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Studies on Preparation and Properties of Vc Nano Liposomes

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Abstract. Vc nano liposomes were prepared using ascorbic acid (Vc) and glyceryl dilaurate by the ultrasound method. The parameters that affected the encapsulation efficiency of ascorbic acid, such as the ultrasound time, the water temperature, and the ratio of materials, have been investigated by single factor tests. The optimal reaction condition was obtained as follows: the ultrasound time 15 min, the water temperature 0℃, the lipid and cholesterol ratio 2:1, the lipid and drug ratio 10:1. The highest encapsulation efficiency was about 35% at optimal experimental conditions.

Introduction

Diglyceride (DG), a kind of saturated neutral diacylglycerol with amphiphilic structure, can form nano liposome vesicles, which encapsulated hydrophilic, lipophilic and amphipathic drugs. DG is natural oil components, non-toxic, good biocompatibility. It is a kind of ideal material which can be applied to replace the conventional phospholipid nano liposomes [1~2]. Under normal temperature, DG is easy to save, transport and use since it is solid. DG has rapid melting and adsorption rate and good chemical stability when temperatures reached body temperature [3]. Because of uniting well-knit and no pin hole, DG [4~5] is kind of excellent suppository, suitable for extensive industrialization produce.

Ascorbic acid is an indispensable vitamin for the human body. Unfortunately, Vc can't been synthesized in the body. It has important significance that ascorbic acid was encapsulated by nano liposomes, to increase the stability of drugs, to extend the time of drug action, reduce the drug dosage.

In this paper, Vc nano liposomes were prepared using self-made diacylglycerol and ascorbic acid. The microstructure of ascorbic acid nano liposomes was observed using a transmission electron microscope. The parameters that affected the entrapment efficiency of ascorbic acid, such as the ultrasound time, the water temperature, and the ratio of material have been investigated by single factor tests.

Experimental sections

A. Reagents

Glyceryl dilaurate was self-made using lipid and cholesterol. Ascorbic acid (AR) and lipid (AR) were purchased from kermel Chemical Reagent Co., Ltd., China and Shanghai biochemical Reagent Co., Ltd, respectively.

B. Apparatus

TECNAI-10 transmission electron microscopy (Philips Co., Ltd, Netherland) was used to observe the morphology of product. The content of ascorbic acid was measured using double-beam UV-spectrophotometer (TU-1901, PuBei Co., Ltd, China). Ultrasonic washing device (CQ250, China) was used to carry out chemistry reaction.

C. *Preparation method*

Firstly, a certain amount of lipid and cholesterol were thoroughly soluble in chloroform solution in a water bath, which used as oil phase. The aqueous solution containing ascorbic acid was added to the oil phase. Then, the W/O emulsion were formed after ultrasound for a certain of time. Secondly, massive distilled water of zero degrees centigrade was quickly poured into the emulsion with stirring. Finally, milky white suspension was formed after the organic solution was completely volatiled.

D. *Determination of encapsulation efficiency of Vc nano liposomes*

The content of ascorbic acid in supernatant was measured after the milky white suspension was centrifuged. The entrapment efficiency was calculated according to the equation (Eq. 1):

$$E\% = M1 / M2 \times 100\%. \quad (1)$$

Where E%, M_1 and M_2 are encapsulation efficiency, the amount of ascorbic acid encapsulated and the total amount of ascorbic acid added in advance, respectively.

Results and discussion

E. *Particle morphology*

Transmission electron microscopy (TEM) images in Fig. 1 show the typical morphology of the Vc nano liposomes. Spherical, ellipsoidal or round shape particles and a uniform size can be seen. The size of these particulate is about 300 nm in diameter. Vc nano liposomes made by ultrasound have good dispersity, no agglomeration.

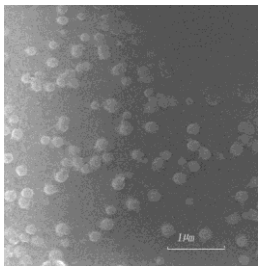

Figure 1. Potographs of Vc liposomes

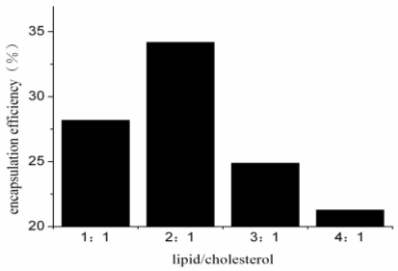

Figure 2. Cholesterol dosage effects on encapsulation efficiency

F. *Influencing factors of encapsulation efficiency*

The factors that affected the encapsulation efficiency, such as the ultrasound time, the water temperature, and cholesterol dosage have been investigated by single factor tests.

G. *Cholesterol dosage*

Lipid and cholesterol was used to prepare glyceryl dilaurate. The mass ratio (lipid/ cholesterol) of mixture used during the reparation was varied from 1:1 to 4:1 for ultrasound 10 min and zero degrees centigrade of distilled water (Fig. 2). When the mass ratio exceeds 2:1, higher cholesterol content have greater encapsulation efficiency. Nevertheless, excessive cholesterol content resulted in asymmetry of product. The kind of asymmetry readily induces loose film, thus encapsulation efficiency become smaller.

H. *Ultrasound time*

Ultrasound time during the reparation was varied from 5 min to 25 min (Fig. 3) for zero degrees centigrade of distilled water and lipid/cholesterol mass ratio 2:1. The encapsulation efficiency gradually increased with time prolonged and reached 33.3% when the ultrasound time was 15 min. Ultrasound time increased continuously. The encapsulation efficiency dropped dramatically and reduced to 8.7% when the ultrasound time was 25 min. According to the experiment result, the optimal ultrasound time was 15 min.

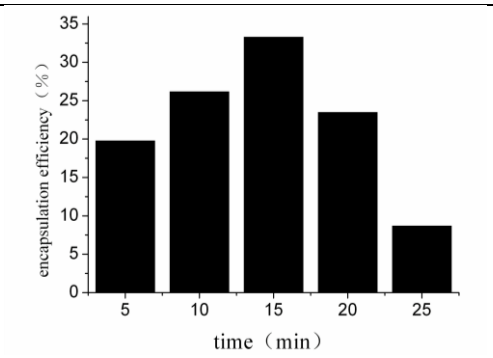

Figure 3. Ultrasound time effects on encapsulation efficiency efficiency

I. Ascorbic acid dosage

Ascorbic acid dosage (mass ratio of lipid/ascorbic acid) was varied from 5:1 to 30:1 (Fig. 4) for zero degrees centigrade of distilled water and lipid/cholesterol mass ratio 2:1 and ultrasound time 15 min. The results showed that, with the lipid and ascorbic acid mass ratio changing, the encapsulation efficiency also had changed in different levels. The encapsulation efficiency increased with the increase of Vc dosage when the lipid/Vc mass ratio exceed 10:1. However, the encapsulation efficiency decreased with the increase of Vc dosage when the lipid/Vc mass ratio less than 10:1. These results promoted us to hypothesize that nano liposome vesicles have limited space and Vc possess saturated sites. We found that some of ascorbic acids precipitated during ultrasound when the nano liposome had too much Vc dosage. Therefore, the encapsulation efficiency decreased to different extent.

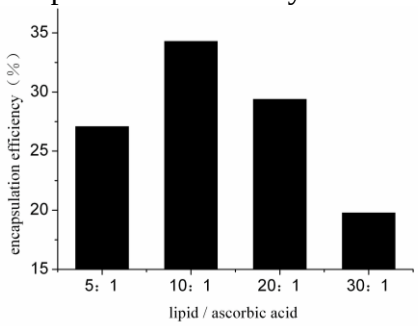

Figure 4. Drug dosage effects on encapsulation efficiency

J. Temperature of aqueous solution

Temperature of aqueous solution was varied from 0 ℃ to 20 ℃ (Fig.5) for lipid/cholesterol mass ratio 2:1 and ultrasound time 15 min. The results show that the encapsulation efficiency decreased with the increase of temperature of aqueous solution. The encapsulation efficiency was about 35.0% and 13.7% at 0 ℃ and 20 ℃ aqueous solutions, respectively. When the temperature of aqueous solution reached 30 ℃, the homogeneous W/O emulsion can't be formed after ultrasound. Thus 0 ℃ was optimum temperature for aqueous solution in this paper.

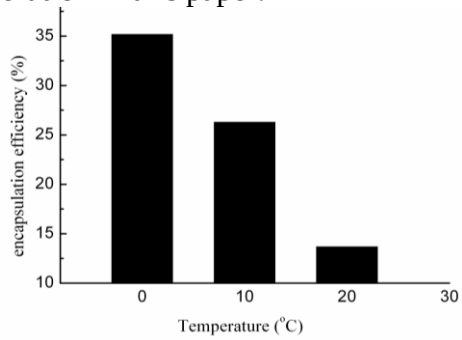

Figure 5. Temperature effects on encapsulation efficiency

Summary

(1) The self-made diacylglycerol, a cheap raw material, was proved that it can be applied in preparing nano liposome drugs carrier with high encapsulation efficiency.

(2) Centrifugal method was used to determine encapsulation efficiency in this paper. The method has the advantages of simple operation, short time. Its disadvantage was difficult to separate from drug adsorbed on liposomes. Multiple centrifugation method was applied in this paper to overcome these shortcomings as far as possible.

(3) The highest encapsulation efficiency was about 35% at optimal experimental conditions. The further study focused on increasing the the encapsulation efficiency.

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