Interfacial Reactions

(Part III)

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7.3.1 Gas–liquid and liquid–liquid interfacial reactions

- Gas–liquid and liquid–liquid reactions can take place in the bulk phase as well as at the interface. To illustrate, a reactive component in a gas, when brought into contact with a liquid that contains another reactant, absorbs at the gas–liquid interface and diffuses through the bulk of the liquid simultaneously undergoing reaction.

- If the reaction is fast (e.g., absorption of CO₂ in aqueous solutions of amines and alkalis, absorption of NO₂ in water, and nitration of toluene by mixed acid at high concentrations of sulfuric acid) or instantaneous (e.g., reaction of H₂S with aqueous NaOH solution and absorption of chlorine in aqueous solutions of Na₂CO₃ or NaOH), the diffusing reactant is completely converted near the interface.

- If the solubility of one reactant is very low in the other phase, the reaction takes place at the interface (e.g., nitration of benzene by mixed acid, because the solubility of benzene in the mixed acid is low). In practice, the reaction occurs in a thin film near the interface. The thickness of the film is approximately 10 nm. The concentration profiles of the reactants for an instantaneous interfacial reaction are shown in Fig. 7.3.1, based on the film theory.

![Fig. 7.3.1 Concentration profiles of the reactants for instantaneous reaction at the interface.](image)

- Reactant A from another phase diffuses into the second phase containing the reactant B. The reaction takes place at a reaction plane, which is depicted by the...
vertical line at $\lambda$. The two reactants, $A$ and $B$, react instantaneously on reaching the front.

- The relative magnitudes of rate of reaction and the rate of physical mass transfer are expressed in terms of Hatta number. It is defined as,

$$Ha = \left( \frac{\text{maximum rate of reaction of } A \text{ in the film per unit interfacial area}}{\text{maximum rate of physical mass transfer per unit interfacial area}} \right)^{1/2}$$  

(7.3.1)

- If the reaction is second-order, the Hatta number is defined as,

$$Ha = \left( \frac{D_A k_2 c_B^{\text{bulk}}}{k_L} \right)^{1/2}$$  

(7.3.2)

where $k_2$ is the reaction rate constant, $D_A$ is the diffusivity of reactant $A$, $D_B$ is the diffusivity of the reactant $B$, and $k_L$ is the liquid phase mass transfer coefficient.

- If $Ha \gg 1$ and $Ha \gg \left( \frac{D_B c_B^{\text{bulk}}}{D_A c_A^{\text{int}}} \right)$, the reaction is instantaneous. On the other hand, if $Ha \gg 1$ and $Ha \ll \left( \frac{D_B c_B^{\text{bulk}}}{D_A c_A^{\text{int}}} \right)$, the reaction is considered as fast.

**Example 7.3.1:** Absorption of oxygen from air into the human blood cells is believed to be instantaneous. The following data are available for absorption of oxygen in hemoglobin, which is accompanied by a chemical reaction. $D_{O_2} = 7 \times 10^{-10}$ m$^2$/s, $D_{\text{Hb}} = 7.5 \times 10^{-12}$ m$^2$/s, $k_L = 1 \times 10^{-4}$ m/s, $c_{\text{Hb}} = 20$ mol/m$^3$ and $k_2 = 1.8 \times 10^3$ m$^3$/mol$^{-1}$ s$^{-1}$. The partial pressure of oxygen is 21278.25 Pa and the Henry’s law constant is $9.277 \times 10^{-6}$ mol m$^{-3}$ Pa$^{-1}$. Calculate the Hatta number and verify whether the reaction is instantaneous or not.

**Solution:** The Hatta number is given by,
\[
\text{Ha} = \left( \frac{D_{O_2} c_{\text{bulk}}^{Hb}}{k_L} \right)^{1/2} = \left( \frac{7 \times 10^{-10} \times 1.8 \times 10^3 \times 20}{1 \times 10^{-4}} \right)^{1/2} = 50.2
\]

\[
c_{\text{int}}^{O_2} = 9.277 \times 10^{-6} \times 21278.25 = 0.1974 \text{ mol/m}^3
\]

\[
\left( \frac{D_{Hb} c_{\text{bulk}}^{Hb}}{D_{O_2} c_{\text{int}}^{O_2}} \right) = \frac{7.5 \times 10^{-12} \times 20}{7 \times 10^{-10} \times 0.1974} = 1.09
\]

\[
\text{Ha} \gg \left( \frac{D_{Hb} c_{\text{bulk}}^{Hb}}{D_{O_2} c_{\text{int}}^{O_2}} \right)
\]

Therefore, the reaction is instantaneous.

### 7.3.2 Reactions at biointerfaces

- A very important category of reactions at biological interfaces is the enzymatic lipolysis reactions. Lipases and phospholipases are water soluble enzymes that can catalyze the hydrolysis of the ester bonds of triglycerides and phospholipids, respectively. They play an important role in the lipid metabolism, and have been found in most organisms from the microbial, plant and animal kingdoms.

- The enzymatic lipolytic reaction is an important example of heterogeneous catalysis. The water soluble lipolytic enzymes act at the interfaces of insoluble lipid substrates, where the catalytic reactions are coupled with various interfacial phenomena.

- The mechanism of the enzymatic lipolysis strongly depends on the mode of organization of the lipid substrate, liposomal dispersions, or oil-in-water dispersions.

- Various kinetic models have been proposed to describe the interfacial mechanisms of enzymatic lipolysis. Verger et al. (1973) proposed the coupling between the Michaelis–Menten chemical step, and penetration and interfacial activation of the enzyme.

- In this model, it is assumed that the reaction products are solubilized instantaneously. Using this simple model, many kinetic experiments on the
hydrolysis of synthetic medium chain lipids which generate water soluble lipolytic products have been analyzed.

- However, the natural substrates of lipolytic enzymes are long chain lipids. They generate water insoluble lipolytic products. The accumulation of the insoluble products at the interface can lead to the dilution of the substrate at the interface and inhibition.

- In some cases, this can lead to autoacceleration of the enzyme binding which enhances the hydrolysis process. Therefore, molecular reorganization at the interface and segregation of the insoluble lipolytic products is important in determining the interfacial lipid structure.

- If water soluble acceptors are present, a process of complexation and solubilization of the lipolytic products into the aqueous subphase takes place. When the lipid substrate is organized in micelles, liposomal dispersions or emulsions, the colloidal features of the system play a vital role in the kinetics of hydrolysis. In such dispersed systems, the interfacial area is very large, which can amplify the interfacial phenomena.

- Let us consider a system consisting of short and medium chain lipids which generate soluble products. The first step is the fixation of a water soluble enzyme ($E$) to the lipid–water interface by reversible adsorption–desorption mechanism.

- The adsorption step involves penetration leading to a more favorable energetic state of the enzyme ($E^*$). This step is followed by a two dimensional Michaelis–Menten catalytic step. The enzyme in the interface ($E^*$) binds a substrate molecule ($S$) to form the complex $E^*S$, followed by its deposition. The reaction product, $P^*$, is assumed to be soluble in the aqueous phase and diffuses away instantly.

- These steps are schematically shown in Fig. 7.3.2. The concentrations of $E$ and $P$ are expressed as their respective bulk concentrations, $c_E$ and $c_P$, respectively, and the concentrations of $E^*$, $S$, $E^*S$ and $P^*$ are expressed as surface concentrations, $\Gamma_{E^*}^*$, $\Gamma_S^*$, $\Gamma_{E^*S}^*$ and $\Gamma_{P^*}^*$, respectively.
The following differential equations mathematically describe this kinetic model.

\[
\frac{d\Gamma^*_{ES}}{dt} = \tilde{k}_2 \Gamma^*_{ES} - \left( \tilde{k}_2 + \tilde{k}_{-1} \right) \Gamma^*_{ES} \tag{7.3.3}
\]

\[
\frac{d\Gamma^*_{E}}{dt} = \tilde{k}_p c_E + \left( \tilde{k}_2 + \tilde{k}_{-1} \right) \Gamma^*_{ES} - \left( \tilde{k}_d + \tilde{k}_4 \Gamma_S \right) \Gamma^*_{E} \tag{7.3.4}
\]

\[
\frac{d\Gamma^*_{P}}{dt} = \tilde{k}_2 \Gamma^*_{ES} \tag{7.3.5}
\]

The partitioning of the enzyme between the aqueous phase and lipid interface is given by,

\[
c_{E0} V = c_E V + \Gamma^*_{E} A + \Gamma^*_{ES} A \tag{7.3.6}
\]

where \( V \) is the volume of the bulk aqueous phase, \( c_{E0} \) is the initial bulk concentration of the enzyme and \( A \) is the area of the interface.

\* At \( t = 0 \), \( \Gamma^*_{P} = 0 \). The substrate concentration at the interface, \( \Gamma_S \), may be assumed to remain constant during hydrolysis. The solution of Eqs. (7.3.3)–(7.3.6) has been given by Verger et al. (1973). The equation for the product surface concentration, \( \Gamma^*_{P} \), is given by,

\[
\Gamma^*_{P} = \frac{\tilde{k}_2 \Gamma_S C_{E0}}{\tilde{K}_m \left( \frac{k_d}{k_p} \right) + \left( \tilde{K}_m + \Gamma_S \right) \left( \frac{A}{V} \right)} \left[ t + \left( \frac{\tau_1^2}{\tau_1 - \tau_2} \right) \left\{ \exp(-t/\tau_1) - 1 \right\} \right] \tag{7.3.7}
\]

where \( \tilde{K}_m \) is the interfacial Michaelis–Menten constant, which is given by,
\[ \tilde{K}_m = \left( \frac{\tilde{k}_2 + \tilde{k}_{-1}}{k_1} \right) \]  

(7.3.8)

- After a long time has elapsed \((t \to \infty)\), the rate of hydrolysis is given by,

\[
\text{Rate} = \frac{d\Gamma_{p^*}}{dt} = \frac{\tilde{k}_2 \Gamma S c_{E0}}{\tilde{K}_m \left( \frac{k_d}{k_p} \right) + \left( \tilde{K}_m \Gamma_S \right) \left( A/V \right)}
\]  

(7.3.9)

- Equation (7.3.9) can be adapted to various interfaces such as, monolayers at the air–water and oil–water interfaces, dispersions of bilayer liposomes, micelles and oil-in-water emulsions.

- The coefficient of proportionality between the rate and \(C_{E0}\) describes the coupling between the enzymatic catalytic action at the lipid interface and the enzyme partitioning between the aqueous bulk phase and the interface.

- This coefficient contains the ratio between the specificity constant, \(\tilde{k}_2/\tilde{K}_m\), and the volume–surface partitioning of the enzyme, \(k_d/k_p\), at steady state.

- If the characteristic time of enzyme desorption is larger than the characteristic time of the catalytic steps, \(k_d \ll \tilde{k}_2/\tilde{K}_m\), the enzyme molecules are confined at the interface during many catalytic turnover cycles. This results in better catalytic efficiency and larger rate of hydrolysis. It is known as scooting mode.

- On the other hand, if the enzyme molecules desorb after a few catalytic cycles, the catalytic efficiency is lower, leading to lower hydrolysis rate. This is known as hopping mode.

- A more general scheme is as follows. The two dimensional Michaelis–Menten catalytic steps, i.e., \(E^* + S \rightleftharpoons E^* S \rightarrow P^*\), are coupled with the following processes: the enzyme partitions between the bulk phase and the surface (i.e., \(E \rightleftharpoons E^*\)), the accumulation and molecular reorganization of the insoluble reaction products (i.e., \(P^* \rightleftharpoons P^{**}\)), the competitive inhibition of the interfacial reaction (i.e., \(E^* + I \rightleftharpoons E^* I\)), and the solubilization of the insoluble lipolytic
products in the presence of acceptors \( \text{i.e., } CD + P^* \rightarrow CD - P \). It is illustrated in Fig. 7.3.3.

\[
\text{Fig. 7.3.3 General model of interfacial enzymatic lipolysis.}
\]

- The general scheme should also take into account the possible exchange of enzyme, substrate and product molecules between the dispersed particles leading to alteration and destabilization of the colloidal reaction system.
- Lipid monolayers at air–water interface have been extensively used as model systems for studying lipolysis. The course of reaction is followed by measuring the decrease of the film area required to maintain the monolayer at constant surface pressure and constant substrate concentration in the Langmuir trough.
- It has been experimentally established that in the monolayer system, a limited number of enzyme molecules penetrate as \( E^* \) and \( E^*S \). After a sufficient amount of time, a typical order of magnitude of bulk–interface enzyme partitioning is, \( \tilde{k}_d / \tilde{k}_p = 10^4 \, \text{m}^{-1} \). The typical value of area–volume ratio is, \( A/V \approx 100 \, \text{m}^{-1} \). Therefore,

\[
\tilde{K}_m \left( \frac{\tilde{k}_d}{\tilde{k}_p} \right) \gg \left( \tilde{K}_m + \Gamma_S \right) \left( \frac{A}{V} \right) \tag{7.3.10}
\]

- With this simplification, Eq. (7.3.9) becomes,

\[
\frac{d\Gamma_{p^*}}{dt} = \frac{\tilde{k}_2 \Gamma_{SE_0}}{\tilde{K}_m \left( \tilde{k}_d / \tilde{k}_p \right)} = \tilde{Q} \Gamma_{SE_0}, \quad \tilde{Q} = \left( \frac{\tilde{k}_2 \tilde{k}_p}{\tilde{k}_d \tilde{K}_m} \right) \tag{7.3.11}
\]
The quantity, $\tilde{Q}$, takes into account the influence of the physicochemical properties of the interface on enzyme activity. It is called interfacial quality.

The hydrolysis of short or medium chain lipids which generate soluble products can be easily followed by measuring the decrease of the film area required to maintain the monolayer at constant surface pressure and constant substrate concentration in a Langmuir trough. During the hydrolysis, each desorbed molecule in the reaction compartment is instantaneously supplied by the other reservoir. The surface substrate concentration, $\Gamma_S$, is maintained constant. The decrease in surface area directly reflects the rate at which the products are formed.

In the presence of insoluble inhibitor, a step for the competitive inhibition of $E^*$ is added to the kinetic scheme. The following equation for the rate of hydrolysis at steady state is obtained.

$$
\frac{d\Gamma_p^*}{dt} = \frac{\tilde{k}_2 \Gamma_S}{\tilde{K}_m \left( \tilde{k}_d \frac{c_k}{k_p} + \left( \tilde{K}_m + \frac{\tilde{K}_I}{\Gamma_I} + \Gamma_S \right) \left( \frac{A}{V} \right) \right)}, \quad \tilde{Q} = \left( \frac{\tilde{k}_2 \tilde{k}_p}{\tilde{k}_d \tilde{K}_m} \right)
$$

where $\Gamma_I$ is the interfacial inhibitor concentration and $\tilde{K}_I$ is the interfacial dissociation constant for the enzyme–inhibitor complex.

Several compounds may interfere with phospholipase activity by interacting either directly with the enzyme or indirectly by affecting the interfacial rate of the lipid substrate. An example is the inhibition of gangliosides of the PLC from *Bacillus cereus* acting upon DLPC monolayers by the modification of the physicochemical state of the lipid monolayer.

For a natural long chain lipid substrate such as dipalmitoylphosphatidylcholine (DPPC), the reaction products are insoluble and remain at the interface in the absence of product acceptors.

Two opposed effects are observed. The accumulation of the insoluble lipolytic products takes place, making the substrate inaccessible to the enzyme and leading to inhibition by the reaction products. Interfacial molecular reorganization and
segregation of the insoluble products, i.e., $P^* \rightarrow P^{**}$, often occur. Such processes modify the microheterogeneous structure of the lipid monolayer during the lipolysis and often lead to increased enzyme binding and lipolytic autoactivation. As a result of the increase of the surface enzyme concentration, biphasic kinetics is often observed. It is characterized by an unusual lag time, which depends on the products accumulating at the interface.

* The morphology of lipid monolayers can be visualized by epifluorescence microscopy, atomic force microscopy or Brewster angle microscopy. The schematic of operation of epifluorescence microscopy is illustrated in Fig. 7.3.4.

![Fig. 7.3.4 Schematic of the experimental set-up for epifluorescence microscopy (Grainger et al., 1989) [adapted by permission from Elsevier Ltd., © 1989].](image)

* Visual observations by epifluorescence microscopy of phospholipid monolayers can distinguish several mechanisms of action of PLA$_2$ on lipid domains. After reaching a substantial level of hydrolysis, highly organized two dimensional domains of enzyme of regular size and morphology can be seen. It is shown in Fig. 7.3.5.

* Morphological differences in the assemblies of the products during the hydrolysis of phospholipid monolayers by PLD from Streptomyces have been reported by Kondo et al. (1994).
Theoretical analysis of the enzyme activity in presence of the insoluble lipolytic products is a more complex task. The assumption that during hydrolysis the surface enzyme and substrate concentrations are constant (because the substrate is in large excess or some regulatory processes are implemented during the experiments to keep it constant) does not hold.

The theoretical description of the interfacial morphology also presents inherent difficulties in the microheterogeneous two dimensional systems in which the thermodynamic parameters associated with the small interfacial phases should be taken into account.

Computer simulations have been performed on gel-to-fluid phase transitions in lipid bilayers (Hønger et al., 1996), which can provide some information on the interfacial heterogeneity.

### 7.3.2.1 Reactions in liposomes

For liposomal dispersions, the $A/V$ ratio is large ($\sim 10^6$–$10^7$ $\text{m}^{-1}$). Therefore, in this case,

$$
\left( \tilde{K}_m + \Gamma_S \right) \left( \frac{A}{V} \right) \gg \tilde{K}_m \left( \frac{k_d}{k_p} \right)
$$

Therefore, from Eq. (7.3.9) we get,
where \( \tilde{Q}_l \) is an overall kinetic constant that takes into account the influence of the surface of the liposomes on enzyme activity.

- It has been experimentally established that in a liposomal dispersion with high interfacial area, practically all enzyme molecules initially present in water are fixed at the interface, i.e., \( V_{cE0} \approx \Gamma_{E*}A \) (Baszkin and Norde, 2000).

**Example 7.3.2:** The kinetic data available for the hydrolysis of a short chain lipid film at air–water interface are as follows: \( \tilde{k}_p = 1 \times 10^{-8} \) m/s, \( \tilde{k}_d = 5 \times 10^{-3} \) s\(^{-1}\), \( \tilde{K}_m = 5 \times 10^{-7} \) mol/m\(^2\) and \( \tilde{Q} = 1.3 \) m\(^3\) mol\(^{-1}\) s\(^{-1}\). The interfacial area is 2 m\(^2\) and the volume of the bulk aqueous phase is 0.01 m\(^3\). The initial bulk concentration of the enzyme is \( 1 \times 10^{-3} \) mol/m\(^3\) and \( \Gamma_S = 1 \times 10^{-6} \) mol/m\(^2\). The products of hydrolysis are soluble in the aqueous phase. Calculate the rate of hydrolysis from these data.

**Solution:** From the given data,

\[
A/V = 2/0.01 = 200 \text{ m}^{-1}
\]

Now,

\[
\tilde{K}_m \left( \frac{\tilde{k}_d}{\tilde{k}_p} \right) = \frac{5 \times 10^{-7} \times 5 \times 10^{-3}}{1 \times 10^{-8}} = 0.25 \text{ mol/m}^3
\]

\[
\left( \tilde{K}_m + \Gamma_S \right) \left( \frac{A}{V} \right) = \left( 5 \times 10^{-7} + 1 \times 10^{-6} \right) \times 200 = 3 \times 10^{-4} \text{ mol/m}^3
\]

Therefore,

\[
\tilde{K}_m \left( \frac{\tilde{k}_d}{\tilde{k}_p} \right) >> \left( \tilde{K}_m + \Gamma_S \right) \left( \frac{A}{V} \right)
\]

The rate of hydrolysis is given by Eq. (7.3.11),

\[
\frac{d\Gamma_p^*}{dt} = \tilde{Q} \Gamma_{SE0} = 1.3 \times 10^{-6} \times 1 \times 10^{-3} = 1.3 \times 10^{-9} \text{ mol m}^{-2} \text{ s}^{-1}
\]
The enzyme molecules are irreversibly bound to the liposomes in the scooting mode, as shown in Fig. 7.3.6.

![Fig. 7.3.6 Scooting mode in interfacial catalysis in liposomes.](image)

A bound enzyme, $E^*$, remains at the interface between many catalytic cycles and hydrolyzes the substrate molecules in the outer monolayer of the liposome.

In the hopping mode, the enzyme molecules can exchange between liposomes as shown in Fig. 7.3.7.

![Fig. 7.3.7 Hopping mode in interfacial catalysis in liposomes.](image)

The binding (i.e., $E \rightarrow E^*$) and desorption (i.e., $E^* \rightarrow E$) of one enzyme molecule occur between the catalytic turnover cycles. The available substrate molecules in the outer monolayers of the liposomes are hydrolyzed by this hopping mechanism.

The kinetics of hydrolysis by the scooting mechanism takes into account the confinement of the enzyme at the interface in a probabilistic manner. The distribution of the enzyme molecules among all liposomes is described by the Poisson distribution.
\[ P_i = \frac{(c_E/c_l)^i}{i!} \exp\left(-\frac{c_E}{c_l}\right) \quad (7.3.15) \]

where \( P_i \) is the probability that a liposome has \( i \) number of bound enzyme molecules, \( c_l \) is the concentration of liposomes and \( c_E/c_l \) represents an average number of enzyme molecules per liposome.

- The probability for liposomes without any bound enzyme is,

\[ P_{i=0} = \exp\left(-\frac{c_E}{c_l}\right) \quad (7.3.16) \]

- At small enzyme/liposome ratios, \( P_{i=0} = 1 - \frac{c_E}{c_l} \).

- For large enzyme/liposome ratios, \( P_{i=0} \approx 0 \), which signifies that there is practically no liposome without any bound enzyme.

- The common experimental techniques for following the course of enzymatic hydrolysis in liposomal dispersions are based on the measurement of the concentration of the products released with time. Spectroscopic methods, pH-stat titration with NaOH and measurement of change in turbidity of dispersion with time by light scattering are some of the widely used experimental methods used in the study of enzymatic hydrolysis.
Exercise

Exercise 7.3.1: Consider a two-phase liquid–liquid reaction: \( P + Q \rightarrow R \). The following mass transfer and kinetic data are available: \( k_L = 4.5 \times 10^{-6} \text{ m/s} \), \( k_2 = 3 \times 10^{-2} \text{ m}^3 \text{ mol}^{-1} \text{s}^{-1} \), \( D_A = 1.3 \times 10^{-9} \text{ m}^2 \text{s} \) and \( D_B = 6.5 \times 10^{-9} \text{ m}^2 \text{s} \). The concentrations of the reactants in the bulk phase and at the interface are: \( c_{A}^{\text{int}} = 60 \text{ mol/m}^3 \) and \( c_{B}^{\text{bulk}} = 2.5 \times 10^3 \text{ mol/m}^3 \). From these data, determine whether the reaction falls in the fast or instantaneous category.

Exercise 7.3.2: Derive Eq. (7.3.7).

Exercise 7.3.3: Compute the probability that a liposome has (i) no bound enzyme, and (ii) 10 bound enzyme molecules, if the average number of enzyme molecules per liposome is 5.

Exercise 7.3.4: Answer the following questions clearly.

1) Give three examples of instantaneous reaction at gas–liquid and liquid–liquid interfaces.
2) Explain the physical significance of Hatta number.
3) Explain the mechanism of enzymatic lipolysis.
4) Explain the terms scooting and hopping in enzymatic lipolysis.
5) Give three techniques by which enzymatic lipolysis can be experimentally monitored.
6) Explain the effect of solubility of the products in the bulk phase in enzymatic lipolysis.
Suggested reading

Textbooks


Reference books

♦ A. Baszkin and W. Norde (Eds.), *Physical Chemistry of Biological Interfaces*, Marcel Dekker, New York, 2000, Chapter 11.

Journal articles