MASS TRANSFER IN HETEROGENEOUS SYSTEM ENZYME–SUBSTRATE; KINETIC MODEL OF ENZYMATIC REACTION IN SYSTEM OF TWO IMMISCIBLE LIQUIDS

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Mathematical model is proposed enabling calculation of enzymatic reaction rates occurring in one phase of a system of two immiscible liquids under conditions of substrate and product transfer over the interphase boundary.

Frequently, it would be advantageous to use for preparation of new compounds an enzymatic reaction, but the substrate has in the aqueous phase, in which the enzymatic reaction takes place, a very low solubility and the designed enzymatic reactors would be too large. It is however possible to use a system of two immiscible liquids (*e.g.* an organic solvent and aqueous buffer) when the substrate is well soluble in the organic phase. The reaction mechanism then includes the following steps: transfer of substrate from the organic into aqueous phase, reaction catalysed by enzyme or mixture of enzymes in the aqueous phase and reverse transfer into the organic phase. The resulting rate of formation of products will depend also on diffusivities of immiscible liquid phases.

There exist a number of enzymatic reactions, which can be realised in the system of two immiscible liquids. With regard to the usually unambiguously defined enzymatic kinetics, formally described by the Michaelis–Menten kinetics, such system can be used also as a model for determination of interfacial liquid–liquid area in reactor or extracor models for the system of two immiscible liquids or in reactors with suspensed solid phase (slurry reactors).

An example of a reaction in the two-phase system is the enzymatic degradation of cholesterole[1] or a more complex system of a selective enzymatic reduction of 20-ketosteroids to 20-hydroxysteroids in the system aqueous buffer–butyl acetate[2], taking place simultaneously with the enzymatic dehydrogenation of ethanol catalysed by a coenzyme, where the initial reaction component and product of reduction reaction are practically insoluble in the aqueous phase, but are well soluble in the organic solvent.

In this study is indicated a method of construction and solution of the kinetic model of an enzymatic reaction taking place in one phase (enzymatic) and affected by the interphase mass transfer (of substrate) from organic into aqueous reaction phase and by reverse product transfer across the interface. At modelling of this situation it is possible to apply the general knowledge from the theory of mass transfer with chemical reaction in two immiscible liquids, see[3].

System of a Simple Enzymatic Reaction

Let us consider following steps:

1) Equilibrium of component A in organic ($\mathscr{A}$) and aqueous (A) phases

$$\mathscr{A} \rightleftharpoons \mathrm{A}\,. \tag{1}$$

2) Fast enzymatic reaction in aqueous phase, which can be expressed by the simple Michaelis–Menten kinetics

$$\mathrm{A} \rightarrow \mathrm{P}\,. \tag{2}$$

3) Extraction of products P from aqueous into organic phase

$$\mathrm{P} \rightleftharpoons \mathscr{P}\,. \tag{3}$$

It is possible to apply for individual steps these relations

$$[\mathrm{A}] = m_{\mathrm{A}}[\mathscr{A}]\,, \tag{1a}$$

$$r_{\mathrm{A}} = \mu_{\max}[\mathrm{A}]/(K_{\mathrm{s}} + [\mathrm{A}])\,, \tag{2a}$$

$$[\mathrm{P}] = m_{\mathrm{p}}[\mathscr{P}]\,. \tag{3a}$$

Let us assume validity of the two-film theory of mass transfer and realisation of enzymatic reaction in the film of liquid phase. Material balance of component A for kinetics (*2a*) can be written in the form of a differential equation

$$D_{\mathrm{A}}(\mathrm{d}^2[\mathrm{A}]/\mathrm{d}x^2) = \mu_{\max}[\mathrm{A}]/(K_{\mathrm{s}} + [\mathrm{A}]) \tag{4}$$

with boundary conditions

$$x = 0\,, \quad -D_{\mathrm{A}}(\mathrm{d}[\mathrm{A}]/\mathrm{d}x) = k_{\mathrm{L0}}([\mathscr{A}] - [\mathscr{A}]_{\mathrm{i}})\,, \quad [\mathrm{A}] = [\mathrm{A}]_{\mathrm{i}} \tag{5}$$

$$x = \delta\,, \quad \mathrm{d}[\mathrm{A}]/\mathrm{d}x = 0\,.$$

Under these boundary conditions (5) the differential equation (4) has an exact analytical solution for flow intensity of component A between the organic and aqueous phases J_A in the form[4]

$$J_A = \left(2D_A \int_{[A]=[A]_L}^{[A]=[A]_i} r_A \, d[A]\right)^{1/2} \tag{6}$$

which after integration becomes

$$J_A = \left\{2D_A\mu_{max}\left[([A]_i - [A]_L) - K_s \ln \frac{(K_s + [A]_i)}{(K_s + [A]_L)}\right]\right\}^{1/2} . \tag{7}$$

For fast enzymatic reactions in the excess of enzyme and low solubility of substrate component in the aqueous phase it is possible to assume the concentration $[A]_L \approx 0$ and relation (7) can be arranged by expressing $[A]_i$ from Eq. (1*a*) into the form

$$J_A = \{2D_A\mu_{max}[m_A[\mathscr{A}]_i - K_s \ln (K_s + m_A[\mathscr{A}]_i)/K_s]\}^{1/2} . \tag{8}$$

For the considered reactions (1)–(3) where the rate controlling step is the mass transfer rate between both phases, the resulting reaction rate is given by intensity of mass flux of component A across the phase boundary

$$R_A = J_A(S/V_0) . \tag{9}$$

After substitution for J_A from Eq. (8) and expressing the concentration $[\mathscr{A}]_i$ by use of the boundary condition (5), Eq. (8) can be arranged into the final form

$$R_A^2 = (S/V_0)^2 \, 2D_A\mu_{max}\left[m_A[\mathscr{A}] - m_A \frac{R_A}{(S/V_0)\, k_{L0}} - K_s \ln\left(1 + (m_A[\mathscr{A}]/K_s) - \right.\right.$$

$$\left.\left. - (m_A/K_s) \frac{R_A}{D_A(S/V_0)\, k_{L0}}\right]\right.,$$

where the dimension of $R_A = [\text{mol/s m}^3 \text{ org. phase}]$, D_A is diffusivity of component A in aqueous phase, S total interfacial area, V_0 volume of organic phase, k_{L0} mass transfer coefficient of component A in organic phase. For number of values R_A and $[\mathscr{A}]$ it is possible to determine, by the optimisation method $(S/V_0)^2$ and k_{L0}. For the case $[A]_i \ll K_s$, Eq. (7) can be simplified to the solution of a quadratic equation. The values K_s and μ_{max} can be determined in the one-phase system or they can be taken from literature.

Enzymatic reactions in a two-phase system with reaction in the aqueous phase can be considered generally in aqueous emulsions as analogous to reactions with immobilised enzyme in aqueous droplets with all consequences resulting from the effect of external or internal diffusion.

Enzymatic Reactions with Coenzyme

A general model of enzymatic reaction with coenzyme and a co-factor is solved, the solution can be easily simplified to the case of simple enzymatic reaction (*1*) to (*3*). As an example can be chosen selective enzymatic reduction of 20-ketosteroids (St-O) to Hydroxysteroids (St-OH), catalysed enzymatically by 20β-hydroxysteroid dehydrogenase (2Oβ-HSDH). With regard to low solubility of the substrate in aqueous phase it is recommended[2] to perform this reaction simultaneously with dehydrogenation of ethanol catalysed by coenzyme alkohol dehydrogenase (ADH) at which simultaneously a reduction of co-factor (NAD^+) to NADH takes place.

$$\text{St-O} + \text{NADH} + \text{H}^+ \overset{\text{20β-HSDH}}{\rightleftharpoons} \text{St-OH} + \text{NAD}^+ \qquad (10)$$

$$\text{C}_2\text{H}_5\text{OH} + \text{NAD}^+ \overset{\text{ADH}}{\rightleftharpoons} \text{CH}_3\text{CHO} + \text{NADH} + \text{H}^+ \qquad (11)$$

In the following derivation $[\mathscr{A}]$ or [A] and $[\mathscr{D}]$ or [D] denote the concentrations of substrate (St-O) and product (St-OH) in organic or aqueous phases, for other reaction components participating in reactions (*10*) and (*11*) following symbols were used, B = NADH, C = H^+, E = NAD^+, F = ethanol, G = acetaldehyde, all in aqueous phase. Similarly as in the case of simple enzymatic reaction (*1*) to (*3*) it is possible to assume that the reaction mechanism consists of the following steps: *1*) Transfer of substrate from organic into aqueous phase

$$\mathscr{A} \rightleftharpoons \text{A}. \qquad (12)$$

2) Reactions (*10*) and (*11*) in the aqueous phase

$$\text{A} + \text{B} + \text{C} \rightleftharpoons \text{D} + \text{E}. \qquad (10')$$

$$\text{F} + \text{E} \rightleftharpoons \text{G} + \text{B} + \text{C}. \qquad (11')$$

3) Extraction of low soluble product D from aqueous into organic phase

$$\text{D} \rightleftharpoons \mathscr{D}, \qquad (13)$$

where for individual steps, equilibrium relations hold

$$[\mathrm{A}] = m_{\mathrm{A}}[\mathscr{A}] \tag{14}$$

$$K_{\mathrm{A}} = [\mathrm{D}] \,.\, [\mathrm{E}]/[\mathrm{A}] \,.\, [\mathrm{B}] \,.\, [\mathrm{C}] \tag{15}$$

$$K_{\mathrm{B}} = [\mathrm{G}] \,.\, [\mathrm{B}] \,.\, [\mathrm{C}]/[\mathrm{F}] \,.\, [\mathrm{E}] \tag{16}$$

$$[\mathrm{D}] = m_{\mathrm{D}}[\mathscr{D}] \,. \tag{17}$$

All reaction steps can be then written in the summarized form

$$\mathscr{A} + \mathrm{F} \rightleftharpoons \mathscr{D} + \mathrm{G} \tag{18}$$

with the equilibrium constant

$$K_{\mathrm{eq}} = m_{\mathrm{A}} \,.\, K_{\mathrm{A}} \,.\, K_{\mathrm{B}}/m_{\mathrm{D}} \tag{19}$$

In the following derivation are then used the next assumptions: *a*) Validity of the two-film theory of mass transfer is assumed, corresponding concentration profiles of components are demonstrated in Fig. 1. *b*) It is assumed that concentrations of components B, C and E are constant in the film and are equal to their values in the bulk of aqueous phase, $[\mathrm{B}] = [\mathrm{B}]_{\mathrm{L}}$, $[\mathrm{C}] = [\mathrm{C}]_{\mathrm{L}}$, $[\mathrm{E}] = [\mathrm{E}]_{\mathrm{L}}$. With regard to buffering properties of the aqueous buffer to usually only catalytic concentrations of components B and E are the relative changes of concentration of these three components negligible and the assumption made is thus justified. *c*) Accumulation of components in the film and in the bulk of aqueous phase does not take place.

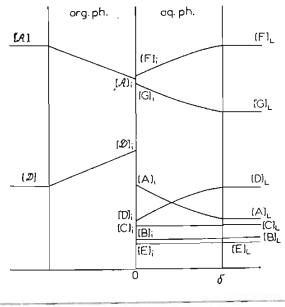

FIG. 1
Concentration profiles of components in close vicinity of interface

Thus there holds

$$J_A = J_D \tag{20}$$

or

$$-D_A \, d[A]/dx = D_D \, d[D]/dx \tag{20'}$$

for $x = 0$, $[A] = [A]_i$, $[D] = [D]_i$;
for $x = \delta$, $[A] = [A]_L$, $[D] = [D]_L$.

From solution of Eq. (*20'*) then results

$$[D] = (D_A/D_D)([A]_i - [A]) + [D]_i \tag{21a}$$

or

$$[D]_L = (D_A/D_D)([A]_i - [A]_L) + [D]_i \tag{21b}$$

d) Equilibrium in the bulk of aqueous phase is assumed

$$K_{eq} = [\mathscr{D}] \cdot [G]/[\mathscr{A}] \cdot [F] \tag{22}$$

or after expression of $[\mathscr{A}]$ and $[\mathscr{D}]$

$$K_{eq} = m_A[D]_L \, [G]_L/m_D[A]_L \, [F]_L \, . \tag{22'}$$

Independence of K_{eq} on pH and on concentration of co-factor and its effective component has been verified experimentally[2]. *e*) Kinetic model assumes validity of the Michaelis–Menten kinetics for the substrate.

In the reaction mechanism with enzymatic catalyst it is always necessary to test both alternative reactions (*10*) and (*11*) as concerns determination of the rate controlling step. With regard to the possibility of positive shift of the reaction equilibrium (*11*) (*e.g.* by independent reaction of component G) it is then possible to consider reaction (*11*) as a relatively fast one and consider Eq. (*10*) as the rate controlling step. The reaction rate of this step can be expressed by relation

$$r_A = \frac{(\mu_{max} \cdot [B])[A]}{K_s + [A]} - \frac{(\mu'_{max} \cdot [E]) \cdot [D]}{K'_s + [D]} \, . \tag{23}$$

For the equilibrium of this reaction there holds from definition

$$K_A = \frac{\mu_{max}(K'_s + [D])}{\mu'_{max}(K_s + [A])} \tag{24}$$

with application of this relation it is then possible to arrange Eq. (*23*) into the form

$$r_A = \frac{\mu_{max}}{K_s + [A]} \left([A][B] - [D][E]/K_A \right). \qquad (23')$$

Relation (*24*) can be expressed as a function of component A only by substitution from Eq. (*21a*). In agreement with the assumption *b*) can be then written in the form

$$r_A = \frac{\mu_{max}}{K_s + [A]} \left[[A] \cdot [B]_L - \frac{[E]_L}{K_A} \left(\frac{D_A}{D_D} \left([A]_i - [A] \right) + [D]_i \right) \right]. \qquad (25)$$

Assuming validity of the two-film mass transfer model, characterised by boundary conditions (*5*) it is possible to derive, similarly as in the case of a simple enzymatic reaction, the relation for flow intensity of component A

$$J_A = \left\{ 2 D_A \mu_{max} \left[\left(m_A [\mathscr{A}]_j - [A]_L \right) \left([B]_L + \frac{[E]_L D_A}{K_A D_D} \right) + \ln \frac{(K_s + [A]_L)}{(K_s + [A]_i)} \cdot \right. \right.$$
$$\left. \left. \cdot \left([B]_L K_s + \frac{[E]_L D_A}{K_A D_D} \left(m_A [\mathscr{A}]_i + K_s + m_D [\mathscr{D}]_i \frac{D_D}{D_A} \right) \right) \right] \right\}^{1/2}. \qquad (26)$$

For substrate concentration in the bulk of aqueous phase it results from relations (*15*), (*16*) and (*22'*)

$$[A]_L = \frac{m_D \cdot [D]_L \cdot [G]_L}{m_A \cdot [F]_L \cdot K_{eq}} \qquad (27)$$

or after substitution into Eqs (*21b*) and (*19*) and further arrangement

$$[A]_L = \left(\frac{D_A}{D_D} m_A [\mathscr{A}]_i + m_D [\mathscr{D}]_i \right) \frac{[E]_L}{K_A [B]_L [C]_L} \Big/ \left(1 + \frac{D_A}{D_D} \frac{[E]_L}{K_A [B]_L [C]_L} \right). \qquad (28)$$

Interfacial concentrations $[\mathscr{A}]_i$ and $[\mathscr{D}]_i$ can be expressed from conditions (*5*) and (*20*)

$$k_{L0,A} \left([\mathscr{A}] - [\mathscr{A}_i] \right) = J_A \qquad (29)$$

$$k l_{L0,D} \left([\mathscr{D}]_i - [\mathscr{D}] \right) = J_D = J_A . \qquad (30)$$

The system of Eqs (*26*), (*28*) to (*30*) then represents a general kinetic model by which can be described composition of substrate D in organic solvent with the effect of enzymatic reaction and simultaneous mass transfer.

LIST OF SYMBOLS

A	component A in aqueous phase
$\mathscr{A}$	component A in organic phase
$[A]_i$	concentration of component A on interphase boundary
B	component B
C	component C
D	component D
D_A, D_D	diffusion coefficients of components A and D
$\mathscr{D}$	component D in organic phase
E	component E
F	component F
G	component G
J_A	flow intensity of component A
k_{L0}	mass transfer coefficient in organic phase
K_A, K_B, K_{eq}	equilibrium constants
K_s	Michaelis–Menten constant
m_A, m_D, m_p	distribution coefficients of components
P	product in aqueous phase
$\mathscr{P}$	product in organic phase
r_A	reaction rate in liquid phase
R_A	reaction rate with diffusion effects
S	interfacial area
V_0	volume of organic phase
x	distance from the film
δ	film thickness
μ_{max}	maximum saturation rate
μ'_{max}	rate of reverse reaction, see Eq. (*23*)

Subscripts

L	in bulk of phase
i	equilibrium value on interface
A	for component A
D	for component D

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