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In vivo evaluation of a self-nanoemulsifying drug delivery system for curcumin

R. Nazari-Vanani[a,b], L. Moezi[b,c], H. Heli[b,*]

[a] *Department of Nanomedicine, School of Advanced Medical Sciences and Technologies, and, Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran*
[b] *Nanomedicine and Nanobiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
[c] *Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

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ABSTRACT

Curcumin has attracted particular attention in recent years due to its great variety of beneficial biological and pharmacological activities. However, its efficacy has been limited due to its low bioavailability, and this limitation can be overcome by novel drug delivery systems. Self-nanoemulsifying drug delivery system (SNEDDS) is a novel route to improve oral bioavailability of lipophilic drugs. SNEDDS spontaneously forms fine oil-in-water nanoemulsion by mild agitation.

An optimal formula for a SNEDDS comprised ethyl oleate:tween 80:PEG 600 (50:40:10% w/w) with 11.2-nm uniform droplets was developed for curcumin delivery. The SNEDDS was characterized and its loading properties for curcumin were orally evaluated in rat. The results showed a significant increment of 3.95 times in C_{max}, and the curcumin bioavailability was enhanced by 194.2%, compared to the curcumin suspension in water. The development of the SNEDDS formulation had a great potential as a possible alternative for curcumin administration.

1. Introduction

Curcumin, 1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6 heptadien-3,5,dione, is a yellow colored phenolic substance extracted from the spice herb Curcuma longa [1]. It has attracted particular attentions in recent years due to its broad spectrum of beneficial biological and pharmacological activities, such as anti-inflammatory [2], anti-oxidant [3], anti-microbial [4], anti-tumor [5], anti-coagulant [6], anti-virus [7] properties. Moreover, curcumin has been shown to have the potential of slowing the progress of Alzheimer's disease [8] and of delaying the onset of its seizures [9]. It also inhibits the formation of brain tumor [10]. Curcumin is a safe, non-toxic and effective alternative for many new drugs [11].

Nevertheless, the majority of the orally administrated curcumin is extracted in the feces and urine, and a few amounts are detected in blood plasma [8]. Although 10 or 12 g/mL of orally administered curcumin in humans leads to a serum curcumin level of ~50 ng/mL, it is lower than a value to achieve curcumin therapeutic effects [12]. The low bioavailability of curcumin is due to its low water solubility [8], instability in low pH values, and resulted in difficult absorption and limited clinical use [13]. At the same time, after oral curcumin dosing, it is rapidly metabolized in the intestine [8]. In order to overcome these limitations, numerous methods have been suggested to improve the curcumin bioavailability [2,14].

Nanomaterials have a high surface area and surface-to-volume ratio causing increasing in the particle surface energy, which can render them into more biological activity [15–20]. Different nanomaterials have been employed in nanomedicine [16–20]. As for nanovehicles for increment in the drugs' solubility, lipid nanoemulsion formulations, and particularly, the self-nanoemulsifying drug delivery system (SNEDDS) has been considered as an ideal alterative for enhanced the oral bioavailability of poorly water-soluble drugs [21]. SNEDDS is an isotropic mixture consisting of oil, surfactant and cosurfactant, which can spontaneously form a fine oil-in-water nanoemulsion with gentle agitation in water [22]. In human, the digestive motility of the stomach and intestine provides the agitation required for self-emulsification *in vivo* [23]. SNEDDS has received particular attention due to its advantage, e.g. reduction in dose [24], protection of drug from the enzymes of the gastrointestinal tract

* Corresponding author.
E-mail addresses: hheli7@yahoo.com, heli@sums.ac.ir (H. Heli).

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[25], reduction of the first-pass effect [26], reduction of side effects [27], controlled and sustained delivery of drugs [28], minimizing gastric irritation [29], increasing maximum concentration (C_{max}) and area under the curve (AUC), and reduction of maximum concentration (T_{max}) [30].

There are reports on the increment in the bioavailability of some poorly water-soluble drugs using SNEDDS such as cilostazol [31], rosuvastatin calcium [32], irbesartan [33], telmisartan [34], cyclosporine [35] and coenzyme Q10 [36]. However, based on our knowledge, enhancement in the curcumin bioavailability using SNEDDS has not been approached, yet. In the present study, a SNEDDS comprising ethyl oleate, tween 80 and polyethylene glycol (PEG) 600 for curcumin was design, characterized, and evaluated *in vivo*.

2. Materials and methods

2.1. Materials

Curcumin and ethyl oleate were bought from Merck (Germany). PEG 600, tween 80, chloroform and absolute ethanol were purchased from Scharlau (Spain). All chemical were of reagent grade. Methanol and glacial acetic acid (HPLC grade) were obtained from Sigma (USA).

2.2. Solubility study and screening of the SNEDDS components

The most important criterion for the screening of components is the solubility of curcumin in oil, surfactant and cosurfactant. Therefore, the solubility of curcumin in ethyl oleate, tween 80 and PEG 600 was determined. 500 μL of these liquids was added to each vial containing an excess amount of curcumin (50 mg) and shaken at ambient temperature for at least 24 h. Then, the vials were centrifuged at 15000 rpm for 20 min in a sealed micro-centrifuge (Eppendorf 5424, Germany) to remove the undissolved curcumin. The supernatants were collected and stored at room temperature. A UV–vis spectrophotometer (Rayleigh UV-2100, China) was employed for determination of the curcumin concentration in the supernatants. The absorption coefficients for curcumin in each liquid were separately obtained by recording the absorbance values of standard curcumin solutions. The solubility values were expressed as mean ± standard deviation.

2.3. Construction of ternary phase diagram

Ternary phase diagram was constructed in order to obtain the construction range of the components that can form self-nano-emulsion upon dilution. Ethyl oleate, tween 80 and PEG 600 were selected as oil, surfactant and cosurfactant, respectively.

Ternary phase diagram was constructed by using a conventional titration technique. Briefly, appropriate amounts of surfactant and cosurfactant were taken in different stoppered test tubes. The ingredients were mixed using a magnetic stirrer until the solution was clear. Oil was then added drop-by-drop to each mixture under proper magnetic stirring at room temperature until the mixture became turbid at a certain point. The amounts of the components were converted to weight per weight percent before constructing the phase diagram. The shaded areas enclosed in the triangle represent the biphasic regain.

2.4. Optimization of SNEDDS formula from the ternary phase diagram

Four different SNEDDS formulations were prepared by selecting the concentrations of oil, surfactant and cosurfactant from single-phase region of the ternary phase diagram denoted as F1 to F4 (Table 1). The best formulation was selected based on the minimum droplet size.

2.5. Preparation of curcumin loaded SNEDDS

Curcumin was firstly dissolved in PEG 600 in a glass vial and the dispersion was gently shaken at for 10 min until perfect dissolution. Then, the required amount of ethyl oleate and tween 80 was added and shaking continued for 5 min. The curcumin loaded SNEDDS were formed following 1:10 dilution with distilled water. These mixtures were stored at room temperature for further studies. A blank SNEDDS formulation was also prepared by the same method without using curcumin.

2.6. Determination of curcumin loading capacity

10 mg curcumin was loaded into 3 mL of SNEDDS as mentioned above followed by liquid-liquid extraction. To the SNEDDS, 1 mL chloroform was added and then shaken for 5 min. The organic layer was then separated and its curcumin content was quantified by HPLC (Waters, USA).

2.7. HPLC analyses

The chromatographic separations were achieved using C18 column (Eurospher, Germany) with 250 mm × 4.6 mm and particle size of 5 μm coupled with a UV–vis detector. The mobile phase was made of a mixture of methanol and water (73:27, v/v) containing 3.6% glacial acetic acid at a flow rate of 1 mL/min [11], and the run time was 7 min. The mobile phase was filtered through a 0.22 μm Millipore membrane filter and degassed by a sonicator (Wise clean, Germany) for 5 min before use. A 60 μL volume was injected into the system and the eluent was monitored at 428 nm. The retention time of curcumin was practically obtained as 4.16 ± 0.23 min in the working conditions in the present study.

From a calibration curve constructed for the curcumin analysis, the concentration of curcumin in the samples was determined. Standard solutions of curcumin were prepared in the mobile phase as a serial concentration in a range of 0.004–1 mg/mL. All data were expressed as mean ± standard deviation.

2.8. Determination of droplet size of SNEDDS and morphological characterizations

SNEDDS formulations (F1-F4, 200 μL) were dispersed in 2 mL distilled water under gentle agitation. The droplet size was measured using particle size analyzer (Scatterscope, Qudix, South Korea). The selected formulation of SNEDDS (F2) was diluted 200 times with distilled water and then mixed by gentle shaking. One drop of the diluted sample was deposited and stained with osmium tetroxide. Field emission scanning electron microscopy (FESEM) images from this sample were recorded (TESCAN Mira 3-XMU, Czech Republic).

Table 1
Compositions of various formulations presented in the phase diagram.

Composition (w/w%)	Ethyl oleate	Tween 80	PEG 600
F1	15	30	55
F2	50	40	10
F3	20	50	30
F4	20	40	40