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Studies on a high encapsulation of colchicine by a niosome system

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Abstract

To prepare niosomes which have high encapsulation capacity for soluble drugs, starting from Span 60 and cholesterol, an improved method, evaporation-sonication method, was proposed. The corresponding niosomes show a good stability at least 40 days. Colchicine was chosen as a model drug for examining the capsulation capacity of these niosomes. To obtain the highest encapsulation efficiency, several factors including the structure of surfactant, level of lipid, content of drug and cholesterol were investigated and optimized. The inner cause was also discussed. The results indicate that the Span 60 is the most ideal surfactant among four kinds of Span. Furthermore, the release studies of colchicine and 5-fluorouracil (5-FU) in vitro from niosomes exhibited a prolonged release profile as studied over a period of 24 h. The results demonstrated that niosomes prepared in this way not only have high encapsulation capacity but also is expected that side effects of drugs may be reduced. It still suggests that this method may be used extensively in the field of encapsulation soluble drugs.

Keywords: Drug carrier; Niosome; Colchicine; 5-Fluorouracil; Span 60; Cholesterol

1. Introduction

Drug delivery systems using colloidal particulate carriers such as liposomes (Betageri and Habib, 1994) or niosomes (Schreier and Bouwstra, 1994) have distinct advantages over conventional dosage forms because the particles can act as drug containing reservoirs. Modification of the particle composition or surface can adjust the affinity for the target site and/or the drug release rate, and the slowing drug release rate may reduce the toxicity of drug. So, these carriers play an increasingly important role in drug delivery. Niosomes are now widely studied as an alternative to liposomes because they alleviate the disadvantages associated with liposomes, such as chemical instability, variable purity of phospholipids and high cost (Vora et al., 1998).

To date, different methods have been reported on preparation of niosomes (Azmin et al., 1985; Baillie et al., 1985; Chopineau et al., 1994; Handjani-Vila et al., 1979; Kiwada et al., 1985; Niemec et al., 1994; Talsma et al., 1994; Wallach and Philippot, 1993; Yoshioka et al., 1994). Because

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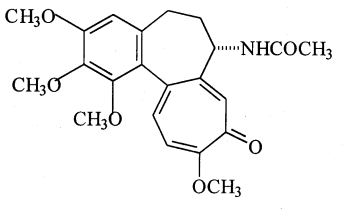
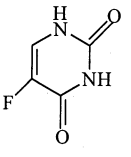
niosomes are capable of encapsulating both hydrophilic and lipophilic drugs, they can serve as effective drug carrier. Though some kinds of niosomes have been studied as drug delivery systems (Chandraprakash et al., 1993; Hunter et al., 1988; Jain and Vyas 1995; Naresh and Udupa, 1996; Ozer et al., 1991; Parthasarathi et al., 1994; Reddy and Udupa, 1993; Uchegbu, 1998; Vanhal et al., 1996), the encapsulation efficiency of soluble solute is very low in some reported niosome systems (Arunothayanun et al., 2000; Azmin et al., 1985; Hu and Rhodes, 1999; Kiwada et al., 1985; Uchegbu and Duncan, 1997; Vyas and Venkatesan, 1999). As expected drug loading is a crucial factor in the formulation of niosomal delivery systems. To improve drug load, the dehydration–rehydration vesicle technique (Kirby and Gregoriadis, 1984) and pH gradients (Mayer et al., 1986) (remote loading procedures) were proposed. Unfortunately, the former method makes niosome size doubled with increased encapsulation efficiency; and the latter one only suits for amine drugs.

Based on the above reasons, the purpose of the current study was to develop a niosome preparation procedure with a high soluble drug loading capacity. Span 60 and cholesterol (CH) were selected as components of niosome and colchicine (chart 1a) as a model drug. Many factors that may affect drug entrapping have been tested and optimized. Additionally, the release studies of colchicine and 5-fluorouracil (5-FU) (chart 1b) in vitro from niosomes were performed as well. The probable inner causes for encapsulation and release were discussed based on experimental results.



(Chart 1a)



(Chart 1b)

2. Material and methods

2.1. Materials

Colchicine, purity > 97% (HPLC), was product of Serva. 5-FU (USP Grade) was purchased from (Amresco, USA) and used as received. Dicetyl phosphate (DCP) was obtained from Sigma (USA). Sorbitan monolaurate (Span 20), monopalmitate (Span 40), monostearate (Span 60), mono-oleate (Span 80), used as received. Cholesterol (CH) was purchased from Beijing Chemical Reagent Company. Dialysis membrane tube (MW cut off 8000–10000) was supplied from Sino-American Biotechnology Company and was treated before using according to the reported method (Fenton et al., 1997). All other reagents were of analytical grade. Double-distilled water was used throughout the study.

2.2. Apparatus

A UV-265 spectrophotometer (Shimadzu, Kyoto, Japan), equipped with 10 mm quartz cell, was employed in absorbance measurements. A RE-52A rotary evaporator (Yarong Biochemistry apparatus factory, Shanghai, China) and an ultrasonic clearer (Kunshan, Jiangsu, China) were used to prepare niosomes. pH values were measured with an 821 acidometer (Zhongshan Univ., Guangzhou, China).

2.3. Preparation of niosomes

Niosomes were prepared by evaporation–sonication method. Appropriate amounts of Span that dissolved in cosolvent of ethanol and chloroform (1:4) and CH (in chloroform) were added in a 100 ml round-bottom flask. The organic solvents were removed by a rotary vacuum evaporator at 60 °C until the film was dried completely. The dried film was hydrated with 10 ml of colchicine solution (or water) by sonication in a water bath at 60 °C for 1 h. The resulting solution was then left to cool to obtain colchicine-containing niosomes (or blank niosomes). 5-FU-containing niosomes were made in the same way.

2.4. Entrapment efficiency

Niosome–entrapped colchicine could be separated from the free drug by dialysis method. The prepared niosomes were filled into dialysis bags and the free colchicine dialyzed for 24 h into 100 ml of phosphate buffer saline (PBS, pH 7.4). The absorbance (A) of the dialysate was measured at 350 nm against PBS buffer, and the absorbance (A_0) of the corresponding blank niosome was measured under the same condition. The concentration of free colchicine could be obtained from absorbance difference ($\Delta A = A - A_0$) based on standard curve. Standard curve was made by measuring absorbance at 350 nm for known concentrations of colchicine solution. The entrapment efficiency of the drug was defined as the ratio of the mass of niosome-associated drug to the total mass of drug. The encapsulation efficiency of 5-FU was determined based on the same method.

2.5. Drug release study

Release of colchicine from niosomes in vitro was performed according to Hu's method with minor modification (Hu and Rhodes, 1999). Dialysis tube containing appropriate volume of colchicine loaded niosome dispersion was placed into a flask containing 100 ml simulated gastric fluid (or simulated intestinal fluid). The flask was placed in a shaker, and shaked at 50 rpm at 37 °C. Aliquots of the dialysate were taken at predetermined time and replenished immediately with the same volume of fresh simulated fluid. Withdrawn samples were assayed spectrophotometrically at 350 nm. At the end of the experiment, 0.5 ml of absolute ethanol was added into the dialysis bag to disrupt the niosomes. An aliquot of dialysate now was sampled to determine the concentration corresponding to 100% release. The fraction of colchicine released at a specific time was determined by comparing the absorbance intensity for the sample to that measured for the 100% release samples. Release of free drug was studied in the same way.

3. Results and discussion

3.1. The influence of surfactant structure on niosomes' properties

A series of niosomes of Span and CH (1:1) was prepared at a same total lipid concentration to investigate the influence of surfactant structure on niosome properties. The encapsulation of different formulations of niosomes is listed in Table 1. Entrapment efficiency in Span 60 was the highest among all of Span formulations. This could be attributed to the structure of surfactant. As is known to all, Span 20, Span 40, Span 60 has the same head group and different alkyl chain. Among these surfactants, only Span 80 has an unsaturated alkyl chain. The introduction of double bonds made the chains bend. This means that the adjacent molecular can not be tight when they form the membrane of niosome. These cause the membrane to be more permeable, which possibly explains the lowest entrapment efficiency of the Span 80 formulation. As for the other three kinds of Span non-ionic surfactants, Span 60 has the longest saturated alkyl chain and shows the highest entrapment. The encapsulation efficiency follows the trend C16 (Span 60) > C14 (Span 40) > C12 (Span 20). It suggests that the length of alkyl chain is a crucial factor of permeability and long chain products high entrapment. The Span 60 was the selected surfactant in the further experiment.

3.2. Effect of cholesterol

With total concentration of lipid fixed at 2×10^{-4} mol/l, niosomes were prepared by changing the ratio of surfactant to CH to test the effects of

Table 1
Surfactant structure on niosomes encapsulation efficiency (EE ± S.D.) (%)

Span	Span 20	Span 40	Span 60	Span 80
EE	88.6 ± 3.0	91.1 ± 2.7	99.0 ± 2.0	71.8 ± 1.8

The concentration of surfactant and colchicine were 2×10^{-4} and 6×10^{-5} mol/l.

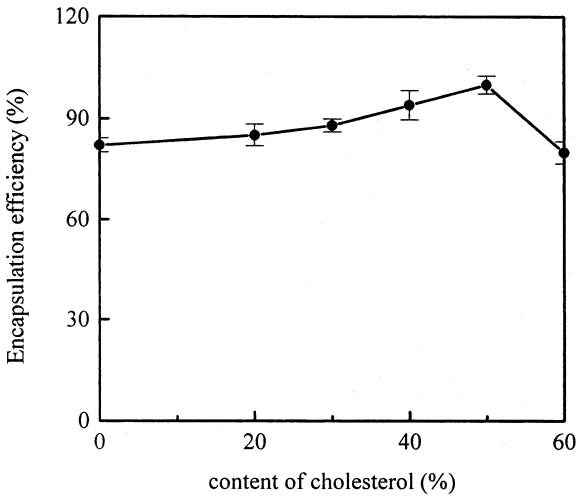


Fig. 1. Influence of cholesterol concentration on entrapment efficiency. Concentration of colchicine is 6×10^{-5} mol/l, total lipid content is 2×10^{-4} mol/l.

different amounts of CH. Cholesterol (CH) is one of the common additives included in the formulation in order to prepare stable niosomes. It is also the essential component in niosome formulation in this study. CH is known to abolish the gel to lipid phase transition of niosome systems (Cable, 1989), which could be able to effectively prevent leakage of drug from niosomes (Rogerson et al., 1987). In this study, niosome without CH has certain encapsulation and the quantity of drug entrapped is increased with increasing CH content. Formulation of the niosome with molar ratio of 1:1 is most beneficial for the efficient encapsulation, and extra CH is unfavorable (Fig. 1). It implies that equal molarity of non-ionic surfactant and CH can make the membrane compact and well organized.

3.3. Impact of surfactant concentration

The experiment indicates that surfactant quantity has an impact on entrapment. Influence of levels of surfactant was examined by altering the total concentration of surfactant meanwhile keeping the other factors invariable. Fig. 2 shows that the concentration of surfactant from 8×10^{-5} to 3.2×10^{-4} mol/l is good for drug entrapment. Low drug entrapment efficiency may be attributed to the small number of niosomes produced by dilute surfactant.

3.4. Effect of colchicine content

Since niosome may be an effective tool for reducing toxicity of drug, colchicine was selected as a model drug in this study. Colchicine has been used in treatment of acute gouty arthritis. However, drug therapy with this agent is associated with several side effects, such as gastrointestinal effect, shock, watery stool or bloody stool, even bone marrow suppression. These adverse drug effects have limited its use.

The influence of drug concentration on encapsulation was examined by varying the amount of colchicine added while keeping the total level of lipid at 2×10^{-4} mol/l. It can be seen that niosomes prepared in this study show a good encapsulation capacity (Fig. 3). With the increasing concentration of solute, the ratio of drug loaded to total lipid shows an increasing trend and reaches 1:1 when concentration of colchicine is 4×10^{-4} mol/l. The ratio keeps constant with more colchicine added. It indicates that the fixed amount of lipid produces the constant number of niosome and has a definite encapsulating capacity. Taking into account hydrophilic solutes, the pro-

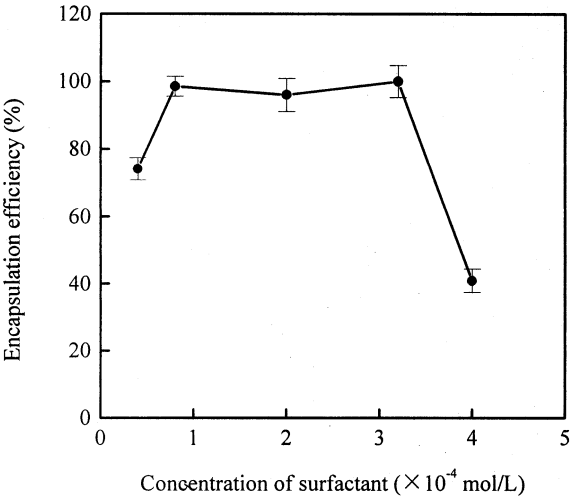


Fig. 2. Influence of total lipid concentration on entrapment efficiency. Concentration of colchicine is 6×10^{-5} mol/l.

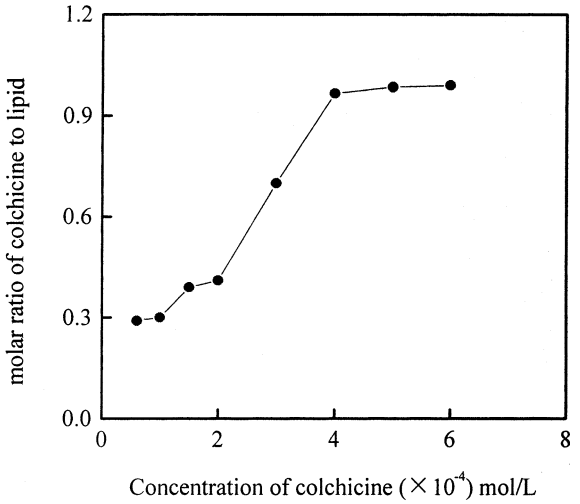


Fig. 3. Molar ratio of colchicine entrapped to total lipid. Concentration of lipid is 2.0×10^{-4} mol/l.

posed method shows a higher encapsulation capacity than some reported ones (Arunothayanun et al., 2000; Azmin et al., 1985; Hu and Rhodes, 1999; Kiwada et al., 1985; Uchegbu and Duncan, 1997; Vyas and Venkatesan, 1999).

3.5. Encapsulation capacity about the two drugs

Under the same preparation condition, same level of 5-FU and colchicine were added with an aim to test the effect of molecular weight on entrapment. The results show that when the content of the solute is 1×10^{-4} mol/l, the encapsulation of these two drugs shows no obvious difference.

Based on the molecular weight of 5-FU and colchicine, it seems that colchicine should have higher encapsulation efficiency. However, similar encapsulation efficiency was achieved. This may be a result of their weight and interaction between drugs and membrane of niosome. The membrane of niosome is made up of Span 60 and CH, both Span 60 and CH have hydroxyl groups. Although 5-FU has a small molecular weight, it possesses two amides, while relative large colchicine has only one amido group. So when hydrogen bonds exist between membrane and these two drugs, the hydrogen bond between 5-FU and membrane is stronger than that of colchicine. From the spectrum study (Section 3.7), it also indicates that a weak interaction exists between drug and membrane. Considering the molecular weight and interaction between solutes and membrane, the encapsulation efficiency of 5-FU and colchicine has no distinctive difference.

3.6. Stability of the system

The encapsulation efficiency of this system after storage for 40 days at ambient temperature has no distinctive difference from that of a freshly prepared sample. The system in this study shows relatively good stability without dicetylphosphate.

3.7. Investigation interaction between niosome membrane and colchicine

The interaction between niosome membrane and colchicine could be seen from two aspects. Characteristics of absorption spectra show that the absorption peak (350 nm) of colchicine after entrapped in niosome has a slight shift towards the short wavelength region till it reached 341 nm. Compared with the free colchicine, there is about 9 nm alteration. This variation may be attributed to a weak interaction between niosome membrane and colchicine.

On the other side, the niosome shows a spontaneous encapsulation capacity and can entrap a certain amount of colchicine after 1 week's store when putting the blank niosome and free colchicine solution into a 10 ml colorimetric tube. It also suggests that weak interaction exists between niosome membrane and colchicine. This is in agreement with the assumption obtained from absorption spectrum changes.

3.8. Effect of other factors

It is found that the hydration temperature has an influence on encapsulation. When the hydration is performed at room temperature, the drug loaded is less than that at 60 °C. The hydration temperature used to make niosomes should usually be above the gel to liquid phase transition temperature of the system. If the hydration proce-

dure is performed below this special temperature may be the transition of the gel to liquid phase could not take place properly and produces some defective niosomes.

DCP, a charged molecule, is often used to prevent niosome aggregation (Cable, 1989) and increase the stability of niosome dispersions (Gianasi et al., 1997). In this study, addition of DCP causes a small increase in encapsulation. Therefore, niosomes were prepared without DCP because of a small increase in this experiment.

3.9. Release of colchicine and 5-FU in vitro

The release rate of colchicine from niosome preparations in simulate intestinal fluid was significantly slowed down compared with free solution (Fig. 4). After 3 h, the released drug reaches the highest and keeps constant for 5 h. By 12 h, about 64% of colchicine were released from free solution, only 12% released in the niosome system. In simulated gastric fluid (Fig. 5), 56% was released in free solution while 15% was released from the niosome system by the end of the experiment.

The release rate of 5-FU from niosome preparations in simulated fluid is significantly lower

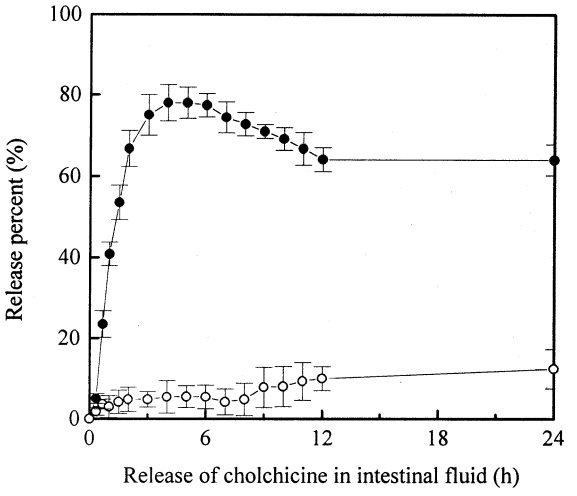


Fig. 4. Release of colchicine (1.0×10^{-4} mol/l) in simulated intestinal fluid from colchicine free solution (●) and from niosome system (○). All data were obtained with 2.0×10^{-4} mol/l niosome.

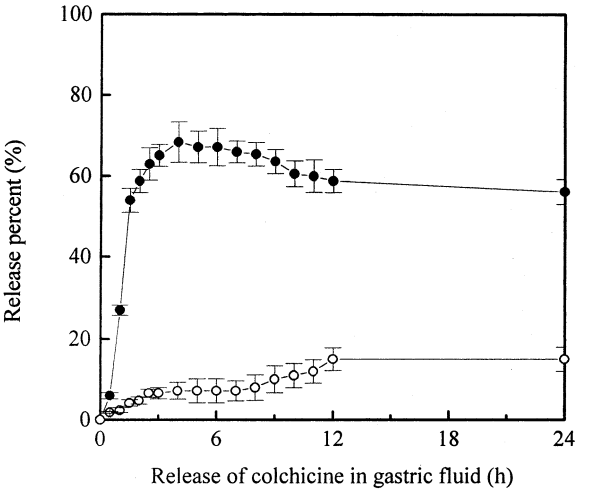


Fig. 5. Release of colchicine (1.0×10^{-4} mol/l) in simulated gastric fluid from colchicine free solution (●) and from niosome system (○).

than that of the free solution. In simulate intestinal fluid (Fig. 6), about 52% of solute was released from free solution while only 13% of 5-FU released from niosomal formulation when the test is over. The release in simulated gastric fluid follows the same trend (Fig. 7).

The release of 5-FU from niosomes is the same as that of colchicine. Their molecular weight and

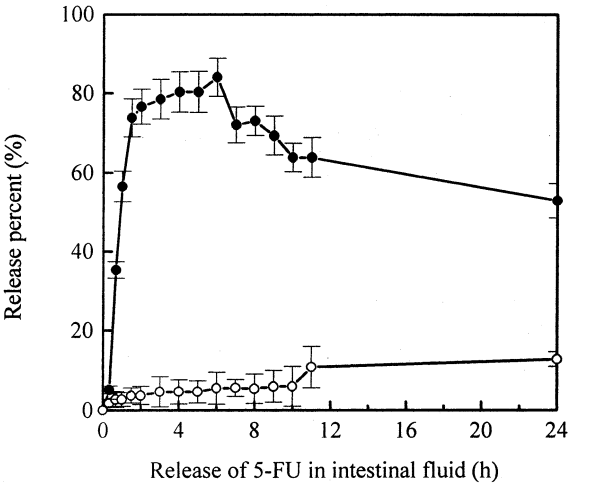


Fig. 6. Release of 5-FU (1.5×10^{-4} mol/l) in simulated intestinal fluid from 5-FU solution (●) and from niosome system (○).

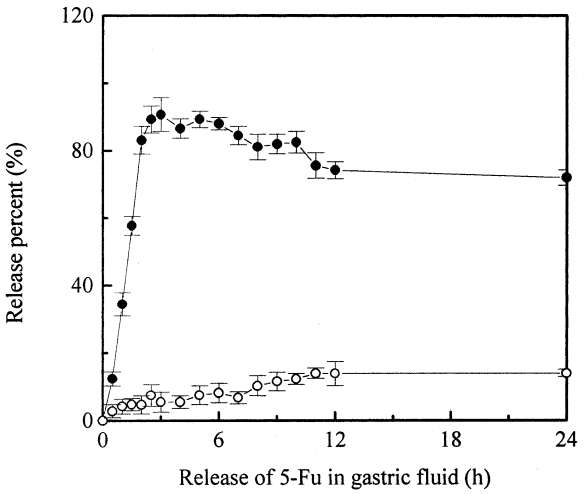


Fig. 7. Release of 5-FU (2.0×10^{-4} mol/l) in simulated gastric fluid from 5-FU solution (●) and from niosome system (○).

interaction between them and membrane also play an important role in release experiments.

The release experiments clearly indicate that the amount of drug released from niosome formulations is effectively retarded. It can be expected that the toxic side effects of both drugs be reduced.

4. Conclusion

It is known that little soluble drug can be entrapped in vesicles (Arunothayanun et al., 2000; Azmin et al., 1985; Brandl et al., 1997; Hu and Rhodes, 1999; Kiwada et al., 1985; Uchegbu and Duncan, 1997; Vyas and Venkatesan, 1999). In this study, an improved niosome preparation method has been proposed for soluble drugs with high encapsulation capacity using Span and CH as initial materials. Several factors were optimized using colchicine as a model drug to obtain high encapsulation efficiency. The results indicate that the best encapsulation efficiency can be obtained when niosomes are made up of Span 60 and CH at molar ratio of 1:1, and concentration of surfactant is kept at the range of 8.0×10^{-5}–3.2×10^{-4} mol/l. Under optimized conditions, some studies were further performed using colchicine and 5-FU as model drugs. Though molecular weight of colchicine is larger than that of 5-FU, the results demonstrated that both 5-FU and colchicine can be encapsulated without obvious differences at rather high encapsulation efficiency. The probable inner causes such as molecular weight and hydrogen bonds is further discussed. Released studies in vitro show that these niosomes can sustain release of both 5-FU and colchicine effectively comparing with their free solution. It can be expected that the toxic side effects of them be reduced. Totally, the proposed niosomes preparation may be a promising candidate and could be used with a potential application.

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