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Nanoencapsulation of green tea catechins by electrospraying technique and its effect on controlled release and *in-vitro* permeability

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ABSTRACT

Zein, a biocompatible, biodegradable macromolecule was employed for nanoencapsulation of green tea catechins by electrospraying technique. For this, the electrohydrodynamic behavior of zein solution was studied and the effective electrospraying concentration of zein was optimized. Later, the influence of nanoencapsulation and core-to-wall ratio on the gastrointestinal stability and permeability of green tea catechins were investigated. Among the various concentrations of zein studied (i.e. 1%–40% w/w), 5% w/w zein solution yielded spherical, monodisperse nanoparticles with mean diameter of 157 ± 36 nm. Nanoencapsulates with 1:50 core-to-wall ratio had highest encapsulation efficiency compared to 1:10 and 1:05 core-to-wall ratio samples. The nanoencapsulated catechins had significantly improved *in-vitro* gastrointestinal stability and Caco-2 cell monolayer permeability compared to unencapsulated catechins. Further, 1:50 and 1:10 samples possessed higher permeability of catechins compared to 1:05 nanoencapsulates. Hence, the study provides a one-step approach for production of green tea catechin nanoencapsulates with sustained release and enhanced permeability properties.

1. Introduction

Green tea (*Camellia sinensis*) catechins are known to possess antioxidant, antiangiogenic, antitumor and antiobesity properties (Cabrera et al., 2006). However, catechins undergo epimerization, degradation and oxidation reactions during food processing and storage, which limits the usage of green tea catechins in functional food development. The stability of catechins both *in-vitro* and *in-vivo* are affected by factors such as temperature, pH and the presence of oxygen or metal ions (Ananingsih et al., 2013). In this context, nanoencapsulation is an effective means of delivering catechins in its chemically active form. Nanoencapsulation is the process of entrapping a bioactive compound in to a protective shell with final particle diameters ranging from 10 nm to 200 nm. Nanoencapsulation improves the chemical stability of the core compound and aids in sustained release in *in-vivo* environment (Quintanilla-Carvajal et al., 2010). Further, nanoencapsulated food bioactives (hydroxycitric acid, vitamin E) are shown to possess improved *in-vivo* bioavailability compared to their micro-encapsulated counterparts (Ezhilarasi et al., 2016; Parthasarathi et al., 2016). Various techniques such as nanoemulsification, coacervation, nanoprecipitation, exist for the production of colloidal nanoparticle dispersions or suspensions. However, food grade nanoparticles in dry powder form are recognized to be suitable for long term storage stability, controlled release and food incorporation applications (Ezhilarasi et al., 2013). Here, need exists in overcoming the challenges associated with the processing and handling of nanoparticles in dry form.

In this context, the use of electrospraying as a nanoencapsulation technique for food bioactives can aid in development of dry nanoencapsulates (Bhushani and Anandharamakrishnan, 2014). Electrospraying is the process of liquid atomization by electrical forces. Recently, electrospraying technique has been used for the encapsulation and stabilization of food bioactives such as curcumin (Gomez-Estaca et al., 2012), folic acid (Bakhshi et al., 2013; Pérez-Masiá et al., 2015a), lycopene (Pérez-Masiá et al., 2015b), epigallocatechin gallate (Gómez-Mascaraque et al., 2015) and also live cells such as *Lactobacillus acidophilus* (Laelorspoen

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et al., 2014). Compared to the commercial spray drying method, electrospraying technique offers various advantages for encapsulation of bioactives (Table 1). However, due to the scalability concerns associated with electrospraying method, its use for production of food-grade nanoencapsulates is still in its laboratory level. Scaling up can be achieved by modifying the nozzle set-up, controlling the temperature and humidity of the electrospraying chamber and increasing the feed flow rate applied. These initiatives would broaden the scope for commercialization of this technology.

Electrospraying technique utilizes a wide range of food grade proteins for encapsulation of bioactives. Among them, zein is a hydrophobic, biocompatible, biodegradable prolamine rich protein from corn. It is classified by the U.S. Food and Drug Administration (FDA) as a generally recognized as safe (GRAS) polymer for pharmaceutical and food applications (Shukla and Cheryan, 2001). It is used as an encapsulating material due to its film forming property, thermal resistance, and oxygen and moisture barrier properties (Corradini et al., 2014). Additionally, the stability and release properties of the core material are largely dependent on the physicochemical properties of wall material and the core-to-wall ratio used for encapsulation purpose (Rajam and Anandharamakrishnan, 2015).

With this background, the major objectives of the study were, (i) to characterize the electrohydrodynamic behavior of zein solution at various concentrations and select the best concentration for nanoencapsulation purpose, and (ii) to study the effect of nanoencapsulation and core-to-wall (catechins-to-zein) ratio on the controlled release and Caco-2 cell monolayer permeability properties of green tea catechins.

2. Materials and methods

2.1. Materials

Dried green tea leaves ('Tetley' green tea, Tata Global Beverages Ltd., Bengaluru, India) were procured from local market. Zein (from maize), (−)-epigallocatechin (EGC), (−)-epicatechin (EC), (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), Corning® Transwell® polycarbonate membrane cell culture inserts (12 mm dia, 0.4 μm pore size with cell growth area of 1.12 cm^2) and lucifer yellow dipotassium salt were obtained from Sigma-Aldrich chemical co. (St. Louis, Missouri, USA). Minimum essential medium eagle, fetal bovine serum, antibiotics, Hank's balanced salt solution (HBSS), non-essential amino acids were obtained from HiMedia chemicals (HiMedia Laboratories Pvt. Ltd., Mumbai, India). Other chemicals, reagents and solvents used were of analytical grade.

2.2. Extraction and isolation of catechins

The extraction of polyphenols from commercial green tea leaves were performed using the method as reported by Bhushani et al. (2016). The green tea leaves were microwave extracted (2450 MHz; 1000 W) at leaves to water ratio of 1:30 at 80 °C for 10 min. The catechins were isolated using the procedure of Dong et al. (2011) with slight modifications. The green tea extract (100 ml) was washed with chloroform (50 ml) thrice and the obtained aqueous layer was extracted thrice with ethyl acetate (50 ml). The green tea catechins isolated powder was obtained by drying the ethyl acetate phases under reduced pressure at 60 °C. This isolated catechin powder was stored at 4 °C until further use and was used as the catechin core material in the study.

2.3. Preparation of electrospraying feed solutions

Zein solutions were prepared at different concentrations ranging from 1.0% to 40% w/w by dissolving it in 80% aqueous ethanol using a magnetic stirrer. For nanoencapsulation, catechins powder was incorporated to the optimized concentration of zein solution at different core-to-wall ratios of 1:50, 1:10 and 1:05.

2.4. Characterization of electrospraying solutions

Viscosity of the emulsions were determined at 25 ± 1 °C using a stress-controlled rheometer (Haake RheoStress 6000, Thermo Scientific, Karlsruhe, Germany) by applying a shear rate in a linear manner from 0.1 to 250 s^{-1}. Analysis was carried out in triplicates and apparent viscosity of the emulsions was calculated at shear rate of 100 s^{-1}. The zero shear rate viscosity of all the solutions was obtained by using the Carreau-Yasuda model. Later, the specific viscosity (η_{sp}) of the solutions was calculated using the following equation:

$$\eta_{sp} = \frac{(\eta_o - \eta_s)}{\eta_s} \quad (1)$$

where, η_o is the zero shear rate viscosity and η_s is the viscosity of dispersing solvent i.e. 80% aqueous ethanol (1.96 ± 0.03 mPa s). According to the *de Gennes's scaling concept*, a theoretical relationship between the polymer rheology and its

Table 1
Comparison of electrospraying and spray drying techniques for encapsulation of food bioactives.

Operational parameters	Electrospraying	Spray drying
Temperature	Non-thermal process	Involves high inlet and outlet temperatures
Use of solvents	Water as well as organic solvents However, solvent recovery at industrial scale set-up is mandatory to avoid explosion risks.	Water Organic solvents not preferred due to possible risk of solvent vapor explosion.
Morphology of particles	Spherical with fine pores, Tailor-made structures	Spherical, dented or with blow holes
Particle size and product recovery	Monodisperse, non-aggregated, nano, sub-micron and micron size particles can be produced. The particle collection and handling system has to be precisely monitored to obtain nanopowders.	Heterogenous particle size distribution due to aggregation, only micron sized particles can be produced. Product collection is simple due to the micron size.
Product quality	No denaturation of proteins or degradation of bioactives	Degradation of bioactives might occur due to high temperature process
Encapsulation efficiency	High	Medium to high
Wall materials used	Wide range of polymers and biopolymers Control on wall material thickness possible using coaxial electrospraying set-up	Polymers and biopolymers suitable for atomization and high temperature drying
Energy consumption	Low energy required	High energy required