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Development and optimization of baicalin-loaded solid lipid nanoparticles prepared by coacervation method using central composite design

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ABSTRACT

The objective of this study was to design and optimize a novel baicalin-loaded solid lipid nanoparticles (SLNs) carrier system composed of a stearic acid alkaline salt as lipid matrix and prepared as per the coacervation method in which fatty acids precipitated from their sodium salt micelles in the presence of polymeric nonionic surfactants. A two-factor five-level central composite design (CCD) was introduced to perform the experiments. A quadratic polynomial model was generated to predict and evaluate the independent variables with respect to the dependent variables. The composition of optimal formulation was determined as 0.69% (w/v) lipid and 26.64% (w/w) drug/lipid ratio. The results showed that the optimal formulation of baicalin-loaded SLN had entrapment efficiency (EE) of 88.29%, particle size of 347.3 nm and polydispersity index (PDI) of 0.169. The morphology of nanoparticles was found to be nearly spherical in shape by scanning electron microscopy (SEM) observation. The differential scanning calorimetry (DSC) analysis indicated that the drug incorporated into SLN was not in an amorphous form but in a crystalline state. The C_{max}, *MRT*, $AUMC_{0\to\infty}$ and $AUC_{0\to\infty}$ values of SLN were approximately 1.6-fold, 1.9-fold, 5.0-fold and 2.6-fold greater than that of reference preparation, respectively.

1. Introduction

Solid lipid nanoparticles (SLNs) are particles with a mean diameter between 50 and 1000 nm, which were developed at the beginning of the 1990s as a drug delivery system in order to overcome the drawbacks associated with the traditional colloidal dispersions (Muller et al., 2000). The development of SLN has attracted an increasing attention in pharmaceutical fields during recent years owing to the excellent tolerability and advantages compared to liposomes or other polymeric nanoparticles (Attama, 2011). Currently SLN has been considered as one of the most promising drug carriers and administrated in many routes for dermal, peroral, parenteral, ocular, pulmonary applications. Several techniques for SLN fabrication have been developed in the last decade, including high-pressure homogenization, microemulsion techniques, and solvent emulsification evaporation methods (Zhang et al., 2009). Each of the mentioned techniques has its own disadvantage, such as the need for technically sophisticated equipment, the high operative temperature, or the use of toxic organic solvent (Corrias and Lai, 2011). The coacervation method is another novel, solvent-free technique that has been reported recently in the literature for the preparation of SLN (Battaglia et al., 2010). This method depends on slowly neutralization of fatty acids alkaline salts with an acidic solution (coacervating solution) in the presence of different stabilizing agents. When the pH of the micellular solution of fatty acid salts is lowered, precipitation of solid lipid micelles consisting of fatty acids occurs due to proton exchange between the acid solution and the soap (Bianco et al., 2010). By offering a feasible and accessible method, at present this technique has opened a promising approach to circumvent the forementioned shortcomings in SLN preparation process.

A coherent strategy for successful evaluation and optimization of the formulation parameters in an efficient approach is necessary. It is generally accepted that the property of SLN is under the influence of the relative amount of lipid and ratio of solid lipid to drugs in the formulation. However, a systematic investigation of the simultaneous influence of multiple formulation variables on the SLN prepared by means of coacervation method has not yet been undertaken. Therefore the objective of the present study was to probe the different variables affecting the physicochemical characterizations of SLN in order to obtain the maximum entrapment efficiency (EE) and the smallest particle size as possible.

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Response surface methodology (RSM), supported by statistical software, is a well-established approach for pharmaceutical formulation development and optimization allowing extraction of maximal information out of few well-designed experiments. Central composite factor design (CCD), one of the techniques in RSM, is suitable for pharmaceutical blending problems allowing investigation with the least number of experiments and selection of the optimal composition for achieving the presetting target (El-Malah et al., 2006). CCD has been extensively adopted to optimize the nanoparticulate formulations (Gonzalez-Mira et al., 2011; JIN et al., 2008). Those studies demonstrated the apparent advantage of CCD utilization in the formula optimization process for drug delivery system design.

Baicalin (7-D-glucuronic acid, 5,6-dihydroxy flavone) (Fig. 1), one of the major bioactive flavone glucuronides present in the radix of *Scutellaria baicalensis*, is generally used in traditional Chinese medicine as a remedy for the treatment of inflammation, fever and allergic diseases (Xing et al., 2005). Due to the glycosyl group on the ring, baicalin is low hydrophilic and poorly absorbed after oral administration, which causes low bioavailability and limits its therapeutic efficacy and clinical application (Shen et al., 2003; Tsai and Tsai, 2004).

It is generally accepted that SLN has been proposed as a significant drug carrier system, thus incorporation of baicalin into SLN carrier may be regarded as a potential approach for oral administration in order to improve the bioavailability. Attempts of enhancement in ocular bioavailability of baicalin using nanoparticulate drug delivery system has been reported in literature (Liu et al., 2010b).

The aim of this study was to design and optimize a novel baicalin-loaded SLN carrier system composed of a stearic acid alkaline salt as lipid matrix and prepared according to the coacervation method in which fatty acids precipitated from their sodium salt micelles in the presence of polymeric nonionic surfactants. After evaluating the main and interaction variables which affect EE, particle size and polydispersity index (PDI), such as the amount of lipid and the drug/lipid ratio, a two-factor five-level central composite design was employed to schedule and perform the experiments. In this study, stearate sodium was selected as a lipid matrix. Optimized SLN formulation was prepared on the basis of the predicted optimum levels of the independent variables of the factorial design. Morphological examination and differential scanning calorimetry (DSC) were also performed to characterize the properties of SLN. Furthermore the pharmacokinetics of free and nanoparticle encapsulated baicalin were also evaluated after oral administration to Wistar rats.

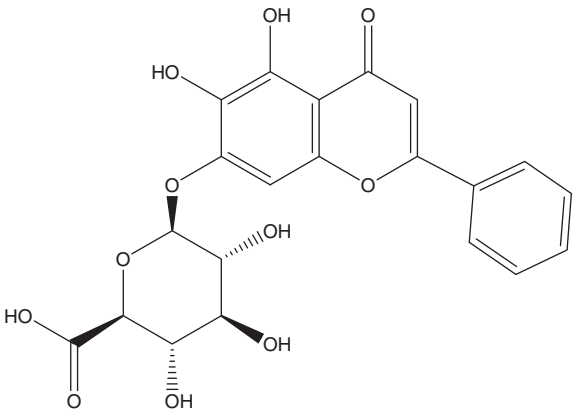

Fig. 1. Chemical structure of baicalin.

2. Materials and methods

2.1. Materials

Baicalin was purchased from Zhucheng Haotian Pharmaceutical Co. Ltd., (>98%, Shandong, China). Baicalin standard was provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Hydroxypropylmethyl cellulose (HPMC, 60RT4000) was supplied by Shandong Ruitai Chemical Co. Ltd. Stearate sodium was purchased from Kemiou Chemical Agent Company (Tianjin, China). Other chemicals and reagents used were chromatographic or analytical grade.

2.2. Preparation of SLN

Baicalin-loaded nanoparticles were prepared as per the coacervation method (Battaglia et al., 2010, 2011). Briefly, stearate sodium was dispersed in the 1% hydroxypropylmethyl cellulose (HPMC) aqueous solution and the mixture was then heated under constant stirring (300 rpm) just above the Krafft point of stearate sodium salt (47.5 °C) to obtain a clear micellar solution. When the temperature of micellar solution was at approximately 60 °C, the model drug baicalin was then added until it was completely dissolved. A 0.1 M solution of HCl, selected as an acidifying agent (coacervating solution), was then added dropwise until pH 4.0 was reached. The obtained suspension was immediately cooled in an ice-water bath under stirring at 1000 rpm until 15 °C was reached.

2.3. Central composite factorial design

After opting for the most important factors influencing the physicochemical properties of the produced baicalin-loaded SLN, a two-factor, five-level CCD was developed to explore the optimum levels of these variables. This methodology consisted of two groups of design points, including two-level factorial design points, axial or star points, and center points. Therefore, two selected independent variables (lipid amount (X_1) and drug/lipid ratio (X_2)) were studied at five different levels coded as $-\alpha$, -1, 0, 1, and $+\alpha$. The value for alpha (1.414) was intended to fulfill the rotatability in the design. Physicochemical properties of the produced SLN, i.e. EE (Y_1), particle size (Y_2) and PDI (Y_3) were selected as dependent variables. The coded and actual values of the variables are given in Table 1. According to the CCD matrix generated by Design-Expert software (Trial Version 7.1.6, Stat-Ease Inc., MN), a total of 13 experiments, including four factorial points, four axial points and five replicated center points for statistical assessment the pure error sum of squares, were constructed (Gonzalez-Mira et al., 2011).

When it came to the prediction of the best suitable formulation, the fitness of the model among the linear, two-factor (2F) interaction model and quadratic model was assessed due to the analysis

Table 1
Independent variables and their levels of experiment design.

Independent variables	Levels				
	−1.414	−1	0	1	1.414
X_1: Amount of lipid (%,w/v)	0.5	0.573	0.75	0.926	1
X_2: drug/lipid ratio(%,w/w)	10	14.39	25	35.6	40
Dependent variables				Constraints	
Y_1 = Entrapment Efficiency (EE%)				Maximize	
Y_2 = Particle size (nm)				Minimize	
Y_3 = Polydispersity index				Minimize	