematical formulation with differential equations and boundary conditions is derived:

$$\left(\frac{\partial E_L}{\partial t}\right) V_L = k_{se} \cdot F \, (E_L - E_R) = D_e \cdot F \left(\frac{\partial E}{\partial x}\right)_R \quad \text{(mol/s)} \qquad (1)$$

(subscript L stands for solution, subscript R means outer particle surface, x is the radial coordinate, t adsorption time, other symbols see table 1)

Mass balance inside the carrier:

$$D_e \left[\frac{\partial^2 E}{\partial x^2} + \frac{2}{x} \cdot \frac{\partial E}{\partial x} \right] = \left(\frac{\partial E}{\partial t} \right)_x + \left(\frac{\partial (EA)}{\partial t} \right)_x \qquad (2)$$

$$\left(\frac{\partial (EA)}{\partial t} \right)_x = k_1 \cdot E \, (A_o - (EA)) + k_{-1} \cdot (EA) \qquad (3)$$

($k_1/k_{-1} = K_a$; the establishment of adsorption equilibrium according to eq. (3) is rapid compared to diffusion) Reaction boundary conditions:

$$V_{br} = k_s \cdot F \, (S_L - S_R) = D_S \cdot F \left(\frac{\partial S}{\partial x} \right)_R \left(\frac{mol}{s} \right) \qquad (4)$$

Mass balance:

$$D_s \left[\frac{\partial^2 S}{\partial x^2} + \frac{2}{x} \cdot \frac{\partial S}{\partial x} \right] = (V)_x + \left(\frac{\partial S}{\partial t} \right)_x \text{(mol /1 sec)} \qquad (5)$$

$$(V)_x = \frac{k_c \cdot (EA) \cdot S}{K_m + S} \qquad (6)$$

Approximate solution

Solution of the differential equations requires major computational capacities, but a simpler approximation proceeds as follows. Both problems (eq. 2 and 5) are solved for non-stationary conditions. The solution for eq (5) approaches stationary values within short reaction time.

The solution is based on the transformation of the differential equations into difference equations. The carrier is subdivided into segments of shells with volume $F_i \cdot \Delta x$. For each segment a mass balance is introduced for ingoing and outgoing transport of enzyme and substrate, for accumulation in the solution volume, and for consumption in the cell by adsorption or reaction. The processes are formulated for a time Δt, selected so that the result becomes almost insensitive to its variation.

From these considerations, the following equations are derived:

Adsorption, boundary condition:

$$\frac{\Delta E_L}{\Delta t} \cdot V_L = k_{se} \cdot F(E_L - E_R) = \frac{\Delta E_{LR}}{\Delta t} \cdot F \cdot \delta + D_e \cdot F \cdot \frac{E_R - E_1}{\Delta x} \quad \text{(mol/sec)} \tag{7}$$

$\Delta E_{LR} = (E_L + E_R)/2$ (The corresponding term in eq.(7) may be neglected).

Mass balance inside the cell of the carrier (cf .7):

$$D_e \cdot 4\Pi x^2 \left(\frac{E_n - E_{n+1}}{\Delta x}\right) - D_e \cdot 4\Pi (x-\Delta x)^2 \left(\frac{E_{n+1} - E_{n+2}}{\Delta x}\right)$$

$$= \frac{\Delta E_{n+1}}{\Delta t} \cdot 4\Pi x^2 \cdot \Delta x + \frac{\Delta (EA)_{n+1}}{\Delta t} \cdot 4\Pi x^2 \cdot \Delta x \text{ mol/sec} \tag{8}$$

$x = (R - n \cdot \Delta x)$; $\Delta E_{n+1} = (E_{n+1})_t - (E_{n+1})_{t-\Delta t}$

when $t > 0$

$$\Delta (EA) = (EA)_t - (EA)_{t-1} \tag{9}$$

$$(EA) = \frac{E \cdot (A_o)}{1/K_a + E} \tag{10}$$

(from eq. (3) assuming very rapid establishment of equilibrium.)
Eq. (8) is solved for E_{n+1}. $(EA)_{n+1}$ is substituted from eq.(10). E_n is taken from the previous solution of eq. (8) for E_n. E_{n+2} is taken from a previous run of calculations at $t - \Delta t$.

Results

Fig.1 shows profiles of adsorbed enzyme as a function of the distance x from the external catalyst surface. The parameters used in the calculation(estimated as above) are summarized in table 1. Clearly the enzyme penetrates the carrier (d_p 100/um) progressively, but on a time scale accessible to experimental control. Even at short adsorption times the concentration of adsorbed enzyme near the external carrier surface (x = o) decreases, due to the decreasing concentration of enzyme in free solution. The profiles are steeper for high equilibrium adsorption constants ($K_a = 5 \cdot 10^6$ mol/1) than for

lower values ($K_a = 5.10^5$ *mol/l, dotted line, fig.]*). Experimentally the adsorption profile is fixed by rapid covalent coupling.

Table 1

Parameters used in calculations, related to experimental conditions with 300 mg porous glass, 75 m^2/g internal surface, 2 ml/g carrier volume,

		Adsorption	Reaction	
De	cm^2/s	5.10^{-7}		Diffusion coefficient
D_s	"		5.10^{-6}	Diffusion coefficient
E_{LO}	mol/ℓ	$8,5.10^{-7}$		Enzyme concentration in free solution at t=O
(EA)	mol/ℓ			Adsorbed enzyme
A_o		2.10^{-3} 1)		Maximum adsorption capacity
F	cm^2	345		Outer particle surface
K_a	ℓ/mol	$5.10^6/5.10^5$	(5.10^5)	Association constant
K_m	mol/ℓ		2.10^{-5}	Michaelis constant
k_c	ℓ/s		12	Kinetic constant
k_{se}	cm/s	2.10^{-3}		External transport coefficient
k	"		0,02	"
d_p	cm	0,01	0,01	Particle diameter
t	s			Time
Δt	s	0,2	0,2	Time interval for calculation
Δx	cm	10^{-4}	10^{-4}	Segment length
S	mol/ℓ		10^{-4}	Substrate concentration

1) based on an adsorption capacity of 0,1 g (Protein) per g (carrier); In several equations, a factor 10^{-3} (l/g) must be introduced for correction of dimensions.

Results for a model calculation are given in fig. 2. Profiles of fixed enzyme (EA) are given for inhomogeneous (i) and homogeneous (h) distribution, with profiles for substrate concentration inside the porous carrier and for the diffusion layer δ at the outer catalyst

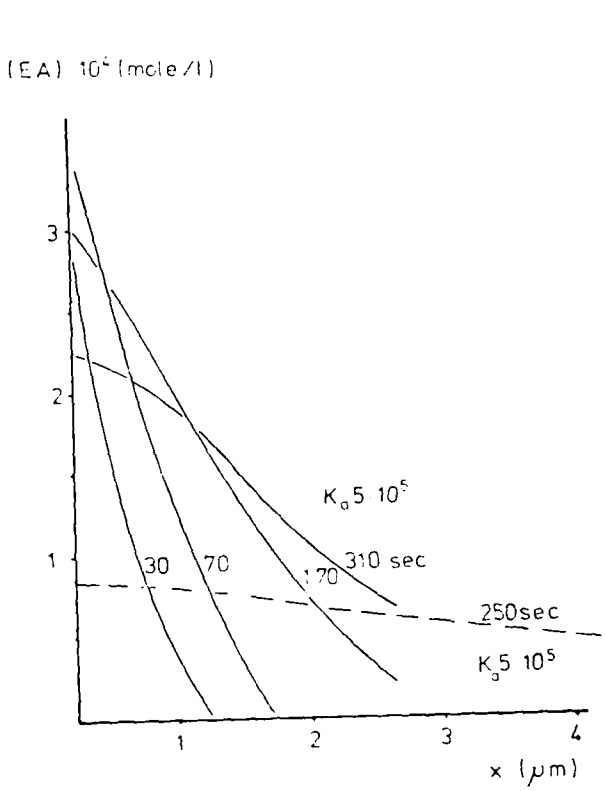

Fig.1 Adsorbed enzyme versus distance from carrier surface, for different adsorption times (30 to 310 sec) and association constants (K_a 5.10^6 or 5.10^5 ℓ/mol).

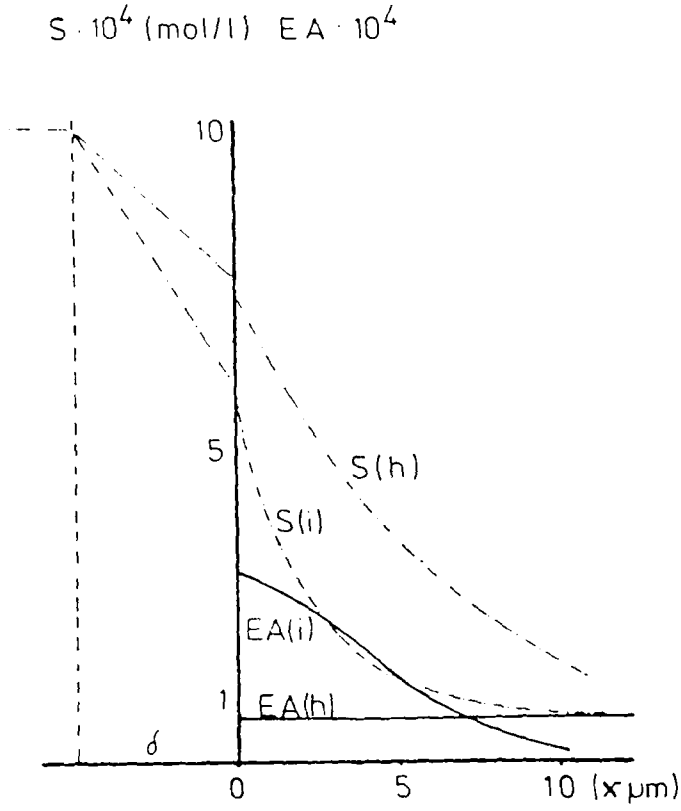

Fig.2 Fixed enzyme density versus distance from carrier surface, and substrate concentrations inside carrier when enzyme distribution is (i) non-uniform, (h) uniform.

surface. In both cases, most of the substrate is converted within a shell of 10 μm of the particle (50% of the volume). However, for the non uniform catalyst (i) the substrate profile is much steeper, and the actual reaction rate is distinctly higher ($2{,}8 \cdot 10^{-7}$ mol/sec) than that for the uniform (h) catalyst ($1{,}7.10^{-7}$ mol/sec). Accordingly the efficiency is higher with the optimized catalyst design, as found experimentally (4).

References

1. Kasche, V., Kapune, A., Schwegler, H., (1979) Enzyme Microb. Technol. 1, 41-46
2. Horvath, C. (1974) Biochim Biophys. A. 358, 164-177
3. Royer, G.P. (1974) Chem Tech 4 No.11, 694-700
4. Borchert, A., Buchholz, K., (1979), Biotechnol. Letters 1, 15-20
5. Buchholz, K., (1978), 1st European Congress on Biotechnology, preprints part 1, p.39.42
6. Gosting, L. (1956), Adv. Protein Chem. Vol. 11, p.516
7. Smith, J. M., Chemical Engineering Kinetics, p.429 McGraw-Hill, New York, (1970)

Support by the Ministry for Research and Technology of the Federal Republic is gratefully acknowledged.