Application of response surface methodology and spectroscopic approach for investigating of curcumin nanoencapsulation using natural biopolymers and nonionic surfactant

Shaghayegh Sheikhzadeh¹ · Mohammad Alizadeh¹ · Mahmoud Rezazad¹ · Hamed Hamishehkar²

Revised: 24 May 2016 / Accepted: 13 October 2016 / Published online: 7 November 2016
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Abstract This study aimed at encapsulation of poor water-soluble curcumin so that it could be utilized in various food products as a functional ingredient. Biopolymer nanoparticles were assembled from sodium caseinate and gum arabic using electrostatic complexation in the presence of nonionic surfactant Tween 20. Fourier transform infrared spectroscopy was used to investigate the interactions of curcumin with protein, polysaccharide and surfactant. The effects of sodium caseinate (A), gum arabic (B), Tween 20 (C) and pH (D) on responses were studied using a three-level four-factor Box–Behnken design. For each response, a second-order polynomial model was developed. For optimum nanoencapsulation of curcumin, the variables concentration of sodium caseinate, concentration of gum arabic, pH and concentration of Tween 20 were 0.21, 0.5, 5 and 0.14 wt% respectively. The particles were characterized by ζ -potential measurement. Spectroscopic results and data modelling, showed interaction of curcumin with sodium caseinate, gum arabic and Tween 20. Also, the nonionic surfactant Tween 20, influenced the electrostatic interaction between sodium caseinate and gum arabic.

Keywords Curcumin · Sodium caseinate · Tween 20 · Nanodispersion · FTIR · Zeta potential

Introduction

There is an increasing interest within the food and pharmaceutical industries in the utilization of nanodispersion as a delivery system for lipophilic active components. Curcumin is a lipophilic low-molecular-weight natural polyphenol that is found in the rhizome of turmeric (*Curcuma longa*). There is considerable interest in making use of curcumin as a functional ingredient in foods and pharmaceuticals because of its potentially beneficial activities, including anti-microbial, anti-oxidant, anti-carcinogenic, anti-inflammatory, anti-parasitic and anti-mutagenic activities (Anitha et al. 2011; Boruah et al. 2012). However, utilization of curcumin as a nutraceutical ingredient is limited due to its low oral bioavailability, poor water solubility, chemical instability in physiological alkaline pH, and susceptibility to thermal treatment, light, metallic ions, enzymes, oxygen and ascorbic acid. To solve these drawbacks, different encapsulation methods have been proposed. Through encapsulation, a bioactive compound can be protected from destructive environmental factors, solubilized and delivered in a controlled manner. One of the most popular and commercially available approaches for the delivery of hydrophobic drugs is polymeric delivery system. Natural biopolymers, such as proteins and polysaccharides, are the most important types of surface-active materials can be preferably used for production of food-grade dispersions in the food industry (Jones and McClements 2010).

One of the main advantages of colloidal delivery systems is their ability to enhance bioaccessibility without compromising the product functionality (e.g. stability, appearance, texture and taste). Sodium caseinate is one of the most common proteins used in encapsulation of nutrients. It presents many desirable

✉ Mohammad Alizadeh
malizadeh@outlook.com

¹ Department of Food Science and Technology, Urmia University, Urmia, Iran

² Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

characteristics as a good encapsulating agent such as biodegradability, biocompatibility, functionality, non-toxicity and inexpensiveness (Liu et al. 2012). The gum arabic (GA) has excellent functional and physical properties including high solubility, pH stability and non-toxicity (Gils et al. 2010). Tween 20 (T20) is a fatty acid ester of sorbitan polyethoxylate, a nonionic and biodegradable surfactant that is widely used in food products (Picone and Cunha 2013).

Even though, the interaction between protein and polysaccharide is well recognized, the influence of surfactant on the electrostatic interaction between protein and polysaccharide has not been fully investigated. According to extensive investigation on interactions between protein and polysaccharide (de Kruif and Tuijnier 2001; Harnsilawat et al. 2006), it is obvious that binary systems show better properties. Recently, Li and McClements (2013), evaluated the effect of T20 on the formation and properties of electrostatic complexes consisting of charged oil droplets and charged biopolymers (β -lactoglobulin, alginate and chitosan). Since Xu et al. (1998) reported that the β -lactoglobulin and α -lactoglobulin had a distinct behavior in the presence of T20, it has been postulated that the behavior of a globular protein (β -lactoglobulin) and a flexible protein (SC) is different in the presence of a non-ionic surfactant (T20). Most of the existing research on nanodispersing techniques have been focused on dispersion stabilization by toxic surfactants, synthetic materials or using high energy equipments. Anarjan and Tan (2013) showed that three component nanodispersion including polysaccharide, protein and small molecular emulsifier could be produced with desirable characteristics and higher physicochemical stability compared to individual components. The objective of this work was to fabricate and optimize a food-grade colloidal delivery system for curcumin using protein, polysaccharide and small molecular surfactant. The solvent displacement method followed by acidification was selected for fabrication because it is cost-effective, uses a nontoxic solvent and does not need any specialized equipment such as additional sonicator or homogenizer. To our knowledge, to date, no studies have been published that investigate the formation of electrostatic complexes between SC and GA in the presence of T20 at various acidic pH. Since, Fourier transform infrared spectroscopy (FTIR) is an appropriate technique to understand functionalization of nanoparticles, and obtain some deeper insights into the effect of surfactant on protein-polysaccharide electrostatic interaction, FTIR and zeta-potential measurement were employed.

Materials and methods

Materials

The sodium caseinate (SC) was obtained from DMV company, Holland. Gum arabic (GA) and ethanol were obtained from Scharlau, Spain. Curcumin and Tween 20 (T20) were purchased from Sigma–Aldrich Chemical Company, USA. Sodium azide, hydrochloric acid (HCl) and all other chemicals with analytical grade were obtained from Merck Darmstadt, Germany.

Preparation of solutions

Curcumin (0.05 wt%) powder was dissolved in absolute ethanol. SC, GA and T20 solutions of different concentration were prepared by dissolving their appropriate proportions in 5 mM phosphate buffer pH 7.0 containing 0.02 wt% sodium azide as a preservative, in separate beakers for solubilization under gentle magnetic stirring. After preparation, solutions were stored at 4 °C overnight to allow complete hydration of the biopolymers. Centrifugation (6000 rpm, 15 min) of the solutions were carried out before use to remove small fractions of insoluble material and air bubbles.

Preparation of nanodispersion

Nanodispersions were formed by dropwise addition of organic phase into aqueous phase containing different proportions of SC, GA and T20 as shown in Table 1 (volume ratio of 1: 1: 1), under moderate magnetic stirring (500 rpm). The ratio of organic phase to aqueous phase was set at 1:10 by volume. After this step, the solutions were acidified by drop wise addition of 0.1 and 0.01 N HCl from pH = 7.0 to pH = 4.8, 5 and 5.2 (Table 1) using a 30-gauge needle syringe at room temperature. The pH of the solution was continuously monitored using a pH meter (pH 510, Eutech instruments, Singapore). The solution was stirred at 500 rpm for 1 h and then, an evaporation step was carried out using a rotary evaporator (R-200, BÜCHI, Switzerland) under reduced pressure to remove ethanol solvent from the dispersion. The samples were stored over night at room temperature prior to analysis.

Fourier transform infrared spectroscopy (FTIR) analysis

Samples were gently mixed with KBr and then compressed into discs by pressing the powders. FTIR spectra in the absorbance mode were recorded using FTIR spectrophotometer (IR Prestige-21, Shimadzu, Japan).

Table 1 Box-Benken design used to evaluate the effects of processing factors on fabrication of nanodispersion

Run	SC (wt%) (A)		GA (wt%) (B)		T20 (wt%) (C)		pH (D)	
	Uncoded value	Coded value	Uncoded value	Coded value	Uncoded value	Coded value	Uncoded value	Coded value
1	0.1	−1	0.75	0	0.2	+1	5	0
2	0.2	0	0.75	0	0.2	+1	5.2	+1
3	0.3	+1	0.75	0	0.1	0	5.2	+1
4	0.1	−1	1	+1	0.1	0	5	0
5	0.2	0	0.75	0	0.2	+1	4.8	−1
6	0.1	−1	0.75	0	0.1	0	4.8	−1
7	0.2	0	1	+1	0.2	+1	5	0
8	0.2	0	0.75	0	0	−1	5.2	+1
9	0.2	0	0.5	−1	0.1	0	4.8	−1
10	0.1	−1	0.5	−1	0.1	0	5	0
11	0.2	0	0.5	−1	0	−1	5	0
12	0.3	+1	0.75	0	0.1	0	4.8	−1
13	0.2	0	0.75	0	0	−1	4.8	−1
14	0.1	−1	0.75	0	0.1	0	5.2	+1
15	0.3	+1	0.5	−1	0.1	0	5	0
16	0.2	0	0.5	−1	0.2	+1	5	0
17	0.2	0	0.75	0	0.1	0	5	0
18	0.2	0	1	+1	0	−1	5	0
19	0.2	0	0.75	0	0.1	0	5	0
20	0.3	+1	0.75	0	0	−1	5	0
21	0.3	+1	1	+1	0.1	0	5	0
22	0.1	−1	0.75	0	0	−1	5	0
23	0.2	0	0.75	0	0.1	0	5	0
24	0.2	0	0.5	−1	0.1	0	5.2	+1
25	0.2	0	1	+1	0.1	0	4.8	−1
26	0.3	+1	0.75	0	0.2	+1	5	0
27	0.2	0	1	+1	0.1	0	5.2	+1
28	0.1	−1	0.75	0	0.1	0	4.8	−1
29	0.1	−1	0.75	0	0.1	0	4.8	−1
30	0.1	−1	0.75	0	0.1	0	4.8	−1

Particle size analysis

Measurements of the mean diameter of the nanoparticles and particle size distribution were carried out by the laser light scattering technique using a particle size analyzer (SALD 2101, Shimadzu, Japan). Measurements are reported as the volume-weighted mean diameter.

$$D[3, 4] = \frac{\sum n_i d_i^4}{\sum n_i d_i^3}$$

where n_i is the number of droplets of diameter d_i .

Turbidity measurement

The turbidity of dispersions was analyzed by a UV–visible spectrophotometer (pharmacia 80-2092-26, LKB Biochrom,

England), at 600 nm. Distilled water was used as a blank reference. The dispersions were vortexed immediately before each measurement to ensure sample homogeneity and prevent any changes in turbidity due to droplet sedimentation (Harnsilawat et al. 2006).

Encapsulation efficiency (EE) (%)

The EE was measured by separation of nanoparticles from the 1 ml of aqueous nanodispersions by centrifugation at 13,000 rpm/min for 30 min (Hettich UNIVERSAL 320 R, Germany). In order to remove the possible residual small particles that do not settle down during centrifugation, a second filtration of the supernatant was done using a PTFE syringe filter with 0.22 μ m pore size. Afterward, the suspension (40 μ l) was diluted to 2 ml with acetone and its

absorbance at 420 nm was measured with a UV/vis spectrophotometer. The EE was calculated as follow:

$$EE (\%) = \frac{\text{Total curcumin amount} - \text{free curcumin amount}}{\text{Total curcumin amount}} \times 100.$$

Experimental design and statistical analysis

The response surface methodology was applied to evaluate the effects of nanoencapsulation parameters and optimize conditions for various responses. The 4 factors, levels and experimental design in terms of coded and uncoded are given in Table 1. The experimental design included three replicates at the center point for evaluation of the experimental error. The experimental data were fitted to a quadratic polynomial model as follows:

$$Y_i = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j$$

where Y_i represents the predicted response, β_0 is constant coefficient of intercept, β_i is the regression coefficient for each linear effect term, β_{ii} and β_{ij} are quadratic and interaction effect terms, respectively; X_i and X_j are the independent variables. The analysis was performed using coded units. Where possible, stepwise deletion of terms was applied to remove the statistically non-significant terms ($P > 0.05$), simplifying the model. However, when the exclusion of such terms from the model decreased R^2 (predicted) and increased the estimator of the variance S , the term was included in the model. The statistically non-significant linear terms also remained in the model when the respective quadratic or interactive effects were statistically significant. The statistical analysis of the data was performed using the SAS Statistical Software (Version 9.2, SAS Institute. Inc.). Optimization was performed using a desirability function to obtain the level of SC (A), GA (B), T20(C) and pH (D) which minimized the particle size and turbidity and maximized the EE.

ζ-Potential measurements

The electrical charge measurements of the particles in the dispersions were determined by the dynamic light scattering technique using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK) at room temperature.

Results and discussion

Fourier transform infrared spectroscopy (FTIR) analysis

The SC FT-IR spectrum including the characteristic absorption peaks of amide I at 1659 cm^{-1} (C–O stretching vibration), amide II at 1522 cm^{-1} (C–N stretch coupled

with N–H bending mode) and amide III at 1240 cm^{-1} (N–H bending mode coupled with C–N stretching) is shown in Fig. 1a.

These values of SC for amide I, II and III peaks were consistent with the values previously reported for SC (Jiménez et al. 2014; Santinho et al. 1999). The principal absorption peaks for GA appeared at 3415 and 2928 cm^{-1} attributed to –OH stretching and –CH stretching, respectively (Nayak et al. 2012). The strong peaks at 1613 and 1425 cm^{-1} are correspond to asymmetric and symmetric –COO[−] stretching vibration, respectively (Fig. 1e). The principal absorption peaks for curcumin appeared at 3509 cm^{-1} due to the phenolic O–H stretching vibrations, at 1627 cm^{-1} corresponds to stretching vibrations of benzene ring, at 2942 and 1428 cm^{-1} were probably attributed to the olefinic C–H bending vibration and deformation of methyl groups (Mohanty and Sahoo 2010). Additionally, the peaks at 1277 , 1154 and 1027 cm^{-1} correspond to the aromatic C–O stretching vibration. The strong peak at 960 cm^{-1} occurred due to the in plane bending mode of the C–H aromatic. These peaks are in close agreement with that reported in literature (Anitha et al. 2011). In the case of SC/GA nanocomplex (Fig. 1c), typical peaks of both SC and GA with significant shifting or deviations were identified. Comparing the spectra of SC with SC/GA nanoparticles, peaks at 1659 and 1522 cm^{-1} corresponding to amide I and amide II groups shifted to 1652 and 1540 cm^{-1} , respectively. The possible explanation for this shifting can be related to interaction between SC and GA. Also, N–H bending mode coupled with C–N stretching bond of SC at 1240 cm^{-1} and asymmetric and symmetric –C–O stretching at 1613 and 1425 cm^{-1} of GA in the FTIR spectra of SC/GA nanoparticles disappeared. The absence of these bonds, could be strong evidence indicating the formation of an electrostatic interaction between the positively charged amine groups (–NH₃⁺) of SC with the negatively charged carboxyl groups (COO[−]) of GA (Fig. 1). This result is in agreement with previous studies that reported interactions between protein and polysaccharide using FTIR (Huang et al. 2012; Luo et al. 2011). The spectrum of SC and GA alone showed characteristic bands at 3324 and 3415 , respectively, attributed to –OH groups of both biopolymers. In the case of SC/GA nanoparticles, these peaks shifted to 3426 cm^{-1} , indicating that hydrogen bonding was involved in the interaction between GA and SC (Huang et al. 2012). In agreement with the results of ζ-potential section that the T20 can be influenced in the electrostatic interaction between SC and GA, for the first time, we have seen through FT-IR spectroscopy that this process was occurred. The FTIR spectra of the nanoparticles obtained upon complexation of SC/GA/T20 showed the changes in wave number of the peaks compared to SC/GA nanoparticles (Fig. 1d). These

Fig. 1 FTIR spectra of individual components and their complex samples. *a* SC, powder; *b* GA, powder; *c* SC/GA nanoparticles, sample; *d* SC/GA/T20 nanoparticles, sample; *e* curcumin, powder; *f*, SC/GA/T20/curcumin nanoparticle, sample

changes of peaks indicate that there is an interaction between the three components. Indeed, with comparing these spectrums, the influence of T20 on the interaction between SC and GA was confirmed by the shift of absorption peaks from 3427, 2927, 1651 and 1420 cm⁻¹ to 3433, 2929, 1638 and 1423 cm⁻¹. In the FTIR spectra of the curcumin nanoparticles (Fig. 1f), the shifting of the C–O stretching vibrational band of amide I (of SC) from 1638 to 1644 cm⁻¹ and C–N stretch coupled with N–H bending mode of amide II (of SC) from 1531 to 1535 cm⁻¹ in the conjugation system was an indication of interaction of curcumin with SC. In the general case, the peaks of curcumin disappeared in the spectrum of curcumin nanoparticle after encapsulation. This result also provided good evidence for prominent interaction of curcumin with biopolymers that are used in the formation of colloidal nanoparticles. Other studies also reported the interaction between curcumin and protein using FTIR (Pan et al. 2013).

Particle size

Particle size is one of the most important parameters to prepare stable nanoparticles and it also impacts both the physicochemical properties and sensory attributes of food products. The pH is one of the most important factors influencing the formation of protein– polysaccharide complexes because it influences the number of charge reactive groups carried by the biopolymers such as amino and carboxylic groups (Ye 2008). As pH decreased from 5.2 to 5, the particle size decreased from 205 to 75 nm. With decreasing pH, further GA concentration was

required. This is because as the pH decreased, the number of the positively charged patches of the SC molecules increased, so the required concentration of GA that had a negatively charged patches, increased in order to interact with them and produce small particles. At pH 4.8, closest to the pI of the SC, particle size was greatly affected by GA concentration. As can be seen in Fig. 2a, b, at high concentrations of SC and GA, the influence of pH on particle size was reduced. The results obtained here were consistent with earlier results (Weinbreck et al. 2003). It can be seen from Fig. 2a that the particle size decreased at first and then increased significantly with the increase of the two variables, and a minimum particle size is achieved when pH and SC concentration were 4.98 and 0.18 wt%, respectively (Fig. 2a). The interaction between GA and pH on the particle size when the T20 and SC concentrations were kept at 0.1 and 0.2 wt%, respectively, indicate that the particle size initially decreased by decreasing the GA concentration from 1 to 0.68 wt% and pH from 5.2 to 4.95, but increased in the low concentration of GA and low pH (Fig. 2b).

It is evident from Fig. 2c that at a constant concentration of GA (0.5 wt%), by increasing of SC concentration from 0.1 to 0.3 wt%, the mean particle size of the system was increased significantly. This phenomenon is attributed to the fact that the number of GA molecules is insufficient to fully cover the surface of protein molecules, and more than one protein molecule simultaneously becomes attached to one polysaccharide chain (Dickinson 1998). On the other hand, at constant concentration of SC when the GA concentration increased from 0.5 to 0.75 wt%, the mean particle size of the system was decreased. In this case, for each

Fig. 2 Contour plot showing the effect of component concentration and pH on Ln (particle size). **a** SC and pH, **b** GA and pH, **c** SC and GA, **d** SC and T20

GA molecule presence, there are fewer SC molecules to associate with them, therefore the particle size of the system was decreased (Ye et al. 2006). The smallest particles were obtained at the intermediate concentration of GA of 0.75 wt%, which can be attributed to formation of a complex between SC and GA molecules, so the SC molecules were not subject to self association (Ye et al. 2006). Similar results were reported by Cho and McClements (2009). The largest particles were obtained at higher and lower concentration of GA and SC, respectively. This may be due to the fact that an excessive amount of polysaccharide molecules lacking sufficient SC molecules, may bind together and form a weak gel network and thus grow the particle size of the system (Dickinson 1998).

The particle size of the system was small in presence of T20. An appreciable increase in the mean particle size in the absence of T20 in all of the SC concentrations was observed Fig. 2d. Similar results have been observed in a

study by Li and McClements (Li and McClements 2013). Interestingly, at low concentration of SC, further increasing the concentration of T20 from 0.1 to 0.2 wt% led to an increase in the particle size and instability of the system. This is probably due to the fact that SC has the ability to tolerate a certain extent of T20. Anarjan and Tan reported that increasing surfactant concentration led to expansion of surfactant-rich regions, restricting the protein or polysaccharide network to a smaller surface area and thicker interfacial layer, so produced bigger particles (Anarjan and Tan 2013). Overall, stabilizing or destabilizing protein structure by binding of surfactant, depends on the type and concentration of surfactant and environmental conditions (McClements 2004) and protein molecules present (Li and McClements 2013).

In the absence of T20 and at high concentration of SC, sedimentation was observed after preparation and even in the preparation stage, but in the presence of T20, this

Table 2 Regression coefficients of the second-order polynomial model for the response variables (analysis has been performed using coded units)

Factors ^a	Ln (particle size)	Ln (turbidity)	Encapsulation efficiency
Constant	4.321	−0.816	0.7467
Linear			
A	−0.0259 ^{ns}	0.576***	0.261***
B	0.573***	0.427***	−0.0179 ^{ns}
C	−0.914***	−0.147*	0.137***
D	0.062	−0.114 ^{ns}	−
Interaction			
AB	−0.718*	−	0.0262 ^{ns}
AC	−1.234***	−	0.0537*
AD	−0.346**	−	−
BD	−0.289 ^{ns}	−	−
CD	0.305*	−	−
Quadratic			
A ²	1.002***	−	−0.103***
B ²	0.668***	−	−
C ²	1.246***	0.333**	−0.118***
D ²	0.486***	0.261*	−
R ²	0.984	0.879	0.985
Adj R ²	0.967	0.848	0.981
Pred R ²	0.873	0.812	0.964
F-value of model	58.955	27.985	215.8
Probability of F	<0.0001	<0.0001	<0.0001

A: SC concentration (wt%); B: GA concentration (wt%); C: T20 concentration (wt%); D: pH

^{ns} non significantSignificant at * $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$

phenomenon did not occur (Fig. 2d). After particle collision due to Brownian motion, according to the system forces (attraction or repulsion) they aggregate or diverge, respectively (Patel et al. 2013). With increasing the concentration of biopolymers, the number of complexes that are formed in the system increases, the frequency of the collisions among particles increased and further approached each other. The repulsive forces, including electrostatic repulsion (due to the high surface charge of particles) and steric stabilization (forming a barrier around the particles), prevent close approach of particles (Patel et al. 2013). Probably due to the insufficient electrostatic repulsion in the absence of T20 after collision, particles were connected, whereas T20 as a barrier (steric stabilization) prevents the approach of particles to a distance that is too close to each other. Table 2 presents the results of fitting quadratic models of data.

Turbidity

Visual appearance is one of the most important quality attributes of food products (Mirhosseini et al. 2008). Turbidity of a colloidal dispersion depends on the average

particle size, particle size distribution, concentration and relative refractive index of any particles that it contains (Chanasattru et al. 2009; Mirhosseini et al. 2008); therefore change in these factors leads to variation in turbidity. The results demonstrated that SC and GA concentrations positively affected the turbidity of dispersions, while it was negatively affected by the liner effect of T20 concentration and pH. Also the quadratic effects of T20 concentration and pH were significant (Table 2). The parallel effect of SC and GA concentrations on turbidity of the system are given in Fig. 3a. It can be seen that transparency of the system was reduced by increasing SC concentration. In general, there was a progressive increase in turbidity when both SC and GA concentration were increased, indicating that there was an increase in the particle size and/or number of complexes formed (Jones et al. 2009; Schmitt et al. 1999). These findings are in agreement with the results reported by Ghorbani Gorji and co-workers (2014), who found that the turbidity of mixtures increased with increasing biopolymer concentrations (Ghorbani Gorji et al. 2014).

Figure 3b shows quadratic effects of T20 concentration and pH on turbidity. Turbidity slightly decreases at first and then increases quickly by decreasing two variables, and a

Fig. 3 Contour plot showing the effect of component concentration and pH on Ln (turbidity). **a** SC and GA, **b** T20 and pH

maximum turbidity is achieved when T20 concentration and pH are 0 and 4.8, respectively. The turbidity of the solutions progressively increased with a further decrease of pH from 5 to 4.8, particularly at high concentration of biopolymers and in the absence of T20, which indicates some aggregates were formed that were large enough to scatter light strongly. At central level of T20, the samples were less turbid, indicating the formation of too small stable complexes unable to scatter light (Fig. 3b). This may be due to retardation in the protein aggregation. Interestingly, with increasing the T20 concentration from 0.1 to 0.2 wt%, the light scattering of particles increased. This phenomenon can be attributed to the change of relative refractive index of the complexes in the presence of surfactant T20. On the other hand, as mentioned above, this is probably due to the fact that the SC was able to tolerate a certain extent of T20. This observation was in agreement

Fig. 4 Contour plot showing the effect of component concentration and pH on EE. **a** SC and GA, **b** SC and T20

with another study on protein solution, in which depletion flocculation occurred at a certain concentration range of the emulsifier, while below this concentration there was negligible flocculation (Dickinson et al. 1999).

Encapsulation efficiency (EE)

As shown in Fig. 4a, the EE of the nanocomplexes increased as the SC concentration increased. Indeed, a higher amount of SC allows more entrapment of curcumin. This could be explained by the increasing protein binding sites resulting in more space for curcumin to be encapsulated. The results obtained in the present study are consistent with the results of Shpigelman (Shpigelman et al. 2012). However, in the constant concentration of SC, the increase of the GA concentration led to slight decrease of EE (Fig. 4a). However, the concentration of GA did not have a significant effect on the EE ($P > 0.05$). It can be seen from the plot (Fig. 4b) that when the T20 concentration increased, the EE of the system increased significantly. High EE occurred probably because the surfactant

(T20) facilitates solubilization of curcumin and therefore leads to more accessibility of SC to connect with curcumin. During the preparation of nanodispersion in the samples without T20, some amount of curcumin was precipitated. Our results are consistent with the literature; thus Boruah et al. (2012), reported that EE of nanoparticles increased dramatically after adding a surfactant, and due to the curcumin interaction with chitosan, it is more pronounced in presence of surfactants (Boruah et al. 2012). Bergonzi et al. (2014) reported that the solubility value of curcumin in T20 is higher compared to oils and other surfactants. This is probably due to the fact that the T20 is an amphiphilic molecule with affinity for both polar and apolar groups of curcumin. The low EE in the high concentration of GA or in the absence of T20 can be related to the fact that the system in these cases has very large particles that are very unstable and sediment immediately, even in the preparation stage. It has been reported that the higher the stability of the dispersion, the higher the EE of the system (Barbosa et al. 2005).

Optimization

To identify the overall optimal processing conditions, the models obtained for different responses were subjected to numerical optimization. For this purpose, the use of desirability functions is one of the useful approaches to the optimization of multiple responses. In this technique, the general approach is to first convert each response Y_i into an individual desirability function d_i that varies over the range, $0 \leq d_i \leq 1$ where if response Y_i is at its target value, then $d_i = 1$, and if it is outside an acceptable region, $d_i = 0$. Then the design variables were chosen to maximize the overall desirability as

$$D = (d_1 \times d_2 \times \dots \times d_n)^{\frac{1}{n}}$$

where n is the number of responses.

This optimum preparation zone was found to be SC concentration 0.21 wt%, GA concentration 0.5 wt%, T20 concentration of 0.14 wt% and pH 5, provided the particle size was 72 nm, the turbidity of nanodispersion was 0.29 and EE of nanodispersion was 81% (Fig. 5a, b).

ζ-potential measurements

The surface charge is an important characteristic of biopolymer nanoparticles, and this was determined by ζ-potential measurements. It can be seen that, at pH 7, negative charge of both SC and GA molecules are responsible for the high and negative ζ-potential value of dispersion. However, by changing pH from 7 to 4.5, the negative charge at surface was reduced (Fig. 6). Because the

Fig. 5 Desirability function plots indicating the region of optimal preparation variables

electronegative charge along the GA chains, such as free carboxyl groups of GA molecules, was decreased, formation of the complexes with positive regions of SC molecules (Ye 2008) led to reduction of the overall net charges on the polysaccharide molecules. However, due to the presence of negative carboxyl groups of GA on the surface of the complexes, the surface charge of the nanoparticles was still negative at all studied pH values and in the absence or presence of nonionic surfactant. Of course, results showed that there was a reduction in the resulting ζ-potentials in the presence of T20, which is consistent with the findings of Li and McClements (2013), though they reported higher reduction relative to our results. The difference in the electrical characteristics of these systems may originate from differences among formulation or processing parameters, especially the arrangement of incorporation of surface active components (protein-polysaccharide-surfactant). Both non-ionic and ionic surfactants could change the protein structures, interactions

Fig. 6 pH-Dependence of the ζ -potential of nanodispersions containing SC/GA and SC/GA/T20

and functional properties by binding to the surfaces of proteins (Li and McClements 2013). Although, nonionic surfactants do not participate directly in the electrostatic interactions, they can influence the structure of the electrostatic complexes (Li and McClements 2014), but the precise mechanism of action is unclear and controversial. Not only, the nonionic surfactants have the ability to bind to proteins through hydrophobic interactions, which may alter the ability of the proteins to interact with other molecules, but nonionic surfactant micelles may also directly connect to hydrophilic polymers such as polysaccharides (Li and McClements 2014), therefore indirectly affected the protein-polysaccharide electrostatic interaction. During the gradual decreasing of pH, due to formation of electrostatic complex between the protein and polysaccharide, the influence of T20 on protein was reduced. In this work, T20 was added after addition of the polysaccharide into protein solution, while Li and McClements (2013) mixed the protein and T20 at the same time and then added the polysaccharide. Likely, in this case, T20 had a greater opportunity to influence on the protein structure. On the other hand, relative to globular proteins such as β -lactoglobulin, flexible proteins such as SC bind more strongly to polysaccharides (Ye 2008) and more weakly to surfactant molecules, which could be another reason for the low impact of T20 on SC and less reduction of ζ -potential in the case of SC. Overall, the protein/polysaccharide/surfactant complexes have lower ζ -potential than the protein/polysaccharide complexes at all pH values. Li and McClements (2014) suggested that this phenomenon can be explained by the fact that there were fewer cationic binding sites present for the anionic

polysaccharide molecules to interact with, due to interaction of protein molecules with surfactant molecules (Li and McClements 2014).

It was noted that the dispersions still had appreciable stability in the presence of T20 even when their surface charge was relatively small (ζ -potential <25). The occurrence of flocculation in a colloidal system depends on the overall balance amongst electrostatic forces, steric forces and van der Waals forces (Dickinson et al. 1999). The attachment of the T20 molecules to the complexes provide an additional steric barrier between the complexes. Therefore, low ζ -potentials of about 20 mV is enough in these dispersions to fully stabilize the system with electrostatic repulsion in combination with steric stabilization, whereas in the case of dispersion stabilized only by electrostatic repulsion (system without T20) a ζ -potential of ± 30 mV is required to physically stabilize the system (Müller et al. 2001).

Conclusion

In this work a model for fabrication of curcumin-loaded biopolymeric nanodispersion with a solvent-displacement method followed by acidification was presented and validated. The technique presents several advantages, such as small particle size, low turbidity, high EE, simplicity and no need for high energy equipment such as a homogenizer. A quadratic polynomial model was employed to optimize the preparation of nanodispersion. In general, regression analysis showed high coefficients of determination (R^2) in the range of 0.88–0.98 for the models, thus ensuring a

satisfactory fitting of experimental data to the polynomial models. On the basis of spectroscopic results and data modeling, interaction among the components namely, curcumin, SC, GA and T20, was concluded and the non-ionic surfactant T20 significantly influenced the electrostatic complex of SC and GA. The optimum process conditions determined by desirability function were as follows: SC 0.21 wt%, GA 0.5 wt%, T20 0.14 wt% and pH 5. In this concentration levels of components and pH, particle size of 72 nm, turbidity of 0.29 and encapsulation efficiency of 81% were obtained. Also, an overall desirability function equal to 0.83 was found that indicates satisfactory level of multiobjective optimization. So, these nanoparticles could be utilized in various food products as functional ingredient.

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