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# Development and evaluation of self–nanoemulsifying drug delivery system of rhubarb anthraquinones

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ABSTRACT

Rhubarb anthraquinones (RhA) as a group were formulated in nanoemulsion based system with the aim of improving its solubility and oral bioavailability. RhA loaded nanoemulsion (RhA–NE) was prepared using spontaneous nanoemulsification method. Solubility of RhA in oils, surfactants and co–surfactants was determined to select nanoemulsion components. Surfactants and co–surfactants were screened for their ability to emulsify selected oily phase. Pseudo–ternary phase diagrams were constructed to identify area of nanoemulsification. A four–factor–five–level central composite design was carried out to attain optimal formulation. The optimized formulations of RhA–NE consisted of capryol 90/ethyl oleate containing RhA as oil phase, cremophor RH 40 as surfactant, transcutol HP as co–surfactant, oleic acid as stabilizer and distilled water as water phase. The RhA–NE was characterized by dynamic light scanning, transmission electron microscope, solubilizing capacity, encapsulation efficiency, drug loading and stability. The optimized RhA–NE can obviously enhance solubilization of RhA in distilled water. The appearance and RhA contents were basically unchanged after 60-day storage at room temperature in brown bottle. More than 50% RhA released from nanoemulsion within 240 h *in vitro*. Pharmacokinetics in rats showed that the $C_{max}$ and AUC of RhA–NE were enhanced compared to that of RhA suspension.


## 1. Introduction

Rhubarb, officially recorded in Chinese, European and Japanese Pharmacopoeia, has been used for thousands of years in China [1]. Among the extracts obtained from the dried rhizome and root of Chinese official rhubarbs, anthraquinone derivatives including emodin, aloe–emodin, rhein, physcion, chrysophanol and their glucosides are commonly accepted as the main active components [2]. Many studies showed that these anthraquinones had many biological and pharmacological properties, for example, anti–bacterial [3,4], anti–fungal [5], anti–viral [6], anti–oxidant [7–9], neuroprotective [10], anti–cancer [11,12], laxative [13–15], hepatoprotective [16], anti–angiogenic [17], anti–inflammatory activities [18–20] and so on.

Pharmacokinetic analysis indicated that the anthraquinones mainly presented as glucuronides/sulfates in serum, intestine and liver, whereas free forms of most anthraquinones were predominant in kidney and liver. In brain, neither free forms nor conjugated metabolites have been detected [21–23]. Glucuronidation generally results in poor gastrointestinal absorption. Apart from the problem of low bioavailability of free anthraquinones, poor water solubility for rhubarb anthraquinones remains a major obstacle to their development and clinical application.

Recently, various nanonization strategies have been made and developed to improve drug solubility and bioavailability, such as, drug nanocrystals, nanoemulsions, polymeric micelles, cyclodextrins, melt extrusion and liposomes [24–26]. Nanoemulsions have uniform and extremely small droplet sizes, typically in the range of 10–100 nm. In addition, high kinetic stability, low viscosity and optical transparency make them very attractive for many industrial applications [27]. Nanosized drug particles can maximize absorption and hence bioavailability of poorly water soluble drug candidates [24,28]. In self–nanoemulsifying drug delivery systems

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(SNEDDS), nanoemulsion can be spontaneously formed following oily phase being mixed with surfactant/co–surfactant mixture (Smix) and water at an optimal proportion. Pseudo–ternary is useful for the screen of surfactant, co–surfactant and Smix ratio. Nanoemulsion composition could affect the physicochemical characteristics of the nanoemulsion, such as mean droplet size (MDS), zeta potential (ZP) and polydispersity index (PDI). Central composite design–response surface methodology (CCD–RSM) is a useful tool to optimize the parameters of rhubarb anthraquinones loaded nanoemulsion and contributes to understand the relationship between independent variables and response variables.

The main objectives of this work were: (i) to screen the components including oil, surfactant, co–surfactant and stabilizer for rhubarb anthraquinones loaded nanoemulsion (RhA–NE) formulation; (ii) to study the effect of processing variables such as drug content, oil, surfactant and stabilizer on MDS, PDI and ZP; (iii) to analyze *in vitro* release properties and pharmacokinetics in rats to validate advantages of the optimized RhA–NE.

## 2. Materials and methods

### 2.1. Materials

The dried radix et rhizoma of *Rheum palmatum* L., produced in Lixian county in Gansu province of China, was purchased from the Lixian Pharmaceutical Company and identified by professor Yongjian Yang, School of Pharmacy, Lanzhou University. A voucher specimen (No. 856002) was deposited in the Institute of Pharmacognosy. Chemical reference substances of aloe–emodin, rhein, emodin, chrysophanol and physcion were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), and their chemical structures were shown in Fig. 1. Capryol 90 and transcutol HP were kindly donated by Gattefossé (Shanghai, China). Cremophor RH 40 and solutol HS 15 were purchased from Beijing Fengli Jingqiu Pharmaceutical Co., Ltd. (Beijing, China). Methanol of HPLC grade was purchased from Shandong Yuwang Industry Company (Jinan, China). All other chemicals and reagents of analytical grade were bought from local commercial company (Lanzhou, China).

### 2.2. Preparation and quantification of the rhubarb extract

In our previous study, a combined procedure for extraction and purification of free anthraquinones from rhubarb has been reported [29]. Briefly, the mixture of rhubarb powder and 20% sulfuric acid solution were heated in water bath at 70 °C, filtered and washed with distilled water until the penetrating water approximately displayed a central pH. Hydrolyzed rhubarb powder was dried and ultrasonically extracted with 90% ethanol. The extraction solution was rotary evaporated to recycle ethanol, and the aqueous solution was extracted triply with chloroform. The chloroform layer was undergone alkali–solution and acid–isolation. The sediment was dissolved in methanol, and the soluble part was dried to obtain brown rhubarb anthraquinones.

The content of total anthraquinones together with five compounds were analyzed by high performance liquid chromatography (HPLC) method. The HPLC system used was Agilent technology 1260 series (Agilent technologies, INC., USA) equipped with an automatic–sampler. Chromatographic separation was achieved on a Diamonsil column (250 × 4.6 mm, 5 μm, C18, Dikma) at 40 °C. The mobile phase consisted of methanol–0.1% phosphoric acid (88:12, v/v) with an isocratic elution. The flow rate was adjusted to 1.0 mL/min. The raw data were acquired at 254 nm and processed with an OpenLAB Software. Calibration curves were generated from series of concentrations of mixed standard solutions containing five reference substances of aloe–emodin, rhein, emodin, chrysophanol and physcion.

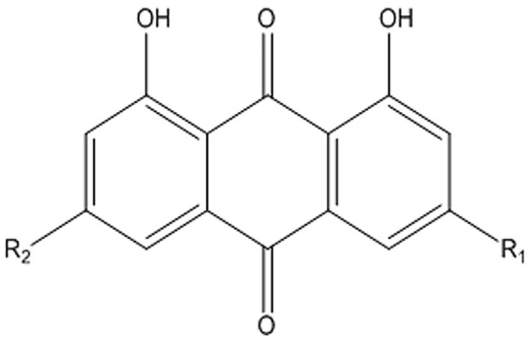
### 2.3. Screening of components

#### 2.3.1. Selection of oily phase

The saturation solubility of RhA in oils (isopropyl myristate, isopropyl palmitate, caprylic/capric triglyceride (GTCC), capryol 90, castor oil, ethyl oleate and mixed oils) was determined by using shake flask method. RhA's saturation solubility in 15% (w/w) surfactant (labrasol, cremophor RH 40, solutol HS 15, tween 20, tween 80, gelucire 44/14 and poloxamer 188) solution and co-surfactants (transcutol HP, 1, 2–propylene glycol, glycerol, n–butanol, PEG 400, PEG 600 and ethanol) was also determined for further reference. Briefly, each kind of oils, aqueous surfactant solutions and co–surfactants of equal mass were transferred to glass tubes and then excess amount of RhA was added, respectively. The tubes were vortexed at 25 °C for 72 h to achieve equilibrium. After attaining equilibrium each tube was centrifuged at 14000 rpm for 20 min. The supernatant was suitably diluted with methanol and anthraquinones' solubility was determined by a validated HPLC method.

#### 2.3.2. Screening of surfactants

Emulsification ability of various surfactants was screened by two kinds of methods. One is turbidimetric method. In brief, equal amounts of surfactant and the selected oily phase were gently vortexed and heated around 45–60 °C. 50 mg of isotropic mixture was subsequently diluted with distilled water to 50 mL to obtain emulsion. The resulting emulsions were visually observed for the relative turbidity, and their transmittance was assessed at 549.2 nm on UV–visible spectrophotometer (Shimadzu, Japan) using double




| Compounds | $R_1$ | $R_2$ |
|---|---|---|
| Aloe–emodin | $CH_2OH$ | H |
| Rhein | COOH | H |
| Emodin | $CH_3$ | OH |
| Chrysophanol | $CH_3$ | H |
| Physcion | $CH_3$ | $OCH_3$ |

**Fig. 1.** The chemical structures of five anthraquinones.