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A green chemistry approach for nanoencapsulation of bioactive compound – Curcumin

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ABSTRACT

It is quite common to use inorganic/organic solvents during the encapsulation of curcumin. Therefore, it is not suitable to realize the food applications of this bioactive compound. In the present work, the objective was to employ a solvent-free green chemistry approach to encapsulate curcumin in nano-form. The milk fat was used as oil medium and the milk protein sodium caseinate as a wall material. Varied concentrations of wall material were systemically studied and the transmission electron microscopic images confirmed the particle size of spray dried powder to be in the range of 40–250 nm. The encapsulation efficiency was calculated to be 91% and loading capacity was 0.9% with respect to 0.99% theoretical loading for the sample having an optimum amount of 5% of wall material. The antioxidant activities of this nanoencapsulated curcumin were found to be higher than its native counterpart.

1. Introduction

The search for a compound with numerous therapeutic properties and almost no side effects on the human body has made researchers focus their attention on curcumin, an orange–yellow colored fluorescent molecule present in the spice turmeric (*Curcuma longa*) which is known since ancient times for many health-beneficial properties (Aggarwal et al., 2006). It has been studied mainly for anti-diabetic (Srinivasan, 1972), cholesterol gall-stone lowering effect (Hussain & Chandrasekhara, 1992; Shubha, Reddy, & Srinivasan, 2011), anti-HIV-activity (Jordan & Drew, 1996), anti-hepatoma (Yen, Wu, Tzeng, Lin, & Lin, 2010), anti-inflammatory properties (Wang et al., 2008), anti-cancer, wound healing, etc. (Darvesh, Altaf, Aggarwal & Bishayee, 2012; Maheshwari, Singh, Gaddipati, & Srimal, 2006). The hydroxyl groups of the benzene rings, double bonds in the alkene part and the central β-diketone moiety (Fig. 1) in curcumin are responsible for many chemical/biochemical reactions such as free-radical scavenging, antioxidant, and anti-microbial activity (Ohara et al., 2005; Priyadarshini et al., 2003; Wang, Lu, Wu, & Lv, 2009).

However, the major limiting factor of curcumin is its lipophilic nature and low stability in gastrointestinal fluids, neutral and basic pH conditions, that reduces its bioavailability. Encapsulation of essential oils/bioactive compounds/functional ingredients at the nanoscale has shown improvement in physical stability of the compounds in and out of the gut and enhanced uptake of components during digestion (Khaled, Khaled & Ashoush, 2014). In order to enhance its bioavailability various techniques like liposome (Niu et al., 2012), O/W emulsion (Wang et al., 2008), organogel-based emulsion (Yu & Huang, 2012), polymer conjugate (Manju & Sreenivasan, 2012), sol–gel process (Trewyn, Slowin, Giri, Chen, & Lin, 2007), and alginate beads synthesis (Song, Wang, Qian, Zhang, & Luo, 2012) have been employed to encapsulate it in nano form and such encapsulated materials have mostly been studied for drug delivery systems (Bitar, Ahmad, Fessi, & Elasissari, 2012; Liu et al., 2013; Ravichandran, 2013). The use of organic solvents, inorganic solutions or polymer based wall materials is quite frequent to nanoencapsulate curcumin for pharmaceutical applications (Bhawana, Bansiwal, Buttar, Jain & Jain, 2011; Esmaili et al., 2011; Song et al., 2011) but is undesirable for food applications. Moreover, a wide range of particle sizes ranging from 100 to 700 nm have been reported using different and cumbersome synthesis processes (Pan, Zhong, & Baek, 2013; Teng, Li, & Wang, 2014). A solvent-free green chemistry approach can help in overcoming this problem by not only enhancing water dispersibility but also making the nanoencapsulated curcumin a suitable candidate for incorporation in food matrices as well. Such functional foods may help in the prevention of many diseases (Srinivasan, 2014). Few reports are available where solvent free approach has been employed for nanoencapsulation of curcumin. Ahmed, Li, McClements, and Xiao (2012) have studied the solubility of curcumin in lipids with different chain lengths (large/medium/short) and the impact of lipid based formulations on curcumin

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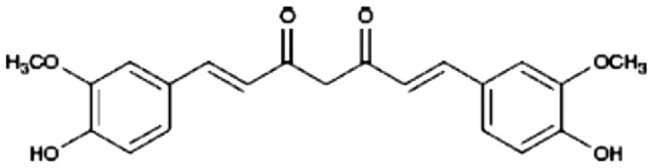

Fig. 1. (1,7-bis(4-hydroxy-3-methoxyphenyl)1,6-heptadiene-3,5-dione).

encapsulation and bioacessibility. Yu, Shi, Liu, and Huang (2012) developed a curcuminoid organogel with high loading and bioacessibility by dissolving Span 20 in medium chain triglyceride and using monostearin as organogelator. In the above processes, either the raw materials were expensive or the process was time consuming. Therefore, a simple and an economical encapsulation process is required which can easily be scaled up for bulk preparation. In the present work, natural, food based and easily available raw materials have been used. Moreover, the process consumes less time and allows tailoring the particle sizes in the range of 50–250 nm.

A green chemistry approach is, basically, an environmental friendly synthesis process that is devoid of harmful chemicals (Sharma, Haranath, Chander, & Singh, 2008). Oil-in-water emulsion technique was employed to encapsulate the curcumin where milk fat was used as solubilizing agent and sodium caseinate as wall material. The criterion to use milk fat was the easy availability and presence of short/medium/long chain triglycerides in different proportion wherein short chain triglycerides form 40% of the mixture (http://www.agroscope.admin.ch/milchfett/index.html?lang=en, 2015). The short chain triglycerides have more polar groups (oxygen) per unit mass facilitating dipole–dipole interactions between carrier lipid and curcumin molecule (Ahmed et al., 2012). In milk fat polar lipid groups are in the range 0.5–1.0% (MacGibbon & Taylor, 2006) and may play a role in solubilisation of curcumin. Sodium caseinate was preferred as wall material because of its purity, small size of casein micelles in the range 40–300 nm with average size 150 nm (Walstra, Wouters, & Geurts, 2005, chap. 3) and easy handling of material at room temperature. The casein micelles are colloidal particles composed of α_{s1}-casein, α_{s2}-casein, β-casein (held together by hydrophobic interactions) and κ-casein (Home, 1986). The first three molecules are primarily covered with κ-casein, which is hydrophilic, charged and has a diffuse surface layer that stabilizes the micelles through intermolecular electrostatic and steric repulsion (De Kruif & Zhulina, 1996). The effect of variation of sodium caseinate amount on the particle size and the antioxidant activity of the encapsulated curcumin have been studied and reported here.

2. Materials and methods

Curcumin (95% pure) was purchased from Salutaris Tech Pvt Ltd., Mysore, India. Milk fat was purchased from Nandini, a dairy cooperative in Mysore, Karnataka, India and sodium caseinate was from Sigma–Aldrich, India. Triple distilled (TD) water was used in all the preparations.

2.1. Protocol for encapsulation

Curcumin was dissolved in hot milk fat and sonicated (Branson, Model-1510E) for 5 min in a water bath set at 60 °C, frequency 40–50 kHz, RF power of 80 W. The milk fat to curcumin concentration was kept constant at 1:0.05% (w/w) for all the samples. The milk fat was 1% of the emulsion while the amounts of sodium caseinate was varied from 0.1% to 10% (w/v; with 2.5% increments) in 200 ml emulsion. The aqueous solution of sodium caseinate was homogenized (IKA T25 digital ULTRA-TURRAX) at 9000 rpm for 10–15 min followed by addition of milk fat containing dispersed curcumin and the mixture was sonicated (Sonics & Mat. Inc., Model-VC 750, Power 750 W, Freq. 20 kHz) for 30 min. The emulsion was spray dried (SD, LSD-48, mini Spray Dryer, JISL) at an inlet temperature of 105 °C, outlet temperature of 66 °C and an air pressure of 1.5 kg/cm^2. The samples were prepared in triplicates and analyzed.

2.2. Crystallinity and melting point determination

The crystallinity and melting point of individual reactants and nanoencapsulated curcumin (NC) were ascertained using differential scanning calorimetry (DSC 8000, Perkin Elmer, USA). 20 mg of powdered sample was sealed in a hermetic aluminum pan and heated from 30 to 250 °C at the rate of 10 °C/min. Nitrogen was used as the transfer gas at a flow rate of 20 ml/min. The results are shown in mW as endotherm upwards (EndoUp).

2.3. Morphology and particle size analysis

To determine the particle size of the NC powder Tri-LASER Diffraction Technology (TLDT; Model – Blue Wave, Microtrac, USA) was used. The three LASER beams in the instrument strike the particles in a 3D angle and detector senses the shape and average size. The FLEX software combines the results obtained per channel and gives the average diameter (d50). The samples weighing 1 g were dissolved in 20 ml of TD water and shaken well or vortexed for analysis. The morphology and particle size of dried powder was evaluated using Transmission Electron Microscopy (TEM, Model: Tecnai G2 Spirit Bio-TWIN operating at 120 kV). 2 μg of sample was dissolved in 5 ml of deionized water and a drop of diluted sample was placed on a 400-mesh copper grid and negatively stained with 1% uranyl acetate.

2.4. UV–VIS absorbance & encapsulation efficiency measurements

The UV–VIS absorbance (UV-1800, Shimadzu, UV-spectrophotometer) of the NC samples and curcumin solutions, each 10 mg in 10 ml of acetone, was scanned from 320 to 500 nm with acetone as blank. To determine the encapsulation efficiency the maximum UV absorbance was recorded at 420 nm with acetone as blank. 10 mg of NC samples was dissolved in 5 ml of water and vortexed for 5 min. The solution was allowed to stand at room temperature (27 ± 1 °C) for 1 h and then centrifuged at 7000 g for 20 min. The precipitates were collected to determine the free curcumin in the sample by dissolving it in acetone and recording the UV absorbance at 420 nm (Teng et al., 2014). The amount of free curcumin was calculated using standard formula (American Spice Trade Association (ASTA) analytical methods, 1997) and the actual curcumin content was determined as given below (ASTA methods 1997; Surassmo, Min, Bejrapha & Choi, 2010; Teng et al., 2014);

$$\text{Actual curcumin content in the yield (g)} = \text{Total amount of curcumin used (g)} - \text{Free curcumin content in the yield as measured by UV(g)}$$