

## Curcumin Microparticles Produced by Electrospraying Technique with Whey Protein Isolate and $\beta$ -Cyclodextrin Complex

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### ABSTRACT

In this research, the potential of the electrospraying technique was used for encapsulation of curcumin in natural polymers such as Whey Protein Isolate (WPI) and mixture of WPI/ $\beta$ -CycloDextrin ( $\beta$ -CD). The encapsulated particles were physicochemically characterized and curcumin release profile was evaluated. At WPI concentration of 25%, more uniform particles were formed and most of them were smaller than 0.7  $\mu$ m in diameter. The encapsulation efficiency of curcumin in WPI and WPI/ $\beta$ -CD solutions was determined as 45.4% and 53.6%, respectively. Differential Scanning Calorimetry (DSC) and ThermoGravimetric Analysis (TGA) of the obtained encapsulated curcumin revealed that WPI and WPI/ $\beta$ -CD polymers could not increase thermal stability of curcumin. Encapsulated curcumin had a better stability than pure curcumin at acidic and alkaline conditions, and the release of curcumin after 7 hours was lower than 40% with the sustained mode in buffer solution conditions (pH= 7.4).

**Keywords:** Differential scanning calorimetry, Encapsulation, Thermal stability, Thermo-gravimetric analysis.

### INTRODUCTION

Curcumin, a natural yellow-orange polyphenol with low molecular weight, is extracted from the rhizome of the herb *Curcuma longa* (turmeric). In addition to food consumption, e.g. as a stabilizer in jellies and a natural colorant, this compound has attracted considerable attention from the pharmaceutical industry due to its extensive pharmacological applications because of its antioxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-parasitic, anti-mutagenic, and anti-immunodeficiency properties (Noorafshan and Ashkani-Esfahani, 2013). However, application of curcumin in food has been restricted due to its low solubility in aqueous media. Other environmental factors such as heating, alkaline condition, enzymes, oxygen, UV irradiation, and metallic ions can also affect

its decomposition (J. Li *et al.*, 2016; Paramera *et al.*, 2011). To resolve the mentioned restrictions, we should use methods such as encapsulation to increase the solubility and protection of curcumin.

According to the proposed definition, encapsulation refers to a process in which a specific component is entrapped within a different kind of matrix that can be made up of one or multiple components. The most common matrices are proteins, polysaccharides, surfactant, lipids, water and/or minerals (Garti and McClements, 2012). Whey protein, one of the two milk protein fractions, is widely used because of its high nutritional value and different functional properties, e.g. the binding of water and flavor, gelation, emulsification, and foaming. Five structural subgroups exist in globular proteins: only  $\alpha$ -helices occur; only  $\beta$ -structure occur;  $\alpha$ -helical and  $\beta$ -

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structure portions occur in separate segments on the peptide chain;  $\alpha$ -helix and  $\beta$ -structure alternate along the peptide chain; and absence of  $\alpha$ -helix and  $\beta$ -structure.

$\beta$ -LactoGlobulin (BLG) and  $\alpha$ -LactAlbumin (ALA) are the major fractions of whey protein with a globular structure. Recently, whey has been considered as a tool for the delivery of bioactive and pharmaceuticals due to the presence of  $\alpha$ -helix and  $\beta$ -sheet in BLG and ALA. The secondary and tertiary structure makes this biopolymer a suitable vehicle for encapsulation. For example, whey protein gels may be used as pH-sensitive hydrogels for the controlled delivery of biologically-active substances. A hydrogel can be defined as a three dimensional network that exhibits the ability to swell in water and retains a significant fraction of water within its structure. The hydrogels ability to absorb water is due to the presence of hydrophilic groups such as  $-\text{OH}$ ,  $-\text{CONH}$ -,  $-\text{CONH}_2$ ,  $-\text{COOH}$  and  $-\text{SO}_3\text{H}$ . (J. Li *et al.*, 2016; Sullivan *et al.*, 2014; Zhong *et al.*, 2018).

$\beta$ -CycloDextrin ( $\beta$ -CD) is one of the common polymers for encapsulation of bioactive compounds.  $\beta$ -CD is a cyclic glucose oligomer with seven glucose units ( $\alpha$ -1,4-glucose), which is formed by the enzymatic modification of starch. The cavity of this oligomer can form an inclusion complex with hydrophobic compounds due to its hydrophobicity. Previous studies showed that encapsulation in  $\beta$ -CD can improve the solubility and stability of insoluble compounds. The cavity of this oligomer fits hydrophobic compounds with molecular weights in the range of 200–800 g mol<sup>-1</sup> (Mangolim *et al.*, 2014; Paramera *et al.*, 2011).

Various approaches have been proposed to fabricate biopolymer-based encapsulated bioactive compounds such as coacervation, liposome, nanoemulsion, nanostructure lipid carriers, spray drying, electrospinning, electrospray, supercritical fluid, emulsion-diffusion, reverse micelle, ultrasonication, emulsification/solvent evaporation, and

high-pressure homogenization. In recent years, the use of electrospray or electrospinning has been considerably developed as a new method for the encapsulation of bioactive compounds (Fathi *et al.*, 2014; Fathi *et al.*, 2012).

Electrospraying is a versatile and cost-effective method to produce particles in the micro, sub-micro, or nano range. Organic solvents and the temperature used in other methods that destroy sensitive encapsulated bioactives can be removed in this method, and it does not have the toxicity problems associated with residual organic agents (López-Rubio and Lagaron, 2012; López-Rubio *et al.*, 2012).

Several studies have been conducted in order to increase the solubility, stability, and bioavailability of curcumin by use of whey protein with spray drying and the antisolvent precipitation method (Aditya *et al.*, 2015; Liu *et al.*, 2016). Complex inclusion with  $\beta$ -CD and loading with biopolymers such as zein from the corn, casein, and amaranth-pullulan are other examples of delivery systems for curcumin (J. Li *et al.*, 2016). The aim of present study was to investigate the possibility of encapsulation of curcumin by electrospraying technique with natural polymer and, then, to investigate characteristics of the encapsulated curcumin in different conditions in comparison to pure state.

## MATERIALS AND METHODS

Curcumin( 65% purity, Mw 368.38, CAS Number 458-37-7) and  $\beta$ -CD (97% purity, Mw 1134.98, CAS No.: 7585-39-9) were obtained from Sigma (St. Louis, MO, USA) and BiPRO Whey Protein Isolate (WPI, purity 98%) was purchased from Davisco Foods Inc. (MN, USA). Ethanol (96%, cas number 64-17-5), HCl (0.1N, cat number 1099730001), NaOH (cat number, 1091411000) and Phosphate Buffer pH 7.4 (cat number 1465920006). Deionized water was used for the preparation of solutions.

### Preparation of WPI/Curcumin Solutions for Electrospraying

In this study, the solution was prepared using the methodology proposed by Gómez-Estaca *et al.* (2015) with little change. Briefly, 20 mL of different concentrations of WPI solutions (25, 30, and 34%, w/v) was prepared by dissolving a precise amount of the material in deionized water. Next, 2 mg of curcumin was dissolved in 500  $\mu$ L of ethanol and, then, added dropwise with continuous stirring to the prepared WPI solutions. The final solution was stirred at 1,000 rpm for 24 hours and at ambient temperature prior to electrospraying.

### Electrospraying Process and Characterization of Electrosprayed WPI/Curcumin Particles

The obtained solutions were loaded into 10 mL of syringe (gauge 18) and set in the electrospraying device (ES1000, Fanavaran Nano-meghyas, Iran, Rotating drum: stainless steel; Length: 30 cm, Diameter: 8 cm). Electrospraying was performed using the following parameters: applied voltage of 24kV; tip-to-collector distance of 150 mm; flow rate of 0.5 mL h<sup>-1</sup>. Due to the emphasis on using water alone as a solvent, the spray particles were not dry properly at a specified interval and wet particles accumulated on the collector. To solve the problem, a temperature of 40°C was used in the chamber.

The morphology and diameter of the particles were determined using Scanning Electron Microscopy (SEM; Model 6400; JEOL, Boston, MA, USA and Mira3-Tescan, USA). Scanning electromicrographs of the particles were obtained after coating the particles with gold for 200 seconds at 60 mA under 0.5 mbar pressure, using a Bal-Tec (Balzers, FL, USA) SCD 005 sputtering system. To draw the particle size distribution, diameters of 300 particles were measured by digimizer software and then the histograms of particle sizes were drawn in Excel 2016.

Fourier-Transform Infrared Spectroscopy (FTIR) was conducted to study the presence of functional groups of curcumin, WPI,  $\beta$ -CD, WPI/curcumin, and WPI/curcumin/ $\beta$ -CD using an FTIR spectrometer, Vertex-70 (Bruker Optik GmbH, Germany) over the scanning range of 500-4,000 cm<sup>-1</sup>. The ThermoGravimetric Analysis (TGA) curves of curcumin, WPI, WPI/curcumin,  $\beta$ -CD, and WPI/curcumin/ $\beta$ -CD were recorded with the TGA/DSC1 equipment (METTLER TOLEDO, Switzerland). Samples were heated from room temperature to 700°C at the heating rate of 5°C min<sup>-1</sup> under argon atmosphere (Blanco-Padilla *et al.*, 2015).

The Differential Scanning Calorimetry (DSC) of curcumin,  $\beta$ -CD, WPI, WPI/curcumin, and WPI/curcumin/ $\beta$ -CD was performed using TGA/DSC1 (METTLER TOLEDO, Switzerland). Samples were heated from 25 to 300°C at the heating rate of 10°C under a constant flow (100 mL min<sup>-1</sup>) of nitrogen gas.

### Preparation of the Inclusion Complex of Curcumin/ $\beta$ -CD

$\beta$ -CD was dissolved in deionized water at 75°C and, after 2 hours, 2 mg of curcumin was dissolved in ethanol and then added. After 24 hours, whey protein isolate was also added to achieve a 25% concentration (w/v). The molar ratio of curcumin and  $\beta$ -CD was selected as 2:1 and then the prepared solution was injected to the electrospraying device (Mangolim *et al.*, 2014).

### Encapsulation Efficiency

First, WPI/curcumin (1g) and WPI/curcumin/ $\beta$ -CD particles were washed twice with 96% ethanol to eliminate the loosely adsorbed curcumin on the surface of encapsulated particles. In the next step, they were re-suspended in 10 mL of ethanol and, after 6 hours of stirring, the solution was centrifuged (Eppendorf 022622501 5804) at



14,000 rpm for 10 minutes. The supernatant was collected and analyzed at 425 nm using a UNICO 4802 UV visible spectrophotometer (Shanghai, China). The amount of encapsulated curcumin was calculated from the calibration curve (Blanco-Padilla *et al.*, 2015).

$$EE(\%) = \frac{C_E}{C_T} \times 100$$

Where,  $C_E$  and  $C_T$  refer to the Encapsulated Curcumin and the Total mass of Curcumin added initially, respectively.

### pH Stability of Encapsulated Curcumin

Precise amounts of pure curcumin, WPI/curcumin, and WPI/curcumin/ $\beta$ -CD were dissolved in the ethanol that was previously adjusted to a pH value in the range of 1, 4, 7.5, 9, and 12, and the absorbance of curcumin was determined after 4 hours.

### In Vitro Curcumin Release Study

The *in vitro* release of curcumin from electrosprayed particles was determined by transferring a precise amount (1 g) of curcumin-loaded particles to vials containing 10 mL of PBS (Phosphate-buffered saline), pH= 7.4. Samples were then maintained at 37°C in a shaking (100 rpm) incubator. At constant intervals, 50  $\mu$ L of aliquots was withdrawn and diluted with equal volumes of ethanol and then analyzed at 425 nm with a UV visible spectrophotometer. The withdrawn aliquots were replaced with equal volumes of fresh PBS, 7.4 buffer, to keep the volume of the release medium constant. The cumulative release (% w/w) was calculated using the standard of curcumin in PBS/ethanol (1:1) (El-Sherbiny and Smyth, 2011).

### DPPH (1,1-diphenyl-2-picrylhydrazyl) Radical Scavenging Activity

According to a previous study (Liu *et al.*, 2016), samples of (WPI/ $\beta$ -CD and WPI/curcumin/ $\beta$ -CD) were dissolved in

0.1N HCL solution to prepare a concentration of 0-10 mg mL<sup>-1</sup>. Then, 1 mL of each solution was mixed with 2 mL of 0.2 mM DPPH ethanol solution and subsequently incubated at room temperature in the dark for 30 minutes. The final solution was centrifuged at 5,000 rpm for 5 minutes and the absorbance was measured at 517 nm. Each test was performed in triplicate.

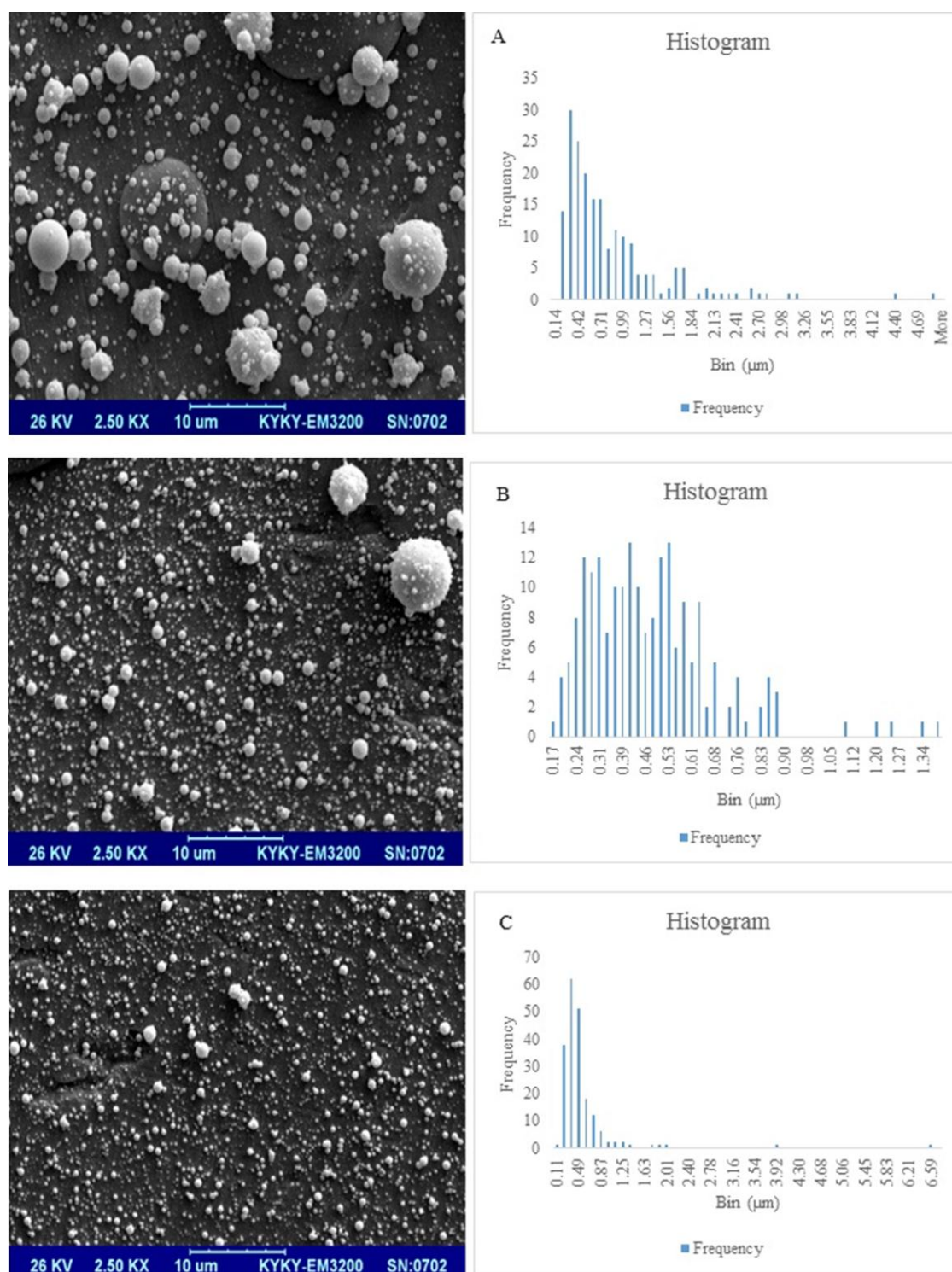
$$\text{Radical scavenging activity (\%)} = \frac{\text{Optical density of control} - \text{Optical density of sample}}{\text{Optical density of control}} \times 100$$

## RESULTS AND DISCUSSION

### Process and Characterization of Particles

For the selection of optimized solution, three WPI concentrations (25, 30, and 34% w/v) were applied to the electrospray device to obtain the best particles for the encapsulation of curcumin. Figure 1 shows the size and distribution of WPI-curcumin particles. At the concentration of 25% (Figure 1-C), electrospraying was performed better than 34 (Figure 1-A) and 30% (Figure 1-B) because the size and distribution of particles were more homogeneous at 25%. Based on Figure 1-C, more uniform particles were formed and most of them were smaller than 0.7  $\mu$ m in diameter. Due to the uniformity and small size of particles, the concentration of 25% was selected as the best concentration for electrospraying.

This result was confirmed by López-Rubio and Lagaron (2012). They utilized Whey Protein Concentrate (WPC) instead of WPI for the encapsulation of  $\beta$ -carotene by electrospraying. They reported that, at concentrations below 35%, only drops of the solution were collected and at concentrations above 60% a gelled structure was collected from the unstable jetting during the electrospraying process. In this research, WPI was used instead of WPC. Only drops





were observed at concentrations below 25% and a gelled structure was collected at concentrations above 34%. (Shenoy *et al.*, 2005) have developed a semi-empirical theory in which a critical parameter, the entanglement number for a solution,  $(\eta e)_{\text{soln}}$ , is defined to indicate whether electrospraying (beads) or electrospinning (fiber) will take place. The value of  $(\eta e)_{\text{soln}}$  can be obtained from the following equation:

$$(\eta e)_{\text{soln}} = \frac{\phi M_w}{M_e}$$

Where,  $M_w$  is the Molecular weight of the polymer in solution and  $M_e$  is the polymer entanglement Molecular weight.

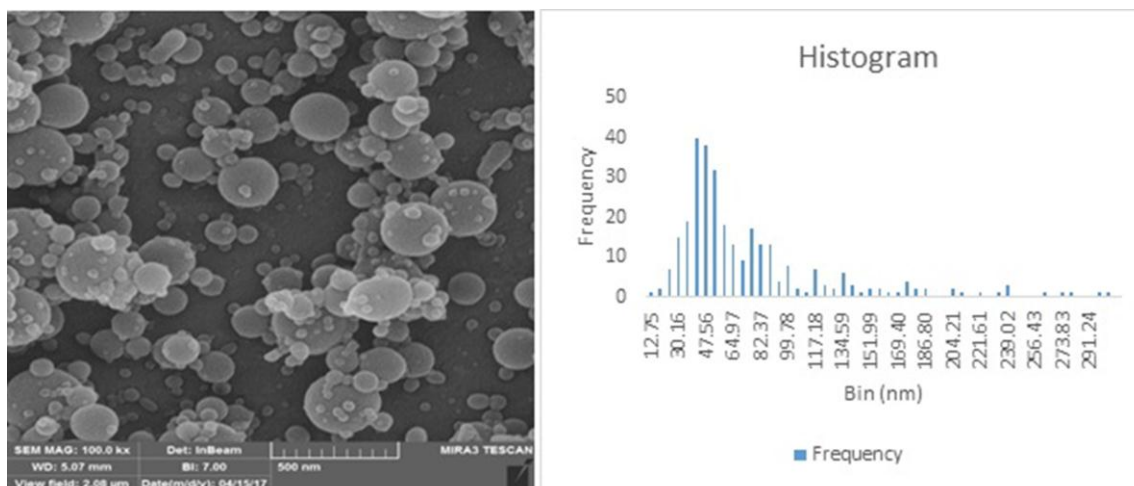
For pure fiber formation by electrospinning, it is typically necessary to use a polymer solution with an entanglement number greater than three and a half. At an entanglement number between two and three and half, there will be a transition from beaded fiber to pure fiber.

At an entanglement number less than two, electrospraying occurs wherein only particles are formed that are visible as beads in the collected material. By reducing the concentration of the solution, the particles size were decreased due to proper spreading of polymer (Ghorani and Tucker, 2015). Also The WPI solutions behaved as Newtonian fluids at concentration ranges <

10% WPI and at higher concentrations up to 50% WPI demonstrated a non-Newtonian behavior and this issue can affect particles size.

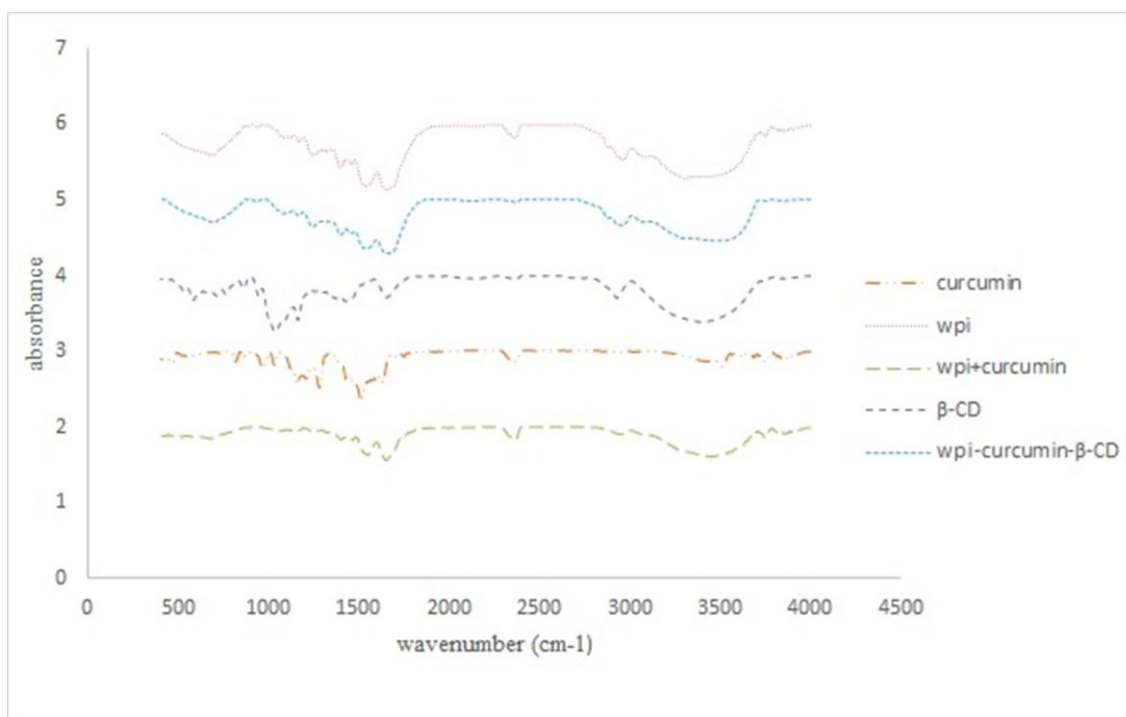
Based on the results, two predominant whey proteins, namely,  $\beta$ -LactoGlobulin (BLG) and  $\alpha$ -LactAlbumin (ALA), have a globular form that prevents them from having sufficient entanglement or interaction with electrospinning, but electrospraying can be created and, consequently, micro- and nanoparticles can be formed (Sullivan *et al.*, 2014; Zhong *et al.*, 2018).

Figure 2 illustrates the size and distribution of the inclusion complex of curcumin in  $\beta$ -CD encapsulated in whey protein isolate solution (25%, w/v). The distribution of particles indicates that  $\beta$ -CD caused more uniform size and much smaller particles. Presumably, the inclusion complex of curcumin in  $\beta$ -CD could help form a better emulsion, leading to the formation of small and uniform particles. Kayaci and Uyar (2012) reported that the addition of  $\beta$ -CD to zein solutions affects electrospinning and bead-free nanofibers. This can be attributed to the reduction of the conductivity and the increase in the viscosity of the solution by adding  $\beta$ -CD (Kayaci and Uyar, 2012).



**Figure 2.** SEM image and particle size distribution of inclusion complex of curcumin in  $\beta$ -CD encapsulated in WPI solution (25% w/v).





**Figure 3.** FT-IR spectra of curcumin, WPI,  $\beta$ -CD, WPI/curcumin and WPI/curcumin/ $\beta$ -CD.

The FTIR spectra for curcumin, WPI,  $\beta$ -CD, WPI/curcumin, and WPI/ $\beta$ -CD/curcumin particles are depicted in Figure 3. For curcumin, the bands observed at 3,511, 1,596, 1,509, 1,427, 1,277, 1,027, and 962  $\text{cm}^{-1}$  are respectively attributed to the phenolic O-H stretching vibration, stretching vibration of benzene ring, C=O and C=C vibration, olefinic C-H bending vibration, and C-O-C stretching vibration, supported by published data (Sun *et al.*, 2013). The polypeptide and protein repeat units of whey protein show special IR absorption bands for amides A and B, and amides I to VII. From among them, Amide I (stretching vibration in the C=O bond at 1,580-1,720  $\text{cm}^{-1}$ ) and Amide II (bending vibrations of the N-H bond at 1,450-1,600  $\text{cm}^{-1}$ ) are the two most important vibrational bands of the protein backbone. However, the secondary structure changes are better observed at the absorption bands of Amide I due to the slight contribution of C-N stretching, C-C-N deformation, and N-H (in-plane) bending (Kong and Yu, 2007; O'Loughlin *et al.*, 2015). For  $\beta$ -CD, the band

at 3,300-3,400  $\text{cm}^{-1}$  belongs to O-H group stretching. An intense peak at 2,900  $\text{cm}^{-1}$  is due to C-H asymmetric/symmetric stretching. Moreover, a peak at 1,650  $\text{cm}^{-1}$  represents the H-O-H deformation bands of water present in  $\beta$ -CD. Peaks at 1,153 and 1,029  $\text{cm}^{-1}$  indicate C-H and that at 1,029  $\text{cm}^{-1}$  indicates C-H, C-O stretching. Furthermore, C-O-C vibration was seen at 1153  $\text{cm}^{-1}$ . In the case of WPI/curcumin, spectrum intensity of WPI at wavelengths under 1,700  $\text{cm}^{-1}$  was decreased, expressing the interaction between curcumin and WPI.

In the case of WPI/curcumin, the FTIR spectra showed that the presence of curcumin reduced the intensity of spectrum, but in the case of WPI/curcumin/ $\beta$ -CD, the FT-IR spectra did not show any extra absorption band or absorption band shifting, indicating the absence of non-covalent interaction between curcumin,  $\beta$ -CD, and whey protein.

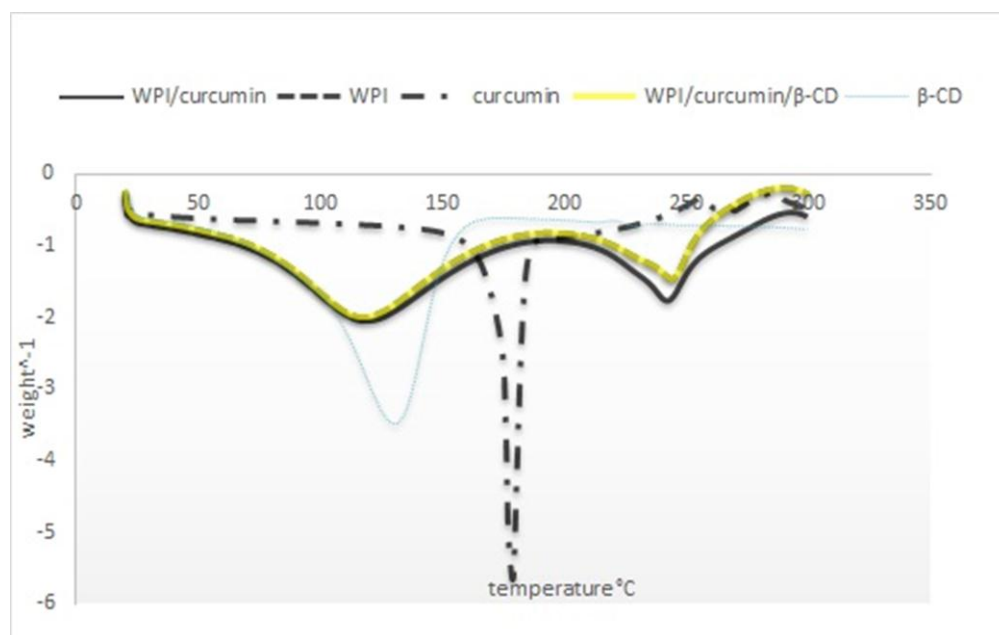
The FTIR spectra of WPI and WPI/curcumin/ $\beta$ -CD are approximately similar, because the inclusion complex of

curcumin in  $\beta$ -CD avoids significant band changes at wavelengths under  $1,700\text{ cm}^{-1}$ .

Figure 4 illustrates the physical state and crystallization properties of curcumin,  $\beta$ -CD, WPI, WPI/curcumin, and WPI/curcumin/ $\beta$ -CD. DSC can be used for identification of encapsulation. As a guest molecule is embedded into  $\beta$ -CD cavities or WPI, melting, boiling and sublimation points may be shifted to different temperatures or simply vanish (Abarca *et al.*, 2016). The DSC scan of curcumin showed an endothermic peak at  $179^\circ\text{C}$  that belongs to the melting point. An exothermic peak began at  $193^\circ\text{C}$ , indicating thermal decomposition. For  $\beta$ -CD, a single endothermic peak at  $125^\circ\text{C}$  was found, which is associated with the release of water (Pinto *et al.*, 2005). The DSC thermogram of WPI showed two specified endothermic peak; the first one was at  $119^\circ\text{C}$  due to the loss of water, and the second one was at  $243^\circ\text{C}$ , corresponding to its melting point. The DSC scan of WPI/curcumin and WPI/curcumin/ $\beta$ -CD revealed that the curcumin crystal melting endothermic peak was vanished, which would indicate molecular encapsulation of the curcumin

within the WPI and cavity of the  $\beta$ -CD. Presumably, crystal melting endothermic peak of  $\beta$ -CD in the WPI/curcumin/ $\beta$ -CD was disappeared because of encapsulation of  $\beta$ -CD itself into WPI molecules.

TGA is an effective way to evaluate changes in physical and chemical properties of material (Zhu *et al.*, 2014). As demonstrated in Figure 5, the thermogravimetric analysis curves of WPI, WPI/curcumin, and WPI/curcumin/ $\beta$ -CD are similar to one another, showing water loss below  $100^\circ\text{C}$  and decomposition starting at  $230^\circ\text{C}$  and continued to  $500^\circ\text{C}$ . The beginning of degradation is at a lower temperature than raw curcumin ( $244^\circ\text{C}$ ), indicating that WPI and  $\beta$ -CD have a weaker thermal stability than curcumin. The TGA of curcumin did not show a stage of water loss due to low hydrophilicity. WPI and WPI/curcumin/ $\beta$ -CD have two stages of degradation. The first one is around  $40\text{--}98^\circ\text{C}$  and showing the loss of water, and the second one ( $288\text{--}450^\circ\text{C}$ ) is associated with protein degradation. Pure curcumin demonstrated more stability than WPI. Loss of water for  $\beta$ -CD happened at  $104^\circ\text{C}$  and degradation started at  $318^\circ\text{C}$ . The  $\beta$ -CD



**Figure 4.** DSC results of curcumin,  $\beta$ -CD, WPI, WPI/curcumin and WPI/curcumin/ $\beta$ -CD.



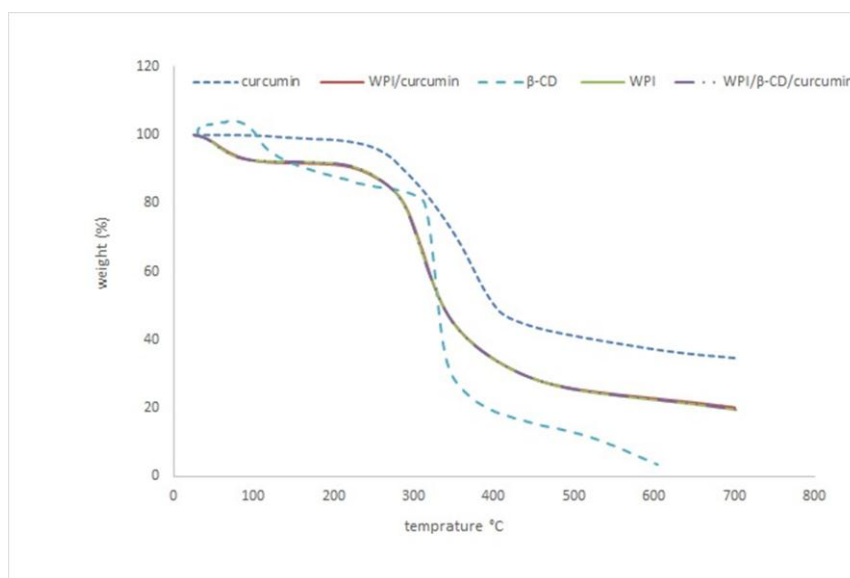


Figure 5. TGA traces of curcumin, WPI,  $\beta$ -CD, WPI/curcumin and WPI/curcumin/ $\beta$ -CD.

thermoanalytical profile generally depicts three parts: (1) Water loss between ambient temperature up to 120°C, (2) Thermal degradation due to oxidation in air, starting above 250°C in solid phase at first and continuing in liquid state after fusion, which occurs approximately at 300°C, and (3) Ignition happening in air above 300°C (Giordano *et al.*, 2001). Based on the thermogravimetric analysis curves, heat resistance of curcumin is higher than WPI and  $\beta$ -CD, therefore, these two polymers are not a suitable option to increase the thermal resistance of curcumin.

### Encapsulation Efficiency

In the present work, 2 mg of curcumin was dissolved in 20 mL of WPI solution (25%, w/v) and the encapsulation of curcumin was determined at about 45.4%. Through the inclusion complex of curcumin with  $\beta$ -CD prior to adding WPI, encapsulation was significantly increased up to 53.6% due to better dispersion and protection of curcumin. Liu *et al.* (2016) studied the effect of three concentrations of WPI (1, 5, and 10%) on the solubility of curcumin. They reported that curcumin saturation linearly increased with WPI concentration. They found the

estimated amount of curcumin loaded in the particles to be about 1.19 mg g<sup>-1</sup> in dry weight. Compared to solubility of 11 ng mL<sup>-1</sup> of curcumin in water, M. Li *et al.* (2015) reported that the solubility of curcumin was increased 15,954-fold through the BLG-curcumin complex.

The tertiary structure of globular whey protein is formed through covalent, electrostatic, hydrogen, and hydrophobic bonds that could encapsulate curcumin in their cavity (Belitz *et al.*, 2008). The driving forces of  $\beta$ -CD for the encapsulation of curcumin are van der Waals and hydrophobic interaction. With regard to encapsulation efficiency, WPI/ $\beta$ -CD can be an appropriate choice for the encapsulation of curcumin as it is an inexpensive and natural by-product. However, in comparison with encapsulation efficiency of curcumin in zein nanofiber (83%), gelatin (100%) and polyvinylpyrrolidone (93%) showed a lower efficiency because of heterogeneous distribution of polymer at the time of preparing the solution (Blanco-Padilla *et al.*, 2015).

pH Stability, DPPH Radical Scavenging Activity, and *in Vitro* Curcumin Release Assay

In acidic and neutral condition (i.e. pH= 3-7), curcumin molecules are in the bis-keto



form (yellow). However, in alkaline condition (i.e. pH > 8), it is in the enolate form (red) (Lee *et al.*, 2013). As shown in Table 1, encapsulated curcumin had a better stability than pure curcumin at acidic and alkaline conditions. The percentage of reduction of curcumin was lower at acidic condition than alkaline one because curcumin is unstable in alkaline medium due to hydrolytic degradation. The pH value of 4.5 is the isoelectric point of WPI where the net charge is close to zero and the swelling rate of gels is minimum. However, at pH levels lower and higher than the isoelectric point, the net charge is increased due to electrostatic repulsion, which increases the swelling rate and solubility (Gunasekaran *et al.*, 2007). It can be concluded that the swelling of WPI, increases curcumin encapsulation. At pH levels close to the isoelectric point, the protective effect of WPI was lower and the percent reduction of curcumin was similar to that of the pure state. Nevertheless, at pH levels lower or higher than the isoelectric point, protection was higher. M. Li *et al.* (2015) reported that the stability of curcumin was increased by forming complex with BLG because more than 95% of curcumin was retained in the pH range from 2 to 8 in 12 hours.

The acidity of the terminal OH-groups of  $\beta$ -CD plays a key role in the complex state.

This ability is stronger in basic condition due to deprotonated OH-group, i.e. O<sup>-</sup> group. Presumably, this reason can provide more protection in alkaline conditions (Gaidamauskas *et al.*, 2009).

The release rate of encapsulated bioactive compound from this delivery system depends on factors such as equilibrium partition coefficient, their original location, the mass transfer coefficients of the bioactive in different phases, mechanical agitation, and the microstructure of the system, e.g. particle size, particle degradation, and layer thickness. Burst, sustained, and delayed are three major models proposed for releasing active ingredients from carrier particles (McClements, 2014). Based on Table 2, the release of curcumin follows the sustained model in intervals of 1.5 hours, it is explosive in the first three hours, and then turns into an almost linear state with a low slope. Cumulative release curcumin (%) was about 40% after 7.5 hours, indicating that WPI can sustain the release of curcumin over time. The inclusion complex of curcumin with  $\beta$ -CD caused a slower release than WPI alone due to the better encapsulation, and only 22% was released after 6 hours.

Figure 6 depicts the antioxidant activity of WPI and WPI/curcumin/ $\beta$ -CD particles

**Table 1.** Percent reduction of curcumin in pure, WPI/curcumin and WPI/curcumin/ $\beta$ -CD state in pH (1, 4, 7, 9 and 12).<sup>a</sup>

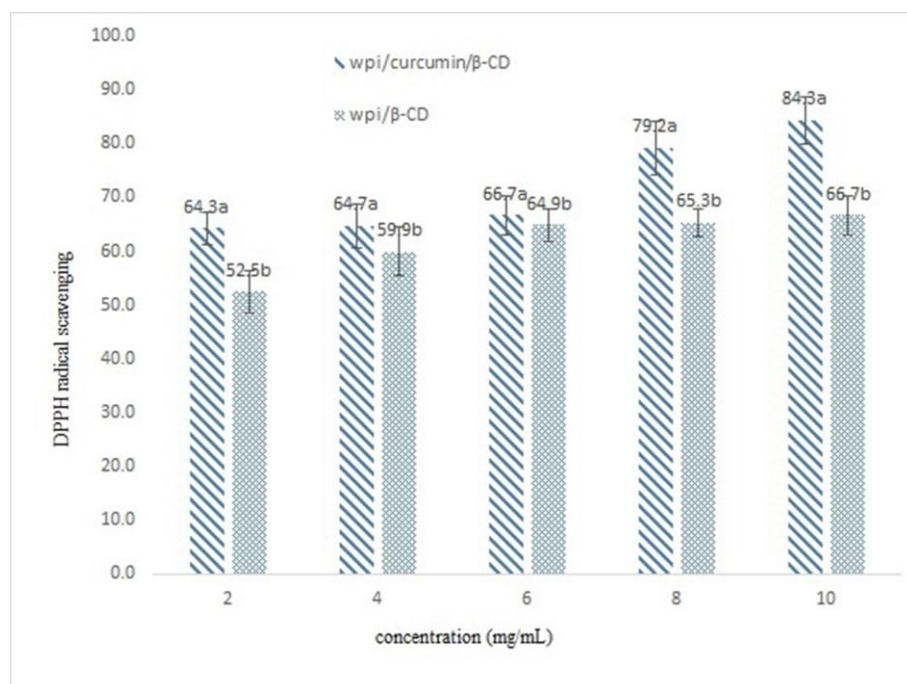
| Polymer                   | Percent of curcumin reduction |                  |                  |                  |                 |
|---------------------------|-------------------------------|------------------|------------------|------------------|-----------------|
|                           | pH= 1                         | pH= 4            | pH= 7            | pH= 9            | pH= 12          |
| WPI/Curcumin              | 1.84 $\pm$ 0.2a               | 35.6 $\pm$ 0.05a | 34.31 $\pm$ 0.8a | 34.3 $\pm$ 10.7b | 39 $\pm$ 13.5a  |
| WPI/Curcumin/ $\beta$ -CD | 2 $\pm$ 0.09a                 | 33.6 $\pm$ 0.07b | 29 $\pm$ 0.04b   | 30 $\pm$ 0.2a    | 38 $\pm$ 0.03a  |
| Curcumin                  | 33.4 $\pm$ 11.3c              | 36.1 $\pm$ 6.9c  | 38.2 $\pm$ 9.21c | 45.1 $\pm$ 0.08c | 70.3 $\pm$ 9.6b |

<sup>a</sup> Different letters in the same column (a, b) indicate differences between samples.

**Table 2.** Cumulative release profile of curcumin from WPI/curcumin and WPI/ $\beta$ -CD/curcumin in phosphate buffer solution at pH 7.4.<sup>a</sup>

| Polymer          | Release of curcumin |                   |                  |                  |                  |                  |                 |
|------------------|---------------------|-------------------|------------------|------------------|------------------|------------------|-----------------|
|                  | 1 (h)               | 2 (h)             | 3 (h)            | 4 (h)            | 5 (h)            | 6 (h)            | 7 (h)           |
| WPI              | 14 $\pm$ 9.3a       | 26.2 $\pm$ 2.63a  | 38.1 $\pm$ 3.32a | 39.5 $\pm$ 0.45a | 39.5 $\pm$ 0.97a | 40.3 $\pm$ 0.69a | 40.3 $\pm$ 1.1a |
| WPI/ $\beta$ -CD | 11.9 $\pm$ 0.076b   | 15.5 $\pm$ 0.067b | 16.9 $\pm$ 0.56b | 18.9 $\pm$ 0.08b | 21.4 $\pm$ 17.1b | 22.2 $\pm$ 0.04b | 22.1 $\pm$ 2.6b |

<sup>a</sup> Different letters in the same column (a, b) indicate significant at 5% (p<0.05), differences between samples.



**Figure 6.** DPPH free radical scavenging activity of WPI/β-CD and electrosprayed WPI/curcumin/β-CD at different concentration.

determined via DPPH radical scavenging assay. In agreement with previous reports (Chiang and Chang, 2005; Kerasioti *et al.*, 2014), WPI alone also had antioxidation activity. The antioxidant activity of WPI is due to the reaction with thiobarbituric acid (Kamau and Lu, 2011). By increasing curcumin to WPI, antioxidation activity was increased due to the donation of hydrogen atom to radicals. Electrospraying prepares a mild condition for the encapsulation of curcumin in comparison with spray drying, and thus the antioxidation activity was better protected.

## CONCLUSIONS

In this study, the electrospraying technique was employed to encapsulate curcumin in a natural polymer for further protection against environmental conditions. The experimental results indicated that the WPI solution with 25% w/v had a suitable spray in test conditions (24kV, 150 mm, 0.5 mL h<sup>-1</sup>) and electrospraying did not occur below and

above this concentration due to dripping and the gelled structure of the solution, respectively. The encapsulation efficiency of curcumin in WPI and WPI/β-CD equaled 45.4 and 53.6%, respectively, because of the specific structure of WPI and β-CD. The release of curcumin in encapsulated state was in the sustained mode in the buffer solution condition, showing that encapsulation can slow down curcumin release. Due to lower thermal resistance than curcumin, WPI and WPI/β-CD could not protect curcumin in severe heat conditions.

## ACKNOWLEDGEMENTS

The authors wish to thank the Tarbiat Modares University for financial support.

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## میکروذرات ایجاد شده با روش الکترواسپری با استفاده از بسپارهای ایزوله پروتئین آب پنیر و کمپلکس بتا سیکلودکستین

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### چکیده

در این مطالعه از پتانسیل روش الکترواسپری برای درونپوشانی کورکومین در بسپارهای ایزوله پروتئین آب پنیر و ترکیب ایزوله پروتئین آب پنیر/ بتاسیکلودکستین استفاده شد. ذرات درونپوشانی شده از لحاظ فیزیکوشیمیایی و نحوه رهایش مورد بررسی قرار گرفت. در غلظت ایزوله پروتئین آب پنیر ۲۵٪، ذرات یکنواخت تری شکل گرفت و بیشتر این ذرات کمتر از ۰/۷ میکرومتر قطر داشتند. کارایی درونپوشانی کورکومین در ایزوله پروتئین آب پنیر و ایزوله پروتئین آب پنیر/ بتاسیکلودکستین به ترتیب ۴۵/۴٪ و ۵۳/۶٪ اندازه گیری شد. گرماسنجی افتراقی و آنالیز توزین حرارتی ذرات درونپوشانی شده نشان داد این دو بسپار توانایی افزایش مقاومت حرارتی کورکومین را ندارند. کورکومین درونپوشانی شده پایداری بالاتری از حالت خالص کورکومین در شرایط اسیدی و قلیایی از خود نشان داد و رهایش کورکومین در حالت درونپوشانی شده در محلول بافر فسفات (۷/۴ pH) پس از مدت ۷ ساعت به میزان ۴۰٪ اندازه گیری شد.