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Optimization of Preparation Conditions for Quercetin Nanoliposomes Using Response Surface Methodology and Evaluation of Their Stability

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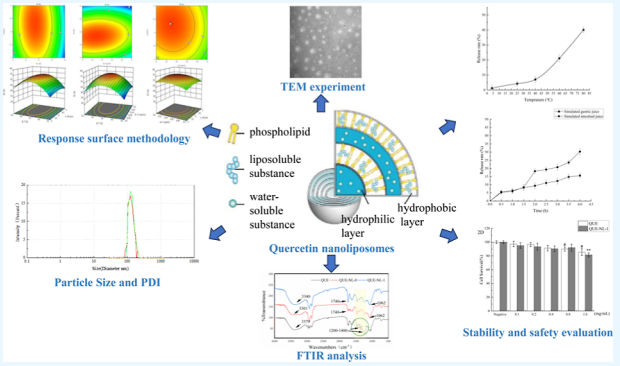
ABSTRACT: Quercetin is a flavonol compound with excellent biological activities. However, quercetin exhibits poor stability and solubility in water, which limits its application. In this study, quercetin nanoliposomes (QUE-NL-1) were prepared using an ultrasonic thin-film dispersion method, and the preparation conditions were optimized using response surface methodology. The optimal conditions for preparing QUE-NL-1 were as follows: an evaporation temperature of 35 °C, a drug concentration of 0.20 mg/mL, and a lipid bile ratio of 4:1. The encapsulation rate of QUE-NL-1 is (63.73 ± 2.09)%, the average particle size is 134.11 nm, and the average absolute value of the zeta potential is 37.50 and PDI = 0.24. By analyzing the storage temperature, storage time, and leakage rate of QUE-NL-1 in simulated gastrointestinal fluid, it was found that quercetin exhibits good stability after embedding and can achieve sustained release in intestinal juice. In addition, the cytotoxicity of QUE-NL-1 was not significant, and the survival rate of Caco-2 cells was >90% when the concentration range of QUE-NL-1 was 0.1−0.4 mg/mL. This study provides an efficient method for preparing QUE-NL-1 with small particle sizes, good stability, and high safety, which is of great significance for expanding the application range of quercetin.



1. INTRODUCTION

Liposomes are microcarriers with a structure similar to that of biofilms, in which phospholipids and cholesterol are used as membrane materials. The hydrophobic tail of phospholipids contains two fatty acid chains, and the hydrophilic head can collect water-soluble molecules.[1] Cholesterol can improve the mechanical stability of the lipid bilayer, regulate its fluidity, and reduce the permeability of water-soluble substances.[2] A liposome vesicle composed of one or more phospholipid bilayers forms under certain conditions. There are two types of liposomes, single-compartment liposomes and multi-compartment liposomes, both of which can be dispersed in the aqueous phase. Liposomes spontaneously aggregate hydrophilic groups under hydrophilic action and cause hydrophobic groups to aggregate under hydrophobic action. After the liposome system stabilizes, a closed circular structure with a "head to head, tail to tail" formation is generated.[3] The hydrophilic and hydrophobic layers are separated by a water film containing drugs, which forms a polymer. Liposoluble drugs are dispersed in multiple bilayers.[4,5] The unique structural characteristics of liposomes enable the encapsulated drugs to function stably and improve the drug bioavailability.[2,6] Liposomes, as drug carriers, can play an effective role in the biological activity of entrapped substances and have good application prospects in the treatment of cancer, atherosclerosis, infectious diseases, eye diseases, and other diseases.[7,8]

Quercetin, also known as quercitron, is a yellow needle-shaped crystalline substance derived from the stems, flowers, leaves, buds, seeds, and fruits of many plants.[9] The chemical name of quercetin is 3,5,7,3′,4′-pentahydroxy flavonoid, which is a polyphenolic compound and also a dietary flavonoid. Quercetin mostly exists in the form of glycosides such as rutin, quercetin, and hyperin. Rutin can be hydrolyzed by acid to obtain quercetin.[10,11] Numerous studies have shown that quercetin can prevent various diseases, such as osteoporosis,[12] liver cancer,[13] tumors,[14] and cardiovascular diseases.[15] The antioxidant effect of quercetin plays an important role in preventing and treating these diseases.[16,17] Quercetin, as a typical flavonoid compound, has shown potential to improve human health due to its in vivo antioxidant activity in the medical field.[18] Some studies have shown that quercetin can be

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used as a nutrient to prevent various diseases.[19] However, the structure of quercetin contains phenolic hydroxyl compounds that bind to various substances, such as sugars, ethers, or phenolic acids, and exhibit a tightly arranged state. As a result, quercetin exhibits poor stability and water solubility. Therefore, as the bioavailability of quercetin decreases, the biological activity also decreases accordingly.[20,21]

The substances embedded in nanoliposomes can be protected from external environmental interference.[8] Nanoliposomes provide a relatively safe environment, improving the biological activity, structure, and stability of the embedded substances. Utilizing nanoliposomes as embedding carriers to encapsulate quercetin and maximize its effectiveness is of great significance. The preparation of traditional QUE-NL-1 mostly focused on its stability. In this paper, an ultrasonic thin-film dispersion method was used to prepare QUE-NL-1. Response surface methodology was used to optimize the preparation conditions of QUE-NL-1. The particle size distribution, morphology, zeta potential, and PDI value of QUE-NL-1 were analyzed. The effects of simulated gastrointestinal fluid, storage temperature, and time on the stability of QUE-NL-1 were studied. Finally, the safety of QUE-NL-1 was evaluated, which is beneficial for the development and utilization of quercetin.

2. EXPERIMENTAL SECTION

2.1. Preparation of Quercetin Nanoliposomes. The preparation method of QUE-NL-1 adopted the ultrasonic thin-film dispersion method. A certain amount of quercetin was dissolved in 10 mL of methanol solution, which was stirred with magnetic force until the solution was particle-free and transparent (at room temperature, 220 rpm). A certain amount of lecithin and cholesterol was dissolved in 20 mL of chloroform, which was stirred with magnetic force until the solution was particle-free and transparent (room temperature, 220 rpm). The above two solutions mix evenly until they appear in an oil-in-water state, and they are transferred to a 250 mL rotating evaporation bottle. The organic solvents were removed by rotary evaporation at 30 °C and 30 rpm until a light yellow film appeared in the rotary evaporation bottle. The deionized water (40 mL) was added to the evaporation bottle for hydration, and the film was removed by ultrasound for 20 min. The quercetin nanoliposomes were labeled QUE-NL-1. Simultaneously unloaded nanoliposomes were prepared, which were labeled as QUE-NL-0. QUE-NL-1 and QUE-NL-0 were stored at 4 °C for later use.

2.2. Single-Factor Experiment on the Preparation of Quercetin Nanoliposomes. Previous studies have found that drug concentration, evaporation temperature, and lipid bile ratio dosage are key factors affecting the encapsulation efficiency of liposomes. Therefore, the drug concentration (0.05, 0.10, 0.15, 0.20, 0.25, 0.30, and 0.35 mg/mL), evaporation temperature (15, 25, 35, 45, and 55 °C), and lipid bile ratio (2, 3, 4, 5, and 6) were optimized separately.

2.3. Response Surface Optimization Experimental Design. Three independent variables were selected: drug concentration (0.2, 0.25, and 0.3 mg/mL), evaporation temperature (25, 35, and 45 °C), and lipid bile ratio (3, 4, and 5). The encapsulation efficiency was used as a key indicator to evaluate the quality of the response surface liposomes. The factors and level design are shown in Table 1.

Table 1. Box Behnken Experimental Design

factors	levels		
	−1	0	+1
drug concentration (mg/mL)	0.2	0.25	0.3
evaporation temperature (°C)	25	35	45
lipid bile ratio	3	4	5

2.4. Determination of Encapsulation Efficiency of Quercetin Nanoliposomes. QUE-NL-1 (0.5 mL) was diluted to 10 mL with a PBS solution. It was centrifuged at 8000 rpm for 30 min. The absorbance of the upper liquid was measured at 370 nm to obtain the free quercetin concentration C_1. QUE-NL-1 (0.5 mL) was dissolved in 4.5 mL of anhydrous ethanol and diluted to 10 mL with PBS solution. The total concentration of quercetin C_2 was obtained through centrifugation and absorbance measurement.[22] Among them, the sample of unloaded nanoliposomes was used as the control group. The encapsulation rate was calculated according to the following formula

$$\text{encapsulation rate } (\%) = (C_1 - C_2) \div C_2 \times 100\%$$

where C_1 is the free quercetin content in QUE-NL-1 and C_2 is the quercetin content after the membrane breaking of QUE-NL-1.

2.5. Fourier Transform Infrared Spectrometer.[23] The freeze-dried quercetin nanoliposome (1.5 mg) was ground and mixed with 1% potassium bromide powder evenly. It is pressed under a film pressing machine to prepare a sample thin sheet with a thickness of approximately 2 mm, which is then measured with an infrared measuring instrument. Obtain infrared spectral information within the frequency range of 4000−400 cm^{-1}.

2.6. Characterization of Quercetin Nanoliposomes. Negative staining of the nanoliposomes was used to observe the microstructure of QUE-NL-1. The quercetin nanoliposome suspension samples were diluted with PBS a certain number of times. The sample is placed on a dedicated copper mesh (diameter of 3 mm, 200 mesh) for transmission electron microscopy (TEM). The 2% phosphotungstic acid solution was added for negative staining at room temperature for 5 min. The excess solution was sucked off. It was placed in a drying lamp for drying and in TEM for observation and recording.

The quercetin nanoliposome solution was diluted. The particle size distribution (polydispersity index, PDI) was detected by a Mastersizer 3000 in the range of 0.1−1000 μm. Then, the zeta potential special detector dish was changed, and potentiometric determination was carried out.

2.7. Effect of Storage Temperature and Storage Time on the Stability of Quercetin Nanoliposomes. The samples of QUE-NL-1 were stored at different temperature conditions (4, 25, 40, 60, and 80 °C) to evaluate the impact of temperature on stability. The leakage rate of QUE-NL-1 was measured every 2 days at 4 °C to evaluate the impact of storage time on stability.

$$\text{leakage rate } (\%) = (1 - EE_t/EE_0) \times 100\%$$

where EE_t is the encapsulation rate of the sample and EE_0 is the encapsulation rate of the freshly prepared liposome.

2.8. Effect of Simulated Gastrointestinal Fluids on the Leakage Rate of Quercetin. The leakage rate of QUE-NL-1 in the simulated gastrointestinal fluids was measured by UV spectrophotometry. Simulated gastric fluid and artificial intestinal juice were purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd. The QUE-NL-1 was mixed with

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simulated gastric fluid and artificial intestinal juice in equal volumes, respectively. The mixtures were incubated in a shaker at 37 °C and 200 rpm for 4 h. Then, samples were taken at intervals of 30 min to determine the leakage rate. The liposomes were used as a control.

2.9. Safety Experiments on Quercetin Nanoliposomes. The survival rate of Caco-2 cells was detected by the CCK-8 method. The toxicity tests of QUE and QUE-NL-1 with concentrations of 0, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/mL were carried out in Caco-2 cell models, respectively. According to the instructions of the CCK-8 kit (Nanjing Jiancheng Technology Co., Ltd.), 10 μL of CCK-8 solution was added to each well and incubated at 37 °C for 2 h. The absorbance value (OD) was measured at 490 nm by using a multifunctional enzyme marker. The cell survival rate was calculated according to the formula

$$\text{cell survival rate} = (OD_e - OD_b)/(OD_n - OD_b) \times 100\%$$

OD_e is the absorbance value of the experimental well, OD_n is the absorbance value of negative wells, and OD_b is the absorbance value of the blank well.

2.10. Statistical Analysis. The experiment assays were carried out with at least three biological replications. Values were given as the means ± standard deviations (n = 3). Statistical analysis was performed using SPSS statistics (version 17.0) to determine significant differences among the means (p < 0.05).

3. RESULTS AND DISCUSSION

3.1. Effect of Different Conditions on the Preparation of Quercetin Nanoliposomes. *3.1.1. Effect of Quercetin Concentration on the Preparation of QUE-NL-1 Process.* Drug concentration is one of the important factors affecting the preparation of liposomes, which can directly respond to the embedding of liposomal systems. When the evaporation temperature and the lipid-to-bile ratio of the prepared QUE-NL-1 were kept constant, the effect of quercetin on the encapsulation rate of nanoliposomes was observed by changing the concentration of quercetin.

The experimental results are shown in Figure 1. When the concentration of quercetin was 0.05−0.25 mg/mL, the encapsulation rate of nanoliposomes increased with the increase in quercetin concentration. When the concentration of quercetin exceeded 0.25 mg/mL, the encapsulation rate showed a gradually decreasing trend. When the concentration of quercetin was 0.25 mg/mL, the encapsulation rate of liposomes reached a maximum. Liposomes are biofilm structures composed of phospholipid bilayers. When the concentration of the transported drug reaches the maximum amount of liposome bearing, the encapsulation of liposomes reaches a saturated state. Therefore, the encapsulation rate gradually decreases with the increase in drug concentration. In addition, the membrane structure of liposomes has a space of hydrophobic and hydrophilic regions. This may also affect the encapsulation rate of quercetin. Therefore, the quercetin concentration range of 0.2−0.3 mg/mL was chosen as the optimal range for the response surface methodology experiment.

3.1.2. Effect of Evaporation Temperature on the Encapsulation Rate of Nanoliposomes. Temperature is also one of the factors affecting film formation in the preparation of QUE-NL-1. The encapsulation rate varies with the evaporation temperature. When the lipid/bile ratio and quercetin concentration were kept constant, the effect of temperature on the encapsulation rate was observed by changing the evaporation temperature. As shown in Figure 2, the encapsulation rate showed a trend of first increasing and then decreasing with the increase in temperature. When the temperature was 35 °C, the encapsulation rate reached a maximum value. The high evaporation temperature can damage the membrane structure of liposomes, which affects stability. The membrane sealing decreases, which leads to leakage of quercetin. The high temperature may hinder the film formation state during rotary evaporation. Film formation is uneven, leading to a decrease in the encapsulation rate. Therefore, 25−45 °C was chosen as the temperature to optimize the preparation of QUE-NL-1.

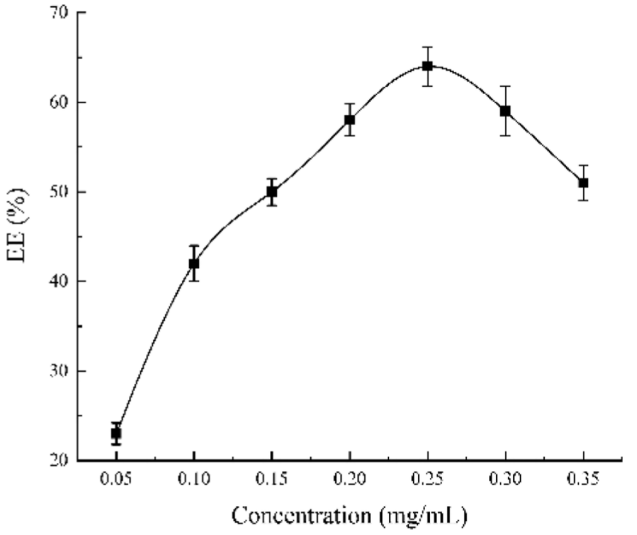

Figure 1. Effect of quercetin concentrations on the encapsulation rate of the nanoliposomes.

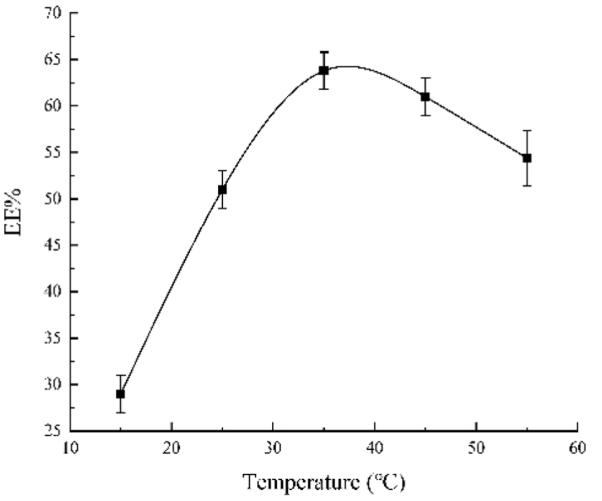

Figure 2. Effect of different temperatures on the encapsulation rate of quercetin.

3.1.3. Effect of Lipid-to-Bile Ratio (PC/CH) on the Encapsulation Rate of Nanoliposomes. Cholesterol and soybean lecithin were used as the main materials for the preparation of the nanoliposome membrane structures. Cholesterol is a stabilizer for liposomes and can improve the stability of phospholipid bilayers in encapsulation systems. The

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encapsulation rate changes with the difference in lipid−cholesterol ratio.[5,24] When the evaporation temperature and quercetin concentration were kept constant, the effect of the lipid-to-bile ratio on the encapsulation rate was observed. As shown in Figure 3, the encapsulation rate of liposomes

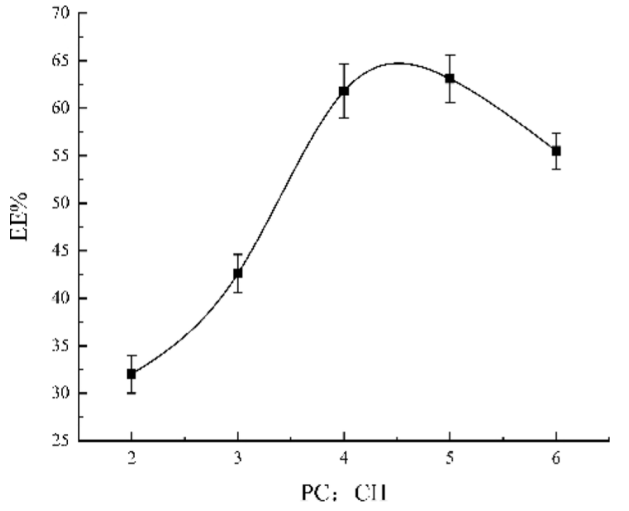

Figure 3. Effect of lipid-to-bile ratios on the encapsulation rate of nanoliposomes.

increased with the elevation of the lipid-to-bile ratio. The encapsulation rate reached a maximum when the lipid-to-bile ratio was 5:1. However, when the lipid-to-bile ratio was greater than 5:1, the encapsulation rate showed a decreasing trend. Soybean lecithin can increase the hydrophobic region in the phospholipid bilayer of nanoliposomes, which is beneficial for embedding more quercetin and increasing the encapsulation rate. Cholesterol plays a stabilizing and protective role for the phospholipid bilayer, which can reduce the mobility of liposomes. When the content of soy lecithin is too high to form a suitable rigid nanoliposome, drug leakage is obvious. Therefore, the lipid-to-bile ratios of 3:1, 4:1, and 5:1 were chosen as the optimal range for the response surface methodology experiment.

3.2. Response Surface Methodology. Drug concentration (C), evaporation temperature (T), and lipid-to-bile ratio (PC/CH) were used as independent variables, and the encapsulation rate of QUE-NL-1 (Y) was used as the response value. A total of 17 experimental designs were presented using Design Expert 10 software, and the results are shown in Table 2. By performing multiple quadratic regression fitting on the experimental results, we obtained the regression equation of Y for independent variables A, B, and C was obtained. Y (EE %) = $73.33 - 1.82A - 2.39B + 1.07C + AB - 0.305AC - 0.45BC - 2.42A^2 - 9.282B^2 - 1.85C^2$.

Using Table 2 response surface methodology factor levels as the basis for ANOVA, the results of the regression model significance analysis are shown in Table 3. The correlation coefficients are R_2 = 0.9951 and R_2Adj = 0.8973. Measured signal-to-noise ratios greater than 4 are desirable. The designed Ades precision = 10.2941 is conducive to simulating the actual level of experimental factors. The P value of the model is 0.0006. It indicates that the model regression is highly significant and statistically significant within the selected range of factors. A coefficient of variation of 2.77 indicates good confidence in the model. All of the above demonstrates

Table 2. Experimental Results of Response Surface Optimization of Liposome Preparation

number	A − C	B − T	C − PC/CH	Y − EE (%)
1	0.20	25	4	65.92
2	0.30	25	4	62.01
3	0.20	45	4	59.25
4	0.30	45	4	59.34
5	0.20	35	3	71.00
6	0.30	35	3	66.22
7	0.20	35	5	72.50
8	0.30	35	5	66.50
9	0.25	25	3	62.50
10	0.25	45	3	58.50
11	0.25	25	5	66.80
12	0.25	45	5	61.00
13	0.25	35	3	72.44
14	0.25	35	5	74.58
15	0.25	35	4	74.25
16	0.25	25	4	75.00
17	0.25	45	4	70.36

Table 3. Significance and Variance Analysis of the Regression Model

source	sum of squares	DF	mean squares	F-value	P-value
model	511.27	9	56.81	16.54	0.0006[b]
A − C	26.64	1	26.64	7.76	0.0271[a]
B − T	45.79	1	45.79	13.33	0.0082[b]
C − PC/CH	9.20	1	9.20	2.68	0.1457
AB	4.00	1	4.00	1.16	0.3163
AC	0.3721	1	0.3721	0.1083	0.7517
BC	0.8100	1	0.8100	0.2358	0.6421
A^2	24.67	1	24.64	7.18	0.0315[a]
B^2	362.25	1	362.25	105.46	<0.0001[b]
C^2	14.42	1	14.42	4.20	0.0797
residual	24.04	7	3.43		
lack of fit	9.23	3	3.08	0.8311	0.5422
pure error	14.81	4	3.70		
cor total	535.31	16			
R_2	0.9519				
R_2Adj	0.8900				
CV	2.77				
Ades precision	10.4921				

[a]Indicates $P < 0.05$. [b]Indicates $P < 0.01$.

that the model can be used to fit the relationship between experimental independent variables and response values. As shown in Table 2, A − C and A^2 have a significant impact on the results, and B − T and B^2 have a highly significant impact on the results. There was a certain main effect relationship between the factors: B − T > A − C > C − PC/CH. The evaporation temperature may be one of the most important factors affecting the encapsulation rate. In order to provide a more intuitive analysis of the effects of drug concentration, evaporation temperature, and lipid-to-bile ratio on the encapsulation rate, corresponding response surface and contour plots were drawn (Figure 4). The curve presented by the interaction between B − T and A − C is steeper. In addition, the contour plot of the interaction between B − T and A − C tends to an elliptical shape. It indicates that the effects of B − T and A − C on the encapsulation rate of QUE-

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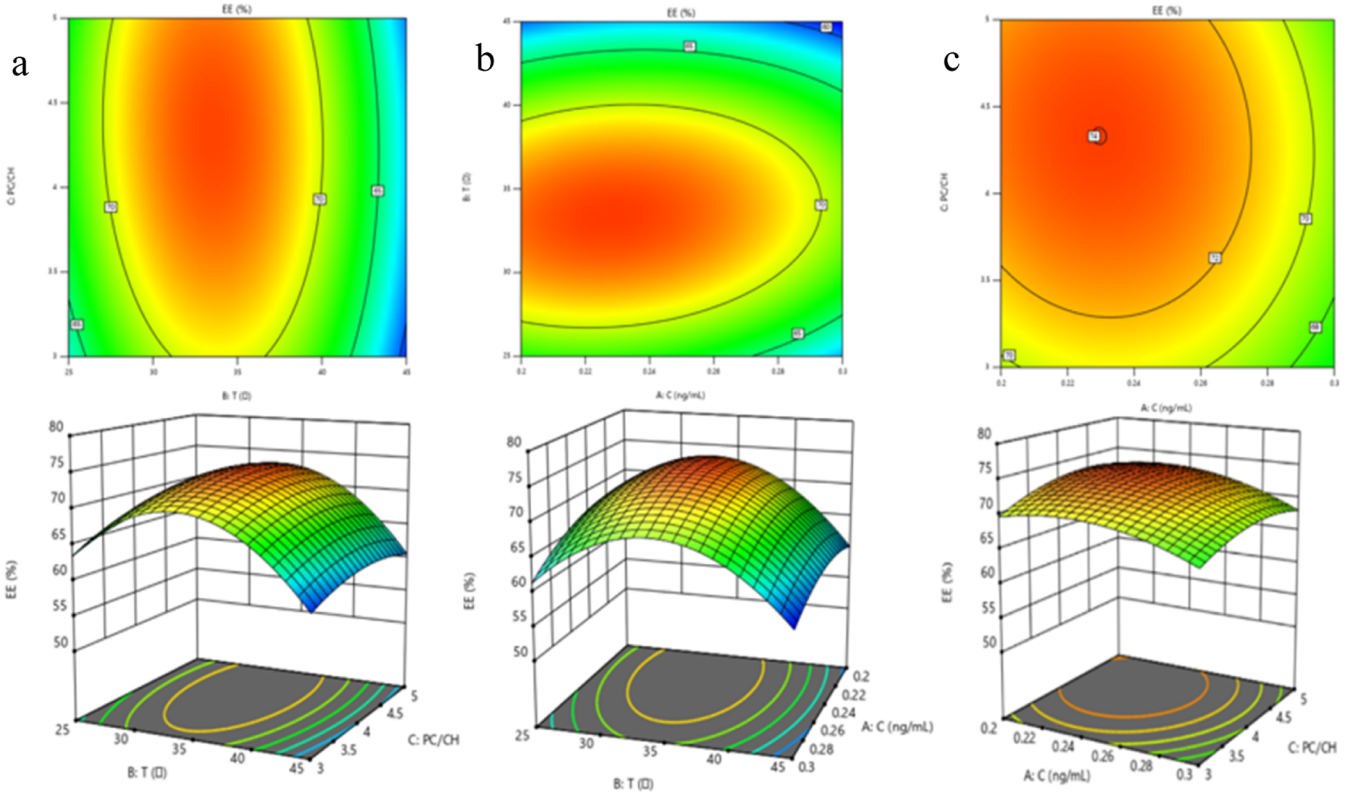

Figure 4. Response surface plots and contour plots of the interaction of $B - T$ and $C -$ PC/CH (a), $B - T$ and $A - C$ (b), and $A - C$ and $C -$ PC/CH (c).

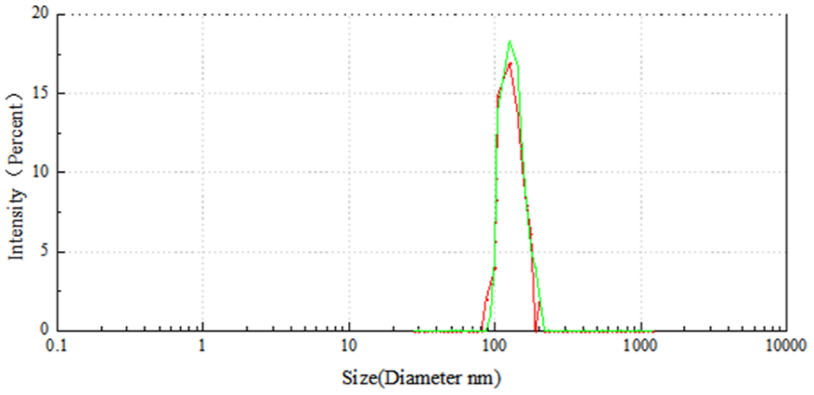

Figure 5. Particle size distribution of QUE-NL-1.

NL-1 are more significant.[25] This result is consistent with the above analysis of the ANOVA.

3.3. Validation of Preparation Conditions for Quercetin Nanoliposomes. The response surface model predicts that the encapsulation rate of QUE-NL-1 is (65.55 ± 3.00)% when the evaporation temperature is 35.85 °C, the drug concentration is 0.24 mg/mL, and the lipid-to-bile ratio is 4.32:1. To verify the accuracy of the response surface prediction results and to take into account the errors existing in the actual operation, the QUE-NL-1 was prepared under the conditions of an evaporation temperature of 35 °C, quercetin concentration of 0.2 mg/mL, and lipid-to-bile ratio of 4:1. The QUE-NL-1 prepared under these conditions is a pale yellow turbid liquid with an encapsulation rate of (63.73 ± 2.09)%, which is not significantly different from the theoretical predicted value.

3.4. Particle Size and PDI of Quercetin Nanoliposomes. The particle sizes of QUE-NL-1 are listed in Figure 5. The average particle size of QUE-NL-1 is 134.11 nm. The absolute value of zeta potential is greater than 30. The average PDI was 0.24. PDI represents the distribution status of nanoparticles. The smaller the PDI value, the narrower the molecular weight distribution, and the more uniform the distribution of nanoliposomes.[26] Therefore, the QUE-NL-1 prepared by the optimized process is more uniformly dispersed.

3.5. Microscopic Evaluation of Quercetin Nanoliposomes. *3.5.1. TEM Observation of Quercetin Nanoliposomes.* The TEM image of QUE-NL-1 is shown in Figure 6. The QUE-NL-1 was uniformly distributed in the medium with a spherical shape. Particles are dispersed separately from each other without adhesion and have a distinct hollow

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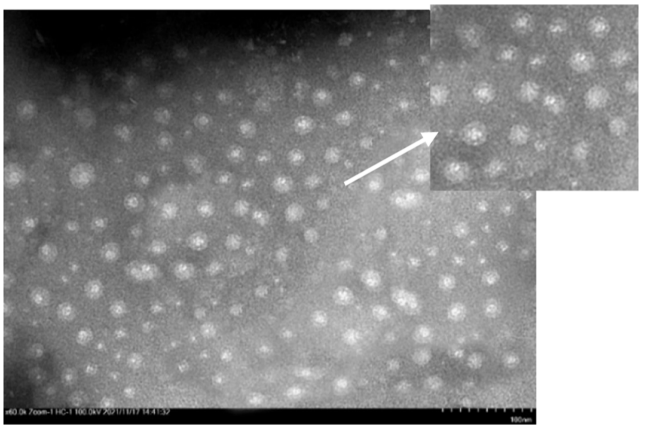

Figure 6. TEM image of QUE-NL-1.

structure. Quercetin is embedded in circular liposomes with vesicular structures.

3.5.2. Fourier Transform Infrared Spectroscopy (FTIR) Analysis. FTIR is mainly used to determine the structure of materials. It is also suitable for studying the potential relationship between nanomaterials and embedded substances. FTIR analysis of QUE-NL-1 is shown in Figure 7. In the region of 4000−500 cm^{-1}, the samples all showed absorption peaks. The region of 3407 and 3309 cm^{-1} is attributed to the presence of the OH groups bonded to the aromatic rings in the quercetin structure. The characteristic absorption peak of the ether C−O group in the frequency region of 1300−1000 cm^{-1} is exhibited. The peak around 1654 cm^{-1} is assigned to the absorption of the ketone C═O group in the phenolic ring of quercetin.[27] Quercetin contains a phenolic hydroxyl group as a characteristic group. The characteristic peak in the range of phenolic hydroxyl telescopic vibration of QUE-NL-1 disappeared when compared with that of QUE, which indicated that quercetin was successfully encapsulated in the nanoliposomes.[28]

3.6. Studies on the Stability of Quercetin Nanoliposomes. *3.6.1. Effect of Storage Temperature on the Stability of Quercetin Nanoliposomes.* The thermal stability of nanoliposomes can be used as an indicator to evaluate the characteristics. The stability of QUE-NL-1 was analyzed by selecting the temperature gradients of 4, 25, 40, and 60 °C. The experimental results are shown in Figure 8. The leakage rate of QUE-NL-1 showed an increasing trend with increasing

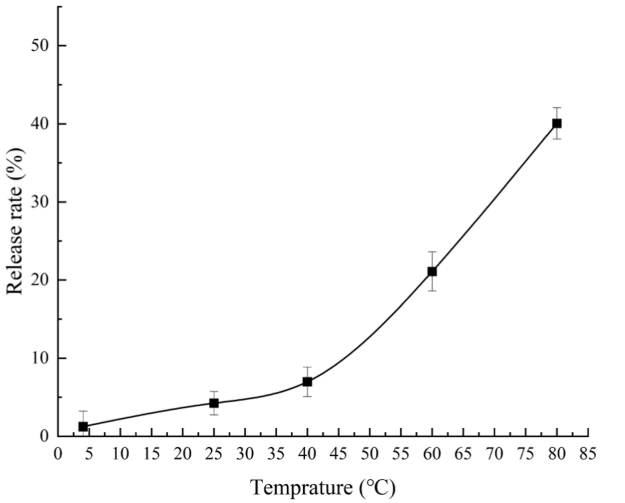

Figure 8. Effect of the storage temperature on the leakage rate of QUE-NL-1.

temperature. According to the results, it is known that it is favorable for quercetin nanoliposome storage under conditions lower than 40. The 4 °C is the optimal storage temperature for nanoliposomes.

3.6.2. Effect of Storage Time on the Stability of Quercetin Nanoliposomes. To investigate the effect of storage time on the stability of QUE-NL-1, the prepared QUE-NL-1 was placed at 4 °C for 14 days. The changes in the particle size and encapsulation rate of the nanoliposomes with the storage time were measured, and the results are shown in Table 4. The average particle size of QUE-NL-1 gradually increased with time. Especially on the eighth day, the particle size of QUE-NL-1 significantly increased. The storage time had no significant effect on the encapsulation rate of QUE-NL-1. The reason for the increase in particle size may be due to the oxidative of liposomes. The wall material of nanoliposomes is prone to oxidation when left in air for too long. A certain degree of aggregation occurs between particles. In addition, the zeta potential on the surface of the nanoliposomes may be

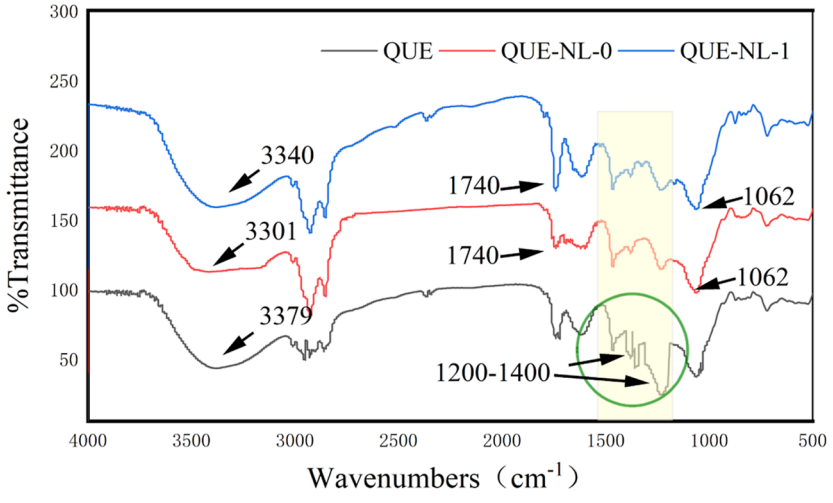

Figure 7. FTIR analysis of QUE-NL-1.

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Table 4. Effect of the Storage Time on the Average Particle Size and Entrapment Rate of QUE-NL-1[a]

days	0	2	4	6	8	10	12	14
average particle size (nm)	132.78±2.51[a]	134.01±3.22[a]	135.20±4.29[a]	141.78±4.11[ab]	153.05±3.52[c]	171.11±5.58[d]	200.05±2.80[e]	222.75±3.71[f]
EE (%)	62.58±2.12[a]	63.00±1.51[a]	62.70±1.22[a]	60.50±1.90[a]	61.23±2.15[a]	60.46±2.56[a]	62.08±1.93[a]	60.55±1.80[a]

[a]The bars labeled with different letters (a–f) indicate protoplast yields are significantly different ($P < 0.05$).

subjected to the effect of the ionic strength of the suspending solution, which leads to an increase in the average size of the QUE-NL-1.

3.6.3. Effect of Gastrointestinal Fluids on the Stability of Quercetin Nanoliposomes. The stability of QUE-NL-1 in the gastrointestinal tract was investigated by simulating gastrointestinal fluid experiments.[29] The results are shown in Figure 9, and the leakage rate of QUE-NL-1 gradually increased with

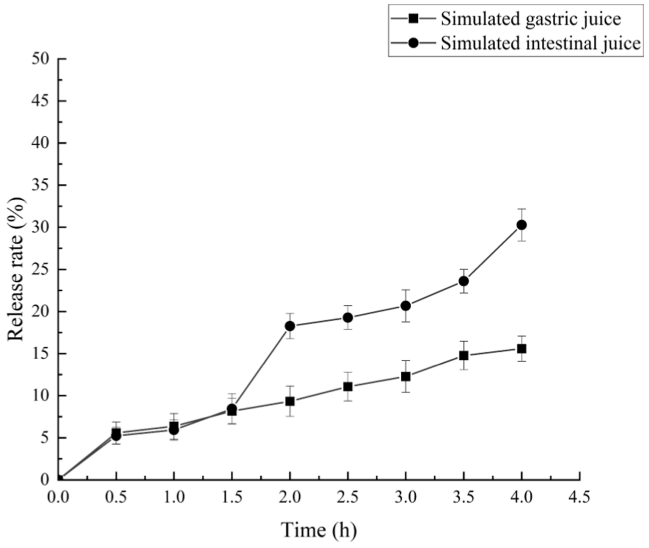

Figure 9. Effect of gastrointestinal fluid on the leakage rate of QUE-NL-1.

the extension of time. The leakage rate of QUE-NL-1 was low within 1.5 h. The leakage rate of QUE-NL-1 in simulated intestinal juice was significantly increased after 1.5 h. It indicates that QUE-NL-1 was gradually released. The results showed that nanoliposomes could effectively protect the effect of gastrointestinal fluids on quercetin. Nanoliposomes increase the stability of quercetin in gastric juice. Quercetin is released upon arrival in the intestinal environment. Therefore, nanoliposomes improve the bioavailability of quercetin.

3.7. Safety Evaluation of Quercetin Nanoliposomes. The safety of QUE-NL-1 was evaluated through Caco-2 cytotoxicity experiments. The experimental results are shown in Figure 10. When the concentrations of quercetin and QUE-NL-1 continue to increase, the cell survival rate shows a decreasing trend. According to the experimental results, statistically insignificant differences were observed in the concentration range of 0.1–0.4 mg/mL. The cell survival rate is greater than 90% within this concentration range, which indicates that quercetin and QUE-NL-1 have no insignificant cytotoxicity. To ensure that the drug concentration remained nontoxic to the cells in subsequent experiments, the quercetin concentration within >90% of cell viability was selected as optimal.

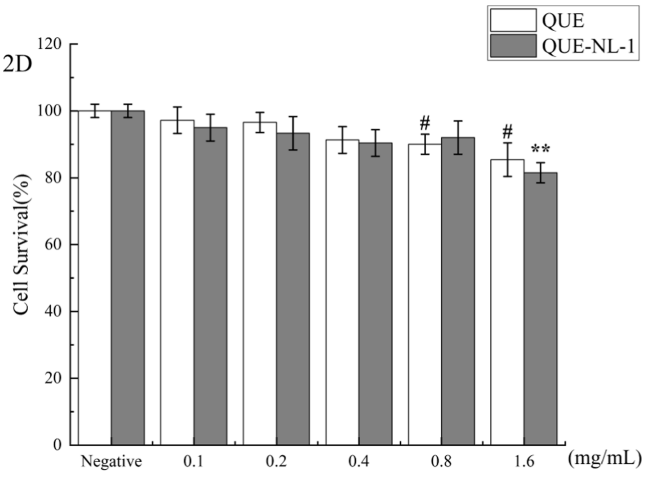

Figure 10. Safety evaluation of QUE-NL-1.

4. CONCLUSIONS

The QUE-NL-1 prepared by response surface methodology optimization showed good reproducibility. The encapsulation rate was (63.73 ± 2.09)%. The average particle size was 134.11 nm, the average absolute value of the zeta potential was 37.50, and the PDI was 0.24. This result indicated that the QUE-NL-1 prepared at a temperature of 35 °C, a drug concentration of 0.20 mg/mL, and a lipid-to-bile ratio of 4:1 could be stably distributed in the medium. The TEM image showed that the QUE-NL-1 was uniformly distributed with an obvious vesicle structure. The measured particle size was basically consistent with the experimental results. FTIR analysis further verified that quercetin was successfully embedded in the nanoliposomes. The excessive storage temperature and prolonged storage time have an impact on the stability of QUE-NL-1. However, storage at 4 °C caused little effect on the leakage rate of QUE-NL-1. In simulated gastrointestinal fluid, the stability of QUE-NL-1 was better, and the leakage rate was lower within 1.5 h. The leakage rate of QUE-NL-1 in simulated intestinal juice was significantly increased after 1.5 h. It indicates that QUE-NL-1 was gradually released. When the concentration range of QUE-NL-1 was 0.1–0.4 mg/mL, the survival rate of Caco-2 cells was >90%, and the cytotoxicity was not significant. This study provides an ideal foundation for the development and application of quercetin in medicine and functional foods.

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L.X.F. and G.R.F. conceived and designed the research. Y.S.Z. and L.X.Q. conducted experiments. Z.Y., Z.H., Z.Z.Y., and G.R.F. contributed new reagents or analytical tools. Z.Y. and W.Y.F. analyzed data. L.X.F., Y.S.Z., and L.X.Q. wrote the manuscript. All authors read and approved the manuscript.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Nsairat, H.; Khater, D.; Sayed, U.; Odeh, F.; Al Bawab, A.; Alshaer, W. Liposomes: Structure, composition, types, and clinical applications. *Heliyon* **2022**, *8* (5), No. e09394.

(2) Guimarães, D.; Cavaco-Paulo, A.; Nogueira, E. Design of liposomes as drug delivery system for therapeutic applications. *Int. J. Pharm.* **2021**, *601*, 120571.

(3) Has, C.; Sunthar, P. A comprehensive review on recent preparation techniques of liposomes. *J. Liposome Res.* **2020**, *30*, 336−365.

(4) Shah, S.; Dhawan, V.; Holm, R.; Nagarsenker, M. S.; Perrie, Y. Liposomes: Advancements and innovation in the manufacturing process. *Adv. Drug Delivery Rev.* **2020**, *154*−*155*, 102−122.

(5) Müller, L. K.; Landfester, K. Natural liposomes and synthetic polymeric structures for biomedical applications. *Biochem. Biophys. Res. Commun.* **2015**, *468* (3), 411−418.

(6) Liu, P.; Chen, G. L.; Zhang, J. C. A review of liposomes as a drug delivery system: Current status of approved products, regulatory environments, and future perspectives. *Molecules* **2022**, *27* (4), 1372.

(7) Crommelin, D. J. A.; van Hoogevest, P.; Storm, G. The role of liposomes in clinical nanomedicine development. What now? Now what? *J. Controlled Release* **2020**, *318*, 256−263.

(8) Li, M.; Du, C.; Guo, N.; Teng, Y.; Meng, X.; Sun, H.; Li, S.; Yu, P.; Galons, H. Composition design and medical application of liposomes. *Eur. J. Med. Chem.* **2019**, *164*, 640−653.

(9) Singh, P.; Arif, Y.; Bajguz, A.; Hayat, S. The role of quercetin in plants. *Plant Physiol. Biochem.* **2021**, *166*, 10−19.

(10) Ulusoy, H. G.; Sanlier, N. A minireview of quercetin: from its metabolism to possible mechanisms of its biological activities. *Crit. Rev. Food Sci. Nutr.* **2020**, *60* (19), 3290−3303.

(11) Hosseini, A.; Razavi, B. M.; Banach, M.; Hosseinzadeh, H. Quercetin and metabolic syndrome: A review. *Phytother. Res.* **2021**, *35* (10), 5352−5364.

(12) Mousavi, S.; Vakili, S.; Zal, F.; Savardashtaki, A.; Jafarinia, M.; Sabetian, S.; Razmjoue, D.; Veisi, A.; Azadbakht, O.; Sabaghan, M.; Behrouj, H. Quercetin potentiates the anti-osteoporotic effects of alendronate through modulation of autophagy and apoptosis mechanisms in ovariectomy-induced bone loss rat model. *Mol. Biol. Rep.* **2023**, *50* (4), 3693−3703.

(13) Liu, X.; Zhang, Y.; Liu, L.; Pan, Y.; Hu, Y.; Yang, P.; Liao, M. Protective and therapeutic effects of nanoliposomal quercetin on acute liver injury in rats. *BMC Pharmacol. Toxicol.* **2020**, *21* (1), 11.

(14) Hsieh, H.-L.; Yu, M.-C.; Cheng, L.-C.; Chu, M.-Y.; Huang, T.-H.; Yeh, T.-S.; Tsai, M.-M. Quercetin exerts anti-inflammatory effects *via* inhibiting tumor necrosis factor-α-induced matrix metalloproteinase-9 expression in normal human gastric epithelial cells. *World J. Gastroenterol.* **2022**, *28* (11), 1139−1158.

(15) Papakyriakopoulou, P.; Velidakis, N.; Khattab, E.; Valsami, G.; Korakianitis, I.; Kadoglou, N. P. Potential pharmaceutical applications of quercetin in cardiovascular diseases. *Pharmaceuticals* **2022**, *15* (8), 1019.

(16) Xu, D.; Hu, M. J.; Wang, Y. Q.; Cui, Y. L. Antioxidant activities of quercetin and its complexes for medicinal application. *Molecules* **2019**, *24*, 1123.

(17) Diao, R.; Gan, H.; Tian, F.; Cai, X.; Zhen, W.; Song, X.; Duan, Y.-G. In vitro antioxidation effect of quercetin on sperm function from the infertile patients with leukocytospermia. *Am. J. Reprod. Immunol.* **2019**, *82*, No. e13155.

(18) Simon, L.; Vincent, M.; Le Saux, S.; Lapinte, V.; Marcotte, N.; Morille, M.; Dorandeu, C.; Devoisselle, J. M.; Bégu, S. Polyoxazolines based mixed micelles as PEG free formulations for an effective quercetin antioxidant topical delivery. *Int. J. Pharm.* **2019**, *570*, 118516.

(19) Deepika; Maurya, P. K. Health benefits of quercetin in age-related diseases. *Molecules* **2022**, *27* (8), 2498.

(20) Chen, W.; Ju, X.; Aluko, R. E.; Zou, Y.; Wang, Z.; Liu, M.; He, R. Rice bran protein-based nanoemulsion carrier for improving stability and bioavailability of quercetin. *Food Hydrocolloids* **2020**, *108*, 106042.

(21) Liu, K.; Zha, X.-Q.; Shen, W.-D.; Li, Q.-M.; Pan, L. H.; Luo, J.-P. The hydrogel of whey protein isolate coated by lotus root amylopectin enhance the stability and bioavailability of quercetin. *Carbohydr. Polym.* **2020**, *236*, 116009.

(22) Ren, G.; He, Y.; Liu, C.; Ni, F.; Luo, X.; Shi, J.; Song, Y.; Li, T.; Huang, M.; Shen, Q.; Xie, H. Encapsulation of curcumin in ZEIN-HTCC complexes: physicochemical characterization, in vitro sustained release behavior and encapsulation mechanism. *LWT* **2022**, *155*, 112909.

(23) Tavares, L.; Noreña, C. P. Z. Encapsulation of ginger essential oil using complex coacervation method: Coacervate formation, rheological property, and physicochemical characterization. *Food Bioprocess Technol.* **2020**, *13*, 1405−1420.

(24) Pinilla, C. M. B.; Reque, P. M.; Brandelli, A. Effect of oleic acid, cholesterol, and octadecylamine on membrane stability of freeze-dried liposomes encapsulating natural antimicrobials. *Food Bioprocess Technol.* **2020**, *13*, 599−610.

(25) Munot, N. M.; Shinde, Y. D.; Shah, P.; Patil, A.; Patil, S. B.; Bhinge, S. D. Formulation and evaluation of chitosan-plga biocomposite scaffolds incorporated with quercetin liposomes made by QbD approach for improved healing of oral lesions. *AAPS PharmSciTech* **2023**, *24*, 147.

(26) Ramli, N. A.; Ali, N.; Hamzah, S. Physicochemical characterization of quercetin-loaded liposomes prepared by sonication for

functional food application. *J. Sustainability Sci. Manage.* **2020**, *15*, 15−27.
(27) Qian, J.; Mo, C.; Yang, H.; Zhang, J. Q.; Zhu, S. M.; Gong, F.; Guo, H. Preparation of myricetin nanoliposomes using film-ultrasonic dispersion method and characterization. *Appl. Nanosci.* **2023**, *13*, 3263−3272.
(28) Javani, R.; Hashemi, F. S.; Ghanbarzadeh, B.; Hamishehkar, H. Quercetin-loaded niosomal nanoparticles prepared by the thin-layer hydration method: Formulation development, colloidal stability, and structural properties. *LWT* **2021**, *141*, 110865.
(29) Rodriguez, E. B.; Almeda, R. A.; Vidallon, M. L. P.; Reyes, C. T. Enhanced bioactivity and efficient delivery of quercetin through nanoliposomal encapsulation using rice bran phospholipids. *J. Sci. Food Agric.* **2019**, *99* (4), 1980−1989.

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