1. Introduction

Lutein, a naturally occurring carotenoid, has numerous benefits on human health such as reducing age-related macular degradation (AMD) (Bian et al. 2012), protecting retinal pigment epithelial (RPE) cells from photo-oxidative (Domingos et al. 2014), providing antioxidant activity, and preventing several diseases (Li et al. 2010). Due to its chemical structure, lutein can be easily oxidized and degraded due to light and heat (Mitri et al. 2011) and also has low water solubility, poor absorption, and low bioavailability (Kotake-Nara and Nagao 2011). To take full advantage of its potential as an antioxidant, novel delivery systems have been developed to enhance its ability to be dispersed in water, as well as its physicochemical stability during processing and storage conditions. Delivery systems developed for antioxidant delivery include solid lipid nanoparticles, nanocrystals, and nanoliposomes (Mitri et al., 2011; Mitri et al. 2011; Tan et al. 2013). All these forms were associated with an increase in the stability of the incorporated drug against physical-chemical degradation. Specially, it has been shown that loss of lutein was faster when entrapped in single-layer (SL) emulsion, compared to layer-by-layer (LBL) emulsion, stabilized by gum Arabic (Lim et al. 2014).

The addition of stabilizing surfactants to the delivery systems is hypothesized as one of the simplest and most effective strategies to sustain the release profiles, to improve the physical stability of the nanodelivery system and chemical stability of entrapped fat-soluble drugs (Podaralla and Perumal 2012). An effective example in this regard is development of a core/shell nanoparticle made with lecithin as the core and pluronic F127 as a shell layer, for delivery of positively charged proteins engineered to provide protection, sustained release, and enhanced stability and functionality of entrapped bioactives (Choi et al. 2010; Oh et al. 2006).

A number of recently published studies provided advancing evidence that protein-based polymeric nanoparticles synthesized from gliadin, soy proteins, lectins, and zein can be successfully made specifically for food applications (Elzoghby et al. 2012). Among these natural potential nanocarriers, zein is particularly interesting as a naturally occurring polymer for synthesis of nanodelivery systems. It is a hydrophobic compound classified as generally recognized as safe (GRAS) as a direct human food ingredient by the Food and Drug Administration (FDA) (Elzoghby et al., 2012). Several attempts have been made to synthesize zein nanoparticles with entrapped drugs, antimicrobial agents,

and bioactive compounds such as 5-fluorouracil (Lai and Guo 2011), thymol (Zhang et al. 2014), curcumin (Gomez-Estaca et al. 2012), and essential oils (Parris et al. 2005)(Wu et al. 2012). Solutions were searched to improve stability of the zein nanoparticles during GI transit. For example, caseinate was used as an electrosteric stabilizer to prevent aggregation of zein nanoparticles in the neutral pH of the intestine (Patel et al. 2010). Even though data is available on characteristics of zein nanoparticles loaded with various antioxidants, little is known about stability of lutein entrapped in zein nanoparticles when exposed to various processing and storage conditions, and on the effect of surfactants on the release and stability of lutein under these conditions.

The objective of this paper was to assess lutein thermal and photo-stability, and lutein release from zein nanoparticles in the presence and absence of lecithin and pluronic F127 co-surfactants. Lutein-loaded zein nanoparticles, made with and without surfactants, were synthesized using a liquid-liquid dispersion method. A combination of phospholipid soybean lecithin and tri-block copolymer pluronic F127 was used in the formulation as surfactants to promote physicochemical stability of the nanoparticles and entrapped bioactives. Lutein emulsions were prepared in parallel to be used as a control. Dynamic light scattering (DLS) and transmission electron microscopy (TEM) were used to characterize particle physical stability. Lutein release from nanoparticles suspended in phosphate-buffered saline (PBS) was quantified in the absence and presence of surfactants; the degradation of released lutein was determined under the same release conditions. In addition, thermal and photo-oxidation of lutein were measured as indicators of lutein chemical stability. The hypothesis was that lutein entrapped in zein nanoparticles was more stable under various storage conditions and that the electrostatic affinity between the zein nanoparticles and surfactants will result in a more sustained release of lutein and improved chemical stability of the entrapped bioactive.

2. Material and Methods

2.1. Material and Reagents

Zein (Z3625), pluronic F127, chloroform, and ethanol were purchased from Sigma Aldrich (St. Louis, MO, USA). Soybean lecithin, hydrochloric acid, and sodium hydroxide were purchased from Fisher Chemical (Fisher Scientific International, Fairlawn, NJ). Lutein was provided by Kemin Foods,L.C. (Iowa, USA). Nanopure water obtained using Nanopure Diamond from Barnstead international (IA, USA) was

used for all solution preparation. 100kDA Spectra/POR cellulose ester Biotech membrane tubing and closures was purchased from Spectrum Laboratories Inc. (CA, USA). All other reagents and components used in this study were of analytical grade.

2.2. Synthesis of Zein Nanoparticles with Entrapped Lutein

Nanoparticles were synthesized by a liquid-liquid dispersion method, as follows. Briefly, 10 mg of zein was dissolved in 1 mL ethanol-aqueous solution (70:30% (v/v)). A lutein solution was prepared at 0.75 mg/mL with 100% ethanol and was added dropwise to the zein solution at a ratio of 1:1 under mild stirring conditions. The mixture was injected into 7.5 mL of an aqueous phase containing a combination of lecithin and pluronic F127 0.045:0.09% (w/v) as surfactants. The sample was then processed in a microfluidizer at 30,000 PSI for 3 cycles (M-110P, Microfluidics, MA, USA). Subsequently, the sample underwent evaporation to remove ethanol under vacuum (at approximately 500-600 mmHg) and nitrogen injection (80 mmHg) in a rotovapor (Buchi R-124, Buchi Analytical Inc., DE, USA). The lutein-loaded zein nanoparticles produced after complete evaporation of ethanol were washed by dialysis using a 100kDa Spectra/POR CE membrane (Spectrum Rancho, CA, USA). The nanoparticle suspension was placed in the membrane and suspended in 1.5 L nanopure water for 48 hours; the dialysis medium was changed every 8 hours to remove free surfactants. The suspension was collected and kept at room temperature for further analysis. Zein nanoparticles without surfactants were prepared in parallel using the same method, with the exception that surfactants were not added to the aqueous phase. The lutein emulsion made with surfactants followed the same protocol was served as a control.

2.3. Particle Size, Polydispersity Index (PDI), and Zeta Potential Analyses

Freshly-made zein nanoparticle samples were characterized by measuring average diameter size, PDI, and zeta potential by dynamic light scattering (DLS), using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, U.K.). Before the measurements were taken, samples were prepared at a final concentration of 0.1-0.3 mg/mL, optimum for the instrument. All measurements were performed in triplicate.

2.4. Morphology Analysis

Morphology of freshly-made zein nanoparticle was observed by transmission electron microscopy (TEM). One droplet of the sample was placed on a copper grid of 400 mesh with a carbon film, and the

excess sample was removed with a filter paper. Uranyl acetate was used as a negative stain to improve the contrast of the sample.

2.5. Entrapment Efficiency (EE) Measurement

One milliliter of the freshly-made lutein-loaded zein nanoparticle sample was centrifuged at 64,000 g for 1 hour, from which 95% of particles were recovered (data not shown). The supernatant and the nanoparticle pellet were collected. Both samples were broken by ethanol and then lutein was extracted with chloroform (1:1 ratio). The relative solubility of lutein in chloroform (6000 mg/L) is 20 times higher than that in ethanol (300 mg/L) (Craft and Soares 1992). The concentration of lutein was measured using a UV/Vis spectrophotometer (GENESYS 6:Thermo Spectronic) with glass cells of 1 cm path length recorded at 445 nm. The absorbance value was converted to lutein concentration based on the standard curve for lutein in 1:1 ethanol and chloroform. Entrapment efficiency (%) was estimated as the ratio of lutein amount in pellet to theoretical lutein entrapped as described by $\frac{\text{Lutein amount in pellet}}{\text{Theoretical amount of available lutein}} \text{ x } 100 = \%\ \text{EE}$. All measurements were performed in triplicate.

2.6. Lutein Release from Zein Nanoparticles in Phosphate-Buffered Saline (PBS)

The release of the entrapped lutein from zein nanoparticles was studied in 0.01 M phosphate-buffered saline (PBS) solution (pH 7.4) at 37°C; 0.5% of Tween 20 was added to PBS to improve the solubility of lutein released. Briefly, 10 mL of freshly-prepared nanoparticles were added to 20 mL of Tween 20 enhanced PBS and mixed thoroughly. The mixture was divided and placed into 1.5 mL centrifuge tubes, placed into a shaking incubator (C25KC incubator shaker, New Brunswick Scientific, NJ, USA) at 37°C and 100 rpm. At pre-defined time intervals, a centrifuge tube was sampled and centrifuged at 64,000 g (Allegra 64R centrifuge, Beckman coulter, Inc., CA, USA) for 1 hour. The supernatant was removed and extracted with ethanol and chloroform (1:1 ratio) and then vortexed for 10 minutes. The extracted lutein was determined in the supernatant by measuring the absorbance at 445 nm using a UV/Vis spectrophotometer as described under entrapment efficiency section. The wavelength was selected to avoid interference from degraded products of lutein, consisting of low-molecular-weight and short-chain aldehydes and ketones, with a maximum absorbance ranging from 270 to 345 nm (Landrum 2009). All measurements were performed in triplicate.

2.7. Degradation of Lutein Entrapped in Zein Nanoparticles

The degradation of lutein entrapped in zein nanoparticles (with and without surfactants) and lutein entrapped in surfactant-stabilized emulsion was determined by measuring and adding the amount of lutein detected in both pellet and supernatant under the same release condition.

2.8. Physical-Chemical Stability of Zein Nanoparticles with Entrapped Lutein

Freshly-made samples were stored in darkness at three different temperatures: 4°C in a refrigerator, 25°C at room temperature and 40°C in an incubator over one month. Samples were monitored for changes in average particle size, surface characteristic, and entrapment efficiency at the sampling time points of 7, 15, and 30 days of storage. All experiments were performed in triplicate.

2.9. Photo-Chemical Stability of Lutein Entrapped in Zein Nanoparticles

Nanoparticle and emulsion samples were placed in transparent glass vials and stored in a lightproof cabinet where they were exposed to 365 nm UV lamps (100 W: Blak-Ray model B 100AP) for up to 10 hours. At exposure time intervals (0.5, 1, 2, 3, 5, 7, and 10 hours), 1 mL was withdrawn from each sample and then extracted and analyzed by measuring lutein concentration using UV-Vis spectrophotometer (GENESYS 6: Thermo Spectronic) at 445 nm. The experiment was performed in triplicate.

2.10. Degradation Reaction Kinetics

A general reaction rate for the lutein degradation and release kinetics can be described by $-\frac{d[C]}{dt} = k[C]^n$: where *C* is the lutein amount (μg), *k* is the reaction rate constant, and *n* is the order of the reaction. The correlation coefficient (R^2) was used as an indicator of the best fitting of the kinetic models for lutein release and degradation studies. The degradation of lutein against UV exposure, followed first-order kinetic as described by $\ln(\frac{[C]}{[C_0]}) = -kt$, similar to the results found in other studies (Abdel-Aal el et al. 2010; Aparicio-Ruiz et al. 2011; Dhuique-Mayer et al. 2007; Lim et al., 2014). Lutein degradations under PBS conditions and storage as a function of time and temperatures followed second-order kinetics as described by $\frac{1}{[C]} = \frac{1}{[C_0]} + kt$: where *C* is the lutein amount (μg) at time *t*, C_0 is the initial amount of lutein (μg), *t* is the time (hours or days) and *k* is the reaction rate derived from the slope of linear regressions.

2.11. Data Statistical Analysis

All experiments were performed in triplicate and the results were reported as the mean ± standard error. Statistical analysis was performed in SAS (version 9.4, SAS Institute Inc., NC, USA). The analysis of variance (ANOVA) was used to determine significant differences between the systems. The significance level (*P*) was set at 0.05.

3. Results and Discussion

3.1. Physicochemical Characterizations

A liquid-liquid dispersion method was successfully used to synthesize lutein-loaded zein nanoparticles in the presence and absence of surfactants. The combination of lecithin and pluronic F127 was used to stabilize the nanoparticles. Pluronic F127 is a hydrophilic non-ionic surfactant copolymer consisting of a hydrophobic block of polypropylene located between two hydrophilic blocks of polyethylene glycol. Gel formation at higher temperatures efficiently overcomes the natural brittleness of zein, supporting its delivery system application (Li et al. 2013). Lecithin, a phospholipid food emulsifier or stabilizer, has a hydrophilic head, phosphatidylcholine (PC) and two hydrophobic tails, phosphatidylethanolamine (PE) and phosphotidylinositol (PI) (Wang and Wang 2008). Because of its partly mixed structure, lecithin can be used as an effective and stable emulsifier to interact simultaneously with both hydrophilic and hydrophobic substances (Choi et al., 2010). One or more layers of lecithin cover the surface of the hydrophobic zein nanoparticles, with lutein entrapped inside the zein matrix by electrostatic interaction. The hydrophilic head of lecithin connects with hydrophilic polyethylene glycol of pluronic F127 and the hydrophobic polypropylene possibly connects with zein matrix resulting in a hydrophilic zein nanoparticle loaded with hydrophobic lutein, which is useful to disperse this bioactive to the aqueous environment while protecting it from degradation (Fig. 1).

[Fig. 1. Near here]

Lutein-loaded zein nanoparticles and unloaded nanoparticles, with and without surfactants, were characterized immediately after purification (Table 1). Average particle size, PDI, and zeta potential of freshly-made samples were measured after 24 hours dialysis in buffer (pH 7.4) (Podaralla and Perumal 2012). The statistical analysis of the data revealed that there was significant difference in size, whereas PDI and zeta potential were not significantly different for zein nanoparticles made with and without

surfactants. The average particle size of lutein loaded in zein nanoparticles with and without surfactants was 216.5±29 nm and 156.1±18 nm, respectively. While zein nanoparticles formed in the presence of surfactants had a relatively small polydispersity (less than 0.3), a higher PDI range of 0.33-0.48 was observed for nanoparticles made without combined surfactants.

[Table 1. Near here]

The size results were confirmed by TEM (Fig. 2). Particles with surfactants showed a spherical shape with a rough surface, with some particles connected in a surfactant mesh (Fig. 2A and 2B). Nanoparticle without surfactants showed a smaller size, with a more spherical morphology, but were less uniform in size and more likely to agglomerate (Fig. 2C and 2D) resulting in higher PDI values as measured by DLS. Similar zein nanoparticle images were reported in other studies (Parris et al., 2005; Zhang et al. 2014).

[Fig. 2. Near here]

Zeta potential has long been accepted as a good measure for assessing stability of a nanoparticles system. A high degree of stability of the nanodelivery system is expected at zeta potential values higher than +30 mV or lower than -30 mV on the basis of charge repulsion between the nanoparticles (Murdock et al. 2008). Particles covered by surfactants were found to be more negatively charged (-47.6±1.6 mV) than particles without surfactants (-31.9±4.3 mV), indicating a good stability of the surfactant stabilized particles. The particles covered only by lecithin showed a strong anionic surface charge (-51.3±1.2 mV) beneficial to inducing electrostatic interactions with positively charged compounds; the negative charge decreased (-47.6±1.6 mV) with the addition of non-ionic pluronic F127, but the charge was indicative of a stable suspension. Entrapment of lutein resulted in a change in zeta potential from -30.9±3.3 mV to a less negative value of -21.0±8.6 mV for particles made without surfactants. The hydrophobic interaction between lutein and zein nanoparticles as nonpolar molecules contributed to the rearrangement in the zein structure to accommodate the entrapped bioactive, resulting in the observed zeta potential change.

The presence of surfactants not only affected average particle size, PDI, and zeta potential, but also lutein entrapment inside the zein matrix. Without surfactants, entrapment efficiency was around 69.1±11.4%; with the addition of surfactants to the system, which resulted in a thicker and denser zein

matrix, the entrapment efficiency increased to 83±5.8%, revealing a statistically significant difference between the two systems. This result was expected; Xu and Hanna (Xu and Hanna 2006), suggested that addition of surfactants to the system can stabilize particles and therefore increase entrapment efficiency.

Both lecithin, an anionic phospholipid (Wang and Wang 2008) which forms an ionic complex with positively charged protein, and the added pluronic F127, which were used simultaneously to stabilize the nanoparticles, were found to confer a negative charge to the particles suitable for inducing the electrostatic interactions that allowed for good nanoparticle stability while increasing lipophilic drug loading (Oh et al. 2006).

3.2. Lutein Release from Zein Nanoparticles in PBS

Phosphate buffered saline (PBS) is a water-based salt buffer solution commonly used in biological research, particularly for testing drug release. The release kinetic of lutein from zein nanoparticles made with and without surfactants in an aqueous buffer PBS was evaluated (Fig. 3). The release of the non-entrapped compounds available on the surface of the particles at the beginning stage of the release is referred to an initial-burst release (Fredenberg et al. 2011). The release profile of zein nanoparticles can be described as a two-phase pattern, with the initial-burst release within 24 hours followed by zero-order release profile (Table 3). For particles made without surfactants (LTZN NSF), lutein released in the initial-burst phase amounted for 43.26%, whereas zein nanoparticles made with surfactants (LTZN SF) only released 19.83% lutein (Fig. 3). The results are not surprising as surface-associated lutein was expected to be released quickly from the surface of the particles when surfactants were not present to inhibit lutein release. Release of lutein after 24 hours followed zero-order kinetics (Table 3) with 51.51% lutein released at 168 hours from nanoparticles without surfactants versus only 42.67% in the presence of surfactants (Fig. 3). Hydrophobic interaction between lecithin, lutein, and the polypropylene chains of pluronic F127 inhibited the hydrolytic degradation of zein and slowed the release of lutein. In the absence of surfactants rapid protein swelling resulted in a faster release of the entrapped bioactive by diffusion through aqueous channels formed in the hydrated swelled zein matrix (Choi et al., 2010). The results supported the hypothesis that the electrostatic affinity between the zein nanoparticles and surfactants; the combined lecithin and pluronic F127, were responsible for a more sustained release

of lutein. Statistical results supported that release of lutein from zein nanoparticles with surfactant was significantly different than release of lutein from zein nanoparticles without surfactant.

[Fig. 3. Near here]

3.3. Entrapped Lutein Degradation in PBS

Lutein is more susceptible to degradation than common carotenes due to conjugated double bonds and the two hydroxyl groups, considered more heat sensitive (Dhuique-Mayer et al., 2007). The degradation of lutein was assessed for lutein entrapped in zein nanoparticles with (LTZN SF) and without surfactants (LTZN NSF) and the result was compared to that of lutein in emulsified form with the same surfactants (LTEM SF) (Fig. 4). Lutein degradation profiles followed second-order kinetics with no significant different values (0.00003-0.00004) of the degradation rate constant (k) among all systems studied (Table 3). Emulsified lutein degraded rapidly to approximately 40% under PBS condition after 168 hours, in accordance with findings reported by Shi and Chen (Shi and Chen 1997), who found that 25-30% pure lutein in distilled water degraded at the same time.

[Fig. 4. Near here]

3.4. Physical-Chemical Stability as a Function of Time and Temperature

Physical stability of zein nanoparticles was investigated at 4°C, 25°C, and 40°C over 30 days by measuring size, PDI, and zeta potential. Chemical stability of entrapped lutein was assessed in parallel, by measuring the absorbance using a UV/Vis spectrophotometer at 445 nm (Table 2). Zein nanoparticles with and without surfactants were stable at low temperature, measuring between 156.1±18 to 216.5±29 nm when stored at 4°C for 30 days. Nanoparticles increased in size over time when stored at higher temperature, especially in the absence of surfactants. For example, while size of nanoparticles with surfactants-increased to 380.5±51 nm over 30 days of storage at 25°C, particles without surfactants measured up to 3103±332 nm at the same temperature. At 40°C, sizes bigger than 1 μm were detected after 7 days of storage for the nanoparticles made without surfactants. The PDI generally increased with temperature and storage time (from 0.27 to 0.80). Zeta potential ranged from -18 mV to -25 mV for nanoparticles without surfactants, and between -15.2 mV to -38 mV for particles made with surfactants.

[Table 2. Near here]

248 The surfactants not only provided long-term storage stability over 30 days for the
249 nanosuspension, but they also delayed the degradation of lutein (Fig. 5). Only 26% of entrapped lutein
250 was degraded after 30 days at 25°C when entrapped in LTZN SF, compared to 54% which degraded at
251 the same time when entrapped in LTZN NSF.

252 [Fig. 5. Near here]

253 Similar trends were found at 40°C in both particles; 13.8% and 7.5% of retained lutein remained
254 in the lutein-zein nanoparticles with and without surfactants, respectively. Emulsified lutein degraded
255 faster than lutein entrapped in zein nanoparticles at all temperatures. Lutein degradation under various
256 temperatures for 30 days followed second-order kinetics, similarly to degradation of lutein in particles
257 suspended in PBS for 7 days (Table 3). The lowest degradation rate (*k*) values of nanoentrapped lutein
258 were also found in the presence of zein nanoparticles in the presence of surfactants at all storage
259 temperatures. Increased temperature resulted in increasing the degradation for all systems. Thus, it is
260 concluded that while the preferred storage condition of lutein in 4°C (Lai and Guo 2011), stability of lutein
261 can be improved by loading it in surfactant covered zein nanoparticles even at higher temperatures.

262 **3.5. Photo-Chemical Stability against UV Exposure**

263 Photochemical stability against UV light of lutein loaded in zein nanoparticles with surfactant and
264 without surfactant was compared to lutein emulsified form made with the same surfactants. There was
265 statistical significance in the surfactants and time as main effects. Emulsified lutein underwent
266 photochemical degradation very quickly (Fig. 6). After 10 hours, only 1.42% entrapped lutein remained in
267 the lutein emulsions whereas 15.91% lutein was protected by entrapment in zein nanoparticles without
268 surfactants (Fig. 6). The zein nanoparticles combined with surfactants were able to provide the greatest
269 protection against UV light-induced lutein degradation, with 46.53% of lutein remaining inside the zein
270 nanoparticles after UV light exposure for 10 hours.

271 [Fig. 6. Near here]

272 The lowest degradation rate constant was found for lutein loaded in zein nanoparticles
273 combined with surfactants (0.0753) whereas zein nanoparticles without surfactants was higher (0.1869)
274 indicating that the rate of lutein degradation in the presence of zein nanoparticles plus the effect of
275 surfactants was noticeably retarded compared with lutein delivered in emulsified form (0.4093) (Table 3).

276 The improved photo-chemical stability of lutein against UV light when entrapped in zein nanoparticles was
277 mainly based on the competitive absorption of UV photons by zein. Zein has been proven to absorb UV
278 due to aromatic amino acids such as phenylalanine in its sequence (Stoscheck 1990). Moreover, the
279 effect of surfactants surrounding zein nanoparticles was another contributor for improved stability of
280 lutein. Lecithin was successfully used as UV protectant previously (Sundaram and Curry 1996). The
281 possible mechanism for the ability of lecithin to protect lutein from degradation was due to energy transfer
282 from the excited lutein species to lecithin. Thus, photo-stability against UV light of the entrapped lutein
283 was significantly improved by the UV absorption of zein nanoparticles with the support of the combined
284 lecithin and pluronic F127 as surfactants.

285 [Table 3. Near here]

286 **4. Conclusion**

287 Zein nanoparticles loaded with 7.5% lutein stabilized by the combined lecithin and pluronic F127
288 surfactants were successfully synthesized using a liquid-liquid dispersion method. The addition of
289 surfactants increased particle size and improved polydispersity index. Zeta potential slightly changed, and
290 entrapment efficiency increased significantly. In the presence of the surfactants, the burst release
291 decreased and the release kinetic was subsequently sustained. Zein nanoparticles showed a great ability
292 to protect lutein from degradation under various storage conditions as compared to the emulsified lutein.
293 The preferred storage condition of lutein-loaded zein nanoparticles with surfactants was 4°C for 30 days.
294 In addition, this complex formulation provided a good protection against UV light for 10 hours. Further
295 studies are under way on the potential of zein nanoparticles to improve the physicochemical stability and
296 functionality of entrapped antioxidants added to liquid food under simulated gastrointestinal (GI)
297 environments. Based on characteristics, release, and stability data, it was suggested that with the
298 addition of surfactants to improve entrapment efficiency of hydrophobic bioactives and to protect lutein
299 against chemical degradation, zein nanoparticles could provide better protection against bioactive losses
300 during thermal and neutral conditions than other delivery systems such as emulsions.

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Zhang, Y., Y. Niu, Y. Luo, M. Ge, T. Yang, L. L. Yu, and Q. Wang (2014) Fabrication, characterization and antimicrobial activities of thymol-loaded zein nanoparticles stabilized by sodium caseinate-chitosan hydrochloride double layers. Food chemistry 142 269-275. doi: 10.1016/j.foodchem.2013.07.058

Figure captions

Fig. 1. Schematic formation of lutein-loaded zein nanoparticle stabilized by lecithin and pluronic F127 surfactants

Fig. 2. Transmission Electron Microscope (TEM) images of zein nanoparticles with surfactants (A and B) and without surfactants (C and D)

Fig. 3. Two-pattern release profiles consisting of an initial burst release within 24 hours and the following zero-order release of lutein from zein nanoparticles made with (LTZN SF) and without surfactants (LTZN NSF) in PBS solution (pH 7.4) at 37°C and 100 rpm for 7 days

Fig. 4. Degradation profiles following second-order reaction of lutein loaded in zein nanoparticles with (LTZN SF) and without (LTZN NSF) surfactants and lutein emulsion made with surfactants (LTEM SF) in PBS solution (pH 7.4) at 37°C and 100 rpm for 7 days

Fig. 5. Degradation profiles following second-order reaction of lutein entrapped in zein nanoparticles at different storage temperatures (A: 4°C, B: 25°C, and C: 40°C) over 30 days

Fig. 6. Photo-chemical stability profiles following first-order reaction of lutein loaded in zein nanoparticles with (LTZN SF) and without surfactants (LTZN NSF) and emulsified lutein with surfactants (LTEM SF) exposed to UV light for 10 hours

Table captions

Table 1. Characteristics of unloaded and lutein-loaded zein nanoparticles made with surfactants (SF) or without surfactants (NSF)

Table 2. Characteristics of lutein-loaded in zein nanoparticles at different storage temperatures over 30 days

Table 3. Fitting model for release and degradation of lutein-loaded zein nanoparticles

Fig. 1.

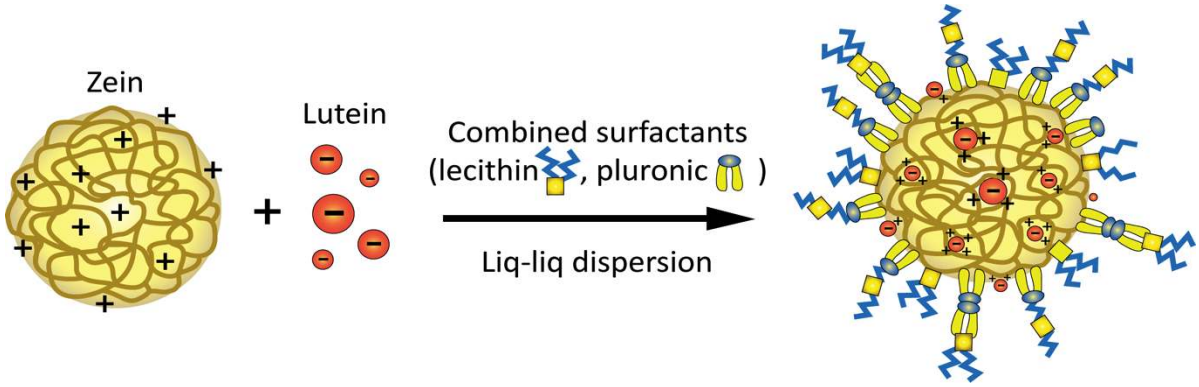
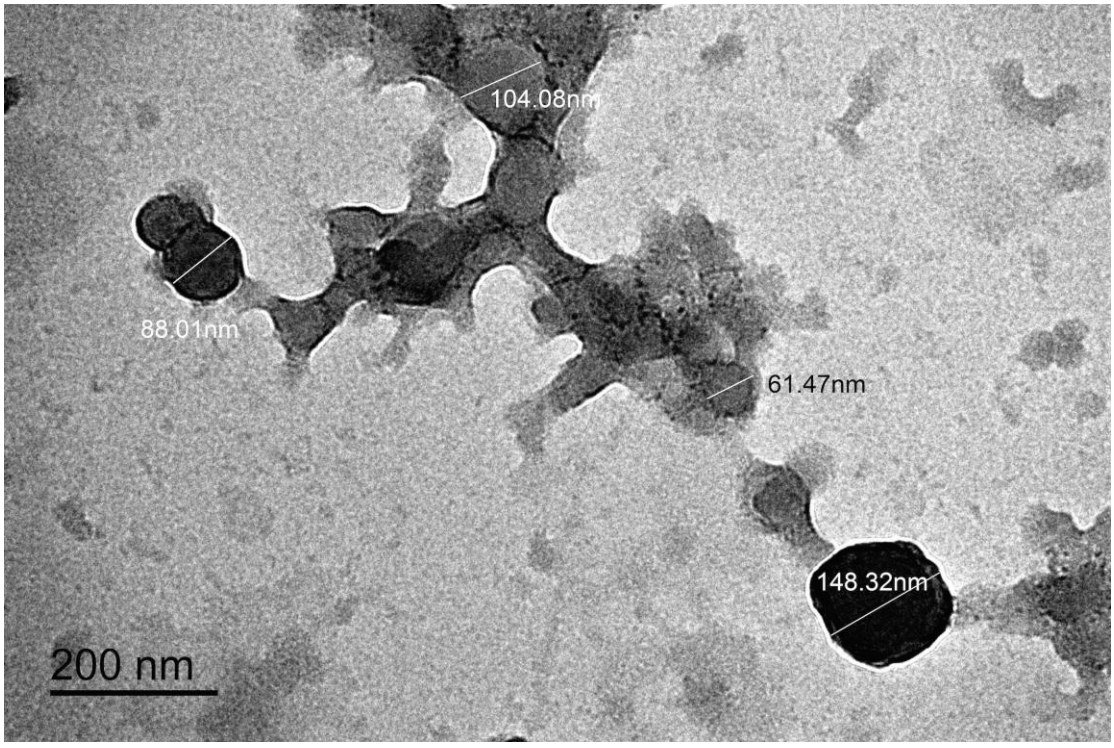
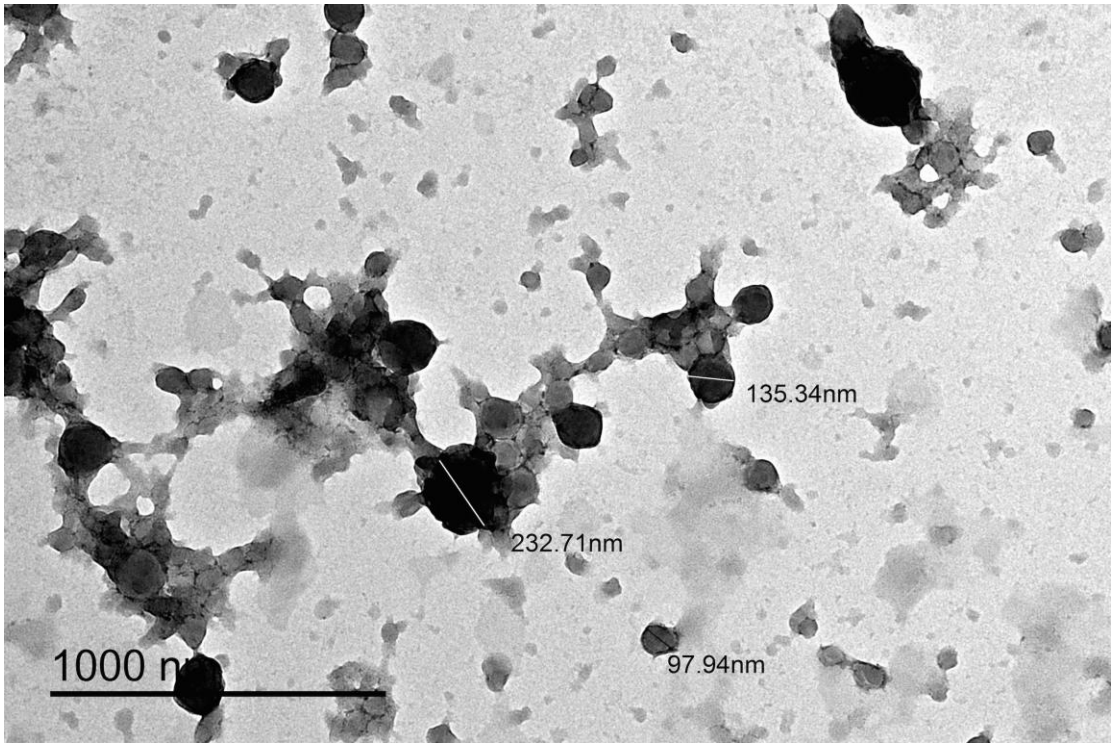

Zein
Lutein
Combined surfactants
(lecithin , pluronic)
Liq-liq dispersion

Fig. 2.


104.08nm
88.01nm
61.47nm
148.32nm
200 nm

A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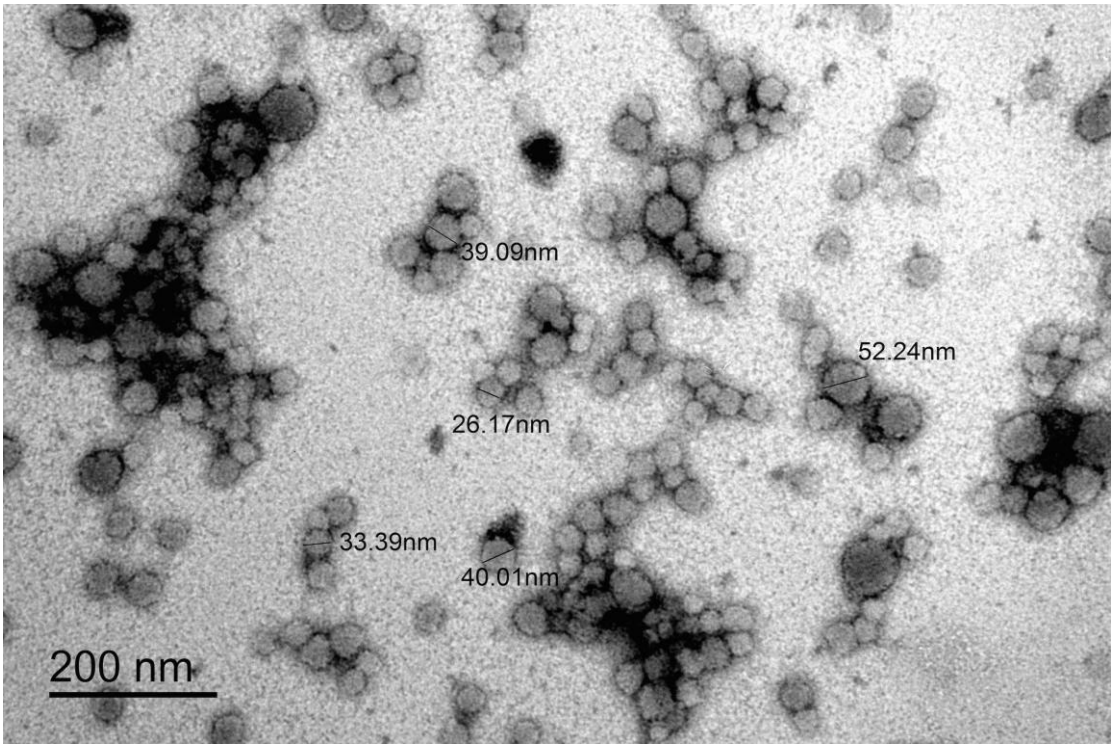
232.71nm
97.94nm
1000 nm

B

C



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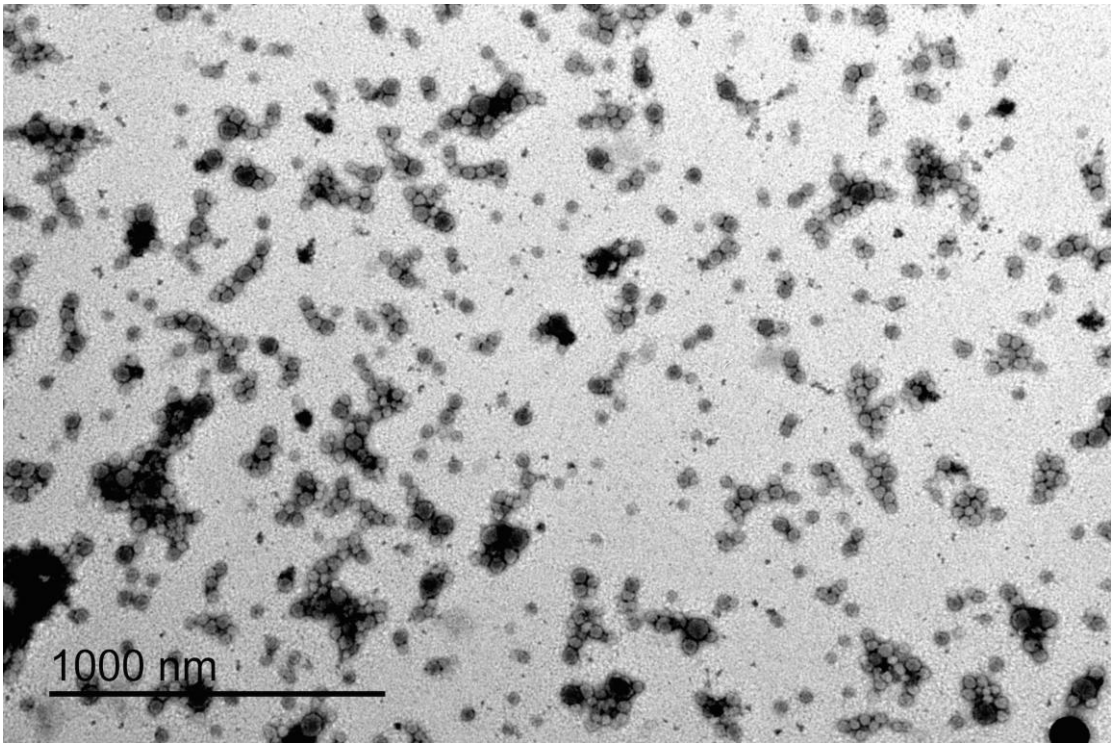

Fig. 3.

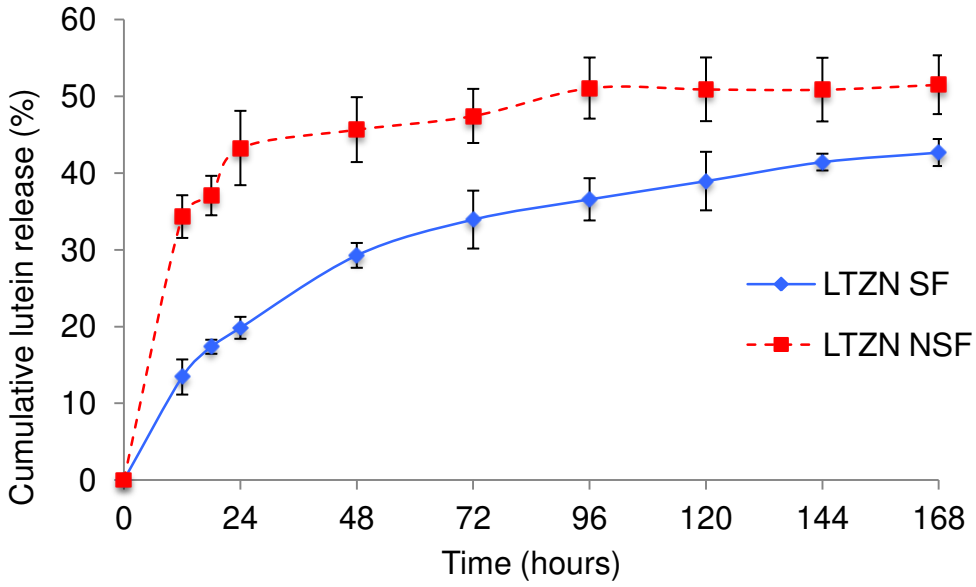
Cumulative lutein release (%)
60
50
40
30
20
10
0
0
24
48
72
96
120
144
168
Time (hours)
LTZN SF
LTZN NSF

Fig. 4.

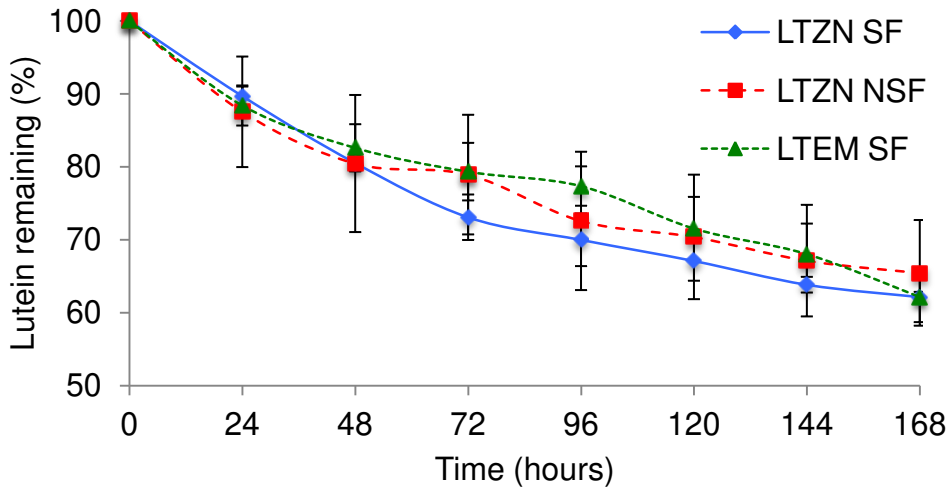
100
90
80
70
60
50
Lutein remaining (%)
0
24
48
72
96
120
144
168
Time (hours)
LTZN SF
LTZN NSF
LTEM SF

Fig. 5.

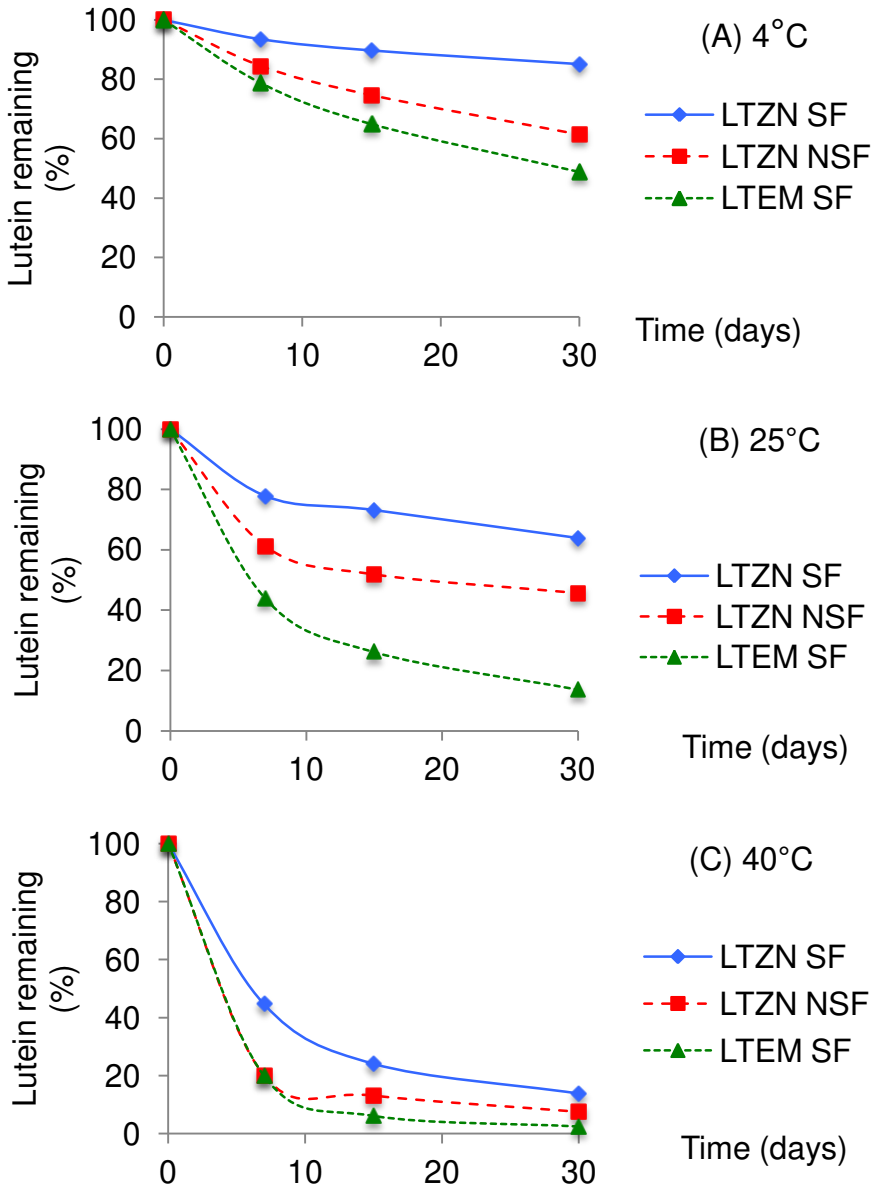
(A) 4°C
Lutein remaining (%)
100
80
60
40
20
0
0
10
20
30
Time (days)
LTZN SF
LTZN NSF
LTEM SF
(B) 25°C
Lutein remaining (%)
Time (days)
(C) 40°C
Lutein remaining (%)
Time (days)

Fig. 6.

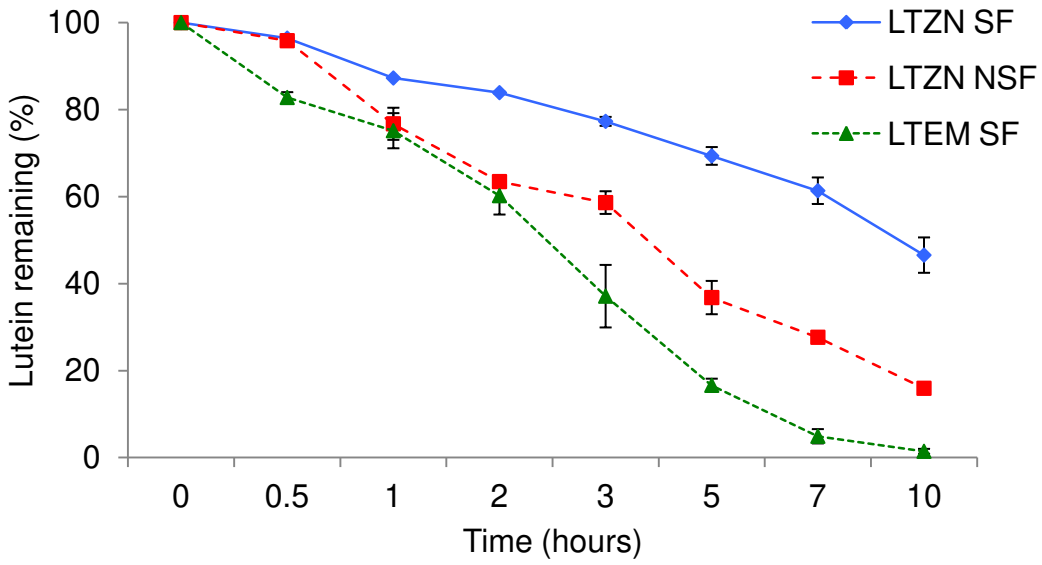
100
80
60
40
20
0
Lutein remaining (%)
0
0.5
1
2
3
5
7
10
Time (hours)
LTZN SF
LTZN NSF
LTEM SF

Table 1.

Sample [a]	Size (nm)	PDI (a.u)	Zeta Potential (mV)	EE (%)
ZN SF	208.8±8.0	0.19±0.04	-47.6±1.6	-
LTZN SF	216.5±29*	0.26±0.09	-30.9±3.3	83.0±5.8*
ZN NSF	149.2±5.5	0.48±0.07	-31.9±4.3	-
LTZN NSF	156.1±18	0.33±0.06	-21.0±8.6	69.1±11.4

Note: Values are expressed as mean ± standard error (n=3). [a] ZN and LTZN represent formulations of zein nanoparticles and lutein-loaded zein nanoparticles, SF and NSF represent formulations with and with no surfactants respectively. Mass ratio of zein:lutein was 1:0.075 (%, w/w) and mass ratio of lecithin:pluronic F127 was 0.045:0.09 (%, w/v). * shows statistically significant difference.

Table 2.

Sample	Temperature	Time (days)	Size (nm)	PDI (a.u)	Zeta Potential (mV)
LTZN SF	4°C	0	216.5±50	0.27±0.05	-30.9±3.3
		7	195.7±19	0.29±0.05	-31.1±10.8
		15	183.0±26	0.27±0.06	-32.2±10.7
		30	168.6±2	0.27±0.04	-33.0±10.8
	25°C	0	216.5±50	0.27±0.05	-30.9±3.3
		7	170.8±65	0.38±0.05	-23.3±2.4
		15	221.0±74	0.35±0.06	-21.8±9.5
		30	380.8±51	0.36±0.07	-15.2±0.3
	40°C	0	216.5±50	0.27±0.05	-30.9±3.3
		7	134.5±40	0.54±0.07	-38.0±1.9
		15	203.1±49	0.24±0.06	-31.8±7.4
		30	229.5±27	0.29±0.03	-29.5±2.9
LTZN NSF	4°C	0	156.1±18	0.26±0.06	-21.0±8.6
		7	142.4±32	0.32±0.11	-23.8±1.0
		15	189.2±55	0.26±0.07	-24.6±1.7
		30	198.9±47	0.39±0.13	-25.0±2.6
	25°C	0	156.1±18	0.26±0.06	-21.0±8.6
		7	567.7±203	0.56±0.07	-24.6±1.7
		15	1406.1±279	0.47±0.13	-23.7±1.5
		30	3103±332	0.58±0.15	-23.6±1.4
	40°C	0	156.1±18	0.26±0.06	-21.0±8.6
		7	1096.1±253	0.58±0.05	-18.0±2.7
		15	2434.5±535	0.73±0.19	-21.0±0.6
		30	3599.5±94	0.80±0.10	-22.7±4.2

Note: Values are expressed as mean ± standard error (n=3).

Table 3.

Experiment	Sample	Time	Kinetic model	K	R^2
Release	LTZN SF	0-24 hours	Zero-order (Initial burst)	0.90930	0.95203
	LTZN NSF			2.02800	0.88626
	LTZN SF	24-168 hours	1st order	0.00120	0.83448
	LTZN NSF			0.01270	0.92693
Degradation	LTZN SF	168 hours	2nd order	0.00004	0.96389
	LTZN NSF			0.00003	0.91575
	LTEM SF			0.00003	0.97666
Physical stability	LTZN SF 4°C	30 days	2nd order	0.00006	0.92430
	LTZN SF 25°C			0.00020	0.84325
	LTZN SF 40°C			0.00210	0.99788
	LTZN NSF 4°C	30 days	2nd order	0.00020	0.99243
	LTZN NSF 25°C			0.00050	0.75685
	LTZN NSF 40°C			0.00420	0.98458
	LTEM SF 4°C	30 days	2nd order	0.00040	0.99878
	LTEM SF 25°C			0.00210	0.99502
	LTEM SF 40°C			0.01210	0.96126
Photo-chemical stability	LTZN SF	10 hours	1st order	0.07530	0.98454
	LTZN NSF			0.18690	0.99256
	LTEM SF			0.40930	0.98301

Note: LTZN and LTEM represent formulations of lutein-loaded zein nanoparticles and emulsified lutein, SF and NSF represent formulations with and with no surfactants respectively. K and R^2 represent the degradation rate constant and correlation coefficient, respectively.

Graphical abstract