Research Article

Influence of Formulation Factors on the Preparation of Zein Nanoparticles

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Abstract. The main objective of the present study was to investigate the influence of various formulation parameters on the preparation of zein nanoparticles. 6,7-dihydroxycoumarin (DHC) was used as a model hydrophobic compound. The influence of pH of the aqueous phase, buffer type, ionic strength, surfactant, and zein concentration on particle size, polydispersity index, and zeta potential of DHC-loaded zein nanoparticles were studied. Smaller nanoparticles were formed when the pH was close to the isoelectric point of zein. DHC-loaded zein nanoparticles prepared using citrate buffer (pH 7.4) was better than phosphate buffer in preventing particle aggregation during lyophilization. The ionic strength did not have a significant influence on the particle size of DHC-loaded zein nanoparticles. A combination of Pluronic F68 and lecithin in 2:1 ratio stabilized the zein nanoparticles. An increase in zein concentration led to increase in particle size of DHC-loaded zein nanoparticles. The use of optimal conditions produced DHC-loaded nanoparticles of 256 ± 30 nm and an encapsulation efficiency of $78\pm7\%$. Overall, the study demonstrated the optimal conditions to prepare zein nanoparticles for drug encapsulation.

KEY WORDS: drug delivery; particle size distribution; pH nanoprecipitation; protein polymers; zein; zeta potential.

INTRODUCTION

Nanoparticles prepared from synthetic polymers have been widely investigated for drug delivery applications (1). Biodegradable protein polymers serve as alternative to synthetic polymers (2). Among the protein polymers, animal-derived proteins such as albumin and gelatin have been studied for drug delivery applications (3,4). On the other hand, there have been limited studies on the use of plant-derived proteins for drug delivery applications (5,6). Plant proteins are a renewable source with wider acceptance compared to animal proteins (5). Furthermore, they do not carry the risk of infection or contamination with biological fluids (5). Most of the protein polymers that have been used for drug delivery are water-soluble proteins with limited sustained-release characteristics (2,5–8). In this regard, it is important to identify water-insoluble hydrophobic proteins for sustained drug delivery applications.

Zein is a water-insoluble plant protein from corn (*Zea mays* L.). It consists of three fractions that vary in molecular weight (MW) and solubility (9). These include α-zein (MW, 19–24 kDa; 75–80% of total protein), β-zein (17–18 kDa, 10–15%), and γ-zein (27 kDa, 5–10%) (9). The large proportion (>50%) of non-polar amino acids (leucine, proline, alanine, and phenylalanine) in zein makes it water insoluble (10). Commercial zein comes in two grades yellow and white zein (10). Yellow zein contains a high concentration of xanthophyll pigments (8–9%) including lutein, zeaxanthin and β-cryptoxanthin (11,12). Some of the undesirable properties of yellow zein have been attributed to xanthophyll (10). The hydrophobic xanthophylls are strongly bound to zein and various extraction methods have been used to decolorize zein (10,11). The purity of yellow zein is around 88–90% (10,11). In contrast to yellow zein, decolorized zein (white zein) contains very negligible amount of xanthophylls (<0.001%) (11,12), and the purity of white zein is >96% (10–12).

Due to its water insolubility and film-forming ability, zein is used in food and packaging industry to form a moisture barrier. Zein is approved by FDA as a generally recognized as safe excipient for film coating of pharmaceuticals (13). Zein microspheres have been studied for sustained drug delivery applications for small drug molecules and macromolecules for oral and parenteral routes (5). Zein microspheres have been compressed into tablets for oral administration of ivermectin to treat parasitic disease in dogs (14). Wang *et al.* investigated the use of zein films loaded with heparin-loaded zein microspheres for cardiovascular stents (15). On the other hand, there are limited drug delivery studies for zein nanoparticles (5,6). Lai *et al.* (16) reported the use of zein nanoparticles for liver targeting of 5-fluorouracil. Zein-chitosan nanoparticles and zein-casein nanoparticles have been used for oral delivery of neutraceuticals (17,18).

The zein particulate systems have been prepared using phase separation based on the differential solubility of zein in ethanol and aqueous solution (19–21). However, most of these methods produce particles with wide size distribution. Many of the studies reported in the literature used yellow zein which produced larger particles, particle aggregation, and lower drug

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encapsulation (19,22–24). Patel *et al.* (25) used casein as a stabilizer to prevent the aggregation of nanoparticles prepared with yellow zein. Recently, we reported a pH-controlled nanoprecipitation method to produce yellow zein nanoparticles using lecithin and Pluronic F68 as stabilizers (Fig. 1) (22). Although our method produced nanoparticles, they were generally >300 nm and had lower drug encapsulation (22). Recently, white zein has been used in the literature for the preparation of nanoparticles (16,20,21). The aim of this investigation is to study the various factors that influence the preparation of nanoparticles using white zein. Given its fluorescence properties, 6,7-dihydroxycoumarin (DHC) was used as a model hydrophobic compound in this study.

EXPERIMENTAL

Materials

Zein F-6000 (white zein) was purchased from Freeman Industries Inc. (Tuckahoe, NY). Ethyl alcohol, DHC, trehalose dihydrate (anhydrous), and egg lecithin were purchased from Sigma (St. Louis, MO). Sodium hydroxide, citric acid, sodium citrate, Pluronic F-68, were from Fisher Scientific, (New Jersey). All other chemicals were reagent grade Sigma (St. Louis, MO).

Methods

Preparation of Zein Nanoparticles Using pH-Controlled Nanoprecipitation

Briefly, 0.0275 g of zein was dissolved in a mixture of ethanol and water (0.1 N NaOH) (92:8% (*v/v*)). Zein solution was added drop wise under ultrasonic shear (750 W and 20 kHz frequency, Sonics® USA) into 15 mL of aqueous phase (buffer or water) containing surfactants lecithin and Pluronic F68 (0.05% to 0.9% (*w/v*)). The temperature of the aqueous phase was maintained at 10°C using an ice bath. Subsequently, the zein suspension was kept on a magnetic stirrer at 500 rpm at room temperature to evaporate the ethanol. After complete evaporation of ethanol, the nanoparticles were purified to remove free DHC. The aqueous suspension of zein nanoparticles were purified by two cycles of differential centrifugation (3,980×*g* for 50 min) using Amicon® centrifugal filters (MW cut off, 10,000 Da). After centrifugation, supernatant was discarded and the pellet was redispersed in 5 mL of buffer. Subsequently the suspension was lyophilized (Virtis™, Bench Top Model, USA) after adding 2% (*w/v*) trehalose. The trehalose concentration was chosen from the literature (26). For preparing DHC-loaded nanoparticles, 0.0135 g of DHC was dissolved in the alcoholic phase.

Determination of Nanoparticle Yield

Nanoparticle yield was determined by BCA assay (Pierce, Thermo Scientific, IL). A weighed amount of nanoparticles (10 mg) was redispersed in deionized water in an Eppendorf tube. The suspension was vortexed for 2 min followed by bath sonication to disperse the sample. The dispersion was centrifuged at 12,000 rpm for 12 min. The supernatant was discarded and the pellet was digested in 90% (*v/v*) ethanol in deionized water. An aliquot of the sample was used for the determination of protein content. The absorbance was read at 562 nm in a microplate reader (SpectromaxM2, Molecular Devices, Sunnyvale, CA), A standard curve was generated by dissolving zein in 90% (*v/v*) ethanol (0.031 to 0.250 mg/mL, $r^2=0.999$). The particle yield was calculated using the following formula where M_a is the actual amount of zein in the nanoparticles and M_t is the amount of zein used for the preparation of nanoparticles.

$$\text{Yield} = \frac{M_a}{M_t} \times 100$$

Encapsulation Efficiency

Approximately 5 mg of the DHC-loaded nanoparticles was dispersed in 1 mL of Milli-Q water, centrifuged at 5,000 relative centrifugal force (rcf) for 10 min at 4°C. An aliquot of the supernatant was diluted with ethanol and used for the determination of concentration of free DHC by measuring the fluorescence at excitation and emission wavelengths of 490 and 520 nm respectively in a micro-plate reader (SpectromaxM2, Molecular Devices, Sunnyvale, CA). The pellet recovered after centrifugation was digested with 90% (*v/v*) ethanol, and an aliquot was used for the determination of zein concentration by BCA assay. An aliquot was diluted with ethanol and the encapsulated DHC content was determined using a standard curve of DHC in 90% (*v/v*) ethanol (0.002 to 0.5 μg/mL, $r^2=0.990$). Free DHC in the supernatant was subtracted from the amount in the nanoparticles. The encapsulation efficiency (EE) was expressed as percent mean of three experiments (±SD) using the following formula where C_a represents the actual amount determined from nanoparticles
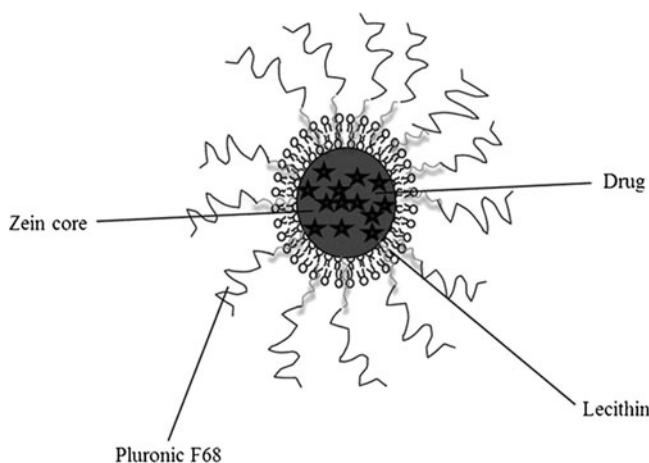

Fig. 1. Proposed structure of the phospholipid stabilized zein nanoparticles. Inner core consists of hydrophobic zein with entrapped drug molecule. Zein core is surrounded by one or more layers of lecithin with polypropylene chains (*inidcated by grey*) of Pluronic F68 and hydrophilic polyethylene oxide chains (*indicated by black*) extend towards the aqueous environment

and C_t represents the amount of DHC added during the preparation of nanoparticles.

$$EE = \frac{C_a}{C_t} \times 100$$

Influence of pH

The pH of the aqueous phase was varied from 2 to 10 using 0.1 N HCl or 0.1 N NaOH during the preparation of DHC-loaded nanoparticles. The effect of pH on the aggregation of nanoparticles was studied by dispersing the pre-formed DHC-loaded zein nanoparticles in deionized water adjusted to pH from 2 to 10 using 0.1 N HCl or 0.1 N NaOH.

Influence of Buffers on Freeze Drying

To study the influence of buffer type on particle growth and aggregation, citrate and phosphate buffers were used to maintain the pH at 7.4 in the aqueous phase. The particle size was measured before and after freeze drying using photon correlation spectroscopy. The phosphate buffer at pH 7.4 (0.1 M) was prepared using dibasic sodium phosphate (1.44 g/L), monobasic potassium phosphate (0.25 g/L), and sodium chloride (10 g/L) in deionized water.
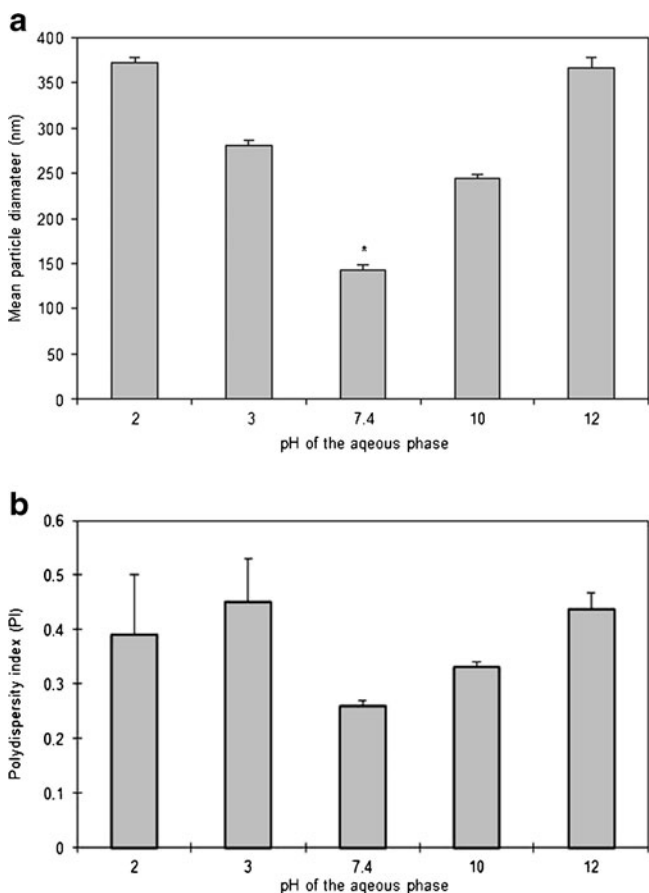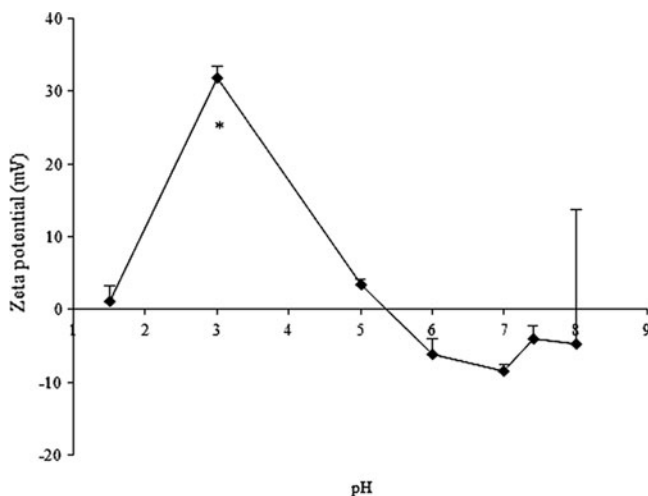

Fig. 3. Influence of the pH on zeta potential of pre-formed DHC-loaded nanoparticles. Zeta potential of the lyophilyzed nanoparticles was measured at different pH (pH adjusted using 0.1 N HCl or 0.1 N NaOH). Each value is an average of three measurements±SD, *$p<$ 0.05, level of significance

Citrate buffer at pH 7.4 (0.1 M) was prepared using citric acid (0.0153 g/L) and sodium citrate (2.91 g/L) in deionized water.

Influence of Ionic Strength

To study the effect of ionic strength on particle size of zein nanoparticles, various concentrations of sodium chloride (0.1 to 1 M) in water was used for the preparation of DHC-loaded zein nanoparticles. To study the role of ionic strength in particle aggregation in physiological conditions, pre-formed DHC-loaded zein nanoparticles were dispersed in deionized water with varying the concentration of sodium chloride (0.01 to 0.1 M) followed by measurement of particle size and zeta potential.

Influence of Surfactants

To study the effect of surfactants on the particle size of DHC-loaded zein nanoparticles, lecithin and Pluronic F68 were used individually or in combination at different concentrations (0.05% to 0.9% (w/v)).
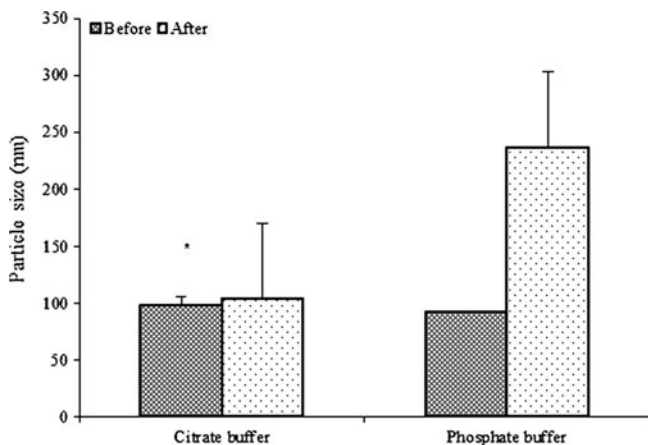

Fig. 2. Influence of pH on **a** particle size and **b** PI of DHC-loaded zein nanoparticles. pH was adjusted using 0.1 N HCl or 0.1 N NaOH during preparation. Each value is an average of three different measurements±SD. *$p<0.05$, level of significance

Fig. 4. Influence of buffer type on the particle size of DHC-loaded zein nanoparticles before and after freeze drying. Each value is an average of three measurements±SD

Table I. Influence of Ionic Strength on the Particle Size of DHC-loaded Zein Nanoparticles

Ionic strength (M)	Particle size (nm)	PI
0.1	309±44.39	0.54±0.042
0.25	305±41.29	0.54±0.083
0.5	299±24.83	0.49±0.056
0.75	250±4.16	0.47±0.032
1	269±32.75	0.45±0.032

Each value is an average of three experiments±SD. NaCl was used to adjust the ionic strength during the preparation of zein nanoparticles
PI polydispersity index, *DHC* 6,7-hydroxycoumarin

Influence of Zein Concentration

To study the effect of zein concentration on particle size of DHC-loaded nanoparticles, zein concentration was varied from 0.09% to 0.5% (*w/v*), while all other parameters were kept constant.

Particle Size Analysis and Zeta Potential

Nanoparticle size, polydispersity index (PI) and zeta potential were measured by photon correlation spectroscopy using NICOMP™ 380 ZLS (Particle Sizing Systems, USA). Briefly 10 mg of nanoparticles were dispersed in 2 mL of deionized water, probe sonicated and centrifuged at 5,000 rcf for 1 min. An aliquot (100 μL) of the supernatant was used for the determination of particle size and zeta potential (diluted at 1:400 in corresponding buffer) at room temperature (25°C). Unless specified, the particle size was measured after lyophilization.

Atomic Force Microscopy

The surface morphology of optimized DHC-loaded zein nanoparticles was analyzed by atomic force microscopy

(AFM; Pacific Nanotechnology, Inc., USA) in tapping mode. Briefly 10 mg of DHC-loaded zein nanoparticles was dispersed in 2 mL of Milli-Q water, and probe sonicated to disperse the particles, and centrifuged at 5,000 rcf for 1 min. About 100 μL of the supernatant was placed on a polyethyleneimine-coated glass cover slip and air dried. Nanorule (Pacific Nanotechnology, Inc., USA) software was used for image processing. Nanoparticles from different fields were counted (50 particles) and the average size was represented as mean±SD.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) analysis of DHC, zein, surfactants, and the nanoparticles were characterized using DSC Q2000 (TA instruments Inc., USA). The sample (10 mg) was weighed in the aluminum pan and hermetically sealed. An empty pan was used as the reference. The sample was heated at 10°C min^{-1} from ambient temperature to 300°C. The thermograms were processed using Universal Analysis 2000 software (TA instruments Inc., USA).

Data Analysis

All the experiments were performed in triplicate, and the results were expressed as mean±SD. One-way ANOVA (Instat, Graph Pad Software, CA) was used to compare the different groups, and the results were considered to be significant at $p < 0.05$.

RESULTS

Influence of pH

To study the effect of pH on precipitation kinetics of zein nanoparticles, pH of the aqueous phase was varied during the preparation of nanoparticles (Fig. 2). The particle size and PI
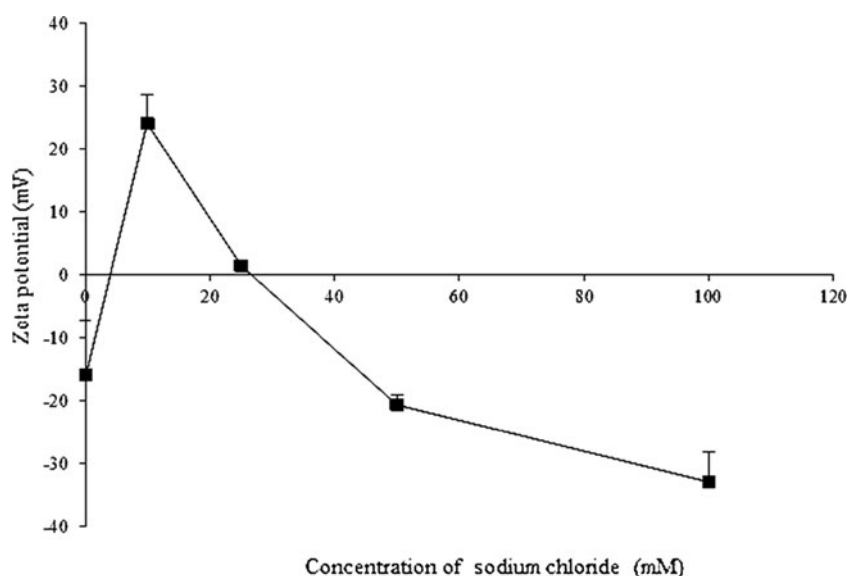

Fig. 5. Influence of ionic strength on zeta potential of pre-formed DHC-loaded zein nanoparticles. Each value is an average of three measurements±SD. *$p < 0.05$, level of significance
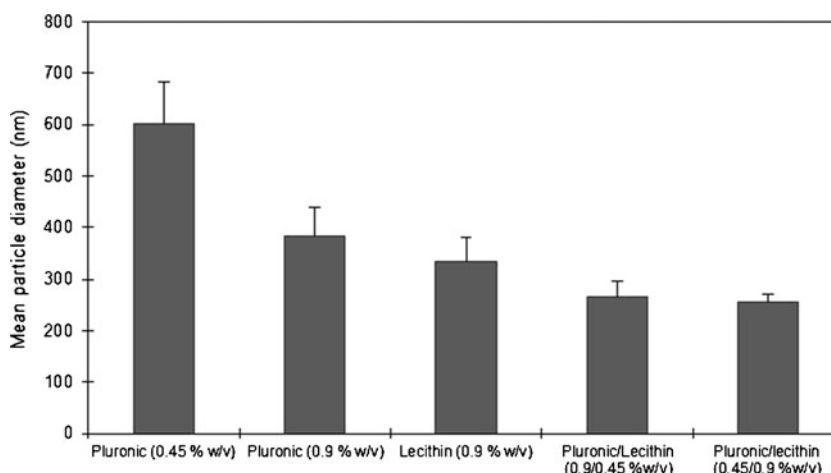
Fig. 6. Effect of the surfactants on particle size of DHC-loaded zein nanoparticles. Each value is an average of three measurements±SD

of DHC-loaded zein nanoparticles were high at extremes of pH. At pH 2 and 12, the particle size and PI were >300 nm and ≥0.4, respectively (Fig. 2). Smaller nanoparticles (143±7 nm) with a low PI (0.26±0.01) were formed at pH 7.4 (Fig. 2). At pH <7.4 and >7.4, larger nanoparticles with a higher PI were formed (Fig. 2). Furthermore, the effect of pH of on particle aggregation was studied by dispersing DHC-loaded zein nanoparticles in aqueous solution varying in pH from 1.5 to 8 and measuring their zeta potential (Fig. 3). The zeta potential was positive at pH <5 while the zeta potential was negative at pH <5. There was a very high variation of zeta potential at highly alkaline pH (pH 8).

Influence of Buffer Type

Figure 4 shows the effect of buffer on the size of DHC-loaded zein nanoparticles before and after lyophilization. Before lyophilization the particle size was comparable in both phosphate and citrate buffers (pH 7.4). However, after lyophilization the particle size increased by twofold in phosphate buffer (pH 7.4) while there was no significant change in particle size with citrate buffer (pH 7.4) (Fig. 4). The PI increased from 0.5±0.03 to 0.72±0.05 in phosphate buffer while it increased from 0.5±0.04 to 0.6±0.06 in citrate buffer.

Influence of Ionic Strength

Table I shows the effect of ionic strength of aqueous phase on the preparation of DHC-loaded zein nanoparticles. The particle size decreased from 309 to 269 nm when the ionic strength was increased from 0.1 to 1 M (Table I). Although the particle size decreased with increase in ionic strength, the change was not statistically significant ($p < 0.05$). Similarly, the change in PI was also not statistically significant (Table I). Similar results were obtained when pre-formed DHC-loaded zein nanoparticles were dispersed in deionized water of varying ionic strength (data not shown). The zeta potential of pre-formed DHC-loaded zein nanoparticles increased with increase in ionic strength (Fig. 5). The zeta potential was positive at low ionic strength and reached zero at 25 mM NaCl and a further increase in ionic strength resulted in a negative zeta potential. Since the zeta potential did not have a significant effect on the particle size, it suggests that the electrostatic interactions may not play a significant role in the stabilization of zein nanoparticles.

Influence of Surfactants

For studying the effect of stabilizers, lecithin and Pluronic F68 were used for the preparation of DHC-loaded zein
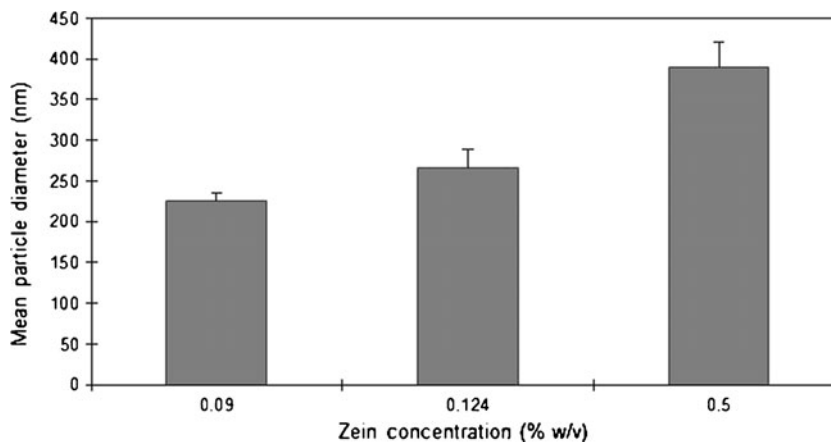

Fig. 7. Effect of zein concentration on particle size of DHC-loaded zein nanoparticles. Each value is an average of three measurements±SD
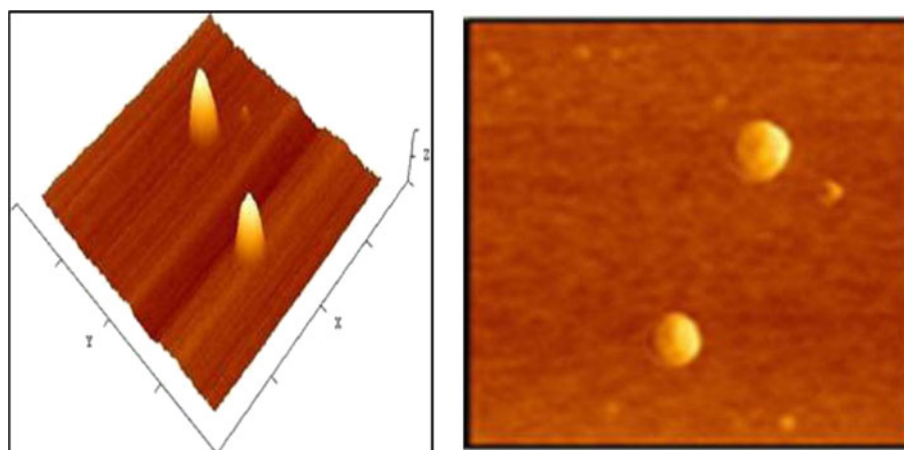
Fig. 8. Representative AFM images of DHC-loaded zein nanoparticles in the tapping mode in air. 3D height (*left*) and 2D phase images (*right*). The scan scale size is 1.14×1.14 μm

nanoparticles. When low concentration (0.05 to 0.45% (*w/v*)) of lecithin or Pluronic was used, aggregates were formed (data not shown). Similarly, the combination of lower concentrations of lecithin (0.45% (*w/v*)) and Pluronic (0.45% (*w/v*)) also formed aggregates (data not shown). On the other hand higher concentration of lecithin (>0.45% (*w/v*)) produced smaller DHC-loaded zein nanoparticles (Fig. 6) but this formulation formed a sticky paste on lyophilization. A higher concentration of Pluronic F68 (>0.45% (*w/v*)) resulted in smaller nanoparticles. However, when a combination of Pluronic F68 (0.9% (*w/v*)) and lecithin (0.45% (*w/v*)) was used, much smaller nanoparticles were obtained (266±30 nm; PI=0.44±0.02). Although the combination of a higher concentration of lecithin (0.9% (*w/v*)) and lower concentration of Pluronic F68 (0.45% (*w/v*)) also produced smaller nanoparticles, the freeze-dried product was a sticky powder. Except for the combination of 0.9% Pluronic F68 and 0.45% lecithin, in all other cases the PI was >0.5 (data not shown).

Influence of Zein Concentration

The influence of zein concentration on particle size and PI is shown in Fig. 7. An increase in zein concentration from 0.09% to 0.5% (*w/v*), increased the particle size of zein nanoparticles while there was no significant effect on PI (varied from 0.4 to 0.5).

Microscopy

Atomic force microscopy pictures show that the optimized DHC-loaded zein nanoparticles were uniformly spherical with a smooth surface as is evident from the 2D phase and 3D topographical images (Fig. 8). The average size of 50 particles was found to be 119±25 nm, and the size was smaller than the particle size measured by photon correlation spectroscopy (Table II).

Differential Scanning Calorimetry

DSC was used to characterize whether DHC was encapsulated or adsorbed on the zein nanoparticles. The glass transition temperature (T_g) of anhydrous zein is reported to be between 162°C and 165°C (27). In our case, zein showed a T_g at around 166°C while Pluronic F68 and lecithin melted at around 50°C and 277°C, respectively (Fig. 9). DHC melted at around 274°C. In case of DHC-loaded zein nanoparticles, the melting point of DHC was not observed which indicates that it is molecularly dispersed in the zein nanoparticle matrix. The melting peak of Pluronic F68 was observed in case of blank and DHC-loaded zein nanoparticles indicating that it is adsorbed on the nanoparticles.

DISCUSSION

Our main goal was to study the influence of the different variables that influence the particle size of zein nanoparticles. The composition and of the starting material has a significant influence on the preparation of nanoparticles (28). Earlier, we have reported the preparation of nanoparticles using yellow zein (22). Xanthophylls which are bound to zein affected the solubility of zein and the drug resulting in relatively larger particles and lower drug encapsulation (22,24). In the present work, decolorized zein (white zein, F-6000) was used for the

Table II. Characteristics of Optimized Zein Nanoparticles

Sample	Particle size (nm)	PI	Zeta potential (mV)	Yield (%)	Encapsulation efficiency (%)
Blank nanoparticles	224±20	0.31±0.06	−16±3	92±5	Not applicable
DHC-loaded nanoparticles	256±30	0.42±0.08	−11.3±1.8	87±10	78±7

Each value is an average of three experiments±SD. Zein concentration of 0.09% (*w/v*), Pluronic F68 and lecithin ratio of 2:1, and citrate buffer at pH 7.4 was used, *PI* polydispersity index, *DHC* 6,7-hydroxycoumarin
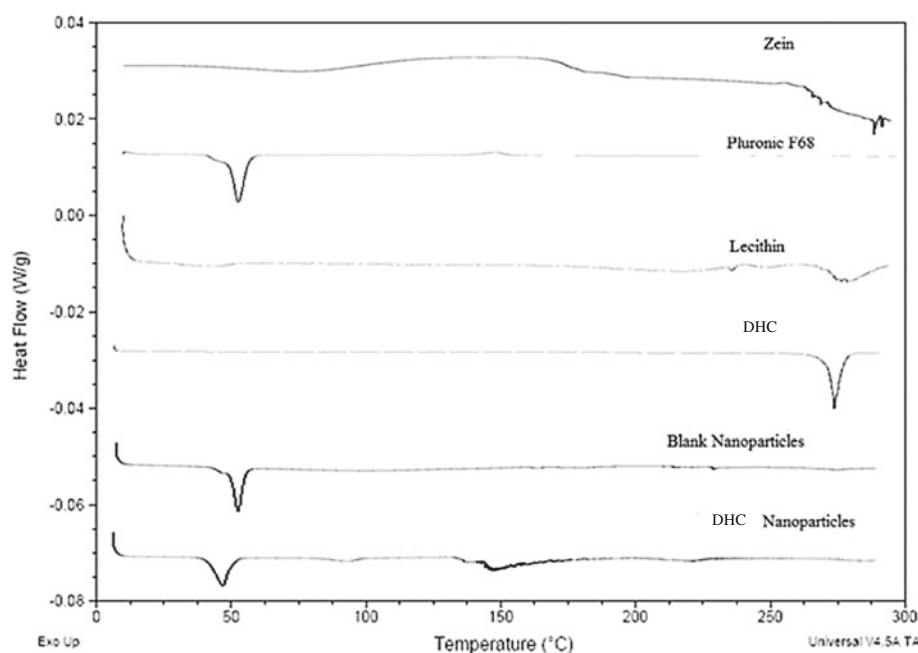
Fig. 9. DSC thermograms of zein, Pluronic F68, lecithin, DHC, and nanoparticles heated at 10°C/min from ambient temperature to 300°C

preparation of zein nanoparticles. The characteristics of nanoparticles are influenced by various formulation factors (3). To this end, we investigated the influence of pH, ionic strength, surfactant concentration and zein concentration, on preparation of zein nanoparticles.

Zein nanoparticles prepared at a pH close to the isoelectric point of zein (pI of 6.8) (29) led to smaller size and low PI. Nanoparticles prepared using legumin, a water insoluble plant protein also showed similar effects of pH on particle size (30). In contrast for albumin nanoparticles, a pH near the isoelectric point of albumin (pI of ~5.05), larger particles were produced (3). Unlike zein and legumin, albumin is a water-soluble protein. The structural changes at different pH are influenced by the amino acids and their charge in the protein. Zein has been reported to form aggregates at pH<pI while it exists in the monomeric form at pH>pI (31). Therefore, the particle size of zein nanoparticle decreases as the pH is increased. The particle size of zein nanoparticles is influenced by the relative rate of precipitation and alcohol evaporation from the aqueous phase (20). The higher solubility of zein at pH ≥8 affects the rate of precipitation resulting in relatively larger nanoparticles at pH ≥8 (31). This also may account for the large variation in zeta potential at pH 8.

The choice of buffering agent is not only critical to maintaining the pH during nanoparticle formation, but is also critical for lyophilization (32). The increase in particle size in phosphate buffer is due to the crystallization of phosphate salt and loss of buffer capacity during freeze drying (33). On the other hand, citrate buffer does not precipitate and therefore effectively resists the changes in pH during freeze drying (33). Further, the amino groups in the zein can be cross-linked by citric acid thus stabilizing and preventing particle aggregation (34). Based on the assay of free amino groups in zein, cross-linking was estimated to be 40% for zein nanoparticles prepared in citrate buffer compared to the nanoparticles

prepared in phosphate buffer (data not shown). The confinement of the protein structure by neutralization of the amino groups due to cross-linking can lead to lower particle size (35).

Ionic strength can influence the particle size of protein nanoparticles. In case of albumin nanoparticles, a lower ionic strength favored smaller particle size, while a higher ionic strength resulted in particle aggregation (3). The zeta potential of DHC-loaded zein nanoparticles was altered by the adsorption of specific salt ions (Na$^+$ and Cl$^-$) on the nanoparticles (36). However there was no pronounced effect of ionic strength on the particle size of zein nanoparticles. Zein has relatively a lesser proportion of charged amino acids compared other proteins such as albumin (9). The results suggest that the zein nanoparticles are sterically stabilized (25) by the surfactants and therefore the electrostatic interactions may not play a predominant role.

A combination of a non-ionic surfactant Pluronic F68 and lecithin (2:1 ratio) sterically stabilized the zein nanoparticles (Fig. 1). When lecithin alone was employed at low concentrations the phospholipid monolayer was not stable enough to prevent particle aggregation (37,38). At higher concentrations, lecithin is known to crystallize during freeze drying and affect the physical characteristics of the freeze-dried cake (39). However, when combined with water-soluble non-ionic surfactant

Table III. Stability of DHC-loaded Zein Nanoparticles at 4°C

Time (weeks)	Particle size (nm)	PI	Zeta potential (mV)
0	251±35	0.37±0.08	−10.5±2
1	260±27	0.41±0.06	−9.4±3.1
6	262±23	0.39±0.05	−12.7±1.6
12	269±31	0.40±0.02	−11.5±2.7

Each value is an average of three experiments±SD
PI polydispersity index, *DHC* 6,7-hydroxycoumarin

Pluronic F68, lecithin monolayer is stabilized (37,38,40). The hydrophobic polypropylene oxide units of Pluronic F68 interact with the phospholipid monolayers of lecithin, while the hydrophilic polyethylene oxide chains overlap with polar groups of the lipid to stabilize the outer shell of zein nanoparticles (37,38) (Fig. 1). The hydrophilic Pluronic shell in addition to prolonging the systemic circulation of zein nanoparticles and can also overcome the drug resistance for delivery of cancer therapeutics (unpublished data).

The increase in zein concentration increases the viscosity of the dispersion and affects the nucleation process leading to a larger size (20). The yield of zein nanoparticles was ≥87% which was higher than zein nanoparticles prepared using yellow zein (25). DHC which is a hydrophobic molecule showed an encapsulation of 78% and is comparable to encapsulation reported in the literature for zein nanoparticles prepared using other stabilizers (17). The encapsulation of DHC in the zein nanoparticles was evident from the absence of its melting peak in the DSC thermogram (16). Since the zeta potential of DHC-loaded zein nanoparticles is only slightly lower than the blank zein nanoparticles, there is very minimal surface adsorption of DHC and hence the burst release is expected to be minimal. The particle size of zein nanoparticles did not increase after DHC encapsulation (Table II). Although the PI is high it is still <0.5 which can be further reduced by optimization of drug/polymer ratio and process parameters. The differences in particle size can be attributed to the large hydrodynamic radius in liquid dispersion as opposed to the dry particles used for AFM (41). Stability studies of zein nanoparticles at 4°C for about 12 weeks indicated that nanoparticles were stable, without any significant changes in the particle size and PI (Table III).

CONCLUSIONS

The results demonstrate that pH plays an important role in controlling zein nanoparticle size. Citrate buffer was identified as a suitable buffer to prevent particle growth during lyophilization. A combination of lecithin and Pluronic F68 stabilized the zein nanoparticles. The particle size was influenced by zein concentration, while ionic strength did not influence the particle size of zein nanoparticles. Overall, the study identified the optimal conditions for preparation of drug-loaded zein nanoparticles.

ACKNOWLEDGMENTS

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