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Nanostructured lipid carriers for oral delivery of baicalin: *In vitro* and *in vivo* evaluation

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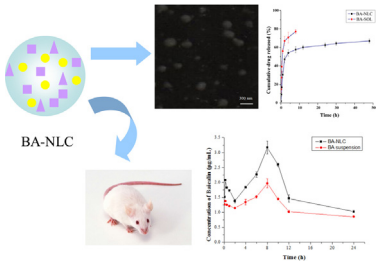
HIGHLIGHTS

- Emulsion–evaporation and low temperature-solidification method was used to prepare baicalin nanostructured lipid carriers.
- Uniform design was utilized for formulation optimization.
- Enhanced drug loading and sustained release were realized by nanostructured lipid carriers.
- The oral bioavailability of baicalin was improved remarkably by nanostructured lipid carriers.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study aimed to develop a novel baicalin-loaded nanostructured lipid carrier (BA-NLC) system for oral delivery to enhance the bioavailability. BA-NLC were prepared by emulsion–evaporation and low temperature-solidification technique and optimized by a five-factor four-level uniform design. The characteristics of BA-NLC including morphology, particle size, zeta potential, entrapment efficiency and drug loading were investigated. The results showed that the optimized BA-NLC was nearly spherical in shape with a mean diameter of 244.7 nm. The entrapment efficiency and drug loading were 59.51 ± 0.57% and 3.54 ± 0.11%, respectively. *In vitro* drug release revealed a pattern with burst release initially and sustained release afterwards for BA-NLC. Moreover, BA-NLC exhibited prolonged MRT and increased AUC compared to pure BA. All the detailed evidence indicated that BA-NLC could be a potential delivery system for the oral administration of BA.

1. Introduction

Baicalin (BA, 7-D-glucuronic acid, 5,6-dihydroxy flavone, Fig. 1) is a flavonoid component isolated from the root of the traditional Chinese medicinal herb *Scutellaria baicalensis* Georgi, which is widely used in the treatments of pneumonia, viral hepatitis, cardiovascular disorders and cancer [1]. According to recent reports, BA possesses a lot of pharmacological and biological effects such as antioxidative [2], anti-inflammatory [3], anticancer [4], antileukemic [5] and antiproliferative activity [6]. Nevertheless, the poor lipid and water solubility of BA results in low oral bioavilibility and limits the clinical application. In order to improve the dissolution and bioavailability, nanocrystal, solid dispersions, emulsions, liposomes, phospholipid complex and nanoparticles were investigated, but new formulations using different technologies should be developed for better application [7,8].

In the beginning of the 1990s, solid lipid nanoparticles (SLN), consisting of solid lipid, were developed as a drug delivery system due to the unique advantages including good biocompatibility and biodegradability, improved solubility, high bioavailability,

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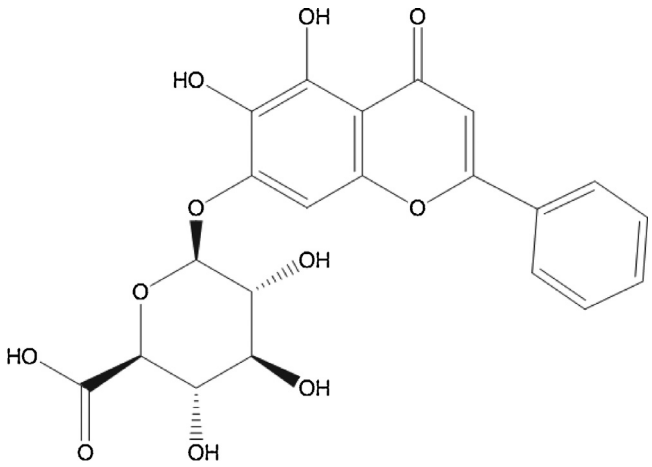

Fig. 1. Chemical structure of baicalin.

controlled drug release, and good stability. The mean particle diameter of SLN is between 50 and 1000 nm. However, the application is limited owing to the restricted loading capacity and the expulsion during storage caused by crystallization of lipid matrix [9,10]. In order to overcome this shortage, a new colloidal delivery system named nanostructured lipid carriers (NLC) were developed on the basic of SLN. NLC, as the second-generation of lipid nanoparticles, consist of the mixture of solid lipid and liquid lipid. They not only possess the outstanding advantages of SLN, but also avoid the disadvantages such as drug leakage and low entrapment efficacy, which can be attributed to the incorporation of liquid lipid that breaks the ordered crystalline state of solid lipid and enlarges drug storage space [11,12].

The development of NLC has attracted more and more attention in the pharmaceutical field during recent years. Currently NLC delivery system is regarded as one of the most promising drug carriers and administrated in many routes for peroral, dermal, ocular, and pulmonary applications. Furthermore, NLC can achieve passive targeting property by altering particle size and acquire active targeting characters by modification of proper materials [13,14]. They can also be PEGylated to realize long circulation [15]. Therefore, incorporation of baicalin into NLC may be an ideal formulation for the efficient oral administration of baicalin.

Several methods for the preparation of NLC have been developed in the last decade, including microemulsion method, solvent diffusion method, solvent evaporation method, film dispersion-ultrasonic method and high pressure homogenization (HPH) method [16,17]. Each of the above techniques has its own disadvantages, such as the need for sophisticated equipment, the high operative temperature or high pressure, the utilization of toxic solvent, *etc*. Based on the traditional methods, emulsion–evaporation and low temperature-solidification technique with simple operation, mild condition, and low energy consumption is developed, which includes emulsification at a high temperature and solidification at 0 °C.

In order to enhance the physical and chemical stability of NLC systems, water should be removed. Methods employed for solidification mainly include freeze drying and spray drying. Freeze drying is more frequently used due to the relatively high yield and low residual moisture content [18,19].

A successful strategy used to evaluate and optimize the formulation parameters in an efficient way is very important. Uniform design (UD), supported by statistical software, is a well-designed method for pharmaceutical formulation development and optimization, which could obtain lots of useful information by a few experiments [20]. UD is utilized to explore the variables influencing the physicochemical characterizations of NLC so as to acquire the maximum drug loading.

In the present work, baicalin-loaded nanostructured lipid carriers (BA-NLC) were prepared by the emulsion–evaporation and low temperature-solidification method and the formulations were well designed by UD. Glycerol monostearate (GMS) and medium chain triglyceride (MCT) were selected as the solid and liquid lipid materials, respectively, with soybean lecithin and poloxamer 188 as emulsifiers. The physicochemical properties of BA-NLC such as morphology, particle size, zeta potential, entrapment efficacy and drug loading were evaluated in detail. Additionally, *in vitro* release and *in vivo* pharmacokinetics were studied.

2. Materials and methods

2.1. Materials

Baicalin was purchased from Shanghai Dibo Chemical technology Co. Ltd., China. Poloxamer 188 (F68) was obtained from Sigma (USA). Soybean lecithin (injection grade) was provided by Shanghai Taiwei Medicine Co. Ltd., China. Glycerol monostearate (GMS) was obtained from Tianjin Damao Chemical agent Co. Ltd., China. Medium chain triglyceride (MCT) was purchased from Croda (Singapore). The methanol (Tianjin Siyou Co., Ltd., China) was of high performance liquid chromatography grade. All the other reagents were of analytical grade.

2.2. Preparation of BA-NLC

The NLC were prepared by emulsion–evaporation and low temperature-solidification method [21]. In brief, GMS, MCT, soybean lecithin (SL) and BA powder were weighed accurately and mixed, then dissolved into adequate ethanol absolutely in a water bath at 75 °C to obtain the organic phase. 10 mL of aqueous solution containing right amount of F68 was heated at the same temperature. Then, the organic phase was injected dropwise into the aqueous solution under a mechanical stirrer (ETS-D4, IKA, Germany) in a water bath for 4 h. The resultant thermal nanoemulsion was dispersed rapidly into 20 mL of distilled water (0–2 °C) in ice bath with stirring at 1000 rpm for 2 h. Consequently, the BA-NLC dispersions were achieved from the supernatant after centrifugation at 3500 rpm for 15 min.

For long term stability, BA-NLC dispersions were freeze-dried. In the freeze-drying process, mannitol (5%, m/v) was utilized as cryoprotectant. First, the NLC dispersions were pre-frozen with an ultra cold freezer (DW-86L, Haier, China) at −80 °C for 24 h. Then, the samples were freeze-dried at −50 °C for 48 h utilizing a freeze dryer (FD-1000, EYELA, Japan). The obtained BA-NLC powders were gathered for further using.

2.3. Optimization of BA-NLC formulation

In order to optimize the formulation of BA-NLC, the uniform design (UD) tests were used after choosing the most important factors influencing the physicochemical properties of the produced BA-NLC. A five factors and three levels UD was developed to probe the optimum levels of these variables. The five factors included the total amount of lipid (g, X_1), the weight ratio of liquid lipid to solid lipid (w/w, X_2), the amount of SL (g, X_3), the amount of F68 (g, X_4), and the rate of stirring (rpm, X_5), which were proved to be the most influential factors by single factor experiments. The DL was taken as an index to evaluate the formulations. The factors and levels of the uniform design were shown in Table 1. All tests were performed in triplicate.