2.0 LITERATURE REVIEW

2.1 Nanoemulsion for oral drug delivery

Chavhan et al., 2013 prepared nanoemulsion (NE) of simvastatin for improving its solubility and/or dissolution rate for enhancing its bioavailability. Simvastatin is poorly bioavailable as it is practically insoluble in water and shows dissolution rate-limited absorption. The developed NEs were evaluated for particle size (PS), zeta potential, transmission electron microscopy (TEM), viscosity, in vitro release and stability studies. The optimized NE showed PS of 132 ± 9 nm and zeta potential of 17.1 ± 1.2 mV. TEM studies demonstrated spherical shape and size of the globules. In vitro release studies showed increased dissolution rate of NE compared with plain drug (PD). Pharmacokinetic studies showed relative bioavailability of simvastatin NE was 369.0% with respect to PD suspension. Pharmacodynamic studies conducted in hyperlipidemic rats showed significant decrease in the total cholesterol and triglyceride levels for NE as compared with PD proving improvement in bioavailability. In conclusion, NE has great potential for improving bioavailability of poorly water-soluble drugs like simvastatin.

Nanotechnologies are being employed to enhance the stability and oral bioavailability of lipophilic substances, such as capsaicin. Considering these facts Choi et al., 2013 examined the pharmacokinetic properties of the formulated capsaicin-loaded nanoemulsions. A pharmacokinetic study was carried out using double-layer nanoemulsions fabricated with alginate and chitosan polymers and triple layer nanoemulsions fabricated with chitosan/alginate polymers. Capsaicin nanoemulsions and capsaicin control (oleoresin capsicum) were administered to the rat at a dose of 10 mg/kg. A statistically significant difference was found in the area under the curve from time zero to time infinity (AUC_{\text{inf}}) among formulations (p < 0.01). In comparison to the control group, the relative bioavailability of formulated nanoemulsions was up to 131.7. The AUC_{\text{inf}} increased in a nano size-dependent manner; as nano size decreased, AUC_{\text{inf}} increased. In comparison to the double-layer nanoemulsions, the triple-layer nanoemulsion showed a significantly increased volume of distribution, resulting in the increased clearance and decreased AUC_{\text{inf}}. They concluded that the formulated nanoemulsions could significantly enhance the bioavailability of capsaicin.
**Li et al., 2013** aimed to prepare nanoemulsions coated with alginate/chitosan for oral insulin delivery. Uncoated nanoemulsions were prepared by homogenization of a water in oil in water (w/o/w) multiple emulsion that was composed of Labrafac® CC, phospholipid, Span™ 80 and Cremorphor® EL. Coating of the nanoemulsions was achieved based on polyelectrolyte cross-linking, with sequential addition of calcium chloride and chitosan to the bulk nanoemulsion dispersion that contained alginate. The particle size of the coated nanoemulsions was about 488 nm and the insulin entrapment ratio was 47.3%. Circular dichroism spectroscopy proved conformational stability of insulin against the preparative stress. *In vitro* leakage study indicated well-preserved integrity of the nanoemulsions in simulated gastric juices. Hypoglycemic effects were observed in both normal and diabetic rats. The relative pharmacological bioavailability of the coated nanoemulsion with 25 and 50 IU/kg insulin were 8.42% and 5.72% in normal rats and 8.19% and 7.84% in diabetic rats, respectively. Moreover, there were significantly prolonged hypoglycemic effects after oral administration of the coated nanoemulsions compared with subcutaneous (sc) insulin. In conclusion, the nanoemulsion coated with alginate/chitosan is a potential delivery system for oral delivery of polypeptides and proteins.

**Karadag et al., 2013** used response surface methodology to optimize the conditions for quercetin (QT) nanoemulsion preparations. The parameters to produce stable coarse emulsion formulations were optimized using pseudoternary phase diagram. The oil phase consisted of limonene oil, emulsifiers as Tween 80 and Span 20 mixture (1:1 weight ratio), and a water phase. Further obtained formulations were treated with high speed homogenization. Subsequently, QT loading was kept constant (0.25%, w/w), and the effects of the oil (10-20%, w/w) and emulsifier (5-15%, w/w) concentrations as well as the homogenization pressure (52-187 MPa) on the particle sizes and emulsion stability were investigated. Experimental data was adequately fit into a second-order polynomial model with a multiple regression coefficient ($R^2$) of 0.9171 for the particle size. $R^2$ values were found to be 0.8545 for the droplet growth ratio during storage and 0.7795 for QT stability. According to the model, major factors affecting particle sizes included the pressure, emulsifier and oil concentrations, and interaction between pressure and oil concentration. The pressure, oil concentration, and interaction terms between the emulsifier and oil concentrations as well as between the pressure and emulsifier concentration had a significant impact on the droplet growth ratio. Regarding the quercetin stability in nanoemulsions, only the oil concentration...
and interaction term between the oil and emulsifier concentrations had a significant effect. Optimum formulation and conditions for minimum particle size and the highest stability were found at 13% mixed emulsifiers, 17% oil content, and 70 MPa homogenization pressure. This study also suggested that the loading of QT in nanoemulsions could significantly affect the particle size and the stability of emulsions depending on the oil: emulsifier ratio in the system.

Zhang et al., 2013a developed a high-drug-loading nanoemulsion by self-assembly to improve the oral absorption of high dosing poorly water-soluble drug. Probucol was selected as a model drug and the probucol-loaded self-assembled nanoemulsion (PSN) was prepared and characterized. Moreover, the intestinal absorption and in vivo pharmacokinetic behavior of PSN were evaluated in rats after oral administration. The experimental results indicated that PSN was nanometer-sized droplets with the mean diameter of 40.32 ± 0.31 nm and polydispersity index of 0.184 ± 0.005. The aqueous solubility of probucol was remarkably increased after its incorporation into PSN. Compared with free drug suspension, the intestinal absorption of PSN was not significantly increased in duodenum, but obviously enhanced 3.62- and 13.1-fold in jejunum and ileum, respectively. In particular, the in vivo pharmacokinetic results indicated that the oral bioavailability of probucol was greatly improved 8.97-fold by PSN. Thereby, the high-drug-loading self-assembled nanoemulsion was very effective in enhancing the oral absorption of high-dosing poorly water-soluble drugs.

Many bioactive compounds are hydrophobic materials that are crystalline at ambient and body temperatures, which reduces their bioavailability and poses challenges to their successful incorporation into pharmaceuticals and functional foods. Considering this statement Pool et al., 2013 designed a study to determine whether a hydrophobic crystalline bioactive component (quercetin) could be successfully incorporated into nanoemulsion-based delivery systems, and evaluated the extent to which these delivery systems altered its bioaccessibility. The maximum amount of soluble quercetin that could be loaded into a carrier oil phase (medium chain triglycerides, MCT) at ambient temperature was ≈ 0.15 mg mL⁻¹ (saturation concentrations, C_{Sat}). At quercetin concentrations <C_{Sat}, nanoemulsions remained stable throughout 30 days storage at 5, 20 and 37 °C, i.e., no droplet growth, droplet creaming, or crystal formation were observed. At quercetin concentrations >C_{Sat}, nanoemulsions remained physically stable (no droplet growth or creaming), but quercetin
crystals formed in the samples during storage. The bioaccessibility of quercetin was determined using an *in vitro* digestion model simulating the mouth, stomach, and small intestine. A higher percentage of quercetin was solubilized in the micelle phase after small intestine digestion when it was incorporated in nanoemulsions than when it was dispersed in either bulk oil or pure water. The bioaccessibility of crystalline quercetin was less than that of dissolved quercetin. The knowledge gained from this study is valuable for the rational design of delivery systems to incorporate crystalline hydrophobic bioactive compounds into pharmaceuticals and functional foods, and to increase their bioaccessibility.

Oil-in-water nanoemulsions are finding increasing use as delivery systems to encapsulate lipophilic bioactive components in functional food, personal care, and pharmaceutical products. Saberi et al., 2013 investigated the influence of system composition and preparation conditions on the particle size of vitamin E acetate (VE)-loaded nanoemulsions prepared by spontaneous emulsification. This method relied on the formation of very fine oil droplets when an oil/surfactant mixture was added to water. The oil-to-emulsion ratio content was kept constant (10 wt%) while the surfactant-to-emulsion ratio (%SER) was varied (from 2.5 to 10 wt.%). Oil phase composition (vitamin E to medium chain triglyceride ratio) had a major effect on particle size, with the smallest droplets being formed at 8 wt% VE and 2 wt% MCT. Surfactant type also had an appreciable impact on particle size, with tween® 80 giving the smallest droplets from a group of food-grade non-ionic surfactants (tween® 20, 40, 60, 80, and 85). Surfactant-to-emulsion ratio also had to be optimized to produce fine droplets, with the smallest droplets being formed at SER=10 wt%. Particle size could also be reduced by increasing the temperature and stirring speed used when the oil/surfactant mixture was added to water. By optimizing system composition and homogenization conditions they were able to form VE-loaded nanoemulsions with small mean droplet diameters (d<50 nm) and low polydispersity indexes (PDI<0.13). The spontaneous emulsification method therefore has great potential for forming nanoemulsion-based delivery systems for food, personal care, and pharmaceutical applications.

Patel et al., 2012 formulated Cefuroxime Axetil loaded nanoemulsion to address the problem of poor oral bioavailability. Formulation was manufactured utilizing Capmul MCM, Soya lecithin, Deoxycholic acid, Pluronic F127 and distilled water. Mean globular size of 121.3 nm was obtained. Drug content of nanoemulsion was found to be 97.12±0.27% w/v. 80.7261% of the drug was diffused from nanoemulsion, as compared with 51.0048% diffused from the
plain Cefuroxime axetil suspension. *In vivo* studies indicated $AUC_{0-24}$ 325.3 for nanoemulsion in comparison to $AUC_{0-24}$ 165.3 for plain suspension.

A new lipid nanoemulsion (LNE) system containing granisetron (GRN) was developed by Doh et al., 2013 and its *in vitro* permeation-enhancing effect was evaluated using Caco-2 cell monolayers. Particle size, polydispersity index (PI) and stability of the prepared GRN-loaded LNE systems were also characterized. The mean diameters of prepared LNEs were around 50 nm with PI<0.2. Developed LNEs were stable at 4°C in the dark place over a period of 12 weeks. *In vitro* drug dissolution and cytotoxicity studies of GRN-loaded LNEs were performed. GRN-loaded LNEs exhibited significantly higher drug dissolution than GRN suspension at pH 6.8 for 2h (P<0.05). *In vitro* permeation study in Caco-2 cell monolayers showed that the LNEs significantly enhanced the drug permeation compared to GRN powder. The *in vivo* toxicity study in the rat jejunum revealed that the prepared GRN-loaded LNE was as safe as the commercial formulation (Kytril). They concluded that LNE could be used as a potential oral liquid formulation of GRN for anti-emetic treatment on the post-operative and chemotherapeutic patients.

Consumption of carotenoids may reduce the incidences of certain chronic diseases, but their use in foods is currently limited because of their poor water-solubility, low bioavailability and chemical instability. Qian et al., 2012 examined the impact of carrier oil type on the bioaccessibility of β-carotene encapsulated with in nanoemulsion-based delivery systems. Oil-in-water nanoemulsions (d<200nm) were formed using a non-ionic surfactant (Tween 20) as emulsifier and long chain triglycerides (LCT), medium chain triglycerides (MCT) or orange oil as carrier oils. The influence of carrier oil type on β-carotene bioaccessibility was established using an *in vitro* model to simulate the oral, gastric and small intestinal phases of the gastrointestinal tract. The rate and extent of free fatty acid production in the intestine decreased in the order LCT≈MCT>>orange oil; whereas β-carotene bioaccessibility decreased in the order LCT>>MCT>orange oil. The bioaccessibility of β-carotene was negligible (~0%) in orange oil nanoemulsions because no mixed micelles were formed to solubilise β-carotene, and was relatively low (~2%) in MCT nanoemulsions because the mixed micelles formed were too small to solubilise β-carotene. In contrast, β-carotene bioaccessibility was relatively high (~66%) in LCT nanoemulsions. They concluded that their results have important implications for the design of effective delivery systems for encapsulation of carotenoids and other lipophilic bioactive components.
Gong et al., 2012 developed a novel and stable nanoemulsion formulation of natural vitamin E with increased oral bioavailability. The natural vitamin E nanoemulsion was prepared by a modified emulsification technique. The physicochemical characteristics of natural vitamin E nanoemulsion were characterized and its pharmacokinetics study was performed as well. The experimental results showed droplet diameters ranging from 20 to 400 nm (average, 87.7 nm) with a negative electrostatic potential (-23.5 ± 1.5 mv). The pharmacokinetics study of this nanoemulsion and corresponding soft capsule was carried out using noncompartment model method. Compared with the marketed soft capsule, the \( C_{\text{max}} \) of the natural vitamin E nanoemulsion was higher, while the \( T_{\text{max}} \) was shorter. Thus, plasma concentration-time profiles in rats dosed with nanoemulsion showed a 1.6-fold enhancement in the area under the curve of natural vitamin E compared with the marketed soft capsule. The antioxidative effects of the natural vitamin E nanoemulsion and the marketed soft capsule were also evaluated by the levels of superoxide dismutase (SOD) activity and malondialdehyde (MDA) concentration in serum and liver tissue. According to the SOD activity and the MDA concentration determined, the nanoemulsion was superior to the marketed soft capsule as an antioxidative agent. The overall results demonstrated that the nanoemulsion drug delivery system could be a promising strategy for the delivery of natural vitamin E, which showed great potential for clinical application.

Coenzyme Q\(_{10}\) (CoQ\(_{10}\)) is an insoluble antioxidant molecule with great biological value but exhibit poor bioavailability. To improve the bioavailability of CoQ\(_{10}\), Belhaj et al., 2012 formulated a nanoemulsion consisting of salmon oil, salmon lecithin, CoQ\(_{10}\) and water. A commercial oily mixture, based on soybean oil and CoQ\(_{10}\), was used for comparison, as well as a second oily mixture, composed of salmon lecithin, salmon oil and CoQ\(_{10}\). Salmon oil and salmon lecithin were used as sources of polyunsaturated fatty acids (PUFA). The maximum solubility of CoQ\(_{10}\) in salmon oil was 81.30 ± 0.08 mg/mL at 37 °C. Mean droplets size of the control and CoQ\(_{10}\) nanoemulsions was 164 and 167 nm, respectively. The nanoemulsion was stable during 30 days at 25 °C. Bioavailability was evaluated as the area under the curve of CoQ\(_{10}\) plasma concentration in male Wistar rats following oral administration of the three formulations of CoQ\(_{10}\). The nanoemulsion increased the bioavailability of CoQ\(_{10}\) at least two folds as compared to the conventional oily formulations regardless of the nature of used fatty acids (soybean and salmon oils). They concluded that nanoemulsion represents a
vectorization of both LC-PUFAs and CoQ(10) and could be an interesting way to increase the absorption of these two bioactive molecules with natural low availability.

Borhade et al., 2012 evaluated the potential of clotrimazole as antimalarial drug. Due to poor aqueous solubility and high lipophilicity, it was formulated in a nanoemulsion based system. The intrinsic effects of nanoemulsion on improvement of antimalarial activity of clotrimazole were assessed in mice infected with Plasmodium berghei and compared to its suspension formulation. In four-day suppressive test, mice treated with 10mg/kg clotrimazole nanoemulsion showed the highest suppression of parasitemia and parasitemia was significantly lower than that of 10mg/kg clotrimazole suspension. In onset of activity and recrudescence test, percent reduction of parasitemia was significantly higher in 10 and 15 mg/kg clotrimazole nanoemulsion groups compared to 15 mg/kg suspension group. In both murine models, survival of mice treated with nanoemulsion was significantly prolonged compared to suspension at equivalent doses. The inhibition of parasite growth by clotrimazole in the nanoemulsion was dose dependent as determined by test for linear trend. In repeated dose oral toxicity, levels of serum liver enzymes and biomarkers of hepatotoxicity did not vary significantly from control. Six-month stability testing of the clotrimazole nanoemulsion exhibited no changes in various physiochemical attributes of drug product compared to initial analysis.

Shen et al., 2011 studied the effect of eugenol on colchicine transport across an isolated rat intestinal membrane using an in vitro diffusion chamber system. They found that eugenol increased the absorptive transport of the drug efficiently. The effect of eugenol on intestinal absorption of colchicine in an oral administrative nanoemulsion formulation was also demonstrated in vivo. The colchicine nanoemulsion was prepared with isopropyl myristate, eugenol, Tween80, ethanol and water, and eugenol was used as an oil phase in the formulation; an average particle size of this nanoemulsion was 41.2 ± 7.2 nm. The permeation of colchicine in the nanoemulsion across the intestinal membrane was significantly different from that of the control group (0.2 mM colchicine). Finally, co-administration of eugenol in colchicine nanoemulsion to enhance the colchicine bioavailability was investigated by an oral administration method. After oral administration of colchicine (8 mg/kg) in the form of either the nanoemulsion or in free colchicine solution, the relative bioavailability of nanoemulsion and eugenol-nanoemulsion were enhanced by about 1.6- and 2.1-fold, respectively, compared with free colchicine solution. The procedure indicated that the
intestinal absorption of colchicine was enhanced significantly by eugenol in the tested nanoemulsion. All the results suggested that eugenol is an efficient component in an oral administrative formulation for improving the intestinal absorption of colchicine. Silymarin, obtained from Silybum marianum is used for hepatoprotection and has poor aqueous solubility and low bioavailability. Parveen et al., 2011 incorporated the drug into oil-in-water (o/w) based nanocarrier to increase its oral bioavailability. In the present study, o/w nanocarrier was prepared by titration method and was characterized for droplet size, viscosity, etc. In vitro drug release was carried out by dialysis membrane method. A pharmacokinetic study was performed to determine maximum plasma concentration (C(max)), area under the curve (AUC), etc. and hepatoprotective activity was evaluated in terms of serum enzyme estimation. The optimized nanoemulsion formulation consisted of sefso-218 as oil, tween 80 as a surfactant and ethanol as a co-surfactant having nano-droplet size and low viscosity. In vitro dissolution studies showed higher drug release from nanoemulsion as compared to bulk drug suspension. The AUC and C(max) of nanoemulsion after oral administration were 4-fold and 6-fold higher than those of drug suspension of silymarin. The results of pharmacokinetic studies showed better effects of developed nanoemulsion than drug suspension and marketed formulation. The study showed that the nanoemulsion being a versatile technology has the potential to improve the biopharmaceutics properties of silymarin.

Chhabra et al., 2011 developed nanoemulsion (NE) of amlodipine besilate (AB) by spontaneous emulsification method with the aim to enhance the solubility and oral bioavailability of AB and to achieve localized delivery of drug at target site. Pseudoternary phase diagrams were constructed to identify the NE region. The selected formulations from NE region were subjected to droplet size analysis, partitioning study and in vitro drug release. The partition coefficient was calculated and correlated with percent dissolution efficiency as a tool to predict in vitro drug release from NEs. The release of drug from NEs was significantly higher (p < 0.01) than the marketed tablet formulation. The optimal formulation contained 15% Labrafil M, 35% [Tween 80: ethanol (2:1)], and 50% by weight aqueous phase (NE3) and was characterized by transmission electron microscopy (TEM) and for thermodynamic stability. The pharmacokinetics and biodistribution studies of the optimized radiolabeled formulation (99mTc-labeled) in mice (p.o.) demonstrated a relative bioavailability of 475% against AB suspension. In almost all the tested organs, the
uptake of AB from NE was significantly higher ($p < 0.05$) than AB suspension especially in heart with a drug targeting index of 44.1%, also confirming the efficacy of nanosized formulation at therapeutic site. A three times increase in the overall residence time of NE further signifies the advantage of NEs as drug carriers for enhancing bioavailability of AB.

Candesartan cilexetil (CC), an inactive prodrug of candesartan, was rapidly hydrolyzed into active candesartan during absorption in the gastrointestinal (GI) tract to achieve antihypertensive effects. However, CC exhibited incomplete intestinal absorption with low oral bioavailability due to its poor aqueous solubility. Gao et al., 2011 designed a novel CC loaded nanoemulsion (CCN) to improve the intestinal absorption. CCN was prepared by a modified emulsification-solvent evaporation technique. The physicochemical characteristics of CCN were characterized, and the intestinal absorption was investigated as well. The experimental results indicated that CCN was nanometer-sized droplets (35.5±5.9 nm) with negative potential (-6.45±0.36mV), and the absorption of CCN was significantly improved in total intestinal tract compared with free CC solution. Moreover, CCN could be internalized into the enterocytes by clathrin-mediated endocytosis pathway, and thereafter transported into systemic circulation via both portal vein and lymphatic pathway. The concentration of active candesartan in rat plasma was determined by LC-MS-MS method. The experimental results showed that the area under the concentration-time curve (AUC(0-t)) of candesartan was improved over 10-fold after CC was incorporated into CCN. The overall results implicated that the nanoemulsion was very effective for enhancing the oral absorption of insoluble CC, and CCN showed the great potential for clinical application.

Bali et al., 2010 developed nanoemulsion of ezetimibe and evaluated its stability, pharmacodynamic and pharmacokinetic potential. Solubility of ezetimibe was determined in various vehicles. Surfactants and cosurfactants were grouped in two different combinations to construct pseudoternary phase diagrams. Formulations were selected from the o/w nanoemulsion region and were subjected to various thermodynamic stability and dispersibility tests. Optimized formulations were characterized for their percentage transmittance, refractive index, viscosity, droplet size and zeta potential. Release rate of optimized formulations was determined using an in vitro dissolution test. The formulation used for assessment of lipid lowering potential and bioavailability contained Capryol 90 (10%, v/v), Tween 20 (33.33%, v/v), PEG 400 (16.67%, v/v), double distilled water (40%, v/v). The release of drug from the nanoemulsion formulations was extremely significant.
(p<0.001) in comparison to the drug suspension. More than 60% of the drug was released in the initial 1h of the dissolution study in comparison to the drug suspension. The value of total cholesterol in the group administered with the formulation PF1 was highly significant (p<0.001) with respect to the group administered with the suspension of the drug. The plasma concentration time profile of ezetimibe from nanoemulsion represented greater improvement of drug absorption than the marketed formulation and simple drug suspension. The shelf life of the nanoemulsion was found to be 5.94 years at room temperature. They concluded that established nanoemulsion formulation could be one of the possible alternatives to traditional oral formulations of ezetimibe to improve its bioavailability.

Singh and Vingkar, 2008 developed nanoemulsion for enhancing the bioavailability and therapeutic efficacy of primaquine. Primaquine (PQ) is one of the most widely used antimalarial and is the only available drug till date to combat relapsing form of malaria especially in case of Plasmodium vivax and Plasmodium ovale. Primaquine acts specifically on the pre-erythrocytic schizonts which are concentrated predominantly in the liver and causes relapse after multiplication. However application of PQ in higher doses is limited by severe tissue toxicity including hematological and GI related side effects which are needed to be minimized. Lipid nanoemulsion has been widely explored for parenteral delivery of drugs. Primaquine when incorporated into oral lipid nanoemulsion having particle size in the range of 10-200 nm showed effective antimalarial activity against Plasmodium berghei infection in swiss albino mice at a 25% lower dose level as compared to conventional oral dose. Lipid nanoemulsion of primaquine exhibited improved oral bioavailability and was taken up preferentially by the liver with drug concentration higher at least by 45% as compared with the plain drug.

Vyas et al., 2008 developed a novel oil-in-water (o/w) nanoemulsions containing Saquinavir (SQV), an anti-HIV protease inhibitor, for enhanced oral bioavailability and brain disposition. SQV was dissolved in different types of edible oils rich in essential polyunsaturated fatty acids (PUFA) to constitute the internal oil phase of the nanoemulsions. The external phase consisted of surfactants Lipoid-80 and deoxycholic acid dissolved in water. The nanoemulsions with an average oil droplet size of 100-200 nm, containing tritiated ([3]H)-SQV, were administered orally and intravenously to male Balb/c mice. The SQV bioavailability as well as distribution in different organ systems was examined. SQV concentrations in the systemic circulation administered in flax-seed oil nanoemulsions were
threefold higher as compared to the control aqueous suspension. The oral bioavailability and distribution to the brain, a potential sanctuary site for HIV, were significantly enhanced with SQV delivered in nanoemulsion formulations. In comparing SQV in flax-seed oil nanoemulsion with aqueous suspension, the maximum concentration (C(max)) and the area-under-the-curve (AUC) values were found to be five- and threefold higher in the brain, respectively, suggesting enhanced rate and extent of SQV absorption following oral administration of nanoemulsions. The results of this study showed that oil-in-water nanoemulsions made with PUFA-rich oils may be very promising for HIV/AIDS therapy, in particular, for reducing the viral load in important anatomical reservoir sites.

2.2 Nanoemulsion for Cancer Therapy

Betulin (Bet), the main component of birch tree bark, has been recently reported to exert anticancer activity in several cell lines. Dehelean et al., 2013 assessed the in vivo effects of betulin administered as nanoemulsion (NE) in two experimental models: (i) the chicken embryo chorioallantoic membrane (CAM) assay for the study of anti-angiogenic effects and (ii) the two-stage model of skin carcinoma induced in mice for the study of anti-tumor and anti-inflammatory effects, respectively. On the CAM of the chicken betulin in nanoemulsion (BetNE) showed a good penetrability at extra-embryonic tissue level, affecting both the chorioallantoic membrane as well as the yolk sac by reducing the capillary density. In the animal model, the potential impact of local application of betulin on the respiratory function of isolated liver mitochondria was further assessed. Topical application of betulin nanoemulsion for 12 weeks together with DMBA (7,12-dimethylbenz[a]anthracene) and TPA (12-O-tetradecanoylphorbol 13-acetate), as tumor initiator and promoter, enhanced the active respiration of isolated liver mitochondria. Betulin also inhibited skin tumor apparition and promotion, proved by histological results and VEGF (vascular endothelial growth factor) expression correlated to non-invasive measurements. Betulin is active in nanoemulsion formulation as a potential inhibitory on the angiogenic process in CAM assay. They concluded that BetNE can be used as a potent anti-inflammatory and anti-carcinogenic formulation with a low toxicity at skin level and can also influence the penetration of carcinogens and reduce damage in main organs (e.g., liver).

In recent years, diverse nanoemulsion vehicles (NEs) have been developed with vast potential for improving therapeutic index of clinically approved and experimental drugs. Using oils rich in omega-3 and omega-6 polyunsaturated fatty acids (PUFA), several promising
nanoemulsion formulations have been developed recently for oral and systemic administration. Jordan et al., 2012 successfully developed, characterize and optimized nanoemulsion platform, using the PUFA-rich argan oil that contain several important anti-inflammatory and antimitotic natural components. Various emulsifying mixtures of polyethoxylated solutol HS-15 and polyethylene glycol Vitamin E succinyl ester (TPGS) was used for the formation of different NEs which showed extended shelf-life stability. The physicochemical properties of prototype argan NEs were analyzed utilizing a 32 full factorial design, followed by biocompatibility screen, using normal vascular myocytes and areolar fibroblasts. While 90-180 day stability of NEs correlated with TPGS:solutol surfactant blend ratios, adverse effects on integrity of test cultures were only noted at high TPGS content in the emulsifier system, exceeding 80%. Finally, the anti-proliferative efficacy of selected stable and acceptably biocompatible nanoscale TPGS-emulsified argan oil formulations was investigated using murine breast and colon carcinoma cells. The IC_{50} values of the combination of argan oil and TPGS (40-80% wt of emulsifiers) were 5-9 folds lower compared to TPGS-free and argan-oil free control NEs. Argan oil NE, stabilized with Vitamin E TPGS and solutol HS mixtures, demonstrated significant pro-apoptotic effect on both test cancer cell lines, indicating built-in anticancer properties for such NE platform, potentially enhancing overall antineoplastic effects of incorporated candidate chemotherapeutic agents.

There is great clinical interest in developing novel nanocarriers for hydrophobic cyanine dyes used as photosensitizing agents in photodynamic therapy (PDT). Bazylińska et al., 2012 employed nanoemulsion-templated oil-core multilayer nanocapsules as robust nanocarriers for a cyanine-type photosensitizer IR-786. These nanoproducts were fabricated via layer-by-layer (LbL) adsorption of oppositely charged polyelectrolytes (PEs), i.e., anionic PSS and cationic PDADMAC on nanoemulsion liquid cores created by dicephalic or bulky saccharide-derived cationic surfactants. All nanocapsules, with different thicknesses of the PE shell and average size <200 nm (measured by DLS) demonstrated good capacity for IR-786 encapsulation. The nanocarriers were visualized by SEM and AFM and their photo-induced anticancer effect and cellular internalization in human breast carcinoma MCF-7/WT cells were determined. Biological response of the cell culture, expressed as dark and photocytotoxicity as well as fluorescence of drug molecules loaded in the multilayer vehicles, analyzed by the FACS and CLSM techniques, indicated that the delivered IR-786 did not
aggregate inside the cells and could, therefore, act as an effective third-generation photosensitizing agent. *In vitro* biological experiments demonstrated that the properties of studied nanostructures depended upon the PE type and the envelope thickness as well as on the surfactant architecture in the nanoemulsion-based templates employed for the nanocapsule fabrication. Similarity of results obtained for stored (three weeks in the dark at room temperature) and freshly-prepared nanocapsules, attests to viability of this stable, promising drug delivery system for poorly water-soluble cyanines useful in PDT.

Ovarian cancer is a debilitating disease, which needs multi-pronged approach of targeted drug delivery and enhanced efficacy with the use of combination therapeutics. Talekar et al., 2012 examined the anticancer activity of PIK75 incorporated in surface functionalized nanoemulsions for targeted delivery to SKOV-3 cells. A pro-apoptotic molecule C₆-ceramide was also co-delivered to augment therapeutic efficacy. EGFR and FR functionalized nanoemulsions incorporating PIK75 and C₆-ceramide were characterized for particle size, surface charge, entrapment efficiency and morphology. Fluorescence and quantitative uptake studies were conducted in SKOV-3 cells to determine intracellular distribution. Cell viability was assessed using MTT assay while mechanism of cytotoxicity was evaluated using capsase-3/7, TUNEL and hROS assay. Cytotoxicity assay showed 57% decrease in IC₅₀ value of PIK75 following treatment with EGFR targeted nanoemulsion and 40% decrease following treatment with FR targeted nanoemulsion. Combination therapy with PIK75 and ceramide enhanced the cytotoxicity of PIK75 compared to therapy with individual formulations. The increase in cytotoxicity was attributed to increase in cellular apoptosis and hROS activity. The results of this study showed that the targeted system improved cytotoxicity of PIK75 compared to the non-targeted system. Combination therapy with ceramide augmented PIK75's therapeutic activity.

The natural flavonoid fisetin (3,3′,4′,7-tetrahydroxyflavone) has shown antitumour activity but its administration is complicated by its low water solubility. Ragelle et al., 2012 incorporated fisetin into a nanoemulsion to improve its pharmacokinetics and therapeutic efficacy. Solubility and emulsification tests allowed to develop an optimal nanoemulsion composed of Miglyol 812N/Labrasol/Tween 80/Lipoid E80/water (10%/10%/2.5%/1.2%/76.3%). The nanoemulsion had an oil droplet diameter of 153 ± 2 nm, a negative zeta potential (-28.4 ± 0.6 mV) and a polydispersity index of 0.129. The nanoemulsion was stable at 4 °C for 30 days, but phase separation occurred at 20 °C.
Pharmacokinetic studies in mice revealed that the fisetin nanoemulsion injected intravenously (13 mg/kg) showed no significant difference in systemic exposure compared to free fisetin. However, when the fisetin nanoemulsion was administered intraperitoneally, a 24-fold increase in fisetin relative bioavailability was noted, compared to free fisetin. Additionally, the antitumour activity of the fisetin nanoemulsion in Lewis lung carcinoma bearing mice occurred at lower doses (36.6 mg/kg) compared to free fisetin (223 mg/kg). In conclusion, they have developed a stable nanoemulsion of fisetin and have shown that it could improve its relative bioavailability and antitumour activity.

Benzyl isothiocyanate (BITC), a compound found in cruciferous vegetables, is an effective chemopreventive agent. Qhattal et al., 2011 developed nanoemulsion formulations for the oral delivery of BITC. Optimized oil-in-water BITC nanoemulsions were prepared by a spontaneous self-nanoemulsification method and a homogenization-sonication method. Both nanoemulsions entrapped high amounts of BITC (15-17 mg/mL), with low polydispersity and good colloidal stability. The BITC nanoemulsions showed enhanced solubility and dissolution compared to pure BITC. These formulations markedly increased the apical to basolateral transport of BITC in Caco-2 cell monolayers. The apparent permeability values were $3.6 \times 10^{-6}$ cm/s for pure BITC and $(1.1-1.3) \times 10^{-5}$ cm/s for BITC nanoemulsions. The nanoemulsions were easily taken up by human cancer cells A549 and SKOV-3 and inhibited tumor growth in vitro. This work showed for the first time that BITC can be formulated into nanoemulsions and may show promise in enhancing absorption and bioavailability.

Paclitaxel is an important anticancer drug and is currently used to treat a variety of cancers, including ovarian carcinomas, breast cancer, non-small cell lung cancer, and AIDS-related Kaposi's sarcoma. Lee et al., 2011 assessed and compared the safety and efficacy of EmPAC (a newly developed nanoemulsion formulation of paclitaxel) versus Taxol (the injectable formulation of paclitaxel involving the use of polyethylated or polyoxyyl castor oil currently used in the clinic). They also investigated the mechanism for the improved safety and efficacy of EmPAC over Taxol. These results showed that EmPAC had better anti-tumor efficacy than Taxol, according to in vitro cell culture studies and studies in animal tumor models. EmPAC had improved anti-tumor efficacy even in tumor cell lines that are known to be multi-drug resistant. Part of the mechanism of action for the improved efficacy may be related to EmPAC inducing greater cellular uptake of paclitaxel into tumor cells than Taxol did, according to the in vitro cell culture radioactive-labeled studies and in vitro cell culture
antibody studies. It may also partly be because EmPAC delivered more paclitaxel to the tumor mass than Taxol, while the delivery of paclitaxel to other tissues (e.g., blood, muscle, liver, spleen, kidney and lung) were similar between the two formulations of paclitaxel, according to studies in animals with tumor xenograft. EmPAC also had better safety than Taxol according to toxicology studies in rabbits. This may be because EmPAC does not contain the toxic ingredients used in formulating Taxol (such as polyethylated or polyoxyl castor oil). These results support the clinical development of the nanoemulsion formulation of paclitaxel.

Tagne et al., 2008a reported the preparation of a water-soluble nanoemulsion of the highly lipid-soluble drug Dacarbazine (DAC). In addition, relative to suspensions of DAC, the nanoemulsion preparation demonstrated a lower zeta-potential (decreased negative charge, less anionic and more cationic) which has previously been associated with influencing drug membrane permeability. This study also reported that, relative to suspensions of DAC with a mean particle size of 5470 nm, nanoemulsions of DAC having mean particle sizes of 131 nm were more efficacious. For example, in a mouse xenograft model using a human melanoma cell line, a topical application of nanoemulsions of DAC compared to the suspension preparation of DAC produced up to 10-fold greater percent (%) reductions of tumor size. The reduction in tumor size by the intramuscular (IM) injection (-61%) and topical application of the nanoemulsion preparations of DAC (-49%) appeared to be comparable in efficacy, although the former was statistically greater (p < 0.05). In addition, 12 weeks after DAC treatment cessation, 98% of the animals given the IM application of the nanoemulsion of DAC remained tumor-free compared to the control or untreated animals. During this drug cessation period, and compared to the suspension preparations, nanoemulsions of DAC showed 5-fold greater efficacies (73% versus 14%) in preventing tumor growth. In conclusion, in this xenograft mouse model of melanoma, nanoemulsion suspensions of DAC were more efficacious in the treatment and prevention of tumor growth.

Tagne et al., 2008b reported the preparation of a water-soluble nanoemulsion of the highly lipid-soluble drug tamoxifen (TAM). In addition, relative to a suspension of TAM, the nanoemulsion preparation demonstrated a greater zeta potential (increased negative charge) which has previously been associated with increasing drug/membrane permeability. This study also reported that relative to suspensions of TAM with particle sizes greater than 6000 nm, nanoemulsions of TAM, having mean particle sizes of 47 nm, inhibited cell proliferation.
20-fold greater and increased cell apoptosis 4-fold greater in the HTB-20 breast cancer cell line. This work suggested that a nanoemulsion compared to a suspension preparation of TAM increased its anticancer properties in breast cancer.

Desai et al., 2008 examined augmentation of therapeutic activity in human glioblastoma cells with combination of paclitaxel (PTX) and the apoptotic signaling molecule, C₆-ceramide (CER), when administered in novel oil-in-water nanoemulsions. The nanoemulsions were formulated with pine-nut oil, which has high concentrations of essential polyunsaturated fatty acid (PUFA). Drug-containing nanoemulsions were characterized for particle size, surface charge, and the particle morphology was examined with transmission electron microscopy (TEM). Epi-fluorescent microscopy was used to analyze nanoemulsion-encapsulated rhodamine-labeled PTX and NBD-labeled CER uptake and distribution in U-118 human glioblastoma cells. Cell viability was assessed with the MTS (formazan) assay, while apoptotic activity of PTX and CER was evaluated with caspase-3/7 activation and flow cytometry. Nanoemulsion formulations with the oil droplet size of approximately 200 nm in diameter were prepared with PTX, CER, and combination of the two agents. When administered to U-118 cells, significant enhancement in cytotoxicity was observed with combination of PTX and CER as compared to administration of individual agents. The increase in cytotoxicity correlated with enhancement in apoptotic activity in cells treated with combination of PTX and CER. The results of these studies showed that oil-in-water nanoemulsions can be designed with combination therapy for enhancement of cytotoxic effect in brain tumor cells. In addition, PTX and CER can be used together to augment therapeutic activity, especially in aggressive tumor models such as glioblastoma.

2.3 Curcumin Drug Deliveries

Gong et al., 2013 developed a biodegradable in situ gel-forming controlled drug delivery system composed of curcumin loaded micelles and thermosensitive hydrogel and applied it for cutaneous wound repair. Curcumin is believed to be a potent antioxidant and anti-inflammatory agent. Due to its high hydrophobicity, curcumin was encapsulated in polymeric micelles (Cur-M) with high drug loading and encapsulation efficiency. Cur-M loaded thermosensitive hydrogel (Cur-M-H) was prepared and applied as wound dressing to enhance the cutaneous wound healing. Cur-M-H was a free-flowing sol at ambient temperature and instantly converted into a non-flowing gel at body temperature. In vitro
studies suggested that Cur-M-H exhibited good tissue adhesiveness and could release curcumin for an extended period. Furthermore, linear incision and full-thickness excision wound models were employed to evaluate the in vivo wound healing activity of Cur-M-H. In incision model, Cur-M-H-treated group showed higher tensile strength and thicker epidermis. In excision model, Cur-M-H group exhibited enhancement of wound closure. Besides, in both models, Cur-M-H-treated groups showed higher collagen content, better granulation, higher wound maturity, dramatic decrease in superoxide dismutase, and slight increase in catalase. Histopathologic examination also implied that Cur-M-H could enhance cutaneous wound repair. In conclusion, biodegradable Cur-M-H composite might have great application for wound healing.

Zhang et al., 2013 developed a novel drug delivery system, curcumin-phytosome-loaded chitosan microspheres (Cur-PS-CMs) by combining polymer- and lipid-based delivery systems. Curcumin exhibits poor water-solubility and is rapidly eliminated from the body. They aimed to use our novel delivery system to improve the bioavailability and prolong the retention time of curcumin in the body. The Cur-PS-CMs were produced by encapsulating curcumin-phytosomes (Cur-PSs) in chitosan microspheres using ionotropic gelation. The final microsphere was spherical, with a mean particle size of $23.21 \pm 6.72 \mu m$ and drug loading efficiency of $2.67 \pm 0.23\%$. Differential scanning calorimetry and Fourier transform infrared spectroscopy demonstrated that the integrity of the phytosomes was preserved within the polymeric matrix of the microspheres. The in vitro release rate of curcumin from the Cur-PS-CMs was slower than that from curcumin-loaded chitosan microspheres (Cur-CMs) in pH 1.0, 4.0, 6.8, and 7.4. Pharmacokinetic studies in rats dosed with Cur-PS-CMs showed a 1.67- and a 1.07-fold increase in absorption of curcumin compared with Cur-PSs and Cur-CMs, respectively. The half-life of curcumin orally administration of Cur-PS-CMs (3.16 h) was longer than those of Cur-PSs (1.73 h) and Cur-CMs (2.34h). These results indicated that the new Cur-PS-CMs system combined the advantages of chitosan microspheres and phytosomes, which had better effects of promoting oral absorption and prolonging retention time of curcumin than single Cur-PSs or Cur-CMs. Therefore, the PS-CMs may be used as a sustained delivery system for lipophilic compounds with poor water-solubility and low oral bioavailability.

Curcumin has shown to be effective against various diabetes related complications. However major limitation with curcumin is its low bioavailability. In this study Joshi et al., 2013
formulated and characterized self nano emulsifying drug delivery system (SNEDDS) curcumin formulation to enhance its bioavailability and then evaluated its efficacy in experimental diabetic neuropathy. Bioavailability studies were performed in male Sprague Dawley rats. Further to evaluate the efficacy of formulation in diabetic neuropathy various parameters like nerve function and sensorimotor perception were assessed along with study of inflammatory proteins (NF-κB, IKK-β, COX-2, iNOS, TNF-α and IL-6). Nanotechnology based formulation resulted in prolonged plasma exposure and bioavailability. SNEDDS curcumin provided good results against functional, behavioural and biochemical deficits in experimental diabetic neuropathy, when compared with naive curcumin. Further western blot analysis confirmed the greater neuroprotective action of SNEDDS curcumin. SNEDDS curcumin formulation due to higher bioavailability was found to afford enhanced protection in diabetic neuropathy.

Curcumin has shown considerable pharmacological activity, including anti-inflammatory, but its poor bioavailability and rapid metabolism have limited its application. Wang et al., 2012 formulated curcumin-solid lipid nanoparticles (curcumin-SLNs) to improve its therapeutic efficacy in an ovalbumin (OVA)-induced allergic rat model of asthma. A solvent injection method was used to prepare the curcumin-SLNs. Physiochemical properties of curcumin-SLNs were characterized, and release experiments were performed in vitro. The pharmacokinetics in tissue distribution was studied in mice, and the therapeutic effect of the formulation was evaluated in the model. The prepared formulation showed an average size of 190 nm with a zeta potential value of -20.7 mV and 75% drug entrapment efficiency. X-ray diffraction analysis revealed the amorphous nature of the encapsulated curcumin. The release profile of curcumin-SLNs was an initial burst followed by sustained release. The curcumin concentration in plasma suspension was significantly higher than those obtained with curcumin alone. Following administration of the curcumin-SLNs, all the tissue concentrations of curcumin increased, especially in lung and liver. In the animal model of asthma, curcumin-SLNs effectively suppressed airway hyper responsiveness and inflammatory cell infiltration and also significantly inhibited the expression of T-helper-2-type cytokines, such as interleukin-4 and interleukin-13, in bronchoalveolar lavage fluid compared to the asthma group and curcumin-treated group. These observations implied that curcumin-SLNs could be a promising candidate for asthma therapy.
The clinical utility of curcumin (CRM) is limited due to its poor oral bioavailability. Lipid based oral formulations (LBOFs) are emerging as useful oral drug delivery systems for 'difficult to deliver' molecules like CRM. In this study, Pawar et al., 2012 reported novel Type IV LBOF for CRM using Gelucire 44/14, Labrasol, Vit. E TPGS and PEG 400 with superior CRM loading and enhanced oral bioavailability. The optimization of LBOF for CRM loading and post dilution droplet size was carried out by design of experiments (DoE) approach with Box-Behnken design. Oral bioavailability of optimized LBOF (O-LBOF) was evaluated in male Sprague-Dawley (SD) rats at a dose of 250 mg/kg. Raw CRM (control) showed $C_{\text{max}}$ and $AUC_{0-\infty}$ of 32.29 ng/ml and 38.07 ng h/ml, respectively. O-LBOF improved $C_{\text{max}}$ and $AUC_{0-\infty}$ by 11.6 and 35.8 folds respectively over control.

Curcumin is a natural bioactive compound with many health-promoting benefits. Its low oral bioavailability limits its application in functional foods. Yu et al., 2012 developed novel organogel-based nanoemulsions for oral delivery of curcumin and improvement of its bioavailability. Developed curcumin organogel was used as the oil phase in the curcumin nanoemulsion formulation. Tween 20 was selected as the emulsifier on the basis of maximum in vitro bioaccessibility of curcumin in the nanoemulsion. In vitro lipolysis profile revealed that the digestion of nanoemulsion was significantly faster and more complete than the organogel. Permeation experiments on Caco-2 cell monolayers suggested that digestion-diffusion was the major absorption mechanism for curcumin in the nanoemulsion. Furthermore, in vivo pharmacokinetics analysis on mice confirmed that the oral bioavailability of curcumin in the nanoemulsion was increased by 9-fold compared with unformulated curcumin. This novel formulation approach may also be used for oral delivery of other poorly soluble nutraceuticals with high loading capacity, which has significant impact in functional foods, dietary supplements and pharmaceutical industries.

Nanodisks (NDs) are nanoscale, disk-shaped phospholipid bilayers whose edge is stabilized by apolipoproteins. In this study, Ghosh et al., 2011 formulated NDs with the bioactive polyphenol curcumin at a 6:1 phospholipid-to-curcumin molar ratio. Atomic force microscopy revealed that curcumin-NDs are particles with diameters <50 nm and the thickness of a phospholipid bilayer. When formulated in NDs, curcumin is water soluble and gives rise to a characteristic absorbance spectrum with a peak centered at 420 nm. Fluorescence spectroscopy of curcumin-NDs provided evidence of self-quenching. Incubation of curcumin-NDs with empty NDs relieved the self-quenching, indicating
redistribution of curcumin between curcumin-loaded and empty NDs. In HepG2 cells, curcumin-NDs mediated enhanced cell growth inhibition as compared to free curcumin. In a cell culture model of mantle cell lymphoma, curcumin-NDs were a more potent inducer of apoptosis than free curcumin. The nanoscale size of the complexes, combined with their ability to solubilize curcumin, indicated that NDs may have in vivo therapeutic applications. Nanodisks (NDs), disk-shaped phospholipid bilayers stabilized by apolipoproteins, showed entrapped curcumin and improve its delivery to HepG2 and mantle cell lymphoma cells in culture. These novel nanocomplexes demonstrated interesting therapeutic application potentials.

Ganta et al., 2010 evaluated the effect of curcumin (CU) in oral bioavailability and therapeutic efficacy of paclitaxel (PTX) administered in nanoemulsion to SKOV3 tumor-bearing nu/nu mice. Oral administration of the mice with CU at 50 mg/kg for 3 consecutive days resulted in a down regulation of intestinal P-glycoprotein (Pgp) and cytochrome P450 3A2 (CYP3A2) protein levels. PTX, a Pgp and CYP3A2 substrate, was administered orally at 20 mg/kg in solution or nanoemulsion either as single agent or upon pretreatment with CUR at 50 mg/kg in tumor-bearing mice. Plasma AUC0-∞ of PTX administered in nanoemulsion to CU pretreated mice showed 4.1-fold increase relative to controls. Similarly, relative PTX bioavailability was increased by 5.2-fold, resulting in a 3.2-fold higher PTX accumulation in the tumor tissue. PTX administered in nanoemulsion to CU pretreated mice also showed significantly enhanced anti-tumor activity. Preliminary safety evaluation showed that CU + PTX combination did not induce any acute toxicity as measured by body weight changes, blood cell counts, liver enzyme levels, and liver histopathology. The results of this study suggested that combination of PTX and CU, administered in nanoemulsions, could improve oral bioavailability and therapeutic efficacy in ovarian adenocarcinoma.

Considerable interest has been focused on curcumin due to its use to treat a wide variety of disorders, however, the therapeutic potential of curcumin could often be limited by its poor solubility, bioavailability, and photostability. To overcome these drawbacks, efficacious formulations of curcumin, including nanocrystal solid dispersion (CSD-Cur), amorphous solid dispersion (ASD-Cur), and nanoemulsion (NE-Cur) was developed by Onoue et al., 2010 with the aim of improving physicochemical and pharmacokinetic properties. Physicochemical properties of the prepared formulations were characterized by scanning/transmission electron microscope for morphological analysis, laser diffraction, and
dynamic light scattering for particle size analysis, and polarized light microscope, powder X-ray diffraction and differential scanning calorimetry for crystallinity assessment. In dissolution tests, all curcumin formulations exhibited marked improvement in the dissolution behavior when compared with crystalline curcumin. Significant improvement in pharmacokinetic behavior was observed in the newly developed formulations, as evidenced by 12- (ASD-Cur), 16- (CSD-Cur), and 9-fold (NE-Cur) increase of oral bioavailability. Upon photochemical characterization, curcumin was found to be photoreactive and photodegradable in the solution state, possibly via type 2 photochemical reaction, whereas high photochemical stability was seen in the solid formulations, especially CSD-Cur. On the basis of these observations, taken together with dissolution and pharmacokinetic behaviors, CSD strategy would be efficacious to enhance bioavailability of curcumin with high photochemical stability.

Takahashi et al., 2009 encapsulated curcumin in liposomes (LEC) for enhancing the delivery of curcumin. They used two kinds of lecithins (SLP-PC70 and SLP-WHITE) for the preparation of liposomes by mechanochemical method using a microfluidizer. They observed that SLP-PC70 LEC solution was stable with encapsulation efficiency for curcumin (68.0 wt %) as compared to SLP-WHITE LEC (less than 10.0 wt %) which became unstable after one day storage. The formulation SLP-PC70 LEC, curcumin and curcumin lecithin mixture at a dose of 100 mg of curcumin/kg body weight was orally administered to Male Sprague-Dawley rats. In case of LEC formulation, a peak plasma level 319.2±70.4 μg/L was achieved in 30 min while for curcumin and its mixture peak plasma level was 64.6±10.7 and 78.3±17.9 μg/L in 120 min respectively. The AUC_{0-120} value of curcumin after oral administration of LEC was 26502.8 μg min/L, which was 4.96 fold greater than that seen after free curcumin administration. The study demonstrated that encapsulation of curcumin in lecithin lead to a substantial improvement in curcumin absorption and systemic bioavailability however little absorption was observed with co administration of curcumin and lecithin mixture. Plasma antioxidant activity was found to be significantly higher for LEC while almost similar for curcumin and its mixture, measured by TEAC assay.

Shaikh et Al., 2009 improved the oral bioavailability of curcumin by encapsulating them in nanoparticles. The PLGA nanoparticle of curcumin was developed by emulsion–diffusion evaporation method using PVA, Pluronic F-68, Vit.E TPGS and CTAB as stabilizer. The maximum encapsulation of curcumin was found with PVA (84.6 ± 1.1%) with largest particle
size (242 ± 2 nm) while lowest encapsulation with CTAB (7.5 ± 0.2%) having smallest particle size (121 ± 3 nm). On increasing drug loading from 5 to 15% using PVA as stabilizer, particles size and PDI (polydispersity index) increased from 242 ± 2 nm and 0.17 ± 0.02 to 264 ± 2 and 0.31 ± 0.05 respectively. *In vitro* drug release from PLGA nanoparticles was biphasic with a rapid release of about 24% in 24 h followed by sustained drug release of about 43% over 20 days. In XRD, no characteristic peak of curcumin was observed when entrapped into nanoparticles, indicating formation of an amorphous complex with the intermolecular interaction occurring within the matrix. After 3 months of storage at accelerated conditions, freeze-dried nanoparticles with 5% sucrose (used as cryoprotectant) were stable without any collapse or shrinkage of the dried cake. Blood levels after oral administration of curcumin nanoparticles (100 mg/kg body weight) to male Sprague Dawley rats were compared with oral curcumin suspension (250 mg/kg body weight) and suspension of curcumin with piperine as absorption enhancer (250 mg/kg+10 mg/kg respectively). A sustained release of curcumin over 48 h was observed from nanoparticles (t\text{max} 2 h), where as in case of simple suspension (t\text{max} 0.5h) and suspension with piperine (t\text{max} 0.75 h), the levels were not detectable beyond 6 h, however the plasma concentration of curcumin decreased rapidly, indicating rapid metabolism of curcumin. In comparison to simple curcumin suspension, curcumin with piperine had a relative bioavailability of 2.8 fold with a high C\text{max} while the nanoparticulate formulation of curcumin had a relative bioavailability of 26 fold compared to the former and 9.2 fold compared to the later.

### 2.4 Analytical Method for Curcumin

Kakkar et al., 2010 developed a simple and sensitive validated LC-MS/MS analytical method for determination of curcumin in rat plasma, using nimesulide as internal standard. Analyses were performed on an Agilent LC-MS/MS system using a Chromolith rod™ and isocratic elution with acetonitrile:10 mM ammonium acetate buffer (pH 3.5) (80:20, v/v) at a flow rate of 0.8 ml/min with a total run time of 3 min and an overall recovery of 77.15%. A triple quadrupole mass spectrometer, equipped with an electrospray ionization interface, operated in the negative mode was used. Calibration curve in plasma spiked with varying concentration of curcumin were linear over the concentration range of 10-2000 ng/ml with determination coefficient >0.99. The lower limit of quantification was 10 ng/ml. Intra and inter-day variability's (RSD) for extraction of curcumin from plasma were less than 10% and 15%
respectively and accuracy was 102.43-108.5%. Multiple reaction monitoring was used to monitor the transition for curcumin (m/z; 367/217 [M-H](-)) and IS (m/z; 307/229). The method was applied for determining curcumin concentration in plasma after peroral administration of 50 mg/kg of free curcumin (C-S) or curcumin loaded solid lipid nanoparticles (C-SLNs) to rats. Results established selectivity and suitability of the method for pharmacokinetic studies of curcumin from C-SLNs.

Cheng et al., 2010 developed a new reversed phase ultra performance liquid chromatography (UPLC) method for the rapid quantification of three curcuminoids (curcumin (C), desmethoxycurcumin (DMC) and bisdesmethoxycurcumin (BDMC)) in Curcuma longa Linn. (C. longa) using a Waters BEH Shield RP C 18, 2.1 mm x 100 mm, 1.7 microm column. The run time was 2 min. The influence of column temperature and mobile phase on resolution was investigated. The method was validated according to the ICH guideline for validation of analytical procedures with respect to precision, accuracy, and linearity. The limits of detection were 40.66, 49.38 and 29.28 pg for C, DMC and BDMC, respectively. Limits of quantitation for C, DMC and BDMC, were 134.18, 164.44 and 97.50 pg, respectively. Linear range was from 3.28 to 46.08 µg/mL. The mean percent recoveries of curcuminoids were 99.47±1.66, 99.50±1.99 and 97.77±2.37 of C, DMC and BDMC, respectively. Comparison of system performance with conventional HPLC was made with respect to analysis time, efficiency and sensitivity. The proposed method was found to be reproducible and convenient for quantitative analysis of three curcuminoids in C. longa. This work provided some references for quality control of C. longa.

Li et al., 2009 described a sensitive, specific and rapid high-performance liquid chromatography (HPLC) method for the determination of curcumin in rat plasma. After a simple step of protein precipitation in 96-well format using acetonitrile containing the internal standard (IS), emodin, plasma samples were analyzed by reverse-phase HPLC. Curcumin and the IS emodin were separated on a Diamonsil C 18 analytical column (4.6 x 100 mm, 5 microm) using acetonitrile-5% acetic acid (75:25, v/v) as mobile phase at a flow rate of 1.0 mL/min. The method was sensitive with a lower limit of quantitation of 1 ng/mL, with good linearity (r² >0.999) over the linear range 1-500 ng/mL. All the validation data, such as accuracy and precision, were within the required limits. A run time of 3.0 min for each sample made high-throughput bioanalysis possible. The assay method was successfully applied to the study of the pharmacokinetics of curcumin liposome in rats.
The bioavailability of the putative cancer chemopreventive agent curcumin is limited, making measurement either in target tissues or in biofluids difficult and variable between studies. Marczylo et al., 2009 developed validated methods of extraction of curcumin from biomatrices and of detection of curcumin and its conjugated metabolites using ultraperformance liquid chromatography (UPLC) and to identify metabolites of curcumin using online tandem mass spectrometry (MS/MS). The limit of detection for curcumin after solid-phase extraction from plasma or urine was 2.5 ng/mL. Extraction efficiencies were 62 and 64% for urine and plasma. Intra- and interday variabilities (RSD) for extraction of curcumin from biofluids were less than 10 and 15%, respectively, and accuracies were 92±10% for plasma and 95 ± 6% for urine. Curcumin was extracted from tissues using protein precipitation with quercetin as internal standard. Curcumin extraction from intestinal mucosa spiked with 0.2, 1, and 5 mug/g curcumin was validated. Extraction efficiency was 65-84%, accuracy was 94-106%, limit of detection was 12.5 ng/g, and intra- and interday variabilities (RSD) were 0.7-4.9 and 4.9-5.5%, respectively. The methods were applied to measure curcumin in tissues from rats that had received oral curcumin (340 mg/kg). Curcumin was found in plasma (16.1 ng/mL), urine (2.0 ng/mL), intestinal mucosa (1.4 mg/g), liver (3671.8 ng/g), and, for the first time, kidney (206.8 ng/g) and heart (807.6 ng/g). Curcumin metabolites identified by UPLC-MS/MS in plasma and urine were phenolic glucuronides and, probably, alcoholic glucuronides. Products of reduction of curcumin and their metabolites were found in the liver.

Pak et al., 2003 developed an HPLC assay method using three methods of plasma sample preparation in order to quantitate curcumin, the main constituent in the herbal dietary supplement turmeric. Each method involved simple and rapid processing of samples (either an ethyl acetate or chloroform extraction) with resulting different quantitation limits for curcumin. The assay was developed in an effort to quantify extremely low curcumin plasma concentrations observed in preliminary in vivo studies. The most sensitive assay can reliably detect concentrations down to 2.5 ng/ml. Plasma quantitation was precise and accurate based on both intra- and inter-day validations as indicated by low values for coefficients of variation and bias, respectively (< or =15%). The analytical validation was reproducible between different analysts.

Curcumin, a derivative of the plant Curcuma longa, is used extensively in the food industry. It is a major component of curry powder, and research has shown that curcumin may prevent
cancer and other chronic diseases. Heath et al., 2003 developed a robust automated analytical method for the determination of curcumin in plasma and urine. The method involved extracting curcumin from 0.2 ml sample volume with ethyl acetate/methanol organic solvents, and use of an internal standard, beta-17-estradiol acetate. Analysis utilized a reversed-phase C$_{18}$ column and UV detection at 262 nm. Performance characteristics were assessed. The assay was linear from 0.2 to 7.0 microgram/ml. The coefficient of variation for intra- and inter-day assays was <7.5%. The average recovery of curcumin from plasma and urine was greater than 96%. The data presented in the report demonstrated that the method provides rapid, sensitive, precise and accurate measurements of curcumin concentrations in plasma and urine.

2.5 Literature Survey Related to Patent

Khamar et al., 2013 invented stable liquid pharmaceutical compositions of curcumin or its pharmaceutically acceptable salts or its derivatives with higher curcumin concentration and improved bioavailability without the use of buffer and/or molecular aggregation inhibitor(s). In accordance with invention the curcumin was in the solubilized form to make a stable liquid pharmaceutical composition. The main claim included a stable pharmaceutical composition of curcumin comprising oil, solvent, surfactant and optionally antioxidant, wherein the ratio of oil to solvent, surfactant to solvent and surfactant to curcumin was in the range of 0.83 to 10, 1 to 60 and 3 to 15, respectively with curcumin in the range of 2 to 20% w/w.

Liu et al., 2013 invented a method of increasing the bioavailability of curcumin. A synergistic combination of excipient polymers provides increased bioavailability thereby increasing the plasma concentration of curcumin and its metabolite curcumin O-glucuronide. The curcumin pharmaceutical compositions are suitable for modifying DNA methylation and treating diseases such as cancer. The main claim included a pharmaceutical composition comprising: curcumin, and at least two excipient polymers selected from the group consisting of a polyethoxylated castor oil, a polyoxyethylene sorbitan ester, and a polyethylene glycol (PEG).

The invention of Endre, 2003 relates to the field of compositions of biologically active agents (nutritional supplements, drugs and pharmaceutical agents), their dosage form and new method of administration into the human body. The invention combines the idea of application of nanoemulsions as transport systems for agents and the oral delivery method of
said compositions by absorption through the buccal mucosa. Said nanoemulsions contained both water-soluble and fat-soluble biologically active agents, which did not need the conventional emulsifier additives in order to show physical stability. The phase separation of the fat and water soluble particles of the solution was avoided by the addition of pure natural phospholipids. The nanoemulsions with mean droplet diameters ranging from 0.00005 to 0.0001 mm were dispersed by a mechanical pump spray into the oral cavity, where they were absorbed through the buccal mucosa and reached the bloodstream and benefited the body.

James et al., 2013 invention provided methods and compositions for the stimulation of immune responses. Specifically, the invention provided nanoemulsion compositions harboring one or more immunogens within the oil phase of the nanoemulsion and methods of using the same for the induction of immune responses (e.g., innate and/or adaptive immune responses (e.g., for generation of host immunity against an environmental pathogen). Compositions and methods of the invention found use in, among other things, clinical (e.g., therapeutic and preventative medicine (e.g., vaccination)) and research applications.

The invention of Hanefeld et al., 2012 related to a lyophilized nanoemulsion comprising a lipophilic phase and one or more saccharose fatty acid esters, to the nanoemulsion that can be obtained by redispersion from the lyophilized nanoemulsion, and to a method for the production of the lyophilized nanoemulsion.

Kohli et al., 2011 disclosed in their invention a pharmaceutical composition in the form of selfnanoemulsifying drug delivery formulation comprising curcuminoids. The pharmaceutical composition of the invention showed an enhanced drug loading ability, better stability and an improved bioavailability. The composition of the invention comprised of a pharmaceutically effective amount of a curcuminoid, an oil phase, a surfactant and a co-surfactant. The main claim included a self-emulsifying drug delivery system for a pharmaceutical composition, comprising, a pharmaceutically effective amount of curcuminoid, an oil phase, a surfactant, wherein said surfactant is a polyoxyethylene or polyethoxyl derivative of a vegetable oil, a co-surfactant, wherein said co-surfactant is different from said surfactant; further wherein said composition is substantially free of polymeric molecular aggregation inhibitor.

Kakumanu et al., 2011 disclosed compositions and methods of forming nanoemulsions, e.g., containing an active component, in combination with lipophilic components such as oils, hydrophilic components such as water, and one or more surfactants capable of causing a
temperature-dependent phase inversion, such as a nonionic polyethoxylated surfactant. Nanoemulsions containing the active component can be produced having average oil droplet sizes of less than 100 nm, 50 nm, or 25 nm without the need for high energy emulsion forming methods (such as microfluidization) by combining the surfactant and the oil in specified weight ratios (e.g., at least 3:1) prior to forming the nanoemulsion.

Sahoo et al., 2011 invented a suitable drug delivery system encompassing a water-insoluble drug curcumin for the treatment of cancer. The composition contained a pharmaceutically acceptable carrier thus providing a biocompatible drug delivery system. The invention further disclosed that the nanoparticles were composed of glycerol monooleate (GMO), polyvinyl alcohol and pluronic F-127 and showed high surface charge (around -32 mV) demonstrating enhanced solubility, stability and bioavailability of entrapped curcumin. The main claim of the method was ‘a novel water soluble curcumin loaded nanoparticulate system for cancer therapy having narrow monodispersed unimodal size distribution (<200 nm) with high zeta potential around -32 mV’. The inventor also claimed the method for the preparation of nanoparticles ‘The method for preparing curcumin loaded nanoparticles system comprised of: incorporating curcumin into the fluid phase of GMO; subjecting the GMO mixture to the step of emulsification with PVA; emulsifying the resultant solution with pluronic F-127 solution; lyophilizing the final emulsion by freeze drying to produce lyophilized powder.

Kumar et al., 2010 invented a method of preparation for curcumin bound to chitosan nanoparticles and methods of producing the same. Bioavailability of curcumin in these formulations was shown to improve by more than 10 fold. The method involved coating of curcumin on the surface of chitosan nanoparticles.

Nair et al., 2010 invented a process for nanomulsification of highly lipophillic polyphenols compounds using non-ionic surfactant and a non-ionic co-solvent with the help of sonar energy, to enhance the aqueous solubility. The said process comprised of following steps:

a. stirring a mixture of curcuminoid and non ionic surfactant polysobate 80 in 1:10 ratio;

b. adding nonionic cosolvent, polyethylene glycol in a ratio of 1:10 with reference to curcumin with continued stirring in order to disperse the curcuminoid particles well in the medium;

c. subjecting the above mixture to sonication for 15-30 min or till the curcuminoids are fully solubilized or until there are no visible particles in the mixture; and
d. dispersing the above mixture into the aqueous phase followed by stirring well to solubilize curcuminoids in water.

Kar et al., 2010 invented a method for preparation of curcumin nanoparticles in which curcumin bound to chitosan nanoparticles. Bioavailability of curcumin in these formulations was improved by more than 10 fold. Nano-sized particles range between 50 to 284 nm of pure curcumin wherein said nano-sized particles comprise about 100% curcumin.

Bansal et al., 2010 invented a pharmaceutical composition of curcuminoids with higher drug loading ability, improved bioavailability having adequate physical and chemical stability as a self nanoemulsifying composition. The main claim included a self nanoemulsifying curcuminoids composition, said composition comprises 0.001% to 30.0% (w/w) of curcumin or curcuminoids, a lipidic carrier system wherein the hydrophilic-lipophilic balance of the lipid carrier system is in the range of 3 to 14, a pH buffer, optionally a polymeric molecular aggregation inhibitor, a surface active agent, a co-solvent and pharmaceutically acceptable excipients, the said composition was capable of spontaneously forming a nanoemulsion when added to aqueous medium with globule size below 200 nm.

Chaniyilparampu et al., 2010 developed nanoemulsified topical formulation comprising curcumin, tetrahydroxycurcumin (BDMC) and curcuminoid either alone or in combinations in an amount of 0.001 % to 50% together with one or more pharmaceutically, nutraceutically or dietetically acceptable excipient(s) or inactive ingredients, useful for the treatment of inflammation, various skin disorders, mucosal disorders and other diseases associated or related thereof.

Robert et al., 2009 disclosure related to compositions and methods of forming nanoemulsions, e.g., containing an active component, in combination with lipophilic components such as oils, hydrophilic components such as water, and one or more surfactants capable of causing a temperature-dependent phase inversion, such as a nonionic polyethoxylated surfactant. Nanoemulsions containing the active component can be produced having average oil droplet sizes of less than 100 nm, 50 nm, or 25 nm without the need for high energy emulsion forming methods (such as microfluidization) by combining the surfactant and the oil in specified weight ratios (e.g., at least 3:1) prior to forming the nanoemulsion.

Kurzrock et al., 2008 invented composition and method which included composition for efficient loading of curcumin, comprising: an amount of curcuminoid: liposome complex
effective to load curcumin into liposomes, wherein the curcuminoid was in between 2 to 9 weight percent of the total composition and the curcuminoid are natural or synthetic. Mora-Gutierrez, 2007 invented a nanoemulsion composition having enhanced oxidative stability, emulsion stability, and health benefits. The composition included individual ingredients or a synergistic blend of non-reducing sugars, sugar polyols, medium-chain triglycerides, polysaccharides, polyphenols, phospholipids, chitosan, and alpha-casein, beta-casein, kappa-casein or protein fragments, glycopeptides, phosphopeptides. The composition may optionally be further utilized for the prevention of hypercholesterolemia or bone (and teeth) mineral loss.