Chapter 1
Introduction

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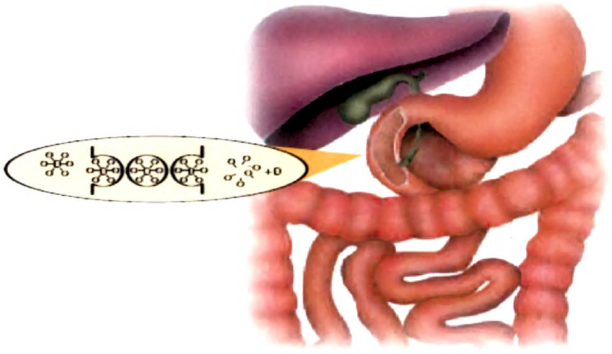


The oral route has been considered as the most convenient route for drug administration. Drug formulations intended for oral administration are preferred over their non-oral alternatives mainly for reasons such as lower production cost, better suitability for self-medication, higher level of patient safety and better patient compliance[1]. In order to be efficient, an orally administered drug must meet some special criteria. For instance, it must possess sufficient solubility in the gastrointestinal (GI) fluids, should withstand acidic and enzymatic degradation in the GI tract, and must be able to permeate the intestinal barrier so as to reach the systemic circulation in sufficient amounts[2]. It has been found that several newly synthesized drugs belong to Biopharmaceutical Classification System (BCS) class II, III and IV. Thus, depending on the physico-chemical properties of the drugs, either solubility or the permeation rate across the intestinal epithelium may be the rate limiting step for the drug to enter systematic circulation. In case of drugs belonging to BCS class II and IV, solubility is a rate limiting factor. These drugs possess low oral bioavailability. As a result, small amount of drug is available in the systematic circulation requiring frequent dose in a day and thus resulting in increased dose dependent systemic side effects into the body. Several physical and chemical approaches, used to overcome the low solubility problem are mentioned in Table 1.1[3]:

Table 1.1.: Different Physical and Chemical Approaches for enhancement of drug solubility.

Physical Modification	Reduction in particle size of the drugs (Nanoparticles, Nanocrystals and Nanosuspension).
	Modification of the crystal habit.
	Polymorphs.
	Pseudopolymorphs (including solvents).
	Complexation/Solubilization (Use of Cyclodextrin and its derivatives/ surfactants).

	Microemulsifying and Self-Microemulsifying Systems.
	Drug dispersion in carriers (Eutectic mixture, Solid dispersion, Solid solution).
Chemical Modification	Soluble prodrugs.
	Salt Formation.

Table 1.1. summarizes various formulation and chemical approaches that can be taken to improve the bioavailability and solubility or to increase the available surface area for dissolution. Of the physical approaches, there has been plenty of prevailing literature on polymorphs[4], amorphous form of the drug[5] and complexation[6,7]. Decreasing the particle size of the compound by milling the drug powder theoretically results in an increase in the available area for dissolution, but in some cases the micronized powder tends to agglomerate, thereby at least partly negating the milling procedure. Presenting the compound as a molecular dispersion combines the benefits of a local increase in the solubility (within the solid solution) and maximizing the surface area of the compound that comes in contact with the dissolution medium as the carrier dissolves.

1.1. Microemulsions

Microemulsions (ME) are clear, thermodynamically stable, isotropic mixtures of oil, water and surfactant, frequently in combination with a co-surfactant. These systems are currently of interest to the pharmaceutical scientist because of their considerable potential to act as drug delivery vehicles by incorporating a wide range of drug molecules[8].Hoar and Schulman (1943)[9] introduced the concept of ME in 1940's. The concept was debated and redefined as a "system of water, oil and amphiphile which is single, optically isotropic and thermodynamically stable liquid solution. The key difference between ME and emulsion is their kinetic and thermodynamic stability. The differences are enlisted in following Table 1.2[10].

Table 1.2. Comparison of emulsions and microemulsions

Sr. No.	Emulsions	Microemulsions
1	Kinetically stable formulations	May or may not be kinetically stable
2	Thermodynamically unstable	Thermodynamically stable
3	Appearance is cloudy	Single, isotropic, clear or translucent solutions

4	Requires large energy input at the time of preparation	Do not require any energy

Microemulsions in broader terms, doesn't contain any microstructure and it includes system that are co-solvents i.e. system wherein the constituents or components are molecularly dispersed. However, most researchers in the field agree that for a ME to be formed it is important that the system contains some definite microstructure. In other words there is a definite boundary between the oil and the water phases at which surfactant is located. It is imperative to consider the structure and properties of surfactants located at the interface between oil and water phases. The conventional surfactants comprise of a polar head group region and a non-polar tail region. The non-polar region is having larger volumes particularly in case of ionic surfactants. On dispersal in water, surfactants self-associate into a variety of equilibrium phases, the nature of which stems directly from the interplay of the various inter and intra-molecular forces as well as entropy considerations. Surfactants also self-associate in non-aqueous solvents, particularly apolar liquids such as alkanes. In this case, the orientation of the surfactant molecules is reversed compared to those adopted in aqueous solution. This reorientation serves to optimize the solvation requirements of the surfactant and minimizes the free energy of the system overall. When surfactants are incorporated into immiscible mixtures of oil and water, the surfactant molecules can locate at the oil/water interface which is thermodynamically very favorable. A number of phases can result which may be structured on the microscopic or macroscopic scale, one example of a phase structured on the microscopic scale is an optically isotropic ME phase. Figure 1.1 shows the variety of such microscopic and macroscopic arrangements of surfactants in presence of water, oil or combinations of all three. Figure 1.2 illustrates the three different types of ME systems which may result due to arrangement of surfactant between the oil and water interface depending upon the composition. It can be seen while the three structures shown are quite different, in each there is an interfacial surfactant in monolayer separating the oil and water domains. Note that while the oil-in-water (o/w) and water-in-oil (w/o) droplets are represented in Figure 1.2 as spheres, they may be asymmetric in shape, frequently adopting the shape of a prolate ellipsoid. The presence of

o/w ME droplets is likely to be a feature in ME where the volume fraction of oil is low. Conversely, w/o droplets are likely when the volume fraction of water is low and systems where the amounts of water and oil are similar, a bicontinuous ME may result. In the latter case, both oil and water exist as a continuous phase in the presence of a continuously fluctuating surfactant-stabilized interface with a net curvature of zero.

In recent years microemulsions systems have found special interest as the oral dosage form to improve drug dissolution as well as drug absorption from G.I.T and Intestine[11]. There are also other advantages with microemulsions compared to other drug delivery systems, including ease of filtration(sterilization) and low viscosity(reducing pain on injection) (Table 1.3.)[13].

Table 1.3. : PHARMACEUTICAL ADVANTAGES OF MICROEMULSION

General Advantages

Ease of Preparation

Clarity

Stability

Ability to filtered

Vehicle for drugs of different lipophilicities in the same systems

Low viscosity(no pain on injection)

Specific Advantages

WATER – IN – OIL (W/O)

Protection of water soluble drugs

Sustained release of water – soluble material

Increased bioavailability

OIL – IN – WATER (O/W)

Increase solubility of lipophilic drugs

Sustained release of oil – soluble material

Increased bioavailability

Biocontinuous

Concentrated formulations of both oil – and water – soluble drugs.

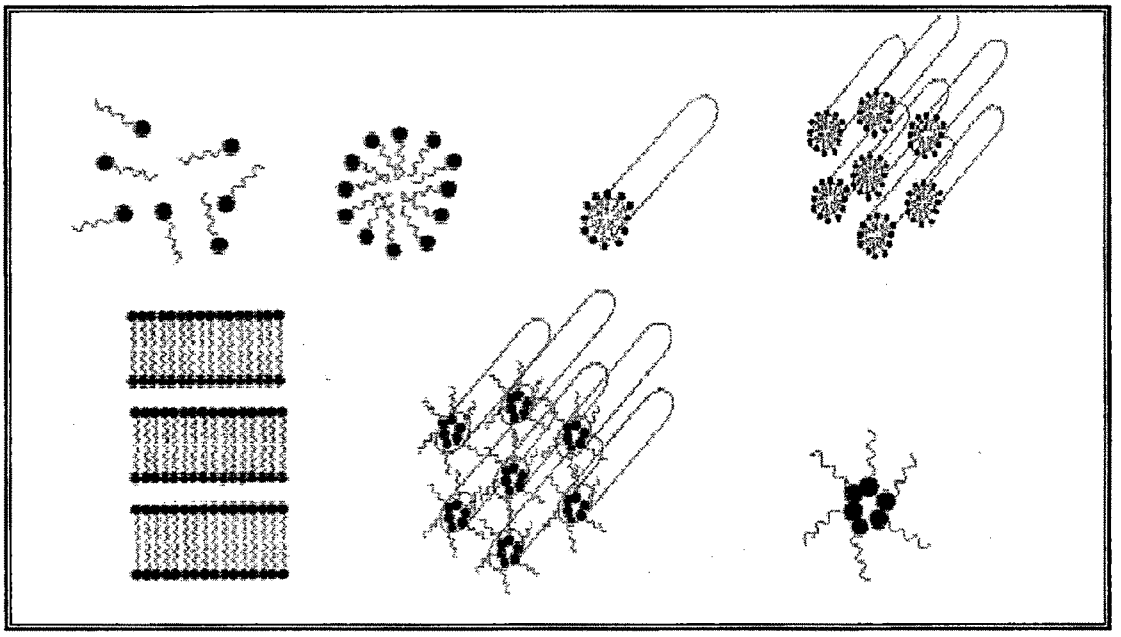


Figure 1.1. Schematic representation of the most common self-association structure resulting by the association of surfactant with oil, water or combinations thereof.

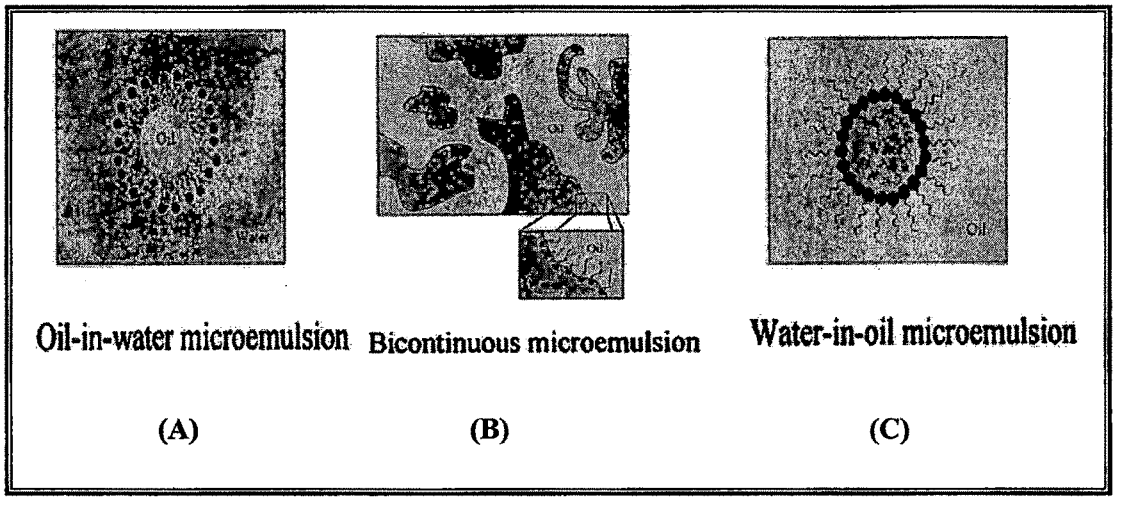


Figure 1.2. Schematic representations of three most commonly observed microemulsion structures (A) oil-in-water, (B) bicontinuous, and (C) water-in-oil microemulsion.

1.1.1. Microemulsion Formation and Phase Behavior:

1.1.1.1. Theories of Microemulsion Formation:

Three approaches can be used to explain the ME formation and stability. These are (1) interfacial or mixed film theories[13,14], (2) solubilization theories[15,16], and (3) thermodynamic treatments[17,18].

The simplified thermodynamic rationalization describing the ME formation can be explained as mentioned below. The free energy of ME formation can be considered to depend on the extent to which surfactant lowers the surface tension of oil-water interface and the change in the entropy of the system such that,

$$\Delta G_f = \gamma \Delta A - T\Delta S$$

Where, ΔG_f - the free energy of formation,

γ - the surface tension of oil-water interface,

ΔA - the change in interfacial area on microemulsification,

ΔS - the change in entropy of the system which is effectively dispersion entropy,

T - the temperature.

When a ME is formed, usually the change in ΔA is very large due to formation of large number of small droplets. Originally workers proposed that in order for a ME to be formed a (transient) the negative value of γ was required, it is now recognized that while value of γ is positive at all times, it is very small (of the order of fractions of mN/m), and is offset by the entropic component. The dominant favorable entropic contribution is the very large dispersion entropy arising from the mixing of one phase in the other in the form of large numbers of small droplets. However, there are also expected to be favorable entropic contributions arising from other dynamic processes such as surfactant diffusion in the interfacial layer and monomer-micelle surfactant exchange. Thus, a negative free energy of formation is achieved when large reduction in the surface tension is accompanied by significant favorable entropic change. In such cases, microemulsification is spontaneous and the resulting dispersion is thermodynamically stable. Several factors determine the formation of w/o or o/w ME. Intuitively, it can be summarized that the most likely ME is that in which the phase with smaller volume fraction forms the droplets and this by no means can be considered the exclusive case. By their very nature, o/w ME droplets generally have larger effective interaction volume than w/o droplets. In the case

of ionic surfactants this is attributable to the presence of an electrical double layer at the surface of the o/w droplet which introduces a strong repulsive term. For o/w ME stabilized by a non-surfactant, although there is hydration shell associated with the polar head groups, the predominant repulsive factor can be attributable to steric interactions. It is also imperative to note that arrangement of surfactant at the interface with high curvature is always easier option for example small droplets, if the surfactant tails extend outwards into continuous oil phase. This also is entropically more favorable as the hydrocarbon tails have more directional freedom which in turn result tends to lower interfacial tension for w/o ME as compare to o/w ME and making their preparation a more easy process. Therefore, it should be noted that while ME are thermodynamically stable, there may be kinetic barriers to their formation. As a consequence, the order of addition of components may impact on the ease of preparation, and in some cases mechanical agitation or the input of heat will assist more rapid microemulsification.

1.1.1.2. Phase Behavior:

The relationship between the phase behavior and ME composition can be captured with the aid of phase diagram. Compositional variables can be studied as a function of temperature and pressure, although the exception of ME prepared using supercritical and near critical solvents or with aid of chlorofluorocarbon and HFA propellants are studied under the ambient pressure conditions. The phase behavior of simple ME systems comprising of an oil, water and surfactant can be studied with the aid of ternary phase diagram in which each corner of the phase diagram represents 100% of that particular phase component. However, in most pharmaceutical preparations, the ME contains drug and/or co-surfactant as additional components. The co-surfaçtant is also amphiphilic with an affinity for both the oil and aqueous phases and partitions to an appreciable extent into the surfactant interfacial monolayer present at the oil-water interface. The co-surfactant does not necessarily be capable of forming association structures in its own right. A wide variety of surfactants can function as co-surfactants such as non-ionic surfactants, alcohols, alkanoic acids, alkanoids and alkyl amines. Reports in the literature reveal that the few studies conducted wherein, large numbers of drug molecules were found to have

surface active properties and can influence the phase behavior[19]. In the case where four or more components are investigated, pseudo ternary phase diagrams are used where a corner will typically represent a binary mixture of two components such as surfactant/co-surfactant, water/drug or oil/drug. The number of different phases present for a particular mixture can be visually assessed. Microstructural features can also be investigated with a wide variety of techniques. A schematic pseudo ternary phase diagram illustrating these features is represented in Figure 1.3.

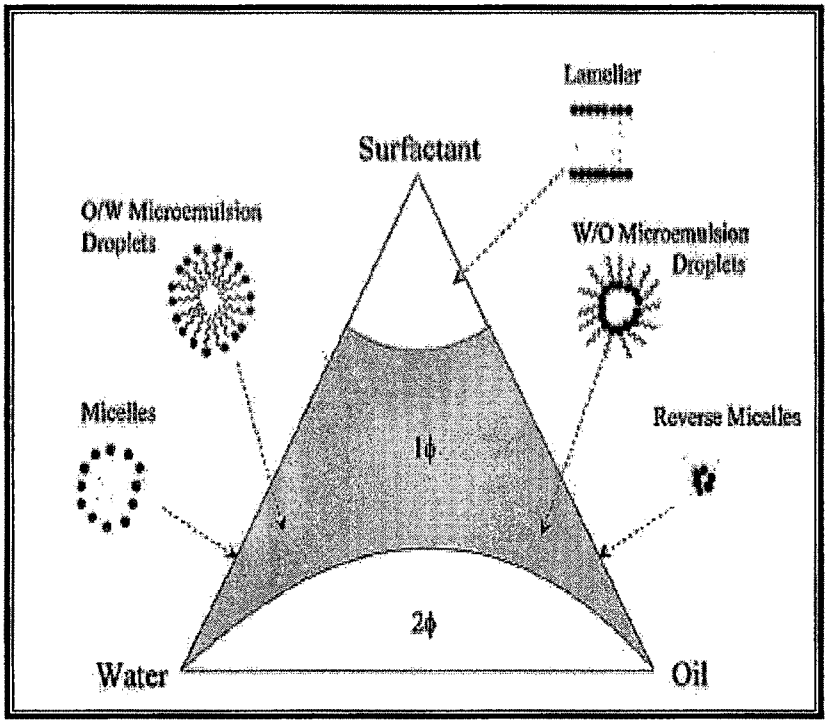


Figure 1.3. A hypothetical Pseudo ternary phase diagram depicting various microstructural features observed during microemulsion formation.

Constructing phase diagrams is time consuming, particularly when the aim is to delineate a phase boundary, as the time taken for the system to equilibrate can be greatly increased as the phase boundary is approached. Heat and sonication are often used, especially when the system contains non ionic surfactants to speed up the process. The procedure most commonly employed is to prepare a series of pseudo binary compositions and titrate with the third component, evaluating the mixture after each addition. Care must be exercised to ensure that the observations are not made on the metastable systems.

However, time constraints impose physical limit on the length of time for which systems can be left to equilibrate and consequently elimination of metastable state. In practice, centrifugation can be useful to speed up any separation. Outside the ME region, particularly for compositions close to the oil-water binary axis, there is insufficient surfactant to facilitate the formation of a single ME phase. In this case multiple phases may exist, the complexity of which increases with increase in the number of components in the mixture. Within this region, and indeed other multiphase regions of the ternary phase diagram, ME can exist in equilibrium with excess water or an oil phase. This multiphase systems can be described using the Winsor classification system. In the Winsor classification system, the one phase ME that is generally explored as drug delivery systems is known as Winsor IV systems.

Transition between the various phases mapped out in this phase diagrams can be driven by the further addition of one of the components, addition of new component such as electrolyte, or by changing the temperature. Transitions from w/o to o/w ME may occur via a number of different structural states including bicontinuous, lamellar and also to multiphase systems. Microemulsions stabilized by non-ionic surfactants, especially those based on polyoxyethylene are very susceptible to temperature because a decrease in surfactant solubility occurs with increasing temperature, and as a result systems stabilized by non-ionic surfactants or mixtures thereof often have characteristic phase inversion temperatures (PITs), with the PIT of the ME varying with a range of experimental factors including the amount and the nature of oil present and the nature of surfactant(s) present.

1.1.1.3. The Role of Surfactant:

The selection criterion of surfactant is also pertinent issue and shall be reviewed critically prior to designing ME[20,21,22]. The single phase ME systems can be classified as Winsor IV. The surfactants used to stabilize such systems may be (1) non-ionic, (2) zwitterionic, (3) cationic, or (4) anionic surfactants. Combination of these, particularly ionic and non-ionic can be very effective at increasing the extent of the ME region. Examples of non-ionic surfactants are polyoxyethylene surfactants (Brij 35) or sugar esters (span 80). Phospholipids are the example of zwitterionic surfactant which exhibits excellent biocompatibility. Soya-lecithin and egg-lecithin are commercially available may contain diacylphosphatidylcholine and its major constituent. Quaternary ammonium

alkyl salts from one of the best known classes of cationic surfactants, with hexadecyltrimethyl ammonium bromide (CTAB), and twin tailed surfactant didodecyammonium bromide (DDAB) amongst the well known. The most widely used anionic surfactant is probably sodium bis-2-ethylhexylsulphosuccinate (AOT) which is twin tailed and is particularly effective stabilizer of w/o ME. Attempts have been made to rationalize the surfactant behavior in terms of hydrophile-lipophile balance (HLB) as well as critical packing parameter. Both approaches are fairly empirical but shall be employed as guide for selection of surfactant. The HLB takes into account the relative contribution of hydrophilic and hydrophobic fragments of the surfactant molecule. It is generally accepted that the low HLB surfactants (3-6) are favored for formation of w/o ME whereas, high HLB surfactants (8-18) are preferred for the formation of o/w ME systems. Ionic surfactants such as sodium dodecyl sulphate which have HLBs greater than 20, often require co-surfactants to reduce their HLB value within the required range for ME formation[23].

In most cases, single-chain surfactants alone are unable to reduce the oil /water interfacial tension sufficiently to enable a ME to form. Medium chain length alcohols which are commonly added as co-surfactants, have the effect of further reducing the interfacial tension, whilst increasing the fluidity of the interface thereby increasing the entropy of the system[24,25,26]. Medium chain length alcohols also increase the mobility of the hydrocarbon tail and also allow greater penetration of the oil into this region. Furthermore, any alcohol present may also influence the solubility properties of the aqueous and oily phases due to its partitioning between these phases. It has also been suggested that some oils, for example the ethyl esters of fatty acids, also act as 'co-surfactants' by penetrating the hydrophobic chain region of the surfactant monolayer. All of the aforementioned mechanisms are considered to facilitate ME formation. In the case of ME stabilized by ionic surfactants, the addition of alkanols also serves to reduce repulsive interactions between the charged head groups. A number of double chain surfactants such as AOT and DDAB are able to form ME without the aid of co-surfactants. These surfactants are characterized by having small head groups in comparison to their hydrocarbon tails. Phosphatidylcholine or lecithin is also a twin-tailed surfactant, but in this case it is generally necessary to include a co-surfactant in

order to disrupt the lamellar structures which characterize its biological behavior. Thus, medium chain alcohols have been successfully used as co-surfactants for the formation of lecithin-based ME. Interestingly w/o ME have been prepared using short diacyl chain lecithins and small molecular volume oils where it is possible that the small molecular volume oils penetrate the hydrophobic chain region thereby facilitating ME formation.

1.1.2. Microemulsion Characterization:

The release of drug(s) from ME system is a function of the microstructure formation within the ME. In contrast to the ease of preparation of ME, it is difficult to characterize microstructure of a ME[27]. However, the knowledge is essential for their successful scaling up for commercial production and exploitation. Microemulsions have been evaluated with a variety of techniques over the years but set of appropriate methods are required in order to fully characterize these systems. At macroscopic levels viscosity, conductivity and dielectric methods provide useful information. For instance, viscosity measurements may provide indication regarding the presence of rod-like or worm-like reverse micelles[28]. Conductivity measurements may provide useful information regarding the type of ME for example oil-continuous or water-continuous[29]. It may also provide means of monitoring percolation or phase-inversion phenomena. Dielectric measurements are powerful tool to probe the structural and dynamic features of ME system. The isoelectric nature and optical clarity makes their study by spectrophotometric techniques particularly in comparison to macroemulsions. Pulse field gradient NMR has been successfully used measure self-diffusion coefficients of the various components and yields information on the mobility and microenvironment. Scattering methods have also been proven invaluable in elucidation of ME structure. It includes method such as dynamic and static light scattering, small-angle neutron scattering (SANS)[30] and small-angle X-ray scattering (SAXS)[31,32]. Tabony et al.have identified presence of cubic phase in bicontinuous ME region using scattering methods[33]. These techniques have been found extremely useful in the development of ME models such as cubic random cell and disordered open connected models. Neutron scattering methods using contrast variation have been used to investigate the nature of the oil penetration into the interfacial surfactant monolayer of ME[34]. Freeze-fracture electron microscopy has also been used to

study ME structure, however extremely rapid cooling of the sample is required in order to maintain structure and minimize the possibility of artifacts[35].

A potentially serious limitation of these methods lies in the requirement to dilute ME systems in order to eliminate particle-particle interactions. It is therefore imperative to work with a method working on high dispersed phase concentration. In spite of above mentioned complications, much of the work reported in the pharmaceutical literature has been conducted using concentrated ME systems. For the most part where particle size is obtained using photon correlation spectroscopy, the measurement quoted remains uncorrected.

1.1.3. Microemulsion Optimization:

Optimization techniques for the dosage forms such as tablets, capsules and injectables have been extensively studied, while, few optimization techniques such as titration technique and pseudo-ternary phase diagram have been reported for optimization of ME[8]. Microemulsions can be optimized using titration method or by constructing a pseudo-ternary phase diagrams at different surfactant: co-surfactant levels. Combinations of both the approaches are frequently used to systematically optimize the ME formulation. Pseudo-ternary phase diagram is usually used to comprehensively study the ME region and its phase behavior, although construction of phase diagram is expensive and time consuming exercise[8].

1.2. Inclusion Complex

An inclusion complex is a unique form of inclusion chemical complex. Here one molecule is enclosed within another molecule or structure of molecules. The combination is characterized by absence of ordinary chemical bonds, the essential criteria is that enclosed molecule or guest should be of a suitable size and shape to fit into a cavity within a solid structure formed by a host molecule. The stereochemistry of host and guest molecules determines whether the inclusion complex can occur. The resulting close fit of the two components produce a combination of significant strength due to total dispersion force between interacting components. This type of spatial complex does not occur by means of ionic, covalent or co-ordinate covalent bond but is dependent upon dispersion forces and possibly highly oriented dipoles for stability differing greatly from chemical complexation. Frank first observed inclusion complexes in hydroquinone and several

volatile compounds[36]. He proposed that two chemical components were interacting without chemical bonding and suggested one molecule was enclosed into another. X-ray studies confirmed those studies showing formation of cage-like structure.

Table 1.4. Classification of inclusion compounds

1) Polymolecular inclusion compounds
2) Compounds forming channel like void space
    - Urea and thiourea
    - Choleic acid
3) Compound forming cage like void spaces
    - Hydroquinone
    - Water
    - Phenol
    - Dianin's compound
    - Cycloveratril
4) **Monomolecular inclusion compounds**
    - **Cyclodextrin**
5) Macromolecular Inclusion compounds
    - Products of Blue-iodine reaction

During last few decades a number of methods to improve the dissolution rate of poorly soluble drugs from solid dosage forms have been described. In the first half of this century the cyclodextrin chemistry was laid down which gained drive potential drive in the field of modified delivery of drugs. Cyclodextrins are widely used in pharmaceutical field owing to their high aqueous solubility and the ability to stabilize insoluble drug molecule[37].

1.2.1. Cyclodextrins

Cyclodextrin is the major product from the reaction between the enzyme cyclodextrin transglycosylate and starch solution pretreated with α-amylase. These produce α, β and γ–cyclodextrins. The use of cyclodextrins as a new family of pharmaceutical excipients and drug carrier become an increasingly successful method to improve the dissolution and general bioavailability of the poorly soluble drugs[38].

The parent cyclodextrins are well known cyclic sugar, with doughnut shaped structure, consisting of α (1-4) joined glucopyranose units. As a result of the hydrophobic inner surface, cyclodextrins are the most important simple organic compound; capable of forming non-covalently bonded inclusion complexes with a variety of drug molecules in aqueous media. There are several unsubstantiated or native Cyclodextrins, the most commonly referenced are:

ALPHA –cyclodextrin (6 cyclo-α(1,4)-anhydroglucose units)

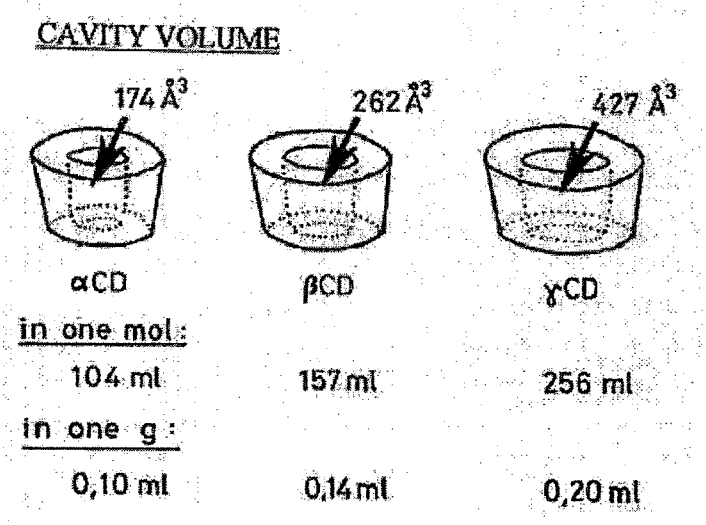
BETA–cyclodextrin (7 cyclo-α(1,4)-anhydroglucose units)

GAMMA–cyclodextrin (8 cyclo-α(1,4)-anhydroglucose units)

The basic characteristics, dimention and hydrophilic/hydrophobic reagions of the cyclodextrins are shown by following table and figure[39].

Table 1.5. Some Characteristics of α, β, γ and δ-cyclodextrins :

	α	β	γ	δ
No. of glucopyrinose units	6	7	8	9
Molecular Weight	92	1135	1297	1459
Central cavity diameter (A°)	4.7-5.3	6.0-6.5	7.5-8.3	10.3-11.2
Water Solubility at 25 °C (g/100mL)	14.5	1.85	23.2	8.19



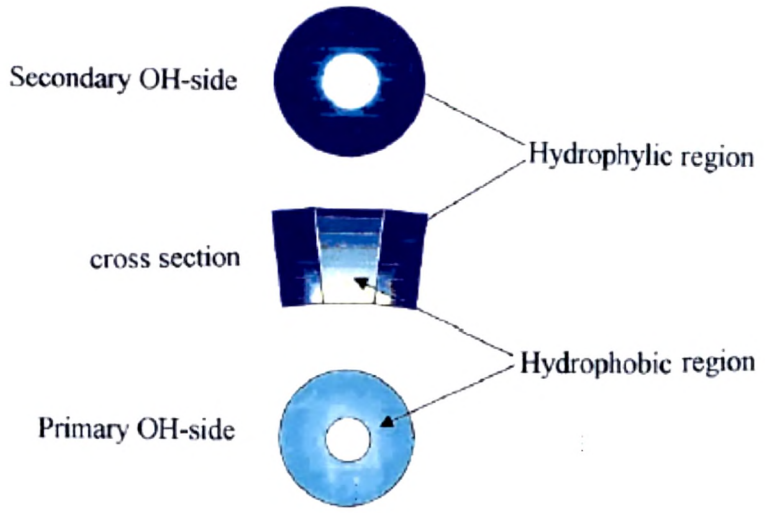


Figure 1.4. Dimensions and Hydrophilic/Hdrophobic reagions of the cyclodextrins molecules.

Recently, various kinds of cyclodextrin derivatives such as hydrophilic, hydrophobic and ionic derivatives have been successfully utilized to extend physicochemical properties and inclusion capacity of natural Cyclodextrins[40-46]. The desirable attribute for the drug carrier is the ability to control the rate and/or time profile of drug release[47-49]. Hydrophilic Cyclodextrins can modify the rate of drug release, which can be used for the enhancement of drug absorption across biological barriers, serving a potent drug carrier in the immediate release formulations. Amorphous Cyclodextrins such as poorly water-soluble drugs during storage, which can consequently maintain the higher dissolution characteristics and oral bioavailability of the drugs[50].

Because of the molecular dimensions (more exactly the cavity diameter),its availability, low price and the better complexion efficiency the **β-cyclodextrin** have significant practical importance in pharmacy

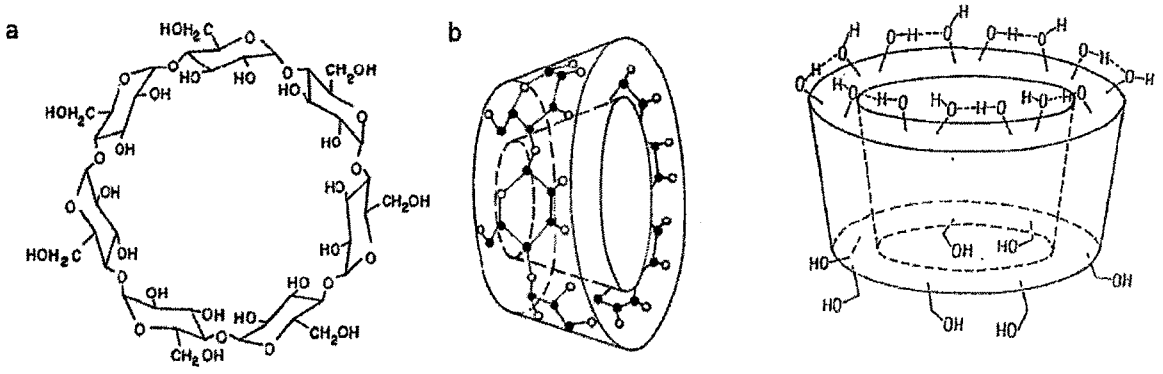


Fig. 1.5. (a) The chemical structure and (b) the toroidal shape of the β-cyclodextrin molecule.

In cyclodextrin every glucopyranose unit has a three free hydroxyl groups(C-2,C-3 and C-6) which can be modified by substituting the hydrogen atoms by variety of groups such as alkyl, hydroxyalkyl, carboxyalkyl, sulphoalkyl, amino and thio, to form derivatised cyclodextrins like, Methylated cyclodextrins, Hydroxypropylated cyclodextrins, Hydroxyethylated cyclodextrins, Carboxymethylated cyclodextrins, Ethylated cyclodextrins and branched cyclodextrins. This derivation improves solubility of cyclodextrins derivatives, fittings and association between cyclodextrin and the guest molecules[51].

There is an infinite number of possible derivatives of cyclodextrins have been synthesized in which the most important chemically modified cyclodextrins are:

(1) HPBCD – hydroxypropyl betacyclodextrin :

These derivatives are highly water soluble, due to both chemical nature and amorphous property. Their dissolution is endothermic so there is no decrease in solubility with increase in temperature. Unlike methyl cyclodextrins, they are not hydrolyzed by gastrointestinal amylase. Main interest is in parentral administration as they have lower hemolytic effect then original cyclodextrins[52].

(2) RAMEB – randomly methylated betacyclodextrin :

Randomly methylated betacyclodextrins are well define into two different derivatives like, heptakis (2,6-di-O-methyl) and heptakis (2,3,6-tri-O-methyl)- β cyclodextrin. This result from methylation of all C-2 secondary hydrolysis and all C-6 primary hydrolysis and trimethyl cyclodextrins result from methylation of all the hydrolysis (C-2,C-3 and

C-6). Their water solubility is much higher as compared to parent cyclodextrins. Their solubility decreased with increasing temperature. On methylation, solubility of beta cyclodextrin increases, but beyond two third of methylation, it decreased again. When administrated by oral route they act as xenobiotics. They exhibit higher hemolytic effect then betacyclodextrins[53].

1.2.2. Inclusion Compounds and Concept :

Cyclodextrins are capable of forming inclusion complex with compounds having a size compatible with the dimensions of the cavity. The hydrophobic cavity of the truncated cone formed by the cyclodextrin ring can be accommodate suitable guest molecules and form"complexes"or inclusion compounds which can be easily isolated as stable amorphous or microcrystalline substance. The conversion of crystalline nature to amorphous or microcrystalline nature is schematically represented in Figure 1.6.[54]

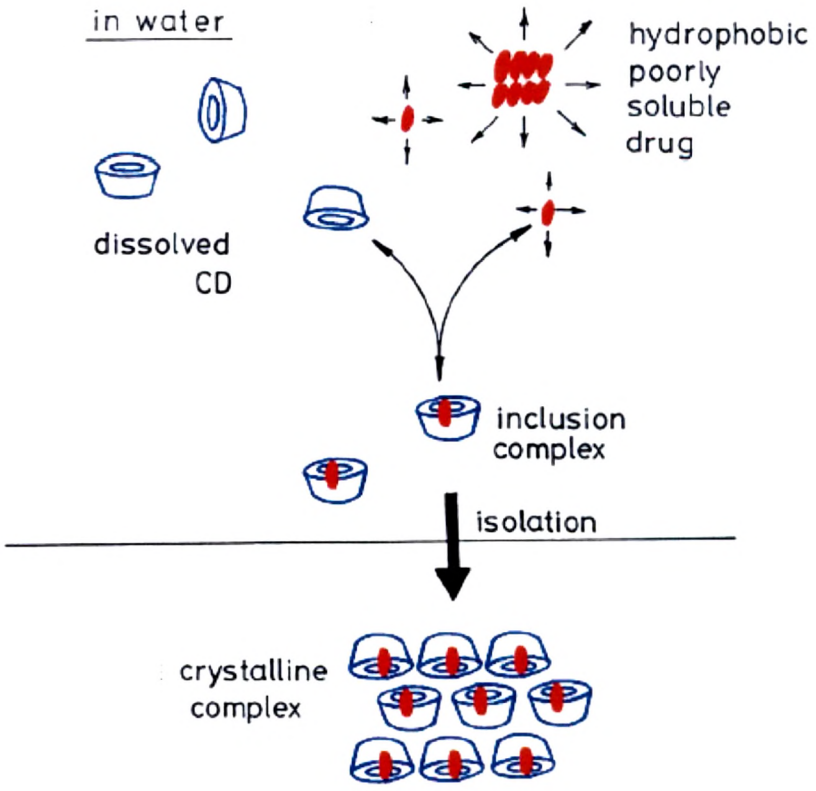


Figure 1.6. Schematic representation of the production of drug-cyclodextrin complex.

An inclusion complex is form of supramolecular complex in which one or part of the molecule the "guest" or substrate is enclosed, entrapped, encapsulated or embedded within another molecular structure. In this relationship no covalent bond is established between the host and guest, and more ever the dissociation-association equilibrium in solution is one of the most characteristic feature of this association.

Inclusion compounds are mainly described into three different classes :

(1) **Polymolecular inclusion compounds :**

The host consist of lattice of several loosely bounded molecules which form a channel (urea, cholic acid) or a cage (hydroquinone) entrapping a guest molecule.

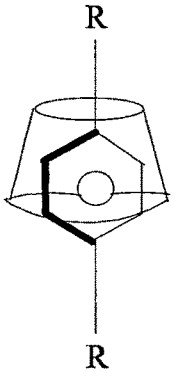
(2) **Macromolecular inclusion compounds :**

The host consist of a three dimensional crystalline lattice capable of retaining molecules. The host compounds, widely used in industry, consist of zeolite, dextrans, polyacrilamine, agarose and silica gels.

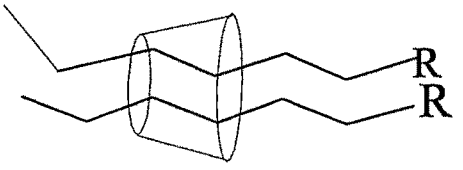
One particular case of inclusion is the product of the blue-iodine solution-the blue in the iodine / starch reaction result from the entrapment of a polymerized chine of iodine by an helix of starch.

(3) **Monomolecular inclusion compounds or molecular encapsulation :**

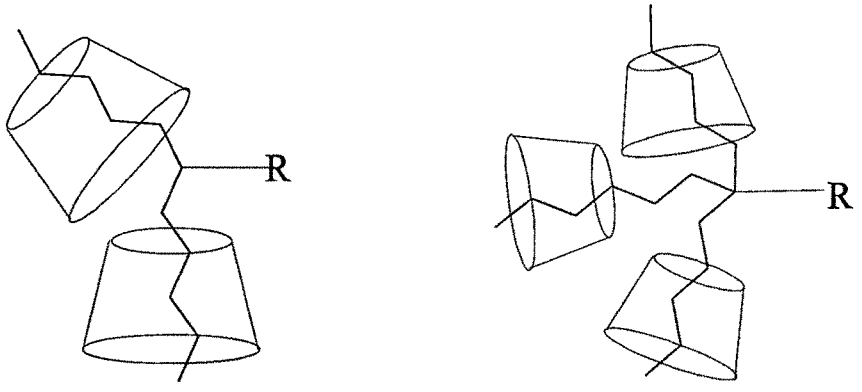
The host is a molecule characterized by the presence of a cavity in which the guest molecule is entrapped or encapsulated.



1:1 complex



1:2 complex



2:1 complex **3:1 complex**

Figure 1.7. Representation of monomolecular encapsulation possibilities.

1.2.3. Mechanism for formation of inclusion complex

The formation of inclusion compounds with cyclodextrin can be described in following six steps:

1. Approach of the guest or substrate molecules to the cyclodextrin molecules.
2. Loss of the water structure within the cyclodextrin cavity with removal of some water molecules.
3. Breakdown of the water structure around the portion of the substrate that will be included and transport of some water molecules in the solution.
4. Interaction of the substitutent groups of the substrate with groups on the rim or inside the cyclodextrin ring.
5. Possible formation of the bonds between the cyclodextrin and the substrate.
6. Re-establishment of the water structure around the exposed parts of the substrate after the inclusion has occured.

1.2.4. Advantages of Inclusion Complex[55]

1. It improves bioavailability from solid and semisolid formulations.
2. Stability and shelf life can be increased.
3. Side effects can be reduced significantly.
4. Aqueous injectable solution from poorly soluble drugs can be prepared.
5. Complexation may be convert oils and liquid drugs into microcrystalline or amorphous powders.

6. Gastrointestinal and ocular irritation may be reduced, minimized or eliminated.
7. Complexation prevent drug-drug or drug-additives intraction.
8. It can be used to mask unpleasant odor and taste.

The inclusion compounds formed with polar molecules are only slightly soluble in water whereas those formed with non-polar molecules are moderately soluble.

Monomolecular inclusion complex formation techniques have been reported by many researchers[56]. These techniques mainly include (1) Co precipitation method (2) Slurry method(3) Kneading method (Paste method) and (4) Dry mixing method.

(1) Co – precipitation method :

Co-precipitation method is the most widely used method in the laboratory. In this method, an organic solution of the drug, was poured under agitation into an aqueous solution of cyclodextrin. It might be necessary to work with a hot solution of cyclodextrin and maintained the heat to about 60°C during agitation. Precipitation of the inclusion compound was obtained either spontaneously or by evaporation. After the precipitation step, a washed with solvent and water, filtration and drying produced a pure inclusion complex.

(2) Slurry method :

In this method, cyclodextrin is suspended in water up to a 45 – 50 % w/w concentration. The drug can have an effect upon the viscosity of the slurry, and concentration are adjusted to allow mixing of the cyclodextrin and drug. It might be necessary to work with a hot solution of cyclodextrin and maintained the heat during mixing procedure. As the cyclodextrin which is in solution forms complexes and the complex precipitates, more of the cyclodextrin dissolves to form more complex. Finally prepared complexes were washed with water, filtration and drying produced a pure inclusion complex.

(3) Kneading method (Paste method) :

The paste method uses as a minimum amount of water, 20 – 30% w/w.It consist of first preparing a paste of cyclodextrin(10 minutes) by introducing into a kneading machine (3 parts of cyclodextrin and only 1 part of water) and then progressive addition of the drug to the paste which is mix for 1 hr. The viscosity of the mixture increase which indicates the formation of the complex. Auxiliary step involves washing the paste with solvent and water followed by filtration. Finally the paste was dried in an oven at 45 °C until dry

then grinding to get a fine powder then pass through sieve mesh no 100. In some cases this method can be done in two steps, a mixing step followed by a holding step to allow completion of the complexation reaction.

(4) Drying Mixing method :

The dry mixing method involves mixing the cyclodextrin with the drug with no added water. This generally not an efficient method of making complexes since mixing time can range from hours to days.

Among all these methods co-precipitation method and kneading method are widely used because of simple, less time consuming, less solvent required and easy to form a inclusion complex.

1.2.5. Study of Cyclodextrin Complexation and Dilution Effects :

The most widely used approach to study inclusion complexation (Fig.6.1.3) is the phase solubility method described by Higuchi and Connors[57], which examines the effect of a solubilizer, i.e., Cyclodextrin or ligand on the drug being solubilized, i.e., the substrate.

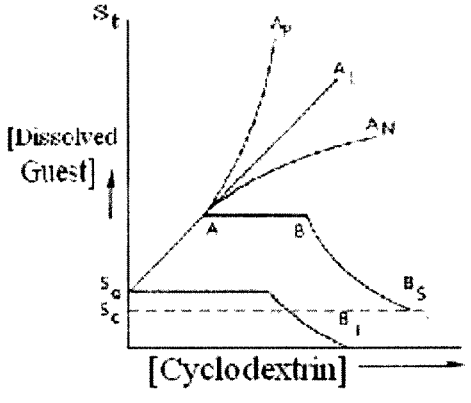


Figure 1.8. Theoretical Phase solubility diagram.

Phase solubility diagrams are categorized into A and B types; A type curves indicate the formation of soluble inclusion complexes while B type suggest the formation of inclusion complexes with poor solubility. A B_S type response denotes complexes of limited solubility and a B_I curve indicates insoluble complexes. 'A' type -curves are subdivided into A_L (linear increases of drug solubility as a function of cyclodextrin

concentration), A_P (positively deviating isotherms) and A_N (negatively deviating isotherms) subtypes. β-cyclodextrin often gives rise to B type curves due to its poor water solubility where as the chemically modified cyclodextrins like HP-β-cyclodextrin and SBE-β-cyclodextrin usually produce soluble complexes and thus give A type systems. In case of a 1:1 complex, using the following equation one can determine the equilibrium binding or association constant, K from the slope of the linear portion of the curve.

$$\text{Stability constant} = \frac{Slope}{S_o(1 - Slope)}$$

Where So is the intrinsic solubility of the drug studied under the conditions. For many drug-cyclodextrin complexes, binding constant values are in the range of 100-20,000M^{-1}. It has been demonstrated that even with tightly bound drugs, a 1:100 dilution reduces the percentage of the complexed drug from 100% to 30%, releasing the free drug that can permeate through biological membranes. A 1:100 dilution can be readily attainable upon injection or dilution in the stomach and intestinal contents. Although the competing lipophiles present at ophthalmic, transmucosal and transdermal delivery sites can displace drugs, the products to these delivery sites are more sensitive to strength of binding or association due to minimal dilution possible. The ratio of free-to-complexed drug upon dilution of a sparingly water-soluble drug/CD complex depends on the phase solubility behavior of the system[58].

1.2.6. Effect on dissolution and bioavailability :

Cyclodextrin complex formation is equivalent to molecular encapsulation; consequently the drug molecules are isolated from each other and are dispersed on a molecular level in an oligosaccharide matrix. Gastric acid or intestinal juices do not disintegrate the crystal lattice of a hydrophobic pharmaceutical agent, but it disintegrates the hydrophilic cyclodextrin lattice. Therefore, the dissolution of cyclodextrin complexed with drug is very rapid. For class II category drug of biopharmaceutical classification (Low solubility - High permeability), K_d is less than K_a. Here dissolution is the rate limiting step in drug absorption. In case of cyclodextrin inclusion complex, following oral dose only the drug and practically no cyclodextrin is absorbed. Cyclodextrin is only a carrier agent. It transports the lipophilic guest molecules through an aqueous media to the lipophilic membrane of cells in the gastrointestinal tract.

The dissociation-association reactions of cyclodextrin complexes in solution are very fast and the equilibrium of free and complexed drug is established instantaneously. Perhaps this may be used to improve medication through use of tablets containing both the hydrophobic drug complexed to cyclodextrin and the same drug in the free form. The former would enter the circulation very rapidly, while dissolution of the latter would occur slowly and form a drug depot. The above application is not limited to oral use. Cyclodextrin complexed drug in suppositories have improved dissolution rates and consequently, higher concentration levels of the drug in circulating blood are obtained.

1.3. RESEARCH ENVISAGED

The aim of this investigation was envisaged to prepare a formulation which can enhance the oral bio availability of poorly water soluble drugs. It was hypothesized that cyclodextrin complex and microemulsion based oral drug delivery system will enhance the drug absorption from G.I. Tract and Intestine. It will help in rapid drug delivery, maximize therapeutic index, reduce the side effect, and reduce dose/frequency of dosing and perhaps the cost of therapy.

Keeping these aims in prospective, the specific objectives of the study were planned through review of literature.

I. Review of literature with special reference to poorly bioavailable drugs, and its market, cyclodextrins and its derivatives, drug-complexes, microemulsions, analytical profiles for selected therapeutic agents, various *in vitro/in vivo* models for evaluation of drug in plasma, tissues and organs.

II. Preparation of solutions containing selected drugs, preparation of cyclodextrin complex containing selected drugs and optimization of cyclodextrin complex with the help of phase solubility and dissolution study.

III. Characterization of drug-cyclodextrin complex using instrumental techniques e.g. I.R. Spectroscopy, D.S.C. data and X.R.D data.

IV. Preparation of solutions containing selected drugs, preparation of microemulsions containing selected drugs and optimization of microemulsions with the help of pseudo ternary phase diagrams and titration technique.

V. Characterization of microemulsions loaded with selected therapeutic agents to evaluate parameters such as globule size determination, zeta potential determination, physical and chemical drug retention assessment at accelerated and under stress conditions.

VI. *In vitro* diffusion studies of drug solutions/microemulsions by using dialysis bag and intestine permeability study method..

VII. *In vivo* drug evaluation of prepare drug-cyclodextrin complex and oral microemulsion by *in vivo* absorption study.

On this basis,

1. Cilostazol and Tadalafil drugs were selected for the investigation.
2. Analytical methods for both the drugs were developed for the estimation of pure drugs, its formulations (microemulsion and Inclusion complexes) and matrix (plasma/urine).
3. A large numbers of inclusion complexes of Cilostazol and Tadalafil with Cyclodextrins and its derivatives were prepared by using different methods.
4. Prepared drugs-Cyclodextrins inclusion complexes were characterized by I.R. Spectroscopy, D.S.C. data and X.R.D data and evaluated by phase solubility and dissolution study.
5. Drug – Cyclodextrins complexes giving best dissolution profile were further selected for *in vivo* pharmacokinetic study
6. Oral microemulsion of Cilostazol and Tadalafil were successfully prepared and optimized by pseudo ternary phase diagrams.
7. Oral microemulsion were characterized by globule size, zeta potential determination, % transmittance study, physical and chemical drug retention assessment at accelerated and under stress condition.
8. Evaluation of oral microemulsions were carried out by *in vitro* diffusion study(Dialysis bag study and intestinal permeability study).
9. Oral microemulsions giving best diffusion profile were further selected for *in vivo* pharmacokinetic study.
10. Finally *in vivo* pharmacokinetic study were carried out in rabbits for both the formulations (Drugs-Cyclodextrins complex and Microemulsions) to study the amount of absorbed drug from its formulations.

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