Evaluation of physiochemical properties of curcumin-loaded Chitosan Tripolyphosphate Nanoparticles

Mirza Ali Mofazzali-Jahromi [1] Majid Pirestani [2] Sharafaldin Al-Musawi [3] Mehdi Fasihi-Rammandi [4] Kazem Ahmadi [5] Zuhair Mohammad Hassan [6] Mahdi Kamali [7] Hajar Rajayi [8] Reza Mirnejad [9]

PhD of Immunology [1] , Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran. Assistant Professor Department of Parasitology [2] , Tarbiat Modares University, Tehran, Iran. PhD of Nanobiotechnology [3] , Tarbiat Modares University, Tehran, Iran. Assistant Professor Department of Biotechnology [4] , Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran. Professor Department of Immunology [5] , Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran. Professor Department of Immunology [6] , Tarbiat Modares University, Tehran, Iran. MSc of Immunology [7] , Tarbiat Modares University, Tehran, Iran. Assistant Professor Department of Nanobiotechnology [8] , Nanobiotechnology Research Center , Baqiyatallah University of Medical Sciences, Tehran, Iran. Associate Professor Department of Medical Bachteriology [9] , Molecular Biology Research Center , Baqiyatallah University of Medical Sciences, Tehran, Iran.

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Original Article

Abstract

Introduction: Curcumin is a water-insoluble agent with some anticancer and antimicrobial effects; while, solubility is essential for a medicine to reach target cells. This study set out to use chitosan tripolyphosphate nanoparticles (CTN) for making curcumin water-soluble, and delivering the required dosage of it to target cells.

Methods: In this study, the nanoparticles were fabricated from chitosan and tripolyphosphate using the ionotropic gelation method. The emulsified solution of curcumin was also loaded during the fabrication of CTNs. Then, the size and loaded amount of the nanoparticles were measured with a zeta potential analyzer; in addition, the shape and size of them were evaluated through atomic-force microscopy. Moreover, the amount of drug loaded chitosan nanoparticles was measured, using a spectrophotometer.

Results: According to zeta analyzer results, the size and charge of the curcumin-contained chitosan tripolyphosphate nanoparticles were 165 ± 5 nm and $+7\pm2$ mV, respectively. According to the AFM findings, the obtained nanoparticles had spherical shape. Spectrophotometer showed that the curcumin loaded into tripolyphosphate nanoparticles was $67\pm1\%$.

Conclusion: Our study suggests that a significant amount of water-soluble curcumin can be loaded into CTNs using ionotropic gelation method.

Key words: Nanosystem, Nanoparticles, Curcumin

Correspondence:
Reza Mirnejad, PhD.
Department of Medical Bacteriology, Molecular Biology Research Center, Baqiyatallah University of Medical Sciences.
Tehran, Iran
Tel:+98 21 82482554
Email:
rmirnejad@bmsu.as.ir

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Introduction:

The treatment of cancer and infections induced by antibiotic-resistant bacteria is a crucial medical issue. Today, the development of nanotechnology has brought the fabrication and optimization of drug release nanosystems to special attention (1). These types of nanosystems are used to deliver medicine to drug-resistant infectious and cancerous cells and tissues. The combination of nanodrugs has offered several therapeutic advantages to drug carrier

nanosystems. Curcumin is a natural yellow phenolic substance that is obtained from the root of Curcuma longa. An impure curcumin composition is used in food industries as turmeric spice for flavoring and coloring. Studies on the therapeutic properties of curcumin have shown that loading thissubstance into nanoparticles improves its antimicrobial (E. coli, S. aureus, B.subtilis, and B. cereus.) and anti-fungal (A. niger, Pe. notatum, and S. cerevisiae) properties (2,3). In addition, curcumin has been used as an antibiotic against Y. enterocolitica, B. cereus, and other microbes (4,5).

Curcumin inhibits the formation and growth of tumors through influencing the courses of mutagenesis, expression of oncogenes, cell cycle regulation, apoptosis, tumor formation and metastasis. This drug inhibits cellular proliferation through the inhibition of some transcription factors (6,7). Despite all aforementioned antimicrobial and anticancer properties of curcumin, it is a water-insoluble drug with short half-life. Therefore, nanotechnology has been used to increase its stability and make it water-soluble (8). Drug carrier nanoparticles are fabricated from substances such as chitosan, albumin, Poly Lactic-co-Glycolic Acid (PLGA), and polyethylene glycol. Among these substances, non-toxic, biodegradable, biocompatible, and non-threatening properties of curcumin have made it a very interesting substance. According to the studies, chitosan can improve the immune system of animals and plants (12). This substance is resistance to microbial infections (9-13). Chitosan has been used as cholesterol reducer and wound healer. Due to the ability of the cationic chitosan molecule in interacting with negatively charged surfaces, it is used to deliver drug to target cells (14). Concerning these properties, chitosan is a good candidate for fabricating a drug delivery system (15,16).

Regarding that previous studies have used different techniques for loading curcumin into chitosan, the present study set out (i) to load higher amounts of curcumin into chitosan nanoparticles by applying the ionotropic gelation method to water-emulsified chitosan tripolyphosphate nanoparticles, and (ii) to evaluate physiochemical properties of curcumin-loaded chitosan tripolyphosphate nanoparticles (CUR-loaded CS/TPP NPs).

Methods:

This descriptive-analytical study evaluated the physiochemical properties of CUR-loaded CS/TPP NPs.

Materials and Methods

The curcumin, chitosan, and tripolyphosphate substances used in this study were the products of Sigma Company (USA). Other employed substances were the products of following companies: hydrochloric acid and tween-80 (Merck, Germany), double distilled water (Zolal, Iran), FAlcon-15 (SPL, Korea), thin aluminum foil (Zarif, Iran), and Amicon cut-off filter 100 (Millipore, Germany). For the measurement and analysis of nanoparticles, Zeta Analyzer (Malvern, UK), AFM (NVB-100, Olympus, Japan), and spectrophotometer (Amersham Biosciences, Uppsala, Sweden) were used.

Preparation of Solutions

To prepare chitosan stock, 1mg chitosan was mixed with 1mL distilled water (pH 30-5) through intense vortex dispersion. In addition, 1mg tripolyphosphate was mixed in 1mL distilled water.

Stocks were wrapped in aluminum foils and placed in a refrigerator at 4°C. In addition, 1 mg curcumin was mixed with 1mL ethanol and/or dimethyl sulfoxide, placed in 1.5mL tubes, wrapped in aluminum foils, and frozen at -20°C (17).

Fabrication of CUR-loaded CS/TPP NPs

Curcumin stock was first melted down. Then, 5µL Tween-80 was added to each mL of curcumin-ethanol or curcumin-dimethyl sulfoxide solutions. While the solution (in a 10mL beaker) was mixing on an electric mixer (500 RPM) in a magnetic dark environment, 1mL distilled water was added. After that, 1mL chitosan stock solution was added to it drop wise. In the next stage, the curcumin/tween-80 solution was also added drop wise to the mixing chitosan. Then, 100µL tripolyphosphate was gradually added to the transparent chitosan-curcumin solution. A period of 45 minutes was given for unique loading of curcumin into the chitosan tripolyphosphate nanoparticles solution. To eliminate large impurities, the solution was centrifuged (4000 RPM) in a dark environment.

The supernatant was stored in a dark environment for condensation in the next stage. To this end, nanoparticles were placed in Amicon filter device (Millipore, Germany) with cut-off points at 100K, and centrifuged at 3,500 RPM. To maintain nanoparticles for a long time, the CUR-loaded CS/TPP NPs were powdered (18).

Evaluation of Size and Charge of CUR-loaded CS/TPP NPs

To measure the size and charge of CUR-loaded CS/TPP NPs, 1µL of it was solved in 1mL double distilled water, analyzed with the Zeta Analyzer (Malven, UK). At the end, results were recorded.

Evaluation of Size and Shape of CUR-loaded CS/TPP NPs

The shape and size of nanoparticles were evaluated with AFM (NVB-100, Olympus, Japan). To perform this test, some drops of CUR-loaded CS/TPP NPs were placed on a clean glass slide and remained to dryness in a dark place at room temperature. The shape and size of CUR-loaded CS/TPP NPs were then investigated using the AFM.

Evaluation of Curcumin Content Loaded into CTN

The CUR-loaded CS/TPP NPs solution was centrifuged at 20,000 RPM for sedimentation of nanoparticles. Then, the supernatant (which did not contain loaded curcumin) was collected and its density was measured with the spectrophotometer (Amersham Biosciences, Uppsala, Sweden). Following formula was used to measure the amount of drug loaded into chitosan tripolyphosphate nanoparticles (19).

Loaded drug (100%) = [(initial amount of drug - unloaded drug)/ (initial amount of drug)] ×100

Statistical Analysis

For the aim of statistical analysis, all experiments were repeated for three times. In addition, means with $P<0.05$ were considered as significant. For statistical analysis, Graphpad (Prism, USA) was used.

Results:

The CUR-loaded CS/TPP NPs are water soluble. Curcumin is a water-insoluble substance that produces a non-transparent yellow solution. The CUR-loaded CS/TPP NPs are water soluble and produce a transparent yellow solution (Figure 1).

Figure 1. Water=soluble CUR-loaded CS/TPP NPs

Curcumin is insoluble in water and its solution is yellowish and turbid (A); whereas, CUR-loaded CS/TPP NPs could be dissolved in water and its solution is transparent (B).

Size and Charge of CUR-loaded CS/TPP NPs

Size and Charge of CUR-loaded CS/TPP NPs were measured with the zeta analyzer. According to zeta analyzer, the charge of the CUR-loaded CS/TPP NPs was +7±2 mV. In addition, the size of the CUR-loaded CS/TPP NPs was 165±2 nm.

Size and Shaper of CUR-loaded CS/TPP NPs

Size and shape of CUR-loaded CS/TPP NPs were investigated with AFM. Results from AFM were similar to zeta analyzer regarding the size of the CUR-loaded CS/TPP NPs (165±2nm). Moreover, the AFM showed that the CUR-loaded CS/TPP NPs had spherical shape (Figure 2).

Amount of Curcumin Loaded into CTN

Our findings showed that a significant content of curcumin was loaded into the chitosan tripolyphosphate nanoparticles. This finding showed that 67±1% of curcumin content was loaded into the tripolyphosphate nanoparticles. Figure 2, indicates probabilistic neural network implemented in MATLAB software.

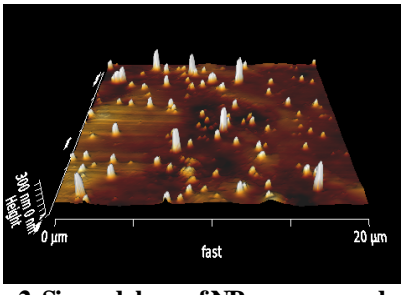

Figure 2. Size and shape of NPs were assessed using AFM

According to AFM, the size and shape of CUR-loaded CS/TPP NPs were 165±5 nm and spherical

Conclusion:

In this study, curcumin was loaded into chitosan tripolyphosphate nanoparticles using ionotropic gelation method. In addition, its physiochemical properties including water-solubility size, shape, charge, and content of loaded drug were measured. According to our findings, the size and charge of the CUR-loaded CS/TPP NPs were 165±5 nm and 7±2mV. Moreover, the CUR-loaded CS/TPP NPs were completely transparent, indicating its water-solubility. It was also observed that 67±1% of curcumin content was loaded into the tripolyphosphate nanoparticles. Das et al. used chitosan to encapsulate curcumin. They used alginate for optimization; whereas, we used tripolyphosphate as the binder, resulting in the fabrication of the CUR-loaded CS/TPP NPs sized 165±5 nm. In comparison to our study, the size of chitosan nanoparticles in Das et al.'s study was smaller (100±20). As it is said in the introduction, curcumin is a fat-soluble and water-insoluble substance; therefore, it is required to become water soluble. In this study, tween-80 was used to emulsify curcumin in water and loading it into chitosan tripolyphosphate nanoparticles. Das et al. used tween-80, as emulsifier, to make curcumin water-soluble. In both studies, the obtained nanoparticles were transparent and yellow, indicating that they were fully solved in water [20]. In consistent with our findings, Tsai et al. loaded 47% of curcumin content into poly (lactic-co-glycolic acid) nanoparticles and produced 163 nm nanoparticles. In addition, the curcumin content loaded into poly (lactic-co-glycolic acid) nanoparticles was 47%. In comparison to our findings, chitosan tripolyphosphate nanoparticles showed better loading efficiency than poly (lactic-co-glycolic acid) nanoparticles. The size of curcumin-loaded chitosan tripolyphosphate nanoparticles was relatively similar to that of curcumin-loaded poly (lactic-co-glycolic acid) nanoparticles. Nevertheless, similar to the curcumin-loaded chitosan tripolyphosphate nanoparticles, the curcumin-loaded poly (lactic-co-glycolic acid) nanoparticles are water-soluble and produce transparent solution (20,21). Boana et al. produced 40nm pure curcumin nanoparticles, which were very smaller than the curcumin-loaded chitosan tripolyphosphate nanoparticles. Their produced nanoparticles were also water soluble (2).

In consistent with our findings, Akhtar et al. also loaded curcumin into chitosan nanoparticles. The size of their produced curcumin-loaded chitosan nanoparticles was 218nm, which was larger than the size of curcumin-loaded chitosan tripolyphosphate nanoparticles (22). Yalapo et al. also loaded curcumin into PLGA and produced 76±5nm nanoparticles. The charge of their produced nanoparticles was +0.06±0.01mV.

They loaded 89% of curcumin content into PLGA nanoparticles. Our findings showed that curcumin was efficiently loaded into PLGA nanoparticles, and produced smaller nanoparticles.

In contrast to the curcumin-loaded chitosan tripolyphosphate nanoparticles with the charge of +7mV, the curcumin-loaded PLGA nanoparticles were almost neutral. The positive charge of curcumin-loaded chitosan tripolyphosphate nanoparticles can facilitate the bind of these nanoparticles to the surface of the negatively charged molecules. It also accelerates the delivery of curcumin to target tissues (23). Curcumin has anticancer and antimicrobial properties. Therefore, the ionotropic gelation method can load a significant content of curcumin into chitosan tripolyphosphate nanoparticles and produce a sphere, positively charged, and water-soluble nanosystem.

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