CHAPTER 1

INTRODUCTION
Noncommunicable diseases (NCDs), such as heart disease, stroke, cancer, chronic respiratory diseases and diabetes, are the leading cause of mortality in the world. Of the estimated 57 million global deaths in 2008, 36 million (63%) were due to noncommunicable diseases. Population growth and increased longevity are leading to a rapid increase in the total number of middle-aged and older adults, with a corresponding increase in the number of deaths caused by NCDs. It is projected that the annual number of deaths due to cardiovascular disease will increase from 17 million in 2008 to 25 million in 2030, with annual cancer deaths increasing from 7.6 million to 13 million. More than two thirds of all cancer deaths occur in low- and middle-income countries, with lung, breast, colorectal, stomach and liver cancers causing the majority of such deaths (http://www.who.int/gho/publications).

**Fig. 1.1: Statistic of death from NCD worldwide**

1.1 Cancer: an overview

According to the World Cancer Report 2008 by WHO, the global cancer burden has doubled in the last thirty years of the twentieth century, and it is estimated that it will double again, between 2000 and 2020 and almost triple by 2030. Although cancer is rare in children between ages 1 and 14 (fatal disease is rare in children), leukaemia is the number one cause...
of death in that age group. About half of those diagnosed with cancer are cured and about half are killed by the cancer. Cancer, which was once considered to be a disease of the westernized, industrialized countries, has now become a common disease of low and medium resource countries (http://www.who.int/healthinfo/global_burden_disease).

In India, the International Agency for Research on Cancer estimated indirectly that about 6,35,000 people died from cancer in 2008, representing about 8% of all estimated global cancer deaths and about 6% of all deaths in India. The absolute number of cancer deaths in India is projected to increase because of population growth and increasing life expectancy. Rate of cancer deaths are expected to rise, particularly, from increases in the age-specific cancer risks of tobacco smoking, which increase the incidence of several types of cancer. The number of oral cancers was more than twice the number of lung cancers in individuals aged 30–69 years, indicating that the range of fatal cancers was caused by tobacco in India. Tobacco is the most important identified cause of cancer and is responsible for 30 to 50% of cancers in men and about 10-15% of cancers in women. In India, among the males, the tobacco related cancers are expected to be 42% of all sites cancers by the year 2020. For the same period, the number of cases for females will be 15% of all sites cancers. Over the years, in spite of decreasing trend in cervix cancer, gynaecological cancers have increased in India and are estimated to be around 182,602 by the year 2020 constituting about 30% of the total cancers among women in India. Among these, cancers of the uterine cervix followed by ovary and corpus uteri are the major contributors. In the year 2010, around 68,903 cases of cervix cancer are estimated to occur which may decrease to 53,654 cases by the year 2020. In women, breast cancer mortality was similar in rural and urban India. Breast cancer has emerged as the leading sites of cancer among women in India. Among males and females, it is the cancer of breast alone which is expected to cross the figure of 100,000 by the year 2020. Breast cancer is likely to be diagnosed at earlier stages in urban women than in rural women and is therefore more treatable (Thanki et al., 2013; Takiar et al., 2010).

The human body is composed of approximately 200 different cell types that are required to accomplish specific functions. Cells in blood, skin, bone, muscle, brain, etc. must perform distinctive tasks to support the organism as a whole, which supplies them with nutrient, oxygen and waste-removal service. Cancer is a disease where abnormal cells divide without control and during metastasis they are able to invade through the blood and lymph systems,
and other tissues. Thus, cancer can spread all over the body. Cancer cells multiply, form lumps called tumors. Tumors which do not spread to other tissues are called benign, may cease to grow and are usually harmless. Cancers are malignant when they spread to other tissues. In fact, the term cancer is typically restricted to refer only to malignant tumours (Hanahan & Weinberg, 2000). Methods of categorizing the development of cancer are called staging. The development of a cancer malignancy occurs in three stages:

- **Initiation** — mutation of a single cell
- **Promotion** — reproduction of the mutant into many cells (a tumor) with the same mutation
- **Progression** — additional mutations in the tumor resulting in malignancy

Anatomically, tumours are highly heterogeneous, showing elevated proliferation along with necrosis or haemorrhages in the core. The tumor-induced blood vessels are highly permeable enhancing macromolecular transport due to the presence of open gaps (inter-endothelial junctions & trans-endothelial channels), vesicular vascular organelles and fenestrations (Vaupel et al., 1989; Jain, 1987; Unezaki, 1996). In vivo fluorescence microscopic studies suggest that the cut-off and the size of these pores are around 400 nm. However, the transport of anticancer drugs in/across interstitium was opposed by physiological (i.e., interstitial pressure) properties of the tumor or physic-chemical properties of the drug molecule (e.g. size, configuration, charge, hydrophobicity) (Kinzler et al., 2002).

Moreover cancer is caused by abnormalities in the genetic material of the transformed cells (Boyland, 1952). These abnormalities are due to mutations in the DNA of a normal cell, changing them to transformed cells or “mutants”. Mutations are mainly caused by exposure to carcinogens; such as tobacco smoke, radiation, chemicals, or infectious agents (Stein et al., 2004). Other cancer-enhancing genetic abnormalities may occur randomly due to errors in DNA replication, or are inherited (Miller et al., 1981). The heritability of cancers is usually affected by complex interactions between carcinogens and the host's genome with cancer-enhancing genetic abnormalities present in cells from birth. Although much progress has been made in cataloguing the environmental causes and cellular and molecular biological basis for this dreaded disease, we still do not have a precise understanding of the differences between a cancer cell and its normal counterpart. Cancer development occurs when cells in a part of the
body begin an ‘out-of-control’ growth of abnormal cells, and instead of dying, they outlive normal cells and continue to form new abnormal cells.

Hanahan and Weinberg, 2011 have highlighted following six hallmarks of most cancer.

1. Evading apoptosis
2. Self-sufficiency in growth signals
3. Insensitivity to anti-growth signals
4. Sustained angiogenesis
5. Limitless replicative potential
6. Tissue invasion and metastasis

Cancer cells acquire autonomy from growth signals, evasion of growth inhibitor signals, evasion of apoptotic cell death, unlimited replication potential, angiogenesis, and invasion and metastasis, of which all are essential for carcinogenesis.

1.2 Role of Nanotechnology in Cancer Therapy

Nanotechnology is an interdisciplinary research field developed with an amalgamation of chemistry, engineering, biology, and medicine, and has various useful applications in cancer biology, such as early detection of tumors, discovery of cancer biomarkers, and development of novel treatments.

1.2.1 Conventional therapies for cancer

The traditional strategies for cancer treatment, includes surgery, radiation, and chemotherapy or combined strategies of these treatments. These are supplemented by some more specialized therapies such as immunotherapy or hormone therapy which can be applied only to some tumor types (Israel, 1978). The oldest form of cancer treatment is surgery. It renders the greatest chance of cure, mainly for solid tumors; particularly those which have not yet metastasized to other parts of the body. It is and will remain in future as one of the most important weapons against cancer. Radiotherapy is the second major weapon against cancer. Radiation therapy involves use of high-energy particle beams or waves (radiation), such as X-rays, gamma rays, neutrons or pi mesons for treating cancer (Kawasaki & Player, 2005). Chemotherapy includes the use of chemicals to treat cancer, particularly suitable for those cancers that have been spread out (metastasized) and cannot be treated any longer by localized methods such as surgery and radiation. One common characteristic of most cancer cells is their rapid rate of cell division. Anticancer drugs like taxol (interferes with the
depolarization of microtubules and hyper stabilizes their structure, doxo- (is thought to intercalates in DNA) or daunorubicin (intercalates, with its daunosamine residue directed toward the minor groove), all adversely affects the process of cell division. The main aim of these drugs is to destroy aggressive cancers. Nevertheless, chemotherapeutics have the same disadvantage like radio-therapeutics. They are also unspecific and therefore do not distinguish between normal and cancerous cell and hence damage normal or healthy cells as well, especially to rapidly dividing cells, e.g. bone marrow cells and cells of the gastrointestinal tract. The normal tissue toxicities routinely occur even when standard therapeutic doses of anticancer drugs are administered. The poor specificity of cytotoxic drugs in terms of both drug biodistribution as well as pharmacology at the cellular level poses a significant challenge to effective anticancer treatment (Kim, 2007; Byrne et al., 2008).

1.2.2 Nanomedicine for cancer

In general terms nanotechnology is the creation, manipulation, and application of structures in the nanometre size range. The term nanoscience is used to infer the study of the phenomena associated with objects somewhat arbitrarily defined as having dimensions between 1 to 100 nm. Nanomedicine refers to the use of nanostructures for the diagnosis and treatment of medical diseases. The specific route of administration (e.g., oral, intravascular, or intratumoral) is chosen according to which method will most safely and effectively deliver nanostructures to the target organ where they can exert their desired effects. Nanostructures have the potential to play a critical role in the future of medicine by serving as carriers for drugs, genes, and imaging agents that will bind to targets on injured or neoplastic tissue. The therapeutic index of nearly all drugs currently being used for cancer would be improved if they were more efficiently delivered to their biological targets through appropriate application of nanotechnologies (Kim, 2007; Byrne et al., 2008).

A tumor is often associated with a defective, leaky vascular architecture as a result of the poorly regulated nature of tumor angiogenesis. In addition, the interstitial fluid within a tumor is usually inadequately drained by a poorly formed lymphatic system. As a result submicron-sized particulate matter may preferentially extravasate into the tumor and be retained there. This is often referred as the “enhanced permeability and retention” (EPR) effect. This EPR effect can be taken advantage of by a properly designed nanoparticulate system such as SLN, nanoemulsion, liposomes etc to achieve passive tumor targeting. Effective targeted cellular
delivery will possibly become a nanomedicine’s ne plus ultra attribute. Many see nanomedicines as having significant potential in enhancing delivery of cancer therapeutics coupled with a proper understanding of the characteristics of tumor biology, tumor targeting has been propelled to the forefront of cancer research. A colloidal nanomedicine, constructed from a drug-loaded core with a peripheral targeting ligand is probably the design with the most potential to achieve these objectives. A target-specific nature of action will then offer tumor size reduction and elimination without damaging healthy tissue. However, the clinical worth of such nanomedicines is determined by an ability to disseminate in the body and reach target sites in therapeutically effective level (Park et al., 2008; Rabinow, 2004).

A number of obstacles may be overcome with various novel applications of nanodrug delivery. For example, many drugs are not very soluble, making it difficult to administer therapeutic doses. These compounds can be solubilized by formulating them into crystalline nanosuspensions that are stabilized by surfactants, or by combining them with organic or lipid nanoparticles, nanoemulsion that keep them in circulation for longer periods. If an efficacious compound has a short half-life in the circulation, its stability can be increased tremendously by encasing it within nanosized liposomes as a drug carrier. In the case of central nervous system cancers, many drugs have difficulty in crossing the blood–brain barrier to attack the tumor. Drug-loaded nanoparticles, nanoemulsion are able to penetrate this barrier, and have been shown to greatly increase therapeutic concentrations of anticancer drugs in brain tumors (Kim, 2007; Torchilin, 2005; Wissing et al., 2004; Koziiara et al., 2004; Steiniger, et al., 2004; Amiji, 2007).

Recently, progress in nanotechnology has allowed the development of several types of nanocarriers such as nanoemulsion, microemulsion, liposomes, solid lipid nanoparticles, polymeric nanoparticles, carbon nanotube, quantum dot, polymeric micelles etc capable of delivering drugs to target tissue and cells. Because each type of nanocarrier has a unique structure and physicochemical characteristics, it exhibits unique pharmacokinetic properties in the body. In order to develop a strategy for establishing a nanocarrier system, it is necessary to understand the pharmacokinetic properties (Johnston, et al., 2007).

1.2.2.1 Liposomes

Among different drug-delivery vehicles, liposomes have been explored for decades due to their biodegradability and potential to load large concentrations of drug. Liposomes are
composed of lipid layers and are either unilamellar, which encapsulate water-soluble drugs, or multilamellar, which are hosts for lipid-soluble drugs, and have the capacity to alter the biodistribution of drugs through delayed clearance and longer intravascular circulation times. Liposome encapsulation has been proven useful for reducing the toxicity of drugs and increasing the solubility of hydrophobic drugs. However, liposomes tend to be trapped by the RES after intravenous injection. Therefore, allowing escape from the RES is one of the key strategies for developing cell-specific carriers because liposomes escaping from the RES accumulate in tumor tissue by a passive mechanism. Drugs with widely varying lipophilicities can be encapsulated in liposomes, either in the phospholipid bilayer, in the entrapped aqueous volume, or at the bilayer interface. Drugs encapsulated within this lipid bilayer are, therefore, protected from extra-liposomal reactions that could alter the effectiveness of the drug. Generally, liposomes have advantages over polymer-based NPs for the formulation of cancer therapeutics. In most of the cases, the lipid membrane structure mimics the most common structure, which provides a remarkable permeability barrier. Moreover, liposome-encapsulated drugs are protected from extraliposomal reactions, which enhance the effectiveness of the said drug. Currently, liposome delivery systems are being utilized for anticancer drugs such as Doxil®, DaunoXome®, DepoCyt® and ONCO-TCS which are liposomal formulations of doxorubicin, daunorubicin, cytarabine, and vincristine, respectively (Johnston, et al., 2007; Fenske, 2008; Kumar et al., 2010; Saberi et al., 2013).

1.2.2.2 Nanoemulsion

An emulsion is generally described as a heterogenous system composed of two immiscible liquids: one dispersed uniformly as fine droplets throughout the other. The majority of conventional emulsions in pharmaceutical use have dispersed particles ranging in diameter from 0.1 to 100 µm. Nanoemulsions are isotropic, highly kinetically stable, transparent (or translucent) systems of oil, water, and surfactant, frequently in combination with a co-surfactant having a droplet size usually in the nanometer range (typically in the range of 20-200 nm). Although nanoemulsions are chiefly seen as vehicles for administering water-insoluble drugs, they have more recently received increasing attention as colloidal carriers for targeted delivery of various anticancer drugs due to their sub-micron size, which makes it possible to target the tumor area for improved efficacy and/or reduced toxicity. Nanoemulsions contain oil phases, surfactants or emulsifiers, active pharmaceutical
ingredients (drugs or diagnostic agents), and additives. The oil phases are mainly natural or synthetic lipids, fatty acids, oils such as medium or long chain triglycerides, or perfluorochemicals. The most widely used oils for parenteral applications are purified soybean, corn, castor, peanut, cotton seed, sesame, and safflower oils. Squalene has been reported to be the choice of oil for formulating stable nanoemulsions with smallest droplet size. Squalene, biocompatible oil, is a linear hydrocarbon precursor of cholesterol found in many tissues, notably the livers of sharks (Squalus) and other fishes. The oil phase of the emulsion systems can act as a solubilizer for the lipophilic compound. Therefore, solubility of lipophilic drugs can be significantly enhanced in a sub micron emulsion system, leading to smaller administration volumes compared to an aqueous solution. In addition, because lipophilic drugs are incorporated within the innermost oil phase, they are sequestered from direct contact with body fluids and tissues (Saberi et al., 2013, Johnston et al., 2007 Beg et al., 2013). If the drug is susceptible to hydrolysis or oxidation, it is protected by the non-aqueous environment. Further-more, incorporation of anti-cancer drugs in submicron emulsions (with droplet size of 50–200 nm) with long circulation properties are expected to enhance the tumor accumulation of the drug by passive targeting through the enhanced permeability and retention effect.

1.2.2.3 Self nanoemulsifying drug delivery

Self-nanoemulsifying drug delivery systems (SNEDDS) have recently gained wide acceptance due to robust formulation perspectives, practical enhancement of solubility and of oral bioavailability of drugs through lymphatic pathways. These are pre-concentrates containing isotropic mixture of oils, surfactants and co-surfactants. They facilitate the dissolution of various poorly water-soluble drug candidates by in situ solubilization in lipidic components. They spontaneously emulsify when exposed to aqueous media or gastrointestinal (GI) fluids to form a stable o/w emulsion with nanometric droplet size ranging between 20 and 200 nm (Saberi et al., 2013; Beg et al., 2013; Driscoll, 2002). They are able to enhance the bioavailability of poorly soluble and permeable drugs by avoiding the dissolution step and enhancing the permeability through biological membranes due to the presence of lipid and surfactant. Other important advantages include high stability, 100% drug entrapment efficiency, decreased dose and dosing frequency (due to improved bioavailability), potential to provide protection to drugs against
degradation in the hostile environment of the gut and ease of manufacturing and scale-up (Pouton, 1997; Mueller et al., 2000; Kaur et al., 2008).

### 1.2.2.4 Solid lipid nanoparticles

Solid lipid nanoparticles (SLN, also referred to as lipospheres or solid lipid nanospheres) are a relatively new class of drug carrier. They are particles of submicron size (50 to 1000 nm) made from lipids that remain in a solid state at room temperature and body temperature. SLN can be conveniently prepared using a wide variety of lipids including lipid acids, mono-, di-, or triglycerides, glyceride mixtures or waxes, and stabilized by the biocompatible surfactant(s) of choice (non-ionic or ionic). They are a comparatively stable colloidal carrier system in which melted lipid is dispersed in an aqueous surfactant by high-pressure homogenization or microemulsification. They are generally made up of a solid hydrophobic core containing the drug dissolved or dispersed. They are safely taken up by brain and exhibit the least toxicity due to the biodegradable nature of the carrier lipid. They have comparatively higher drug entrapment efficiency, render the drug more stable in their lipid matrix, and provide a controlled release lasting up to several weeks. Their production can be scaled up with excellent reproducibility. Surface coating of SLNs with hydrophilic polymers or surfactants, such as poly(ethylene glycol) (PEG), minimizes their uptake in liver cells and results in improved bioavailability. Stearic acid–PEG 2000 has been used for their stearic stabilization, whereas the use of complex lipids (mono-, di-, triglycerides of different chain lengths) results in an increased loading efficiency. Many cytotoxic compounds are reactive and relatively unstable. Incorporation of drug molecules in solid lipids may minimize their exposure to the aqueous environment and partly immobilize the drug molecules within the lipid matrix — both of which may protect the encapsulated drugs against degradation (Kaur et al., 2008; Wissing et al., 2004; Mishra et al., 2010; Soppimath et al., 2001).

### 1.2.2.5 Polymeric nanoparticles

Polymeric nanoparticles (PNPs) are prepared from a synthetic polymeric block to increase the circulation half-life and to reduce phagocytic uptake and inactivation of the therapeutic moiety and can be used to deliver and target therapeutic agents. They are formulated by incorporation of biodegradable polymers to maximize tissue compatibility and minimize cytotoxicity. Polymers approved by the U.S. Food and Drug Administration (FDA) for administration in human beings are polylactic acid (PLA), poly(glycolic acid) (PGA), PLGA,
poly-e-caprolactone, and poly(methyl methacrylate). For example, PLA and PLGA can easily be hydrolyzed into individual monomers (lactic acid or glycolic acid), which are removed from the body via normal metabolic pathways. Methods of preparation of PNPs fall into two major classes: one deals with the polymerization of monomers (e.g., emulsion and dispersion polymerization), whereas the other essentially involves dispersion of polymers (e.g., salting out, emulsification, diffusion, and nanoprecipitation). Drug release takes place through their simultaneous biodegradation, followed by desorption, diffusion, or erosion. A variety of therapeutic agents like anticancer drugs, protein and peptides, vaccine, and so forth, have been effectively delivered and targeted through PNPs (Moghimi, et al., 2005).

1.2.2.6 Polymeric Micelles

Polymeric micelles are nanosized assemblies of amphiphilic block copolymers that are suitable for the delivery of hydrophobic and amphiphilic agents. In an aqueous medium, micelles consist of a hydrophilic shell that minimizes clearance by the mononuclear phagocytic system (MPS) and a hydrophobic core that functions as a reservoir for hydrophobic drugs. The hydrophilic ends of the molecules are then in contact with the liquid environment surrounding the micelle structure and form a mantle. Micelles are useful for delivery of water insoluble drugs carried in the hydrophobic central core. The results from the clinical studies have indicated that the polymeric micelle formulations reduce the toxicity associated with conventional formulations of these drugs that, in turn, results in a higher therapeutic index. Many preclinical fundamental studies have evaluated the relationships between the composition of the copolymers and the physico-chemical properties of the micelles. The properties of the micelles such as polymer–drug compatibility, thermodynamic and kinetic stability, and the drug release profiles have been shown to influences the in vivo performance and therapeutic effectiveness of the micelle-formulated drugs. These studies serve as guidelines for the optimization of polymeric micelles for clinical applications (Johnston et al., 2007; Kataoka, et al., 1993; Siddiqui et al., 2012).

Cancer nanotechnology has been aggressively evaluated and implemented in cancer management and therapeutics, with suggestions that it might lead to major advances in diagnosis, detection, and treatment of the disease (Thanki, et al., 2013). Nanotechnology currently is being evaluated in cancer in two broad areas of nanovectors, ie, nanoparticles which can be loaded with drugs or imaging agents and then targeted to tumors, and high
throughput nanosensor devices for detecting the biological signatures of cancer. Nanotechnology-mediated delivery of bioactive food components is very effective because of the fact that nanoparticles rarely pose any toxicity to normal cells (Thanki, et al., 2013). A number of nanotechnology-based constructs are currently in clinical or preclinical development, and several of these are already approved by the Food and Drug Administration (FDA). Some of the nanotechnology-based drugs that are currently available in the market are listed in Table 1.1.

**Table 1.1: Marketed formulation based on Nanoparticulate system**

<table>
<thead>
<tr>
<th>Nanocarrier Approach</th>
<th>Drug/Active ingredients</th>
<th>Trade Name</th>
<th>Indication</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanoparticles</td>
<td>Adenosine deaminase</td>
<td>Adagen</td>
<td>Adenosine deaminase enzyme deficiency</td>
<td>Enzon Pharmaceutical Inc. NJ, USA</td>
</tr>
<tr>
<td></td>
<td>Onscaspar</td>
<td>L-asparaginase</td>
<td>Acute lymphoblastic leukaemia</td>
<td>Enzon Pharmaceutical Inc. NJ, USA</td>
</tr>
<tr>
<td></td>
<td>Copaxone</td>
<td>Glatiramer acetate</td>
<td>Relapsing remitting multiple sclerosis</td>
<td>Teva Pharmaceutical, Tikva, Israel</td>
</tr>
<tr>
<td></td>
<td>Pegasys</td>
<td>Pegylated interferon Alfa -2a</td>
<td>Hepatitis C</td>
<td>Nektar Therapeutics, CA, USA</td>
</tr>
<tr>
<td></td>
<td>Abraxane®</td>
<td>Paclitaxel</td>
<td>Breast Cancer</td>
<td>Abraxis Bioscience, USA</td>
</tr>
<tr>
<td></td>
<td>PEG-INTRON</td>
<td>Peginterferon alfa 2 b</td>
<td>Hepatitis C</td>
<td>Nektar Therapeutics, CA, USA</td>
</tr>
<tr>
<td></td>
<td>Somavert</td>
<td>Pegvisomant</td>
<td>Acromegaly</td>
<td>Nektar Therapeutics, CA, USA</td>
</tr>
<tr>
<td>Liposomes</td>
<td>Abelecet</td>
<td>Amphotericin B</td>
<td>Fungal infection</td>
<td>Enzon Pharmaceutical Inc. NJ, USA</td>
</tr>
<tr>
<td></td>
<td>Depocyt</td>
<td>Cytarabine</td>
<td>Lymphomatous meningitis</td>
<td>Enzon Pharmaceutical Inc. NJ, USA</td>
</tr>
<tr>
<td></td>
<td>Daunoxome</td>
<td>Daunorubicin</td>
<td>Kaposi’s sarcoma</td>
<td>Gilead Pharmaceuticals Inc. NJ, USA</td>
</tr>
</tbody>
</table>
Nanocarrier Approach | Drug/Active ingredients | Trade Name | Indication | Company |
--- | --- | --- | --- | --- |
**Liposomes** | Myocet | Doxorubicin | Advanced Breast cancer | Zeneus/Cephalon, Inc, Frazer, PA, USA |
| Epaxal | Inactivated influenza virus | Hepatitis A | Bema Biotech, Bern, Switzerland |
| Doxil | Doxorubicin | Ovarian cancer and Kaposi sarcoma | Ortho Biotech, NJ, USA |

**SNEDDS** | Caelyx | Doxorubicin | Ovarian cancer, Kaposi sarcoma and Breast Cancer | Schering Plough, Kenilworth, NJ, USA |
| Marqibo Kit | Vincristine sulfate | Acute lymphoblastic leukemia | Talon Therapeutics Inc.CA, USA |

**Nanoemulsion** | Neoral® | Cyclosporin | Immunosuppressant | Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA |
| Gengraf® | Cyclosporin | Immunosuppressant | Abbott Laboratories, North Chicago, USA |
| Aptivus® | Tipranavir | HIV infections | Boehringer Ingelheim Pharmaceuticals, Inc. Ridgefield, CT 06877 USA |
| Norvir® | Ritonavir | HIV infections | Abbott Laboratories, North Chicago, USA |

**Nanoemulsion** | Estrasorb® | 17-β-Estradiol hemihydrates | Postmenopausal woman for endometrial cancer | Novavax, Inc Columbia, MD, USA |
| Restasis® | Cyclosporine | Increase tear production | Allergan, Inc. Irvine, CA, USA |

Nanotechnology can be used as an inexpensive, tolerable, and readily applicable approach for cancer control and management. In addition, the advancement in nano chemoprevention might help us to achieve higher concentrations of phytochemicals which are unattainable when the agents are provided as part of a regular diet. If everything falls into place at the right
time with nanotechnology and its existing and forthcoming applications for cancer success in near future can be anticipated.

1.3. Lipid based drug delivery

Drug delivery using lipid-based formulations is one of the emerging strategies to design pharmaceutical dosage forms with improved therapeutic benefits. In recent times, many new chemical entities have been designed based on the structure of their target receptors using combinatorial chemistry and high-throughput screening, which has resulted in the discovery of very large molecules with greater degree of lipophilicity. For drugs to be well absorbed across the gastrointestinal tract (GIT) and provide good oral exposure, a number of potentially limiting factors must be overcome. These include appropriate stability and solubility in the GI fluids, reasonable intestinal permeability, and resistance to metabolism both within the enterocyte and the liver. The aqueous solubility of a drug is a critical determinant of its dissolution rate. The limited dissolution rate arising from low solubility frequently results in the low bioavailability of orally administered drugs, and compounds with aqueous solubility lower than 100 µg/mL generally present dissolution-limited absorption. Their poor aqueous solubility leads to poor solubilization in gastrointestinal fluid, low and variable bioavailability and poor in vitro/in vivo correlation. (Stegemann et al., 2007, Lipinski et al., 2000, Chakraborty et al., 2009)

Though high lipophilic drugs possess great therapeutic advantage, they pose a great challenge for the formulation scientists in their effective delivery. To overcome these issues, lipid-based formulations are now considered as a valuable alternative. These formulations have gained popularity because of the unique physicochemical properties of the lipidic excipients and their resemblance to the in vivo components, which help to enhance the in vivo solubility and thus bioavailability of hydrophobic drugs. Two critical quality attributes for development of lipid based drug delivery systems have been identified which include drug solubility within lipidic system and subsequent physiological processing of drug loaded lipidic system to achieve adequate drug absorption (Fig.1.2) The process of digestion and absorption that the lipids undergo in the gastrointestinal tract significantly enhances uptake of associated drugs into the lymphatic system, which helps to bypass the liver and drain them into the systemic circulation by means of the thoracic lymph duct (Lipinski et al., 2000; Odeberg et al., 2003).
Realisation that the oral bioavailability of poorly water-soluble, lipophilic drugs may be enhanced when co-administered with a meal rich in fat has led to increasing recent interest in the formulation of poorly water-soluble drugs (PWSD) in lipids as a means to enhance drug solubilisation in the gastrointestinal tract. Lipids such as fatty acids, triglycerides, vegetable oils and their derivatives, used for developing multiparticulate dosage forms, are available in solid, semi-solid or liquid state. The solid lipids with high melting point (above body temperature) can be directly converted into solid dosage forms, whereas the semi-solid or the liquid lipids may be converted into multiparticulate forms by the process of adsorption on suitable pharmaceutical additives. The process of conversion of liquid into solid dosage forms is advantageous in terms of handling, patient compliance, accurate dosing as well as for improving shelf life of the product. At present, lipids are being widely used for the preparation of different formulations by innovative adaptations and modifications of conventional equipment with relative ease and process simplicity, using methods such as melt-granulation, adsorption on solid support, spray-cooling, melt-extrusion/spheronization, and soon. These techniques facilitate dramatic improvement of the flow properties of the bulk lipids by transforming them into solid particles of definite dimension (powders, granules or pellets), which could subsequently be filled into capsules, sachets or even compressed into dispersible tablets (Odeberg et al., 2003; Shukla et al., 2011).

The Lipid Formulation Classification System (LFCS) was introduced as a working model in 2000 (Pouton, 2000) and an extra ‘type’ of formulation was added in 2006 (Pouton, 2006, Pouton, 2000). In recent years the LFCS has been discussed more widely within the pharmaceutical industry to seek a consensus which can be adopted as a framework for comparing the performance of lipid-based formulations. The main purpose of the LFCS is to enable in vivo studies to be interpreted more readily, and subsequently to facilitate the identification of the most appropriate formulations for specific drugs, i.e. with reference to their physicochemical properties. Table 1.2 indicates the fundamental differences between Type I, II, III and IV formulations. Whilst the defining properties of each Type are easy to understand, as yet there are few studies which link the LFCS with in vitro or in vivo performance.
Fig. 1.2 Key advantages associated with lipid based drug delivery systems (Modified from Thanki et al., 2013)

Many of the marketed products are Type III systems but this group is particularly diverse as a result of the wide variation in the proportions of oily and water-soluble materials used. This group has been further divided into Type IIIA and Type IIIB, to distinguish between formulations which contain a significant proportion of oils (Type IIIA) and those which are predominantly water-soluble (Type IIIB). The distinction between Types IIIA and IIIB was based arbitrarily, as a starting point for discussion, on the proportions of typical excipients in formulations. At present the sub-classification of Types III formulations is ill-defined, particularly when one considers that a Type III formulation could contain 3–5 excipients, including water insoluble and water-soluble surfactants, as well as water miscible cosolvents. In the immediate future it will be important to establish in vitro performance criteria which can be determined experimentally to distinguish between various Type III formulations, because this group is likely to contain formulations which have very different performance characteristics. The rate of crystallisation of drug from the prototype Type IIIB formulation is much more rapid than from the Type IIIA formulation, a property which is likely to affect the
physical from of the drug in the intestine and, as a consequence, affect the rate of absorption of the drug.

1.3.1 Excipients for lipid formulations

A wide range of triglycerides, partial glycerides, semi-synthetic oily esters, and semi-synthetic non-ionic surfactants esters are available from excipient suppliers. These excipients are selected basically on the basis of safety and ability to solubilise the drugs.

Table 1.2: The Lipid Formulation Classification System: characteristic features, advantages and disadvantages of the four essential types of ‘lipid’ formulations

<table>
<thead>
<tr>
<th>Formulation type</th>
<th>Materials</th>
<th>Characteristics</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Oils without surfactants (e.g. tri-, di- and monoglycerides)</td>
<td>Non-dispersing, requires digestion</td>
<td>GRAS status; simple; excellent capsule compatibility</td>
<td>Formulation has poor solvent capacity unless drug is highly lipophilic</td>
</tr>
<tr>
<td>Type II</td>
<td>Oils and water-insoluble surfactants</td>
<td>SEDDS formed without water-soluble components</td>
<td>Unlikely to lose solvent capacity on dispersion</td>
<td>Turbid o/w dispersion (particle size 0.25–2 μm)</td>
</tr>
<tr>
<td>Type III</td>
<td>Oils, surfactants, cosolvents (both water-insoluble and water-soluble excipients)</td>
<td>SEDDS/SMEDDS formed with water-soluble components</td>
<td>Clear or almost clear dispersion; drug absorption without digestion</td>
<td>Possible loss of solvent capacity on dispersion; less easily digested</td>
</tr>
<tr>
<td>Type IV</td>
<td>Water-soluble surfactants and cosolvents (no oils)</td>
<td>Formulation disperses typically to form a micellar solution</td>
<td>Formulation has good solvent capacity for many drugs</td>
<td>Likely loss of solvent capacity on dispersion; may not be digestible</td>
</tr>
</tbody>
</table>

Toxicity is an independent issue, and is important with regard to the choice of surfactants. Water-insoluble surfactants penetrate and fluidize biological membranes and water-soluble surfactants have the potential to solubilise membrane components. All surfactants are potentially irritant or poorly tolerated as a result of these non-specific effects. In general terms cationic surfactants are more toxic than anionic surfactants which in turn are more toxic.
than non-ionic surfactants. Lipid-based delivery systems usually only include non-ionic surfactants so it is pertinent to compare the toxicity of non-ionic surfactants. In general bulky surfactants such as polysorbates or polyethoxylated vegetable oils are less toxic than single-chain surfactants, and esters are less toxic than ethers (which are non-digestible). Non-ionic surfactants are generally considered to be acceptable for oral ingestion and the emergence of several successful marketed products has given the industry confidence in lipid-based products. The oral and intravenous LD50 values for most non-ionic surfactants are in excess of 50 g/Kg and 5 g/Kg respectively, so 1 g surfactant in a formulation is well-tolerated for uses in acute oral drug administration. More careful consideration needs to be given to formulation of a product which is intended for chronic use, and it is noteworthy that marketed lipid products for chronic use generally do not include surfactants. Although most non-ionic surfactants have similar LD50 values, in practice formulators are predictably cautious when choosing surfactants and usually turn to one of a few tried and tested materials which have been used in marketed products (Strickley, 2007; 1983; Pouton and Porter, 2008).

1.3.1.1 Triglycerides

Naturally occurring oils and fats are comprised of mixtures of various triglycerides (TG) which are more correctly (but rarely) referred to as triacylglycerols, since chemically they are fatty acid tri-esters of glycerol. Triglyceride vegetable oils have many advantages as the foundation of lipid-based delivery systems. They are commonly ingested in food, fully digested and absorbed, and therefore do not present any safety issues. Naturally occurring triglycerides contain fatty acids of varying chain lengths and degrees of unsaturation. Based on the hydrocarbon chain length of their component fatty acids, triglycerides can be classified as short (less than five carbons), medium (6 to 12 carbons), or long chain (more than 12 carbons). Vegetable oils are glyceride esters of mixed unsaturated long-chain fatty acids, commonly known as long-chain triglycerides (LCT). Oils from different vegetable sources have different proportions of each fatty acid. The fatty acid compositions of coconut and palm kernel oils are important in that they are unusually rich in saturated medium-chain oils (C8, C10 and particularly C12). Coconut oil is distilled to produce the generic product ‘medium-chain triglycerides’ (MCT) (also known as glyceryl tricaprylate/caprate) which is available from several suppliers and commonly comprises glyceryl esters with predominantly saturated C8 (50–80%) and C10 (20–45%) fatty acids. Triglycerides are highly lipophilic and
their solvent capacity for drugs is commonly a function of the effective concentration of the ester groups, thus on a weight basis MCT generally has higher solvent capacity than LCT. In addition MCT is not subject to oxidation, so MCT is a popular choice for use in lipid-based products. Castor oil is noteworthy as the only common source of glyceryl ricinoleate, which uniquely has a hydroxyl group coupled to the alkyl chain (Cao et al., 2004; Anderson., 1999; Jannin et al., 2008; Gibson., 2007).

### 1.3.1.2 Water-insoluble surfactants

Non-ionic esters which are not polyethoxylated or polyglycerylated can be considered to be polar oils. In the context of oral lipid-based formulations, group of excipients of intermediate HLB (8–12), which adsorb strongly at oil–water interfaces, as ‘water-insoluble surfactants’. These materials are insufficiently hydrophilic to dissolve in water and form micelles but nevertheless are sufficiently hydrophilic to be capable of driving self-emulsification. The constituents of water-insoluble surfactants will have a finite solubility in water depending on their degree of ethoxylation, but solubility is generally very low. These surfactants are sometimes described as ‘dispersible’ in water, meaning that they can form an emulsion if subject to shear. These materials typically are predominantly oleate esters, such as polyoxyethylene (20) sorbitan trioleate (polysorbate 85—‘Tween 85’) or polyoxyethylene (25) glyceryl trioleate (‘Tagat TO’). These two examples have HLB values between 11 and 11.5 and are particularly useful for formulation of Type II systems. Polysorbate 80 can be blended with sorbitan monooleate (i.e. classical Tween 80/Span80 mixtures) to give an average HLB of 11 but such a blend will contain a mixture of water-soluble and water-insoluble molecules, and will not behave in the same way as Tween 85 which consists predominantly of water-insoluble molecules. Tween 85 is polymeric so it will contain a finite fraction of water-soluble components, but this fraction will not dominate the fate of the formulation components after dispersion or digestion (Pouton, 1985; Pouton, 1997; Pouton, 2000).

### 1.3.1.3 Water-soluble surfactants

The most commonly used surfactants for formulation of nanoemulsion, microemulsion, SEDDS or SMEDDS are water-soluble, though by definition these materials can only be used in Type III or Type IV formulations. Above their critical micelle concentration these materials dissolve in pure water at low concentrations to form micellar solutions. This implies
an HLB value of approximately 12 or greater. The fatty acid components can be either unsaturated or saturated. The popular castor oil derivative Cremophor RH 40, is a typical example of a product with saturated alkyl chains resulting from hydrogenation of materials derived from a vegetable oil. Its close relative Cremophor EL, which has also been used widely, has a slightly lower degree of ethoxylation but is not hydrogenated and is therefore unsaturated. Relatively few of the available water-soluble ester surfactants have been used in pharmaceutical products. This is a function of their proven safety profile rather than particular advantages they offer in physicochemical performance (Pouton, 1997).

1.3.1.4 Co-solvents/Co-surfactant

Several marketed lipid-based products contain water soluble co-solvents. The most popular materials have been PEG 400, propylene glycol, ethanol and glycerol, though other approved co-solvents have been used in experimental studies. There are at least three reasons why co-solvents have been included in lipid-based formulations. Ethanol was used in early cyclosporine products at a low concentration to aid dissolution of the drug during manufacture. More commonly it has been assumed that co-solvents could be included to increase the solvent capacity of the formulation for drugs which dissolve freely in co-solvents. However to enhance the solvent capacity significantly the co-solvent must be present at high concentration and this is associated with the risk of drug precipitation when the formulation is dispersed in water. Co-solvents lose their solvent capacity quickly following dilution. For many drugs the relationship between co-solvent concentration and solubility is near to logarithmic. A third reason for inclusion of co-solvents is to aid dispersion of systems which contain a high proportion of water-soluble surfactants. There are practical limits on the concentrations of co-solvents which can be used, governed by issues of immiscibility with oil components and also possible incompatibilities of low molecular weight co-solvents with capsule shells (Cole et al., 2008; Strickley., 2007).

1.3.1.5 Hydrophilic–Lipophilic Balance

The “hydrophilic–lipophilic balance” (HLB) is a measure of the relative hydrophilicity and lipophilicity of amphiphilic molecules (e.g., surfactants and emulsifiers), which possess both a hydrophilic and a lipophilic region. HLB values can be calculated from the relative size and strength of hydrophilic and lipophilic portions of the molecule or determined experimentally by various methods. Relative hydrophilicity increases with increasing HLB value; excipients
with HLB values < 9 are considered to be hydrophobic, whereas those with values >11 are considered hydrophilic. Preparation of an oil-in-water emulsion of a particular lipophilic excipient requires that the HLB value of the surfactant be matched to the requirements of the excipient. Table 3 shows the required surfactant HLB values for several lipid excipients commonly used to prepare oil-in-water emulsion formulations of poorly water-soluble drugs. The required surfactant HLB value for emulsifying a particular excipient can be determined empirically by preparing several emulsions of the excipient over a range of surfactant HLB values produced by mixtures of various proportions of a hydrophobic and a hydrophilic surfactant of the same chemical type [e.g., hydrophobic SPAN 80 (HLB = 4.3) and hydrophilic TWEEN 80 (HLB = 15), both of which are oleates]. The required HLB is that producing the best quality emulsion (e.g., resistance to phase separation and high degree of dispersion). The relative proportions of the surfactants required to prepare a two-component mixture of a specific HLB may be calculated from the following relationships:

\[
\text{Percentage of surfactant } A = \frac{100(\text{Desired HLB} - \text{HLB of surfactant B})}{\text{HLB of surfactant A} - \text{HLB of surfactant B}}
\]

\[
\text{Percentage of surfactant } B = 100 - \text{Percentage of surfactant } A
\]

The chosen surfactant pair must include one surfactant with an HLB higher, and the other with an HLB lower, than the desired HLB of the surfactant blend. The chemical type of the surfactants used to make an emulsion also affects the quality of the emulsion. After the required HLB for emulsification of the excipient is determined, emulsions of the excipient are prepared with several binary mixtures of hydrophobic and hydrophilic surfactants mixed in the ratio which produces the required HLB. Each surfactant of a given pair is of the same chemical type, but each pair of surfactants should be of different chemical types. For example, if the required HLB for emulsification of an excipient is 12, emulsions containing mixtures of the surfactants, SPAN 20 and TWEEN 20 (laurates), SPAN 40 and TWEEN 40 (palmitates), SPAN 60 and TWEEN 60 (stearates), or SPAN 80 and TWEEN 80 (oleates), in proportions yielding an HLB of 12, can be prepared and the best emulsion selected as the final formulation. Screening for the ideal surfactant pair is empiric, with the ultimate selection being determined by the quality of the resulting emulsion (Shinoda et al., 1983; Gibson., 2007).
### Table 1.3: Required surfactant Hydrophilic–Lipophilic Balance for oil-in-water emulsification of various lipids (Gibson, 2007)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Materials</th>
<th>Required hydrophilic-lipophilic balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Caprylic/capric triglycerides (medium chain triglycerides)</td>
<td>5</td>
</tr>
<tr>
<td>2.</td>
<td>Castor oil</td>
<td>14</td>
</tr>
<tr>
<td>3.</td>
<td>Cholesterol</td>
<td>10–11</td>
</tr>
<tr>
<td>4.</td>
<td>Corn oil</td>
<td>8</td>
</tr>
<tr>
<td>5.</td>
<td>Coconut oil</td>
<td>5</td>
</tr>
<tr>
<td>6.</td>
<td>Cottonseed oil</td>
<td>6</td>
</tr>
<tr>
<td>7.</td>
<td>Ethyl oleate</td>
<td>11</td>
</tr>
<tr>
<td>8.</td>
<td>Hydrogenated castor oil</td>
<td>8</td>
</tr>
<tr>
<td>9.</td>
<td>Isopropyl myristate</td>
<td>12</td>
</tr>
<tr>
<td>10.</td>
<td>Isostearic acid</td>
<td>15–16</td>
</tr>
<tr>
<td>11.</td>
<td>Lauric acid</td>
<td>16</td>
</tr>
<tr>
<td>12.</td>
<td>Linoleic acid</td>
<td>16</td>
</tr>
<tr>
<td>13.</td>
<td>Palm oil</td>
<td>7</td>
</tr>
<tr>
<td>14.</td>
<td>Oleic acid</td>
<td>17</td>
</tr>
<tr>
<td>15.</td>
<td>Olive oil</td>
<td>7–8</td>
</tr>
<tr>
<td>16.</td>
<td>Ricinoleic acid</td>
<td>16</td>
</tr>
<tr>
<td>17.</td>
<td>Safflower oil</td>
<td>7</td>
</tr>
<tr>
<td>18.</td>
<td>Sesame oil</td>
<td>7–8</td>
</tr>
<tr>
<td>19.</td>
<td>Soybean oil</td>
<td>6</td>
</tr>
</tbody>
</table>

#### 1.3.2 Considerations in design of isotropic solutions

When selecting among the various isotropic solution options available for a poorly soluble drug (e.g., lipid solutions, Microemulsion, Nanoemulsion, SEDDS, and SMEDDS), careful considerations of the drug physicochemical properties and the choice of lipid excipients is
essential. Drug physicochemical parameters that influence the design of isotropic solutions include solubility, HLB, partition coefficient, dielectric constant, and molecular weight (MW). Critical excipient properties to consider include surface tension and degree of lipid fatty acid saturation. Ternary phase diagrams are very useful in determining the optimal ratios of drug, lipid, surfactant, and co-surfactant to use when developing a microemulsion, nanoemulsion SEDDS or SMEDDS formulation.

1.3.2.1 Physical–Chemical Considerations

1.3.2.1.1 Solubility

Solubility of the drug in the formulation is one of the most critical parameters controlling the absorption-enhancing performance of a lipid-based formulation. Insufficient solubility of the drug in the excipient matrix requires administration of multiple dosage units for a single dose and is generally viewed as unacceptable. This situation is most frequently encountered in situations dealing with moderate potent compounds requiring doses in the range of 100 to 200 mg. During initial excipient screening activities, drug solubility is routinely determined in various oils, surfactants, and co-surfactants.

A typical screening protocol involves combining an excess of the drug (approximately 500 mg) with 1 to 2 mL of the excipient in a screw-capped glass vial followed by heating to 60°C in a water-bath, brief agitation with a vortex mixer, holding for 48 hours at 25°C to 30°C, and centrifugation at 2000 to 3000 g to separate the undissolved drug. The supernatant containing the solubilized drug should be a clear, monophasic liquid at ambient room temperature, and should be of sufficiently low viscosity to allow for facile dispersion upon dilution in aqueous media. There are some factors that could be useful in predicting drug solubility in a particular excipient (Shah et al., 2007).

1.3.2.1.2 Solubility parameters

The solubility parameter of a substance is defined as the square root of its cohesive energy density, expressed as the energy of vaporization.

When the solubility parameters of two materials are similar, one would expect them to be miscible, and thus provide some guidance in selecting an appropriate lipid vehicle for maximum solubilization of a drug. The Hildebrand solubility parameter (HSP) is useful for estimating the solubility of hydrophobic drugs in lipid excipients. The HSP is derived from
several fundamental molecular properties, including boiling point (expressed in °K), MW, and specific gravity (SG).

\[ \text{HSP (d)} = \sqrt{(23.7 \times B.P. + 0.02 \times B.P.^2 - 2950) - 1.98 \times T} \]

MW/SG

As the difference between the HSP values for two substances increases, their miscibility decreases (Hancock et al., 1997; Shah et al., 2007).

1.3.2.1.3 Partition Coefficient

The lipophilicity of a molecule can be quantified by its Log P value, which describes (as the common logarithm) its degree of partitioning between an aqueous and a lipophilic phase (usually water and n-1-octanol, respectively). The partition coefficient is the concentration of the drug in the organic layer divided by that in the aqueous one. In many instances, the partition coefficients of a drug and its melting point have been shown to be key factors in determining solubility in lipids. While drugs with Log P 4 tend to possess greater solubility in lipid vehicles than those of lower lipophilicity, there are several exceptions to this rule (Bachynsky et al., 1997).

1.3.2.1.4 Dielectric Constant (\(\varepsilon\))

The dielectric constant that increases in proportion to the relative polarity of a molecule, is determined by oscillometry and is defined as the ratio of the capacity of a condenser (made with the test substance as the dielectric material) to the capacity of the same condenser with air as the dielectric, as determined at a frequency of 1MHz (Shah et al., 2007). Given the general assumption that “like dissolves in like,” substances with similar dielectric constants are typically miscible with one another.

1.3.2.2 Formulation Considerations

1.3.2.2.1 In Vivo Performance of Lipids

An understanding of the GI processing of lipid excipients is critical in that this phenomenon can exert a considerable influence on drug absorption from lipid-based formulations. This processing involves lipid digestion, in which lipids are degraded into component fatty acids and monoglycerides followed by emulsification by endogenous bile acids, leading to the creation of complex micellar species, which are intimately related to drug absorption. In general, lipids that are nondigestible, should be avoided due to the poor drug absorption
typically associated with these excipients. In addition, the fatty acid chain length of glyceride excipients controls the rate of lipolysis and consequently, drug absorption. Glyceride excipients comprised of long chain fatty acids are lipolysed relatively slowly, whereas lipolysis of medium chain glycerides occurs more readily, which can be associated with more rapid and complete drug absorption. In addition, the surfactant component of a formulation can adversely affect the digestion process by sterically hindering the attachment of lipase enzymes at the oil–water interface, resulting in sub-optimal drug release (MacGregor et al., 1997; Hutchison, 1994).

1.3.2.2.2 Drug Loading

Drug loading can influence both the physical characteristics and long-term physical stability of a lipid-based formulation. Thus, it is important to determine the saturation solubility of the drug in the chosen formulation. Excessive drug loading in a lipid solution can result in gelling or drug crystallization under shear conditions or during storage, as well as drastic changes in the formulation dispersibility, which is associated with an increase in the mean emulsion droplet diameter (MEDD) due to droplet coalescence and or aggregation (Shah et al., 2007).

1.3.2.2.3 Optimal Drug: Surfactant Ratio (Phase Diagram Construction)

The stability, maximum drug loading, and self-emulsifying behaviour of a binary nonionic surfactant–oil mixture is dependent on both temperature and surfactant. Pseudo-ternary phase diagrams are useful for identifying the optimum concentrations, or concentration ranges, of oil, surfactant and cosurfactant necessary to form an efficient self-emulsifying formulation. The self-emulsifying properties of any nanoemulsion or SNEDDS formulation will be influenced by the physicochemical properties of the incorporated drug (e.g., polarity and surface activity) and its concentration. Hence, in the search for a formulation with optimal self-emulsifying properties (as determined by the size of the dispersed lipid droplets), the phase diagram should be constructed by varying not only the concentrations of the excipients but also that of the drug. In addition to oil droplet size, an acceptable SEDDS will exhibit the following characteristics:

- Facile formation of a fine emulsion with a lipid droplet size of less than 200 nm upon dilution with aqueous media following mild agitation.
• Dispersed oil droplets possess sufficient polarity to promote rapid transfer of drug into the aqueous phase.

Phase diagrams can be constructed with relatively small quantities of drug and excipients. The test formulations, containing varying concentrations of the drug and excipients are sequentially diluted with water and allowed to equilibrate. The resulting dispersions are next examined with optical microscopy under crossed polar filters to determine lipid droplet size, check for drug precipitation, verify the emulsion isotropic behaviour and detect liquid crystalline behaviour, which will be indicated by birefringence. The information so gathered is used to construct the phase diagram, which allows determination of the optimal ratios of drug, lipid, surfactant, and cosurfactant to use when developing a nanoemulsion or SNEDDS formulation (Shah et al., 1994; Charman et al., 1992).

1.3.3 Role of exogenous lipids in bioavailability enhancement

High solubility and permeability are considered prerequisites for oral absorption, and many drugs have been identified to exhibit poor and variable bioavailability due to high dose to solubility ratio. The bioavailability of such drugs is frequently increased by co-administration of food (Charman et al., 1997; Winstanley et al., 1989; Amidon et al., 1995; Horter et al., 2001). Crounse 1961 was the first to report the food-dependent bioavailability of drugs, wherein the absorption of a water-insoluble drug, griseofulvin, was significantly enhanced on administration with a high-fat meal. Among all the food constituents, lipid component of food is of particular importance in stimulating the physiological responses for the absorption of lipophilic or poorly water-soluble drugs (Feinle et al., 2001; Hunt et al., 1968; Ladas et al., 1984). Intake of a high-fat meal results in stimulation of biliary and pancreatic secretions, prolongation of GI tract residence time, stimulation of lymphatic transport, changes in mesenteric and liver blood flow, increased intestinal wall permeability and reduced metabolism and efflux activity which significantly contribute in improving bioavailability (Wagnera et al., 2001). Studies on healthy human subjects have shown that in addition to already well-characterized parameters (such as pH and bile salt levels), some other parameters like buffer capacity, surface tension, osmolality and food components, which significantly change pre/postprandially, can also affect the intraluminal performance of dosage forms. Ingestion of meals containing 10–25 g of lipid has been demonstrated to promote emptying of the gallbladder and its maximal contraction (Kalantzi et al., 2006; Stone
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et al., 1992). The presence of quantitatively most important lipid component in the human diet – triglycerides (TGs), which may amount to 100 g per day or more in the small intestine and long-chain (rather than medium-chain fatty acids) fatty acids (FAs) appear to be most effective in driving food-related inhibition of motility which would help in improving GI residence time (Mu et al., 2005; Raybould et al., 1998). For particularly lipophilic drugs or large molecular weight macromolecules, lymphatic uptake can be increased in the presence of a high-fat meal. Gershkovich and Hoffman, 2007, suggested that changes in drug disposition for certain lipophilic compounds may occur when the drug interacts with TG-rich lipoproteins (TRL), the concentration of which elevate as a result of consumption of high-fat meals. It was perhaps the result of these interesting outcomes which infused the idea of lipid-based oral drug delivery system for bioavailability enhancement, among modern researchers.

Several cases of food effects on bioavailability of drugs have been reported in the literature in correlation to class of the drug as per biopharmaceutics classification system (BCS). It has been observed that the bioavailability of Class 1 drugs is not affected, while that of Class 2 and 3 drugs increases and decreases, respectively, in the presence of food. The probable reason for such observations can be explained on the basis of solubility, permeability and inhibition of efflux transporters in the presence of food (Fleisher et al., 1999; Benet et al., 2004). Class 1 drugs being of high solubility and permeability can easily cross the membrane by passive diffusion and also are capable of saturating any cellular transporter, both efflux and absorptive. As the absorption process is dominated by passive diffusion, transporter drug interaction is minimal and therefore, no significant effect on the extent of bioavailability is observed for Class 1 compounds in the presence of a high-fat meal. Similarly, for Class 2 drugs, absorption is primarily through passive diffusion due to their lipophilicity and high permeability. However, the low solubility of these compounds prevents saturation of the efflux transporters. Consequently, a dual effect of inhibition of efflux transporters and increase in the drug’s solubility by micellar solubilization in the presence of food increases the extent of oral bioavailability and the rate of absorption of these drugs. Class 3 compounds, though are sufficiently available in the gut lumen due to good solubility, they are poorly metabolized and poorly permeable and therefore are majorly dependent on the cellular uptake transporters for penetration into the enterocyte. With high-fat meals, these drugs could show lower bioavailability due to inhibition of uptake transporters in the intestine (Fleisher et al., 1999; Custodio et al., 2008). Though it is difficult to predict the fate of Class 4 drugs, they
may behave as Class 3 drugs due to increase in their solubility in the presence of high-fat meal. The association of postprandial TG-rich lipoprotein with lipophilic drugs within the enterocyte has been found to be prone to both intestinal lymphatic transport and post-absorptive changes in disposition following a high-fat meal. This association of drug results in decrease in the volume of distribution and clearance and thus possibly changes the kinetics of the pharmacological action of the lipophilic drug (Gershkovich et al., 2007). Therefore, several challenges exist in the development of compounds that exhibit food effects. If a high-fat meal is required to obtain efficacious drug levels, there is serious concern of sub-therapeutic plasma drug concentration in patients taking the drug without food. The situation may worsen with compounds of narrow therapeutic index as changes in bioavailability, particularly in the positive direction, may precipitate unwanted side effects. As a result, the clinical plan may require control and/or monitoring of food intake in relation to dosing. However, the above issue may be addressed by administering such drugs as lipid-based formulations. Although the nature and quantity of lipids contained in a high-fat meal would be significantly different to what would be included in a pharmaceutical formulation, design of lipid-based formulations can reduce the inherent imitations of slow and incomplete dissolution of poorly soluble drugs and facilitate the formation of solubilized phases from which absorption may occur. This can offer a prospective approach to reduce their food-dependent bioavailability and will also be functional in reducing the dose (Humberstone et al., 1997). Some examples of drugs that exhibit enhanced bioavailability when administered in combination with food include griseofulvin (Aoyagi et al., 1982), danazol, halofantrine, atovaquone and troglitazone (Nicolaides et al., 2001; Schmidt et al., 2002).

1.3.4 In vivo fate of lipid in human body

A normal adult diet includes a daily intake of about 60–80 g of fat. Additionally, 40–60 g of fat is of endogenous origin, which consists of phospholipids, cholesterol and membrane lipids from desquamated intestinal cells and bacteria (Hinsberger et al., 2004). This indicates that an adult digestive system is powerful enough to hydrolyze approximately 100–140 g of lipid everyday. The solubilization of drug in the GI tract and its bioavailability depend predominantly on the intraluminal processing to which lipids are subjected prior to absorption. Therefore, knowledge of the journey of lipids from the GI lumen to the circulatory system in the presence of a powerful digestive system is of great significance for
interpretation of the biopharmaceutical properties of oral lipid-based formulations and successful product development (Wahren et al., 2005). The processing of lipid-based formulations in the human body is highly complex, and the exact mechanism of drug absorption and its fate in association with the administered lipid are still not clear (Porter et al., 2007; Trevaskis et al., 2008). Therefore, the focus of this section is to simplify the understanding of the entire process by dividing it into three distinct phases:

- digestive phase
- absorption phase
- circulatory uptake

1.3.4.1 Digestive phase

The digestive phase initiates with the physical breakdown of lipid formulation into a coarse emulsion (lipid droplets <0.5µm) of high surface area due to shear produced by antral contraction, retropulsion and gastric emptying. This is accompanied with hydrolysis of the fatty acid glyceryl esters by gastric lipase secreted from chief cells in the stomach (capable of functioning in an acidic environment) which act at the oil/water interface. The enzymatic hydrolysis reduces the TGs into its more polar products monoglycerides (MGs) and FAs. Lipase cleaves the two ester bonds of the TG molecule, producing a molecule of diglyceride and one free FA first, and then two molecules of free FAs and one molecule of MG (Fig.1.3). The dispersed lipid digestion products along with the undigested lipids then empty into the duodenum.

As the acidic gastric content reaches the duodenum, the low pH causes the release of secretin from the duodenal mucosa into the portal circulation, which drains the digestive organs, spleen, and pancreas and delivers the blood to the liver via hepatic portal vein. This stimulates the pancreas to produce and secrete bicarbonate (along with lipase and co-lipase) into the duodenum to create a pH-neutral environment, which in turn maximizes the activity of pancreatic lipase and co-lipase. In the presence of FAs, cholecystokinin is released into the portal circulation which additionally stimulates the pancreas to release TG lipase and co-lipase required to facilitate the TGs digestion within emulsified particles. Being partially ionized, FAs and MGs are also potent emulsifiers which promote binding of the co-lipase–lipase complex to the emulsion surface (Borgstrom 1980; Bernback et al, 1989). Thus, the lipolysis is an autocatalytic process capable of enhancing the emulsification when lipolytic
products are produced. Both enzymes being water soluble act at the water/lipid interface of the particles and hydrolyze TGs to MGs and FAs (Kozlovz et al., 1992; Embleton & Pouton 1997). The digestion phase ends with the formation of mixed micelles by the interaction of FAs and MGs with bile salts, while a part of the TGs and FAs may form vesicles after digestion in this pre absorptive phase (Ollivon et al., 1988). It is at this phase that the drug released from the formulation due to either precipitation or dissolution into the gastric media is re solubilized as micelles or mixed micelles by emulsifications, which can play a significant role on the performance of the formulation. The overall in vivo solubilization capacity depends on both the lipophilicity and chemical structure of the drug and the nature of the endogenous and exogenous lipids involved in the formation of colloidal species (Fatourosa et al., 2007; Kossena et al., 2007).

Quantities even in the range of 2 g of long-chain lipid stimulate gall bladder contraction and elevate intestinal biliary lipid accumulation without any significant alteration in gastric emptying time. However, similar quantity of medium-chain lipid has been demonstrated to have little effect on gallbladder contraction and elevation in intestinal concentrations of biliary-derived lipids. It has been shown that a lipid emulsion containing 10 g of glyceryl monooleate is capable of stimulating the same increase in drug absorption of danazol in healthy volunteers as that observed after administration with a large meal (Kossena et al., 2007; Humberstone et al. 1996).

The enzymatic action being an interfacial process, the rate of lipolysis is enhanced in formulations with good dispersibility like self-nano/microemulsifying drug delivery systems. These types of formulations maximize the rate of drug partitioning into the aqueous intestinal fluids and provide consistent bioavailability, as seen in the case of Sandimmune and Sandimmune Neoral formulation (Ptachcinsky et al., 1986; Kovarik et al., 1994).

1.3.4.2 Absorption phase

The colloidal species produced, in the form of micelles, mixed micelles, vesicles and free FAs as a result of lipid digestion, are taken up by passive diffusion, facilitated diffusion and active transport through the enterocyte membrane. In the cytosol, a fatty acid-binding protein transports them from the apical membrane to the smooth endoplasmic reticulum (ER). Thereby, a concentration gradient facilitates the uptake of FAs into the cell by a carrier-mediated process (Stremmel, 1988). In the smooth ER, FAs and MGs are resynthesized into
TGs and phospholipids, respectively, which are transferred to the golgi apparatus and stored into secretory vesicles to be released by exocytosis into the extracellular space via basolateral membrane. Another critical step is the association of the absorbed free drug with the intestinal lipoproteins (chylomicrons) within the enterocyte. These chylomicrons are relatively large (<1 μm in diameter) and colloidal in nature which eventually lead to selective intestinal lymphatic transport of the lipophilic compound (Charman and Porter, 1996; Harrison., 2005; Ichihsdhi et al., 1992). The formed chylomicrons are then subjected to lymphatic transport system via mesenteric lymph and ultimately enter the systemic circulation by lymphatic drainage at thoracic duct.

Fig. 1.3: A diagrammatic representation of the role of lipase/co-lipase and mixed bile salt micelles in digestion of triglycerides and solubilization of the digestion products. (Modified from Pouton, 2006)

Various types of lipid based nanocarriers implemented for improving the oral delivery of drugs include microemulsions, nanoemulsions, lipid nanocapsules, self-emulsifying systems, lipid nanoparticles, hybrid lipid nanoparticles, liposomes and surface engineered liposomes to name a few (Thanki et al., 2013). Fig. 1.4 depicts various absorption mechanisms by which lipid nanocarriers improve the oral bioavailability of drug substances.
During the absorption phase, the drug molecules are usually exposed to the activity of major phase I drug metabolizing enzyme, Cytochrome P450 3A4 (CYP 3A4), present at high concentrations in enterocytes located at the villus tip of the small intestine in humans. Studies conducted across different laboratories have accounted the role of these enzymes in increasing the bioavailability of drugs when co-administered with lipid, which is indicative of an additional pathway by which lipids enhance oral drug bioavailability (Trevaskis et al., 2005; Wacher et al., 2001; Parkinson., 1996). However, the exact mechanism behind this phenomenon is still unclear. Few workers are of the opinion that the lipids attenuate the expression and activity of these enzymes, while others have proposed that the lipid shields the drug molecules from the enzymes (Reubsaet et al., 1988; Haunerland., 1994).

Fig.1.4 Absorption mechanisms followed by lipidic nanocarriers for enhancing the oral bioavailability of drug substances (Modified from Thanki et al., 2013)
1.3.4.3 Circulatory uptake

The majority of orally administered drugs gain access to the systemic circulation by absorption into the portal blood. However, some extremely lipophilic drugs (log P > 5, solubility in TG > 50 mg/ml) gain access to the systemic circulation via lymphatic route, which avoids hepatic first-pass metabolism. Therefore, highly metabolized lipophilic drugs may be potential candidates for lipid based drug delivery. Compounds showing increased bioavailability in the presence of lipids (dietary or lipid-based formulation) are absorbed via the intestinal lymph as they are generally transported in association with the long-chain TGs lipid core of intestinal lipoproteins formed in the enterocyte after re-esterification of free FAs and MGs. Short-chain TGs are primarily absorbed directly in the portal blood. Drug transport via the lymphatics, therefore, requires co-administration of lipid to stimulate lipoprotein formation (Charman and Stella, 1986; Thomson et al., 1989).

Direct uptake of TGs and phospholipids into the bloodstream is not possible, though the portal blood is approximately 500-fold higher than that of the intestinal lymph. This is because their large molecular size restricts them to pass through capillary fenestration spaces. The walls of lymphatic capillaries consist of a single layer of squamous epithelial cells, and this thin wall makes it possible for tissue fluid (interstitial fluid) from the interstitial space to enter the lymphatic capillary. Moreover, the endothelial architecture of the lymphatic vessels facilitates the size-selective transport of high molecular weight substances like chylomicrons for which facile access across the blood capillary endothelium is restricted (Leak, 1976). Studies have shown that the free FA chain length and the composition and size of the lymph lipid precursor pool in the enterocyte play major role in lymphatic drug transport. In general, free fatty acids (FFA) of chain length 612 carbons are absorbed primarily by means of the portal blood, whereas FFA with chain lengths >12 carbons are re-esterified and transported via intestinal lymph (Trevaskis et al., 2005). Additionally, increase in the degree of unsaturation produces larger size lymph lipoproteins and selectively enhances lymphatic uptake The lymph fluid is then emptied (average 3 L per day) via thoracic duct into the subclavian vein, thus protecting the drug from hepatic first-pass metabolism (Zuther, 2005). The lymphatic system, being the principal systemic transport pathway for B and T lymphocytes as well as the primary route of metastatic spread of a number of solid tumours and several viruses (Pouton, 2006), is a potential drug delivery target for immunomodulatory,
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 anticancer compounds and other related drugs (Cense et al., 2006; Garzon-Aburbeh et al., 1983; Pantaleo et al., 1983).

The drug being transported in the circulatory system, in the form of either micelles or mixed micelles, may then be available in its free form, since upon dilution with a large volume of the lymph/blood, surfactant concentration may reduce below its cmc value and micelle may dissociate into monomers. The drug transported as lipid vesicles may remain intact for extended periods and, thereby, can result in prolonged release of the encapsulated drug (Yokoyama., 1992; Hwang et al., 1977). Fig. 1.5 is a diagrammatic presentation of the various mechanisms by which lipids enhance the bioavailability of drug.

Fig. 1.5: Different mechanisms of enhancement of drug bioavailability in the presence of lipids (Modified from Porter et al., 2007)

Following are the step involved in absorption (Indicated in the Fig 1.5) (Porter et al., 2007)
(i) Solubilization of drug in the intestinal fluid by formation of colloidal species viz., vesicles, mixed micelles and micelles;
(ii) Interference with enterocyte-based transport and metabolic processes, thereby potentially changing drug uptake, efflux, disposition and the formation of metabolites (M) within the enterocyte;

(iii) By selective lymphatic uptake which reduces first-pass drug metabolism as intestinal lymph travels directly to the systemic circulation.

1.3.5 Morphology of lipid digestion products

The GI-tract basically influences the performance of lipid-based formulations as triglycerides and their derivatives are quantitatively hydrolysed prior to absorption. After a meal, the presence of lipid digestion products induces secretion of pancreatic and biliary fluids which alter the luminal environment of the small intestine. Lipid digestion in the intestine results in the formation of several colloidal species including vesicles, micelles and mixed micelles. Vesicles are self-assembled lamellar phases composed of water-insoluble phospholipids (such as phosphatidylcholine). Micelles are composed of surfactant molecules which solubilize as monomers in water up to the critical micellar concentration above which the monomers self assemble to form micelles. Mixed micelles are micelles composed of mixed surfactant systems. The chemistry and nature of the interaction of lipid digestion products with the aqueous contents of the GI-tract change as a function of digestion and solubilization (Kossena et al., 2004). The vesicles represent intermediate products of the interactions between oil droplets and bile salt media in the presence of lipase activity. During the digestive process, bilamellar vesicles are generated which usually transform into unilamellar vesicles. These spontaneously dissolve into micellar and/or mixed micellar phases with increase in the surfactant (bile salt)-to-lipid ratio. The phase transition produces the thermodynamic condition most favourable for effective lipid absorption from the upper small intestine, while the lipolytic products dispersed as unilamellar and multilamellar vesicles are responsible for fat absorption along the later part of small intestine in bile salt deficiency states (Andelman et al., 1994; Rigler et al., 1986).

1.3.5.1 Digestion of triglycerides

Intestinal absorption of the products of lipid digestion is the result of three sequential processes, which involve

(i) Dispersion of fat globules (ingested lipids) to yield a coarse emulsion of high surface area.
(ii) Enzymatic hydrolysis of the fatty acid glyceryl esters (primarily triglyceride lipids) at the oil/water interface.

(iii) Dispersion of the lipid digestion products into an absorbable form.

Salivary glands secrete lingual lipase and gastric mucosa secretes gastric lipase, which initiate triglyceride (TG) hydrolysis to the corresponding diglyceride (DG) and fatty acid (FA) within the stomach. The pH-optimum ranges for lingual and gastric lipase from 3 to 6, and medium chain triglycerides are hydrolyzed at a faster rate than long chain triglycerides (Dahan et al., 2008).

TG are preferably hydrolysed by pancreatic lipase, an interfacial enzyme, which preferentially acts at the surface of emulsified TG droplets to quantitatively convert TG into the corresponding 2-monoglyceride (MG) and two fatty acids (FA). Optimum activity is observed when oleate is the fatty acid, and it decreases towards shorter chain triacylglycerols and soluble carboxyl esters substrates. Bile salts at concentrations usually present in the duodenum inhibit binding of pancreatic lipase at the oil-water interface.

Further classes of biliary-derived lipids are the phospholipids (e.g., phosphatidylcholine, PC) playing an important role as solubilisers for lipid digestion products. Prior to absorption, PC is hydrolyzed by phospholipase A2 to lysophosphatidylcholine (lyso-PC), with the majority of PC present in the intestine secreted in bile (10–20 g/day) with only modest dietary influence (Chakraborty et al., 2009; Dahan et al., 2008; Porter and Charman, 2001; Nanjwade et al., 2011).

Bile plays a key role in solubilisation of lipid digestion products and poorly water-soluble drugs. The presence of lipid digestion products as well as cholecystokinin-mediated contraction of the gall bladder and relaxation of the sphincter of Oddi leads to expulsion of bile, with peak flow occurring approximately 30 min after ingestion of a meal (Charman et al., 1997).

Typical concentrations of bile salts in the fasted intestine are 4–6 mM compared with postprandial concentrations of 10–20 mM. The inclusion of the lipidic components decreases the critical micelle concentration (CMC) and an increase the size and solubilisation capacity of the micelles, e.g., the inclusion of lecithin and MG decreases the CMC of mixed micellar systems to values less than 1 mM. Therefore, it is likely that the CMC of mixed bile salt systems present in the intestine is surpassed even in the fasted state. Although the specific mechanisms of absorption of the lipid digestion products have not been elucidated yet, the
common role of the intestinal mixed micellar phase to solubilise these poorly water-soluble compounds and to provide a concentration gradient for absorption of lipids and presumably of drugs solubilised in this phase is generally accepted. Micelles are not absorbed intact, and lipids are suggested to be absorbed from a monomolecular intermicellar phase. The dissociation of monomolecular lipids from the mixed micellar phase prior to absorption may be stimulated by a microclimate of lower pH at the intestinal absorptive site (Simmonds, 1972; Westergaard et al., 1976; Keleman et al., 1990).

Quasielastic light scattering analysis has revealed that ex vivo mean hydrodynamic radii of micelles are 640 Å, whereas unilamellar vesicles are in the range of 200–600 Å, and lipids were largely solubilized by micelles than were dispersed as unilamellar vesicles. Cryogenic transmission electron microscopy of in vitro digestion of a self-nanoemulsifying drug delivery system revealed the entire sequence of the phase changes in the digestive process, wherein micelles of around 10 nm were present at all time points (Fatourosa et al., 2007). The structures provided evidence to the previously proposed model in which unilamellar and multilamellar vesicles co-exist with micelles (Rigler et al., 1986). A liquid lamellar crystalline phase containing calcium and ionized FAs called calcium soaps followed by “a viscous isotropic phase” has been identified with light microscopy after hydrolysis of an oil emulsion (Patton and Carey, 1979). Small-angle X-ray scattering measurements have also been employed efficiently as a screening tool to elucidate the processes encountered during the digestion of lipid-based formulations in an in vitro dynamic lipolysis. Further investigations into the morphological characteristics of the structural changes occurring during lipid digestion process can help to understand the partitioning of the drugs to the lipolytic products and consequently the in vivo performance of the formulation (Fatouros et al., 2007).

1.4 Nanoemulsion formulations for drug delivery

An emulsion is generally described as a heterogeneous system composed of two immiscible liquids: one dispersed uniformly as fine droplets throughout the other. The majority of conventional emulsions in pharmaceutical use have dispersed particles ranging in diameter from 0.1 to 100 mm. As with other dispersed systems such as suspensions, emulsions are thermodynamically unstable, as a result of the excess free energy associated with the surface of the droplets in the internal phase. The dispersed droplets strive to come together rand
reduce the surface area. In addition to this flocculation effect, the dispersed particles can coalesce, or fuse, and this can result in the eventual destruction (phase separation) of the emulsion. To minimize this effect, a third component, the emulsifying agent or surface active agent (surfactant), is added to the system to improve the stability. The liquid present as small globules is called the dispersed or internal phase, where as the liquid where the droplets are dispersed is called the dispersion, continuous, or external phase.

In general, the immiscible liquids are described as oil and water phases. The oil phase may be any hydrophobic non-polar liquid, and the water phase may be any hydrophilic polar liquid. The term macroemulsion is sometimes employed to distinguish the ordinary emulsions defined above from microemulsions, nanoemulsions, and micellar systems. Whereas nanoemulsions are two-phase systems where the dispersed phase droplet size has been made in the nanometer size range, the microemulsions, and micellar systems are single-phase systems (Salager, 2000; Tenjarla, 1999; Keipert, 1989). All these systems are a result of interfacial phenomenon brought out by surface active agents. Therefore, it is essential to understand the molecular mechanisms responsible for the creation of these systems and distinctions between them.

Surface active compounds contain hydrophilic and hydrophobic moieties in the same molecule. When these surfactants are added to the water phase or the oil phase, one portion of the molecule is always incompatible with the other solvent molecules. Surfactants overcome this incompatibility by adsorption at interfaces (air–water, oil–water, or solid–water) and/or by formation of aggregates called micelles. Micelles are colloidal aggregates that form above a specific concentration, the so-called critical micelle concentration (CMC). When the solvent is water, a micellar aggregate exhibit the structure where the surfactant molecules are oriented in such a way that the hydrophilic groups are in contact with the water molecules, and the hydrophobic tails are located in the micellar core away from the aqueous environment. In this case, the core of a micelle resembles a tiny pool of liquid hydrocarbon where compounds that are poorly soluble in water but soluble in non-polar solvents can be solubilized. When the solvent is oil phase, inverse (or reverse) micelles are formed where the surfactant’s hydrophilic groups are located inside. In this case, the compounds that are soluble in water phase can be solubilized in the micellar core. The process of solubilization takes place well above the CMC, and because of solubilization, micelles become swollen and may attain the size of a small droplet (~100nm). Consider, for example, that water is solvent, the surfactant
concentration is well above CMC, and the oil phase is also present; the micelles would solubilize large amounts of the oil phase and become swollen until they start interacting through a phenomenon called percolation. Such packed swollen micelle structures that could solubilize large amounts of both oil- and water-soluble compounds have been called microemulsion because they were first thought to be extremely small droplet emulsions (Sarker, 2005; Solans & Aramaki, 2008).

There is, however, general agreement that microemulsions are optically isotropic, thermodynamically stable systems and are not amenable to dilution with the external phase as normal emulsions. Application of microemulsion is usually limited to dermal and peroral application because of their high surfactant concentration that tends to prove toxic through other routes of administration. Also, they exist in narrow regions of the phase diagrams and, as such, they are very sensitive to quantitative changes in formulation (Maali and Hamed, 2012; Anton and Vandamme, 2011).

Fig.1.6 Different kind of emulsions (Modified from Lawrence and Rees, 2000)

Two types of emulsion systems are possible depending on if the dispersed droplets are oil or water (i.e., oil-in-water (O/W) or water-in-oil (W/O) emulsion). O/W nanoemulsion systems are of particular pharmaceutical importance from a parenteral drug delivery point of view. W/O emulsions are also parenterally used; however, their applications are limited for obtaining the prolonged release of the water soluble compounds on intramuscular application.
Multiple emulsion or double emulsion systems are also possible where preformed emulsion is re-emulsified with either oil or water. For example, multiple emulsions of oil-in-water-in-oil (O/W/O) are W/O emulsions where the water droplets contain dispersed oil globules. Similarly, water-in-oil-in-water (W/O/W) multiple emulsions are those where the internal and external water phases are separated by an oil phase (Solans and Solé, 2012).

1.4.1 Theories of Nanoemulsion formation

Many approaches have been used to explore the mechanisms of nanoemulsion formation and stability. Some emphasize on the formation of an interfacial film and the production of ultra low interfacial tension (mixed film theories); others emphasize on the monophasic nature of many nanoemulsions (solubilization theories). Thermodynamic theories take into consideration the free energy of formation of the nanoemulsions and the bending elasticity of the film.

![Fig.1.7 Different step involved in the formation of nanoemulsion (Modified from Kota et al., 2013)](image)

Eccleston, 1994 reviewed the various approaches that have been postulated for nanoemulsion formation. Although not a single approach alone covers all aspects of nanoemulsion structure and stability but all have a place in the overall understanding of nano or microemulsions. The attractive interactions between the molecules of the two liquid phases are different since an interfacial tension, $\sigma$, exists between the two liquids in their contact point (Myers, 1999; Meleson et al., 2004). The energy required to create an additional interfacial area ‘A’ between
the two liquid phases is $\sigma A$. Interfacial tension always acts to minimize the interfacial area. Therefore, the interface between two immiscible liquid is like a planar sheet at their contact point (Fig 1.7i). Usually the oil phase remains in the upper side since it has a low density than the water phase. And so the system is under thermodynamic equilibrium in the absence of any surfactants. When we add surfactants, they remain at the interface so as to reduce the interfacial tension (Fig. 1.7ii). Surfactants that are highly soluble in any one of the phases especially in the dispersed phase can reduce the interfacial tension significantly. If oil--water interface that is coated with surfactants brought in close to each other, a thin film of water will remain at the interface. Therefore, the interface repels each other due to the like or similar charges of the surfactants (Fig. 1.7iii).

At a very low volume fraction of dispersed phase ($\phi$), the droplets remains spherical with a radius of ‘$a$’, the curved interface exerts a pressure on the molecules inside the droplet, called the Laplace pressure (Mason, 1999).

$$\text{Laplace pressure} \Pi_L = 2\sigma/a$$

Since the Laplace pressure is inversely proportional to the radius, smaller droplets experience a higher Laplace pressure than larger ones. So to deform a droplet, the applied shear must overcome Laplace pressure. Therefore, a larger shear stress ($\tau$) is to be applied to rupture the droplets into smaller size

$$\text{shear stress, } \tau = \eta_c \gamma'$$

where $\eta_c$ is the viscosity of the continuous phase and $\gamma'$ is the shear rate.

Since a large number of surfactant molecules are present in the continuous phase, they form a coating on the interface immediately when an additional interfacial area is formed and thus stabilizes the system from coalescence. In the last century, Taylor developed a relationship between how an isolated droplet is ruptured further smaller by the applied shear stress. According to Taylor, droplet size is inversely proportional to shear stress.

$$a \propto \frac{\sigma}{\tau}$$

$$a = \frac{\sigma}{\eta_c \gamma'}$$

The size distribution of the ruptured droplets is determined by the history of applied shear, whether the shear is applied to an emulsion having either high or low-volume fraction of dispersed phase (Taylor, 1934).
1.4.2 Preparation of Nanoemulsion

Both high-energy and low-energy methods can produce stable NEs. High-pressure homogenizer or ultrasound generator can be used for the preparation of NE by high-energy emulsification method. Phase inversion methods such as phase inversion temperature (PIT) and phase inversion composition (PIC) are low-energy method for the preparation of NEs. Extreme emulsification methods such as microfluidic and ultrasonic techniques can be used to produce nanoscale dispersions of droplets of one liquid in another immiscible liquid by rupturing larger microscale droplets into nanoscale droplets (Landfester et al., 2000). The apparatus used for high-energy emulsification method should supply homogeneous flow and high energy in the shortest time in order to produce the smallest size. Classical ‘metastable emulsions’ are formed by applying mechanical shear to the continuous phase in order to break larger droplets of the dispersed phase into smaller droplets. Low-energy methods such as PIT and PIC methods are collectively called as condensation method; they make use of stored chemical energy instead of mechanical energy in high-energy methods (Mason et al., 1996; Hinch and Acrivos, 1980; Rallison, 1984).

1.4.2.1 High-pressure microfluidic nanoemulsification method

High-pressure homogenizers are most widely used and accepted equipment since they meet all requirements (Becher, 1965). To create a tremendously strong extensional flow for making NE by rupturing droplets in a concentrated emulsion, rapidly flowing streams of a premixed emulsion of droplet sizes typically less than 10 µm are forced through rigid stainless steel micro channels of dimensions typically closer to 100 µm. High-pressure air around 100 psi is mechanically amplified by a piston to produce liquid pressures that can reach as high as about 30,000 psi. Micro scale emulsion droplets are taken at a rate of about 3 ml sec$^{-1}$ into the channel and routed into an interaction area where an extreme extensional shear flow is created. Recirculation of the emulsion through the region of high shear is essential to maintain relatively low polydispersibility due to the in homogeneities of the pulsed microfluidic flow. Due to the higher shear rates, the emulsion is heated above room temperature. NE that leaves the region of extreme shear can be cooled by a heat exchanger without affecting the size distribution or stability. The volume rate of production of the NE is high about many liters per hour. The advantage of this ‘high-throughput’ microfluidic nanoemulsification method is the combination of extremely high shear rates, high-volume...
throughput of nanoscale droplets and reasonably uniform droplet size distributions (Solans et al., 2005; Floury et al., 2003).

1.4.2.2 Ultrasonication method

Another method for producing NEs is by ultrasonic agitation of a premixed emulsion of microscale droplets. In this method, a vibrating solid surface agitates the premixed emulsion at ultrasonic frequencies about 20 kHz or larger. This high power produces extreme shear and cavitations that breaks up microscale droplets to nanoscale. The devices contain focusing horns and pointing tips. In most of the ultrasonic devices, recirculation is necessary like high-pressure homogenizers since the emitted sound field is normally in homogeneous. Practically uniform droplet size distributions at dilute concentrations can be obtained if the emulsion is recirculated many times through the region of high shear (Mason et al., 2006). According to Landfester, the efficiency of the dispersion process is strongly dependent on the ultrasonication time at different amplitudes. And also it was found that the more hydrophobic the monomer is, the longer will be the sonication time required (Landfester et al., 2004).

1.4.2.3 Phase Inversion Temperature (PIT) method

According to PIT introduced by Shinoda and associates, fine dispersion can also be obtained by chemical energy, due to the phase transitions taking place through emulsification path (Shinoda & Saito, 1968). Adequate phase transitions can be produced by varying the composition at constant temperature or by varying the temperature at constant composition (Kunieda et al., 1996). This method is based on the changes in solubility of nonionic surfactants with temperature. The polyoxyethylene surfactants become lipophilic with increasing temperature due to dehydration of the polyoxyethylene chains. The surfactant monolayer has a large positive spontaneous curvature at low temperature. It forms an oil-swollen micellar solution phases or microemulsions of o/w type, which coexist with an excess of oil phase. As the temperature increases, the curvature becomes negative and water-swollen reverse micelles or w/o microemulsions will form and they coexist with excess water phase. At intermediate temperatures (the HLB temperature), the spontaneous curvature becomes close to zero. At this temperature, a bicontinuous-phase microemulsion containing similar amounts of water and oil phases coexists with excess water and oil phase (Rang et al., 1999; Sharma et al., 2010).
1.4.2.4 Phase Inversion Composition (PIC) method

PIC method, that is phase inversion composition method, involves the change in composition at a constant temperature. The preparation of w/o NEs by a low-energy method consisting of slow addition of the oil to surfactant/water mixtures is first reported by Uson and associates. The droplet size was a function of the surfactant mixing ratio and water concentration (Uson et al., 2004). Porras and associates also described the formation of water in oil NEs by the same low-energy method stabilized with mixtures of sorbitan ester surfactants. In experiments where the surfactant:oil ratio is constant, droplet size increases as water concentration increases. In addition, under conditions of constant water concentration, droplet size decreases when the surfactant: oil ratio increases (Porras et al., 2004).

1.4.2.5 Low energy emulsification method

Self-emulsification or spontaneous emulsification methods make use of the chemical energy released due to a dilution process with the continuous phase, generally at constant temperature, without any phase transitions (no change in the surfactant spontaneous curvature) taking place in the system during emulsification. When diluting, diffusion of water-miscible component(s) (solvent, surfactant and/or cosurfactant) from the organic phase into the aqueous phase (to obtain O/W nanoemulsions) is produced, which results in a dramatic increase of the interfacial area, giving rise to the metastable emulsion state. The experimental conditions reported in the literature in order to obtain droplets in the nanoscale range with this spontaneous emulsification method are related to a very high solvent/oil ratio. The solvent diffusion is hence even quicker and the turbulence generated causes nano-scaled droplets to form (Miller, 1988; Bouchemal et al., 2004).

1.4.3 Characterization of nanoemulsion systems

Nanoemulsion has been characterized using a wide variety of techniques. The characterization of nanoemulsions is a difficult task due to their complexity, variety of structures and components involved in these systems, as well as the limitation associated with each technique, but such knowledge is essential for their successful commercial exploitation. The rate of release of sodium salicylate from a lecithin-based nanoemulsion, is dependent upon their nanostructure (Khoshnevis et al., 1997). Nanoemulsions have been evaluated using
a wide range of different techniques over the years, but a complementarily of methods is generally required in order to fully characterize these systems.

1.4.3.1 Viscosity measurements

These measurements can indicate the presence of rod like or worm like reverse micelle (Yu et al., 1995; Angelico et al., 1998).

1.4.3.2 Conductivity measurement

It determines the type of nanoemulsion and detects the phase inversion phenomenon (Yu et al., 1995; Angelico et al., 1998).

1.4.3.3 Dielectric measurements

They are powerful means of probing both structural and dynamic features of nanoemulsions systems (Angelico et al., 1998).

1.4.3.4 Electron microscopic studies

Freeze fracture transmission electron microscopy (FFTEM) and Transmission electron microscopy (TEM) are the most important techniques for the study of nanostructures because it directly produces images at high resolution and it can capture any coexistent structure and nanostructure transitions (Gulik-Krzywicki et al., 1984; Vinson et al., 1991; Bolzinger et al., 1999). However, extreme rapid cooling of the sample is required in order to maintain structure and minimize the possibility of artifacts in FFTEM studies. Recent developments in the cryofixation technique have overcome many problems associated with artifact formation in early studies.

A complementary technique is of direct imaging, in which thin portions of the specimen are directly investigated in the frozen hydrated state by using a cryostage in the TEM. The development of glass forming nanoemulsions that does not breakdown during cooling and in which neither disperse nor matrix phase crystallizes has provided a way for direct studies of nanoemulsion and nanoemulsion structures. The first type of such systems to be reported were w/o nanoemulsions with a non-crystallizing aqueous matrix obtained by adding propylene glycol to water in the ratio 1:3 (Dubochet et al., 1984).
1.4.4 Advantages of Nanoemulsions

Nanoemulsions exhibit several advantages as a drug delivery system. They are listed as:

1. Nanoemulsions are kinetically stable systems and the stability allows self-emulsification of the system whose properties are not dependent on the process followed.

2. They act as supersolvent of the drug. They can solubilize hydrophilic and lipophilic drugs and improve the bioavailability of the poorly soluble drugs. This is due to the existence of nanodomains of different polarity within the same single-phase solution.

3. The dispersed phase, lipophilic or hydrophilic (o/w or w/o nanoemulsions) can behave as a potential reservoir of lipophilic or hydrophilic drugs respectively. The drug partition between dispersed and continuous phase. When the system comes into contact with a semipermeable membrane, the drug can be transported through the barrier. Drug release with pseudo zero order kinetics can be obtained, depending on the volume of the dispersed phase, the partition of the drug and the transport of the drug.

4. The mean diameter of droplets in nanoemulsion is below 0.1µm and therefore they can be sterilized by filtration. The small size of droplets in nanoemulsion e.g., below 100 nm, yields very large interfacial area, from which the drug can quickly be released into the external phase, when absorption (in vitro or in vivo) takes place, maintaining the concentration in the external phase close to the initial levels.

5. Same nanoemulsion can carry both lipophilic and hydrophilic drugs.

6. Because of thermodynamic stability, nanoemulsions are easy to prepare and require no significant energy contribution during preparation.

7. Nanoemulsions have low viscosity compared to other emulsions.

8. The use of nanoemulsion as drug delivery systems can improve the efficacy of drug, allowing the total dose to be reduced and thus minimizing side effects.

9. The formation of nanoemulsions is reversible. They may become unstable at low or high temperature, but when temperature returns to the stability range, the nanoemulsion reforms.

10. Hydrophilic peptide drugs which are susceptible to proteolysis in the GI tract can be successfully incorporated into the dispersed aqueous phase of w/o nanoemulsion
droplets where they are afforded some protection from enzymatic degradation when administered orally.

11. Water in oil nanoezmulsions can be employed as intramuscular injections.

1.5. Curcumin: Herbal bioactive

In recent years, extensive research has been carried out to study the health promotion properties of different phytochemicals which are food supplements and commonly used as part of the daily diet. Pharmacological properties are associated to several vegetables, fruits and herbs, which are known to be full sources of potential molecules for treatment of several malignancies (Espín et al., 2007; Faller and Fialho, 2010). The chemical structure of polyphenolic compounds have at least one aromatic ring with a reactive hydroxyl group, being divided into different classes according to the number of phenolic rings and the structural elements linking this rings, e.g. phenolic acids and flavonoids. One of the reasons for the growing interest in studying these compounds resides on their protector role against different type of cancer (Santos et al., 2012; Verma et al., 2009). However, the proven benefits of natural anti-oxidants, e.g. polyphenols, which are healthy substances, sometimes, have some targeting delivery and bioavailability issues. The therapeutic efficacies of these dietary polyphenols were reduced because of restricted bioavailability which is not only due to the physiochemical properties (e.g. solubility, molecular weight etc) of the bioactive compound, but also because of enzyme and microbial-mediated biotransformation and active efflux (Kumar et al., 2010; Santos et al., 2012). The bioavailability of phytochemicals can be enhanced by encapsulating them into lipid based system which can change their pharmacokinetics (PK) and biodistribution (BD) (Huang et al., 2010).

1.5.1 Potential Biological Properties of Curcumin

Since times immemorial, herbal plants have been regarded as a potential source of therapeutic compounds or medicine, responsible for the treatment of various disorders from ‘head to toe’. Turmeric (Curcuma longa) is one of them, and its dried rhizome is the source of curcumin called as ‘Indian solid gold’. The characteristic yellow colour of the turmeric is due to the curcuminoids present in it. The rhizome or root of turmeric is processed into turmeric powder which contains 2% to 5% curcuminoid, and has been used in traditional medicine from centuries as a house hold remedy for various diseases, including biliary disorders, diabetic
wounds, cough, hepatic disorders, AIDS, sinusitis and as a blood purifier (Chattopadhyay et al., 2004; Aggarwal et al., 2007; Han et al., 1999; LoTempio et al., 2005; Babu et al., 1997).

**Fig.1.8 Turmeric (Curcuma longa) and its dried rhizome with curcumin**

In addition to household remedy for various diseases, curcumin is currently used in perfumes and as a natural yellow coloring agent, as well as an approved food additive to flavor various types of curries and mustards. When curcumin is mixed with natural compounds such as slaked lime then it is used topically for the treatment of inflammation, wound, tumors and various skin disorders. Fig.1.9 representing use of curcumin against variety of cancers.

**Fig.1.9 Role of curcumin against different type of cancer**
1.5.2 Chemical Properties of Curcumin

Curcumin was first isolated from turmeric in 1815 by Vogel, obtained in crystalline form in 1870 and its structure was delineated in 1910 as diferulolymethane or 1,6-heptadiene-3,5-dione-1,7-bis(4-hydroxy-3-methoxyphenyl)-(1E,6E) (Goel et al., 2008) The orange yellow colour of crystalline curcumin is due to a polyphenolic compound known as diferulolymethane which is hydrophobic in nature and practically insoluble in water and ether. It is soluble in organic solvents such as ethanol, dimethylsulfoxide (DMSO), acetone and dimethyl formamide (DMF). The solubility of curcumin in acetone is approximately 20 mg/ml but in the remaining solvent such as DMSO and DMF it is 1 mg/ml approximately. Commercial preparation of curcumin (curcuminoids) comprises of 77% curcumin I (diferulolymethane), 17% curcumin II (demethoxycurcumin) and 6% curcumin III (bisdemethoxycurcumin). The maximum absorption of curcumin in methanol occurs at 430 nm and in acetone at 415 to 420 nm. Curcumin exists in enolic and β-diketonic (Fig.1.10) forms due to tautomerism between enol- and keto- structures (Shen et al., 2007; Tonnesen et al., 1985). In solution curcumin exists primarily in its enolic form characterized by strong intramolecular hydrogen bond which has an important bearing on the radical-scavenging ability of curcumin. The physicochemical properties of curcumin are due to formation/disruption of both intra and inter-molecular H-bonds along with charge delocalization which are responsible for its therapeutic potential (Zsila et al., 2003, Lin et al., 2000).
Curcumin is stable at acidic pH (extremely low degradation) but unstable at neutral and basic pH and under these conditions it is degraded to trans-6-(4´-hydroxy-3´-methoxyphenyl)-2,4-dioxo-5-hexanal, ferulic acid and feruloylmethane (Loftsson & Brewster 1996; Tomren et al., 2007). Most of the curcumin (>90%) is rapidly degraded within 30 min of placement in phosphate buffer system of pH 7.2. This degradation was inhibited by the addition of foetal calf serum, human blood, or addition of antioxidants such as N-acetylcysteine or glutathione and ascorbic acid in culture media or phosphate buffer (above pH 7) (Wang et al., 1997, Sharma et al., 2005).

When curcumin is orally administered it undergoes phase II metabolism, predominately, glucuronidation and sulfation. Most of the ingested curcumin is excreted in the faeces and only trace amounts of curcumin (or its metabolites) appear in the blood. The absorbed curcumin is rapidly metabolized in the intestinal mucosa and liver to several reduction products (di-, tetra-, and hexahydrocurcumin and hexahydrocurcuminol) and their glucuronide or sulfate conjugates (Fig.1.11) (Pan et al., 1999).

![Fig.1.11 Structure of curcumin and its metabolite](image)

**1.5.3 Bioavailability of curcumin**

Over the last few years, a number of research studies have revealed that curcumin has a surprisingly wide range of beneficial properties, including anti-inflammatory, antioxidant, chemopreventive, radiosensitizing, wound healing activities, antimicrobial, antiviral, antifungal and chemotherapeutic activity. These activities have been demonstrated both in
cultured cells and in animal models, and have paved the way for ongoing human clinical trials (Duvoix et al., 2003; Arora et al., 1971). Numerous preclinical and clinical studies (Table 1.4) have suggested the lower serum and tissue levels of curcumin, depending on the route of administration. An early study in 1978 by Wahlstrom and Blennow (Wahlstrom and Blennow, 1978) reported that 75% curcumin was excreted in faeces and negligible amount reached in systemic circulation after oral administration to Sprague Dawley rats. When curcumin was given by i.v. route to the rats at a dose of 10 mg/kg, a maximum serum curcumin level of $0.36 \pm 0.05 \, \mu g/mL$ was observed as compared to $0.06 \pm 0.01 \, \mu g/mL$ obtained for 50 fold dose of curcumin with oral administration (Yang et al., 2007). When 2 gm of curcumin was administered orally to the human with piperine, 2000% increase in bioavailability was observed suggesting the role of piperine in bioavailability enhancement (Shoba et al., 1998).

**Table 1.4: Preclinical/clinical pharmacokinetics of curcumin after oral administration**

<table>
<thead>
<tr>
<th>Human/Animal</th>
<th>Dose</th>
<th>Conclusion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprague Dawley rats</td>
<td>1g/kg</td>
<td>Negligible amounts of curcumin in blood plasma</td>
<td>(Wahlstrom and Blennow, 1978)</td>
</tr>
<tr>
<td>Rats</td>
<td>2g/kg</td>
<td>Serum conc. was observed 1.35 ± 0.23 µg/mL at time 0.83 h</td>
<td>Shoba et al., 1998</td>
</tr>
<tr>
<td>Humans</td>
<td>2g/kg</td>
<td>Either undetectable or extremely low (0.006± 0.005 µg/mL at 1 h) serum levels.</td>
<td>Shoba et al., 1998</td>
</tr>
<tr>
<td>Rat</td>
<td>340 mg/kg</td>
<td>Serum level 6.5±4.5 nM at 0.5 h</td>
<td>Marczylo et al., 2007</td>
</tr>
<tr>
<td>Rats</td>
<td>400 mg</td>
<td>Trace amount (less than (5µg/mL) was found in the portal blood from 15 min to 24 h</td>
<td>Ravindranath et al., 1980</td>
</tr>
<tr>
<td>Humans</td>
<td>4–8 g</td>
<td>Peak plasma levels of 0.41–1.75 µM after1 h</td>
<td>Cheng et al., 2001</td>
</tr>
<tr>
<td>Humans</td>
<td>36-180 mg/kg</td>
<td>Mostly in faces and almost none in urine or blood</td>
<td>Sharma et al., 2001</td>
</tr>
</tbody>
</table>
1.5.4 Marketed Dosage form of curcumin

To date, a plethora of products are available in the market in different dosage forms (Fig. 1.12 and Table 1.5) containing either turmeric or curcumin in combination or without combination with some natural products including piperine, bromelain and others as an adjutants for enhancement of therapeutic efficacy of curcumin. These products are widely used in every corner of the world for the treatment of various ailments such as cough, psoriasis, wound healing, skin infections and as a dietary supplement. In spite of its efficacy and safety, curcumin has not yet been approved as a therapeutic agent because of rapid metabolism, rapid elimination and poor absorption which further leads to poor bioavailability. The use of curcumin is also limited due to low water solubility under acidic or neutral conditions, high decomposition rate in alkaline media and photo degradation in organic solvents. According to literature survey, most of the published review articles on curcumin have emphasized on the mechanism of action of curcumin in different diseases and no review has been published on drug delivery. However in a review article of Anand et al., 2007 a small subheading dealing with drug delivery technique for bioavailability enhancement of curcumin has been reported. Given the explosive growth of interest in the extensive literature the purpose of the current review is to present an appraisal of the current level of knowledge regarding the potential of curcumin in drug delivery and bioavailability enhancement. The current review also focuses on the cell line study used in cancer targeting by different drug delivery technique of curcumin.

Table 1.5: Marketed dosage form of curcumin

<table>
<thead>
<tr>
<th>Active Ingredient</th>
<th>Dosage form</th>
<th>Brand Name</th>
<th>Marketed By</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcuminoids and BioPerine</td>
<td>Tablet</td>
<td>Curcumin C₃ Complex</td>
<td>Sabinsa corporation, USA</td>
</tr>
<tr>
<td>Curcumin, Bromelain and Bioperine</td>
<td>Tablet</td>
<td>Turmeric Extract - 95% Curcumin</td>
<td>Source naturals, California, USA</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Capsule</td>
<td>Turmeric</td>
<td>Swanson health product, USA</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Capsules</td>
<td>Curcumin 95</td>
<td>Jarrow Formulas, USA</td>
</tr>
</tbody>
</table>
### Active Ingredient | Dosage form | Brand Name | Marketed By
--- | --- | --- | ---
95% Curcuminoids | Capsule | Turmeric/Curcumin | Nature's Bounty, USA
95% Curcumin | Capsule | Turmeric Extract | Planetary Herbals, USA
Curcumin and Bioperine | Capsule | Super curcumin | Life Extension, USA
95% curcumin | Capsule | Turmeric Extract | Vitamin Shoppe, North Bergen, NJ
Curcumin | Capsule | Pure Encapsulations Curcumin | Pure encapsulation, USA
Curcumin | Capsule | Turmeric Curcumin | Thompson, Chula Vista, CA
Curcumin | Capsules | Turmeric Curcumin | Good 'N Natural, Steinbach, Manitoba
Curcumin and Black pepper | Capsule | BioActive Nutrients Curcumin and Black Pepper | BioActive NUTRIENTS, USA
Curcumin, dl Phenylalanine, Boswellia and Nattokinase | Capsule | Phenocane with Curcumin and DLP A | OxyLife, USA
Curcumin, Fermented Soy and Bioperene | Capsules | Jiva Curcumin & Fermented Soy | Jiva, USA
Curcuminoids Complex | Softgels | Curcu-Gel Ultra™ | Tishcon Corp, USA
Curcumin | Softgels | Curcu-Gel™ | Tishcon Corp, USA
Curcumin | Softgels | Curcumin | Solaray Nutritional Supplement, UK
Curcumin Extract, Aloe Vera | Gel | PSORIA-GOLD CURCUMIN GEL | Albi Naturals, Canada
Aloe Vera, Curcumin | Gel | Psoria Gold Curcumin Gel | Omnicure, USA
Curcumin | Softgel | Curcu-Gel Rx-95 | Phyto Therapy,
### INTRODUCTION

<table>
<thead>
<tr>
<th>Active Ingredient</th>
<th>Dosage form</th>
<th>Brand Name</th>
<th>Marketed By</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin</td>
<td>Cream</td>
<td>Vicco Turmeric</td>
<td>Vicco Labrotries, India</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Gel</td>
<td>Curcuma Herbal Bath Gel</td>
<td>Bynature, Thailand</td>
</tr>
</tbody>
</table>

#### Fig. 1.12 Different dosage form of curcumin available in the market alone or with combination

1.5.5 Clinical study of curcumin against cancer

In preclinical studies, curcumin exhibits anticancer effects by modulating a variety of molecules involved in cancer progression. Curcumin can also potentiate anticancer effects of cytotoxic agents. Based on these promising preclinical data, several investigators, have tested this agent in clinical trials. Some clinical benefits were reported; however, plasma curcumin levels remained low, despite taking gram doses of curcumin. Administration of more than 8 g of curcumin failed to increase plasma curcumin levels in a dose-dependent manner in healthy volunteers. Thus, poor bioavailability has been a challenging problem for the clinical application of curcumin. To overcome this problem, many attempts are being made including...
the application of innovative drug delivery system (liposome, nanoparticle, phospholipids, etc.) or the development of new curcumin analogues (Bansal et al. 2011; Li et al., 2004; Li et al., 2005).

Several pilot clinical trials have been reported using curcumin against cancer. These clinical studies were performed in patients of cancer and in healthy volunteer for prevention of cancer. These studies were compiled in Table 1.6.

**Table 1.6: Human clinical data with respect to the use of curcumin against cancer**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Study</th>
<th>Subject</th>
<th>Dose</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prospective Phase I trial of curcumin against Head and Neck cancers</td>
<td>25 patients</td>
<td>500-1200 mg/day</td>
<td>3 months Cheng et al., 2001</td>
</tr>
<tr>
<td>2</td>
<td>Prospective Phase I trial of curcumin against colorectal cancer</td>
<td>15 patients</td>
<td>36-180 mg</td>
<td>4 months Sharma et al., 2001</td>
</tr>
<tr>
<td>3</td>
<td>Prospective Phase I trial of curcumin against Prevention of colon cancer</td>
<td>24 healthy volunteers</td>
<td>500-12,000 mg single oral dose</td>
<td>Lao et al., 2006</td>
</tr>
<tr>
<td>4</td>
<td>Phase II clinical trial of curcumin against Pancreatic cancer</td>
<td>17 patients</td>
<td>8000 mg/daily orally for 2 months</td>
<td>Dhillon et al., 2006</td>
</tr>
<tr>
<td>5</td>
<td>Phase IIa Clinical Trial of Curcumin for the Prevention of Colorectal Neoplasia</td>
<td>40 patients</td>
<td>2g-4g/day for 30 days</td>
<td>Carroll et al., 2011</td>
</tr>
<tr>
<td>6</td>
<td>Phase II Trial of Curcumin in Patients with Advanced Pancreatic Cancer</td>
<td>25 patients</td>
<td>8 g curcumin by mouth daily for 2 month</td>
<td>Dhillon et al., 2008</td>
</tr>
</tbody>
</table>