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Original article

Curcumin and piperine loaded zein-chitosan nanoparticles: Development and in-vitro characterisation

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ABSTRACT

Curcumin as the active compound of turmeric has antioxidative, antiinflammatory, antimicrobial and anticancer properties among others. However, its disadvantageous properties like low solubility, poor bioavailability and rapid degradation under neutral or alkaline pH conditions or when exposed to light limit its clinical application. These problems can be solved by a smart combination of using a natural enhancer like piperine and preparing nanoparticles by a proper method like electrospray.

Due to these facts it was aimed in this study to develop curcumin and piperine loaded zein-chitosan nanoparticles step by step. For that purpose various formulation parameters like the concentrations of zein, curcumin, piperine and chitosan and the preparation parameters like the applied voltage and the nozzle diameter were investigated step by step. The nanoparticles were characterised by investigating their shapes, morphologies, particle sizes with help of SEM images and the cytotoxicity on neuroblastoma cells.

It was succeeded to prepare curcumin and piperine loaded zein-chitosan nanoparticles having a mean particle size of approximately 500 nm and high encapsulation efficencies for curcumin (89%) and piperine (87%). Using a curcumin concentration of 10–25 μg/ml resulted in reduction of the viability of approximately 50% of the neuroblastoma cells. The here developed nanoparticle formulation consisting of solely natural compounds showed good cytotoxic effects and is a promising approach with appropriate properties for final consumption.

1. Introduction

The preparation of curcumin and piperine loaded zein-chitosan nanoparticles, having a particle size of approximately 500 nm and appropriate properties for final consumption is the main objective of this study. Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3,5-dione] is a diphenolic, active compound of turmeric (*Curcuma longa*), with antioxidative (Jayaprakasha et al., 2006), antiinflammatory (Jurenka, 2009), antimicrobial (Wang et al., 2009a) and anticancer (Villegas et al., 2008; Yoysungnoen et al., 2008) properties. Unfortunately, curcumin has some disadvantageous properties such as low solubility and poor bioavailability (Wang et al., 2009b; Yang et al., 2007), which limit its clinical application. This combination of low solubility and poor bioavailability negatively affects its biological efficacy (Shaikh et al., 2009). One important approach to improve the poor biopharmaceutical properties of curcumin is to improve its aqueous solubility using nanotechnology and nanoparticles, having a small size in the nanometer range (Torchilin, 2009; Ruenraroengsak et al., 2010; Sultana et al., 2013).

Due to these facts the starting-point to solve these problems consists of two crucial factors: nanoencapsulation of curcumin to enhance its poor solubility and to protect it against degradation, and combination of curcumin with the natural enhancers like piperin, which was proven to enhance the absorption of curcumin (Han et al., 2008), and chitosan in order to enhance its poor bioavailability.

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Nanoencapsulation systems exhibit a high potential as carriers of bioactive substances due to their subcellular size, allowing relatively higher intracellular uptake (Mora-Huertas et al., 2010) and improved stability and protection of labile substances against degradation (Preetz et al., 2008).

Encapsulation includes the immobilization of a particular compound in a material that coats it or in which it is dispersed. Encapsulation, in general, can improve disadvantageous properties of a substance, like a low aqueous solubility and protect a molecule from degradation or loss of functionality due to the effects of light, oxygen, pH or moisture. All these properties are occurring with curcumin. Encapsulating curcumin by several methods has been described like incorporating into cyclodextrins (Baglole et al., 2005), liposomes (Li et al., 2007a), microemulsions (Lin et al., 2009) and micelles (Yu and Huang, 2010). In addition, the development of micro- or nanoparticles by spray-drying (Wang et al., 2009b), solvent emulsion-evaporation (Shaikh et al., 2009; Mukerjee and Viswanatha, 2009; Prajakta et al., 2009), electrohydrodynamic atomization and so-called electrospray (Gomez-Estaca et al., 2012) were referred. A simple and one-step method had been developed to encapsulate actives (Jaworek, 2008; Zhang et al., 2012; Cao et al., 2014), which is called electrospray or electrohydrodynamic atomization. Electrospray is a well-known method for preparing polymeric nanoparticles and for encapsulating drugs with poor aqueous solubility, just like curcumin. Electrospray is based on the break-up of a liquid into fine charged droplets under the action of an electric field. When a liquid is passed through a thin metal tube such as a nozzle, needle or capillary and the liquid meniscus located at the tip of the tube is electrically stressed by applying a potential difference between the tube and the counter electrode, the electrospray is generated. Electrospray offers several advantages compared to other nanoparticle manufacturing methods, like monodispersed particles, the fact that the particle size can be easily controlled by adjusting the preparation parameters (Yun et al., 2009; Ding et al., 2005), a high encapsulation efficiency and no need for a tedious separation process to remove the particles from the solvent, like for many encapsulation methods. Furthermore, hydrophobic or hydrophilic drugs can be loaded into electrosprayed particles with high entrapment efficiency (Kim and Kim, 2011; Valo et al., 2009) and core–shell structured particles can be conveniently obtained (Zhang et al., 2011). The complex electrospraying process is affected by many variables like the electrostatic field strength, needle/nozzle diameter, the solution flow rate and concentration of the compounds. Although the size of electrosprayed particles was always within micron to submicron range many strategies have been developed to decrease the particle size. Particles with sizes ranging from 275 to 860 nm were obtained by varying the polymer concentration, feed rate and applied voltage (Chang et al., 2010). Chitosan particles with an average diameter of 124 nm were prepared by decreasing the polymer concentration and reducing the fluid flow rate (Zhang and Kawakami, 2010). It must be pointed out that decreasing the particle size by decreasing the solution concentration or flow rate can lead to a low particle production capacity. Nanoparticles showed an enhanced colon bioadhesion and increased oral bioavailability compared to microparticles (Lamprecht et al., 2001; Sigfridsson et al., 2011).

Due to previous experiences of encapsulating lycopene with zein (Kose and Bayraktar, 2016) this maize protein was selected as the encapsulating material for dispersing curcumin. Zein comprises a group of alcohol soluble but aqueous insoluble proteins (Shukla and Cheryan, 2001). There are many reports about the use of zein as a wall material for encapsulating a variety of compounds (Patel et al., 2010; Liu et al., 2005; Zhong and Jin, 2009). Biofunctionality and the use of non-toxic materials are the most fundamental conditions for the release of bioactive compounds in the pharmaceutical industry to be possible. Therefore, natural polymers like zein or polyelectrolytes such as chitosan are competitive candidates as materials for the formation of nanoparticles. After zein was certained as matrix for curcumin, a second multifunctional biopolymer for the shell of the nanoparticles was appreciated. There are several studies in which viscosity modified macromolecules such as sodium alginate (Tonnesen, 2006), chitosan (Mazzarino et al., 2012), acrylic derivates of cellulose (Jaiswal et al., 2010) alone or in combination, were tested as a curcumin bioavailability promoter, because of their ability to increase water solubility, their affinity for the biological membranes, time release and membrane permeation. The biocompatible and biodegradable polymer chitosan is currently employed to prepare nanoparticles with mucoadhesive properties. This cationic copolymer can form nanoparticles by different methods like ionotropic gelation, microemulsion, emulsification solvent diffusion, and polyelectrolytes complex formation (Nagpal et al., 2010). Chitosan exhibits some promising properties like being nontoxic, biodegradable and biocompatible (Helander et al., 2001). Chitosan is a naturally positively-charged polymer with the propensity to easily interact with negatively-charged sites on a cell surface (Gordon et al., 2008). These beneficial properties made chitosan to one of the most popular biopolymers for the development of bioactive compounds delivery systems for a wide range of applications (Luo and Wang, 2014). Due to the fact that the surface of all physiological membranes including the intestine, have a negative surface charge (Rojanasakul et al., 1992), positively charged nanoparticles containing chitosan, proven to enhance the absorption (Thanou et al., 2001), makes them very attractive.

The major component of black pepper (Piper nigrum L.) piperine is the first and most potent bioenhancer till date (Atal and Bedi, 2010), enhances the bioavailability of many drugs by increasing the absorption from the intestine, suppresses the drug metabolism via inhibiting CYP3A4 and P-glycoprotein (P-gp) (Makhov et al., 2012). The ATP-dependent efflux transporter P-gp pumps various drug molecules out of the cells (Zhu et al., 2007) and is responsible for multidrug resistance in cancer cells (Ampasavate et al., 2010). Piperine has diverse important effecst like antimicrobial and anti-parasitic (Raay et al., 1999), antiinflammatory (Vaibhav et al., 2012), antidepressant (Li et al., 2007b), antiangiogenic (Doucette et al., 2013) and anticancer activities (Pradeep and Kuttan, 2002). Although piperine suppresses tumor growth and metastasis in vitro as well as in vivo (Lai et al., 2012), its use alone as chemotherapeutic molecule for cancer therapeutics is limited because of its high concentration requirement, due to its hydrophobic nature. The co-administered piperine (10 mg) increased the plasma concentration and delayed the elimination of drugs like phenytoin (Velpandian et al., 2001) and rifampin (Zutshi et al., 1985), both P-gp substrates (Schuetz et al., 1996).

In another study it was shown that piperine abolished the P-gp function in Caco-2 and L-MDR1 cells at a concentration of 50 μM. Piperine was non-cytotoxic at concentrations up to 100 μM to the Caco-2 and L-MDR1 cells after 4 h exposure, shown with the in vitro MTT assay (Han et al., 2008). Piperine suppressed tumor growth and metastasis in vitro and in vivo in a 4T1 murine breast cancer model (Lai et al., 2012). Co-administration with piperin is one of the strategies to increase the bioavailability of curcumin. The application of 2 g of curcumin with 20 mg piperine increased the curcumin bioavailability in human and in rats (Shoba et al., 1998). However, this was not a fixed combination, like it was aimed in this study. As the way of action for piperine, it was postulated that it acts as an apolar molecule forming an apolar complex with drugs. The activity of piperine against P-gp mediated efflux was shown in several studies (Khajuria et al., 1998; Okura et al., 2010). In a clinical trial it could be shown that twice daily application of 5 mg piperine enhanced the absorption of 2 g curcumin (Han et al., 2008).

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