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Optimization of madecassoside liposomes using response surface methodology and evaluation of its stability

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ABSTRACT

Polar compounds with large molecular weight have poor membrane permeability, liposomes can promote drugs to penetrate epidermis and remain or release at dermis. Madecassoside (MA) exhibits powerful potency in treatment of skin disorders such as wound healing, scar management, and psoriasis, but it is not easy to penetrate epidermis for its hydrophilic nature. The aim of this work is to get the optimum process conditions and evaluate physicochemical properties and physical stability of MA liposomes. In order to avoid this disadvantage and maintain long term drug storage, MA Liposomes were designed to achieve optimum preparation conditions using response surface methodology (RSM) in our experiment. The process and formulation variables were optimized by achieving maximum drug encapsulation efficiency. The optimum conditions were 0.4424 g of madecassoside, 8.174 of ratio of egg yolk lecithin to cholesterol, 65 s of ultrasonic time. The results of particle size, zeta potential and encapsulation efficiency of madecassoside liposomes were 293 nm, −35.6 mV, and 40.90%, respectively, on the basis of the above optimum conditions. According to the morphology of liposomes and encapsulation efficiency of triplicate experiments conducted at optimum conditions, MA liposomes obtained by this optimized formulation had characters of favorable repeatability and proper particle size. The physical stability tests of MA liposomes indicated that its suitable storage temperature was at 4 °C with higher encapsulation efficiency.

1. Introduction

Madecassoside (MA, $C_{48}H_{78}O_{20}$, Fig. 1) is a triterpenoid saponin existed in *Centella asiatica*, a stoloniferous perennial herb, which grows commonly in the damp areas in China, Southeastern Asian, India, and Africa (Randriamampionona et al., 2007; Inamdar et al., 1996). MA was reported to be the highest content ingredient among the main bioactive triterpene constituents of *C. asiatica* (Liu et al., 2008).

Previous studies demonstrated that MA possessed various biological effects on skin disorders including anti-psoriasis, anti-inflammatory, skin ageing prevention, and wound healing (Haftek et al., 2008; Lee et al., 2012). These therapeutic effects might be related to protection against lipid peroxidation, enhancement of collagen synthesis and expression, and promotion of angiogenesis in wound skin tissues (Li et al., 2009; Jin et al., 2013).

In order to exert sufficient effects against skin disorders, it is necessary for the drug to have a hydrophobic nature which would facilitate the penetration of the drug into the epidermis and maintenance of the therapeutic concentration for a certain time in the local skin tissues. However, the glycoside chain group and several hydroxyl substitutes render polarity to MA, which is a major impediment in epidermal penetration. Polar chemicals with large molecular weight have poor membrane permeability, MA wrapped in the lipid bilayer of liposomes was expected to avoid some external uncertainties damage, such as light, air, acid, and alkali, which might improve membrane permeability and the stability of liposomes, sustain more drugs to stay from epidermis to dermis. Until now, there were no report and patent about formulation research on avoiding the hydrophilic disadvantage of MA to yield selected membrane permeability, considerable stability and controlled release, so MA liposomes were designed and preformed by response surface methodology (RSM) to get

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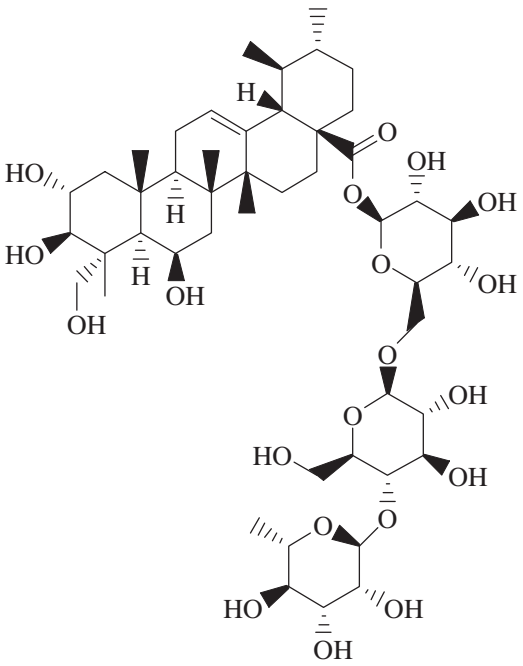

Fig. 1. Chemical structure of MA.

optimization of process conditions and favorable particle size for transdermal characters in our experiments.

Hydration of phospholipids, control of appropriate particle size, and drug loading were three main key processes in liposomes procedure. Optimization of process conditions is one of the strictest stages in the development of an efficient and economic bioprocess. Response surface methodology (RSM) was a powerful statistical mathematical model with a collection of statistical techniques and always used to optimize process conditions. Wherein, interactions between multiple processes variables can be identified with fewer experimental trials. In our present study, MA liposomes prepared by film-dispersion methods were investigated with particle size, zeta potential, and encapsulation efficiency. We optimized the preparation conditions and repeated the optimal process formulations to obtain liposomes with high encapsulation efficiency by RSM. In addition, the effects of three important factors on encapsulation efficiency of MA liposomes were studied in detail. On the basis of the regression model and three-dimensional plots, we optimized the formulation to obtain liposomes with high encapsulation efficiency and proper particle size.

2. Materials and methods

2.1. Materials

Egg yolk lecithin (72–85%) was purchased from Guangzhou Hanfang Pharmaceutical Co., Ltd., cholesterol and sodium dihydrogen phosphate were purchased from Sinopharm Chemical Reagent Co., Ltd., vitamin E acetate was purchased from Tongxiang Tianhecheng Food Science and Technology, double distilled water was used in all experiments, and all other chemicals were of analytical grade.

2.2. Preparation of liposomes

MA liposomes were prepared by film-dispersion method (Traikia et al., 2000). Briefly, MA liposomes were obtained by mixing phospholipid and cholesterol at the desired ratio in chloroform. Then chloroform was removed by rotary vacuum evaporation at 32 °C. Residual organic solvent was removed by pumping in a vacuum for 3–4 h. Lipid film was then hydrated with a certain mass of MA's phosphate buffer solution (PBS, pH 7.0), the final concentration of lipid mixture was 50 mg/ml. Lipid mixture was homogenized by water bath ultrasonic for 65 s to form well-distributed liposomes. Ultimately, the resulting suspensions of MA liposomes were stored at 4 °C (Zhao et al., 2012). The neat liposomes were prepared by the same way as MA liposomes except for adding MA.

2.3. Response surface methodology (RSM)

Based on the single-factor experiments, central composite design (CCD) in RSM experiment involved three different factors, namely mass of MA (m), ratio of egg yolk lecithin to cholesterol (w/w), ultrasonic time (s). Among the total of 15 experimental trials in CCD, the first 12 were organized in a factorial design, and experimental trials from the 13th to the 15th were the replications of central points.

Once the experiments were performed, a second order polynomial Eq. (1) shown below was used to describe the effect of variables of liner, quadratic and cross product.

$$Y = \mu_0 + \sum_{i=1}^{\lambda} \mu_i X_i + \sum_{i=1}^{\lambda} \mu_i X_i^2 + \sum_i \sum_j \mu_{ij} X_i X_j \quad (1)$$

Y is the encapsulation efficiency, while μ is regression coefficient, λ is the number of factors studied and optimized in the experiment. Fit quality of the second order equation was expressed by coefficient of R^2, and its statistical significance was determined by F-test. The significance of each coefficient was determined using Student's t-test. The coefficients of the equation were determined by employing SAS 9.0 software. Analysis of variance (ANOVA) for the predictive equation was analyzed using the same software package.

2.4. Physiochemical characterization of MA liposomes

2.4.1. Encapsulation efficiency of MA liposomes

Dialysis method (Fan et al., 2013) was used in assay of encapsulation efficiency of MA liposomes. 2 ml of liposomes suspension was placed in a dialysis tube (MWCO 14,000) and sealed tightly. Then, the tube was immersed in 200 ml of water used as release medium. The release medium was stirred at 480 rpm using a magnetic stirrer. Samples (2 ml) were taken from the release medium at 11 h. Concentration of MA was determined by HPLC (Agilent 1100, USA) after being appropriately diluted by adding water. The total and free amounts of MA were signed C_0 and C_i, respectively. Encapsulation efficiency (EE%) was calculated as followed:

$$\mathrm{EE}\% = \left(1 - \frac{C_i}{C_0}\right) \times 100\% \quad (2)$$

2.4.2. Determination of particle size and zeta-potential of liposomes

Average size and zeta-potential of MA liposomes and neat liposomes were measured using Malvern zeta-size Nano ZS (Malvern Instrument Ltd., UK). The samples with appropriate concentration were measured at 25 °C in triplicate to yield mean value.

2.4.3. Scanning electron microscopy (SEM) of MA liposomes

Liposomes prepared at the optimum conditions were diluted appropriately and placed on a glass slide, and air-dried at room