CHAPTER 1

INTRODUCTION

1.1 CANCER

Cancer, a life threatening disease, is caused by abnormal cell growth that invades nearby tissue, blood or lymph (Cancer library 2008). The cell expands continuously by losing its normal control mechanism and travels to other parts of the body, stimulating the growth of new blood vessels there by drawing on their nutrients. Cancerous cells proliferate throughout the body through the primary site (Overview of cancer 2013).

1.1.1 Origin of Cancer

Cells in our body have a mechanism such that when the cells became old or damaged, they die and are replaced by newer ones. Sometimes, this ordinary process is disturbed and goes wrong. The genetic material (DNA) of a cell is damaged or changed due to mutations, which may affect normal cell growth and division. Generally four mutations are enough to develop a tumor across time as shown in Figure 1.1. The exact number of mutations required for the development of a fully malignant cell from a normal cell is still unknown, but it is known that the number of mutations required is less than ten. When mutations occur, the altered cells grow and divide excessively to yield abnormal growth of malignant cells. At this stage, the cells do not die and new cells are formed. These extra cells may develop a mass of tissue termed as a “tumor”. Not all tumors are cancerous; tumors can
be benign or malignant. Benign tumors are often removed, and mostly do not recur so they are non-cancerous. Benign tumor cells do not spread to other parts of the body. Malignant tumors are said to be cancerous and invade nearby tissues and also spread to other parts of the body, a process known as metastasis.

There are more than 100 types of cancer, including adrenal cancer, anal cancer, brain tumor, breast cancer, cardiac tumors, colon cancer, ductal carcinoma, ovarian cancer, skin cancer, leukemia, lung cancer, lymphoma, pancreatic cancer, prostate cancer, throat cancer, and vaginal cancer. Among the different types of cancers, breast cancer is the second leading cause of female mortality. (Siegel et al 2014).

1.2 BREAST CANCER

Breast cancer is a malignant tumor that grows from cells of the breast and is manifested in serious conditions like changed breast shape, changed nipple and leakage, skin dimpling and red patch of skin which
ultimately leads to bone pain, swollen lymph nodes, shortness of breath or yellow skin. The incidence of breast cancer is increasing worldwide, with a tremendous growth of new cases in developing countries. Breast cancer is the most common type of cancer occurring in Indian women. In urban India, cervical cancer had high morbidity rates among female cancers for the past 15 years, but now breast cancer has overtaken it with a higher morbidity rate. According to the American Cancer Society, globally around 1.7 million women are diagnosed with breast cancer annually and about 4,65,000 are likely to die from the disease. In India 1,44,937 women are affected by breast cancer every year, out of which 70,218 women die each year (Breast Cancer India, PINK Indian Statistics 2014).

The breast cancer that occurs in women is ductal carcinoma and lobular carcinoma. Ductal carcinoma, the most common type of breast cancer, develops from the cells of the milk ducts. Around 80% of breast cancers are invasive ductal carcinomas. Lobular carcinoma is another type of breast cancer that grows from lobes or lobules, and it affects both breasts. It is the second most common type of breast cancer after ductal carcinoma. Noninvasive breast cancers do not spread to the surrounding tissues; they are restricted to the ducts or lobules by themselves; but invasive breast cancers invade and spread to the lymph node and to the surrounding areas of tissues by breaking the normal barriers. (Understanding Breast Cancer 2013).

1.2.1 Treatment Methods for Breast Cancer

Currently, breast cancer is being treated by various methods such as surgery, radiation, use of hormonal drugs, and chemotherapy. There are two types of surgery in breast cancer treatment. In lumpectomy, surgery is carried out to remove the tumor (lump) along with a small amount of normal tissue surrounding it; whereas, in mastectomy, surgery is carried out to remove the cancer affected part of the breast with some normal tissues surrounding it.
In radiation therapy, high-energy x-rays (external beam radiation therapy (EBRT), conformal radiotherapy (3D-CRT), and intensity-modulated radiation therapy (IMRT) are used to kill the cancer cells and shrink the tumors. The genetic materials of cancer are damaged when exposed to radiation and the growth of the cancer cells is stopped. Radiation also affects the normal cells near the cancer cells. But normal cells usually repair themselves, while cancer cells cannot repair themselves.

In hormonal therapy, hormones are removed / blocked such that they can stop cancer cells from growing further. For e.g. endocrine therapy is used to slow down/ stop the growth of prostate and breast cancer. In general, estrogen and progesterone promote the growth of some breast cancer cells in women. Common hormonal drugs used for the treatment of cancer are tamoxifen, aromasin, anastrozole, letrozole, lutenizing androgen deprivation therapy, and luteinizing hormone-releasing hormone (LHRH) analogs, or agonists.

Chemotherapy is a treatment in which drugs are used to stop the growth of cancer cells either by killing the cells or by preventing them from dividing. The chemotherapeutic agents used in conventional treatment have poor specificity to reach the tumor cells. When a drug is administrated into the body intravenously, it is circulated throughout the body and passes many biological barriers to reach the target site, by the time the drug may become inactivated. To overcome this problem, large doses are required to reach therapeutic concentration at the target site; these large doses affect the healthy cells also and result in many negative side effects.

Cancer drugs are delivered to targeted cancer cells effectively by the following methods.
1.2.1.1 Direct introduction of anticancer drugs into the tumor

- Injection directly into the tumor
- Tumor necrosis therapy
- Injection into the arterial blood supply to the cancer
- Local injection into the tumor for radiopotentiation
- Localized delivery of anticancer drugs by electroporation (Electro chemotherapy)
- Local delivery by anticancer drug implants

1.2.1.2 Systemic delivery of drugs to the targeted tumor

- Heat-activated targeted delivery
- Tissue-selective targeted delivery using carrier-mediated transport systems
- Tumor-activated prodrug therapy for targeted delivery of chemotherapy
- Pressure-induced filtration of drugs through vessels to the tumor
- Promoting selective permeation of the anticancer agent into the tumor
- Two-step targeting using bispecific antibody
- Site-specific delivery and light-activation of anticancer proteins
1.2.1.3 Drug delivery targeted to blood vessels of the tumor

- Antiangiogenesis therapy
- Angiolytic therapy
- Drugs to induce clotting in the blood vessels of the tumor
- Vascular targeting agents

1.2.1.4 Special formulation and carriers of anticancer drugs

- Albumin-based drug carriers
- Carbohydrate-enhanced chemotherapy
- Delivery of proteins and peptides for cancer therapy
- Fatty acids as targeting vectors linked to active drugs
- Microspheres
- Monoclonal antibodies
- Nanoparticles
- PEGylated liposome’s
- Polyethylene glycol (PEG) technology
- Single-chain antigen-binding technology

1.2.1.5 Transmembrane drug delivery to intracellular targets

- Cytoporter
- Receptor-mediated endocytosis
- Transduction of proteins and peptides
- Vitamins as carriers for anticancer agents
1.2.1.6 Biological therapies

- Antisense therapy
- Cell therapy
- Gene therapy
- Genetically modified bacteria
- Oncolytic viruses
- RNA interference

The delivery of conventional chemotherapeutic drugs was improved by adopting a combination of controlled release technology and efficient targeted drug delivery. It allows preferential distribution of a drug to the cancer cells (Frank et al. 2007, Lübbe et al. 2001), and a maximum fraction of the delivered drug molecule reacts exclusively with the cancer cells without harming the adjacent normal cells. Recent research is focussed mainly on the development of a delivery vehicle that has the capacity to target and control the release of chemotherapeutic agents in nano scale. Since, the majority of solid tumors exhibit a vascular pore cutoff size between 380 and 780 nm (Hobbs et al. 1998) that facilitates the greater permeability of larger particles (Ganta & Amiji 2009) and greater internalization of drugs as a result of the EPR effect. The nanocarrier containing chemotherapeutic drugs can increase the efficacy, reduce the dosing interval, and maximize patient compliance and targeted delivery through tumur vasculature permeability (Gua et al. 2007).

1.3 CANCER TARGETING BY NANOCARRIERS

For a system to be an ideal targeting system, it has to exist at the target site in appropriate concentration, able to release the drug at a
predetermined rate so that the therapeutic efficacy can be improved for an extended period of time, and produce little or no toxic side effects. The nanocarrier used for targeted delivery should be greater than the intercellular gap of the healthy tissue but smaller than the pores found within the tumor vasculature. The nanocarrier for cancerous tissues uses active or passive target delivery system. In active targeting, the drug carrying nanocarrier is conjugated with a cancerous tissue or cell-specific ligand, whereas in passive targeting, the drug or therapeutic agent incorporated in a nanocarrier reaches the target cancer tissues passively (Gua et al 2007).

1.3.1 Passive Targeting

The tumor microenvironment that favours passive targeting is a leaky tumor vasculature, which is highly permeable to macromolecules relative to normal tissue and the dysfunctional lymphatic drainage system, and, therefore, results in enhanced fluid retention in the interstitial space of the tumor (Gua et al 2007).

Tumor angiogenesis is characterized by vessels with irregular diameters, branching, lacking defining structures of vasculature such as arterioles, capillaries, or venues, and leakiness of tumor vessels. Tumor angiogenesis is caused by openings between defective endothelial cells, wide interendothelial junctions, absent or incomplete basement membrane, absent or loosely attached pericytes (cells that provide support for the endothelial cells), and large numbers of transendothelial channels or pores (Kim 2007) because of abnormal secretion of vascular endothelium growth factor (VEGF), bradykinin, nitric oxide, prostaglandins, matrix metalloproteinases, and other vasoactive factors that cause vasodilatation. Tumor leaky vasculature exhibit enhanced permeability and retention of extravasated large molecules in tumor cells (Koo et al 2005a, Alexis et al 2008).
The tumor lymphatic system is also abnormal, which results in fluid retention in tumors and high interstitial pressure. This characteristic promotes tumor cell intravasation. The broken lymphatic system also leads to retention of nanocarriers in the tumor interstitium as these particles are not cleared from the interstitium quickly. Therefore, the leaky microvasculature and the lack of an intact lymphatic system help in enhancing the permeation and retention effect (tumor-specific deposition) and “passive” cancer targeting by accumulation of the nanocarriers in the tumor at a higher concentration (Figure 1.2) (Alexis et al 2008).

The extent of nanocarrier extravasation is inversely proportional to its size. As the pore size of transport pathways such as open interendothelial gap junctions and transendothelial channels is between 380 and 780nm, the particles should be much smaller than the cutoff pore diameter so that they can reach the target tumor site. The normal healthy vasculature is impermeable for drug-associated carriers larger than 2 to 4 nm (Koo et al 2005a). So the nanocarriers are more effective for the tumor microvasculature (Alexis et al 2008). This extravasation provides the chance of increasing drug accumulation and local concentration in the tumor site, which might reduce drug distribution and toxicity to normal tissues.

The nanocarriers should circulate in the blood for a prolonged time to achieve successful passive targeting. Nanocarriers usually have short circulation half-lives due to the natural defence mechanisms of the body to eliminate them after opsonization by the mononuclear phagocytic system (MPS), termed as reticuloendothelial system. So the particle surfaces are to be modified such that they are “invisible” to opsonization using hydrophilic polymer such as chitosan, polyethylene glycol, and poloxamers (Koo et al 2005a).
1.3.2 Active Targeting

In passive targeting, pharmacokinetic manipulation and size reduction of the nanocarrier takes place, but in active targeting, it is achieved by delivering the drug-encapsulated nanocarrier to the target site using site specific ligands. Cancer tissues have the character of overexpressing some epitopes or receptors, which are used as targets in active targeting. In the local drug delivery system, the nanocarrier is developed by encapsulating the drug; this delivers the drug directly to the cancer cell and also reduces the harmful toxicity to non-cancerous cells adjacent to the target tissue (Ferrari 2005). For metastatic cancers, the location, abundance, and size of tumor metastasis within the body limits its visualization or accessibility, thus making local delivery approaches unfeasible. In this case, the drug delivery vehicle would be administered systemically (Anderson et al 2004). Hence, nanocarriers can be used to actively target the sites by both local and systemic administration by coupling them with ligands such as antibodies, aptamers, peptides, tumor-specific small molecules, or tumor-associated antigens as they are overexpressed at target cancer sites (Figure 1.3). When nanocarriers are
targeted at the tumor, they may be specifically taken up into cancer cells through receptor-mediated endocytosis. The specific targeting, intracellular uptake, and regulated therapeutic delivery of a drug are properties that are achieved through a rational design of nanocarriers (Alexis et al 2008).

Figure 1.3 Active Targeting of anticancer drug-loaded nanocarrier

Currently biodegradable polymeric nanoparticles, hydrogels, micelles, liposomes, microemulsions, nanoemulsions, dendrimers, nanoshells, nanotubes, nanomaterials, polymersomes, nucleic-acid-based nanoparticles, magnetic nanoparticles, polynucleotide nanoparticles and virus nanoparticles are some classes of materials that have been developed and utilized for targeting drugs to the site of cancerous organs (Singh & Lillard 2009, Alexis et al 2008).

1.4 MAGNETIC NANO CARRIERS FOR TARGETED DRUG DELIVERY TO CANCERS

Among the various nanocarriers, magnetic nanoparticles have been used for selective and quantitative accumulation of chemotherapeutic agents
at the target sites with minimal toxicity toward normal cells. The magnetic nanocarriers contain ferromagnetic nanoparticles encapsulating biodegradable polymer(s) along with the drug(s). The ferromagnetic particle should have a low-oxidizing nature and maintain a stable magnetic response such as magnetite and maghemite (Kumar et al 2010). The drug is to be delivered either encapsulated or conjugated on the surface of the magnetic nanocarrier and administered through the IV route, thereby accumulating and delivering the drug locally in the targeted area, using an externally applied magnetic field.

The efficiency for accumulation of magnetic nanocarriers at target site may be affected by various parameters such as particle size, surface characteristic, field strength and geometry, depth of the target tissue, vascular supply, and rate of blood flow. The magnetic nanocarriers, with the help of the magnetic field, extravasate into the tumor area by the discontinuous or “leaky” nature of the tumor microvasculature. Furthermore, the site-specificity of the magnetic nanoparticle is enhanced by attaching high affinity ligand for active targeting combined with external magnetic field guidance to the targeted site (Sun et al 2008).

Magnetism and magnetite are safe for biological systems and adaptable to all parts of the body (Vyas & Khar 2004, Varma et al 2013). Magnetic nanoparticles have magnetism only when an external magnetic field is applied. They are physiologically inert, with no measurable LD$_{50}$ and possess super paramagnetic behavior. Therefore, aggregation and blockage in micro capillaries were avoided. When they enter the bloodstream, by opsonisation, they are coated rapidly with plasma proteins. The reticuloendothelial system recognizes the opsonized particle and may remove it by phagocytosis. Reticulo Endothelial System (RES) evasion could
be accomplished by incorporating the Vitamin E TPGS -like substances into the nanocarriers (Gao et al 2008).

The effectiveness of magnetic nanoparticles depends on the following factors: a) high magnetic susceptibility for an effective magnetic enrichment, b) size, c) super paramagnetic behavior, and d) tailored surface chemistry for specific biomedical application. Super paramagnetic nanocarriers with their unique mesoscopic physical, chemical, thermal, and mechanical properties, offer a high potential for several biomedical applications, such as cellular therapy, tissue repairing, drug delivery, contrast agent in magnetic resonance imaging (MRI), hyperthermia, magnetofection, and detoxification of biological fluids (Gupta & Gupta 2005).

The drawbacks of magnetic nanocarriers include the following: an encapsulated drug cannot be targeted to deep seated organs in the body, an external magnet should have relatively constant gradients to avoid local overdosing with toxic drugs, and an encapsulated drug needs a specialized magnet for targeting, an advanced technique for monitoring, trained personnel to perform the procedure, and a permanent deposition of a large fraction (40-60%) of the magnetite in the target tissues (Vyas & Khar 2004).

1.5 MICROEMULSIONS

In recent years, microemulsions (MEs) have become one of the promising drug-delivery systems because of their unique solubilization property for the delivery of poorly soluble drugs with enhanced bioavailability. ME is characterized as a non-equilibrium, heterogeneous system of two immiscible liquids, where the nanodroplets of oil are dispersed in aqueous continuous phase and stabilized by appropriate surfactants under homogenization (Mason et al 2006). The oil phase in the ME serves as the reservoir for the lipophilic drugs, protects the drug molecule from the external
physiological environment, offers controlled drug release, and can deliver the drug through various routes of administration such as oral, topical, ocular, pulmonary, and intravenous routes. (Lu et al 2013, Shah et al 2010, Kumar & Sinha 2014, Shende et al 2007, Li et al 2011, Chen et al 2011).

Hoar and Schulman obtained the ME by titrating a milky emulsion with hexanol. (Hoar & Schulman 1943). Many authors have defined the term ME in their own words. Danielsson and Lindman (1981) defined microemulsion as a system of water, oil, and surfactant(s), which is a transparent, single optically isotropic and thermodynamically stable liquid solution, which was accepted as the definition of ME worldwide. This definition excludes the aqueous solutions of surfactants without additives, liquid crystalline phases, coarse emulsions, surfactant free systems, and aqueous surfactant solutions containing only water soluble non-electrolytes.

The MEs and macroemulsions differ with respect to their size and the shape of the particles dispersed in continuous phase. An emulsion is nothing but spherical shaped droplets of size ranging from 1 to 20 µm dispersed from one phase into another; however, the ME can have a varied structure with droplet-like swollen micelles as well as a bicontinuous state in the form of chains, clusters, or noodle shaped state, making the usual oil-in-water (o/w) and water-in-oil (w/o) distinction irrelevant. MEs are transparent liquids as their droplet size ranges between 10 and 200 nm. The formulation of macroemulsions needs a high input of energy, which is not required for the formulation of the MEs. (Jayakrisnan et al 1983, Tenjarla 1999, Ghosh et al 2006, Kale & Patravale 2008).

The formulation of MEs can happen only if the following conditions are met: the tension at the oil/water interface is very low, the region is highly flexible and soft, and the penetration and association of oil molecules to the interfacial surfactant film is possible (Schulman et al 1959 ).
To bring the interface tension to a low level, we need to select carefully and accurately the components in appropriate proportions. The flexibility can be altered by adding the cosurfactants. Introduction of a cosurfactant may also expand the monophasic zone of systems, which are capable of forming a ME without a cosurfactant (Aboofazeli & Lawrence 1994, Karasulu 2008). A surfactant containing a short lipophilic chain length or fluidizing groups (e.g., unsaturated group) is capable of producing a ME without addition of a cosurfactant.

1.5.1 Phase Manifestation and Structure of the ME

The ME system has multi component composition; so it is regarded as a complex process. The mixing of oil, water, and amphiphile depends on their proportions, nature, temperature, and additives, and these may form a variety of isotropic or mesomorphic solutions as well as complex multiphase equilibria. The nature of the system can be studied by identifying the phase equilibria and determining the phase boundaries. (Li et al 1996, Richardson et al 1997, Aarra et al 1999, Baran, 2001, Silas et al 2001, Dizaj 2013). By examining its physical appearance, we can easily predict the phase and structure. (e.g., emulsions are opaque and phase separation takes place after a while; lamellar structure and cubic phases are highly viscous). These can be shown by examining with a polarized light (crystalline phases) and thus can be recognized from an actual ME.

Different types of equilibria can be achieved by adjusting the constituents’ proportions. The phase forming behavior (Paul & Moulik 1991, 1997, Yamaguchi et al 1999, Zarur et al 2000, Arvidsson & Soderman 2001) of ternary and quaternary ME forming combinations depends on the concentration and chemical nature of the surfactant(s), oil, types of polar medium (water, glycerol, glycol etc.), the presence of additives (especially electrolytes), the temperature, the pressure, and so on (John & Rakshit 1993,
Binks et al 1997, Ferdinand et al 2000). The extent of the phases and their internal structure is obviously influenced by the above mentioned intrinsic and extrinsic factors. The MEs formed can be one of three types: 1) o/w, in which water is the continuous phase; 2) w/o, in which oil is the continuous phase; 3) bicontinuous, in which approximately equal volumes of water and oil exist.

The interface of the MEs changes continuously since the ME is a dynamic system (Lam & Schechter 1987). ME structures possibly approach regular or reverse ‘swollen micelle’ droplet-like shapes in a diluted system with a minor % of an oil or water phase. (Lawrence 1994). However, the components inside the ME usually form nonspherical aggregates, which may be more or less continuous in the phase with highest volume fraction (Stilbs et al 1980, Lindman et al 1981, Stilbs & Lindman 1984). When these nonspherical aggregates are titrated with the phase of the lowest volume fraction, they get transformed into bicontinuous structures, which easily invert to ‘reversed’ aggregates. Thus, ME systems do not often display emulsion-like behaviour with sudden inversion of the ‘swollen micelle’, and the emulsion terminology of the systems as o/w or w/o is, therefore, in many situations not applicable to MEs.

Some MEs show emulsion-like behavior by forming small droplet-like ‘swollen micelle’ structures with a dispersed and a continuous phase (Aboofazeli et al 1995, Constantinides & Yiv 1995, Narang et al 2007, Cao et al 2011). These MEs lose their characteristic structures by swelling and forming colloidal structures (typically regular macroemulsions) by adding the dispersed phase continuously. Separate regions of existence for o/w and w/o droplet-like MEs can also be observed in some systems (Aboofazeli & Lawrence 1993, 1994, Aboofazeli et al 1995).
The exact mechanism for the structural formations and transitions and its relation to the physico-chemical properties of the components is still not recognized. An important factor that determines the ME structures and the structural transitions by changing the component ratios is the flexibility of the surfactant film. A very rigid surfactant film will result in droplet-like shapes, which will not allow the bicontinuous structure and will limit the existence of the ME structures.

A more flexible surfactant film will result in the formation of several different structures like aggregates and bicontinuous structures, which will broaden the range of their existence. The internal structure of an ME vehicle plays a significant role in the diffusivity of the phases and also in the diffusion of a drug in the respective phases (Kreilgaard 2001).

1.5.2 Formation of MEs

The theories that explain formation and the stability mechanism of MEs are:

1. Interfacial mixed film (Schulman et al 1959, Prince 1967)
The simplified thermodynamic equations are as follows:

The free energy of ME formation $F$ can be divided into three contributions, that is,

$$F = F_i + F_b + F_s$$  \hspace{1cm} (1.1)

where $F_i$, $F_b$, and $F_s$ are the contributions of interfacial energy, the bending energy of the surfactant layer, and the mixing entropy, respectively. The interfacial energy is the product of interfacial tension ($\gamma$) and interfacial area ($a$). Therefore, the term $F_i$ is written as

$$F_i = \gamma a$$  \hspace{1cm} (1.2)

The term $F_i$ is dominant in normal water-oil-surfactant systems because of the high interfacial tension. Concurrently, it leads to phase separation as a result of the increase in the size of the dispersed phase. Here ‘$a$’ has to be as small as possible. The term $F_i$ is dominant in normal water-oil-surfactant systems due to the high interfacial tension. Concurrently, it leads to phase separation due to increase in the size of the dispersed phase. Here ‘$a$’ has to be as small as possible.

In the case of the ME system, the contribution of the term $F_i$ is not comparable to the others because of a very low value, and thus, there can be an optimal droplet size. The surfactant layer of the ME droplets has been known to show elastic behavior. Its contributions can be expressed as

$$F_b = \frac{k_b}{2} \left( \frac{1}{R_1} + \frac{1}{R_2} + \frac{1}{R_0} \right)^2 + \frac{k_s}{R_1 R_2}$$  \hspace{1cm} (1.3)

where $R_1$ and $R_2$ are the principal radius and $R_0$ is the spontaneous radius, (Helfrich 1973, Szleifer et al 1990), $k_b$ is called the bending constant and it is
on the order of kT (Szleifer et al 1990), where k and T have their usual meanings, for microemulsion droplets, and $k_s$ is called the saddle bending constant. It has the opposite sign to $k_b$, and its absolute value is much smaller than that of $k_b$. The spontaneous radius $R_0$ is dominated mainly by the molecular shapes of both surfactants and solvents, but some physical interactions, such as electrostatic interaction and hydration force, also play important roles. The shape of a surfactant molecule is expressed quantitatively by the “critical packing parameter” P, that is

$$P = \frac{v}{a_0 l_c}$$  \hspace{1cm} (1.4)

where $v$, $a_0$, and $l_c$ are the volume of the hydrophobic part, the cross sectional area of hydrophilic/hydrophobic interface, and the length of the hydrophobic chain, respectively (Israelachvilli et al 1976, Mitchell & Ninham 1981). When P is between 0 and 1, o/w systems are likely to be formed, and when the P is >1, w/o MEs are expected. However, P does not take into consideration the penetration of oil and cosurfactant molecules into the surfactant interface and the hydration of the surfactant head groups. This parameter is very useful to explain the investigated changes in the morphology of MEs. This parameter cannot be used to obtain the desired structures at all times, because each value depends on various factors such as salt concentration, the species of organic solvent, and temperature.

The biggest contribution to $F_S$ is the mixing entropy of each component. The ideal mixing of each component is the most favourable state for this term. Therefore, the larger structure is favourable for $F_i$, but the smaller structure is favorable for $F_S$. There is an optimal aggregate size for the $F_b$. If the contributions of these terms are comparable, an equilibrium structure can be obtained. However, this structure may be destroyed by
additional components such as drugs and may not be formed in physiological environments (Kawakami & Yoshikawa 2001).

1.5.3 Formulation of MEs

The ME is formulated with a combination of three to five components such as an oil phase, an aqueous phase, a primary surfactant, and in many cases, a secondary surfactant (cosurfactant), and sometimes electrolytes. These isotropic systems are formed by spontaneous interaction between the components, and so they are more difficult to formulate than an ordinary emulsion. The formation of an associated structure of MEs is not only determined by the chemical nature of the components but also by the ratio of the components. Therefore, construction of the phase diagram is a key to study the system and to identify the ME existence zones. Selections of components, the right proportion of right components, plays a major role in the formulation of the MEs. Only few excipients are acceptable for the development of parenteral ME products. The excipients chosen for the parenteral route should be biocompatible, sterilizable, nonpyrogenic, and nonirritant to nerves and nonhaemolytic. For example, sugar surfactant is biocompatible and its solubilization potential is quite good, but it is hemolytic (S¨oderlind et al 2003). The components to be considered in the formulation of MEs are as follows.

1.5.3.1 Oil phase

In the formulation of ME, the selection of a proper oil phase plays a major role as it influences the choice of other ingredients mainly in the case of the o/w ME. The selection of the oily phase will be such that the oil has maximum solubilizing potential for the drug chosen. This supports in attaining the maximal drug loading capacity in the formulation. At the same time, the ability of the selected oil to yield systems with a larger ME region is
also important. A single oily component fulfilling both requirements is very difficult to find. It is known that the chain length of the oily phase is directly proportional to the solubilization capacity of lipophilic moieties (Vandamme 2002) but inversely proportional to microemulsification. That is, oil with a very long hydrocarbon chain (high molecular volume), such as soybean oil, is difficult to microemulsify, whereas oils with shorter chains (low molecular volume), such as medium chain triglycerides (MCTs) and fatty acid esters (like ethyl oleate), are easy to microemulsify (Malcolmson et al 1998a, 1998b, Warisnoicharoen et al 2000).

The selection of the oily phase correlates with the capacity to solubilize the drug and assist formation of the ME. In some cases, a mixture of oils were used to satisfy both requirements. For example, a mixture of fixed oil and MCT is used to have a better balance between drug loading and emulsification (Jumaa & Mueller 2002a). The mixtures of different types of oils are capable of forming stable MEs as evident from the commercial formulation Neoral®. The oil phase of Neoral® is a mixture of mono-, di-, and triglycerides. Gelucire® is a well-known material for preparing ME (Kawakami & Yoshikawa 2001). The regulatory acceptability of various potential carriers is the restricting factor in practical use. Most of the recently established excipients (chemically modified glycerides), such as medium chain glycerides, hydrogenated glycerides, and glycerol esters, propylene glycol esters of fatty acids, and mixtures of propylene glycol esters/glycerol esters, are receiving wider regulatory acceptance. Recently, MEs based on medium chain mono- and di-glycerides have also been reported (Nornoo et al 2008, Nornoo & Chow 2008). Capmul® MCM, which has a higher solubilization potential than that of the fixed oils and MCT, are easy to microemulsify. However, parenteral safety of these excipients for long-term administration should be considered.
Emulsions based on Vitamin E (α-tocopherol) have been projected recently as they have good solubilizing potential (Constantinides et al 2004). It is also reported that Vitamin E has the ability to solubilize active pharmaceutical ingredients (API) that are difficult to solubilize by standard oily components, for example, itraconazole, saquinavir (Constantinides et al 2004).

1.5.3.2 Surfactants

Pharmaceutical scientists face a great challenge in the selection of surfactant(s) looking at their clinical acceptability for a particular application from the large number of surfactants (Swenson & Curatolo 1992). The hydrophilic-lipophilic balance (HLB) value is considered as the basic value for selecting the surfactant used in a microemulsion. For w/o MEs, the HLB value for emulsifiers should be in the range of three to eight, whereas, for o/w microemulsions, the HLB value should be in the range of 8–18. The emulsifier is selected by the average HLB requirement of the proposed ME (Eccleston 1992, Attwood 1994). Critical packing parameters can be also adopted for the selection of surfactants (Israelachvilli et al 1976, Mitchell & Ninham 1981). The surfactants used in the ME formulations will be one of the following (i) non-ionic, (ii) zwitterionic, (iii) cationic, or (iv) anionic.

Most of the pharmaceutical ME formulations use zwitterionic or nonionic surfactants as these are less toxic when compared to the charged ones (Osborne et al 1988, Swenson & Curatolo 1992, Bansal et al 2011) and also less sensitive to changes in environment, whereas the charged MEs are sensitive to changes in salt concentration, which affects the electrostatic interaction. The electrostatic interaction plays the main role in maintaining the microemulsion structure. The salt concentration is inversely proportional to electrostatic repulsion, which makes changes in the shape of surfactant
assemblies. Therefore, using only charged surfactant for pharmaceutical products is very rare (Kawakami & Yoshikawa 2001).

However, when the charged surfactant is mixed with nonionic surfactants, it is effective in certain cases. It has been proven that when a charged surfactant is added to the o/w ME, the oil capacity of the droplet increases (Kawakami et al 2002). In addition, there seems to be an optimum-mixing ratio of two surfactants, which seems to be because of the heterogeneous mixing of two surfactants. Such nonideal mixing of surfactants has been reported (Haque et al 1999).

Phospholipids are notable examples of zwitterionic surfactants and exhibit excellent biocompatibility. Lecithin-based MEs, both o/w and w/o, have been recently well explored as alternative drug delivery systems that avoid problems of toxicity associated with some of the nonionic surfactants. Usefulness of lecithin in topical and parenteral MEs have been well demonstrated by several recent studies (Brime et al 2002, Paolino et al 2002, Hwang et al 2004, Rhee et al 2007, Yuan et al 2008, Raut et al 2012, Lin et al 2014).

The most preferred nonionic surfactants are poloxamers, polysorbates, spans, vitamin E TPGS, and solutol HS 15 (Strickley 2004). Recently, polyethoxylated castor oil derivatives (Cremophore®EL, Cremophore® RH 40, and Cremophore® RH 60) have been used in marketed co-solvent based formulations (Akers 2002). However, the polyethoxylated castor oil derivative, Cremophore®EL (PEG-35-castor oil), a surfactant with very good parenteral acceptability causes several adverse effects such as anaphylactic shocks and histamine release, which limits its use (Tije et al 2003). Surfactants such as polyoxyethylene alkyl ethers at higher concentrations lead to haemolysis on parenteral administration (Söderlind et al 2003). Among poloxamers, poloxamer 188 should be selected since
Poloxamer 407 may lead to hyperlipidemia with long-term administration (Palmer et al. 1997, Blonder et al. 1999). So the above mentioned factor should be considered while selecting the surfactants. Biodegradability of nonionic surfactants raises issues with regard to long-term toxicity, especially in chronic use.

Currently, Solutol® HS 15 (PEG-660-12-hydroxystearate) has appeared as replacement for Cremophore® EL since it has more tolerance over parenteral administration in comparison to Cremophore® EL and possesses the ability to withstand freeze–thaw cycling very efficiently (Jumaa & Mueller 2002b). The parenteral ME formulation with use of Solutol®HS 15 has been reported. (Zhao et al. 2005, Rhee et al. 2007, Date & Nagarsenker 2008).

Safe surfactants other than lecithin and nonionic surfactants have also been reported. The potential of a biodegradable version of n-alkyl amine N-oxides either in isolation or in combination with lecithin and amine N-oxide surfactant, di-methyldecylamine N-oxide, for use in ME formation has been demonstrated (Satra et al. 1995, Tolle et al. 2000, Warisnoicharoen et al. 2000). Sugar surfactants such as alkyl glucosides are widely considered as biodegradable, but they still exhibit a level of hemolytic activity at par with that exhibited by the polyoxyethylene n-alkyl ethers (Desai 1990, Kahlweit et al. 1996, Ryan et al. 1997, Stubenrauch et al. 1997, Ryan & Kaler 1997). The use of sucrose fatty acid esters and polyglycerol fatty acid esters as surfactants in the stabilization of ME phases has also been investigated (Keipert & Schulz 1994, Thevenin et al. 1996, Bolzinger et al. 1998, 1999, Garti et al. 1999, Sahle et al. 2012). Monosorbitan glyceride has also been used as a surfactant in its own right to stabilize a triglyceride-in-water ME system and used in self-microemulsifying systems (Constantinides et al. 1996).
1.5.3.3 Co-surfactants

In most of the cases, a surfactant alone is unable to reduce the oil/water interfacial tension to form an ME that leads to the addition of an amphiphilic short chain molecule or cosurfactant to reduce the surface tension close to zero. Short chain alcohols have been used as cosurfactants. The range of short chain length is from \( \text{C}_2 \) – \( \text{C}_{10} \), and the amphiphilic nature of these agents allow them to collaborate with surfactant monolayers at the interface such that it affects their packing (Lawrence & Rees 2000, Vandamme 2002). On addition of a cosurfactant, the chemical composition and relative hydro/lipophilicity of the system is altered as they distribute themselves between an aqueous and an oily phase.

The use of cosurfactants in the formulation of MEs raises acceptability issues in the pharmaceutical field because of their significant toxicity and irritancy issues. On the other hand, the aqueous solubility of cosurfactants in o/w ME systems is higher than that of the principal surfactant. As a result, when diluting the o/w ME, the cosurfactant partitions more strongly to the aqueous phase. This character affects the stabilization of microemulsion droplets by reducing the cosurfactant concentration at the oil/water interface. Short chain amines (Wormuth & Kaler 1987) and alkanoic acids (Aboofazeli et al 1994) have been proposed as alternatives to medium chain alcohols as they act like alcohols.

Ethanol, benzyl alcohol, propylene glycol, glycerol, PEG 400 (Lawrence & Rees 2000, Bagwe et al 2001, Vandamme 2002), sodium caprylate (Morey et al 2004), 2-Pyrrolidone (Soluphor® P), and N-methyl pyrrolidone (Pharmasolve®) (Akers 2002) were used as cosurfactants in parenteral ME formulations. Glycofurol (Tetrahydrofurfuryl alcohol PEG ether or Tetraglycol) is an amphiphilic liquid that has ability to behave as a

A higher concentration of polyhydric alcohols (propylene glycol and glycerol) is needed to be used as additives in o/w ME to help microemulsification. However, a higher concentration of propylene glycol leads to pain during injection and hemolysis. Further, the MEs made with polyhydric alcohols are unstable when diluted with water because of their high aqueous solubility (Attwood et al 1992, Joubran et al 1994).

Benzyl alcohol, short chain amphiphile, up to 1% (w/v) was used as cosurfactants for the formation of MEs (Ryoo et al 2005, Rhee et al 2007). It is partially miscible with water and possesses local anesthetic and preservative properties (Rowe et al 2006b). However, in most of the cases, it has been found to be inferior when compared to ethanol.

1.5.3.4 Aqueous phase

The nature of the aqueous phase also plays a major role in the formulation of MEs, particularly in the case of parenteral MEs. The aqueous phase used should be isosmotic to the blood. The isosmotics can be achieved by the use of additives like electrolytes (sodium chloride), glycerol, dextrose, and sorbitol. The area of existence of MEs can be affected by the use of additives. The pH of the aqueous phase is also another factor which might influence the phase behavior of the ME.

1.5.4 Phase Diagram and Preparation of ME

The ternary phase diagrams of systems containing oil-surfactant-water are used to recognize ME existence regions. A hypothetical pseudo-ternary phase diagram schematically representing conventional micelles,
reverse micelles or w/o MEs, o/w microemulsions, and coarse emulsions is shown in Figure 1.4. Depending on the nature of the oil and surfactant and their mixing ratio, oil-surfactant mixtures can be either clear and isotropic solutions or oily dispersions in the absence of water. Since w/o MEs are also known as reverse micelles, these two phases are represented by the same field on the phase diagram. Coarse emulsions, which are thermodynamically unstable two-phase dispersions, are represented along the oil-water line.

**Figure 1.4** A hypothetical pseudo-ternary phase diagram of an oil/surfactant/water system with emphasis on microemulsion and emulsion phases

Within phase diagrams, existence fields are shown where conventional micelles, reverse micelles, or w/o MEs and o/w MEs are formed along with bicontinuous MEs and coarse emulsions.

Self-emulsifying o/w and w/o ME formulation needs four basic components, namely, a blend of two surfactants, oil, and an aqueous phase. As mentioned above, these systems can be best explained by pseudo-ternary phase diagrams where a constant ratio of two of the components is used and
the others are varied. For example, the mixture of the oil and oil soluble low HLB surfactant can be kept, fixed, and titrated against with known amounts of the high HLB surfactant and water. As the ME formation is thermodynamically favored, the order in which the components are mixed should not have any influence on the final size and stability of the particle. To prepare the ME one can just blend the oil, water, surfactant, and cosurfactant with mild agitation (Eccleston 1994, Attwood 1994).

The ME formed from long chain glycerides, such as soybean oil and monoolein, can be prepared by mixing the components at temperatures between 40–60°C to reduce viscosity. The components that are solid at room temperature, such as monoolein, can either be premelted at the appropriate temperature or should be dissolved in one of the liquid components before mixing with the oil and other surfactants. In addition to improving their stability, these MEs are equilibrated at 40–50°C for about 24 h. MEs of MCTs can be prepared spontaneously at room temperature over a wide range of compositions. It is advantageous to prepare thermoliable drugs, particularly peptides, at an ambient temperature (Constantinides 1995).

1.5.5 Characterization of MEs

The structure of water containing MEs is detected after identifying a monophasic region by simple tests such as dye solubilization, dilutability by an excess of the dispersed phase, viscosity, and conductance measurements. The characterization of MEs includes the following: measurement of interfacial tension, determination of density, viscosity, refractive index, and particle size. From viscosity measurements, we can conclude the presence of rod-like or worm-like reverse micelles (Yu & Neuman 1995, Angelico et al 1998), and from conductivity measurements we can verify whether an ME is oil-continuous or water-continuous as well as monitor percolation or phase inversion phenomena (Yu & Neuman 1995, D’Angelo et al 1996,

Scattering methods play a vital role in interpreting ME structure, and the methods employed include dynamic and static light scattering or photon-correlation spectroscopy (PCS) (Constantinides & Scalart 1997, Ktistis 1997), small-angle neutron scattering (SANS) (Bolzinger et al 1999), and small-angle X-ray Scattering (SAXS) (Nakamura et al 1999, Hirai et al 1999). By scattering techniques, we can determine structural dimensions but cannot discriminate between unicontinuous and bicontinuous structures. As the MEs are reproduced as a result of reversible self-assembly, this depends upon the concentrations of the components in the system, so it is not advisable to adopt dilution procedures. (Chang & Kaler 1986, Zemb et al 1987). To study the internal physicochemical states of ME, many methods are used such as transmission electron microscopy (TEM), time resolved fluorescence quenching, ultrasonic interferometry, ultrasonic absorption, transit electrical birefringence, thermal conductivity, infrared spectroscopy, and calorimetry. A detailed study on the use of different methods was recently presented by Moulik & Pal (1998).

The ME used for pharmaceutical uses was composed of water and isopropyl myristate with a constant amount of Tween-40 and Imwitor 308 at a mass ratio of one, characterized by the following techniques for identification of its type and structure: density and surface tension, viscometry, electric conductivity, differential scanning calorimetry (DSC),
and SAXS (Podlogar et al 2004). ME based on monodisperse hard spheres effectively fitted the SAXS data in w/o MEs and projected that, depending on composition of ME, elongated and/or spherical droplets are formed. It also revealed the involvement of strong attractive interactions in o/w systems. Results of conductivity, viscosity, density, and surface tension measurements also established the prediction of a percolation transition to a bicontinuous structure. The type of ME is identified by DSC by detecting the degree of water interaction with surfactants. The study concluded that even for complex systems, the above techniques can be used to determine the type and structure of such ME systems and to predict the partitioning and release rates of drugs from MEs (Podlogar et al 2004). The electrical conductivity and apparent viscosity study is used to characterize the influence of drug incorporation in the structure of MEs (Djordjevic et al 2004).

Drug release is the most important characteristic based on the selection of proper excipients. The factors that influence drug release are oil-water partition coefficient, phase volume ratio, droplet size of the dispersed phase, distribution of the drug in the various phases of the system, potential interaction between the drug and the excipients, and the rate of drug diffusion in both phases of the system. Hydrophilic drug release from the o/w ME is influenced by the composition of the ME. The drug release rate shows a linear relationship with oil-water partition coefficient in the absence of interactions between the drug and the surfactant. In addition, the partition coefficient of a drug among oil, water, and cosurfactant is used to establish the drug partition between the dispersed and continuous phases.

The amount of cosurfactant in an o/w ME is directly proportional to partitioning of the lipophilic drug into the dispersed oil phase and inversely proportional to the rate of drug release. The interaction between the drug and surfactant will also slow down the drug release rate (Hu et al 2011).
1.5.6 Drug Delivery Potential of MEs

The potential of MEs to be used as drug delivery vehicles for different routes of administration, viz, oral, topical, parenteral, ocular, and pulmonary, has been explained in several studies. (Butani et al 2014, Lu et al 2013, Ren et al 2012, Shah et al 2010, Tayade et al 2010, Lawrence & Rees 2000). The use of MEs as drug delivery vehicles was first reported in 1974 (Attwood et al 1974). Research on MEs in the pharmaceutical field commenced only after the publication of a review article by Bhargava and coworkers (Bhargava et al 1987). After 1987, the research papers published on this topic increased. In 1955, ME as a drug delivery vehicle for oral administration of therapeutic peptides was studied. (Sarciaux et al 1995). To enhance drug dissolution and oral absorption, the physical and biopharmaceutical characteristics of lipid MEs were evaluated by Constantinides (1995). Tenjarla in 1999, studied MEs and its pharmaceutical applications, highlighting the factors affecting drug delivery by these systems. Formation, phase behavior, and characterization of MEs with recent developments and new opportunities were presented in a recent review article (Lawrence & Rees 2000). Kreilgaard studied cutaneous delivery of drugs using MEs and the influence of their composition, components, and structure on their drug delivery potential. The details of important works carried out to demonstrate the potential applications of MEs as drug delivery vehicles for different routes of administration such as oral, topical, and parenteral are presented in the following sections.

1.5.7 Oral Delivery

Scientific innovations in drug discovery and the biotechnology process have developed many new drugs. Many of these drugs have poor water solubility and, as a result, have poor dissolution in the gastro intestinal (GI) tract and erratic and unpredictable drug absorption after oral
administration. Nowadays, peptide drugs have been used as effective therapeutic agents; but their physical nature is a disadvantage, and the enzymatic barrier imposed by the GI tract limits oral administration. Peptide drugs are generally specific in their action with a short biological half-life and also undergo degradation in the GI tract, which necessitates frequent administration of the peptides via the parenteral route. So the delivery of peptides via other routes of administration (oral and/or nasal) is gaining importance; this also provides commercial success for peptide formulations. To overcome these problems and to increase the bioavailability of both hydrophilic and lipophilic drugs as well as hydrophilic peptides and proteins, the drugs can be incorporated into ME vehicles (Sarciaux et al 1995, Tenjarla 1999).

Recently, self-microemulsifying drug delivery systems (SMEDDS) were developed for several drugs such as raloxifen, paclitaxel, simvastatin, and 9- nitro camptothecin (CPT) (He et al 2003, Kang et al 2004, Lu et al 2008, Thakkar et al 2011) to improve the oral bioavailability of the encapsulated hydrophobic drugs.

Self-emulsifying drug delivery systems (SEDDS) was used for the oral administration of the drug using ME systems. SMEDDS and SEDDS can be described as isotropic solutions of drug(s), oil, and surfactant(s), which form o/w (micro) emulsions spontaneously in aqueous media under mild agitation. The absorption of the drug from these systems is influenced by a number of factors such as phase volume ratios, particle size of the systems in the GI tract, partition coefficient of the drug, emulsification capacity of systems, metabolism of the oil present in the systems, effect of lipid components on gastric emptying, drug solubilization potential of the systems, and the influence of individual components of the systems on absorption
enhancement. Designing of systems depends on the physical nature of a drug and the particular need to be fulfilled (Tenjarla 1999).

1.5.7.1 W/O microemulsion

Water soluble drugs, particularly peptides and proteins, are formulated in w/o ME because these systems are capable of overcoming metabolic and physical barriers to water soluble compounds. Further, these systems do not require high temperature and /or homogenization for their preparation, which is an undesirable condition for thermolabile peptides and proteins. O/w microemulsion system comprises of a fatty ester, a sorbitan ester-polyoxyethylene glycol monoether mixture of surfactant, short chain alcohol, and water. These systems have higher absorption of cyclosporine A than a macroemulsion. It has been proposed that the oral bioavailability of the drug increases when the particle size of the regular emulsion decreases (Tarr & Yalkowsky 1989) and proved that bioavailability of cyclosporine A with an ME is enhanced when compared to the solution (Ritschel et al 1989). When compared with ME, long chain fatty acid showed with branched-chain fatty acid ester, ME with long chain fatty acid higher bioavailability of vasopressin and insulin (Ritschel 1991). It is likely that the observed differences in bioavailability are related, at least in part, to the large reduction in lipolytic activity exhibited by lipases toward branched chain fatty acid substrates (Rees et al 1991). MEs formulated with straight chain fatty acid esters will disintegrate because of rapid enzymatic hydrolysis being degraded in the GI tract. As a result, the products formed from disintegration are surface active and will stabilize any (micro) emulsion that may form and also act as membrane permeation enhancers (Yeh et al 1994). It was also observed that cyclosporine A is better absorbed rectally when delivered as ME gel (Ritschel et al 1990).
Highly water soluble and poorly absorbed RGD peptide and SKF106760 with MEs of different compositions and particle sizes were observed to have enhanced intraduodenal bioavailability than the solution formulations. SKF 106760 is stable in enzymatic hydrolysis in the GI tract and has low membrane permeability. It is understood that the lipid composition of the formulation is responsible for an increase in bioavailability. The relationship between the particle size of the microemulsion and increased bioavailability was not established. This concept was further confirmed with marker molecule calcein (Constantinides et al 1995, 1996). However, the smaller particle size improved bioavailability of lipophilic dissolution rate limited drugs (Myers & stella 1992). The mechanism by which w/o microemulsions enhance the oral bioavailability of water soluble drugs is not yet well elucidated. The proposed mechanisms are lipid absorption pathways or enhancement of permeability (Constantinides et al 1995, 1996). It was reported that w/o microemulsions that convert to o/w systems upon exposure to water delivered the peptides. Improved stability of proteins occurred when medium chain glycerides were present in the ME formulation (Owen et al 1992).

1.5.7.2 O/W microemulsions


The bioavailability of drugs that are not easily soluble in water is enhanced by SMEDDS by solubilization in the excipient matrix or interface and dispersion in the GI tract. The small size of the dispersed oil droplets in the nanometer range and a very high surface area to volume ratio of MEs are
also responsible for faster drug release, which can be designed further to make the release characteristics independent of the GI physiology and the fed/fasting state of the patient. In some cases, an important factor to enhance the bioavailability is to protect the drug from degradation. Further enhancement may be due to inhibition of P-glycoprotein and/or cytochrome P450 to increase intracellular concentration and residence time. In some cases, reduction in intestinal first-pass metabolism by stimulation of lipoprotein/ chylomicron production also enhances the bioavailability (Shah et al 1994, Constantinides 1995, Pouton 1997, Hauss 2002, Itoh et al 2002).

SMEDDS is formed by mixing the lipid mixtures with higher surfactant and cosurfactant/oil ratios (Constantinides 1995). The droplet size depends on the concentration of the surfactant being used.

In certain cases, the increased surfactant concentration leads to droplets of smaller sizes because of localization of the surfactant molecules at the oil–water interfaces. The oil droplets will be stabilized, for example, as in a mixture of saturated C₈-C₁₀ polyglycolized glycerides (Levy & Benita 1990). Alternatively, the droplet size may increase with increasing surfactant concentrations (Kommuru et al 2001). This phenomenon could be attributed to the interfacial disruption augmented by the enhanced water penetration into the oil droplets mediated by the increased surfactant concentration and leading to ejection of oil droplets into the aqueous phase (Pouton 1997).

It has been reported that the bioavailability of a drug is improved with small droplets penetrating between the pleats of the brush border membrane easily (Kawakami & Yoshikawa 2001). However, Gershanik et al (1998) found that for SEDDS, the optimal droplet size was 100–500 nm with the lesser effect of small droplets being explained as an immediate neutralization of positive charges on the droplets by mucin. The absorption
behavior of drugs depends on the physical stability and the components of ME formulations, mainly oils and surfactants.

In addition, a high concentration surfactant leads to micellar solubilization of lipophilic drugs, which in turn affects the amount of free drug and the extent of absorption (Chiu et al 2003). This was demonstrated by the intestinal absorption of griseofulvin in rats, which decrease in the presence of 20 milli molar taurocholate. It has also been shown that in-vitro permeability studies conducted utilizing the Caco-2 cell line a decrease in the permeability of cyclosporin A in the presence of surfactants such as Cremophor EL RH40 and D-alpha-tocopherol polyethylene glycol 1000 succinate at concentrations above 0.02% w/v, was seen, which was attributed to micellar solubilization (Chiu et al 2003).

The effect of bile on an ME was demonstrated, and it showed that when bile was added to ester oil of Tween-80 ME, and polyoxyethylene -9- monolauric ether ME, the former led to faster degradation than the latter, suggesting that the latter ME was too stable against bile and thus absorption did not improve at all. It indicated that too stable an ME, that is, one that forms very quickly and has sterically protected interfacial structure, is not favorable to the formulation (Kawakami & Yoshikawa 2001). The improved solubility and bioavailability of biphenyl dimethyl dicarboxylate, a drug that is not easily soluble in water and is used in treating liver diseases, when formulated as premicroemulsion concentrate composed of Tween 80 and Neobee M-5 in the ratio 2:1 and 35% of triacetin has been reported. This improvement was possibly due to the increase in solubility and immediate dispersion of the drug in the GI tract (Kim et al 2001). A study carried out for the assessment of oral bioavailability of the lipophilic cholesterol lowering agent simvastatin formulated in SMEDDS in fasting dogs demonstrated 1.5-fold increase in bioavailability compared to
the conventional tablet (Kang et al 2004). An increase in the bioavailability of the 5α-reductase inhibitor that is not easily soluble in water in beagle dogs has also been reported (Matuszewska et al 1990).

In another study done on dogs, the oral bioavailability of the lipid soluble compound coenzyme Q₁₀ was substantially improved when this compound was administered as SEDDS in comparison to the powder-filled capsule formulation. Coenzyme Q₁₀ is used as an antioxidant and in the treatment of cardiovascular disorders including angina pectoris, hypertension, and congestive heart failure. The formulation used for the study contained acetylated monoglycerids, Labrafac CM-10, and propylene glycol monolaurate (Kommuru et al 2001).

Oral ME of berberine was developed by Gui et al (2008) in order to improve the bioavailability using oleic acid, Tween 80 and PEG 400. They characterized the developed MEs for its viscosity, refractive index, electrical conductivity, and particle size. The formulated MEs were uniform in size (24 nm). The results of an in vivo study indicate that the oral bioavailability of the berberine ME was 6.47-fold greater than berberine tablet suspensions.

Shen et al (2011) developed microemulsion of daidzein incorporating ethyl oleate, cremophor RH40, PEG 400, and water as components, and they compared this ME with borneol/menthol eutectic mixture. The pharmacokinetic study in rats after oral administration showed a 1.5 to 3.5 fold enhanced oral bioavailability of daidzein from the microemulsion compared to the daidzein suspension.

Hintzen et al (2014) developed a self-microemulsifying drug delivery system for the model peptide drug leuprolelin to prove a protective effect against luminal enzymatic metabolism. They developed the microemulsion of leuprolelin using 30% (m/m) cremophor EL, 30% (m/m)
capmul MCM, 10% (m/m) propylene glycol, and 30% (m/m) captex 355) at a concentration of 4 mg/g. They found that the microemulsion was able to shield the leuprolide oleate from enzymatic degradation by trypsin and α-chymotrypsin. The study concluded that microemulsions could be likely a novel platform technology to improve the oral bioavailability of peptide drugs.

*Ganoderma lucidum* triterpene loaded microemulsion was developed by Qu et al (2014) using Coix lacryma jobi (adlay) seed oil for anti-tumor effect. The formulation developed showed an encapsulation of around 80.0 to 84.0 % of drug. The antiproliferative effect of the ME developed was checked in A549 cells, and it was found that the half maximal cytotoxic inhibitory concentration was 0.62 mg/mL of the crude extract. This suggested that the ME-based formulations can improve the solubility of triterpenes.

### 1.5.8 Topical Delivery

ME as a topical drug delivery system increases percutaneous absorption of both hydrophilic and lipophilic drugs based on the constituents used in the formulation of the microemulsions. Moreover, the transdermal drug delivery potential of microemulsions is directly dependent on the ratio of the respective components rather than their individual characteristics.

MEs enhance the transdermal permeation of drugs due to:

1. Their greater solubility potential in both lipophilic and hydrophilic drugs, which may generate an increased thermodynamic activity toward the skin;
2. Of their components being capable of permeation enhancer activity; and Use of components possessing permeation enhancer activity.

3. Of their capability to alter the affinity of a drug to the internal phase thereby favouring partitioning into stratum corneum (Kreilgaard 2002).

MEs containing drugs, such as indomethacin, flurbiprofen, aceclofenac, celecoxib, rofecoxib, ibuprofen, piroxicam, and nimesulide, have been delivered topically to treat inflammations. Topical celecoxib microemulsion was developed to treat UV-B mediated inflammation (Subramanian et al 2004, Desai 2004, Park et al 2005, Ambade et al 2008, El Maghraby 2010, Shah et al 2010, Pawar 2011, Hu et al 2014).

Topical microemulsions that were developed for drugs such as 5-aminolevulinic acid, evodiamine and rutaecarpine, cyclosporine A, nicotinic acid, tolterodine tartrate, and octylmethoxycinnamate showed enhanced permeation of encapsulated drugs across the skin membrane. (Lopes et al 2006, Elshafeey et al 2009, Araujo et al 2010, Montenegro et al 2011, Zhang et al 2011, Tashtous et al 2013).

Gannu et al (2010) had developed and optimized lacidipine loaded topical ME to improve the solubility and bioavailability of lacidipine, using isopropyl myristate, Tween-80, and labrasol. They optimized the developed formulation using Box–Behnken design by altering the concentrations of isopropyl myristate, surfactant mixture, and water. The results of the bioavailability studies in rabbits show improvement of the transdermal application of ME gel in comparison to oral suspension of lacidipine (3.5 times statistically significant). They justify the use of the developed ME in the management of hypertension.
Sertaconazole topical ME was developed by Sahoo et al (2014) for cutaneous fungal infection. They prepared the hydrogel of sertaconazole ME using carbopol 940 (0.75%, w/w). The permeation rate of optimized formulation composed of oleic acid (8.75%, w/w), Tween-80 (33.35%, w/w), propylene glycol (33.35%, w/w), and water (24.55%, w/w) was observed to be higher in comparison with other HSMs and commercial creams with three times higher drug retention capacity in the skin than commercial creams, and did not cause any erythema or edema based on a skin sensitivity study on the rabbit. Moreover the developed formulation possesses significant antifungal activity against Candida albicans.

Recently Butani et al (2014) developed a ME formulation of amphotericin B for the treatment of invasive fungal infections; they screened the oil phase based on the drug solubilizing capacity. Moreover they checked the influence of the surfactant and cosurfactant mass ratio (S\text{mix}) on the ME formation and permeation of ME through excised rat skin. Their final optimized formulation was 0.1% (w/w) Amp B, 5% (w/w) isopropyl myristate, and 35% (w/w) S\text{mix} (3:1, Tween-80 and propylene glycol). The in vitro characterization results shows a globule of size 84.20 ± 2.13 nm, a polydispersity index of 0.164 ± 0.031, pH of 7.36 ± 0.02, and conductance of 229.3 ± 1.95 \mu S. The results of in vitro anti-fungal activity (trichophyton rubrum) fungal species have shown that developed ME has a higher zone of inhibition.

1.5.9 Parenteral Drug Delivery

The excellent thermodynamic stability, high solubilization capacity, low-viscosity and ability to withstand sterilization techniques makes ME a better delivery system for the parenteral route. The ME has been reported to be a painless technique (Lee et al 2002). The nanostructure of the microemulsions ensures that the probability of embolisms forming in the
blood is insignificant. In addition, the small size of the microemulsions leads to higher blood circulation time. As the drugs are being encapsulated in microemulsions, the toxicity of certain drugs, for example amphotericin B, is reduced (Moreno et al 2001). The controlled delivery of hydrophilic therapeutic actives like aminoglycoside antibiotics was also achieved by the w/o microemulsions. Certain excipients such as PEGylated phospholipids are used to enhance the circulation time of the therapeutic agents in the blood, which will be helpful in infections like malaria and in cancer treatment (Jain 2010).

Self-microemulsifying drug delivery systems (SMEDDS) are those in which the drugs are stored in the form of anhydrous preconcentrates. These SMEDDS can be diluted with the i.v fluids such as 0.9% saline or 5% dextrose while administering to give microemulsions spontaneously. The preconcentrates can be employed for the drugs susceptible to hydrolysis and can easily be autoclaved if the therapeutic agent is heat stable.

Microemulsions as vehicles for parenteral delivery of a number of drugs, such as felodipine, flurbiprofen, clonixic acid, amphotericin B, all-trans-retinoic acid, docetaxel, artemether, etoposide, lorazepam, propofol, rutaecarpine, paclitaxel, and vincristine, have been reported.

1.5.9.1 Delivery of anti-cancer agents

Most anticancer agents need to be administered by the parenteral route. But, factors such as poor water-solubility and high degree of toxic side effects restrict their delivery. Anticancer agents are mostly formulated as a mixture of co-solvents and surfactants and thus undergo common problems associated cosolvent-based parenteral formulations. An interesting substitute for delivering the cytotoxic agent is microemulsions. Phospholipid-based
MEs containing etoposide, docetaxel, vincristine, and paclitaxel have been developed as parenteral MEs.

Paclitaxel is derived from a plant, acts as an antineoplastic agent, and has dramatic clinical activity against ovarian, breast, nonsmall cell lung carcinomas, and AIDS-related Kaposi’s sarcoma (Wall & Wani 1996). Paclitaxel is administered as an intravenous (i.v.) infusion to patients as Taxol® (Trissel 1997) as they have very low oral bioavailability because of their limited aqueous solubility (10.8 g/ml) and high lipophilicity (KO/W = 311). Taxol® is comprised of Cremophor® EL (polyoxyethylated castor oil) and ethanol. The adverse-effects of Cremophore® EL limit the clinical utility of the product. Several ME formulations were used to improve its clinical use.

The parenteral ME of paclitaxel was first proposed by He et al (2003), and was composed of lecithin, ethanol, and poloxamer 188 with a minimal amount of Cremophore® E when compared with Taxol®. The potential of ME and Taxol® to produce a hypersensitivity reaction was evaluated in rabbits and it was found that paclitaxel ME showed less hypersensitivity reaction as compared to Taxol®. Additionally, paclitaxel ME and Taxol® were subjected to pharmacokinetic evaluation and the result showed that MEs have higher AUC values for paclitaxel (34.98 gml⁻¹ h) than Taxol® (21.98 gml⁻¹ h).

The possibility of developing controlled release SMEDDS of paclitaxel was also investigated by Kang et al (2004). The controlled release paclitaxel SMEDDS were formulated in which polylactide-co-glycolide (PLGA) was dissolved with the help of glycofurol. The paclitaxel SMEDDS contains Cremophore® ELP, glycofurol, and Labrafil 1944 CS. The controlled release SMEDDS in body fluid would release paclitaxel at a slower rate by forming a ME gel (because of the presence of PLGA) that contains paclitaxel in solubilized form. Furthermore, in dissolution studies the
controlled release SMEDDS showed sustained release of paclitaxel and also resulted in sustained cytotoxic action in human breast cancer cell line MCF7 and human ovarian cancer cell line SKOV-3. The study proved that controlled release paclitaxel SMEDDS were effective in reduction of tumor volume as compared to PLGA free ME.

Zhang et al (2006) successfully developed and evaluated the paclitaxel SMEDDS, which was devoid of Cremophore® EL. The developed SMEDDS comprises tributyrin, tricaprin, ethanol, lecithin, and poloxamer 188. The dilution of SMEDDS with saline resulted in ME globules as small as 16nm in size. The pharmacokinetic parameters of paclitaxel SMEDDS were compared with Taxol®. This showed higher AUC value for SMEDDS and longer circulation times when compared to Taxol®.

Nornoo et al (2008) and Nornoo & Chow (2008) have reported the potential of w/o ME of paclitaxel free from Cremophor and also determined the effect of medium chain mono- and diglycerides such as Capmul® MCM and Myvacet® in the development of parenteral microemulsions. Determining the erythrocyte toxicity showed that the ME was found to be safer. The pharmacokinetic studies of paclitaxel MEs showed higher circulation time and wider tissue distribution in comparison to Taxol®.

Vincristine is a hydrophobic cytotoxic alkaloid obtained from *Catharanthus roseus* and used in the treatment of leukaemia, Hodgkin’s disease, nonHodgkin’s lymphomas, breast and lung cancers. Junping et al (2003) developed microemulsions of vincristine with the help of PEGylated phospholipids, vitamin E, cholesterol, and oleic acid. The microemulsions showed higher efficacy, tolerability, and survival rate in tumor bearing mice when compared to the free drug. In the case of MEs, the concentration of vincristine was found to be higher in tumors compared to other organs, which
indicates a less adverse effect. This study clearly illustrates the potential of MEs in enhancing the delivery of the anticancer agent.

A phospholipid-based ME containing etoposide was successfully developed by Jain et al (2010) and investigated the potential of parenteral delivery of the same. To prevent hypotension it is generally recommended that etoposide is administered by intravenous infusion over a period of 30 to 60 min. Stable ME formulation having globule size less than 100 nm has been developed using the composition of 40% PEG 400, 5% capmul MCM, 22.5% Tween-80, and 2.5% w/v of epikuron 135 F (phosphatidylcholine enriched liquid fraction of soybe lecithin). They found that the ME was easy to sterilize by autoclaving and that the dilution was robust with intravenous fluids without any precipitation over infinite dilution. The in vitro erythrocyte toxicity study demonstrated that the capmul MCM and Tween-80 have less haemolytic activity and indicated the safety and parenteral acceptability of the ME.

Parenterally acceptable docetaxel containing self-microemulsifying drug delivery system (SMEDDS) was developed by Yao et al (2012) with the optimal formulation composition of 35% of Solutol® HS15, 35% of GTCC, 14% of soybean lecithin, 15% of ethanol, and 1% w/w of cholesterol sulfate sodium. The pharmacokinetic profiles of docetaxel SMEDDS were studied by the administration of intravenous infusion in rats. They found a 1.55-fold increase in the bioavailability of SMEDDS when compared to the conventional formulations. The evaluation of in vivo antitumor efficacy of docetaxel SMEDDS in Kunming mice-bearing S180 sarcoma showed effective tumor growth inhibition with preferable tolerance compared to the conventional formulation.
1.5.9.2 Delivery of anti-fungal agents

The parenteral dosage of antifungal agents is important for systemic fungal infection in immunocompromised patients. Various methods such as liposomes, mixed micelles, lipid complexes, and cyclodextrin complexation have been implemented for enhancing the parenteral delivery of antifungal agents. Recently there have been reports on the potential of microemulsions in parenteral delivery of antifungal agents.

Lyophilized lecithin-based amphotericin B (AmB)-loaded microemulsions were developed and evaluated by Moreno et al. (2001) for their in vivo toxicity to validate them as new and low toxic drug delivery systems. AmB in the ME formed a complex with lecithin, which was observed on the oil-water interface of the developed formulation. When lyophilized, an oily cake of ME that can be easily reconstituted was produced and found to be more stable. The single dose acute toxicity of the formulation showed that the AmB ME LD$_{50}$ was 4 mg/kg of animal weight, when compared with 1 mg/kg for Fungizone. The developed lyophilized parenteral lipid formulations make them suitable for large doses and intravenous administration.

A parenteral ME containing itraconazole (ITZ) was formulated by Rhee et al. (2007) using the mixture of benzyl alcohol and medium chain triglyceride as oil phase, polyoxyethylene (50) hydrogenated castor oil, and ethanol as Smix. They found that the phenolic and hydroxyl group of the benzyl alcohol (BA) can form a hydrogen bond with the itraconazole, which is responsible for stability and solubility. Higher affinity towards the PEO ring of the polyethylene glycol (PEG) can give rise to the hydrogen bond, which can increase the solubility. Since the solubility was increased with the combination of the BA: MCT and PEGs, was chosen as oil and surfactant phase. Pseudoternary construct was used to develop the stable parenteral ME
with a parenterally acceptable droplet size (< 150 nm), which is also nontoxic to red blood cells.

### 1.5.9.3 Delivery of antimalarial agent

Recently antimalarial MEs were developed for parenteral delivery of artemether. Parenteral MEs containing artemether were prepared, and the antimalarial activity evaluated by Tayade et al (2010). The developed ME shows a globule size of 113 nm and the method of sterilization did not affect the drug content and globule size of the ME. They found there was a 1.5-fold higher antimalarial activity with increased survival with good stability compared to the marketed oily artemether injection.

### 1.5.9.4 Delivery of Antianxiety drug

Kale et al (2008) developed cosolvent-free lorazepam parenteral MEs and evaluated the globule size, compatibility with parenteral fluids, in vitro haemolysis, and stability. The highest solubility of LZM in Capmul MCM was seen only when used as an oily phase. They developed the MEs using Tween-80 as the surfactant phase with an average globule size less than 200 nm, which is most compatible with parenteral dilution fluids with less haemolytic activity.

### 1.5.9.5 Delivery of anesthetic drug

Propofol MEs were prepared and their anesthetic properties were evaluated by Morey et al (2006). They hypothesized that the transparent propofol MEs preserved their anesthetic properties compared to the conventional macroemulsions, and by altering the surfactant type and concentration, the dose–response relation can be selectively modified. They formulated the propofol ME with the help of the pseudoternary diagram using
propofol itself as oil core, poloxamer 188 (3%, 5%, 7%), and sodium salt of fatty acids in saline, further characterized its globule size and stability upon dilution and in vivo anesthetic properties of induction (dose, righting reflex stunned, loss of lash reflex, and withdrawal to toe pinch) and recovery (lash, righting, and withdrawal reflexes). They found that propofol MEs cause anesthetic induction with great recovery, and that a significant dose of propofol is required to produce anesthesia with MEs rather than the surfactant type/concentration with macroemulsions. It reported that the impulsive destabilization and anesthetic induction were affected by the surfactant type and concentration.

The potential of the parenteral delivery of propofol loaded MEs was investigated by Date et al (2008). Pseudo-ternary phase diagrams were constructed for identification of the microemulsification region. The developed ME shows the droplet size is less than 25 nm and demonstrated good physicochemical stability. The haemolytic potential of the developed ME caused negligible (1%) haemolysis after 2 h of the incubation with human blood. The rat paw-lick test showed that the developed propofol MEs were significantly less painful when compared to the marketed formulation. They revealed that the anesthetic activity of the MEs was similar to that of the marketed propofol formulation. They concluded that the developed propofol ME as parenteral delivery is a novel painless and commercially feasible approach.

1.5.9.6 Liquid crystals MEs

Recently liquid crystal forming microemulsions were developed for parenteral delivery.

Prolonged parenteral delivery of liquid crystal forming ME was developed by Ren et al (2012) and evaluated for its rheological behaviour.
especially with regard to its phase transition, globule size, and drug release. They developed the in situ phase transition ME from the phase construct using miglyol 812N as the oil phase and a mixture of surfactants consisting of solutol HS 15 and span 80 with ethanol. Liquid crystal (LC) and coarse emulsion (CE) areas were identified closer to the ME forming region with high water content present in the phase construct. They found that the phase transition behaviour of the LC forming ME allowed it to remain a contracted region at the water/ME interface, whereas the CE-forming ME easily dispersed in the aqueous environment. Gamma-scintigraphy results revealed that the LC-forming ME possesses minimal spreadability with slow release of 99mTc and suggests phase transition at the interface. They concluded that the developed LC forming MEs, could be used as a potential parenteral drug delivery vehicle for prolonged drug release.

Wu et al (2014) studied the suitability of ME for the prolonged release of injectable ME through in situ phase transition. Three different ME formulations of both w/o and o/w types were developed using the pseudoternary phase diagram and they investigated the phase transition behavior, and in vivo drug release of 99mTc. The LC forming MEs formed depots in water because of the in situ phase transition with higher viscosities at the site of injection upon the absorption of water. They found that the formulations that formed LCs showed pseudo plastic properties. The LC-MEs exhibited prolonged release compared to the CE-MEs, and it was seen that the developed formulations had great potential for both hydrophilic and lipophilic drugs because of the sustained release behaviour. Wu et al concluded that the retention of vehicles at the site of injection was determined by its in situ phase transition behavior, instead of the oil content or ME type.
1.5.9.7 Delivery of anti-inflammatory agents

The parenteral route is considered to be the best route for some of the anti-inflammatory agents when there is severe pain and in emergencies. The parenteral formulation is ideal as there is quick onset of action and a longer duration, which is desirable for optimal treatment.

Park & Kim (1999) prepared and evaluated the flurbiprofen-loaded parenteral ME. They achieved 8-fold increased solubility of flurbiprofen with ethyl oleate and Tween-20 (around 10 mg/ml) in the o/w type of parenteral MEs when compared with the PBS solution, which ultimately reduces the total injection volume. They formulated the parenteral ME with globule size less than 100 nm using less than 1% (w/w) of flurbiprofen. After an intravenous administration of flurbiprofen ME to a rat, the pharmacokinetics profile of flurbiprofen ME was not significantly different from that of flurbiprofen in PBS. Therefore, the authors suggest that the developed ME system can be used as a carrier for parenteral delivery of lipophilic drugs without any chemical modification, with long-term stability of flurbiprofen.

The steadily increasing number of research workers engaged in studies on MEs, in view of their unique properties and different applications, have made significant contributions to pharmaceutical and cosmetic technology, demonstrating the potential of MEs as novel compartmentalized liquids for parenteral delivery of hydrophobic drugs.

1.6 CAMPTOTHECIN (CPT)

CPT is a natural cytotoxic quinoline alkaloid drug derived from the Chinese plant Camptotheca acuminata by Wall and co-workers in 1966. (Wall et al 1966). CPT showed promising anticancer activity against a wide range of cancers including the breast, lung, ovarian, pancreatic, and colon
cancers in animal models leading to the clinical evaluation of the same. Currently thousands of CPT derivatives have been synthesized; among them, Topotecan and Irinotecan are commercially approved antitumor agents that are still in clinical trials. Therapeutic use of the unchanged parent camptothecin molecule is hindered by its poor solubility in aqueous and oil phases and high toxicity.

CPT (Figure 1.5) is a pale yellow color crystalline powder, with a planar pentacyclic ring structure, empirical formula C_{20}H_{16}N_{2}O_{4}, a molecular mass of 348.36 g/mol, and a melting point of 264–267 °C. It is an optically active compound; under UV it gives intense blue fluorescence. Its IUPAC name is (S)-4-ethyl-4-hydroxy-1H-pyrano-[3′, 4′, 6, 7] indolizino [1, 2-b] quinoline-3,14 (4H,12H)-dione. Camptothecin should be stored in air tight containers at 2–8 °C.

![Chemical structure of camptothecin](image)

**Figure 1.5 Chemical structure of camptothecin**

CPT can exist either as lactone (at acidic pH) or carboxylate (at alkaline pH) based on the pH of the surrounding medium. It is susceptible to spontaneous reversible hydrolysis in such a way that the biologically active lactone form predominates at acidic pH, and the inactive open-ring carboxylate species is favored at neutral / alkaline pH. Hence CPT should be delivered in such a way that the lactone ring should predominate at the site of action for effective cancer treatment.
1.6.1 Mechanism of Action

The camptothecins act by binding to the DNA-topoisomerase I cleavable complex and blocking the movement of the DNA replication fork. (Fan et al 1998, Laco et al 2002). It inhibits the relegation step that leads to accumulation of single-stranded breaks in the DNA. This ternary drug–enzyme–DNA complex will collide with the advancing DNA replication fork. This results in an irreversible double-strand break that eventually leads to cell death. (Tsao et al 1993). CPTs can be considered S phase-cytotoxic drugs, since the ongoing DNA synthesis is essential to the series of events that lead to cytotoxicity. (Gerrits et al 1997, Takimoto et al 1998). However, CPT-induced cytotoxicity has also been observed in cells that are not actively synthesizing DNA. This may involve the induction of serine proteases and endonucleases. (Boroviskaya & D’Arpa 1998).

1.6.2 Structural Activity Relationship

Two-fold or more decreased membrane association occurs in the carboxylate form when compared to membrane affinity of CPT. Charged drug species obtained when the ring opening occurs in CPT alters and limits the drug diffusibility through the cell lipid bilayer of low dielectric constant. The lipophilicity of CPT enhances the intracellular accumulation and minimizes the lactone hydrolysis by improving partitioning of lactone into red blood cells. Ring opening results decrease the intrinsic potency and reduce the topoisomerase I target. Decreased membrane binding, diffusibility, and decreased potency led to the reduction in cytotoxic activity associated with the lactone ring opening of CPT (Thomas et al 2004, Rahier et al 2004, Li et al 2006).
1.6.3 Pharmacokinetics

The equilibrium between the closed ring of lactone form and open ring of carboxylate form of CPTs is pH-dependent and ring closure is happen with increasing acidity. The binding serum albumin to the salt form also affects the equilibrium (Mi & Burke 1994, Fleury et al 1997). Serum albumin adhere the CPT resulting the rapid ring opening. Others such as erythrocyte membranes and lipoproteins bind to CPTs, favoring the closed lactone form (Mi & Burke 1994, Fleury et al 1997, Scott et al 1993). Chemotherapeutic activity of CPT is less with open salt whereas closed lactone ring is considered as an essential pharmacophore for activity against cancer cells. The sodium salt of CPT posses the low anticancer activity in the animals is consistent with the failure of clinical trials (Muggia & Creaven 1972) due to the association of the chemotherapeutic activity of CPT with lactone and toxic responses with the open salt form. The target of pharmacokinetic and metabolic studies should contribute to the development of dosing regimens optimal for tumor uptake of CPTs. (Rowinsky et al 1992). The large dosage of CPT and its derivatives was found to be ineffective when given as large intervals. CPT needs to be administrated as a prolonged schedule with continuous low doses or frequently fractioned dosing schedules to maintain normal hematopoietic cells and mucosal progenitor cells with low topoisomerase- I levels while preserving efficacy (O’Leary & Muggia 1998). Different types of delivery systems have been developed to achieve the prolonged schedule of CPT. The CPT undergoes hydrolysis with an enzyme carboxylesterase present in rat liver and is converted into an active metabolite of 7-ethyl-10-hydroxy-camptothecin. It is conjugated with the hepatic enzyme UDP-glucuronyl transferase and converted into a β-glucuronide form, which is excreted into the bile along with the other components (Platzer et al 2000).
1.6.4 Delivery Approaches of Camptothecin

Several formulation approaches, such as nanoconjugates, nanocrystalline suspension, solid lipid nanoparticles, micelles, liposomes, nanoparticles, microparticles, miniemulsions, dendritic polymers, prodrug approaches, and drug-polymer conjugates, have been attempted for delivering camptothecin and its derivatives by increasing their solubility and/or protecting the lactone ring hydrolysis in the physiological pH (Hatefi & Amsden 2002).

CPT was first incorporated into micelles derived from negatively charged surfactants (Tyner et al 2004). The negatively charged micelles were then encapsulated in nanoparticles of magnesium–aluminium layered double hydroxides (LDHs) by an ion exchange process. The resulting nanobiohybrids released CPT rapidly with complete release within 10 min at both pH 4.8 and 7.2. When administered to glioma cells in vitro, the nanobiohybrid containing CPT resulted in significantly lower survival times compared to untreated cells, or to cells incubated with the surfactant, the pristine LDH, or water (delivery medium). The encapsulation method allowed for approximately 3 folds increase in solubility of CPT. In addition, the modification of the surface of the LDH provided potential site-direction of the nanohybrids.

Alvarez-Lorenzo et al (2004) studied the micellar solutions of pluronic-g-poly(acrylic acid) copolymers for enhanced solubilisation and stabilization of CPT. The solubility of camptothecin in polymer micellar solutions was reported as being three to four-fold higher than that in water at pH 5. The drug was not only solubilized by the hydrophobic core of PPO, but also by the hydrophilic POE-PAA shells of the micelles. They also reported that the lactone form of CPT was 10-fold more stable in polymeric micelles, compare to the free drug in physiological pH buffer solution.
Injectable thermoplastic biodegradable polymer CPT depot system was developed by Hatefi et al (2004) for localized action and improvement of the stability of CPT. Ring-opening polymerization method was used to prepare ε-Caprolactone oligomers, the drug was loaded over it, and diffusion-controlled CPT release was observed from the oligomers. The lactone stability of unreleased CPT was found to last up to 16 weeks. This was possible because of the hydrophobic environment of the polymer, which provides high drug loading efficiency and maintains the stability of the lipophilic hydrolyzable drug prior to release.

Barreiro-Iglesias et al (2004) studied the capability of a family of copolymers comprising Pluronic R (PEO-PPO-PEO) surfactants covalently conjugated with poly (acrylic acid) (Pluronic-PAA) to enhance the aqueous solubility and stability of the lactone form of CPT. The unprotected lactone form of CPT, which possesses cytotoxic activity, is rapidly converted to the ring-opened carboxylate form under physiological conditions. Barreiro-Iglesias et al characterized the critical micellization concentration (CMC) of Pluronic-PAA copolymers, equilibrium solubility partitioning, and hydrolysis of the lactone form of CPT in the presence of Pluronic-PAA in water and in human serum. They found three- to four-fold higher solubility of CPT in polymer micellar solutions compared to that in water at pH 5. The amount of CPT solubilized per PPO was considerably greater in the Pluronic-PAA solutions than in the parent PluronicR solution, which suggests that the drug is not only solubilized by the hydrophobic cores but also by the hydrophilic POE-PAA shells of the micelles. The equilibrium partition coefficient of the CPT lactone between Pluronic-PAA solutions and water exceeded (2–3) × 10³. The complete solubilization of CPT and the absence of chemical interactions between CPT and Pluronic-PAA were confirmed by modulated temperature differential scanning calorimetry, infrared spectroscopy, and X-ray diffraction of films. The loading of CPT into the Pluronic-PAA
micelles helped to prevent the hydrolysis of the lactone group of the drug for 2 h at pH 8 in water. When compared to the unprotected CPT, the kinetics of the CPT hydrolysis in human serum was about 10-fold slower in the Pluronic-PAA formulations.

Safe biocompatible nanocarrier systems were developed by Koo et al (2005) for improved parenteral delivery of CPT as sterically stabilized micelles (CPT-SSM) consisting of polyethylene glycol (PEGylated) phospholipids. They studied the solubilization potential, stability, and in vitro cytotoxicity of CPT in SSM. The CPT-SSM showed that with a size of ~14 nm there was 25-fold higher solubilization of CPT in the micelles than in the CPT solution. They confirmed that the developed formulation was three times more stable and three-fold more cytotoxic to MCF-7 cells than CPT alone, without addition of lyoprotectants to the CPT-SSM and lyophilized and reconstituted without any significant change in properties.

Opanasopit et al (2006) investigated N-phthaloyl chitosan-g-mPEGself-assembly micellar system for CPT, to improve the aqueous solubility and stability of the lactone form of CPT. The CPT was loaded into the self-assembled micelles by dialysis method, with an effective loading of 40% CPT. The hydrophobic inner core of the micelles could protect the CPT from hydrolysis in physiological pH and help retain the active lactone form for a longer period of time, thereby increasing in half-life from 94 min to 76.15 h. The in vitro drug release from the micelles also showed sustained release of nearly 70% of the lactone form of the drug in about 95 h.

Han et al (2009) developed a novel 10-methoxy-9-nitrocamptothecin (MONCPT) nanoemulsion (o/w type) to enhance its solubility, stability, and antitumor activity. The development of the nanoemulsion was achieved by using lipoid E80 and cremophor EL as main emulsifiers by microfluidization method. Solubility and internalization of the
The lactone form of MONCPT in nanoemulsion in the interfacial surfactant layer of the nanoemulsion was 200-fold higher than in water. MOCPT nanoemulsion shows no obvious haemolysis in rabbit erythrocytes. MONCPT nanoemulsion showed increased cytotoxicity with a suppression rate of 93.6%, whereas only 24.2% was observed for MONCPT injection at the same dosage. In vivo imaging of tumor in S180-bearing mice showed more accumulation in tumor cells.

CPT nanocrystals were prepared by Zhang et al (2011) using a sonication–precipitation method without adding stabilizing surfactants to overcome the solublization issue. The developed nanocrystals were found to be more potent to MCF-7 cells than the CPT solution. It has also exhibited significant tumor growth suppression in MCF-7 xenografted BALB/c mice compared to the drug salt solution.

CPT nanosuspension (Nano-CPT) was developed by Yao et al (2012) using a novel supercritical antisolvent (SAS) system and high pressure homogenization technique. The in vitro, in vivo antitumor efficacy and dose dependent toxicity were accessed for CPT nanosuspensions and were compared with topotecan (TPT). The developed Nano-CPT produces a higher level of cytotoxicity than TPT against the MCF-7 cell lines, and similar in vivo antitumor activity with TPT with minimal toxicity.

Recently Yu et al (2012) developed a novel intravenous emulsion containing 0.05% w/v of CPT, soybean lecithin, poloxamer 188, MCT, oleic acid, vitamin E, soybean oil, acetic acid, and glycerol by homogenization in which CPT was converted into a drug-phospholipid complex. Intravenous administration of the emulsion to rats showed higher accumulation of CPT in the liver. However, apart from difficulty in maintaining physical stability, sterility, and aseptic handling this lipid based emulsion, the long-term
infusion of soybean oil containing emulsions produces hyperlipidemia and
causes pain at the site of injection (Theilen et al 2002).

CPT-loaded liposomal formulation was developed and the
incorporation and retention efficacy of CPT in liposomes using radiolabeled
CPT were studied (Flaten et al 2013). The retention ability of different CPT
liposomes in buffer was influenced by the concentration of lipid and the
drug/lipid ratio, rather than lipid composition. The composition of lipid did
not influence the retention of CPT, and the biodistribution of liposomes
provides longer circulation than free drug, but premature CPT release from
liposomes occurred.

Yin et al (2014) developed camptothecin-N-poly (lactic acid)
nanoconjugates by polymerization conjugation method. CPT conjugates were
obtained by a facile hydrolysable amino ester linker with the terminal
carboxylate group of polylactide (PLA). Self-assembled CPT-N-PLA
conjugates showed a size range between 50–100 nm and better in vitro and
enhanced in vivo therapeutic efficacy against Lewis lung carcinoma (LLC)
induced in C57BL/6 mice.

The limited success of currently available drug delivery systems for
CPT makes it necessary to search for and develop of newer effective drug
delivery systems for CPT.