CHAPTER 3
OBJECTIVE AND RATIONAL
3.0 OBJECTIVE AND RATIONAL

3.1 Objective of the Study

Turmeric, the source of the polyphenolic active compound curcumin (diferuloylmethane), has been used extensively in traditional medicine since ancient times as a household remedy against various diseases, including hepatic disorders, cough, sinusitis, rheumatism, and biliary disorders. Recently a large number of studies have shown that curcumin (CU) has a surprising array of antioxidant, antitumor, anti-inflammatory, anticancer, and other desirable medicinal properties. There is scientific evidence suggesting that CU has a highly pleotropic structure, that physically interacts with a wide range of molecular targets, e.g., transcription factors, growth factors and their receptors, cytokines, enzymes, and genes regulating cell proliferation. Curcumin (CU) functions as an anti-cancer agent by activating apoptosis signaling and inhibiting cell proliferation. CU has been shown to suppress the expression of epidermal growth receptor and estrogen receptors, which are cancer-associated growth factors (Duvoix et al., 2003; Tapal and Tiku, 2012). However, the main drawback associated with CU is its poor aqueous solubility (11 ng/mL) (Kaminaga et al., 2003), stability in gastrointestinal fluids, low permeability and first pass metabolism which leads to poor bioavailability (~1% in rat) (Pan et al., 1999; Yang et al., 2007). CU is a BCS Class IV (low solubility low permeability) molecule (Wahlang et al., 2011). Thus, the study has been designed to augment the in vitro release by improving solubility and permeability using lipid NE and to enhance the bioavailability of CU by delivering it at the molecular level in the form of nano globule via lymphatic portal system through oral route.

In order to deliver CU to targeted organs, its hydrophobic property needs to be modified. The small size of carriers is very important for the biodistribution in the body. The capillaries are so small that red blood cells can only travel through them in single file. The capillaries measure approximately 5–10 μm in diameter. Particles larger than this size cannot be circulated in the body and become entrapped in the capillary bed. Thus, the particle diameter should be generally smaller than micrometers for the particles to be circulated in the blood vessels. In addition, reduction in the particle diameter to less than 100 nm is thought to decrease their removal by the reticuloendothelial system and increase their extravasations from the smallest capillaries. Thus, nanotechnology is one of the effective methods to be used for the delivery of CU.
With this background the specific objectives of the present study were

- to enhance the solubility and permeability of the poorly soluble drug CU, by lipid based nanoemulsion (NE) as the formulation technique.
- to optimize the NE drug delivery systems on the basis of globule size distribution, drug loading, and emulsification potential.
- to evaluate the solubility and drug release in *in vitro* models.
- to investigate the bioaccessibility of CU from lipid NE
- to investigate the stability of CU in lipid NE at intestinal pH
- to investigate NE for increased or more effective absorption of drugs *in vivo* (pharmacokinetic studies).
- to investigate the efficacy of CU by performing cytotoxicity studies using MTT assay method against glioma cells (U-87).
- to evaluate stability of CU in NE formulation.
3.2 Rationale of the Study

The rational for the present study was to increase the bioavailability of CU, an anticancer drug, for increasing the therapeutic effectiveness against cancer using lipid NE as the formulation approach via lymphatic portal system through oral route as the drug delivery approach.

3.2.1. Cancer

Cancer is defined as a complex series of disease condition caused by persistent tissue injury and host environment interactions. The repeated exposure of carcinogens such as tobacco, ultraviolet light and infections leads to various genetic (mutations), epigenetic (loss of heterozygosity) and global transcriptome changes (via inflammation pathways) and is associated with increased cancer risk. Owing to increased occurrence of cancer and worldwide prevalence during the last decade, it has posed a great challenge to the health care professionals. The latest WHO statistics suggests about 45% increase in the global cancer deaths by 2030, of which 70% would be contributed from developing countries like India (Thanki et al., 2013).

3.2.2. Bioavailability of curcumin

Curcumin (bis-α,β-unsaturated β-diketone), commonly called as diferuloylmethane, is a low-molecular-weight, natural polyphenolic compound found in the rhizome of turmeric (Curcuma longa). The major drawback associated with the applicability of CU as a health promoting agent is its extremely low solubility in aqueous solution, poor stability in gastrointestinal fluids, low permeability and first pass metabolism which leads to its poor bioavailability (Anand et al., 2007). The low systemic bioavailability of CU following oral dosing seems to limit the tissues that it can reach at efficacious concentrations to exert beneficial effects, the attainment of such levels in the gastrointestinal tract, particularly the colon and rectum, has been demonstrated in both animals and humans (Anand et al., 2007). The first-pass effect or first-pass metabolism is a phenomenon of drug metabolism whereby the concentration of a drug is greatly reduced before it reaches to the systemic circulation. There are four primary systems that affect the first pass effect of a drug including the enzymes of the gastrointestinal lumen, gut wall enzymes, bacterial enzymes, and hepatic enzymes. Drug delivery using lipid-based formulations is one of the emerging strategies to
design pharmaceutical dosage forms with improved bioavailability and therapeutic benefits. The process of digestion and absorption that the lipids undergo in the gastrointestinal tract significantly enhances uptake of associated drugs into the lymphatic system, which helps to bypass the liver and drain them into the systemic circulation by means of the thoracic lymph duct. To improve bioavailability of CU, numerous approaches have been used. Nanoparticles, liposome, micelle, and phospholipid-complexes are the promising novel formulations, which appear to provide longer circulation, better permeability, and resistance to metabolic processes (Anand et al., 2007; Kumar et al., 2010).

3.2.3 Formulation approach for bioavailability enhancement and cancer targeting

Design and development of lipid based drug delivery of therapeutic entities with improved bioavailability is the main research aspect on which extensive work had been done in the past few decades for different therapeutic drugs. One of the promising and exciting drug delivery system which can meet the above mentioned requirements is NE. NEs are isotropic, highly kinetically stable, transparent (or translucent) systems of oil, water, and surfactant, frequently in combination with a co-surfactant having a droplet size usually in the nanometer range (typically in the range of 20-200 nm). The lipid and surfactant-based NE offers a practical advantage by improving the oral bioavailability of poorly water-soluble drugs by presenting and maintaining the drug in a dissolved state, at the molecular level, in small droplets of oil (<200 nm), throughout the gastrointestinal tract during transit in GI tract. NE has certain advantages such as good biocompatibility, biodegradability, physical stability, and ease of large-scale production. In addition, they can incorporate hydrophobic and amphipathic drugs because of their structural characteristics. Since CU has a hydrophobic nature, it can be the payload of a lipid emulsion. Thus, a lipid NE may be a promising approach for the delivery of CU for bioavailability enhancement by promoting lymphatic uptake through oral route.
3.3 Drug Profile of Curcumin

Curcumin is the main colouring component of turmeric. It is used as a colouring agent for medicines, foodstuffs, and cosmetics. CU was first isolated from turmeric in 1815 by Vogel. It was obtained in crystalline form in 1870, and its structure was delineated in 1910 as diferulolylmethane (Fig. 3.1) or 1,6-heptadiene-3,5-dione-1,7- bis(4-hydroxy-3-methoxyphenyl)-(1E,6E).

![Fig. 3.1 Chemical structure of CU](image)

3.1 Physicochemical properties of CU (USP 36, 2012)

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3.3.2. Pharmacology

3.3.2.1. Pharmacological Effects of CU

Curcumin has been traditionally used in prevention and treatment of several conditions and diseases. Several of these effects have been already well documented scientifically. Studies indicated that CU exerts hepatoprotective and nephroprotective, thrombosis suppressing, myocardial infarction-protective properties. Additionally, its strong antioxidant, antimicrobial, anticarcinogenic and anti-inflammatory activities were also reported (Aggarwal and Harikumar, 2009).

- The mechanisms implicated in the anti-inflammatory and anticarcinogenic potential of CU may include:
- Suppression of the activation of the transcription factor NF-κB, which regulates the expression of pro-inflammatory gene products.
- Down-regulation of the expression of cyclooxygenase-2 (COX-2), an enzyme linked with most types of inflammations.
- Decreasing the activity and protein levels of inducible nitric oxide synthase (iNOS) enzymes through reducing the expression of iNOS genes.
- Inhibition of arachidonic acid metabolism via lipoxygenase and scavenging the free radicals generated in this pathway.
- Down-regulation of the expression of various cell surface adhesion molecules that have been linked with inflammation.
- Decreasing the expression of various inflammatory cytokines, including TNF, IL-1, IL-6, IL-8, and chemokines.
- CU is a potent antioxidant, which contributes to its anti-inflammatory action.

3.3.2.2. Pharmacokinetics

In summary, the vast majority of these studies have demonstrated that CU exhibits extremely poor gastrointestinal absorption/oral bioavailability, and undergoes metabolism to form several chemical species, including CU glucuronide, CU sulfate, hexahydrocurcumin, tetrahydrocurcumin, and dihydrocurcumin. Pan et al., 1999 for example, investigated the pharmacokinetic properties of CU administered either orally or intraperitoneal (i.p.) in mice.
With oral administration of 1.0 g/kg of CU, low plasma levels of 0.13μg/mL appeared in plasma after 15 min, while a maximum plasma level of 0.22μ g/mL was obtained at 1 h; plasma concentrations then declined below the detection limit by 6 h. Entirely different plasma CU levels were found after i.p. administration of 0.1 g/kg. Plasma CU levels peaked (2.25 μg/mL) within 15 min of administration and declined rapidly within 1h. Ravindranath et al., 1980 showed that after oral administration of 400 mg of CU to rats, no CU was found in heart blood, whereas a trace amount (less than 5μg/mL) was found in the portal blood from 15 min to 24 h after administration of CU.

3.3.2.3 Side effects

Clinical studies in humans with high doses (2–12 grams) of CU have shown few side effects, with some subjects reporting mild nausea or diarrhea (Cheng et al., 2001). More recently, CU was found to alter iron metabolism by chelating iron and suppressing the protein hepcidin, potentially causing iron deficiency in susceptible patients (Jiao et al., 2009).