Chapter 1

Introduction
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1. INTRODUCTION

1.1 Background

Despite tremendous research and innovation in drug delivery methods in past few decades, the oral route still remains as the most preferred route for the administration of most new chemical entities (NCE) as well as existing drug molecules. The oral route is mostly preferred by virtue of its convenience, economical and high patient compliance compared to other alternate routes. The pharmaceutical market place continually reinforces its preference for orally administered products. In fact over 80% of all pharmaceutical products are administered by the oral route. With the explosive growth in the fields of genomics and combinatorial chemistry coupled with technological innovations in the last few years, synthesizing a new molecule has become easier, however there are certain existing molecule which are very promising and need to be delivered in an efficient way to have optimum activity (Balimane et al., 2000). Though the advent of high throughput discovery technique has taken drug discovery to new heights and has increased number of drug candidates but has done little to address the inherent need for these compounds to be orally active. Self administration of drugs for systemic absorption and therapeutic effect, however, critically depends on the rate and extent of drug absorbed from the gastrointestinal (GI) tract. Optimization of the dose fraction that reaches the systemic circulation in chemically unchanged form is a central factor during drug development. Although the relationship between a dose and the corresponding systemic exposure as determined pharmacokinetically by the total area under time concentration curve (AUC) is highly complex, a host of variable have been identified that limit oral absorption and, hence, lead to poor oral bioavailability. This includes:

- poor dissolution or low aqueous solubility
- degradation of the drug in gastric or intestinal fluids
- poor intestinal membrane permeation
- presystemic intestinal or hepatic elimination
Compounds intended for oral administration must have adequate solubility and permeability in order to achieve optimum therapeutic concentration. Nearly 40% of NCE are rejected due to their poor biopharmaceutical properties like solubility and lipophilicity during drug evolution (Panchaghunula and Thomas, 2000). The problem of poor permeability is much worse than the poor solubility as no formulation fix exists (Lipinski, 2000). There is always a need to develop a variable oral controlled release systems capable of delivering a drug at a therapeutically effective rate to a desirable site for a duration required a drug at a for optimal treatment it should even modulate the GI transit time so that the system developed can be transported to a target site or to the vicinity of an absorption site and reside there for a long time to enable the efficient drug absorption. Preventive measures should be advised to either bypass or minimize the extent of hepatic metabolic effect. There is great need for improving the oral bioavailability of various poorly bioavailable drugs.

1.1.1 Oral Bioavailability

Low oral bioavailability of systemically acting drugs is often associated with variable plasma concentrations and poorly controlled pharmacologic and toxic effects. In addition, incomplete oral bioavailability results in the wasting of much of an oral dose, and adds to the cost of drug therapy, especially when the active drug substance is expensive. Therefore, the maximization of oral bioavailability is a common goal during drug selection and development, and in clinical therapy.

According to Biopharmaceutics Classification System (based aqueous solubility and intestinal permeability of drug), the drug substances are classified as follows:

Class I: High Solubility – High Permeability
Class II: Low Solubility – High Permeability
Class III: High Solubility – Low Permeability
Class IV: Low Solubility – Low Permeability
Cyclosporine belongs to Class IV category drug under BCS. Either low solubility or poor membrane permeation or presystemic metabolism could cause low oral bioavailability.

Bioavailability is the fraction of a dose that reaches the systemic circulation intact, when not directly injected into the circulation. Bioavailability is clinically important because pharmacologic and toxic effects are proportional to both the dose and bioavailability. When bioavailability is very low (e.g., <20%), inter- and intra-subject variability in bioavailability are magnified and incomplete oral bioavailability can become a great concern.

![Diagram of drug fate processes](image)

Scheme I- Scheme outlining the possible fate of a drug administered orally in a solid dosage form. The processes represented in italics result in incomplete bioavailability.

Several approaches can be taken toward solving an oral bioavailability problem. The cause or causes of incomplete bioavailability must be first understood.

1.1.2 Intestinal Absorption Process

If the solid doesn’t dissolve within the gastrointestinal transit time, it will be eliminated in the feces. If a dissolved drug permeates through the intestinal membrane too slowly, it will be mostly eliminated in feces. Some drugs may be rapidly degraded at certain pH, or by brush border enzymes. To be absorbed, these
compounds must avoid degradation and metabolism, and must permeate through the membrane within the limited time they are exposed to the membrane. Even after having permeated the intestinal membrane, many compounds do not achieve complete systemic absorption because of extraction and metabolism on the first pass through the liver.

1.1.3 Categories of Bioavailability Problems-
Based on the Scheme I, various causes of poor oral bioavailability, method to test the particular problem and possible solutions of these problems are categorized in Table 1.

Table 1: Various causes, test methods and possible solutions of poor bioavailability

<table>
<thead>
<tr>
<th>Problem</th>
<th>Test method</th>
<th>Possible solutions</th>
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<tbody>
<tr>
<td>Solubility/dissolution rate</td>
<td>Aqueous solubility</td>
<td>Particle size reduction</td>
</tr>
<tr>
<td></td>
<td>F with alternative solid formsᵃ</td>
<td>Solubilizing or wetting agents</td>
</tr>
<tr>
<td></td>
<td>F with non-aqueous solutionsᵃ</td>
<td>Alternative solid forms</td>
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<td></td>
<td></td>
<td>Non-aqueous vehicles</td>
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<td></td>
<td></td>
<td>Solid dispersions</td>
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<tr>
<td>Degradation/metabolism in GI lumen</td>
<td>Stability in gastric &amp; intestinal fluids</td>
<td>Enteric coating</td>
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<td></td>
<td></td>
<td>Encapsulation</td>
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<tr>
<td></td>
<td></td>
<td>Lipid or surfactant vehicle</td>
</tr>
<tr>
<td>Poor membrane permeation</td>
<td>In vitro, in situ permeation rates</td>
<td>Permeation enhancers</td>
</tr>
<tr>
<td>• Poor partitioning</td>
<td>Octanol/buffer distribution</td>
<td>Ion pairing complexation</td>
</tr>
<tr>
<td>• Low diffusivity</td>
<td></td>
<td>Lipid or surfactant vehicle</td>
</tr>
<tr>
<td>• Binding to the mucus membrane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presystemic elimination</td>
<td>Everted intestine</td>
<td>Metabolism inhibitors</td>
</tr>
<tr>
<td>• By brush border</td>
<td>Intestinal homogenate</td>
<td>Lipid or surfactant vehicles</td>
</tr>
<tr>
<td>• By intestinal cells</td>
<td>F after portal dosingᵃ</td>
<td></td>
</tr>
<tr>
<td>• Hepatic</td>
<td></td>
<td></td>
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</tbody>
</table>

ᵃ F is percentage oral bioavailability.

The approaches to solving these problems are quite different from one category to other, emphasizing again the importance of understanding the cause of poor bioavailability. For some compounds there may be more than one contributing cause.
Poor membrane permeation is most commonly due to either poor partitioning into the lipid membrane or low membrane diffusivity. Membrane diffusivity is inversely related to the molecular size (molecular weight) of the diffusing compound. Apart from partitioning, poor membrane permeation may also be associated with the compound binding to the contents of the intestinal lumen, within the mucus layer, or to the cellular elements. Thus, in effect, lowers the free drug concentration and reduces the diffusion rate. Apart from the various possible solutions listed above, another approach is to modify the drug structure to increase the lipophilicity (as done in case of cefuroxime to cefuroxime axetil), reduce molecular weight or replace hydrogen bonding groups. Prodrugs are one way to modify an active compound to manipulate some physical, chemical or pharmacokinetic property.

1.1.4 Intestinal Permeation Enhancers

In-vivo intestinal absorption is influenced by many factors, and absorption enhancement could be related to various mechanisms, of which permeation enhancement is one. The term ‘permeation enhancement’ will be used to refer to cases in which it is clear that the operative mechanism is a change in intestinal permeability. The less specific term absorption enhancement is used when other mechanisms are likely to be involved. For example, with lipid or surfactant vehicles the mechanisms possibly involved could include increased drug solubility, altering gastric or intestinal transit time and stimulations of bile flow, in addition to increased intestinal permeability.

The three most important criteria for evaluation of absorption enhancers (table 2) are:

1. how effective the absorption enhancer is for the drug of interest
2. the potential to cause toxicity
3. The mechanism by which absorption is enhanced.
### Table 2: Different types of absorption enhancers

<table>
<thead>
<tr>
<th>Absorption enhancers</th>
<th>Examples</th>
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<tbody>
<tr>
<td>Bile salts</td>
<td>Sodium cholate</td>
</tr>
<tr>
<td></td>
<td>Sodium desoxycholate</td>
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<tr>
<td>Non-ionic surfactants</td>
<td>Polyoxylethylene alkyl ethers</td>
</tr>
<tr>
<td></td>
<td>Polyoxylethylene alkyl esters</td>
</tr>
<tr>
<td></td>
<td>Polysorbates</td>
</tr>
<tr>
<td>Ionic surfactants</td>
<td>Sodium lauryl sulphate</td>
</tr>
<tr>
<td></td>
<td>Dioctyl sodium sulphosuccinate</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>Sodium caprate</td>
</tr>
<tr>
<td></td>
<td>Oleic acid</td>
</tr>
<tr>
<td>Glycerides</td>
<td>Natural oils</td>
</tr>
<tr>
<td></td>
<td>Medium chain glycerides</td>
</tr>
<tr>
<td></td>
<td>Phospholipids</td>
</tr>
<tr>
<td></td>
<td>Polyoxylethylene glyceryl esters</td>
</tr>
<tr>
<td>Acyl carnitines and cholines</td>
<td>Palmitoyl carnitine</td>
</tr>
<tr>
<td></td>
<td>Lauroyl choline</td>
</tr>
<tr>
<td>Salicylates</td>
<td>Sodium salicylate</td>
</tr>
<tr>
<td></td>
<td>Sodium methoxysalicylate</td>
</tr>
<tr>
<td>Chelating agents</td>
<td>EDTA</td>
</tr>
<tr>
<td>Swellable polymers</td>
<td>Starch</td>
</tr>
<tr>
<td></td>
<td>Polycarbophil</td>
</tr>
<tr>
<td></td>
<td>Chitostan</td>
</tr>
</tbody>
</table>

This group of formulations includes oils, surfactant vesicles or additives, and formulations in which the lipid or surfactant are structured, as with micelles, mixed micelles, emulsions and liposomes. The lipid and/or surfactant component of these formulations could affect GI absorption in a number of ways that are listed in Table 3. But more than one mechanism is probably involved with most lipid or surfactant formulations. The mechanism involved in increasing intestinal permeation in-vitro or in-situ may be different from what happens in-vivo. The structure of these formulations probably changes in the environment of the GI lumen, where fats are digested and absorbed: oils will be emulsified, and micelles, emulsions, and liposomes could be broken and restructured.
Table 3: Possible mechanism by which lipid and surfactant formulation could increase oral bioavailability

<table>
<thead>
<tr>
<th>S. NO</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Solubilization/wetting</td>
</tr>
<tr>
<td>2</td>
<td>Prolonging GI residence time (decreased gastric emptying, decreased gastric motility).</td>
</tr>
<tr>
<td>3</td>
<td>Protection from luminal degradation</td>
</tr>
<tr>
<td>4</td>
<td>Protection from brush border or cellular metabolism&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>Enhanced membrane permeability</td>
</tr>
<tr>
<td>6</td>
<td>Increased membrane contact/absorption by lipid absorption mechanism&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>Absorption via lymphatics</td>
</tr>
<tr>
<td>8</td>
<td>Decreased hepatic extraction&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> These mechanisms would seem to require the drug and lipid or surfactant to remain associated during or after absorption through the epithelium.

Non-ionic surfactants increase the absorption by increasing the membrane permeability. The mode of action of these surfactants appears to be linked to their ability to increase membrane fluidity and their capacity to solubilize and extract membrane components. Low concentrations of surfactants may reversibly increase permeation via tight junctions.

Doluisio <i>et al</i>, (1969) demonstrated that the in-situ method using rat intestine is useful method for obtaining realistic drug absorption rate. Doluisio <i>et al</i>, (1969) has also found that if the fasting period of rat exceeds 20 hrs, then absorption rates were found to decrease significantly and decrease was dependent on the duration of fasting period. Berggren <i>et al</i>, (2004) evidenced that carrier-mediated intestinal efflux is a process appeared not to have a significant influence on the rate and extent of in vivo intestinal absorption. But there is a report which indicates that cells may become drug-resistant by several different mechanisms, but one major type of MDR is linked to the overexpression of a 170 kDa plasma membrane glycoprotein, known as the P-glycoprotein (P-gp) (Sharom, 1997). It has also been evaluated whether efflux-mediated and saturable mechanisms play any role toward poor and variable intestinal absorption of rifampicin (Mariappan and Singh 2004). The effect of self-microemulsifying drug delivery systems containing Labrasol on tight junctions in Caco-2 cells has been studied by Xianyi Sha <i>et al</i>, (2005). Postolache <i>et al</i>, (2002) reported...
the comparative bioavailability of two cyclosporine capsule products with different pharmaceutical formulation an open randomized two-period cross over study was conducted in 24 healthy volunteers.

Nathalie et al (1997) indicated that the structure of the CsA carriers greatly affects drug bioavailability and that aqueous micellar solutions of CsA-TUDC-monooolein constitute efficient vehicles, thus providing for CsA high absorption with low variability. There is a report that nonionic surfactants and biodegradation of Polycyclic Aromatic Hydrocarbons increases bioavailability (Frank et al, 1995). The oligopeptides can be made enterally absorbable by coupling to modified bile acid molecules making use of the specific intestinal absorption pathway for bile acids (Werner et al, 1993). This finding may be of great importance for the design and development of orally active peptide drugs.

Shabouri, (2004) reported relative bioavailability of CsA from chitosan nanoparticles was increased by about 73%. (Tomas, 2001) has reported no difference among pharmacokinetic parameters of novel drug delivery system and microemulsion system have been observed in spite of fact that average particle size of novel system is almost 1000x bigger.

Pouton et al, (1997) reported strategies used for formulation of SEDDS, and mentioned about practical considerations regarding the use of SEDDS for enhancement of the bioavailability of drugs from the gastro-intestinal tract. The absolute bioavailability of cyclosporine loaded in microemulsion system was increased about 3.3 and 1.25 fold compared with Sandimmun and Sandimmun Neoral (Zhong et al, 1998).

Calum and Celesta, (2000) has reported new impetus in surfactant self-assembly objects as agents for drug delivery that are an alternative to micellar, lamellar liposome, niosome and transfersome or microemulsion-based vehicles. Toos et al, (2005) has reported specific targeting and delivery as well as the display of antigens
on the surface of professional antigen-presenting cells (APCs) are key issues in the design and development of new-generation vaccines aimed at the induction of both humoral and cell mediated immunity. Various methods of niosome preparation and the numbers of different morphologies that have been identified are detailed (Ijeoma et al, 1998).

Johanna et al, (2003) has reported that fractionated oat oil (FOO) and medium chain monoglycerides (60:30:10 mono-, di- and tri-glycerides) promoted absorption. (Sudaxshina et al, 2005) has found that all the formulations tested, except for the hydrophilic amphiphilologel and its aqueous dispersion, were bioequivalent to Neoral, the commercially available preparation.

Michael et al, (2003) has reported about P-gp inhibition, and it was assessed using RH123 accumulation into LS180V cells as well as Rh123 transport across Caco-2 monolayers. The relative P-gp expression (P-gp/villin integrated optical density ratio) progressively increased from proximal to distal regions. Among individuals, relative P-gp levels varied 2.1-fold in the duodenal/proximal jejunal region, 1.5- to 2.0-fold in the middle/distal jejunal region, and 1.2- to 1.9-fold in the ileal region. Within-donor variation was somewhat greater, from 1.5- to 3.0-fold (Stephane and Mary, 2003).

Matthew and Dhiren, (2003) described a set of P-gp substrates was determined in absorptive and secretory directions in Madine–Darby Canine kidney (MDCK), Caco-2, and MDR-MDCK monolayers. Yu-Yuan Chiu et al, (2003) has reported and suggested with his experiments that, the effects of surfactants via micellar solubilization and inhibition of P-gp efflux on CsA transport in Caco-2 cells are significant.

Aranya et al, (2003) has reported the stearyl chain (C18) non-ionic surfactant vesicles showed higher entrapment efficiency than the lauryl chain (C12) non-ionic surfactant vesicles. Cholesterol was used to complete the hydrophobic moiety of single alkyl chain nonionic surfactants for vesicle formation. The passive diffusion and influx transporter markers gave similar profiles with a plateau of permeability along the
jejunum, and with the exception of l-Dopa showing lower permeability in the ileum. Permeability in the duodenum was two to three times lower than the jejunum for all compounds (Olivier et al., 2004). The effect of sodium caprate on drug oral bioavailability was studied by Teodoro et al., (2004) and reported increase in absorption rate constant (ka) of acamprosate in the mid-intestine of the rats. Rama Prasad et al., (2004) has reported that, labrasol increased the intestinal absorption of Low molecular weight heparin, and jejunum was found to be the best site of absorption. Berggren et al., (2004) has reported the jejunal permeability of ropivacaine, lidocaine and bupivacaine was high and it has been observed that carrier-mediated intestinal efflux did not have significant influence on the rate and extent of in vivo intestinal absorption. Rather, passive diffusion of these agents seems to be the major mechanism for the intestinal absorption.

1.2 Delivery Systems

A considerable attention has been focused on the development of new drug delivery systems. There are number of reasons for the intense interest in new system:

a) Recognition of the possibility of re-patenting successful drugs by applying the concepts and techniques of controlled drug delivery system, coupled with the increasing expense in bringing new drug entities to the market, has encouraged the development of new drug delivery system.

b) New systems are needed to deliver the novel, genetically engineered pharmaceuticals, i.e. peptides and proteins, to their sites of action without increasing significant immunogenicity or biological inactivation.

c) Enzyme deficient diseases and cancer therapies can be improved by temporal placement within the body, thereby reducing both the size and frequency of doses.

d) Therapeutic efficacy and safety of drugs can be improved by precise targeting.

The new drug delivery system developed should behave ideally fulfilling two prerequisites:
a) To deliver the drug at a rate dictated by the needs of the body over a period of treatment.
b) To channel the active entity to the site of action.

Conventional dosage forms, including prolonged release dosage forms, are unable to control either the rate or site of action. At present, no available drug delivery system behave ideally in achieving all the goals, but sincere attempts have been made to achieve them through various novel approaches in drug delivery.

Controlled drug delivery attempts to:

- Sustain drug action at a predetermined rate by maintaining relatively constant, effective drug delivery level in the body with concomitant minimization of undesirable side effects.
- It can also localize drug action by spatial placement of controlled release system adjacent to or in the diseased tissue or organ.
- Target drug action by using carriers, chemical derivatization to deliver drugs to a particular target cell type.

New generation dosage form mainly comprises of colloidal drug carrier systems. The carrier serves to protect the drug from premature degradation, inactivation and protect the host from unwanted immunological or pharmacological effects.

Colloidal drug delivery system can be of two types

1. Soluble carriers
2. Particulate carriers

Particulate carrier system includes: Nanoparticles, Microparticles, Microspheres, Microemulsion, Magnetic microspheres, Resealed erythrocytes, Liposomes, Niosomes, transferosomes and bilosomes (other vesicular systems) as described in figure 1.
Ideally, a colloidal drug delivery system should possess the following characteristics:

1. It should be pharmaceutically acceptable with regards to stability and ease of administration.
2. It must be sterilizable, if required.
3. It must transport a drug to its desired site of action.
4. It must release the drug at a required rate at the site of action.
5. The carrier should be non-toxic and biodegradable.

In fact, any colloidal drug delivery systems do not comply with these characteristics absolutely.

1.3 NONIONIC SURFACTANT BASED DELIVERY SYSTEMS

The delivery of drug candidates using nonionic surfactants seems to ideal as variety of nonionic surfactants belongs to GRAS and the reported toxicity profile is pretty low. In addition to this the nonionic surfactants enjoys number of advantages as they are able to improve permeability through variety of mechanisms. In the proposed
study the following structured vehicles comprising nonionic surfactants has been taken up for delivery of CsA,

1.3.1. Vesicular System

1.3.2. Microemulsions

1.3.1 Vesicular System

Encapsulation of a drug in vesicular structures can be predicted to prolong the existence of the drug in systemic circulation and perhaps reduce toxicity if selective uptake can be achieved. Vesicular systems are of considerable interest because they can be used as membrane models, in chemical reactivity or in drug delivery and targeting.

Some advantages of vesicular system are as follows:

1. Solve the problems of drug insolubility, instability and rapid degradation.
2. Both hydrophilic and lipophilic drugs can be incorporated.
3. Function as sustained release systems.
4. Improve the bioavailability of poorly soluble drugs and also reduces the cost of therapy.
5. Reduce irritancy and ensures patient compliance.
7. The phagocytic uptake of the systemic delivery of drug loaded vesicular delivery system provide efficient method for delivery of drug directly to the site of infection leading to reduction of drug toxicity with no adverse effects.

The most prevalent vesicular systems investigated for drug delivery are liposomes and niosomes.

Liposomes

Liposomes are simple microscopic vesicles where an aqueous space is enclosed by a lipid bilayer structures. These are formed when phospholipids are dispersed in an aqueous medium, the hydrophilic interaction of the lipid head groups with water
results in the formation of multilamellar and unilamellar systems (vesicles). These vesicles consist of a simple lipid bilayer that resembles biological membranes in the form of spherical shell. Because of their entrapping ability liposomes are being considered as drug delivery structures or vesicles. Liposomes are advantageous in fulfillment of the aspects related to protection of the drug, controlled release of the active moiety along with the target delivery and cellular uptake via endocytosis. But liposomes possess certain disadvantages associated with degradation by hydrolysis or oxidation and sedimentation, leaching of drugs, aggregation or fusion of liposomes during storage. Some problems associated with clinical applications of liposomes are difficulties experienced in sterilization and large scale production. Moreover, it is difficult to obtain large quantities of sterile products with defined and reproducible properties that display adequate chemical and physical stability. The cost and purity of phospholipids is another limiting factor. They are suitable for parenteral administration but oral administration is not possible because of inability of liposomes to survive the action of bile salts and phospholipids. Tedious conditions dealt in handling liposomes under cryogenic atmosphere have prompted the use of non-ionic surfactant vesicles or niosomes as an alternative to liposomes.

Niosomes
Niosomes were first reported as a feature of cosmetic industry but, Handjani-vila et al., (1979) introduced niosomes as oral drug delivery system and drug targeting agents. Niosomes or non-ionic surfactant vesicles are microscopic vesicles that are formed from self assembly of non-ionic surfactant in aqueous media resulting in closed bilayer structure. Niosomes consisting of one bilayer are designated as small unilamellar vesicles (SUV) or large unilamellar vesicles (LUV) and one with more bilayers are called multilamellar vesicles (MUV). The bilayered vesicular structure is an assembly of hydrophobic tail of surfactant monomer shielded away from aqueous space located in the center and hydrophilic groups in contact with same. Figure 2 depicts unilamellar niosomes showing molecular structure of the lipid bilayer.

Though niosomes exhibit a behavior similar to liposomes in vivo, niosomes have certain advantages over liposomes:
a) Niosomes are quite stable even in the emulsified form.
b) Niosomes require no special conditions such as low temperature or inert atmosphere for protection or storage.
c) Niosomes are chemically stable.
d) Relatively low cost of materials makes it suitable for industrial manufacture.

![Figure 2: Unilamellar niosome showing bilayer](image)

**Advantages of Niosomes**

1. Niosomes can entrap both hydrophilic and lipophilic drugs, either in aqueous layer or in vesicular membrane made of lipid materials.
2. Niosomes are osmotically active and stable as well as they increase the stability of entrapped drug.
3. Handling and storage of surfactants require no special conditions.
4. Niosomes exhibit flexibility in their structural characteristics (composition, fluidity, size, lamellarity, trapped volume, surface charge etc.) and can be designed according to the desired situation.

5. Niosomes can improve oral bioavailability of poorly absorbed drugs and enhance skin penetration of drugs.

6. They can be made to reach the site of action by oral, parenteral as well as topical routes.

7. They allow their surface for attachment of hydrophilic groups and can incorporate hydrophobic moieties in bilayers to bring about changes in the in-vivo behavior of niosomes. These moieties can be attached or anchored to the head groups of surfactant monomer in form of a positive, neutral or negative charge on their surface (surface ligands e.g. carbohydrate, glycoprotein, etc). The nature and density of charge of niosomes influence the stability, permeability as well as interactions with RES (Reticuloendothelial system).

8. Niosomal dispersion in aqueous phase can be emulsified in non-aqueous phase to regulate the delivery rate of drug and administer normal vesicles in external non-queous phase.

9. Niosomal surfactants are biodegradable, biocompatible and non-immunogenic.

10. Niosomes improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells.

11. Compounds having wide range of molecular weight can be entrapped into niosomes i.e. from simple ions to complex DNA molecules.

12. Niosomes can penetrate in the lipid bilayers of stratum corneum and epidermis, and presence of surfactants may serve as penetration enhancer and facilitate dermal delivery leading to higher localized drug concentration.
13. When injected intravenously they are dispatched to RES (Reticuloendothelial system). The passive targeting nature can well be exploited in the condition where RES is involved e.g. Leishmaniasis, metal poisoning in liver, inflammatory conditions, hepatotoxicity, etc.

14. Niosomes improve the performance of the drug molecules by:

- Serving as a local depot for sustained release of dermally active compounds.
- Increasing the residence time of entrapped drug and hence increases the availability at the absorption site.
- Serving as a rate limiting membrane barrier for modulation of systemic absorption.

Chandraprakash et al, (1990; 1997) made non-ionic surfactant vesicles using lipophilic surfactants like Span 40, 60 and 80 entrapped with methotrexate. The tissue distribution of methotrexate was improved after entrapping with niosomes. Entrapment of methotrexate was better with Span 60 compared to Tween 80, which may be due to increased lipophilicity. These vesicles were also found to be osmotically stable.

Bilosomes

Bilosomes are made of nonionic surfactants they are similar to niosomes or non-ionic surfactant vesicles except they contain the one of the bile salt as the integral component of the vesicular system, off course these are microscopic vesicles that is formed from self assembly of non-ionic surfactant in aqueous media resulting in closed bilayer structure. The bile component having similar structure to cholesterol is anticipated to get fit into closed bilayer structure and at the same time is able to impart its own functions. The concentration of bile component can be optimized in order to have vesicular structure without affecting its integrity.
Advantages of Bilosomes

The bilosomes enjoys all the advantages of niosomes in addition to it provides the system the following benefits:

1. Variety of bile salts could be incorporated to facilitate absorption through different mechanisms.
2. Absorption of drug candidates can be enhanced.
3. The stability of vesicles in GI tract can be improved and oral delivery of vesicles can be made possible.
4. Fluidity of the membranes can be improved for enhanced delivery.
5. Oral delivery of vaccine incorporated into vesicles is possible.

1.3.1.1 CLASSIFICATION OF VESICULAR SYSTEMS

Vesicular systems are usually classified according to the number of bilayers present. They are of three types:

1. Multi-lamellar vesicles (MLV) 2µm - 5µm
2. Small unilamellar vesicles (SUV) 100nm - 500nm
3. Large unilamellar vesicles (LUV) 500nm - 900nm

1.3.1.2 MECHANISM OF UPTAKE OF NIOSOMES

In general large niosomes are cleared more readily than small ones. At the cellular levels niosomes can interact with cells in atleast 4 ways:

- Fusion of outer bilayer of the niosomes with the plasma membrane.
- Stable adsorption to the cell surface either non-specific or via ligand.
- Transfer lipid molecule between outer monolayer and cell without direct association of two entities.
- Endocytosis

Many factors, including the size, chemical composition and surface charge of niosomes can alter their properties. Positive, negative or neutral charge that the niosomes carry on surface can alter their pattern of distribution in the body. The glycolipid composition of liposomes/niosomes can also alter their affinity for various tissues. Coating the outermost surface of niosomes with polysaccharide derivatives is
another way for niosome to be utilized as targetable drug carriers. Furthermore depending on their structure niosome can be made to release entrapped drug at higher temperature thus increasing the potential for regional therapy because parts of the body can be made hyperthermic with various techniques because of the pH of the tumors base being low, niosomes were prepared that would release their content efficiently at pH 5-6 rather than at physiological pH of 7.4 with the intent of releasing drug only at specific site of tumors (Connor et al, 1986). The local release of drug is also triggered by the enzymatic degradation of niosomes.

In early 1960's (Bangham and Home, 1964) reported that on dispersion in water, phospholipids form multilayer vesicles, which enclose aqueous compartment. Each layer of these vesicles is a bimolecular lipid membrane is termed as liposomes. Kunitake & Okahata, (1976); Okahata et al, (1981) had reported that most of the amphiphiles (ionic) are toxic and are unsuitable for use as drug carriers.

Handjani-vila et al, (1979) has reported that hydration of a mixture of cholesterol and single chain non-ionic surfactant, vesicular system was formed, Okahata et al, (1981) who observed the formation of vesicle by dialkylpolyoxyethylene ether (non-ionic surfactant) supported this. The resultant vesicle was termed as Niosomes. Niosomes are osmotically active and better alternative to phospholipid vesicles and are relatively more chemically stable. Azmin et al, (1985) and Rogerson et al, (1988) used non-ionic hydrophilic surfactant like Tween-80 for making Niosomes entrapped with methotrexate and studied the pharmacokinetics of methotrexate after i.v injection of niosomes to the mice.

Rogerson et al, (1988) and Baillie et al, (1985) have used niosome as drug carrier for doxorubicin and sodium stibogluconate for better targeting property. Cook and Florence (1988) have shown that Tween-80 enhance the cytotoxic effect of podophylotoxin derivative, etoposide. Nonionic surfactants are reported to increase fluidity and permeability of biological membrane.

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Chitnis et al, (1984) has reported the effect of adriamycin dissolved in Tween-80 and compared its antitumour activity with adriamycin dissolved in water on mice bearing lymphocyte leukemia and found that antitumour activity of adriamycin dissolved in aqueous solution of Tween 80 was higher.

Design and development of Vesicles

Niosomes can be prepared by different types of non-ionic surfactants. Non-ionic surfactants consist of a hydrophilic head group and hydrophobic tail. They have no charge unlike anionic and cationic surfactants. These are also used as emulsifying, solubilizing and wetting agents depending upon their HLB (Hydrophilic Lipophilic Balance) value. The various non-ionic surfactants that are used for the preparation of niosomes are listed in Table 4.

The hydrophobic moiety of the surfactant may be alkyl (Tanaka, 1990; Polidiri et al, 1994; Talsma et al, 1994), fluoroalkyl or steroidal in nature (Guedj et al, 1994; Darwish and Uchegbu, 1997). The number of hydrophobic moiety at present are limited but a wide variety of hydrophilic head groups are available in vesicle forming surfactants. It is in this area of vesicle forming surfactant design, a considerable scope for new formulations still exist. The two portions may be linked via ether, amide or ester bonds. The hydrophilic head groups include glyceryl head groups (Kerr et al, 1988), ethylene oxide head groups (Okahata et al, 1981; Chauhan et al, 1989), crown ether head groups (Montserrat et al, 1980; Echegoyen et al, 1988), sugar head group and amino groups (Guedj et al, 1994; Zarif et al, 1993) and sorbitan head groups (Chandraprakash et al, 1990; Raja Naresh et al, 1994).

Various additives are included in the formulation in order to prepare stable niosomes. The most common is additive is cholesterol. Cholesterol is known to abolish gel to liquid phase transition of niosomes system resulting in niosomes that are less leaky (Cable, 1989). The aggregation of niosomes can be prevented by inclusion of molecules like solulan C24 cholesteryl poly-24-oxyethylene ether). Solulan C24 provides steric stabilization (Uchegbu et al, 1995). Electrostatic
stabilization can also be achieved by inclusion of dicetyl phosphate, stearylamine and diacylglycerol that are the charge inducers (Yoshioka and Florence, 1994).

Methods of Preparation

1. Ether injection method

In this method, a lipid solution in diethyl ether is slowly introduced into warm water. Typically the lipid mixture is injected into an aqueous solution of the material to be encapsulated (using syringe type infusion pump) at 55-65°C and under reduced pressure. Vaporization of ether leads to the formation of single layered vesicles (SLV). Depending upon the condition used, the diameter of vesicles varies (Baillie et al, 1985) and used this method for entrapment of 5, 6 carboxyfluorescein whereas (Hunter et al, 1988; Carter et al, 1989) and even used it for the entrapment of sodium stibogluconate.
Table 4: Various non-ionic surfactants used in preparation of niosomes

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Class</th>
<th>Definitions</th>
<th>Example</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Macrogol ethers</td>
<td>Condensation products of ethylene oxide and fatty alcohols</td>
<td>Hexadecyl diglycerol ether</td>
<td>Arunothayanun et al, 1999.</td>
</tr>
</tbody>
</table>

2. Lipid film formation (Hand shaking method)

Baillie et al, (1986) reported hand shaking method for the entrapment of 5, 6 carboxyfluorescein. Methotrexate was entrapped in niosomes and prepared by hand shaking method using lipophilic surfactants like Span 40, Span60 and Span 80, cholesterol and diacetyl phosphate in the ratio of 47.5:47.5:5. The tissue distribution of Methotrexate was improved after entrapping with Niosomes Chandraprakash et al, (1990). Rogerson et al, (1988) prepared Doxorubicin entrapped Niosomes using pure surfactant or a mixture of surfactants and cholesterol. Azim et al, (1985) modified this method for the preparation of methotrexate entrapped niosomes.
3. Sonication method


Methods for controlling the size of niosomes

Size distribution of niosomes is an important parameter to study the physical properties and biological fate of niosomes and their entrapped substances. In order to maintain uniformity and reproducibility of niosome preparation, vesicle size homogeneity is a mandatory requirement. Control of size distribution makes niosome formulation a promising industrial product. Uniformity in vesicle size is essential for dose calculation when niosome is administered for clinical indication. The variation in size influence the pattern of niosome disposal from blood; large size vesicles may reside in lung due to alveolar retention and effect of alveolar phagocytic cells, while small sized vesicles which can pass through fenestrations in liver sinusoidal epithelium, have better access to spleen (Carter et al, 1989; Rogerson et al, 1988).

Vesicle size distribution of the preparation can be controlled by the following methods:

a) Fractionation of the size of interest from a heterogeneous population that includes centrifugation and size exclusion chromatography (Uchegbu et al, 1995).

b) Homogenization of a polydisperse dispersion to yield a population of small vesicles with a narrow size distribution.

c) Extrusion of a heterogeneous preparation through capillary pore diameter (Stafford et al, 1988).

d) Probe sonication (Baille et al, 1985; Azmin et al, 1985).
Factors affecting vesicle size and entrapment efficiency

I) Drug:

a) **Entrapment of drug**

Entrapment of drug in niosomes increases vesicle size, probably by interaction of solute with surfactant head groups, increasing the charge and mutual repulsion of the surfactant bilayers thereby increasing vesicle size. In polyoxyethylene (PEG) coated vesicles, some drug are entrapped in long PEG chain thus reducing the tendency to increase the size.

b) **Hydrophilic-Lipophillic Balance (HLB)**

The hydrophilic- lipophilic balance of a drug affects degree of entrapment. For a series of Spans and Tweens, reported maximum entrapment of water-soluble drug diclofenac sodium in hydrophilic surfactant, Tween 60, and reported maximum entrapment of slightly water-soluble drug methotrexate in lipophilic surfactant, Span 60 (Raja Naresh et al, 1994; Chandraprakash et al, 1990).

II) Cholesterol content: Baillie *et al*, (1986) has reported that incorporation of 50% cholesterol in surfactant composition reduces vesicle permeability of 5, 6-carboxyfluorescein by a factor of 10. Cholesterol has decreasing effect on gel-liquid transition temperature, at which rapid efflux of vesicle content occurs, it converts well defined gel-liquid transition temperature of a pure surfactant to gel-liquid transition range. It is reported that total amount of cholesterol should not exceed 33%. Upto 33%, it provides stabilization to vesicles, after that destabilization starts.

III) Method of preparation:

- Hand Shaking method form large vesicles (0.35-13μm) compared to those prepared by Ether injection method (50-1000nm) (Arunothyam *et al*, 1999)
- Sonication of MLV prepared by other methods either with probe sonicator or bath type sonicator forms unilamellar vesicles with considerably reduced diameter.
- Increase in sonication time generally results, reduction in vesicle diameter.
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• Hydrating the lipid above phase transition temperature of surfactant and vortexing during hydration helps to reduce the size of vesicles prepared by hand shaking method.

• Stafford et al, 1988 extruded the niosomal dispersion prepared by Hand Shaking method through 0.1 or 0.22\(\mu\)m Nucleopore polycarbonate membranes in series. After 8 such extrusions average diameter of vesicles was 135-340 nm.

• Small sized vesicles can also be produced by Reverse Phase Evaporation method (Baillie et al, 1985). In this, emulsion of aqueous phase in organic solvent containing lipid is prepared by sonication, followed by evaporation of organic solvent, resulting in formation of vesicles.

• Microfluidization method gives greater uniformity and small sized vesicles.

• Parthasarthy et al, (1994) has prepared niosomes by transmembrane pH gradient or remote loading. Niosomes formed by this method showed greater entrapment efficiency and better retention of drug.

• Niosomes bearing 5,6-carboxyfluorescein prepared by Ether injection method showed entrapment efficiency significantly higher than those prepared by Hand shaking method or Sonication compared to Hand shaking and Reverse Phase Evaporation methods. Niosomes prepared by Remote loading method show greater entrapment efficiency and slower release of drug (Baillie et al, 1986).

IV) Osmotic effect: Addition of hypertonic salt solution to suspension of niosomes brings about reduction in vesicle diameter with concomitant water efflux, which may be due to pumping out of vesicle content whereas in hypotonic salt solution, there is initial slow release with swelling of vesicles probably due to inhibition of eluting fluid from vesicle, followed by faster release, which may due to mechanical loosening of vesicles under osmotic stress (Kunitake and Okahata, 1976).

1.3.1.3 Modified non-ionic surfactant vesicles

1) Discomes: These are disc shaped structures formed by mixing of cholesterol, polyoxyethylene (Solulans) and vesicular dispersion. Dispersion in discome phase consists of large “Discomes” (30 to 60 micrometer mean volume diameter). Lipid in
the proportion (i.e. surfactant: cholesterol: Solulan C24: DCP: 49: 19.5: 29.5: 2) produce discome on sonication. Discomes entrap water-soluble solute. Entrapment of 5(6) -carboxyfluorescien showed aqueous entrapment value of 3.603 ± 2.916% and release of 50% solute after 24 hours at room temperature. Large volume carrying capacity allows them to be used in ophthalmology (Jain, 1997).

2) Polymerized non-ionic surfactant vesicles: Since vesicle system are more or less thermodynamically unstable, proximity and regular orientation of surface active molecules at interface has been exploited to increase the stability by control polymerization of vesicle forming non-ionic surfactant bearing a polymerizable residue. Polymerizable surfactant used was:

1) Dimethyl n-hexadecyl \{1- isocyanoethyl\} carbonyloxy methyl ammonium bromide.
2) N, N (dihexadecanoyloxyethyl) maleyl amide.
3) Dihexadecyl N, methyl N-maleyl ammonium bromide.

The vesicles prepared from this surfactant were polymerized by radiation or radical initiation. Polymerization restricts mobility of hydrocarbon core and hence improves the stability of niosomes. Size of the vesicles on polymerization remains unchanged while change in appearance depends upon location of polymerizable group.

3) Emulsified niosomal dispersion: Vesicles-in-water-in-oil (v/w/o) emulsion from niosomes made from Spans (20, 40, 60, 80) in the size range 600nm-3.5μm, dispersed in water droplets of around 5-25μm, themselves dispersed in an oil (octane, hexadecane, isopropyl myristate). The release was affected by the HLB of surfactant, nature of oil and temperature of dialysis media. Thus by their appropriate choice, delivery rate of drug could be regulated.

This system allowed administration or application of vesicles in an external non-aqueous phase while maintaining normal vesicular structure in aqueous phase and can be of potential use in drug delivery or as vaccine vehicle.
4) **Proniosomes**: This non-ionic surfactant based vesicular system overcomes the problems of physical instability of aqueous niosomal dispersions (aggregation, fusion and leakage) or any other vesicular drug delivery system. The encapsulation efficiency of proniosomes is high and they are capable of incorporating lipophilic, hydrophilic and amphiphilic drugs. The particle size distribution can easily be controlled. A vacuum or nitrogen atmosphere is not required for stability maintenance. All these characteristics are pointer to the commercial viability of the proniosomes. Proniosomes can be divided into two category i.e. Dry granular and liquid crystalline proniosomes, However the dry granular proniosomes seems to feasible for oral delivery.

Dry granular type of proniosomes involves coating of water soluble carrier such as sorbitol or maltodextrin with surfactant. The result of coating process is a dry formulation in which each water soluble particle is covered with thin film of surfactant. The proniosomes are reconstituted by addition of aqueous phase at a temperature greater than transition temperature and brief agitation. It is essential to prepare vesicles at a temperature above the transition temperature of non-ionic surfactant being used in the formulation. Successful rehydration of the surfactant to produce niosomes from dried film requires that the film should be as thin as possible to avoid clumping and precipitation that occurs when pure granular surfactant is hydrated directly. Inefficient rehydration leads to coarse broken surface on proniosomes and occurrence of aggregation and precipitation in final niosomal preparation/suspension. An explanation for efficient niosome formulation from proniosome is based on dissolution of the carrier to facilitate hydration of the surfactant.

According to type of carrier and method of preparation of dry granular proniosomes they are further divided into:

1. Sorbitol based proniosomes
2. Maltodextrin based proniosomes

Sorbitol based proniosomes is a dry formulation that involves sorbitol as a carrier, which is further coated with non-ionic surfactant and is used as niosomes within minutes by addition of hot water followed by agitation.
Advantages of Sorbitol Based Proniosomes:

a) Very similar to the niosomes prepared by conventional method.
b) Size distribution is very uniform.
c) It provides a suitable method for formulating hydrophobic drugs in a lipid suspension.
d) It is also useful in case where the active ingredient is susceptible to hydrolysis.

Maltodextrin based proniosomes have maltodextrin as the carrier and preparation involves fast slurry method (Welsh & Rhodes, 2001a). The time required to produce proniosomes by the slurry method is independent of the surfactant solution to carrier ratio. Proniosomes of high surface to carrier ratio can be prepared. The flexibility of proniosomes preparation permits the optimization of the drug in the final formulation based on the type and amount of maltodextrin. This formulation of proniosomes is practical and simple method for producing niosomes at point of use.
for drug delivery. An analogous process with sorbitol results in a solid surfactant sorbitol cake. Since maltodextrin morphology is preserved, hollow blown maltodextrin particles can be used for significant gain in surface area. The high surface area results in thinner surfactant coating which makes the rehydration process efficient. For drugs, where maltodextrin is found to affect the encapsulation efficiency, the quantity of maltodextrin can be minimized by producing proniosomes with greater surfactant loading (Welsh and Rhodes, 2001b). The preparation has a potential for application to deliver hydrophobic, hydrophilic and amphiphilic drugs. When the surfactant molecules are kept in contact with water, there are three ways by which lipophilic chains of surfactant can be transformed into a disordered, liquid crystalline state: increasing temperature at Kraft point (Tc), addition of solvent which dissolves lipids, use of both temperature and solvent.

1.3.1.4 BIOMEDICAL AND THERAPEUTIC APPLICATIONS

A wide variety of drugs can be encapsulated in niosomes. Table 5 summarizes the various agents encapsulated in the niosomes and results obtained.
Table 5: Agents encapsulated in Vesicular system

<table>
<thead>
<tr>
<th>System</th>
<th>Drug</th>
<th>Category</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niosome</td>
<td>Adriamycin</td>
<td>Anticancer</td>
<td>Kerr et al., 1988</td>
</tr>
<tr>
<td>Niosome</td>
<td>Amarogenetin</td>
<td>Antileishmanial</td>
<td>Medda et al., 1999</td>
</tr>
<tr>
<td>Niosome</td>
<td>Bacopasaponin</td>
<td>Antileishmanial</td>
<td>Sinha et al., 2002</td>
</tr>
<tr>
<td>Niosome</td>
<td>Bleomycin</td>
<td>Anticancer</td>
<td>Sludden et al., 2000</td>
</tr>
<tr>
<td>Niosome</td>
<td>Centchroman</td>
<td>Contraceptive</td>
<td>Shenoy et al., 1999</td>
</tr>
<tr>
<td>Niosome</td>
<td>Ciproflloxacin</td>
<td>Antimicrobial</td>
<td>D souza et al., 1997</td>
</tr>
<tr>
<td>Niosome</td>
<td>Cisplastin</td>
<td>Anticancer</td>
<td>Gude et al., 2002</td>
</tr>
<tr>
<td>Niosome</td>
<td>Colchicine</td>
<td>Anticancer</td>
<td>Hao et al., 2002</td>
</tr>
<tr>
<td>Niosome</td>
<td>Cyatarabine</td>
<td>Anticancer</td>
<td>Ruckmani et al., 2000</td>
</tr>
<tr>
<td>Niosome</td>
<td>Doxorubicin</td>
<td>Anticancer</td>
<td>Rogerson et al., 1988</td>
</tr>
<tr>
<td>Niosome</td>
<td>5-Flurouracil</td>
<td>Anticancer</td>
<td>Namdeo &amp; Jain, 1999</td>
</tr>
<tr>
<td>Niosome</td>
<td>Methotrexate</td>
<td>Anticancer</td>
<td>Azmin et al., 1985</td>
</tr>
<tr>
<td>Niosome</td>
<td>Pentoxifylline</td>
<td>Anticancer</td>
<td>Gaekwad et al., 2000</td>
</tr>
<tr>
<td>Niosome</td>
<td>Enoxacin</td>
<td>Antimicrobial</td>
<td>Fang et al., 2001</td>
</tr>
<tr>
<td>Niosome</td>
<td>Flurbiprofen</td>
<td>Anti-inflammatory</td>
<td>Reddy &amp; Udupa, 1993</td>
</tr>
<tr>
<td>Niosome</td>
<td>Indomethacin</td>
<td>Anti-inflammatory</td>
<td>Pillai &amp; Salim, 1999</td>
</tr>
<tr>
<td>Niosome</td>
<td>Insulin</td>
<td>Hypoglycemic</td>
<td>Khaska et al., 2000</td>
</tr>
<tr>
<td>Niosome</td>
<td>Iobiterdol</td>
<td>Radio-opaque</td>
<td>Muller et al., 2000</td>
</tr>
<tr>
<td>Niosome</td>
<td>Ketoprofen</td>
<td>Anti-inflammatory</td>
<td>Khandare et al., 2004</td>
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<td>Norfloxacin</td>
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<td>D souza et al., 1997</td>
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<td>Niosome</td>
<td>Piroxicam</td>
<td>Anti-inflammatory</td>
<td>Reddy &amp; Udupa, 1993</td>
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<tr>
<td>Niosome</td>
<td>Plumbagin</td>
<td>Antitumor</td>
<td>Kinni et al., 1997</td>
</tr>
<tr>
<td>Niosome</td>
<td>Propanolol</td>
<td>Antihypertensive</td>
<td>Sihorkar &amp; Vyas, 2000</td>
</tr>
<tr>
<td>Niosome</td>
<td>Quercitin</td>
<td>Antileishmanial</td>
<td>Sarkar et al., 2002</td>
</tr>
<tr>
<td>Niosome</td>
<td>Retinoic acid</td>
<td>Vitamin</td>
<td>Desai &amp; Finlay, 2002</td>
</tr>
<tr>
<td>Niosome</td>
<td>Rifamycin</td>
<td>Antitubercular</td>
<td>Jain &amp; Vyas, 1995</td>
</tr>
<tr>
<td>Niosome</td>
<td>Sumatriptan</td>
<td>Antimigraine</td>
<td>Gayatrivedi et al., 2000</td>
</tr>
<tr>
<td>Niosome</td>
<td>Tretinoína</td>
<td>Marker</td>
<td>Manconi et al., 1997</td>
</tr>
<tr>
<td>Niosome</td>
<td>Vincristine</td>
<td>Anticancer</td>
<td>Parthasaradhi et al., 1994</td>
</tr>
<tr>
<td>Proniosome</td>
<td>Estradiol</td>
<td>Harmonal</td>
<td>Fang et al., 2001</td>
</tr>
<tr>
<td>Proniosome</td>
<td>Ibuprofen</td>
<td>Analgesic</td>
<td>Hu &amp; Rhodes, 1999</td>
</tr>
<tr>
<td>Proniosome</td>
<td>Levonorgestrel</td>
<td>Contraceptive</td>
<td>Vora et al., 1998</td>
</tr>
</tbody>
</table>

1.3.2 MICROEMULSION

When water is mixed with an oil and amphiphile, a turbid milky emulsion is obtained. Emulsion is a heterogeneous system of one immiscible liquid dispersed in another in the form of droplets. The droplet size for ordinary emulsion (macro emulsions) is more than 1 μm (Forgiarini et al, 2000). Emulsion has thermodynamic instability,
thus after some time separates again into an aqueous and an organic phase. In 1950s Shulman observed that these unstable water oil dispersions can be converted into optically transparent and thermodynamically stable mixtures by adding alcohol (Shulman et al, 1959). These mixtures were called microemulsion. A microemulsion is a thermodynamically stable dispersion of one liquid phase into another, stabilized by an interfacial film of surfactant. This dispersion may be either oil-in-water (o/w) or water-in-oil (w/o). The size of the droplet ranges from 4 to 100 nanometers; hence the microemulsion solutions are apparently transparent. The droplet can have structures such as spheres, rods or disks, depending on the composition and types of surfactant and oil used. The interfacial tension between the two phases is extremely low. The microemulsions are characterized by the weight fraction of surfactant, $\gamma$, in the ternary mixture, the weight fraction of oil, $\alpha$ in the mixture of oil and water and the molar ratio of water to surfactant $W_0$ (Schomacker & Braun, 1996).

\begin{align*}
(1) \quad & \gamma = m_{\text{surfactant}}/m_{\text{surfactant}} + m_{\text{water}} + m_{\text{oil}} \\
(2) \quad & \alpha = m_{\text{oil}} /m_{\text{water}} + m_{\text{oil}} \\
(3) \quad & W_0 = n_{\text{water}} /n_{\text{surfactant}}
\end{align*}

Because of the presence of three entirely different polarity domains: polar aqueous, nonpolar, and interfacial regions, the microemulsion have been found to be very useful as reaction, extraction, and separation media. The aqueous core of microemulsion contributes to the solubilization of water soluble compounds in nonpolar organic phase whereas the organic bulk phase offers the advantages of dissolving organic solutes. It is noted that the size of the aqueous core can easily be tuned by varying the water content of the microemulsion to host a variety of solutes ranging from dye to large protein molecules (Kumar & Mittal, 1999). From the formulation point of view, microemulsions find several application fields such as nanoparticle preparation, and polymerization. The study of phase behavior of the ternary mixture is a matter of common scientific interest from both theoretical and experimental point of view. Because types (either water or oil continuous type),
stability, phase inversion, and preparation method of ordinary ternary mixture are related to the phase behavior in surfactant system.

1.3.2.1 Surfactants
Surfactants, also known as wetting agents, is a molecule that, when added to a liquid at low concentration, changes the properties of that liquid at a surface or interface and lower the surface tension of a liquid, allowing easier spreading. The term surfactant is a compression of “Surface active agent”. Surfactants are usually organic compounds that contain both hydrophobic and hydrophilic groups, and are thus semi-soluble in both organic and aqueous solvents. Surfactants are also known as amphipathic compounds, meaning that they would prefer to be in neither phase (water or organic). For this reason they locate at the phase boundary between the organic or oil and water phase, or, if there is no more room there, they will congregate together and form micelles. The hydrophilic end is water-soluble and is usually a polar or ionic group. The hydrophobic end is water-insoluble and is usually a long fatty or hydrocarbon chain. This dual functionality, hydrophobic and hydrophilic, provides the basis for characteristics useful as detergent in formulations, including surface tension modification, emulsification, foam, and cloud point. Surfactants are generally characterized by the hydrophilic group into the following categories: anionic, cationic, nonionic and amphoteric based on ionic character.

1.3.2.2 Phase Behavior in a Surfactant System
Consider a ternary mixture of water, a hydrocarbon and a nonionic surfactant. Phase behavior of the ternary system as a function of field variable can be described by a Gibbs prism with oil-water-surfactant triangle as its base and the field variable as the ordinate (Kahlweit et al, 1990). At constant pressure the ternary system is specified by setting three independent variables. These are generally the temperature T, the weight fraction of oil α, and the weight fraction of surfactant Y. Figure 4 represents the Gibbs prism with T as the ordinate. In the ternary nonionic microemulsion system common phase sequence as a function of temperature is given as Type I! Type II!
Type III (Winsor, 1968; Shinoda and Saito, 1968). The notation describes the change in the surfactant solubility from more water soluble (o/w, 2, Winsor type I) to more oil soluble (Winsor type II). The progress of the phases can be illustrated on isothermal sections of the phase prism (Figure 5).

When the surfactant is more hydrophilic at low temperatures, the surfactant dissolves mainly in water and forms oil-in-water (o/w) type microemulsion that is in equilibrium with an excess oil phase (2). On the other hand, when the surfactant is more lipophilic at high temperatures, the surfactant dissolves mainly in oil phase and forms water-in-oil (w/o) type microemulsion in equilibrium with an excess water phase (2). At intermediate temperatures, the surfactant separates from both water and oil and forms a bicontinuous type microemulsion phase that is equilibrated with excess water and oil phases (3). In surfactant systems, temperature is a tuning parameter of this kind of phase transition and the temperature at which an o/w type microemulsion inverts to a w/o type microemulsion is called phase inversion temperature (PIT). On the other hand the formation of the microemulsion or aggregation of reverse micelle is depending on the surfactant concentration known as critical micelle concentration (cmc).

Figure 4: Schematic phase prism of a ternary mixture of water, oil and surfactant.
Figure 5: Isothermal sections of the phase prism.

The shape of the phase diagram obtained at equal amounts of water and oil ($\alpha = 0.5$) is that of a fish. The extent of the fish and the temperature at which it is located provides key information about the particular system of water, oil and surfactant (Schubert & Kaler, 1996). Figure 6 shows the fish shape phase diagram (see also in Figure 4, the vertical section at $\alpha$, $\alpha = 0.5$).

Figure 6: Section of the Gibbs prism at equal amounts of oil and water.
At low surfactant concentrations the phase sequence is \(2 \rightarrow 3 \rightarrow 2\) as a function of temperature. Increasing the amount of surfactant result in the formation of single phase microemulsion surrounded by two phase regions finally, at higher surfactant concentrations a single homogeneous microemulsion phase is observed. The surfactant concentration \(\gamma\) represents a measure of efficiency of the surfactant. That is the amount of surfactant required to completely solubilize equal amounts of water and oil. The temperature \((T)\) is the phase inversion temperature.

Another characteristic section of the Gibbs prism is a vertical plane at constant surfactant concentration (for a value of \(\gamma\) higher than) (Figure 7). This plane is perpendicular to the plane on which the fish are seen (Figure 3). A region of isotropic single phase solution is observed extending from water-rich to oil-rich side of the phase prism and this single phase region is surrounded by two two-phase regions. On the oil-rich side, the mixtures consist of stable dispersions of water droplets in oil (w/o microemulsion) and the reverse micelles are formed. On the water-rich side, the mixture consists of stable dispersion of oil droplets in water (o/w microemulsion) and the micelles are observed.
Chapter 1

Introduction

Marquez et al, (2002) has reported the partitioning of the surfactant oligomers in the oil and water phases of a Winsor III system was determined. The effect of different salts on the surfactant partitioning was discussed. Indranil et al, (2003) have reported isopropyl myristate based microemulsion systems alone could increase the solubility values of progesterone and indomethacin up to 3300-fold and 500-fold, respectively. Kyung-Mi Park and Chang, (1999) has prepared microemulsions of flurbiprofen with ethyl oleate and Tween 20 and concluded with his studies saying that, it can be used as a parenteral drug carrier, and can be applied to other poorly water-soluble drugs.

The transport rates of model drugs in emulsions increased with an increase in Brij 97 micellar concentrations up to 1.0% w/v and then decreased at higher surfactant concentrations (Chidambaram and Burgess, 2000). Kenji Aramaki et al, (1997) had reported the change in the HLB composition is well described by increasing the monomeric solubility and decreasing the mixing fraction of nonionic surfactant in the surfactant layer. As a result, temperature insensitive microemulsions are formed in the SDS–C12E03 system. Hironobu et al, (1999) had shown the surfactant molecules are tightly packed in the aggregates since the reduction in repulsion force takes place in the excess EO chain part of the hydrophilic surfactant longer than the short EO chain of the lipophilic one.

Warisnoicharoen et al, (2000) has reported that higher molecular volume oils were incorporated into the microemulsions prepared using the polyoxyethylene-based surfactants in a different manner. Stig et al, (1997) has reported relaxation time for the formation of vesicles varied from 0.09s to several seconds depending on the concentration of the vesicle forming surfactant, while the corresponding time for the formation of hydrotrope stabilized micelles from the vesicular solution was found to be 0.24 s irrespective of the concentrations involved.

Johan et al, (1996) has reported utilization of microemulsions as systems in chemical reactions. The effect of concentrations of co-surfactant and gelling agent on
emulsification process and in vitro drug diffusion was studied using $3^2$ factorial design (Patil et al, 2004). Hanan, (2003) has described particle size and polydispersity using photon correlation spectroscopy after dilution with excess of the continuous phase proved the efficiency of the microemulsion system as a drug carrier that ensures an infinitely dilutable, homogeneous, and thermodynamically stable system.

Benita and Neslihan, (2004) has reported efficiency of oral absorption of the drug compounds from the SEDDS depends on many formulations related parameters, such as surfactant concentration, oil/surfactant ratio, and polarity of the emulsion, droplet size and charge, all of which in essence determine the self-emulsification ability. Reza et al, (2000) suggested that a microemulsion was formed only when sufficient water was present to satisfy the hydration of both the phospholipid head groups and the hydroxyl groups of the co-surfactant associated with the droplet. At water concentrations lower than this minimum value, a co-solvent system was observed.

1.4 TOXICITY AND STABILITY

Non-ionic surfactants used in niosomes are non-toxic and no toxic reports have been reported so far in animal studies due to the use of niosomes as drug carrier. Rogerson et al, (1988) has reported in an experiment carried on 70 male NMRI mice, didn’t report any fatalities that could be attributed to the safe preparation. The toxic side effects directly related to the drug are also reduced. Niosomes are stable structures; Jain et al, (1997) has reported that, there is no change in gross morphologically on storage for three months. (Yoshioka & Florence, 1994) found them stable even in the emulsified form and determined the stability in buffer and suggested that a substantial amount of entrapped solute would be retained under long term storage conditions (Baillie et al, 1986).

Nonionic surfactant based delivery systems are systems in which nonionic surfactant agent would be added as integral component of the delivery system in which the core part would be active constituent. According to biopharmaceutical
classification system of drugs, class iv drugs are those which are poorly soluble and poorly permeable so, therefore this work has taken up to develop the platform for all those drugs which belong to this category, cyclosporine A is the model drug, in this work by developing this delivery system for this drug can be extrapolated for those all drugs that belong to this category according to BCS.

Other delivery systems bearing cyclosporine

Tarr & Yalkowsky, (1989) has reported the improved intestinal absorption of cyclosporine (in-situ) in rats using an olive oil emulsion prepared by either stirring or homogenization. Abdallah, (1991) have reported tablet formulation prepared by direct compression and compared with the commercial oil solution of cyclosporine placed into soft gelatin capsules.

Benmoussa, (1994) has reported effect of non-absorbable fat substitutes, sucrose polyester (SPE) and tricarballylate triester (TCTE)) on cyclosporine A (CsA) intestinal absorption. Ferrea, (1994) has reported oral microemulsion formulation substitutes for intravenous cyclosporine in child with graft-versus-host disease. Reymond, (1988) has reported influence of lipid vehicles on the intestinal absorption of Cyclosporine and, it was studied in-vivo and resulted with digested vehicles significantly promoted the absorption compared to non-digested vehicles.

Manconi et al, (1997) has reported tretinoin loaded niosomes prepared from polyoxyethylene lauryl ether, sorbitan esters and a commercial mixture of octyl/decyl polyglucosides, in the presence of cholesterol and dicetyl phosphate. Release data showed that tretinoin delivery is mainly affected by the vesicular structure and that tretinoin delivery increased from MLVs to LUVs to SUVs. Multilamellar acetazolamide loaded in niosomes which was formulated with Span 60 and cholesterol in a 7:4 molar ratio were found to be the most effective and showed prolonged decrease in Intraocular pressure (IOP) (Guinedi et al, 2005).

Ahn et al, (1995) has proposed the preparation of proliposomes and found that, they are free flowing particles which are composed of drugs, phospholipids and a water
soluble porous powder, and immediately form a liposomal dispersion upon hydration. The rat was used as a suitable model for pharmacokinetic and bioavailability studies of cyclosporine A (CsA). Two peroral formulations in the form of microemulsions were compared with a commercially available P.O. solution (to be diluted for administration) and a solution for intravenous administration (Ritschel, 1990). Nicholas et al, (1988) has reported the procedure for the preparation of a dry, free-flowing granular product which, on addition of water, disperses/dissolves to form an isotonic liposomal suspension, suitable for administration either intravenously or by other routes.

Baillie et al, (1986) has reported advantages of non-ionic surfactant vesicles, niosomes, and developed a delivery system for the anti-leishmanial drug, sodium stibogluconate. Hunter et al, (1988) has reported vesicular systems for delivery of sodium stibogluconate in experimental murine visceral leishmaniasis. Improved doxorubicin pharmacokinetics and tumoricidal activity has been reported by (Uchegbu et al, 1995; 1997). Fang et al, (2001) has developed proveisicular formulation that encapsulated (%) of estradiol in proniosomes with span surfactants showed a very high value of about 100%. Proniosomes with Span 40 and Span 60 increased the permeation of estradiol across skin.

Conacher et al, (2001) has reported that non-ionic surfactant vesicles have an ability to induce systemic immune responses in mice following oral immunization and effectiveness of this formulation was significantly increased by incorporating bile salts (in particular deoxycholate) into the formulation. Mann et al, (2006) has reported the bilosomes act as protein antigens administered via the oral route when they are exposed to a hostile environment in the gastrointestinal tract, consisting of digestive enzymes and a range of pH (1-7.5). Margaret et al, (2001) even reported that, the ability of non-ionic surfactant vesicles to induce systemic immune responses in mice. The effectiveness of this formulation was significantly increased by incorporating bile salts (deoxycholate) into the formulation.
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Jamie et al, (2004) has proposed a delivery system, which overcomes disadvantages of liposomes, that is a lipid vesicle containing bile salts (bilosome), which prevents antigen degradation and enhances mucosal penetration. Singh et al, (2004) has reported the bile salt stabilized vesicles; bilosomes appear to be a promising and potential carrier system for oral delivery of peptides and proteins. Jamie et al, (2006) has reported the comparative study in which mice were immunized orally with tetanus toxoid (40 or 200g dose/mouse, four doses in total) entrapped in non-ionic surfactant vesicles formulated with bile salts (bilosomes). The permeation of drug into the intestinal mucosal of GIT and the availability of drug into the portal system or the fraction of drug absorbed into the portal system, or intestinal mucosal, represents an upper limit to the amount of the drug that can reach the systemic circulation (Lipka and Amidon, 1999).

1.5 DRUG PROFILE

Cyclosporine A is a fat soluble cyclic undecapeptide antibiotic produced by the fungus Tolypocladium inflatum. Cyclosporine A is the only Cyclosporine analog to have been extensively used in the clinical practice. It is the prototype of a new generation of the immunosuppressive agent that selectively suppresses the activation of T lymphocytes, primarily by impairing the autocrine production of T lymphocytes growth factors.

Description USP, 2000, Merc Index, 12th Ed.

Solubility: Table 6

Table 6: Solubility of Cyclosporine A in different solvents (U.S Pharmacopoeia)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Very slightly soluble</td>
</tr>
<tr>
<td>Methanol</td>
<td>Soluble</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Soluble</td>
</tr>
<tr>
<td>Acetone</td>
<td>Soluble</td>
</tr>
<tr>
<td>Ether</td>
<td>Soluble</td>
</tr>
<tr>
<td>Saturated Hydrocarbon</td>
<td>Slightly Soluble</td>
</tr>
</tbody>
</table>

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Chemical name

Structure Fig 8

Figure 8: Chemical Structure of Cyclosporine A

Molecular formula \( \text{C}_{62}\text{H}_{111}\text{N}_{11}\text{O}_{12} \)

Molecular Weight 1202.64 Da

Percentage Purity Cyclosporine A contains not less than 98.5% and not more than 101.5% of Cyclosporine A \( (\text{C}_{62}\text{H}_{111}\text{N}_{11}\text{O}_{12}) \) calculated on the dried basis.

Chemical abstract registry number [599865-13-3]
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- **Category**: Immunosuppressant
- **Appearance**: Antipsoriatic and Antirheumatic
- **Melting Point**: It is white prismatic needle like odorless powder with bitter taste.
- **PKa**: 148-151° C
- **Octane/ Water partition coefficient**: Unionized
- **Packaging and Storage**: 120
- **Preserve in tight, Light resistant containers**

#### Mechanism of action

(Ref: Expert reviews in molecular medicine) (Figure 9)
Mechanism of action of cyclosporine

In the cytoplasm, cyclosporine (CsA) binds to its immunophilin, cyclophylin (CpN), forming a complex between cyclosporine and CpN. The cyclosporine CpN complex binds and blocks the function of the enzyme calcineurin (CaN), which has a serine/threonine phosphatase activity. As a result, CaN fails to dephosphorylate the cytoplasmic component of the nuclear factor of activated T-cells, (NF-ATc), and thereby the transport of NF-ATc to the nucleus and the binding of NF-ATc to the nuclear component of the nuclear factor of activated T cells (NF-ATn). The NF-ATc–NF-ATn complex binds to the promoter of the interleukin 2 (IL-2) gene and initiates IL-2 production. Consequently, T cells do not produce IL-2, which is necessary for full T-cell activation.

Pharmacokinetics (Dollary, 1997)

(CsA) Cyclosporine A is variable absorbed after oral administration (25-35%) of conventional soft gelatin capsule. The mean systemic bioavailability is 30% (range 20-40%). Peak Plasma concentration is seen 2-4 hr after oral administration. The plasma clearance of drug is biphasic, with mean elimination half life of 1-2 hr (α phase) and 27 hrs (β phase) reported. The absorption of Cyclosporine A is known to get enhanced by micro-emulsification of the drug in presence of water. Cyclosporine A is a lipid soluble. Animal and human data show high tissue to blood ratios ranging from 2-10hr after oral administration. Highest levels occur in kidney, liver endocrine glands and high levels occur in lymph nodes spleen and bone marrow and in breast milk.

Cyclosporine A is extensively metabolized in liver. There are 24 known metabolites. Among the metabolic reactions that occur involves hydroxylation of the terminal carbon of the C9 amino acid and the gamma position of the methyl leucine and N-demethylation of the methyl leucine at the position 4. In the bile metabolite M8, M26 and M17 predominate, rather than the parent drug. The most active metabolite is abundantly found in urine. Pre-existing hepatic dysfunction may reduce the rate of metabolism and excretion of Cyclosporine A requiring marked dosage reductions.
particularly in liver transplant patients. Administration of anabolic steroids, ketoconazole, and erythromycin may significantly reduce the hepatic clearance of Cyclosporine A in absence of biochemical abnormalities of hepatic function.

**Indications:** Solid organ transplantation, Bone marrow Transplantation, Severe Psoriasis, Atopic dermatitis and Severe, active rheumatoid arthritis.

**Contraindications:** Known hypersensitivity to cyclosporine A, hypersensitivity to Polyethoxylated castor oils (iv concentrate only)

- Abnormal renal function, uncontrolled hypertension, uncontrolled infections, Malignancy, Breast feeding mothers, Special precautions in case of children and older patients.

**Pharmacokinetics Parameters**

**Table 7: Pharmacokinetic parameters of CsA (Cyclosporine A)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral absorption</td>
<td>40%</td>
</tr>
<tr>
<td>Presystemic metabolism</td>
<td>30%</td>
</tr>
<tr>
<td>Plasma Half Life</td>
<td>27 hrs</td>
</tr>
<tr>
<td>Volume of distribution</td>
<td>1.5-7.0/kg (mean 3.5 l/kg)</td>
</tr>
<tr>
<td>Plasma protein binding</td>
<td>90-95%</td>
</tr>
<tr>
<td>Peak plasma concentration after Oral administration</td>
<td>0.60 ± 0.17 µg/ml</td>
</tr>
<tr>
<td>Time for attaining peak plasma concentration</td>
<td>2-4 hrs</td>
</tr>
</tbody>
</table>

**Adverse effects:** Impairs liver function, Nephrotoxicity, Anorexia, Lethargy, Hyperkalemia, Sustained rise in BP, Viral infections, Hirsutism, Gum hyperplasia and Tremor & Seizures
Drugs Interactions: (Dollary, 1997)
Potentially hazardous interactions: Ketoconazole, erythromycin, anabolic steroids, norethindone, bromocryptine, clarithromycin, diltiazem, fluconazole, itraconazole, methylprednisolone, metoclopramide, nicardipine, virapamil, phenytoin, Phenobarbital, refampin, carbamazipine, isoniazid, aminoglycosides, vancomycin, amphotericin B.

Other significant Interactions: Corticosteroids, Non steroidal Anti-inflammatory drugs (NSAIDS). The combined use of cyclosporine and corticosteroids has been associated with an increased incidence of fluid retention, hypertension convulsions in bone marrow transplant recipient. Likewise combined use of cyclosporine A and NSAIDS results in adverse effects on renal function.

Useful interactions: Ketoconazole. The interactions with ketoconazole have been used to elevate the systemic levels of Cyclosporine A and save on prescribing costs.

Dose, dosage form and leading brands

- Oral formulation
  Sandimmune (Sandoz, USA) and Sandimmun (Sandoz, UK) capsules contain 25 mg, 50 mg, and 100 mg.
  Neoral soft Gelatin capsules (Sandoz, USA, UK), contains 25 mg, 50 mg and 100 mg of cyclosporine A.

- Sandimmune (Sandoz, USA) Sandimmune (Sandoz, UK) oral solution contains Cyclosporine A 100 mg.

- Intravenous Form
  Sandimmune (Sandoz, USA) sandimmun (Sandoz, UK) concentrate for intravenous infusion containing Cyclosporine 50mg/ml in 1 ml (UK only) or 5ml ampoules and after dilution, is intended for infusions. Sandimmune/Sandimmun products should be stored at not more than 30°C protected from light; Neoral capsules should be stored below 25°C and Neoral oral solutions between 15°C and 25°C. The Oral solutions should be used within 2 months of opening the bottle and should not be refrigerated.
Other leading brands are Immunosol (Dabur), Immunospore (Cipla), and Panimum Bioral (Panacea Biotech Ltd).

1.6 ANALYTICAL METHODS OF CYCLOSPORINE

Assay procedures

USP23/NF19 (2000) describes the HPLC method for the assay of CsA is describes below.

Mobile phase- Prepare a mixture of water, acetonitrile, teri-butyl methyl ether, and phosphoric acid (520:430:50:1) make adjustment if necessary.

Diluent- Prepare a mixture of acetonitrile and water (1:1).

Standard preparation- 1- Dissolve an accurately weight quantity of USP Cyclosporine RS in diluent to obtain a solution having a known concentration of about 1.25 mg/ml.

Standard preparation-2- Transfer 2.0 ml of standard preparation 1 to 250 ml volumetric flask, dilute with diluent to volume and mix. This solution contains about 0.01 mg of USP Cyclosporine RS per ml.

Assay preparation: Dissolve about 25 mg of Cyclosporine accurately weight, in diluent, dilute with diluent to 20.0 ml and mix.

Resolution solution- Dissolve about 25 mg of cyclosporine in 10 ml of diluent added 5ml of solution of USP CsA RS in diluent containing 0.6 mg/ml, add 5ml of diluent, and mix.

Chromatographic System- The liquid chromatography is equipped with a 210 nm detector, a 0.25 mm X 1m stainless steel tube connected to a 4 mm x 25cm column that contain 3to 5 micrometer packing L1. The tube and column are maintained at 80°C. The flow rate is about 1.2/ml min.

Procedure- separately inject the equal volume ( about 20 ul ) of standard preparation 1, standard preparation 2, and the assay preparation in to the chromatograph record the chromatograph and measure the peak response , calculate the percentage of the Cyclosporine( C_{62}H_{111}N_{11}O_{12}) in the CsA taken by the formula:

\[(CP/10U)(r_u/r_s)\]

In which C is the concentration, in mg/ml, of USP Cyclosporine RS in standard preparation, P is the specific purity, ug/ml, of specimen in assay preparation, and ru
and r₅ are the main Cyclosporine peak responses obtained from the assay preparation and standard preparation 1, respectively.

Thermo spray HPLC-MS determination of Cyclosporine A in blood has been reported by employing ODS spherosorb (5um) column kept at 75°C with gradient elution (1.0ml/min) and detection done at 214 nm.

Abian et al, (1992) has reported the method for cyclosporine estimation using triple quadruple MS connected to the UV detection via a 0.5 um online HPLC filter, mobile phase comprised of 45% solvents B (0.05 M ammonium acetate solution in methanol-acetonitrile 1:1) and 55% solvent A (0.005 M ammonium acetate solution in methanol-acetonitrile 5:4). Nazir et al, (1997) has described determination of Cyclosporine A in formulation through HPLC method using relatively a new stationary phase of porous graphite carbon (PGC). Comparison of HPLC technique and monoclonal fluorescence polarization immunoassay for the determination of whole blood CsA in liver and heart transplant patient has been performed by (Taylor et al, 1994). Quantitative measurement of Cyclosporine A and its metabolites has been reported by time of flight mass spectrometry and matrix Assisted laser desorption ionization Mass spectrometry utilizing data analysis techniques. Its comparison was done with HPLC (Muddiman et al, 1994, 1995)

Automated simultaneous quantification of CsA in blood has been performed with electrospray-mass spectrometric detection using Nucleosil 100 C₁₈ column maintained at 35°C. Mobile phase used was aqueous 90% methanol (0.2 ml/min) (Vidal et al, 1998). (Lachno et al, 1990) describe improved HPLC method for analysis of Cyclosporine A has been performed on a column of CPS hypersil (5um at 53°C with hexane and ethanol 91:9 as the mobile phase (0.7 ml/min) and detection at 210nm.

Determination of Cyclosporine A in human whole blood by HPLC has been done on a spherosorb C₈ (7um) column at 65°C with acetonitrile/methanol/ waterpropane-2-ol (114:36:50:3) as the mobile phase (1:4 ml/min) and detection at 208 nm (Li et al,
1996). HPLC determination of Cyclosporine A in whole blood has been carried by (Zhuo, 1989) with ultra sphere CN (5μm) operated at 50°C with water/acetonitrile/methanol (10:7:3) as mobile phase (1.05ml/min) and detection done at 210nm. A novel fast HPLC method has been developed for determination of Cyclosporine A and its metabolites with separation carried out using microbore RP column under isocratic condition with acetonitrile/methanol/water (200:180:140:v/v/v) at 70°C and detector kept at 205 nm (Hana et al, 2000).

CsA and its metabolites have been determined with Nucleosil RP-2 column kept at 80°C component A of the mobile phase is 0.8 ml of ortho phosphoric acid in 1000ml of water, while component B of the mobile phase was prepared by adding 0.8 ml of orthophosphoric acid in 1000 ml of acetonitrile (Kumar and Mittal, 1999).

Christian et al, (2000) has reported LC/LC-MS method for estimation of Cyclosporine A in blood. Cyclosporine A has been analyzed by RP HPLC method using shim-pack C18 column under isocratic mode with acetonitrile /water 70:30 as the mobile phase (2ml/min) kept at 215nm at 70°C (Sharma et al, 2002).

Determination of Cyclosporine A and its metabolites has been reported using a molecularly imprinted polymer based radioligand binding assay (Senholdt et al, 1997). Determination of Cyclosporine A with aryl oxylate chemiluminescences has been reported by (Katayama et al, 1995). Cyclosporine A determination in whole blood has been reported by monoclonal florescence polarization immunoassay technique through Axsym and monoclonal Enzyme Multiplied Immunoassay Technique through cobs Farr (Murthy et al, 1998).

Non specific determination of Cyclosporine A can be done through Folin- Ciocalteu phenol reagent (lowery et al, 1951).
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Introduction

1.7 RESEARCH ENVISAGED AND RATIONALE FOR PROPOSED DELIVERY SYSTEM

Cyclosporine, a group of nonpolar cyclic oligopeptides with immunosuppressant activity widely used in organ transplantation. Its potent immunosuppressive properties, subsequently demonstrated by prior research, result from its interference with IL-2 gene transcription that occurs through the inhibition of calcineurin-dependent activation of NF-AT, a nuclear promoter of gene transcription for substances including IL-2. Although this confers high efficacy on CsA, early experience indicated that the agent also had less desirable characteristics. The CsA is known to be very poorly soluble in water and are also, known P-gp substrate, thus exhibit low therapeutic index with poor and variable bioavailability when administered orally. Furthermore, P-glycoprotein (P-gp), an ATP-dependent efflux transporter has been proposed as a factor in the poor absorption of CsA via various experiments, including single nucleotide polymorphism of P-gp. It is also the case that blood/blood-serum cyclosporine levels achieved using available dosage systems exhibit extreme variation between peak and trough levels. That is, for each patient, effective cyclosporine levels in the blood vary widely between administrations of individual dosages. This variation in patient response has been found to be attributable to a significant extent to variation in the availability of naturally occurring surfactant components, e.g. bile acids and salts, within the gastro-intestinal tract of the subject treated.

The daily dose of CsA is 15mg/kg body weight was given as initial dose in transplantation, and the absolute bioavailability of cyclosporine is reported to be 20-30% of this dose. Along with the low absolute bioavailability, the drug also shows variable inter subject bioavailability. This kind of response in human volunteers as been attributed low solubility and permeability, metabolism and to presence of P-gp (P-glycoprotein) which is an established efflux transporter, which is supposed to the absorption modulator of CsA, and is considered to be mainly responsible for variable bioavailability.

In order to enhance bioavailability and to reduce inter-subject variability of cyclosporine by improving solubility, permeability and reducing the efflux
transportation of cyclosporine from intestinal membrane, an attempt has been made to develop the surfactant based formulations for improved bioavailability of CsA. It is also anticipated that the application of nonionic surfactant in formulations would render CsA to get absorbed efficiently by overcoming various hindering factors as mentioned above since nonionic surfactants has ability to enhance intestinal permeation and to increase quantum of solubilized drug at absorption site. The preparations of CsA available in the market contains cremophor and other solvents which is reported to be potentially toxic and therefore it has been envisaged that having a formulation of CsA devoid of cremophor and solvents would be more desirable. It is also envisaged that the market formulations are not independent of bile flow and its absorption and bioavailability is affected by variability in bile flow within gastrointestinal tract. Nonionic surfactant based delivery systems are delivery systems, in which nonionic surfactants (spans and tweens) are used as an in-active ingredients, the basic reason for using nonionic surfactants in formulations is due to the fact that, they are safe, and inert in nature, and other in-active ingredients are selected based on physiological function of immune suppression carried out by CsA which is regulated by bile salt (sodium deoxycholate or sodium taurocholate) secretion and synthesis in the body.

It is anticipated to develop nonionic surfactant based formulations which include niosomes, bilosomes, proniosomes and microemulsions for oral administration, which mainly consist of nonionic surfactants, cholesterol, bile salt (sodium deoxycholate or sodium taurocholate) and charge inducer (stearylamine or dicetyl phosphate) for oral administration of CsA, which are free from oil, ethanol (organic solvents) triglycerides, and toxic excipients like Cremophor varieties. Nonionic surfactant based delivery systems can be used as viable technology for all those drugs or peptides which behave similarly to that of cyclosporine, and this technology can used be utilized for an improvement of bioavailability of cyclosporine after optimization of formulations.
1.8 OBJECTIVES

Cyclosporine A, a group of nonpolar cyclic oligopeptides with immunosuppressant activity widely used in organ transplantation, are known to be very poorly soluble in water and are also, known P-gp substrate, thus exhibit low therapeutic index with poor and variable bioavailability (20-30%) when administered orally. Because of poor and variable bioavailability daily dosages needed to achieve the desired blood levels need to be varied considerably in the existing dosage forms of cyclosporine and a concomitant monitoring of blood levels is essential. This adds an additional cost to the therapy. This variation in patient response has been found to be attributable to a significant extent to variation in the availability of naturally occurring surfactant components, e.g. bile acids and salts, within the gastro-intestinal tract of the subject treated, since cyclosporine is reported to have inhibitory effect on bile salt synthesis and moreover it reduces the efflux of bile from liver which is required for efficient absorption of cyclosporine. For the proposed niosomal / proniosomal formulations, the presence of such natural surfactants in sufficient quantity as an integral component along with other nonionic surfactants and at least one lipid may help to achieve satisfactory absorption of cyclosporine.

1. To design and develop nonionic surfactant based formulation of CsA (Cyclosporine) for oral delivery in such way that it releases the drug at controlled rate at absorption site, so that it will maintain the effective concentration of drug within therapeutic range.

2. To develop oral alternative formulation of cyclosporine made of nonionic surfactants which are free of cremophor EL or RH and alcohol. In addition to this, it is devoid of natural vegetable oil, which is prone to form flakes or jelly like structures on storage.

3. To develop an alternative, safe, nontoxic, stable delivery system of cyclosporine using an novel drug delivery approach (Vesicular and microemulsion System) which
could anticipate to enhance bioavailability and reduce the inter-subject variability (existing in the marketed formulation). Development of nonionic surfactant based delivery system of cyclosporine can probably extrapolated to other drugs which are of same category of cyclosporine (class iv drugs).

4. To develop nonionic surfactant based formulations containing bile salts, relevant experiments would be carried out to understand the role of bile salts involved in mechanism of absorption in relation to P-gp activity.

1.9 PLAN OF WORK
1. Selection of materials: Drugs, surfactants, lipids and vehicles
2. Preformulation studies: Identification of drugs, % purity, solubility analysis, Partition coefficient, Drug-excipient interactions and characterization of other additives
3. Analytical techniques: Method of analysis will be developed /selected for In-vitro and In-vivo studies. Calibration curves in different vehicles and biological fluids will be prepared.
4. Formulation Development:
   a. Selection of surfactants based on stability of formulation (Span 20, 40, 60, 80).
   b. Preparation of different types of surfactant based delivery system i.e vesicles or microemulsions using different methods
   c. Modification of system by
      *Incorporation of charge inducer like stearylamine or dicetyl phosphate.
      *Incorporation of bile salts and other absorption enhancing agents.

An attempt will be made to prepare free flowing dry granular proniosomes which is anticipated to eliminate the problem of aggregation, transportation and leakage. These proniosome may represent unit dosage form.
5. Optimization of process variables: The developed system bearing CsA (cyclosporine A) will be optimized as follows
   a. Optimization for surfactant concentration
   b. Optimization of hydration time
   c. Optimization of drug concentration
   d. Optimization of cholesterol and bile salts.
   e. Optimization of oils and surfactants using phase diagram (in microemulsion)

6. Characterization of developed system
   a. Shape and surface morphology (Phase contrast or Transmission electron microscopy),
   b. Average vesicle size and size distribution (using Malvern particle size analyzer), Drug entrapment studies
   c. Content uniformity
   d. In-vitro drug release studies.
   e. Dispersibility etc.

7. Stability studies:
   Stability study will be conducted to assess the shelf-life of product at 4°C and at room temperature under following headings
   (i) Effect of ageing on storage at 4°C and at room temperature.
   (ii) Stress testing e.g. Centrifugation etc.

   This will be assessed on the basis of
   a. Aggregation if any,
   b. Residual drug content.
   c. Change of vesicle size

8. In-vitro everted intestinal sac method for absorption study. Effect of formulation on P-gp functioning will also be carried out
   a. Using modified ussing apparatus
b. Intestinal rings (everted sac)

9. In-situ intestinal loop method will be used for assessing drug absorption and to measure absorption half-life and permeability rate constant.

10. Effect of formulation on proliferation of T lymphocytes: This will be carried out by inducing proliferation of T lymphocytes in spleenocytes of mice. The degree of proliferation will be carried out either by
   a. using MTT
   b. using Tritiated thymidine

11. Toxicity studies:
   Toxicity studies for surfactant delivery system will be conducted at two levels:
   (a) Histopathological studies of intestine will be conducted on formalin fixed tissues embedded in paraffin wax and sectioned at 5 micrometer are mounted on a glass slides and stained with haematoxylin eosin.
   (b) Assessment of lactate dehydrogenase leakage after incubation with the formulation.

12. In vivo studies
   (a) Suitable animal model will be selected. Animal studies will be conducted as per protocol, approved by (Institutional Animal Ethics Committee).
   (b) The bioavailability studies will be carried out by monitoring of plasma drug concentration.
   (c) Various pharmacokinetic parameters will be calculated.