



Investigations of curcumin release from chitosan nanoparticles by ultrasound waves and TPP concentration effects

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Abstract

The major active agent of the turmeric plant, which has extensive pharmacological activities, is curcumin, a dietary polyphenolic compound. As a potential medium for drug delivery, a nanoparticle chitosan polymer has been created. Chitosan is a low-toxic, biodegradable, biocompatible and secure polymer used to produce nanoparticles. Chitosan nanoparticles have been prepared using the process of ionic crosslinking. The mean particle size of chitosan nanoparticles was 180-200 nm in the range and curcumin encapsulation efficiencies were 82%. Ultrasound is a non-invasive means to administer medicines to the darkest areas of the human body. Cumulative release was studied in order to study how ultrasound allows curcumin to be extracted from chitosan nanoparticles. The main aim is to release curcumin, as an anti-inflammatory drug, from the Chitosan nanoparticles using the ultrasonic waves. Based on the results, utilizing the ultrasonic waves improved the drug release from the chitosan nanoparticles. The effect of ultrasound and tripolyphosphate (TPP) concentration on the release behavior of curcumin from chitosan nanoparticles is discussed in this paper. Using the ultrasound wave, drug release from chitosan nanoparticles was improved

Key words: Chitosan; nanoparticles; ultrasound; release; curcumin

1. Introduction

Because of its unusual biological properties, chitosan is a polysaccharide that is commonly used in medicinal and biomedical preparations. It is a polymer that is secure, biocompatible and biodegradable. It was approved for wound dressing by the Food and Drug Administration (FDA) [1-7]. Composed of β -2-amino-2-deoxy-D-glucopyranose, this biopolymer is (glucosamine units). The monomer units are joined by glycosidic linkages of β (1-4) (Fig. 1) [3,4].

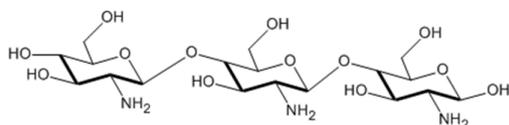


Fig. 1. Chitosan polymer chain

Chitosan has distinctive characteristics such as biocompatibility, biodegradability, safety, and muco adhesiveness. Chitosan's medicinal use is due to the amino groups on its chain. Much focus has been

given to the application of chitosan as a biomaterial to provide controlled release systems [5,6]. In the delivery of therapeutic agents, including genetic material, proteins, and chemotherapeutic agents, ultrasound has a promising function. The mediators by which the energy of comparatively non-interactive pressure waves is concentrated are cavitating gas bodies such as microbubbles to create forces that are capable of raising cell membrane permeability and can shear the vesicles carrying drugs. In specific, the presence of microbubbles thus increases the delivery of genetic materials, proteins and drugs. It is generated by exciting at a proper frequency an ultrasonic transducer (usually based on a piezoelectric component or on an electromagnetic inductor) able to convert the electrical signal into a mechanical displacement [6-7]. Turmeric, a spice that has long been recognized for its medicinal properties, has received interest from both the medical/scientific world and from culinary enthusiasts, as it is the major source of the

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Receive Date: 05 February 2021, Revise Date: 25 February 2021, Accept Date: 18 April 2021

DOI: 10.21608/EJCHEM.2021.61591.3326

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polyphenol curcumin. It aids in the management of oxidative and inflammatory conditions, metabolic syndrome, arthritis, anxiety, and hyperlipidemia. It may also help in the management of exercise-induced inflammation and muscle soreness, thus enhancing recovery and performance in active people [5,7]. Ultrasound devices are usually composed by a generator, a compensating amplifier and a transducer [8-9]. The degradation rate of bioerodible polymers [9] is impaired by ultrasound. Curcumin is derived from *Curcuma longa*'s rhizome as a hydrophobic polyphenol. For the prevention of inflammatory diseases in Asia, curcumin is commonly used as a culinary seasoning and a topical medicine. Curcumin has antioxidant, anti-inflammatory, anti-carcinogenic, and antimicrobial properties, blocking the development of a broad spectrum of tumor cells [7].

Canavese et al. used of ultrasound in the presence of both soft and solid-state nanoparticles (NPs) against tumor cells or tissues and with a special emphasis on the sono dynamic treatment (SDT) [10]. Furthermore ultrasound was used for the treatment of numerous pathologies, such as a remedy of soft tissue injuries, for the acceleration of wound healing, for the resolution of edema, or for the softening of scar tissues [11-15].

Several cancer-related clinical trials have discussed the pharmacokinetics, protection and effectiveness of human curcumin [15-18].

The main aim is to release curcumin, as an anti-inflammatory drug, from the Chitosan nanoparticles using the ultrasonic waves. Based on the results, utilizing the ultrasonic waves improved the drug release from the Chitosan nanoparticles. The effect of ultrasound and TPP concentration on curcumin release as a drug model loaded onto chitosan nanoparticles was analyzed in this research.

2. Experimental

2.1. Materials

Chitosan was purchased with a MW of 60*10³ from Sigma-Aldrich (USA) and the degree of deacetylation was 93%. Acquired curcumin. Merck also purchased tripolyphosphate (TPP), polysorbate 80 (Tween 80), acetic acid and other reagents.

2.2. Preparation of TPP-Chitosan nanoparticles

In ethanol, curcumin, a badly soluble drug in water, was dissolved (2:10). The ionotropic gelation process for the preparation of nanoparticles is based on the electrostatic interaction of negatively and positively charged molecules, such as polyanionic and cationic polymers [19].

By dissolving it in 1 percent acetic acid and polysorbate 20, the chitosan solution was prepared as an emulsifier, then ethanol curcumin (2 percent v/v) was blended into the chitosan solution. The aqueous phase (chitosan solution) was combined with this oil phase by probe sonication, applying a frequency of 30 kHz for 5 min. TPP solutions were lowered into the chitosan solution using a spray cartridge. The nanoparticles were then washed repeatedly with purified water, accompanied by vacuum drying for 12 h [20-22].

2.3. Drug content of nanoparticles

Drug loading was calculated by ultracentrifugation of the solution of nanoparticles at 40000 rpm for 45 min, and then the volume of curcumin in the supernatant was measured at 423 nm using a spectrophotometer (Agilent 8453). It is possible to quantify loading utility (DL) percent as follows:

$$DL \% = [(total\ amount\ of\ drug - free\ amount\ of\ drug) / total\ amount\ of\ drug] \times 100 [23].$$

2.4. In vitro release of curcumin from chitosan nanoparticles

The experiment was conducted to determine the volume of released curcumin using a static horizontal Franz diffusion cell. At a molecular weight cutoff of 1200 D with a surface area of 2.0 cm², the cellulose acetate membrane was used and placed on the Franz diffusion cell. The accurate amount of the receptor medium was 50 ml and consisted of an aqueous solution of physiological saline, phosphate buffer saline (PBS) and 20% ethanol at pH 7.4, combined with a magnetic bar at 500 rpm to homogenize the medium. 2 ml of chitosan nanoparticles is mounted into the donor compartment separately. Using a syringe needle, 1 ml of the release medium was sampled and the same amount of fresh receptor medium was substituted at some time intervals. As

previously described [24-16], the samples were analyzed using a spectroscopic process.

2.5. Morphological characterization of chitosan nanoparticles

Until being analyzed, the samples were sonicated for 5 min in the Wisd bath, WUC-M sonicator (LOVO, China) and were immediately used for measurements. Photon correlation spectroscopy (PCS) using the Malvern Zetasizer ZS series and the Scattering Particle Size Analyzer has defined the size and zeta potential of prepared nanoparticles (Malvern Co, UK). The samples of nanoparticles contaminated with 2% (w/v) phosphotungstic acid were used to capture transmission electron microscopy (TEM) (Zeiss-EM 10C- Germany) microphotographs. Surface morphology study of nanoparticles was carried out using electron microscopy scanning (SEM, KYKY-EM3200, China).

3. Results and discussion

3.1. Size of chitosan nanoparticles study

In this analysis, because of ionic interactions between positively charged amino groups of chitosan and negatively charged counter ions of TPP, TPP-Chitosan nanoparticles were manufactured. It is important to remember that TPP is a non-toxic and multivalent anion capable of forming cross-links [3, 4]. Chitosan-TPP nanoparticles loaded with curcumin were prepared and investigated in terms of bulk, charge and form. The typical nanoparticles were 160 ± 10 nm in diameter.

The SEM image is seen in the figure of the samples containing drug-loaded chitosan nanoparticle. The picture revealed a spherical shape with a smooth surface with a nanometric scale of particle size. The particle size displayed by SEM photos of curcumin-loaded chitosan nanoparticle concurs with the findings obtained by TEM (Fig. 3) and the PCS outcome. The spherical structure and no aggregation of the nanoparticles are representative of all applied techniques. It was observed that the average diameter determined by both the SEM and TEM was about 200 nm. The drug release findings have been seen in (Fig. 4).

3.2. Degradation rate of chitosan nanoparticles

The degradation rate of chitosan nanoparticles was affected by ultrasound. Ultrasound boosted the amount of nanoparticles produced by the drug. Ultrasound-generated cavitating gas is a moderator by which the energy of comparatively non-interactive pressure waves is concentrated to create forces that disrupt cell membranes and shear the vesicle-containing drug. The involvement of microbubbles therefore significantly enhances the drug's delivery [25]. As stated in the fig.4. Using ultrasound, drug release from nanoparticles has improved by up to 90%. Therefore, ultrasound increases the volume of diffused curcumin molecules from the polymer matrix during the process of drug release. At the end of the ultrasonic application, the curcumin was released by the polymer matrix as drug molecules to maintain balance with the environment [27-28].

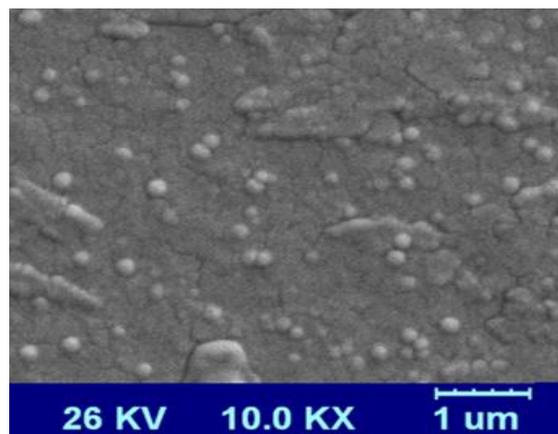


Fig. 2. SEM image of chitosan nanoparticles.

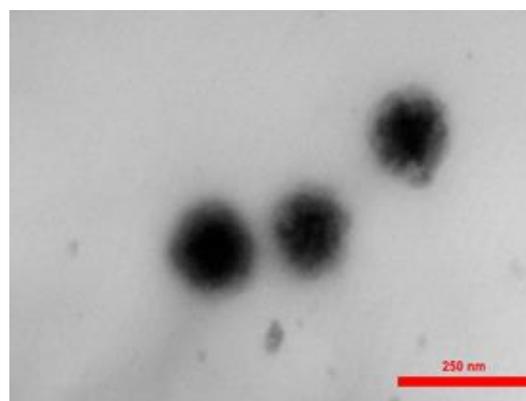


Fig. 3. TEM image of chitosan nanoparticles

Various TPP concentrations were used and their effects on the release of curcumin from the nanoparticles were investigated (Fig. 5). With the addition of TPP concentration, the curcumin release of the NPs decreased as the volume of TPP used in the formulation increased (Fig. 5). The sum of TPP was increased in this analysis, while the quantity of chitosan and the liquid volumes were stable. In general, crosslinking agents have been used to decrease the mobility of chitosan in the formulation of Chitosan NPs and improve its physicochemical properties, such as stability. TPP is a polyanion that interacts in chitosan with the amino groups to form a gel during the ionic gelation procedure by inter- and intra-molecular cross-linkages [28-31].

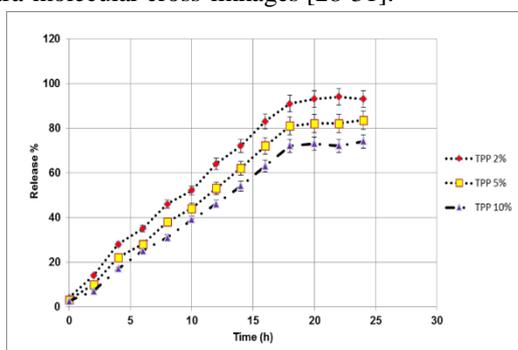


Fig. 5. Profiles Release of curcumin from chitosan nanoparticles with different percent of TPP in phosphate buffer in pH=7.4

4. Conclusion

Nanomedicine has created a groundbreaking new field in medicine for the use of nanoscale drug delivery materials over the past decade. Tripolyphosphate (TPP) chitosan nanoparticles were prepared using an ionic crosslinking process. The size and morphology of the chitosan nanoparticles obtained in the range of 180-200 nm were determined by TEM and SEM. Curcumin encapsulation efficiencies have been estimated to be 82%. By adding guided energy, e.g. ultrasound, the performance of drug delivery has been improved. Ultrasound increased the volume of curcumin molecules diffused at a higher rate, up to 90%, from the polymer matrix. Ultrasound-based therapies and diagnostics play a fundamental role in the scientific research panorama and in the clinical context. Several kinds of both soft and hard-material NPs were proposed for assisting US-based therapies. With TPP concentration applied, the curcumin release of the NPs decreased. We therefore propose that the use of ultrasound can increase the release of drugs

from polymeric nanoparticles, which can be used for selective systems of drug delivery.

5. Conflicts of interest

The authors declare no conflict of interest. This article does not contain description of studies with the involvement of humans or animals performed by any of the authors.

6. Acknowledgments

This work was supported by the Shahid Chamran University of Ahvaz (project no. SCU.SC98.31316).

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