Review Article

Current Status and Future Prospects of Application Specific Engineered Nanocurcumin Compounds

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ABSTRACT

Establishing chemical conjugations among complex macromolecules and nanostructured material species with the help of activity-specific chemical moieties either acquired during chemical synthesis of the backbone structures or attaching them later along with the targeting ligands on to the nanoparticulate surfaces by surface functionalization are currently being used in preparing functional compounds appropriate for targeted drug/gene delivery applications. Current drug discoveries involving phytochemicals in place of purely synthetic molecules are getting translated faster into relatively cost-effective formulations that are free from meeting most of the requirements of the stringent test-conditions set by the regulatory authorities as they use the constituents with known toxicity profiles already established through traditional experience of using them as medicine over a long period of time in the past. Moreover, the knowledge of their physico-chemical attributes like hydrophilicity, hydrophobicity, and amphiphilicity of these constituents is easy to put to use in controlling the shape and size of the nanoparticulate formulations through self-organized nano/microstructures like micelles and other hierarchical supramolecular assemblies in different solvent media as discussed in this review. The structure activity relationships (SARs) of curcumins have shown enough indications that they are going to emerge as an intelligent drug design platforms where the synergistic combinations with natural and benign phytochemicals with numerous biomedical activities would certainly produce smarter but affordable treatments of human ailments in near future.

KEYWORDS: Nanoparticulate Preparations of Curcumin, Smart and Intelligent Drug Design Platform, Pharmaceutical Efficacy of Curcumin against Human Diseases.

Introduction

Based on the well-known principle of ‘materials by design’ of nanoscience and technology for materials discoveries and the detailed examination of the structure activity relationships (SARs) of curcumins, as considered in another earlier publication, the pharmaceutical activities associated with curcumin compounds have already been clearly established through cell line assays as well as actual trials conducted on animal and human models involving almost all kinds of ailments and as a result of that they are foreseen to emerge as disease specific formulations applicable in numerous cases once the associated bioavailability issues are adequately addressed by various approaches that are particularly discussed in this current review (Ahmad, 2005; Ahmad, 2016c). Of course, for maximizing the benefits to be derived from these curcumin compounds there are also possibilities of either supplementing them with other phytosomal compounds derived from natural sources or modifying them in such a way that their metabolic problems are well taken care of before they are put to use. In this context, the basic principles of nanoscience and technology are expected to play major roles in offering numerous useful solutions, as there are well established ways of modifying the characteristic properties of the nanostructured material entities over a wide range under varying conditions of the modifications with special reference to their applications in semiconductor devices (Scher, et al, 2003; Talapin, et al, 2010; Diaconescu, et al, 2013; Ahmad, 2015). Applications of these modifications incorporated into curcumin and its compounds are discussed in this review.

The extension of the basic concept of modifying the physical, chemical and biological activities of nanostructured material species, already examined in the domain of nanoscience and technologies, are currently being employed in synthesizing nanostructured materials building blocks for arriving at macromolecular complexes involving covalent and noncovalent bondings to offer the requisite pharmaceutical properties for their further integrations into chemical conjugations meant for offering not only the desired set of the engineered pharmaceutical characteristics but also acquiring single/multiple stimuli responsive features, which combined with their self-organizing behaviors would be useable in

incorporating smart and intelligent features in an autonomous manner quite similar to those seen in the living organisms (Ahmad, 2016a; b). The concurrent developments taking place in the different domains of drug and gene deliveries, nanobiomaterials, genetic engineering (pathways involved in influencing the biochemical responses) in connection with providing effective treatment of numerous disease have been found extremely useful and the current developments of life sciences are especially providing sufficient impetus to acquire better understanding and develop better remedies to human sufferings.

In this context of drug and gene delivery developments, it is also important to note that instead of starting with completely new synthetic molecules as drug development platform it will be far better and safer to start with phytochemicals derived from various natural herbs and plant species available to provide better drug design platforms which will be far easier to translate into a successful drug because of their known toxicity profiles as compared to completely unknown ones of the altogether newer molecules as mentioned. Even the regulatory contraints will be much simpler in the cases of phytochemicals than other unknown compounds, which would be difficult to charter over a short period of time. So, instead of taking risk of unknowns, it would be better to start with known phytochemicals and then apply all kinds of nanoformulations based modifications and conjugations to realize the ultimate target goals. In this approach, a number of the natural compounds could also be used in form of adjuvants or conjugated complexes with proper chemical moieties based bridges for improving their pharmaceutical activities appropriately.

In addition, there are numerous kinds of nanoformulations that are possible to develop while combining with a large variety of inorganic, organic and biopolymeric nanoparticulates offering properties, which would certainly be synergistic combinations of all the constituents used. Some of the examples are included in case of curcumin in this review (Ahmad, 2016a).

While exploring all the facets of curcumin, the problem of producing them in large quantities with controlled features must be addressed to as included in the current review. There are different concerns, which require further developments to arrive at a commercially viable green process, as far it is possible to put minimum load on the environment. Having observed the versatile activity chart of the curcumin in affecting numerous diseases, it is possibly a question of time to reach to a stage where curcumin will not only be popular as spice or coloring agent but also in the form of disease specific remedies for a number of diseases.

**Bioavailability and Efficacy Improvements of Curcumin Compounds**

Numerous attempts have been made in recent past in modifying the overall performance of curcumin either by using them along with some other similar phytochemicals derived from natural resources or modify their chemical properties by incorporating changes at their different chemical bond locations wherever it is feasible as discussed in the followings.

**Curcumin as Adjuvant/Supplement Therapies**

Pharmaceutical activities of curcumin are found significantly improved in the presence of few other natural phytochemicals like piperine owing to their improved bioavailability as discussed here. This appears somewhat similar to the basic objectives of the conventional formulations of natural medicines used in Unani and Ayurvedic systems of medicines practiced in India since long. In the present context of studying these adjuvants through proper understanding of the pathways involved in these cases a better way of exploring the synergism is seen to exist among the phytochemical compounds can be put together to proper uses after knowing the basic mechanism of interactions involved therein.

Curcumin has been used as an adjuvant therapy in a number of cases to overcome part of its poor bioavailability by getting some metabolic pathways blocked. Some of the known compounds in this context are discussed below, in addition to considering numerous types of nanoformulations involving nanoparticulates, liposomes, micelles, and phospholipid complexes that are found as very promising alternatives to provide prolonged circulations, better permeability, and resistance to metabolic processes as discussed separately later.

Piperine - black pepper extract, for example, was the first compound of hepatic and intestinal glucuronidation inhibitor that when combined with curcumin showed significant influence in rats and humans. For instance, curcumin (i.e. 2 g/kg BW) given alone and in combination with piperine (i.e. 20 mg/kg BW) produced a maximum serum-level of 0.23 μg/mL after 0.83 hour of dosing in contrast to increased bioavailability of 154% with piperine. In humans, curcumin (2 g) along with piperine showed 2000% increase in its bioavailability as compared to that of curcumin alone. In another crossover design of human trials curcumin (2 g) was given with and without piperine (5 mg) for one week followed by cross over to the opposite therapies showed doubling of the curcumin absorption in the presence of piperine. While studying the influence of piperine on tissue uptake of radio labeled fluoro-propyl-substituted curcumin in mice, it showed significant increase (48%) in brain uptake after 2 minutes. However, in the other organs, the intake was found unaffected possibly due to its poor solubility in ethanolic saline. The glucuronidation inhibiting effect of piperine and lower activity of curcumin glucorinides show that glucuronidation inhibition by piperine may be the mechanism involved in increasing the curcumin bioavailability (Shoba, et al, 1998; Ireson, et al, 2001; Ryu, et al, 2006).

The other compounds exhibiting synergistic effects with curcumin include quercetin, genistein, eugenol/terpeneol, and epigallocatechin-3-gallate (EGCG). For example, colectomy patients receiving oral curcumin
wild-type EGFR both in-vitro and in-vivo models where it led to improved response of gefitinib to et al, 2011). Similarly, in lung cancer patients, using therapeutic activity of curcumin was discussed (Wilken, where the in-vitro and in-vivo data supported the chemotherapy and radiation causing patient morbidity example, treatment of head and neck squamous cell here to highlight the importance of curcumin. For observations a number of attempts were made as given properties with 2.2 and 2.5-fold increase of curcumin levels in the skin after 8 hours of application (Fang, et al, 2012). Other recent studies employed EGCG (epigallocatechin-3-gallate) from green tea in counteracting some curcumin activities (Balasubramanian, and Eckert, 2004). These observations made in numerous studies indicate, in general, towards the fact that the activity of curcumin can be modulated both at the cellular as well as organismic levels, and expect beneficial regulations by using different adjuvants along with.

The recent findings of anti-cancer activities in curcumin via their interactions with a variety of biological pathways involved in mutagenesis, oncogene expression, cell cycle regulation, apoptosis, tumorigenesis and metastasis in which curcumin have exhibited antiproliferative effect in multiple cancers, and as an inhibitor of the transcription factor NF-xB and downstream gene products (including c-myc, Bel-2, COX-2, NOS, Cyclin D1, TNF-α, interleukins and MMP-9) besides affecting a variety of growth factor receptors and cell adhesion molecules involved in tumor growth, angiogenesis and metastasis. Based on these observations a number of attempts were made as given here to highlight the importance of curcumin. For example, treatment of head and neck squamous cell carcinoma (HNSCC) involving surgery, platinum-based chemotherapy and radiation causing patient morbidity was supplemented with curcumin adjuvant therapy where the in-vitro and in-vivo data supported the therapeutic activity of curcumin was discussed (Wilken, et al, 2011). Similarly, in lung cancer patients, using curcumin led to improved response of gefitinib to wild-type EGFR both in-vitro and in-vivo models where it not only inhibited cell proliferations but also down-regulated (concentration-dependent) EGFR phosphorylation through promoting EGFR degradation with wild type EGFR or T790 M EGFR besides enhancing the anti-tumor activity of gefitinib by blocking EGFR activation and inducing apoptosis. The combined treatment with curcumin and gefitinib exhibited significant inhibition in the CL1-5, A549 and H1975 xenografts tumor growth in SCID mice through reducing EGFR, c-MET, cyclin D1 expression, and inducing apoptosis activation through caspases-8, 9 and PARP leading to better survival rate and less intestinal mucosal damage compared to gefitinib-alone therapy causing attenuation of cell proliferation, inhibition, and apoptosis through altering p38 mitogen-activated protein kinase (MAPK) activation in intestinal epithelia cell. These findings confirmed curcumin as an adjuvant to enhance the efficacy of gefitinib by overcoming its inefficiency (Lee, et al, 2011). The problems arising due to side effects on kidney, liver and GI system with the prolonged use of non-steroidal anti-inflammatory drug like diclofenac in knee osteoarthritis because of its analgesic and anti-inflammatoryary features (i.e. inhibiting the prostaglandin synthesis via COX-1 and COX-2 isoenzyme) curcumin, with anti-inflammatory properties causing down regulation of NF-κB and pro inflammatory cytokines such as Tumor Necrotic Factor-α, Interleukin-1, Interleukin-8, and Nitric Oxide Synatse, was found helpful with lesser side effects as confirmed in a double blind randomized trial administering diclofenac (75 mg/d + placebo) and diclofenac 75 mg/d with curcumin 1,000 mg/d for 3 months. The data collected in terms of visual analog scale (VAS) for pain and knee injury and osteoarthritis outcome score (KOOS) showed better results in terms of pain and function in daily living (Pinsornsak, and Niempoo, 2012).

Although, curcumin has extensively been studied but to date only few of these cases were devoted to its potential antiproliferation effects and resistance reversal in antiestrogen-resistant breast cancers as investigated in a study related to examining the efficacy of curcumin alone and in combination with tamoxifen in the established antiestrogen-resistant breast cancer cell lines MCF-7/LCC2 and MCF-7/LCC9. Curcumin treatment showed anti-proliferative and pro-apoptotic activities by inducing cell cycle arrest at G2/M phase confirming that the combination of curcumin and tamoxifen showed a synergistic inhibitions in MCF-7/LCC2 and MCF-7/LCC9 cells where curcumin could suppress expression of pro-growth and anti-apoptosis molecules, induce inactivation of NF-κB, Src and Akt/mTOR pathways and down regulate the key epigenetic modifier EZH2 suggesting that curcumin alone and combinations of curcumin with endocrine therapy may be beneficial in endocrine-resistant breast cancer treatment (Jiang, et al, 2013).

The pain relieving property of curcumin noted in traditional Chinese medicine and also observed in preclinical studies was put to use of its antinociceptive effects in inflammatory and neuropathic pains by conducting animal trials of postoperative pain by making surgical incisions on the right hind paw to induce a sustained mechanical hyperalgesia lasting for 5 days. Curcumin treatment (10-40 mg/kg) was noted to reverse the mechanical hyperalgesia in addition to facilitating the recovery from surgery while repeated curcumin treatment before surgery did not affect the postoperative pain threshold and recovery rate (Zhu, et al, 2014).

Inferior therapeutic efficacy of Temozolomide (TMZ) in glioblastoma treatment due to resistance was found affected by curcumin sensitization as observed in in-vitro/in-vivo experiments performed for evaluating the interaction of CUM and TMZ on the inhibition of glioblastoma in U87 MG cell lines and xenograft mouse
models that showed enhanced performance in U87MG glioblastoma via ROS route causing synergistic effect. From these findings the blockage of AKT/mTOR signaling appeared to contribute to the elevated apoptosis caused by combined treatment (Yin, et al, 2014).

The need for low toxicity adjuvants to standard chemotherapy in inoperable colorectal cancer for improving the outcomes and decreasing the side-effect burden was addressed to by adding curcumin to oxaliplatin therapy showing promising results observed in pre-clinical studies by combining oral curcumin with FOLFOX-based (5-fluorouracil, folinic acid and oxaliplatin) chemotherapy in colorectal cancer patients with inoperable liver metastases. Primary and secondary objectives of this trial were to determine safe and tolerable dose for long-term use by individuals receiving oxaliplatin-based chemotherapy for inoperable colorectal cancer and observe any changes in neuropathic side-effects of chemotherapy, improvement to progression-free or overall survival and identification of putative efficacy biomarkers in plasma (Irving, et al, 2015).

In a recent review, different mechanisms including cell cycle arrest; G0/G1 and/or G2/M phase cell cycle arrest by up-regulating Cdk inhibitor, p21/WAF/CIPI and p53, inhibition of transcriptional factors; NFκB, AP-1, TNFα, IL, STAT-3, and PPAR-γ, downstream gene regulation; c-myc, Bcl-2, COX-2, NOS, Cyclin D1, TNFα, interleukins and MMP-9, growth factors; bFGF, EGF, GCSF, IL-8, PDGF, TGFα, TNF, VEGF and cell adhesion molecules; fibronectin, vitronectin, and collagen involved in angiogenesis and metastasis were discussed in case of curcumin compounds in combination with cyclophosphamide, doxorubicin, mitomycin, and other chemotherapies, was found effective in treating breast cancer as reviewed recently (Kumar, et al, 2015).

The adjuvant curcumin therapy to anti-diabetic therapy was explored recently on the basis of the known interplay involving insulin resistance, oxidative stress, dyslipidemia, and inflammation in type-2 diabetes mellitus, by considering subjects receiving metformin therapy without and with turmeric (2g) supplements for 4 weeks. Curcumin + Metformin treated patients showed significantly decreased fasting glucose (95 ± 11.4 mg/dl) and HbA1c levels (7.4 ± 0.9 %) besides causing reductions in lipid peroxidation, MDA (0.51 ± 0.11 μmol/l); enhancing total antioxidant status (511 ± 70 μmol/l), and rendering beneficial effects on dyslipidemia LDL cholesterol (113.2 ±153.5 mg/dl), non HDL cholesterol (138.3 ±121.5 mg/dl) and LDL/HDL ratio (3.01 ± 0.61) and reducing the inflammatory marker, hsCRP (3.4 ± 2.0 mg/dl). Turmeric was thus proven to be an effective adjuvant to T2DM treatment with beneficial influence on blood glucose, oxidative stress and inflammation (Selvi, et al, 2015).

In another study of cisplatin-induced ototoxicity in head and neck squamous cell carcinoma and in rat models, curcumin was tried as an adjuvant molecule by analyzing its influence on the molecular targets, signal transducer and activator of transcription 3 (STAT3) and NF-E2 p45-related factor 2 (Nrf-2), in tumor progression and cisplatin resistance in-vitro and the adverse effect ototoxicity in-vivo. It was thus shown that curcumin attenuated all the stages of tumor progression (survival, proliferation) by targeting pSTAT3 and Nrf-2 signalling pathways, providing chemo sensitization to cisplatin in-vitro, and protecting from its adverse ototoxic effects in-vivo. This study of head and neck cancer treatment showed modulation of therapeutic targets (STAT3 and Nrf2) and, at the same time, reduced cisplatin-related ototoxic adverse effects (Fetoni, et al, 2015).

In a still continuing search for better cure of human cancers after witnessing the inadequacies of the available anti-cancer drugs exhibiting limited efficacies, causing severe side effects, besides being costly despite many significant advances made in the recent past, curcumin was extensively studied over the last few decades for its potential anti-inflammatory and/or anti-cancer effects exhibiting suppressed initiation, progression, and metastasis of a variety of tumors predominantly mediated through its negative regulation of various transcription factors, growth factors, inflammatory cytokines, protein kinases, and other oncogenic molecules besides abrogating the proliferation of cancer cells by arresting them at different phases of the cell cycle and/or by inducing their apoptosis. These related aspects of curcumin were examined in a current review by taking into account the diverse molecular targets modulated by curcumin make it work against human cancers (Shanmugam, et al, 2015).

Although, methotrexate (low doses) is commonly used in rheumatoid arthritis with its major drawback of hepatotoxicity, which were attempted to minimize using
curcumin (30 and 100 mg/Kg BW) along with sub
therapeutic dose of methotrexate (1 mg/kg) to reduce
oxidative stress and produce synergistic anti-arthritic
action in rats. Animals, given sub therapeutic dose of
methotrexate followed by half an hour later with 30 and
(100 mg/kg) of curcumin showed significant reduction in
hepatotoxicity as confirmed by a significant change in

Molecular signalling pathways based treatments of
hepatocellular carcinoma and hepatoblastoma involving
chemotherapy and surgery that are known to cause
higher morbidity and mortality due to advanced stages,
the associated adverse effects and recurrence were found
affected by curcumin adjuvant therapy showing further
improvements in terms of cancer cell apoptosis, anti-
proliferative activity, anti-angiogenic action, preventing
tumor invasiveness and metastasis and preventing
recurrence besides reducing the adverse effects of
chemotherapeutics and synergizing the anticancer
action. Curcumin being active at the molecular level by
affecting the metabolic pathways involved in
tumorigenesis promoted healing due to its anti-
inflammatory, anti-oxidant and anti-infective activities
(Sharma, et al, 2016).

Attempts were made to augment curcumin with
turmerones for enhancing its anti-tumor activities in
tumor-bearing mice based on the fact that curcumin
accumulates in the presence of turmerones inside colonic
cells and accordingly, the combined efficacy of curcumin
+ bevacizumab was investigated in HT29 colon tumor-
bearing mice to show the highest level of plasma
curcumin in extract-fod mice. Besides, the combined
treatment was noted to inhibit the tumor growth. Such
inhibitory effects were stronger than those of curcumin
plus bevacizumab or bevacizumab alone and were
comparable with those of 5-fluorouracil + leucovorin
+ oxaliplatin (FOLFOX) plus bevacizumab with no
observable side effect induced by turmeric extract while
significant side effects were there in FOLFOX-treated
mice (Yue, et al, 2016).

**Curcumin Analogues**

In order to overcome the disadvantages associated
with poor bioavailability of curcumin that limit its
utility, attempts are continuously going on to enhance its
aqueous solubility and intestinal absorption by
formulating curcumin analogues besides converting it in
various nanoformulations involving polymeric, liposomal
nanoparticulates, cyclodextrin-complexes, conjugates
with a variety of molecular complexes, solid dispersions
and many similar other preparations as discussed in a
number of publications in detail (Li, et al, 2005; Lao,

Curcumin with a number of functional groups in
β-diketonic form with two aromatic rings of phenolic
groups connected via unsaturated carbonyl groups of
diketone moiety being available in keto-enol tautomeric
form, where more stable enol-form exists in solid phase
and acidic solutions, it is metabolized very fast due to
de-protonation in mild alkaline medium resulting in the
enolate moiety. Although, availability of α, β-unsaturated
carbonyl groups as Michael acceptor causing nucleophilic
additions under biological conditions was explored for
improved bioavailability but it met with limited success
in modulating curcumin metabolisms resulting in ill-

Keeping this background in view, a number of
structural modifications in terms of incorporating
aryl-side-chains, functional changes in diketo-groups,
modifications of double bonds, methylene functionality,
and formation of metal complexes, were explored recently
for preparing useful analogues of curcumin particularly
for slowing-down their metabolisms and improving their
potencies/efficacies against cancer treatments. For
example, a bio-conjugate of Luteinizing Hormone
Releasing Hormone (LHRH) with curcumin employing
the interactions of LHRH receptors and MIAPaCa-2,
Panc-1 and BxPC-3 pancreatic cancer cells produced a
conjugate capable of inhibiting cell growth and inducing
cell-death via cleavage of polyadenosine-5′-diphosphate-
ribose-polymerase and caspase-3 besides causing
reductions in tumor weight/volume in pancreatic cancer
models (Vyas, et al, 2013). While examining the
anticancer attributes of the two analogues named - EAC
and PAC the latter one was found 5-times more effective
in inducing apoptosis and causing delay in the cell cycle
besides enhancing bio-distribution and bioavailability in
mice. Another class of curcumin with ethyl-curcumin
bioconjugates was reported with better efficacy compared
to the parent compounds while being benign to non-
cancerous cells. Compounds from another study, for
instance, were found more cytotoxic against human
malignant melanoma and breast cancers while one
compound showed highest cytotoxicity against human
ovary cancer cells (Aggarwal, et al, 2011; Al-Hujaily,

The protective influence of curcumin +
salicylcurcumin conjugate was studied by giving an
intra-gastric dose to rats where it exhibited significant
reductions in number and size of colon tumors; lowered
the lipid peroxidation and enhanced the activities of GPx,
GST, SOD and CAT in liver during 1, 2-dimethyl-
hydrazine (DMH)-induced colon carcinogenesis via
modulation of hepatic enzymes and showing antioxidant
behavior due to hydroxyl group in the aromatic ring that
are responsible for protective properties of these
compounds in place of the methoxy-group as thought
earlier (Devasena, et al, 2002; 2006).

Numerous studies have confirmed the efficacies of
curcumin derivatives including β-glucosides against
human colon cancer cells; monoesters for anti-
proliferative activities against KB and HeLa cells; newer
compounds with improved chemical properties by
glycosylation of aromatic ring resulting in better water
solubility/stability; acyl-derivative and glycosyl-
curuminoids against cisplatin (cDDP)-sensitive human
ovarian cancer cells and its resistant counterpart cancer
cells with improved performance as compared to
curcumin alone. These observations clearly point towards
the need for developing still better compounds as also
highlighted in several publications by research groups (Dubey, et al, 2008; Ferrari, et al, 2009; Arafa, 2010).

Several types of curcumin analogues were consequently explored for using them against prostate/breast cancer cells in which some specific compounds were found highly cytotoxic against androgen-dependent/ independent LNCaP, prostate and breast cancer cells besides offering another cytotoxic taxoid conjugate for possible treatment of a panel of cancer cells including human lung, ovarian, colon, epidermoid skin/nasopharynx, and KB variant expressing P-glycoprotein (KBVIN) cancer cells. Synthesis of 2-hydroxycurcuminoid (HCC-7) having 2-hydroxy-cinnamaldehyde motif showed growth inhibition capability against colon tumor cells by inducing apoptosis through reactive oxygen species-mitochondria pathway and cell cycle arrest at G2/M phase, in addition to the other analogue with highest inhibition potency of COX-1/2 enzymes. A three-dimensional structure of human P-12-LOX was also visualized from docking simulations of the curcumin derivatives to identify the inhibitors that are superior to curcumin with >75% docking into the active site of P-12-LOX.

From the list of such curcuminoids, two variants named E22C and E26C were noted to inhibit human lipoxygenase whereas false-positives curcuminoids showed poor water solubility with no inhibitory activity as discussed in the publications (Jankun, et al, 2006; Handler, et al, 2007; Nakagawa-Goto, et al, 2007; Fuchs, et al, 2009; Han, et al, 2011). A tetra-glycine conjugate of curcumin synthesized by linking of an oligonucleotide (5′-GTTAGGTTAG-3′) to a human telomerase RNA template through phosphate/C-2 linker and transfected into KB and HeLa cells was found to influence cell growth by targeting to anti-sense mechanism in telomerase. Aromatic/heterocyclic aromatic curcuminoids were also synthesized with anti-inflammatory activities against acute carrageenan-induced paw edema model and chronic adjuvant arthritis in which the compounds designated as RK-97, 103, 104 and 106 were found active in anti-arthritis assays with very little gastric/systemic toxicity especially the compound RK-106 inhibited TNF-α and IL-1β in a monocytic cell-line (Kapoor, et al, 2007; Khan, et al, 2012).

The anti-proliferative capability of PEGylated curcumin through suppression of Jab1/CSN activity was confirmed in a water-soluble conjugates with enhanced solubility, targeted delivery and greater reduction of cell growth in pancreatic cancer cells by arresting mitotic phase during formation of abnormal multi-nucleated cells, and this synthetic compound was found to affect cell cycle progression to inhibit cell growth besides increasing protein stability in pancreatic cancer cells and inhibiting Jab1/CSN-associated kinases activities (Li, et al, 2009). Another polyvinyl pyrrolidone-curcumin conjugate was reported to self-assemble in aqueous solution forming nano-sized micelles that were found more toxic against L929 fibroblast cells due to enhanced aqueous solubility and polymer-mediated drug internalization (Manju, and Sreenivasan, 2011). The PEGylated curcumin compounds are, thus, noted to activate Nrf2 several times more than free curcumin wherein the copolymer is the most potent Nrf2 activator that induces Nrf2-driven NQO1 expression in a concentration dependent manner as confirmed in gene assays (Pandey, et al, 2011).

The pro and anti-oxidant behavior of dipiperoyl and diglycinoyl derivatives cause higher cell death rates at lower concentrations than di-acetyl-curcumin while down-regulating Bcl-2 protein and activating caspase-3 in apoptotic death of tumor cells (Mishra, et al, 2005). A number of derivatives subsequently synthesized with modified aryl-rings exhibited inhibitory activities on thioredoxin reductase (TrxR) where most of the analogues were effective in low concentration (Qi, et al, 2008). The SAR analysis further revealed that analogues with furan-moiety showed strong inhibitory action on TrxR in an irreversible manner, indicating that the furan-moiety served as a possible pharmacophore during the interaction of curcumin analogues with TrxR in which the compound 46 inhibited the different TrxR over-expressed A549/R and MCF-7/R cancer cells and the conjugates with two PEG-derivatives showed improved water solubility and cytotoxicity against cancer cells including LS-174T, MIAPaCa-2 and BxPC-3 (Safavy, et al, 2007).

Some of the generic modifications incorporated in curcumin skeleton are described here in the following to highlight their importance in exploring their improved pharmaceutical efficacies.

### Chemical Modifications of Curcumin

With the help of adequate information collected from SARs of various structural moieties and their interconnecting bonds in curcumin, synthetic chemists have been trying to modify curcumin structures to produce compounds endowed with certain characteristic features considerably improved. This seems another interesting route to modify the existing chemical structure of the curcuminoids to have the desired features enhanced and/or incorporated as discussed below.

#### Aryl-Side-Chain Modification

While attempting to modify the aryl-side-chain in curcuminoids, dimethoxy-curcumin (DMC) with modified aryl-side-chain showed better potency in colon cancer because of its improved metabolic profile (Tamvakopoulos, et al, 2007). Several synthetic analogues were similarly found effective against Caco-2, murine leukemia and human lymphoblast, and human chorionic gona-dotropin-β (hCG-β)/hCG cells with growth inhibiting properties against MOLT-4 and U-937 cells (Youssef, et al, 2007; Vyas, et al, 2009; Wichitnithad, et al, 2011). DMC is known to inhibit adhesion, migration and invasion of breast cancer cells by reducing matrix metallo-proteinase-9, membrane type-1 matrix metallo-proteinase, urokinase plasminogen activator and uPA receptor along with up-regulating PAI-1 levels besides reducing expression of intercellular adhesion molecule-1 and chemokine-receptor 4 that mediates the tumor metastasis and inhibits DNA-binding of NF-κB that mediates the expression of MMPs, uPA, uPAR, ICAM-1,
and CXCR-4 proteins. DMC and bis-demethoxycurcumin (BDMC) suppress β-catenin response transcription activated by Wnt3a conditioned medium without changing intra-cellular β-catenin levels, and inhibiting colon cancer cell growth in addition to down-regulating p300, which is a positive regulator of the Wnt-β-catenin pathway (Yodkeeree, et al, 2010).

**Diketo Functionality Modifications**

By considering the influence of carbonyl/hydroxyl groups in PKC-binding the synthesis of pyrazole/isoxazole compounds were examined for higher binding to PKC-0-C1B compared to PKC-α-C1B and PKC-ε-C1B; and the role of compound-63 and semi-carbazone derivatives of curcumin in increasing cell growth inhibitory and pro-apoptotic effects in liver cancer and other tumor cells; in breast cancer and multi-drug resistant variant MCF-7R cells with over-expression of P-glycoprotein leading to inhibition of apoptosis and COX-2 proteins; and antioxidant, anti-proliferative, and radical scavenging activities in radiation induced lipid peroxidation inhibition in the rat liver microsomes affected by steric hindrance due to semi-carbazide side chains as reported in different corresponding publications (Dutta, et al, 2005; Poma, et al, 2007; Labbozzetta, et al, 2009; Sahoo, et al, 2009; Das, et al, 2011).

Multi-component-one-pot-condensation protocols of preparing curcumin, substituted aromatic aldehydes and urea/thiourea (solvent free conditions using SnCl₂, 2H₂O catalyst) were developed for synthesizing 3,4-di-hydropyrimidinones that showed cytotoxic profile in which specifically compound-67-69 showed more improved cytotoxic activity against human Hep-G2, HCT-116 and QG-56 cancer cells (Lal, et al, 2012). Other anti-proliferative curcumin compounds like hydrazine-curcumin (HC), and benzoyl-hydrazino-curcumin (HBC) derivatives in case of bovine aortic endothelial cells (BAECs) helped in identifying HC as a new anti-angiogenic agent. Identification of Ca²⁺/calmodulin (Ca²⁺/CaM) as a direct target protein of HBC using phage display biopanning was specifically bound to immobilize HBC where binding was Ca²⁺dependent (Shim, et al, 2008; Yodkeeree, et al, 2010).

**Double Bond Modification**

In connection with modifying the double-bonds, a curcumin derivative called tetra-hydro-curcumin (THC) was synthesized causing autophagic cell-death in leukemia cells by increasing the cell-death marker, forming acidic vascular organelle by down-regulating phosphatidylinositol 3-kinase/protein kinase B and mitogen-activated protein kinase signaling and decreasing phosphorylation of glycogen synthase kinase 3β and p70 ribosomal protein S6 kinase besides dose dependent reduction of HT1080 cell invasion and migration by down regulating enzymes as well as inhibiting cell adhesion to ECM proteins, and attenuating pathological features of angiogenesis including micro-vascular dilatation, and hyper-permeability leading to significant reduction in capillary vasularity (Yodkeeree, et al, 2008; Yoysungnoen, et al, 2008; Wu, et al, 2011).

**Methylenic Group Modifications**

Modifying the active methylenic groups for preparing curcumin analogues was attempted to realize an androgen receptor (AR) antagonist against two prostate cancer cells - PC-3 and DU-145 transfected with AR and androgen receptor co-activator, ARA70 constructs and several compounds from this study showed anti-angiogenic activities superior to hydroxylflutamide, which is the standard treatment of prostate cancer. For instance, specifically compound-23 did show reduction in di-hydro-testosterone (DHT)-induced AR activity in PC-3 cells, while in DU-145 cells transfected with wild-type AR and ARA70 compounds-81 and 82 were almost equipotent and were slightly more active than compound-23. SAR studies confirmed the importance of bis-(3, 4-dimethoxyphenyl) conjugated β-diketone moiety and the symmetry of the molecules for their anti-angiogenic activity by suggesting that co-planarity of the β-diketone moiety and strong hydrogen bond are crucial for the anti-angiogenic activity. After superimposing DHT and compound-23, the resultant analogues might function like 17α-substituted DHT (Ohtsu, et al, 2002). Subsequently, some compounds like 4-fluoro-4-ethoxy-carbonyl ethyl and 4-ethoxycarbonyl ethyl carbonyl curcumin were designed to overcome the inherent problems in the tautomerism of 4-ethoxycarbonyl ethyl curcumin in which the keto-enol analogues showed varying anti-androgen potencies whereas the diketo compounds were practically...
ineffective. Tetrahydropyranylation of the phenoxyl groups in curcumin is noted to have a positive impact on the anti-androgenic activity of 4-ethoxy carbonylpyryl-4'ethoxycarbonyl-6-phenylcurcumin possessing anti-androgenic activity. Five new compounds were SAR-designed and subsequently synthesized and out of which, compound-86 was found most potent anti-androgenic agent that was considered as a promising drug candidate for the treatment of prostate cancer. Further analogues were synthesized containing mono-phenyl and substituted phenyl/heterocyclic moieties at the methylenic position of curcumin with various linkers showing cytotoxicity against two prostate cancer cells - LNCaP and aPC-3 (Lin, et al, 2006).

Another group of derivatives prepared by substituting methyleneic groups with pyrazole and isoxazole pharmacophores was found to exhibit anticancer activity against MCF-7 and SKBR3 cancer cells (Amolins, et al, 2009). Similarly, ferrocenyl curcumin conjugates were synthesized and found cytotoxic to B16 melanoma and normal NIH 3T3 cells as well as inhibiting tubulin polymerization and affecting the morphology of endothelial cells whereas compounds 101-105 were found cytotoxic to B16 melanoma cells, and compound 102 was found to inhibit tubulin polymerization (Arezki, et al, 2011). Some of the compounds were noted to bind selectively to Janus kinase-2 and the STAT3 Src homology-2 domain, which is important for STAT3 dimerization and signal transduction and these compounds are known to inhibit STAT3 phosphorylation, DNA-binding activity, and transactivation leading to the impediment of multiple oncogenic processes by inducing apoptosis in pancreatic and breast cancer cells (Lin, et al, 2010). It was however, observed that administration of FLLL32 could inhibit tumor growth and vascularity in chicken embryo as well as substantial reduction in tumor volumes in mice and these findings indicate about their positive potentials in targeting pancreatic and breast cancers that exhibit constitutive STAT3 signaling and it was shown that the phosphorylated/activated form of STAT3 was expressed in colon cancer stem-like cells, which was attenuated by STAT3 selective inhibitor FLLL32 while these compounds showed specificity for STAT3 over other homologous proteins (Bill, et al, 2010; Lin, et al, 2011).

Curcumin was noted to be less effective while examining the tubulin binding affinities and self-assembly based inhibitors as a bifunctional ligand with substitutions at the diketone functionality or acetylation of the terminal phenolic groups in which benzylidene derivative is more potent in inhibiting tubulin self-assembly by showing that curcumin binds tubulin away from the colchicine-binding site. Docking simulation studies indicated that the curcumin-binding sites being closer to the vinblastine-binding sites made the compound 108 to possess higher affinity (Chakrabarti, et al, 2011). Various effects of C086 on different molecular entities were examined in connection with the growth inhibition and NF-kB regulation in colon cancer and tumors due to anti-proliferative activity against HT29, SW480, KM12, SW1116, WiDr and Colon26 cancer cells whereas its oral intakes showed growth suppression of SW480 tumors with reduced expression in tissues besides inhibiting IxB-a phosphorylation, and its subsequent degradation causing suppression of the nuclear translocation and DNA binding activity of NF-kB and noting its influence on NF-kB-regulated gene products-C-myc, cyclin D1 and Bcl-2, involved in cellular proliferation and anti-apoptosis in C086 treated group (Chen, et al, 2011).

The curcumin analogues (e.g. compound 115/116) were found cytotoxic against Ehrlich Ascites Carcinoma (EAC) (Fadda, et al, 2009). Ester and acid series based analogues gave IC50 values lower than curcumin when assessed in ovarian and colon cancer cells having selectivity against colon cells due to their high lipophilicity favoring a greater and faster cellular uptake and thereby overcoming their apparently higher instability under physiological condition (Ferrari, et al, 2011). A series of new 4-arylidene curcumin analogues was found active against the growth of a panel of lung cancer cells in which several compounds (e.g. serialized as compounds-117-126) were found exerting superior inhibition properties against A549 cells through NF-kB due to inhibition of IxB phosphorylation and degradation via IKK blockage (Qiu, et al, 2010). Out of several β-ionone-curcumin conjugates, compound-131 was found active against T877A, W741C, and H874Y mutated AR cells besides showing cytotoxicity towards LNCaP, PCa-2b, 22Rv1, C4-2B and PC-3 prostate cancer cells (Zhou, et al, 2009; Sertel, et al, 2012).

A ‘Knoevenagel Condensation’ based conversion of enolic diketones of curcumin into non-enolizable moieties using a bioactive thio-semi-carbazone pharmacophores was found effective in inhibiting TNF-induced NF-kB activation and proliferation in human leukemic KBM-5 cells with extension to include fluoro-substituted condensates to act against colon and pancreatic cancer cells. Out of these studies, di-fluorinated curcumin (CDF) compounds were reported having superior anticancer activity in colon, prostate and pancreatic cancer cells with no steric changes in the parent curcumin molecules while facilitating more H-bonding interactions.

A combination of curcumin and CDF is although noted to down-regulate the expression of NF-kB in MIAPaCa-2 cells but the latter did it at minimal concentration while both reduced the PGE2 levels showed the superiority of CDF targeting of COX-2 (Zambre, et al, 2006; Padhye, et al, 2009). For instance, a combination of CDF and gemcitabine exhibits better efficacy than CDF in inducing apoptosis in pancreatic BxPC-3, MIAPaCa-E and MIAPaCa-M cancer cells by reducing cell viability and eliminating pancreatosphere formation after treatment. Such a study recommended CDF as a significant therapeutic agent for eliminating cancer stem-like cells (CSCs) besides attenuating CD44 and EpCAM expressions in pancreatospheres. The in-vivo subcutaneous tumor models in mice showed that the combination of CDF and gemcitabine inhibits tumor
growth in MIA PaCa-2 tumors more than either of the individual agents with no weight-loss during treatment. In another investigation of orthotopic model of human pancreatic cancer cells, CDF was noted to inhibit tumor growth through reduced expression of histone methyltransferase and Nanog signaling pathways, and this observation is found consistent with the increased expression of let-7, miR-26a, and miR-101 (Ali, et al, 2010; Bao, et al, 2011). The effect of CDF along with 5-fluorouracil and oxaliplatin against colon cancer was assessed against chemo-resistant HCT-116 and HT-29 cells showing inhibition of cellular growth during incubation where the experimental results indicated that the combination of CDF and conventional chemo-therapeutics agents might be a good source of preventing the emergence of chemo-resistant colon cancer cells. It is noted that CDF treatment (i.e. in-vitro studies or MIA PaCa-2 induced tumors in-vivo) causes re-expression of let-7 and miR-143 along with down regulation of miR-21 expression, which is consistent with loss of Ras expression and inhibition of tumor growth. The effects of CDF on the regulation of AR/TMPRSS2-ERG/Wnt signaling were explored by observing the activation of AR resulting in the induction of ERG expression through TMPRSS2-ERG fusion (Li, et al, 2011; Kanwar, et al, 2011; Ali, et al, 2012).

Curcumin and Nanocurcumin

For comparing the different forms of curcumin in one of the earlier studies, combinations of control solution (curcumin-H), curcumin in DMSO (curcumin-D) and curcumin NPs (curcumin-NP) were prepared and tested. The control solution was prepared by curcumin sonication in water followed by centrifuge to retain the supernatant as stock solution after separating from the undissolved part whereas the curcumin-D was prepared by dissolving in DMSO, and the curcumin-NP was synthesized by nano precipitation in the presence of PVP for improving solubility and bioavailability by delaying crystallization via molecular adducts formations. The mean values of PS in these samples were ~2632, and ~143nm in case of curcumin-H and curcumin-NP, respectively, with the general observation that smaller size particles along with more uniform particle size distributions were obtained due to PVP reducing the particle size as well as spread in size distribution consistent with earlier findings. PVP is considered as an optimal polymer for preparing curcumin-NP with the process yield and encapsulation efficiency of 92 and 99.9%, respectively. XRD and DSC measurements showed characteristic peaks (angle 2θ) @ 8.89, 14.48, 17.22, 18.18, 23.33, 24.60, and 25.52° confirming its crystalline structure.

In contrast, observing no characteristic peaks in lyophilized curcumin-NP confirmed the conversion from crystalline to amorphous phase due to suppression of the curcumin aggregation. The melting of curcumin is reflected in an endothermic peak at 175°C, whereas PVP shows a broad endothermic peak @ 80°C. The physical mixture of curcumin exhibits a weak endothermic peak representing the presence of crystalline curcumin in the physical mixture. The endothermic peak of curcumin missing in the lyophilized curcumin-NP indicates that amorphous form of curcumin is finally dispersed during nano precipitation (Wu, et al, 2009; Yen, et al, 2010). The FTIR spectra of curcumin, PVP, and curcumin-NPs display the characteristic intensities of the O-H stretch @ 3508 cm⁻¹, PVP absorption band at @1662 cm⁻¹, but in case of curcumin-NP the absorption band of PVP is shifted to a lower wavenumber and O-H absorption band of curcumin is missing due to the formation of intermolecular hydrogen bonds between O-H band of curcumin and PVP where the hydrogen bond influences the conversion of the crystalline form. The dissolution profiles of curcumin and curcumin-NPs in simulated gastric medium showed dissolution of <1% and 99%, respectively @ 60 minutes under the same conditions possibly due to enhanced surface wetting arising from the absorption of PVP onto the surface of the crystalline curcumin besides the reduction in particle size resulting in stronger intermolecular interactions as confirmed by the FT-IR measurements (Gupta, et al, 2007; Yen, et al, 2008).

In one of the most recent investigations of exploring antimicrobial properties of nanocurcumin, samples were prepared by dissolving curcumin powder in dichloromethane followed by drop-by-drop adding to boiling water under constant stirring followed by continued stirring for another 20 minutes till the orange colored precipitate was obtained from which removing the supernatant gave curcumin pellet for characterization. Nanocurcumin, so prepared, gave absorption spectra peak @ 419 nm with average nanoparticle size of 110 nm and FTIR peaks at 1626, 1454, 1146 and 1037 cm⁻¹ with ZP of -18mV (i.e. showing moderate stability). TEM analysis confirmed spherical shaped poly-dispersed NPs having the size in the range of 60-80 nm. X-ray diffraction analyses showed characteristic peaks (2θ) at 23.03, 24.60, and 25.55°. The antimicrobial activity of nano curcumin was tested against E. coli (ATCC 14948), S. aureus (ATCC 333591), and P. aeruginosa (MTCC 4676) using Kierby-Bauer disc diffusion method where nano curcumin was found more effective against P. aeruginosa. A newly formulated nano curcumin cream was found more effective against P. aeruginosa but less effective against S. aureus as reported (Pandit, et al, 2015).

The antioxidant behavior of curcumin is generally attributed to its basic structure involving two methoxylated phenols and enol from a β-diketone moiety in which the phenolic OH-group and hydrogen abstraction from methylene (CH₂)-group impart its antioxidant activity. The experimental results obtained from the examination of three forms of curcumin e.g. curcumin-H, curcumin-D and curcumin-NP as described earlier, put the nanoparticulate curcumin at the same level of DPPH free radical scavenging efficiency as that of curcumin-D, but comparison with curcumin-H reflects a 1034-fold enhanced activity confirming the improved efficacy of water-soluble nanoparticulate curcumin at low
Curcumin complexes, for example, and Se $^{2+}$ were found to be readily soluble in water-concentration. The inhibitory effect on FeCl$_2$/ascorbate-induced lipid peroxidation in rat liver homogenate was used for evaluating the anti-lipid peroxidation activity of the different curcumin forms, which showed similar efficiencies in anti-lipid peroxidation activity of curcumin-NPs against curcumin-D but a 87-fold enhanced efficiency compared to that of curcumin-H (Schaffazick, et al, 2005).

Curcumin is known to induce apoptosis (cell death) in human cancer cells, including gastric, lung, pancreatic, hepatocellular, epithelial, breast, and lymphocytic leukemia cells by inducing damage in mitochondrial and nuclear DNAs in the hepatoma cells. A recent clinical study showed that a single dose of 8 g/day in humans produced a biological effect sufficient for the chemoprevention of cancer despite poor bioavailability of oral intake. For further investigations of hepatoma cell proliferations, HepG2, PLC/PRF/5, and Hep3B cells were treated with curcumin-H, curcumin-D and curcumin-NP for 48 hours in which the cytotoxicity assays clearly showed that curcumin-NP suppressed HepG2, PLC/PRF/5, and Hep3B cell growth with similar efficiency as curcumin-D whereas curcumin-H did not show any cytotoxicity in all the cell lines. The enhanced efficiency in arresting hepatoma cell growth by curcumin-NPs was also observed in another study, in which the paclitaxel NPs system synergistically inhibited the growth of HepG2 cells (Liang, et al, 2006; Cao, et al, 2007).

Despite showing similar behavior curcumin-D is not preferred in clinical applications due to several unwanted side effects, including vomiting, diarrhea, hemolysis, hypertension, bradycardia, and heart block. In contrast, PVP based curcumin-NP being readily soluble in water exhibit equally efficient profile in terms of anti-oxidant and anti-cancer activities in comparison to DMSO-dissolved curcumin. In light of the observations made in connection with this kind of curcumin-NP development there seems sufficient merit in furthering this study to other forms of nanoparticulate curcumin for better clinical applications (Swanson, 1985).

Curcumin Nanoformulations (Nanocurcumin)

Although, classical techniques involving control of physical parameters like - thermal treatment, pH-control, and complex formations with metal ions, polymers or serum have been used in the past to prepare better soluble curcumin formulations with improved bioavailability but introduction of nanotechnology in recent times has made significant impact in improving the overall bio-availability of curcuminoids explored in disease management. In addition, curcumin has also been chemically modified for preparing its derivatives or analogues, which ultimately led to 12, and 3-fold improved solubility using thermal treatment without any disintegration besides attempting heat-dissolved curcumin in water for in-vivo delivery (Kurien, and Scofield, 2009). Curcumin complexes, for example, prepared involving metal ions including Zn$^{2+}$, Cu$^{2+}$, Mg$^{2+}$ and Se$^{2+}$ were found to be readily soluble in water-glycerol and were quite stable towards light and heat (Zebib, et al, 2010). Complexes with serum albumin enjoy enhanced solubility while lowering amphotericin B toxicity by delaying the erythrocyte membrane damage (Kudva, et al, 2011). Chemically modified 4-arylidene curcumin derivatives, and water-soluble conjugates with two differently sized poly (ethylene glycol) molecules, for instance, show improved solubility and enhanced efficacy against different types of cancer treatments (Lin, et al, 2006b; Zambre, et al, 2006; Weber, et al, 2006a; Safavy, et al, 2007; Padhye, et al, 2010; Qiu, et al, 2010).

A series of sixteen curcumin derivatives were designed and assessed for their anti-bacterial/anti-fungal activities by recognizing the importance of lipophilicity and presence of pharmacophore sites as the factors that govern their orientations leading to anti-bacterial/anti-fungal activity. This study showed that POM analysis, a suitable method to correlate the structural features of curcumin derivatives with their promising combined antibacterial/antifungal activity, may be helpful in the development of novel anti microbial agents against drug-resistant human pathogens (Youssoufi, et al, 2015).

Different forms of curcumin deliveries were identified as briefly mentioned in the followings depending upon the method of preparation and materials used. For example, subcutaneous polymeric implant of curcumin and poly-($\varepsilon$-caprolactone) compounds in rats not only produced maximum concentration in liver but also confirmed its use in avoiding oral route still with sustained release. On the other hand, micellar curcumin retained its cytotoxicity when injected along with poly (ethyleneoxide)-b-poly (\varepsilon\textendash caprolactone) in mouse melanoma and human mantle cell lymphoma cells. In-vivo experiments conducted on curcumin NPs + poly (D, L-lactide-co-glycolide)-b-poly (ethylene glycol)-b-poly (DL-lactide-co-glycolide; PLGA-PEG-PLGA) showed reduced liver and spleen uptakes with enhanced distribution simultaneously in lung and brain. In-vivo testing of curcumin NPs confirmed a 9-fold increase in oral bio-availability as compared to curcumin administered with piperine. PLGA based curcumin nanof ormulation gave 22-fold higher oral bioavailability in rats as compared to conventional curcumin. Similarly, curcumin + dextran sulphate-chitosan NPs showed preferential killing of cancer cells compared to normal cells (Shaikh, et al, 2009; Anitha, et al, 2011; Bansal, et al, 2011; Song, et al, 2011; Tsai, et al, 2011).

Polymeric NPs (with embedded curcumin) not only dissolve easily in water but also exhibit anti-cancer properties in preclinical trials and prove to be a prominent delivery system where the nanocurcumin retains its mechanical specificity of free form that inhibits the activation of the seminal transcription factor NF-$\kappa$B, and reduces levels of pro-inflammatory cytokines like ILs and TNF-$\alpha$. Curcumin NPs (e.g. 2-40 nm, wet milled) show stronger antimicrobial properties although its anticancer activity is yet to be ascertained. A similar approach of employing reduced size curcumin crystals was proposed for preparing nanosuspensions for...

Water-dispersible nanogels for intracellular curcumin delivery combining Au/Ag bimetallic NPs, polystyrene gel layer, and polyethylene gel showed cytotoxicity against B16F10 cells in a combined chemo-photothermal treatment. Enhanced anti-inflammatory property was shown in case of o/w nanoemulsions, as evaluated in mouse ear inflammation model where conventional formulations did not show the same effect (Wang, et al, 2008; Wu et al, 2011a).

Another formulation of tocopheryl polyethylene glycol sucinate (TPGS) based curcumin nanosuspension was reported having ~4 and ~11-fold enhancements in bioavailability and mean residence time against the simple curcumin intake after it’s intravenous delivery in rabbits and mice. Further developments in the area of polymer-drug conjugates were also explored as alternative therapeutics by employing the existence of two phenolic rings and active methylene groups to conjugate biomacromolecules onto them. A family of nucleoside-curcumin bio-conjugates thus, became available with higher levels of curcumin glucuronide and sulfate conjugates in healthy humans. In continuation, some hormone releasing curcumin-conjugates, and mono/diester bioconjugates using urethane chemistry were reported with enhanced antitumor effects suitable for treating pancreatic and other forms of cancer (Donsi, et al, 2010; Setthacheewakul, et al, 2010; Kumar, et al, 2000; Vareed, et al, 2008; Dubey, et al, 2008).

The presence of methyl jasmonate and salicylic acid in cell culture was noted to enhance glucoside formations with curcumin (curcumin-4′, 4′-O-beta-D-digentiobioside), which showed higher solubility of curcumin (0.65 mmol/ml). Similarly, the formation of mono-glutathionyl-curcumin conjugates was reported in biological systems when catalyzed in presence of human glutathione S-transferase. Enhanced bioavailability and biological effects of these curcumin-conjugates are assigned to the excretion through intestinal lumen (Kaminaga, et al, 2003; Usta, et al, 2007). Another novel PEGylated-curcumin analogue was proposed as a potent nuclear factor erythroid-2 related factor2 (Nrf2) activator to regulate the anti-oxidant behavior and act as modifier for inflammatory diseases. These analogues not only improve the curcumin solubility (e.g. from 0.6 to 0.98 x 10^−5g/ml) but are also critical in deciding their growth inhibitory effects on human pancreatic cancer cells due to enhanced suppression of Jun activation domain-binding protein-1 (Jab1) activity, as confirmed by a significant elevation of the protein levels and reduction of c-Jun. The emerging preparations of PEG-curcumin nanoconjugates are thus found very useful in achieving a higher cytotoxicity for cancer cells. The observed synergy is dependent on the type of linker chain terminal functionality and molecular weight. For example, a cationic poly (vinyl pyrrolidone)-curcumin-conjugate formulation with stable particle size and ZP over a pH range of 3-9 is found more potent in L929 fibroblast cells over free curcumin. Polycatocol-curcumin-conjugates - synthesized by condensation polymerization of curcumin and anhydrides, like PCure 8 - an optimized commercial polycatocol-curcumin conjugate was found highly cytotoxic to ovarian, and breast cancer cell lines in which these conjugates were taken up efficiently by cancer cells, hydrolyzed and released in an active form of curcumin in lysosomes. The cancer cell growth was arrested and apoptosis was induced through the caspase-3 dependent pathway leading to 68% decrease in tumor growth observed with intravenous injection of PCure 8 in the SKOV-3 intra-peritoneal tumor xenograft mouse models compared with the control group. Polyamine conjugated curcumin analogues are noted to transport efficiently to mitochondria. A direct conjugation of curcumin molecules to carboxylic acid groups of a hyaluronic acid polymer is used for producing nanosize micelles in aqueous solution through hydrophobic interactions and in turn an equivalent of 13μg of conjugated curcumin is able to kill 80% of the L929 cells. Hyaluronic acid-curcumin conjugates are specifically targeted to cell-specific surface markers like CD44. The related discussions are given in several publications (Manju, and Sreenivasan, 2011; Tang, et al, 2010; Simoni, et al, 2010; Pandey, et al, 2011).

Curcumin-diclofenac conjugates were synthesized using esterification of phenolic group of curcumin with the acid moiety of diclofenac, and characterized. The relative solubility of curcumin-diclofenac conjugates, curcumin and diclofenac; stability of curcumin-diclofenac conjugate in intestinal extract; permeability study of curcumin-diclofenac conjugates using the everted rat intestinal sac method; stability of curcumin-diclofenac conjugate in gastrointestinal fluids and in-vitro efficacy were evaluated subsequently. In-vivo bioavailability of curcumin-diclofenac conjugate and curcumin in rats, and anti-arthritic activity of curcumin-diclofenac conjugate, curcumin and diclofenac in modified streptococcal cell wall-induced arthritis model in mice to mimic rheumatoid arthritis in humans were also studied. The curcumin-diclofenac conjugate exhibited enhanced stability as compared to curcumin; its activity was twice that of diclofenac in inhibiting thermal protein denaturation taken as a measure of in-vitro anti-inflammatory activity; it enhanced the bioavailability of curcumin by more than five folds, and significantly alleviating the arthritis symptoms in streptococcal cell wall-induced arthritis model (Jain, et al, 2014).

Some of the basic formulations involving a variety of processes including precipitations, micro/nano emulsions, homogenization and sonication, complex formation, vesicular phospholipid formation, and solid lipid nanoparticle formation are described in the followings with the current state of technologies highlighted accordingly.

**Nanoprecipitated Chemical Formulations**

One of the most popular protocols of preparing nanoparticulate-formulations of curcumin is based on emulsion-diffusion-evaporation that starts with solubilizing the drug and/or polymer (PLGA) in an
organic solvent (ethyl acetate), followed by its drop-by-drop addition into a suitable aqueous phase stabilizer for preparing an emulsion that is homogenized and diluted with a large quantity of water so that solvent diffusion leads to nanoprecipitation resulting in uniformly sized spherical curcumin NPs in the range of 120-240 nm. Since the drug solubility is critically important in controlling drug encapsulation efficiency, stabilizers with lower drug solubility are preferred for higher values of drug encapsulation. *In-vitro* studies carried out in this context in rats showed that curcumin NPs exhibit enhanced bioavailability by 26-fold as compared to oral suspension and by 9-fold as compared to curcumin suspension administered in conjunction with piperine (Shaikh, et al, 2009). Although, PLGA-NPs, prepared using F-68 solubilizer, show similar efficacy as free curcumin in killing tumor cells but otherwise exhibit higher potency in inhibiting NF-kB activation in cell cultures (Anand, et al, 2010).

A detailed investigation of preparing curcumin and piperine based NPs was carried out by examining a number of processes for meeting the final objective of 100nm particle size, 0.2 polydispersity index and ± 30mV ZP by examining the influence of various process parameters on average particle size, polydispersity index and zeta potential to find out the appropriate method of preparation. The different protocols that were examined included thin film hydration, solid dispersion, emulsion polymerization and Fessi methods. Most of the observations noted in this significantly important study are summarized below (Moorthi, et al, 2012).

The NPs were spontaneously formed in each method owing to polymer deposition on the interface between the organic phase and aqueous phase when aqueous miscible organic solvent diffused out into the aqueous phase from each of the transient particle intermediate. Using acetone or ethanol as solvent had no significant effect on NPs but surfactants did help in reducing the particle size due to the adsorption on the surface of NPs that prevented the aggregation due to the static repulsion and special hindrance. Addition of anionic surfactant was the best choice, however, to reduce the aggregation than the cationic and non-ionic surfactant. Out of a number of non-ionic surfactants like Poloxamer 188 and Poloxamer 407, anionic surfactant like SLS and a combination of non-ionic and anionic surfactants with cationic polymer (Eudragit E 100), it was SLS that produced a maximum ZP around -50 mV with average particle size above 150 nm while non-ionic surfactants produced a maximum ZP below -10 mV with average particle size above 250nm. However, Poloxamer 188/Eudragit E 100 combination produced a maximum ZP around 30 mV with average particle size 100nm. Similarly, the use of SLS/Eudragit E 100 combination produced a maximum ZP = 53.1 mV with the average particle size >100nm. Fully spherical shaped particles were formed above critical stirring speed. Further increase in the rotational speed of the mixer, significantly reduced the PS with a wide and random size distributions narrowed down by the stirring speed. It was confirmed that increase in stirring speed from 100 to 500 rpm significantly reduced the average PS to around 700 nm and further increase in stirring speed in Fessi method (i.e. from 500 to 1000 rpm) reduced the average particle size <100 nm, which is mainly due to high shear rate. Using medium or high viscosity binder caused marked reduction in heterogeneities of average particle size. However, increase in viscosity promoted agglomerations. Use of Poloxamer (188 or 407) produced ZP < -10 mV, which may lead to aggregation on prolonged storage and in the gut, which may decrease the bioavailability. Studies have shown that use of mixed copolymer led to average particle size reduction, producing optimal zeta potential and high entrapment efficiency, increasing the drug encapsulation, leading to sustained release, prolonged circulation time of the drug, significantly enhancing the bioavailability and also increased stability. A marked reduction in average particle size and optimal zeta potential was obtained with the combination of Poloxamer 188 with Eudragit E 100 (Moorthi, et al, 2012).

Antiamyloid and antioxidant activities of curcumin were found useful for the treatment of Alzheimer's disease (AD). With the objective of improving the curcumin bioavailability in this context, water-soluble PLGA coated-curcumin NPs were synthesized and characterized while coupling the NPs with Tet-1 peptide that is known to have affinity to neurons and possess retrograde transportation properties. The curcumin encapsulated-PLGA NPs were able to destroy amyloid aggregates, exhibit anti-oxidative property and were non-cytotoxic (Mathew, et al, 2012).

Curcumin-NPs are also prepared by using other co-polymers like N-iso-propylacrylamide (NIPAM), Nvinyl-2-pyrrolidone (VP), and polyethylene glycol monoacrylate [NIPAM (VP/PEG A) having very low polydispersion index and average size ~50 nm that makes them easily permeate into different pancreatic cancer cells. Although, these NPs show equal efficacy as free curcumin in cell culture but have the advantage of directly injecting them into systemic circulations, thereby by passing the oral route (Bisht, et al, 2007). Another method of preparation is to use anionic polymerization-solvent-evaporation in which a drop-by-drop addition of a butyl-cyanoacrylate monomer solution into a constantly stirred acidic ethanol solution containing a suitable surfactant and sodium sulfate makes the surfactant molecules aggregate together to form micellar structures with multiple monomer units. Polymerizations of these monomers inside the micelles form the primary polymer particles that grow in size to finally form the NPs. Curcumin or any other chemopreventive could, thus, be easily added during or after the addition of monomer solution to achieve efficient encapsulation during such a growth phase. This protocol could produce uniform 160-240 nm size NPs with lower polydispersion index and these particle sizes are directly related to the monomer concentration and inversely proportional to the surfactant concentration. Further more, it also leads to the formation of highly porous surface topology with very large surface area that
enables them to easily load the hydrophobic drugs including curcumin (Mulik, et al, 2009; Petri, et al, 2007). These NPs provide higher drug release under in-vitro conditions (acidic pH compared with physiologic pH) and show their ability to efficiently deliver their cargos inside the cells after degradation by the lysosomes that have more acidic environment. The other associated advantage of using polymeric NPs is the ease in changing their surface properties by employing functional groups like thiols that easily conjugate with the polymeric chains to increase or decrease the mean residence time of the NPs in the gastrointestinal mucosal environment. Such thiolated chitosan modified PLGA-NPs are, for instance, prepared successfully as thiolated chitosans with their SH-groups interacting well with the mucus to form disulfide-linkages conferring them with highly mucoadhesive properties and hence resulting in an increased residence time. Furthermore, the presence of various kinds of inter and intra-molecular disulfide bonds established between chitosan molecules leads to forming a 3-d structure that provides better controlled release functions. The phenomenon of thiolation not only increases the mean residence time of these coated NPs on the mucosa but also enhances their average particle size with decreased encapsulation efficiency of drugs as compared with unmodified NPs. The average size of curcumin NPs is consequently noted to increase from 284-420 nm to 817-960 nm after chitosan coating with half the entrapment efficiency limiting the drug-loading capacity (Werle, et al, 2009; Werle, and Hoffer, 2006).

Another variant is in form of a multilayered polyionic/polymeric shell encapsulating NPs that contain drug in which the polyelectrolyte shells are formed as layer/layers over the surface to alter their cell-uptake; to attach tumor-targeting radicals; to increase stability; and/or to control their loading/release characteristics. These layered NPs are produced using gelatin as polymer in a 2-step de-solvation process followed by formation of layered polyionic-shells (Shutava, et al, 2009). It starts with the formation of gelatin NPs by precipitating gelatin from an acidified solution by slowly adding acetone followed by adding glutaraldehyde for cross-linking. The aqueous solution of these NPs that are coated with polyionic shells by the sequential addition of polystyrene sulfonate, poly-L-glutamic acid, or dextran sulfate and polyallylamine HCl, poly-L-lysine, or protamine sulfate enabling the formation of a polyanionic layer first due to positively charged gelatin at acidic pH, and by a polycationic layer later. Once done, these NPs are further added to curcumin solution to adsorb curcumin at their surface via hydrophobic interactions that is developed between phenol groups of curcumin and amino acids of gelatin like proline (Ai, et al, 2003). Such multilayered NPs are able to modify their targeted deliveries involving many other chemopreventives as well. In such nanostructured polymeric layers with the entrapped chemopreventives, it is possible to encapsulate a magnetic iron core as well to provide further enhanced targeting features. Efficacy of such a system of multilayered curcumin-nanoparticulates was confirmed using poly (NIPAAM) and PLGA as polymers. In this approach, NIPAAM undergoes free-radical polymerization onto the magnetic core via covalent coupling with a silane reagent. The resultant NPs are then coated with PLGA using a double emulsion solvent evaporation method, yielding particles of 500-1,000 nm in size that enable the deliveries of both hydrophilic and hydrophobic chemopreventive compounds, simultaneously in which hydrophilic compounds are loaded into the poly (NIPAAM) layer and the hydrophobic drugs into the outer PLGA layer (Koppolu, et al, 2010).

The attachment and encapsulation of multiple poly (NIPAAM) particles at the surface as well as in the PLGA layer used for encapsulation of the individual particles, however, is known to cause problems in precise control and successful completion of the protocol. Targeted delivery of chemopreventives is also possible to achieve by conjugating NPs or drugs with ligands like folic acid that can recognize some specific surface attributes of the target cells. Different cancer types often over-express some of these specific epitopes or receptors and bioconjugation of chemopreventives to these ligands having high specificity for these unique surface receptors may lead to their targeted-delivery to any cancer type. For example, targeted delivery of curcumin NPs, in this context, prepared by combining folic acid and the polymeric carrier by covalently linking PEG to folic acid on one end with isocyanate group of hexamethylene, which is further linked to a cyclodextrin curcumin complex on the other end, this kind of configuration enabled them to undergo clathrin-independent endocytosis into cells that specifically over-expressed the folic acid receptors. Hexamethylene was used as a linker to decrease the steric hindrance of bulky PEG-chains with cyclodextrin curcumin complex where cyclodextrin was employed to bind curcumin into its cavity resulting into enhanced solubility. These conjugated curcumin-complexes are found 3,200-times more soluble, 12-times more stable, twice more specific, and 45-times less degradable @ pH = 7.2 compared to using curcumin alone. An insufficient cell uptake leading to limited effects of this bio-conjugate needs further investigations to develop efficient drug releases from the conjugates into the tumor cells (Salmaso, et al, 2007).

A temperature-responsive self-assembled nanoparticulate system was developed involving ionic modifications of hydroxypropyl cellulose resulting in cationic and anionic form that spontaneously self-assembled into NPs in water. The size and surface charge of the NPs were possible to modify by the poly-cation/anion ratio and these spherical NPs (150-250 nm) grew in size at elevated temperatures. Curcumin was successfully entrapped inside these nanospheres with temperature dependent release profile (Bielska, et al, 2013).

The therapeutic activity of curcumin loaded poly (lactic-co-glycolic acid)-CUR-NPs (PLGA-CUR-NPs) was studied where efficient internalization of curcumin was observed from PLGA-CUR-NPs in prostate cancer cells along with cytosolic cell compartment. Tests conducted via cell proliferation (MTS), colon genic, and Western
blot analyses revealed effective inhibition of the proliferation and colony formation ability of prostate cancer cells than free curcumin while causing superior tumor regression compared to curcumin in xenograft mice. Further investigations revealed inhibition of nuclear β-catenin and AR expression in cells and in tumor xenograft tissues while suppressing STAT3 and AKT phosphorylation leading to apoptosis via inhibition of key anti-apoptotic proteins, Mcl-1, Bcl-xL and caused induction of PARP cleavage with down-regulation of oncogenic miR21 and up-regulation of miR-205. Superior anti-cancer potentials of PSMA antibody conjugated to PLGA-CUR-NPs and superior tumor targeting of (131) I labeled PSMA antibodies were achieved in prostate cancer xenograft mice models (Yallapu, et al, 2014).

Dextrin coated iron oxide (Dx-Fe 3O 4) aqueous colloidal NPs were synthesized followed by liposomal encapsulation resulting in magnetic liposomes (MLs) that were chemically and structurally stable with uniform dispersal in aqueous solution, with successful surface conjugation of dextrin, lipid, and curcumin. Curcumin release from these MLs was ~ 56% at elevated temperatures, following magnetic hyperthermia. Treatment of human cervical cancer derived cell lines (HeLa) in-vitro demonstrated that the curcumin-loaded MLs were effective in inhibiting cell proliferation with an IC 50 value of 2.09 mg/ml and these MLs appear promising for controlled release of curcumin for the treatment of cervical cancer (Nigam, et al, 2014).

Fairly good understanding has already been acquired about various mechanisms involved in controlling the features of curcumin-solid-lipid-nanoparticles (C-SLNPs) along with chitosan and two different stabilizers while preparing curcumin nanoemulsions using sonication and HPH followed by lyophilization and detailed characterization of parameters like PS and ZP to assess the overall stability by extending the observations over two months duration. UPLC measurement of in-vitro release of curcumin-NPs @ 37°C in buffer with Tween 80, and assessment of cellular-uptake of curcumin from the solution as well as curcumin-NPs in Caco-2 cells after 30, 60 and 90 minutes of treatment were carried out specifically in this context. The general observations made during this particular study are summarized here as follows.

The average PS of curcumin-loaded chitosan-nanoemulsion is noted to reduce by 50-65% after three cycles of HPH and Poloxamer turns out to be a better stabilizer in comparison to PVA in stabilizing these chitosan-NPs, as it requires only one-fifth of the stabilizer concentration compared to that of PVA based formulations. Poloxamer-NPs produce more sustained release compared to that from the PVA-based formulations (e.g. cumulative release of 73.93 ± 5.25% and 53.15 ± 5.84% of curcumin were noted from PVA and poloxamer based nanoemulsion after 171hours). Cellular uptake of curcumin is 2.5-times more in case of PVA-NPs compared to poloxamer-NPs among all the samples considered. Nanoemulsion measurements carried out during this study showed that the average PS increased with drug loading from 0% to 8% in both PVA as well as poloxamer based NPs. The general trend of average PS reductions observed in nanoemulsions appeared to saturate around 50-65% after 3rd cycle of HPH; however, further reductions acquired were only marginal even after 10 cycles. Further, the average PS and ZPs of all the formulations stored at room temperature over a period of two months did not exhibit any noticeable change confirming very good stability (Mistry, 2011).

The effect of process parameters and stabilizers on the chitosan NP-system was studied in detail using curcumin as a model hydrophobic drug in which HPH processing is found very effective in reducing the average PS of nanoemulsions by 50 to 65%, which could not be achieved by sonication alone. A process of 3 HPH cycles was found optimal for the preparation of GMO/chitosan NPs with a hydrophobic drug loading. Poloxamer was found better stabilizer compared to PVA for chitosan system containing curcumin because it gives more stable nanoemulsions at one-fifth % (w/v) concentration of PVA. These experimental observations establish that the process parameters and the type of stabilizer used for preparing chitosan assisted NPs affect several physicochemical properties of the NP-system such as average PS, ZP as well as cellular uptakes in the Caco-2 cells.

GMO/chitosan system is another surface-modified NP-system comprising of lipid as GMO and chitosan as the coating polymer. The GMO/chitosan delivery system is required in sustained and targeted delivery of both hydrophobic drugs like paclitaxel and dexamethasone as well as hydrophilic drugs like gemcitabine prepared by multiple emulsion/solvent evaporation technique using large volume of 0.5% (w/v) PVA as stabilizer. Recently, a sustained delivery system with increased stability of ifosfamide in GMO/chitosan combination was reported using 0.15% (w/v) tocopherol polyethylene glycol succinate (TPGS), oleic acid and low volumes of 0.5% (w/v) PVA for emulsification and stabilization of the nanoparticles. Due to the associated toxicity of PVA, there are still some concerns about the parenteral applications of this system with the possibility of changing the stabilizer for modifying its physicochemical and/or biological properties accordingly. The effects of different stabilizers on nanoparticles were previously studied on different systems along with exploring the influence of parameters like pressure and number of cycles during high-pressure homogenization processes on the physicochemical properties of various nanoparticulate systems as discussed in several publications (Trickler, et al, 2010; Pandit, and Dash, 2011; Dinda, et al, 2011).

The process of lyophilization of a nanoemulsion in general show 20-60% increase in average PS but, PVA based nanoemulsions show more positive ZPs as compared to those of poloxamer ones. The surface morphology of the lyophilized curcumin-PVA-NPs is of porous nature due to the phenomenon of aggregation as opposed to smooth surfaces of the poloxamer-based NPs. Besides observing a peak at 179.5°C (corresponding to
melting point of curcumin) in the thermograms of different nanoemulsions, the blank PVA-NPs showed diffused peaks at 100 and 184°C possibly due to dehydration and chitosan degradation, respectively. Some kind of characteristic signature pattern of peaks are seen in the overlays of the powder XRD patterns of curcumin (pure drug), blank PVA-NPs and curcumin loaded PVA-NPs in which several identical peaks are seen in case of curcumin loaded PVA and polaxomer-NPs. Curcumin nanoemulsions are prepared by using chitosan-based system by introducing some changes like starting with primary emulsion formation involving lipid phase and chitosan coating along with TPGS surfactant that is further stabilized by adding either 0.5% (w/v) PVA or 0.1% (w/v) poloxamer. Reductions in PS are observed after sonication as well as HPH of the primary emulsion. HPH is found more efficient method of preparing submicron size solid lipid dispersions as compared to sonication alone or along with high shear mixing. While trying to increase curcumin loadings from 4 to 10% (w/w) difficulties are faced to pass through HPH in case of 10% loaded nanoemulsions. Thus, 8% (w/w) curcumin loading is found optimal in curcumin-chitosan system (Pandit, and Dash, 2011; Mistry, 2011).

The average size of the curcumin loaded-NPs is significantly larger than the blank-NPs in both PVA and poloxamer based preparations and this is possible by considering the increase in hydrophobicity and viscosity with drug concentration during loading, which make the stabilizers less effective. Simultaneously, there is a reduction in interfacial coverage of the surfactant on the dispersed particles, which in turn affects the stability adversely due to flocculation/agglomeration. Further, any increase in drug loading also increases the viscosity of the emulsion and due to this effect the droplets turn out to be more difficult to break. The increase in average PS of primary emulsion after addition of chitosan is understood by taking into account the chitosan coating onto the particles causing increase in their diameters. Only small reduction observed in the average PS after 10 cycles of HPH as compared to that after 3 cycles indicates that 3 cycles of HPH producing 50 to 65% of reduction is almost optimal for the preparation of nanoemulsions. The negative ZP of the primary emulsion is possibly due to chitosan with a positive charge in acidic solutions arising out of the presence of protonated amino-groups. The physical stability data indicates that the blank as well as curcumin-loaded nanoemulsions are stable at 25°C for a period of at least 60 days, irrespective of the stabilizer used (Tamilvanan, et al., 2010; Qian, 2011).

The increase in PS of the drug-loaded NPs as compared to the blank-NPs is possible to explain by considering the increase in droplet size of the curcumin nanoemulsions. No significant change was noted in PS of both blank as well as curcumin-loaded NPs on replacing 0.5% (w/v) PVA with 0.1% (w/v) poloxamer and this suggested that poloxamer 407 was more efficient stabilizer for GMO/chitosan system as compared to PVA as the stabilizer. PVA being toxic in use, any reduction in the concentration of this stabilizer is not only considered better but also safer (Lacasse, et al, 1998). However, ZP of PVA-NPs is significantly higher compared to that of poloxamer-NPs possibly due to significant difference in surface morphologies. The surfaces of curcumin-PVA-NPs were highly porous compared to smooth surfaces of the curcumin-poloxamer-NPs. The surface morphology being dependent on process and stabilizer concentration used, the difference in morphology of the two formulations is possible to explain by considering change in the stabilizer of the lyophilized NPs from PVA to poloxamer (Sun, et al, 2009; Lee, et al, 2011).

**Curcumin Polymersomes**

A family of polymeric nanoparticulate curcumin (PNPC) was developed and assessed in one of the animal models of breast cancer by observing significant suppression of mammary and hepatocellular carcinoma cells proliferation while it was safe at 31.25mg/kg BW and lower doses but higher doses showed minimal hepatocellular and renal toxicity in paraclinical/histopathological tests where tumor take rate in PNPC-treated group was 37.5% compared with 87.5% in control. These findings provided evidence for superior biocompatibility of the polymeric nanocarriers with excellent tumor-suppressing responses (Alizadeh, et al, 2015).

Polymersomes, owing to their intrinsic hollow and compartmentalized nano domains with diverse functionalities are now finding increasing applications in the biomedical field, including drug/gene delivery, magnetic resonance imaging, theranostics, and other fields (Ahmad, and Hashim, 2012). In a recent review, a number of issues related to the rational designs and syntheses of polymer vesicles based on different polymeric building blocks, followed by an insight into the structure and formation mechanism of polymer vesicles, as well as the recently developed method of determining the exact thickness of the vesicle membrane were addressed to. Polymer vesicles are becoming ‘smarter’ owing to their improved responses to the additional stimuli like electrical field, magnetic field, sugar molecules, gas, ultrasound, and other similar radicals leading to their uses as novel nanoreactors, nano sensors, and water remediation materials besides others (Zhu, et al, 2016).

Considering that the sensitization of cancer cell is dependent on curcumin-mediated inhibition of thioredoxin reductase 1, knocking down of TnRxRd1 expression was shown to ameliorate this sensitization by curcumin and over-expression of TnRxRd1 in cells with low endogenous levels enhancing the cisplatin sensitivity for which curcumin encapsulation into polymersome NPs was found necessary comprising of poly(ethylene oxide)-block-polycaprolactone co-polymer to improve drug delivery involving drugs in the membrane and aqueous core allowing for simultaneous delivery of curcumin and cisplatin (Daurio, et al, 2013). Curcumin loaded Alginate/Pluronic® tri-block copolymer micelles were prepared in which the inner core was found to
encapsulate curcumin within an average spherical size of 50-100 nm. Curcumin release profile in aqueous PBS solution exhibited Korsmeyer-Peppas model suggesting diffusion assisted sustained release when tested with L929 cancer cells with comparable anticancer activity (John, and George, 2014).

Redox-responsive polymersomes were prepared by self-assembly of keratin employing a water addition/solvent evaporation method with PEG-40 stearate (PEG-40ST) as hydrophobic block grafted to keratin. These vesicles loaded with curcumin showed high entrapment efficiencies, and GSH-dependent drug release rate as observed in HeLa cells with the evidence of efficient internalization. Polymersomes loaded with curcumin inhibited HeLa and CHO-K1 cancer cells proliferation confirming the keratin polymersomes as efficient nanocarriers for chemotherapeutic agents (Curcio, et al, 2015).

A new delivery system was synthesized from reactive oxygen species (ROS)-responsive poly (propylene sulfide) (PPS) and tested for on-demand delivery of curcumin by exploiting the phase change of PPS from hydrophobic to hydrophilic upon oxidation. PPS microspheres efficiently encapsulated curcumin through oil-in-water emulsion and provided sustained on-demand release that was modulated in-vitro by hydrogen peroxide concentration. The cyto-compatible curcumin-loaded formulation was found preferentially targeting and scavenging intracellular ROS in activated macrophages, reducing in-vitro cell death in the presence of cytotoxic levels of ROS, and decreasing tissue-level ROS in-vivo in diabetic mouse hind limb ischemia model of peripheral arterial disease. The local delivery of curcumin-PPS microspheres accelerated recovery from hind limb ischemia in diabetic mice, as demonstrated using imaging (Poole, et al, 2015).

A series of amphiphilic polyaspartamide poly electrolytes (PEs) were synthesized to solubilize hydrophobic drugs in aqueous medium and enhance their cellular uptake through controlled (~20 mol %) derivatization of polysuccinimide (PSI) precursor polymer with hydrophobic amines, while the remaining succinimide residues of PSI were opened using a protonable and hydrophilic amine, 2-(2-amino-ethyl amino) ethanol (AE). Curcumin was loaded as representative drug to explore the drug-delivery potential with unprecedented enhancement in the aqueous solubility. In case of oleyl/dodecyl residues based PEs, the solubility was enhanced > 65,000-fold without any organic solvent while retaining stability over 2 weeks. Stable PECs with average size of 150-170 nm (unloaded) and 220-270 nm (loaded) were realized with ZP from + 36 to + 43 mV. Improved cyto-compatible PEs exhibiting 40 wt% drug loading got easily internalized by mammalian cells like HEK-293T, MDA-MB-231, and U2OS, through clathrin-mediated endocytosis besides significantly improving curcumin transport in cancer cells causing their death by apoptosis while noncancerous cells remained unaffected (Fatima, et al, 2016).

A micellar system with compartments for curcumin and platinum was prepared using tri-block copolymer involving polycaprolactone PCL, and an amine-bearing polymer as the interphase for the conjugation of platinum by combining ring opening and RAFT polymerizations. Curcumin was loaded into the self-assembled onion-type micelle by physical encapsulation into the PCL core with an entrapment capacity of 6wt%. The size of the dual drug micelles was ~38 nm and the toxicity results tested against A2780 human ovarian cancer cell line showed synergy with a combination index ranging from 0.4 to 0.8, the combined delivery in one nanoparticle did enhance the synergistic effects resulting in a combination index of approximately 0.2-0.35 (Scarano, et al, 2015).

The effects of temperature and additives on the photo-initiation efficiency of curcumin-based systems were investigated showing highest photo-initiation efficiency @ 25°C. The curcumin/ diphenyliodonium hexafluorophosphate/triarylphosphine combination is a multicolor photoinitiating system capable of initiating free radical photo-polymerization under air upon UV, blue, green, yellow, red, and warm white household LED lights besides causing reversible addition-fragmentation chain transfer (RAFT) photo-polymerization of N-isopropylacrylamide by using curcumin-based system under irradiation of blue LED bulb. The polymer sample produced through photo-polymerization process using curcumin-based photo-initiating system demonstrated no toxicity to human fibroblast Hs-27 cells endowing this photo-initiating system with great potential for fabrication of biocompatible polymeric materials (Zhao, et al, 2015).

Inhalable formulations of curcumin with hydroxypropyl-β-cyclodextrin and polyvinylpyrrolidone were produced by a newly developed anti-solvent micronization technique based on the supercritical fluid (SCF) technology. The micronization process used is the atomized rapid injection solvent extraction process (ARISE), which utilizes high-pressure carbon dioxide as the anti-solvent. Curcumin formulations with aerodynamic performance suitable for inhalation delivery were produced and tested in-vitro using normal and lung cancer cells - MRC-5 and H1299 cells. The cytotoxicity study revealed that the encapsulation of curcumin improved its bio-distribution and solubility for lung cancer cells. A rapid cell uptake was observed for all formulations by confocal microscopy and flow cytometry (Kurniawansyah, et al, 2015).

**Micellar Curcuminas**

Curcumin conjugated to polyactic acid (PLA) via tris (hydroxymethyl) aminomethane (Tris) linker producing hydrophobic drug-binding block and methoxy-PEG (mPEG) as hydrophilic block in micellar form was characterized by the resultant particle size, loading capacity, stability, and critical micelle concentration (CMC) before testing against the hepatocellular carcinoma (HepG2) cells. The mPEG-PLA-Tris-Cur micelles with average size of <100 nm showed 1/10th of
the CMC of mPEG-PLA micelles. Curcumin loading in mPEG-PLA-Tris-Cur micelles reached 18.5 ± 1.3% (w/w), compared to traditional mPEG-PLA micelles at 3.6 ± 0.4% (w/w). IC_{50} of mPEG-PLA-Tris-Cur micelles (~22 μg/mL @ curcumin-equivalent dose) was found similar to unmodified curcumin. This showed the potentials of micelle-forming polymer-drug conjugates containing multiple curcumin molecules as an efficient mean to increase loading and intracellular delivery of low-potency curcumin (Yang, et al, 2012).

Curcumin loaded mono-methoxy PEG-oleate (mPEG-OA) micelles were tested for anticancer activity against U87MG brain carcinoma and HFSF-PI3 cells as normal human fibroblasts with significantly enhanced in-vitro bioavailability than that of free curcumin by showing half maximal inhibitory concentrations of free curcumin and curcumin-loaded mPEG-OA as 48 and 24μM, respectively, along with dose-dependent apoptosis induction (Efrafani-Moghadam, et al, 2014).

The therapeutic potential of transferrin (TF)-targeted micelles of PEG-PE+Vitamin E/Curcumin (CUR) was examined as single as well as in combination with PCL against SK-OV-3 human ovarian adenocarcinoma along with its multi-drug resistant (MDR) version SK-OV-3TR cells in-vitro indicating that the TF-targeted combination micelles were able to improve the net cytotoxic effect of CUR and PCL synergistically against the SK-OV-3 cells. While keeping curcumin concentration constant and varying PCL concentrations, the PCL IC_{50} decreased from ~1.78 to 0.68 μM for the non-targeted formulations to ~0.74 and 0.1 μM for the TF-targeted ones, respectively (Abouzeid, et al, 2014).

A new curcumin micellar formulation was reported showing activity in mitochondrial function in-vitro in PC12 cells and ex-vivo in isolated mouse brain mitochondria with an enhanced bioavailability of 10-40-fold (in plasma/mice brain) while incubation with native curcumin and curcumin micelles prevented isolated mouse brain mitochondria from swelling, indicating less mitochondrial permeability transition pore opening and prevention of the injury. Micellar curcumin proved more efficient in preventing mitochondrial swelling in isolated mouse brain mitochondria and protecting PC12 cells from nitrosative stress than native curcumin (Hagl, et al, 2015).

Two versions of curcumin loaded Cur/PLA-PEG and Cur/PLA-PEG-Fol micelles were reported for cancer therapy in which the solubility of curcumin was increased from 0.38 to 0.73 mg/ml after loading in an average size of micelles from 60-69 nm with encapsulating efficiency of 49-91%. Comparatively, the size of Cur/PLA-PEG-Fol micelles was 80-86 nm with better in-vitro uptake and cytotoxicity against HepG2 cells. The cytotoxicity of the NPs, however, depends much on the PEG component and these test results demonstrated that folate-modified micelles could serve as potential nanocarriers to improve solubility, anti-cancer activity of curcumin along with targeting (Phan, et al, 2016).

Curcumin Liposomes

Liposomes are most promising alternate nanocarriers having highly organized internal structures comprising of concentrically situated phospholipid bilayers including water-volume between them, and also a central cavity for curcumin delivery. Liposomes with dimensions in the range of 20 nm to several microns and 4-7 nm thick membranes can easily encapsulate hydrophilic/lipophilic compounds (Thakur, et al, 2012).

In further investigations, it was noted that phospholipid vesicles and lipid-nanospheres with curcumin improved its intravenous delivery to tissue macrophages, especially bone marrow and spleen macrophages. Solid lipid nanoparticles (SLNPs) were also proposed for enhancing oral bioavailability of curcumin as pharmacokinetic profile of curcumin in rats showed significant improvement as compared to solubilized curcumin. In order to further enhance anti-cancer potential of curcumin, transferrin-mediated SLNPs containing curcumin were proposed and their superiority was tested in breast cancer cells. The SLNPs were, thus, proposed for topical application of curcumin as well. The SLNPs tested in rat models of inflammatory bowel disease exhibited enhanced anti-angiogenic and anti-inflammatory activities (Yadav, et al, 2009; Mulik, et al, 2010; Kakkar, et al, 2011).

Based on known capabilities of the liposomal delivery systems to incorporate poorly soluble drugs in preparing their aqueous deliveries, it is naturally expected to accommodate curcumin inside the hydrophobic interior of liposomes, resulting in higher drug loading capacity with improved stability than those of the free curcumin in phosphate buffer saline, human blood, plasma and RPMI-1640 medium with 10% calf-serum. Liposomal curcumin suppresses NF-κB binding and reduces the expression of NF-κB-regulated gene products, including COX-2 and IL-8 that are responsible for tumor growth/ invasiveness. Suppression of antitumor/antiangiogenesis effects in-vivo made the liposomal curcumin appropriate for the treatment of pancreatic cancer patients. Liposomal curcumin formulations show dose-dependent growth inhibition and apoptosis as observed in the two human colorectal cancer cell lines and synergetic effect with oxaliplatin based chemotherapy for the malignancy. In-vivo study of liposomal curcumin showed tumor growth reductions in Colo205 and LoVo xenografts in mice as reported in the related publications (Chen, et al, 2009; di Cagno, et al, 2011).

Liposomes coated with prostate membrane specific antigen specific antibodies could achieve drug targeting in a typical liposomal delivery system that were tested in two human prostate cancer cell lines exhibiting 10-fold better antiproliferative activity in human prostate cancer cell lines (LNCaP and C4-2B) compared to nonliposomal curcumin. It was also observed that LNCaP cells were relatively more sensitive to liposomal curcumin than C4-2B cells. Another liposomal curcumin formulation was noted to suppress the growth of head and neck squamous cell carcinoma (HNSCC) besides reducing the
activation of NF-κB without affecting the expression of pAKT and reducing the expression of cyclin D1, COX-2, MMP-9, Bel-2, Bel-xL, Mcl-1L and Mcl-1S. Suppression of mice tumors after 3.5 weeks treatment using intravenous liposomal curcumin with no noticeable toxicity suggested that liposomal curcumin is a viable non-toxic therapeutic agent for HNSCC. Another oral liposomal delivery system was suggested incorporating up to 68% of curcumin and offering faster rate and better absorption in rats as compared to simple curcumin. These results indicate that liposomal encapsulations certainly enhance the gastrointestinal absorptions of curcumin. A liposomal intravenous formulation of bis-demethoxy curcumin analogue showed better hepatoprotective activity compared to its free form. A combination of liposomal curcumin + resveratrol was reported to significantly decrease the prostatic adenocarcinoma by effectively inhibiting cell growth and inducing apoptosis. These observations confirmed that liposomal phytochemicals-in-combination might reduce prostate cancer incidence. Another novel system of curcumin-decorated nano-liposomes was proposed with very high affinity for amyloid-β peptide as vectors for targeted delivery of Alzheimer disease treatment that suggested about the possibility of exploring vesicle-surface available curcumin in various cancer treatments (Narayanan, et al, 2009; Aukunuru, et al, 2009; Mourtas, et al, 2011).

It was noted that DMPC-based liposomes show better encapsulation efficiency with a more desirable particle size in the range of 100 to 150 nm as compared with liposomes prepared with dipalmitoyl phosphatidylcholine (DPPC) and egg phosphatidylcholine (PC). Furthermore, DMPC liposomes inhibit (70%-80%) cellular proliferation of the human prostate LNCaP and C4-2B cancer cells (i.e. 5 - 10 mmol/L concentration) as compared with free curcumin that required 10-fold higher doses to exhibit similar inhibition properties. In-vitro/in-vivo studies have shown that liposomal-curcumin is more effective than free curcumin at equimolar concentrations emphasizing that liposomal delivery certainly enhances their uptakes and hence bioavailability/activity into the cells. A curcumin liposomal formulation using dimyristoyl-sn-glycero-3-phosphocholine was also tested for its positive influence on the modulation of signaling pathways involving proliferation, apoptosis, and angiogenesis of human pancreatic carcinoma cells. When administered (e.g. 40 mg/kg BW; three times/week) it suppressed the growth of BXPC3 and MiaPaCa2 tumors in a murine model indicating its efficacy.

Transcutaneous delivery of liposomal chemo-preventives is also feasible through hair follicles providing a reservoir for locally applied substances and to enable topical administration. The penetration depth of a novel class of amphoteric liposomes having isoelectric point at slightly acidic pH was studied for determining the efficiency of trans-follicular delivery of curcumin to penetrate about 35% to 69% of the follicle length depending upon the charge on the liposomes, showing their ability for topical delivery of lipophilic chemo-preventives for both therapeutic and chemo-preventive purposes. However, rapid elimination of these liposomal vesicles by active opsonization is also known to limit their overall efficacies, which can be avoided by modifying the liposomal surface with PEG to confer stealth properties. Similar liposomal delivery systems were also reported for active curcumin metabolites like tetra-hydro-curcumin (THC) in form of a cream formulation (developed particularly in Thailand) using phospholipid-derived THC-liposomes, which confirmed its safety along with a significantly lower irritation potential compared to the reference during dermatologic tests for irritation, conducted on female volunteers. Furthermore, a corneometry analysis of the skin above antecubital fossa revealed a higher moisturizing effect, which further showed that topical delivery of liposomal curcumin was possible to use in various skin ailments. However, some of the major problems of this delivery system include its lack of stability, poor batch-to-batch reproducibility, sterilization difficulties, and low drug loading as described in the related publications (Li, et al, 2005; Jung, et al, 2006; Wattanakrai, et al, 2007; Mitsopoulos, et al, 2008; Campbell, et al, 2009).

The liposomal membrane consisting of natural phospholipids and lipids like 1,2-dipalmitoyl-sn-glycerol-3-phosphatidyl choline (DPPC), 1, 2-dioleoyl-sn-glycerol-3-phosphatidyl choline (DSPC), egg phosphatidyl choline (EPC), dimyristoylphosphatidyl choline (DMPC), soybean phosphatidylethanolamine (SPC), hydrogenated soybean phosphatidylcholine (HSPC) and cholesterol were examined in detail for optimizing pharmacokinetics of liposomal curcumin as reported. It was shown that treatment of rats afflicted with pancreatic cancer with curcumin loaded DPPC-liposomes provided prolonged life of mice reflecting almost 3-fold increased bioavailability. Another study showed more than 74% prolongation of the life of mice with implanted tumor after treatment with liposomal curcumin. Liposomal curcumin shows dose-dependent increase in fraction of apoptotic cancer cells with suppression of NFκB activity. Enhanced cell-internalization of liposomal curcumin compared to that loaded in albumin associates in lymphocytes and EL4 lymphoma cells indicated that liposomes are capable of producing better inner-cell-delivery than serum albumin. Despite so many plus points in favor of liposomal curcumin delivery, one of the main problems faced is its lower entrapment capacity owing to the localization of the curcumin molecules primarily in phospholipid membranes (Li, et al, et al, 2005; Chen, et al, 2008; Shi, et al, 2012; Drakalska, et al, 2014; 2015).

The self-associating features of phospholipids arising out of their natural tendency to shield hydrophobic groups from aqueous environment while interacting with the aqueous phase with their hydrophilic groups enable them to form as spherical bilayer vesicles with an aqueous interior comprising of amphiphilic phospholipids and cholesterol molecules. Depending upon their structure and size, the liposomes are formed as multilamellar, and large/small unilamellar systems. Alternately, depending upon the driving force for drug
release, they are also classified as conventional, pH-sensitive, cationic, immune, and long-circulating liposomes. These lipid-based carriers significantly enhance the solubility of poorly water-soluble chemopreventives. Different drugs based upon their lipophilic character are distributed either inside the phospholipid bilayer, in the interior aqueous phase, or at the bilayer-water interface. These lipophilic chemopreventives including curcumin, and resveratrol, oryzanol, and N-acetyl cysteine, make them useful for liposomal drug delivery where lipophilic cores provide an optimum environment for drug entrapment. A liposomal system for the targeted delivery by adding prostate-specific antigen antibodies of curcumin was also reported while studying its partitioning potentials (Kristl, et al, 2009; Viriyaroj, et al, 2009).

Liposomal and HSA vehicles were further studied for transferring curcumin to spleen lymphocyte cells, EL4 lymphoma cell line and compared with aqueous DMSO vehicles and it was shown from there that liposomal vehicle is capable of loading more curcumin into cells than HSA or aqueous-DMSO, and lymphoma cells show preferential uptake of curcumin to lymphocytes. The present study demonstrated a simple and quantitative method of estimating curcumin delivered to cells using absorption and fluorescence spectroscopy (Kunwar, et al, 2006).

A curcumin-loaded liposomal formulation was found better owing to its improved absorption and fluorescence levels in lymphoma cells compared with normal cells. The influence of different combinations of lipid and curcumin was accordingly studied on various pancreatic carcinoma cells demonstrating an inhibition concentration of \( IC_{50} \) @ 2.0-37.8 \( \mu M \), whereas \( IC_{50} \) was 6.75-94.5 \( \mu M \), as assessed by cytotoxicity (Li, et al, 2005). In another study, curcumin was incorporated in egg phosphatidylcholine (EPC) liposomes at a 1:14 molar ratio (curcumin and liposome). A two-fold increase in the concentration of curcumin was found in rat plasma with the lecithin-curcumin formulation compared with a curcumin and curcumin-lecithin mixture after administration of a single oral dose of 100 mg curcumin/kg BW (Pandelidou, et al, 2011). Interesting observations were made while examining the use of curcumin and resveratrol in liposome and evaluating their combined effects on: cell growth, apoptosis and the cell cycle; and on activated p-Akt, cyclin D1, mammalian target of rapamycin (m-TOR) and androgen receptor (AR) proteins involved in tumor progression of PTEM-CaP8 prostate cancer cells. This combined formulation was found effective in reducing the prostatic adenocarcinoma (Narayanan, et al, 2009).

Liposomes comprising of artificial phospholipid vesicles are considered biologically safe, and biocompatible while offering protection to the drugs from external stimuli as an extended absorption capacity of curcumin was observed by dissolving/mixing/complexing it with different types of phospholipids. A curcumin-lipid formulation was reported using 1,2-dimyrstoyl-sn-glycero-3-phosphocholine (DMPC) and an anionic amphiphile, L-glutamic acid, N-(3-carboxy-1-oxopropyl)-, 1,5-dihexadecyl ester (SA), which on intravenous administration in rats showed no acute response in circulating blood cells, and much of the curcumin was found accumulated in bone marrow and spleen tissues (Sou, et al, 2008).

A systematic study was conducted using egg yolk phosphatidylcholine (EYPC) one-component monolayers and bilayers, as well as mixed systems containing additionally dihexadecyl phosphate (DHP) and cholesterol for having a better understanding of the mechanism of improving the curcumin delivery to cells as the curcumin attachment was noted to impact the size and stability of the liposomal carriers significantly. The EYPC/DHP/cholesterol liposomal bilayer curcumin was found stabilizing the system proportionally to its content, while the EYPC/DPH system was destabilized upon drug loading. A three-component lipid composition of the liposome was seen to be the most efficient for curcumin delivery (Karewicz, et al, 2011).

Skin permeation and anti-neoplastic activities of curcumin were investigated in form of liposomal transdermal drug-delivery system comprising of soybean, egg yolk, and hydrogenated soybean phospholipids producing curcumin-loaded liposomes (named as: C-SPCL, C-EPCL, and C-HSCPCL, respectively) with the particle sizes of ~82 nm (C-SPCL), ~83 nm (C-EPCL), and ~92 nm (C-HSCPCL) and corresponding encapsulation efficiency values as ~ 82, 82, and 81%, respectively. In-vitro skin penetration assessment put their activities in the following order - C-SPCL > C-EPCL > C-HSCPCL > curcumin solution. Thus, C-SPCL displayed highest activity to inhibit the growth of B16BL6 melanoma cells. A significant effect on anti-melanoma activity was observed with C-SPC-L, as compared to treatment with curcumin solution in-vivo and these results identified C-SPCL as a promising transdermal carrier for curcumin in cancer treatment (Chen, et al, 2012).

The potentials of curcumin loaded liposomal formulations were evaluated against cancer models of mesenchymal (OS) and epithelial origin (breast cancer) by noting promising anticancer activities both in-vitro and in-vivo against KHOS OS and MCF-7 breast cancer cells. The liposomal curcumin was found to initiate the caspase cascade leading to apoptotic cell death in-vitro in comparison to the DMSO-curcumin induced cell death. Curcumin-loaded γ-cyclodextrin liposomes were found very promising delivery vehicles for the treatment of cancers of different tissue origins (Dhule, et al, 2012).

The liposomal delivery of artemisinin and artesiminin + curcumin was investigated by assessing the in-vivo antimalarial activity of artesiminin-based liposomal formulations in Plasmodium berghei NK-65 infected animals. PEGylated liposomes were given to animals at a dosage of 50 mg/kg BW/day alone or plus curcumin as partner drug, administered at the dosage of 100 mg/kg BW/day. Treatments with several combinations of the formulations such as: artesiminin-loaded conventional liposomes (A-CL), artesiminin + curcumin-loaded conventional liposomes (AC-CL), artesiminin-loaded...
PEGylated liposomes (A-PL), and artemisinin–curcumin-loaded PEGylated liposomes (AC-PL) appeared to have an immediate antimalarial effect as all the formulations cured the malaria-infected mice within the same post-inoculation period of time along with showing less variability in artemisinin plasma concentrations, which suggested that A-CL, AC-CL, A-PL and AC-PL produced a modified release of drug (s) and, as a consequence, a constant antimalarial effect. In particular, A-PL was found most effective in this murine model of malaria (Isaachi, et al, 2012).

Silica-coated curcumin loaded flexible liposomes (CUR-SLs) were prepared by a thin-film method combined with homogenization, followed by the formation of a silica shell by the sol-gel process with a mean diameter of 157nm and entrapment efficiency of 91%. Compared with curcumin-loaded flexible liposomes (CUR-FLs) without silica-coatings, CUR-SLs exhibited higher stability against artificial gastric fluid and more sustained drug-release in artificial intestinal fluid as determined by assays. CUR-SLs and CUR-FLs had 7.76 and 2.35-fold higher bioavailability, respectively, than that of the curcumin suspension where silica coating improved the stability of flexible liposomes, and CUR-SLs exhibited a 3.31-fold increase in bioavailability compared with CUR-FLs, indicating that silica-coated flexible liposomes may be employed as a potential carrier to deliver drugs (Li, et al, 2012).

Curcumin-loaded long-circulating liposomes (CurLCL) were reported using ethanol injection method showing mean diameter of 110 nm (-5.8 mV ZP) with entrapment efficiency and drug loading of 80, and 2%, respectively. The release profile confirmed 49 and 89% releases from Cur-LCL in 7 and 24 hour, respectively. These results indicate that Cur-LCL formulation produces controlled drug release with increased circulation time (You, et al, 2014).

Curcumin was loaded in liposomes as free drug or after formation of hydropropyl-β- or hydroxypropyl-γ-cyclodextrin (HPβCD or HPγCD) complexes and characterized. Curcumin stability (at 0.01 and 0.05 mg/mL) in 80% (v/v) fetal bovine serum (FBS) was evaluated at 37°C, which demonstrated that HPβCD stabilized curcumin more than HPγCD, but liposome encapsulation provided substantially better protection, than CDs. There was 23-times more loading observed depending on the lipid composition of liposomes and the CD used, resulting in higher solubility. The stability profile of curcumin in serum was found to depend on the composition of liposomes and curcumin concentration, since at lower concentrations larger curcumin fractions were protected due to protein bindings (Matloob, et al, 2014).

The liposomal route of curcumin delivery was modified with three components of DDAB, cholesterol and non-ionic surfactant to determine their cellular responses against two types of cell lines (HeLa and SiHa), which showed DDAB as a potent inducer of cell uptake and cell death in both the cell lines. However, the cytotoxicity of DDAB-containing liposomes was higher and needed to be optimized. In addition, the anticancer efficiency and apoptosis effect of the liposomal curcumin formulations with DDAB was higher than those of DDAB-free liposomes (Saengkrit, et al, 2014).

An approach of curcumin encapsulation by nanoliposomes was reported with improved bioavailability by using salmon’s lecithin compared to those constituted of rapeseed and soya lecithin. A real-time label-free cell analysis was used to investigate in-vitro cytotoxicity of the formulation developed (Hasan, et al, 2014).

Knowing well about the anti-inflammatory potentials of curcumin (Cur) and bisdemethoxycurcumin (BDMC), these compounds were put to use for treating osteoarthritis by loading them in soybean phosphatidylcholines based liposome formulation, which showed ~70% entrapment efficiency and stable particle sizes. Both these liposomes inhibited macrophage inflammation and osteoclast differential activities besides being less cytotoxic and expressing high cellular uptake of the drugs. Curloaded liposomes prevented liposome-dependent inhibition of osteoblast differentiation and mineralization, but BDMC-loaded liposomes could not. With interleukin (IL)-1β stimulation, curcuminoid-loaded liposomes were able to down-regulate the expression of inflammatory markers on osteoblasts showing a high osteoprotegerin (OPG)/receptor activator of nuclear factor κB ligand (RANKL) ratio to prevent osteoclastogenesis. This showed the use of curcumin loaded liposomal formulation in slowing down the osteoarthritis progression (Yeh, et al, 2015).

In one of the recent studies, curcumin was entrapped in a water soluble complex PEGylated tertbutylicalix[4]arene, which allowed the drug to occupy both the phospholipid membrane and the aqueous core of liposomes forming dipalmitoylphosphatidylcholine: cholesterol liposomes, whose membranes were grafted with a poly(isoprene-b-acrylic acid) di-block copolymer to offer pH-sensitivity. This kind of curcumin-loaded formulation was found superior cytotoxic and apoptotic agent compared to the free drug. The related assays confirmed that the pro-apoptotic effects of pH-sensitive liposomal curcumin were mediated via recruitment of both extrinsic and intrinsic apoptotic pathways in HL-60 and HL-60/CDP cells (Jelezova, et al, 2015).

A curcumin-phospholipid complex (CPC) was reported showing that the CPC was amorphous, which formed hydrogel with higher erosion rates due to the amorphous nature of CPC causing enhanced dissolution. The CPC showed higher wound healing effect than the control on the rat skin wound model especially in the early phase (Du, et al, 2016).

Curcumin Solid-Lipid-Nanoparticles (SLNPs)

Pharmaceutical preparations involving solid-lipid nanoparticles (SLNPs) in form of colloidal drug carriers comprising of dispersed solid-lipid nanoparticles (e.g. in the size range of 10-1000 nm) are currently finding more and more applications in intravenous, intramuscular,
oral, rectal, ophthalmic, dermal and other routes of administration with the added advantages of other colloidal systems such as polymeric NPs, fat-emulsions, liposomes and micelles. SLNPs based drug deliveries are consistently providing enhanced stability, and oral bioavailability of poorly soluble drugs along with their controlled/targeted and sustained releases with improved drug loading efficiency, minimum use of organic solvents, and associated toxicity besides being easy to upscale for production.

These SLNPs contain emulsifier(s), water, and solid-lipid(s) like tristearin, and tripalmitin; partial glycerides like glyceryl monostearate, and glyceryl monooleate; fatty acids such as stearic acid, and palmitic acid; steroids like cholesterol and cetyl palmitate wax. Soybean lecithin, polysorbates, and PVA are the emulsifiers and stabilizers of the lipid-dispersions by appropriately combining features like different charges and molecular weights. The surface properties of these SLNPs are easy to modify by surface functionalization or coating with polymers for avoiding phagocytic uptake by macrophages while improving their pharmacokinetics. Detailed discussions on various aspects of SLNPs are available in some related publications (Garcia-Fuentes, et al, 2005; Uner and Yener, 2007).

Examining the details of SLNPs reveals the presence of a high value of interfacial tension at the surface of the lipid particles dispersed in aqueous medium. Smaller size lipid particles with larger surface to volume ratios possessing higher values of free energy that causes thermodynamic instability. Consequently, the phenomenon of flocculation/aggregation of the colloidal NPs sets in to relax the above said unstable system by reducing the associated surface free energy by promoting the formation of more stable crystalline nanostructures. Consequently, stabilizers are found helpful in reducing the free surface energy of these NPs by decreasing the interfacial tensions and checking the inter-particle aggregation by electronic repulsions or steric stabilizations. A number of surface-active agents like bilesalts, phospholipids, poloxamers, and polysorbates have been employed successfully in stabilizing the SLNP-assemblies as reported in various publications (Rosenblatt, and Bunjes, 2009; Verma, et al, 2011).

While looking for an appropriate stabilizer in this context, a compound named as - D-a-tocopheryl polyethylene glycol 1000 succinate (vitamin E TPGS or TPGS) was found as a good emulsifier derived from Vitamin E (a-tocopherol), which is not only water-soluble but also approved by FDA as a food supplement and drug solubilizer in oral, parenteral, topical, nasal as well as rectal/vaginal formulations. TPGS has often been used in preparing paclitaxel-PLGA-NPs with high drug entrapment efficiency against PVA. In case used alone, TPGS-NPs are generally of larger sizes as compared to PVA-NPs. The smallest NPs are reported when TPGS and PVA are used together. PVA (empirical formula of (C_{12}H_{22}O_{7}), n ~ 500 - 5000 represented by a MW ~ 20,000 - 200,000) is a water-soluble stabilizer for preparing emulsions in concentrations varying from 0.25% to 3.0% (w/v). Although, PVA based NPs are relatively smaller in size with uniform size distribution but several studies have reported about its associated toxicity, as it is carcinogenic in case administered parenterally in a long-term therapy. Therefore, PVA stabilized NPs are not favored due to its systemic toxicity (Rosenblatt, and Bunjes, 2009; Xie, et al, 2011). On the other hand, ‘Poloxamers 407’ is another stabilizer of ethylene and propylene oxides based co-polymer available in a variety of grades differing in the number of ethylene and propylene-oxide-units. For example, ‘Poloxamer 407’ (Pluronic F127) with an average molecular weight of 12600 and comprising of 196 units of ethylene oxide and 67 units of propylene oxide is water-soluble and approved by FDA as stabilizer for oral, parenteral, ophthalmic and dermal formulations. It is also used as a stabilizing agent for preventing aggregation in nanoemulsions, NPs as well as micro particles. Castor oil based nanoemulsions with poloxamer 188 and chitosan emulsifier produce stable formulations. Additionally, glyceryl monooleate (GMO) and ‘poloxamer 407’ are also used for producing stable simvastatin-NPs with a sub-200 nm mean particle sizes. The salient points involved in these protocol developments are covered in the related references (Dumortier, et al, 2006; Lai, et al, 2009; Tamilvanan, et al, 2010).

A number of protocols have extensively been described in the literature for SLNPs in which the specific choice depends on the properties of the drug as well as the formulation parameters including concentration of the lipid, and the stabilizer type used. Some of the widely used methods include solvent emulsification/evaporation; microemulsion based preparation; high pressure homogenization; shear homogenization and/or sonication; and high-pressure homogenization.

Lipids dissolved in water-immiscible organic solvents like cyclohexane are emulsified in aqueous phase followed by solvent evaporation that causes lipids to precipitate in the aqueous phase leading to colloidal NP-dispersions. This protocol involving room temperature processing suits well in case of heat-sensitive drugs. However, this method requires a large volume of organic solvents that raises not only the toxicity issues but also the environmental concerns. Scaling up of this technique for large-scale production is also not very easy as one of its minus points.

Stirring the low melting lipid, emulsifier and co-emulsifier in an aqueous phase at 65-70°C forms microemulsion that is dispersed in cold water (2-3°C) under continuous stir leading to the formation of SLNPs. The typical volume ratios of the hot microemulsion to cold water (e.g. kept @ 1:25-1:50) along with the parameters like composition of microemulsion and temperature gradient were noted to influence the SLNP-formations in this method. For example, high temperature gradient facilitated rapid crystallization of lipid preventing its aggregation. However, owing to the dilution step required in this method, the lipid contents achievable were rather considerably low.
SLNPs exhibit significant potentials for the delivery of lipophilic compounds like curcumin as first introduced in mid 1990s in form of a novel drug delivery system capable of protecting the labile drugs from light/pH/heat-mediated degradation, controlled release, and excellent bio-compatibility and tolerability. SLNPs are spherical lipid NPs that can be easily modified to attain a favorable zeta potential (ZPs), pseudo zero-order kinetics, rapid internalization by cancer cells, and imparting stealth properties to lessen uptake by the reticulo-endothelial system (RES), which make them highly useful drug delivery systems for a variety of compounds with different physicochemical/pharmacological properties. They are able to cross the blood-brain-barrier (BBB), providing a viable alternative vehicle for the delivery of less lipophilic drugs that cannot cross the BBB. Furthermore, the lipid component of these SLNPs having biological origin renders them lesser toxic as compared with polymeric ones. These drug delivery carriers not only protect the entrapped drug from photochemical or pH-mediated degradations but also enable drug targeting and easy large-scale production. Such characteristics make these SLNPs as suitable drug delivery carriers for curcumin and other chemopreventives like resveratrol, and b-carotene, which due to their lipid solubility not only get localized in the bilayer membrane of lipid vesicles/NPs but also improve their bioavailabilities. Initially, hot homogenization and warm microemulsion techniques were used for the preparation of these SLNPs but later other advanced techniques like high-pressure homogenization (HPH), solvent emulsification evaporation/diffusion, high-speed stirring, double emulsion method, and ultra-sonication were also introduced and explored accordingly as discussed in the referred publications (Kaur, et al, 2008; Bawarski, et al, 2016).

In one of the protocols, curcumin SLNPs were synthesized using dimyristoyl phosphatidylcholine (DMPC) via extrusion through a 0.2 micron filter and these vesicles were surface modified by L-glutamic acid, N-(3-carboxy-1-oxopropyl)-1, 5-dihexadecyl ester, and PEG to increase their uptake by macrophages. Because macrophages produce ROS that leads to oxidative damages and inflammatory responses, curcumin delivery to these macrophages can give rise to its maximal anti-inflammatory actions. It was reported that localization of curcumin SLNPs in macrophage rich sites such as bone marrow, spleen, and liver even 6-hours after the injection, showed their preferential uptakes by macrophages and their considerable ROS-scavenging potentials equivalent to 160 to 1,050 superoxide dismutase (SOD) units when analyzed by a hypoxanthine and xanthine oxidase system. Although initial reductions in white/red blood cells (W/RBCs), and platelets were observed with these vesicular NPs, the recovery of these blood components within 3 hours showed absence of any acute toxic response toward these vehicles. The potential of curcumin deliveries to different tissues was further evidenced by the presence of fluorescence of curcumin in tissue samples of animals, as detected in confocal microscopy experiments. One concern with this approach, however, was increased curcumin release from these vesicles at room temperature that suggested a possible problem with the retention of entrapped curcumin during storage (Sou, et al, 2008).

Curcumin encapsulations in SLNPs were found helpful not only in improving their aqueous solubility and instability but also in enhancing their cytotoxicity as aqueous dispersions in which oil phase was substituted with a solid biocompatible/degradable lipid-matrix was more stable against liposomes that enabled easy entrapment of many hydrophobic drugs that were enzyme protected in different application routes in which their smaller size and higher surface to volume ratio made them more appropriate for systemic administration (Garud, et al, 2012; Patil, et al, 2013; Drakalaske, et al, 2014; 2015).

Curcumin-SLNPs based microemulsions, reported recently as strong monoamine oxidase inhibitors with improved bioavailability and higher anti-depressant features in treated mice, are also noted to be effective in asthma treatment in rat models by blocking Nfkb proteins and causing higher plasma concentration of curcumin into the lungs and liver with significant inhibition of the cytokines, especially IL-4 and IL-2, which makes them promising nanocarriers besides attenuating the pain and immune modulatory cascade in rats having rheumatic arthritis (Kaur, and Kakkar, 2012; Wang, et al, 2012; Arora, et al, 2015).

Various examples of modifications introduced by using curcumin-SLNPs such as those reaching IMR 32 neuroblastaoma cells, causing reduced cytotoxicity in HL-60, A549, and PC3 cancer cells followed by significant reduction in malondialdehyde, enhancing cognition along with inhibition of acetylcholine esterase in ischemic rat models with increased levels of enzymes like glutathione, superoxide dismutase, catalase and mitochondrial complex enzyme in a brain delivery, and causing improvements in behavior as well as biochemical/histochemical responses in mice, did show its enhanced bioavailability. However, these all-useful curcumin-SLNPs do possess some limitations in terms of showing initial burst type drug release, tendency of gelation and unpredictable particle growth, which are not desirable in nanodelivery applications as indicated by many groups (Kakkar, and Kaur, 2012; Kakkar, et al, 2013; Kaur, and Slavcev, 2013; Yadav, et al, 2013; Rahman, et al, 2014).

A recent administration of SLNPs in patients with osteosarcoma reported (i.e. a level of 31.42-41.15 ng/ml of curcumin within 4 hours of 2000-4000 mg oral dose) no adverse effects (Gota, et al, 2010). In another study, 134 nm SLNPs with 84% encapsulation efficiency exhibited slow release over a week resulting in high oral bioavailability in rat plasma (Kakkar, et al, 2011). The pharmacokinetics of curcumin SLNPs after single oral doses of 1, 12.5, 25 and 50 mg/kg BW in rats exhibited a maximum concentration in serum as: 1.00 ± 0.01, 7.87 ± 3.02, 8.00 ± 1.87, and 14.29 ± 0.15 μg/ml, respectively, whereas free curcumin was only present at...
0.292 ± 0.06 μg/ml, even at a dose of 50 mg/kg BW, and such enhancement in bioavailability was due to the increased curcumin absorption. A comparative study revealed the uptake of liposome and serum albumin-loaded curcumin formations in normal spleen lymphocytes and EL4 lymphoma cells occurring via fluid phase pinocytosis and membrane fusion, respectively (Kunwar, et al, 2006).

SLNP formulations involving many fatty acids such as myristic, palmitic, stearic and behenic acids were prepared using co-acervation technique in the presence of polymeric non-ionic surfactants resulting in loading from 28 to 81% in 500 nm diameter particles, which were reduced to 300 nm size after hydrolysis (Chirio, et al, 2011).

Curcumin SLNPs (C-SLNPs) were prepared using stearic acid as lipid carrier during nano precipitation where the SLNPs were incorporated in hydro gel of Carbopol ® 934 with the mean particle size of 527.6nm, poly dispersity of 0.383 and 82.73% entrapment efficiency while in-situ testing exhibited a concentration dependent increase in firmness, consistancy, cohesiveness and viscosity in which SLNPs influenced the swelling properties. In-situ hydrogels also exhibited uniform and extended release of curcumin, along with higher permeation characteristics (Chawla, 2016).

Mono-stearin was used as lipid carrier in another optimization study of preparing curcumin-loaded SLNPs by using emulsion-evaporation and low temperature solidification method that resulted in the optimized formulation with 100 nm particles having −19.9mV ZP, 97.86% encapsulation efficiency and, 4.35% drug-loading capacity. The in-vitro release kinetics demonstrated curcumin-loaded SLNPs to be efficient in controlling the drug release (Chen, et al, 2013).

Curcumin loaded mono-methoxy PEGolate (mPEG-OA) micelles and polymersomes were tested for their anticancer activity against U87MG brain carcinoma and HFSF-PI3 cells as normal human fibroblasts in which its in-vitro bioavailability was found significantly enhanced. The half maximal inhibitory concentrations of curcumin and curcumin-loaded mPEG-OA for the U87MG cancer cell line were 48 and 24 μM, respectively with dose dependent apoptosis induction in the curcumin-loaded mPEG-OA treatments (Erfani-Moghadam, et al, 2014).

C-SLNPs were tested in forced swim model of depression in mice exhibiting 47.42%, 67.39%, 31.67% and 36.2% reduction in immobility time after administration of 1, 2.5, 5, 10 mg/kg BW doses, respectively, whereas free curcumin did not show any significant reduction. These results were primarily assigned to the therapeutic quantity of curcumin that crossed the blood-brain barrier, and thus recommended these C-SLNPs to be an effective anti-depressant treatment requiring administration of a single and a much lower dose when compared to free curcumin (Kakkar, and Kaur, 2012).

A simultaneous loading of curcumin with lipophilic gold nanorods (GNRs) into polymeric micelles made of biocompatible PLGA-b-PEG copolymer through a double re-emulsification process was reported after testing on Barret's esophagus and esophageal adenocarcinoma cell lines that demonstrated a significant reduction in cell viability with curcumin and GNRs exposure. Animal models confirmed these results with successful in-vivo eradication of all high-grade dysplastic premalignant cancer cells (Martin, et al, 2015).

C-SLNPs were also prepared by using ‘Compritol 888 ATO’ and ‘Precirol ATO5’ along with HHP followed by sonication resulting in average particle size in the range of 200-300nm with higher entrapment efficiency. The in-vitro release studies conducted in phosphate buffer not only showed improved solubility but also showed sustained release from these NPs as a function of parameters like physiochemical properties of drug/polymer, particle size, surfactant/stabilizer used for particle preparation, drug loading and nature of the release medium. It was also noted that curcumin encapsulation in SLNPs enhanced its stability compared to native curcumin, which was found degrading by 97% in only 3 hours, whereas freeze-dried curcumin loaded SLNPs were stable for 6 months at 2-8 °C. These findings also suggest that the transformation of SLNPs into a dry powder prevents the NPs aggregation and improves the stability against light and oxygen. In-vivo pharmacokinetics studies in rat plasma revealed that encapsulation of curcumin into SLNPs increased relative bioavailability of curcumin by 12 folds. SLNPs offer advantages over polymeric NPs as they are more biologically accepted because they are made from biodegradable solid lipids that offer higher potentials for sustained release as confirmed experimentally (Shelat, et al, 2015).

Curcumin Dendrosomes

In order to explore about the interactions taking place between curcumin and PAMAM-C12 25% a detailed systematic study was undertaken showing that the formation of PAMAM-C12 25%@curcumin non-covalent adduct induced the fluorescence quenching of PAMAM-C12 25%; curcumin entered the interface of PAMAM-C12 25% with mainly five classes of binding sites by hydrophobic, hydrogen bonds, and van der Waals force interactions where higher values of binding constants indicated that PAMAM-C12 25% held the curcumin tightly (Cao, et al, 2013).

Curcumin based 142 nm size dendrosomal formulation exhibited inhibitory effect against U87MG cell proliferation in a glioblastoma model by involving master genes of pluripotency/regulatory miRNA. Flow cytometry, methylthiazol tetrazolium, annexin-V-FLUOS and caspase assays confirmed their anti-proliferative properties by quantifying apoptosis and suppressing U87MG cells growth with no cytotoxicity related to dendrosomes. Non-neoplastic cells being not affected by this formulation, the relative expression of CT4A, OCT4B1, SOX-2, and Nanog combined with miR-145 over-expression were found to decrease. The dendrosomal curcumin was, thus, found to reduce the proliferation of U87MG cells through the down-regulation of OCT4 variants and SOX-2 (SRY [sex determining region Y]-box 2) in a miR-145-dependent manner (Mirgani, et al, 2013).
The solubility of nanoparticulate curcumin was 1.5 × 10^5 amorphous forms were synthesized and evaluated from entrapment efficiency and 12.84% of drug loading in caprolactone. Curcumin-loaded micelles with 92.54% of methoxy-poly (ethylene glycol), epichlorohydrin and hydrolysis and ring-opening polymerization reaction with copolymer synthesized through O-alkylation, basic mouse and human cancer cells including fibrosarcoma, to increase aqueous solubility and higher bioavailability encapsulate curcumin in a micellar structure, which led from esterification of oleic acid and PEG residues to and polymer chain relaxation (Song, et al, 2015).

A curcumin loaded dendrimer formulation called 3,4-difluoro-benzylidene curcumin (CDF) was reported for CD44 targeted therapy of pancreatic cancer by employing amine terminated fourth generation poly (amidoamine) (PAMAM) dendrimer and hyaluronic acid (HA) as a targeting ligand resulting in a dendrimer nanosystem (HA-PAMAM-CDF) with particle size and surface charge of 9.3 ± 1.5 nm and −7.02 ± 9.53 mV, respectively, and exhibiting dose-dependent cytotoxicity against CD44 receptor overexpressing MiaPaCa-2 and AsPC-1 human pancreatic cancer cells. A HA-PAMAM-CDF formulation displayed higher cellular uptake in MiaPaCa-2 cancer cell lines (Kesharwani, et al, 2015).

Micellar encapsulation for curcumin by thin-film hydration was examined using a linear-dendrimer methoxy-poly (ethylene glycol)-b-poly (ε-caprolactone) copolymer synthesized through O-alkylation, basic hydrolysis and ring-opening polymerization reaction with methoxy-poly (ethylene glycol), epichlorohydrin and ε-caprolactone. Curcumin-loaded micelles with 92.54% of entrapment efficiency and 12.84% of drug loading in amorphous forms were synthesized and evaluated from in-vitro cytotoxic activities against Hela and HT-29 cells. The solubility of nanoparticulate curcumin was 1.5 × 10^5 times higher than that of curcumin in water, and the associated drug release was a combination of diffusion and polymer chain relaxation (Song, et al, 2015).

A novel generation of dendrosomes was synthesized from esterification of oleic acid and PEG residues to encapsulate curcumin in a micellar structure, which led to increase aqueous solubility and higher bioavailability and its anticancer properties were confirmed in different mouse and human cancer cells including fibrosarcoma, colon, glioblastoma, bladder, gastric, breast and hepatocellular carcinoma in-vitro/vivo with no cytotoxicity on normal cells (Birgani, et al, 2015).

In one of the recent studies, the hepatocellular carcinoma curing activity of curcumin was observed by inducing apoptosis in cancerous cells, and was explored with the help of dendrosomal curcumin (DNC) employed in preventing hepatocarcinoma in both RNA and protein levels as confirmed by observing cell cycle distribution and apoptosis (i.e. by going through flow cytometry, Annexin-V-FLUOS/PI staining, real-time PCR, and Western blot to analyze p53, BAX, Bcl-2, p21 and Noxa) in DNC-treated cells. DNC-treated hepatocellular carcinoma cells are noted to undergo apoptosis by changing the expression of genes involved in the apoptosis and proliferation processes and these observations suggest that DNC is a suitable formulation for cancer treatment (Montazeri, et al, 2016).

A poly-amido-amine dendrimer (G0.5) was reported with cytotoxicity against human breast cancer cells (MCF-7). G0.5 in form of spherical NPs of ~150nm were loaded with curcumin in two different formulations. Formulation 1 was tested in-vitro drug release exhibiting encapsulation efficiency (62%) and loading capacity (32%). The solubility of curcumin was increased ~ 415 and 150-fold with respect to the unformulated drug, respectively, for formulation 1 and formulation 2 (Falconieri, et al, 2016).

**Host-Guest and Supramolecular Complexes of Curcumin**

In the context of overcoming some of the limitations of liposomal curcumin, macromolecules like cyclodextrines and calix[n]arenes were examined systematically in detail with the objective of influencing the pharmacokinetic characteristics of the hydrophobic drugs by specific 'host-guest' type complex formations. For instance, cyclodextrins are cyclic oligosaccharides consisting of D(1,4) glucopyranose units linked through α-(1,4) glucosidal linkages that divide cyclodextrins into three groups namely - α, β and γ-cyclodextrins, with 6, 7 and 8 glucopyranose units, respectively. These complexes are found not only to enhance the curcumin solubility, but also show anti-tumor activities in-vitro as well as optimized pharmacokinetic profiles in-vivo. For example, curcumin: β-cyclodextrin complex showed 31-fold increase in water and >18% more stability along with 2-fold higher transdermal permeability. Anti-proliferative and anti-inflammatory activities of curcumin as a result of suppression of the THP-induced activation of NF-xB were found more in curcumin-cyclodextrins displaying higher half-life and cell internalization. Oral administration of curcumin + cyclodextrin complexes was found suppressing the tumor growth in mice with lung cancer. Although, curcumin loaded liposomes composed of DPPC and DMPC in 1:1 molar ratio have poor encapsulation efficiency but it could be improved by adding into the aqueous cavity of liposomes in form of water-soluble inclusion complex with γ-cyclodextrins by hydration of thin liposomal film leading to double encapsulation efficiency. The cyto-toxicities of free agent in DMSO and loaded into liposomal platforms were evaluated on human cancer cell lines like KHOS, MCF-7 and skin fibroblast showing increase in antitumor activity against KHOS cells stemming from the osteosarcoma and MCF and tumor breast cells. However, γ-cyclodextrins also possess some negative features in terms of being expensive besides having lower aqueous solubility and posing problems in dissolving enzyme in starch. Moreover, using organic solvents like toluene or acetone also causes unwanted immunological or toxic effects. All these observations were substantiated in the related publications accordingly (Yadav, et al, 2009; Dandawate, et al, 2012; Rahman, et al, 2012; Mangolim, et al, 2014; Rachmawati, et al, 2013; Yallapu, et al, 2015).
Various options of curcumin deliveries have been attempted using calix[n]arene family, comprising of phenol units linked via methylene groups in 2,6 position as an alternative endowed with 'cup'-like structure of well-defined lower and upper rims and a hydrophobic cavity that is suitable for incorporation of guest molecules and ions. Although this form has low aqueous solubility, but it has been possible to synthesize octopus-shaped polyoxethylated calix[4]arenes for removing this limitation as well while showing none of the cytotoxic, hemolytic and immunological activities. These macromolecules participating in the phenomenon of self-association in water forming supramolecular aggregates provide attractive platforms for curcumin due to improvement in its water-solubility (Rocks, et al, 2012).

Curcumin release profile from supramolecular aggregates, however, exhibit an initial burst type of release, which is somehow possible to take care of by incorporating curcumin in DPPC: CHOL liposomal membrane and in the aqueous cavity of liposomes as inclusion complex of curcumin: polyoxethylated calix[4]arene. The critical influence of liposomes on curcumin nanocarriers was evaluated in terms of phospholipid content of phospholipidic micelles (ZP = -27.7 ± 1.7mV) and loading efficiency of ~1.2mg/ml concentrations of PCL and curcumin were successfully loaded respectively resulting in 15-20nm aggregates, however, exhibit an initial burst type of release, which is somehow possible to take care of by incorporating curcumin in DPPC: CHOL liposomal membrane and in the aqueous cavity of liposomes as inclusion complex of curcumin: polyoxethylated calix[4]arene. The critical influence of liposomes on curcumin nanocarriers was evaluated in terms of phospholipid content of phospholipidic micelles (ZP = -27.7 ± 1.7mV) and loading efficiency of ~1.2mg/ml concentrations of PCL and curcumin were successfully loaded respectively resulting in 15-20nm

A supramolecular nano-assembly of electrostatically interacting two oppositely charged lipid and polymer was prepared as nanocarrier having spherical shape with high positive zeta potential (>30 mV), monodisperse (polydispersity index <0.3), amorphous in nature, stable in the pH range of 2–6 and having enhanced antioxidant potency in comparison to crystalline curcumin in aqueous media (Pathak, et al, 2015).

**Curcumin-Metal-Complexes**

The presence of α, β-unsaturated diketomoiety in curcumin causes strong metal-chelation to form complexes with metals/nonmetals in different stoichiometric ratios of 1:1, 2:1 and 3:1. The metal coordination of curcumin group causes replacement of enolic-proton of the curcumin by metal ion while the o-methoxy phenolic part remains intact. In turn, the metal-oxygen bond is characterized by IR peak @ 455 cm⁻¹ accompanied by smaller shifts ~10cm⁻¹ in the carbonyl peaks after metal complex formation. A large number of curcumin complexes are already reported involving transition metals like Fe³⁺, Mn²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Pb²⁺, Cd²⁺, Ru³⁺, Re³⁺, non-transition metal ions and rare earth ions like Al³⁺, Ga³⁺, Sm³⁺, Eu³⁺, Dy³⁺, Y³⁺, Se²⁺ and metal oxides like VO²⁺. Stable complexes of some transition metals were prepared by mixing stoichiometric quantities of curcumin and metal salts in organic solvents and refluxing for few hours to have precipitates that are purified by column chromatography and crystallization. Curcumin-metal complexes modify both the physico-chemical properties of curcumin as well as the biological reactivity of metals. For example, metal complexation with curcumin reduces the toxicity of the metals as seen in curcumin complexes of Cu²⁺, and Mn²⁺ that are good anti-oxidants besides forming superoxide dismutase enzyme mimics caused by electron transfer reactions with superoxide ions (Barik, et al, 2005; 2007; Kunwar, et al, 2007; Koiram, et al, 2007; Leung, et al, 2013).

Curcumin-metal complexes are considered significantly important for treating Alzheimer’s disease, where these lipophilic complexes are able to cross the blood brain barrier and chelate with metal ions that are toxic to the neurons. It is quite likely that general observation of significantly reduced incidence of AD among the turmeric consuming people could be due to this reason as curcumin forms stable complexes with all the metals involved in Alzheimer’s disease (Jiang, et al, 2011; 2012).

Curcumin and Al form three types of complexes out of which 1:1 Al₃⁺-curcumin complex show lesser affinity to DNA than free Al³⁺ that is found to reduce development of Al³⁺-induced Alzheimer’s disease. More applications of curcumin-metal complexes are reported to include complexes of Ga, Zn, Au, and V in different contexts such as preparing bio-ceramics, anti-cancer agents with gastro-protective and anti-depressant effects in rats, anti-artrhitic agent, and anti-oxidant/anti-rheumatic agents, respectively (Gianluca, et al, 2011; Mei, et al, 2011; Pucci, et al, 2012).
Although in metal co-ordination, curcumin reduces the toxicity of heavy metals like Hg²⁺, Cd²⁺, and Pb²⁺ where significant reduction in heavy metal-induced oxidative stress is reported through complex formation. Owing to their positive charges, most of the metal complexes of curcumin bind to DNA and show pro-oxidant behavior leading to anti-cancer activity (Valentini, et al, 2009; Agarwal, et al, 2010; Rennolds, et al, 2012; Oguzturk, et al, 2012).


A number of curcumin-metal complexes have been explored for imaging of cancer cells based on fluorescence and radiochemical properties. For instance, Re(CO)₃ (Curcumin) (H₂O), ⁹⁹Tb(CO)₃(curcumin) (H₂O), and ⁶⁸Ga³⁺ complexes find several imaging applications due to the presence of fluorescent and radiochemical activities with affinity to beta-amyloid plaques and related systems in connection with Alzheimer’s treatment and diagnostics. Detailed studies of chemistry and spectroscopy of different curcumin-metal complexes would be helpful for future development of curcumin-metal complexes as imaging agents (Song, et al, 2009; Sagnou, et al, 2011; Asti, et al, 2014).

Curcumin-metal complexes involving Eu, Ce, La, Y, Cr, and Pd metals showed coordinated metal ions in bidentate mode in deprotonated form exhibiting anti-bacterial activity against Klebsiella pneumonia and Escherichia coli having good interaction with genomic DNA (Subhan, et al, 2014).

Curcumin complexed with barium (Ba²⁺) formed a supramolecular multimer in which Ba²⁺ got attached to di-phenolic groups of curcumin to form an end-to-end complex with reduced overall crystallinity without degrading curcumin and producing < 200 nm NPs. Curcumin stability was improved by > 50% after complexation with Ba²⁺ with 70% curcumin stability in water. These Ba-Cur supramolecular nanoparticles can be considered as a new class of pro-drugs with improved solubility and stability as the conversion of curcumin to a metal-organic supramolecular prodrug improved the solubility, stability and release profile of curcumin (Kamalasanan, et al, 2014).

**Miscellaneous Curcumin Formulations**

Curcumin transfersomes were prepared for better entrapment and permeation by optimizing the parameters like effect of lecithin, surfactant ratio, effect of various solvents and influence of surfactants like Tween 80 and Span 80 in varying concentrations. Higher entrapment and permeation were observed (T8 formulation) with an average vesicle size of 340nm. This study suggested that transfersomes made from PC: Span 80 in the ratio 85:15 (mmol) is a promising approach to improve the permeability of curcumin in period of time (Patel, et al, 2009). Chitosan-PVA-silver nanocomposite films were prepared for their applications in antimicrobial packaging, wound dressing and antibacterial materials by reduction of silver NPs (particle size ~ 16.5 nm). The anti-microbial/anti-fungal activity of the chitosan-PVA-silver-NP-films was found to significantly react against Escherichia coli, Pseudomonas, Staphylococcus, Micrococcus, Candida albicans, and Pseudomonas aeruginosa (P. aeruginosa).

Curcumin loaded chitosan-PVA-Ag-NP-films showed enormous growth inhibition of E. coli compared to curcumin and chitosan-PVA-Ag-NP-film alone (Vimala, et al, 2011).

A variety of PEG-cross-linked-acrylic-polymers were synthesized for curcumin delivery that showed sustained release depending on pH of the medium before testing them against human cervical cancer cell lines. These results confirmed the acrylic polymers as an efficient vectors for pH-sensitive, controlled delivery of hydrophobic drugs (Deepa, et al, 2012). Curcumin, loaded onto starch NPs during in-situ precipitation in water-in-oil microemulsion system exhibited enhanced solubility in aqueous solution depending upon the formulation parameters such as types of reaction medium, types of surfactant, surfactant concentrations, oil/ethanol ratios, loading time, and initial curcumin concentration. Under optimum conditions, curcumin loaded starch NPs (~87 nm) exhibited maximum loading efficiency of 78% leading to a sustained release over 10 days (Chin, et al, 2014).

Curcumin loaded cubosomes are novel carriers that were developed for topical delivery by homogenization method by using carboxyl as gelling agent and evaluated for appearance, pH, viscosity, entrapment efficiency, in-vitro drug diffuse studies, skin irritation studies and anti-bacterial activity. The particle size of ~75.2 nm and ZP of -24 mV gave rise to stable, nano-sized vesicles, able to improve curcumin anti-bacterial activity in topical drug delivery (Archana, et al, 2015).

Curcuminoid noisome formulation was reported using a series of non-ionic surfactants to enhance the skin permeation of curcuminoinds. Optimal noisome formulation including sorbitan monoooleate, cholesterol, and Solulan C-24 was loaded with curcuminoids having entrapment efficiency of 83% with particle size ~12 μm. In contrast to methanol solution 4% of entrapped curcuminoids traversed the shed snakeskin (Rungphanichkul, et al, 2011).

For enhancing the poor bioavailability of curcumin, solution-enhanced dispersion by supercritical carbon dioxide was developed for determining the optimal parameters and their influence on the size of the curcumin NPs. Particle size was noted to increase with temperature or solution flow rate, or with reducing precipitation pressure, under processing conditions. Curcumin NPs with a spherical shape and smallest mean...
particle size of 325 nm were reported using processing conditions comprising of $P = 20$ MPa, $T = 35^\circ$C, flow rate $= 0.5$ mL/min, and solution concentration of 0.5%. The chemical composition of curcumin was found unchanged whereas the crystalline state was affected adversely. The solubility and dissolution were found higher than that of the original curcumin powder ($\sim 1.4 \, \mu g/mL$ in 3 hours) (Zhao, et al, 2015).

Highly porous structure of poly (HEMA-MAPA) membranes, prepared by UV-polymerization technique, was used for controlled-release of curcumin triggered by pH and temperature changes. Optimum drug release efficiency was 70% @ pH=7.4 and 37°C within 2 hour. Time-dependent curcumin release was noted to be a slow release from the membrane demonstrated within 48 hour (Caka, et al, 2016).

Several groups extensively studied the preparations of curcumin-phospholipid complexes for improving its properties in terms of enhanced bioavailability, improved pharmacokinetics and increased hepatoprotective activity in comparison to its physical mixture of curcumin and phosphotidylcholine. A typical curcumin formulation named as Meriva® (phosphatidylcholine) showed significantly improved rat bioavailability. Curcumin phospholipid complexes administered orally result in higher serum concentrations of curcumin as compared to simple curcumin. However, the curcumin content in complexes are limited to about 17 and 32% (w/w), respectively, which is much lower than what could be achieved by liposomal encapsulation of curcumin (Gupta, and Dixit, 2011).

**Biomimetic Smart Formulations**

Biomimetic or biologically inspired methods of synthesizing biohybrid systems or biomimetic materials capable of mimicking biological recognition for drug delivery, drug targeting, and tissue engineering devices are currently under active consideration as the synthesis and characterization of biomimetic gels and molecularly imprinted drug release and protein delivery systems form a significant part of the recent research. For example, configurational biomimetic imprinting of an important analyte on an intelligent gel is possible to use for preparing new biomaterials that not only recognize the analyte but also act therapeutically by locally or systemically releasing an appropriate drug.

**Stimuli Responsive Nanoparticulate Systems**

Stimuli-responsive nanoparticulate systems have been explored for drug delivery applications by influencing their physico-chemical features at the target site when exposed to external stimuli arising out of a differing physico-chemical parameter inside the tumor in comparison to normal tissues (e.g. pH and protease expression) or the changes caused by the external conditions like applications of heat, ultrasound, light, and magnetic/electric fields. Such NPs ultimately help in improving the drug accumulations, penetrations and internalization with better efficacy (MacEwan, et al, 2010).

A number of pH-responsive nanocarriers that are reported for cancer treatment use the characteristic values of pH in the human tumors or those encountered in the endosomal/lysosomal compartments to trigger the drug release. Consequently, the involved polymer-NPs either swell/shrink or change their solubility due to protonation that causes hydrophobic-to-hydrophilic conversion. These pH-sensitive polymer-NPs are as such stable at neutral pH but cleave under mild acidic conditions exposing the hydroxyl groups to promote swelling and subsequently they behave like sources of drug in cells or tumors (Zubris, et al, 2011).

A pH-sensitive combination of curcumin + celecoxib-NPs exhibited synergistic activities in terms of improved delivery while pH-sensitive polymer helped in reducing the toxicity and enhancing mitigation efficacy of ulcerative colitis (UC) (Gugulothu, et al, 2014). A micellar system comprising of curcumin and amphiphilic/pH-sensitive methoxy PEG-poly (lactide)-poly(β-amino ester) (MPEG-PLA-PAE) was reported where the micelles remained stable in murine plasma at 37°C but shrank in size (i.e. 171 to 22.6 nm) with pH changing from 7.4 to 5.5 causing significant improvements in cell uptake and increased growth inhibitions by 65.6% in human breast cancer cells (Yu, et al, 2014).

Layer-by-layer assembly of CaCO$_3$ NPs template was prepared for synthesizing nanocapsules from two oppositely charged polyelectrolytes poly (diallyldimethyl-ammonium chloride), and poly (sodium 4-styrene-sulfonate sodium salt) films after core dissolution. The loading and release kinetics of curcumin inside these hexagonal shaped 350-400 nm capsules were controlled by changing the interactions between the drug and the polyelectrolyte matrix of the hollow nanocapsules when they reached the cytoplasm and nucleus compartment of Hela cancer cells after 24 hour of incubation without any toxicity to fibroblast cells (Kittitheeranun, et al, 2015).

PEG-DOX-Cur-NPs were found helpful in co-delivery of doxorubicin (DOX) and curcumin (Cur) in cancer treatment by conjugating DOX to PEG that led to NPs self-assembly while curcumin got embedded into the core. During internalization of these NPs by the tumor cells, the acidic environment breaks the PEG-DOX-linkers causing release of both DOX and curcumin into the nuclei and cytoplasm resulting in higher anti-tumor activity due to prolonged blood circulation, elevated drug accumulation and increased tumor penetration (Zhang, et al, 2016).

**Hydro/Nanogel Formulations**

Before examining the recent progress made in the area of preparing highly stabilized curcumin loaded gels (hydrogels, and nanogels), and emulsions (micro and nanoemulsions), especially for their drug delivery applications, it will be helpful to have a quick look into their general features and the mechanisms involved therein while processing them for different applications.

Hydrogels are 3-d cross-linked polymer networks that are held together by covalent/ionic/hydrogen/hydrophobic
interactions among the constituents, which in turn cause swelling in presence of water leading to large quantity water retention owing to the presence of hydrophilic pendant groups like: –OH, –CONH–, –CONH2–, and –SO3H. Because of these characteristic features, hydrogels are currently being explored extensively in tissue engineering, biomedical implants and drug delivery. The internal constituents cross-linked by physical, chemical, covalent, hydrogen bonding, van der Waals interactions, or physical entanglements not only provide water absorption and retention quality but also keep the network intact without dissolution. The characteristic features of hydrogels in terms of their high water content, soft and rubbery consistency, and low interfacial tension with water or biological fluids make them to resemble very much with the living organisms.

The drug release from hydrogels involving diffusion-controlled, swelling-controlled, and chemical-controlled modes are primarily decided by the mesh size within the gel matrix that is in turn controlled by parameters like the monomers involved, degree of cross-linking and the intensity of external stimuli (pH, and temperature) besides providing mechanical strength, diffusivity, and bio-degradability.

The physico-chemical and biological properties of hydrogels like swelling and water retaining, protecting the loaded drugs from hostile environment due to enzymes, pH, and other factors, via undergoing phase transitions with respect to the environmental conditions during controlled drug release in response to the external stimuli like pH, temperature, ionic strength and electric field have been explored in preparing numerous drug delivery formulations reported in literature. Current examples of curcumin hydrogels will be examined in brief in the current review later.

Nanogels, an extension of hydrogels in the nano domain, are cross-linked networks of nanosize hydrophilic/amphiphilic polymer chains that are useable as drug delivery carriers that can be made to absorb biologically active molecules through formation of various types of bonds including hydrogen bonds, or hydrophobic interactions. Interactions of polyelectrolyte nanogels with oppositely charged smaller drug molecules/bio-macromolecules such as oligonucleotides, siRNA, DNA and proteins that are attached to the ionogenic chains and phase separate within the finite nanogel volume, make the loading capacity of nanogels superior to the other drug carriers. Although drug binding causes volume reductions (e.g. one order of magnitude) but drug-nanogel-dispersions are maintained due to the lyophilizing effect of hydrophilic polymer chains in nanogels. Multiple chemical functionalities of nanogels have been exploited for introducing imaging labels, targeting molecules along with triggered drug release capabilities such as stimuli-responsive and degradable cross-links. Recent studies have suggested several promising applications of nanogels, including drug delivery of phosphorylated nucleoside analogs, oligonucleotides or siRNA for anticancer or anti-viral treatment, encapsulation of bioactive proteins, fabrication of nano metallic or nano ceramic composites, imaging agents, oral and CNS drug delivery (Kabanov, and Vinogradov, 2009).

A number of single/multiple stimuli responsive nanogels are currently being explored as smart drug delivery systems for controlled drug release in therapeutic applications offering longer durability and better control of drug administration. Responsive behavior of the nanosystems undergoes a series of changes in response to an external signal (physical or chemical) causing chemical reaction of the material or changes of the material’s properties, which in turn trigger drug release. Studies regarding pH, temperature, ionic strength, and light-triggered release of the drugs from the inner volume of the nanogels have been reported (Maya, et al, 2013).

Curcumin Loaded Hydrogels

The hydrogels are prepared using a combination of hydroxyl propyl methylcellulose and polyvinyl pyrrolidone as reported while exploring the influence of size and hydrophilic nature of the nanocarriers to enhance absorption and prolong the rapid clearance of curcumin. Reproducible ~100 nm NPs (encapsulation efficiency ~ 72%) were produced by solvent emulsion-evaporation technique followed by freeze-drying where the freeze-dried sample was readily reconstituted with distilled water. In-vivo anti-malarial studies revealed superior action of these nanoparticulates over curcumin control while acute/sub-acute toxicity studies confirmed its oral safety (Dandekar, et al, 2010).

Curcumin-peptide-hydrogels were reported in which the processes of curcumin mixing and peptide gelation were realized simultaneously together combining the features of minimally invasive delivery of stabilized curcumin with the provision of controlling its release by changing the peptide concentration. This β-hairpin peptide hydrogel displayed solid-like properties after shear thinning and returned quickly to stiffness close to pre-shear values. Stiffer features of the loaded hydrogel could be assigned to curcumin residing in core of the fibrils causing stiffening or bridging different fibrils thus producing additional crosslinks. In-vitro tests with medulloblastoma cells showed curcumin causing cell death and these results indicated the potential applications of curcumin-loaded β-hairpin hydrogels as injectable formulation for local curcumin delivery (Altunbas, et al, 2011).

A number of curcumin loaded transdermal hydrogel formulations containing carboxy polymethylene were reported showing good consistency, homogeneity, and spreading for better topical applications. The formulations with curcumin and olive oil showed good drug permeation across artificial as well as rabbit skins. The anti-inflammatory activity of 2% w/w curcumin hydrogel in the rat hind paw edema model revealed that the drug was delivered to the inflammation sites at a controlled rate over a period of about 3 hours (Nawaz, et al, 2012).
A curcumin-loaded hydrogel (25-75 mol% content) was reported using curcumin attachment to the hydrogel backbone and cross-linked through biodegradable carbonate linkages where curcumin was protected from oxidation and degradation, and hydrolysis resulted in the active curcumin release. Nontoxic PEG and desaminotyrosyl-tyrosine ethyl ester were used to tune the hydrophilic/hydrophobic hydrogel properties. In this way, hydrogels with a wide range of physical properties including water-uptake (100-550%) and compression moduli (7-10 kPa) were obtained. Swelling-controlled curcumin release could be extended up to 80 days. Curcumin-loaded hydrogels showed selective cytotoxicity against MDA-MB-231 breast cancer cells in-vitro but no cytotoxicity to noncancerous human dermal fibroblasts even at high curcumin concentrations (160μM). One possible application of these curcumin-derived hydrogels is as soft tissue filler after surgical removal of cancerous tissues (Shpaisman, et al, 2012).

The physico-chemical properties of a curcumin/xanthan: galactomannan hydrogel were investigated for developing nasal/topical pharmaceutical applications, in cosmetics and foods. Curcumin-loading in the hydrogel, although, influences the hydrogel characteristics but without affecting the structure of the gel network. Highly biocompatible nature of this hydrogels was confirmed by noting no CAM tissue injury during application (Da-Lozzo, et al, 2013).

Curcumin binary hydrogel was reported using xanthan and galactomannan from Schizolobium parahybae (guapuruvu) offering gel characteristics, stable pH values and mechanical stress resistance even after 45 days of heat exposures (45°C). In-vitro cytotoxicity analysis showed non-cutaneous membrane irritation, and the in-vitro skin permeation analysis indicated 2.15-2.50 μg/mL curcumin at the stratum corneum, epidermal and dermal levels. This binary hydrogel with curcumin solubilized in ethanol presented 76.8% inhibition of topical inflammation. Chemical stability and non-cytotoxicity analysis confirmed its safety during prolonged exposure of the skin in the topical treatment, offering long-lasting XGEC and XGMC actions (Koop, et al, 2015).

A series of injectable chitosan-based hydrogels were prepared by cross-linking of chitosan and genipin with the co-operation of ionic bonds between chitosan and sodium salts at room temperature. Curcumin was incorporated into the above formulation that exhibited the profiles of sustained release with initial burst for all the hydrogels with ~3-6 times higher cumulative release than the gel control (Songkroh, et al, 2015).

Charge-modified citrus pectins form hydrogels owing to interaction between calcium ions and free carboxyl groups of pectins, which were explored for encapsulating poorly water-soluble drugs and taking them through the gastro-intestinal tract for site-specific targeted delivery. Modified pectins show different gel strengths at similar total charge suggesting that they may be a highly effective delivery carrier. Low methoxy pectin (LMP) is a favorable drug delivery vehicle due to cross-linking of calcium ions with polymers leading to charge neutralization of the drug that affects the rate of drug release by limiting the interaction with dissolution media. The encapsulation efficiency (EE) of all pectin hydrogels studied in a master’s program showed values >99%, which is very high compared to the published results, which may be due to higher Ca²⁺ level forming stronger hydrogels by increasing the number of crosslinks within the matrix (Lee, 2015). The curcumin release rates from all pectin samples were less than 0.3% at pH 1.2. For both pH=1.2 and 6.5 stages, pectin hydrogels with 400 mg curcumin had lower release rates than those with 100 mg of curcumin. The effect of pectin on inhibition of adipogenesis of 3T3-L1 adipocytes showed high-methoxyl pectin (HMP) at 100 μg/mL had inhibitory effects on lipid content (Lee, 2015).

One-pot synthesis of curcumin-PEG–polyurethane (PU-CUR) hydrogels was reported using PEG-4000, 4, 4’-methylenebis (cyclohexyl isocyanate), and curcumin in presence of a cross-linker, 1,2,6 hexanetriol (HT) where the degree of swelling could be controlled by varying the amount of HT and curcumin. Cryogenic cooling and lyophilization led to the formation of inter-connected pores in the hydrogels. The tensile and breaking compression strengths of swollen gels were in the range of 0.22-0.73 MPa and 1.65-4.6 MPa, respectively while detailed in-vitro experiments showed the biocompatibility of gels, cytostatic dosage of curcumin, selective toxicity toward cancer cell lines, and antibacterial property (Divakaran, et al, 2016).

Multi-domain peptides (MDPs) based bio-compatible hydrogel scaffolds were reported using SN-38, daunorubicin, dflunisal, etodolac, levofloxacin, and norfloxacin in which the steady-state fluorescence and drug release studies showed that hydrogels loaded with SN-38, dflunisal, and etodolac exhibit prolonged drug release profiles due to intra-fibrillar drug encapsulation (Li, et al, 2016).

**Curcumin Nanogels**

A core-shell structured nanogels were synthesized by coating the Ag/Au bimetallic NPs with a hydrophobic polystyrene (PS) gel layer as inner shell, and a subsequent thin hydrophilic nonlinear PEG-based gel layer as outer shell in which the Ag/Au core NPs not only emit strong fluorescence for imaging and monitoring at the cellular level, but also exhibit strong absorption in the near-infrared (NIR) region for photo-thermal conversion. While the inner PS gel layer is introduced to provide strong hydrophobic interactions with curcumin for good drug loading, the external nontoxic and thermo-responsive PEG gel layer triggers the release of the loaded curcumin either by variation of surrounding temperature or exogenous irradiation with NIR light (Wu, et al, 2011).

In one of the studies, curcumin loaded dextrin nanogel was reported with nanogel/curcumin ratio dependent stability and loading features that showed useful in-vitro release profile in HeLa cells. Curcumin-chitin nanogels, in another study, showed enhanced
release in acidic pH compared to the neutral besides specific toxicity towards melanoma cells (~0.1-1.0 mg/mL), no toxicity to normal cells even at higher uptake in human melanoma cells with 4-fold increase in transdermal flux appropriate for melanoma treatment. Alternately, a water-dispersible nanogel was prepared for intracellular delivery by combining curcumin with poly styrene coated Ag/Au NPs where bimetallic NPs offered good absorption in near-IR for photo-thermal therapy besides offering imaging and monitoring possibilities. Hyaluronic acid nanogel conjugates were developed for CD44-positive drug-resistant tumors by employing membranotropic cholesteryl-HA for targeting and inhibiting tumor growth with increased bioavailability and sustained release following ester linkages hydrolysis producing 2-7-fold more cytotoxicity in human breast/pancreatic cancer cells. Overall, these drug loaded nanogel designs show significantly enhanced drug bioavailability/stability, loading efficiency, transdermal penetration, cell targeting, and treatment efficacy against cancer cells and multicellular spheroids as discussed in the related publications (Yallapu, et al, 2011; Wu, et al, 2011; Mangalathillam, et al, 2012; Gonçalves, et al, 2012; Wei, et al, 2013; Maya, et al, 2013; Ghalandarlaki, et al, 2014).

Micro/Nanoemulsions

Microemulsions, being one of the most widely used drug delivery systems, are capable of providing high drug entrapment efficiency with long-term stability of the hydrophobic molecules. These stable, optically isotropic, transparent formulations are characterized by a dynamic microstructure that results spontaneously by mixing lipophilic and hydrophilic constituents in presence of suitable surfactants. Such microstructures result into high drug solubilization capacity along with free and fast drug diffusion that coupled with lipophilic nature endow them with a high potential for delivering lipophilic compounds like curcumin not only across lipophilic cell membranes but also through skin (Teichmann, et al, 2007; Lee, et al, 2008; Santos, et al, 2008).

Further studies showed that curcumin is easy to deliver through the stratum corneum and into the complete follicular infundibula via o/w microemulsions. These microemulsions can be further formulated into hydrogel patches of chitosan/chitosan-starch blends to protect the drug from the detrimental effects of pH, light, and/or oxygen-mediated degradations. Their stability improves significantly and controlled release at a desirable site can be obtained after these compounds are microemulsified and entrapped into a hydrogel like matrix. Studies have shown that even after 2 months of storage at room temperature, mean hydrodynamic diameter of the oily internal phase increases only slightly, showing the high stability and efficiency of such hydrogels. In addition, the external aqueous phase of these emulsions provides hydration to the stratum corneum and moisturizes the skin. Drug release from microemulsified droplets are further possible to augment by using ultrasonic energy externally due to structural reorganization that result in the phase separation of oil droplets from the aqueous vehicles releasing the compound. Similarly, a curcumin based microemulsion cream formulation was described with entrapment efficiency of 35% to 70% (curcumin + SLNPs) with a diffusion-mediated controlled release pattern. In addition, the formulation was found to increase the photo-stability of curcumin even after 6 months of storage with no significant change in the viscosity or color of the formulation. These observations were made during several studies conducted and reported (Jung, et al, 2006; Tiyaboonchai, et al, 2007; Teichmann, et al, 2007; Boriwanwattanarak, et al, 2008).

Although this approach seems promising in enhancing the delivery of potent therapeutics, its usefulness for chemo-preventives has not yet been established in animal and human clinical studies. Microcapsules of curcumin with gelatin using ethanol/acetone as co-acervating agents to separate the two phases that result in precipitation of the drug in spherical microcapsules was reported sometime back by preparing curcumin dispersion in the gelatin solution followed by its addition to ethanol, which was mixed with a formaldehyde solution (37% v/v) to provide rigidity to gelatin coating. Enhanced microencapsulation yield, drug loading, and entrapment efficiency were affected by the solubility of curcumin in the co-acervating solvents. Acetone was better to dissolve curcumin as compared to ethanol in which curcumin tend to disperse at high concentrations used for loading into microemulsions. Furthermore, the microcapsules prepared by using acetone were found to possess better flow and higher stability with retention of their spherical shape (Aziz, et al, 2007). A similar injectable microparticulate curcumin formulation was prepared using PLGA for breast cancer chemo-prevention study providing sustained deliveries in blood and tissues for around a month by a single subcutaneous injection with tissue levels of 10- to 30-fold higher in brain and lung as compared to that in plasma, suggesting their potentials to sustain drug levels on subcutaneous administration (Shahani, et al, 2010).

Isotropic mixtures of oil + water + surfactant/co-surfactant in which the oil phase is a mixture of different hydrocarbons and olefins are called microemulsions (MEs) that are thermodynamically stable structures and therefore do not require high inputs of energy or shear stress for their formation as compared to ordinary emulsions that are kinetically stable but thermodynamically unstable and always try to phase separate. Besides being commercially more viable due to its lower energy requirements, MEs are also getting examined as drug delivery vehicles in food and pharmaceutical industry applications, as well as in the petrochemical industry (Lee, 2010).

Nanoemulsions (NEs), as an extension of microemulsion in nano domain, prepared either in form of ‘oil-in-water’ or ‘water-in-oil’ using GRAS specified surfactants for dissolving large quantities of hydrophobic drugs with their mutual compatibilities while protecting them from hydrolysis/enzymatic degradations, have been
qualified as parenteral transport vehicles in drug delivery to ensure sustained/control drug release leading to significant reductions in frequency and dosage of the injections to be given during the treatments. The lack of flocculation, sedimentation and creaming, combined with large surface area/free energy – all combined offer advantages over emulsions of larger particle size as very large interfacial area of these droplets positively affects the drug transport and delivery along with site-specific targeting. Droplet size reductions during processing not only impart optical transparency but also modify the elastic behavior holding promise as useful dispersions of deformable nanosize droplets. NEs are, therefore, expected to play significantly important roles in future owing to smaller quantity of surfactant required in preparation than those in lyotropic microemulsion phases in commercial productions (Lovelyn, and Attama, 2011). NEs differ from macro/microemulsions not only in being transparent/translucent (droplet sizes ~ 20-200 nm) but also allow transport/solubilization of hydrophobic drugs within the aqueous phase. Consuming extra energy in their preparations makes NEs metastable in contrast to thermodynamically stable microemulsions. Out of several methods of applying energy like stirring, high-pressure homogenization, and ultrasonics, the processing based on ultrasonic energy is becoming more cost effective. In terms of their stability, micro/nanoemulsions may be stabilized over a period of few hours to years depending upon the process parameters used. The important feature of NEs is manifested in the mutual solubilization of the two phases and the amount and types of surfactants used (Delmas, et al, 2011).

Curcumin Microemulsions

Curcumin loaded microemulsions, comprising of surfactants, oil, and water, are stable solutions for improving curcumin delivery via local and transdermal routes for scleroderma, psoriasis and skin cancer (Yallapu, et al, 2012). Eucalyptol-based curcumin microemulsions, for instance, have very high permeability and flux with moderate solubility of curcumin compared with many oleic acid and estern oil-based microemulsions (Liu, and Chang, 2011). Enhanced penetration capacity of curcumin microemulsions, as well as it’s effect on the cellular structure of skin, already known, are possible to enhance further by using a new nanoemulsion formulation comprising of limonene, polysorbate 80, ethanol and water to promote curcumin presence in the skin (9.3-30 μg in an area of 0.785 cm²) (Liu, et al, 2011). A stable self-micro-emulsifying drug delivery system comprising of 20% ethanol, 60% Cremophor RH40® and 20% isopropyl myristate has been found to encapsulate 50 mg/ml of curcumin and to release it completely in 10 minutes (Wu, et al, 2011). Curcumin microemulsions, prepared in the presence of ethyl oleate and isopropyl myristate, result in homogeneous, yellow, transparent solutions, whereas soybean and peppermint oil based formulations are turbid in appearance (Lin, et al, 2009). Highly smooth surface gelatin microspheres with 75.5% curcumin encapsulation efficiency are formulated by the emulsion crosslinking method suitable for lung targeting where these 5-30 μm diameter microspheres release within 22 hours for immediate therapeutic effects (Cao, et al, 2009). Co-administration of curcumin and paclitaxel nanoemulsion formulations are capable of overcoming multidrug resistances in SKOV3 (TR) human ovarian adenocarcinoma cells by inhibiting NF-κB activity, down regulating P-gp and promoting apoptotic responses (Ganta, and Amiji, 2009). Additionally, curcumin nanoemulsions are noted to increase the bioavailability of paclitaxel up to 5.2-fold, and a 3.2-fold in its tumor site accumulation in an oral administration to mice models resulting from down regulation of intestinal P-gp and cytochrome P450 3A2 (CYP3A2) protein levels (Ganta, et al, 2010).

The formulations prepared from glyceryl monooleate (GMO) and cremophor involving self-nano-emulsified curcumin exhibit improved transdermal permeability (Rachmawati, et al, 2013). In another case, chemotherapy resistance of human ovarian adenocarcinoma cells was taken care of by administering simultaneously loaded with curcumin and paclitaxel in nanoemulsion resulting in enhanced cytotoxicity and sensitivity to chemotherapy of the treated cells (Ganta, and Amiji, 2009). Similar results were reported in few more cases, where nanoemulsions loaded with curcumin and etoposide-loaded nanoemulsions (<150 nm diameter and high entrapment efficiency) were used successfully in treating prostate cancers (Wang, et al, 2014). However, nanoemulsions are generally expensive formulations to prepare with pH and temperature dependent stability involving high concentration of surfactants and co-surfactants (Prashant, et al, 2014).

A new formulation of curcumin based lipid nanoemulsion was prepared by modifying a thin-film hydration method followed by sonication and had the smallest particle size, the highest drug loading efficiency, and a good physical stability for cancer chemotherapy. Curcumin lipid nanoemulsions employed soybean oil, hydrogenated L-a-phosphatidylcholine from egg yolk, and co-surfactants to formulate the emulsions with mean particle diameter of 47–55 nm, and incorporating 23-28 mg curcumin per 30 mL, and were stable in particle size for 60 days @ 4°C. The cytotoxicity studies of curcumin solution and curcumin-loaded nanoemulsion using B16F10 and leukemic cell lines showed IC₅₀ values ranging from 3.5 - 30.1 and 22.2 to 53.7μM, respectively. These results demonstrated the utility of curcumin loaded nanoemulsion particles with small particle size, high loading capacity, good physical stability, and preserved cytotoxicity (Anuchapreeda, et al, 2012).

An exponential dependence of droplet size in time as a function of ultrasonic energy was observed while studying various aspects of preparing highly stabilized NEs (droplets ≤150 nm) as discussed in a publication by tracking the droplet size evolution during sonication. The trapping of the species within the droplets can be utilized to oppose Ostwald ripening, and this was put to use in
preparing highly stabilized NEs by noting that in surfactant rich regime, the droplet diameter decreases exponentially as a function of the input energy density. The saturated size obtained at the end of sonication is governed by the efficiency of the ultrasonic cavitation process in creating high local shear rates and on the characteristic interfacial energy of the droplets. The resulting NEs can be stabilized against Ostwald ripening by incorporating insoluble species inside the core and/or in the membrane compartment. Compared to conventional emulsions, the small size of the droplets inhibits the formation of droplet-droplet contact zones large enough to promote fusion. Stabilization against coalescence is readily obtained in NEs by using enough surfactant (Delmas, et al, 2011).

Nanoemulsions, being quite similar to micro-emulsions, contain oils/fats dispersed into aqueous phase with the help of emulsifiers and surfactants, approved for human administration by FDA and possessing droplets size in the range of 20-200 nm, with great potentials for hydrophobic drug encapsulations. Nanoemulsions are finding more and more applications as nanocarriers in cancer treatments, diagnostics and transdermal applications (Shah, et al, 2010). For instance, curcumin-nanoemulsions prepared by thin-film-hydration, using Tween-80 as a co-surfactant resulting in 50-75 nm particle sizes exhibit improved oral bioavailability while suppressing NFκB activity in-vivo besides showing cytotoxicity in mice melanoma and human leukemic cells (Young, et al, 2014; Anuchapreeda, et al, 2012).

Curcumin Nanoemulsions

A curcumin nanoemulsion formulation was developed for ophthalmic applications by screening a number of surfactants and oils based on their ability to solubilize curcumin. Using a ratio of curcumin: Acconon: Tween 80: water (0.12:1:7:1) a novel formulation was reported with spherical droplets sizes in the range of 8-22 nm with adequate stability. This study ensured the stability and formation of characteristic nanodroplets with curcumin entrapment as a result of the developed formulation (Anjana, et al, 2012).

The impact of different lipid-based formulations was investigated in one of the studies of curcumin encapsulation for bio-accessibility enhancements by preparing oil-in-water nanoemulsions involving long, medium, and short chain triacylglycerols - LCT, MCT and SCT, respectively. This study revealed the initial digestion rate to decrease in the order SCT > MCT> LCT, while the final digestion decrease was in the order MCT>SCT> LCT. The bio-accessibility of curcumin decreased in the order MCT > LCT >> SCT and appeared to be slightly higher in conventional emulsions than in nanoemulsions (Ahmad, et al, 2012).

A nanoemulsion based drug delivery via olfactory route was reported involving artemether and curcumin for treating cerebral malaria involving drug-loaded chitosan-NP nanoemulsion (average globule size - 32-70nm; ZP= -12 to -28 mV) exhibiting high electro-conductivity owing to their oil-in-water nature and exhibiting lower toxicity tested in Vero cell lines. The ex-vivo release studies in sheep nasal mucosa incomparison to drug suspension in simulated nasal fluid revealed that release rate was biphasic and faster than that from the drug suspension. The antimalarial efficacy tested in *Plasmodium berghei* ANKA murine model of cerebral malaria showed promising results (Jain, et al, 2012).

In one of the studies, curcumin nanoemulsion was prepared using high-pressure homogenization of corn oil and 3 different emulsifiers namely - Tween 20 (non-ionic), Sodium Dodecyl Sulphate (SDS, anionic) and Dodecyl Tri-methyl Ammonium Bromide (DTAB, cationic) revealing that the positively charged DTAB-stabilized emulsions were least stable during the digestion process, and it exhibited the largest increase in droplet size followed by phase separation causing lower bioavailability. Emulsions stabilized with Tween 20 showed retention of emulsion structure and greater free fatty acid production leading to increased bioavailability where the emulsifier charge influenced the lipid digestion and the bioavailability of the bioactive compound incorporated, probably by altering the ability of bile salts and digestive enzymes to adsorb onto the emulsion surfaces, thus altering the droplet size due to droplet breakup or coalescence within the digestive tract. The importance of subjecting the emulsions to a simulated gastric environment was highlighted in this study since parameters like changes in pH, ionic strength, gastric enzyme activity and shear - all were noted to impact the emulsion properties in the small-intestine (Pinheiro, et al, 2013).

A curcumin loaded cassava starch based NP-formulation for improving its cellular absorption was reported by preparing cassava starch-NPs by acid hydrolysis followed by mixing of acetone-dissolved curcumin. Curcumin cytotoxicity and cellular uptake were studied in L929 fibroblast cell lines confirming that curcumin was successfully loaded onto starch NPs and its antioxidant activity was on par with pure curcumin but its cellular absorption was significantly higher than that of pure curcumin (Athira, and Jyothi, 2014).

The process optimization was carried out to produce curcumin nanoemulsions for intranasal delivery by considering parameters like oil, surfactant, and co-surfactant concentration in deciding the globule size and ZP. The formulations, so prepared, were subjected to *in vitro* cytotoxicity using SK-N-SH cell line and nasal ciliotoxicity studies showing no toxicity besides being safe for intranasal delivery for brain targeting. *In vitro* diffusion studies revealed that nanoemulsions had a significantly higher release compared to drug solution and ex-*vivo* diffusion studies showing higher flux and permeation across sheep nasal mucosa (Sood, et al, 2014).

Oral delivery in tablet form was reported containing curcumin nanoemulsion prepared from oil phase (glycerylmonoooleate), surfactant (cremophor RH 40), and co-surfactant (PEG 400), followed by tablet formulation using 9% PEG 6000 (lubricant), polyvinylpyrrolidone 5%...

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substantial reversal of arthritic symptoms in rat models incorporated into gel using carbopol-980 demonstrating formulation with droplet size of 41 nm (-33 mV ZP) was permeation as compared to curcumin in oil. One resulting in several formulations with an average droplet size of 100 nm has the maximum potential for particle size of 134 nm when dispersed in water (Rachmawati, et al, 2014a).

Curcumin nanoemulsion comprising of glyceryl monooleate, cremophor RH40, and PEG 400 (1:8:1) was prepared with varying curcumin loading to obtain different droplet size of nanoemulsion. The plasma kinetic profile of curcumin nanoemulsion with various sizes (50, 150, 200, and 300 nm) was studied to determine the relationship between particle size to the plasma kinetic profile of curcumin after intravenous and oral administrations to male healthy rats (dose: 10 mg/kg and 50 mg/kg BW for intravenous and oral routes, respectively). In-vivo release profile of curcumin from the oil droplet was found inversely proportional to the size of the curcumin nanoemulsion, regardless to the route of administration suggesting that size is one of important parameter in designing nanocarrier influencing the biological responses (Rachmawati, et al, 2014b).

Curcumin-loaded lipid nanoemulsions (50, 100 and 200 nm droplet size) were prepared by a modified thin-film hydration method followed by sonication. For finding out the optimal particle size, which exhibited the strongest physiological activity, the inhibitory effects of the nanoemulsions were carried out against inflammatory and allergic activities. In-vitro cell culture experiments revealed 100-nm curcumin lipid nanoemulsion showed the most prominent inhibitory effect on the production of tumor necrosis factor-α (TNF-α) induced by lipopolysaccharide (LPS) in RAW264.7 murine macrophages, and on the release of β-hexosaminidase induced by the calcium ionophore, A23187, in rat basophilic leukemia RBL-2H3 cells. In-vivo experiments showed 100-nm curcumin lipid nanoemulsion to possess the most prominent anti-inflammatory and anti-allergic effects, inhibiting 12-O-tetradecanoylphorbol-13-acetate-induced inflammatory ear edema and immunoglobulin E (IgE)-induced passive cutaneous anaphylactic (PCA) reaction. These results suggested that the physiological activities of curcumin lipid nanoemulsions depended on particle size and a particle size of 100 nm has the maximum potential for use in enhancing the bioavailability and medical value of curcumin (Onodera, et al, 2015).

Curcumin nanoemulsion (CR-NE) was also prepared by involving oil (Labrafac PG/glyceryl triacetate), surfactant/co-surfactant (tween 80, PEG400), and water resulting in several formulations with an average droplet size of ≤ 70 nm, which showed a four-fold increase in skin permeation as compared to curcumin in oil. One formulation with droplet size of 41 nm (-33 mV ZP) was incorporated into gel using carbopol-980 demonstrating substantial reversal of arthritic symptoms in rat models (Naz, and Ahmad, 2015).

Curcumin was encapsulated in triglyceride oil droplets of nanoemulsion prepared by ultra-sonication using whey protein concentrate-70 and Tween-80 as emulsifiers with an encapsulation efficiency of 91% with average PS ~142 nm (~6.9 mV ZP). In-vitro release kinetics showed that the curcumin nanoemulsion was relatively resistant to pepsin digestion but pancreatin caused release of curcumin from nanoemulsion. The antioxidant activity of curcumin nanoemulsion was reduced from 3.53 to 3.33 μM/mg of curcumin after encapsulation. The prepared nanoemulsion was stable to pasteurization, different ionic strengths (0.1-1 M) and pH ranging from 3.0 to 7.0 (Sari, et al, 2015).

A curcumin nanoemulsion formulation appropriate for transdermal delivery was reported by incorporating curcumin inside nanoglobules prepared from an oil phase of glyceryl monooleate, Cremophor RH40 and PEG 400 showing loading capacity of 350 mg curcumin/10g of oil phase with mean droplet diameter, polydispersity index and zeta potential of optimized nanoemulsion of 85 nm, 0.18 and -5.9 mV, respectively that resulted in improved permeation flux from the matrix gel (Rachmawati, et al, 2015).

A novel technique of preparing melt sonocrystallized curcumin (MSC CMN) was described with its in-vitro cytotoxicity profile determined against human oral cancer cell-line KB before going for tablet formulation that exhibited 2.36-fold and 2.40-fold solubility enhancement in distilled water and phosphate buffer (pH=4.5), respectively, in addition to better flow properties and intrinsic dissolution rates (0.242 and 0.195 mg/cm²/min) in comparison to its original form. The GI50 value of MSC CMN was found to be less than 10, specifying inhibition of growth more effectively at its least concentration by half. A gastroretentive-floating tablet prepared from Formulation-F4 displayed controlled drug release (96.22%) for over 12 hour and thus confirmed the utility of melt sonocrystallized curcumin to produce particles with superior biopharmaceutical properties without the use of organic solvents or other excipients (Khan, et al, 2015).

Curcumin nanoemulsions (Cur-NEs) were developed using high-pressure homogenization and finally applied to the commercial milk system and its antioxidant and in-vitro digestion activities were tested using 2,2-diphenyl-1-picrylhydrazyl, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt, pH-stat method, and thiobarbituric acid reactive substances assays. These Cur-NE formulations were physically stable for 1 month @ room temperature. The mean droplet size decreased from 122 to 90 nm when surfactant concentration was increased by 3-times while showing effective oxygen scavenging activity. Cur-NEs-fortified milk showed significantly lower lipid oxidation than control (Joung, et al, 2016).

Lactoferrin stabilized curcumin nanoemulsions were prepared by homogenizing 5 wt.% corn oil containing curcumin (0.1 wt.%) and 95 wt.% aqueous emulsifier solution (2% lactoferrin) for 2 minutes before going to high-pressure homogenizer (20,000 Psi; 20 cycles).
Curcumin multilayer nanoemulsions (lactoferrin/alginate stabilized) were prepared by adding the curcumin nanoemulsion (@ pH=4) drop-by-drop to an alginate solution (pH=7) under stirring conditions for 30 minutes followed by sonication to disrupt flocculated droplets formed during its preparation. A digestion model (simulating digestion in stomach, duodenum, jejunum and ileum) was employed in evaluating the impact of alginate coating on the digestion of curcumin nanoemulsions stabilized by lactoferrin. The interfacial characteristics of curcumin nanoemulsions had a significant impact on their physicochemical stability within the simulated GI tract: lactoferrin-stabilized nanoemulsions were stable in the stomach but formed aggregates in the small intestine, while lactoferrin/alginate-stabilized nanoemulsions were unstable under both gastric and small intestinal conditions. These results suggested that alginate coating may be instrumental in the control of the rate of lipid digestion and FFA adsorption within the GI tract, but the encapsulated lipid was digested at the same extent, releasing the lipophilic bioactive compound, which was then taken up by mixed micelles (Pinheiro, et al, 2016).

Physicochemical profiles of homogenized nanoemulsions of (cottonseed oil + surfactants (sodium dodecyl sulphate-anionic, dodecyltrimethylammonium bromide-cationic, poloxamer-407 and tween-20–nonionic) + hydrophilic additives (ethanol, glycerol)) were determined by using sound velocity, isentropic compressibility, acoustic impedance, electrical conductivity, particle size and zeta potential. Zeta potentials observed in the range of – 144.21 to 149.60 mV and FTIR stretching frequencies (1300-1600, 2800-3100 and 3513 cm⁻¹) confirmed the stability, intramolecular hydrogen bonding, a presence of carbon-carbon bonding and the phenyl ring on stabilized micelle formation with curcumin (Malik, et al, 2016).

**Pharmaceutical and Other Applications of Nanocurcuminis**

While exploring pharmaceutical applications of curcumin in various forms discussed earlier, it is most critical to know about the exhaustively studied toxicity profile assessed by standard procedures and assays conducted on cell lines as well as human and animal models. This exercise is considered most stringent in the whole process of drug discovery according to the norms laid down by the regulatory authorities involved. Before proceeding towards the developments taking place in the application areas of curcumin it will be better to know the current status of its toxicity profiles as evaluated by several groups in past and discussed in the followings.

**Toxicity Profile**

The toxicity profile of curcumin loaded polymeric NPs was assessed in human pancreatic cancer cell lines without any evidence of toxicity in cell viability (assays spanning over a 20-fold dose range). Similarly, despite using relatively large dosages, the mice being administered with curcumin loaded polymer NPs demonstrated no evidence of weight loss, gross organ changes, or behavioral changes in the mice during investigation. Before examining the toxicity profile of nano encapsulated curcumin in polymeric NPs, blank experiments were conducted simultaneously to check the toxicity profile of polymer based NPs alone using human pancreatic cancer cells as a model, and comparing its efficacy to that of free curcumin. It was noted that curcumin loaded polymeric NPs were taken up well by pancreatic cancer cells, and in cell viability assays while interacting with a series of pancreatic cancer lines, these curcumin loaded polymeric NPs demonstrated comparable efficacy against the free curcumin, although some cell lines were resistant. Curcumin loaded polymeric NPs were found to block clonogenicity of the MiaPaca pancreatic cancer cell line in soft agar assays. In comparison to untreated cells, or cells exposed to void polymeric NPs, both free curcumin and nanocurcumin caused inhibition of clonogenicity where the effect with NPs was more pronounced at the lower doses. Activated nuclear factor kappa B (NFκB) is the cellular target of curcumin in cancer cells where many of the pleiotropic effects of curcumin are ascribed to inhibiting this transcription factor. Curcumin NPs are found to robustly inhibit NFκB in pancreatic cancer cell lines BxPC3 and MiaPaca as observed in gel shift assays for assessing the DNA binding ability of NFκB in BxPC3 cell lines, inhibition of NFκB was seen as early as 1-2hours post exposure to both free and nanocurcumin. In MiaPaca cell lines, a persistent activation of NFκB in cells exposed to free curcumin was seen after overnight incubation, while a perceptible gel-shift was noted in curcumin loaded polymeric NPs treated cells. Incubation of stimulated PBMCs with both free and curcumin NPs decreased steady-state mRNA levels of IL-6, IL-8 and TNFa, compared to DMSO and void nanoparticle-treated cells with the evidence of dose dependent reduction of IL-6 by both agents (Bisht, et al, 2007).

The MTT toxicity assays on Caco-2 cells showed higher toxicity of the NP-system containing both PVA as well as poloxamer as compared to the simple solution. The increased toxicity of curcumin NPs is possibly due to higher accumulation of curcumin within the Caco-2 cells as compared to that in case of the simple solution. At higher concentrations, the blank NPs also cause cell death that was approximately constant in 24, 48 and 72 hours, and this suggested that the chitosan based NP-formulation by itself possibly caused necrotic cell deaths. Also, the survival of Caco-2 cells was found to decrease with time in curcumin formulations. For instance, the percent cell-survival was lesser after 72 hours as compared to that after 24 hours incubation suggesting the apoptosis caused by curcumin in Caco-2 cells. The percentage cell survival of the intestinal Caco-2 cells after treatment with curcumin solution and NP-formulations demonstrated that at higher concentrations of chitosan based NPs containing PVA and poloxamer are more cytotoxic as compared to simple curcumin solution along with higher cell death rates seen with blank NPs.
indicating the toxicity to the Caco-2 cells even from the blank formulations (Wahlang, et al, 2011).

Improved efficacy of nanocurcumin (encapsulated in polylactic-co-glycolic acid PLGA) in comparison to free curcumin was confirmed in case of arsenic-induced immune dysfunction in rats. Curcumin NPs (130 nm size) were found to significantly attenuate the arsenic-mediated effects with clear evidence of better ameliorative potential than free curcumin at the equivalent dose levels (Sankar, et al, 2013).

Magnetic field aided nanocurcumin was shown to be effective in enhancing cellular uptake, improving bioavailability, and resulting in ultimate efficiency of curcumin against prostate cancer cell line (PC3), and this scheme was even tested in four bacteria strains (two Gram positive: Micrococcus luteus ATCC 9341, Staphylococcus aureus ATCC 29213 and two Gram negative: Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853) mammalian cell line (HEK) and human erythrocytes (RBC) showing three times higher efficiency compared to the curcumin combined with magnetic field. Presence of magnetic field was shown to reduce MBCs for all tested bacteria compared with control confirming the advantages of using magnetic field in enhancing cellular uptake for PC3 and enhancing the penetration of drug into the cells with fixing mild cytotoxic profile for HEK and RBC (Aldahoun, et al, 2016).

The immune-suppressive effects of nanoemulsion curcumin (NEC) were assessed in animal models harboring an NFxB-luciferase reporter gene, with the suppression of LPS-induced NFxB activity with NEC compared to an equivalent amount of curcumin in aqueous solution. Oral NEC caused reduction of blood monocytes decreasing the levels of both TLR4 and RAGE expressions, and inhibiting secretion of MCP-1 while blocking LPS-induced phosphorylation of the p65 subunit of NFxB and IxBB in murine macrophages. In a mouse model of peritonitis, NEC significantly reduced macrophage recruitment, but not T or B-cell levels, and these results indicated that curcumin is able to suppress inflammation by inhibiting macrophage migration via NFxB and MCP-1 inhibition and established NEC as an effective therapeutic formulation with improved bioavailability (Young, et al, 2014).

Chronic lead exposures are known to give rise to many health problems in mammals as its exposure forms reactive oxygen species leading to oxidative stress that reduces body anti-oxidant enzymes inflicting injury to numerous macromolecules or cell necrosis in several organs like hematopoietic, skeletal, renal, cardiac, hepatic, and reproductive, and central nervous system. Higher bioavailability combined with chelating property, and enhanced retention time of nanocurcumin over bulk is found to remove lead toxicity from various organ systems (Pal, et al, 2015).

In one of the recent studies of assessing the toxic effects of dendronosomal nanocurcumin on 4T1 cells derived from BALB/c mice breast tumors, MTT assay was conducted by exposing the mouse skin normal fibroblasts (MEF) cells to nanocurcumin, and the results indicated that nanocurcumin treatment led to significant reductions in the viability of cancer cells in a dose and time-dependent manner with no significant effect on normal cells. The data further clarified that nanocurcumin exerted toxic effects similar to that of doxorubicin on cancer cells. Given less toxicity of nanocurcumin on normal cells in comparison with doxorubicin, this finding is of significant importance (Farhangi, et al, 2014).

The study of pharmacokinetics and metabolism of curcumin encapsulated in polymeric NPs, and solvent solubilized curcumin formulations revealed enhanced plasma concentration of curcumin (1749-fold) relative to the solvent solubilized curcumin besides observing increased relative abundance of curcumin and glucuronides in bile, but not altering urine and tissue metabolite profiles. The noted increase in biliary and urinary excretion of curcumin and metabolites in case of nanocurcumin formulation suggested a rapid “burst” type release. Although the burst release is not desirable for targeted tumor delivery, nanocurcumin still exhibits major advantages over solvent solubilized curcumin, as the nano formulation does not result in the lung accumulation observed for the solvent solubilized curcumin and increasing overall systemic curcumin exposure (Zou, et al, 2013).

It is a known practice in drug discovery that cardiac toxicity consideration is the foremost reason for discontinuing a drug development program leading to clinical evaluation and post market surveillance culminating into final FDA rejection of many potential pharmaceutical compounds due to QT prolongation effects. In case safe and potent drugs are formulated in a way that mitigates the associated QT prolongation, it may get FDA approval for clinical trials. In one of the studies in this context, dealing with QT prolongation characteristics of curcumin formulations, efforts were made to examine them in terms of their QT prolonging effect by inhibiting the hERG channel and its low bioavailability. It was found that lipids offer protection against hERG channel inhibition and therefore QT prolongation. The manual patch clamp assay of HEK 293 cells illustrated that the hybrid nanocurcumin formulation, developed in a particular study, was able to prevent the curcumin induced inhibition of hERG channel at concentrations higher than the therapeutic concentrations of the curcumin. Thus, it was demonstrated that a curcumin encapsulated lipopolymeric hybrid NP formulation was able to protect against QT prolongation and also render increased bioavailability and stability thereby overcoming the limitations associated with curcumin (Ranjan, et al, 2013).

The toxicity of liposomal curcumin formulations explored for taking care of the poor absorption of curcumin was in-vitro examined in human red blood cells (RBCs) and while examining the morphology and mean cellular volume (MCV), dose-dependent echinocyte formation was observed after incubation with free, and
liposomal curcumin, with a threshold concentration of 10 μg/mL and peak effect after 30 minutes and concomitant increase in mean cellular volume (Storka, et al, 2013).

In one of the recent studies of curcumin toxicity, evidence was found in proliferating bovine aortic endothelial cells at dietary concentrations and below those reported in earlier cancer models. After confirming the capability of curcumin to up-regulate hemeoxygenase-1 in a dose-dependent manner, curcumin concentration that was sufficient to inhibit endothelial cell DNA synthesis was determined to be 0.1μM causing disproportionate DNA segregation, karyorrhexis, and micronucleation in proliferating endothelial cells (Jackson, et al, 2013).

A particular type of curcumin loaded polymeric NPs (Eudragit S100) were subjected to various toxicological evaluations including acute-toxicity study, sub-acute-toxicity study 28 days and various genotoxicity studies like in-vivo Micronucleus, Chromosomal Aberration, and Comet assays and reported that the formulation used was non-toxic at the dose equivalent to 2000 mg/kg BW of curcumin in the acute-toxicity study. Sub-acute-toxicity study confirmed the safety of the formulation for prolonged administration at the commonly used therapeutic dose of 100 mg/kg BW of curcumin and at twice the therapeutic dose. Genotoxicity studies confirmed the cellular safety of the developed formulation at the therapeutic dose, as well as at equivalent to thrice the therapeutic dose. Thus, the curcumin loaded polymeric NPs of Eudragit S100 were found to be safe for oral administration for a short as well as a prolonged duration (Dandekar, et al, 2010).

**Curcumin Compounds - Large-Scale Production and Market Forecast**

Knowing well about the pharmaceutical activities of curcumin in terms of its physiological interactions, food values, coloring capability and cosmetic properties, it is expected that the market for curcumin based products will certainly grow fast in time. Another basic reason for higher demand of curcumin is expected from its poor bioavailability and solubility in water. In order the meet fast growth of its production, purification, and translation into useable products in near future. Because of these reasons it will be useful to have quick look into the attempts made in past to develop relevant technologies although a commercially viable process of producing nanoparticulate compounds of curcumin and its conjugates is still awaited.

Before proceeding for the direct synthesis of curcumins in nanoparticulate form it would be perhaps better to go first for the supercritical fluid (SCF) based extraction of curcumins. In one of the recent studies conducted in this context, a net improvement in extraction yield of 25% was reported in case of SCF CO₂ modified by ethanol based extraction of curcumins from turmeric powder as briefly mentioned here (Pyo, and Kim, 2014). Knowing the fact that the pressure and temperature of the supercritical fluid are the critically important parameters to be optimized, for efficient SFE experiment. Extraction efficiency of curcumins was, therefore, investigated as a function of extraction pressure, temperature, modifier, and extraction time as briefly mentioned here. Extending the extraction pressure over a range of 200 to 275 atm, the optimum pressure was experimentally determined to be 250 atm as an increase in extraction pressure is supposed to increase the fluid density, which causes increase in the solvating power of the supercritical fluid. However, at pressures above 250 atm the extraction efficiency was found to decrease because of the onset of other compound extractions along with those of curcumins. Following a similar method for determining the optimum extraction temperature, the process was conducted over a range of 40 to 70°C @ 250 atm pressure according to the previous observation. From the experimental observation an extraction at 60°C gave the highest yield, as the extraction at still higher temperature was found less efficient due to decrease in the density of SCF. Relatively poor extraction efficiency (i.e. 4.53 mg/g) was noted with SCF CO₂ alone as the curcumins have phenolic and ketonic polar functional groups, which can be profoundly influenced by adding a modifier compound like ethanol in increasing the solubility levels of polar solutes in supercritical fluids. When the flow rate of modifier was increased, for instance, from 0.1 to 0.3 mL/min, the extraction yield increased from 14.29 to 31.07 mg/g. But at still higher flow rate of modifier (i.e. 0.4 mL/min), the extraction yield of curcumins was found decreasing, which could be due to some solute-modifier interactions weakening the solute-supercritical CO₂ interactions. For determining the optimal extraction time, the process was carried from 90 minutes to 150 minutes with an interval of 30 minutes. When the extraction time was increased from 90 minutes to 120 minutes, the extraction yield increased from 26.45 to 31.07 mg/g but started showing saturating behavior from 120 minutes onward to 150 minutes. From these observations, the optimum extraction time was found to be 120 minutes. Finally, for comparison of SCF extraction yield with that of conventional solvent extraction, using methanol as the solvent gave the cumulative yield of 24.73 mg/g (e.g. three components namely - curcumin, DMC, and BDMC yields were 17.82, 4.09, and 2.82 mg/g, respectively), which is ~25% lower than the SCF extraction yield.
The general process of upscaling is not only critical but also difficult as it involves various formulation parameters to be optimized for the desired outcome. In one of the studies devoted to this aspect of considerations, curcumin loaded poly (lactic acid-co-glycolic acid) nanoparticles (PLGA-CURC) were reported with improved bioavailability, and thus making it suitable for cancer therapy. Process optimization was carried out using Central Composite Design (CCD) Methodology for up-scaling the process in four stages with final outcome of producing 5 g of curcumin loaded NPs with mean-size of 158.5 ± 9.8 nm exhibiting drug loading efficiency of 10.32 ± 1.4% and exhibiting a slow but sustained release corresponding to 75% drug over a period of 10 days where Gamma sterilization showed no significant change in the particle size or drug loading of the NPs. Stability analysis revealed long-term stability of the PLGA-CURC formulation (Ranjan, et al, 2012).

Besides converting curcumin and its analogues into nanoparticulate forms as discussed already, the other alternative worth exploring approach would be to go for curcumin encapsulation in some suitable nanocarrier as attempted in case of chitosan-NPs. Some of these experimental results are given here to highlight the importance of this route of development. In order to avoid repetitions of the protocols already mentioned earlier, some specific methods that are especially amenable to producing commercially viable quantities are included in the following discussions.

A simple process known as spinning disc processing (SDP) was reported, sometime back in the context of process up-scaling, for the production of chitosan NP-based drug delivery platform by mixing chitosan solution (0.25%, w/v, in dilute acid, 27.5 mL, 1.5 mL/s) with sodium tri-poly-phosphate solution (0.10%, w/v, in water, 20 mL, 1.1mL/s) on a 1000-rpm spinning disc that produced 20 ± 3 nm size NPs. Even larger size NPs (131 ± 5 nm) were produced by increasing the chitosan and TPP feed concentrations to 0.5% and 0.125%, respectively besides noting similar increase in particle size with different drug loadings. For example, loading of N-acetyl cysteine (NAC) and paracetamol produced 403 ± 4 nm and 165 ± 4 nm diameter NPs, respectively whereas co-loading of both the drugs increased the size to micron range. SDP was found to be a viable technology of producing blank and drug-loaded chitosan NPs for the biomedical and pharmaceutical industries (Loh, et al, 2010).

Having noted the inherent potentials of above-mentioned SDP technique, it was further modified into another form called multiple stepwise SDP technique to develop aggregates of uniformly sized poly (methyl acrylates) - coated chitosan-diclofenac sodium nanocores (CS-PMA NPs) for colonic drug deliveries using Box–Behnken design technique of process optimization, and producing 10 μm agglomerates of CS-PMA NPs comprising of 10 nm NPs with 88% drug loading efficiency exhibiting >90% of the drug load released into simulated colonic fluid within Shour. Drug uptake from CS-PMA NPs into Caco-2 cells was found three-fold higher than that from a control drug solution, with no apparent cytotoxicity observed at the doses administered. The collective data suggested that this modified SDP was yet another robust manufacturing method that could potentially be used for up-scaling the production of composite nanoparticulate colon-targeted drug delivery systems besides many similar other routes as well (Huanbutta, et al, 2013).

In the process of screening a large variety of phytochemicals possessing inherent potential pharmaceutical activities involving in terms of their activities like lowering blood pressure, reducing cancer risk-factors, regulating digestive-tract activity, strengthening immune systems, regulating cellular growth, controlling blood sugar, lowering cholesterol and serving as antioxidants; systematic studies are currently going on to synthesize chitosan-tri-polyphosphate (TPP) based NPs for encapsulating the active ingredients from one or more of these phytochemicals. In a typical example of preparing nanocarriers from tea catechins, the influence of the modulating conditions like-chitosan concentration, chitosan-TPP mass ratios, pH of the chitosan solution, and concentration of tea catechins, was examined in detail for improving not only the encapsulation efficiency but also modulating the release profile from these nanoparticulates. From this study, the encapsulation efficiency of tea catechins was found in the range of 24 to 53% in the presence of covalent and hydrogen bonds between tea catechins and chitosan-NPs possessing reasonably controlled release profile. By optimizing various parameters mentioned above, the minimum particle size of chitosan-NPs was found ~ 42 ± 5 nm that was further put to use in rapidly increasing number of applications in the food and biochemical industries as practically any active ingredient can be encapsulated therein (whether hydrophobic, hydrophilic, or even bacterial) (Zhao, et al, 2011). From these useful results, it is concluded that micro/nanostructured chitosan can be used as carrier for bioactive ingredients as they can be put to use in developing novel encapsulation or immobilization carriers. Due to their favorable biological properties such as non-toxicity, biocompatibility, biodegradability and anti-bacterial abilities, they are also promising candidates as drug delivery carriers and cell proliferation enhancers.

In another example of preparing curcumin nanoparticulates bovine β-casein, a milk protein that is highly amphiphilic leading to self-assembly into stable micellar NPs in aqueous solution and can solubilize curcumin molecules, was reported to produce a drug-delivery system comprising of curcumin anticancer drug encapsulated in β-casein-NPs. The experimental assessments showed that at pH = 7, curcumin molecules while binding to β-casein molecules form micelles leading to complex formation through hydrophobic interactions. The cytotoxicity study of this nano carrier revealed that the curcumin-β-casein complex exhibited better effects on MCF7 cells compared to equivalent dose of free curcumin (Mehranfar, et al, 2015).
In an attempt to reduce the cost of manufacture in case of curcuminoids, pressurized liquid extraction (PLE) from the de-flavored (i.e. after a supercritical CO₂ extraction) turmeric rhizomes was reported using ethanol solvent in a static extraction time of 20 minute by varying the independent variables of temperature and pressure over the range of 333-353 K, and 10-35 MPa, respectively. It was found feasible to arrive at the optimum extraction temperature and pressure of 333 K and 10 MPa, respectively, which took six times less extraction-time than that of low-pressure solvent and Soxhlet extractions for the similar extraction yield. The cost of curcumin manufacturing was noted to decrease by 7% for increasing the capacity of extraction by 10 times and 82% with reduction in the cost of raw materials used (Osorio-Tobón, and Meireles, 2013; Osorio-Tobón, et al, 2014). These observations show enough promises for future development of commercially viable processing technology.

Microwave-assisted extraction (MAE) of curcumin from turmeric was investigated to compare with the conventional heat-assisted extraction (CHAE) by varying the experimental conditions like solvent concentration (0-100%, v/v), temperature (30-130°C) and time (0-20 minutes) for optimizing the extractions. Antioxidant potential and radical scavenging abilities of MAET and CHAET were evaluated in the cost of raw materials used (Osorio-Tobón, and Meireles, 2013; Osorio-Tobón, et al, 2014). Microwave-assisted extraction (MAE) of curcumin showed high antioxidant activity, but the antioxidant properties of MAET were stronger than those of CHAET (Bener, et al, 2016).

Attempts were also made to prepare curcuminoids by following a biosynthetic pathway, including reactions of phenylalanine ammonia-lyase (PAL) from the yeast Rhodotorula rubra, 4-coumarate: CoA ligase (4CL) from Lithospermum erythrorhizon and curcuminoid synthase (CUS) from rice (Oryza sativa), a type-III polyketide synthase, was constructed in Escherichia coli for the production of curcuminoids and thus cultivation of the recombinant E. coli cells in the presence of tyrosine or phenylalanine, or both, led to production of bis-demethoxy-curcumin, dicinnamoylmethane and cinnamoyl-p-coumaroylmethane. In another E. coli system, carrying 4CL and CUS genes, a high-yield production of curcuminoids was realized from exogenously supplemented phenylpropanoid acids: p-coumaric acid, cinnamic acid and ferulic acid. The yields of curcuminoids were up to approximately 100 mg/l giving approximately 60 mg curcumin/l from 10 g rice bran pitch, an industrial waste discharged during edible oil production from rice bran, as a source of ferulic acid (Katsuyama, et al, 2008).

Curcumin Market - Forecast

Curcumin market is expected to reach beyond US$ 94.3 million by 2022 according to a market forecast made last year (Market Forecast, 2015). Curcumin demand is expected to grow significantly because of growing consumer awareness regarding therapeutic, food, and cosmetics properties of curcumin that make it extremely useful in food and medical applications. The demand of herbal and ayurvedic skin care products is also expected to go up accordingly. The demand of curcumin in food applications has been growing continuously on account of growing demand for natural coloring and flavoring substances. Growing demand of herbal skin care products is expected to augment the curcumin market growth.

Some Other Applications of Nanocurcumin

Besides pharmaceutical applications of curcumin and its related compounds, there are other applications, which are also being studied and explored for their numerous beneficial applications in many diverse fields. This shows the importance of the chemical structure of curcumin that is responsible in these cases as can be seen from the brief descriptions included here in the followings.

Curcumin-Protein Conjugates

Having noted the enhanced levels of curcumin in serum after the oral intake of a curcumin-peptide complex, systematic attempts were made to explore this route of improving the bioavailability of curcumin. The peptide compounds, in this context, could be chosen from a whole host of amino acids, proteins, or peptides (i. e., synthetic or natural with chemical or enzymatic post translational modifications) by considering either direct binding to an amino acid part of the peptide, or through a linker involving alky/alkenyl/alkynyl moiety that are also being studied and explored for their involving linkages via hydrogen, electrostatic, lipophilic interactions (van der Waal's or pi-stacking), or covalent bonds to the peptides (Payne, et al, 2015).

For instance, a curcumin-whey protein complex was prepared by mixing whey protein powder to form a clear solution (i.e. prepared from mixing 5 g curcumin powder with 1000mL ethanol @ 50 °C) followed by rotary evaporator treatment @ 50 °C until 90% of the ethanol was evaporated before going to final lyophilization. The curcumin/WPI complex so prepared was a fluffy powder that mixed well with water, forming a suspension. Similarly, for amino acid complex, curcumin was dissolved in 95% ethanol, followed by the addition of N-acetyl cysteine (NAC). For appropriate dosing purposes around 100 mg of curcumin was complexed to 1000mg of cysteine in a 0.5% (w/v) solution of curcumin in ethanol. Administering 1.8 g of the cysteine-curcumin complex in healthy human volunteers exhibited 4-fold increase in blood concentration of the cysteine-curcumin complex compared to that observed in case of curcumin-whey protein complex (Payne, et al, 2015).
pharmacokinetics of diclofenac in rats, subcutaneous injection of formalin were given into the dorsal surface of the right hind-paw followed by oral administration of diclofenac (1-31 mg/kg BW), curcumin (3.1–100 mg/kg BW) or the diclofenac + curcumin combination (2.4-38.4 mg/kg BW). It was clearly noted in this exercise that diclofenac, curcumin, or diclofenac + curcumin combination produced an anti-nociceptive effect with the evidence of synergistic interaction between diclofenac and curcumin. The experimental data collected in this study suggested that the diclofenac–curcumin combination exhibited positive interactions at the systemic level that could be used with therapeutic advantages for the clinical treatment of inflammatory pain.

In another case, a combination of SH-Aspirin + curcumin was co-encapsulated into methoxy poly (ethylene glycol)-poly (lactide-coglycolide) (mPEG-PLGA)-NPs through a modified oil-in-water single-emulsion solvent evaporation process in form of a mono-dispersed mean particle size of 122.3 ± 6.8 nm in water with high drug-loading capacity and stability. These NPs showed synergistic anticancer effects on ES-2 and SKOV3 human ovarian cancer cells observed in-vitro, and activation of the mitochondrial apoptosis pathway (Zhou, et al, 2015).

**Curcumins and Fermentation**

In one of the studies of exploring novel applications of curcumin in food sector, milk was fermented using a mixed strain culture (Bifido bacterium bifidus, Streptococcus thermophilus, Lactobacillus acidophilus), and its physicochemical properties were characterized along with determining the inflammatory cytokine-modulating effects of the fermented milk. The growth rate of lactic acid bacteria in fermented milk containing curcumin was found much faster than that in the control sample besides observing hydrolysis of caseins and whey proteins causing the formation of lactic and acetic acids in larger amounts. Although, RAW 264.7 cells treated with curcumin fermented milk supernatant showed no cytotoxicity, inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were significantly produced by fermented milk while the nitric oxide (NO) secretion from RAW 264.7 cells was significantly enhanced relative to the control. These results suggested the possibility of using curcumin as a natural immune-modulating compound for preparing yogurts, beverages, and other food products (Gereltuya, et al, 2015).

**Photodynamic Therapy**

Examining the possibility of using the maximum light absorption properties of curcuminoids near blue, a methodology of employing photodynamic therapy (PDT) was proposed for inducing cell-death via the formation of ROS under illumination and using curcuminoids as potential photosensitizers. This kind of curcuminoids-PDT was found to exhibit significant inhibition of cell viability in breast cancer cell lines; in particular DMC-PDT has the highest anti-proliferative effect where autophagy is an early event followed by apoptosis later and ROS generation by exciting the photosensitizer in PDT activates the MAPK pathway. These results indicate that DMC may be considered as a new photosensitizer in PDT for cancer treatment (Lin, et al, 2015).

For further enhancing the phototoxic effects of curcumin on bacteria under <450 nm irradiation, curcumin-NPs conjugates were synthesized using polyethylene-imine to combine curcumin with a typical composition of lanthanide nitrates [i.e. Re(NO)₃₉Y:Yb:Er = 78:20:2] linked by ethylene-diamine-tetra-acetic acid in sodium fluoride (NaF) matrix, to up-convert NIR to 432 nm light for excitation of the curcumin samples. The results from this experiment revealed that these stable NPs with average size of 179.5 nm (zeta potential was -33.7 mV) in normal saline were able to produce singlet oxygen alleviating MRSA-induced pneumonia and reduced bacterial counts in lungs after 980 nm irradiation in mice (Ye, et al, 2014).

**Curcumin Dyes in Photovoltaic Solar Cells**

Photosensitization capability of curcumin was put to use in developing curcumin-based dyes namely BCMoxo and BCTCM that were used in preparing ZnO-NP-based dye-sensitized solar cells (DSSCs) in which boron-complex dyes modified with di-carboxylic anchor groups (BCTCM) provided surface attachment with a strong UV/Visible-region absorption than the dye molecule without anchor groups (BCMoxo). Photo-anodes with 5-80nm diameter poly-dispersed ZnO-NPs specifically optimized for the critical thickness, sensitization time and concentration using a solvent-free ionic electrolyte produced $J_{sc}$ ~1.66 mA/cm² (@ 80 mW/cm² irradiation). This kind of photo-response observed in case of curcumin-derived dyes as photosensitizer in DSSCs is seen as a future potential for enhancing conversion efficiencies of the PVSCs (Ganesh, et al, 2010).

For studying the influence of curcumin yellow dye as an additive to blend with PMMA polymer dye-system, curcumin yellow extract was mixed with PMMA-acrylic polyl blended dye in different volume ratios and applied on pre-treated cold-roll mild steel panels for characterization (Abidin, et al, 2013). All the coated samples were subjected to potential time measurement (PTM), rapid impact deformation, differential scanning calorimetry (DSC), cross hatch and FTIR tests. The addition of curcumin dye was found to improve upon the adhesion, flexibilities and resistance against electrolytes penetration of the blended PMMA - acrylic polyl paint system. Cross hatch tests showed that high amount of curcumin dye colorant had the lowest peel-off coating area from the substrate. The FTIR tests confirmed high concentration of hydroxyl group in the sample causing hydrogen bonding between coating and substrate interface. The samples exhibited the highest coating flexibilities when tested with rapid impact test as it was due to the lowest glass transition value $T_g$ indicating

**Curcumin Nanodyes for Fibers and Textiles**

In a systematic study of one of the natural dye development programs, curcumin/curcuminoids were extracted from turmeric root and high purity curcumin/curcuminoids were isolated, characterized in detail before preparing stable and reproducible nanocurcumin formulations. In the application part of the program, cotton fabric samples were colored using nanocurcumin so prepared and compared with those using curcumin in water solution.

Dissolving turmeric powder in ethanol by mechanical stirring and drop-by-drop mixing of Poloxamer 188 dissolved in water was developed as a simple method of preparing nanoparticulate curcumin. Stirring was prolonged further to remove ethanol by evaporation before storing in a closed container. For assessing the dyeing capability of above prepared nanocurcumin, cotton cloth samples were dyed using commercially available curcumin for comparison with the samples dyed using nanocurcumin along with chitosan mordanting. On comparison, the dyeing capability of nano curcumin exhibited 10 times better performance when measured using standard procedures. These results confirmed that the curcumin NPs are found to disperse uniformly preventing dye spot formations and have sufficiently smaller size to penetrate deep inside the fabric, which ultimately gives stronger fixing (Rawal, and Ahmad, 2014).

**Other Applications**

In one of the experiments of exploring the interaction of curcumin nanoparticulates with other inorganic type of NPs, highly stabilized Au-NPs were prepared using turmeric/curcumin/curcumin glycoside based reduction/stabilization. In all the three sets of experiments, the size of the Au-NPs was, in general, found decreasing with increasing concentration of reducing/stabilizing agent providing better density of the functional groups required for more stability to smaller NPs. For example, the average particle size of 20-50 nm was reduced to 15-30 nm by doubling the concentration of turmeric solution in the synthesis of Au-NPs. The average particle size of curcumin stabilized Au-NPs was further reduced to 5-20 nm with similar trend as in case of turmeric reduction and stabilization and further decreasing to <10 nm with 1mL of curcumin solution. Finally, curcumin glycoside stabilized Au-NPs turned out to be still smaller in size with fine dispersion but exhibiting significant cytotoxicity against cancer cells. From SAR considerations, perhaps carbonyl group of enolic curcumin helped in reducing as well as capping to the Au-NP surface, and increasing the curcumin content for stabilizing regular and smaller sized Au-NPs as compared to that of lower loadings. Detailed stability studies confirmed **in-vivo** stability of the Au-NPs in aqueous solution besides showing no influence of dilution on the characteristics of Au-NPs on dilution in the range of 0.1 to 0.01 M. These results show that the stability of the Au-NPs is certainly possible by encapsulating them with glycosylated curcumin. Further evaluation of their cytotoxic effects on three different cancer cell lines (DU145, A549 and MDA-MB-231) the observed results revealed that the cytotoxicity of curcumin encapsulated NPs decreased in DU145 and A549 cells as compared to MDA-MB-231 cells. This kind of observed curcumin concentration dependent toxicity could possibly be due to the increased concentration of curcumin that form fully encapsulated Au-NPs, which are not accessible to growing cells in culture (Sreelakshmi, et al, 2013). Glycyrrhetin acid-modified curcumin-loaded nanostructured lipid carriers (Cur-GA-PEG-NLC) were prepared by the film ultrasound method with encapsulation efficiency in the range of 90-95% and particle size in the range of 123-133nm. An **in-vitro** MTT assay showed that Cur-GA10%-PEG-NLC had significantly higher cellular uptake and cytotoxicity against HepG2 cells compared with other groups (Chu, et al, 2014).

Attempts were made recently to enhance the solubility of curcumin by loading onto starch maleate (SM)-NPs under mild conditions by simply mixing the dissolved curcumin and SM NPs separately in absolute ethanol and ethanol/aqueous media, respectively. Curcumin-loaded starch-maleate-NPs were subsequently precipitated from a homogeneous mixture of these solutions in absolute ethanol based on the solvent exchange method with average PS of 50 nm spread over 30-110 nm exhibiting loading capacity of 15 mg/g that reached within 12 hours. These NPs exhibited 300-times higher solubility than that of pure curcumin (Pang, et al, 2014).

Another novel route of curcumin conjugation with sugar was evolved by attaching a sugar moiety to curcumin to retain all the characteristics of curcumin pharmacophore where sugar would help in increasing its water/plasma solubility and the hydroxyl groups of sugar would participate in competitive hydrogen bondings. Though loss in antioxidant potential caused by blocking one of the phenolic groups during this conjugation would be compensated by the vastly increased water/plasma solubility but the sugar–curcumin conjugate would retain other structural characteristics of the curcumin pharmacophore such as its metal chelating ability. Synthesis of water/plasma soluble sugar-curcumin conjugate with amplified bioavailability was thus reported inhibiting amyloid-β and tau peptide aggregations at very low concentrations (i.e. 8 and 0.1 nM, respectively). This observation has put this conjugate in the list of promising candidate for the treatment of Alzheimer’s disease (Dolai, et al, 2011).

Assessing the influence of water-soluble curcumin in erectile dysfunction was studied by determining the elevation of cyclic guanosine monophosphate (cGMP) by employing estimations of gene expression of endothelial nitric oxide synthase (eNOS), neuronal NOS (nNOS), inducible NOS (iNOS), heme oxygenase-1 (HO-1), nuclear transcription factor-erythroid2 (Nrf2), NF-Kβ,
and p38 besides cavernous tissue levels of HO and NOS enzyme activities, cGMP and intra-cavernosal pressure (ICP) in animals under trial. Twelve weeks after induction of diabetes, erectile dysfunction was confirmed by the significant decrease in ICP followed by a significant decrease in cGMP, NOS, HO enzymes; eNOS, nNOS, HO-1, NF-κB, p38, and iNOS genes. Administration of pure curcumin or its water-soluble conjugate led to a significant elevation in ICP, cGMP and intra-cavernosal pressure (MAP), and HO-1 genes in the diabetic groups. Water-soluble curcumin showed significant superiority and more prolonged duration of action while administration of ZnPP significantly reducing HO enzyme, cGMP, ICP/ mean arterial pressure (MAP), and HO-1 genes in the diabetic groups. Water-soluble curcumin was shown to enhance erectile function with more effectiveness and with more prolonged duration of action (Abdel Aziz, et al, 2012).

In a recent study, glutaraldehyde-chitosan-pectin-NPs were examined for curcumin encapsulation in enhancing its bioavailability besides the already explored routes of using a variety of nanocarriers including palmitat, formaldehyde, sodium tripolyphosphate, and genipin based cross-linking reported earlier. After confirming the interaction between the carboxyl group of pectin and the prototonated amino groups by FTIR, 40nm diameter spherical chitosan-NPs were characterized without observing any change between NPs before and after the release where the involved amorphous state of the encapsulated curcumin was found influenced by the concentration of polymer matrix and glutaraldehyde. The in-vitro release kinetics studies confirmed an initial burst followed by a steady state release and concluding that glutaraldehyde-crosslinked chitosan-pectin-NPs are promising carriers for effective delivery of curcumin (Abdel Aziz, et al, 2012).

In an attempt to improve drug release characteristics, and approach based on preparing a nano composite by blending of guar gum, sodium alginate with cloisite 30B was reported where it confirmed the presence of an intercalated or partially exfoliated nanocomposite with significant improvement in its properties. The drug release kinetics was investigated in case of curcumin as the drug by plotting the cumulative release data vs. time by fitting to an exponential equation, which indicated the non-Fickian type of kinetics (Chahatray, et al, 2013).

A simplified and cost effective approach of producing quantity of nanocurcumin formulations is necessary for its widespread applications in human healthcare. In order to explore about the possible future developments of curcumin nanoformulations it would be useful to keep in view the positive aspects of using approaches based on lipids, cyclodextrins, PLGA, chitosan and magnetic NP-formulations. Oral and intra-peritoneal administrations of these nano-formulations appear to be a preferred approach to reduce the number of patient visits and also the cost.

In another case, a treatment of disease called *Trichomonas vaginalis* was found very effective in a study conducted for assessing the efficacy of curcumin against *Trichomonas* (i.e. a diseases caused by protozoan parasite - *Trichomonas vaginalis*), which is the most common sexually transmitted disease (e.g. 174 million cases reported annually worldwide). A complete eradication of all the trichomonal cells within 24 hour could be realized after administering a dose of 400 μg/ml curcumin (Wachtler, et al, 2014).

The curcumin loaded chitosan/poly(lactic acid) (PLA) nanofibers were produced using electro spinning by exploring the optimization of variables like chitosan/PLA strength (% w/v), curcumin strength (% w/v) and applied voltage (kV) to obtain uniform fiber diameter. Molecular interactions and the presence of each chemical compound of curcumin loaded chitosan/PLA fibers were characterized by FTIR and EDX analysis. Antioxidant, drug release and in-vitro cytotoxicity tests were performed to evaluate the suitability of nanofibers that would be used for wound healing. *In vivo* wound healing studies on excision and incision wounds created on rat model showed significant reduction of wound area when compared to the untreated ones in which the better healing efficiency could be attributed to the presence of curcumin and chitosan (Dhurai, et al, 2013).

The effects of curcumin on human bladder cancer cell lines and intra-vesical activity in a rat bladder tumor model were studied and found to induce apoptotic cell death and cause cell arrests in the G2/M phase by down regulating the anti-apoptotic Bcl-2 and Survivin protein together with enhancement of the Bax and p53 expression. Curcumin was found stronger than cisplatin as it could not be prevented by catalase pretreatment in T24 cells. Clonal assay revealed that short-term administration of larger doses of curcumin was lethal to bladder cancer cells in addition to *in-situ* apoptosis leading to slower development of bladder cancer (Tian, et al, 2008).

The anti-atherogenic potentials of extracts of ginger (*Zingiber officinale*) and curcuminoids from turmeric (*Curcuma longa*), were compared in rabbit models of hyper-cholesterolaemia. The anti-atherogenic effects of curcuminoids and ginger were correlated with their ability to lower cholesteryl ester transfer protein activity. Ginger extract exerted preferential effects on plasma lipids, reverse cholesterol transport, cholesterol synthesis and inflammatory status. Curcuminoids, however, showed superior antioxidant activity (Elseweidy, et al, 2015).

The effect of curcumin on Gelatinase B (MMP-9), an ECM remodeling regulatory enzyme, in NE-induced cardiac stress was studied in a recent work where curcumin is found as a cardio-protectant compound. H9c2 cardio-myocytes were subjected to NE and curcumin treatments to study the response in stress conditions by examining its influence on total collagen content using Picosirius red staining, assessing Gelatinase B activity through Gel-Diffusion Assay and Zymographic techniques, and involving RT-PCR, Western Blotting and Immuno-cyto-chemistry for assessing the effect on expression of gelatinase B besides
examining the effect of curcumin on the localization of gelatinase B regulating NF-κB. Curcumin was found to suppress the increase in the total collagen content under hypertrophic stress and inhibit the in-gel and in-situ gelatinolytic activity of gelatinase B besides suppressing the mRNA and protein expression of gelatinase B. This study provided evidence for an overall inhibitory effect of curcumin on Gelatinase B in NE-induced hypertrophic stress in H9c2 cardio-myocytes, which may contribute in the prevention of ECM remodeling (Kohli, et al, 2013).

For finding out a suitable remedy for recurrent aphthous stomatitis (RAS)—a most common disease of oral cavity, the patients were treated with curcumin with Triamcinolone acetonide in gel form and the findings from this trial showed significant difference in size, pain, number, and duration of ulcers within a period of 7 days showing that curcumin could be used as an effective alternative to steroids in treatment of RAS (Deshmukh, and Bagewadi, 2014).

In lung cancer, TRF1 is a modulator for telomerase activity that is responsible for cancerous cells, and is a suitable target for therapy. Using PLGA-PEG-NPs was found effective on telomerase and TRF1 expressions in lung cancer cell lines while assessing the cytotoxic effects determined by MTT assay, and real-time PCR. The experimental data, so gathered, indicated curcumin cytotoxicity to be dose and time-dependent whereas curcumin loaded NPs showed IC50 values in lower concentration in comparison to free curcumin. Curcumin loaded PLGA-PEG-NPs were found to decrease hTERT expression and increased TRF1 expression more than pure curcumin (Anganeh, et al, 2014).

The effects of dietary curcuminoids were investigated on lipid metabolism in male Sprague-Dawley rats in three diet groups in which control group did not receive any supplements, while the others were administered with 0.2 g (group-1), and 1.0 g (group-2)/ 100 g of diet curcuminoids. Liver triacylglycerol, cholesterol concentrations, plasma triacylglycerols in the VLDL fraction were significantly low in group-1 rats than in control rats. Hepatic acyl-CoA oxidase activity of both the group-1, 2 rats were significantly higher than that of control rats. Furthermore, epididymal adipose tissue weight was significantly reduced with curcuminoid intake in a dose-dependent manner indicating that dietary curcuminoids have lipid-lowering potency in-vivo, probably due to alterations in fatty acid metabolism (Asai, and Miyazawa, 2001).

**Discussions**

Recent investigations carried out in relation to improving the bioavailability of scarcely water-soluble curcumin compounds have clearly shown that nanoparticulate formulations in amorphous phase are certainly better than the crystalline counterpart. Of course, one has to be careful about the problems arising during conversion from one phase to the other besides influencing the overall stability of the formulation due to associated inherent aggregation/coagulation properties in which case the surface modification based stabilization becomes an additional necessity. For implementing appropriate surface passivation of a given nanoparticulate formulation along with the processing for surface activation for anticipated target-specific chemical conjugation, an appropriate form of a combined process of fluidized bed chemical vapor deposition (including atomic/molecular layer epitaxy) along with supercritical fluid conditions may be considered seriously even up to production levels. Precisely controlled depositions of few monolayers of specific surface passivating compounds onto the surface of nanoparticulates are realizable using a combination of atomic/molecular layer epitaxy along with fluidized bed CVD as explored in the domain of semiconductor device structures (Kikic, and Vecchione, 2003; Czok, and Werther, 2005; Spence, et al, 2007; Ahmad, 1998).

In spite of several recent reports about considerable improvements realized in bioavailability of curcumin and their derivatives in nanoparticulate forms, there are still some reservations. It is, therefore, important to examine the reported claims and counter-claims in regard to the bioavailability of curcumin and the metabolites in presence of hydrolysis caused by enzymes acting against the half-life of the curcuminoid molecules. In this context, it is significantly important to take note of the most recent contribution in bioavailability enhancement attempt by using poly electrolytic complexes that raises the hope of overcoming this problem considerably (Fatima, et al, 2016). While examining various problems of limited bioavailability and its possible enhancement by different approaches, as discussed already earlier in the main text, it is still important to recognize the fact as pointed out by M/S Sabinsa that practically every work on curcumin bioavailability enhancement study is found to estimate the ‘metabolites of curcumin’ instead of curcumin. The hydrolysis of metabolites by glucuronidase and sulfatase enzymes takes place well before it is experimentally possible to determine the curcumin concentration. Thus, most of the bioavailability studies reflect the estimates of only curcumin metabolites. It is however, significantly important to note that according to a recent report the curcumin metabolites are not effective against cancer cells and thus cannot be considered as pharmaceutically active anti-inflammatory agent (Pal, et al, 2014). These observations ultimately do clarify that the excipients simply increase the non-bioactive curcumin metabolites in systemic circulations. The author of a paper claiming very high bioavailability reported earlier did concede to the fact that there is a challenge in determining the pharmacokinetics of free curcumin because of their short half-life and limited window of observation in plasma/serum (Jaeger, et al, 2014). However, these conjugates do represent the curcumin bioavailability and their efficacies although indirectly. Yet, in another investigation, it was shown that a dose of 10/12 g curcumin generated mainly curcumin glucuronides and sulfates and the concentrations of...
curcumin conjugates observed in human plasma after consumption of such single doses did result in plasma concentrations sufficient to evidence pharmacologic activity in-vitro once de-conjugated (Vareed, et al, 2008).

The other important point to resolve clearly is the difference in physiological activities of amorphous and crystalline states of curcumin nanoparticulates. A recent study conducted in this context by comparing the crystalline and amorphous forms of curcumin-NPs by preparing gel formulations out of both the types of the curcumin nanoparticulates and characterizing them in terms of their different physical stress tests, followed by in-vitro release study, zeta potential, viscosity, transmittance, globule size distribution and ex-vivo studies. Nanoemulsions (o/w) were prepared using water titration method that produced spherical shaped NanoCur NPs determined by TEM. With optimum composition of Curcumin: Carbopol934: Ethanol: Tween20: Capryol90: PEG2000 = 0.154: 0.702: 0.013: 0.076: 0.015: 0.038 %w/w, and distilled water, and anti-inflammatory effects of both the formulations were assessed in carrageenan-induced paw edema in rats using Diclofenac as a reference. The anti-inflammatory effect of NanoCur was significantly higher compared to Cryscur but comparable with the standard Diclofenac. Short-term storage stability showed insignificant changes in the droplet size and zeta potential, confirming its high shelf life. Finally, it was concluded that NanoCur could be a promising tool in the management of topical inflammation (Al-Rohaimi, 2015).

It appears logical from the observation of the results reported on various aspects of pharmaceutical activity of curcumin to assume that the processes that are available there are clearly capable of offering sufficient pharmaceutical advantages especially in case of nanoparticulate curcumin, curcuminoids embedded in lipids and other polymeric, surface modified nanoparticulates using fluidized bed assisted atomic/molecular layer epitaxy for creating a strong data base for putting the same to use in SAR based designs of molecular complexes with desired features.

Although, nanoparticulate preparations of the phytochemicals in general and curcuminoids in particular involving a physical process like conventional milling is found unsatisfactory in comparison to the conventional method of chemical precipitation of inorganic NPs, but there are several other green methods available like high power homogenization, ultra-sonication, ultrasonic energy supported fluidized bed based chemical reaction, microwave, and laser assisted nanonation that are found helpful in this context. Moreover, most of these green methods are easy to up-scale for commercial production as well.

There is another area that needs further exploration is using supercritical fluid based nanoparticulate formulations involving properly designed copolymers (Kikic, and Vecchione, 2003; Hamidi, et al, 2012), which are rather easy to up-scale for commercial production but have shown inherent limitations by exhibiting poor control of mono-dispersions due to the nature of the supercritical condition involved therein. This is the reason that one would prefer using supercritical extraction of high purity curcuminoids from the turmeric powder with improved efficiency instead of going for nanoparticulate formulations. In this context, the route based on different combinations of nano emulsification combined with selective precipitation/evaporation may be more appropriate to finally use micellar processes based on SLNPs to control their mono-dispersions in a better way. Exploring the phenomenon of self-limiting organizational behavior of nanostructured assemblies should, in principle, be preferred for preparing curcumin nanoparticulates and its conjugates, analogues and complex formations. Even exploring the possibility of combining curcumin NPs with inorganic NPs with specific target of achieving some kind of homing capability to a desired site combined with external excitation from outside along with multimodal imaging explored in nano-theranostics is another area open for much wider applications, in future, in which especially magnetic NPs based conjugates would be more appropriate for making the interaction through external triggering.

In addition, a simple concept of attaching some appropriate hydrophilic radicals to the curcuminoid backbones should in principle be sufficient to take care of their solubility including the numerous possible combinations with block co-polymers (Hamidi, et al, 2012), which has been attempted but with the caution that this process should not interfere with the availability of the moieties that are responsible for providing the inherent beneficial activities to the parent molecule. A good molecular modeling based design is therefore recommended, in this context, to carry out the desired modifications.

Exploring the possibility of using dendrimer molecules for improving the bioavailability of curcuminoids is another attractive area where synthetic organic chemistry as well as supramolecular chemistry has to offer a number of novel solutions to produce better formulations in this context. Using supramolecular complexes with appropriate combinations of low energy hydrogen, van der Waal’s, and other forms of chemical bondings are there to offer intelligent characteristics by involving protein, enzymes and whole host of bioactive molecules already known so far (Gao, et al, 2013; Ahmad, 2015). Having seen the partial success of nanoparticulate formulations of curcuminoids, the next phase of development should be directed towards using the SAR considerations for designing newer and intelligent molecular complexes that would accordingly respond specifically to different diseases under optimal conditions. As far as the pharmacological activities of curcuminoids are concerned, there are ample evidences showing their highly interactive nature in almost all human diseases starting from simple to more complex forms like cancers. Now, the next step should be planned to target for designing supramolecular intelligent structures involving specific proteins/genes as marker/target molecules for extracting the optimum benefit from
these all pharmaceutically powerful curcuminoid molecules.

Extending the argument further, it is equally likely that curcuminoids are chemically conjugated to some other compounds, which have their inherent improved water solubility as in the case of combining sugar molecules with curcuminoids (Dolai, et al, 2011). Here, such a list may include large number of entries from the family of proteins and other bioactive molecules especially derived from phytochemicals for obvious reasons (Teiten, et al, 2010; 2014). Combining the bioavailability enhancing properties of adjuvants is another area worth examining in continuation to have multi-component drugs quite similar to natural medicines practiced in Unani and Ayurvedic formulations of Indian system of medicines. In these cases there is a strong possibility of having synergy, which is missing in most of the single molecule drugs.

Knowing very well about broad-spectrum interactions of curcuminoids in almost all human diseases as highlighted in the main text of this review, all the indications are there for using curcumin as a promising intelligent nanocarrier besides using it in the treatment of various diseases as such. Exploiting synergistic interactions of curcumin in presence of many other useful chemical moieties may be a better approach to adapt than expecting curcumin perhaps to work in each case equally optimally. This kind of synergistic considerations combined with directions derived from the traditional knowledge base of natural and herbal medicines may provide better solutions to human healthcare problems in future.

In order to introduce smart and intelligent features in future drug and gene delivery nanocarriers, several useful schemes of realizing single and multi stimuli triggered release profiles were explored recently and some of their salient features are re-examined here for their possible applications in developing intelligent delivery system involving curcumin family of drugs (Gao, et al, 2013; Ahmad, 2015).

In this context, two significantly prominent schemes of charge reversals in nanogels (inside the tumor), and using acid-labile linkers for conjugating drugs were explored using species like ester, hydrazone, dimethylmaleic anhydride, orthoester, imine, vinyl ether, and phosphoramidate for linker applications (Gao, et al, 2010). Hydrazone linker was, for instance, used to attach cisplatin to polyethylene glycol-b-poly(L-lactide) and DOX to PEG chain before transferring to the surface of gold-NPs that could release the drugs after NPs were endocytosed (Aryal et al., 2010; Wang, et al, 2011).

Alternatively, co-polymer (poly-β-aminoester ketal-2) based NPs were reported to switch from hydrophobic to hydrophilic state at some specific pH causing rapid degradation to end up in increased cyto-plasmically efficient gene delivery. Similarly, employing pH-labile linkers with charge-reversal polymers were used for nuclear-targeted drug deliveries based on modification of positive amines in the cationic polymers (e.g. PLL and PAMAM dendrimers) with negatively charged acid-labile amides that made the polymer negatively charged at physiological condition, but hydrolyzed at lower pH to restore its positive charge leading to efficient nuclear-targeted gene delivery (Shen, et al, 2010; Morachis, et al, 2012). For gene expression, pH-labile linkers were employed in synthesizing smart nanoassemblies capable of self-de-PEGylation in which the lipid envelopes of PEG-vinyl etherDOPE self-removed PEG and recovered the fusogenic ability of DOPE at lower pH, resulting in higher transfection efficiency and much lower cytotoxicity than that of commercial Lipofectamine 2000 in cancer cell lines (Xu, et al, 2008). Over-expressed cancer-associated enzymes were also used in form of liposomes, polymer-based NPs, MSNPs and gold-NPs designed with appropriately higher specificity for enzyme stimulus in which enzyme-responsive NPs with biological motifs were degraded by enzymes to release the encapsulated or conjugated drugs (Andresen et al., 2010; Ghadiali and Stevens, 2008; Ulijn, 2006). Similarly, the NPs were so designed that their physical properties underwent changes by enzymatic stimulation facilitating cellular uptake/intracellular delivery. Metallo-proteinase matrices (MMPs) (MMP-2 and MMP-9) have been up regulated in the tumor microenvironment by integrating MMP-cleavable lipopeptide into the formulation to prepare MMP-sensitive liposomes that rapidly release their cargos by MMP-9 (Roy, et al, 2009). In another approach, PEG-MSNPs responding to MMPs were engineered in which the proteases in the tumors triggered DOX release resulting in significant cellular apoptosis (Singh, et al, 2011). Peptides incorporated into a sequence capable of getting recognized by the cancer-associated proteases have been used for preparing liposome formulations that were more stable and resistant to osmotic swelling, but they released payload triggered by the protease at the tumor site (Basel, et al, 2011). For example, the higher concentration of phospholipase (PLA2) in the tumors was found showing PLA2 triggered degradation of liposomes comprising of prodru antitumor ether lipids (proAELs) resulting in the release of antitumor ether lipids (AELs) that possess the ability to enhance transmembrane drug diffusion, encapsulation of the conventional chemotherapeutic drugs in liposomes containing proAELs to enhance intracellular drug distribution in the cancer cells (Andresen, et al, 2004). Saccharide derivative based nanocarriers were found to be lyzosomal amylase sensitive while modifying the MSNP-pores for enzyme triggered drug release in the tumors due to opening of the molecular gates by lyzosomal amylase, which caused the release of cytotoxic agent reducing the cell-viability. Besides, differences in the reducing potentials of the extra and intracellular environment provided another stimulus for drug delivery. The disulfide linkage was, for example, employed extensively in designing redox sensitive nanocarriers due to their stability in the oxidizing extracellular environment and their elevated lability in the reducing intracellular environment (high glutathione concentration) to efficiently release entrapped cargos (Saito, et al, 2003). A linear cationic...
click polymer was synthesized with disulfide bonds via “click chemistry” for facilitating efficient gene delivery through efficient DNA release by the cleavage of disulfide bonds involved in linking poly(epsilon-caprolactone) and poly(ethylene phosphate) in diblock copolymer under reduction (Gao, et al, 2011). The diblock copolymer based micelles were able to load the drug molecules in the inner core in aqueous solution and could subsequently release the drugs rapidly under glutathione stimulus leading to enhanced toxicity to the tumor cells (Tang, et al, 2009). The disulfide linkage based micellar species were also synthesized with the capability to self-remove the PEG-chains under the intracellular reducing environment where PEG detachment in the endosome was found beneficial for endosomal escape of micelles and improved gene transfection efficiency (Takae, et al, 2008).

Besides the above-mentioned schemes, an extrinsic stimulus could also be used for enhancing the drug distribution in the tumor or improve intracellular drug accumulation by selective release of its payload. Thermosensitive polymers exhibiting temperature specific volume phase transitions were, thus, developed in this temperature and electric field for programmed drug matrix (PLGA-PEG-PLGA) that responded well to the than one stimulus by combining a conducting polymer (Husseini and Pitt, 2008, 2009). The polymeric micelles at definite time and space trigger DOX and other hydrophobic drug releases from 2013). Similarly, ultrasound could also be employed to super-paramagnetic iron oxide NPs (Oliveira, et al, 2011; Hoare, et al, 2011). Inducing a local hyperthermia by magnetic field in the polymersome “click polymer was synthesized with disulfide bonds via “click chemistry” for facilitating efficient gene delivery through efficient DNA release by the cleavage of disulfide bonds involved in linking poly(epsilon-caprolactone) and poly(ethylene phosphate) in diblock copolymer under reduction (Gao, et al, 2011). The diblock copolymer based micelles were able to load the drug molecules in the inner core in aqueous solution and could subsequently release the drugs rapidly under glutathione stimulus leading to enhanced toxicity to the tumor cells (Tang, et al, 2009). The disulfide linkage based micellar species were also synthesized with the capability to self-remove the PEG-chains under the intracellular reducing environment where PEG detachment in the endosome was found beneficial for endosomal escape of micelles and improved gene transfection efficiency (Takae, et al, 2008).

Besides the above-mentioned schemes, an extrinsic stimulus could also be used for enhancing the drug distribution in the tumor or improve intracellular drug accumulation by selective release of its payload. Thermosensitive polymers exhibiting temperature specific volume phase transitions were, thus, developed in this context for triggering drug release and local accumulation by application of heat besides using ultrasound and electromagnetic radiations as external stimuli to produce heat (O’Neill and Rapoport, 2011). Knowing about the lower critical solution temperature (LCST) of polymers involving N-isopropylacrylamide (NIPAm), N, N-diethylacrylamide and N-vinylcaprolactam monomers, and upper critical solution temperature (UCST) of acrylamide and acrylic acid monomers based polymers; curcumin loaded thermo-responsive chitosan-g-poly (N-vinylcaprolactam) polymer based NPs were prepared by wrapping super-paramagnetic iron oxide NPs with a film of PNIPAm-based nanogels and ethyl cellulose in which the entrapped drug molecules were able to transport across the film through heating the super-paramagnetic NPs after the dissolution of the PNIPAm showing to kill cancer cells at above their LCST (Schmaljohann, 2006; Rejinold, et al, 2011; Hoare, et al, 2011). Inducing a local hyperthermia by magnetic field in the polymersome membrane could also be used for triggering drug release from polymericomes encapsulated DOX together with super-paramagnetic iron oxide NPs (Oliveira, et al, 2013). Similarly, ultrasound could also be employed to trigger DOX and other hydrophobic drug releases from the polymeric micelles at definite time and space (Husseini and Pitt, 2008, 2009).

Recently, NPs were synthesized responding to more than one stimulus by combining a conducting polymer (polypyrrole) and a temperature-sensitive hydrogel matrix (PLGA-PEG-PLGA) that responded well to the temperature and electric field for programmed drug delivery (Ge, et al, 2012). Super paramagnetic maghemite (γ-Fe₂O₃) NPs modified with poly (2-(dimethylamino) ethyl methacrylate) (pDMAEMA) were found to exhibit pH and temperature-dependent reversible agglomeration showing efficient gene delivery in CHO-K1 cells (Majewski, et al, 2012). Peptide modified poly(2-(pyridin-2-yldisulfanyl) ethyl acrylate NPs loaded with DOX were shown to possess pH/redox sensitive drug release properties in which the RPDSG/DOX NPs were stable in physiological condition but caused DOX release triggered by acidic pH/redox potential (Bahadur, et al, 2012). The micelles formed by block co-polymer comprising of acid-sensitive tetrahydroxypuran-protected 2-hydroxyethyl methacrylate (HEMA) hydrophobic and temperature-sensitive poly (N-isopropylacrylamide) (PNIPAM) hydrophilic chain were found to respond well to temperature, pH and redox potential (Klaikherd, et al, 2009).

There is yet another alternative route of realizing smart and intelligent deliveries by taking care of various steps like the drug delivery to the blood vessels, transport across the vessel wall into the interstitium, and finally migration through the interstitium to the cancer cells in the tumor that are encountered during the journey of the nanoparticles administered intravenously.

In this context, a properly designed multistage vector could possibly be employed for circumventing the biological barriers (BB) met during the drug transport in which a number of tasks are taken care of by proper design of the nanostructured system called nano cell that are prepared using drug conjugated polymer-NPs having pegylated-lipid envelope to entrap anti-angiogenesis agent. Such multistage release profiles are feasible to realize using mesoporous silicon (first stage) loaded with appropriate nanoparticulates (second stage) along with anticancer therapeutics (third stage) within a tumor producing improved therapeutic effects and reduced toxicity. The mesoporous silicon is found to transport the drugs while protecting them in ferrying the inner nanoparticulates within until it reaches the tumor vasculature before the cargo is released from the MSP due to physiological degradation and the drug nanoparticulates are able to extravasate through fenestrations of vessels and enter the tumor parenchyma, and concentrate the diagnostic and therapeutic loads within the target site. Liposomal dioleoyl phosphatidylcholine with siRNA targeted against EphA2 oncprotein (second-stage carriers) were loaded, in an example, into MSPs for sustained targeted delivery causing sustained EphA2 gene silencing that lasted for 3 weeks after a single intravenous administration compared to one-stage neutral nano liposome delivery that required twice-weekly injections to achieve similar gene silencing. Similarly, super-paramagnetic CaCO₃ mesocrystals encapsulating DOX, Au-DNA, and Fe₃O₄@silica NPs for the co-delivery of drug and gene via a multistage method was realized for treatment of cancer (Tanaka, et al, 2010; Zhao, et al, 2010). In another version of multistage delivery system, size-shrinking property was incorporated using 100 nm shrinking nanoparticulates of gelatin core and surface covered with 10 nm QDs. After exposure to the tumor micro-environment, the 100 nm nanoparticulates were found to shrink to 10 nm NPs due to the hydrolysis of gelatin by
the MMPs that are suitable for the EPR effect, besides being able to diffuse in the collagen matrix of the interstitial space and penetrate into the tumor parenchyma (Wong, et al, 2011; Stylianopoulos, et al, 2012).

Besides targeted delivery, multistage vectors were also developed for their theranostics applications combining therapeutics and diagnostics especially in cancer treatments where it was found feasible to visualize in real time the blood circulation, and biodistribution of drugs with noninvasive assessment of drug accumulation/release besides facilitating triggered release as well as monitoring distribution according to the responses for drug efficacy evaluations (Sumer, and Gao, 2008; Lammers, et al., 2010). The integration of imaging features of MRI, single photon emission computed tomography (SPECT), positron emission tomography (PET), computer tomography (CT), and ultrasonography (US) by either attaching appropriate moieties to the nanocarriers or employing the intrinsic properties of the specific NPs such as SPIOs (MRI) and QDs (fluorescence) into the design made it possible to evaluate the systemic response of NPs that allowed for dosing adjustments of the drugs for more personalized disease managements (Diou, et al, 2012; Mura, and Couvreur, 2012). A number of such systems combining anti-cancer drugs with imaging techniques were thus evolved accordingly employing liposomes, micelles, polymeric, gold, magnetic, carbon, and silica-based nanomaterials in combination with coating of dextran, dendrimer, polyaniline, and polyvinylpyrrolidone on the surface of magnetic NPs. For example, coating of pluronic polymer F127 and β-cyclodextrin (β-CD) onto Fe3O4-NPs for encapsulating anti-cancer drugs produced water-dispersable formulation causing improved MRI and therapeutic effects that were further extended to even triggered release by tuning the polymer coatings. In another case, a poly (β-amino ester) (PBAE) copolymer was co-polymerized to trap SPIO and DOX for cancer detection/ treatment using pH-controlled drug release. The magnetic-NPs loaded with imaging moieties were also used in multimodal imaging such as near-infrared dyes loaded into a magnetic NPs stabilized by amphiphilic block copolymer provided for both tumor MRI and optical imaging (Yallapu, et al, 2011; Fang, et al, 2012; Foy, et al, 2010). For realizing targeted delivery, a variety of ligands were attached to the outside layer of MNPs such as folate, cRGD, and antibody. A number of examples including FR-targeted vesicular NPs with SPIO and DOX, antibody and fluorescence-labeled nano theranostics, cyclo(Arg-Gly-Asp-d-Phe-Cys) (c(RGDfC)) peptides are already there for modifying nanocarriers for targeted delivery as well as dual PET/MRI imaging, MRI visible gene delivery (SPIO NCs + shell of biodegradable stearic acid modified low molecular weight PEI called Stearic-LWPEI) via self-assembly showing synergistic advantages in the effective transfection of mRNA and non-invasive MRI of gene delivery reported recently (Yang, et al, 2010; Zou, et al, 2010; Yang, et al, 2011; Wan, et al, 2013).

In addition, due to size and shape specific optical properties, gold NPs have been explored extensively based on the supported surface plasmon resonance and photo-thermal characteristics, which enable them not only for imaging applications but also to induce photo-thermal effects for therapeutic purposes (Dykman and Khlebtsov, 2012; Saha, et al, 2012). For instance, optical/thermal properties of gold-NPs including spherical, rod-like, shell and cage-type nanostructures were easily tuned by changing their morphology and surface properties. Different kinds of gold-NPs like high-photoluminescence-yield nano cubes, gold nano rod-in-shell nanostructures, silica-modified gold nanorods, and metalloproteinase sensitive gold nano rods have been reported for successful imaging as well as photo-thermal cancer therapy (Hu, et al, 2009; Wu, et al, 2010; Yi, et al, 2010; Huang, et al, 2011). There are few specific examples like – gold-NP + PEG, biotin, PTX and rhodamine B linked β-CD and gold-NP + PSMA RNA aptamers where biotin is noted to interact with cancer cells to release PTX and for CT imaging/prostate cancer therapy with improved CT intensity and DOX efficacy in LNCaP cells than that of non-targeted PC3 cells (Kim, et al, 2010; Heo, et al, 2012). Similarly, MSNs provide an excellent theranostic platform owing to their high surface to pore volume ratios, tunable porosity, and facile functionalization that all suit well for encapsulating both imaging and therapeutic agents (Ambrogio, et al, 2011; Lee, et al, 2011). Nanostructured C and Si species embedded into MSNP matrix, dye-doped silica shell prepared with single Fe3O4-NC-core, and MSNPs loaded with contrast agent/drug and conjugated with cRGDYK peptides were reported to offer higher efficiency loading of insoluble drugs with NIR-to-Vis luminescence imaging features, drug delivery with dual MRI/fluorescence imaging, and for targeted delivery to the over-expressed αvβ3 integrins and tumor imaging, respectively, besides offering multimodal imaging and treatment with gold composites (Cheng, et al, 2010; He, et al, 2012). AuNRs-capped magnetic core and mesoporous silica shell based NPs could achieve synchronous chemotherapy, photothermtherapy, in-vivo MR, infrared thermal and optical imaging into one single system (Ma, et al, 2012). Nanostructured species including AuNPs, SPIO, and MSNs have special features for theranostics applications frequently involving lipid and polymer-based formulations by loading them with a variety of contrast agents like SPIOs and gadolinium compounds for MRI applications where conjugation with radionuclide agents such as 64Cu, 99mTc, and 111In for radionuclide imaging, or loading with fluorescent molecules or QDs for fluorescence imaging (Luk, et al, 2012). Some of the lipid and polymer-based nano theranostics are made environmentally sensitive by incorporating thermo-sensitive polymer chains or pH-sensitive linkages within polymer backbone (Kono, et al, 2011; Liu, et al, 2012). Single walled carbon nanotube (SWCNT) is another potential candidate as it generates significant amount of heat upon excitation with near-infrared light for the photo-thermal destruction of tumors (Moon, et al, 2009).
In addition, due to its strong optical absorbance, it has also been used as contrast agents for photo-acoustic imaging, microwave-induced thermo-acoustic imaging, and hyperthermia treatment (Mashal, et al, 2010). Other nanostructured nanomaterials, such as, upperconversion NPs (UCNPs), QDs, silver-NPs, and drug-loaded NPs with perfluoropentane (PFPE) nano/ micro bubbles have also been explored extensively for their theranostic applications (Wu, et al, 2010; Cheng, et al, 2011; Mitra, et al, 2012).

Conclusions

Having confirmed the feasibility of significant enhancements in bioavailability, absorption and sustained release of curcumin and other curcuminoids in amorphous nanoparticulate form (100 nm average PS) in combination with polymeric (block copolymers), liposomal, dendrismol, supramolecular stimuli responsive complexes, metal complexes, hybrid formulations and numerous types of conjugates with a variety of inorganic, organic, bio-polymeric compounds, synthesis of an engineered version of smart nanocurcumin is expected to be there soon employing the SAR considerations in a systematic manner for specific diseases before a more generic intelligent drug development platform is available for its varied applications in pharmacy, nutraceutical and cosmeceutical sectors. With the background of sufficient experience in preparing nanoparticulate formulations of curcumin in different configurations as already mentioned earlier the availability of cost effective formulations in future might not be anticipated as a problem at all. Full potentials of curcumin will be possible to explore with intelligent designs of nanoparticulate conjugates in due course of time at affordable cost. Curcumin is bound to revolutionize the concept of intelligent nanocarrier for drug and gene deliveries.

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Sahin Ahmed: Current Status and Future Prospects of Application Specific Engineered Nanocurcumin Compounds


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