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Preparation, characterization, release and antioxidant activity of curcumin-loaded amorphous calcium phosphate nanoparticles

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ABSTRACT

In the study, amorphous calcium phosphate (ACP) nanoparticles were prepared by the coprecipitation method to optimize release profile of curcumin (Cur) and avoid burst releases, which were used to overcome the weakness of Cur, such as poor chemical stability and bioavailability. To find the best preparation condition, the influence of reaction concentration, temperature, time and pH on crystal phase of the samples was investigated by XRD and FTIR. The results showed that amorphous calcium phosphate (ACP) was obtained, when pH value and the concentration of PO_4^{3-} were 8 and 0.024 mM, prepared at 30 °C for 10 min. In vitro drug release assay, ACP nanoparticles showed high loading capacity of Cur and favorable pH-responsive drug release properties. Furthermore, the Cur-load ACP nanoparticles showed an excellent ability to scavenge free radical and damage A549 cells, resulting in a high antioxidant properties and low cell viability. Therefore, the as-prepared nanoparticles have promising applications in the food and biomedical fields.

1. Introduction

Although reactive oxygen species (ROS) is beneficial for human body at low/moderate concentrations, such as defence against infectious agents and contribution to cellular signalling systems, it also exerts many harmful effects to living systems [1]. Oxidative stress caused by excess ROS disturbs the equilibrium status of prooxidant/antioxidant reactions in living organisms, which damages cellular lipids, proteins or DNA and inhibits their normal functions [2]. Therefore, scavenging ROS is one of the most effective means of decreasing the level of oxidative stress, which may be achieved using antioxidant compounds extracted from natural plants, such as catechins, isoflavones, anthocyanins, phenolic acids and vitamins. Particularly, antioxidant compounds extracted from natural plants gradually have attracted more attention [2]. Curcumin (Cur), a natural lipophilic polyphenol found in the rhizomes of turmeric [3], shows a wide variety of pharmacological properties, including antioxidant, anti-inflammatory and anticancer [4], which endows it with applications in antioxidant therapy. However, its bioavailability is significantly restricted because of its poor chemical stability. Employing carriers is a feasible strategy to overcome the drawbacks of Cur. Therefore, various efficient carriers have been appiled, such as dendrimers, liposome, and micro/nano-particles [5]. Compared with other delivery systems, nanoparticle delivery system has been widely investigated due to their distinct advantages, such as high encapsulation efficiency, slower degradation rate, small particle size and effective penetration ability [6].

Amorphous calcium phosphate (ACP), one of the calcium phosphate materials, is regarded as a metastable phase with a short range order [7]. Compared with other calcium phosphate materials, ACP nanoparticles are promising drug carriers owing to their advantages including large specific surface area, high drug loading capacity and controlled drug release behavior [8]. Furthermore, ACP nanoparticles have good biodegradability and could promote osteoblast adhesion and osteconductivity [9]. In previous studies, ACP nanoparticles were prepared as cements, which were used in bone consolidation or reconstruction and were commercially available since the 1990s [10]. Moreover, the solubility of synthetic ACP nanoparticles increases with the decrease of pH value in aqueous solution [11]. Consequently, the

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ACP nanoparticles could be designed and used as pH sensitive drug nano-carriers, which give an almost entire prevention of premature drug release in physiological condition of plasma (pH 7.4). Above all, nanoparticle delivery system could lower or annihilate the plasmatic drug concentration, thus avoiding secondary effects or general toxicity [12]. In recent years, much attention has been paid to the preparation of ACP nanoparticles using different methods. Yang et al. [13] investigated the synthesis of novel gallium-doped ACP nanoparticles by sol-gel method. Zhu et al. [14] fabricated ACP porous nanospheres by using a microwave-assisted hydrothermal method with adenosine 5′-triphosphate disodium salt (ATP) as the phosphorus source and stabilizer. The co-precipitation method to synthesize nanostructured materials has attracted much interest and been growing fast because of its excellent advantages such as rapidness, facile productive process and low-cost. Moreover, ACP nanoparticles have been used for the investigation of silybin loading and release [15]. However, to date, a few studies have been reported about the specific preparation by co-precipitation method as well as and release, antioxidant activity and anticancer activity of Cur-loaded ACP nanoparticles, which is referred in this paper.

The present study aimed to synthesize ACP nanoparticles as novel delivery systems to encapsulate, stabilize and slowly release Cur by co-precipitation method. In addition to the encapsulation efficiency and loading efficiency of Cur and the micro-morphology of the ACP nanoparticles were evaluated. Furthermore, the ability of ACP nanoparticles to control the release of Cur was also investigated in this work, which was useful for the development of potential carriers for bioactive compounds. The effects of the temperature, time, pH and concentrations of $(NH_4)_2HPO_4$ on the crystal phase of the product were researched. The prepared ACP nanoparticles have relatively high specific surface area and are efficient for drug loading and release using Cur as a model drug. The Cur-load ACP nanoparticles show an outstanding ability to damage free radical and cancer cells. Hence, they are promising for the application in drug delivery.

2. Experiment

2.1. Materials

Curcumin (99%), 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH), 2, 2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Butyl hydroxy anisd (BHA), butylated hydroxytoluene (BHT) were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. Calcium nitrate ($Ca(NO_3)_2$), ammonium hydrogen phosphate ($(NH_4)_2HPO_4$) and sodium hydroxide (NaOH) were purchased from Sinopharm Chemical Reagent Co. Ltd. All the other reagents obtained from Tianjin Kemiou Chemical Reagent Co., Ltd. were analytical grade and all solutions were prepared by distilled water.

2.2. Synthesis

ACP nanoparticles were formed by coprecipitation method [15]. Briefly, $Ca(NO_3)_2{\cdot}4H_2O$ was dissolved in 29 mL deionized water to form 1 mM aqueous solution. 29 mL $(NH_4)_2HPO_4$ phosphate solution was added dropwise to the above solution and the aqueous solution quickly turned to white suspension. The raw materials were designed with a Ca/P molar ratio from 1.5 and the pH value was maintained within 8 by addition of 1 M NaOH solution. The resulting mixture was agitated at 30 °C for 10 min. Finally, the obtained nanoparticles were washed three times with deionized water to remove any residual ions, and the sample was collected by centrifugation and freeze-dried.

Drug loading: Cur (5 mg/mL) was added to the solution of Ca $(NO_3)_2$. The mixture was stirred for 1 h, followed by slow addition of an aqueous solution of Na_2HPO_4 (0.024 mM). The mixture was gently stirred at 30 °C for another 15 min. After the suspension was centrifuged at high speed to collect the products, the concentration of unloaded drug in the supernatant was determined by UV–vis spectrophotometer. The drug concentration in the supernatant was quantified on the basis of a linear standard curve by ultraviolet (UV) absorption at 424 nm, a strong absorption band of Cur. The encapsulation efficiency (EE) and loading capacity (LC) were calculated according to equation [3, 16]:

$$EE = \frac{total\ weight\ of\ Cur - weight\ of\ unloaded\ Cur^*}{total\ weight\ of\ Cur}\ 100\% \quad (1)$$

$$LE = \frac{total\ \text{weight}\ of\ Cur - weight\ of\ unloaded\ Cur^*}{weight\ of\ microspheres}\ 100\% \quad (2)$$

2.3. Characterization of nanoparticles

The X-ray powder diffraction (XRD) of the sample was characterized using X-ray diffractometer (Rigaku D/max 2500 V, Cu Kα radiation, k = 1.54178 Å). Fourier transform infrared (FTIR) spectra were recorded using a Fourier Transform IR Spectrometer (Model: Nicolet-710 spectrometer) by the KBr pellet at wavelengths ranging from 400 to 4000 cm^{-1}. The UV–vis spectroscopy was carried out on a UV–vis spectrophotometer (UV1800, Shimadzu Corporation) in the wavelength range of 200-500 nm. The morphology of the samples was examined by a scanning electron microscope (SEM, FEI Magellan 400, USA) and a transmission electron microscope (TEM, JEM2100F, Japan). Energy Dispersive Spectrometer (EDS) was used to estimate the Ca/P molar ratio of the ACP nanoparticles. Brunauer-Emmett-Teller (BET) specific surface area and pore size distribution were obtained by a surface area and pore size analyzer (V-Sorb 2800P, Gold APP China). Dynamic light scattering (DLS) was used to measure diameter of sample (Malvern, UK).

2.4. In vitro drug release assay

The drug release was studied using the membrane dialysis method against phosphate buffered saline (PBS, pH 7.4) and acetate buffers (pH 5.4) at 37 °C, which were used as the drug-release media to simulate normal blood/tissue and tumor environments. Before the experiment, the Cur-loaded ACP nanoparticles were re-suspended with PBS (pH 7.4, 5.4). Firstly, 5 mL of the Cur-loaded ACP nanoparticles (2 mg/mL) was placed in dialysis bags with a molecular weight cutoff of 34 kDa. Next, the dialysis bags were immersed in 100 mL of the beakers, which was shaken (70 rpm) at 37 °C while shielded from light. The release medium was withdrawn at various intervals and replenished with an equal volume of fresh medium. The amount of released Cur was estimated by measuring the absorbance at 424 nm.

2.5. Cur protection

To elucidate the effect of encapsulation on the stability of Cur against external severe processing, we compared with remnant content of free Cur with that of entrapped Cur in ACP nanoparticles after thermal treatment and ultraviolet radiation. Free and encapsulated Cur with quality of 20 mg by thermal treatment (60 °C, 30 min) and ultraviolet radiation was taken into account for the protective effect. For free Cur and Cur-loaded ACP nanoparticles, ethyl alcohol was added and stirred for 30 min [17]. The existent Cur was calculated through the absorption value at 424 nm.

2.6. Determination of antioxidant activity in vitro

2.6.1. 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) radical-scavenging activity assay

The antioxidant properties of Cur and Cur-load ACP nanoparticles were evaluated by determination of scavenging effect on DPPH radicals, and scavenging capacity of the samples was determined using a method reported previously [18, 19]. In this assay 1 mL samples at