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Research Paper

Curcumin bioactive nanosizing: Increase of bioavailability

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ABSTRACT

Curcumin is a natural compound existing in *Curcuma longa* Linn, featuring great medicinal activity, including antitumor, and playing a role in the battle against Alzheimer's disease. However, it has low solubility and consequently its bioavailability is limited in aqueous medium. For such, several studies in the nanotechnology field are being performed aiming the improvement of its solubility. Nanotechnology is an important branch of materials engineering with several studies focused on making feasible formulations and drug or bioactive substance delivery systems. In this study curcumin nanoparticles were obtained by two methods and using two solvents, which were characterized by infrared spectroscopy (FTIR), atomic force microscopy (AFM), solubility test, and stability tests by Dynamic Light Scattering (DLS) and Zeta potential analyses. DLS and AFM results evidence that particles were obtained in nanometric scale. Zeta potential shows stable nanoparticles in aqueous medium for 24 months. FTIR results point out that nanoparticles obtained have the same chemical features of commercial curcumin. Solubility trials show an increase of over 30 times in nanoparticle solubility, without polymer conjugation. Hence, the processes used to obtain curcumin nanoparticles may allow the use of this substance in clinical trials.

1. Introduction

Curcumin is a polyphenol extracted from *Curcuma longa* Linn (*Zingiberaceae*) rhizome, popularly known in Indian cuisine as turmeric. From ancient times, this seasoning is used in Indian traditional medicine (*Ayurveda*) against several diseases, such as rheumatism, skin diseases, body pains, hepatic diseases, intermittent fever, inflammation, sinusitis and asthma (Basnet and Škalko-Basnet, 2011; Prasad et al., 2014). Currently it is used in the food industry as flavoring, preservative and coloring agent. *Curcuma longa* alcoholic extract contains three main curcuminoids, namely curcumin, desmethoxycurcumin and bis-desmethoxycurcumin, which comprise approximately 3–5% of most turmeric preparations (Basnet and Škalko-Basnet, 2011). Commercially available curcumin is comprised of a mixture of 75–80% curcumin, 15–20% desmethoxycurcumin and 3–5% bis-desmethoxycurcumin (Ahmed and Gilani, 2014). Studies have pointed out synergistic effects among them, enhancing nematicidal activity as well as slowing the progression of Alzheimer's disease (Ahmed and Gilani, 2014; Anderson et al., 2000; Kiuchi et al., 1993). Ahmed and Gilani's (2014) study has also shown that isolated curcumin could be less effective than the mixture of the three curcuminoids, since the compound acts on specific targets, supplementing the pharmacological action of each other, thus allowing a multiple action originating cumulative or synergic properties. Thus, commercial curcumin provides better therapeutic potential to treat Alzheimer's disease compared to isolated curcumin (Ahmed and Gilani, 2014). Several studies evidence the curcumin's antioxidant (Basnet and Škalko-Basnet, 2011; Pizzo et al., 2010), anti-inflammatory (Aggarwal and Harikumar, 2009; Vishvakarma, 2014), antimicrobial (Aziz et al., 2012; Wang et al., 2009), anti-diabetic (Aziz et al., 2012) and antitumor (Ji et al., 2012; Lee et al., 2009; Milac et al., 2008; Naksuriya et al., 2014; Vishvakarma, 2014) activities.

While containing several medicinal properties, clinical application of this compound is highly limited due to its poor water solubility, low bioavailability and fast metabolization, and susceptibility to degradation in alkaline medium and when exposed to light (Anand et al., 2007; Naksuriya et al., 2014; Souza et al., 1997). New studies in the nanotechnology field are being carried out to make feasible the clinical application of curcumin, such as the formation of liposomes, nanoparticles, nanogels, nanocrystals and nanoemulsions (Sun et al., 2012).

Nanotechnology is an area undergoing rapid expansion, being considered one the most relevant fields of materials science. Based on nanotechnology, researchers are able to understand and manipulate materials in atomic and molecular scale for application in a variety of fields (Safari and Zarnegar, 2014; Tian et al., 2013). Structures with

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nanometric scale – nanomaterials – are designed by procedures that allow a high control over their chemical and physical characteristics, creating unique properties that bring benefits to their application (Safari and Zarnegar, 2014; Thorley and Tetley, 2013; Tian et al., 2013). A particular field in which the application of nanomaterials may bring about a potential breakthrough in medicine. Nanomedicine may spur technological advances both in the development of new drugs and reformulation of existing ones, as well as to enhance their efficacy, targeting and reducing side effects (Drbohlavova et al., 2013; Thorley and Tetley, 2013), and allowing the use of bioactive substances such as curcumin.

Based on these information the obtention of high water solubility nanoparticles is of great interest for biological applications. This study aims to obtain commercial curcumin nanoparticles, that present low cost and have the same pharmacological effects as isolated curcumin. With the purpose of obtaining water-soluble nanoparticles two different methods (stirring and sonication) with solvent variation (ethanol and chloroform) were tested, without polymer addition. Therefore the prepared nanoparticles can be easily used in biological tests without the need of using commonly organic solvents such as dimethyl sulfoxide (DMSO) which can be harmful.

2. Materials and methods

2.1. Materials

Commercial curcumin, Kampo de Ervas Produtos Naturais, batch 2014-2/1; denatured ethyl alcohol 40B, J. T. Baker; chloroform, Merck; acetone, Aldrich; deionized water. All reagents and solvents used were PA grade products.

2.2. Obtaining curcumin nanoparticles

The commercial curcumin solution was prepared at 0.05 g mL^{-1} in ethanol and at 0.10 g mL^{-1} in chloroform. 100 mL of solution were added to a predefined volume of deionized water subject to agitation (200–1000 rpm) at 50 °C in a Fisatom 752A stirrer or sonication (120 W) at 50 °C using a Sonics Ultrassom Vibra-cell, originating four samples. Both methods were performed during 2 h. Nanoparticle samples obtained from ethanol and agitation and from sonication processes were named NEA and NES, respectively. Nanoparticle samples obtained from chloroform and agitation and sonication processes were named NCA and NCS, respectively. The curcumin nanoparticle solutions were separated in two parts: the first one was lyophilized obtaining a yellow powder and the second part was maintained as solution. The nanoparticles yield was calculated following the Eq. (1):

$$Yield\ (\%) = \frac{m_f}{m_i}\ X\ 100 \quad (1)$$

Where m_f is the final mass and m_i corresponds to inicial mass.

2.3. Infrared spectroscopy (FTIR)

Spectra were obtained in KBr disks at 400–4000 cm^{-1} interval, with 4 cm^{-1} resolution and 32 scans, using a Shimadzu IR Affinity-1 equipment.

2.4. Atomic force microscopy (AFM) particle analysis

For this analysis, 10 μL of each sample, in a 0.8 mg mL^{-1} solution, were deposited onto a mica substrate. After drying, topography images for a morphology study and phase contrast images for differentiation with other compounds were made on Nanosurf FlexAFM atomic force microscope in ambient atmospheric pressure chamber, 23 °C temperature and 30% humidity. All measurements were taken with a silicon cantilever operating on dynamic mode, with a frequency of 300 kHz and elastic constant of 40 N m^{-1}. Free software Gwyddion was used for image treatment and analysis. Diameters of sample particles were obtained with the aid of UTHSCA Image Tool® software.

2.5. Solubility test

A supersaturated solution of each nanoparticle was prepared in deionized water and stirred constantly (500 rpm) at 25 °C for 24 h. After this period, solutions were filtered with 1.2 μm filters and 3.0 mL of filtrate were lyophilized and weighted. The experiments were performed in quintuplicate.

2.6. Stability studies

Freshly made and reconstituted from dry powder curcumin nanoparticles were subjected to dynamic light scattering (DLS) size analysis and Zeta potential measurement. The dried powder was stored at temperature of 4 °C for 24 months and reconstituted with de-ionized water prior to the above test.

The size analysis was performed by means of DLS using a Zetasizer Nano ZS (Malvern Instruments) at 25 °C. Samples were suspended in water at 0.8 mg mL^{-1}. For Zeta potential, Zetasizer Nano ZS (Malvern Instruments) equipment was used, DTS1070 cuvette, at 25 °C. Three independent determinations were made for both DLS and Zeta potential.

3. Results

3.1. Obtaining curcumin nanoparticles

Curcumin nanoparticles were obtained by nanosizing methods. The process yield was 78.8 ± 1.0% for NEA, 76.8 ± 2.4% for NES, 72.3 ± 1.4% for NCA, and 61.4 ± 1.6% for NCS.

3.2. Infrared spectroscopy (FTIR)

FTIR spectra have shown characteristic bands on 3300–3400 cm^{-1} region regarding vibrations of phenol −OH free group. Intense bands on 1640, 1647 and 1650 cm^{-1} related to vibration of carbonyl group. Bands on 1380–1470 cm^{-1} region refer to C=C vibrations of the aromatic ring and elongation of C−O bond. Bands on 1020–1155 cm^{-1} region are associated with ether C−O group stretching. On 700–900 cm^{-1} region, they refer to C−H of alkene groups. The results obtained from FTIR are showed in Fig. 1.

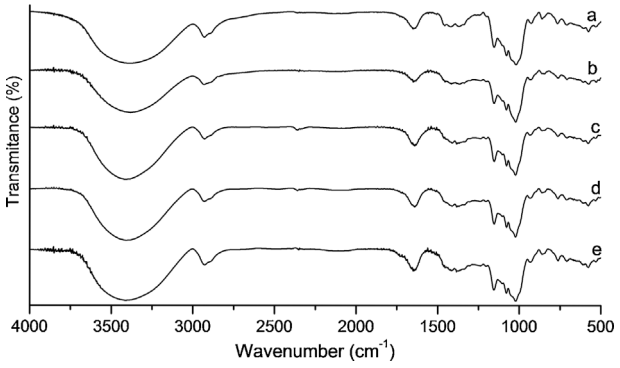

Fig. 1. Infrared spectrum: (a) commercial curcumin, (b) Nanoparticle obtained from ethanol and agitation (NEA), (c) Nanoparticle obtained from ethanol and sonication (NES), (d) Nanoparticle obtained from chloroform and agitation (NCA), and (e) Nanoparticle obtained from chloroform and sonication (NCS).