ARTICLE TYPE

Chitosan nanoparticles potentiate the *in vitro* and *in vivo* effects of curcumin and other natural compounds

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ARTICLE HISTORY

Received:
Revised:
Accepted:

DOI:

Abstract: The development of biodegradable nanoparticles is an important tool for the biological transport of chemical compounds. The nanoencapsulation reduces the biopharmaceutical and pharmacokinetic drawbacks of compounds and enhances their biological properties. Naturally occurring polymers such as proteins and polysaccharides have been widely applied in the development of nanostructured systems of several therapeutic agents. Among them is chitosan, a crustacean-carapace-chitin derived biopolymer. In addition to its biocompatibility and biodegradability, chitosan is known for its mucoadhesion properties. Chitosan-based nanostructured systems potentiate most of aspects of the loaded drugs, including cellular transport and other biological effects. The use of chitosan nanoparticles enhances permeation, stability and bioactivity of natural compounds. In this review, an overview of the main features of chitosan nanoparticles that improved *in vitro* and *in vivo* effects of bioactive natural molecules is given, emphasizing the results obtained with curcumin.

Keywords: chitosan, nanoparticles, curcumin, bioavailability.

1. INTRODUCTION

Naturally occurring compounds obtained from several sources, especially plants, have great medical potentials due to their wide variety of chemical structures [1, 2]. Despite their biological potential, verified *in vitro* and *in vivo* assays, these compounds have not achieved meaningful results in pre-clinical or clinical trials [3,4]. This outcome is mainly attributed to the reduction of the solubility and permeability properties of these molecules [5, 6].

Curcumin, a natural compound with elevated therapeutic potential and derived from *Curcuma* sp., has been extensively studied due to its broad spectrum of *in vitro* effects, such as antioxidant [7], anti-inflammatory [8], and antimicrobial activities [9]. Also, *in vivo* evaluations have shown anticancer activity [10, 11] and protection against neurological [12] and cardiovascular diseases [13].

However, pharmacokinetic restrictions of curcumin limit its therapeutic application [14] due to its poor absorption, fast metabolism and elimination [15, 16, 17]. Pre-clinical studies demonstrate this limitation in rats [18, 19] and humans, confirming the limited bioavailability of curcumin on oral application [20, 21].

Nanotechnology arises as an excellent tool for the pharmacokinetic and pharmacodynamic enhancing of natural compounds, optimizing their therapeutic effectiveness and minimizing the problems associated with conventional delivery. [22]. The reduced size of the nanoparticles (NPs) allows not only the improvement of cellular traffic of the drugs, but also provide a sustained release, increasing the drug half-life. The enhancement of drug biodistribution due to its higher absorption leads to the reduction of therapeutic doses and, consequently, the side effects associated to drug use [23, 24, 25].

Among the polymers employed for the natural compounds' nanoencapsulation, is chitosan (CS), a natural polymer derived from chitin deacetylation. CS is widely applied in the nanoencapsulation of natural products due to its biocompatibility and biodegradability, required for its application in humans, besides its sustainability. Additionally, its cationic nature favors the adsorption on cell surfaces, which contributes to cell-particle interactions, as well as transport/permeation through cells [26, 27].

This review highlights the main *in vitro* and *in vivo* studies of CS nanoparticles loaded with natural compounds, especially curcumin (Cur), over the past 5 years.

2.0 CHITOSAN NANOPARTICLES: APPLICABILITY AND BENEFITS

Nanomedicine is the application of nanotechnology for the diagnosis, prevention, and treatment of diseases at cellular and molecular levels. NPs refer to particles that present singular physicochemical properties, emphasizing the reduced size (<1000 nm) and high proportion between their surface area and volume. These characteristics are exploited to design controlled/sustained release systems based on natural compounds, since they increase the biopharmaceutical, pharmacokinetic and pharmacodynamic properties of the loaded compound [28, 29, 30].

Natural and synthetic polymers are used to guarantee specific targeting and to increase the effectiveness, bioavailability, and stability of the encapsulated compounds [31, 32]. Given the nature of polymeric NPs, it is possible to obtain by different preparation methods, nanospheres, where the compounds are spread or adsorbed on the surface [33], and nanocapsules, in which the compound is trapped in the oil core surrounded by the polymer wall [34].

Several approaches can be employed to obtain NPs, such as solvent emulsification-evaporation, coacervation, nanoprecipitation and ionic gelation [35]. Nevertheless, the choice of the method should consider some important factors, including

compound and polymer characteristics, administration route, and application constancy. Furthermore, the NPs must possess biocompatible and biodegradable properties [36].

NPs made of polysaccharides have been extensively developed due to their physical, chemical and biological properties. CS stands out as a biocompatible, biodegradable and non-toxic biopolymer, susceptible to surface modifications aiming to enhance the stability and permeability in the biological system [37, 38, 39].

CS is a biopolymeric derivative of chitin occurring in crustacean shells. CS forms a linear polycation insoluble in neutral and alkaline aqueous solutions. Differently, in acidic solution, CS shows a high density of positive charges, with hydroxyl and amino groups forming hydrogen bonds, conferring CS the ability to act as a carrier for different therapeutic agents when adsorbing onto negatively charged surfaces. Therefore, CS proved to be an excellent polysaccharide to compose the matrix of nanoparticles [40, 41, 42].

The bioadhesive properties of CS have been demonstrated in several studies [43, 44]. This characteristic stems from the abundance of amino groups in the CS main chain, resulting in increased bioavailability and extended drug release. To enhance the retention, the membrane permeability of charged particles may be facilitated by size and surface binders addition [45, 46].

Considering the wide application of CS in nanostructured systems, some specific characteristics need to be evaluated, for instance, deacetylation degree and molecular mass. An increase on the molecular mass and deacetylation degree leads to elevated reticulation yield, producing larger particles when high CS concentrations are employed [47, 48].

Regarding industrial-scale feasibility, CS-polymer nanostructured systems are promising carriers for drugs such as anticancer, anti-inflammatory, and antihistamine compounds, as well as other classes of macromolecules like hormones, proteins, enzymes, and vaccines. In view of this, CS has vast applicability in food, cosmetics, and pharmaceutical industries [49, 50, 51, 52].

In addition, CS NPs can also be applied to carry nucleic acids for gene therapy e. g. plasmid DNA. For this, the polyelectrolyte complex technique (PEC) is employed. PEC consists on the addition of a DNA solution to CS dissolved in acetic acid under mechanical stirring, leading to the self-assembly of NPs as a result of the ionic interactions between the cationic polymer and the phosphate groups of the DNA molecule [53, 54].

Among the adopted methods for CS-NPs preparation, ionic gelation technique, produced by the ionic interaction between CS and tripolyphosphate (TPP) (Figure 1), is frequently used. During this process, particle self-assembly occurs due to the interactions between amino and phosphate groups, as well as hydrogen bonds coming from several hydroxyls in the CS chain. As a result, CS cationic polysaccharides create a reticulated structure in the presence of anionic molecules [55]. Therefore, there is a predominance of electrostatic interactions in this structure, so NPs are spontaneously formed under mechanic agitation, which results in small diameter NPs (200 – 500 nm) and allows easy reproducibility [56].

Figure 1: Representative scheme of the ionic gelation process for Chitosan nanoparticles preparation. Images edited using resources made available by LES LABORATORIES SERVIER

Another method described for CS/NPs preparation is the emulsification from organic solvent to isolate particles. However, organic solvents may cause toxicity and are difficult to remove. In the microemulsion method, a surfactant is solubilized into an organic solvent, and then, a solution containing CS with acetic acid and glutaraldehyde is added to form NPs under mechanic stirring [57, 58, 59]. The CS/NPs can be applied in several administration routes, such as parenteral [60], oral [61], nasal, ocular [62], topical and transdermal [63], increasing the solubility and permeability of different therapeutic agents, ensuring higher bioavailability and maintenance of these compounds in the body [64].

2.1 CS NANOPARTICLES FOR NATURAL COMPOUNDS DELIVERY

Phenolic compounds have been used in search for new alternatives for several disease treatments, such as cancer [65], inflammatory [66] and central nervous system illness [67]. Despite attractive results, it is still necessary to overcome delivery and bioavailability limitations of these drugs [68, 69]. In this respect, CS nanoparticles have been used to potentiate their biological activities [70].

Anthocyanins (ACNs) are known for their potential features regarding health. However, these compounds are thermosensitive, with low bioavailability and gastrointestinal stability, which limit their medicinal and food applications [71]. He et al. [72] obtained CS NPs with slow ACNs release in simulated gastrointestinal fluid (GIF), increasing the stability of the encapsulated compound during the storage in a food drink model.

Corroborating these results, CS hydrochloride (CHC) and carboxymethyl CS (CMC) NPs containing ACNs were produced in a study led by Ge et al. [73] with an average size of 178 nm and encapsulation efficiency of 44%. After storage at 40 °C for 72 h, the encapsulated compounds presented thermal stability of 88%, while the nonencapsulated compound kept only 10% of the remnant content. Hence, these results had shown the beneficial characteristics of CS encapsulated ACNs for use in the food industry. Ferulic acid

(FA) is a natural product with considerable biological properties, but its photosensitivity and low aqueous solubility limit its application as a free drug. El-Marakby et al [74] demonstrated the selective release of FA into HepG2 (hepatocellular carcinoma) cells in its nanostructured CS form modified with glycyrrhizin (GL) during an *in vivo* study. Besides improving the bioavailability and absorption of FA, the NPs also exhibited higher cytotoxicity to HepG2 cells when compared to the nonencapsulated compound. The authors suggested that GL enabled the internalization of the NPs, mediated via receptors and selective absorption by hepatocytes, along with the passive endocytosis due to the small size of the particles (<200 nm).

It is important to note that, in this study, AF NPs containing GL reached IC₅₀ of 60 µg/mL on human hepatocellular carcinoma HepG2 cells and were biocompatible to normal cells. These results are promising for clinical studies based on chemotherapeutics.

Another application of FA NPs is as a substitute for methylprednisolone on initial spinal cord injury (SCI) neurodegeneration, since this medicine has shown several side effects and controversial effectiveness [75]. Wu et al [76] developed glycol CS (GC) NPs to encapsulate AF aiming to ascertain their neuroprotector effect against SCI in glutamate-induced cytotoxicity model in mice, since glutamate increase is a pathological indicative of SCI. Compared to the nonencapsulated FA, the NPs presented better effects on neuronal loss and prevented neuritis disintegration. In view of the neuroprotector effect and 98% neuronal survival obtained with pretreatment using AF NPs, these results have great clinical relevance concerning SCI. Moreover, the NPs demonstrated locomotive function restoration two hours after injury, while the regular methylprednisolone medicine did not lead to any improvement.

Dong et al [77] observed the efficacy of AF CS hydrogel after traumatic brain injury (TBI) in neuronal cells (Neuro -2a) *in vitro*. As stress after TBI is an important factor for triggering additional brain deficiencies, the inhibition of oxygen-reactive species (ORS) prevent secondary brain

injuries, indicating the neuroprotective effect of the NPs.

Piperine, present in black pepper, was investigated due to its anticonvulsant action for the treatment of neurological diseases, such as Parkinson and epilepsy [78]. Anissan et al [79] evaluated the *in vivo* neuroprotector effect of piperine CS NPs through a pentylenetetrazole-induced epilepsy model. In their study, the NPs reduced cell loss and the astrocytes activation compared to the nonencapsulated compound.

Krill oil (KO) is rich in polyunsaturated fatty acids, has antioxidant properties and is indicated to prevent cardiovascular diseases, but low solubility and oxidative instability of KO limit its biological action [80]. In view of this, CS nanoparticles can represent an alternative to increase utilization of KO. Haider et al [81] developed a CS NPs obtaining 59% encapsulation efficiency. The NPs prevent KO oxidation during two weeks of storage.

Scutellaria (SCU), a plant rich in flavonoids, is widely utilized as treatment for cerebral ischemic disease in Chinese medicine. Liu & Ho [82] developed CS NPs containing SCU for oral and intranasal administration in an adult male C57BL mice model. The verified pharmacokinetic parameters showed increased amounts of SCU in the brain after intranasal administration, indicating the promising application of SCU NPs for sustained release into the brain.

The anticancer action of CS NPs modified with diethylethylamine hydrochloride containing the flavonoid quercetin (QCT) was evaluated *in vitro* by Pedro et al [83]. The 410 nm NPs exhibited cytotoxicity effect against breast cancer cells (MCF-7), providing 15% cellular viability on NPs treat samples, while the samples treated with the nonencapsulated compound showed 50% cellular viability.

Aluani et al [84] evaluated *in vitro* and *in vivo* CS/QCT NPs cytotoxicity effect against HepG2 cells (human and mice hepatocytes). The results indicated lack of cell lysis and the histological, hematological and biochemical samples remained normal, proving the biocompatibility, absence of toxicity and a protector effect of the NPs against HepG2 cells. Baksi et al [85] demonstrated the

greater antioxidant activity and tumor size reduction in lung and breast cancer (A549 and MDA MB 468 cells) of QCT NPs compared to the nonencapsulated compound, indicating the QCT NPs potential as chemotherapy agent.

The synergic effect obtained with the co-encapsulation of QCT and resveratrol (RES) was verified during an *in vivo* study with rabbits by Natesan et al [86]. Because IOP represents a risk factor to glaucoma development and can cause serious eye problems, such as optic neuropathy CS NPs were modified with polyethylene glycol (PEG) for intraocular pressure (IOP) reduction and glaucoma treatment [87]. The results attested better tissue permeation, increase of bioavailability and the prolonged effect of RES on NPs. Also, QCT inclusion increased the RES bioavailability since it competitively inhibits the transmembrane protein P-gp which is associated with multiple drug resistance [88]. The authors attributed these results to CS bioadhesive properties and the PEG role on the action and circulation time improvement of QCT. Also, the nanosystem allowed sustained reduction (8 h) of IOP, with the highest peak at 4 h: 5.5 ± 0.5 mmHg. Therefore, PEG-modified NPs were more effective in RES releasing in comparison, without QCT, the PIO reduction was 48 ± 0.5 mmHg.

. Another co-encapsulation study was performed by Ramaswamy et al [89] involving rutin and curcumin. The *in vitro* results demonstrated sustained release and *in vivo* studies showed an increase in both compounds bioavailability in relation to the nonencapsulated compounds. According to the authors, this fact can be attributed to the size reduction and surface area increase in NPs, leading to greater permeability and elimination rate decrease.

Hesperidin (Hesp) is a compound with anticancer properties and restricted clinical use due to its low water solubility. Lazer et al [90] synthesized CS NPs conjugated with folic acid to carry Hesp, justifying the folate addition by its ability to mediate the internalization of NPs. The system proved to be seven times more effective in inhibiting colorectal cancer cells (HCT 15) proliferation in relation to the free compound. Besides that, the molecular analysis showed

induced apoptosis via pro-apoptotic genes expression.

To increase the solubility and antioxidant effect of Hesp, Cao et al [91] developed CS NPs to carry Hesp using the spray-drying method, which increased about four times Hesp solubility. In addition, the antioxidant activity increased 422% and 238% shown by ABTS and DPPH radical scavenger methods, respectively. The results demonstrated an alternative to Hesp applications in food, biomedicine and medicine areas.

Kumar et al [92] prepared naringenin (Na) CS NPs using the ionic gelation method to improve Na bioavailability after oral administration. The produced NPs showed resistance in SGI, antioxidant activity determined by the DPPH method, and *in vitro* cytotoxicity over lung cancer cells (A549), as well as the absence of toxicity on normal fibroblasts cells (3T3).

Propolis is widely used since ancient times due to its antioxidant [93], antimicrobial [94], anticancer [95] and wound healing [96] activities. Nevertheless, its low bioavailability and aqueous solubility are significant limitations [97]. In this regard, CS nanoparticles containing propolis have been designed in order to improve the propolis bioactive properties. Ong et al [98] evaluated the antibacterial activity of propolis CS NP against *Staphylococcus epidermidis* and *Enterococcus faecalis*. Their results showed biofilm and cellular inhibition by 25% and 70%, respectively, at the concentration of 100 $\mu\text{g/mL}$ for *S. epidermidis*. For *E. faecalis*, the CS NP containing propolis extract was even able to inhibit 90% of bacteria biofilm at the concentration of 100 $\mu\text{g/mL}$.

Elbaz et al [99] explored the anticancer activity of propolis CS NPs with higher solubility and controlled release. The observed cytotoxicity effect on HepG2 cells (human liver cancer) were attributed to apoptosis induction.

Apocynin has been shown therapeutic potential in combating oxidative stress and inflammatory processes, however it has a quick debugging and low bioavailability. Aman, Hashim and Meshali [100] formulated lipidic CS NPs containing apocynin by the emulsification method and observed an increase in oral and intravenous

bioavailability and in the average residence time compared to the non-encapsulated compound.

Accordingly, CS has proved to be a promising polymer to be used in pharmaceutical nanotechnology for the transport and release of different natural products (Figure 2), showing excellent results related to their absorption, bioavailability, and solubility improvement. Moreover, CS NPs are obtained by simple techniques to enhance the biological and physicochemical properties of various compounds and can be administrated via different routes.

Figure 1: Scheme of chitosan nanoparticles obtained by ionic gelling and representation of loaded natural compounds .

3.0. CS NANOPARTICLES FOR CURCUMIN DELIVERY

Cur is one of the most studied natural products for cancer treatment, selected by the *U.S. National Cancer Institute* as a third-generation chemopreventive agent [101]. Even though there are lots of effort to improve Cur therapeutic

effectiveness, Cur is photosensitive and its low solubility, bioavailability, and stability in basic pH hamper clinical advances with the compound [102].

Chuah et al [103] observed CS NPs containing Cur mucoadhesive effect in an *ex vivo* study in colorectal cancer cells (HT-29). The Cur NPs displayed stronger internalization compared to the nonencapsulated compound. Chuah et al [104] reported that Cur may interact with the membrane glycoproteins and CS NPs can loosen the cellular occlusive junctions. Also, the positive charged NPs led to high association rate and cellular internalization as a result of their interaction with the negatively charged cellular membranes

Jardim et al [105] designed CS NPs containing Cur using the ionic gelation method and investigated their *in vitro* cytotoxicity against lung tumors cells (A549). After 72 h, the NPs where inhibited 60% of the cells, while the nonencapsulated compound inhibition rate was 41%.

Udompornmongkol & Chiang [106] found similar results for CS NPs containing Cur in the *in vitro* treatment of rectal colon cancer cells (HT29 and HCT116). The NPs not only demonstrated resistance to GIF, but also showed greater cytotoxicity than the non-encapsulated compound in all tested concentrations. These results were attributed to the higher cell uptake reached due to NPs resistance in the GIF, which permitted the NPs to reach the colon region without significant loses. The promising therapeutic potential of Cur NPs was also demonstrated *in vitro* against cerebral glioma (C6 cells) [107]. The hyaluronic acid coated CS NPs were able to enter the cells via active endocytosis mediated by the trans-membrane glycoprotein CD44, leading to a two-fold dose and absorption dependent cytotoxicity in relation to free Cur.

Zheng et al [108] designed CS NPs with Cur to evaluate their cytotoxicity and genotoxicity on renal embryonic cells (HEK 293). The results indicated the safety of NPs, since neither cytotoxic nor genotoxic effects were observed. To evaluate the *in vitro* apoptotic activity of Cur in different HPV positive cervical cancer cells (SiHa, HeLa, Caski, and C33A), Khan et al [109] synthesized

CS NPs by the emulsion cross-linking technique. Despite the negative cytotoxicity effect on SiHa cell line, the NPs presented a time and concentration dependent antiproliferative activity against Hela, Caski, and C33A cells. Differently, Fachi et al [110] designed NPs with trimethyl CS (TMC) employing an emulsification-solvent evaporation method to carry Cur, which proved to be resistant to GIF and possessed cytotoxicity to cervical tumor cells (SiHa) and biocompatibility to VERO cells derivative from African green monkey kidney [111].

Abruzzo et al [112] investigated, by means of fluorescence techniques, the internalization of Cur in normal intestinal cell lines (I407) and colorectal cancer cells (HT29), noting no toxicity to I407 cells while HT29 cells were inhibited. These results demonstrated the selective Cur release in cancer cells compared to normal ones.

Duse et al [113] verified Cur CS NPs toxicity against A59 lung cancer cells after 4 h incubation and subsequent exposure to LED light with 132.1 J cm^{-2} using photodynamic therapy (PDT), in which the light excitement of specific photosensitizers produce ORSs, allowing the verification of the cytotoxicity effect with tissue specificity [114]. This result revealed Cur as an efficient photosensitizer, since it led to ORS generation, damaging the tumoral cells and led to cell apoptosis.

Hashemian et al [115] led an *in vivo* study to evaluate the neuroprotector effect of CS and sodium alginate encapsulated Cur in a pentylenetetrazole-induced model of chronic epilepsy. The results showed the anticonvulsant activity of NPs and the histologic tests indicated that the NPs reduced the cell death rate and affected neuroglia, confirming the improved neuroprotective property of Cur in its nanoencapsulated form.

Yadav et al [116] performed *in vivo* and *in vitro* studies with Cur CS NPs, observing the sustained release of the compound and dose-dependent cytotoxicity activity against HeLa cells. The cellular capture led to DNA damage, cell cycle blockage and high level of ORS generation, followed by cell apoptosis. In addition, tests on

zebrafish embryo models showed the absence of toxicity to healthy cells, proving the NPs safety.

Beyond the studies that report CS as the main polymer in NPs formulation, there are also those that use CS as a coating agent. Polycaprolactone (PCL) NPs coated with CS were designed to encapsulate Cur via the nanoprecipitation technique and were evaluated for *in vitro* treatment of oral cancer. The author confirmed the NPs mucoadhesiveness through permeation studies using esophageal mucosa. Also, antiproliferative effects were observed after 48 h and the nonencapsulated compound presented greater cytotoxicity effect against oral squamous cell carcinoma (SCC-9) compared to the NPs [117].

Loch-Neckel et al [118] also developed CS-coated PCL NPs containing Cur to assess their *in vitro* cytotoxicity against melanoma cells (B16F10). The results indicated a concentration-dependent reduction in cell viability via apoptosis and a decrease in the expression of metalloproteinases associated with the migration and proliferation of cancer cells. *In vivo*, the NPs were effective in the reduction of tumor nodules in metastasis of lung cancer cells (C57BL/6).

Cur CS NPs evaluation studies were conducted to improve Cur properties for oral administration, since this compound has low bioavailability and aqueous solubility [119]. Vijayakurup et al [120] led *in vivo* pharmacokinetic studies with Cur CS NPs, which presented greater cellular uptake and Cur retention comparing to the nonencapsulated form, verifying the sustained release of Cur for 180 h. The NPs also demonstrated pharmacological safety, due to their nontoxic properties and, moreover, were able to reduce tumoral incidence and multiplication in pulmonary adenocarcinoma induced by benzo(a)pyrene.

The slow and sustained release of CS NPs were also observed in the studies conducted by Hasan et al [121], in which the authors evidenced CS NPs potential as Cur carriers, aiming at the oral administration, since the obtained nanosystem allowed 90% of compound retention on simulated gastrointestinal fluids.

Besides the prolonged and sustained release, the antitumor effectiveness of CS NPs containing Cur was also proven by Elbially & Elfatah & Khalil

[122]. The *in vitro* results showed the NPs therapeutic effect against colon (HCT-116) and breast (MCF-7) carcinogenic cells. The morphologic analysis of the cancer cells submitted to NPs demonstrated Cur internalization through cellular aggregates and condensed cytoplasm, confirming the NPs anticancer activity.

Another concerning medical issue is antimicrobial resistance, since the number of available drugs for its treatment has decreased continuously, highlighting the importance of new alternatives for the control of microbial infections [123]. An important effect of antibacterial activity is the synergism between different antimicrobials that can potentiate pathogen inhibition [124].

Among the vast therapeutic properties of Cur, its antibacterial effect stands out. The ORS production promoted by Cur leads to cell death via membrane transport interruption [125] and cell wall and DNA damage [126].

Pseudomonas aeruginosa is an opportunistic microorganism common in immunosuppressed, neutropenic patients or diseases like cystic fibrosis. Furthermore, its adaptive antibiotic resistance mechanisms enable recurrent infections. Therefore, alternative therapies for *P. aeruginosa* infections prevention and treatment are needed [127, 128]. To achieve this, Samrot et al [129] produced CS/Cur NPs smaller than 50 nm, which exhibited *in vitro* prolonged release and antibacterial activity against *P. aeruginosa*.

Niranjan et al [130] demonstrated the *in vitro* and *in vivo* antibacterial activity of CS/Cur NPs on transdermal adhesive. The NPs were tested against Gram-positive and Gram-negative bacteria often found in wounds, such as *Bacillus subtilis*; *Staphylococcus aureus*; *Escherichia coli* and *P. aeruginosa*, and demonstrated activity against all tested microorganisms. Likewise, the NPs showed biocompatibility to fibroblast cell lines (NIH3T3) and were able to absorb exudate from wounds, reducing microbial colonization and reaching a healing rate higher than the mupirocin treated control group.

Kumar et al [131] also obtained antibacterial CS/Cur NPs and tested them against *S. aureus*, *E. coli*, and *P. aeruginosa* strains, demonstrating NPs activities higher than the commercial drug

tetracycline. The NPs promoted the prolonged release of Cur, enabling a connection with the protein receptors on bacteria surface that facilitated the entry into the bacterial cells.

Vishwakarma et al [132] reported a higher *in vitro* antimicrobial activity of CS/Cur NPs against *E. coli* and *S. aureus* compared to a commercial drug. In their study, vancomycin was effective only against *S. aureus*, while the Cur NPs inhibited both strains in lower concentrations than the nonencapsulated compound. Also, there was not significant difference between NPs and vancomycin on *S. aureus* treatment.

Agel et al (2019) [133] tested the *in vitro* antibacterial activity of CS/Cur NPs against *Staphylococcus saprophyticus* and *E. coli* through PDT. The antibacterial phototoxicity of the CS/Cur NPs was verified through bacterial growth reduction (survival rate lower than 0,1% in both strains). Additionally, the morphological alterations after PDT onto bacterial structures were observed with electron microscopy.

Barbinta-Petrascu et al [134] developed hybrid folic acid-CS NPs containing Cur and obtained NPs with size of 41 nm and zeta potential of -59,5 mV. The antimicrobial activity was evaluated against *E. coli* and membrane morphological alterations, cytoplasmatic content loss, and consequent apoptosis were observed.

Another study involving hybrid CS NPs was developed by Rao-Kummara et al [135], employing silver and sodium hyaluronate by complexation. This structure inhibited *E. coli* growth at higher rates compared to Cur and sodium hyaluronate.

CS/Cur NPs were designed by self-assembly for inhalation aerosol administration of Cur and their antimicrobial activity was evaluated by microdilution in broth. The formulation showed biocompatibility to pulmonary epithelium cells and was effective against *Stephanomonas maltophilia* and *Burkholderia cepacia* in a minimum inhibitory concentration (MIC) of 100 µg/mL, and *Klebsiella pneumonia* and *P. aeruginosa* in MIC of 150 µg/mL [136].

Silver nitrate-CS NPs containing Cur were produced using copolymerization and acrylamine reticulation in CS. The study evaluated the NPs

antibacterial activity with the method of disc diffusion against *E. coli* and *S. aureus*, verifying bactericidal effect on 99.99% [137]. CS/Cur NPs with alginate and starch were designed by desolvation, and the antimicrobial activity evaluation was assessed against *Streptococcus mutans*, achieving an inhibitory effect of 95.28% [138].

The mentioned works evidenced the importance of the alternative therapy developments regarding bacterial resistance and demonstrated the great potential of CS nanostructures containing Cur both *in vitro* and *in vivo*. Another drug carrier types with therapeutic application are CS nanohydrogels. These systems have properties that justify their use for biomedical applications, including biocompatibility, biodegradability and physicochemical improvements of the compounds [139]. According to El-banna et al [140], CS presents hydrogel-based applications potential. It can be employed as gel absorption intensifier, mainly due to its mucoadhesive characteristics that facilitate the permeation into the targeted cells. This way, CS hydrogels have been tested as an alternative for the Cur application in healing processes [1].

Niranjan. et al [130] demonstrated the CS/Cur NPs action on biofilms on epidermal wounds using both *in vivo* and *in vitro* methods. The NPs had an average diameter of 20 nm and presented biocompatibility, injury healing capacity and tissue regeneration superior to the commercial ointment mupirocin. The effect was attributed to the cell proliferation increase, collagen deposition, and antibacterial activity against bacteria found in cutaneous wounds.

Liu et al [141] also designed CS/Cur NPs biofilm and the *in vitro* and *in vivo* tests demonstrated Cur sustained release, wounds repairing and greater healing rate compared to the free compound. The biofilm potential is ascribed to their transdermal adhesives that promotes wound healing and tissue regeneration. Rezaii, Oryan & Javeri [142] tested CS/Cur NPs using an *in vivo* model of cutaneous wound healing. The authors described the healing via regulation of the expression of the cell growth factor TGF- β 1 and its antagonist Smad7. In their study, the topic

application of CS/Cur NPs provided positive regulation of $\uparrow$ TGF- β 1 and $\downarrow$ Smad7 factors, resulting in the acceleration of the cellular growth via new blood vessel formation, collagen matrix deposition and therefore, increased epidermis thickness.

Cheng et al [143] designed a CS/Cur nanohydrogel aiming at its topic application for glaucoma treatment. The studies demonstrated that the NPs at concentrations of 20 μ M did not cause any toxicity and reduced *in vitro* oxidative stress. The effect was ascribed to apoptosis reduction and inflammation-related genes (*TNF*; *IL-6*; *IL-1 α*) expression. According to the authors, the oxidative stress provided to the ocular trabecular mesh cells plays an important role in glaucoma pathogenesis, since it compromises the aqueous humor drainage, leading to an eye pressure increase.

CS NPs for Cur delivery proved to be promising, since they improve its solubility, bioavailability and therapeutic effectiveness of the compound. Both *in vitro* and *in vivo* results referenced in this review evidence the high potential of CS NPs, which constitute a viable strategy to add value to the widely known and commercialized Cur. Table 1 shows some recently described biological activities of Cur CS NPs.

Table 1- Chitosan nanoparticles designed for curcumin delivery and main results.

Conducted Study	Assay	Main results	Reference
<i>In vitro, in vivo and ex vivo</i>	Gel physicochemical characterization; permeation and skin irritability tests	Size: 84 nm; Expressive permeability; absence of skin irritation; Wound healing <i>ex vivo</i>	[144]
<i>In vitro and ex vivo</i>	Physicochemical characterization and antitumoral activity against MCF7 cells (breast cancer)	Size: 119-127nm; EE%: 96%; release profile (7 days/ pH= 5.0): 90%; cytotoxicity effect greater than the free compound	[145]
<i>In vivo</i>	<i>In vivo</i> oral bioavailability evaluation and gastrointestinal release <i>in vitro</i> (FGS/FIS) of nanocapsules	Size: 197nm; EE%: 92%; greater bioavailability compared to the free Cur; Low resistance to SGF/SIF	[102]
<i>In vivo</i>	Antioxidant activity against cadmium induced oxidative stress	Antioxidant action due to inhibited toxicity by cadmium and enzymatic index increase (catalase and superoxide dismutase)	[146]
<i>In vivo</i>	Cytotoxicity activity against cervical tumoral cells	Increase of cell capture and antitumoral activity against SiHa, CaSKi and HeLa	[147]
<i>In vivo</i>	Antifungal activity against <i>Candida albicans</i>	Stronger anti- <i>Candida albicans</i> effect than chlorhexidine; mouth ulcer decrease and better healing compared to benzidamine	[148]
<i>In vivo</i>	Cytotoxicity effect against lung cancer (H1299 cells) in animal model induced by benzopyrene	Size: 170-200 nm; Sustained release; increase of bioavailability; expressive cell capture; greater reduction of H1299 cells viability	[120]

compared to free Cur			
<i>In vivo</i>	Healing and cytotoxicity activity evaluation on human keratinocytes cells (HaCaT)	Size: 140 nm; EE%: 78%; high healing activity (closing > than 90%); absence of cytotoxicity over keratinocytes	[149]
<i>In vitro</i>	Transdermal permeability and cytotoxicity tests on human keratinocytes cells (HaCaT)	Size: 167-251 nm; EE%: 80%; greater transdermal permeation and cellular viability than free compound	[150]
<i>In vivo</i>	Cytotoxicity assays in breast cancer cells (MCF-7)	Size: 130 nm; EE%: 87.5%; cytotoxicity in MCF-7 cells: NPs (70%); free Cur (71.6%)	[151]

This review gathered encouraging results of CS NPs for the nanoencapsulation of natural compounds. The described works embraced improvements on drug solubility, permeability, stability, bioavailability, biodistribution, and long-lasting residence in the body. The differentiated intracellular traffic of drug-loaded CS NPs compared to free drug explains the NPs potentiation of the recognized biological effects of natural compounds and represents a new pathway for the pharmaceutical industry.

More research is needed on the pharmacokinetics of drug release from CS NPs under different conditions and in different organs, on the synergetic effects of drugs encapsulated in NPs or the specificities of CS NPs on cancer cells. However, high expectations for clinical applications are justified from what we already know from the reports of chitosan nanoparticles loaded with various drugs.

CONCLUDING REMARKS

Concerning the biological properties of natural compounds, several studies have reported the design of nanoparticles, especially the ones containing chitosan, to improve their delivery and targeting, aiming to circumvent the biological barriers and enhance compound absorption and bioavailability, thereby potentiating their therapeutic effect.

The high potential of natural compounds on prevention and treatment of age-related diseases and cancer is evident. However, their potentiality is suppressed in conventional delivery and dosage forms. Low solubility and cellular permeability, as well as chemical instability of the natural compounds negatively modulate the biopharmaceutical phase. As a consequence, the pharmacokinetic parameters are often poor, evidencing low bioavailability and short half-life, hindering the *in vivo* therapeutic efficacy. Here, the nanoencapsulation represents an important tool for effectively exploiting the potential of natural compounds.

CONSENT FOR PUBLICATION

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The authors would like to thank CAPES for the scholarship given to L. de A. Campos (88882.464225/2019-01).

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