The duration of action of a drug is known as its half life \( (t_{1/2}) \). This is the period of time required for the concentration or amount of drug in the body to be reduced by one-half. We usually consider the half life of a drug in relation to the amount of the drug in plasma. A drug’s plasma half-life depends on how quickly the drug is eliminated from the plasma. A drug molecule that leaves plasma may have any of several fates. It can be eliminated from the body or it can be translocated to another body fluid compartment such as the intracellular fluid or it can be destroyed in the blood. The removal of a drug from the plasma is known as clearance and the distribution of the drug in various body tissues is known as the volume of distribution. Both of these pharmacokinetic parameters are important in determining the half life of a drug.

Experimentally the half life can be determined by giving a single dose, usually intravenously and then the concentration of the drug in the plasma is measured at regular intervals. The concentration of the drug will reach a peak value in the plasma and will then fall as the drug clearance progresses.

The time taken for the plasma concentration to halve is the half life of that drug. Some drugs like ibuprofen have very short half lives, others like warfarin and digoxin, take much longer time to eliminate from the plasma resulting in a long half life. So drugs like ibuprofen that are cleared from the blood more rapidly than others need to be given in regular doses to build up and maintain a high enough concentration in the blood to be therapeutically effective.

As repeated doses of a drug are administered its plasma concentration builds up and reaches what is known as a steady state. This is when the amount of drug in the plasma has built up to a concentration level that is therapeutically effective and as long as regular doses are administered to balance the amount of drug being cleared the drug will continue to be active. The time taken to reach the steady state is about five times the half life of a drug. Drugs like digoxin and warfarin with a long half life will take longer to reach a steady state than drugs with a shorter half life.
Sometimes a loading dose may be administered so that a steady state is reached more quickly than smaller ‘maintenance’ doses are given to ensure that the drug levels stay within the steady state.

**1.1 PHARMACOKINETIC PARAMETERS AFFECTING DRUG’S HALF-LIFE**

Absorption, distribution, and elimination processes begin when a dose is administered and may govern the appearance of any therapeutic effect (Fig. 1.1). Pharmacokinetics is used to quantitate these processes. Apart from its usefulness in explaining the safety and toxicity assessment data, pharmacokinetic analysis is used primarily to design appropriate dosing regimens and also to quantitatively define drug disposition. The term pharmacokinetics can be defined as what the body does to the drug. The parallel term, pharmacodynamics, can be defined as what the drug does to the body. A fundamental hypothesis of pharmacokinetics is that a relationship exists between a pharmacologic or toxic effect of a drug and the concentration of the drug in a readily accessible site of the body (e.g., blood). This hypothesis has been documented for many drugs\(^1\),\(^2\); although for some drugs no clear relationship has been found between pharmacologic effect and plasma or blood concentrations.

Among many pharmacokinetic parameters, that can be determined when defining pharmacokinetics of a new drug substance are clearance, effective concentration range, extent of availability, fraction of the available dose excreted unchanged, concentration ratio, toxic concentration, extent of protein binding, volume of distribution and rate of availability.
Figure 1.1 Fate of drug administered in body
1.1.1 Clearance
Clearance (CL) is the measure of the ability of the body to eliminate a drug. Initially, clearance will be looked at from a physiological point of view. The rate of presentation of a drug to a drug elimination organ is the product of organ blood flow (Q) and the concentration of drug in the arterial blood entering the organ (C_A). The rate of exit of a drug from the drug-elimination organ is the product of the organ blood flow (Q) and the concentration of the drug in the venous blood leaving the organ (C_v). By mass balance, the rate of elimination (or extraction) of a drug by a drug-elimination organ is the difference between the rate of presentation and the rate of exit.

\[
\text{Rate of presentation} = QC_A \quad \text{………………..1}
\]

\[
\text{Rate of exit} = QC_V \quad \text{………………..2}
\]

\[
\text{Rate of elimination} = QCA - QC_V
\]

\[
= Q (C_A - C_V) \quad \text{………………..3}
\]

The extraction ratio (ER) of an organ can be defined as the ratio of the rate of elimination to the rate of presentation.

\[
\text{Extraction ratio} = \frac{\text{rate of elimination}}{\text{rate of presentation}}
\]

\[
ER = \frac{Q (C_A - C_V)}{QC_A} \quad \text{……………………………………..4}
\]

The maximum possible extraction ratio is 1.0 when no drug emerges into the venous blood upon presentation to the eliminating organ (i.e., C_V = 0). The lowest possible extraction ratio is zero, when the entire drug passing through the potential drug-eliminating organ appears in the venous blood (i.e., C_V = C_A). Drugs with an extraction ratio of more than 0.7 are by convention considered as high extraction ratio drugs, whereas those with an extraction ratio of less than 0.3 are considered as low extraction ratio drugs.
The product of organ blood flow and extraction ratio of an organ represents a rate at which a certain volume of blood is completely cleared of a drug. This expression defines the organ clearance (CL$_{\text{organ}}$) of a drug.

\[ \text{CL}_{\text{organ}} = \text{QER} = \frac{Q (C_A - C_V)}{C_A} \]  

From equation 3 and 5, one can see that clearance is proportionality constant between rate of elimination and the arterial drug concentration.

At steady state, by definition, rate in equals rate out. Rate is given by the dosing rate (Dose/τ, i.e., dose divided by dosing interval τ) multiplied by the drug availability F, whereas rate out is the rate of elimination (clearance multiplied by the systemic concentration C).

\[ \text{Rate in} = \text{Rate out} \]  

\[ F \left( \frac{\text{Dose}}{\tau} \right) = \text{CL} \left( C \right) \]  

When equation 7 is integrated over time from zero to infinity, equation 8 results.

\[ F \left( \text{Dose} \right) = \text{CL} \left( \text{AUC} \right) \]  

Where AUC is the area under the concentration time curve and F is the fraction of dose available to the systemic circulation.

Clearance may be calculated as the available dose divided by the area under the systemic drug concentration curve.

\[ \text{CL} = \frac{F \left( \text{Dose} \right)}{\text{AUC}} \]  

The maximum value for organ clearance is limited by the blood flow to the organ (i.e., extraction ratio is 1). The average blood flow to the kidneys is approximately 66 L/h and
to the liver, approximately 81 L/h Clearance can occur in many sites in the body and is generally additive. Elimination of a drug may occur as a result of processes occurring in the liver, the kidney, and other organs. The total systemic clearance will be the sum of the individual organ clearances.

\[ \text{CL}_{\text{total}} = \text{CL}_{\text{hepatic}} + \text{CL}_{\text{renal}} + \text{CL}_{\text{other}} \] \hspace{1cm} 10

Among the many organs that are capable of eliminating drugs, the liver, in general, has the highest metabolic capability. Drug molecules in blood are bound to blood cells and plasma proteins such as albumin and alpha-acid glycoprotein. Yet only unbound drug molecules can pass through hepatic membranes into hepatocytes, where they are metabolized by hepatic enzymes or transported into the bile. Thus to be eliminated, drug molecules must partition out of the red blood cells and dissociate from plasma proteins to become unbound or free drug molecules. Because unbound drug molecules are free to partition into and out of the blood cells and hepatocytes, there is equilibrium of free drug concentration between the blood cells, the plasma, and the hepatocytes. The ratio between unbound drug concentration and the total drug concentration constitutes the fraction unbound \((f_u)\)

\[
\text{Fraction unbound} = \frac{\text{unbound drug concentration}}{\text{total drug concentration}}
\]

Or

\[
F_u = \frac{C_u}{C} \hspace{1cm} 11
\]

Because an equilibrium exists between the unbound drug molecules in the blood cells and the plasma, the rate of elimination of unbound drugs is the same in the whole blood as in the plasma at steady state. Thus,

\[
\text{CL}_p C_p = \text{CL}_b C_b = \text{CL}_u C_u \hspace{1cm} 12
\]
Where the subscripts p, b, and u refer to plasma, blood, and unbound, respectively. From the material presented so far, one may intuitively imagine that hepatic drug clearance will be influenced by hepatic blood flow, fraction unbound, and intrinsic clearance; that is, the intrinsic ability of the organ to clear unbound drug. The simplest model that describes hepatic clearance in terms of these physiological parameters is the well-stirred model. Assuming instantaneous and complete mixing, the well-stirred model states that hepatic clearance (with respect to blood concentration) is

\[
CL_{hep} = \frac{Q_{hep} f_u CL_{int}}{Q_{hep} + (f_u CL_{int})} 
\]

where \(f_u\) is calculated from unbound and total concentration in whole blood. Equation 5 advises that hepatic clearance is the product of hepatic blood flow and hepatic extraction ratio. Therefore, as shown in Equation 13, the hepatic extraction ratio is

\[
ER_{hep} = \frac{f_u CL_{int}}{Q_{hep} + (f_u CL_{int})} 
\]

By examining Equations 13 and 14, one finds that for drugs with a high extraction ratio (i.e., ER approaches 1.0), \(f_u CL_{int}\) is much greater than \(Q_{hep}\) and clearance approaches \(Q_{hep}\). In other words, the clearance for a high extraction ratio drug, imipramine for example, is perfusion rate limited. For drugs with a low extraction ratio (i.e., ER approaches zero), \(Q_{hep}\) is much greater than \(f_u CL_{int}\) and clearance is approximated by \(f_u CL_{int}\). An example of a low extraction ratio drug is acetaminophen.

The intrinsic ability of an organ to clear a drug is directly proportional to the activity of the metabolic enzymes in the organ. Such metabolic processes, both in vitro and in vivo, are characterized by Michaelis-Menten kinetics:

\[
\text{Rate of metabolism} = \frac{V_{MAX} C}{K_M + C} 
\]

In which \(V_{MAX}\), the maximum rate at which metabolism can proceed, is proportional to the total concentration of enzyme. \(K_M\) is the Michaelis-Menten constant corresponding to
the drug concentration that yields one-half of the maximum rate of metabolism. Dividing both sides of Equation 15 by the systemic concentration C yields

\[
\frac{\text{Rate of metabolism}}{C} = \frac{V_{\text{MAX}}C}{(K_M + C)}
\]

Because identification of a saturable process occurs only for low extraction ratio compounds (i.e., \( CL_{\text{metabolism}} = f_u \cdot CL_{\text{int}} \)), the relationship between classical enzyme and pharmacokinetics is revealed:

\[
f_u \cdot CL_{\text{int}} = \frac{V_{\text{MAX}}}{(K_M + C)}
\]

Because \( V_{\text{MAX}} \) and \( K_M \) can be obtained from in vitro metabolism experiments, development scientists may reasonably predict the in vivo clearance parameter of a low or intermediate extraction ratio drug from in vitro data, by use of Equations 13 and 17. By use of appropriate scaling factors, the in vivo clearance parameter in humans can be approximated from in vitro metabolism data from other species 4, 5.

The kidneys are also important drug eliminating organs. Renal clearance (\( CL_\tau \)) is a proportionality term between urinary excretion rate and systemic concentration. By integrating Equation 18a over time from zero to infinity, one obtains renal clearance, which is the ratio of the total amount of the drug excreted unchanged to the area under the systemic concentration curve. The total amount of drug excreted unchanged can be measured experimentally or can be calculated from the dose, if the fraction of the available dose excreted unchanged (\( f_e \)) is known.

\[
CL_\tau = \frac{\text{Urinary excretion rate}}{C} = \frac{\text{Total amount of drug excreted unchanged}}{AUC} = \frac{f_e [F(Dose)]}{AUC}
\]

It is obvious from the preceding arguments that the conceptual approach to clearance requires measurements of blood concentrations. Yet, bioanalytical measurements are often carried out in plasma because of the ease of sample handling. However, measuring
the blood to plasma ratio allows one to convert clearance values, determined in plasma, to their corresponding blood values by use of Equation 12.

1.1.2 Bioavailability

The bioavailability of a drug product through various routes of administration is defined as the fraction of unchanged drug that is absorbed intact and then reaches the site of action; or the systemic circulation following administration by any route. For an intravenous dose of a drug, bioavailability is defined as unity. For drugs administered by other routes of administration, bioavailability is often less than unity. Incomplete bioavailability may be attributed to a number of factors that can be subdivided into categories of dosage-form effects, membrane effects, and site of administration effects. Obviously, the route of administration that offers maximum bioavailability is the direct input at the site of action for which the drug is developed. This arrangement may be difficult to achieve because the site of action is not known for disease states and, in other cases, the site of action may be completely inaccessible, even when the drug is placed into the bloodstream. The most commonly used route is oral administration. However, orally administered drugs may decompose in the fluids of the gastrointestinal lumen, or be metabolized as they pass through the gastrointestinal membrane. In addition, once a drug passes into the hepatic portal vein, it may be cleared by the liver before entering into the general circulation. The loss of drug as it passes through drug-eliminating organs for the time is known as the first-pass effect.

The fraction of an oral dose available to the systemic circulation, considering both absorption and the first-pass effect, can be found by comparing the ratio of AUCs after oral and intravenous dosing.

\[ F = \frac{AUC_{\text{oral}}}{AUC_{\text{i.v.}}} \]

If an assumption is made that all of a drug dose is absorbed through the gastrointestinal tract intact, and that the only extraction takes place at the liver, then the maximum bioavailability \( F_{\text{max}} \) is
From Equations 5 and 20, one can derive the following relationship for $F_{\text{max}}$

$$F_{\text{max}} = 1 - (\text{CL}_{\text{hep}} / Q_{\text{hep}})$$

For high extraction ratio drugs, where $\text{CL}_{\text{hep}}$ approaches $Q_{\text{hep}}, F_{\text{max}}$ will be small. For low extraction ratio drugs, $Q_{\text{hep}}$ is much greater than $\text{CL}_{\text{hep}}$ and $F_{\text{max}}$ will approximate one. Recently bioavailability and clearance data obtained from a crossover study of cyclosporine kinetics before and after rifampin dosing revealed a new understanding of drug metabolism and disposition of the compound. Healthy volunteers were given cyclosporine, intravenously and orally, before and after CYP3A (P450 3A) enzymes were introduced by rifampin. As expected, the blood clearance of cyclosporine increased from 0.31 to 0.42 L h$^{-1}$ Kg$^{-1}$ as a result of the induction of the drug’s metabolizing enzymes (i.e., an increase in $V_{\text{max}}$ in Equation 15). There was no change in volume of distribution, but there was a dramatic decrease in bioavailability from 27% to 10% in these individuals.

A decrease in bioavailability is to be expected because cyclosporine undergoes some first-pass metabolism as it goes through the liver after oral dosing. However, if one predicts on the basis of pharmacokinetics what the maximum bioavailability [as calculated by Equation 21 would be before and after rifampin dosing, the maximum bioavailability would decrease from 77% to 68%. Thus, there would be an expected cyclosporine bioavailability decrease of approximately 12% just on the basis of clearance changes resulting from inducing CYP3A enzymes in the liver. In fact, there was a bioavailability decrease of 60%. Furthermore, bioavailability was significantly less than would be predicted at maximum. Although some of that lower bioavailability was attributed to formulation effects, the discrepancy between the theoretical maximum availability and the achievable bioavailability of cyclosporine remained a question. However, on the basis of new findings during the last several years about the high prevalence of CYP3A isozymes in the gut, significant metabolism of cyclosporine in the
gut as well as in the liver was speculated. This hypothesis can consistently explain the significantly lower bioavailability that would be predicted, even if all of the drug could be absorbed into the blood stream. This finding, particularly quantification of the magnitude of gut metabolism (more than two-thirds of the total metabolism for an oral dose of cyclosporine occurs in the gut), would not have been realized, had pharmacokinetics not been used in the analysis of the given data.

1.1.3 Volume of Distribution

The volume of distribution (V) relates the amount of drug in the body to the concentration of drug in the blood or plasma, depending on the fluid measured. At its simplest, this relationship is defined by Equation 22:

\[ V = \text{amount of drug in body}/C \]  

At equilibrium, the distribution of a drug within the body depends on binding to blood cells, plasma proteins, and tissue components. Only the unbound drug is capable of entering and leaving the plasma and tissue compartments. A memory aid for this relationship can be summarized by the expression

\[ V = V_P + V_{TW} f_u / f_u_T \]

Where \( V_P \) is the volume of plasma, \( V_{TW} \) is the aqueous volume outside plasma, \( f_u \) is the fraction unbound in plasma, and \( f_u_T \) is the fraction unbound in tissue. Thus, a drug that has a high degree of binding to plasma proteins (i.e., low \( f_u \)) will generally exhibit a small of distribution. Unlike plasma protein binding, tissue binding of a drug cannot be measured directly. Generally, this parameter is assumed to be constant unless indicated otherwise.
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Figure 1.2 Mean serum IFN-α concentrations after a single $36 \times 10^6$ U dose as an intravenous infusion (O) or an intramuscular (X) or subcutaneous (Δ) injection\(^7\).

In Equation 22, the body is considered as a single homogeneous pool of body fluids as described above for digoxin. For most drugs, however, two or three distinct pools of distribution space appear to exist. This condition results in a time-dependent decrease in the measurable blood or plasma concentration, which reflects distribution into other body pools independent of the body’s ability to eliminate the drug. Figure 1.2 describes mean serum IFN-α concentrations after a 40-min intravenous infusion as well as after intramuscular and subcutaneous injections of the same dose. Note the logarithmic biphasic nature of the mean plasma concentration-time curve after the intravenous infusion. This biphasic nature represents both the distribution and elimination processes.

Generally, comparisons of volume of distribution are made by use of a parameter designated as the volume of distribution at steady state (Vss), which reflects the sum of the volumes of all the pools into which a drug may distribute. Vss can be calculated from the area under the moment curve (AUMC) and the area under the curve (AUC) as defined by Benet and Galeazzi\(^8\):
AUMC can be calculated from areas under a plot of concentration vs. time. Both AUC and AUMC may be calculated from the coefficients and exponents of the equations used to describe the multicompartment nature of drug kinetics as depicted in Fig.1.2. In addition to the three parameters, clearance (CL), bioavailability(F) and volume of distribution (V) discussed previously, a fourth parameter, half-life \( t_{1/2} \), is also crucial in therapeutics. Clearance defines the dosing rate, bioavailability defines dose adjustment, and half-life defines the dosing interval. Volume of distribution defines the loading dose.

### 1.1.4 Dosing Rate

As discussed in Equation 6, rate of presentation equals rate of exit at steady rate. Whereas rate of presentation is the product of bioavailability and dosing rate, rate of exit is the product of clearance and average (steady-state) concentration. By replacing the average concentration with the target concentration, the dosing rate can be computed with known values of bioavailability and clearance.

\[
\text{Rate in} = \text{rate out} \quad \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdOTS
administer either a completely bioavailable intravenous (i.v.) infusion at 100 mg/min or a sustained release oral dosage form with 50% bioavailability at 200 mg/min. As shown in Equations 7a and 7b, the actual dosing rate depends on the bioavailability of the dosage form. For proper dosing adjustment, the bioavailability (F) of a given dosage form stands as a must-know parameter in therapeutics.

1.1.6 Dosing Interval

Half-life ($t_{1/2}$) is an extremely useful kinetic parameter in terms of therapeutics, given that this parameter, together with therapeutic index, helps define the dosing interval at which drugs should be administered. By definition, half-life is the time required for 50% of the drug remaining in the body to be eliminated. In three and one-third half-lives, 90% of the dose would have been eliminated. Table 2 shows the percentage of dose lost in different numbers of half-lives.

**Table 1.1 Percentage of Dose Lost as a Function of Number of Half-Lives**

<table>
<thead>
<tr>
<th>Number of Half-Lives</th>
<th>Dose Lost (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>87.5</td>
</tr>
<tr>
<td>3.3</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>93.8</td>
</tr>
<tr>
<td>5</td>
<td>96.9</td>
</tr>
</tbody>
</table>

If the dosing interval is long relative to the half-life, large fluctuations in drug concentration will occur. On the other hand, if the dosing interval is short relative to half-life, significant accumulation will occur. The half-life parameter also allows one to predict drug accumulation within the body and quantifies the approach to plateau that occurs with multiple dosing and constant rates of infusion. Conventionally, three and one third half-lives are used as the time required achieving steady state under constant
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infusion. The concentration level achieved after this time is already 90% of the steady-state concentration and, clinically, it is difficult to distinguish a 10% difference in concentrations.

After determining the extent of oral drug bioavailability, half-life is probably the next most important parameter in terms of deciding the appropriateness of a drug for further development. Drugs with very short half-lives create problems in maintaining steady-state concentrations in the therapeutic range. Thus, a successful drug product with a short half-life will require a dosage form that allows a relatively constant prolonged input. Drugs with a very long half-life are favored in terms of efficacy considerations; however, this long half-life may be a negative characteristic in terms of toxicity considerations. Figure 1.3 illustrates the importance of half-life in defining the dosing interval.

Figure 1.3 Relationship between frequency of dosing and maximum plasma concentrations when a steady-state plasma level of 15 µg/mL is desired. The time course of plasma concentrations for 43.2 mg/h intravenous infusion, and 8-hourly 340 mg oral dose, and a 24-hourly 1020 mg oral dose are depicted.
Figure 1.3 depicts the relationship between the frequency of theophylline dosing and the plasma concentration time course when a steady-state theophylline plasma level of 15 μg/mL is desired. The smoothly rising curve shows the plasma concentration achieved with an i.v. infusion of 43.2 mg/h to a patient exhibiting an average theophylline clearance of 0.69 mL/min.kg. The steady state theophylline plasma level achieved is midway within the therapeutic concentration range of 10-20 μg/mL. The figure also depicts the time courses for 8-hourly administration of 340 mg and 24 hourly administration of 1020 mg, assuming that these dose are administered in an immediate release dosage form. In each case the mean steady state concentration is 15 μg/mL. However the peak to trough ratio and the concentrations achieved with once daily dosing (i.e., 1020 mg) of a rapidly released formulation would result in concentrations in the toxic range, exceeding 20 μg/mL for certain periods of time as well as concentration less than 10 μg/mL, for significant periods during each dosing interval. In contrast the 8 hourly dosing, which is approximately equivalent to the half-life of theophylline, shows a twofold range in peak to trough, and theophylline levels stay within the therapeutic plasma concentration range.

Although half-life is a very important parameter in therapeutics for defining the dosing interval, half-life can be a very misleading parameter when one is attempting to use pharmacokinetics as a tool in defining drug disposition. As depicted in Equation 25, half-life varies as a function of the two physiologically related parameters, volume and clearance.

\[ t_{1/2} = 0.693 \frac{V}{CL} \]  

As clearance decreases, because of a disease process, half-life would be expected to increase. However, this reciprocal relationship is exact only when the disease does not change the volume of distribution. For example, as Klotz and coworkers have shown, the increase in half-life of diazepam with age does not result from a decrease in clearance but rather results from an increase in volume as the patient ages.\(^9\) Clearance, a measure of the body's ability to eliminate the drug, does not significantly decrease with age for diazepam. However, when volume increases, fewer drugs is in the blood flowing to the liver and elimination can occur only for those molecules that come
into contact with the liver. Thus the time that the drug remains in the body is increased. The half-life of Tolbutamide decreases in patients with acute viral hepatitis; that is; the drug appears to be eliminated faster by a diseased liver, the exact opposite from what one might expect. Here, the disease appears to decrease protein binding in both plasma and tissues, causing no change in volume of distribution but an increase in free fraction in the plasma, which results in an increase in total clearance and, subsequently, a decrease in half-life\textsuperscript{10}.

Equation 25 describes the half-life relationship for a drug that appears to follow compartment body kinetics; that is, when the body is considered to be a single homogeneous pool of body fluids. However, many drugs appear to exhibit multiple distribution pools and therefore may have multiple half-lives (as was depicted in Fig. 1.2 for IFN-a). Drugs with multiple half-lives are usually reported in the literature as having "distribution" and "terminal elimination" half-lives. Defining the "relevant" half-life in such situations has been addressed by Benet and coworkers\textsuperscript{11, 12}.

Consider the situation in which a drug is best described by a two-pool model, as has been suggested for IFN-a by Wills et al.\textsuperscript{7}. The data in the Wills study were recalculated to represent the equation describing the concentration (ng/mL) of the drug after a 228 µg dose of IFN-a as a function of time (h), as given in equation 26.

\[
C = 14.13e^{-0.04t} + 0.545e^{-0.136t} \tag{26}
\]

The disposition constants in the exponents of Equation 26 correspond to half-lives of 0.667 h (40 min) and 5.1 h. In the interferon literature, the 5.1-h half-life is generally referred to as the mean elimination half-life, whereas the 40-min half-life, if mentioned at all, is generally referred to as a distribution half-life. These representations may not be accurate; the relevance of a particular half-life may be defined in terms of the fraction of the clearance that is related to each half-life. Note in Equation 8 that clearance is inversely related to area under the drug concentration time curve (AUC). When the equation describing the time course of drug concentrations requires more than one exponential term, this circumstance can be represented by Equation 27. For an n-compartment pharmacokinetic model,
\[ C = L_1 e^{-\lambda_1 t} + L_2 e^{-\lambda_2 t} + \ldots + L_n e^{-\lambda_n t} \]  

AUC can be calculated as the ratio of coefficients and exponents as in Equation 28.

\[ \text{AUC} = \frac{L_1}{\lambda_1} + \frac{L_2}{\lambda_2} + \ldots + \frac{L_n}{\lambda_n} \]  

Calculating AUC for IFN-a as described in equation 26 yields

\[ \text{AUC (ng h}^{-1} \text{mL}^{-1}) = 13.59 + 4.01 \]

\[ (77\%) \quad (23\%) \]

Note that 77% of the AUC relates to the coefficient and exponent for the 40-min half-life, which suggests that the 5.1-h half-life is in fact a minor contributor to the prediction of steady-state concentrations of IFN-a. The importance of these fractional areas can be observed if Equation 7 is rearranged to predict steady-state concentrations (C_{SS}):

\[ C_{SS} = \frac{F \text{ (Dose)}/ (\tau \text{CL})}{\ldots} \]  

Now, given that CL can be defined as given in Equation 9 as the relationship between an available single dose and AUC, Equation 7c becomes

\[ C_{SS} = \frac{[F \text{ (Dose)}] \text{ AUC}/\tau [F \text{ (Dose)}]}{\ldots} \]  

Thus, the ability to correctly predict C_{SS} is dependent on the accuracy of the measurement of AUC. If the 5.1-h half-life for IFN-a is ignored, the data of Wills et al. suggest that the value for AUC, and therefore the steady state concentration, will be underestimated by only 23%, given that this longer half-life represents a relatively small fraction of the total IFN-a clearance. This error in drug concentrations could probably be ignored with confidence because a 23% difference may often be within analytical error for protein drugs, as well as within the day-to-day variability in a particular patient. The above calculations assume that drug concentrations are important in defining the
efficacy or toxicity of IFN-a. If this is true, the clinician can safely ignore the 5.1-h half-life in patients with normal elimination characteristics because little change in steady-state drug levels will be observed. However, it may be that the response, particularly toxicity, is related to the amount of drug in the body, rather than the systemic concentration. The amount in the body at steady state (Ass) is the product of the systemic concentration and the steady state volume of distribution:

\[ \text{Ass} = \text{Css} \times \text{Vss} \]

As can be seen from Equation 24, the accurate calculation of \( \text{Vss} \) requires an understanding of how AUMC is related to the fractional area of each half-life. The complications of this relationship will not be discussed in this introductory chapter but the correct estimation of \( \text{Vss} \) is always significantly determined by the terminal half-life. More recently, Benet called multiple dosing half-lives, the half-life for a drug that is equivalent to the dosing interval to choose so that plasma concentrations (Equation 31) or amounts of drug in the body (Equation 32) will show a 50% drop during a dosing interval at a steady state. These parameters are defined in terms of the mean residence time in the central compartment (MRTC) and the mean residence time in the body (MRT).

\[ t_{1/2\text{MD}^{\text{plasma}}} = 0.693 \times \text{MRTC} \] \[ t_{1/2\text{MD}^{\text{amount}}} = 0.693 \times \text{MRTC} \]

MRTC in a one-compartment body model is the inverse of the rate constant for elimination. In a multiple-compartment model, where the multiple dosing plasma half-life is useful, MTRC is given by the volume of the central compartment where drug concentrations are measured divided by clearance. MRT in Equation 32 is the ratio of AUMC/AUC.
1.1.7 Loading Dose

For drugs with long half-lives, the time to reach steady state is substantial. In these instances, it may be desirable to administer a loading dose that promptly raises the concentration of a drug in plasma to the projected steady-state value. In theory, only the amount of the loading dose needs to be computed, not the rate of its administration. To a first approximation, this is true. The amount of drug required to achieve a given steady state concentration in the plasma is the amount of drug that must be in the body when the desired steady state is reached. For intermittent dosage schemes, the amount is that at the average concentration. The volume of distribution is the proportionality factor that relates the total amount of drug in the body to the concentration in the plasma. If a loading dose is administered to achieve the desired steady-state concentration, then

\[
\text{Loading dose} = \text{amount in the body at steady state} = C_{P, \text{SS}} V_{\text{SS}}
\]

For most drugs, the loading dose can be given as a single dose by the chosen route of administration. However, for drugs that follow complicated a multicompartment model, the distribution phase cannot be ignored in the calculation of the loading dose. If the rate of absorption is rapid relative to distribution (this circumstance is always true for i.v. bolus administration), the concentration of drug in plasma that results from an appropriate loading dose can initially be considerably higher than desired. Severe toxicity may occur, albeit transiently. This may be particularly important, for example, in the administration of anti arrhythmic drugs, where an almost immediate toxic response is obtained when plasma concentrations exceed a particular level. Thus, although estimation of the amount of the loading dose may be quite correct, the rate of administration can be crucial in preventing excessive drug concentrations, and slow administration of an i.v. drug (over minutes rather than seconds) is almost always wise.
1.2 STRATEGIES TO MANAGE DRUG METABOLISM FOR ENHANCING HALF-LIFE

A variety of methods have been used to circumvent the rapid metabolism of certain drugs. These methods seek to improve drug therapy decreasing the overall extent of metabolism and increasing the duration of action. In some instances, these methods have provided increased site specificity.

1.2.1. Pharmaceutical strategies involve the use of different dosage forms to either avoid or compensate for rapid metabolism\(^ {13} \).

**Sublingual tablets** are useful for delivering drugs directly into the systemic circulation and bypassing hepatic first-pass metabolism. **Nitroglycerin**, a rapidly acting antianginal agent, is essentially ineffective when administered orally due to an extremely high first pass effect but is very effective in treating acute attacks of angina if given sublingually.

**Transdermal patches** and **ointment formulations** provide a continuous supply of drug over an extended period of time and are useful for rapidly metabolizing compounds such as nitroglycerin. These delivery systems, while not suited to treat acute anginal symptoms are effective in providing prophylactic concentrations of nitroglycerin.

**Intramuscular depot injections** also provide a continuous supply of drug over an extended period of time. Highly lipid soluble esters of **estradiol** and **testosterone** (e.g., Estradiol benzoate, testosterone enanthate) are slowly absorbed from their administration site. Hydrolysis of these Prodrugs produces a steady supply of these rapidly metabolized hormones.

**Enteric-coated formulations** can protect acid sensitive drugs as they pass through the acidic environment of the stomach. **Methamine**, **erythromycin**, and **omeprazole** are examples of acid sensitive agents that are available as enteric coated preparations.
Nasal administration allow for the delivery of peptides, such as calcitonin salmon, which have very low (if any) oral bioavailability. Characteristics of lung make it ideal for the administration of peptides. Aerosolized drugs only need to penetrate a thin epithelial layer to reach abundant capillary beds. Additionally the lungs contain protease inhibitors, which allow for greater stability of the peptides.

1.2.2. Pharmacological strategies involve the concurrent use of enzyme inhibitors to decrease drug metabolism. In some instances, the concurrent use of additional agent does not prevent the toxicity caused by metabolites of therapeutic agent.

Levodopa (L-dopa), the amino acid precursor of dopamine, is used in the treatment of Parkinsonism. Unlike dopamine, L-dopa can penetrate the blood brain barrier and reach the central nervous system. When in the brain, it is decarboxylated to dopamine. To ensure that adequate concentrations of L-dopa reach the CNS, peripheral metabolism of the drug must be blocked. The concurrent administration of carbidopa, a dopa decarboxylase inhibitor that can not penetrate the blood brain barrier, prevents peripheral formation of dopamine and allows site specific delivery of dopamine to the CNS.

β-Lactam antibiotics. The antibacterial activity of a number of β-Lactam antibiotics is reduced by microorganisms capable of secreting the enzyme β-Lactamase. This enzyme hydrolyzes the β-Lactam ring and inactivates the antibiotic. To counter this resistance mechanism, a β-Lactamase inhibitor, such as clavulanic acid, is used in conjunction with penicillin, such as amoxicillin, to successfully treat infections caused by β-Lactamase-producing bacteria.

Ifosfamide is an alkylating agent that must undergo in vivo metabolism to produce active nitrogen mustard. In the process of this metabolic activation, significant concentration of acrolein is produced. These acrolein molecules react with nucleophiles on renal proteins and produce hemorrhagic cystitis. To prevent this toxicity, ifosfamide is always
coadministered with mensa, a sulphydryl containing compound that reacts with and neutralizes any acrolein that is present in the kidney.

**HIV protease inhibitors** are extensively metabolized by CYP3A isozymes. In addition, compounds within this class can inhibit these same isozymes. This latter action has been used to optimize therapy. Ritonavir is an HIV protease inhibitor that is known to cause hepatotoxicity at therapeutic doses. Lopinavir is an HIV protease inhibitor that is ineffective if used alone due to rapid CYP3A oxidation. A new strategy combines a low dose of ritonavir with a therapeutic dose of lopinavir. This results in an inhibition of CYP3A, the establishment of adequate plasma levels of lopinavir and therapeutic efficacy without hepatotoxicity.

1.2.3. **Chemical strategies** involve the addition, deletion, or isosteric modification of key functional groups. These molecular modifications hinder or completely eliminate metabolic transformations\(^\text{13}\) (figure 1.4)

![Chemical structures](image)

**Testosterone**  
**Methyltestosterone**

**Figure 1.4 Testosterone hydroxyl group is protected by methyl group from metabolism**

Testosterone is not orally active due to rapid oxidation of its 17- hydroxyl group to a ketone. Addition of a 17α-methyl group converts the labile secondary alcohol to a stable tertiary alcohol. The resulting compound, Methyltestosterone, is only half as potent as
testosterone; however it is not subject to rapid first pass metabolism and can be used orally. A similar strategy has been used to make orally active estradiol analogues.

Isoproterenol is a potent β-adrenergic agonist used for the relief of bronchospasm associated with bronchial asthma. Because it is catechol (i.e., 3, 4-dihydroxy-substituted benzene ring), isoproterenol is subject to rapid metabolism by catechol O-methyl transferase (COMT) and thus has poor oral activity. Alteration of the 3, 4-dihydroxy substitution to a 3, 5-dihydroxy substitution produces metaproterenol, a bronchodilator that is not susceptible to COMT, is orally active, and has a longer duration of action than isoproterenol.

Octreotide is a synthetic octapeptide used to suppress or inhibit severe diarrhea associated with certain tumors. Octreotide mimics the actions of somatostatin, a naturally occurring 14-amino acid peptide. Somatostatin undergoes rapid proteolysis, has a half life of 1-3 minutes, and must be administered as a continuous intravenous infusion. Octreotide contains the amino acid essential for clinical efficacy but replaces two of the amino acids with their D-enantiomers. These unnatural D-amino acids are more resistant to hydrolysis. As a result, octreotide has an increased half life and can be administered as a subcutaneous injection.

There are various strategies that can be used to make drugs more resistant to hydrolysis and drug metabolism, and thus prolong their activity.

1.2.3.1 Steric shields

Some functional groups are more susceptible to chemical and enzymatic degradation than others. For example esters and amides are particularly prone to hydrolysis. A common strategy that is used to protect such groups is to add steric shields, designed to hinder the approach of a nucleophile or an enzyme to the susceptible group. These usually involve the addition of a bulky alkyl group close to the functional group. For example, the t-butyl group in the antirheumatic agent D1927 serves as steric shield and block hydrolysis of
terminal peptide bond (fig. 1.5)

![Steric shield diagram](image)

**Figure 1.5** The use of a steric shield to protect the antirheumatic agent D 1927

Steric shields have also been used to protect penicillins from Lactamases and to prevent drug interacting with cytochrome P450 enzymes.

### 1.2.3.2 Electronic effect of bioisosteres

Another popular tactic used to protect a labile functional group is to stabilize the group electronically using a bioisostere, a chemical group used to replace another chemical group within a drug, without affecting the important biological activity. Other features such as the drugs stability may also be improved. Isosteres and non classic isosteres are frequently used as bioisosteres. For example, replacing the methyl group of an ethanoate ester with an NH$_2$ result in a urethane functional group which is more stable than the original ester (fig. 1.6)

![Isostere diagram](image)

**Figure 1.6** Isosteric replacement of a methyl group with an amino group
The NH$_2$ group is the same valency and size as the methyl group and therefore has no steric effect, but it has totally different electronic properties since it can feed electrons into the carboxyl group and stabilize it from hydrolysis. The cholinergic agonist carbachol is stabilized in this way, as is the cephalosporin cefoxitin.

\[
\text{H}_2\text{N-CO-O-CH}_2\text{-CH}_2\text{-NMe}_3
\]

Carbachol

Alternatively, a labile ester group could be replaced by an amide group (NH replacing O). Amides are more resistant to chemical hydrolysis, due again to the lone pair of nitrogen feeding its electrons into the carbonyl group and making it less electrophilic.

It is important to realize that bioisosteres are not general and are often specific to a particular field. Replacing an ester with a urethane or an amide may work in one category of drugs but not another. One must also appreciate that bioisosteres are different from isosteres. It is the retention of important biological activity that determines whether a group is a bioisostere, not the valency. Therefore non isosteric group can be used as bioisosteres. For example, a pyrrole ring was used as a bioisostere for an amide bond in the development of the dopamine antagonist Du 122290 from sulitopride.
Similarly, thiazoyl rings were used as bioisosteres for pyridine ring in the development of ritonavir.

One is not confined to the use of bioisosteres to increase stability. Groups or substituents having an inductive electronic effect have frequently been incorporated into molecules to increase the stability of a labile functional group. For example, electron withdrawing groups were incorporated into the side chain of penicillins to increase their resistance to acid hydrolysis. Because electron withdrawing group could draw electrons away from the carbonyl oxygen and reduce its tendency to act as a nucleophile (fig. 1.8)
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1.2.3.3 Stereochemical modifications

Steric hindrance and electronic stabilization have often been used together to stabilize labile groups. For example, procaine (Fig. 1.9) is a good local anaesthetic, but it is short lasting because its ester group is quickly hydrolyzed. Changing the ester group to the less reactive amide group reduces chemical hydrolysis. Furthermore, the presence of two ortho- methyl groups on the aromatic ring helps to shield the carbonyl group from attack by nucleophiles or enzymes. This result in longer acting, local anaesthetic lidocaine. Since steric and electronic influences are both involved, the modifications are defined as stereoelectronic. Further successful examples of stereoelectronic modifications are demonstrated by oxacillin and bethanechol.
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Figure 1.9 Stereoelectronic modifications which make lidocaine a longer lasting, local anaesthetic compared with procaine

1.2.3.4 Metabolic blockers

Some drugs are metabolized by the introduction of polar groups at particular positions in their skeleton. For example, the oral contraceptive megesterol acetate is oxidized at position 6 to give a hydroxyl group at that position (Fig. 1.10). The introduction of polar hydroxyl group allows the formation of polar conjugates which can be quickly eliminated from the system. By introducing a methyl group at position 6, metabolism is blocked and the activity of the drug is prolonged\(^{14}\).

Figure 1.10 Metabolic blocking by the addition of a methyl substituent
On the same lines, a popular method of protecting aromatic rings from metabolism at the para-position is to introduce a fluoro substituent. For example, CGP 52411 (Fig. 1.11) is an enzyme inhibitor which acts on the kinase active site of the epidermal growth factor receptor. It went forward for clinical trials as an anticancer agent and was found to undergo oxidative metabolism at the para position of the aromatic rings. Fluoro substituents were successfully added to block this metabolism and the analogue CGP 53353 was also put forward for clinical trials. This tactic was also applied successfully in the design of gefitinib.

\[ \text{Figure 1.11 The use of fluorine substituents as metabolic blockers.} \]

\[
\begin{array}{c}
\text{X = H, CGP 52411} \\
\text{X = OH, metabolite} \\
\text{X = F, CGP 53353}
\end{array}
\]

1.2.3.5 Removal or replacement of susceptible metabolic groups

Certain chemical groups are particularly susceptible to metabolic enzymes. For example, methyl groups on aromatic rings are often oxidized to carboxylic acids. These acids then are quickly eliminated from body. Other common metabolic reaction includes aliphatic and aromatic C-hydroxylation, N- and S-oxidations, O- and S-dealkylations, and deaminations\textsuperscript{14}. Susceptible groups can sometimes be removed or replaced by groups that are stable to oxidation, in order to prolong the lifetime of the drug. For example, the aromatic methyl
substituent of the antidiabetic Tolbutamide was replaced by a chloro substituent to give Chlorpropamide, which is much longer lasting (Fig. 1.12).

![Tolbutamide](image1.png)

**Tolbutamide**

![Chlorpropamide](image2.png)

**Chlorpropamide**

**Figure 1.12 Replacing metabolically labile groups**

This tactic was also used in the design of gefitinib. An alternative strategy which is often tried is to replace the susceptible methyl group with CF₃, CHF₂ or CH₂F. The fluorine atoms alter the oxidation potential of the methyl group and make it more resistant to oxidation.

Another example, where a susceptible ester in cephalosporins is replaced with metabolically stable groups to give cephaloridine and cefalexin.

**1.2.3.6 Group shifts**

Removing or replacing a metabolically vulnerable group is feasible if the group concerned is not involved in important binding interactions with the binding site. If the group is important, then we have to use a different strategy.

There are two possible solutions. We can either mask the vulnerable group on a temporary basis by using a prodrug or we can try shifting the vulnerable group within the
molecular skeleton. The latter tactic was used in the development of salbutamol (fig. 1.13)
Salbutamol was introduced in 1969 for the treatment of asthma, and is an analogue of the
neurotransmitter noradrenaline- a catechol structure containing two ortho phenolic
groups\textsuperscript{14}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{salbutamol_noradrenaline}
\caption{Salbutamol and noradrenaline}
\end{figure}

One of the problems faced by catechol compounds is metabolic methylation of one of the
phenolic groups. Since both phenol groups are involved in hydrogen bonds to the
receptor, methylation of one of the phenol groups disrupts the hydrogen bonding and
makes the compound inactive. For example, the noradrenaline analogue (I in Fig. 1.14)
has useful anti asthmatic activity, but the effect is of short duration because the
compound is rapidly metabolized to the inactive methyl ether (II in Fig. 1.14)

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{metabolic_methylation}
\caption{Metabolic methylation of a noradrenaline analogue}
\end{figure}
Removing the OH or replacing it with a methyl group prevents metabolism, but also prevents the important hydrogen bonding interactions with the binding site. Solution of this problem was to move the vulnerable hydroxyl group out from the ring by one carbon unit. This was enough to make the compound unrecognizable to the metabolic enzyme but not to the receptor binding site.

Fortunately, the receptor appears to be quite lenient over the position of this hydrogen bonding group and it is interesting to note that a hydroxyethyl group is also acceptable. Beyond that, activity is lost because the OH group is out of range, or too large to fit. These results demonstrate that it is better to consider a binding region within the receptor binding site as an available volume, rather than imagining it as being fixed at one spot. A drug can then be designed such that the relevant binding group is positioned into any part of that available volume.

Shifting an important binding but metabolically susceptible group worked for salbutamol, but one cannot guarantee that the same tactic will always be successful. Shifting the group may make the molecule unrecognizable both to its target and to the metabolic enzyme.

### 1.2.3.7 Ring variation and ring substituents

Certain ring systems may be susceptible to metabolism, and so varying the ring might improve metabolic stability. This can be done by adding nitrogen into the ring to lower the electron density of the ring system. For example, the imidazole ring of the antifungal agent toconazole is susceptible to metabolism, but replacement with a 1, 2, 4-triazole ring as in fluconazole results in improved stability\(^\text{14}\) (Fig. 1.15)
**Introduction**

Electron rich aromatic rings such as phenyl groups are particularly prone to oxidative metabolism, but can be stabilized by replacing them with nitrogen containing heterocyclic rings such as pyridine or pyrimidine. Alternatively, electron withdrawing substituents could be added to the aromatic ring to lower the electron density.

Ring variation can also help to stabilize metabolically susceptible aromatic or heteroaromatic methyl substituents. Such substituents could be replaced with more stable substituents, but sometimes the methyl substituent is to be retained for good activity. In such cases, introducing a nitrogen atom into the aromatic/heteroaromatic ring can be beneficial, since lowering the electron density in the ring also helps to make the methyl substituent more resistant to metabolism. For example, F13640 is undergoing phase II clinical trials as an analgesic (Fig. 1.16).

The methyl substituent on the pyridine ring is susceptible to oxidation and is converted to carboxylic acid which is inactive. The methyl group plays an important binding role and has to be present. Therefore the pyridine ring was changed to a pyrimidine ring resulting in a compound (F15599) that has increased metabolic stability without affecting binding affinity.

---

**Figure 1.15 Increasing stability in antifungal agent**

Ticonazole  
Fluconazole
Susceptible to metabolism

Figure 1.16 Stabilizing an aromatic or heteroaromatic methyl substituent by adding a nitrogen to the ring
1.3 DRUG DELIVERY SYSTEMS (DDS) FOR ENHANCING HALF-LIFE

DDS are classified as a function of the structure and the release mechanism into: (a) membrane based systems, where the drug is dispersed inside of a polymer membrane and the release can be controlled by drug diffusion or by osmotic pressure, (b) matrix based systems, where the drug is dispersed in a polymeric matrix and its release can be controlled by drug diffusion or matrix erosion, (c) hydrophilic matrices where the drug release is controlled either by matrix swelling or by the matrix slow dissolution, (d) stimuli responsive systems where drug release is controlled by changes in stimuli such as temperature or pH, and (e), polymer-drug conjugates where drug release is chemically controlled.

Covalent polymer-drug conjugates are a special type of DDS where the drug or bioactive compound (peptides, proteins, growth factors, hormones, enzymes, etc.) is covalently linked to the macromolecular backbone through a physiologically labile bond. The possibility of linking any bioactive molecule to a macromolecular chain makes polymeric conjugated systems very useful for applications not only related to medication, but also in fields as tissue engineering, biosensors, affinity separations, enzymatic processes, cell culture, etc.

1.3.1 Synthetic polymer-drug conjugates (the ringsdorf model)

The concept of covalent polymer-drug conjugates was firstly introduced by Helmut Ringsdorf in 1975, who called them synthetic polymeric drugs or pharmacologically active polymers. His model is based on the covalent link between the drug and a macromolecular backbone through a labile bond.

**Polymer backbone (biodegradable or biostable)**

![Diagram of polymer backbone](https://via.placeholder.com/150)

**Figure 1.17** Ringsdorf model of synthetic polymer drugs.
Figure 1.17 shows the scheme of the Ringsdorf model where a biostable or biodegradable polymer backbone carries three different units. A hydrophilic area used to make the whole macromolecule soluble and non-toxic, a second region in which the drug is linked to the polymer chain, and a third area that incorporates a transport system whose function is to carry the whole polymer to the target cells or site of pharmacological action. The separation of the different areas along the macromolecular chain may be accomplished by statistical terpolymerization or block copolymerization.

In addition, polymer properties can be induced by the polymer specific structural characteristics such as high molecular weight, coil structure, neighbouring groups’ effects, copolymer composition, variable polyelectrolyte charges, flexibility of polymer chains, and microstructure. In this sense, the advantages of this type of DDS are their diversity in composition, molecular structure and molecular weight, being predominantly used hydrophilic systems with polar or ionic groups, which lead to polymer water solubility and reactivity for conjugation with bioactive molecules. The different ways that can be used to chemically link the bioactive molecules to the polymer chains by hydrolysable or biodegradable bonds are multiple and are shown in Figure 18. As it will be discussed below, the reactive groups can be linked at the end of the polymer chain (end groups), or in the side chain (pendant groups).

![Biodegradable bonds diagram](image)

**Figure 1.18** Biodegradable bonds used to conjugate bioactive molecules to polymer backbones.
Other important aspects that are also some limitations of the synthetic polymers used in this type of applications are: a) non-toxic and non-immunogenic, b) clearable from the body without any accumulations in tissues or organs, c) controlled average molecular weight and molecular weight distribution avoiding undesirable responses of low or high Mw polymer-drug conjugates, d) high purity of polymers, e) possible sterilization of polymers with biodegradable character.

These types of DDSs are probably the most attractive devices because they are designed on the molecular basis. However, in despite of the high versatility of this approach, it is difficult to find marketed products mainly because these conjugates are considered as new drug molecules and the path needed to be approved is arduous and expensive.

The three areas of the Ringsdorf model exposed in Figure 1.17 provide the different possibilities from the chemical point of view to design and prepare covalent polymer-drug conjugates with specific applications. The most important requirement of the solubilizer area is to provide non-toxic and non-immunogenic character, as well as to give a soluble character to the polymer chain. Water solubilizing units have generally been introduced with co-monomers such as vinyl pyrrolidone (VP) or acrylamides, whereas in the case of lipid-solubilizing units co-monomers bearing alkyl side chains increase the possible absorption at lipid phases and cell membranes. As mentioned above, the polymer molecular weight and molecular weight distribution are significant as soluble polymers can be excreted via the glomerular kidney membrane which are below 50,000 Da.

The second area of Figure 1.17 is that of the drug bonded to the polymer chain, where aspects as fixing the drug in mild conditions, the nature of the chemical fixing groups (see Figure 1.18), and the nature of the drug are of great interest when designing this type of DDS. The drug fixation must be mild enough to allow attachment without any adverse effect on its biological activity. In this sense, polymerizable active esters and amides have been widely used. Connected to this area is the release of the drug and its relationship with the spacer groups between the polymer and the drug. The drug might be required to be released fast or slow and the release rate can be modulated by the type of the spacer group. A permanent spacer separates the drug from the polymer backbone, and interferes to the biological activity of the bound material, being an example the direct fixation of
enzymes which leads to loss its enzymatic activity when directly bonded to the polymer, activity which is restored when the enzyme is attached through a long alkyl group. Temporary spacer groups are a type of spacers applied to release the active molecule. A typical example is the presence of peptides groups bounded to the polymer chain and to the drug$^{17}$.

The third area of Figure 1.17 is the transport system which function is to induce specific or non-specific resorption at the target cells or local site of pharmacological activity. Specific resorption at the biological target can be achieved by using homing devices, i.e. receptor-active components like immunoglobulin, enzymes, hormones or special drugs, as it has been demonstrated in cancer chemotherapy$^{16}$. On the other hand, non-specific resorption enhancers can be produced by inducing variations in the normal body distribution of polymers that can be expected in surface, membrane and skin active systems with, for example, sulfoxides or formamide groups which enhance the uptake of certain drugs through the skin. Other special type of transport systems are those based on the fixation via reactive esters that can be obtained by local injections into specific areas (tumours or tissues), or by simply fixation of the reactive ester to the intestinal wall.

**Types of polymer-drug conjugates**

The classification of this type of DDS is based on the conjugation sites of the mentioned reactive groups. These reactive groups can be used for conjugations via end groups or pendant polymer groups. In the pendant polymer groups the same or different biomolecules can be conjugated to a given polymer chain by controlling the number of the reactive pendant groups, including spacers between them and the bioactive molecule and maintaining the biodegradable character of the links, whereas in the polymer end group model, the conjugation only can be produced in one or two extremes of the chain ends.

Despite the great versatility in composition, structure and molecular weight of synthetic polymers, one of the simplest polymers, polyethylene glycol, PEG, has been the most widely used in end group synthetic polymer-biomolecule conjugates. This approach has found an important application in the so-called protein PEGylation (see Figure 19), based
on the conjugation of the PEG to protein drugs to protect them from recognition by the immune body system, and to prolong their circulation time in the body, as described by Abuchowski et al. in 1977 about the alterations of immunological properties of bovine serum albumin (BSA), covalently attached to PEG\textsuperscript{18}. All the modifications on PEG have been based on the replacement of the hydroxyl end group, also taken into consideration the influence of the molecular weight of the PEG chains and the site of conjugation, that influence the final properties of the conjugates\textsuperscript{19}. In this sense, PEG with average molecular weight of several thousands have generally been used, and the majority of the PEG-protein conjugations have been performed via the lysine amino group of the protein, although other reactive sites of histidine and cysteine can be employed\textsuperscript{20}. Other type of conjugation developed with PEG are some interesting branched, star and comb-like polymers that have been conjugated with proteins\textsuperscript{21}.

**Figure 1.19** Typical end-group polymer-drug conjugate based on polyethylene glycol (PEG) modifications.

![Typical end-group polymer-drug conjugate based on polyethylene glycol (PEG) modifications.](image)

Many efforts have been made to achieve an efficient and stable coupling of PEG chains to the biopharmaceutical. Very successful developments have been achieved with regard to the variability of the coupling chemistry and the availability of specialized linkers\textsuperscript{22}. Site-specific monoPEGylation is of significant relevance in order to provide highly reproducible products maintaining maximum activity. In the majority of cases, high molecular weight PEG chains (10 – 40kDa) are used for the monoPEGylation of proteins. At best, it is possible to attach one single PEG chain to the N-terminal amino group of a protein by reductive amination. Especially with small proteins, such as cytokines, it is possible to apply genetic methods to introduce rare amino acids, which then can be used for the coupling of PEG\textsuperscript{23}. Preferred for this purpose is a cystein residue which can be
specifically PEGylated at the thiol group by maleimide coupling. Some examples have also been published in which the site-directed PEGylation has been achieved by an enzyme catalyzed reaction with a transglutaminase\textsuperscript{24}.

Branched PEG reagents showed superior protecting properties compared with linear molecules. Today there are only a few commercial examples of such branched PEG molecules, because very often their synthesis is complex and time-consuming. However, some important progress in this field is expected in the next few years.

Meanwhile, there are some successful commercial examples of PEGylated drugs. Many others are under clinical development or close to approval. The first ever PEGylated drug on the market was ADAGEN\textsuperscript{®} (PEGylated Adenosine deaminase), produced by Enzon, which is used for the treatment of a severe combined immune deficiency in children and adolescents (SCID-disease), caused mainly by the lack of the body's own Adenosine deaminase. ADAGEN\textsuperscript{®} was approved under the orphan drug act in 1990 on the basis of a clinical trial with 6 patients. This method of therapy, called enzyme replacement therapy, is the starting point for the treatment of many genetically caused diseases of the metabolism\textsuperscript{25}. The market success of such an enzyme drug for human therapy very often heavily depends on an efficient PEGylation.

A further established PEGylated enzyme drug is, for example, PEG-Aspargase (ONCASPAR\textsuperscript{®}) for the treatment of acute lymphoblastic leukemia in children. The major advantage of ONCASPAR\textsuperscript{®} is a prolonged plasma half-life and a strongly reduced immunogenicity compared to the nonPEGylated Asparaginase. Other PEGylated enzymes to be mentioned are Uricase, Glutaminase, Arginine deiminase and Glucocerebrosidase. These drugs are still under clinical development.

Cytokines are commercially the most important biopharmaceuticals today. Even their efficiency has been improved by PEGylation. Blockbuster products are PEG-\textalpha-Interferon 2b (PEG-INTRON) and PEG-\textalpha-Interferon 2a (PEGASYS)\textsuperscript{26}, which are used for the treatment of infections with hepatitis C virus. Both proteins have a very similar amino acid sequence,
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however they may be distinguished by the kind of PEG chains used for PEGylation. PEG-INTRON has been conjugated with a linear PEG molecule with a molecular weight of 10 kDa, while PEGASYS has been modified with a branched reagent consisting of two 20kDa PEG chains. PEGASYS is superior to PEG-INTRON because of a higher specific activity and a prolonged plasma half-life.

After two decades of experience, PEGylation technology can be regarded as a proved and tested method for the drug delivery of biopharmaceuticals, especially proteins. Already today, biopharmaceuticals are a multibillion-dollar market which is expected to grow rapidly within the next decade. The sales of several PEGylated drugs such as PEG-INTRON have reached 3 billion US $, comparable to the sales of conventional synthetic blockbusters. However, the development of PEG technology is by no means finished. Recent publications reveal that some questions still remain to be resolved. In particular, there is a requirement for new synthetic routes to provide functionally expanded PEG reagents of reproducible high quality. Site-specific conjugation will be one of the major topics for the coming years, especially with regard to technically applicable and commercially reasonable solutions.

Many investigations are under way to explore new fields for the application of PEGylation. These are manifold and not limited to protein drugs or biopharmaceuticals. In the area of oncology, for example, the coupling of conventional synthetic drugs (so-called "small molecules"), such as Ara-C or Camptothecin, to polymers resulted in a significant reduction of toxicity and an increased selectivity for tumour tissue. The latter phenomenon is based on a nonspecific accumulation of large molecules in malignant tissue. This effect is called the EPR effect: enhanced permeability and retention. Other promising applications for PEGylation are PEGylation of liposomes, nonviral gene shuttles, nanoparticles, oligonucleotides and surfaces for technical or medical devices.

Due to the increasing relevance of biopharmaceuticals and the high regulatory demands for their approval, innovative and specialized drug delivery systems have gained considerable importance. Thus, intelligent drug delivery systems such as PEGylation will
Introduction
determine the commercial success of the pharmaceutical industry in the future. With its broad applicability, high efficiency and comparably low costs, PEGylation will maintain a leading position. As a result, PEGylation, alone or in combination with other drug delivery technologies, will be an essential part of a successful strategy for the development of biopharmaceutical drugs.

In a nut shell main advantages of biomolecules conjugated with PEG (PEGylation) are based on the following aspects:

a) PEG is essentially non-toxic (approved for human use)

b) Is easily activated for conjugation and inexpensive

c) Conjugates have improved solubility and stability

d) Posses higher resistance to surface adsorption

e) Exhibit prolonged circulation time in the bloodstream due to increase in the hydrodynamic size (size in solution) of the agent

f) Reduced dosage frequency, without diminished efficacy with potentially reduced toxicity

g) Have reduced immunogenicity and antigenicity because covalent attachment of PEG to a drug or therapeutic protein can mask the agent from the host’s immune system.

h) Have controlled permeability through biological barriers.

i) Increased bioavailability
One of the most important limitations is that many different sites of the protein may be conjugated to the polymer molecules, interfering sometimes with the active recognition pocket and reducing the bioactivity of the protein\textsuperscript{30}. Despite this aspect, there are many examples in the literature of increase biological activity of biomolecules after their end group conjugation to polymer macromolecules\textsuperscript{31}.

1.3.2 Dendrimer as drug carrier

Nanoparticle drug-delivery systems are the popular ones as are able to increase the selectivity and stability of therapeutic agents. However reticuloendothelial system (RES) uptake, drug leakage, immunogenicity, hemolytic toxicity, cytotoxicity, hydrophobicity restrict the use of these nanostructures. These shortcomings are overcome by surface engineering the dendrimer such as Polyester dendrimer, Citric acid dendrimer, Arginine dendrimer, Glycodendrimers, PEGylated dendrimers, etc. The bioactive agents can be easily encapsulated into the interior of the dendrimers or chemically attached i.e. conjugated or physically adsorbed onto the dendrimer surface, serving the desired properties of the carrier to the specific needs of the active material and its therapeutic applications. In addition to supplying a multivalent backbone for drug attachment, dendrimers also provide access to various new polymer architectures that are potentially relevant to drug delivery applications.

A. Description\textsuperscript{32,33,34}

A dendrimer is generally described as a macromolecule, which is characterized by its highly branched 3D structure that provides a high degree of surface functionality and versatility. Dendrimers have often been referred to as the “Polymers of the 21\textsuperscript{st} century”. Dendrimer chemistry was first introduced in 1978 by Fritz Vogtle and coworkers. He synthesized the first “cascade molecules”. In 1985, Donald A. Tomalia, synthesized the first family of dendrimers. The word “dendrimer” originated from two words, the Greek word \textit{dendron}, meaning tree, and \textit{meros}, meaning part. At the same time, Newkome’s group independently reported synthesis of similar macromolecules. They called them \textit{arborols} from the Latin word ‘arbor’ also meaning a tree. The term \textit{cascade molecule} is
also used, but ‘dendrimer’ is the best established one. Due to their multivalent and monodisperse character, dendrimers have stimulated wide interest in the field of chemistry and biology, especially in applications like drug delivery, gene therapy and chemotherapy.

B. Structure 35, 36, 37

Dendrimers are built from a starting atom, such as nitrogen, to which carbon and other elements are added by a repeating series of chemical reactions that produce a spherical branching structure. As the process repeats, successive layers are added, and the sphere can be expanded to the size required by the investigator. The result is a spherical macromolecular structure whose size is similar to albumin and hemoglobin, but smaller than such multimers as the gigantic IgM antibody complex.

Dendrimers possess three distinguished architectural components, namely

(i) An initiator core.

(ii) Interior layers (generations) composed of repeating units, radically attached to the interior core.

(iii) Exterior (terminal functionality) attached to the outermost interior generations.

Figure 1.20: The Dendritic Structure
Components of a Dendrimer Structure

Generation

It is the hyperbranching when going from the centre of the dendrimer towards the periphery, resulting in homostructural layers between the focal points (branching points). The number of focal points when going from the core towards the dendrimer surface is the generation number. That is a dendrimer having five focal points when going from the centre to the periphery is denoted as the 5th generation dendrimer. Here, we abbreviate this term to simply a G5-dendrimer, e.g. a 5th generation polypropylene imine is abbreviated to a “G5-PPI-” dendrimer, The core part of the dendrimer is sometimes denoted generation “zero”, or in the terminology presented here “G0”. The core structure thus presents no focal points, as hydrogen substituents are not considered focal points. Intermediates during the dendrimer synthesis are sometimes denoted half-generations; a well-known example is the carboxylic acid-terminated Poly (amidoamine) dendrimers (PAMAM) dendrimers.

Shell

The dendrimer shell is the homo-structural spatial segment between the focal points, the “generation space”. The “outer shell” is the space between the last outer branching point and the surface. The “inner shells” are generally referred to as the dendrimer interior.

Pincer

In dendrimers, the outer shell consists of a varying number of pincers created by the last focal point before reaching the dendrimer surface. In PPI and PAMAM dendrimers the number of pincers is half the number of surface groups (because in these dendrimers the chain divides into two chains in each focal point).
End-group

It is also generally referred to as the “terminal group” or the “surface group” of the dendrimer. Dendrimers having amine end-groups are termed “amino-terminated dendrimers”.

Types of Dendrimers

Pamam Dendrimer

Poly (amidoamine) dendrimers (PAMAM) are synthesized by the divergent method starting from ammonia or ethylenediamine initiator core reagents. Products up to generation 10\(^7\) (a molecular weight of over 9, 30,000 g/mol) have been obtained (by comparison, the molecular weight of human hemoglobin is approximately 65,000 g/mol). PAMAM dendrimers are commercially available, usually as methanol solutions. Starburst dendrimers is applied as a trademark name for a sub-class of PAMAM dendrimers based on a tris-aminoethylene-imine core. The name refers to the star like pattern observed when looking at the structure of the high-generation dendrimers of this type in two-dimensions.

Pamamos Dendrimer

Radially layered poly (amidoamine-organosilicon) dendrimers (PAMAMOS) are inverted unimolecular micelles that consist of hydrophilic, nucleophilic polyamidoamine (PAMAM) interiors and hydrophobic organosilicon (OS) exteriors. These dendrimers are exceptionally useful precursors for the preparation of honeycomb-like networks with nanoscopic PAMAM and OS domains.

PPI Dendrimer

PPI-dendrimers stand for “Poly (Propylene Imine)” describing the propylamine spacer moieties in the oldest known dendrimer type developed initially by Vögtle. These dendrimers are generally poly-alkyl amines having primary amines as end groups, the dendrimer interior consists of numerous of tertiary tris-propylene amines. PPI dendrimers
are commercially available up to G5, and has found widespread applications in material science as well as in biology. As an alternative name to PPI, POPAM is sometimes used to describe this class of dendrimers. POPAM stands for Poly (Propylene Amine), which closely resembles the PPI abbreviation. In addition, these dendrimers are also sometimes denoted “DAB-dendrimers” where DAB refers to the core structure, which is usually based on Diamino butane.

**Tecto Dendrimer**

These are composed of a core dendrimer, surrounded by dendrimers of several steps (each type design) to perform a function necessary for a smart therapeutic nanodevice. Different compounds perform varied functions ranging from diseased cell recognition, diagnosis of disease state drug delivery, reporting location to reporting outcomes of therapy.

**Multilingual Dendrimers**

In these dendrimers, the surface contains multiple copies of a particular functional group.

**Chiral Dendrimers**

The chirality in these dendrimers is based upon the construction of constitutionally different but chemically similar branches to chiral core.

**Hybrid Dendrimers Linear Polymers**

These are hybrids (block or graft polymers) of dendritic and linear polymers.

**Amphiphilic Dendrimers**

They are built with two segregated sites of chain end, one half is electron donating and the other half is electron withdrawing.
Micellar Dendrimers

These are unimolecular micelles of water soluble hyper branched polyphenylenes.

Multiple Antigen Peptide Dendrimers

It is a dendron-like molecular construct based upon a polylysine skeleton. Lysine with its alkyl amino side-chain serves as a good monomer for the introduction of numerous of branching points. This type of dendrimer was introduced by J. P. Tam in 1988, has predominantly found its use in biological applications, e.g. vaccine and diagnostic research.

Fréchet-Type Dendrimers

It is a more recent type of dendrimer developed by Hawker and Fréchet based on polybenzyl ether hyper branched skeleton. These dendrimers usually have carboxylic acid groups as surface groups, serving as a good anchoring point for further surface functionalisation, and as polar surface groups to increase the solubility of this hydrophobic dendrimer type in polar solvents or aqueous media.

C. Syntheses 41, 43, 44

The syntheses used for dendrimer preparation permit almost entire control over the critical molecular design parameters such as size, shape, surface/interior chemistry, flexibility, and topology. Many dendrimer syntheses rely upon traditional reactions, such as the Michael reaction or the Williamson ether synthesis whilst others involve the use of modern techniques and chemistry, such as solid-phase synthesis, organo-transition-metal chemistry, organosilicon chemistry, organo-phosphorus chemistry, or other contemporary organic methodologies. The choice of the growth reaction dictates the way in which the branching should be introduced into the dendrimer. Branching may either be present in the building blocks as is more often the case or it can be created as a function of the growth reaction, as is the case with the poly (amidoamine)s and the poly (propylene imine)s.
Introduction

‘Divergent’ Dendrimer Growth \(^41,44\)

The synthetic methodology employed in the early dendrimer syntheses came to be known as the ‘divergent’ approach. This name comes from the way in which the dendrimer grows outwards from the core, diverging into space. Starting from a reactive core, a generation is grown, and then the new periphery of the molecule is activated for reaction with more monomers. The two steps can be repeated. The divergent approach is successful for the production of large quantities of dendrimers since, in each generation-adding step, the molar mass of the dendrimer is doubled. Divergently grown dendrimers are virtually impossible to isolate pure from their side products. The synthetic chemist must rely on extremely efficient reactions in order to ensure low polydispersities. The first synthesized dendrimers were polyamidoamines (PAMAMs). They are also known as starburst dendrimers. Ammonia is used as the core molecule & In the presence of methanol, it reacts with methylacrylate and then ethylenediamine is added: \(^44\)

\[
\text{NH}_3 + 3\text{CH}_2\text{CHCOOCH}_3 \rightarrow \text{N(}\text{CH}_2\text{CH}_2\text{COOCH}_3\text{)}_3
\]

\[
3\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2 + \text{N(}\text{CH}_2\text{CH}_2\text{COOCH}_3\text{)}_3 \rightarrow \text{N(}\text{CH}_2\text{CH}_2\text{CONHCH}_2\text{CH}_2\text{NH}_2\text{)}_3 + 3\text{CH}_3\text{OH}
\]

At the end of each branch there is a free amino group that can react with 2 methyl acrylate monomers and 2 ethylenediamine molecules. Each complete reaction sequence results in a new dendrimer generation. The number of reactive surface sites is doubled with every generation the mass increases more than twice \(^41\).
The 'convergent' approach was developed as a response to the weaknesses of divergent syntheses. Convergent growth begins at what will end up being the surface of the dendrimer, and works inwards by gradually linking surface units together with more. When the growing wedges are large enough, several units are attached to a suitable core to give a complete dendrimer. The advantages of convergent growth over divergent growth stem are that only two simultaneous reactions are required for any generation-adding step\(^\text{36}\). The convergent methodology also suffers from low yields in the synthesis of large structures. The convergent growth method has several advantages:

1. Relatively easy to purify the desired product and the occurrence of defects in the final structure is minimized.

2. Possible to introduce subtle engineering into the dendritic structure by precise placement of functional groups at the periphery of the macromolecules.

3. Approach does not allow the formation of high generation dendrimer because steric problems occur in the reactions of the dendrons and the core molecule\(^\text{33}\).
D. Properties of Dendrimers

![Figure 1.22: Correlation between intrinsic viscosity and Molecular weight](image-url)

The classical polymerization process, which results in linear polymers, is usually random in nature and produces molecules of different sizes, whereas size and molecular mass of dendrimers can be specifically controlled during synthesis. Dendrimers are monodisperse macromolecules, unlike linear polymers. Because of their molecular architecture, dendrimers show some significantly improved physical and chemical properties when compared to traditional linear polymers. In solution, linear chains exist as flexible coils; in contrast, dendrimers form a tightly packed ball. This has a great impact on their rheological properties. Lower generation dendrimers, which are large enough to be spherical but do not form a tightly packed surface, have enormous surface areas in relation to volume. In contrast to linear polymers the intrinsic viscosity of dendrimer solutions does not increase linearly with mass but shows a maximum at a specific generation and then begins to decline. Such behaviors are unlike that of linear polymers. This is likely to be because of the way in which dendrimer shape changes with generation, i.e. lower generations adopt a more open planar–elliptical shape with transition to a more compact spherical shape for higher generations. The presence of many chain ends is responsible for high solubility and miscibility and for high reactivity. In the structure of dendrimer (Fig. 1.23) the molecular density is theoretically highest in the periphery of the dendrimers. It has been suggested that back folding of the terminal
branches leads to a more uniform or even reverse density profile \(^{47}\) (Fig. 1.23). In nature tree-like structures have evolved to maximize the exposed surface area, e.g. to maximize the light exposure/number of leaves of a tree. In a similar fashion dendritic architecture creates molecules where a large proportion of the groups are exposed at the surface and which can have very high molecular surface to volume ratios (up to 1000 m\(^2\)/g). The presence of numerous terminal groups in dendrimers facilitates multiple simultaneous interactions of surface groups with the solvent, surfaces or other molecules and, as a consequence, dendrimers tend to show high solubility, reactivity, and binding.

### Table 1.2: Properties of Dendrimer and linear polymers

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Property</th>
<th>Dendrimers</th>
<th>Linear Polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Structure</td>
<td>Compact, Globular</td>
<td>Not compact</td>
</tr>
<tr>
<td>2</td>
<td>Synthesis</td>
<td>Careful &amp; stepwise growth</td>
<td>Single step polycondensation</td>
</tr>
<tr>
<td>3</td>
<td>Structural control</td>
<td>Very high</td>
<td>Low</td>
</tr>
<tr>
<td>4</td>
<td>Architecture</td>
<td>Regular</td>
<td>Irregular</td>
</tr>
<tr>
<td>5</td>
<td>Shape</td>
<td>Spherical</td>
<td>Random coil</td>
</tr>
<tr>
<td>6</td>
<td>Crystallinity</td>
<td>Non-crystalline, amorphous</td>
<td>Semi crystalline/crystalline</td>
</tr>
<tr>
<td></td>
<td></td>
<td>materials</td>
<td>materials</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-lower glass temperatures</td>
<td>-Higher glass temperatures</td>
</tr>
<tr>
<td>7</td>
<td>Aqueous solubility</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>8</td>
<td>Nonpolar solubility</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>9</td>
<td>Viscosity</td>
<td>Non linear relationship with</td>
<td>Linear relation with molecular</td>
</tr>
<tr>
<td></td>
<td></td>
<td>molecular weight</td>
<td>weight</td>
</tr>
<tr>
<td>10</td>
<td>Reactivity</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>11</td>
<td>Compressibility</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>12</td>
<td>Polydispersity</td>
<td>Monodisperse</td>
<td>Polydisperse</td>
</tr>
</tbody>
</table>
This multivalency is of particular importance for biomedical applications. The multiple interactions between surface amines and nucleic acid phosphates are also important for the formation of dendrimers and DNA complexes. Dendrimers’ solubility is strongly influenced by the nature of surface groups. Dendrimers terminated in hydrophilic groups are soluble in polar solvents, while dendrimers having hydrophobic end groups are soluble in nonpolar solvents. In a solubility test with tetrahydrofuran (THF) as the solvent, the solubility of dendritic polyester was found remarkably higher than that of analogous linear polyester. A marked difference was also observed in chemical reactivity.

Effect of Various Factors on the Properties of Dendrimers

Effect Of pH

Amino-terminated PPI and PAMAM dendrimers have basic surface groups as well as a basic interior. For these types of dendrimers with interiors containing tertiary amines, the low pH region generally leads to extended conformations due to electrostatic repulsion between the positively charged ammonium groups. Applying molecular dynamics to predict the structural behaviour of PAMAM dendrimers as a function of pH show that the dendrimer has an extended conformation, based on a highly ordered
structure at low pH (pH<4). At this pH, the interior is getting increasingly “hollow” as the generation number increases as a result of repulsion between the positively charged amines both at the dendrimer surface and the tertiary amines in the interior. At neutral pH, back-folding occurs which may be a consequence of hydrogen bonding between the uncharged tertiary amines in the interior and the positively charged surface amines. At higher pH (pH>10) the dendrimer contracts as the charge of the molecule becomes neutral, acquiring a more spherical (globular) structure, where the repulsive forces between the dendrimer arms and between the surface groups reaches a minimum. At this pH, the conformation has a higher degree of back-folding as a consequence of the weak “inter-dendron” repulsive forces (Figure 1.24).49

![Figure 1.24: Three-dimensional structure of a G6-PAMAM dendrimer, under different pH.](image)

When looking at the pH-dependent conformational changes of PPI dendrimers having acidic (carboxylic acid) end-groups, the picture is somewhat different compared to what is observed for their amino-terminated counterparts (Figure 1.25). Small angle neutron scattering (SANS) and NMR measurements of self-diffusion coefficients at different pH values show that at pH 2 the dendrimer core has the most extended conformation due to the electrostatic repulsion between the positively charged protonated tertiary amines, leading to a large radius of the core, whereas the dendrimer reaches its minimum radius at pH 6, where the amount of positively charged amines equals the amount of negatively charged carboxylic groups (isoelectric point) resulting in a “dense
core” conformation more subjective to back-folding. Thus, at pH 6 some degree of back-folding occurs as a result of attractive interactions between the negatively charged surface carboxy-groups and the positively charged tertiary amines in the inner shells of the dendrimer. At pH 11 the electrostatic repulsion between the negative charged forces the surface groups apart to give a more extended conformation with a highly expanded surface area (Figure 1.25).

![Figure 1.25: Two-dimensional depiction of conformational changes upon different pH of a carboxy-terminated PPI-dendrimer.](image)

**Effect of Solvent**

The ability of the solvent to solvate the dendrimer structure is a very important parameter when investigating the conformational state of a dendrimer. Dendrimers of all generations generally experience a larger extent of back-folding with decreasing solvent quality, *i.e.* decreasing solvation. However, being more flexible, the low generation dendrimers show the highest tendency towards back-folding as a result of poor solvation compared to the higher generation dendrimers. NMR studies performed on PPI dendrimers conclude that a nonpolar solvent like benzene, poorly solvates the dendrons favouring intramolecular interactions between the dendrimer segments and back-folding. However, a weakly acidic solvent like chloroform can act as a hydrogen donor for the interior amines in a basic dendrimer like PPI, leading to an extended conformation of the dendrimer because of extensive hydrogen bonding between the solvent and the dendrimer amines.\(^7\) Both experimental as well as theoretical studies on amino-terminated PPI and PAMAM dendrimers (polar dendrimers) show the tendency that nonpolar aprotic (poor)
solvents induce higher molecular densities in the core region as a result of back-folding, whereas polar (good) solvents solvate the dendrimer arms and induce a higher molecular density on the dendrimer surface. Back-folding of the polar surface groups may expose the more hydrophobic dendrimer parts to the surroundings leading to a decreased surface polarity of the back-folded dendrimer.

![Proposed scheme for solvation of a dendrimer under different solvent conditions.](image)

**Figure 1.26:** Proposed scheme for solvation of a dendrimer under different solvent conditions. (a) Solvation of a polar dendrimer in a protic solvent (“good”) leading to extended conformation exposing a polar surface. (b) Solvation of a polar dendrimer in an apolar aprotic solvent (“poor”) leading to exposure of an apolar surface consisting of alkyl chains by back-folding.

### Effect of Salt

High ionic strength (high concentration of salts) has a strong effect on charged PPI dendrimers and favours a contracted conformation of dendrimers, with a high degree of back-folding somewhat similar to what is observed upon increasing pH or poor salvation. At low salt conditions, the repulsive forces between the charged dendrimer segments results in an extended conformation in order to minimize charge repulsion in the structure (Figure 1.27).
Effect of Concentration

In dendrimers with flexible structures the conformation is not only affected by small molecules like solvents, salts or protons, but may also be sensitive to larger objects, such as other dendrimers or surfaces which can have a great effect on the molecular density and conformation of the dendrimer. Small angle X-ray scattering (SAXS) experiments performed on PPI dendrimers (G4, G5) in a polar solvent like methanol show that the molecular conformation of dendrimers upon increasing concentration becomes increasingly contracted. This molecular contraction may minimize the repulsive forces between the dendrimer molecules and increase the ability of the dendrimers to exhibit a more tight intermolecular packing.
Introduction

Biological Properties

Biological properties of dendrimers are crucial because of the growing interest in using them in biomedical applications. Size is a key determinant of dendrimer cytotoxicity for both PAMAM and PPI dendrimers. Cytotoxicity of PAMAM dendrimers increases with generation for both full generation cationic dendrimers (G2–G4) and the “half-generation” anionic intermediates (G2.5, G3.5). The nature and density of charged groups are other factors that determine dendrimer toxicity. Cationic (surface) charges are in general more toxic but details depend on the specific groups involved, that is, for amines it has been proposed that primary amines are relatively more toxic than secondary or tertiary amines. A concentration dependent tendency to cause haemolysis and changes in erythrocyte morphology has been linked to the presence of –NH$_2$ groups. In contrast to PAMAM dendrimers PPI dendrimers with DAB and DAE cores did not show generation dependence for the haemolytic effect. Quaternisation has previously been used as a strategy to reduce toxicity of polymers. The approach also seems to be beneficial for higher generation PPI dendrimers but for complexes the effects of quaternisation are complex and can include changes of complex morphology and physical chemistry, which are difficult to deconvolute. By contrast anionic dendrimers,
e.g. those bearing a carboxylate surface are not cytotoxic over a broad concentration range. So despite their broad applicability of commercially available polyamidoamine (PAMAM) dendrimers and Polypropyleneimine dendrimers (PPI), it is generally necessary to modify the surface amine groups of these dendrimers with neutral or anionic moieties to avoid the toxicity and liver accumulation associated with their polycationic surfaces. Polyaryl ether dendrimers (Figure 1.29b), developed by Fréchet and Hawker, have been tested for drug delivery applications, but their poor water solubility necessitates the extensive use of solubilizing groups at their periphery. In recent years, much effort has been devoted to the preparation of dendrimers that are designed to be highly biocompatible and water soluble. In addition, some dendrimers have been designed to be biodegradable, and monomer units that are chemical intermediates or products in metabolic pathways have been incorporated. For example, several peptide-based dendrimers, such as those based on polylysine (Figure 1.29c), have been reported, and have been developed as promising vaccine, antiviral and antibacterial candidates after suitable peripheral modifications. Fréchet and co-workers have recently prepared a dendritic analog of the highly biocompatible PEO as a promising backbone for biological applications. Various polyester dendrimers incorporating monomers such as glycerol, succinic acid, phenylalanine and lactic acid (Figure 1.29d) have been prepared by Grinstaff et al., and their potential use in tissue engineering has been demonstrated. Dendritic polymers incorporating glycerol monomers have been developed by Haag. Several other dendrimer families, such as the amides synthesized by Schluter et al. and the triazines produced by Simanek et al., might also prove to be useful for biological applications.
Figure 1.29: Structures of biocompatible dendrimers that have been tested for drug delivery applications. (a) PAMAM dendrimer. (b) Polyaryl ether dendrimer. (c) Polylysine dendron. (d) Polyester dendrimer based on glycerol and succinic acid.
E. Applications 41, 44, 45, 59, 68-71

Many potential applications of dendrimers are based on their unparalleled molecular uniformity, multifunctional surface and presence of internal cavities. These specific properties make dendrimers suitable for a variety of high technology uses and are as follows:

**Pharmaceutical Application**

**Dendrimers Drug Delivery: Targeted and Controlled Release Drug Delivery** 41, 45, 59

Dendrimers have attracted attention as possible drug carriers because of their unique properties namely their well defined three-dimensional structure, the availability of many functional surface groups, their low Polydispersity and their ability to mimic. Drug molecules can be loaded both in the interior of the dendrimers as well as attached to the surface groups. Dendrimers can function as drug carriers either by encapsulating drugs within the dendritic structure, or by inter-acting with drugs at their terminal functional groups via electrostatic or covalent bonds (prodrug).

**Delivery of Anticancer Drugs by Dendrimers and Dendritic Polymers** 41, 59

The star polymer gave the most promising results regarding cytotoxicity and systemic circulatory half-life (72 h). In addition to improving drug properties such as solubility and plasma circulation time polymeric carriers can also facilitate the passive targeting of drugs to solid tumors. Combined, these factors lead to the selective accumulation of macromolecules in tumor tissue – a phenomenon termed the ‘Enhanced Permeation and Retention’ (EPR) effect 51. Therefore, the anticancer drug doxorubicin was covalently bound to this carrier via an acid-labile hydrazone linkage. The cytotoxicity of doxorubicin was significantly reduced (80–98%), and the drug was successfully taken up by several cancer cell lines. The encapsulation behavior of these compounds for the anticancer drugs adriamycin and methotrexate was studied. The highest encapsulation efficiency, with on average 6.5 adriamycin molecules and 26 methotrexate molecules per dendrimer, was found for the G = 4 PAMAM terminated with PEG2000 chains. The
anticancer drug 5-fluorouracil encapsulated into G = 4 PAMAM dendrimers with carboxymethyl PEG5000 surface chains revealed reasonable drug loading, and reduced release rate and hemolytic toxicity compared to the non-PEGylated dendrimer\(^{(10)}\). In contrast, up to 24 drug molecules were encapsulated into the hyper branched polyl. The drug was successfully transported into lung epithelial carcinoma cells by the dendrimers. Recent studies using Caco-2 cell lines have indicated that low generation PAMAM dendrimers cross cell membranes presumably through a combination of two processes, i.e., paracellular transport and adsorptive endocytosis, while cell efflux systems have a minor effect.

Figure 1.30: The encapsulation of anticancer drugs methotrexate (left) and 5-fluorouracil (right) into PEGylated generation 3 and 4 PAMAM dendrimers\(^{59}\).

**Mechanisms of Drug Delivery**\(^{45,59}\)

Dendrimers are particularly attractive as they offer a high drug-loading capacity. 2 methods of dendrimer drug delivery are encapsulation of drugs and dendrimer –drug conjugates.
Noncovalent Encapsulation of Drugs / Host–Guest Relation

Encapsulation of drugs uses the satiric bulk of the exterior of the dendrimer or interactions between the dendrimer and drug to trap the drug inside the dendrimer. Maciejewski introduced the concept of encapsulating guest molecules into special, egg-shell-like structures. Such a system can be used to encapsulate drugs and provide controlled delivery. Initial studies of dendrimers as potential delivery systems focused on their use as unimolecular micelles and ‘dendritic boxes’ for the noncovalent encapsulation of drug molecules. For example, in early studies, DNA was complexed with PAMAM dendrimers for gene delivery applications, and hydrophobic drugs and dye molecules were incorporated into various dendrimer cores. An advantage of using dendritic unimolecular micelles rather than conventional polymeric micelles is that the micellar structure is maintained at all concentrations because the hydrophobic segments are covalently connected. Although the introduction of stabilizing PEO chains on the dendrimer periphery has expanded the scope of dendritic unimolecular micelles to incorporate anticancer drugs such as 5-fluorouracil methotrexate and doxorubicin and can slow the drug release rates in these systems to some extent. A promising new approach to controlling the release of drugs from the encapsulating micellar compartment involves the use of hybrids of PEO and dendrimers with pH-sensitive hydrophobic acetyl groups on the dendrimer periphery.

Covalent Dendrimer–Drug Conjugates

An alternative approach to the development of dendrimers as anticancer drug carriers is to exploit their well-defined multivalency for the covalent attachment of drug molecules to the dendrimer periphery. In dendrimer–drug conjugates, the drug is attached through a covalent bond either directly or via a linker/spacer to the surface groups of a dendrimer. Dendrimers have been conjugated to various biologically active molecules such as drugs, antibodies, sugar moieties and lipids. The drug loading can be tuned by varying the generation number of the dendrimer, and release of the drug can be controlled by incorporating degradable linkages between the drug and dendrimer. Conjugates of PAMAM dendrimers with cisplatin, a potent anticancer drug with non-specific toxicity
and poor water solubility. The conjugates show increased solubility, decreased systemic toxicity and selective accumulation in solid tumors. Furthermore, the dendrimer–platinum complex has been found to show increased efficacy relative to cisplatin in the treatment of subcutaneous B16F10 melanoma. By using a careful synthetic strategy with two different chain end functionalities, it is also possible to attach both hydrophobic model drugs and PEO moieties to the dendrimer periphery in a controlled manner. Aliphatic polyester dendrimers based on 2,2-bis(hydroxyethyl)propionic acid are promising dendrimer backbones for the development of anticancer drug conjugates.

**Dendrimer as Solubility Enhancers**

There are many substances, which have a strong therapeutic activity but due to their lack of solubility in pharmaceutically acceptable solvents have not been used for therapeutic purposes. Water soluble dendrimers are capable of binding and solubilizing small acidic hydrophobic molecules with antifungal or antibacterial properties. Dendrimers having a hydrophobic core and a hydrophilic surface layer have been termed unimolecular micelles. Unlike traditional micelles, dendrimers do not have a critical micelle concentration. This characteristic offers the opportunity to soluble poorly soluble drugs by encapsulating them within the dendritic structure at all concentrations of dendrimer. A hydrophilic–hydrophobic core-shell dendrimer with PAMAM interior and long alkane chain exterior was shown to bind 5-flourouracil, a water-soluble anti-tumor drug. After phospholipid coating of the dendrimer–fatty-acid macromolecule, oral bioavailability in rats of 5-flourouracil was nearly twice the level of free 5-flourouracil. Dendrimer-based carriers could offer the opportunity to enhance the oral bioavailability of problematic drugs. Propranolol, conjugated to surface-modified G3 PAMAM dendrimer, the solubility of propranolol increased by over two orders of magnitude. Thus, dendrimer nanocarriers offer the potential to enhance the bioavailability of drugs that are poorly soluble and/or substrates for efflux transporters.
Kannan et al. studied the dynamics of cellular entry into A549 human lung epithelial carcinoma cells of a range of PAMAM dendrimers (G4-NH₂, G3-NH₂, G4-OH, PEGylated G3 [G3-PEG]) and a hyper branched polymer (polyol). G4-NH₂ and G4-OH entered cells more rapidly than did G3-NH₂, polyol or G3-PEG. It was suggested that the rapid entry of G4-NH₂ might be a result of the cationic nature of the amine surface groups, which may interact electrostatically with negatively charged epithelial cells and enter via fluid phase pinocytosis. The lower rate of cellular entry of G3-NH₂ compared with G4-NH₂ may be a result of fewer surface charges on the G3-NH₂ dendrimer. Because polyol and G3-PEG do not have cationic surface groups, their cellular entry may result from non-specific adsorption to the cell membrane and subsequent endocytosis. Dendrimer–ibuprofen complexes entered the cells rapidly compared with pure drug (1 hr versus >3 hr), suggesting that dendrimers can efficiently carry the complexed drug inside cells. PAMAM dendrimers were surface-engineered with lauryl chains to reduce toxicity and enhance cellular uptake.

**Dendrimers as Nano-Drugs**

Poly(lysine) dendrimers modified with sulfonated naphthyl groups have been found to be useful as antiviral drugs against the herpes simplex virus can potentially prevent/reduce transmission of HIV and other sexually transmitted diseases (STDs). In earlier studies, it was found that PAMAM dendrimers covalently modified with naphthyl sulfonate residues on the surface, also exhibited antiviral activity against HIV. This dendrimer-based nano-drug inhibited early stage virus/cell adsorption and later stage viral replication by interfering with reverse transcriptase and/or integrase enzyme activities. PPI dendrimers with tertiary alkyl ammonium groups attached to the surface have been shown to be potent antibacterial biocides against Gram positive and Gram negative bacteria. Poly (lysine) dendrimers with mannosyl surface groups are effective inhibitors of the adhesion of E. coli to horse blood cells in a haemagglutination assay, making these structures promising antibacterial agents. Chitosan–dendrimer hybrids have been found to
be useful as antibacterial agents, carriers in drug delivery systems, and in other biomedical applications.

**Dendrimers in Photodynamic Therapy (PDT)**

The photosensitizer 5-aminolevulinic acid has been attached to the surface of dendrimers and studied as an agent for PDT of tumorigenic keratinocytes. Photosensitive dyes have been incorporated into dendrimers and utilized in PDT devices. This cancer treatment involves the administration of a light-activated photosensitizing drug that selectively concentrates in diseased tissue. The possibility of improving the properties of dendrimers through appropriate unfunctionalization of their periphery makes dendrimers promising carriers for photosensitizers. ALA is a natural precursor of the photosensitizer protoporphyrin IX (PIX), and its administration is known to increase cellular concentrations of PIX.

**Dendrimers in Gene Transfection**

Dendrimers can act as vectors, in gene therapy. PAMAM dendrimers have been tested as genetic material carriers. Numerous reports have been published describing the use of amino-terminated PAMAM or PPI dendrimers as non-viral gene transfer agents, enhancing the transfection of DNA by endocytosis and, ultimately, into the cell nucleus. A transfection reagent called SuperFectTM consisting of activated dendrimers is commercially available. Activated dendrimers can carry a larger amount of genetic material than viruses. SuperFect–DNA complexes are characterized by high stability and provide more efficient transport of DNA into the nucleus than liposomes. The high transfection efficiency of dendrimers may not only be due to their well-defined shape but may also be caused by the low pH of the amines (3.9 and 6.9). The low pH permit the dendrimer to buffer the pH change in the endosomal Compartment. PAMAM dendrimers functionalized with cyclodextrin showed luciferase gene expression about 100 times higher than for unfunctionalized PAMAM or for non-covalent mixtures of PAMAM and cyclodextrin. It should be noted that dendrimers of high structural flexibility and partially degraded high-generation dendrimers (i.e., hyper branched
architectures) appear to be better suited for certain gene delivery operations than intact high-generation symmetrical Dendrimers. 

Figure 1.31: Dendrimer involved in gene transfection

In nutshell dendrimers holds a promising future in various pharmaceutical applications and diagnostic field in the coming years as they possess unique properties, such as high degree of branching, multivalency, globular architecture and well-defined molecular weight, thereby offering new scaffolds for drug delivery. An increasingly large number of drugs being developed today facing problems of short half-life, poor solubility, bioavailability and permeability. Dendrimers can work as a useful tool for optimizing drug delivery of such problematic drugs. Also the problem of biocompatibility and toxicity can be overcome by careful surface engineering. Recent successes in simplifying and optimizing the synthesis of dendrimers provide a large variety of structures with reduced cost of their production. Also as research progresses, newer applications of dendrimers will emerge and the future should witness an increasing numbers of commercialized dendrimer based drug delivery systems.
1.4 ORIGIN AND OBJECTIVES OF RESEARCH WORK

Half life is the period of time required for the concentration or amount of drug in the body to be reduced by one-half. We usually consider the half life of a drug in relation to the amount of the drug in plasma.

Therapeutically active agents, however, often suffer from number of shortcomings which limit and complicate their use. A particular problem is short half life of drugs, after administration to the patient; a drug may be so rapidly cleared from the body by metabolic or other pathways or otherwise biologically inactivated so that only a relatively small percentage of the drug administered actually has a therapeutic effect for example many over the counter pain relievers like ibuprofen and medications that are used as tranquilizers like benzodiazepines have notoriously short half lives.

To compensate for this problem, it is common practice to increase the dose of the drug and or to prolong its period of administration and or to shorten the interval between doses so that the therapeutically effective concentration of the drug in the patient is maintained for a period sufficient to achieve the desired result.

These procedures are useful but have their own limitations. Increasing the dose may be limited, for example, in the case of intramuscular administration, by the bolus which can be tolerated. Many drugs have toxic side effects which may limit the dose duration or interval which can be safely used. In some cases, promising drugs cannot be used because side reactions are so severe that an effective therapeutic dose cannot be safely administered. The need to administer multiple small doses of a drug or to use continuous infusion techniques increases the cost of treatment and the burden on hospital personnel, and of course, adds to the patient’s discomfort. Accordingly there exists a need for means by which the therapeutically active concentration of a drug after administration is maintained for a longer time.
Objectives

The main objectives of the study are as under:

1. To reduce the dosing frequency.
2. To improve patient convenience and compliance.
3. To minimize dose by maximum utilization of drug for therapeutic effect via reduction of unavoidable wastage of drug through fast elimination.
4. To extend the duration of action of drug.
5. To reduce health care cost and improves safe use of drug.

In literature survey it is found that there are mainly two approaches to increase serum half-life ($t_{1/2}$) of a drug, which are as following-

1.) By decreasing drug metabolism (pharmaceutical, pharmacological and chemical approaches described in section 1.2)

A simple example of this type of modification is Chlorpropamide which is derived from Tolbutamide having short half life used as oral hypoglycemic.

\[
\text{SO}_2\text{-NH-CO-NH-(CH}_2\text{)}_3\text{CH}_3 \quad \text{Cl-SO}_2\text{-NH-CO-NH-C}_3\text{H}_7
\]

$\text{H}_3\text{C-SO}_2\text{-NH-CO-NH-(CH}_2\text{)}_3\text{CH}_3 \quad (t_{1/2} = 4.5 \text{ to } 6.5 \text{ hrs})$

$\text{Tolbutamide}$

$\text{Cl-SO}_2\text{-NH-CO-NH-C}_3\text{H}_7 \quad (t_{1/2} = 36 \text{ hrs})$

$\text{Chlorpropamide}$

2.) By increasing molecular weight of drug by polymer binding to drug-

a) It decreases glomerulus filtration of drug due to high molecular weight and increases serum half life of drugs

b) It decreases the approach of drug metabolizing enzymes to their site of action on the drug molecule due to steric hindrance of polymer bound drug thus half life of drug increases.
1.5 PLAN OF WORK

The research is planned and would be executed on the following lines:

- Selection & Authentication of target drugs for Derivatization
- Selection of reaction and kind of derivatives to be prepared
- Structural Derivatization / modification in the selected drugs reducing its elimination through the body to increase its half-life.
- Characterization of the derivatized drugs.
- *In-vitro* studies of derivatized drugs.
- *In-vivo* studies of derivatized drugs.
REFERENCES


Introduction


