RESEARCH ON THE FORMULATION OF NANOPARTICLES CONTAINING MANGIFERIN USING SELF-ASSEMBLY METHOD

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ABSTRACT

Background: *The use of natural-origin active compounds in disease treatment is currently a prevailing trend. Mangiferin, a major component found in mangoes, is present in relatively high proportions. However, mangiferin's disadvantages include low solubility and poor permeability. There are numerous methods available to improve solubility and permeability, among which self-assembly is noteworthy. Self-assembly involves the combination of certain molecules or macromolecules to form three-dimensional networks or other structures with new characteristics, phospholipids and chitosan are often used as raw materials for the self-assembly process to create nanoparticles. This method offers advantages such as high efficiency, simple implementation, short execution time, high retention efficiency, and increased permeability through biological membranes.* ***Objectives:*** *To develop a formulation and process for producing nano mangiferin using the self-assembly method with phosphatidylcholine and chitosan.* ***Materials and methods:*** *The solubility of mangiferin in high-proof ethanol was investigated, followed by an assessment of the influence of formulation factors and processes on the characteristics of nano-sized mangiferin particles.* ***Results:*** *The average solubility of mangiferin in 96% ethanol was approximately 0.5529±0.0003 mg/mL. The mole ratio of mangiferin to Lipoid S100 was 1:1, and the mass ratio of Lipoid S100 to chitosan was 20:1. A stirring speed of 1.000 revolutions per minute, reflux temperature of 70°C, reflux time of 2 hours. homogenization speed of 1,000 revolutions per minute, and homogenization time of 15 minutes produced the smallest nanoparticles (103.44±0.46 nm), with a low polydispersity index (0.281±0.009≤ 0.3) and zeta potential (31.95±0.08 mV ≥ +30 mV), encapsulation efficiency of 82.42±0.53%; loading capacity of 32.97±0.95%.* ***Conclusions:*** *A successful formulation of nano-sized mangiferin particles was achieved using the self-assembly method, resulting in particles that meet the criteria of small size, uniformity, and durability. Moreover, nearly 100% mangiferin*

release was achieved after 60 minutes, indicating the promising potential for developing highly bioavailable oral formulations.
Keywords: *self-assembly, mangiferin, Lipoid S100, chitosan*

I. INTRODUCTION

Mangiferin is a potential compound for development in the pharmaceutical industry, particularly due to its hypoglycemic and lipid-lowering effects. However, mangiferin belongs to group IV compounds (classified according to the biopharmaceutics classification system) with poor water solubility and low permeability, leading to low oral bioavailability [1].

Several methods have been explored to improve the solubility and permeability of mangiferin, such as the study by Alkholifi FK and colleagues (2023) "Study on the formulation of nano-hydrogel containing mangiferin for transdermal use" [2]. Ma H. et al. (2014) "Improving the permeability and absorption of mangiferin orally by forming phospholipid complexes", or the research by Khurana RK et al. (2018) "Enhancing the bioavailability of mangiferin by formulating nano-sized particles using the self-assembly method" [3], [4]. In Vietnam, research on improving the solubility of mangiferin is still limited, such as the study by Le Dinh Nguyen, Nguyen Duc Hanh, and Do Quang Duong (2018) "Research on causal relationship and formula optimization of lipid nanocarriers containing mangiferin". Le Dinh Nguyen and Nguyen Duc Hanh (2019) "Research on the formulation of In Situ nano lipid gel containing mangiferin", or the study by Nguyen Truong Giang (2018) "Research on the transformation of mangiferin into calcium mangiferin with high water solubility" [5], [6], [7]. However, there is currently no research published on the formulation of nano-sized particles using the self-assembly method. Therefore, this study aims to develop nano-sized particle systems containing mangiferin using the self-assembly method, to improve its solubility and thereby increase mangiferin's bioavailability.

II. MATERIALS AND METHODS

2.1. Materials

Materials: Mangiferin (95.16%) was bought from China. Lipoid S100. Chitosan. and Poloxamer 407 were made in Germany. Acetic acid was purchased from China. Double distilled water and 96% ethanol were made in Vietnam. Mangiferin standard substance with a purity of 97% $C_{19}H_{18}O_{11}$. based on the dried substance. provided by the Institute for Drug Quality Control Ho Chi Minh City. batch number QT339 011020.

Equipment: The main equipment includes UV-Vis spectrophotometer (Japan). magnetic stirrer (USA). particle size and zeta potential analyzer (UK). centrifuge (China). Kern electronic balance (Germany) and scanning electron microscope SEM (Japan).

2.2. Methods

2.2.1. Determination of mangiferin solubility in ethanol

An excess amount of mangiferin was accurately weighed into a 50 mL volumetric flask, and then 96% ethanol was added to the mark. The mixture was stirred continuously on a magnetic stirrer for 24 hours, and then the solution was stabilized for 8 hours. The solution was centrifuged at 2.000 rpm for 20 minutes to remove insoluble mangiferin. The solution was quantified after centrifugation using UV-Vis spectrophotometry at a wavelength of 258 nm [8], n = 3. Requirement: RSD (%) value of solubility ≤ 2.0%.

2.2.2. Investigation of the effects of formulation and process factors on the characteristics of nano-sized particles containing mangiferin

The components in the formulation are shown in Table 1.

Table 1. Proposed formulation for the preparation of 100 mL nano-sized particle system

Phase	Components	Role in the formula	Content
Alcohol phase	Mangiferin	Active ingredient	A
	Lipoid S100	Nano-sized particle former	X
	Ethanol 96%	Solvent	Sufficient to dissolve A
Aqueous phase	Chitosan	Nano-sized particle former	Y
	Poloxame 407	Suspending agent. system stabilizer	0.3%
	Glacial acetic acid	pH adjustment	Sufficient to adjust pH
	Double distilled water	Solvent	Sufficient to make up 100 mL

Alcohol phase: Mangiferin and Lipoid S100 were weighed and placed into a round-bottom flask. and then absolute ethanol was added. The mixture was refluxed for 2-4 hours at 60-70°C until the solution became clear (1).

Aqueous phase: Chitosan was weighed into a 50 mL beaker. Double distilled water was added, and then glacial acetic acid was added while stirring with a glass rod. The mixture was transferred to a volumetric flask. Double distilled water was added twice. When Chitosan was completely dissolved, double distilled water was added to the mark. The solution was shaken well to obtain a Chitosan solution with a base concentration (2). An appropriate amount of solution (2) was taken according to the ratio of Lipoid S100 and Chitosan into a beaker. Poloxamer 407 and double distilled water were added twice. The beaker was cooled, and then it was stirred with a magnetic stirrer until Poloxamer 407 was completely dissolved (3).

Nano formation: the alcohol phase (1) was slowly pumped into the aqueous phase (3), while stirring continuously until all of the alcohol phase (1) was pumped in. at a pumping rate of 1 mL/minute, and the stirring speed of the magnetic stirrer was 400-1000 rpm. Then, it was continued stirring for 15 minutes, and then double distilled water was added twice to make up to 100 mL.

Spray drying for nano powder formation: 0.9 g of maltodextrin and 0.1 g of Aerosil were added into 100 mL of nano-sized particle system solution, and they were stirred well. It proceeded with spray drying with an inlet temperature of 140°C, outlet temperature of 57.5°C, and a feeding rate of 10 mL/minute. The powder formed was then analyzed for particle morphology under a scanning electron microscope (SEM).

Based on the results of the mangiferin solubility study in 96% ethanol, further investigation was conducted on the ratio of Mangiferin to Lipoid S100, and the ratio of chitosan to Lipoid S100 as shown in Table 2. The parameters such as reflux temperature, reflux time, homogenization speed, and homogenization time were kept constant.

Table 2. Investigation of components in the formula (n=3)

Form-ula	The molar ratio of Mgf: Lipoid S100	Ratio of Lipoid S100: chitosan (w:w)	Poloxamer 407 concentration (%)	Stirring speed (rpm)	Reflux boiling temperat-ure (°C)	Reflux boiling time (hours)	Homog-enizati-on speed	Hom-ogeni-zation time
F 1	1:3	20:1	0.3	1000	60	2	1000	15
F 2	1:5	20:1	0.3	1000	60	2	1000	15

Form-ula	The molar ratio of Mgf: Lipoid S100	Ratio of Lipoid S100: chitosan (w:w)	Poloxamer 407 concentration (%)	Stirring speed (rpm)	Reflux boiling temperat-ure (°C)	Reflux boiling time (hours)	Homog-enizati-on speed	Hom-ogeni-zation time
F 3	1:10	20:1	0.3	1000	60	2	1000	15
F 4	1:3	10:1	0.3	1000	60	2	1000	15
F 5	1:5	10:1	0.3	1000	60	2	1000	15
F 6	1:10	10:1	0.3	1000	60	2	1000	15
F 7	1:3	5: 1	0.3	1000	60	2	1000	15
F 8	1:5	5: 1	0.3	1000	60	2	1000	15
F 9	1:10	5: 1	0.3	1000	60	2	1000	15

Evaluation of nanoemulsion includes the average particle size, polydispersity index (PDI), and zeta potential.

Particle size and polydispersity index (PDI): were determined based on the dynamic,light scattering mechanism on the Zetasizer instrument, at 25°C with a backscattering angle of 173°. Results were reported as the average particle size ± standard deviation and the average PDI ± standard deviation. Requirement: particle size < 200 nm, PDI ≤ 0.3.

Zeta potential: was determined on the Zetasizer instrument with five cycles and ten measurements per sample. The rest time between cycles was 5 seconds. Samples were appropriately diluted and measured under the same conditions at 25°C. The measured zeta potential was reported as the average zeta potential ± standard deviation. Requirement: |zeta potential| ≥ +30 mV.

After determining the ratios of components in the formula, the technical parameters in the process of formulating a nanoemulsion system were investigated in Table 3.

Table 3. Investigation of technical parameters in the formula (n=3)

Formula	The molar ratio of Mgf: Lipoid S100	Ratio of Lipoid S100: chitosan (w:w)	Poloxa-mer 407 concentr-ation (%)	Stirring speed (rpm)	Reflux boiling temper-ature (°C)	Reflux boiling time (hours)	Hom-ogeni-zation speed	Hom-ogeni-zation time
F 10	X	Y	0.3	800	60	2	1000	15
F 11	X	Y	0.3	400	60	2	1000	15
F 12	X	Y	0.3	Z	70	2	1000	15
F 13	X	Y	0.3	Z	P	4	1000	15
F 14	X	Y	0.3	Z	P	Q	400	15
F 15	X	Y	0.3	Z	P	Q	800	15
F 16	X	Y	0.3	Z	P	Q	R	10
F 17	X	Y	0.3	Z	P	Q	R	20
F 18	X	Y	0.3	Z	P	Q	R	25
F 19	X	Y	0.3	Z	P	Q	R	30

Evaluation of nanoemulsion includes the average particle size, polydispersity index, and zeta potential.

2.2.3. Examination of the physicochemical parameters of mangiferin nanoemulsion

The experiment was repeated 3 times with the best formula selected from Table 3. The particle size, polydispersity index, zeta potential, encapsulation efficiency, loading capacity, particle morphology, and dissolution ability were investigated.

Encapsulation efficiency (EE): was determined by an indirect method. The procedure was as follows: (1) The total mangiferin content in the nanoemulsion system was quantified (w). (2) To separate the crystalline mangiferin, the mixture was centrifuged at 4000 rpm for 15 minutes. The supernatant was quantified to determine the total concentration of free and encapsulated mangiferin (w_1). (3) The supernatant was filtered through a 10 kDa centrifugal filter tube, and then it was centrifuged at 4000 rpm for 30 minutes. The concentration of free mangiferin in the filtered supernatant was quantified (w_2). The encapsulation efficiency is calculated by the formula:

$$EE(\%) = \frac{w1-w2}{w} \times 100\% \qquad (2.1)$$

Drug loading (DL): was calculated from the concentration of mangiferin in the nanoemulsion obtained from the encapsulation efficiency. The drug loading of the nanosystem was calculated by the formula:

$$DL(\%) = \frac{m1}{m2} \times 100\% \qquad (2.2)$$

Where: m_1 was the mass of mangiferin in the nanoparticle (mg), m_2 was the mass of nanoparticle obtained (mg)

Where the mass of nanoparticle obtained was indirectly determined through the total mass of mangiferin in the nanoparticle and carrier components (Lipoid S100, chitosan).

Particle morphology (SEM): The sample was placed in the vacuum chamber and surface images of the particles were captured using a scanning electron microscope.

Dissolution test: the dissolution test was performed using a Pharmatest dissolution tester, paddle type. An amount equivalent to 80 mg of mangiferin was weighed into 500 mL of pH 1.2 dissolution medium at a stirring speed of 100 rpm at 37±0.5°C. After intervals of 5, 15, 30, 45, and 60 minutes. 10 mL of the dissolution solution was withdrawn and filtered. 1 mL of the filtered solution was taken into a 10 mL volumetric flask and pH 1.2 buffer solution was added to reach the volume. The absorbance was measured at 258 nm. The blank sample was a pH 1.2 buffer.

The unadjusted mangiferin concentration at the nth time was calculated as follows:

$$C_n = C_{no} + \frac{V_o}{V} \times C_{n-1} \qquad (2.3)$$

C_n and C_{no} were adjusted concentration and unadjusted concentration at the n^{th} time (μg/mL). respectively. C_{n-1} was adjusted concentration at the $(n-1)^{th}$ time (μg/mL). V_o was the volume of the withdrawn dissolution solution (V_o = 10 mL). V was the volume of the dissolution medium (V = 500 mL).

The percentage of dissolved mangiferin at time t was calculated by the formula:

$$\%\text{mangiferin} = \frac{C_n \times 500}{m \times 1000} \times 100 \qquad (2.4)$$

m was the amount of mangiferin in the sample (mg).

III. RESULTS

3.1. Determination of the solubility of mangiferin in ethanol

The actual solubility of mangiferin in 96% ethanol is presented in Table 4.

Table 4. Solubility of mangiferin in 96% ethanol

Number	Dilution Factor	Absorbance	Mangiferin Concentration (mg/mL)
1	50	0.7430	0.5533
2	50	0.7423	0.5528
3	50	0.7421	0.5527
Average			0.5529±0.0003
RSD (%)			0.06

The average solubility of mangiferin in 96% ethanol is approximately 0.5529±0.0003 mg/mL after three repetitions with an RSD of 0.06%.

3.2. Results of investigating the effects of formulation factors and processes on the characteristics of nanoemulsions containing mangiferin and selecting parameters.

The results of investigating the components in the nanoemulsion formula and the formulation parameters are presented in Table 5 and Table 6.

Table 5. Results of investigating the components of the formula (n=3)

Formula	Average particle size (nm)	Polydispersity index	Zeta potential (mV)
F 1	140.53±3.85	0.353±0.015	26.74±0.77
F 2	151.86±3.25 ^{c}	0.317±0.018 ^{c}	29.76±1.00 ^{c}
F 3	*116.53±4.90*	*0.290±0.016*	*31.30±2.01*
F 4	126.41±2.50	0.327±0.012	28.10±1.16
F 5	123.87±2.04 ^{d}	0.366±0.006 ^{d}	25.57±1.08 ^{d}
F 6	122.72±1.67	0.305±0.020	31.96±2.00
F 7	139.23±1.30	0.311±0.025	27.50±1.39
F 8	139.55±1.55	0.293±0.027	28.80±1.31
F 9	136.06±1.71	0.298±0.019	30.34±1.02

**Values with different letters within the same column of each formula indicate statistically significant differences with p < 0.05. while values with the same letters within the same column of each formula indicate no statistically significant difference with p > 0.05.*

Using one-way ANOVA analysis, CT3 obtained the smallest nanoparticle size, with PDI ≤ 0.3, indicating the stability of the nanoemulsion system, and the highest zeta.

Table 6. Results of investigating the influence of technical parameters (n=3)

Formula	Average particle size (nm)	Polydispersity index	Zeta potential (mV)
F 10	143.13±2.56	0.318±0.013	29.79±0.74
F 11	154.18±4.00 ^{e}	0.358±0.016 ^{e}	27.46±1.29 ^{e}
F 12	*103.81±5.19* ^{f}	*0.288±0.007* ^{a}	*31.77±1.01* ^{a}
F 13	108.14±2.39	0.319±0.013 ^{a}	29.37±0.62 ^{a}
F 14	123.37±2.47 ^{a}	0.282±0.004 ^{a}	30.28±0.70 ^{a}
F 15	107.46±6.46	0.268±0.015	32.69±2.13
F 16	113.14±3.91	0.311±0.009	30.62±0.66
F 17	118.51±2.47	0.318±0.006	30.12±1.23
F 18	123.61±1.97	0.305±0.010	31.12±0.92
F 19	126.73±2.10 ^{a}	0.312±0.003 ^{a}	30.76±0.73 ^{a}

**Values with different letters in the same column of each formula represent statistically significant differences with p < 0.05. while values with the same letters in the same column of each formula represent no statistically significant difference with p > 0.05.*

Using one-way ANOVA analysis. CT12 yielded the smallest particle size, PDI of ≤ 0.3, indicating stable nanoparticle dispersion, and the highest zeta potential.

3.3. Results of physicochemical characterization of nano mangiferin

Three batches of CT12 were prepared. and the results are presented in Table 7.

Table 7. Physicochemical parameters of nano mangiferin prepared from CT12

Formula	Average particle size (nm)	Polydispersity index	Zeta potential (mV)	Encapsulation efficiency (%)	Loading capacity (%)
1	103.89	0.288	31.87	82.13	33.82
2	103.46	0.271	31.95	85.11	31.94
3	102.97	0.283	32.03	83.03	33.15
Average	**103.44±0.46**	**0.281±0.009**	**31.95±0.08**	**82.42±0.53**	**32.97±0.95**

The results show high repeatability, with all particles meeting the specified requirements.

Morphological results

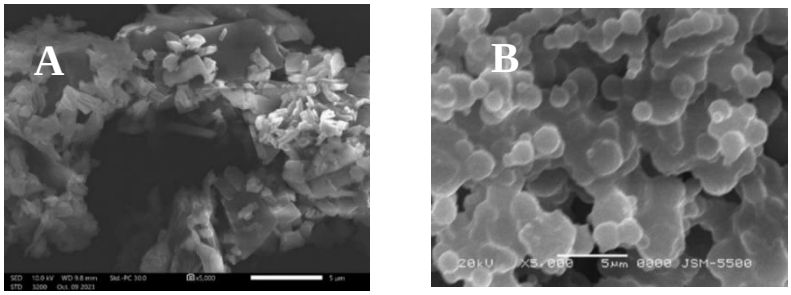

Figure 1. Morphology of mangiferin (A) and nano mangiferin (B) observed under scanning electron microscopy.

The SEM observation results of mangiferin raw material (A) reveal mangiferin existing in a crystalline rod-like form with a smooth surface. while nano mangiferin (B) appears spherical with a porous surface. This indicates an interaction between mangiferin, Lipoid S100, and Chitosan causing a change in particle shape and surface.

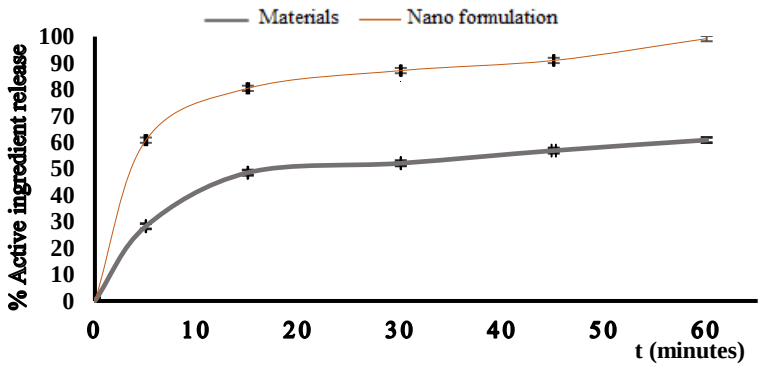

Figure 2. Graph of the percentage release of mangiferin from raw material and nanoformulation containing mangiferin.

The results indicate that the percentage release of the active ingredient mangiferin dissolved in the nanoformulation at 60 minutes is 99.23±0.03%, which is higher than that of the raw material at 60.87±0.21%.

IV. DISCUSSION

From the solubility study in 96% ethanol. the result of mangiferin solubility in

ethanol at a concentration of 0.5529±0.0003 mg/mL is consistent with the study by Jhoany A. et al. (2016) [9] investigating mangiferin solubility in commonly used solvents. It shows that mangiferin solubility decreases in the order ethanol > methanol > water > diethyl ether > acetone > n-hexane, indicating the suitability of using ethanol as the solvent for mangiferin dissolution.

The average size of the nanoformulation is significantly affected by the ratio of mangiferin to Lipoid S100. At a ratio of 1:5, the nanoformulation size decreases from 151.86±3.25 nm to 116.53±4.90 nm at a ratio of 1:10 with $p = 0.0001 < 0.05$. The reduction in particle size is attributed to direct binding between Lipoid S100 and mangiferin via electrostatic or hydrophilic interactions at the hydrophilic head of phosphatidylcholine in Lipoid S100 with mangiferin. Moreover, the particle size of the nanoformulation is significantly influenced by the ratio of chitosan to Lipoid S100. When this ratio changes with ratios of 20:1. 10:1. and 5:1, there is a significant change in particle size ($p = 0.004 < 0.05$), increasing from 116.53±4.90 nm to 131.78±2.18 nm. This result is consistent with the study by Ma Q. et al. (2020) [10], where the weight ratio of phospholipid to chitosan ranges from 5:1 to 20:1. resulting in nanoparticles with smaller sizes (below 280 nm) and narrow size distribution.

The polydispersity index (PDI) is used to assess the uniformity of particle size distribution. with a lower PDI indicating a more uniform particle size distribution. When the ratio of mangiferin to Lipoid S100 changes from 1:3 to 1:10. the PDI decreases. Moreover, the PDI is also affected by the ratio of chitosan to Lipoid S100, where an increase in the chitosan ratio leads to a decrease in PDI, though not significantly. The results of PDI are similar to the study by Ma Q. et al. (2020) [10] where the weight ratio between phosphatidylcholine and chitosan is 20:1. yielding a PDI of 0.290±0.016.

Reflux temperature significantly influences the size of the nanoformulation. with an increase in temperature from 60°C to 70°C resulting in a decrease in size from 116.53±4.90 nm to 103.81±5.19 nm ($p = 0.04 < 0.05$), a statistically significant difference. Temperature helps improve mangiferin solubility in alcohol, facilitating the bonding process between components. However, excessively high temperatures may disrupt the nanostructure, causing nanoparticles to aggregate into larger particles. This result differs from the study by Dai L. et al. (2016) [11], where the size of nano adhesive particles synthesized from zein and zein-lecithin was determined to be 312 nm after heating at 90°C, compared to 130 nm at 50°C. Conversely, the reflux time parameter does not significantly affect the size of the nanoformulation.

Stirring rate, reflux time, and homogenization time significantly affect the polydispersity index. Results show that a higher stirring rate leads to a lower polydispersity index. When alcohol encounters water, nanoparticles are formed if the stirring rate is too low, resulting in poor particle cutting during the self-assembly process, leading to uneven particle sizes. Reflux time from 2 hours to 4 hours increases the polydispersity index, indicating that longer refluxing causes some particles to break and reaggregate into larger particles, affecting the polydispersity index. A homogenization time of 15 minutes yields a lower polydispersity index compared to homogenization times of 10 minutes and 30 minutes. This can be explained by insufficient time for uniform particle formation at shorter homogenization times, but at longer times, both particle breakage and aggregation occur. Only reflux time significantly affects the zeta potential, while other technical parameters

have little influence. The reason is that longer refluxing causes the oppositely charged substances to move further apart. resulting in a decreased zeta potential.

Higher encapsulation efficiency and drug loading capacity make application in dosage forms easier. Results show an encapsulation efficiency of approximately 33% and a drug loading capacity of about 83% for the nanoformulation, which is higher than the study by Khan M.M. et al. (2019) [12] on "Lipid-chitosan hybrid nanoparticles for controlled cisplatin delivery" where the encapsulation efficiency was 89 ± 2.3% but the cisplatin loading capacity was very low at about 2%.

V. CONCLUSIONS

Successfully formulated nano mangiferin with the following formula composition: Mangiferin (4.4 mg), Lipoid S 100 (82 mg), 96% ethanol (8 mL), chitosan (4.1 mg), Poloxame 407 (300 mg), glacial acetic acid (1 mL), distilled water (100 mL), maltodextrin (900 mg), Aerosil (100 mg). Using a stirring rate of 1000 rpm, reflux temperature of 70°C, refluxing time of 2 hours, homogenization speed of 1000 rpm, and homogenization time of 15 minutes. The resulting nanoparticles have an average size of 103.44±0.46 nm; polydispersity index of 0.281±0.009; zeta potential of 31.95±0.08 mV; encapsulation efficiency of 82.42±0.53%; loading capacity of 32.97±0.95%; and 99.23±0.03% mangiferin release after 60 minutes.

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