KINETICS

Syllabus

Kinetics

(a) Mathematical expression for zero , first, second order reactions and their applications in rate processes inherent to biological systems with special emphasis on transport of drug across biological membrane.

(b) Temperature dependency of degradation and its application in chemical and physical stability testing of drug and dosage form.

RATES AND ORDERS OF REACTIONS

Rate

The rate of a chemical reaction of process is the velocity with which the reaction occurs. Let us consider the following reaction:

drug A $\rightarrow$ drug B

If the amount of the drug A is decreasing with respect to time (i.e. the reaction is going in a forward direction), then the rate of this reaction can be expressed as follows:

$-\frac{dA}{dt}$ The negative sign appears because concentration of drug A decreases with time.

Since the amount of drug B is increasing with respect to time, the rate of the reaction can also be expressed as:

$+\frac{dB}{dt}$ The positive sign appears because the concentration of the product B increases with time.

Usually, in pharmacokinetics, only the parent (or pharmacologically active) drug is measured experimentally. The metabolites of the drug or the products of the decomposition of the drug may not be known or may be very difficult to quantitate. Hence, the rate of a reaction is determined experimentally by measuring the disappearance of drug A at given time intervals.

Order of a reaction

If C is the concentration of drug A, the rate of decrease in C (of drug A) can be expressed by a general expression as function of time, t as: $\frac{dC}{dt} = -kC^n$ .....eqn. 1

Where k = rate constant and n = **order of the reaction**.

If n = 0 then the reaction is called a zero-order reaction,

if n = 1 then the reaction is called a first-order reaction.

if n = 2 then the reaction is called a second order reaction.

If a reaction is: $aA + bB \rightarrow$ Product

and if the reaction rate = $-k [A]^a [B]^b$ then the reaction is said to be (a + b) order.

***Example*:** In the reaction of ethyl acetate and sodium hydroxide in aqueous solution, for example,

$$CH_3COOC_2H_5 + NaOH_{soln} \rightarrow CH_3COONa + C_2H_5OH$$

the rate expression is

$$Rate = -\frac{d[CH_3COOC_2H_5]}{dt} = -\frac{d[NaOH]}{dt} = k[CH_3COOC_2H_5]^1[NaOH]^1$$

The reaction is first-order (a = 1) with respect to ethyl acetate and first-order (b = 1) with respect to sodium hydroxide solution; overall the reaction is second-order (a + b = 2).

Suppose that in this reaction sodium hydroxide and water was in great excess and ethyl acetate was in a relatively low concentration. In this case the concentration of sodium hydroxide may be taken as constant and the rate equation can then be written as:

$$Rate = -\frac{d[CH_3COOC_2H_5]}{dt} = k'[CH_3COOC_2H_5]^1$$

in which k' = k [NaOH]. The reaction is then said to be *pseudo-first order*, because it only depends on the first power (a = 1) of the concentration of ethyl acetate.

Zero-Order Reactions

If the concentration of drug A is decreasing at a constant time interval t. then the rate of disappearance of drug A is expressed as:

$$\frac{dC}{dt} = -k_0 \quad \text{.....eqn. 2}$$

The term k_0 is the zero-order rate constant and is expressed in units of concentration / time [e.g. (mg/ml)/ min).]

or $dC = -k_0\, dt$

Integrating both sides: $\int dC = -k_0 \int dt$

or, $\boxed{C = -k_0 t + C_0}$ eqn.3

where C_0 is the concentration of drug at t= 0.

Half life of the reaction*:*

At $t = t_{1/2}$ $C = \frac{1}{2} C_0$.

Replacing t and C in eqn. 3 $\frac{1}{2} C_0. = - k_0 t_{1/2} + C_0.$

or, $k_0 t_{1/2} = \frac{1}{2} C_0.$

or, $\boxed{t_{1/2} = \frac{1}{2}\frac{C_0}{k_0}}$

Unit of k $\frac{mg}{ml \text{ min}}$

Graphical representation

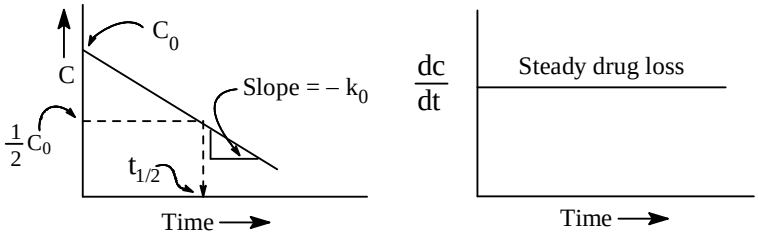


Fig: Plot of Conc vs. time Fig. Rate vs. time

Example

A drug in suspension follows apparent zero-order kinetics in which the concentration of the drug in the solution remains constant with time. When the drug in the solution degrades or lost by any means new drug molecules from the suspended solid particles dissolve in the solution to keep the concentration constant at the **equilibrium solubility**. That is the solid suspended particles acts as reservoir of drug.

1st Order Reactions

In first order reaction the rate of reaction is proportional to the concentration of the drug remaining and can be expressed as:

$\frac{dC}{dt} = - kC^1$ or simply $\frac{dC}{dt} = - kC$

$or, \frac{dC}{C} = - k\,dt$ ..... eqn 4

Integrating eqn 4 we get,

$\ln C = -kt + A$ where is a constant

Initially when t = 0, C = C_0. Hence, the equation will be

$\boxed{\ln C = -kt + \ln C_0.}$ ..... eqn. 5

or, $\ln \frac{C}{C_0} = -k\,t$ or, $\frac{C}{C_{.0}} = e^{-k\,t}$ or, $\boxed{C = C_0\, e^{-k\,t}}$ ..... eqn. 6

Another form of eqn. 5 is the expression in $\log_{10}$ format:

$$\boxed{\log C = -\frac{k}{2.303}t + \log C_0 .}$$ ..... eqn. 7

Half life of the reaction

At $t = t_{1/2}$ $C = \frac{1}{2} C_0$.

Replacing t and C in eqn. 7

$$\log \frac{1}{2} C_0 = -\frac{k}{2.303} t_{1/2} + \log C_0 .$$

$$\text{or,} \quad \frac{k}{2.303} t_{1/2} = \log C_0 . - \log \frac{1}{2} C_0.$$

$$\text{or,} \quad t_{1/2} = \frac{2.303}{k} \log \frac{C_0}{C_0/2} = \frac{2.303 \log 2}{k} = \frac{2.303 x\ 0.30103}{k} = \frac{0.693}{k}$$

$$\boxed{\text{or, } t_{1/2} = \frac{0.693}{k}}$$

Graphical representation

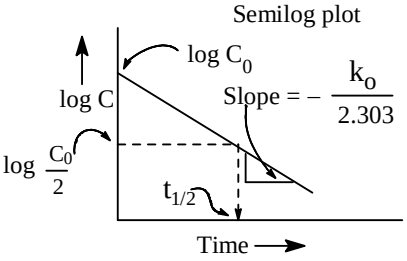


Fig : Plot of eqn. 7

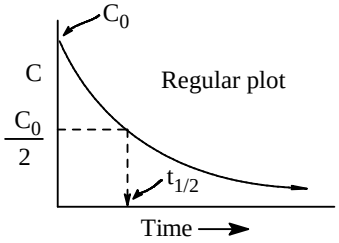


Fig: Plot of eqn.6

Unit of k : min^{-1}.

Example

All the passive transport of drug molecules through the biological membranes follows first order kinetics.

Second order reaction

The rates of bimolecular reactions, which occurs when two molecules come together

$$A + B \rightarrow \text{Products}$$

are frequently described by the second order rate equation.

The rate of the reaction = $-\frac{dA}{dt} = -\frac{dB}{dt} = k[A][B]$

If a and b are the initial concentrations of A and B and x is the concentration of each species reacting in time t, the rate law may be written

	Conc. A	Conc. B
At time t = 0	a	b
At time t = t	a – x	b – x

$\therefore$ Rate = $\frac{dx}{dt} = k(a - x)(b - x)$ ..... eqn 8

[Here dx/dt is a positive term because as the reaction proceeds x increases.]

in which dx/dt is the rate of reaction and (a – x) and (b – x) are the concentrations of each reactants remaining at time t

Case-I: When A and B are remaining is the same concentration

That is a = b

then $\frac{dx}{dt} = k(a-x)^2$ ..... eqn 9

Equation 9 is integrated using the conditions that x = 0 at t = 0 and x = x at t = t.

$$\int_0^x \frac{dx}{(a-x)^2} = k\int_0^t dt$$

or, $\frac{1}{a - x} - \frac{1}{a - 0} = kt$

or, $\frac{x}{a(a-x)} = kt$ ..... eqn. 9.1

or, $\boxed{k = \frac{1}{at}\left(\frac{x}{a - x}\right)}$ ..... eqn 10

Case -II: When A and B are remaining in different concentration

Integration of eqn. 8 yields

$$\int_0^x \frac{dx}{(a-x)(b-x)} = k\int_0^t dt$$

or, $$\frac{1}{(a-b)}\left(\int_0^x \frac{dx}{(b-x)} - \int_0^x \frac{dx}{(a-x)}\right) = k\int_0^t dt$$

or, $$\frac{2.303}{(a-b)} \log\frac{b(a-x)}{a(b-x)} = kt$$ ..... eqn 10.1

or, $$\boxed{k = \frac{2.303}{t(a-b)} \log\frac{b(a-x)}{a(b-x)}}$$ ..... eqn 11.

Graphical representation

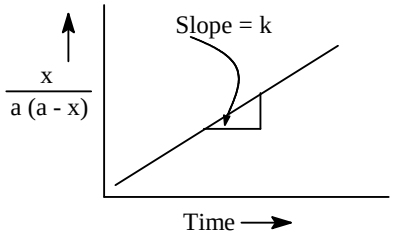


Fig: Plot of equation 9.1

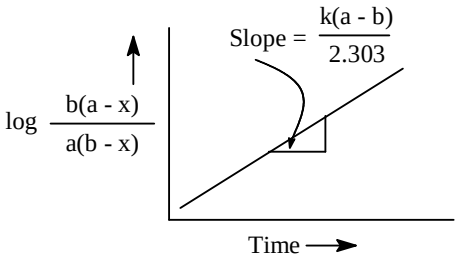


Fig. Plot of eqn 10.1

Determination of Order of a reaction

The order of a reaction may be determined by several methods:

Substitution method:

The data accumulated in a kinetic study may be substituted in the integrated form of the equations that describe the various orders. When the equation is found in which the calculated k values remain constant within the limits of experimental variation, the reaction is considered to be of that order.

Order of reaction	**Integrated rate equation**	
0	$C = C_0 - kt$	$k = \frac{C_0 - C}{t}$
1	$\log C = \log C_0 - kt$	$k = \frac{1}{t} \log \frac{C_0}{C}$
2	$\frac{x}{a(a-x)} = kt$	$k = \frac{1}{at}\left(\frac{x}{a-x}\right)$
	or, $\frac{2.303}{(a-b)} \log \frac{b(a-x)}{a(b-x)} = kt$	$k = \frac{2.303}{t(a-b)} \log \frac{b(a-x)}{a(b-x)}$

Graphical method

A plot of the data in the form of the equations of the reaction or various order may be used to ascertain the order of the reaction. Straight line will result if the data set obeys the following conditions:

The reaction is of zero order if C vs t produce straight line.

The reaction is of 1st order if log C vs t produce straight line.

The reaction is of 2nd order if either $x/\{a(a-x)\}$ vs. t produces straight line

or

$\log \{b(a-x)\}/\{a(b-x)\}$ vs. t produces straight line.

Half life method

First the half lives are obtained graphically plotting a vs. t at two different initial concentrations and reading the time at $a_1/2$ and $a_2/2$.

In zero-order the half life is proportional to the initial concentration i.e. $t_{1/2} = a/2k$

In first-order reaction the half life is independent of the initial concentration i.e. $t_{1/2} = 0.693 / k$

In second-order reaction where the initial concentration of all the reactants are same (i.e. a = b) the half life of the reaction is proportional to (1/a) . i.e. $t_{1/2} = (1 / ak)$.

TRANSPORT OF DRUG ACROSS CELL MEMBRANES

For systemic absorption, a drug must pass from the absorption site through one or more layers of cells to gain access into the general circulation. For absorption into the cells, a drug must traverse the cell membrane.

STRUCTURE OF CELL MEMBRANE

Cell membrane surrounds the entire cells and acts as a boundary between cell and interstitial fluid. Cell membrane acts as a selective barrier to the passage of molecules. Water, some small molecules, and lipid-soluble molecules pass through such membrane; whereas highly charged molecules and large molecules, such as proteins and protein-bound drugs, do not.

Structure

Cell membrane are generally thin, approximately 70 to 100 $\overset{0}{A}$ in thickness. They are primarily composed of phospholipids in the form of bilayer. Some carbohydrates and proteins are interdispersed within this lipid bilayer.

Lipid bilayer or Unit membrane theory (Proposed by Davson & Danielli; 1952)

According to this theory the cell membrane is composed of two layers of phospholipids between two surface layers of proteins. The hydrophilic "head" groups of the phospholipids facing the protein layers and the hydrophobic "tail" groups of the phospholipids aligned towards the interior.

* This theory can explain:

the observations that lipid-soluble drugs tend to penetrate cell membranes more easily than polar molecules.

* This theory cannot explain:

the diffusion of water, small molecules such as urea, and certain charged ions through this lipid- bilayer.

Fluid mosaic model (Proposed by Singer & Nicolson 1972)

According to this model the cell membrane consists of globular proteins embedded in a dynamic fluid, lipid-bilayer matrix.

Integral proteins are embedded in the lipid bilayer;

Peripheral proteins are associated with the inner surface of the membrane.

The carbohydrates consist of monosaccharides attached together in chains that are attached to proteins (forming glycoproteins) or to lipids (forming glycolipids).

Carbohydrates are always on the exterior side and peripheral proteins are always on the cytoplasmic or inner surface.

The integral proteins provide a pathway for selective transfer of certain polar molecules and charged ion through the lipid membrane.

The principal mechanisms of transport of drug molecules across the cell membrane are :

1. Passive diffusion
2. Carrier mediated transport
    (a) Active transport
    (b) Facilitated transport

3. Vesicular transport
    (a) Pinocytosis
    (b) Phagocytosis
4. Pore transport
5. Ion pair formation

1. PASSIVE TRANSPORT

Passive diffusion is the process by which molecules spontaneously diffuse from a region of higher concentration to a region of lower concentration. This process is passive because no external energy is expended.

Characteristics of passive transport

1. Drug molecules moves from a region of relatively high concentration to one of lower concentration.
2. The rate of transfer is proportional to the concentration gradient between the compartments involved in the transfer.
3. The transfer process achieves equilibrium when the concentration of the *transferable species* is equal on both sides of the membrane.
4. Drugs which are capable of existing in both charged and a non-charged form approach an equilibrium state primarily by transfer of the non-charged species across the membrane.
5. Greater the membrane/water partition coefficient of drug faster the absorption [since the membrane is lipoidal in nature, a lipophilic drug diffuses at a faster rate by solubilising in the lipid layer of the membrane]

Mathematical expression

Passive diffusion is best expressed by *Fick's first law of diffusion* which can be expressed mathematically:

$$\frac{dQ}{dt} = \frac{DAK_{m/w}}{h}\left(C_{GIT} - C_P\right)$$

where, dQ/dt = rate of drug diffusion (mass/time)

D = diffusion coefficient of the drug through the membrane (area/time)

A = surface area of the membrane through which rug diffusion is taking place (area)

$K_{m/w}$ = Partition coefficient of the drug between the lipoidal membrane and the GI-fluids (no units).

Several factors influence the passive diffusion of the drug:

1. The degree of lipid solubility of the drug (Km/w)

Highly lipid soluble drug has large value of Km/w and hence has higher rate of transport.

2. the surface area of the membrane (A)

Duodenal area shows most rapid drug absorption than that of other places of intestine because duodenal area has villi and microvilli, which provide a large surface area. This villi are less abundant in other area of the GIT.

3. thickness of the membrane (h)

Drugs usually diffuses very rapidly through the capillary cell membrane except through the cell membranes present in the capillaries of the brain. In the brain, the capillaries are densely lined with glial cells, so a drug diffuses slowly into the brain.

2. CARRIER MEDIATED TRANSPORT

Some polar molecules cross the membrane more readily than can be predicted from their concentration gradient and partition coefficient values. This suggests the presence of some specialized transport mechanisms without which many essential water-soluble nutrients like monosaccharides, amino acids and vitamins will be poorly absorbed. The mechanism is thought to involve a component of the membrane called as the *carrier* that binds reversibly or noncovalently with the solute molecules to be transported. This carrier-solute complex traverses across the membrane to the other side where it dissociates and discharges the solute molecule. The carrier then returns to its original site to complete the cycle by accepting a fresh molecule of solute. The carrier nay be an enzyme or some other component of the membrane.

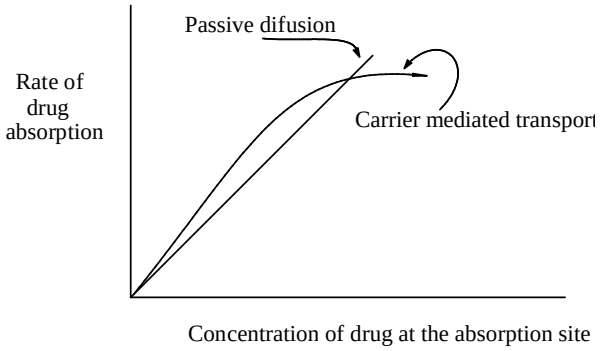
Characteristics of Carrier Mediated Transport:

1. The transport is structure specific i.e. the carrier can bind with a specific chemical structure only. Since the system is structure-specific, drugs having structure similar to essential nutrients, called *false-nutrients* are absorbed by the same carrier system.

e.g. 5-fluorouracil and 5-bromouracil serves as false nutrients.

2. As the number of carrier systems are limited there will be *competition* between similar chemical structures for the carrier molecules.

1. Since there are a finite number of carriers available, the system is *capacity limited*. If the total number of transferable molecules exceeds the number of carrier sites available for transfer, the system will become *saturated*. The system will then be working in full capacity and the transfer of drug may thus occur at a constant rate until the concentration of drug falls below that of the capacity limit of the system.



2. For a drug absorbed by passive diffusion the rate of absorption increases linearly with the concentration but in case of carrier mediated process, the drug absorption increases linearly with concentration until the carriers become saturated after which it becomes curvilinear and approach a constant value at higher doses. Such a capacity limited process can be adequately described by **mixed order kinetics** also called as **Michaelis-Menten saturation** or **non-linear kinetics**.

The process is called mixed order because it is first order at subsaturation drug concentration but apparent zero order at and above saturation levels.

N.B. The bioavailability of a drug absorbed by such a system decrease with increasing dose – for example vitamins like B_1, B_2 and B_{12}. Hence administration of large dose of such vitamins is irrational.

5. Carrier-mediated absorption generally occurs from specific sites of the intestinal tract which are rich in number of carriers. Such *an area in which the carrier system is most dense* is called as **absorption window**. Drugs absorbed through such absorption windows are poor candidates for controlled release formulations.

Active Transport

1. The drug is transported from a region of lower concentration to a region of higher concentration, i.e. against the concentration gradient.
2. Since the process is occurring against the concentration gradient hence, energy is required in the work done by the carrier.
3. As the process requires expenditure of energy it can be inhibited by metabolic poisons that interfere with energy production like fluorides, cyanide and dinitrophenol and lack of oxygen.

4. It is a capacity limited process. When all the carriers become saturated the drug is carried at a constant rate.

Endogeneous substances that are transported actively include

sodium, potassium, calcium, iron in ionic state;

certain amino acids and

vitamins like niacin, pyridoxine and ascorbic acid.

Drugs having structural similarity to such agents are absorbed actively, particularly the agents useful in cancer chemotherapy.

Examples: Absorption of 5-fluorouracil and 5-bromouracil via pyrimidine transport system,

Absorption of methyldopa and levodopa via L-amino acid transport system

Absorption of angiotensin converting enzyme (ACE) inhibitor (e.g. enalapril)via the small peptide carrier system

Facilitated diffusion

Facilitated diffusion is also a *carrier mediated* transport system but it *moves along a concentration gradient* (i.e from higher to lower concentration) and hence it *does not require any energy*.

Characteristics: It is a carrier mediated transport system.

The carriers are saturable and structurally selective for a drug and shows competition kinetics for drugs having similar structures.

It does not require any energy expenditure.

3. VESICULAR TRANSPORT

Vesicular transport is the process of engulfing particles or dissolved materials by the cell.

There are two types of vesicular transport – **Pinocytosis** and **Phagocytosis**.

Pinocytosis refers to the engulfment of small solutes or fluid.

Phagocytosis refers to the engulfment of larger particles or macromolecules, generally by macrophages.

Endocytosis and *exocytosis* are the processes of moving macromolecules into and out of a cell, respectively.

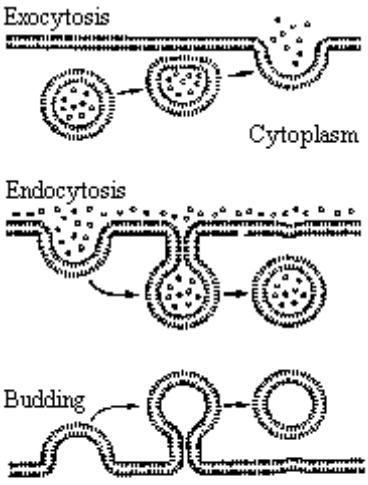


Fig. Diagram showing exocytosis and endocytosis

During pinocytosis or phagocytosis, the cell membrane invaginates to surround the material and then engulfs the material, incorporating into the cell (fig). subsequently the cell membrane containing the material forms a vesicle or vacuole within the cell.

e.g.

- Vesicular transport is the proposed process for the absorption of orally administered *Sabin polio* vaccine and large proteins.
- Transport of proteins, polypeptides like insulin from insulin producing cells of the pancreas into the extracellular space.

4. PORE TRANSPORT

Very small molecules (such as urea, water, and sugars) are able to rapidly cross cell membranes as if the membrane contains channels or pores. [although pores are not evident microscopically]. A certain type of protein called transport protein may form an open channel across the lipid membrane of the cell.

e.g.

- Drug permeation through aqueous pores is used to explain the renal excretion of drugs and the uptake of drugs into the liver.

5. ION PAIR FORMATION

Strong electrolyte drugs are highly ionized or charged molecules, such as quaternary nitrogen compounds with extreme pKa values. Strong electrolyte drugs maintain their charge at all physiologic pH values and penetrate the membrane very poorly.

When ionized drugs is linked up with an oppositely charged ion, an ion pair is formed in which the overall charge of the pair is neutral. This neutral drug-complex diffuses more easily across the membrane.

e.g.

- Propranolol, a basic drug, forms an ion pair with oleic acid.
- Quinine forms an ion pair with hexylsalicylate.

Thermal degradation of drugs:

A number of factors other than concentration may affect the reaction velocity. Among these are temperature, solvents, catalysts and light. The speed of many reaction increases about two to three times with each 10^0 C rise in temperature. The effect of temperature on a rate constant of a reaction is given by the equation, first suggested by Arrhenius,

$$k = A e^{-\frac{Ea}{RT}}$$

$$\log k = \log A - \frac{Ea}{2.303R}\frac{1}{T}$$

in which **k** is the specific reaction rate,

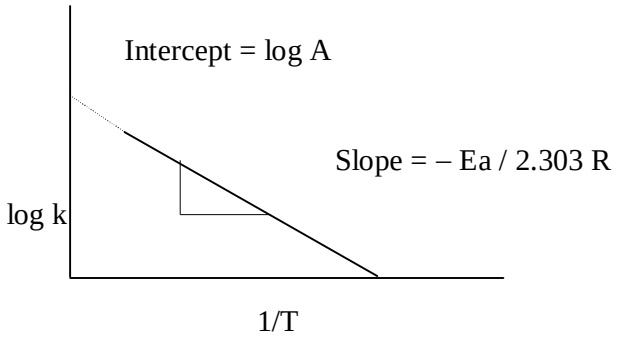
A is a constant known as the Arrhenius factor or Frequency factor

E_a is the energy of activation

R is the universal gas constant

T is the absolute temperature

A plot of log k vs 1/T yields a slope equal to – Ea / 2.303 R from which the value for the energy of activation (Ea) and Arrhenius factor (A) can be calculated.



So, Ea = Slope x 2.303 R

And A = $10^{\text{intercept}}$

Accelerated stability testing

Instabilities in modern formulations are often detectable only after considerable storage periods under normal conditions. To reduce the time required to obtain information, various tests that

involve storage of the products under conditions that accelerate decomposition have been introduced.

Objectives of accelerated stability tests:

(i) The rapid detection of deterioration in different initial formulations of the same product – this is used in selecting the best formulation from a series of possible choices;

(ii) The prediction of shelf life, which is the time a product will remain satisfactory when stored under expected or directed storage condition; and

(iii) The provision of rapid means of quality control, which ensures that no unexpected change has occurred in the stored product.

All these objectives are based on obtaining a more rapid rate of decomposition by applying to the product a storage condition that places a higher stress or challenge to it when compared with normal storage conditions.

Common high stresses or challenges:

(a) Temperature

An increase in temperature causes an increase in the rate of chemical reactions. The products are therefore stored at room temperatures greater than room temperature. The nature of the product often determines the range covered in the accelerated test.

Samples are removed at various time intervals and the extent of decomposition is determined by analysis.

(b) Humidity

Storage of the product in atmospheres of high humidity will accelerate decomposition that result from hydrolysis. Marked acceleration will be obtained if a “naked” product (i.e. not enclosed in a container) is subjected to these tests. This type of stability tests are useful in determining the degree of protection that should be afforded by the container.

(c) Light

A source of artificial light is used to accelerated the effect of sunlight or sky light. the source should emit a similar distribution of radiant energy to that in sunlight because photochemical reactions involve the absorption of light of definite wavelengths.

“Day light” fluorescent lamps provide a satisfactory source.

The prediction of shelf-life:

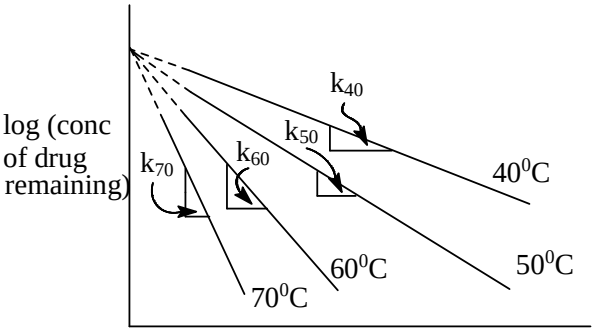
Say, the room temperature = 25^0C

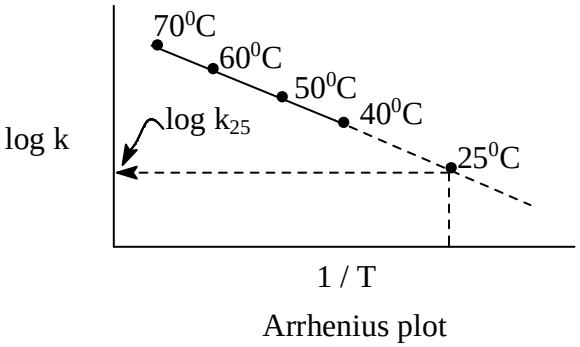
Method 1: Prediction from Arrhenius plot:

Concentration of undecomposed drug is plotted against time (hr) at various temperature above room temperature (25^0C)

The stability constants at various temperatures are plotted in Arrhenius plot (i.e. log k vs 1/T).

From the Arrhenius plot the stability constant at room temperature i.e. (k_{25}) is determined by





extrapolation.

Let us assume that when the drug is 10% decomposed it is to be said that the product has expired.

i.e. at time t = 0 hour drug concentration remaining = 100%

at time = t hour drug concentration remaining = 90%

Now we have to calculate the time 't'.

If the product is kept at room temperature (25^0C)then the following equation from 1st order kinetics may be used:

$\log C = \log Co - (k_{25} / 2.303) \times t$

or, $t = (2.303 / k_{25})$

$= (2.303/k_{25}) \log (Co / C)$

$= (2.303/k_{25}) \log (100/90)$

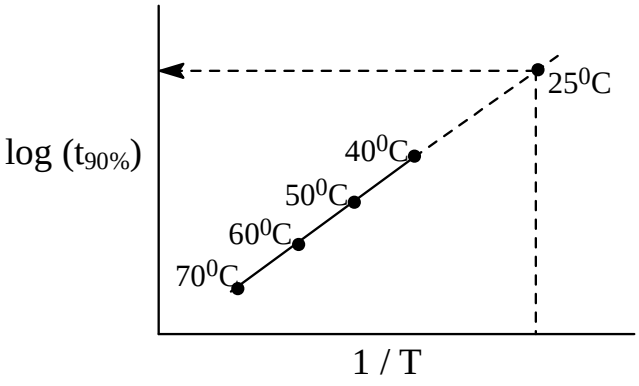
Since k_{25} value is known, therefore **t** can be calculated.

Method -II: Simplified techniques for stability prediction:

Free and Blythe describe such technique for liquid products where the decomposition behaves according to the general kinetic laws.

In this case log(% of drug remaining) is plotted against time (in days).

From the graph the time for the potency (concentration) to fall to 90% of the original value (i.e $t_{90\%}$) are read at different temperature.



Then the log ($t_{90\%}$) is plotted against (1/T) and the time at 25^{0}C gives the shelf life of the product (in days).