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Preparation of puerarin-loaded zein nanoparticles: characterization and stability study

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Abstract

The Thai herb *Pueraria mirifica* has been used in cosmetics, dietary supplements and natural health products for a long time because of its rejuvenation properties. In this study, zein was used to enhance the stability during four-month storage of puerarin, the glycosylic isoflavone in the *Pueraria* extract (PE). Zein nanoparticles (ZNs) were prepared by the liquid-liquid dispersion method. The process was based on dissolving zein in 85% aqueous ethanol, followed by shearing the stock solution into citrate solution, and ethanol was removed by rotary vacuum evaporation. Three different loading percentages (10, 20 and 30%) of PE were applied, and the characteristics of puerarin loaded-zein nanoparticles (PuZNs) were investigated for their size, per cent yield, per cent encapsulation efficiency (% EE) and stability. The results showed that the particles size and zeta potential of PuZNs varied from 165.3 ± 2.4 nm to 232.8 ± 1.9 nm and -26.7 ± 0.8 mV to -31.3 ± 1.3 mV, respectively. Increasing the % PE loading increased % yield and % EE of puerarin. Scanning electron microscopy images showed that the PuZNs were spherical with an average size of less than 200 nm. The puerarin of *Pueraria* extract in the ZNs from 20 and 30% PE loading presented stability during four months of storage at 4 ± 1°C. The results indicated the potential use of zein for encapsulation of puerarin to maintain its stability for food applications.

Keywords*: encapsulation efficiency; Pueraria extract; puerarin; Pueraria mirifica; zein nanoparticles; zeta potential*.

1. Introduction

Pueraria candollei var. *mirifica* (syn. *Pueraria mirifica*, PM) or white 'Kwao Krua' belongs to the family Leguminosae and is indigenous to Thailand, especially in the north, west and northeast (Van der Maesen, 2002). This plant's tuber has been applied in traditional Thai medicine as a rejuvenating tonic with oestrogenic activity (Kerr, 1932; Wanandorn, 1933). PM contains phytoestrogens and glycosides such as puerarin, daidzein, genistein, genistin, kwakhurin, kwakhurin hydrate and mirificin. These compounds exhibit estrogenic activity (Chansakaow et al., 2000; Trisomboon et al., 2004), bone loss prevention (Urasopon, Hamada, Asaoka, Cherdshewasart, & Malaivijitnond, 2007) and antioxidant activity (Cherdshewasart & Sutjit, 2008).

PM is used in food supplements, natural health products and cosmetics (Bodner & Hymowitz, 2002; Malaivijitnond, 2012) for relieving the symptoms of oestrogenic deficiency, such as bone loss, sagging breasts and wrinkled

skin. More than 200 herbal products containing PM are registered by private herbal manufacturers (Office of Agricultural Economics, 2 0 0 7) in different formulations such as tablets, capsules, creams and gels to the Thai market, and these are continuously widespread throughout the global market. PM components have been extensively studied. It was found that 95% ethanol extract of this plant contained puerarin as a major compound (Peerakam et al., 2018). Similarly, the main flavonoids and isoflavones in the *Pueraria tuberosa* LINN. were puerarin (8.31%), daidzein (1.70%) and genistein (1. 37%) (Rastogi et al., 2013; Maji, Pandit, Banerji, & Banerjee, 2014). Puerarin has been widely used in many disease treatments such as diabetes, liver fibrosis, cardiovascular diseases, neurotoxicity and Alzheimer' s disease (Zhou, Zhang & Peng, 2014). Puerarin was also shown to exhibit various protective effects against fever, osteonecrosis, inflammation and oxidative damage (Zhou et al., 2014; Wei, Chen, & Xu, 2014). Puerarin prevents epithelial tight junction dysfunction induced by ethanol in the Caco-2 cell model (Che et al., 2020). However, puerarin is practically water-insoluble with a low bioavailability, which limits its application.

Nanoencapsulation is a promising approach to overcome the water-insolubility and low oral bioavailability of bioactive compounds. The development of nanosized drug carriers (in the size range of 10–1000 nm) could improve the anti-oxidation, increase the ease of handling, provide better solubility (Padua & Wang, 2009; Raharjo, Purwandari, Hastuti, & Olsen, 2019) and enhance the stability of active chemicals for drug formulation (Huang et al., 2020; Thao & Niwat, 2018). The nanosized delivery systems significantly improve the absorption profiles of the loaded molecules because the nanoparticle size, shape and surface properties affect intestine uptake. The nanoparticle size of 50–300 nm was found to have preferential uptake from the gastrointestinal tract (Roger, Lagarce, Garcion, & Benoit, 2010). Various techniques have been used for puerarin micro/nanoencapsulation, such as self-micro-emulsifying drug delivery systems (Zhang, Wang, Wu, & Shen, 2012) and solid lipid nanoparticles, by which the relative bioavailability of loaded puerarin was improved (Luo et al., 2011a).

Zein is one of the few hydrophobic, water-insoluble biomaterials that are generally recognised as safe by the FDA (Patel & Velikov, 2014, Tapia-Hernández et al., 2019). Zein presents several properties that allow it to self-assemble into various structures such as film, bioplastics and spheres (Wang & Padua, 2010). Zein has slow digestibility and a mucoadhesive nature. It can be developed into various nano/microstructures by many methods to encapsulate and deliver bioactive compounds, such as polyphenols, vitamins and omega-3 fatty acids (Penalva, González-Navarro, Gamazo, Esparza, & Irache, 2017; Donsì, Voudouris, Veen, & Velikov, 2017; Tapia-Hernández et al., 2019) and gamma oryzanol (Rodsuwan et al., 2020). Nanoencapsulation was able to improve the stability and processing ease of the water-insoluble gamma oryzanol (GO). An increase of zein concentration and % GO loading exhibited an increase in yield and encapsulation efficiency. Consequently, zein was used for the nanoencapsulation of puerarin by liquid-liquid extraction in this research.

2. Objectives

This research aimed to 1) prepare the nanoparticle for the encapsulation of puerarin from the *Pueraria* extract using zein protein, 2) study the structure and determine the properties of the puerarin-loaded zein nanoparticles (PuZNs), and 3) measure the stability of puerarin in the nanoparticles during long-term storage.

3. Materials and methods

3.1 Materials and chemicals

Pueraria extract (PE), which contained 65% (by weight) puerarin was provided from Thai-China Flavours and Fragrances Industry Co., Ltd. Zein protein was a product from Sigma Aldrich Co., Ltd. (USA). Tween 80 and lecithin were food-grade, brought from Union Chemical 1986 Co., Ltd. (Thailand). All other chemicals and solvents were analytical grade.

3.2 Effect of the ratio between zein and surfactant on zein nanoparticle (ZN) properties

The ZNs were prepared using a liquid-liquid dispersion method modified from Rodsuwan et al. (2020). Tween 80 was mixed with lecithin at 1:2 (w/w) and dissolved at 0.1% (w/v) in 0.1 M citrate solution adjusted to pH 8. Twenty ml of 0.4% zein solution (w/v) in 85% ethanol was mixed with the surfactant solution (60 ml) and stirred using a homogeniser (Ultra-Turrax T25, IKA, Germany) operated at 15,000 rpm for 10 min in an ice bath. The final concentration of zein in the emulsion was

0.1% (w/v), and three ratios between zein and surfactant were studied: 1:0.2, 1:0.01, and 1:0.05 (w/w). The ethanol removal was done using a vacuum rotary evaporator by centrifuging at 5,000 rpm for 10 min. At lower temperatures, degradation of the bioactive compound could be prolonged, and consequently, the stability study was carried out in the storage at 4 ± 1ºC. Freshly prepared ZNs in the dispersion and those of 30 days storage were measured for their particle size and zeta potential using the dynamic light scattering (DLS) technique and the laser Doppler velocimetry (LDV) technique with a Zetasizer Nano (Malvern Instrument, UK), respectively.

3.3 Effect of % PE loading on PuZN properties, yield and encapsulation efficiency (EE)

Puerarin-loaded zein nanoparticles (PuZNs) were fabricated following Rodsuwan et al. (2020) with slight modification. The PE was added dropwise into 20 ml of zein solution in ethanol using a magnetic stirrer at 450 rpm for 10 min. The sample was then added to the citrate solution containing the surfactant with the ratio of zein to surfactant at 1:0.05 (w/w), and the final concentration of zein was 0.1% (w/v). Three levels of PE loading at 10, 20 and 30% (w/w) of zein were performed, and the properties of PuZNs were investigated. The mixture was prepared using a homogeniser operated at 15,000 rpm for 10 min in an ice bath. After evaporation, the suspension of PuZNs obtained was centrifuged at 9,000 × *g* for 50 min to remove the free puerarin, washed with 0.1 M citrate solution and dried using a freeze drier (Alpha 1-4 LSCplus, Christ, Germany) at −50ºC under a pressure of 0.01 bar. The particle size and zeta potential of freshly prepared PuZNs were measured. Encapsulation efficiency and yield were also determined. The encapsulation efficiency of puerarin in PuZNs was defined as the puerarin content encapsulated in the ZNs following Maji et al. (2012). Zein in PuZNs was dissolved in ethanol 85% (w/w), and puerarin encapsulated in the ZNs was separated by centrifugation and filtered through a nylon syringe filter (pore diameter 0.45 µm) before injection. The puerarin content in the filtrate was measured by HPLC as described above. The percentage of yield and EE were calculated as follows:

$$\%Yield = (A/B) \times 100 \quad (1)$$

where *A* is the dry weight of nanoparticles and **B** is the total initial weight of the zein and PE used for nanoparticle preparation and

$$\% EE = (C/D) \times 100 \quad (2)$$

where *C* is the total puerarin amount in nanoparticles and *D* is the total PE amount added to the nanoparticle preparation.

The puerarin content in the particles was measured by the high- performance liquid chromatography, HPLC (Agilent Technology 1200 series, Germany) equipped with an Eclipse Zorbax XDB-C 18 (Agilent, 250 mm × 4.6 mm, 5µm) analytical column and a Zorbax XDB-C18 (Agilent, 12.5 mm × 4.6 mm, 5µm) guard column. A mixture of 0.1 % acetic acid in acetonitrile mixed with 0.1 % acetic acid at 90:10 (v/v) was used as a mobile phase with 1 ml/min for 15 min in each injection. The column temperature was controlled at 25ºC with a UV detector at 254 nm. The retention time of puerarin was 2.211 min, as shown in Figure 1.

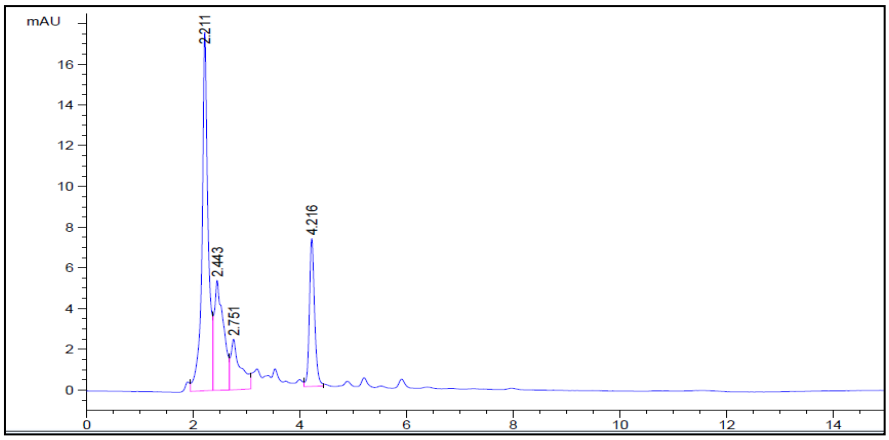


Figure 1 HPLC chromatogram of puerarin extract.

3.4 Properties of ZNs and PuZNs

3.4.1 Particle size

Particle size, polydispersity index (PDI), distribution of the ZNs and PuZNs were measured using a dynamic light scattering (DLS) with a DelsaTM nanoparticle analyser (Beckman Coulter, Fullerton, CA). The laser light from particle size was operated at 658 nm, and the scattered angles emitted were 15, 30 and 160 θ.

3.4.2 Zeta potential

The Laser Doppler Velocimeter (Zetasizer Nano ZS90, Malvern, UK) was used for determining the surface charges of different samples with a folding capillary cuvette. The surface charge was presented by zeta potential, which was converted from each sample's electrophoretic mobility. All measurements of DLS were performed at 25ºC.

3.5 Scanning electron microscopy (SEM) and Transmission electron microscope (TEM)

Image study on morphological structures of the zein protein, ZNs and freeze-dried PuZNs prepared from PE loading at 10, 20, and 30% were performed using a scanning electron microscope, SEM (JSM-6610LV, J L, EOJapan), with a voltage of 5 kV. Samples were attached to conductive carbon tape before they were mounted on specimen stubs and coated with a thin conductive platinum layer (<20 nm) using a sputter coater.

The morphology of PuZNs was observed using TEM (Transmission Electron Microscope, JEM-1400 Electron Microscope, JEOL, Japan). The suspension of PUZNs from PE loading at 30% was prepared, and one drop of the sample was placed on a coated carbon copper grid (400-mesh) for 2–3 min. The specimen was stained with 1% (v/v) uranyl acetate (Sigma-Aldrich, St. Louis, Mo, USA) for 30 s and dried. Photographs were taken under TEM and observed at 10 kV and 50 kV.

3.6 Retention of puerarin during storage

The freeze-dried PuZNs prepared from the PE loading at 10, 20 and 30% were sealed in a glass vial (20 ml) and stored at 4 ± 1°C for four months. Samples were taken every month to determine the puerarin retention by HPLC following the methods described above.

3.7 Statistical Analysis

The results are shown as the mean ± standard deviation (SD). Analysis of variance (ANOVA) was used to analyse the statistical significance using SPSS 22.0 for Windows (SPSS Inc., Chicago, III, USA). The data were subjected to ANOVA, and a comparison of means was carried out by Duncan's multiple range test (DMRT).

4. Results and discussion

4.1 Effect of the ratio between zein and surfactant on ZNs properties

Table 1 shows the size and zeta potential of ZNs prepared by liquid-liquid extraction using different zein: surfactant. In the absence of surfactant, the smallest zein nanoparticles at 129.5 ± 5.0 nm were obtained. However, when the surfactant ratio increased from zero to 0.05, 0.1 and 0.2, the particle size of the ZNs began to rise from 129.5 ± 5.0 nm to 157.2-± 22.6 nm, 180.8 ± 18.5 nm and 238.1 ± 5.8 nm, respectively. The particle sizes of ZNs were increased with an increase in the

surfactant ratio. Phospholipids have been extensively used to disperse oil in water as emulsions for food and pharmaceutical applications. Lecithin-Tween 80 self-assemblies can emulsify the PE and disperse PE under a high-speed homogeniser (Rocchio et al., 2017). A higher ratio of the surfactant resulted in a more complete coverage of the PE droplet interface and larger particle size. The particles' surface charge was enhanced for all preparations with the surfactants, -34.6 to (-35.80) mV, and did not change after 30 days of storage. Zeta potential is an essential parameter for understanding the nanoparticle surface and predicting particle stability in the dispersion. A large positive or negative zeta potential value of the nanoparticle presents good physical stability of the nanoemulsion due to the electrostatic repulsion of individual particles (Jiang, Oberdörster, & Biswas, 2009). Generally, a value of zeta potential other than −30 to +30 mV is considered to have sufficient repulsive force to maintain better physical colloidal stability. ZNs prepared with the surfactants exhibited a high degree of colloidal stability, and the surface charge did not change even after the storage at 4 ±1ºC for 30 days. The surface charge of the ZNs in control (without surfactant) indicated lower stability since its surface charge was reduced from -31.97 mV to -27.5 mV after 30 days at 4 ± 1ºC. The ratio of zein to surfactant of 1:0.05 was selected for further study since the condition yielded the particle size not significantly different from the control ($p < 0.05$) and more stable dispersion than the control.

Zein, divided into four classes: α, β, γ and δ zein, has a molecular weight of 22 to 27 kDa with isoelectric pH of 6.8 (Elzoghby, Elgohary, & Kamel, 2015). Zein contains roughly hydrophobic (two-thirds) and hydrophilic (one-thirds) amino acid residues. The amino acid composition of zein is rich in glutamine (21.4%), leucine (19.3%), proline (8.3%) and alanine (6.8%), but low in the essential amino acids lysine and tryptophan. Zein presents a biopolymeric structure with water-insoluble groups; therefore, it is freely soluble in many solvents such as ethanol, methanol, acetone and isopropanol (Paliwal & Palakurthi, 2014; Patel & Velikov, 2014). The use of the non-ionic surfactant, Tween 80 and anionic surfactant lecithin (1:2 ratios by weight) could stabilise the zein nanoparticles. Lecithin increased the electrostatic interactions between zein particles and enhanced the surface charge of the dispersion.

4.2 Effect of % PE loading on PuZNs property, % yield and % EE

The particle size of PuZNs tended to increase with an increase of % PE loading, as shown in Table 2. The particle sizes increased from 165.4 ± 2.4 nm to 185.5 ± 4.8 and 232.9 ± 1.9 nm with the PE loading at 10, 20 and 30%, respectively. The particle sizes corresponded with the increase of the percentage of encapsulation efficiency of puerarin in the zein nanoparticles. The surface charges of the smaller PuZNs (165.4 ± 2.4 and 185.5 ± 4.8 nm) at -31.4±1.3 and -30.1±0.7 mV indicated higher stability of the nanoparticle dispersion than larger PuZNs from 30% PE loading (-26.7 ± 0.8 mV). Colloidal dispersion is an unstable system since there is a natural tendency for a solid-liquid system to separate and reduce its interfacial energy. Determining the micro/nanodispersion colloidal stability is required since the food processing or formulating process usually comprises a complicated procedure.

Table 1 Size and zeta potential of zein nanoparticles using different ratio of zein: surfactant

Zein: surfactant*	Particle size (nm)-Day 0	Zeta potential (mV)-Day 0	Zeta potential (mV)-Day 30
1:0 (control)	129.5 ± 5.0^{a}	-31.97 ± 1.2^{b}	-27.5 ± 0.2^{b}
1:0.05	157.2 ± 22.6^{a}	-35.8 ± 0.7^{a}	-34.3 ± 0.2^{a}
1:0.1	180.8 ± 18.5^{b}	-34.6 ± 0.6^{a}	-32.3 ± 0.3^{a}
1:0.2	238.1 ± 5.8^{c}	-35.3 ± 0.2^{a}	-33.3 ± 0.8^{a}

Different superscript letters in the same column mean significantly different ($p < 0.05$).*Final concentration of zein in the emulsion was 0.1% (w/v).

Zein has an inter-particle hydrophobic attraction with a formation of a thin layer of particles; consequently, zein can be aggregated easily during extended storage (Donsì et al., 2017). Generally, attractive inter-particulate forces predominated over repulsive inter-particulate forces, causing system instability and resulting in increased particle size and particle aggregation, respectively (Guo, Heinämäki, & Yliruusi, 2008). However, a very slight change in the particle size was observed for PE loading at 10 and 20%, while the surface charge of PuZNs from 10, and 20% loading slightly decreased during the first two months of storage (Table 3). A combination of both surfactants and the nanoparticle size could help stabilise the PuZNs colloid system during extended storage. Comparatively, all PuZNs suspensions indicated a stable colloidal system during four months of storage. Tween 80 and lecithin were used as surfactants to stabilise the colloid obtained from the encapsulation. This agreed with gamma oryzanol (GO) encapsulation in the zein nanoparticles in our previous study (Rodsuwan et al., 2020). The experiments presented a stable dispersion with no precipitation observed with the nanoparticles of GO loaded zein nanoparticles as small as 127.8, 145.0 and 144.1 nm, while precipitation after 24 h appeared with the zein particles larger than 350 nm.

In other studies on puerarin nanoencapsulation, Zhang et al. (2012) presented the fabrication of self-microemulsifying drug delivery systems (SMEDDS) in sustained-release pellets to improve oral bioavailability. Castor oil was used as the oil phase with Cremophor® EL and 1,2-propanediol as the emulsifier and co-emulsifier. The mean particle size of the encapsulated products was 50 ± 8 nm and 2.6-fold enhanced the bioavailability of the puerarin-SMEDDS compared to that of the puerarin tablet. Besides, Luo et al. (2011b) reported the solid lipid nanoparticles (SLN) of puerarin. SLNs with an average particle size of 160 nm and zeta potential of −35.4 mV were formulated using monostearin, soya lecithin and poloxamer 188. The relative bioavailability of puerarin loaded in SLN improved more than three-fold.

An increase of the % PE loading to 20 and 30% led to a higher yield of PuZNs at 68.8–70.6% compared to 10% PE loading (63.8% yield). A similar trend for the % EE was obtained. The highest % EE at 65.4% was obtained with the % PE loading at 30%. Similar results were detected with an increase of % GO loading in the nanoencapsulation of GO by zein (Rodsuwan et al., 2020). The highest yield of nanoparticles, at 75%, was obtained with 0.5% zein concentrations at 50% GO loading. An optimisation for maximum per cent loading of %PE in the future would be interesting since higher % EE might be obtained.

Table 2 Some properties and encapsulation efficiency of puerarin in zein nanoparticles using different percentages of *Pueraria* extract loading

Pueraria extract loading (%)	Particle size (nm)	Zeta potential (mV)	Yield (%)	Encapsulation efficiency (%)
10	165.4 ± 2.4^{a}	-31.4 ± 1.3^{a}	63.8 ± 1.0^{a}	55.5 ± 1.8^{a}
20	185.5 ± 4.8^{b}	-30.1 ± 0.7^{a}	70.6 ± 0.4^{b}	57.3 ± 1.9^{b}
30	232.9 ± 1.9^{c}	-26.7 ± 0.8^{b}	68.8 ± 1.5^{b}	65.4 ± 0.9^{c}

Different superscript letters in the same column mean significantly different ($p<0.05$).
*The ratio of zein: surfactant was 1: 0.05 and the final concentration of zein in the emulsion was 0.1% (w/v).

Table 3 Particle size and zeta potential of puerarin loaded zein nanoparticles in the suspension during three months of storage at 4 ± 1°C

Puerarin extract loading (%)	1 month		2 month		3 month	
	particle size (nm)	zeta potential (mV)	particle size (nm)	zeta potential (mV)	particle size (nm)	zeta potential (mV)
10	168.3 ± 2.4^{aA}	-31.3 ± 1.3^{aA}	172.3 ± 1.7^{aB}	-26.6 ± 0.4^{bB}	174.1 ± 1.4^{aB}	-26.0 ± 0.6^{aB}
20	185.7 ± 4.2^{bA}	-30.0 ± 0.7^{aA}	186.2 ± 0.6^{bA}	-26.4 ± 0.5^{cB}	189.2 ± 3.9^{bA}	-26.8 ± 0.3^{bB}
30	234.8 ± 1.6^{cA}	-26.7 ± 0.8^{bA}	240.5 ± 3.7^{cB}	-27.9 ± 0.6^{aA}	242.0 ± 2.5^{cB}	-26.7 ± 0.8^{aA}

Different superscript letters (a–c) in the same column mean significantly different ($p <0.05$) for particle size and zeta potential at each loading, and letters (A, B) in the same row mean significantly different ($p <0.05$) for particle size and zeta potential at each month.

4.3 SEM and TEM images

SEM images of pure zein and ZNs are presented in Figure 2, while Figure 3 shows the structure of PuZNs from three PE loading percentages. Most particles of PuZNs present spherical shapes upon the evaporation of the ethanol and citrate solution. Increasing the % PE loading tended to expose hydrophobic residues with higher encapsulation efficiency, resulting in larger particles in the dispersion, as detailed in Table 2. However, SEM pictures of the freeze-dried PuZNs from 30% PE loading seemed similar to the other two loadings. This result might be due to shrinkage of the nanoparticles during the freeze-drying process.

TEM is a powerful tool to characterise the morphology of encapsulated nanoparticles using a focused beam of electrons transmitted through the specimen, the ZNs shell and the core of the PuZNs. The low-density material of puerarin is seen in the core section as a light shade and surrounded by the high density of ZNs in a darker shade. The TEM image indicates that ZNs encapsulated puerarin at around 200 nm in diameter (Figure 4).

4.4 Retention of puerarin during long storage

Figure 5 presents the per cent retention or remaining puerarin in the PuZNs over four months of storage at 4 ± 1°C. The puerarin encapsulated in the ZNs from 20 and 30% PE loading was very stable. It did not change during the long storage, and a very slight decrease of puerarin from 10% PE loading was presented after four months of storage. The data obtained might due to the low content of the encapsulated puerarin inside the nanoparticles. The results showed that encapsulation in zein nanoparticles could maintain puerarin stability for four months storage. However, a study on the stability for a longer time, both at 4ºC and room temperature, would give more results beneficial for the food and pharmaceutical applications.

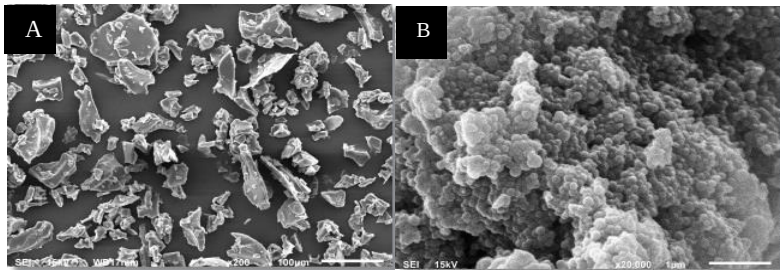
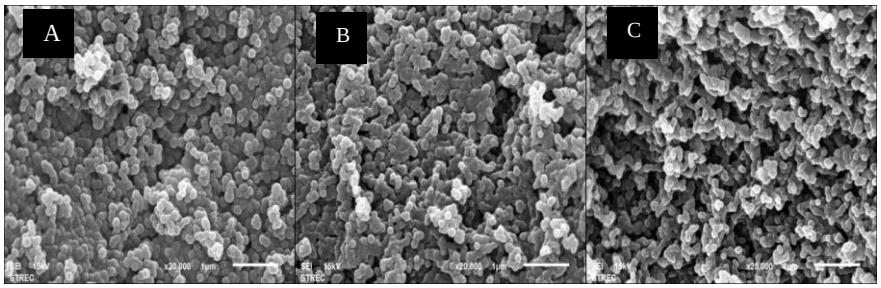


Figure 2 SEM images of zein protein, (200X; A) and zein nanoparticles prepared from zein: surfactant at 1:0.05 (20,000X; B).



10% PE loading 20% PE loading 30% PE loading

Figure 3 SEM images of PuZNs prepared using different % PE loading (x 20,000X).

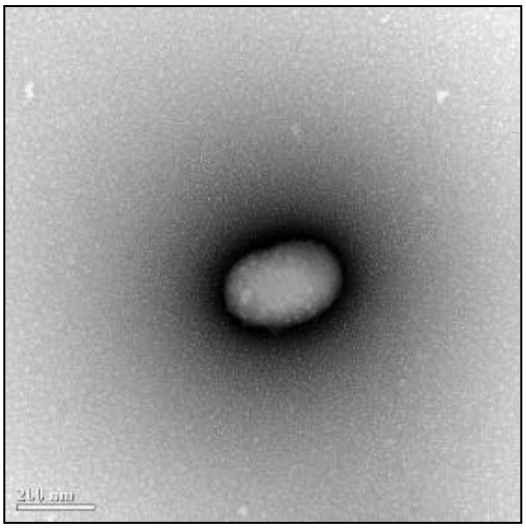


Figure 4 TEM image of puerarin loaded zein nanoparticles (120,000X).

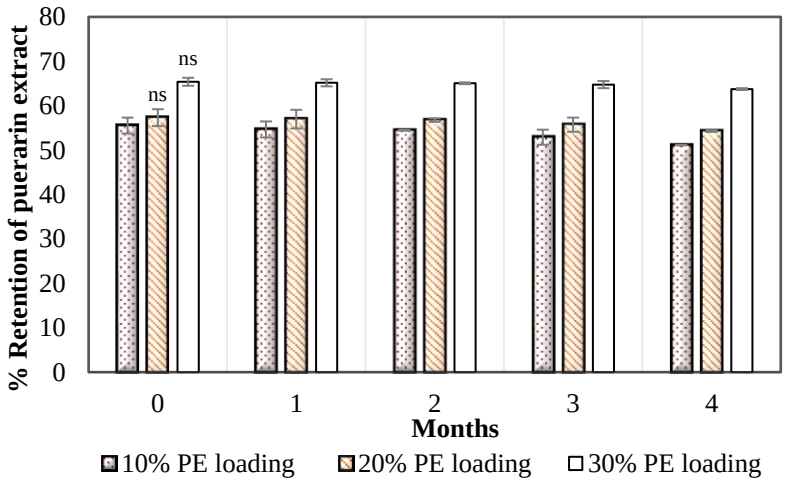


Figure 5 % Retention of puerarin in the PuZNs from different per cent PE loadings during the storage at 4 ± 1°C for four months.

5. Conclusion

Puerarin was successfully encapsulated in the zein nanoparticles using liquid-liquid extraction. The results demonstrated that the ratio of zein to the surfactant, lecithin and tween 80, significantly affected the size of ZNs. The increase of per cent PE loading (10 to 30%) led to larger PuZN sizes (165.4 ± 2.4–232.9 ± 1.9 nm) with comparatively stable colloidal systems and higher percentages of encapsulation efficiency and yield. The per cent yield and per cent EE for 30% PE loading were 68.8 ± 1.5 and 65.4 ± 0.9%, respectively. The SEM and TEM images exhibited a spherical shape for PuZNs with an average size of less than 200 nm. The puerarin in the ZNs from 20 and 30% PE loading was stable during four months of storage at 4 ± 1°C. Our work shows that ZNs have the potential for use as a wall material for encapsulation to preserve puerarin stability from oxidation during storage. This result could widen the potential applications of puerarin in functional and food industries.

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