Pharmacology & Pharmacy, 2022, 13, 199-211
https://www.scirp.org/journal/pp
ISSN Online: 2157-9431
ISSN Print: 2157-9423

Study on the Preparation and Characterization of Glycyrrhizic Acid Liposomes

Bing Wang*, Xiang Luo*, Jue Wang, Yibao Jin, Tiejie Wang, Xiean Yu#, Guo Yin#

NMPA Key Laboratory for Bioequivalence Research of Generic Drug Evaluation, Shenzhen Institute for Drug Control, Shenzhen, China

Email: #yuxieanalj@126.com, #ayinguoa@126.com

How to cite this paper: Wang, B., Luo, X., Wang, J., Jin, Y.B. Wang, T.J., Yu, X.A. and Yin, G. (2022) Study on the Preparation and Characterization of Glycyrrhizic Acid Liposomes. *Pharmacology & Pharmacy*, **13**, 199-211.
https://doi.org/10.4236/pp.2022.136016

Received: May 6, 2022
Accepted: June 27, 2022
Published: June 30, 2022

Abstract

The purpose of this study was to prepare glycyrrhizic acid nanoliposomes and evaluate the encapsulation efficiency and other properties of glycyrrhizic acid nanoliposomes, which would provide a reference for further research on Glycyrrhizic Acid in the treatment of liver diseases. Firstly, the preparation conditions of glycyrrhizic acid liposomes were optimized by the orthogonal design method. Then, glycyrrhizic acid liposomes were prepared by ultrasonic-film dispersion method and the encapsulation efficiency was determined by the HPLC method. Finally, the properties of glycyrrhizic acid nanoliposomes were also studied. The results showed that the optimal preparation conditions of liposomes were as follows: the ratio of drug to lipid was 1:30; Lecithin: cholesterol = 1:1; Hydration medium: pure water; Ultrasonic time: 120 s. The encapsulation efficiency of liposomes was about 90%. The final liposomes were round and uniform in distribution, with an average particle size of about 50 nm and absolute zeta potential of −28.9 mV. In this study, glycyrrhizic acid liposomes were prepared and the optimal preparation conditions were optimized. The encapsulation efficiency of the liposomes under the optimized conditions was determined. The evaluation of the morphology, size, particle size and stability of the liposomes was completed.

Keywords

Glycyrrhizic Acid, Liposome, Orthogonal Design, Quality Evaluation

1. Introduction

Hepatology mainly includes the clinical diagnosis and treatment of viral hepatitis, chemical damage liver disease, autoimmune and cholestatic liver disease, ge-

*These authors contributed equally to this work.
#Corresponding authors.

netic metabolic liver disease and vascular abnormal liver disease [1] [2]. The chemical injury liver disease has arisen from the stimulation of alcohol, drugs and poisons. There was still a gap in clinical diagnosis and treatment of non-communicable liver disease compared with developed countries. Viral hepatitis has always been the most common liver disease in China, including hepatitis A, B, C, D and E. Among them, hepatitis C and hepatitis B were the main liver diseases. Hepatitis B was a highly infectious disease with a high incidence rate and was seriously harmful to human health. China was the hardest hit area by hepatitis B. It was said that about 100 million people carried hepatitis B virus, accounting for about one-third of the global hepatitis B virus carriers. The number of people who die of viral hepatitis even accounted for half of the global number. Moreover, hepatitis B could cause a variety of acute and chronic liver diseases, such as liver failure, liver cirrhosis, liver cancer, etc. More than 1 million patients die of these complications every year [3] [4] [5]. Therefore, chronic hepatitis B was still the main liver disease endangering the health of Chinese people [6].

Glycyrrhizic acid was the most abundant triterpenoid saponin in Glycyrrhiza uralensis Fisch. It was also the main active component of Glycyrrhiza uralensis Fisch. It had anti-inflammatory, anti-tumor, anti-virus, anti-oxidation, immune regulation and other functions [7] [8]. The most widely used was the antiviral effect, which had the most significant effect on the liver. It could significantly reduce the necrosis of hepatocytes and the inflammatory reaction between hepatocytes, prevent hepatocyte steatosis, inhibit the proliferation of the liver fibrous layer and promote the regeneration of hepatocytes, which could effectively improve various kinds of liver diseases [9]. As a first-line drug for anti-inflammatory and liver protection treatment in the field of liver disease, the safety and effectiveness of glycyrrhizic acid have been confirmed in several clinical studies [10] [11].

However, Glycyrrhizic acid was difficult to dissolve in water and had low bioavailability, which led to its failure to exert its maximum efficacy in the treatment process. Moreover, if glycyrrhizic acid was directly used in clinical treatment, it had poor targeting and was easy to produce many adverse reactions [12] [13]. The main adverse reactions were followed: allergic reactions including skin allergic reactions, rashes, itching and anaphylactic shock; Digestive system adverse reactions such as nausea, vomiting, diarrhea and other symptoms; Endocrine system response: longer medication cycles can lead to a series of chronic diseases such as hypokalemia, hypertension, water and sodium retention [14] [15]. These problems greatly limited the practical application of glycyrrhizic acid in clinical practice, resulting in that glycyrrhizic acid could not play an important role in anti-inflammatory and liver protection.

In view of the above adverse reactions, the most effective way was to change the dosage form. Liposome was a kind of artificial nano-targeted preparation. By using the structural characteristics of its spherical phospholipid bilayer, drugs

can be encapsulated in it and then delivered to the target organs to play the therapeutic role. Targeted preparation had the characteristics of location concentration, controlled drug release, low toxicity and side effects, improving the bioavailability of drug preparation [16]. Now there are many drugs that use these characteristics of targeted agents to improve the efficacy and reduce adverse reactions. All of these excellent characteristics would be attributed to the natural tendency of liposomes. After being encapsulated by liposomes, general drugs were mainly concentrated in liver, spleen, bone marrow and other organs. And the natural target organ of liposomes was mainly liver [17] [18]. Therefore, the liposome as a carrier to construct liver targeted glycyrrhizic acid nanoliposomes was helpful in achieving the targeted and precise treatment of glycyrrhizic acid, reducing the occurrence of adverse reactions, improving the bioavailability of glycyrrhizic acid and provides a new strategy for the clinical application of glycyrrhizic acid.

Therefore, this experiment would combine the anti-inflammatory, liver protective effects of glycyrrhizic acid and the natural targeting of liposomes to prepare liver targeted glycyrrhizic acid liposomes. The entrapment efficiency was selected as the index to optimize the best preparation conditions of glycyrrhizic acid nanoliposomes via orthogonal design. The evaluation indexes include encapsulation efficiency, morphology, particle size and stability. High-performance liquid chromatography was used to determine entrapment efficiency. The best preparation methods: Precision weighing glycyrrhizic acid 1 mg, lecithin 30 mg, cholesterol 30 mg, and the mixture of 2 mL trichloromethane methanol (1:2) mixed solution to dissolve. Transfer the mixed solution into the flask of the round bottom, and the uniform honeycomb film is formed by vacuum evaporation. Add 10 mL of the same temperature ultra-pure water into the round bottom flask, and the best liposome is obtained after emulsion ultrasonic 120 s. Finally, the entrapment efficiency of this batch of liposomes was about 90%.

2. Reagents and Apparatus

2.1. Reagents

Soybean lecithin and Cholesterol were offered by Xi'an Ruixi Biotechnology Co., Ltd. Glycyrrhizic acid standard (content ≥ 93.0%, Tokyo Chemical Industry Co., Ltd.); Acetonitrile (chromatographic purity, Merck Co., Ltd.); Methanol (chromatographic purity, Merck Co., Ltd.); Ultra pure water; Chloroform (analytically pure, Guangdong Guangshi reagent Technology Co., Ltd., batch No.: 2020040115); Phosphotungstic acid (content ≥ 99%, Shanghai Aladdin Biochemical Technology Co., Ltd.).

2.2. Apparatus

LC-20a Shimadzu HPLC; Fresco 21 micro centrifuge; Ms204s electronic analytical balance; Kq-500de ultrasonic cleaning machine; Milli-q academic water purifier; MS-3 digital display vortex mixer; N-1100 rotary evaporator; A-1000s wa-

ter flow ventilator; Ca-1111 cooling water circulating device; Q700 ultrasonic crusher; Seven Easy S20 pH meter; Dl-5-b low-speed large capacity centrifuge; Q700 ultrasonic crusher; Mastersizer micro Malvern laser particle sizer.

2.3. Chromatographic Conditions

Thermo acclaim TM 120 C18 (4.6 mm × 250 mm, 5 μm) Chromatographic column was selected for separation. The mobile phase was acetonitrile-0.1% phosphoric acid aqueous solution. The flow rate was set as 0.8 $mL \cdot min^{-1}$. The detection wavelength was 254 nm with a column temperature 25˚C. According to the literature, the maximum absorption wavelength of glycyrrhizic acid was 249 nm, while the excipient had no obvious absorption at 254 nm, so the detection wavelength was 254 nm.

2.4. Preparation of Reference Solutions

Accurately weigh 1.5 mg of a glycyrrhizic acid reference substance, place it in a 2 mL centrifuge tube, add 1.5 mL of 30% methanol, and shake it evenly to prepare as the mother liquor of glycyrrhizic acid reference substance.

2.5. Preparation of Test Solution

Precisely extracted 1 mL of glycyrrhizic acid nanoliposome and placed it in a 5 mL volumetric flask, added methanol as demulsifier and solvent to demulsify and dilute it to 5 mL, shook it evenly and used it as the test solution of a glycyrrhizic acid liposome. The blank liposome was made into a blank liposome solution by the same method.

3. Methods and Results

3.1. Preparation of Glycyrrhizic Acid Nanoliposomes and Optimization of Preparation Conditions

3.1.1. Selection of Preparation Method

Liposomes were formed by the hydrophobic interaction of phospholipid molecules in the aqueous phase. The preparation methods were different. The particle size of liposomes can be from tens of nanometers to several microns, and the structure is not the same. There were many methods to prepare liposomes, such as multiple emulsion method, solvent injection method, reverse evaporation method and film dispersion method. Through the preliminary investigation, the particle size of liposomes prepared by the ultrasonic film dispersion method was more in line with the requirements and the operation was convenient, so the ultrasonic film dispersion method was selected to prepare glycyrrhizic acid nano liposomes.

3.1.2. Encapsulation Efficiency of Liposomes

The entrapment efficiency of liposomes refers to the percentage of the encapsulated substance (such as a certain drug) in the liposomal suspension of the total amount of drug. It was an important indicator of quality control of liposomes

and nanoparticles, which would reflect the extent of drugs encapsulated by carriers [19]. Determination of liposome entrapment efficiency was usually by first separating drug-containing liposomes from free drugs and then calculating their concentration directly or indirectly by other methods [20]. Ultracentrifugation was chosen for this study, considering the nature of the liposomes containing glycyrrhizin and the operating conditions.

Determination method: 1 mL of glycyrrhizic acid liposome solution was added into an ultrafiltration centrifuge tube and centrifuged at 14,000 rpm for 10 min to collect the filtrate. After demulsification with methanol, the microporous membrane was filtered. In the same way, glycyrrhizic acid nanoliposomes were demulsified, centrifuged and diluted at the same time. The two diluted solutions were filtered by microporous membrane and then analyzed to calculate the content of glycyrrhizic acid. The formula of drug entrapment efficiency was as follows:

$$\text{Encapsulation efficiency} = (1 - C_f / C_t) \times 100\%.$$

where C_f is the amount encapsulated in the microparticle formulation and C_t is the total drug amount encapsulated versus unencapsulated in the microparticle formulation.

3.1.3. Prescription Optimization

By consulting the relevant literature, we found that the main factors influencing the encapsulation rate of liposomes were: drug to lipid ratio, $m_{lecithin}$:$m_{cholesterol}$, hydrating medium type, dosage of hydration medium, hydration time, ultrasonic time, etc. [21]. In this study, the drug encapsulation rate was used as the evaluation index, and three levels of three factors (drug lipid ratio, $m_{lecithin}$:$m_{cholesterol}$, hydrating medium type) were investigated. L9 (33) orthogonal design experiment was used to design and screen the prescription, as shown in **Table 1**. Nine batches of glycyrrhizic acid nanoliposomes were prepared and the encapsulation rate was determined according to the above methods. The results are shown in **Table 2** and **Table 3**.

The results showed that the choice of hydration medium had a significant effect on the encapsulation rate of liposomes. According to the effect chart (**Figure 1**), the optimal technological conditions are drug to fat ratio = 1:30, lecithin: cholesterol = 1:1, hydration medium as pure water.

3.2. Methodological Validation of Chromatography for Glycyrrhizic Acid Determination

3.2.1. Specific Experiment

The blank methanol, glycyrrhizic acid reference solution and blank liposome test solution were detected. The spectrum results showed that the peak shape of glycyrrhizic acid was good under the chromatographic conditions, without the interference of miscellaneous peaks, and the retention time was 22.956 min. Compared with blank methanol and blank liposome, the chromatographic conditions were specific, and the results are shown in **Figure 2**.

Table 1. Factor level of orthogonal test.

Level	Factor		
	Drug lipid ratio	Material ratio	Hydrated medium
1	1:20	1:1	Water
2	1:30	1:2	PBS (pH = 6.8)
3	1:50	2:1	PBS (pH = 7.2)

Table 2. Orthogonal test results.

Serial number	Drug lipid ratio	Material ratio	Hydrated medium	Encapsulation efficiency (%)
1	1	1	1	76.69
2	1	2	2	49.90
3	1	3	3	73.03
4	2	1	2	89.65
5	2	2	3	83.81
6	2	3	1	92.00
7	3	1	3	92.24
8	3	2	1	84.10
9	3	3	2	76.54
Average 1	66.540	86.193	84.263	
Average 2	88.487	72.603	72.030	
Average 3	84.293	80.523	83.027	
Range	21.947	13.590	4.213	

Table 3. Analysis of variance.

Factor	Sum of squares of deviations	Degree of freedom	F retio	F critical value	Significance
Drug lipid ratio	814.421	2	2.335	4.460	
Material ratio	279.563	2	0.801	4.460	
Hydrated medium	272.110	2	0.780	4.460	
Error	1395.32	8			

3.2.2. Limit of Detection

The mother liquor of glycyrrhizic acid reference substance was diluted to different concentrations and the liquid chromatogram was recorded. When S/N = 3, the concentration was the lowest detection limit. The results showed that the detection limit of glycyrrhizic acid in vitro was 0.78 μg·mL^{-1}.

3.2.3. Linearity and Range

Accurately measure the appropriate amount of glycyrrhizic acid reference stock solution, and dilute with 30% methanol to glycyrrhizic acid test solution with the

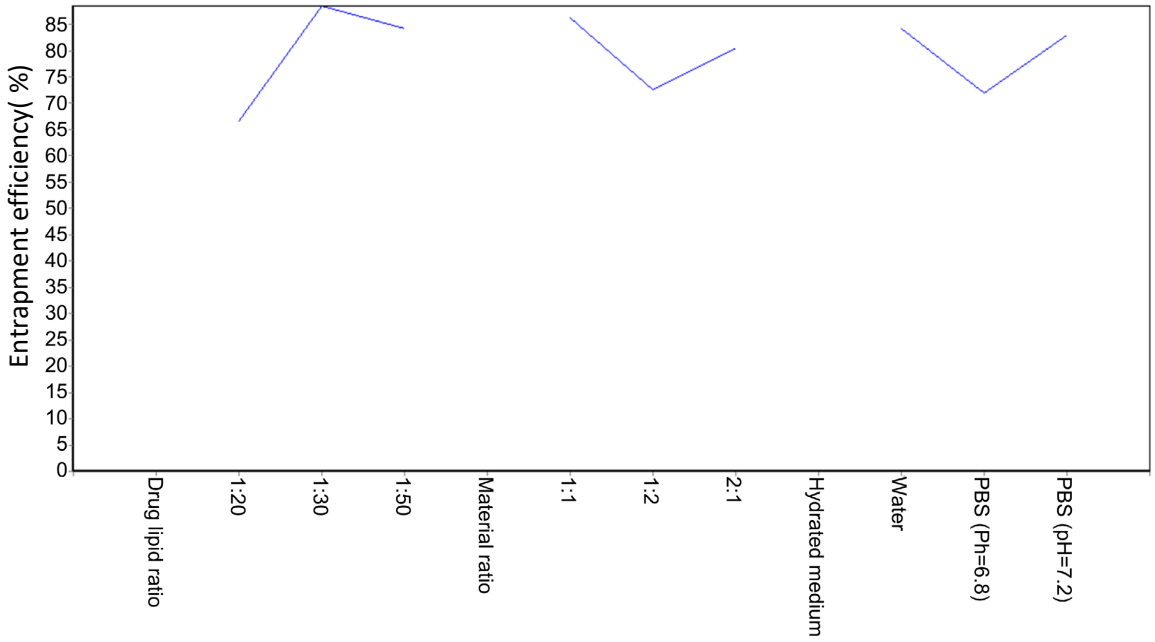

Figure 1. Orthogonal effect graph.

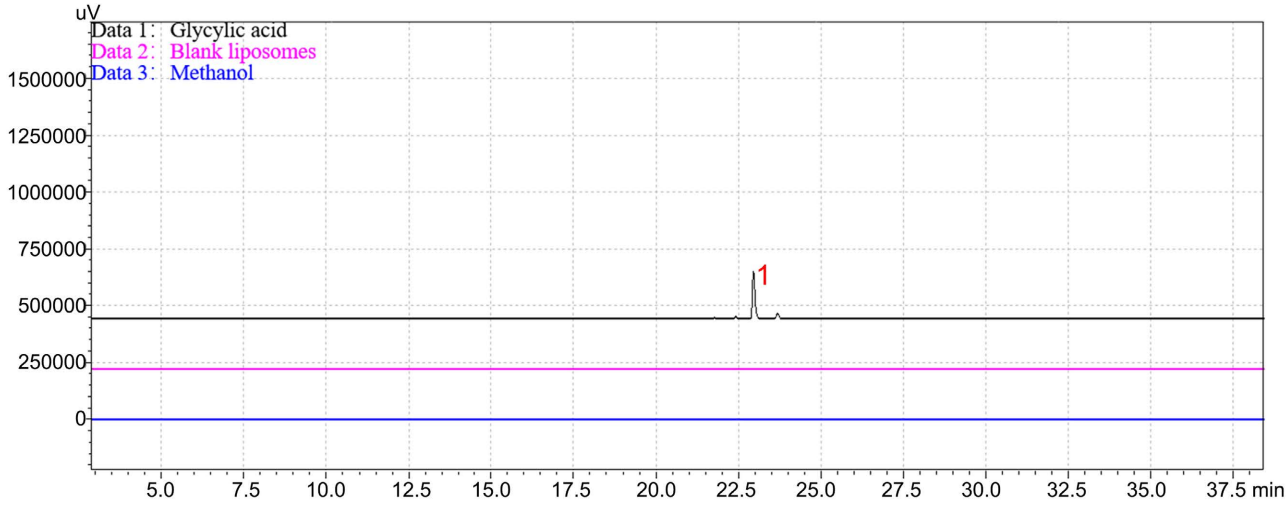

Figure 2. Typical chromatogram of glycyrrhizic acid.

concentration of 2.5, 5, 10, 20, 40, 80, 160 μg·mL^{-1} respectively. The regression equation of glycyrrhizic acid concentration (c) to absorption peak area (A) was obtained as follows: $y = 7076.9x - 10633$, $R^2 = 0.9999$. The results were shown in **Figure 3**, which indicated that there was a good linear relationship between glycyrrhizic acid concentration and peak area in the range of 2.5 - 160 μg·mL^{-1}.

3.2.4. Precision Test

Prepare glycyrrhizic acid reference solution with high (125 μg·mL^{-1}), medium (25 μg·mL^{-1}) and low (5 μg·mL^{-1}) concentrations. Each concentration was determined 6 times a day for 3 days. The intra-day precision and inter-day precision were obtained in **Table 4**, **Table 5**. The results showed that the intra-day

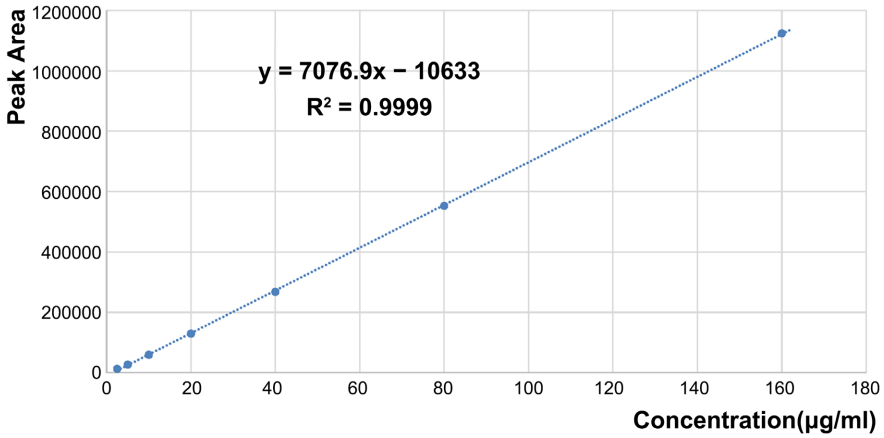

Figure 3. Standard curve of glycyrrhizic acid.

Table 4. Intra-day precision (n = 6).

Sample	Concentration (μg/mL)	Accuracy (%)	RSD (%)
glycyrrhizic acid	5	100	0.43
	25	104	0.28
	125	102	0.41

Table 5. Inter-day precision (n = 6).

Sample	Concentration (μg/mL)	Accuracy (%)	RSD (%)
glycyrrhizic acid	5	96.3	0.35
	25	105	0.28
	125	103	0.38

and inter day precision of high (125 μg·mL^{-1}), medium (25 μg·mL^{-1}) and low (5 μg·mL^{-1}) glycyrrhizic acid were less than 1.0%, indicating that the precision of the test was good and met the requirements of methodology.

3.2.5 Stability Test

Take the prepared glycyrrhizic acid reference materials with high (125 μg·mL^{-1}), medium (25 μg·mL^{-1}) and low (5 μg·mL^{-1}) concentrations to determine the peak area. The results were shown in Table 6, and the relative standard deviation was less than 1.0%, indicating that the test was good stability.

3.2.6. Repeatability Test

Take the prepared 125 μg·mL glycyrrhizic acid reference substance and operate six copies in parallel, then record the chromatographic peak area. The results are shown in Table 7. The relative standard deviation was within the allowable range, indicating that the test was good repeatability.

3.2.7. Sample Recovery Test

1 mL glycyrrhizic acid nanoliposome solution was used for testing recovery. The results are shown in Table 8. The average recoveries were above 90% and the

Table 6. Stability test (n = 6).

Sample	Concentration (μg/mL)	Accuracy (%)	RSD (%)
glycyrrhizic acid	5	92.0	2.93
	25	103	1.84
	125	104	0.45

Table 7. Repeatability test (n = 6).

Sample	Concentration (μg/mL)	Accuracy (%)	RSD (%)
glycyrrhizic acid	5	100	0.62
	25	94.5	0.38
	125	105	0.27

Table 8. Sample recovery rate test (n = 6).

Sample	Concentration (μg/mL)	Average recovery (%)	RSD (%)
glycyrrhizic acid	5	93.9	0.51
	25	94.6	0.47
	125	94.8	0.31

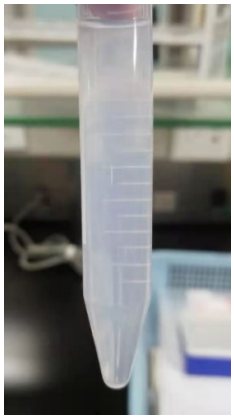

Figure 4. Glycyrrhizic acid nanoliposomes.

relative standard deviations were less than 1%, indicating that the excipients had no significant effect on the determination of glycyrrhizic acid in glycyrrhizic acid nanoliposomes.

3.3. Evaluation of Glycyrrhizic Acid Nanoliposomes

3.3.1. The Morphology and Distribution of the Liposomes

A batch of glycyrrhizic acid liposomes was prepared according to the optimized formula and uniform and transparent milky white suspension was observed (**Figure 4**). Take an appropriate amount of the glycyrrhizic acid liposome suspension, dilute it with ultrapure water, drop it on the special copper mesh, place it for two minutes, absorb the excess water with filter paper, dye it with phosphotungstic acid for 2 minutes, dry it naturally, observe it under the transmis-

sion electron microscope and take photos. The results are shown in Figure 5.

3.3.2. Particle Size and Zeta Potential of the Liposomes

Analyze the optimized glycyrrhizic acid nanoliposomes by Malvern laser particle size analyzer. The results were as follows: the hydrodynamic diameter was about 50 nm (Figure 6). The record of the particle size and Zeta potential were −28.9 mV (Figure 7).

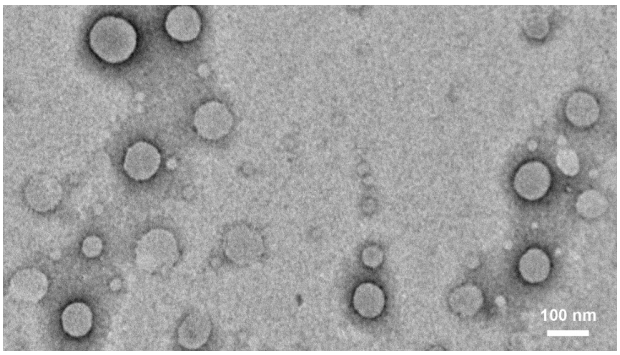

Figure 5. Electron micrograph of glycyrrhizic acid nanoliposomes.

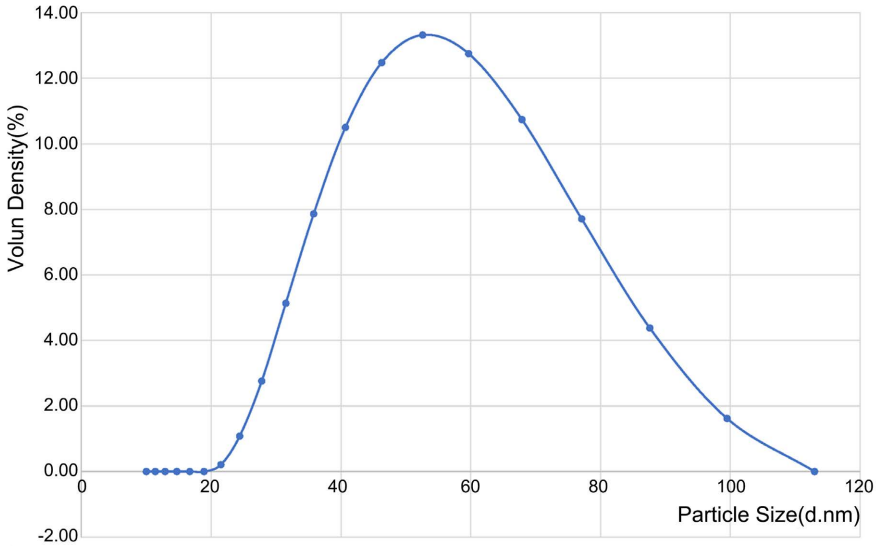

Figure 6. Particle size distribution of glycyrrhizic acid liposomes.

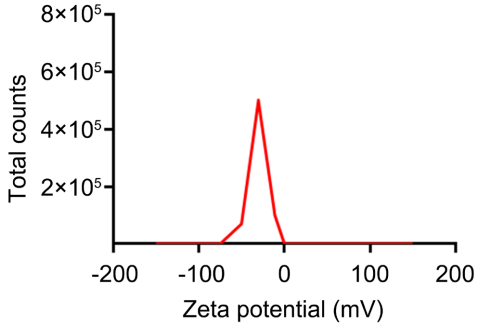

Figure 7. Zeta potential diagram of glycyrrhizic acid liposome.

4. Summary and Discussion

In this project, glycyrrhizic acid liposomes were successfully prepared by the film dispersion method. The best preparation conditions were selected by an orthogonal design experiment. The best preparation method was as follows: accurately weigh 1 mg glycyrrhizic acid, 30 mg lecithin and 30 mg cholesterol, add 2 mL chloroform methanol (1:2) mixed solution to dissolve, transfer the mixed solution into a round bottom flask, A uniform honeycomb membrane was formed by vacuum evaporation, then O/W emulsion was added to 10 mL ultra pure water at the same temperature. The best liposomes were obtained after ultrasonic emulsion of 120 s. Finally, the entrapment efficiency of this batch of liposomes was about 90%.

The stability and morphological properties of liposomes were preliminarily investigated. The Glycyrrhizic nano liposomes were observed to be a homogeneous and transparent emulsion. Transmission electron microscopy showed that glycyrrhizic acid nano liposomes were spherical vesicles with a particle size of 100 nm. Zeta potential, also known as surface potential, is the characterization of the amount of charge on the surface of particles, which is related to the stability of the particle system. Zeta potential can be positive or negative, and it is related to the excipients used, such as the surface potential of lipid emulsion prepared with lecithin as emulsifier is negative. In general, the higher the absolute value of zeta potential, the greater the electrostatic repulsion between particles and the better the physical stability. Generally, the system is stable when the absolute value of zeta potential reaches 25 mV. The particle size was about 50 nm and the zeta potential was −28.8 mV, which indicated that the liposome was stable. The establishment of this method provides a new strategy for the further study of glycyrrhizic acid in the treatment of viral hepatitis.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Valenti, L., Aghemo, A. and Forner, A. (2019) Liver International: Anticipating the Future of Hepatology Worldwide. *Liver International*, **39**, 1796-1797. https://doi.org/10.1111/liv.14240

[2] Berk, P.D. (1995) Hepatology and Hepatology: the Trends Continue. *Hepatology*, **21**, 875-878. https://doi.org/10.1016/0270-9139(95)90543-X

[3] Tandon, R.K. (2006) Journal of Gastroenterology and Hepatology: 20 Years and Gaining Momentum. *Journal of Gastroenterology and Hepatology*, **21**, 3-5. https://doi.org/10.1111/j.1440-1746.2005.04235.x

[4] Teufel, A. (2015) Bioinformatics and Database Resources in Hepatology. *Journal of Hepatology*, **62**, 712-719. https://doi.org/10.1016/j.jhep.2014.10.036

[5] Rustgi, V.K., Davis, G.L., Herrine, S.K., McCullough, A.J., Friedman, S.L. and Gores, G.J. (2008) Future Trends in Hepatology: Challenges and Opportunities.

Hepatology, **48**, 655-661. https://doi.org/10.1002/hep.22451

[6] Bliuger, A.F. (1987) Sovremennye problemy klinicheskoĭ gepatologii [Current Problems of Clinical Hepatology]. *Klinicheskaia Meditsina*, **65**, 51-59.

[7] Selyutina, O.Y. and Polyakov, N.E. (2019) Glycyrrhizic Acid as a Multifunctional Drug Carrier—From Physicochemical Properties to Biomedical Applications: A Modern Insight on the Ancient Drug. *International Journal of Pharmaceutics*, **559**, 271-279. https://doi.org/10.1016/j.ijpharm.2019.01.047

[8] Sun, Z.G., Zhao, T.T., Lu, N., Yang, Y.A. and Zhu, H.L. (2019) Research Progress of Glycyrrhizic Acid on Antiviral Activity. *Mini Reviews in Medicinal Chemistry*, **19**, 826-832. https://doi.org/10.2174/1389557519666190119111125

[9] Sato, H., Goto, W., Yamamura, J., Kurokawa, M., Kageyama, S., Takahara, T., Watanabe, A. and Shiraki, K. (1996) Therapeutic Basis of Glycyrrhizin on Chronic Hepatitis B. *Antiviral Research*, **30**, 171-177. https://doi.org/10.1016/0166-3542(96)00942-4

[10] Chen, K., Yang, R., Shen, F.Q. and Zhu, H.L. (2020) Advances in Pharmacological Activities and Mechanisms of Glycyrrhizic Acid. *Current Medicinal Chemistry*, **27**, 6219-6243. https://doi.org/10.2174/0929867325666191011115407

[11] Li, J.Y., Cao, H.Y., Liu, P., Cheng, G.H. and Sun, M.Y. (2014) Glycyrrhizic Acid in the Treatment of Liver Diseases: Literature Review. *BioMed Research International*, **2014**, Article ID: 872139. https://doi.org/10.1155/2014/872139

[12] Ming, L.J. and Yin, A.C. (2013) Therapeutic Effects of Glycyrrhizic Acid. *Natural Product Communications*, **8**, 415-418. https://doi.org/10.1177/1934578X1300800335

[13] Ling, Q., Jin, H., Zheng, J. and Shi, G. (2014) A Meta-Analysis of Diammonium Glycyrrhizinate Enteric-Coated Capsules versus Diammonium Glycyrrihizinate in Patients with Chronic Viral Hepatitis. *Chinese Journal of Hepatology*, **22**, 411-415. https://doi.org/10.3760/cma.j.issn.1007-3418.2014.06.003

[14] Wang, H.N., Liang, S.B., Yao, X.L., Lai, B.Y., Wen, T.Y. and Su, N. (2021) Systematic Review and Meta-Analysis of Efficacy and Safety of Compound Glycyrrhizin Injection in Improving Chronic Hepatitis B Liver Damage. *China Journal of Chinese Materia Medica*, **46**, 694-702. https://doi.org/10.19540/j.cnki.cjcmm.20200705.501

[15] Allen, T.M. and Cullis, P.R. (2013) Liposomal Drug Delivery Systems: From Concept to Clinical Applications. *Advanced Drug Delivery Reviews*, **65**, 36-48. https://doi.org/10.1016/j.addr.2012.09.037

[16] Abu Lila, A.S. and Ishida, T. (2017) Liposomal Delivery Systems: Design Optimization and Current Applications. *Biological and Pharmaceutical Bulletin*, **40**, 1-10. https://doi.org/10.1248/bpb.b16-00624

[17] Rahman, M., Beg, S., Anwar, F., Kumar, V., Ubale, R., Addo, R.T., Ali, R. and Akhter, S. (2017) Liposome-Based Nanomedicine Therapeutics for Rheumatoid Arthritis. *Critical Reviews in Therapeutic Drug Carrier Systems*, **34**, 283-316. https://doi.org/10.1615/CritRevTherDrugCarrierSyst.2017016067

[18] Zhao, Y.Z., Zhang, L., Gupta, P.K., Tian, F.R., Mao, K.L., Qiu, K.Y., Yang, W., Lv, C.Z. and Lu, C.T. (2016) Using PG-Liposome-Based System to Enhance Puerarin Liver-Targeted Therapy for Alcohol-Induced Liver Disease. *AAPS PharmSciTech* **17**, 1376-1382. https://doi.org/10.1208/s12249-015-0427-5

[19] Schwendener, R.A. and Schott, H. (2010) Liposome Formulations of Hydrophobic Drugs. *Methods in Molecular Biology* , **605**, 129-138.

https://doi.org/10.1007/978-1-60327-360-2_8

[20] Patil, Y.P. and Jadhav, S. (2014) Novel Methods for Liposome Preparation. *Chemistry and Physics of Lipids*, **177**, 8-18. https://doi.org/10.1016/j.chemphyslip.2013.10.011

[21] Yamamoto, E., Miyazaki, S., Aoyama, C. and Kato, M. (2018) A Simple and Rapid Measurement Method of Encapsulation Efficiency of Doxorubicin Loaded Liposomes by Direct Injection of the Liposomal Suspension to Liquid Chromatography. *International Journal of Pharmaceutics*, **536**, 21-28. https://doi.org/10.1016/j.ijpharm.2017.11.035