CHAPTER

1 Preformulation Concepts

Preformulation Concepts – Drug Excipient interactions different methods, kinetics of stability, Stability testing. Theories of dispersion and pharmaceutical Dispersion (Emulsion and Suspension, SMEDDS) preparation and stability Large and small volume parental – physiological and formulation consideration, Manufacturing and evaluation

Different Methods of Drug – Excipient Interactions

Pharmaceutical formulation is a mixture of drug substance, commonly called as API and excipients which do not have any therapeutic effect. Each excipient is used for a definite purpose – (1) to facilitate the manufacturing process. (2) to facilitate administration or absorption of the drug. In general, the excipient used in the formulation should have following properties:

- Should not alter the dose,
- Should not alter stability features of the formulation,
- Should not alter the release profile of the drug from the formulation, and
- Should be therapeutically inactive
- Should not have any toxicity

But some excipients can participate in chemical or physical interactions with drug substance, without compromising the therapeutic effectiveness of a formulation. Excipients although expected to be highly pure, but are not available/used in those form. Commonly, the materials used as excipients are minerals, synthetic, semi-synthetic or natural origin. The materials used as starting materials, reagents and solvents may remain present in the product as residue, may be in very minimum quantities. These residual substances may interact with the drug substance. It is necessary to know the process(s) involved in the manufacture of excipient, so that the type of impurity likely to be present and how they can interact with the API can be identified.

Excipients having functional groups can interact directly with the active pharmaceutical ingredient. In some cases, excipients or drug substance can decompose or degrade producing impurities which may interact with either the drug substance or excipients present in the formulation.

Therefore, following three major points must be considered when a proposed pharmaceutical dosage form is being manufactured[1]:

- Properties and shortcomings of API
- Properties and shortcomings of excipients
- Advantage and disadvantages of the method(s) used

For development of a dosage form, all three points of equally important are to be considered. As mentioned above excipients are the substances other than drug substance which for certain definite reasons are incorporated into pharmaceutical dosage form[2] for the following purposes; for example,

- To improve the stability of the drug substance in the dosage form,
- For modification of the bioavailability of the drug substance,
- To maintain the pH of the liquid formulation,
- To maintain the desired rheology of the suspension and semisolid dosage form,
- To act as tablet binders, tablet disintegrant,
- To increase the bulk weight,
- To act as antioxidant and emulsifying agents,
- To allow the recommended administration,
- To facilitate the manufacturing of dosage form,
- For aesthetic reason, and
- For identification

The IPEC (International Pharmaceutical Excipients Council) has defined the term excipient as the substance(s) other than the API which has been appropriately evaluated for safety and is included in a drug delivery system to either help processing of the formulation during manufacturing or protect, support or enhance stability, bioavailability or patients compliances or to help in the identification of the product and to enhance any other attributes of overall safety and effectiveness of the formulation during its storage or use.

According to the functions[3] in a tablet formulation, the excipients can be classified as:

- Diluent
- Binders,
- Disintegrants
- Lubricants
- Glidants

- Compression aids
- Colors
- Sweeteners
- Preservatives
- Flavors
- Film formers/coatings
- Suspending/dispersing agents/surfactants

Therefore, the knowledge of drug-excipients interactions is very important during selection of appropriate excipients for a proposed dosage form.

Mode of drug decomposition

Most of the drugs are organic compounds; hence, in most cases the degradation of drug takes place in similar way with normal organic compounds. Reactive agents such as thionyl chloride, lithium aluminium hydride are used in relatively high concentrations. Generally, more than 10% is kept under accelerated conditions such as heating at boiling temperature toreflux or heat in a pressure bomb. Reactions take place in relatively short period of time. For example, the concentration of dexamethasone sodium phosphate in its injection is 0.4%, in topical cream is 0.1%, and in its ophthalmic ointment is 0.05%. The decomposition of a drug is probably not intervened by another drug present in the formulation, commonly by water, oxygen, or light. Measurable decomposition takes place in months or years' time; not in hours or days. The substances used medicinally must have structural features for which they interact with the receptors or facilitate metabolic functioning. These make them susceptible to degradation or interaction with other materials. The common methods of degradation are:

Hydrolysis

Drugs having functional groups such as esters, amides, lactones, or lactams are generally susceptible to hydrolytic degradation. Most commonly the drug degrades by hydrolysis because of presence of groups susceptible to hydrolysis. Such groups are prevalent in the drug molecule and degrade due to omnipresence of water in form of moisture. Water acts as a vehicle for interactions and facilitates microbial growth.

Oxidation

Next to hydrolysis, oxidative degradation is the common method of drug degradation. The mechanism of oxidative degradation is relatively complex. In this method, an electropositive atom, radical or electron is removed or an electronegative moiety is added. Oxygen, heavy metal ions and light, leading to free radical formation can catalyze the oxidation reaction. The free radicals react with oxygen and produce

peroxy radicals. On the contrary, these peroxy radicals react with oxidizable compound to produce additional free radicals to initiate further reactions. Compounds having aldehydes, alcohols, phenols, alkaloids and unsaturated fats and oils are susceptible to oxidation.

Isomerization

In this process a chemical compound is converted into its optical or geometric isomer. The isomers have same molecular weight but are structurally different. As a result, the isomers may be pharmacologically or toxicologically different among themselves. Optical isomers can rotate the plane of polarized light (sodium light) differently, either clockwise or anticlockwise. For example, the therapeutic activity of levorotatory (L) form of adrenaline is 15-20 times greater than the dextrorotatory (D) adrenaline.

Photolysis

On exposure to sunlight or artificial light some of the reactions such as polymerization, alteration of ring, oxidation-reduction is catalyzed, or their rate of reactions are increased by absorbing the energy from the light. This process of catalysis or acceleration of reaction is called photolysis. When the wavelength of light is low, the absorption of energy would be more. Most of the drugs which are susceptible to photolysis can absorb UV light. These drugs degrade commonly when they are exposed to radiation of lower wavelength. Degradation of color (discoloration) is the most common example of photolysis in most of the compounds.

Polymerization

When two or more molecules react together can form additive compounds such as dimmer or molecules of higher molecular weight. For example, when the concentration of solution of ampicillin (amino penicillin) is increased, ampicillin forms gradually its dimmer, trimmer, and finally to its polymeric degradation products. The medicinal agents which commonly degrade under such conditions and polymerize are presented in Table 1.1.

Table 1.1 Mechanism of drug degradation

Mechanism	Drug	Mechanism	Drug
Hydrolysis	Methyl dopa Procaine Penicillin	Oxidation	Calcitonin Ascorbic acid Isoprenaline
Isomerization	Tetracycline Vitamin A Adrenaline	Photolysis	Folic acid Riboflavin Nifedipine
Polymerization	Ampicillin Ceftazidime		

Degradation of drugs indicates about their susceptibility to environmental stressful conditions such as heat, humidity, light, or drug–drug interactions. The process of degradation can be encouraged or facilitated by excipients having the functional groups suitable for such interaction. The substances that contain residual impurities can catalyze/participate in degradation or polymerization process. Thus, an excipient containing such functional groups or residual impurity and is also susceptible to undergo change. These can provide additional possibilities to produce species that can participate in the degradation processes.

Mechanism of drug-excipients interaction

The mechanism by which a drug can interact with the excipients is not accurately known. However, the literatures survey speaks about various well documented mechanisms. The frequency of occurrence of drug-excipient interaction is more than excipient-excipient interaction[4,5]. There may be the advantages or disadvantages of drug-excipients interaction. These can be categorized as[1]

- Physical interactions
- Chemical interactions

Physical interactions

The physical interactions occur very commonly. But, their detection is very difficult. Generally, no chemical change is observed in physical interaction. Physical interactions can be commonly observed during manufacturing of pharmaceutical dosage forms; for example, dissolution characteristics of a drug can be modified. Whatever may be the physical change occurs, no such change is desired. These changes create problem. However, some changes may be advantageous for performance of a product. Most common example of physical interaction between the drug and excipients is the interaction between primary amine drugs and microcrystalline cellulose. During dissolution in water a small amount of drug is found to be bound to the microcrystalline cellulose and is not released. This interaction may become a major problem in dissolution of drug from the dosage form containing higher amount of drug. Such problem can be overcome by using 0.05M HCl as the dissolution medium.

Physical interaction may take place during mixing (interactive mixing). Generally, the smaller particles of the drug particles interact with the surface of the larger particles of excipients through physical forces[6]. As a result, a homogeneous blend of powders is obtained. These are the examples of beneficial effects of physical interaction.

There are damaging effects of physical interactions; for example, use of magnesium stearate reduces the hardness, dissolution rate of the tablets

and capsules. The bioavailability of drug is reduced due to adsorption of drug molecules over the surface of excipient and the drug becomes unavailable for dissolution and diffusion. Similarly, the use of magnesium stearate as lubricant reduces the antibacterial activity of cetylpyridinium chloride due to adsorption of cetylpyridinium cation over stearate anion. Degradation of nitrazepam in tablet dosage form can be catalyzed by colloidal silica possibly due to adsorptive interactions. This alters the electron density in the vicinity of the labile azo group and facilitates the attack by hydrolyzing entities. Complexing agent such as cyclodextrin is sometimes used to increase the bioavailability of poorly water-soluble drugs. It has been found that complexation of cyclodextrin with non-steroidal anti-inflammatory drug (NSAID) naproxen and tolbutamide increases the dissolution, but there was no corresponding increase in bioavailability. Phenobarbital can produce an insoluble complex with PEG-400, which can decrease the absorption due to slower dissolution. Water soluble excipients can make complexes with prednisolone, a steroid, which increases the dissolution of prednisolone. But in-vivo bioavailability of the drug may be less since the complexes have high molecular weight for which their rate of diffusion through GI membrane becomes slow.

Polymorphism

Same compound with different crystal structures is called *polymorphs*. Polymorphs are commonly obtained during crystallization of the drug using different solvents under different conditions. When the compound is exposed to different temperature, pressure, relative humidity, and comminution the polymorphs undergo transformation called *polymorphic transformation*. Thus, the polymorphic transformation takes place during drying, milling, granulation, and compression. Steroids, sulphonamides, and barbiturates are well known for polymorphic transformation. In case of sulphacetamide, polymorphism does not occur due to stronger hydrogen bonds formed with the amide hydrogens in the molecule. These hydrogen bonds are neither stretched nor broken to make alternate crystalline structure (polymorphs). Crystal energies of different polymorphs are different; hence, most energetic form will try to transform to the most stable form. Physicochemical properties of different polymorphs are different. Pseudo-polymorphism has also been observed after crystallization. Crystallization in solvent with one or more molecules of the solvent may be found in the solvated crystal of the polymorph; the polymorphs then can transform from solvated to nonsolvated form, hydrate to anhydrous, and vice versa showing changes in the properties of solid-state. There is evidence that crystalline forms of drugs are more stable than their amorphous forms[7];

for example, cyclophosphamide[8]. However, amorphous biosynthetic insulin has been found more stable than its crystalline form[9].

Vaporization

Even at room temperature some drugs and excipients have sufficiently high vapor pressure; these compounds are called volatile substances. Thus, their vaporization can cause a loss of the active pharmaceutical ingredient. For example, nitroglycerin has a vapor pressure of 0.00026 mm at 20ºC and 0.31 mm at 93ºC. When the patient stores the drug product and use, significant amount of the drug may be lost. Flavors are composed of mainly aldehydes, ketones, esters, and cosolvents such as ethyl alcohol. Hence, loss of flavor would occur even at room temperature. Vapor pressure of the substance, and thus, the volatility of it such as nitroglycerin can be reduced by making its dispersion in macromolecules such as polyethylene glycol, polyvinylpyrrolidone, microcrystalline cellulose, etc. These macromolecules interact with the volatile component and stabilize the preparation. For the same reason, β-cyclodextrin-nitroglycerin tablets can be prepared and used.

Aging

Probably the most interesting and least reported area is aging of pharmaceutical preparation. Through aging the disintegration and/or dissolution profiles of a tablet, capsule formulation changes. This type of change may take place due to the delicate and unknown physicochemical properties of the inert excipients or of the drug. Due to this, the bioavailability of the drug can change. For example, suppositories of aminophylline when stored at 22ºC for about 6 months, the melting period of the suppositories changes from 20 min to 60 min; this results in the decrease in bioavailability of the drug. Probably the ethylenediamine in aminophylline interact with the free fatty acids of suppository base. This may increase the melting point of the suppository and decreases the bioavailability of the drug. But, the melting period of the suppositories does not change if the suppository is stored at 4ºC for 15 months.

Thus, due to aging at storage condition the in-vitro dissolution of a product may change; but it may not necessarily change the in-vivo absorption of the drug. For example, methaqualone tablet after storing at 80% relative humidity for about 8 months does not indicate any change in its in-vivo absorption.

Adsorption

This has been observed that interaction between drug and plastic made of polyvinyl chloride (PVC) has been increasing and showing major problem when the solution is preserved in plastic bags and being injected or infused through an administration set. For example, if

nitroglycerin is stored in PVC infusion bag for 7 days at room temperature, 50% of the drug would be lost. Such loss of drug is not due to its degradation, it is due to adsorption. The drug can be recovered by rinsing the bag with less polar solvent such as methanol. Similarly, 40% of quinidine gluconate may be lost, if the drug is administered with traditional PVC iv-administration set. However, the loss of drug can be reduced by using shorter tubing and a winged iv-catheter[10]. The tendency of adsorption is mostly related to oil/water partition coefficient of the drug.

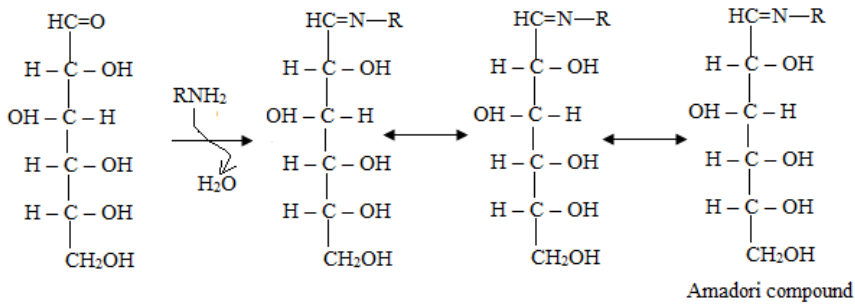
Chemical interactions

The reactions between drug and excipient, between drug and impurities or between drugs and residues present have been found to occur; such chemical interactions are harmful. The ICH guideline ICHQ3B[11] has classified these degradation products.

Chemical interactions between drug and excipients

When primary amine group of chlorpromazine reacts with glycosidic hydroxyl group of a reducing sugar, such as dextrose, an imine is formed (Maillard reaction). Finally, the imine breaks down to form Amadori compounds[12] as shown below. A study said that chitosan (natural polymer) can inhibit the release of diclofenac sodium from a matrix tablet at low pH. This might be due to the formation of ionic complex of diclofenac sodium with ionized polymer (cationic). Like Michael addition reaction, primary amines may interact with double bonds; for example, primary amine group of fluxamine when reacts with the double bond of maleic acid (counterion), fluvoxamine maleate is formed. The excipients containing double bond, such as sodium stearyl fumarate and sorbitan monooleate, are expected to undergo similar reactions.

Some APIs such as atorvastatin and cytidine nucleoside analogues are susceptible to oxidation. Oxidation reactions may be promoted by fumed metal oxides such as fumed silica, fumed titanium, and fumed zirconium. It is very difficult to predict these reactions because they are relatively complex in nature.



Water soluble suspending agents such as sodium alginate forms large negatively charged anions in its aqueous solution. When sodium alginate is mixed with drugs such as neomycin and polymyxin (positively charged) precipitates are formed. Silicon dioxide can catalyze the oxidation of diethylstilbestrol and forms peroxide and conjugated quinone degradation products. Colloidal silicon dioxide can catalyze auto-oxidation of methyl linoleate to form peroxides and the peroxides thus formed, subsequently decompose to aldehydes[13]. On grinding of a mixture of chloramphenicol stearate and colloidal silica, chloramphenicol undergoes polymorphic transformation. This shows that unwanted effects of drug-excipient interaction are not limited to chemical transformation only[14].

Incompatibilities

Two drugs or drug and excipient when remain present in the same formulation may interact with each other. For example, in an IV admixture kanamycin or gentamicin, cationic aminoglycoside antibiotic interacts with anionic penicillins, and thus kanamycin or gentamicin becomes inactivated. This is an example of incompatibilities between two drugs. These two classes of antibiotics form inactive complex, and it occurs in *in-vitro* as well as *in-vivo* in patients with severe renal failure.

The biological half-life of gentamicin sulphate (GS) when administered alone is about 60 hr; but when it is administered with carbenicillin disodium (CD) at a ratio of 1:80 (CD:GS), the half-life of gentamicin sulphate is reduced to about 24 hr.

In most of the solid dosage forms incompatibilities can be observed. For making tablet dosage forms diluents, binders, lubricants, disintegrants are used. Before preparing the required dosage form, it becomes necessary to select a set of suitable excipients based on their incompatibilities with the drug. Thereafter, the dosage form with desired properties is prepared. A model for selection of excipients has been predicted by Serajuddin et al[15]. Due to interaction of the amine functional group of a component, pharmaceutical incompatibilities can occur. Potential drug incompatibilities have been summarized below in Fig 1.1.

Solvolysis

This takes place between a drug and the solvent. Water is used as the universal solvent. Of course, the cosolvents may also involve in solvolysis. For example, ethyl alcohol and polyethylene glycol (PEG) commonly used as cosolvents, are found to take part in this type of reaction. These cosolvents can operate as nucleophiles that can attack the electropositive centers in drug molecule. Commonly, solvolysis

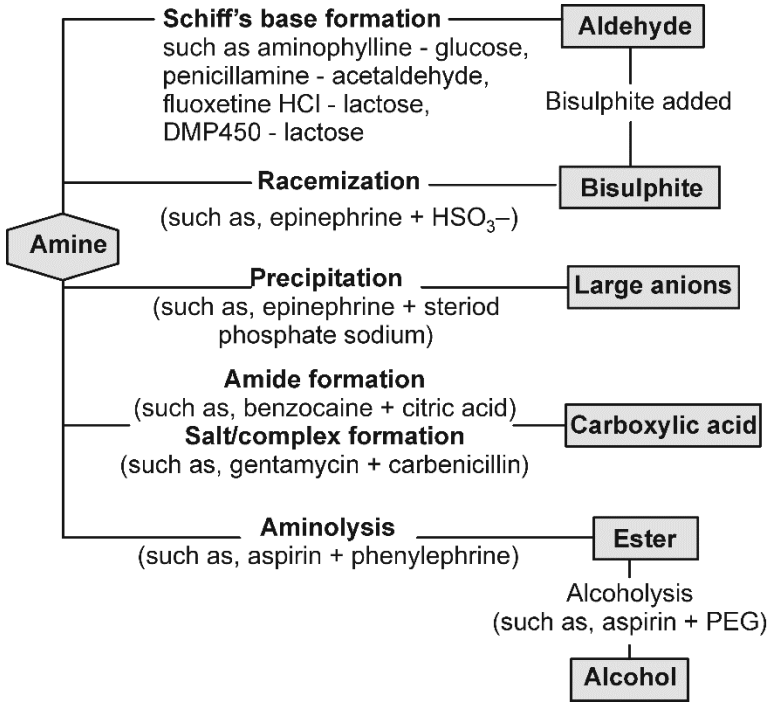


Fig. 1.1 Some potential drug incompatibilities

reactionsengage the labile carbonyl compounds, such as esters, lactones, and lactams as shown in Table 1.2. All the functional groups mentioned in the table are involved in solvolysis but their rates of reaction are different. For example, β-lactam ring can undergo hydrolysis at a higher rate than its linear analogue. For example, the half-life of β-lactam in potassium phenithicilline at 35°C and at pH 1.5 is about 1 hr; while that of penicillin G is about 4 min.

Table 1.2 Some functional groups involved in hydrolysis

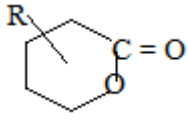
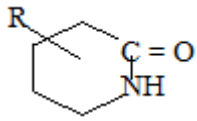
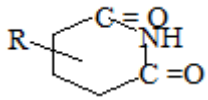
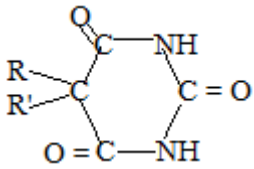
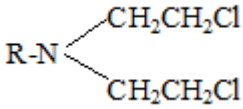
Types of Drug	Structure	Examples
Esters	RCCR' $ROPO_3M_x$ $ROSO_3M_x$ $RONO_2$	Aspirins, Alkaloids Dexamethasone sodium phosphate Estrone sulphate Nitroglycerine
Lactones		Pilocarpine Spiromolactone
Amides	$RCONR'_2$	Thiacinamide Chloramphenical

Table 1.2 *Contd...*

Types of Drug	Structure	Examples
Lactams		Lactams Penicillins Cephalosporins
Oximes	$R_2C = NOR$	Steriodoximers
Imides		Gluethimide Ethosuximide
Malonic ureas		Barbiturates
Nitrogens mustards		Melphanlan

On the other hand, in 0.18 molal H_2SO_4 at 25°C the half-life for hydrolysis of propionamide (simple amide) is about 58 hr. Generally, the normal amides undergo slow nonenzymatic hydrolysis; but the rate of this reaction increases at extremely high pH and at highest temperature. The N-C(O) is inherently stable; although amine is a good leaving group particularly if the pKa of it is more than 4.5. Even then at normal temperature, the amides are found susceptible to hydrolysis.

Sometimes, the hydrolysis of esters has been found as the reason for instability of a drug. If properly formulated, certain esters remain stable for many years. The substituents can influence the rate of degradation of esters radically. For example, *tert*-butyl ester of acetic acid is about 120 times more stable than the methyl ester and the methyl ester is about 60 times more stable than vinyl ester. This has been observed that the substituent groups may put forth the electronic, steric, and/or hydrogen-bonding effects, and this can strictly affect the stability of the compounds[16]. Intermolecular catalysis can also reduce the stability of the ester to a great extent, particularly if the neighboring groups can behave as acid-base, such as $–NH_2$, –OH, –COOH, $–COO^–$, etc.

Interaction of drug with excipient residues/ impurities

Commercially used pharmaceutical excipients are not completely pure, whether the materials are of minerals, synthetic, semi-synthetic or

natural origin.They contain residual amount of starting materials, reagents and solvents. These residues remaining with the material can be identified but if removed, the cost of material becomes so high that economy does not permit its commercial use. Thus, in some cases, materials with least amount of residual impurity have been allowed to be used in the dosage form. However, such impurities can act as catalyst in the suitable decomposition reaction. Table 1.3 shows the impurities of commonly used pharmaceutical excipients.

Table 1.3 Impurities of commonly used pharmaceutical excipients

Excipients	Impurities
Povidone, crospovidone, polysorbates	Peroxides
Magnesium stearate, fixed oils, lipids	Antioxidants
Lactose	Aldehydes, reducing sugars
Benzyl alcohol	Benzaldehyde
Polyethylene glycol	Aldehydes, peroxides, organic acids
Microcrystalline cellulose	Lignin, hemicelluloses, water
Starch	Formaldehyde
Talc	Heavy metals
Dibasic calcium phosphate dihydrate	Alkaline residues
Stearate lubricants	Alkaline residues
Hydroxypropyl methyl/ethyl celluloses	Glyoxal

In the parenteral dosage form, dextrose is used to adjust the tonicity of the product as well as to provide the adequate nutrition. Such solution of dextrose when terminally sterilized by autoclaving, isomerization of dextrose takes place; the reaction produces fructose and an aldehyde (5-hydroxymethyl furfuraldehyde). This aldehyde can react with primary amino group to form a base and develops the yellow color. It has been found that the mixture of lactose and fluoxetine when filled in capsule, Maillard reaction takes place[17]. Lactose is a disaccharide; it contains the reducing sugars such as glucose and galactose which are found in spray-dried lactose. The heat in spray drying causes the degradation of the hexose and production of 5-hydroxymethyl furfural. The hydrolytic degradation of the hexose can be accelerated by the presence of pH modifier. Most drugs are the salts of weak organic acids or bases. When the drugs are stored for long time, the residues/impurities may be converted to free acids or bases. Such products may be volatile and lost, if the formulation is subjected to sublimation.

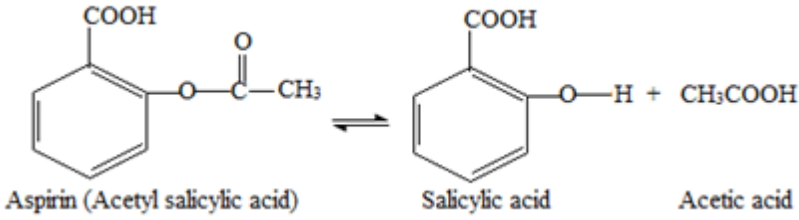


Fig. 1.2 Hydrolysis of aspirin

These problems can be avoided by thorough characterizing the drug and impurities. Various studies on the drug substances have revealed that the bound water can be removed from the substances by the operation such as grinding and drying. These become free to take part in hydrolytic reactions.

Under such stress conditions of the process, the bound water of the excipients may become loose, which degrade the moisture sensitive drugs present in the formulation. Hence, testing of drug-excipient mixtures is necessary for selection of appropriate excipient. Other stress conditions are compression, attrition, or crystal disruption. These stress conditions are catalyzed for interaction. For example, polyvinyl pyrrolidone and urea catalyzes the hydrolysis reaction of aspirin. Moreover, the excipients can form hydrates which enhance the degradation of drugs during grinding by releasing their water of crystallization. For example, during grinding, 4-methylphenylamino acetate hydrochloride is degraded by lactose hydrate.

Kinetics of Stability

Stability indicates whether, how and to what extent the purity of a drug or its product is affected under the influence of environmental factors such as moisture, heat and light when the formulations are stored for considerable period. Testing is done to determine the shelf-life of the drug product, appropriate storage condition and to quantify the extent of variation of purity under different environmental conditions.

The patients desire that they must get the products having good quality with adequate shelf-life. The pharmaceutical manufacturers provide label which claim the date of expiry on each product to guide the patients who commonly store the products in a cabinet placed in their bathroom where humidity is high. Storing in bathroom cabinet affects the stability of the product.

The assessment of degradation of new drug is not simple. Chemical kinetics needs to be applied to generate more reliable results and to prepare stable drug products based on the scientific basis of storage of

dosage form. A pharmacist enables to help the physician and the patients for proper storage and utilization of medicines.

For logical design and evaluation of dosage form of a drug, the stability of the drug must be considered a major factor in determining their suitability. The rejection of the dosage form may be caused due to various types of instability.

A chemical degradation of the drug reduces the quantity of the drug in the dosage form. This will then cause reduction of therapeutic effect of the drug. There are some drugs which undergo chemical degradation; for example, carbazepine, digoxin, theophylline, 5-fluorouracil, etc. These drugs have smaller therapeutic windows and hence, require careful development of dosage form; otherwise, their plasma concentration become too high to exert toxic effect or too less to make them therapeutically ineffective. The decomposed material of a drug may produce the toxic effects in the body. There are various drugs whose decomposition products are more toxic than their parent molecules. For example, epianhydrotetracycline obtained from tetracycline, arsphenamine obtained from oxophenarsine, p-amino salicylic acid from m-aminophenol is more toxic. Thus, sufficient care should be taken to avoid such degradation and subsequent ingestion. It has been reported that chloroquine, an antimalarial drug undergoes photochemical degradation, and its degraded product can produce toxic effects. During administration, chlordiazepoxide and nitrozepam show phototoxicity. Degraded penicillin G when injected sensitizes lymphocytes and form antipenicilloyl antibodies.

Degradation of a drug in its product can decrease the bioavailability along with decrease in amount of drug and can form the toxic product; thereby decreasing the therapeutic efficacy of a drug.The physical and/or chemical changes of the excipients present in a formulation can cause such effects irrespective of the changes in the drug.

Physical appearance of a formulation may change substantially due to physical interaction; for example, mottling in tablets, creaming in emulsions, and caking in suspensions. However, the therapeutic efficacy of the drug may not change due to physical changes. But it would affect the patient's confidence and the product may be rejected ultimately.

In some cases, the drug substance does not degrade and retains its potency; but the excipient such as antimicrobial preservative, solubilizer, emulsifying or suspending agent may degrade. As a result, the formulation of the drug loses its integrity.

A drug product must satisfy the stability criteria – physical, chemical, toxicological, and therapeutic. Anticipation and quantification of undesirable products produced by degradation of drug and/or excipient is necessary for formulation development; so that appropriate

stabilization technique can be applied to improve the stability of the product.

The expression $\frac{dc}{dt}$ is generally used to express the rate, velocity, or speed of a chemical reaction; where dc indicates the increase or decrease of concentration of the drug over an infinitesimal time interval, dt. The law of mass action can be defined as *the rate of a chemical reaction is proportional to the product of the molar concentration of the reactants each raised to a power usually equal to the number of moles*. If *a* and b, are the molar concentrations of two substances A and B, respectively, are undergoing the reaction, then the rate equation can be written as

$$aA + bB + \ldots\ldots = \text{Product} \qquad \ldots..(1)$$

Then, the rate of reaction $= \frac{1}{a}\frac{d[A]}{dt} = \frac{1}{b}\frac{d[B]}{dt}$

$$= \ldots.k[A]^a\,[B]^b\ldots\ldots \qquad \ldots..(2)$$

Where, k is the rate constant.

According to equation 2, the overall order of the reaction would be (*a*+b). With respect to one of the reactants, A or B, the order would be either *a* or b of that particular concentration term. For example, in the reaction of ethyl acetate with sodium hydroxide in water the rate of reaction can be expressed as;

$$CH_3COOC_2H_5 + NaOH_{soln} = CH_3COONa + C_2H_5OH$$

Rate of the reaction $= -\frac{d[CH_3COOC_2H_5]}{dt}$

$$= -\frac{d[NaOH]}{dt}$$

$$= k[CH_3COOC_2H_5]^1[NaOH]^1 \qquad \ldots..(3)$$

The reaction is first order (since *a* = 1) with respect to ethyl acetate and first order (since b = 1) with respect to sodium hydroxide solution. The overall order would be (*a*+b = 2).

Since in this reaction, the concentration of sodium hydroxide is in great excess and that of ethyl acetate is less, the concentration of ethyl acetate decreases as the reaction proceeds. The concentration of sodium hydroxide remains almost constant.

Hence, the rate of this reaction can be written as;

$$\frac{d[CH_3COOC_2H_5]}{dt} = k'[CH_3COOC_2H_5]$$

Where k' = k[NaOH].

The reaction can be said to be a pseudo–first-order reaction; because it depends only on the first power,*a* = 1, of the concentration of ethyl acetate. Generally, when one of the reactants is present in much great

excess so that its concentration may be considered constant, the reaction is said to be pseudo-order. When a chemical reaction takes place by more than a single step, its overall rate is expressed by the *slowest step*. This slowest step is called *the rate determining step*.

For example, when acetic anhydride reacts with ethyl alcohol, ethyl acetate and water are formed as shown below

$$(CH_3CO)_2O + 2C_2H_5OH = 2CH_3COOC_2H_5 + H_2O$$

The rate of reaction $= -\frac{d[(CH_3CO)_2O]}{dt}$

$$= k[(CH_3CO)_2O]^1[\ C_2H_5OH]^2$$

In the above reaction ethyl alcohol acts as the solvent, the concentration of alcohol does not change appreciably, since it is present in large excess in comparison to acetic anhydride.

Hence, the reaction appears to be first order with respect to acetic anhydride and second order with respect to ethyl alcohol. The overall order of the reaction would be 1+2 = 3. Since, ethyl alcohol is present in large excess, its concentration does not change appreciably and the rate of reaction can be written as

Rate of reaction $= -\frac{d[(CH_3CO)_2O]}{dt} = k'[(CH_3CO)_2O]$

Kinetically the reaction is said to be pseudo-first order.

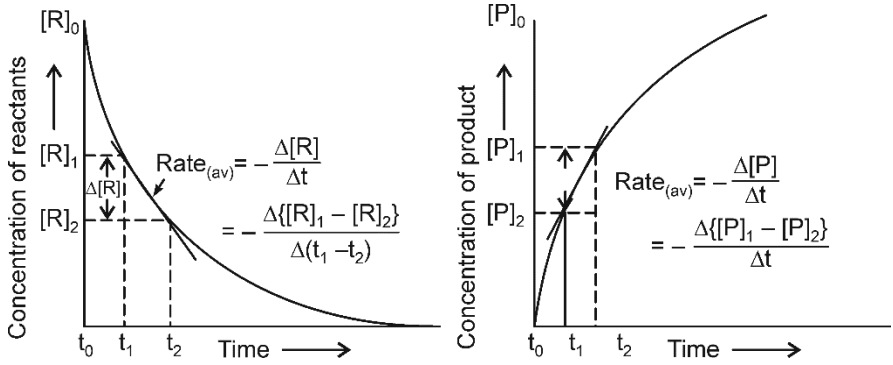


Fig. 1.3 Average rate of a reaction with respect to concentration of reactants or products

Zero-Order Reactions

The change in color of a multisulfa drug product can be measured using a spectrophotometer. The absorbance at a wavelength of 500 nm decreases with the loss in color. This degradation follows a zero-order rate. The rate expression for the change of absorbance, A, with time can therefore be expressed as:

$$-\frac{dA}{dt} = k_o \qquad \text{.....(4)}$$

Where, the negative sign indicates that the intensity of the color is decreasing (fading) with time. The rate at which the color fades is constant and independent of the concentration of the colorant used. Integrating between the initial absorbance A_o, corresponding to the original color of the preparation at t = 0, and A_t, the absorbance after t hours, we get:

$$\int_{A_o}^{A_t} dA = -k_o \int_0^t dt$$

or, $A_t - A_o = -k_o t$

thus, $A_t = A_o - k_o t$ .....(5)

Usually, the initial concentration is written as *a* (A_o) and the concentration at time t is c (A_t).

When this linear equation 5 is plotted with c against t, the slope of the line is equal to $-k_o$.

The value for k is 0.00082 absorbance decrease per hour at 60°C, indicating that the color is fading at this constant rate independent of concentration. Because *the half-life is the time required for one-half of the material to disappear*, in the present case A_o = 0.470 and ½A_o = 0.235

Thus, half-life of the present case would be, $t_{\frac{1}{2}} = \frac{\frac{1}{2}A_o}{k_o}$

$$= \frac{0.235}{8.2 \times 10^{-4}} = 2.9 \times 10^2 \text{ hr}$$

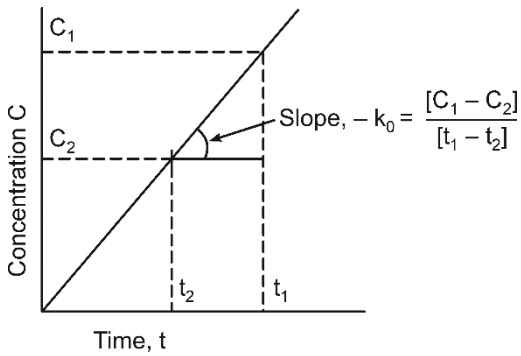


Fig. 1.4 Determination of slope of zero-order reaction

Apparent Zero-Order Kinetics

Generally, drug suspended in a suspensions indicate zero-order kinetics for its degradation. The amount of drug going into solution actually degrades. The concentration in solution depends on the solubility of drug. As the drug decomposes in solution, more amount of drug would be released from the suspended particles to keep the concentration constant. This concentration is equal to the equilibrium solubility of the drug in a particular solvent at a particular temperature. Thus, the amount of drug in solution remains constant despite its decomposition with time. The reservoir of solid drug in suspension is responsible for this constant concentration. The equation for an ordinary solution, with no reservoir of drug to replace that depleted, is the first-order expression, equation 6:

$$\frac{-d[A]}{dt} = k[A] \qquad \text{.....(6)}$$

Where, [A] is the concentration of drug remaining undecomposed at time t, and k is the first-order rate constant. When the concentration [A] is constant, as in the case of a suspension, we can write

$$k[A] = k_o$$

So, the first-order rate law 6 can be written as

$$\frac{-d[A]}{dt} = k_o \qquad \text{.....(7)}$$

Equation 7 obviously represents a zero-order equation. It is referred to as an apparent zero-order equation, because of the suspended drug reservoir. This ensures constant concentration. Once all the suspended drug particles go into solution, the system changes to a first-order reaction.

First-Order Reactions

In 1918, Harmed observed that the rate of decomposition of hydrogen peroxide catalyzed by 0.02 M KI solution was proportional to the concentration of hydrogen peroxide remaining in the reaction mixture at any time. The decomposition reaction of H_2O_2 is expressed as

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Although as per the stoichiometric equation, two molecules of hydrogen peroxide are participating in the reaction, the reaction was found to be first order. The rate equation can be written as

$$-\frac{dc}{dt} = kc \qquad \text{.....(8)}$$

Where, c is the concentration of hydrogen peroxide remaining undecomposed at time t and k is the first-order rate constant. After integrating the equation 8 between concentration c_o at time t = 0 and concentration c at time, t, we get

$$\int_{c_o}^{c} \frac{dc}{c} = -k \int_{o}^{t} dt$$

or, $\ln c - \ln c_o = -k(t - 0)$

Thus, $\ln c = \ln c_o - kt$ …..(9)

On conversion to common logarithm,

$$\log c = \log c_o - \frac{kt}{2.303} \quad \text{…..(10)}$$

or, $$k = \frac{2.303}{t} \log \frac{c_o}{c} \quad \text{…..(11)}$$

The equation 9 can be written in exponential form as

$$c = c_o e^{-kt}$$

The equation 10 can be written as

$$c = c_o 10^{-kt/2.303}$$

If the concentration starts with c_o and decreases as the reaction becomes progressively slower. The concentration approaches a final value c_∞ at infinity, then the equation 11 can be written as

$$k = \frac{2.303}{t} \log \frac{a}{a-x}$$

Where, *a* replaces c_o, *x* is the decrease of concentration in time t, and *a* - *x* = c.

Molecularity

The Molecularity of a reaction can be defined as *the number of molecules or ions participating in the rate determining step*. If the concentration of a single species or entity changes or takes part in the transition state, the reaction is called *unimolecular*. If two chemical species or entities react or combine in the transition state of the rate determining step, it is called *bimolecular*. If three independent species react together in the transition state, it would be called as *termolecular*. Termolecular reactions are rarely taking place.

When a reaction occurs through several steps or elementary reactions, each of the elementary reactions has a particular *stoichiometry* which indicates the number of molecules taking part in that reaction step. Since, *the order of an elementary reaction is indicated by the number of molecules reacting together in that step*, it is generally referred to the order as the molecularity of that particular elementary reaction. The term molecularity is not used in the rate law.

For example, the unimolecular reaction

$$Br_2 \rightarrow 2Br$$

Similarly, bimolecular reaction can be represented as

Chemical reactions that occur by more than one step are called as *complex reactions*. However, the overall order of the reaction determined kinetically may not exactly match with the molecularity; because the reaction consists of several steps, and each step has its own

molecularity. For example, the overall order of the following reaction has been determined as 2.

$$2NO + O_2 \rightarrow 2NO_2$$

The reaction is not termolecular; two molecules of NO would not be able to collide simultaneously with one molecule of O_2. The mechanism is assumed to have two elementary steps, each being bimolecular:

$$2NO \rightarrow N_2O_2$$

$$N_2O_2 + O_2 \rightarrow 2NO_2$$

Stability Testing

The patients expect that the medicines being consumed must be elegant, safe, therapeutically effective, approved by the regulatory authorities, and of a reputed company.

Purpose of stability testing

Thus, the purpose of conducting stability studies can be categorized as:

- **Safety of the consumer:** The primary objective of conducting stability studies by a manufacturer before marketing or sale of the product is to provide a safe medicine to the consumer; so that a confidence in the minds of consumer is built up. The drugs are chemical compounds which may undergo decomposition if not properly manufactured and stored. The purity of the drug must be retained by the product throughout the shelf-life of it. A medicine must elicit its therapeutic effect for a right period, for a specific duration, and does not show any side effect other than those inherited. A stable product does not lose its elegance acquired at the time of manufacture, during its shelf-life. The instability of the drug may cause change of elegance[18,19].
- **Reputation of the manufacturer:** The reputation of the manufacturer depends on the safety and confidence of the consumer on a medicine. The quality of the product should be consistent, and it should never change if it is properly stored. The manufacture, import, export, and sale of the drug in the internal markets are being regulated by the Drug Control Authority; hence, a manufacturer must convince the regulatory authority with proper justification that the product manufactured and sold, is of desired quality and is safe. Once permitted, it becomes the responsibility of the manufacturer to maintain the quality uninterruptedly; because the reputation of the company is directly related to the quality of the product[18,20,21].
- **Requirement of the regulatory authority:** Since all the medicines being sold in the national markets, whether manufactured by national manufacturer or are imported must be regulated by the regulatory authority. The authority has stipulated some rules for

getting the approval for manufacture and sale of any medicine. All manufacturers are supposed to comply with these conditions. Out of all these conditions, submission of stability data is one. Therefore, it is mandatory to conduct stability studies on the product supposed to be manufactured and sold[20,21].

- **Stability testing and formulation development:** The main purpose of stability testing is to generate the data to know the pathways for variation of the quality of a drug or its product with time under the influence of different environmental conditions such as temperature, humidity, and light. The stability testing is done also to determine the retest period for the drug or the shelf life for the drug product and to recommend the necessary storage conditions[22] to maintain desired quality. The storage and testing during stability studies must be performed as per the current good manufacturing practices (cGMPs)/ ICH guidelines as shown in table 1.4. All these activities are to be performed before marketing the product. The intrinsic characteristics of the drug will help in designing the plan for formulation and to manufacture the stable finished product that would retain all the desired characteristics till it is expired[23]. The issues related to the stability testing can exert significant impact on various stages in development of product as mentioned below:
- **Development of analytical method:** To determine the stability of a drug or its product a suitable analytical method is to be developed and used. The methods used for routine determination of stability are usually called as stability indicating assay methods (SIAMs). Under various stress conditions the drug will degrade and there should be suitable analytical procedure for quantification of degradation products. Sometimes, the degradation products are produced after stressing parent drug during real time stability studies[24,25].
- **Stress testing of drug substance:** It is necessary to know the nature of the drug before starting any formulation activity. The drug/drug product is put under various stress conditions as per the predetermined guidelines. Testing of drug under stress produces the required information about intrinsic stability of the drug. This includes determination of the effects of temperature, humidity, oxidation, light on stability of the drug. At the same time, the purity of the drug is also checked and change in impurity at different storage time is determined. Degradation may take place during manufacturing or processing steps. This provides the detail idea about the conditions that keep the drug most stable or the factors which initiate or increase the degradation of the drug. This information is very important and helps in designing appropriate conditions during manufacturing, processing, and storage to ensure maximum stability of drug[20,26].

- **Preformulation study:** Preformulation study is very much necessary to formulate a stable pharmaceutical product. Selection of suitable excipients for formulation is also an important activity. Assessment of possible incompatibilities between the drug and different excipients is a part of preformulation study. In most cases, the formulation of a drug involves, blending of drug with different excipients to examine their interaction, and to maximize the product's ability to be administered effectively. The excipients used should be inert. Physical and chemical interactions may occur between the drug and excipients. These interactions between drugs and the excipients can affect the chemical nature, the stability and bioavailability of drugs and their therapeutic efficacy and safety[27,28].
- **Shelf-life determination:** Appropriate storage conditions for a drug or its product requires knowledge about the conditions that encourage degradation and the mechanisms of degradation. Degradation studies are performed on the drug provides the required idea about its intrinsic stability and susceptibility towards degradation. Most of the information can be achieved from stress-testing studies combined with accelerated stability testing. In fact, accurate shelf-life can be predicted best with data generated from actual long-term stability studies[28].
- **Packaging development of final formulation:** Appropriately conducted accelerated stability studies give an idea about intrinsic stability of the substance. It also helps to determine the susceptibility of drug towards various chemicals. The results obtained in the stress testing helps very much to select appropriate packaging materials to pack the final product. If after accelerated stability studies the drug is found to be susceptible for acid degradation, then enteric coated formulation is recommended. If the drug is found to be sensitive to hydrolysis, proper packaging is designed, so that the permeation of water vapor or moisture from primary packaging material does not take place. Thus, the drug can be protected from hydrolysis. This will ensure long term stability of product[28].
- **Selection of appropriate storage conditions for final product:** Extrapolated stability data may be used to support the product registration, and real time data must be obtained to determine the actual expiry date of the product. After receiving approval from the authority in favor of the drug product, stability studies are routinely continued to determine the stability of the marketed drug product. Representative batches are put on long term stability for annual product monitoring.
- **Safety and Toxicological concern:** If after accelerated stability testing a known toxic compound is produced, necessary steps should

be taken to avoid the formation of such toxic degradant. Sometimes formation of degradation product is a complex process, in such cases it is very difficult to synthesize degradation product. The toxic substance produced should be identified and analyzed properly using a sophisticated and reliable analytical method[18,29].

Table 1.4 Guidelines available for and applicable to stability studies of pharmaceutical products

Guideline	Title	Guideline	Title
ICH Q1A(R2)	Stability testing of new drug substances and products	ICH Q1B	Photostability testing of new drug substances and products
ICH Q3A(R)	Impurities in new drug substances	ICH Q3B(R2)	Impurities in new drug products
WHO	Stability testing of active pharmaceutical ingredients and finished pharmaceutical products		

Different Guidelines for Stability Testing

In very simple words, the method used for analysis of the samples to assess their stability is called *stability indicating assays*. In 1987 the US-FDA first time defined that, stability indicating method is the quantitative analytical method that are based on the structural, chemical, or biological characteristics/ properties of each active ingredient of a drug product and that can distinguish each active ingredient from its degradation products so that the active ingredient content can be accurately measured. Later on in 1998, the US-FDA defined the term in its draft stability guideline as *validated quantitative analytical methods that can detect the changes in the chemical, physical, or microbiological properties of the drug substance and drug product with time, and that are specific so that the contents of active ingredient, degradation products, and other components of interest can be accurately measured without interference*.

Thus, the stability indicating assay method should be capable to distinguish between the drug in its pure form or from its product and degradation product generated during shelf life. The sensitivity of the method should be such that it can identify as well as quantify one or more degradation product present in the sample[24,30].

Types of stability indicating assays

Principally there are two types of stability indicating assay:

A. Specific: The method is used to measure only and accurately the drug/drugs. All the degradation products, the excipients, and additives, likely to be present in the formulation should not interfere with the determination.

B. Selective: These methods are used to clearly measure the drug(s)and all degradation products in the presence of excipients and additives, which are generallypresent in the drug product.

Steps involved in development of stability indicating methods

The basic steps to be followed for the development and validation of stability-indicating assay methods are not given in the regulatory guidelines, as well as not in the Pharmacopoeias. Therefore, following 6 steps are involved in the development of stability indicating assay methods:

Assessment of possible routes of decomposition thorough study of the structure of the drug

- Compilation of the information about physicochemical properties of the drug(s),
- Conducting accelerated stability studies to generate the degradation products,
- Groundwork on the studies separately on the samples under stress,
- Development and optimization of methods to be used finally,
- Identification and characterization of the degradation products and preparation of the standards.

Hydrolytic degradation

Hydrolytic degradation is one of the most common pathways for degradation of drug over wide range of pH. In hydrolysis, water reacts with the drug and produces the degradation products of various chemical compositions. Water is present either as a solvent or moisture in the air. The source of water either may be as a solvent or as moisture can cause the degradation. In general, such degradation occurs in acidic, alkaline or in neutral medium. The degradation results give indication about the stability of drug in aqueous medium as a function of pH. This study can also help in ADME studies of a drug. The degradation study can be performed by refluxing the drug in 0.1N HCl or in 0.1N NaOH. If optimum degradation is found, exposure should be stopped at that point. Under preliminary conditions, if no degradation is found to occur, the drug should be refluxed in acid/alkali of higher strength for longer period. Otherwise, if the total degradation is observed after allowing the drug to initial condition, acid/alkali strength should be decreased at lower temperature. In general, the temperature and pH are the major determinants in stability of the drug susceptible to hydrolytic decomposition.

The experiment performed at pH 1–2 can help to develop the proper formulation strategy. If drug is acid labile and it is to be administered

orally and it must be made an enteric coated formulation. Degradation studies performed under neutral to moderately basic conditions, that is, at pH 6–9. This can help in assessing the possibilities for non-enzymatic degradation of the compound under physiologically relevant pH conditions such as pH 7.5. Such information would be useful not only for the study of metabolism, but also for pharmacokinetic studies; that is, to understand how long a compound might remain intact in vivo and under such conditions what would be the degradation products. Estimation of rate of degradation under different pHs will also provide necessary information related to analytical importance.

Suppose an analyte is separated at specific pH of mobile phase; at the same pH there should not be any degradation of that analyte. These studies would also help in selection of solvent, pH, and sample preparation. Therefore, hydrolytic degradation studies are very decisive[24,25,31].

Accelerated Stability Studies

Primary explanation of carrying out the stress testing of a drug or its product is– to expose to an accelerated stability condition, to examine whether there is any degradation, hydrolysis of drug with base or acid, and photo-stability of drug is taking place or not. Moreover, a suitable method of analysis can be developed to indicate the stability of the drug/drug product and to use for both. Such studies may also provide information about the degradation pathways and the selectivity of the applied analytical methods. According to the ICH and FDA guidelines, stress testing is conducted to fulfill three main purposes[31]:

1. To obtain a stability assessment of the drug or its product.
2. To know the possible degradation pathways of the drug or the active pharmaceutical ingredient in the drug product.
3. To examine the stability-indicating power of the analytical procedures applied for the drug and its product.

Studies should be repeated when the methods, processes, or formulations are changed. Table 1.5 provides the general protocol of tests and conditions that may be used to obtain data for submissions to the regulatory authority.

In general, the degradation varies between 5 – 20%. This range includes the permissible 10% degradation limit for small molecule of pharmaceutical drug products, for which the stability limit is 90% – 110% of the label claim[31].

Table 1.5 General procedure for degradation study of drug and drug product

Condition	Drug		Drug Product	
	Solid	Solution/ suspension	Solids Tablet/ Capsules	Solution (IV, Oral Suspension)
Acid/Base		Recommended		Optional
Oxidative	Optional	Recommended	Recommended	Recommended
Photostability	Recommended	Recommended	Recommended	Recommended
Thermal	Recommended		Recommended	Recommended
Thermal/ Humidity	Recommended		Recommended	

Oxidative stress testing

Oxidative degradation reactions can be complex in nature. Sometimes it may be difficult to predict the susceptibility of drug towards oxidation. This is partly because of oxidative mechanisms, that can be quite varied and complex. The oxidative degradation frequently does not follow typical Arrhenius kinetic models. Many drugs undergo autoxidation, that is, oxidize under normal storage condition involving elemental oxygen at its ground state. Autoxidation is a free radical reaction that requires a free radical initiator to start the chain reaction. Commonly the agent used to carry out oxidation study is hydrogen peroxide. It imitates possible presence of peroxides in the excipients. Other oxidizing agents such as metal ions, oxygen, and radicals behave as initiators, such as azobisisobutyronitrile can also be used. Selection of an oxidizing agent, its concentration, and stress conditions to be applied or maintained depends on the drug. Samples can be analyzed at different time intervals to determine the desired level of degradation. The type and extent of degradation depend on the nature of the functional groups present in drug molecule.

The mechanism of oxidative degradation of drug generally involves an electron transfer mechanism to form reactive anions and cations. In general, the susceptible functional groups are amines, sulphides, and phenols which subsequently produce N-oxides, sulphoxidehydroxyl-amine, sulphones. Hydrogen peroxide can be used in the concentration of 3–30% at a temperature not more than 40˚C for 2–8 days[32,33].

Thermal degradation studies

Generally, the rate of a reaction increases proportionally with increase in temperature. That is, the drugs degrade at higher temperature. Degradation which is caused by heat is commonly called as '*thermal degradation*'. The temperature at which degradation takes place is sufficient high to break the bond which is commonly called as *pyrolysis*. Common degradations caused by heat include hydrolysis, dehydration, isomerization, epimerization, decarboxylation,

rearrangements, and certain types of polymerization reactions. In accordance with ICH guidelines, drug samples of solid state and samples of drug products should be exposed to dry and wet heat, whereas the liquid drug products may be exposed to dry heat. It is customary that the effect of temperature is to be studied in increments of 10°C and above for routine accelerated stability testing and relative humidity should be kept at 75% or greater. If sufficient degradation is not found, the studies may be carried out at higher temperatures for a shorter period of time. Sampling done at different time intervals could provide information not only about the rate but it can give an idea about the primary and secondary degradation products. Sometimes, due to inherent stability of a drug molecule, the applied stress conditions produce little or no degradation. Therefore it is necessary to apply more stress in thermal degradation during accelerated stability studies, for example, 40°C for 6 months, before end of the stress study[25, 32,33].

Photo degradation

Stability testing for assessment of photostability must be carried out. This is an indispensable part of stress testing for examining the effect on photo labile compounds. On exposure to light, the drug molecules may produce photolytic degraded products. Some recommended conditions for photostability studies are mentioned in ICH Q1B Photostability Testing of New Drug Substances and their Products. The extent and rate of photo degradation depend on

- The intensity of incident light,
- Quantity of light absorbed by the drug molecule,
- Duration of exposure,
- Temperature maintained, and
- Type of light to which the sample is exposed

Photolytic degradation is generally carried out by exposing the drug either, in solid or in the solution, or its formulation to a combination of visible and UV light. Samples of drug substance, and solid/liquid drug product, should be exposed to a minimum of 1.2 million lux hours and at 200 watt hours per square meter. Routinely accepted wavelength of light for exposure of sample is in the range of 300-800 nm which can cause the photolytic degradation. The photolytic degradation takes place through non- oxidative or oxidative photolytic reaction.

The different types of non-oxidative photolytic reaction commonly occur are isomerization, dimerization, cyclization, rearrangements, decarboxylation and hemolytic cleavage of hetero bonds, N-alkyl bond, SO_2-C bonds etc.

Oxidative photolytic reactions occur through either singlet oxygen or triplet oxygen mechanism. The singlet oxygen reacts with the

unsaturated bond, such as alkene, diene, and polynuclear aromatic hydrocarbon to form photo oxidative degradation products. On the other hand, the triplet oxygen reacts with free radical of the drug molecule, which then reacts with a triplet oxygen molecule and forms peroxide. Therefore, light can also act as a catalyst to oxidative reactions. Thus, characterization of photo degradation process involves the study of the transient species and the interaction between precursor and products. It is an important way to understand the potential photo toxicity of a drug and to determine it. To reduce the effect of change in temperature during exposure to light, temperature control may be necessary. The samples exposed to photo degradation may be analyzed for significant changes in physical properties such as, appearance, clarity, color of solution, and for assay and identification of degradation product, if any[25,32,33].

Therefore, stress testing is essential to predict and resolve the issues related to instability of drug or its product. Reporting threshold for degradation product as per ICH guidelines has been summarized in the Table 1.6.

Table 1.6 Threshold for degradation products in new drug products as per ICH guidelines (Q3A(R2)ICH)

Reporting Thresholds	
Maximum Daily Dose[1]	**Threshold[2,3]**
≤ 1 g	0.1%
> 1 g	0.05%
Identification Thresholds	
Maximum Daily Dose[1]	**Threshold[2,3]**
< 1 mg	1.0% or 5 µg TDI, whichever is lower
1 mg - 10 mg	0.5% or 20 µg TDI, whichever is lower
>10 mg - 2 g	0.2% or 2 mg TDI, whichever is lower
> 2 g	0.10%
Qualification Thresholds	
Maximum Daily Dose[1]	**Threshold[2,3]**
< 10 mg	1.0% or 50 µg TDI, whichever is lower
10 mg - 100 mg	0.5% or 200 µg TDI, whichever is lower
>100 mg - 2 g	0.2% or 3 mg TDI, whichever is lower
> 2 g	0.15%

Note: 1. The amount of drug substance administered per day. 2. Thresholds for degradation products are expressed either as a percentage of the drug substance or as total daily intake (TDI) of the degradation product. Lower thresholds can be appropriate if the degradation product is unusually toxic. 3. Higher thresholds should be scientifically justified.

Theories of Dispersion

Dispersion is a heterogeneous system. In this system, *one phase remains dispersed in another phase*. The dispersed phase may be a gas, solid or liquid. The dispersion medium in which the gas, solid, or liquid particles remain suspended may be either oil or water. Again, the particles dispersed may be very fine, fine, or coarse. Therefore, the dispersion systems can be categorized in different ways.

1. Based on the state of dispersed phase as:
   - Foam, a gas is the dispersed phase,
   - Suspension, solid particles are dispersed,
   - Emulsion, a liquid either pure or solution is dispersed.
2. Based on size of the particles dispersed:
   - Coarse dispersion, the particle size is greater than 0.5μm
   - Colloidal dispersion, the particles are in the colloidal size range from 1 nm to 0.5μm.
   - Emulsion, the particle or globule size remains within 0.2 to 5μm.
   - Microemulsion, the particle or globule size remains within0.15 to 2μm
   - Nanoemulsion, the particle or globule size remains within 2 – 100nm
3. Based on the type of dispersion medium:
   - Oil in water, an oil is dispersed in aqueous phase
   - Water in oil, an aqueous phase is dispersed in an oil phase

Among all the various types of pharmaceutical systems, the dispersion system is the most complex one, difficult to manufacture and difficult to stabilize. Various environmental factors such as temperature, holding time, moisture in the circulating air, etc. can affect the bioavailability, elegance, and stability of the drug product.

Although a dispersion system consists of two phases, the numbers of components used in the system are many. Based on the state of dispersed phase and of the dispersion medium, various types of dispersions can be obtained as shown in the Table 1.7.

Apart from the active components present in the two phases, other components present in the system are called dispersion aids. Disperse systems are not thermodynamically stable. Huge numbers of in equilibrium states are possible in this system. Simple change in design can result in thermodynamic instability. The concept of thermodynamic equilibrium is mainly related to

- Surface free energy of the system,
- Particle size,
- Particle shape,

- Number of particles distributed,
- Wettability of the particles, and
- Adsorption

Table 1.7 Different types of disperse system

Dispersed phase	Dispersion Medium	Dispersion	Examples
Liquid	Gas	Liquid aerosol	Fog, Liquid spray
Solid	Gas	Solid aerosol	Smoke, Dust
Gas	Liquid	Foam	
Liquid	Liquid	Emulsion	Milk
Solid	Liquid	Sol, Paste	Tooth paste, Paint, suspensions
Gas	Solid	Solid foam	
Liquid	Solid	Solid emulsion	Pearl
Solid	Solid	Solid dispersion	Plastics

The surface or interfacial free energy is the energy existing at the boundary between two phases. If the particles to be dispersed are micronized by pulverization, the surface free energy of the particles increases. The particle interaction and electrical properties at the interface contribute to the stability of the system. Due to this surface free energy the system may be unstable. The stability of the system depends on the properties of the dispersed particles; that is, their size, shape and numbers; its wettability and adsorption properties are also equally important. Therefore, these characteristics of a disperse system are to be understood.

Pharmaceutical Dispersion

The drugs which are poorly soluble in aqueous medium show poor bioavailability from oral dosage form; hence, appear as a major challenge for developing a successful therapeutic product. It is reported that about more than 70% of the drugs and active ingredients are poorly soluble in water; they belong to BCS II or BCS IV [34]. Various methods have been tried to solve the aqueous solubility of drugs, such as

- Micronization[35],
- Nanocrystallization[36],
- Salification[37],
- Cyclodextrin inclusion[38],
- Cocrystallization[39],
- Micelle solubilization[40],

- Solid dispersion[41],
- Lipid-based dispersion,
- Liquisolid technique[42], and
- Encapsulation in nanoparticles[43].

Among all these, the well-developed and more commonly used pharmaceutical dispersion are solid dispersion (SDs), lipid-based dispersion and liquisolid dispersion. These dispersion systems have been widely used to formulate poorly water-soluble drugs to satisfy the issues of the solubility and permeability of the drug substance. In case of an orally administrable poorly water-soluble drug, improved bioavailability can be achieved through a liquisolid formulation; because the drug already remains in solution. However, other dispersion methods are also actively developed, such as co-precipitation, concomitant crystallization and inclusion complexation. These methods offer options to deal with the drugs of low bioavailability due to the poor aqueous solubility.

Dispersion is a method by which a substance is dispersed or embedded in another molecule or in a continuous phase. Dispersion can be classified according to the size and the state of dispersed substance. Generally, there are three main types of dispersions:

1. Coarse dispersions (suspensions);
2. Colloidal dispersions (nanoparticles); and
3. Molecular dispersions (true solution, liquid or solid state).

Dispersions generally produce a reversible agglomeration of two or more substances by van der Waals forces, hydrogen bonding, hydrophobic interaction and/or physical entanglement[44]. To overcome the intermolecular force between the drug molecules and to achieve quick dissolution, dispersion can be prepared asan effective method. Dissolution of drug is a process of interaction between the drug molecules and the dissolution medium. If the intermolecular force between drug molecules is decreased, the dissolution will be appreciably improved. Weak interactions between drug and the carrier formed in the dispersions not only maintain well the dispersion state of the drug in a carrier, but also produce a higher internal energy between drug and carrier than between drug molecules. This high-energy state greatly contributes to the drug dissolution. Drug dissolution from formulations is mostly important for those drugs with a short absorption window. They might have passed their absorptive sites by the time they have dissolved. Dissolution is a precondition of drug absorption in the gastrointestinal tract. Low aqueous solubility always brings about a slow rate of drug dissolution in the coarse dispersion systems. Formulation of poorly soluble drugs into ultrafine dispersions can lower the energy barrier for dissolution and thus, enhances the dissolution

rate. For BCS II drugs, it is possible to promote the oral absorption by preparing dissolution-based dispersions. However, for BCS IV drugs, it is not sufficient to improve the extent of absorption utilizing a dispersion approach that simply overcomes the dissolution limit[45]. Simultaneously it must overcome the dissolution and absorption barriers. Formulation techniques that have the function of dispersion plus absorption-promoting effect are essentially required to be developed. Lipid-based formulations have demonstrated great potential in betterment in absorption due to high biocompatibility and interaction with cell membrane[46].

Solid dispersions are the dispersion of a single active ingredient or mixture of active ingredients in an inert polymeric carrier. The dispersion remains in the solid state. It is prepared by melting, dissolving in solvent, solvent-melting, or by other methods. The drug is then dispersed within solid polymer. The rate of dissolution of a drug as per Noyes-Whitney equation depends on the concentration difference between the bulk solution and dissolving interface. In case of poorly water-soluble drugs, the rate of dissolution on the interface depends on the particle size of the dispersed drug, particularly above 100 nm[47].

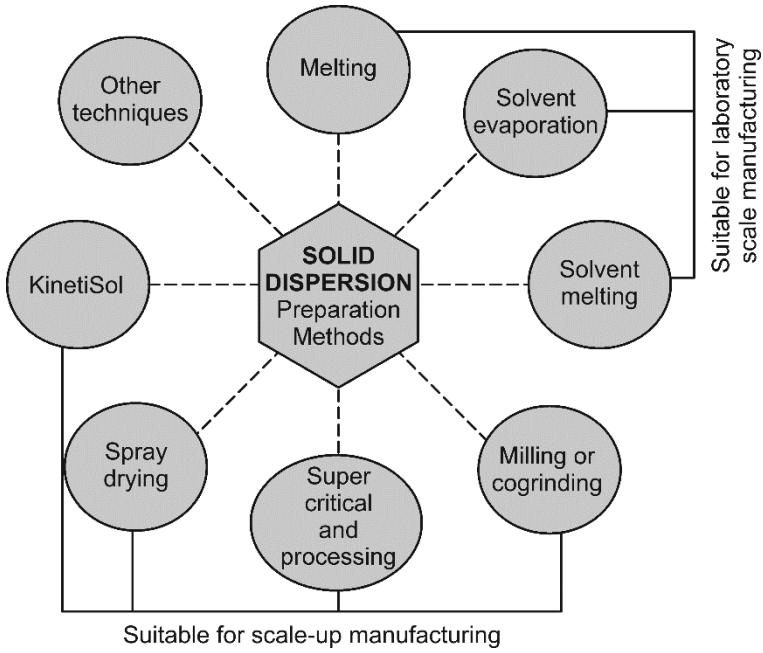


Fig. 1.5 Commonly used methods for preparing solid dispersion

Lipid dispersion is a dispersion in which the nanoparticles are formed by using lipid excipients. It is different from solid dispersion. The liquid dispersions are the products prepared by using lipid excipients.

Even after solidification by spray-drying or lyophilization, they exhibit little powder property, less stability during storage, and very difficult to pulverize. However, formulation of drug into lipid carriers indicates an effective dispersion method that can improve the dispersibility of drug and produce a supersaturated solution in the gastrointestinal lumen for absorption of drug. Solubilization and release describe the characteristics of the drug lipid dispersions. Lipid dispersion technique has been found more suitable for preparation of highly lipophilic, low-melting, and poorly permeable drugs. These drugs are easily prepared into lipid nanoparticles with satisfactory physiochemical stability.

The dispersed particles are expected to remain uniformly dispersed throughout the medium even on storage, so that dose uniformity can be maintained easily. But this is rarely observed, because the suspension intended for oral administration is a coarse dispersion which on standing is likely to form sediment. In case of formation of sediment, the sediment should be easily re-dispersed on mild shaking. For this reason, in the label of dispersion system it is mentioned that: shake well before use. The most important properties of a suspension/dispersion system are:

- Particle shape,
- Mean particle size,
- Particle size distribution, and
- Physical and chemical properties of the dispersed particles.

Particle shape

Even after size reduction and classification, insoluble drug particles have been rarely prepared spherical in shape. For example, precipitation and mechanical comminution produce particles of randomly shape, if the solids being micronized do not possess distinct crystal habits. Commonly the shapes of pharmaceutical solids are spherical, cylindrical, rod shaped, needles shaped, and crystalline shapes. Spherical droplets of internal phase are formed by emulsification processes. This reduces the interfacial area between two phases. To understand the behavior of suspension during storage, the information about the particle shape should be understood. The particle size and the shape of suspended particles can influence the packing of sediment such as density of the packing and settling characteristics. Polymeric particles of pseudo latexes can be prepared from preformed polymers either by solvent evaporation or by inverse emulsification processes. These particles are spherical or almost spherical. There are pharmaceutical excipients which have characteristic particle morphologies based on their molecular structure and the arrangement of

the molecules. For example, bentonite and kaolin clay particles have plate-like structures with straight edges and hexagonal angles. Other clays have particles are of lath-like or rod-like structures. The overall shape of a particle can influence the particle properties such as surface properties and volume property. Understanding of particle shape is necessary for thorough understanding of the behavior of suspension during storage. In case of a high-density suspension, wide particle size distribution, variation in particle shape can affect the density of cake/sediment and their dispersibilities. The particle shape may be plates, needles, filaments, and prisms. If the particles are of these types of shapes, low-density slurries can be formed. This has been found that symmetrical barrel-shaped particles of calcium carbonate form stable suspension which does not form cake on storage; while a firm sediment cake can be formed by asymmetrical needle shaped particles of calcium carbonate and the cake/sediment cannot be re-dispersed easily[48].

Particle shape can affect the viscosity of a colloidal suspension. The salvation of the dispersed particles depends on the relation between the particle shape and viscosity of the dispersion[49].

Particle size and size distribution

As stated earlier, particle size and size distribution in the dispersed phase is highly essential for thorough understanding of any disperse system. The absorption property of a drug depends on the particle size of the dispersed drug. Some dosage forms require specific size range of the particles. For example, suspensions for aerosols require particle size of the range of 1 – 5 μm for delivery of drug into respiratory tract directly. Such product must not have any particle more than 10 μm. The size of a spherical particle (symmetric) is expressed in terms of its diameter. If the particles are found asymmetric, the equivalent spherical diameter can be used to express the particle size and surface area. With the increase in asymmetry, an equivalent spherical diameter can correlate the size of the particle to the diameter of a perfect sphere having the same surface area (Surface diameter, d_s) or the same observed area in its most stable plane (projected diameter, d_p). However, the size of a particle can be expressed in terms of Stokes diameter, d_{st}. It refers to the diameter of an equivalent sphere that can deposit at the same rate. The type of equipment required or the method to be used depends on the type of diameter to be determined. Most of the collections of particles are polydisperse; that is, they contain particles of different size ranges along with mean size and the size distribution is important to be known. Theoretically, there are two types of distribution:

- Normal, and
- Gaussian,

The most common and simple type is the normal distribution. The data on particle size can be expressed either graphically or digitally. Either the number or weight fraction of the particles lying within a certain size range is plotted against the size range or mean particle size. This produces either a bar graph (histogram) or a frequency distribution curve. To study the sedimentation properties of a suspension/dispersion, the particle-weight data would be useful; while to study the surface properties the particle number data would be useful[50,51].

Surface properties

The particles in a dispersed phase being fine, their surface area is large; hence, the surface properties as well as interfacial phenomenon are most important to understand. When these are studied two main properties become important:

Surface free energy; that is, the surface free energy increases with decrease in particle size (size reduction),

Zeta potential; that is, the electrical charge present on the surface of a particle.

To understand the behavior of the dispersed particles, these two properties are to be understood.

Interface

Interface refers to the boundary between two phases of a suspension, solid/ liquid. In case of an aerosol, solid/ gas interface is important. The relation between surface free energy, ΔG and the total surface area, ΔA can be expressed as:

$\Delta G = \gamma_{SL} \times \Delta A$; where γ_{SL}represents the interfacial tension at solid/ liquid interface of the suspension. The fineness of the dispersed particles makes the large surface area and makes the system thermodynamically unstable due to large surface free energy. Very fine particles are highly energetic. To attain a stable state, the surface free energy is required to be reduced and hence, these fine particles are required to be regrouped. At equilibrium, ΔG can be brought to zero by reducing the interfacial tension, γ_{SL}or by decreasing the surface area, ΔA.

Particle interaction

In fact, the interaction between the particles may be similar or dissimilar particles, and dispersion medium is difficult to be expressed; but is an important parameter for the dispersion/ suspension stability. The interaction may be attractive or repulsive. These forces depend on –

- Nature of the dispersed substance,
- Size of the dispersed particles,

- Orientation of the particles,
- Distance among the dispersed particles, and
- Distance between the external phase and internal medium.

The balance between these forces determines the overall status of the suspension/ dispersion. In a dispersion/ suspension fine particles undergo Brownian movement. These particles may collide each other. When the particles come closure to one another both attractive forces and repulsive forces become operative. If the particles come within the attractive force, they agglomerate and the system becomes unstable. If repulsive force prevails, the particles remain separated and the system becomes stable. There are four types of attractive forces –

Dipole – dipole force(Keesom orientation force),

Dipole – induced dipole force(Debye induction force),

Induced dipole – induced dipole force(London force), and

Electrostatic forces between the charged particles.

Among all these forces, the electrostatic force has been found the strongest one.

Electrical properties at the particle surface

When dispersed in an aqueous medium, most of the dispersed solid particles or liquid globules acquire a surface charge. The charge at the surface of the solid particles or liquid globules may develop due to various reasons; for example, ionization of the functional groups present at the surface of the particles such as amine or carboxylic groups, the pH of dispersion/ suspension medium and ionization of the groups, adsorption of protons by the dispersed particles, such as polymers and metal oxides (surface –OH groups of aluminium hydroxides can adsorb protons and become positively charged). Desorption/ donation of protons from the surface can make it negatively charged.

- Preferential adsorption of specific ions over the surface,
- Use of ionic (anionic or cationic) surfactants.
- Ion deficiencies in the crystal lattice or interior of the particles.

Due to isomorphic substitution, many mineral types of clay possess negative charges on their surfaces.

Particles of any of these categories when dispersed in aqueous medium, they become ionic in the dissolved state and start interacting with the surface charge of the dispersed phase. Later on an overall equilibrium is established. Grossly, the distribution of electric charges takes place in double layers–

1. The first layer is tightly bound, and
2. The second layer is relatively not tight, is diffusible.

The zeta potential of the dispersion can be defined as the potential between the shear plane (interface layer that moves with the particle) and the bulk of the medium. According to the DLVO theory, the stability of a disperse system depends on the height of the maximum in the potential energy curve, V_{max}, also called as potential energy barrier. The disperse system becomes stable, when overall kinetic energy of the dispersed particles is sufficiently less than the potential energy barrier. The potential energy barrier reduces when a substance on addition to the system,

- The surface charge of the dispersed particle starts neutralizing,
- The hydration layer starts losing,
- The double layer is being compressed, and/or
- Adsorbed species such as surfactants get deadsorbed from the surface of the particles[50,51].

This has been observed that electrolyte is required for flocculation. If the monovalent electrolyte, such as Na^+ is replaced by a divalent electrolyte such as Mg^{2+}, the amount of electrolyte required to cause flocculation decreases. The efficiency of an electrolyte in precipitating a dispersion depends on how fast the electrolyte gets hydrated. According to the Hofmeister or Lyotropic series, the monovalent and divalent ions can be arranged in order of their decreasing hydration and decreasing efficiency in destabilization of the dispersion:

Monovalent ions: $Li^+ > Na^+ > NH_4^+ > Rb^+ > Cs^+$

Divalent ions: $Mg^{2+} > Ca^{2+} > Sr^{2+} > Ba^{2+}$

Wetting

For making any dispersion, wetting of the particles to be dispersed is highly essential, the individual particles must be properly wetted by the dispersion medium. If thorough wetting is done, the air at the surface of the particles would be removed completely by the medium. The degree of wetting of a powder can be expressed in terms of the contact angle, θ. The contact angle can be defined *as the angle between the boundaries of the solid surface and the tangent to the curvature of the liquid drop*. The contact angle is the result of three interfacial tensions acting at – liquid/ air, air/ solid, and solid/ liquid interfaces. When the value of θ is less than 90°, the solid is wettable. When the value of θ is more than 90°, the solid becomes non-wettable and particularly when the value of θ approaches 180°, the solid is completely non-wettable by the liquid.

Generally, hydrophobic materials are non-wettable in aqueous medium. This problem of non-wettability can be solved by using anionic or nonionic surfactant as wetting agent. The mechanism of wetting of hydrophobic powder by a surfactant is that the hydrophobic powder gets adsorbed by the hydrophobic (lipophilic) part of the surfactant and

towards the aqueous medium the polar part of the surfactant remains forwarded. The efficiency of a wetting agent can be measured as follows:

A narrow lyophobic trough is used. One end of the trough holds a compressed bed of the hydrophobic powder and in the other end, a solution of wetting agent is placed. The rate of penetration of the solution is measured as the degree of wettability. A hydrophobic powder is supported by gauze and the solution of the wetting agent is dropped onto the gauze. The amount of drug carried by solution through the gauze indicates the wettability of the solution.

Adsorption

Adsorption is *the tendency of holding of atoms, molecules, ions to be placed at the surface/ interface*. It occurs due to the unequal distribution of forces at the interfaces. The particles adsorbed may be of solids, liquids, or gases, and the interfaces may be solid/ solid, solid/ liquid, solid/ gas, liquid/ liquid, liquid/ gas, etc. Based on the nature of interaction, adsorption may be of two types –

1. Physical adsorption or physisorption, where the forces and processes are reversible, nonspecific, and involve relatively low energy.
2. Chemisorptions, where the forces and processes are irreversible, specific, and involve relatively higher energy.

Adsorption may be of monolayer or multilayer. Various properties of a dispersion system may be responsible for the adsorption process. Adsorption of surfactants changes the properties at the interface. The adsorption of ionic surfactants usually increases the charge density on the surface of the dispersed particles and improves the stability of the dispersion[48].

Onto the surface of the dispersed particles protective colloids or polymers can be adsorbed. Sometimes the preservative materials can be adsorbed by the dispersed phase; this may cause insufficiency of preservative action. Hence, selection of amount of preservative should be made according to the extent of adsorption, and required preservative efficiency.

Rheological properties

The study of flow and deformation of material under the influence of external force is called rheology. It speaks about the viscosity of powders, liquids, and semisolids. In industrial production this concept is applied to plastic material, coating, lubricants, inks, adhesives, and food products. Flow properties of pharmaceutical disperse systems particularly of topical products are of great importance. Many fundamental investigations have been conducted to demonstrate the rheological properties of these systems.

Newton's law and Newtonian flow

It is necessary to know some basic terms before describing the rheological properties of materials. Let us assume that a liquid consists of a series parallel layers having the surface area of A, the layer below the upper layer is fixed; that is, the distance between two consecutive layers is fixed. If an external force is applied on the top layer, it starts moving with a velocity directly proportional to the distance from its bottom stationary layer. Thus, the velocity d_v/d_r is called the *rate of shear*, G. Where, d_v is the differential velocity and d_r is the distance of separation between the top layer and just immediately bottom layer.

Hence, $$G = \frac{d_v}{d_r}$$

If the force applied on unit area is F'/A, and is required to effect the flow (velocity dv/dr), it is called the *shearing stress*, F. Thus, $F = \frac{F'}{A}$.

Since the rate of shear, G is directly proportional to the applied shearing stress, F.

This is expressed as

$$\frac{F'}{A} \alpha \frac{dv}{dr} \quad \text{or, } F = \eta G$$

$$\eta = \frac{F}{G}$$

Where, η is the coefficient of viscosity or *viscosity*. Thus, if a liquid is more viscous, greater shearing stress would be required to produce a desired rate of shear. The unit of viscosity is poise which is defined as *the shearing force required to produce a velocity of 1 cm^2/sec of a plane of 1 cm^2 area and separated by a distance of 1 cm.* However, most used unit of viscosity is centipoises (cp).

Fluidity, Ø is the reciprocal of viscosity, η. Thus, $Ø = \frac{1}{\eta}$.

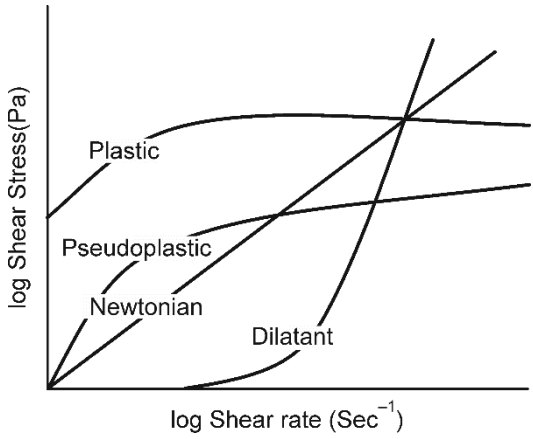


Fig. 1.6 Flow profile of Newtonian and non-Newtonian fluids

The U.S.P. has introduced one term called kinematic viscosity which is the ratio of viscosity to density of the liquid, ρ.

So, kinematic viscosity $= \frac{\eta}{\rho}$

In case of simple Newtonian fluids such as pure water, glycerin if shear stress is plotted against rate of shear in log scale, a straight line would be obtained. This indicates that the viscosity of a Newtonian fluid is constant at a particular temperature; it does not depend either on shear rate or on time. Pharmaceutical disperse systems this is not observed. Their viscosity changes with time and/or shear rate. According to the change of viscosity on shear rate these may be classified into three types – plastic, pseudoplastic and dilatants. Based on the time dependency, they may show thixotropic or rheopective characteristics.

Non-Newtonian flow

Pseudoplastic flow refers to the fluids whose viscosity decreases with increasing shear rate at a particular temperature. This type of behavior is called shear thinning; that is, the resistant of the material to flow decreases with increasing shear rate and require less energy to sustain the flow. The material at rest forms a structural network. The fluids or materials which exhibit this property are called pseudoplastic. It is assumed that the linear polymers orient themselves in the direction of flow as shear stress is increased. Pharmaceutical suspensions, emulsions, and polymers (thickening agent) show the pseudoplastic flow property.

Plastic flow is demonstrated by the non-Newtonian fluids which exhibits yield value. Due to their inter-particle association, they behave like a solid at rest. The attractive forces between the particles must be overcome to interrupt the structure. Beyond this point, the material behaves as a fluid. The viscosity may be constant (ideal Bingham liquid) or reduces with increasing shear rate. Initially the viscosity may decrease, then becomes constant (ideal Bingham liquid) or continuously decrease as in case of pseudoplastic flow. Flocculated suspensions sometimes show plastic flow.

Dilatant flow is observed when the viscosity increases with increasing rate of shear. The system seems to be more structured and more viscous with increasing shear stress; for example, pastes containing plasticizers, ionic polymers, or suspension containing high solid content, paint containing high pigmented system, etc.

Emulsion

Emulsions are thermodynamically unstable, heterogeneous, biphasic system. It consists of immiscible liquids, one phase is dispersed as fine

globules (dispersed phase) into the other liquid phase (continuous/external phase). The system is stabilized by addition of emulsifying agent. Most of the emulsions contain the droplets of size varying from 0.1 – 100 μm[49]. An emulsion becomes stable and uniform when

- Its internal phase contains fine and mono-sized droplets,
- The emulsion does not aggregate,
- The emulsion does not form large droplet due to coalescences,
- The internal droplets do not cream up or cream down,
- The cream layer formed due to coalescences of the droplets should be re-dispersible,
- The emulsion should remain in its original type without phase inversion,
- The emulsion does not degrade due to microbial contamination during storage,
- The emulsion does not become rancid or degrade due to oxidation of an oil or fat, and
- The emulsion remains stable at different temperatures such as 5°C, 40°C and at 50°C.

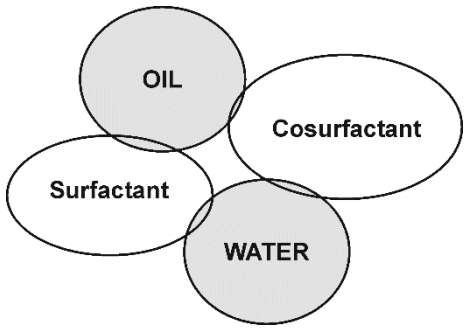


Fig. 1.7 Principal components of microemulsion

Emulsion can be of two types –

1. Oil in water (O/W), and
2. Water in oil (W/O).

If in the aqueous phase a hydrophilic emulsifier such as sodium laurel sulfate, sodium oleate, or glyceryl monostearate, is present in more than 45% of total weight, oil in water emulsion is formed. Similarly, spans, cholesterol and wool fats are hydrophobic emulsifier.

Microemulsion is a low viscous homogenous and transparent system. It contains higher amounts of oil, water and also the emulsifier[50]. Microemulsion may be of two types – O/W/O and W/O/W. These are widely used in cosmetics. Microemulsions are of scientific interest

because they can incorporate both hydrophilic and hydrophobic drugs[52]. There are different principles by which the formation, stability and phase behavior of microemulsions can be explained. For example, their thermodynamic stability can be explained as: the oil can be dispersed in water due to the presence of surfactant. The elastic properties of the surfactant film formed at the interface can help the formation of dispersion. Probably the curvature and rigidity of the film of the surfactant film is utilized as the parameters. The dependence on pressure, temperature, and/or salinity of the aqueous phase can be measured. The values can be used to decide the region of stability of the microemulsion or can be used to clarify the region where three phases coexist. Interfacial tension of the microemulsion with coexisting oil or aqueous phase can be used to explain their formulation. Coexistence of the three phases has been depicted in the Fig 1.8.

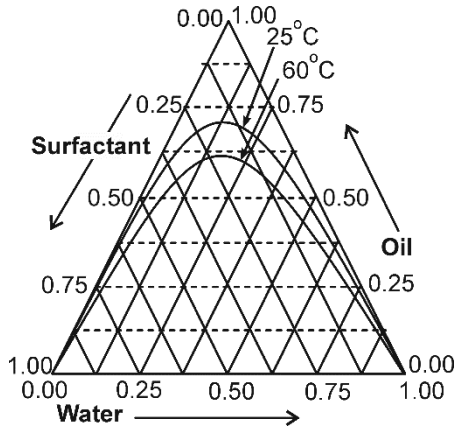


Fig. 1.8 Three-phase diagram at different temperature

Phase Diagram

The ternary-phase diagrams help to illustrate the microemulsion region. To prepare a microemulsion three components – an oil phase, aqueous phase and surfactant-cosurfactant mixture would be required. Sometimes, cosurfactant used at a fixed ratio to the surfactant as a single pseudo-component. The ternary phase diagram represents the relative amounts of these three components. To demonstrate the influence of changes in the volume fractions of the different phases on the phase behavior of the system, Gibbs phase diagrams can be used. The diagram looks like a triangle and each apex of the triangle represents the volume of 100% of a surfactant. From one corner it is moved away to the other, the concentration of a respective component reduces, and the other component increase as shown in the Fig 1.7. Thus, any point within the triangle can used to calculate the possible

composition of the mixture of components or pseudo-components. According to the Gibb's phase rule, the point represents one, two or three phases. When these points are combined, it forms a region with boundary, representing the phase behavior of the system at a particular temperature and pressure. However, the Gibb's phase diagram is an experiential visual observation of the state of the system. It may or may not state the actual number of phases within a given composition. The systems having high volume fractions of both the immiscible phases can be destabilized by anything that can change the equilibrium; that is at high or low temperature, or by addition of substance that can modify the surface tension.

Multiple Emulsions

In the field of emulsion technology, the multiple emulsion systems are newly developed dispersed systems. These systems cannot not be easily prepared. In this type of emulsion systems smaller droplets are dispersed. The composition of the internal or dispersed phase is same as that of external phase. The multiple emulsions have gained popularity because they can be used as sustained drug delivery system such as in cancer chemotherapy[53]. Such emulsions are prepared by double emulsification; hence these systems are also called as *double emulsion*. Like simple emulsion the multiple emulsions are of two types – O/W/O and W/O/W.

In Oil-in-Water-in-Oil (O/W/O) emulsion system, the aqueous (hydrophilic) phase remains separated from the internal and external oil phase. That is, the aqueous droplets enclosing the oil droplets remain surrounded by oil phase. On the other hand, in the Water-in-Oil-in-Water (W/O/W) emulsion system, the immiscible oil phase keeps two miscible aqueous phases separated from it and is called *liquid membrane.* This acts as a barrier and semi-permeable membrane for the drug entrapped in the internal aqueous phase. An organic substance (hydrophobic) present in the oil phase remains separated internally and externally by an aqueous phase. That is, the oil droplets enclose the aqueous droplets and again these oil droplets remain surrounded by aqueous droplets. Among the multiple emulsions these systems have been more studied. The o/w/o systems are better formed by lipophilic and non-ionic surfactants while in w/o/w systems both hydrophilic and hydrophobic emulsifiers are used. However, the non-ionic surfactant can form the w/o/w emulsions. Various methods are used to prepare multiple emulsions; for example,

- Two-step emulsification,
- Modified two-step emulsification,
- Phase inversion,
- Membrane emulsification, and

- Micro channel emulsification.

In general, two types of emulsifiers are used in the preparation of multiple emulsions. In two step emulsification method, the first step is to prepare the primary emulsion either W/ O or O/W type, then the primary emulsion is re-emulsified with an excess of aqueous phase or oil phase in presence of second emulsifier to obtain the multiple emulsions of w/o/w or o/w/o respectively as shown in Fig 1.9. Out of two surfactants used one is lipophilic and other is hydrophilic surfactant.

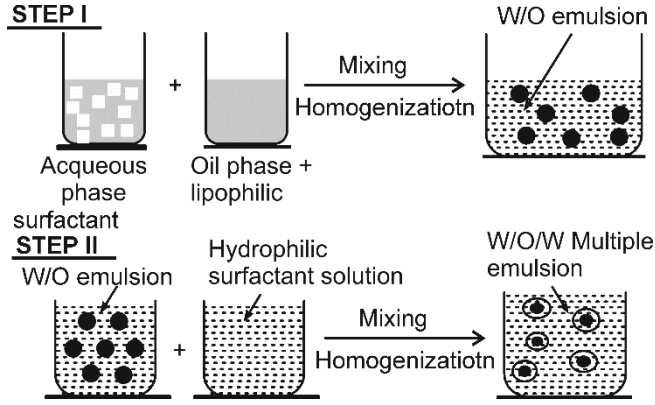


Fig. 1.9 Two-step multiple emulsion preparation technique

Non-aqueous Emulsion

Non-aqueous systems are widely used as solvents for suspension making vehicles and oleo gels. Most of these are emulsions containing water as one phase and are used in pharmaceutical industries. This has extensively found that these emulsions are used for delivery of drug other than topical delivery. Anhydrous, non-aqueous, oil-in-oil emulsions are generally used as drug reservoirs for preparation of microspheres and nanoparticles. The important factors that determine the emulsifying ability of a solvent depends on hydrogen bonding, and non-polarity[54]. Anhydrous or non-aqueous, or oil-polar solvent, or oil-in-oil emulsions have restricted application; hence, limited attention is being paid. But, where the presence of water is not required, this type of emulsions can replace a traditional emulsion[55]. The selection of the solvent and prediction of their respective miscibility and behavior of a surfactant would be necessary for selection of a suitable solvent for developing an emulsion. On the polarity of the solvents, the selection of the two phases mainly depends. Stable oil emulsions in formamide or in polyethylene glycol are prepared by using commercially available non-ionic surfactants. It is difficult to predict the use of other polar liquids as the continuous phase. In fact, the hydrogen bonding and dielectric

constant are very close to formamide and water, and hence, are used as the external phase. While searching for stable non-aqueous emulsions, two basic strategies are found. The surfactants selected should have two incompatible blocks; each of the blocks is selectively soluble in either of the immiscible liquids. To search for a suitable oil-immiscible polar liquid to replace water substantially using existing surfactants, a suitable method is to be found out. For example, non-ionic surfactants having HLB numbers around 12 have been found to stabilize the oil dispersed in formamide. Formamide can replace water during emulsification when non-ionic surfactants are used as stabilizer. In most of the studies, linear alkanes (C_6 – C_{16}) have been dispersed in formamide used as continuous phase using polysorbates 20 as stabilizing surfactant. The mean globule size varied in a non-linear manner with alkyl chain length, the minimum chain length was between C_{10} and C_{12}. Even sonication for 30 sec resulted in smaller difference in the mean globule size. Concentration of surfactant, solvophilicity can influence the function of non-aqueous emulsions. The compositions of some common non-aqueous emulsions are given[56] in Table 1.8.

Table 1.8 Compositions of some common non-aqueous emulsions

Internal phase	External phase	Surfactant used
Castor oil	Dimethicone	Non-ionic
Castor oil	Silicone oil	Non-ionic
Dodecane	Formamide	Ionic, Anionic, Cationic
Dodecane	Dimethyl sulphoxide	Non-ionic
Dodecane	Polyoxy ethylene glycol	Non-ionic
Dimethicone	Castor oil	Non-ionic
Liquid crystals	Silicone oil	Non-ionic
Olive oil	Glycerin	Anionic, Cationic
Silicone oils	Liquid crystals	Non-ionic

Liposome Emulsion

For safe drug targeting to its target site in the precise time period, liposomes are administered. This is done to achieve a controlled release and maximum therapeutic effect. Liposomes provide several advantages in delivering genes to cells. These can be complex both with negatively and positively charged molecules. These offer a degree of protection to the DNA from degradative processes. Large pieces of DNA can be carried. The liposomes may be potentially as large as a chromosome and can be targeted to specific cells or tissues[57].

Suspension

A suspension may be defined as *a disperse system containing solid particles dispersed (dispersed phase) in a liquid phase (dispersion medium)*. It is desired that the internal phase (suspensoid, suspended phase) generally containing solid particles of almost specific size range should remain uniformly distributed throughout the suspending medium or vehicle in which it is practically insoluble. Coarse suspension intended for oral administration usually contains the particles within about 1µm – 75µm in size. Most good pharmaceutical suspensions contain the particles within 50µm in size. In general, pharmaceutical suspensions contain more than one ingredient.

There are various advantages of pharmaceutical suspensions. Compared to an aqueous solution, suspension is reasonably resistant to hydrolysis and oxidation. Thus, pharmaceutical suspensions should have physicochemical stability. Oral suspension have appreciably good elegance and of good palatability. Despite having advantages, suspensions suffer from certain important disadvantages. For example, sedimentation, caking, crystal growth, etc.

A number of formulation components can be included to prepare a physically stable pharmaceutical suspension in which solid particles can remain suspended uniformly. These components can be classified as, (1) components of the suspending system including wetting agents, dispersants or deflocculating agents, flocculating agents, and thickeners, (2) components of the external phase (suspending vehicle) including pH maintaining agents, buffers, osmotic agents, preservatives, coloring and flavoring agents, and the liquid vehicle. Each of the components is selected for their use in oral, topical, or parenteral suspensions.

Oral suspensions of wide ranges of drugs such as antibiotics, antacids, etc. are mostly important. In this group of suspensions, the solid content varies considerably. For example, an antibiotic suspension may contain 125mg to 500mg per 5ml (2.5% to 10%), while a drop concentrate may contain the same amount of drug only in 1 – 2ml. Antacid or radiopaque suspensions intended for oral administration contain relatively higher amounts of suspended material.

Suspending vehicle can be syrup, sorbitol solution, and gum-thickened water with artificial sweeteners. For oral suspensions, taste masking and mouth-feel are important characteristics. Many antibiotics are not stable in aqueous medium; hence, these are supplied as dry powder mixtures which are constituted at the time of dispensing. Generally, this type of products are formulated either in form of powders or granules which on dilution with adequate amount of water and agitation, forms a suspension suitable for administration[58]. Such formulation is categorized in the USP as ‘For Oral Suspension’, while the ready-to –

use preparations are categorized as 'Oral Suspension'. The dry mix products generally contain drugs, colorants, flavors, sweeteners, stabilizing agents (citric acid or sodium citrate), suspending agents (guar gum, xanthan gum, methylcellulose), and preservatives (parabens, sodium benzoate).

Formulation of oral sustained-release suspensions show limited success due to the instability of sustained-release particles in the medium. For this purpose, coated beads, drug impregnated wax matrix, microencapsulation, ion-exchange resins have been used[59-61]. The Penn kinetic system, combination of ion-exchange resin complex with particle coating, has been used to produce commercial products. In this method ionic drugs are first complexed with ion-exchange resins, and the drug-resin complex particles are then treated with impregnating polymer (polyethylene glycol 4000). Finally, these impregnated particles are coated with a sustaining polymer (ethyl cellulose)[62,63]. In the liquid suspensions, the dispersion medium is generally free of ions that could replace drug ions in the resin complex. When swallowed, ions from the gastrointestinal fluid can penetrate the particles and replace the drug ions, which subsequently diffuse out of the system at a controlled or slow rate. The drug release from this system depends on

The type of drug-resin complex provides the ionic environment of the GI tract (pH and electrolyte concentration in the GI fluid).

The properties of the resin used are:

Most of the ion-exchange resins currently used in preparation of sustained-release product contains sulphonic acid groups that exchange cationic drugs containing amine functional groups.

Topical suspensions are prepared for external application; for example, calamine lotion, shake lotion. Safety and toxicity of such products are determined in terms of dermatological acceptability; many new suspending agents have been used in topical formulations. The protective action and cosmetic properties of topical lotions usually require high concentrations of disperse phase.

On the other hand, in parenteral suspensions the concentration of disperse phase is relatively less (low solid content), usually between 0.5% and 5%. However, in penicillin injections the solid content goes beyond 30%. For these sterile preparations the syringability is an important characteristic. Viscosity of these preparations should be sufficiently low to facilitate injection. Commonly, isotonic preserved saline solution or parenterally acceptable vegetable oils are used as suspending medium.

Ophthalmic or optic suspensions are instilled into the eye or ear must also be sterile and are prepared under sterile environment. The vehicles generally are aqueous and isotonic.

SMEDDS

Most of the drugs recently developed are poorly water soluble and their bioavailability is dissolution rate limited. Among all the routes, oral route of drug administration is the most popular and preferred. Failure to achieve the intended therapeutic effect of these drugs by the oral route has forced to develop the self-micro emulsifying drug delivery system (SMEDDS). This system fulfils the therapeutic requirement with minimum dose. There are many formulation approaches such as solid dispersion, complexation, pH modification, and cocrystallization. However, lipid based drug delivery system has gained increased application for increased drug absorption. Among all lipid-based formulations, self-micro emulsifying drug delivery systems, having droplet size less than 100 μm, have been found to increase the oral bioavailability of hydrophobic drugs; because these systems can efficiently solubilize the hydrophobic drugs. By presenting the solubilized drug for absorption, the problem in dissolution is overcome. Various components used to prepare this type of dosage form include surfactants and lipids; these excipients improve oral bioavailability through lymphatic transport of the drug. Thus, hepatic first pass metabolism can be avoided.

Mechanism of self-emulsification

The free energy of an emulsion can be expressed by the following equation:

$$\Delta G = \sum Nr2\sigma$$

Where,

ΔG = free energy,

N = number of droplets,

r = radius of droplet, and

σ = interfacial energy

According to this equation, it is understood that the free energy becomes less when the interfacial energy is less. Self-emulsification takes place when the energy involved in the dispersion is more than the energy required for the formation of droplets[64]. Since, high energy is required to form new surface between two immiscible phases such as water and oil, the free energy of traditional emulsion is high. Probably this may be the reason why an emulsion is usually an unstable preparation and in SMEDDS, emulsion forms easily. Sometimes, the SMEDDS has negative free energy most probably due to the presence of flexible interface.

Mild agitation of an oil and surfactant/cosurfactant mixture with water, an interface is formed immediately between two phases. The aqueous phase starts penetrating and gets solubilized within the oil phase as per

the solubilization limit. If penetration of water is increased, it forms the crystalline phase of dispersed liquid on the basis of concentration of the surfactant. If SMEDDS is mildly agitated, penetration of water starts rapidly and results in the disruption of interface and the droplets are formed[65]. Since, the microemulsions are thermodynamically stable; equilibrium can exist within the system. Although there will be continuous exchange of matter between the two phases. This exchange of matter usually takes place in two different ways –

1. Fusion of small droplets followed by the fission of larger droplet into small droplets, and
2. Disintegration of droplets which subsequently coagulate with other droplets[66].

Drug

When for insufficient absorption of the drug the major cause is its poor solubility, lipid based formulations become the formulations of choice[67]. The drug suitable for this type of formulation must have adequate lipid solubility[68]; that is, should be sufficiently hydrophobic. The partition coefficient in octanol: water must be high. Most of the hydrophobic drugs are soluble in synthetic oils and surfactant than in natural oils. The drugs having low dose show greater bioavailability from the SMEDDS. Thus, the absorption of a drug from SMEDDS depends on its solubility in water and in lipid phase[69]. Because of presystemeic metabolism can be prepared as SMEDDS, if the drug are highly soluble in long chain triglyceride (more than 50mg/ml) and partition coefficient in octanol: water is more than five[70].

Selection of excipients

Based on solubility

For preparation of SMEDDS selection of suitable excipients must be performed. It helps to predict in-vivo precipitation of drug. Thus, solubility of drugs should be done in various oils, surfactants, and cosurfactants should be performed[71,72]. Solubility studies are generally conducted by shaker-flask method at room temperature for about 48 hr. The supernatant solution is first centrifuged, and then filtered through membrane filter of 0.45µm. The filtrate obtained is analyzed for determining drug content per ml.

The purpose of conducting solubility studies are: (1) selection of oil that can solubilize maximum amount of drug and surfactant/cosurfactant having ability to solubilize maximum amount of drug, (2) achieving optimum drug loading capacity with minimum amount of total volume of the formulation[73].

Screening of surfactant/cosurfactant

The ability surfactants to emulsify can be determined by mixing the equal amounts of selected oil and surfactant to obtain a homogenized

mixture after homogenization. To assess the ease of emulsification, the homogenized mixture is added to the double distilled water, and the number of flask inversions required to form homogeneous emulsion is noted. This gives the idea about the ease of emulsification. This resultant microemulsion is tested for clarity, turbidity, and transmittance. The surfactants showing highest percentage transmittance and requiring the lowest flask inversions should be selected[74]. The cosurfactants should be selected following the same method by mixing the selected surfactants, oil phase, and cosurfactant[75].

Preparation

SMEDDS can be prepared by adding the required amount of drug to the mixture of selected oil, surfactant, and cosurfactant and then the total mixture is stirred well until a clear solution is obtained[76]. In some cases, the drug is dissolved in the oil by mixing; then surfactant/cosurfactant mixture is added and mixed well[77]. The solution is then tested for turbidity, kept at room temperature for 48 hr. If required the solution is heated to make it clear solution. The solution is filled in capsules as per the dose size and is subjected to evaluation.

Stability Studies

A stable dosage form in a specific closed container should be capable of retaining its physical, chemical, microbiological, therapeutic, and toxicological specifications throughout its shelf-life. Officially the term stability can be defined as, *the duration of time during which the drug product (dosage form) retains the same properties and characteristics that is found at the time of its manufacture*. In stability testing the effects of environmental factors on the quality of a drug or its formulation is evaluated to

- Predict its shelf life,
- Determine its storage conditions, and
- Labeling instructions

The stability study is designed to assess the effects of variation in temperature, time, humidity, intensity of light and partial vapor pressure on the drug or its product to be tested. When conditions during manufacturing the formulation are not maintained as specified in cGMP, the formulation must be investigated for its stability. In fact, as per regulatory language the formulation is adulterated. Stability testing of a formulation assures the patient's well-being. During formulation development stage, performing stability studies help to select

appropriate container-closure system, storage conditions, and approximate shelf life.

Stability studies can evaluate the physical characteristics of the product such as color, hardness of a tablet, phase separation of an emulsion, caking in suspensions, redispersibility of the cake in a suspension; chemical properties such as purity, impurity, identification of the drug, and therapeutic efficacy, etc. However, the testing parameters will vary with different dosage forms. The major aim of pharmaceutical stability testing is to assure that the products remain at an acceptable level of quality throughout the period during which they are in the marketplace until the patient uses the last unit of the product[78].

Types of Stability Testing

In general, there are four types of stability tests conducted: (1) real-time stability testing, (2) accelerated stability testing, (3) retained sample stability testing, and (4) cyclic temperature stress testing.

Real-Time Stability Tests

Real-time stability test refers to testing a product for long period of time. The product is stored at recommended storage conditions and tested at specific time interval. Usually the stability test is carried out at 0, 3, 6, 9, 12 months in the first year, at every 6 months in the second year and once every year afterwards. The results are collected and tabulated after each of the tests. The reliability of interpretation of the test data can be increased when a reference batch is tested parallely. The stability of the reference batch will also include the stability of the reagents used and performance of the instruments is consistent or not. The performance of reagents as well as of instruments should be regularly monitored[79].

Accelerated Stability Tests

In accelerated stability testing, a product is stored under stress conditions. Generally, the product is exposed to different high temperature (warmer than normal) conditions to accelerate the rate of degradation. The accelerated stability testing is done at 0, 3 and 6 months interval. However, there is ICH guidelines for different climate zones to conduct this study. Degradation at the recommended storage conditions can then be detected by using known relationships between the acceleration factor and the degradation rate. This information can be used to predict the shelf life, probable storage conditions of the product. In addition to temperature, the effect of moisture, light, agitation, gravity, pH, and package should also be measured. Depending on the type, the product kept under stress conditions is refrigerated and analyzed. Since the duration of testing is short, the chance of instability measurement system is reduced in comparison to the real-time stability

testing. In accelerated stability testing, the results of analysis of stressed material are compared with that of unstressed material. For statistical analysis, it is recommended to conduct stability study at four different stress temperatures. The concept of accelerated stability testing is based on the Arrhenius equation expressed as;

$$\ln K = \ln A + \frac{\Delta E}{RT} \qquad \text{.....(1)}$$

Where,

K = rate of degradation,

A = frequency factor,

ΔE = energy of activation (kJ/mol),

R = universal gas constant (0.00831kJ/mol), and

T = absolute temperature (K)

In logarithmic form, the above equation can be written as

$$\left(\frac{k_1}{k_2}\right) = \frac{-E_a}{2.303R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \qquad \text{.....(2)}$$

Where k_1 and k_2 are rate constants at T_1 and T_2 respectively in degree Kelvin, E_a is the activation energy, and R is the universal gas constant.

Both the equation1 and 2 represent the relationship between the rate of degradation changes and temperature. Thus, by using Arrhenius equation the stability of a substance (drug product) can be determined. By drawing a graph of rate of degradation against temperature, the slope of the straight line obtained gives the value of activation energy (E_a). Once the value of activation energy is known, the rate of degradation at lower temperature can be known[80].

Commonly pharmaceutical manufacturing industries for prediction of shelf life of the products use various shortcuts such as Q rule and bracket tables. But these shortcuts are not officially accepted either by ICH or FDA. According to the Q rule, the rate of degradation decreases by a constant factor Q_{10} when the storage temperature is decreased by 10°C. The value of Q_{10} is set at 2, 3, and 4 because these correspond to reasonable activation energy. The assumption of this model that the value of Q does not vary with temperature is not correct.

As per the bracket table concept, the activation energy is between two limits such as 10 – 20 kcal, for a given analyte. Thus, a table may be drawn indicating days of stress at various stress temperatures. The bracket table of 10 – 20 kcal is reasonably useful because broad experience indicates that most analytes and reagents used in pharmaceutical clinical laboratories have activation energies in this range.

Retained sample stability testing

This is a usual testing practice done for every marketed product which requires stability data. In this method, at least one batch in a year is stored and the retained samples of this batch are subjected to stability studies. If the number of marketed batches is more than 50, the samples from two batches are recommended for stability studies. When the product is first time introduced in the market, the samples from every batch may be taken, which may be decreased to only 2% to 5% of marketed batches. In this study, the stability testing is conducted at predetermined intervals. For example, the product has shelf life of 5 years, then the testing would be done at 3, 6, 9, 12, 18, 24, 36, 48, and 60 months. This method is alternatively called as *constant interval method*[81]. Evaluation of marketed samples by stability testing is a modified method. In this method, the samples of the batch already in the market are drawn and evaluated. This type stability study is more practical.

Cyclic temperature stress testing

This is not a method routinely done to evaluate marketed products because cyclic temperature stress is designed to mimic the possible conditions of storage in the market place. Generally, the period of cycle is maintained at 24 hours because the diurnal rhythm on earth is 24 hours. This is most likely experienced by the marketed drug during storage. For cyclic stress testing the minimum and maximum temperatures are selected on product-by-product basis and also on considering the factors such as recommended storage temperature for the product and specific physical and chemical degradation properties of the products. Normally the recommended test should have 20 cycles[82].

Stability of Small Volume Parenterals

The parenterals are divided into two categories – (1) small volume parenteral (SVP) and (2) large volume parenteral (LVP). According to USP the SVPs are 100ml and less and LVPs are more than 100ml in volume. The guidelines for stability of small volume parenterals are presented in Table 1.9.

Table 1.9 FDA stability guidelines for small volume parenterals

A	All products Strength Appearance Color Clarity pH Sterility Pyrogens Particulate matter Store inverted or on sidewise
B	Powder and freeze-dried products Residual moisture Stability after reconstitution

For a new product containing new molecule (chemical entity) the product stability studies can be divided into three categories:

1. Initial accelerated stability testing to screen various formulas and obtain a general profile of the stability of the formulated drug,
2. Longer term pre-market testing of products to establish the storage conditions and expiry date
3. Post-marketing stability on products commercially manufactured to assure no difference between initial stability data and current stability data.

Storage temperature

The definition of 'controlled room temperature' varies from pharmacopoeia to pharmacopoeia. For example, as per USP, it is 15° to 30°C; as per European Pharmacopoeia it is 15° to 25°C. For this reason, FDA has mentioned the temperature in the stability guidelines. Many pharmaceutical companies market parenteral products in other countries. Since there is wide variation in ambient temperature in countries throughout the world, the world has been classified into four climatic zones: (1) Temperate, (2) Mediterranean/Subtropical, (3) Hot and dry, and (4) Hot and humid[83]. For all countries climatic data are available. Physical, chemical, and microbiological properties of drug products depend markedly on temperature and humidity.

In fact, for worldwide marketing, the product should be stored at 30°C and 75%RH. However, a practical method is to build this variation in temperature into one test station (25°C) rather than two (25° and 30°C). Usually, stability testing is conducted at 25°C, 40°C, 50°C and at 60°C.

Solutions

Most of the injectable preparations are solutions and their stability should be evaluated by various parameters such as pH, color, clarity, odor, stopper appearance, particulate matter, toxicity, integrity of

container/closure, and preservative effectiveness. Like other dosage forms, stability of injectable preparations should be assessed based on the effects of duration, temperature, and humidity. As per the compendia or the government, most of the injectable products must contain 90% of the label claim of the active constituents on the day of expiry. Presently the HPLC method has been developed to estimate the drug content. The HPLC method can also be used in quantitative isolation and identification of the drug and its degradation products.

During stability testing, change in the pH of a drug solution can indicate either degradation of the active ingredient or interaction of the constituents of solution with either container/closure or the rubber closure. For example, a significant increase in the pH of a neutral or acidic solution indicates leaching of alkaline material in the solution from the glass container, and it is observed when type III glass (soda-lime) container is used in place of type I (borosilicate) glass container. On the other hand, the pH of highly alkaline sodium barbiturate solution can be reduced causing precipitation of free acid form of the drug. Thus, to control the pH of an injection within a narrow range, suitable buffer should be used.

Color of a solution of injectable drug may change if the drug is stored at high temperature (> 40ºC). This accelerated decomposition of drug happens, if there is oxidative degradation or interaction with metallic component of the rubber closure. Color change of the injectable solution can be prevented by: (1) addition of a chelating agent such as EDTA, (2) replacing air in headspace and in bulk by bubbling with an inert gas such as nitrogen.

Due to Tyndall effect a solution may appear as turbid. The suspended particles may adsorb or scatter diffracted, reflected or refracted light. When transmission of light is reduced by suspended particles through a solution, turbidity is resulted. Usually, an injectable solution during its shelf life transmits 70% or more light. If a solution immediately after manufacture transmits light up to 92 – 97%, and maintains the transmission not less than 70% during 3 – 5 years, it is accepted. The reasons for developing turbidity in solution is particulate matter usually derived from a solution-container interaction, precipitation of a constituent of the solution due to reaction between drug and preservative, drug and closure, preservative and closure, or similar reaction, or growth of microorganism; generally, due to loss of preservative.

Occasionally, a container should be opened and examined for change in odor. Change in odor indicates decomposition if the solution is of sulphur-containing drug or antioxidant. The rubber closure should be removed occasionally to examine for any change in color and surface texture. Many drug solution, particularly those of high or low pH or oil-

based, can interact with rubber closures. Such interaction can be accelerated by storing the vials inverted under different temperatures. If there is any change in appearance of the stopper is noticed, it should be investigated to detect the actual reason for such change.

Samples of different batches should be studied for stability and characterized for particulate matter of 10μm or larger. These batches should be stored under normal storage conditions that is, at 5°C and 25°C, and periodically reexamined using similar methods to check the number and size of particulate matter. Most likely the number of particulate matter may increase due to solution-container interaction, instability of the drug in solution at a particular pH, technique used to solubilize the drug, etc.

Safety tests data (LD_{50} or LD_0) should be collected initially and at intervals during storage, to see whether there is any development of toxic materials being formed. If there is significant change in LD_{50} data, the reason must be explained logically.

Suitable preservatives are added in sufficient quantity in products in multiple-dose vials so that microorganisms unintentionally introduced during use. Therefore, a microbial challenge test must be conducted initially and after every year on the samples stored under ambient condition. Procedures to be followed are given in official compendia. The loss of preservative occurs due to decomposition of antimicrobial agent present in solution and ineffectiveness of the preservative. Due to change in pH of the solution or chelation with metal ions leached from rubber closure, or absorption of metal ions into the rubber closure, microbial challenge may occur. During formulation development of the product, the minimum effective concentration (MEC) and minimum inhibitory concentration (MIC) of preservatives have been determined and the formulation has been prepared. A stability indicating assay method is to be developed to determine the amount of preservative present in samples stored under normal storage conditions. The samples should be tested at regular intervals to quantify the amount of preservative present in samples. Generally, loss of preservative below the MIC is observed during the shelf life of the product.

Sterile solids

Sterile solids (powders) intended for injection should be tested as stated above after reconstitution. That is, potency, color, clarity, pH, odor, appearance of stopper, particulate matter, and toxicity should be checked at regular intervals. The dry powders should be checked for uniformity of color, moisture content, and reconstitution rate (solubilization rate). Besides moisture content and reconstitution rate, other attributes have already been discussed.

Most of the dry powders, whether dry fill, spray dried, or freeze-dried, have optimal moisture content for their stability. In very exceptional

case, vials containing dry, sterile, powders require butyl rubber closures with low MVT. Generally, presence of excess moisture can accelerate the process of chemical degradation; but polymorphic or hydrate form of the drug can accelerate the degradation process. Hence, along with moisture content, the property of polymorphism or hydration of the drug should be tested. In most cases, crystalline form of the drug is found to be chemically stable, for example, cephalosporin antibiotic.

The sterile re-constitutable powders must be immediately (within 1 min or less) go into solution. However, sometimes chemical changes of the drug such as formation of less soluble degradation products or a different crystalline form can delay in reconstitution; that is, the reconstitution rate is decreased. Hence, the powders should be tested for reconstitution and the data for reconstitution rate should be generated.

Suspensions

Parenteral suspensions show instability in a different way as discussed for solution and dry solids. Physical properties such as viscosity, rheological behavior, suspendability, syringability, and particle size distribution are very important and monitored in stability program.

The viscosity of a suspension is expressed as the resistance to flow on shear. With the help of a recording viscometer, the rheological behavior and viscosity of a fluid can be measured. The flow behavior of prednisolone acetate suspension is almost Newtonian with very small hysteresis loop. The linear and consistent sedimentation rate indicates stability of particle size and particle size distribution. Aggregation of particles is either very insignificant. A consistent force is required to eject the suspension from a syringe.

Thus, very fine particles are necessary and the viscosity of the suspension should be such as ejection from a syringe is not affected.

Stability Protocol

The final formulation needs to be evaluated completely for assessment of stability. The representative samples packed in proposed container/ closure system are to be stored at three elevated temperatures and at room temperature and in the light for 12 weeks. A schedule for accelerated stability study is shown in Table 1.10.

Table 1.10 A typical schedule for accelerated stability study

Time (weeks)	Roomtemperature	40°C	50°C	60°C	Light
0	X				
1			X	X	X
3	X	X	X	X	
6	X	X	X	X	X
12	X	X			X

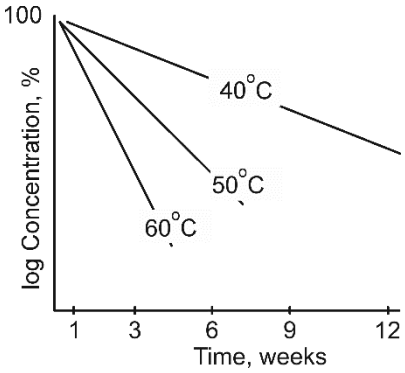


Fig. 1.10 Typical first-order plot of log concentration vs time at three temperatures

After 12 weeks, the results collected can indicate whether the study will continue. Since most of the degradations are hydrolytic in nature follow the first order or pseudo-first order kinetics. By plotting log temperature against time at different temperature as shown in Fig 1.10, the chemical stability of the drug substance can be predicted. Each line will indicate its slope and rate constant can be calculated at a particular temperature.

$$\text{Rate of degradation} = \frac{dC}{dt} = kC$$

Where,

C is the concentration of the reactants,

k is the rate of degradation reaction, and

t is the time

The above equation shows that the rate of reaction is directly proportional to the concentration of the reactants.

The above equation can be written as $\frac{dC}{C} = -k.dt$

Or, $$\log C = \frac{k}{2.303} t + \text{constant}$$

Hence, by plotting the concentration against time on semi log graph paper, the reaction rate can be determined from the slope of the line.

The effect of temperature on the rate of reaction can be expressed as

$$\log k = -\frac{\Delta H_a}{2.303R}\frac{1}{T} + \log S$$

Where,

R is the gas constant (1.987 cal. deg^{-1} mol^{-1})

T is the absolute temperature,

S is the frequency factor, and

ΔH_a is heat of activation.

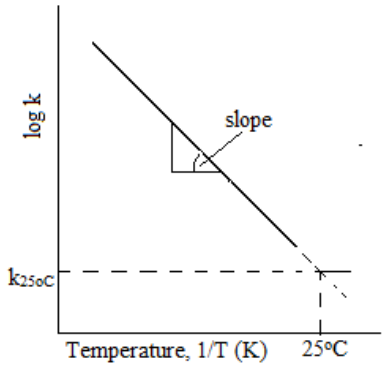


Fig. 1.11 Plot of log k vs. reciprocal of absolute temperature (1/T)

Now, by plotting the rate constants at respective accelerated temperatures against reciprocal of absolute temperature, heat of activation can be calculated from the slope as shown in Fig 1.11.

The value indicates the energy required by the molecule to react. By extrapolating the line as shown in Fig 1.11, the rate constant at room temperature (25°C) can be determined.

By using the following equation the time required for 10% degradation can be calculated.

$$t_{10\%} = \frac{2.303}{k_{25^oc}} \log \frac{100}{90}$$

In the same manner, loss of preservative and loss of antioxidant can also be determined. Thus, the data generated in accelerated stability study can help to take decisions and actions to be taken on the product[84].

Stability of Large Volume Parenterals

The label pasted on each LVP should inform about the date of expiry. Similar to other drug products, a LVP should have an expiry date to ensure that the standards for identity, strength, quality, and purity of the LVP product are maintained at the time of use. The testing is done to establish the date of expiration for the product. The total stability program includes (1) initial studies, (2) proposed product studies, (3) new product studies, (4) established product studies, and (5) special studies.

Preliminary information about the suitability of the combination of solution, container, and closure can be obtained by analyzing the samples monthly during a period of 3 months after storing these at 50°C. If the pH of the solution in a glass container and particle count are increased, the results are examined and necessary action such as

using a suitable buffer to control the pH within limits, changing to a more resistant glass container, or use of a plastic container are taken.

Decrease in mass or increase in assay value of the solution filled in plastic container shall indicate higher moisture vapor transmission. The solution should be tested for any known or anticipated degradation products, and leachable item identified in early test.

If there is change in color or formation of degradation product, either a suitable antioxidant should be added, or the dissolved air and air in head space should be replaced by an inert gas such as nitrogen.

Sensitivity of light can be tested by exposing the samples to a specified number of lumens for a specified time, and the samples should be tested for color and degradation. The results of this test should be compared with the results obtained with the samples kept away from light.

In some cases, the sample is autoclaved at 121^{o}C for 1 hour to get information about the stability of the product during sterilization. The changes in color, pH, assay values, and number and size of particulate matter should be noted and examined whether these are within the limits.

Before introducing into market, the proposed product should undergo stability testing. The data generated in the test should be used to correlate with initial data and decision is to be taken on further course of action. An adequate number of batches should be tested to examine statistical validity of the proposed shelf life. The accelerated stability should be conducted as per ICH guidelines. The samples stored at 30^{o}, 40^{o}, and 50^{o}C would be tested at 30-, 60-, and 90-days intervals. The sample stored under room temperature would be tested initially and quarterly in the first year, half-yearly in the second year and thereafter yearly. The LVP may be considered established, if additional batches are manufactured; however, stability testing should continue to demonstrate its consistent quality. The shelf life of LVPs such as electrolyte solution can be extended up to 5 years, while amino acid or dextrose solutions will have only 2 to 3 years depending on stability characteristics of the product. At this stage, lesser number of batches is to be tested, and during interim test periods, all the tests as per specifications are not to be tested. Complete tests are to be carried out at initial and final stages. Interim tests are planned only to conduct the tests that can track degradation of the product such as pH, color, odor, clarity, chromatography, and assay. The following might indicate the reasons for instability of the product:

- Source or process for an ingredient,
- Changes in the manufacturing process,
- Direct contact of packaging materials with the product,

- Changes or modification of the formula,
- Change in size of package, and
- Changes in the sterilization cycle

The revision of a formula or studying a revised product should be a part of the protocol for the stability program.

To get the answer to the specific question about the product, manufacturing processes, or conditions outside the control of the manufacturer, special studies are conducted. These studies are conducted to help labeling information related to shipping and storage at extreme allowable temperatures, regarding prevention from exposure to light. When the packages are stored upside down, when other drugs are added, or when the user asks questions about the use under special circumstances, all these become the part of special studies program. The data obtained from this study are compared with those obtained from initial studies.

Stability studies include many features such as change of color or formation of a precipitate (physical), change in pH or assay (chemical), or packaging. These must not be reactive and must protect the product during its shelf life. Each of the studies must be well documented and report should be reviewed for decision making, or as a part of quality assurance program. These reports are reviewed by the investigators of FDA[85]. The following reports are to submitted to the FDA for consideration and approve the product.

Table 1.11 Content of stability report

General information about the product	Name of the drug and drug product Dosage form and strength Labeling and formulation
Specifications and methods of testing	Physical, chemical, and microbiological characteristics and prior submission specifications (or specific references to NDA or USP). Methods used for testing (or cited by reference) for each sample tested. Information on specificity, accuracy, precision, and suitability of the methodology (cited by reference).
Study designs and study conditions	Description of the sampling plan, including Batches and number selected. Containers and number selected. Number of dosage units and whether tests were done on individual units or composites. Time of sampling.
	Expected duration of the study. Conditions of storage of the product under study (light, temperature, humidity).

Contd…

Stability data or information	Lot number (research, pilot plant, production) and manufacturing date. Analytical data and source of each data point such as lot, container, and composite. Pooled estimated may be submitted if individual data points are provided. Relevant information on previous formulations or container-closure systems should be included.
Data analysis and conclusions	Documentation of appropriate statistical methods and formulas used in the analysis. Evaluation of data, including calculations, statistical analysis, plots, or graphics. Proposed shelf life and its justification. Release specifications (establishment of acceptable minimum potency at the time of release for full expiration dating period to be warranted).

Physiological Consideration

Depending on the intended use of the formulation the physiological factors vary and the desired action also varies. When a drug for systemic activity is administered, it must be absorbed completely from the site of administration; while a drug is used for its local action, should be negligibly available in the systemic circulation. Again, extended-release drug product show slow release of the drug at a predetermined rate from the site of application and the rest amount remains there for predetermined period[86,87]. Hence, there are various factors that influence the release parameters and rate of absorption of a drug from its product.

Circulatory system

To understand the physiological factors related to parenteral absorption, it is necessary to review the gross anatomy of the circulatory system. There are major differences in blood vessels at the various sites of injection. It becomes important when the environment under which the physiological processes occur during the absorption of drug administered. A drug can exert its expected pharmacological activity, but it must make its own way into the complex circulatory system either by being administered intravenously or by undergoing absorption from extravascular site. The drug is distributed then within the body tissues through capillary beds which eventually connect the arteriovenous system. After exerting its pharmacological effect it is ultimately excreted and/or metabolized. The deoxygenated blood (solid lines) from venous system enters the heart at right atrium and passes to the right ventricle, from which it is pumped to the lungs. Oxygenated blood returns from the lungs into the left atrium and passes through the left ventricle, from where it is pumped into the aorta. The aorta branches into smaller arterioles and finally into microscopic capillaries which exist to varying degrees in all tissues of the body. Ones in the

capillary bed, the process reverses and blood passes from the capillaries into the ventricles, which finally lead to the venous system and ultimately to the vena cava, which empties blood to the right atrium where the loop is completed.

The major function of blood circulation is to supply oxygen, nutrients, any drug administered to the tissues, to remove carbon dioxide, and other metabolic by-products. It is only through capillaries this exchange occurs. Arteries and veins act as channels to and from capillaries. The volume, rate, and pressure for the various sections of the arteriovenous system are different. The capillaries carry only 250ml or 7% of the blood at any time with a flow rate from 50 to 400 times slower than that of aorta and vena cava. During this time blood pressure decreases about 50-fold times.

The human circulatory system contains about 3.6 billion capillaries with a total cross-sectional area of about 4500 sq. cm^{88}. Hence, an average capillary cross-sectional area of only 1.25×10^{-6} sq. cm. Due to this very small cross-sectional area, blood flow is so slow. However, due to this slow blood flow, transfer of nutrients and drugs become possible. During this process of capillary microcirculation, fluid diffuses out containing extremely low concentration of protein besides lipids, nutrients, and drug. The capillaries become more permeable allowing protein molecules to leak out due to slight trauma, anoxia (lack of oxygen) and other factors. When the fluid diffuses back to the capillaries, little amount of protein may be carried with it. Thus, under various conditions, protein can accumulate in the tissue spaces. If such fluids are allowed to accumulate unchecked, swelling would occur. The main function of the lymphatic system is to remove this protein and any other high molecular weight molecules including drug and macromolecular formulation excipients.

Lymphatic vessels begin as blind-ending capillaries. These collect lymph from tissue spaces. These vessels coalesce into larger channels, called lymph ducts. These ducts finally drain into major veins. The walls of lymphatic capillaries are very thin and highly permeable. Proteins and other and larger foreign particles can easily enter. These fluids are of three types: (1) blood plasma, (2) interstitial fluid, and (3) lymph. Interstitial fluid passes from tissues to lymph capillaries by a passive action controlled by pressure difference. Muscle movement, closely associated arteries and their pulsation, and differential pressure occurring during breathing process, regulate the flow of lymph. Lymph returning to the venous system is filtered by lymph nodes. These nodes are imposed along the chain of vessels.

To the various organs and tissues of the body the quantitative blood supply varies according to their functional requirements. Muscle can require maximum up to 10 times the amount of blood during vigorous

contraction at the resting stage[88]. To serve these requirements blood vessels supplying muscle divide into a large number of capillaries. On the other hand, glandular tissues require only a minimal blood supply and have blood vessels with a few capillaries associated with it.

Drug products placed in extravascular tissues must enter the capillaries of the venous and lymphatic systems in the general manner. Once a drug enters the bloodstream it makes its way out through capillaries into the tissues, where it produces its pharmacological action. All drugs administered by injection must traverse this system before they can exert their activity either locally or at some distant organs.

Route of administration

Drugs can be administered through different routes such as enteral route (oral, sublingual, and rectal), parenteral route (intravenous, intramuscular, subcutaneous, etc.), topical, inhalational, intranasal, and transdermal[89]. There are two major factors that control the selection of the route: (1) desired therapeutic properties, (2) properties of the drug[90]. Therapeutic properties include rate of onset of action, duration of action, ready accessibility of the target site, and patient compliance. The properties of drug governing the route of administration is physicochemical properties including molecular size, ionization status, solubility in lipid, etc. and the plasma concentration-time profile[91]. However, the route of administration can be changed based on the disease condition – acute or chronic, emergency. Generally, in case of chronic conditions, oral, topical, transdermal route is used.

Talbe 1.12 Circulation times of intravenous route

Between two sites of the body	Circulation time (sec)
Arm to toe	24.8 $\pm$ 4.4
Arm to finger	17.5 $\pm$ 4.4
Arm to ear	8 – 14
Lung to ear	3 – 5
Rigjt ventricle to Left ventricle	2 – 4

Intravenous route means no absorption step is there. There is no physiological factor which can affect the absorption. Among various sites of human body, the intravenous circulation time is maximum about 25 sec when the drug reaches toe from arm as shown in Table 1.12.

Depending on the property of the drug injected and the site of action, the actual onset of action of a drug varies.

Extravascular route is used to inject a drug. The speed at which the drug enters the blood stream can vary greatly and is influenced by a

number of physiological factors. For absorption, a fraction of drug present in solution is required to be transferred through the capillary wall membrane. It is thought that drugs are absorbed only through this mechanism. No evidence has been reported indicating the ability for drugs to become absorbed directly through the walls of the larger blood vessels.

The capillary cell membrane is very thin and elastic, 75 – 100 Å(1Å = 1×10^{-8} cm). The elastic material contains about 55% of proteins, 40% of lipid, and 5% of polysaccharide substances[92]. However, its exact molecular structure is not uncertain. It is considered that a central layer of lipids is covered by protein layers and then a thin layer of polysaccharide substance covers the outer surface. The central lipid layer makes it impermeable to lipid insoluble substances. This is a significant factor for diffusion of drugs extra vascularly injected. However, very small lipid-insoluble substances such as water and urea can pass through the capillary membrane. These can pass through pores by a process of filtration. The concentration gradient is the driving force. Since the drug does not leave the aqueous solution during filtration, physicochemical factors such as pKa and partition coefficient do not influence the rate of transport. The pores are only about 30Å in radius and total area is less than about 0.1% area of the capillary wall. This process is considered insignificant to the overall membrane transfer process because of its small size.

Anatomical characteristics

Anatomical characteristics of the extravascular injection site considerably influence the rate of absorption of a drug. The surface area available for absorption depends on the number of capillaries at the site. If the number of capillaries is more, greater surface area would be available and more absorption will occur.

Muscle movement

Muscle movement is directly related to the rate of flow through lymphatic vessels. This can be demonstrated by injecting the rabbits subcutaneously. It has been seen that when venom was injected with immobilized limb, it took about 8 hrs to cause death, where as unrestrained rabbits died in 2.5 hr. Absorption of drug increases with increased activity or increased blood flow. Muscular contraction occurs during exercise increases both dispersion of drug and local blood flow. Muscle becomes more vascular than subcutaneous tissue; hence uptake becomes faster.

Tissue condition

Tissue condition indicates the tissue vascularity and hence, affects the absorption of drug. In other words, changes in tissue vascularity caused by scarring can influence the drug absorption. In rabbits the rate of

clearance of hippuran became slow when injection was given at a site, previously mechanically traumatized by injecting sodium chloride injection twice daily for a week. Scar tissue can be formed by injecting at one site repeatedly. Rotation of injection sites can provide more favorable absorption in maintaining good absorption of a drug.

Body temperature

Body temperature is also directly related to the rate of absorption of a drug, as does the rate of metabolism and most other physical and chemical reactions. It is considered that both diffusion and blood flow, and other physiological activities, can be affected by changes in body temperature. Generally, vasoconstriction takes place during the seasons when external temperature is cold. This is the way how the body reduces the loss of heat through surface vessels. On the other hand, during summer when external temperature is more, vasodilation occurs. However, the amount of body temperature changes from normal is usually not so important or controllable factor for designing of a dosage form. Application of heat at the site of injection definitely causes faster absorption due to local vasodilation.

Age of the patient

The factors such as reduced adipose tissue, lower renal clearance are related to the age. However, the effect of age on the absorption of drug from injection sites is not well known, and whether these factors play a definite role is not certain also. Reduced adipose tissue causes a greater availability of drug to the systemic circulation because the ability of retention of drug by such tissue is reduced, particularly if the drug has a high partition coefficient. Usually, lower drug-blood level is found in neonates and children than in adults when the drug is injected based on 1 mg/kg of body weight. This is attributed to high volumes of distribution of drug into body tissues other than blood. This is due to greater relative amounts of total body water per kilogram of body weight in neonates compared to adults[93]. It has been suggested that the thickness and composition of subcutaneous adipose tissue may change with age. May be for this reason, partition coefficient in younger and older tissues changes and there would be variation in absorption of drug. This has also been observed that relative percentages of C_{12} to C_{18} triglycerides between adult and children showed marked difference; this further supports the possibility changes in subcutaneous adipose tissue with age.

Disease state

Absorption of penicillin G from the intramuscular injection site is reduced in case aqueous suspension of penicillin G is injected to patients suffered from heart failure. This can be justified by the fact that a damaged heart cannot pump blood as efficiently as a healthy heart; thus, volumes of blood available for absorption of drug are different. In

another study, it has been found that the metabolic function of cancer cells is higher than normal cells; for this reason, when intravenous or intra-articular injection of methotrexate is administered, drug tissue level is much lower in cancer patients than in normal patients. Thus, rapid metabolism of drug lowers the drug tissue level in cancer patients.

Mediator enzymes

The onset of drug absorption is increased by the enzymes hyaluronidase, even when a drug is administered through intramuscular injection, is a known fact. This enzyme hydrolyzes hyaluronic acid, a component of tissue ground substance. This controls the spread of fluids at the site of injection. After hydrolysis the area of drug distribution in the tissue is increased and as a result, the absorption rate is also increased. Chymotrypsin can increase the rate of elimination of tetracycline from the body from 13% to 37%, the actual mechanism for such effect is not known.

Vasoactive agents

Epinephrine has been found to influence the rate of absorption of radioactive ^{24}Na administered subcutaneously. Epinephrine constricts the local blood vessels present in the area of absorption. As a result, the blood flow is reduced and absorption of drug is also reduced. When epinephrine is co-administered with benzyl penicillin solution, absorption would be significantly reduced. This principle has been used particularly for the administration of local anesthetics. Similarly, it was found that when prostaglandin E_2 was co-administered, its vasodilating effect increased the blood flow and increased absorption of benzyl penicillin[94].

Formulation Consideration

There are various factors which are to be considered during formulation development of injectables to distribute the drug effectively when the drug is administered through subcutaneous and intramuscular injection.

Solubility of drug

The drugs should be completely solubilized before they penetrate the muscle or tissue barriers and enter the circulatory system. Two types of solubility are important – (1) solubility in the vehicle of the dosage form, and (2) solubility in body fluids.

For drugs administered as solution, dosage form solubility is avoided. However, for drugs administered as suspensions, the rate of dissolution of drug from the dosage form vehicle, and the rate of dissolution in tissue fluids at the injection site, mainly determines the rate of absorption of the drug. The dissolution rate of injected drug suspensions depend on

1. The size of drug particles,
2. pH of the fluids at injection site,
3. The polymorphic character of the drug crystals, and
4. The diffusion coefficient of the drug.

At the higher viscosities the diffusion coefficient of the drug is less. Once the drug goes into solution from the dosage form, its solubility in fluids in the subcutaneous tissues or muscles depends on its partition coefficient and its degree of ionization as per the pH of the fluids present at the site of injection.

Partition coefficient of the drug

If the drug is less soluble in lipids, its partition coefficient will be less. Thus, the absorption of the drug into the blood stream from the site of injection would also be slow.

Rate of blood flow at the injection site

It is known that if the blood flow in the capillary network to the site of injection and from there to the capillaries is higher, the rate of absorption of drug would be high. If the drug is injected into the muscle of the lateral thighs or buttocks, the absorption of drug would be less and slow; because of less vascularity and higher fat content. When the drug is injected in the deltoid muscles, absorption of drug would be comparatively higher. Factors that increase the blood flow such as exercise, increase absorption of drug after intramuscular or subcutaneous injection. On the other hand, factors that delay blood flow, such as epinephrine (vasoconstrictors), when administered concurrently at the site of injection, decrease the rate of drug absorption.

Degradation of drug at the injection site

The distribution of drug may be retarded if the drug is metabolized or degraded at the site of injection.

Particle size of the drug

The size of the suspended drug particles can influence the rate of dissolution of the drug in its dosage form vehicle. If the size of the suspended particle is larger, the rate of dissolution would be slower, since less surface area of the drug becomes available for interaction with the body fluids. The precipitated particles may dissolve ultimately, but the rate of dissolution would be slow.

Formulation ingredients

The materials those are added to the formulation for different reasons such as cellulose derivatives for suspendability, glycerin for solubilization, antioxidants for improvement of stability, can potentially affect the distribution of drug from the site of administration. These effects may be apparent in different ways, such as complexation

reduces the rate of drug dissolution and increased viscosity can slow down the passage of drug from the site of injection to the systemic circulation.

Manufacturing

Manufacturing of a parenteral formulation involves the mixing of one or more ingredients with a drug and a suitable vehicle to improve the convenience, acceptability, or effectiveness of the formulation. Only when a liquid preparation is either not stable or possible to formulate, dry sterile powder is dispensed. Drug is a chemical compound having definite physical and chemical properties, it is necessary to screen the excipients and investigate for any interaction, before mixing them. If any interaction occurs, the formulation requires to be modified. As such preparation of a sterile formulation is very challenging[95].

In case of a new therapeutically active compound, the amount of information available to the formulator regarding the physical and chemical properties of the compound and its interaction with other compounds, sometimes, may appear insufficient. As regards the new compound, information about the basic properties such as purity, molecular weight, solubility, colligative properties, and chemical reactivity must be known. Improvement in a formulation is a continuous process because important properties of a drug or of its product cannot be known unless the drug product has been stored or used for a long period of time. Because of the extensive test documents are required by the USFDA, only the outstanding formulations would be allowed to continue the marketing the product. The major excipient for parenteral product is the solvent or vehicle; others are antioxidants, preservatives (for SVPs), solubilizer, mild viscosity imparting agents, buffers, etc.

Solvent system

Most of the parenteral preparations are solutions. If it is aqueous, the solution should be physiologically compatible with body tissues, and the biological response to be exhibited should be logically predictable.

The universal solvent, water has high dielectric constant and hence, can dissolve electrolytes. It can form hydrogen bonds with the organic compounds such as alcohols, aldehydes, ketones, and amines. On the other hand, it can poorly dissolve nonpolar compounds such as alkaloidal bases. In fact, nonpolar substances are soluble in nonpolar solvents. The drug substances (therapeutically active compounds) administered by injection may be highly polar to nonpolar; accordingly the polarity of the solvent should be used to dissolve the drug so that a complete solution is achieved[96].

Therefore, a solvent selected or used for injection must be of low toxicity to body tissue. Sometimes, to achieve required solubility cosolvents such as ethanol is used. For example, 40% ethanol in water is used to dissolve digitalis glycosides. Compounds that are solubilized in water can undergo degradation such as hydrolysis, oxidation, decarboxylation, and racemization.

In such cases, formulation should be designed in such a way that the degradative effects are as minimum as possible. In most cases the pH of the solution becomes highly affected; hence, pH is also required to be maintained to reduce degradation. For example, in solution epinephrine undergoes racemization and oxidation, if the pH of the solution is maintained at 3.0 or less, little reaction can occur. Oxidative degradation can be avoided by replacing dissolved oxygen by an inert gas such as nitrogen and by adding 0.1% sodium metabisulphite as an antioxidant. Atropine sulphate in water rapidly hydrolyzes; this can be avoided significantly by adding a suitable buffer to maintain pH at about 3.5 to 4.0.

Sometimes, the mixed solvents can reduce the degradative reaction. For example, derivatives of barbituric acid hydrolyze readily in water, particularly at low pH; but by dissolving pentobarbital sodium in a mixed solvent system containing 60% polyethylene glycol 400 and 10% ethanol in water at pH of about 8.0, the solution can be made stable. However, degradation reactions can be avoided by using anhydrous, nonpolar solvent such as fixed oil. In cold seasons, oleaginous injections cannot be used due to their viscosity.

Additives

To achieve required stability and therapeutic efficacy, various additives such as antioxidants, antibacterial agents (preservatives), buffers, and tonicity modifiers are added to injection formulations. In some cases, chelating agents such as tetra sodium or calcium disodium salt of ethylenediamine tetra acetic acid may be added to the formulations to bind the free heavy metals which can catalyze the degradation reactions. Most commonly the above chelating agent is used in a concentration of about 0.05%. For example, this chelating agent is used to stabilize thimerosal in poliomyelitis vaccine. Thimerosal is used as bacteriostatic agent in the vaccine but it is not stable in the presence of cupric ions. If thimerosal degrades, its degradation products destroy the antigenicity of the vaccine. Thus, stabilization of thimerosal means stabilization of vaccine. Sometimes, heavy metals leached out from the rubber closure can be bound by the chelating agent, and hence can reduce the chance of interaction between the product and rubber closure.

In case of suspensions of a compound can be stabilized by adding slightly soluble salt through common ion effect. For example, procaine

hydrochloride reduces the solubility of procaine benzylpenicillin and helps to stabilize the crystals in aqueous suspension of the antibiotic.

Sometimes, complexation takes place between the added ingredient and macromolecule in the formulation. For example, methyl and propyl parabens (preservatives) can form complex with polysorbate 80, and decrease the antibacterial activity. Hence, the preservative efficiency is reduced. The preservative efficiency can be regained by compensating the quantity of preservative bound with non ionic surface active agent.

The manufacturing process consists of all the steps involved starting from accumulation of data to packaging of finished product and making ready for distribution. Personnel are intimately associated with these processes and they complete the processes using the facilities available or provided for operation. Ideally, planned processes can be completed ineffective by the personnel who do not possess the right attitude and training, or by utilizing the facilities that do not provide an effective and controlled required environment.

To assure successful manufacturing operations, all process-steps must be written after being revealed to be effective. All these steps when made available as written methods, these are called standard operating process (SOPs). These standard operating procedures (SOPs) need to be approved by the authority and cannot be changed without any permission and justification. For any change in the original SOPs to be made, permission from the authority would be required with proper justification. According to the FDA's Good Manufacturing Practices, the documented evidences to be produced indicating that these SOPs have been followed critically and the quality of finished product are as per specification. In fact, in-process control is essential to assure the quality of the product. In-process quality control is more significant than the final testing of a finished product. The production of a quality product is the outcome of continuous, dedicated effort in developing, performing, and compliance with the SOPs by the QA, Production and QC personnel within the manufacturing house. A flow diagram for each product is to be provided to improve observation how the materials are being moved during the manufacturing process. A typical flow diagram for material movement is shown in Fig 1.12.

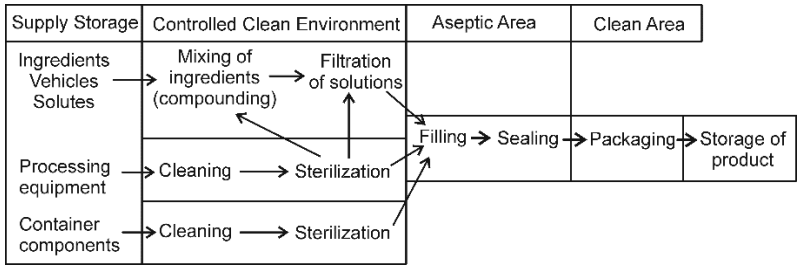


Fig. 1.12 Typical flow diagram of materials in manufacturing department

The first step is to collect all materials (raw and packaging materials, equipment) necessary for a particular batch as per the formula from their respective storage area. Of course, all these materials are approved and released for use. According to the master formula or batch formula the ingredients are mixed or compounded in a desired or required environment so that highest degree of cleanliness and safety can be maintained. In case of a solution, the bulk solution is filtered in such a way that it becomes free from bacteria. That is, it is filtered first through a filter press and then through a membrane filter. The filtered solution must reach to the filling room maintained aseptic. The process equipment and containers used in manufacture of the product must be thoroughly cleaned according to the cleaning process for meeting the specifications.

Process equipment and container components are cleaned thoroughly according to the cleaning process guidelines. All these should meet the required specifications, are assembled under a clean environment. If suitable, the materials and depyrogenated before use.

All equipment and supplies to be used in filling area must be sterile. It would be better to have one door of a double door sterilizer should be facing towards the filling area so that sterility of the articles could be better maintained. When this is not available, all the articles sterilized such as hose lines from equipment, other small size articles should be passed to the filling area after sterilization through as small as possible opening. The opening should be reclosed immediately after passage of the articles and under aseptic condition so that least chance of contamination remains there. All the articles for their sterilization should be wrapped properly. It is recommended that the outer wrapping should be wiped with a suitable disinfectant solution, since these have been transferred from non-aseptic area to aseptic area. The sterility of the aseptic area must be maintained all along. The outer wrappings are loosened, and inner wrappings are opened and contents are received.

Once the filling operation is completed simultaneously sealing is done, so that sterility of the content of the container is not affected. As shown in Fig 1.14 both filling and sealing areas are the aseptic; thus, when the product is filled in every container and the container is sealed thereafter. As per the schedule the sterility of the area should be checked periodically. Similarly, the cleanliness of the non-sterile areas is checked to ensure the cleanliness as per specification. The final filled product is then transferred to the packaging area maintained clean. However, it is necessary to maintain the cleanliness as per the standard maintained in aseptic room. The packed products are then transferred to

quarantine storage area until the batch is completely tested by QC department and finally approved by QA department after evaluating all the in-process control test reports. After getting the approval of QA department, the batch becomes ready for distribution.

Evaluation

Parenteral products include injections and implanted drug products which are administered through injection. The injections are of following types

Injection (solution)

Powder for injection or Infusion

Intravenous Infusion – small volume and large volume

Suspension for injections

Emulsions for injection

Irrespective of the type of parenterals the following tests are applied to evaluate parenteral products. The quality of an injectable is assessed by evaluating the product for following tests as per the USP. All the following parameters are considered responsible for safety and therapeutic efficacy of the drug.

- Uniformity of content
- Extractable volume
- Particulate matter in the injections
- Bacterial endotoxin present
- Presence of pyrogen
- Sterility

1. **Uniformity of content & weight:** The amount of the active ingredient (drug)present in each of the 10 containers which have been sampled randomly is analyzed. If all of the individual values determined are found within 85 to 115% of the stated value, the sample can be considered to comply with the test. If one value is outside the limits of 75 to 125% of the stated value, the sample would be considered as failed. If one individual value is outside of 85 to 115% but within 75 to 125 percent of the stated value, the test is repeated using another 20 containers sampled randomly. The preparation under investigation complies with the test if among the total sample of 30 containers if only one individual value is outside the limits of 85 to 115 percent and none are outside the limits of 75 to 125 percent of the stated value. The limits for uniformity in weight as per the USP are shown in table 1.13.

Table 1.13 Limits for uniformity in weight

Parenteral formulation	Stated mass (mg)	Percentage deviation (%)
Powders for parenteral use	More than 40	10
Powders for eye drops	Less than 300	10
Powders for eye lotions	300 or more	7.5

2. **Extractable volume**
   Method I: In this test 6 containers are to be used, when the nominal volume is within 5ml; 5 containers for the tests and 1is used for rinsing the syringe. A syringe with appropriate capacity is rinsed with the sample and then, the content from each container is withdrawn as much as possible, transferred into a dry graduated cylinder of such capacity that the total combined volume to be measured occupies not less than 40% of the nominal volume of the cylinder. During each transfer the needle is not emptied. The process is repeated until the contents of the 5 containers have been transferred and the average volume of the content is measured. The average volume of the content of the 5 containers should be within the 100% to 115% of the nominal volume. Alternatively, the volume of contents in milliliter can be calculated by dividing the mass (grams) by the density (gm/ml).
   Method II: This method is used when the nominal volume is more than 5ml.The content of not less than 3 containers is transferred separately into a dry graduated cylinders as stated above so that the volume to be measured occupies not less than 40% of the nominal volume of the cylinder and the volume transferred is to be measured. The contents of each container should not be less than the nominal volume and not more than 110% of the nominal volume.
3. **Particulate matter in injections:** The test for particulate matter can be alternatively called the test for clarity. The test is done to detect the presence of any particulate matter, either floating or sedimented. In solution injections and parenteral infusions the particulate matter represents extraneous mobile undissolved particles, not any bubble of air/gas, present in the solutions unintentionally. Solutions for injection to be administered by the intramuscular or subcutaneous route must meet the requirements of Particulate Matter in Injections.
   For indefinite period this requirement has been postponed for products for veterinary use.
   The parenterals packed and labeled exclusively for use as irrigating solutions are also exempted from the requirements of Particulate Matter in Injections.
   Radiopharmaceutical preparations are also exempted from this test.

When the parenteral products (injections) require final filter before administration are exempted from the tests for *Particulate Matter*, provided that scientific data are available to justify this exemption.

Method: Before opening, wash the outer surface of the container by using particle free water-jet; remove the closure carefully so that no contamination could occur. Mix the contents by inverting the container slowly for 20 times successively. Stop the bubbling of the gas by suitable method such as keeping the sample undisturbed for 2 min or by sonication. For large volume parenterals, single unit may be sufficient for the test; while for small volume parenterals, a mixture of 10 units or more that makes a volume of 25 ml may be taken for the test. The contents are mixed in a clean container. The test solution can be prepared by mixing the contents of a suitable number of units such as vial or ampoules and diluting to 25 ml with particle free water or with particle free solvent, if particle-free water is not suitable. When each unit of small volume parenterals contains 25 ml or more, the product can be tested individually. If an appropriate sampling plan is used, less than 10 units may be sufficient for the test.

Remove four portions, not less than (NLT) 5 ml each, and count the number of particles equal to or greater than 10 μm and 25 μm. Do not consider the result for the first portion. Calculate the mean number of particles for the preparation to be examined.

Primarily, there are two methods used to determine the particulate matter in the sample:

1. Method I (Light Obscuration Particle Count Test), and
2. Method II (Microscopic Particle Count Test).

Method I is the most suitable for examining injections for sub-visible particles. However, in some cases both methods are used to arrive at doubt free conclusion for conformance to the requirements.

The criteria of Test 1.A is applicable to the preparations filled in containers with a nominal volume of more than 100 ml.

The criteria of Test 1.B is applicable to the preparations filled in containers with a nominal volume of less than 100 ml or equal to 100 ml.

If the average number of particles is more than the limits, the sample is examined by the Microscopic Particle Count Test 1.A: This test is applied to the solutions for parenteral infusion or solutions for injection supplied in containers with a nominal volume of more than 100 ml. The sample must comply with the test, if the average number of particles present in the unit tested does not exceed 25 per ml or greater than 10 μm and does not exceed 3 per ml or greater than 25 μm.

Test 1.B: This test is applied to solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of less than 100 ml. The preparation complies with the test if the average number of particles present in the units tested is not more than 6000 per container or greater than 10 µm and is not more than 600 per container or greater than 25 µm (effective spherical diameter). The limits for particle number and their size are presented in Table 1.14.

Table 1.14 Limits for particulate matter as per IP, BP, EP.

Volume of solution	Particle size≥ 10µm	Particle size≥ 25µm
Small volume injections (< 100 ml)	3000 per container	300 per container
Large volume injections (> 100 ml)	12 per ml	2 per ml

4. **Bacterial endotoxin test or LAL (Limulus Amebocyte Lysate) test:** The bacterial endotoxins test (BET) is performed to find out or to quantify the endotoxins of Gram negative bacteria using amoebocyte lysate from the horseshoe crab (*Limulus Polyphemus* or *Tachypleustridentatus*). For this test there are three methods: A, B, and C.
   - Method A is the gel-clot technique, which is based on the concept of gel formation.
   - Method B is the turbidimetric technique. It is based on the development of turbidity after cleavage of an endogenous substrate.
   - Method C is the chromogenic technique. The method is based on the development of color after cleavage of a synthetic peptide-chromogen complex.

   Unless otherwise stated in the individual monograph, the Method A as indicated below should be followed. This test is conducted to know whether there is any bacterial endotoxin in the sample or not. The USP reference standard contains 10,000 USP endotoxins per vial. The LAL reagent is used to form a gel-clot. According to the test, a stated volumes of products, standard, positive control, and negative control of endotoxin are taken. The tubes are incubated without any vibration at 37±1ºC for 60 ±2 minutes.

   The integrity of the gel for tests is carried out in tubes as follows: take out each tube in sequence directly from the incubator and invert it at approximately 180 degrees in one single smooth motion. If the gel formed is firm, it remains ‘as it is’ upon inversion, the result is then recorded as positive. A result is negative if an intact gel is not

formed. The LAL test is not considered valid unless the lowest concentration of the standard solutions shows a negative result in all replicate tests.

The endpoint would be the lowest concentration of the standard endotoxin that clots the lysate. Determine the geometric mean of the endpoint concentration by calculating the mean of the logarithms of the endpoint concentrations of the four dilution series; take the antilogarithm of this value, as indicated in the following formula:

Geometric Mean Endpoint Concentration = antilog $\frac{\sum e}{f}$

Where, $\sum e$ = the sum of the log endpoint concentrations of the dilution series used, and

f = the number of replicate test tubes

The geometric mean endpoint concentration is the measured sensitivity of the lysate (IU/ml). If this is not less than 0.5λ and not more than 2λ, the labeled sensitivity is confirmed and is used in tests performed with this lysate. The test must be carried out in a manner that avoids endotoxin contamination.

5. **Pyrogen test:** The test is performed on the rabbits (test animals). The volume of sample solution to be injected should be of 10 ml per kg body weight of the rabbit and should be injected through ear vein when the animal's body temperature is 37±2ºC. The sample should be made isotonic either by adding required amount of Pyrogen free sodium chloride. The rabbits before and during the test should not be excited. The temperatures are recorded at 1, 2 and 3 hours after injection.

   If no rabbit individually should show any rise in temperature of 0.6ºC or more thanthe respective control temperature, and the sum of three temperature-rises should not exceed 1.4ºC;if this is observed, the test material passes the test for the absence of pyrogens. Otherwise the product fails and the test should be repeated as per the method described in the respective Pharmacopoeia.

6. **Sterility test:** The test for sterility must be carried out under sterile environment. Sufficient care must be taken to prevent contamination. Thus, the sterility of theenvironment needs to be checked frequently. The type of medium to facilitate growth of aerobic, anaerobic bacteria and fungi are selected. Generally, fluid Thioglycollate medium is used for growth of anaerobic bacteria. However, this medium can detect the presence of aerobic bacteria. Soybean-Casein Digest medium is used for culture of fungi and aerobic bacteria. The pH of the medium after sterilization should be maintained at 7.1 ±0.2. the filtration assembly is sterilized before use.

Filtration of the solution is done through 0.45μm cellulose nitrate membrane having diameter of 47mm. The filtration rate is adjusted to 55–75 ml of water per min under 70mm of Hg. After filtration the membrane is cut into two pieces for inoculation of the respective media for bacterial growth and fugal growth. The liquids, soluble powders containing bacteriostatic or fungistatic properties, oils, creams and ointments after being suitably diluted with suitable solvent should be filtered by using membrane filtration technique. For bacterial growth the inoculated medium is incubated at 30°–35°C, and for fungal growth at 20°– 25°C for 14 days.

Sterility test can be done by using direct inoculation method; that is, directly by aseptic transfer of specified volume of sample from the container to the culture medium; after inoculation the sample is incubated for 14 days and visually inspected on 3rd, 4th, 5th, 7th, 8th and 14th day for any growth. The test is met when no growth is observed on any day. If growth is observed, the test should be repeated with double numbers of samples (under test) in first stage when the test was found to be conducted under faulty or inadequate aseptic techniques.

Oily liquids, ointments and creams are emulsified with suitable emulsifying agent such as polysorbate 80 before dilution and filtration.

The test results as per different pharmacopoeias are shown in Table 1.15 below.

Table 1.15 Results of the sterility test (temperature limit) as per IP, USP, BP and EP[97]

Pharmacopoeia	No. of rabbits in a group	Approved, if the Temperature (°C) is ≤	Failed, if the Temperature (°C) is ≥
IP	3	1.4	-
	8	3.7	Temp should not rise more than 0.6°C for each rabbit
	3	-	-
USP	8	3.3	-
	3	1.15	2.65
	6	2.80	4.30
BP & EP	9	4.45	5.85
	12	6.6	6.6
	3	1.3	2.5

7. **Clarity of Solution:** Test for 'clarity of solution' is performed to make sure that the injectable under test is free from foreign particles. The injection is to be reconstituted as per the direction given in the label: a) The solid must dissolve completely, so that no visible residue as left undissolved. b) Clarity of the constituted injection is not significantly less than that of an equal volume of diluents in water for injection contained in a similar container and examined in the same manner.
8. **Leak Test:** This test is performed only on filled ampoules sealed by fusion of glass to make sure that no ampoules have any leakage that may result in contamination. The test is conducted by using a) Vacuum Chamber Test, b) Dye Bath Test.
9. **Identification tests:** As directed in individual monograph, the tests are to be carried out to ensure the identity of the substance.
10. **pH of the preparation:** In case of aqueous solution, the pH of the preparation is directly measured. The nonaqueous preparations should be prepared as directed in individual monograph.
11. **Assay:** The drug content of the preparation is determined by specific method as described in respective monograph[98].

The specifications for injections and powders for injection as per different pharmacopoeias are given in table 1.16 below.

Table 1.16 Specifications for powders for injection and injections as given by IP, BP, EP and USP

Test	IP	BP	EP	USP
Uniformity of Content	85 –115%	85 –115%	85 –115%	85 –115%
Extractable Volume	100 – 110%	–	100 – 110%	100 – 110%
Particulate matter in injections	≥ 25 µm – 2% can be present	≥ 25 – 4dss 2% can be present	–	≥ 25 µm – 2% can be present
Bacterial endotoxin Test	Shall not show positive result			
Pyrogen test	Temperature should not rise more than 0.6°C for each rabbit	Summed temperature of 3 rabbits should not be more than 1.15°C	Summed temperature of 3 rabbits should not be more than 1.15°C	Temperature should not increase more than 0.6°C for each rabbit
Sterility Test	No growth shall be found in 14 days			

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Exercises

Multiple Choice Questions Carrying 01 Mark

1. An excipient is used in a formulation for which of the following purpose?
   (a) To alter the dose.
   (b) To facilitate the manufacturing process
   (c) To alter stability characteristics of the formulation
   (d) None of the above
2. During manufacture of a product which of the following point must be kept in mind?
   (a) Properties and limitation of excipients used
   (b) Properties and limitation of the drug substance
   (c) Advantages and limitations of the method used
   (d) All of the above
3. Which of the following components is not generally used in a capsule dosage form?
   (a) Sucrose powder (b) Dry starch powder
   (b) Starch powder (d) Talcum
4. Complexing agent is generally used for the purpose of
   (a) Decreasing the bioavailability of poorly water soluble drugs.
   (b) Increasing the bioavailability of water soluble drugs.
   (c) Increasing the bioavailability of drugs which are very soluble in water.
   (d) Increasing the bioavailability of poorly water soluble drugs.
5. A volatile substance can be formulated by using
   (a) Macromolecule
   (b) Maintaining a particular pH

(c) Suitable viscosity imparting agent
(d) Diluent

6. Pseudo polymorphism generally occurs
(a) On grinding (b) After crystallization
(b) Before crystallization (d) After heating

7. Solvolysis is which type of interaction between two or more components in the formulation
(a) Physical interaction between drug and solvent
(b) Physical interaction between drug and cosolvent
(c) Chemical interaction between drug and solvent
(d) Chemical interaction between solvent and cosolvent

8. Which of the functional groups mentioned are not involved in solvolysis
(a) Imide (b) Amide
(b) Lactam (d) Carboxylic acid

9. Which of the following impurities is commonly found in talcum powder?
(a) Heavy metals (b) Peroxides
(b) Chlorides (d) Formaldehyde

10. A chemical degradation of the drug having smaller therapeutic windows, causes
(a) The plasma concentration become too high to exert toxic effect
(b) The plasma concentration become too less to make them therapeutically ineffective.
(c) All of the above
(d) None of the above

11. Which of the following dosage form is subjected to degradation under humid condition?
(a) Solution (b) Tablets
(b) Suspension (d) Aerosol

12. Which of the following statements is correct?
(a) Pearl is a dispersion of solid in solid
(b) Pearl is a dispersion of solid in liquid
(c) Pearl is a dispersion of liquid in liquid
(d) Pearl is a dispersion of liquid in solid

13. Thermodynamic equilibrium of a dispersion system depends on
(a) Surface free energy of the system
(b) Surface tension of the dispersed phase
(c) Surface tension of the dispersion medium
(d) Interfacial tension of the system

14. The size of the dispersed particles/globules is the lowest, in case of
    (a) Microemulsion (b) Nanoemulsion
    (b) Colloidal emulsion (d) Emulsion
15. Which of the following is not considered a reason for instability of drug?
    (a) Keeping the size of package unchanged
    (b) No modification of the formula
    (c) Changes in the sterilization method
    (d) Changes in the manufacturing process
16. In an injection the color of the injectable solution may changes due to
    (a) Presence of nitrogen in the head space
    (b) Addition of EDTA in the product
    (c) Presence of air in the head space
    (d) None of the above
17. The free energy of a self- emulsifying formulation can be calculated as
    (a) Free energy=$\sum$(Number of droplets × diameter of droplet × interfacial energy)
    (b) Free energy=$\sum$(Number of droplets × radius of droplet × interfacial energy)
    (c) Free energy=$\sum$(Number of droplets × diameter of droplet × interfacial energy)/4
    (d) Free energy=$\sum$(Number of droplets × diameter of droplet × interfacial energy)/2
18. Which of the following ingredients should be present in dispersed phase of a suspension?
    (a) Buffers (b) Thickeners
    (b) Osmotic agents (d) Preservatives
19. Non-aqueous emulsion systems are widely used
    (a) For making topical emulsion only
    (b) As a dosage form only
    (c) As solvents for drugs
    (d) None of the above
20. An emulsion becomes stable and uniform when
    (a) The particle size of the droplets varies from 1 – 1000 μm
    (b) The particle size of the droplets varies from 10 – 1000 μm
    (c) Its internal phase contains the droplets varies from 0.10 – 1000 μm
    (d) Its internal phase contains fine and mono-sized droplets,

Short Questions Carrying 3-5 Marks

1. What are the reasons for which different excipients are incorporated in a pharmaceutical formulation?
2. What are different classes of excipients in solid dosage forms and mention the function of each.
3. What is pseudopolymorphism? Give an example of pseudopolymorphism
4. What is oxidative stress testing in stability testing?
5. What are different classes of the dispersion?
6. What are factors related to thermodynamic equilibrium of dispersion?
7. How the wetting of particles is related to dispersion?
8. Explain briefly the Newtonian flow of liquid.
9. Under what circumstances an emulsion remains stable and uniform?
10. What is the principle behind the formulation of self-micro emulsifying drug delivery system?

Long Questions Carrying 7-9 Marks

1. Explain how oxygen and water can decompose a drug in its formulation?
2. Explain in brief the mechanisms of drug degradation.
3. Explain in short how the drug and excipient physically interact.
4. Discuss briefly what happens in photo degradation.
5. Describe briefly salient features of different types of dispersion classified on the basis of particle size.
6. How the non-Newtonian flows of liquid is related to stability of suspensions?
7. Explain the mechanism of self emulsifying drug delivery system.
8. On what basis the excipients for SMEDDS are selected?

Answers

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
b	d	a	d	a	b	c	d	a	c	b	d	a	b	d	c	a	b	c	d