



of topics related to reaction kinetics that have a significant bearing on the development of mathematical models of cellular networks.

A chemical reaction is usually depicted in the form of a chemical equation which describes the transformation of one or more **reactants** into one or more **products**. The reactants appear on the left of the equation and the products on the right. Both sides are separated by an arrow indicating the positive direction of the transformation. The simplest possible reaction is the conversion of a single reactant,  $A$ , into a single product,  $B$ , as depicted in the following way:



Such a reaction can be studied by observing the change in concentration of  $A$  and/or  $B$  in time. Experimentally there are a variety of ways to do this, for example by observing the emission or absorption of light at a specific wavelength, the change in pH, or the incorporation of a radioactive or heavy isotope into the product. An example of an actual biochemical reaction is the familiar interconversion of the adenine nucleotides:



This describes two molecules of ADP being transformed into one molecule of ATP and one molecule of AMP. Sometimes a double arrow is used to explicitly indicate that a reaction is reversible, as in:

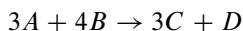


If a reaction is reversible (as almost all reactions are to some extent), then the reaction rate can be positive or negative. By convention, a positive rate means that the reaction progresses from left to right, whereas a negative rate indicates a right to left reaction.

### *Example 1.1*

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What does the following reaction notation mean:



This notation means that during a reaction event, 3 molecules of  $A$  and 4 molecules of  $B$  react to form 3 molecules of  $C$  and one molecule of  $D$ .

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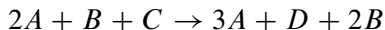
We now need to define a number of terms: the stoichiometric amount, rate of change, stoichiometric coefficient, and reaction rate.

## Stoichiometric Amount

The **stoichiometric amount** is defined as the number of molecules of a particular reactant or product taking part in a reaction. Stoichiometric amounts will always be **positive** numbers. For example, in the reaction:



ADP has a stoichiometric amount of two, ATP a stoichiometric amount of one, and AMP also with a stoichiometric amount of one. If the same species occurs on the reactant and product side of a reaction then it must be treated separately. For example, in the reaction:



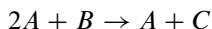
The stoichiometric amounts on the reactant side include: A with two, B with one and C with one. On the product side the stoichiometric amounts include: A with three, D with one and B with two.

The **stoichiometric amount** is the number of molecules of a particular reactant or product taking part in a reaction.

### Example 1.2

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List the stoichiometric amounts in the following reaction:



On the reactant side the stoichiometric amount for A is two and for B is one. On the product side, the stoichiometric amount for A is one and for C one.

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## 1.1 Rates of Change

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The rate of change can be defined as the rate of change in concentration or amount (depending on units) of a designated species. If  $S$  is the species then the rate of change is given by:

$$\text{Rate} = \frac{\Delta S}{\Delta t}$$

Because rates change as reactants are consumed and products made, the rate of change is better defined as the instantaneous change in concentration, or a derivative:

$$\text{Rate} = \frac{dS}{dt}$$

If we were to plot the rate of product formation as a function of time, the rate of reaction would be given by the slope of the curve (Fig. 1.1). If concentrations are measured in moles per liter (L) and time in seconds (sec), then the rate of reaction is expressed in  $\text{mol L}^{-1} \text{sec}^{-1}$ .

When reporting a rate of change, it is important to give the name of the species that was used to make the measurement. For example, in the reaction  $2A \rightarrow B$ , the rate of change of  $A$  is twice the rate of change of  $B$ . In addition, the rate of change of  $A$  is negative because it is consumed, whereas the rate of change of  $B$  is positive because it is being made.

### Stoichiometric Coefficients

Stoichiometry deals with static information about the amounts of substances involved in a chemical transformation, whereas kinetics relates rates of change that occur in these amounts. To paraphrase a statement made by Aris [4], stoichiometry provides the framework within which chemical change takes place irrespective of the forces that bring them about, and by kinetics the speed of chemical change. Aris then went on to state, “Just as the latter can only be built on a proper understanding of the kinematics, so the analysis of stoichiometry must precede that of kinetics”. We will do the same here.

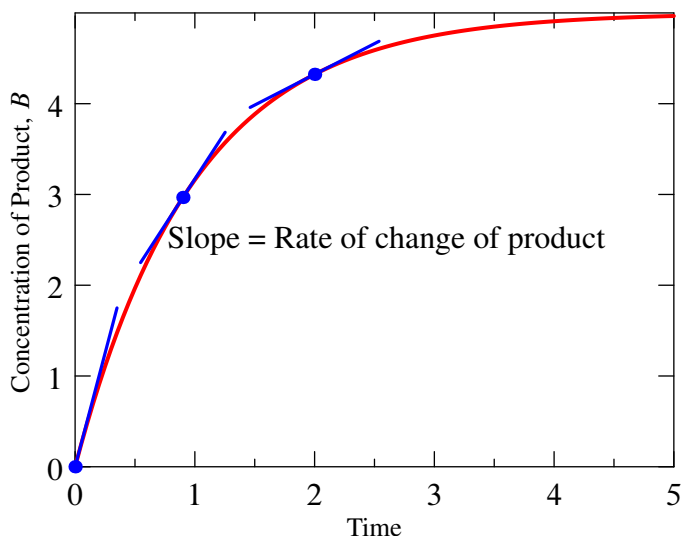
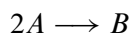


Figure 1.1: Progress curve for a simple irreversible reaction,  $A \rightarrow B$ . Initial reactant concentration,  $A$ , is set at 5 units. The plot shows the accumulation of product,  $B$ , as the reaction proceeds. The rate of change of product is given by the slope of the curve which changes over the course of the reaction.

The stoichiometry coefficient refers to the **relative** amount of substance that is consumed and/or produced by a reaction. Given a reaction such as:

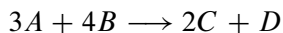


the stoichiometric amount of  $A$  is 2 and for  $B$ , 1. The species stoichiometry or **stoichiometric coefficient** however, is the difference between the stoichiometric amounts of a given species on the product side and the stoichiometric amount of the same species on the reactant side. The definition below summarizes this more clearly.

The **stoichiometric coefficient**,  $c_i$ , for a molecular species  $A_i$ , is the difference between the stoichiometric amount of the species on the product side and the stoichiometric amount of the same species on the reactant side, that is:

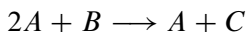
$$c_i = \text{Stoichiometric Amount of Product, } A_i \\ - \text{Stoichiometric Amount of Reactant, } A_i$$

In the reaction,  $2A \longrightarrow B$ , the stoichiometric amount of  $A$  on the product side is **zero** while on the reactant side it is two. Therefore the stoichiometric coefficient of  $A$  is given by  $0 - 2 = -2$ . In many cases a particular species will only occur on the reactant or product side and it is relatively uncommon to find situations where a species occurs simultaneously as a product and a reactant. As a result, reactant stoichiometric coefficients tend to be **negative** and product stoichiometric coefficients tend to be **positive**. To illustrate this further consider the more complex reaction:



Since  $A$  only appears on the reactant side, its stoichiometric coefficient will be  $-3$ , similarly for  $B$  which will have a stoichiometric coefficient of  $-4$ . Species  $C$  only occurs on the product side, therefore its stoichiometric coefficient is  $+2$ , and similarly for  $D$  which will have a stoichiometric coefficient of  $+1$ . In these cases the stoichiometric amounts and the stoichiometric coefficients are the same except for the sign difference on the reactant stoichiometric coefficients.

Finally consider the following reaction where a species occurs on both the reactant and product side:



The stoichiometric coefficient of  $A$  must take into account the fact that  $A$  appears both as a reactant and a product. The overall stoichiometric coefficient of  $A$  is therefore  $+1 - 2$  which gives  $-1$ .

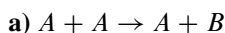
The last example highlights how information can be lost when computing stoichiometric coefficients. It is not possible to recreate the original reaction equation from the stoichiometric coefficients alone, and therefore

underscores the danger of just supplying stoichiometric coefficients when communicating information on reaction equations to other researchers. One option is to store the stoichiometric amounts together with the associated reactant or product. Computer exchange formats, such as the Systems Biology Markup Language (SBML) [34] are specifically designed to preserve complete reaction equation information for this very reason.

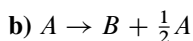
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**Example 1.3**

Write down the stoichiometric coefficients for the following reactions:



The stoichiometric amount of  $A$  on the reactant side is 2 and on the product side, 1. Therefore the stoichiometric coefficient for  $A$  is  $1 - 2 = -1$ . The stoichiometric amount of  $B$  on the product side is 1 and on the reactant side, 0, therefore the stoichiometric coefficient for  $B$  is  $1 - 0 = 1$ .



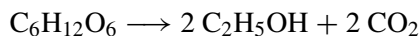
The stoichiometric amount of  $A$  on the reactant side is 1 and on the product side  $\frac{1}{2}$ , therefore the stoichiometric coefficient for  $A$  is  $1/2 - 1 = -1/2$ . The stoichiometric amount of  $B$  on the reactant side is 0 and on the product side, 1, therefore the stoichiometric coefficient for  $B$  is  $1 - 0 = 1$ .

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Example 1.3 (b) highlights another fact about stoichiometric coefficients. The coefficients can be fractional amounts, often represented as rational fractions.

## Reaction Yields

One application of stoichiometry is to compute maximum theoretical yields for a given reaction. Consider the yeast fermentation of glucose to ethanol:



If a yeast culture is started with 10g of glucose, what is the maximum amount of ethanol that can be produced if all the glucose is consumed? The

stoichiometric amount for ethanol is 2, that is for every one mole of glucose consumed, two moles of ethanol are formed. The molar mass of glucose is 180, therefore the number of moles of glucose in 10g is  $10/180 = 0.055$  moles. From the stoichiometry, this means that 0.111 moles of ethanol will be formed. If the molar mass of ethanol is 46, then 5.2g of ethanol are formed. The same calculation can be made for  $\text{CO}_2$  yielding a mass of 4.8g of carbon dioxide. As a final check it is evident that the total mass of product is  $5.2+4.8$  or 10g, exactly the amount of initial glucose. Therefore mass is conserved, as expected.

The percentage yield of ethanol can be calculated from the mass of ethanol produced compared with the mass of glucose consumed. If a 100% conversion is assumed, so all the glucose is converted and no side reactions occur, then the percentage yield for ethanol is 52% ( $5.2\text{g} / 100\text{g}$ ) with the remainder lost as carbon dioxide. However, in reality maximum yields are never achieved because some of the glucose is diverted to produce biomass. In anaerobic growth for example, fermentation of glucose to ethanol in yeast will typically yield about 0.46-0.48 g ethanol per gram of glucose, that is 90-94% of the theoretical yield [39].

#### Definitions:

*Intensive Property:* A property that does not depend on the quantity of substance. Examples include temperature, density and concentration.

*Extensive Property:* A property that does depend on the quantity of substance. Examples include mass and volume.

## Reaction Rates

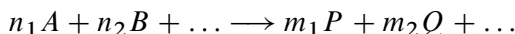
In this section we will introduce the concept of a **reaction rate**, denoted by  $v$ . The standard unit for the reaction rate is amount per volume per time. This is an intensive property, which does not depend on the amount of substance, for example  $\text{mol L}^{-1} \text{sec}^{-1}$ . In a previous section we introduced the rate of change. In practice it is the rate of change that we measure experimentally. We also briefly mentioned that in the reaction  $2A \rightarrow B$ ,

$A$  is consumed twice as fast as the production of product,  $B$ . This means that the sign and magnitude of the rates of change will vary depending on which species we choose to measure.

A simple way to avoid these differences is to divide each rate of change by the species **stoichiometric coefficient**. In this case the stoichiometric coefficient of  $A$  is  $-2$  and for  $B$  is  $+1$ . If we do this we obtain:

$$\frac{1}{-2} \frac{dA}{dt} = \frac{1}{1} \frac{dB}{dt} = v$$

In general, for a reaction of the form



where  $n_1, n_2, \dots$  and  $m_1, m_2, \dots$  represent the stoichiometric coefficients, the reaction rate is given by:

$$\text{Rate} = v \equiv -\frac{1}{n_1} \frac{dA}{dt} = -\frac{1}{n_2} \frac{dB}{dt} \dots = \frac{1}{m_1} \frac{dP}{dt} = \frac{1}{m_2} \frac{dQ}{dt} \dots \quad (1.1)$$

Defined this way, a reaction rate is independent of the species used to measure it. The same applies if a given species appears on both sides of a reaction. For example, in the reaction  $A \rightarrow 2A$ , the stoichiometric coefficient is  $+1$  so that the reaction rate,  $v$ , is:

$$v = \frac{1}{+1} \frac{dA}{dt}$$

To make the definition of the reaction rate more formal, let us introduce the **extent of reaction**, indicated by the symbol,  $\xi$ . We define a change from  $\xi$  to  $\xi + d\xi$  in time  $dt$  to mean that  $c_1 d\xi$  moles of  $A_1$ ,  $c_2 d\xi$  moles of  $A_2$  etc, react to form  $c_n d\xi$  moles of  $A_n$  etc. By this definition we can state that for any component  $i$ , the following is true for the time interval  $dt$ :

$$dn_i = c_i d\xi \quad (1.2)$$

or

$$\frac{dn_i}{dt} = c_i \frac{d\xi}{dt}$$

where  $n_i$  equals the amount in moles of species  $i$ . From this relation we **define the extensive rate of reaction**,  $v_E$ , to be:

$$v_E \equiv \frac{d\xi}{dt}$$

In other words

$$\frac{dn_i}{dt} = c_i v_E \quad (1.3)$$

For the moment we will use  $v_E$  and  $v_I$  to distinguish the extensive and intensive reaction rates. Note that  $\xi$  has units of **amount** and  $v_E$  has units of **amount per unit time** and is therefore an **extensive property**, being dependent on the size of the system. The advantage of introducing the extent of reaction is that it allows us to formally define the rate of reaction independently of the species we use to measure the rate. This convenient property can be expressed as:

$$v_E \equiv \frac{d\xi}{dt} = -\frac{1}{c_1} \frac{dn_1}{dt} = -\frac{1}{c_2} \frac{dn_2}{dt} \dots = \frac{1}{c_n} \frac{dn_n}{dt} = \frac{1}{c_{n+1}} \frac{dn_{n+1}}{dt} \dots$$

#### Example 1.4

Express the rate of reaction and the rates of change for the following biochemical reaction:  $2 \text{ADP} \rightarrow \text{ATP} + \text{AMP}$  The rate of reaction is given by

$$\begin{aligned} v &= \frac{d\xi}{dt} = \frac{dn(\text{ATP})}{dt} = \frac{dn(\text{AMP})}{dt} \\ &= -\frac{1}{2} \frac{dn(\text{ADP})}{dt} \end{aligned}$$

If the volume,  $V$ , of the system is constant we can also express the rate in terms of concentration,  $C_i = n_i/V$ .

We can therefore rewrite the rate of reaction in the form:

$$\frac{v_E}{V} = -\frac{1}{c_1} \frac{dC_1}{dt} = \dots$$

where  $v_E$  has units of amount per unit time ( $\text{mol s}^{-1}$ ). The relation  $v_E/V$  is the intensive version of the rate,  $v_I$ , with units of concentration per unit time ( $\text{mol L}^{-1} \text{s}^{-1}$ ) and is the most commonly used form in biochemistry.

$$v_I = \frac{v_E}{V} = \frac{1}{c_i} \frac{dC_i}{dt}$$

or

$$\frac{dC_i}{dt} = c_i v_I \quad (1.4)$$

where  $C_i$  is the concentration of species  $i$  and  $v_I$  is the **intensive rate of reaction**. For constant volume, single compartment systems, this is a commonly encountered equation in models of cellular networks. The above equation may also be expressed as:

$$\frac{1}{V} \frac{dn_i}{dt} = c_i v_I \quad (1.5)$$

to emphasize the change in mass that accompanies a reaction. Recall that  $v_I$  is expressed as  $\text{mol L}^{-1} \text{s}^{-1}$ . If a  $E$  or  $I$  subscript is not used on  $v$  then the specific form should be clear from the context. In this book, where we use  $v$ , we will generally mean  $v_I$ , the intensive form.

In some simulation situations, for example those involving multiple compartments of different volumes or where there are specific mass conservation laws at work, the intensive rate is not appropriate. This is because the intensive version is unable to keep track of the total number of moles undergoing transformation. In these situations it is necessary to deal explicitly with the extensive rate of reaction, in other words:

$$\frac{dn_i}{dt} = V c_i v_I$$

### A Word on Notation

In many texts, the concentration (molarity) of a substance,  $X$ , is denoted using square brackets, as in  $[X]$ . To avoid unnecessary clutter in the current text, the use of square brackets to indicate molarity will be relaxed.

## 1.2 Elementary Rate Kinetics

Up to now, we have not discussed how  $v$  might be calculated other than by experimental measurement. In this section we introduce mass-action kinetics. Chemical reactions that involve no reaction intermediates are called **elementary reactions**. Such reactions often have simple kinetic properties and empirical studies have shown that the rate of reaction is often proportional to the product of the molar concentration of the reactants raised to some power.

For a simple elementary monomolecular reaction such as:



the rate of reaction,  $v$ , is often found to be proportional to the concentration of species  $A$ , or:

$$v = kA$$

This property is often called the law of **mass-action** and the corresponding kinetics called **mass-action kinetics**. The proportionality constant,  $k$ , is called the **rate constant**.  $A$  is the concentration of reactant and  $v$  is the rate of reaction with units of  $\text{mol L}^{-1} \text{t}^{-1}$ . Recall that the rate of change of  $A$  is the reaction rate times the stoichiometry coefficient (1.4), since the stoichiometry coefficient of  $A$  is  $-1$ , the rate of change is given by:

$$\frac{dA}{dt} = -v = -kA \quad (1.6)$$

The units for  $k$  are  $t^{-1}$  and for the concentration of  $A$ , moles  $L^{-1}$ . The rate of change of  $A$  therefore has units of moles  $L^{-1}t^{-1}$ . By convention,

a rate that is proportional to a reactant raised to the first power is called **first-order** with respect to the reactant. Similarly, reactants raised to the zeroth power are called **zero-order**, and reactants raised to the power of two are called **second-order** (See Figure 1.2).

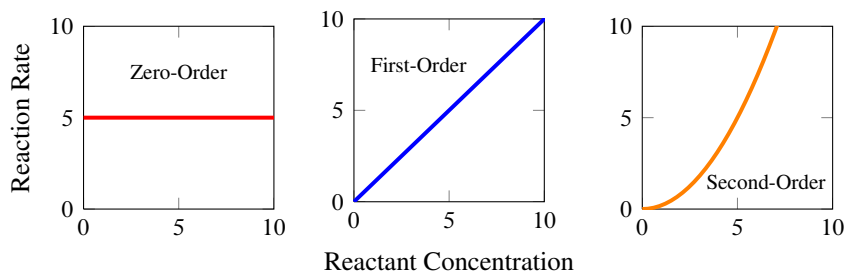


Figure 1.2: Curves illustrating zero-order, first-order and second-order kinetics.

Equation (1.6) is a differential equation that can be solved using standard methods in differential calculus to describe the change in concentration of  $A$  over time. This solution is shown in Figure 1.3 and is described by the equation:

$$A(t) = A(0) e^{-kt} \quad (1.7)$$

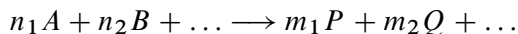
where  $A(0)$  is the initial concentration of  $A$  and  $t$  the time.

A common metric that is used to judge the rate of different first-order reactions is the **half-life**. This quantity measures the time taken for half the level of substance,  $A$ , to be transformed into product. When half of  $A$  has been consumed we can set  $A(t)$  in equation (1.7) to  $\frac{1}{2}A(0)$  so that

$$\frac{1}{2} = e^{-kt_{1/2}}$$

The time,  $t_{1/2}$ , is called the half-life and by suitable rearrangement is given by,  $t_{1/2} = \ln(2)/k$ . For example if  $k = 0.5 \text{ sec}^{-1}$ , the half life is equal to  $\ln(2)/0.5 \simeq 1.4 \text{ sec}$ , that is, after 1.4 sec, half the concentration of substance has been consumed.

For the general reaction:



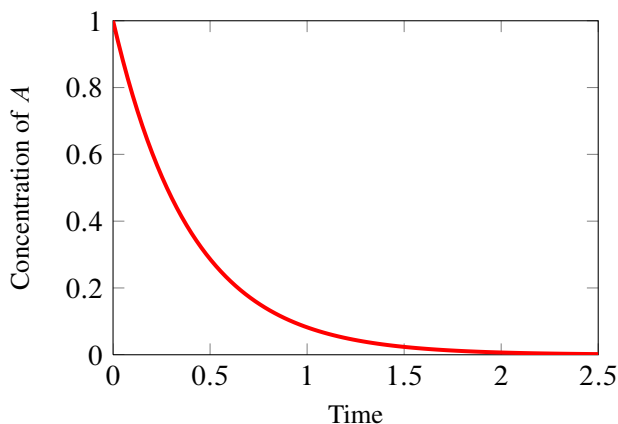


Figure 1.3: Progress curve for species  $A$  in the irreversible reaction  $A \rightarrow B$ , computed using  $A(t) = A(0) \exp(-kt)$  where  $A(0)$  is the initial concentration equal to 1.0,  $k$  the rate constant equal to 2.5, and  $t$  the time. The change in  $B$  is given by  $B(t) = A(0) - A(t)$ .

the rate law has been found through empirical observation to often have the form:

$$v = kA^{n_1}B^{n_2} \dots$$

or more generally:

$$v = k \prod_i S_i^{n_i} \quad (1.8)$$

where each reactant is raised to the power of its stoichiometric amount. For example, the forward reaction rate for the following uncatalyzed reaction:



can be written as:

$$v = k \text{ADP}^2$$

If the reaction is reversible then the rate law is appended with the reverse rate. In general a reversible mass-action rate law is given by:

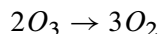
$$v = k_1 A^{n_1} B^{n_2} \dots - k_2 P^{m_1} Q^{m_2} \dots \quad (1.10)$$

In the case of reaction (1.9), this would mean:

$$v = k_1 \text{ADP}^2 - k_2 \text{ATP AMP}$$

In all mass-action rate laws, the units for the reactant and product terms must be expressed in concentration. The units for the rate constants,  $k$  will depend on the exact form of the rate law but must be set to ensure that the rate of reaction is expressed in units  $\text{moles } L^{-1} t^{-1}$ .

Although in many cases one will often assume a rate law of the form (1.10), this need not always be the case. For example, the gas reaction



has been found experimentally to follow the rate law:

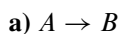
$$v = \frac{1}{2} k \frac{(O_3)^2}{O_2}$$

rather than the expected,  $v = 1/2 k (O_3)^2$ . The reason for the discrepancy is that the decomposition of ozone into oxygen occurs via a series of elementary reactions and it is the combination of these elementary reactions that gives rise to the non-elementary rate law. In biochemistry this effect is readily seen in enzyme kinetics where the rate laws are far from elementary.

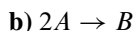
### Example 1.5

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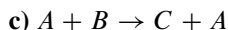
Write down the mass-action rate laws for the following reversible reactions. Assume that the forward and reverse rate constants are  $k_1$  and  $k_2$  respectively.



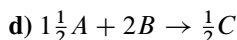
$$v = k_1 A - k_2 B$$



$$v = k_1 A^2 - k_2 B$$



$$v = k_1 A B - k_2 C A = A(k_1 B - k_2 C)$$



$$v = k_1 A^3 B^4 - k_2 C$$

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### 1.3 Chemical Equilibrium

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In principle all reactions are reversible, meaning transformations can occur from reactant to product or product to reactant. The net rate of a reversible reaction is the difference between the forward and reverse rates. We can write down the forward rate,  $v_f$ , and reverse rate,  $v_r$  for the simple unimolecular reaction  $A \rightleftharpoons B$  as:

$$\begin{aligned} v_f &= k_1 A \\ v_r &= k_2 B \end{aligned} \tag{1.11}$$

The net rate of reaction,  $v$ , is then given by the difference between the forward and reverse rates:

$$v = v_f - v_r$$

Furthermore, all reactions in a closed system (See Table 10.1), that is a system which is isolated from the surroundings, will tend to **thermodynamic equilibrium** (Figure 1.4).

At equilibrium the forward and reverse rates will be equal and the net rate zero:

$$v_f - v_r = 0$$

Inserting equations (1.11) into the above yields the ratio:

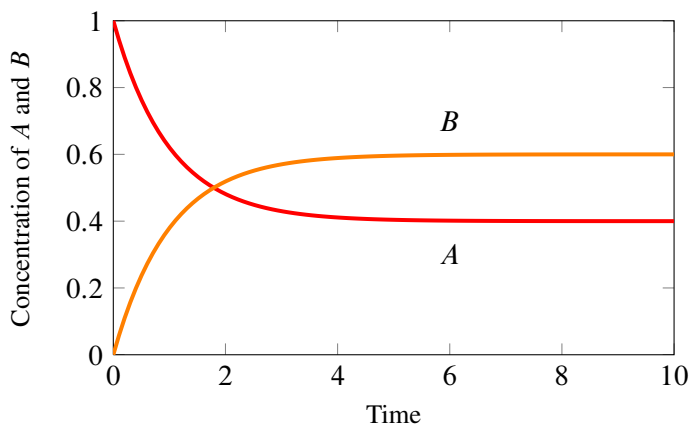
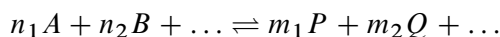


Figure 1.4: Approach to equilibrium for the reaction  $A \rightleftharpoons B$ ,  $k_1 = 0.6$ ,  $k_2 = 0.4$ ,  $A(0) = 1$ ,  $B(0) = 0$ . Progress curves calculated from the solution to the differential equation  $dA/dt = k_2B - k_1A$ .

$$\frac{k_1}{k_2} = \frac{B}{A} = K_{eq} \quad (1.12)$$

This ratio has special significance and is called the **equilibrium constant**, denoted by  $K_{eq}$ . The equilibrium constant is also related to the ratio of the rate constants,  $k_1/k_2$ . For a general reversible reaction such as:



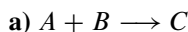
and using arguments similar to those described above, the ratio of the rate constants can be easily shown to be:

$$K_{eq} = \frac{P^{m_1} Q^{m_2} \dots}{A^{n_1} B^{n_2} \dots} = \frac{k_1}{k_2} \quad (1.13)$$

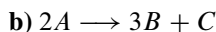
where the exponents are the stoichiometric **amounts** for each species.

**Example 1.6**

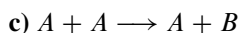
Write out the equilibrium relationship for the following reactions



$$K_{eq} = \frac{C}{AB}$$

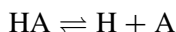


$$K_{eq} = \frac{B^3 C}{A^2}$$



$$K_{eq} = \frac{A B}{A^2} = \frac{B}{A}$$

For a bimolecular reaction such as:



chemists and biochemists will often distinguish between two kinds of equilibrium constants called association and dissociation constants. Thus the equilibrium constant for the above bimolecular reaction is often called the **dissociation constant**,  $K_d$ :

$$K_d = \frac{H \cdot A}{HA}$$

to indicate the degree that the complex is dissociated into its component molecules at equilibrium. The **association constant**,  $K_a$ , though less commonly used, describes the equilibrium constant for the reverse process  $H + A \rightleftharpoons HA$ , that is the formation of a complex from component molecules:

$$K_a = \frac{HA}{H \cdot A}$$

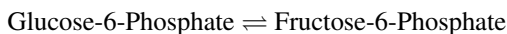
It should be evident that:

$$K_d = \frac{1}{K_a} \quad (1.14)$$

**Example 1.7**

The equilibrium constant for the reaction between glucose-6-phosphate and fructose-6-phosphate catalyzed by glucose-6-phosphatase isomerase (EC 5.3.1.9) is known to have a value of 0.395 at 25°C. The concentration of glucose-6-phosphate in liver cells is estimated to be 4.9 mM. Assuming the reaction is at equilibrium, estimate the concentration of fructose-6-phosphate.

The reaction is described by



and the equilibrium constant is therefore given by

$$K_{eq} = \frac{\text{Fructose-6-phosphate}}{\text{Glucose-6-phosphate}}$$

By simple rearrangement the Fructose-6-Phosphate concentration is equal to

$$\begin{aligned} \text{Fructose-6-phosphate} &= K_{eq} \text{ Glucose-6-phosphate} = \\ &0.395 \times 4.9\text{mM} = \\ &1.94 \text{ mM} \end{aligned}$$

**Example 1.8**

The previous problem can be made more difficult by stating that the *total* concentration of glucose-6-phosphate and fructose-6-phosphate is 4.9 mM. The question now is to compute the equilibrium concentration of both species. The calculation begins by constructing an equilibrium table:

Species	G6P	F6P
Initial concentration	4.9	0
Equilibrium concentration	4.9 - x	x

G6P ≡ Glucose-6-Phosphate

F6P ≡ Fructose-6-Phosphate

From the equilibrium constant and the above table we derive the following

$$K_{eq} = \frac{x}{4.9 - x}$$

Solving for  $x$  and hence the equilibrium concentration, yields

$$x = \frac{K_{eq} 4.9}{1 + K_{eq}}$$

Therefore the equilibrium concentration of Glucose-6-Phosphate is 1.387 mM and for Fructose-6-Phosphate, 3.514 mM. A simple check that the ratio, 1.387/3.514 equals the equilibrium constant will confirm the result. With more complex reactions the above method yields polynomial solutions which can have multiple solutions, usually one negative and the other positive. It should be clear however that the negative solution is physically impossible which leaves the other as the solution we seek.

---

## Principle of Detailed Balance

In its simplest form, the principle of detailed balance says that the forward and reverse rates must be equal at thermodynamic equilibrium. For the simple reversible reaction:



where the forward rate  $v_f$  is given by  $v_f = k_f A$ , and the reverse rate,  $v_r$  by  $v_r = k_r B$ , detailed balance states that  $v_f = v_r$  at equilibrium, or

$$k_f A_{eq} = k_r B_{eq}$$

From the definition of the equilibrium constant we see that

$$K_{eq} = \frac{B_{eq}}{A_{eq}} = \frac{k_f}{k_r}$$

Detailed balance is more useful when applied to more complex systems. Consider the system shown in Figure 1.5 which is comprised of three species linked by three reversible reactions. Each reaction has a forward and reverse rate constant.

At equilibrium the following must be true:

$$\frac{k_{1f}}{k_{1r}} = \frac{B}{A} = K_{eq1}, \quad \frac{k_{2f}}{k_{2r}} = \frac{C}{B} = K_{eq2}, \quad \text{and} \quad \frac{k_{3f}}{k_{3r}} = \frac{A}{C} = K_{eq3}$$

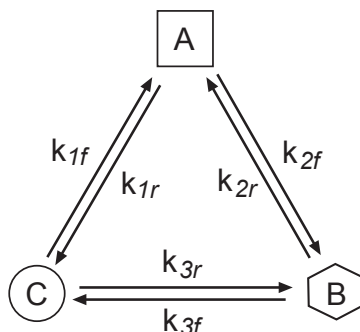


Figure 1.5: The principle of detailed balance.

Combining the three equations and eliminating  $A$ ,  $B$ , and  $C$  yields the following relation among the rate constants:

$$k_{1f}k_{2f}k_{3f} = k_{1r}k_{2r}k_{3r}$$

The product of rate constants in one direction around the loop is equal to the product of rate constants in the opposite direction around the loop. A restatement of the above relation is that the product of the equilibrium constants in a loop is one:

$$\frac{B}{A} \frac{C}{B} \frac{A}{C} = K_{eq1} K_{eq2} K_{eq3} = 1 \quad (1.15)$$

Equation (1.15) applies irrespective of the actual reaction mechanism. Detailed balance applies a constraint on the allowable rate and equilibrium constants in a reaction loop. In addition it means that the change in free energy (See chapter 10) around the loop is zero. By analogy, one can compare detailed balance to a hike over a mountain range, where a hiker traverses one peak after another. If we assume that the hiking route eventually returns him to the original starting point, the net change in height is zero. Detailed balance also precludes the construction of a perpetual motion machine.

## Mass-action and Disequilibrium Ratio

In closed systems, reactions will tend to equilibrium whereas reactions occurring in open living cells are generally out of equilibrium. The ratio of the products to the reactants *in vivo* is called the **mass-action ratio**,  $\Gamma$ . For the system,  $A \rightarrow B$ :

$$\Gamma = \frac{B_{in\ vivo}}{A_{in\ vivo}}$$

At equilibrium  $\Gamma = K_{eq}$ . The ratio of the mass-action ratio to the equilibrium constant is often called the **disequilibrium ratio** and denoted by the symbol,  $\rho$ .

$$\rho = \frac{\Gamma}{K_{eq}} \quad (1.16)$$

At equilibrium, the mass-action ratio is equal to the equilibrium constant and  $\rho = 1$ . If the reaction is far from equilibrium ( $B/A < K_{eq}$ ) then  $\rho < 1$ .

For a simple unimolecular reaction it was shown previously that the equilibrium ratio of product to reactant,  $B/A$ , is equal to the ratio of the forward and reverse rate constants. Substituting this into the disequilibrium ratio gives:

$$\rho = \Gamma \frac{k_2}{k_1} = \frac{B}{A} \frac{k_2}{k_1}$$

Therefore:

$$\rho = \frac{v_r}{v_f} \quad (1.17)$$

Thus the disequilibrium ratio is the ratio of the reverse and forward rates. This relationship clearly shows how the disequilibrium ratio tells us whether the reaction is going forward, is at equilibrium or whether it is in reverse. If  $\rho < 1$ , then the net reaction must be in the direction of product formation

$\ln(\rho)$	Direction of Reaction	$v$	$\Delta G$
$< 0$	Forward Direction	$v > 0$	$\Delta G < 0$
$= 0$	Equilibrium	$v = 0$	$\Delta G = 0$
$> 0$	Reverse Direction	$v < 0$	$\Delta G > 0$

Table 1.1: Relationship between  $\rho$  and  $\Delta G$ .

since  $v_f > v_r$ . If  $\rho = 1$  then  $v_r = v_f$ , and the system is at equilibrium. Finally if  $\rho > 1$  then  $v_r > v_f$ , the reaction must be going in reverse.

If we take the natural log of equation (1.16) on both sides we get:

$$\ln(\rho) = \ln(\Gamma) - \ln(K_{\text{eq}}) \quad (1.18)$$

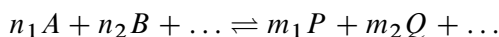
With this transformation, if  $\ln(\rho)$  is negative the reaction must be in the forward direction, zero if the reaction is at equilibrium and greater than zero if the reaction is in the reverse direction. This form of the equation will appear again in the chapter on thermodynamics (chapter 10) where it will be possible to determine  $\rho$  from more fundamental concepts such as entropy and enthalpy.

Those who are already familiar with the concept of free energy ( $\Delta G$ ) may realize that equation (1.18) is closely related to the free energy equation:

$$\Delta_r G = \Delta_r G^\circ + RT \ln \Gamma$$

where  $\Delta_r G = RT \ln(\rho)$  and  $\Delta_r G^\circ = -RT \ln K_{\text{eq}}$ . Because all the rate information has been lost in the derivation of the equation (1.16), the value of  $\ln(\rho)$  tells us nothing about how fast the reaction will proceed, only the direction it proceeds.

Relation (1.17) is actually much more general and applies to any reaction of the form:



The disequilibrium ratio is an important quantity and reappears in later sections and chapters when to discuss enzymatic reactions. It is particularly relevant when one considers the control of cellular pathways.

## Modified Mass-Action Rate Laws

A typical reversible mass-action rate law will require both the forward and the reverse rate constants to be fully defined. Often however, only one rate constant may be known. In these circumstances it is possible to express the reverse rate constant in terms of the equilibrium constant.

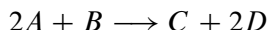
For example, given the simple unimolecular reaction,  $A \rightleftharpoons B$ , it is possible to derive the following:

$$\begin{aligned}
 v &= k_1 A - k_2 B \\
 v &= k_1 A \left( 1 - \frac{k_2 B}{k_1 A} \right) \\
 \text{Since } K_{eq} &= \frac{k_1}{k_2} \\
 v &= k_1 A \left( 1 - \frac{\Gamma}{K_{eq}} \right) \tag{1.19}
 \end{aligned}$$

where  $\Gamma$  is the mass-action ratio. This can be generalized to an arbitrary mass-action reaction to give:

$$v = k_1 A^{n_1} B^{n_2} \dots \left( 1 - \frac{\Gamma}{K_{eq}} \right) = k_1 A^{n_1} B^{n_2} \dots (1 - \rho)$$

where  $A^{n_1} B^{n_2} \dots$  represents the product of all reactant species,  $n_1$  and  $n_2$  are the **corresponding** stoichiometric amounts, and  $\rho$  is the disequilibrium ratio. For example, for the reaction:



where  $k_1$  is the forward rate constant, the modified reversible rate law is:

$$v = k_1 A^2 B (1 - \rho)$$

The modified formulation demonstrates how a rate expression can be divided up into functional parts that include both kinetic and thermodynamic components [31]. The kinetic component is represented by the term  $k_1 A^{n_1} B^{n_2} \dots$  while the thermodynamic component is represented by the expression  $1 - \rho$ . We will see this pattern repeated again and again, particularly in enzyme rate laws where additional components appear in the form of effector regulation.

We can also derive the modified rate law in the following way. Given the net rate of reaction  $v = v_f - v_r$ , we can write this expression in the following way:

$$v = v_f \left( 1 - \frac{v_r}{v_f} \right)$$

That is:

$$v = v_f(1 - \rho)$$

## 1.4 Kinetics across Membranes

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In this section let us briefly consider the kinetics of simple membrane diffusion. Consider two compartments  $A$  and  $B$  as shown in figure 1.6 connected by a thin membrane that allows diffusion of substance from one compartment to another.

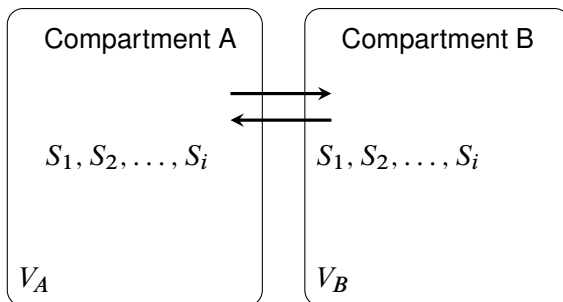


Figure 1.6: Compartmental Analysis. Two compartment, A and B where volumes  $V_A$  and  $V_B$  exchange mass across a membrane.

A membrane that separates two compartments is a two dimensional surface. As a result the kinetic formulation is slightly different compared to bulk solution reaction kinetics. While concentration changes in the bulk solution are often expressed in terms of moles of substance transformed per unit *volume* per unit time (moles  $V^{-1} t^{-1}$ ), transport across a membrane is expressed in units of moles per unit *area* per unit time (moles  $A^{-1} t^{-1}$ ) and is called the flux,  $J$ .

According to Fick's first law of diffusion, the flux is proportional to the concentration gradient across the membrane:

$$J_A = -D_A \frac{dS}{dx}$$

The negative sign ensures that the flux is positive when the concentration gradient is negative, that is declining left to right.  $J_A$  is the flux in units of moles  $l^{-2} t^{-1}$  (moles per unit area per time),  $D_A$  the **diffusion coefficient** has units of  $l^2 t^{-1}$  (area per unit time),  $S$  is the concentration and  $dS/dx$  the concentration gradient in units of moles  $l^{-3} l^{-1}$ , that is moles per volume per length.

If the zone of diffusion has a width  $\delta$ , we can approximate Fick's law:

$$J_A = -D_A \frac{S_{\text{out}} - S_{\text{in}}}{\delta}$$

or

$$J_A = P_A(S_{\text{in}} - S_{\text{out}}) \quad (1.20)$$

where  $P_A$  equals  $D_A/\delta$  and is called the permeability coefficient with units of length per unit time (often  $\text{cm } t^{-1}$ ). The units of flux at this stage are moles per unit area per unit time (moles  $\text{cm}^{-2} t^{-1}$ ). To obtain the total amount of mass that moves from one compartment to another we must multiply the flux,  $J_A$ , by the cross-sectional area of the membrane area, thus:

$$J = AJ_A$$

where  $J$  is the total amount of substance crossing the membrane and  $A$  the area of the membrane. If this substance is moving into a volume  $V$ , then the rate of change of concentration in the compartment is given by:

$$\frac{dS}{dt} = -\frac{J}{V}$$

The negative sign indicates that mass is leaving the compartment.

## 1.5 Temperature Dependence

---

The rates of most chemical reactions increase as the temperature is raised. As a rule of thumb, a typical reaction rate will double for every ten degree Celsius increase. This increase can be measured as a change in the rate constants for the reaction. Some reactions show a more complex response to an increase in temperature. For example, enzyme catalyzed reactions tend to increase in rate but at a certain temperature (often around 43° C), the reaction rapidly falls as the enzyme denatures.

In many cases it has been found that the temperature dependence of a reaction's rate constant follows the **Arrhenius equation**:

$$k = Ae^{-E_a/RT}$$

where  $k$  is the reaction rate constant,  $A$  is the pre-exponential factor,  $E_a$  is the activation energy,  $R$  is the gas constant and  $T$  the temperature. This relation was proposed by the Swedish chemist Svante Arrhenius in 1889.

Figure 1.7 shows the rate constant as a function of the activation energy. The lower the  $E_a$  the higher the forward and reverse rate constants. By taking natural logarithms on both sides, the equation can be expressed as:

$$\ln k = \ln A - \frac{E_a}{RT}$$

Thus, a plot of  $\ln k$  versus  $1/T$  is linear with a slope of  $-E_a/R$  and the  $y$  intercept,  $\ln A$ .

The constants that appear in the Arrhenius equation can be interpreted in terms of collision theory. Chemical reactions occur between two molecules

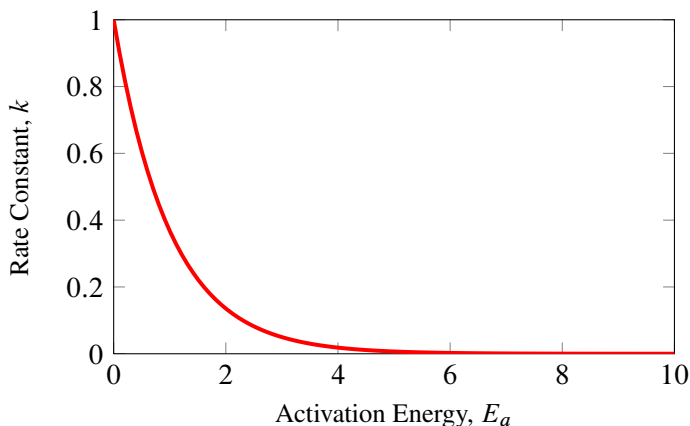


Figure 1.7: Reaction rate constant as a function of the activation energy,  $E_a$ .

when they collide. However, not all collisions lead to a reaction. In particular, a collision must occur at a sufficiently high energy (and orientation) in order for a reaction to occur. The activation energy,  $E_a$  is the minimum kinetic energy that reactants must have during a collision in order to form products while the pre-exponential factor can be interpreted as the rate at which collisions occur per unit time and volume.

## Catalysis

A catalyst is a substance that can accelerate a chemical reaction without itself being consumed. Catalysts often operate by lowering the activation energy,  $E_a$ , of a reaction. This means that a catalyst cannot change the equilibrium constant of the reaction. A catalyst will only accelerate a reaction towards its equilibrium point. Many catalysts are highly specific and in biology the most common catalysts are enzymes. Catalysts work by enabling alternative reaction paths that require less activation energy, for example by changing bond polarity or orientating molecules into a more favorable position.

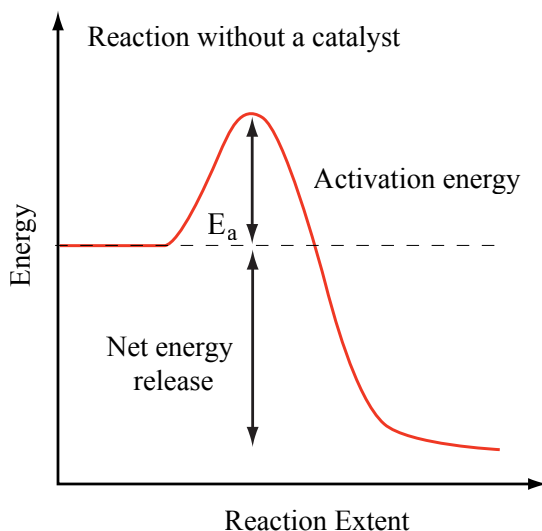


Figure 1.8: The energy profile of a reaction. The graph shows the potential energy profile as the reaction proceeds. The activation energy is the height of the barrier.

A common feature of catalysts is that the degree of reaction acceleration depends on the concentration of catalyst. This means a catalyst will appear in a rate law as an additional concentration factor that is not part of the stoichiometric component. Given a mass-action rate law, the simplest way to introduce a catalyst,  $E_i$ , is as a linear multiplier. For example:

$$v = E_i(k_1 A - k_2 B)$$

In this form the catalyst obeys the usual catalytic rules, that is both forward and reverse rates are equally affected and the catalyst appears as an additional concentration factor, independent of the reaction stoichiometry.

## Chapter Highlights

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This chapter provides a brief introduction to chemical kinetics. It is not meant to be comprehensive and omits a number of important topics nor-

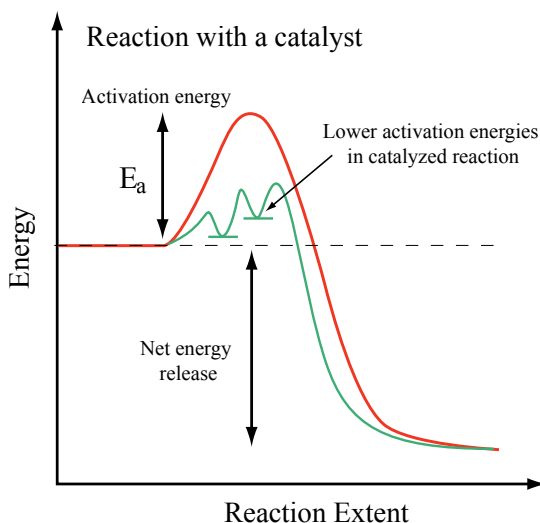


Figure 1.9: The effect of a catalyst on the activation energy of a reaction.

mally associated with chemical kinetics. For example, no mention is made of how one can distinguishing between first and second order kinetics. Little is provided on experimental methods available to measure rates of change and how such data can be used to determine reaction mechanisms using either log plots or integrated rate laws. What is presented is an introduction to reaction rates, the difference between stoichiometric amounts, stoichiometric coefficients, reaction rates and rates of change. The difference between these terms is subtle and the reader should take note as to their distinct meaning.

The **stoichiometric amount** refers to the number of reactant and product molecules involved in a particular reaction. The **stoichiometric coefficient** refers to the net stoichiometry of a given species and takes into account whether a species occurs as both a reactant and product. The **reaction rate** is defined the rate of change of a given species, normalized by the stoichiometric coefficient. Conversely, the **rate of change** of a particular species is defined as the reaction rate multiplied by the stoichiometric coefficient. Strictly speaking only the rate of change can be directly measured

by experiment.

Reactions can be classified as elementary or non-elementary. Elementary reactions involve no reaction intermediates and can be classified according to the order of reaction for the species involved. Common reaction orders include zero, first and second-order depending on the power to which the reaction species is raised. Unless there is specific mechanistic information, it is possible to assume as a first approximation, that the rate of an elementary reaction is proportional to the product of each reactant species, raised to its stoichiometric amount.

Chemical equilibrium is a fundamental aspect of any chemical reaction and describes the state when the net reaction rate is zero. The equilibrium constant is defined as the ratio of the product species (raised to their stoichiometric amounts) and reactant species at equilibrium. Two convenient measures, called the **mass-action ratio** and the **disequilibrium ratio** allow us to easily summarize whether a reaction is at equilibrium, is progressing from left to right, or from right to left. These measures also allow us to modify elementary reaction rate laws by eliminating the reverse rate constant and instead use the more readily available equilibrium constant.

## Further Reading

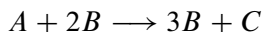
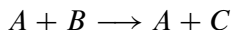
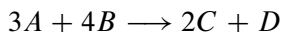
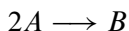
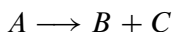
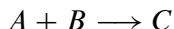
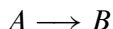
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1. Atkins P and de Paula J (2006) *Physical Chemistry for the Life Sciences*. Oxford University Press, W. H. Freeman and Company, New York. ISBN: 0-7167-8628-1
2. Chang R (2005) *Physical Chemistry for the Biosciences*. University Science Books. ISBN-10: 1891389335

## Exercises

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1. Determine the stoichiometric amount and stoichiometric coefficient for each species in the following reactions:



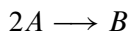
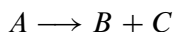
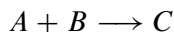
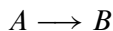
2. A culture of a newly engineered microorganism that can convert glucose to butyric acid is started with 50 gms of glucose. Calculate the maximum amount of butyric acid that could be produced if all the glucose were consumed. Determine the amount of butyric acid produced and the percentage yield.



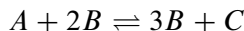
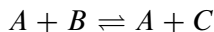
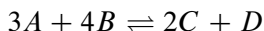
3. The following table shows data from an experiment that measures the concentration of product over time. Use the data to estimate the average reaction rate.

Time (mins)	Concentration (M)
0	0
1	0.09
2	0.18
3	0.27
4	0.35

- Define the terms: Stoichiometric amount, Stoichiometric coefficient, reaction rate, rate constant
- Assuming that the following reactions are irreversible elementary reactions, write out the reaction rate laws for each:

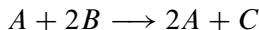


Assuming the following reactions are reversible, write out the reaction rate laws:



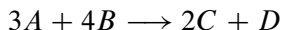
- A reaction mix starts with an initial concentration of substance  $A$  of 200 mM. The reaction is known to follow first-order kinetics. After 45 seconds the concentration of reactant is 100 mM. Assuming that the product has no effect on the reaction rate, estimate the rate constant of the reaction.
- In the following two reactions what are the rates of change for each species? Assume a reaction rate of  $3.5 \text{ mol t}^{-1}$  in each case.
  - $A + 2B \longrightarrow 3C$
  - $2A + B \longrightarrow 3B + A + C$
- What does it mean when we say that a reaction  $A \rightarrow B$  has a reaction rate of  $-6 \text{ mol t}^{-1}$ ?

9. In the following reaction, the rate of change of species  $A$  was found to be  $+5.0 \text{ mol } t^{-1}$ . Assume that the reaction is going from left to right.



What is the rate of change of the species  $B$  and  $C$  and what is the rate of reaction?

10. The *total* concentration of glucose-6-phosphate and fructose-6-phosphate is  $4.9 \text{ mM}$ . Given that the equilibrium constant between the two species is  $0.395$ , calculate their equilibrium concentrations.
11. For the following reaction, show that the equilibrium constant is the ratio of the forward and reverse rate constants. Assume mass-action kinetics.



12. The permeability coefficient of glucose across a lipid membrane is  $2 \times 10^{-4} \text{ cm s}^{-1}$ . The flux across the membrane, left to right, is  $5 \times 10^{-6} \text{ mol cm}^{-2} \text{ s}^{-1}$ . If the concentration of glucose to the right of the membrane is  $0.1 \text{ mM}$ , what is the concentration of glucose on the left side of the membrane?
13. A reaction rate law for a unimolecular and reversible reaction that incorporates a catalyst,  $E$ , is given by the equation:

$$v = E(k_1A - k_2B)$$

where  $A$  and  $B$  are the reactant and product respectively. Show that the catalyst  $E$  has no effect on the equilibrium constant of the reaction.