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REVIEW PAPER

Enhancement of bioactivity and bioavailability of curcumin with chitosan based materials

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Abstract−Curcumin (CUR) has been investigated for its poor accessibility to a site of action or absorption and rapid metabolism to cope with the limited medication and cure applications. This article reviews numerous approaches, such as encapsulated surfactant/polymeric micelles, liposomes, micro/nano-spheres, nano-suspensions/composites, nano-complex, films, and hydrogels for effective transfer of CUR to target sites. Chitosan (CS), and chitosan derivatives have been found to enhance therapeutic efficacy of CUR. CS/modified-CS based alginate, cyclodextrin, starch, dextran sulfate, ZnO, phytosomes, and poly(butyl) cyanoacrylate drug delivery matrices improved bioavailability, prolonged drug loading and permeability, sustained release rate, improved solubility and stability (prevent metabolic degradation) of CUR, consequently promoting various clinical applications. CS based polysaccharide, protein, and metal oxide drug delivery nano formulations advantageously participated to improve biological activities of CUR. We have attempted to summarize these delivery approaches, and reviewed future trends/strategies to permit the introduction of CUR as practical therapeutic drug.

Keywords: Chitosan, Curcumin, Bioactivity, Bioavailability, Drug Delivery Systems

INTRODUCTION

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, (Fig. 1(a)) is one of the bioactive components of the Indian spice curry Turmeric (Curcuma longa Linn) that has long been recognized by its historical dietary use and clinical trials [1-4]. Curcumin exhibits keto-enol tautomerism due to the presence of α, β-unsaturated β-diketone moiety in the center of the molecule [1]. Curcumin presents a wide range of therapeutic properties, such as anticancer [5-11], antioxidant [6,12], anti-arthritic [13], antiamyloid [14], anti-ischaemic [15], and anti-inflammatory [16-23] regarded as a safe compound according to FDA department of USA [24]. Despite its highly promising health-promoting features, the clinical efficacy of CUR is hindered owing to poor aqueous solubility of curcumin (~11 ng/ml in plain aqueous buffer pH 5.0), photodegradation in organic solvents and unstable metabolic system [25-30]. Hence, several efforts have been made to circumvent these drawbacks and to raise the stability and bioavailability of CUR following methods such as encapsulation in surfactant micelles [31-33] phospholipids [34-36], cyclodextrine [37], hydrogel [38], liposomes [39,40], polymeric micelles [41], nanoparticles or polymeric nanoparticles [42-45], biodegradable microsphere [46]. Adjuvants such as piperine, which can block metabolic pathways of curcumin, are also used to improve bioavailability of curcumin [47]. The curcumin solubility and stability are promoted by chemical alteration [25], complexation or interaction with macromolecules [48,49], according, improve bioavailability and therapeutic efficacy. CUR in the bound state retains its medicinal activity through a variety of drug delivery matrix systems [50]. Drug delivery systems based on previously mentioned approaches are evolving as one of the feasible substituents that facilitate to transport therapeutic concentrations of several effective chemo-preventives (such as CUR) into the systemic circulation by increasing their bioavailability [51].

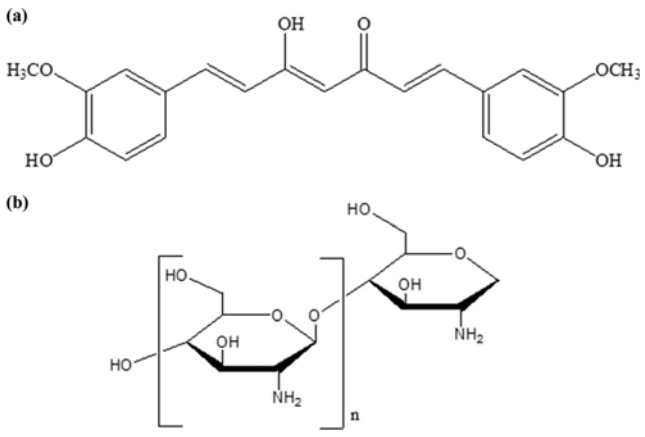

Fig. 1. The chemical structure of (a) the enol form of curcumin [1]; (b) the chitosan [52].

Natural/synthetic polymers have been investigated in drug delivery systems. Among them chitosan (CS) exhibits special advantages in drug delivery because of cationic (owing to primary -NH_2) groups [52,53]. CS is a linear copolymer of β-(1, 4)-linked 2-acet-

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amido-2-deoxy-β-d-glucopyranose and 2-amino-2-deoxy-β-d-glycopyranose (Fig. 1(b), and is chemically prepared by N-deacetylation of naturally occurring chitin [54,55]. A trademark of CS is its properties, e.g., polyoxysalt creation, solubility in various media, metal chelations, polyelectrolyte behavior, and structural uniqueness [56]. It presents wide ranging applications (wound healing/dressings, scaffolds for cell regeneration and nutraceuticals) [7,57-60] because of its favorable characteristics like biocompatibility [61], biodegradability [62], mucoadhesiveness [63] and non-toxicity [64]. To further improve the solubility, mucoadhesive and permeation enhancing properties, various derivatives such as chitosan oligosaccharides, carboxymethyl chitosan were developed [65]. Chitosan oligosaccharide (COS) synthesized by the decomposition of CS is a low molecular weight cationic polymer, while carboxymethyl chitosan (CMCS) was manufactured by incorporating carboxymethyl to the CS structure, has overcome the poor aqueous solubility of CS [66-68] and emerged as a promising functional biomaterial for efficient delivery of many of the highly potent hydrophobic anticancer drugs [69-73]. As compared to CS, CMCS increased the characteristic features of CS like water solubility, moisture retention ability [74], antioxidant property [75], antibacterial [76-78] and antifungal [79] activity.

Yoshioka et al. [80] reported the amphiphilic modification of natural polymers. Chitosan micellar structure arises due to attachment of various lengths of normal alkyl chains (2-16 carbon atoms) to the amino group (-NH_2) of chitosan by amide bonding to offer the hydrophobic part of the surfactant. Afterward, a sulfate group was linked to the -OH group to constitute the hydrophilic group. The hydrophobic region of the polymer micelle showed similarity to conventional LMW surfactants and had high solubilizing power toward hydrophobic substances [80].

Reported results also indicated that fluorescence intensity of CUR can be significantly enhanced in the presence of CS by bovine and human serum albumin [81], resulting due to synergic effects of beneficial hydrophobic micro-environment offered by bovine serum albumin and CS and effective intermolecular energy transfer between bovine serum albumin and CUR [81]. A decrease in degradation rate of curcumin was examined when bonded to chitosan nanoparticles. Much improvement in uptake efficiency of curcumin-loaded chitosan nanoparticles (NPs) in red blood cells (RBC) of mouse was observed than neat curcumin [82]. Oral delivery of curcumin-loaded chitosan NPs enhanced the bio-availability of CUR both in plasma and in RBC. Chitosan and its derivatives increase drug stability when it is encapsulated in chitosan nanoparticles and chitosan film, leading to increment in drug accumulation and toxicity to cancer cells [83-85]. CUR encapsulated in polymer NPs with CS and Gold-NPs was produced by solvent evaporation method and released CUR in a steady and controlled way in HCl and PBS media [86].

Currently, polysaccharide based NPs have displayed huge potential in pharmaceutical, biological, and food uses [87]. CS modified CUR carrier systems, including various polysaccharides (alginate, carboxymethyl cellulose, dextran sulfate, heparin and hyaluronan), protein (kafarin) and metal oxide effectively transferred CUR to the target site. These systems not only controlled the CUR release rate but also enhanced the bioactive properties. In view of this perspective, the present review gives a concise summary of the current status, clinical evidence, and future trends and projections of CS/modified CS based curcumin carrying nano/micro formulations.

CHALLENGES AND IMPROVEMENTS IN THE TOPICAL AND ORAL DELIVERY OF CURCUMIN

Due to its hydrophobic nature, curcumin is poorly absorbed following oral administration and only traces of the compound appear in the blood serum [88]. Curcumin also undergoes extensive first-pass metabolism and is light-sensitive [89]. Low serum and tissue levels of CUR are seen regardless of the route of administration as a result of massive hepatic and intestinal metabolism accompanied by prompt elimination (less circulation time), therefore confining bioavailability of CUR [1,90]. Due to rapid metabolism and weak aqueous solubility in the gastrointestinal (GI) environment, curcumin is often reported to exhibit low oral bioavailability. Merely trace amount of CUR was identified following oral administration up to 8 g/day, while, the oral bioavailability was found to be only 1% in rats [90-93].

However, low water-solubility and extensive first-pass metabolism made CUR a suitable candidate for topical applications. Topical application of curcumin has more pronounced effects on wound healing compared to its oral administration owing to the greater accessibility of the drug at the wound site [94-96]. Keep in mind, many groups have developed new formulations of curcumin to achieve better topical application at the wound site, including chitosan-alginate sponges [97], polymeric bandages [98], alginate foams [99], collagen films [100], and creams [101]. When compared to free curcumin, it was found that the bioactivity of curcumin increased when bonded/encapsulated into these formulations. However, no significant difference in curcumin's wound healing effect was found between these different formulations as they all exhibit similar wound healing profiles. While the above-mentioned formulations enhanced the topical application of curcumin, the infiltration of curcumin into cells at the wound site still needs to be improved through nano-formulations. In traditional Chinese medicines (TCM) that confront similar limitations in curcumin application, successfully formulated curcumin nanoparticles have resulted in substantially improved therapeutical effects [102]. Therapeutic advantages of curcumin nanoparticles are due to the higher total surface area and smaller size that facilitate the cellular uptake of curcumin [102]. Nano-formulation of curcumin was found to enhance cellular uptake into cancer cells and reduce their proliferation compared to raw curcumin [103,104], thus improving the oral/topical bioavailability and longer half-life of curcumin [105]. Curcumin nano-formulation enhances water dispersion [104] property and allows curcumin to be made into an aqueous formulation such as cream [101]. Up till now no report has tested the effect of curcumin nanoparticles on wound healing. The future studies demand to focus on these formulations in order to improve curcumin delivery to wound sites.

Curcumin delivery systems with improved stability, accessibility and solubility in or to the GI tract, were established. Nano-suspensions, solid-lipid nanoparticles, and phosphatidylcholine complexes are already studied and introduced in clinical profiles. However, their bio-availability is far beyond that of the usual dosage forms. Moreover, other systems, for example, polymer particles/

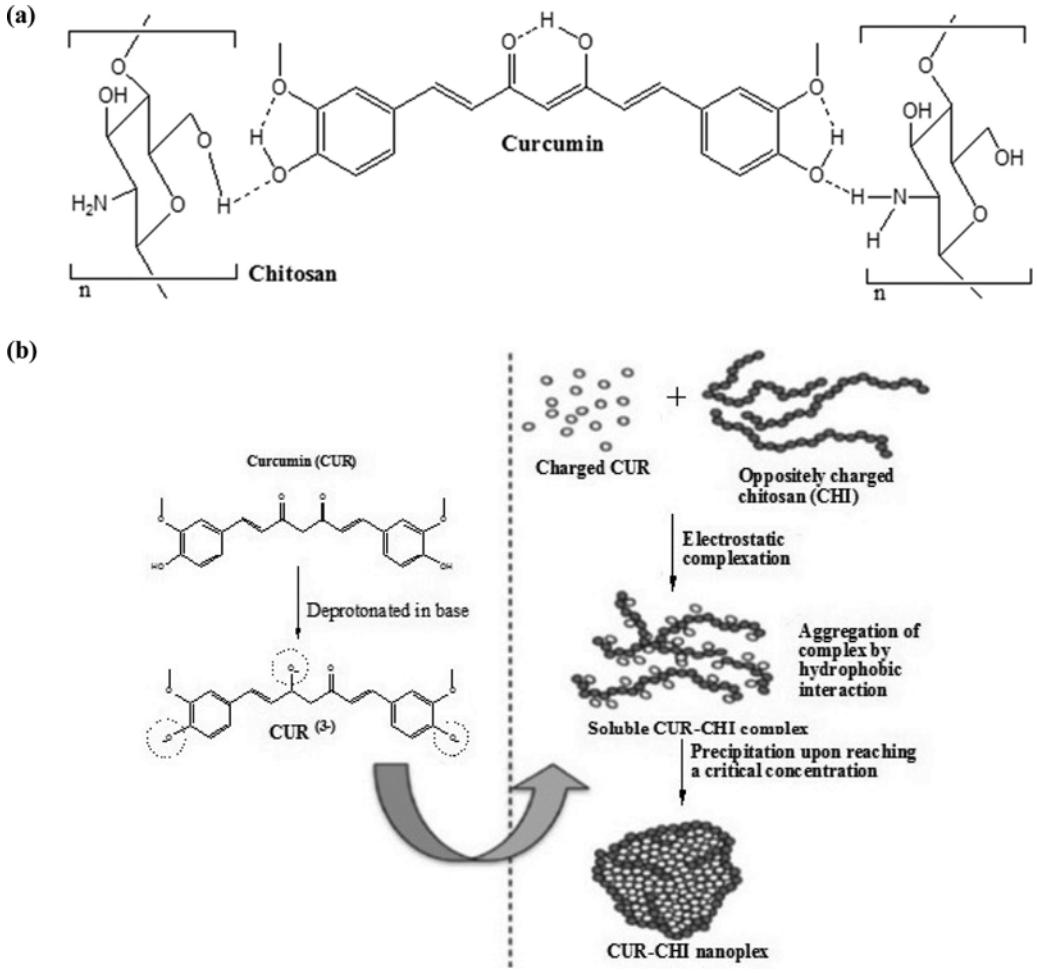

Fig. 2. (a) Hydrogen bonding interactions between curcumin and chitosan molecules [109]; (b) preparation of amorphous curcumin-chitosan nanoparticle complex [145].

nano-emulsions, are being widely investigated [106].

Biodegradable polymeric nanoparticles are also a good option for developing drug delivery matrices of curcumin. Chitosan shows mucoadhesiveness, in situ gelling, permeation enhancing, and efflux pump inhibitory features. Furthermore, a sustained or regulated drug release and nanoparticulate delivery systems for DNA-based drugs and siRNA can be attained by employing ionic interactions [107,108]. Actually, the majority of these properties of chitosan, yet, are practically weak providing simply not an adequate value that would rationalize product enhancements based on it [107]. Despite these beneficial properties, both chitosan and its derivatives show low stability, poor absorption, and short lifetime of drug into support matrix. So, efforts have been made to develop suitable chitosan/chitosan derivative based support matrices by mixing natural and synthetic polymers and metal oxide that provide better environment for the efficient drug adsorption, improve solubility and stability subsequently promote bioavailability and maintain bioactivity.

INTERACTION BETWEEN CURCUMIN (CUR) CARRYING CHITOSAN (CS) MATERIALS

Molecular mimicry is used to investigate the interaction between curcumin and chitosan molecules. The optimized geometries of chitosan and curcumin molecules exhibited hydrogen bonding interactions as shown in Fig. 2(a) [109]. Chitosan combines the herbal pigment curcumin with great affinity, because of its glucosamine unit, at significantly high pH: 7.0-10.5 [110]. For the curcumin molecule, intramolecular hydrogen bond is the primary interaction, consisting of two hydroxyl groups (-OH) in the benzene ring and the hydroxyl near keto group (C=O). Especially, the hydrogen bond between -OH and C=O has been investigated by many researchers [111]. Curcumin exhibits characteristic keto-enol tautomerism and the enolate form predominantly prevails in the pH range of 3-7 [109]. A clear diagrammatic sketch is shown in Fig. 2(a).

The reported results show that a more pronounced/strong interaction is observed in the presence of surfactants between CUR and chitosan even at the physiological pH of the system. Thermodynamic studies (indicated by both enthalpy and entropy) revealed that the binding process is driven by hydrophobic, electrostatic and hydrogen bond formation between curcumin and chitosan [112]. Chitosan-coated nanoparticles interact with mucin due to electrostatic interaction between amino groups (protonated NH_3^+) of chitosan and negatively charged moities, i.e., carboxylate or sulfonate groups, of protein carbohydrate chains [113], [114]; consequently,

this interaction can be useful to prolong the contact time and performance of drug (curcumin or others) carrying systems at target site.

CUR CARRYING CS SYSTEMS AND THEIR POTENTIAL PROPERTIES

In vitro and in vivo evaluation of curcumin indicated chemopreventive and chemotherapeutic effects on various cancer cell types and animal models [140,141]. But there is a medical need for improving the oral bioavailability of curcumin because a major portion of a dose never reaches the plasma or exerts its pharmacologic effect. Improvement in oral bioavailability is therapeutically important because the extent of bioavailability directly influences plasma concentrations, as well as the therapeutic and toxic effects resulting after oral drug administration. The most common approach for oral curcumin delivery is the encapsulation using mucoadhesive polymers like chitosan that physically protect the CUR from enzymatic degradation. Moreover, chitosan based nanoformulations showed numerous advantages, including protection and release of the drug over an extended period in a controlled way [142].

CUR-loaded chitosan based nanoparticles increase the bioavailability and delay the retention time of CUR because of accumulation of nanoparticles in endoplasmic reticulum system. In vivo tests predicted better wound healing after application of curcumin-loaded chitosan nanoparticles polymers by way of better collagen deposition, and re-epithelialization of epidermis CUR-loaded chitosan based complex proved fatal for the parasite on account of inhibiting hemozoin synthesis [84] and could also be administered in order to detoxify arsenic [133] through better antioxidant and chelating potential. These materials act as potential drug carriers in cancer therapy as well. The NPs of this mucoadhesive polymer (chitosan) interact with the negatively charged mucosal surface and can be beneficial to lengthen the contact time of drug delivery system in the mucosa, which would increase the therapeutic functioning of CUR or/and other drugs [136].

1. Chitosan Nano/Micro-particles

CUR dissolved in ethanol was incubated with chitosan nanoparticle suspension prepared by ionic gelation method in Millipore water at three different pH (4.0, 7.0, and 8.5) adjusted by adding either HCl or NaOH followed vacuum drying. Curcumin-bonded chitosan nanoparticles facilitated the enhancement of bioavailability and metabolic stability, while, binding is pH dependent and maximum at pH. An additional advantage of binding at low pH is the stability of curcumin at a lower pH value 4 [143]. These nanoparticles prevented degradation of bound curcumin in mouse plasma in comparison to free curcumin. Oral delivery of these particles to normal mice showed that they can cross the mucosal barrier intact and confocal microscopy detected the curcumin bound chitosan nanoparticles in the blood one hour after feeding. Oral administration of curcumin bonded chitosan nanoparticles cured mice from *P. yoelii* infection [82]. Consequently, binding of Cur to chitosan nanoparticles increased its bioavailability and chemical stability, and in vitro details also propose that this complex can inhibit hemozoin synthesis, which is harmful for the parasite.

Wan et al. [144] reported TPP crosslinked chitosan microparticles prepared by ionic gelation method and studied their feasibility to enhance the dissolution and oral bioavailability of Cur. The cumulative drug release behavior of Cur was found to be 90% following the diffusion mechanism. As it was noted that microparticles sank immediately, whereas pure drug kept floating on the surface of the dissolution medium for a longer period of time, signifying the improved hydrophilicity of the drug incorporated into microparticles. The open pore structure of microparticles in TPP was also responsible for the improved drug dissolution. The microparticles upon oral administration present longer T_{max}, lower K_e, higher C_{max}, and larger AUC. It was suggested that the microparticles could sustain the absorption of Cur, delay the drug transit time in vivo, inhibit degradation of the prototype molecules and metabolism, and ultimately, improve the bioavailability of the drug. Consequently, the therapeutic ability was also enhanced [144].

2. Chitosan Nanoplex Carrier

In the preparation of drug-polysaccharide complex, CUR was first dissolved in base (0.1 M KOH, pH 13) to produce charged CUR molecules, after which the CUR solution was mixed with aqueous acetic acid solution of oppositely charged CHI, resulting in the formation of soluble CUR-CHI complex, as illustrated in Fig. 2(b). Aggregates of the soluble complex were subsequently formed due to hydrophobic interactions among the bonded CUR molecules. The complex aggregates later precipitated out to form the CUR-CHI nanoplex upon reaching a critical aggregate concentration whose value was dictated by the hydrophobicity of CUR and CHI. Strong electrostatic interactions between CUR and CHI molecules suppressed the former from assembling into ordered crystalline structures during precipitation, resulting in the formation of amorphous CUR-CHI nanoplex [145].

CUR having pKa of 8.4, 9.9, and 10.5 was fully deprotonated at pH 13 to form the negatively charged $CUR^{(3-)}$ with a charge density of 8.14×10^{-6} mol-charge/mg [146], whereas the NH_2 group of CHI (pKa 6.5) was protonated upon its dissolution in acetic acid with a charge density of 5.58×10^{-6} mol-charge/mg [147]. After eight hours the concentration of CUR was at approximately 76% of the saturation solubility nearly 24% degradation of CUR in a period of 6.5 hours. This demonstrated that the CUR released from the nanoplex did not undergo significant degradation in phosphate-buffered saline (PBS) in contrast to previously reported work [148].

3. Lipid-chitosan Microsphere

A new polymer-lipid based delivery system was developed by synthesizing CUR-phytosome-loaded chitosan microspheres to enhance the bioavailability and sustainable retention time of CUR in a controlled way using ionotropic gelation method [125]. Characterization techniques such as DSC and FTIR revealed that the integrity of the phytosomes was protected within the polymeric matrix of the microspheres. A slower sustained pH dependent release rate of CUR in curcumin phytosome-loaded chitosan microspheres was observed than curcumin-loaded chitosan microspheres. This was first due to the inhibited diffusion of phytosomes from the microsphere arises by force of attraction between the negative charge of phytosomes and the positive charge of chitosan and second due to complex formation between curcumin and phospholipids, requiring some time for CUR to release from curcumin-

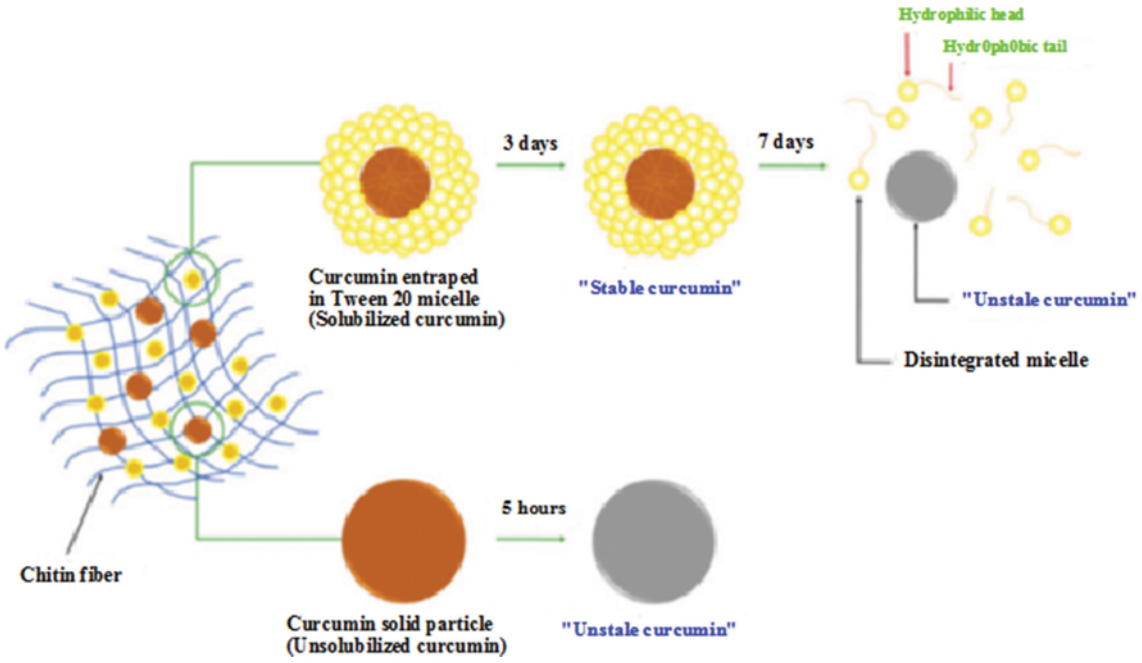

Fig. 3. Proposed scheme illustrates the presence of the solubilized and unsolubilized curcumin in the chitin sheet in a phosphate buffer solution (pH 7.4) at 37 °C [152].

phytosomes. Pharmacokinetic findings showed enhancement in CUR absorption in CUR-phytosome-loaded chitosan microspheres compared with CUR-phytosomes and CUR-loaded chitosan microspheres. Additionally, CUR-phytosome-loaded chitosan microspheres showed a longer half-life in oral delivery when compared to former. This suggested the new CUR-phytosome-loaded chitosan microsphere combined system was used advantageously in oral drug delivery systems [125].

4. Polysaccharide-chitosan Nano-systems

Complexation of CUR with cyclodextrins (CD) is another approach to overcome the limited properties of CUR. Complexation with CD is one of the most widely studied methods to enhance the solubility of CUR, especially for oral delivery. Reported results confirmed that by forming CUR-CD inclusion complex, the solubility of CUR is significantly enhanced [149,150]. How-ever, this system suffered from issues such as low yield, non-scalability and low increment in solubility [149-151]. To address the above challenges, a novel design of CUR nano-formulation was presented using both CD and CS as excipients [121]. In the first step, a simple and scalable spray drying method was adopted for the preparation of CUR-CD hollow particles with high solubility. Highly soluble CUR-CD system made it possible to encapsulate it in CS nanoparticles (CUR-CD-CS) with the aid of aqueous synthesis approach. Higher solubility of CUR-CD complex in aqueous solution directed to greater interaction between chitosan (positively charged) and CUR-CD (negatively charged) complex resulted in high loading increment in nanoparticles. It is demonstrated that CUR-CD-CS nanoparticles showed to be superior in vitro drug release performance and high toxicity against human skin cancer cell line (SCC25), leading to nearly 100% apoptotic cell death [51].

β-Chitin films containing CUR were developed via a water-based system to direct a condition of being safe. It is indicated that chitin sheets are made up of small fibers and could be used as a carrier for Tween 20/curcumin micelles. The *β*-chitin non-woven fibrous sheets containing curcumin could be a promising candidate as a drug delivery device applied for human skin [152]. Stability of the solubilized CUR is sustained for three days since the CUR is entrapped in the Tween 20 micelles. Proposed scheme illustrated the presence of the solubilized and un-solubilized curcumin in the chitin sheet as well as the mechanism for the loss of stability of the curcumin is also shown in Fig. 3.

Crosslinked chitosan materials prepared through various crosslinkers, such as glutaraldehyde, suberoyl chloride, asgenipin, and epichlorohydrin imparted controllable water absorption and drug release properties and use for biomedical applications. But the toxicity of these crosslinkers is a great issue. So chitosan films with controllable physic-chemical properties were obtained using cellulose micro crystals which were dispersed uniformly into chitosan film by vapor induced phase inversion approach [153]. An almost diffusion-controlled release performance Ch/CMC film samples was attributed to crosslinking and hydrogen bonding interaction between surface -OH groups of cellulose crystals and hydroxyl groups of chitosan. It was found that unlike chitosan, cellulose crystals appeared as a diffusion barrier against the release of Cur molecules from the film samples [153].

It is mentioned that addition of starch granules enhanced the mechanical strength of chitosan-TPP beads [154]. Bajer and coworkers [155] discussed the inter-molecular interactions between the chitosan and starch in their blend [155]. The chitosan-starch composite carriers aimed to provide high efficiency release of anticancer drug. In this study, bis-demethoxy curcumin analog loaded chitosan-starch (BDMCA-CS) nanocomposite particles were developed using different ratios of chitosan and starch by ionic gelation method. The entrapment efficiency, 86.60%, and drug loading capac-

ity, 24.25%, were highest for the formulation with the ratio 3 : 1 of BDMCA : CS. These formulations showed sustained release of BDMCA. Subsequently, formulation of BDMCA-CS (3 : 1) exhibited anti-cancer activity against the breast cancer cell lines (MCF-7). Kinetic studies of drug release pattern revealed that the release of BDMCA from the CS nanocomposite followed both diffusion and polymeric erosion pathways. On the whole, it was found that the CS nanocomposite are used as a better delivery tool for BDMCA to treat breast cancer [156].

Curcumin loaded dextran sulfate-chitosan NPs were prepared by simple co-acervation method. Investigations on entrapment efficiency; in vitro drug release, cell uptake and cytotoxicity of the prepared NPs were conducted. The NPs have a spherical morphology with negative zeta potential and good colloidal stability. In this study 70% of curcumin was released after 120 h and the reported drug release rate was better than previously stated system of curcumin loaded O-carboxymethyl chitosan NPs [126]. These results suggest that dextran sulfate-chitosan NPs are an ideal carrier to deliver hydrophobic drugs like curcumin, especially for the treatment of cancer [130].

An in situ injectable N,O-carboxymethyl chitosan/oxidized alginate nanocomposite hydrogel was effectively developed for slow in vitro release of encapsulated nanocurcumin with the controllable diffusion behavior, used for the treatment of dermal wound [118]. Wound healing is a complex process resulting in the contraction and closure of wound tissue and restoration of a functional barrier [94,157]. Repair of injured tissues proceeds in three interrelated dynamic and overlapping phases, including inflammation, granulation and remodeling [158]. Since 1980s, numerous wound dressings based on natural polymers such as hyaluronic acid (HA), and chitosan (CS) have been developed to promote the wound healing [159]. Studies showed that use of nano-Cur/N,Ocarboxymethyl chitosan/oxidized alginate hydrogel could significantly amend the re-epithelialization of epidermis and collagen deposition on dorsal wounds of rat. Presence of protein, DNA, and hydroxyproline content in wound tissue site directed that combination of nanocurcumin and N,Ocarboxymethyl chitosan/oxidized alginate hydrogel could considerably speed up the process of wound curing [118].

In situ injectable CS-based hydrogels comprised of chitosan, genipin and sodium salts by the cooperation crosslinking between chemical and physical bonds were reported [160]. In vitro Cur release profiles of hydrogels showed sustained release of Cur about 3 to 6 times higher than the other comparable systems beside initial fast release at the first 24 h. These hydrogels demonstrated their potential as Cur delivery carriers.

5. O-carboxymethyl CS nanocarrier

The potential application of chitosan is hindered by its limited solubility in aqueous media. Thus, CS is chemically modified to improve the processability, solubility, antimicrobial activity and the ability to interact with other substances [161]. Introducing a carboxymethyl group is the most advantageous method of increasing the solubility of chitosan at neutral and alkaline pH without affecting other important characteristics. The reaction carried out at room temperature favors O-substitution, while higher temperature favors N-substitution, indicating temperature as a critical factor in carboxymethylation of chitosan [162]. The ability of O-CMC

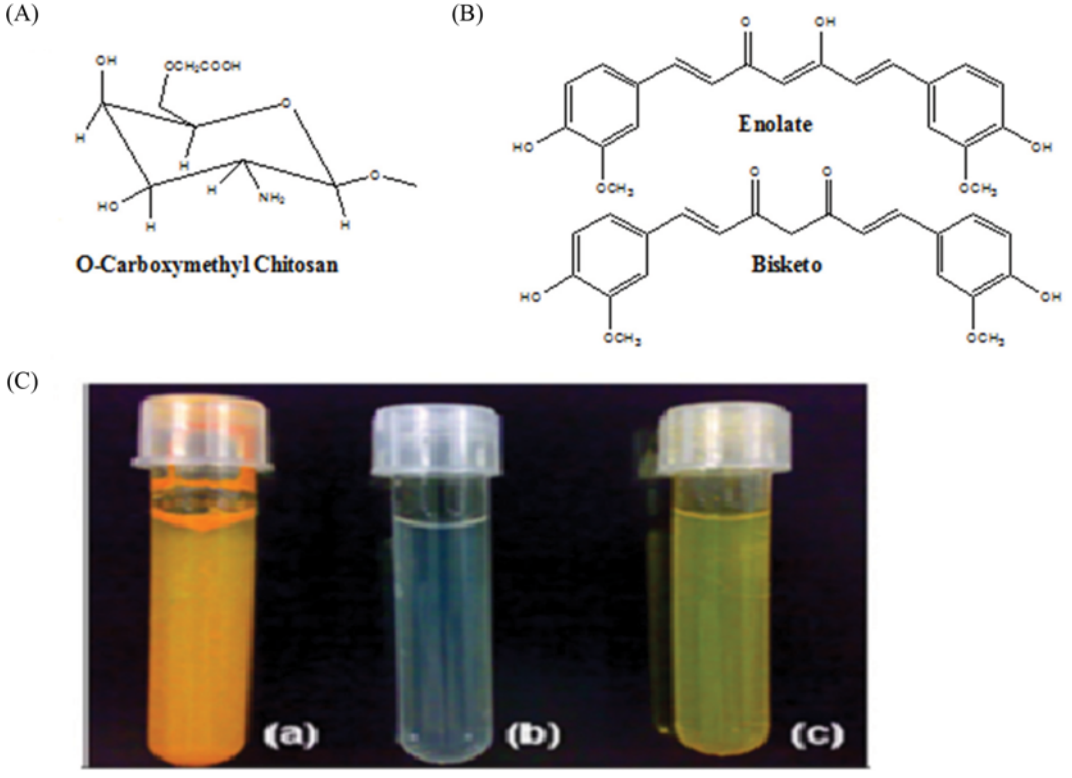

Fig. 4. Chemical structure of (A) O-CMC, (B) curcumin; (C) Solubility of curcumin (a) curcumin in water, O-CMC Nps and curcumin-O-CMC Nps [126].

as a carrier for hydrophobic drugs in cancer targeted drug delivery applications was evaluated [126]. A simple method of solvent evaporation followed by ionic gelation was developed to form a nanoformulation based on O-CMC into which the hydrophobic anticancer drug, Cur, was loaded (Fig. 4). This approach tried to solubilize Cur and thereafter to stabilize it in O-CMC NPs owing to hydrogen bonding interaction between the -COOH group of O-CMC and -OH group of Cur [126,163]. The negative value of zeta potential indicated the stability of the NPs system. Cur-O-CMC Nps exhibited no crystalline peaks when compared to neat Cur. The amorphous configuration of complex was assigned to intermolecular interaction between O-CMC and Cur within the NPs matrix. Enhancement in both cellular uptake and cell apoptosis was reported with increasing concentration of Cur loaded in O-CMC nanoparticles [126].

6. Amphiphilic-chitosan Microparticles

Among CS derivatives, amphiphilic CS derivatives are types of molecules which have intensively been studied due to their interesting behavior. Chitosan molecule in its native form has no amphiphilicity. Various amphiphilic chitosan derivatives like carboxymethyl hexanoyl chitosan [126], water soluble N-methylated chitosan possessing hydrophobic -N(CH_3)$_2$, -NH(CH_3) and hydrophilic -N-(CH_3)$_3$ groups [164] etc. were suggested to enhance the mucoadhesive and solubility properties of chitosan. Spectrophotometric results after the dissolution of curcumin and LSCS-CUR in aqueous solution reported enhancement in solubility and bioavailability of curcumin after encapsulation procedure [129]. Pharmacokinetic findings indicated 11.5-fold increased pharmacological availability

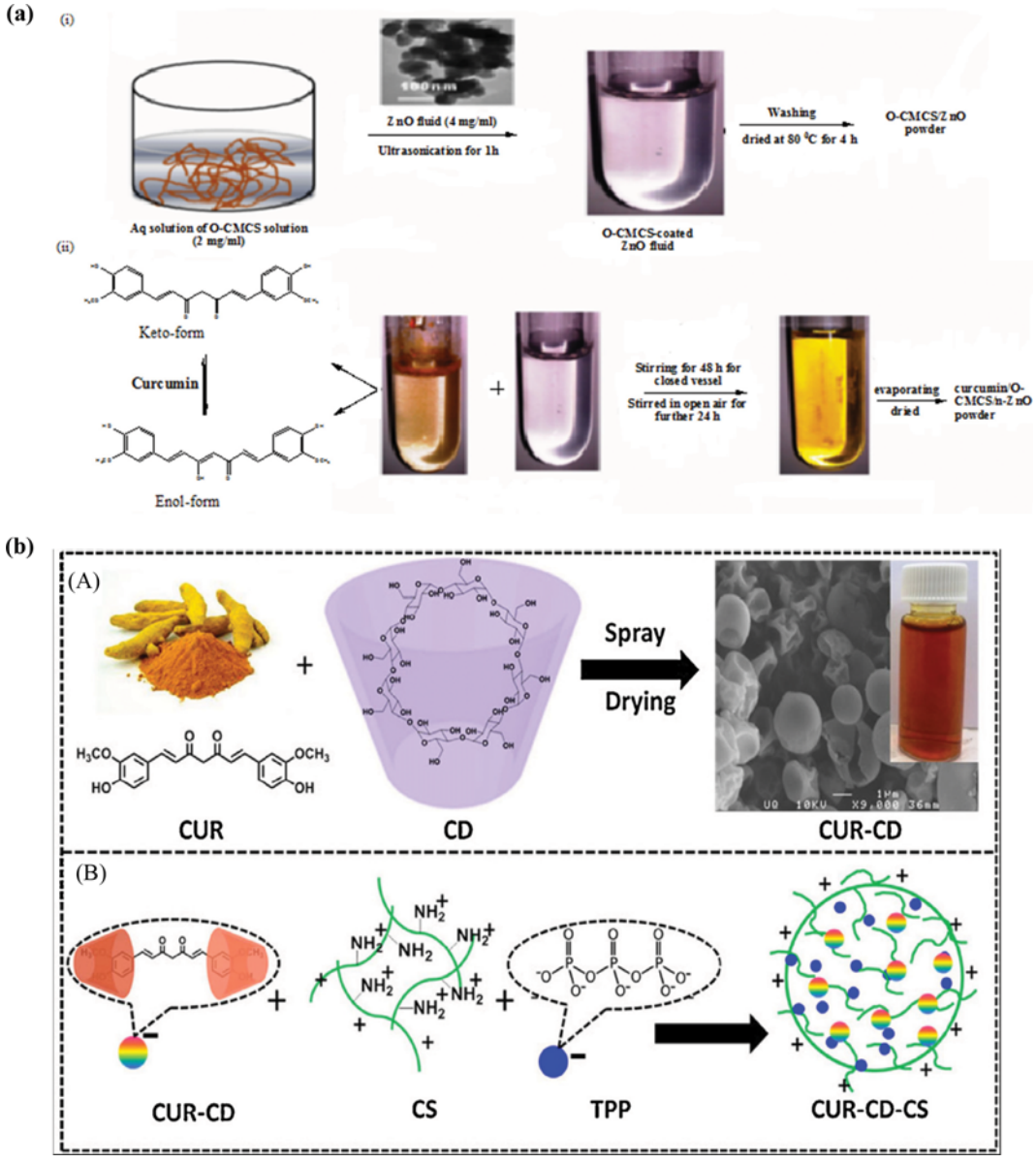

Fig. 5. (a) Pictorial representation for O-CMCS/n-ZnO nanocomposites (i) and O-CMCS/n-ZnO/curcumin nanocomposite (ii) [116]; (b) schematic representation of (A) the preparation of highly soluble CUR-CD complex by a novel sprays drying method; (B) the preparation method of CUR-CD-CS nanoparticles [121].

of Cur with encapsulated curcumin compared with native Cur after oral administration in rats [129]. In this study [131], hydrophilic and hydrophobic drugs were encapsulated onto the amphiphilic CS, i.e., Lauroyl sulfated chitosan micro particles and its release properties were compared. Insulin was chosen as a hydrophilic drug and curcumin as a hydrophobic one. Due to the inconvenience of insulin injections, various approaches have been attempted to formulate insulin for administration by oral routes such as encapsulation of insulin within polymeric particles [131]. Lauroyl sulfated chitosan matrix showed a pH sensitive mechanism for both drugs and capable to transport insulin to the absorptive site. These microparticles with increased mucoadhesion property improve bioavailability, drug residence time in GI and enhanced contact between drug and absorbing surface. A slow and controlled release of CUR in 30 days from the microparticles was probably due to the hydrophobic interactions between CUR and lauroyl groups on chitosan. Therefore, it could prevent the pre-mature degradation of incorporated drug [131].

7. Metal Oxide-chitosan Nanocomposite

Cancer treatment still remains a major challenge in the medicine world as chemotherapies and radiotherapies are aggressive and less effective. These conventional anticancer therapies pose side effects such as fatigue, nausea, insomnia, delirium and vomiting, which are common troubles for cancer patients [165,166]. Efficient water soluble O-carboxymethyl chitosan (O-CMCS) based nanocomposites (NCs) with nanostructured zinc oxide (n-ZnO) were synthesized by ex-situ grafting method for the delivery of anticancer drug curcumin (Cr) [116]. The pictorial representation for the preparation of O-CMCS/n-ZnO nanocomposites and (i) O-CMCS/n-ZnO/curcumin nanocomposite (ii) is shown in Fig. 5(a).

The electrostatic bonding between ZnO and O-CMCS and curcumin with O-CMCS in Cr/O-CMCS/n-ZnO NCs was confirmed by FT-IR study and exposed homogeneous morphology reported by SEM. The corresponding entrapment and loading efficiency of curcumin in O-CMCS/n-ZnO nanocomposites are 74% and 43%, which increased with amount of O-CMCS/n-ZnO nanocomposites that facilitated controlled increase in drug loading and entrapment and increased the particle size as well. A pH dependent release response was observed and is faster in acidic pH due to protonation of -NH_2 groups of CMCS, which actually cause swelling of polymer. Cr loading into O-CMCS/n-Zn nanocomposites enhanced the drug solubility in water. The anticancer activity of curcumin and Cr/O-CMCS/n-ZnO nanocomposites showed that curcumin maintained its anticancer activity into polymer matrix after loading and the anticancer effects were improved in the presence of ZnO [116].

8. Protein-chitosan Nanosuspension

Kafirin-based NPs enabled protective delivery of biologically

Table 1. List of different types of curcumin loaded chitosan based materials, their prospective application and the effect of the material on the properties of curcumin [115-139]

Sr. no.	Name	Type of material	Delivery route	Effect on properties of curcumin	Applications	Reference
1	Curcumin-N,O-carboxymethyl chitosan	Nanoparticles	-	Enhanced plasma half-life and anticancer efficacy	For colon cancer treatment	[115]
2	Chitosan/curcumin-ZnO	Nanocomposites	-	Improved cellular uptake and sustainability	For anticancer therapy and biomedical field	[116]
3	Curcumin-kafirin Carboxymethyl-chitosan	Nanoparticles	-	Enhancement in cellular uptake of curcumin	Gastrointestinal tract targeted drug delivery	[117]
4	Curcumin-N,O-carboxymethyl chitosan-alginate	Nanocomposite hydrogel	Transdermal delivery	Stimulate fibroblast proliferation, capillary formation and collagen production	Wound healing dressings applications	[118]
5	Curcumin-thiolated chitosan	Nanoparticles	-	Improve bioavailability, anticancer activity	Colon cancer treatment	[119]
6	Curcumin-chitosan	Nanoparticles	Mucoadhesive drug delivery	Improved cellular uptake and plasma half life	For colorectal cancer treatment	[120]
7	Curcumin-cyclodextrin-chitosan	Nano-conjugates	-	Enhanced solubility and cell cytotoxicity	For gene delivery application	[121]
8	Curcumin-O-carboxymethyl chitosan/fucoidn	Nanoparticles	Oral delivery system	Increase cellular uptake, in vitro stability	Used for oral drug delivery applications	[122]
9	Curcumin-chitosan/tripolyphosphate	Nanoparticles emulsion	-	Improve stability and sustained release	For the delivery of bioactive compounds	[123]
10	Curcumin-chitosan	Cryogel	-	Controlled release rate	Food and pharmaceutical industry	[124]

Table 1. Continued

Sr. no.	Name	Type of material	Delivery route	Effect on properties of curcumin	Applications	Reference
11	Curcumin-phytosome-chitosan	Microspheres	Oral drug delivery	Prolong retention time and promote oral absorption	For drug delivery systems	[125]
12	Curcumin-chitosan	Nanoparticles	Oral drug delivery	Increases chemical stability and bioavailability	Prevent hemozoin synthesis to kill animal parasites	[84]
13	Curcumin-carboxymethyl chitosan	Nanoparticles	-	Enhance solubility and sustainability	For cancer drug delivery applications	[126]
14	Amorphous Curcumin-chitosan	Nanoparticle Complex	Oral delivery	Enhance bioavailability and chemical stability	Clinical application for oral drug delivery system	[127]
15	Curcumin-galactosylated chitosan-polycaprolactone	Nanoparticles	-	Enhance cell apoptosis ability and bioavailability	As promising carriers for hepatocyte-targeted drug delivery	[128]
16	Curcumin-lauroyl sulphated Chitosan	Sub-microparticles	Oral delivery	For enhancing bioavailability and prolong sustainability	Treatment of carcinoma patients	[129]
17	Curcumin-dextran sulphate-chitosan	Nanoparticles	-	Improved elution rate	Cancer therapy	[130]
18	Curcumin-insulin-chitosan	Microparticles	Oral delivery	Improve release sustainability	For insulin and curcumin delivery applications	[131]
19	Curcumin-chitosan Carrageenan Carboxymethyl cellulose	Ternary cryogel	-	Improve controlled release rate	For controlled release delivery applications	[132]
20	Curcumin-chitosan	Nanoparticles	Oral delivery	Improve solubility and bioavailability	For the treatment of arsenic toxicity	[133]
21	Curcumin-N-trimethyl chitosan-sodium alginate	Complex beads	-	Improve solubility and bioactivity	For intestinal and gastric fluid targeted drug delivery	[134]
22	Curcumin-chitosan	Nanoparticles	-	Increase cellular uptake	For cancer drug delivery	[135]
23	Curcumin-chitosan	Nanoparticles	Buccal drug delivery	Prolong the cellular contact time	For mucoadhesive applications	[136]
24	Curcumin-chitosan-coated poly(butyl) cyanoacrylate	Nanoparticles	-	Improve solubility of curcumin and prevents RES uptake	Inhibit hepatic-carcinoma growth for clinical uses	[137]
25	Curcumin-N-trimethyl chitosan coated lipid	Nanoparticles	Oral delivery	Improve bioavailability and release rate	Used for clinical applications	[138]
26	Curcumin-alginate-chitosan-pluronic	Composite nanoparticles	-	Improve release rate	For hydrophobic drug delivery systems	[139]

active components as it is less prone to severe GI tract environs. Furthermore, curcumin represents the polyphenolic compounds that are particularly suitable for kafirin encapsulation, since they are known to have a strong affinity with prolamin residues via hydrophobic interaction and hydrogen bonding [167]. In addition, Cur can emit florescence under excitation, which makes monitoring the fate of nanoparticle after absorption without external labeling possible. Kafirin based nanoparticles containing curcumin were first prepared (cc-kaf) followed by introduction of CM-chitosan by acid-induced aggregation (cc-kaf/CMC). During nanopar-

ticles synthesis process kaf or/and CM-chitosan inhibited the crystal aggregates of curcumin, stimulating the amorphous complex arrangement. Amorphous curcumin can diffuse through the polymeric matrix easily, thus facilitating the encapsulated drug to be released at a controlled rate. CM-chitosan self-aggregated particles existed, with glucose backbone of CM-chitosan serving as the inner hydrophobic domains while the -COOH groups and the protonated NH_3^+ groups formed the outer hydrophilic shell. Therefore, CM-chitosan encapsulates the curcumin effectively and acts as cellular uptake enhancer and provides a physical barrier against photo-degradation and enzymatic digestion. The incorporation of CM-chitosan increases the average encapsulation and loading efficiency of cc-kaf from 55% to 86% and 5% to 6.1% that could be attributed to trapping of free curcumin by self-aggregates of CM-chitosan particles in solution, or CM-chitosan helped encapsulated surface Cur in kaf nanoparticles. Compared to pure curcumin, curcumin embedded in polymer matrix was found to be more stable to UV treatment. Contrary to this, pure curcumin solution when constantly exposed to direct UV treatment exhibited almost linear degradation kinetics. During the first 60 min of treatment, over 90% of curcumin was unchanged in cc-kaf and cc-kaf/CMC suspensions as compared to less than 55% unchanged fraction in native suspension. At the end of UV treatment, 45% and 50% of curcumin was found to be unchanged in cc-kaf and cc-kaf/CMC suspension, respectively, as compared to 25% for pure curcumin solution [117].

In in vitro release profiles of native curcumin, cc-kaf, cc-kaf/CMC, only 10% of pure curcumin was detected at the first 30 min of SGF treatment; after that pure curcumin showed a slow dissolution rate with less than 15% of curcumin detected at the end of 6 h. This clearly indicates that when passing through the GI tract only small faction of pure curcumin approximately 13 mg/ ml, which is the saturated solubility in water medium with 0.5% tween 20, exists in the format of solubilized curcumin, which can then be effectively absorbed by the small intestinal [47]. The kinetic dissolution of cc-kaf and cc-kaf/CMC showed similar biphasic profiles in SGF and SIF. Burst effect at the beginning of each fluid addition process was observed for both formulations, probably due to the rapid dissolution of peripherally encapsulated curcumin [117].

FUTURE TRENDS IN CUR LOADED CS BASED DELIVERY SYSTEMS

The usual chitosan/chitosan derivative based drug delivery systems are based on ionic and hydrogen bond interactions, relatively providing week mucoadhesion. Thus, systems are developed with covalent bond interactions, e.g., thiolated chitosan (TCS) is able to form covalent bonds with cysteine-rich subdomains of mucus as well [168]. Recently, Cur was encapsulated in CS-N-acetyl-L-cysteine conjugate by melt-emulsification technique [67]. CS-N-acetyl-L-cysteine was formed by immobilizing the -COOH group of N-acetyl-L-cysteine on the pri-amino groups of CS. Chemical modification of the thiol functions through coating of CMCS and chitosan oligosaccharides improved its efficacy to encapsulate and load Cur.

Another important aspect of the Cur carrying delivery systems is their stability, especially in case of nano-emulsions. Chitosan coated nano-liposomes demonstrated prolonged absorption in the GI, which could be contributed to its mucoadhesive properties. MCT oil, Tween 80 and lecithin based nano-emulsions were used to encapsulate curcumin at high loading ability and efficiency [169]. CS coating could prevent degradation of Cur during thermal/UV irradiation treatment. Though, nano-emulsions with middle and high molecular weight CS during the in vitro digestion interfere with the lipolysis of nano-emulsions, which to some extent reduced its bio-accessibility. CS based polysaccharide-NPs as novel delivery systems are effective to encapsulate, stabilize and deliver Cur. Recent studies [170] also reported the electrostatic complex between gum Arabic and CS containing emulsifying agents, i.e., EYPC and Tween 80 as carriers for Cur. These hydrophilic nanoparticles were supposed to make curcumin water-soluble and able to be dispersed in aqueous formulations. As a result, the DPPH scavenging and FRAP activities of Cur were notably improved by encapsulation. Actually, the strong matrix biopolymer network around the Cur-emulsifier droplets can amend the delayed release of Cur in a simulated GI tract. Another approach is the preparation of BSA protein based CS nanoparticles by nano-precipitaion method for targeted delivery of Cur to human colorectal adenocarcinoma (DLD-1) cells [171]. SLN surface-modified trimethyl derivative of CS was successfully prepared [172] and used to develop as novel nanocarrier system to increase the oral bioavailability and brain distribution of the Cur. But still, the development and improvement in these matrices, especially for the medicinal/therapeutic applications, required a more precise choice of mixing polymers that should be investigated further. Additionally, human trials are required to prove the established effectiveness of CUR in medical/clinical applications as a better therapeutic modality for curing of various diseases using chitosan based systems.

Among the most recent approaches, nanostructured lipid and polymer carriers showed pronounced improvements in Cur drug release systems. Encapsulation efficiency of conjugated curcumin is also enhanced by employing polyelectrolyte complex, nanocomposite and pickling emulsion formulation techniques. CS-Nanoformulation actually provided a more advanced way to get better solubility (up to 1000 folds), absorption due to greater surface area and pronounced therapeutic properties of Cur.

CONCLUSIONS

Potentially, CUR loaded CS blend materials accelerate wound healing, increase DNA and protein content, provide better mucoadhesion, preventing cancer causing HT-29, SCC25, Caco-2 cell lines by apoptosis and arresting the cell cycle. Pharmacokinetic study of CUR loaded CS based drug delivery system ensures an extended therapeutic period, slows increase and sustained plasma concentration of CUR, prolongs drug transit time in vivo with lower K_e and longer $T_{1/2}$, protects from degradation, improves solubility and absorption, controls slow and sustained release of CUR, increases AUC value of CUR after oral administration, reflecting an increased bioavailability. These chitosan/chitosan derivatives based materials significantly rectify the limiting factors as matrix material by combining natural/synthetic polymeric materials or metal oxides. Poor long-term stability is a considerable problem in the scaling-up of CS pharmaceutical uses that is now addressed by the

addition of a stabilizing agent, mixing hydrophilic polymer, and incorporation of ionic/chemical cross-linkers. So there is a promising future for chitosan/chitosan derivative based drug delivery systems for targeted delivery of CUR.

LIST OF ABBREVIATION

AFM : atomic force microscopy
ANOVA : analysis of variance
AUC : area under the curve from 0 to t
BDMCA-CS : bis-demethoxy curcumin analog loaded chitosan-starch
C_{max} : peak plasma concentration
CC/Cr/CUR : curcumin
CCS-OA : carboxymethyl chitosan-oxidized alginate
CD : cyclodextrins
CMs : chitosan microsphere
CS : chitosan
DIC : differential interference contrast
DLS : dynamic light scattering
DSC : differential scanning calorimetry
DTA : differential thermal analysis
ELISA : enzyme linked immunosorbent assays
FDA : food and drug administration
FT-IR : fourier transform infrared spectroscopy
GI : gastrointestinal
^{1}HNMR : proton nuclear magnetic resonance
HPLC : high-performance liquid chromatography
K_e : elimination rate constant
Kaf : kafirin
LSCS : lauroyl sulfated chitosan
MALDI : matrix assisted laser desorption spectroscopy
MCF-7 : human breast adenocarcinoma cell line
NCs : nanocomposites
NMR : nuclear magnetic resonance spectroscopy
NPs : nanoparticles
O-CMCS : O-carboxymethyl chitosan
PCS : photon correlation spectroscopy
PS : phytosome
SEM : scanning electron microscopy
SGF : simulated gastric fluid
SIF : simulated intestinal fluid
SLN : solid lipid nanoparticles
T_{max} : time to reach maximum plasma concentration
$T_{1/2}$: half life
TCM : traditional chinese medicines
TEM : transmission electron microscopy
TGA : thermogravimetric analysis
TPP : triphosphate
USA : united States of America
UV-visible : ultraviolet visible spectroscopy
VIPI : vapor induced phase inversion

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