

Communication

Preparation of solid lipid nanoparticles for Enhancement of oral bioavailability of Curcumin

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Abstract: Purpose: To prepare solid lipid nanoparticles (SLN) of curcumin (CRM) by microemulsion method and assessing its effect on oral bioavailability in Sprague-Dawley rats. Methods: CRM SLN composed of Gelucire® 50/13 were prepared by microemulsion method followed by freeze drying and characterized for mean particle size by Photon Correlation spectroscopy. The SLN were administered (p.o., 100 mg/kg) to male Sprague-Dawley rats (225-275g) and plasma samples were analyzed using a validated HPLC-UV/VIS method. An aqueous suspension of CRM was used as the reference (administered p.o. 250 mg/kg). The pharmacokinetic parameters $AUC_{(0-5h)}$, C_{max} and T_{max} were calculated using non compartmental modeling. Results: The mean particle size of the SLN was found to be 375 nm. The results for $AUC_{(0-5h)}$, C_{max} and T_{max} were found to be 28.0 ngh/mL, 28.9 ng/mL and 0.5 h, respectively for reference; and 75.5 ngh/mL, 31.3 ng/mL and 1.0 h, respectively for SLN. A statistically significant increase ($P= 0.035$, 2 sample unpaired t-test) of 647% was observed after dose normalization in the oral bioavailability of CRM (in terms of $AUC_{(0-5h)}$). Conclusion: The oral bioavailability of CRM can be significantly improved by developing SLN of CRM.

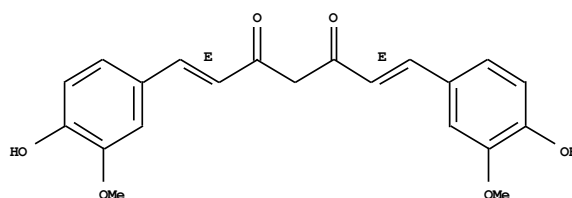
Keywords: curcumin; solid lipid nanoparticles; bioavailability.

1. Introduction

Curcumin (CRM, figure 1), the active ingredient obtained *Curcuma longa*, is undergoing clinical trials for several diseases like ulcerative colitis, colon cancer, pancreatic cancer, hypercholesterolemia, atherosclerosis, pancreatitis, psoriasis, Crohn's disease and neurological diseases (Goel et al., 2008) owing to its multiple pharmacological actions (Maheshwari et al., 2006) and lack of major side effects. However, the limiting factor in preventing the clinical development of CRM into a 'medicine' is its extremely poor oral bioavailability (Anand et al., 2007). Poor aqueous solubility, degradation in gastrointestinal tract (GIT) at neutral and alkaline pH, high pre-systemic metabolism in the intestinal wall are the reasons responsible for its poor oral bioavailability leading to short half life and rapid systemic elimination (Anand et al., 2007; Sharma et al., 2005; Yang et al., 2007). Solid lipid nanoparticles (SLN) have been explored to improve the oral bioavailability of several drugs for example retinoic acid (Hu et al., 2004), cyclosporine (Muller et al., 2006), Vinpocetine (Luo et al., 2006).

The present work deals with the preparation of Gelucire® 50/13 based SLN of CRM and determination of its oral bioavailability in male Sprague Dawley rats.

Figure 1. Chemical structure of curcumin



2. Results and Discussion

Formulation development and characterization

In order to incorporate a drug into SLN, the drug needs to possess a sufficiently high solubility in the lipid used for SLN production (Muller et al., 2006). Prior to the production of the SLN dispersion a lipid screening was performed by dissolving CRM in various molten lipids. Monostearin, stearic acid, Gelucire® 50/13, Compritol and Precirol were tried and the maximum solubility of CRM was found in Gelucire® 50/13 and was selected. As the lipid concentration was increased from 5% to 10%, particle size increased drastically giving microparticle instead of nanoparticles (figure 2). Hence, the maximum concentration of lipid was restricted to 5%.

The maximum loading achieved for SLN preparation was 6.5% w/w of the lipid. Above this concentration, the particle size increased drastically leading to the development of microparticles (figure 3).

Surfactants are incorporated into SLNs to enhance the surface stabilization of the nanoparticles. Various surfactant like Tween 80, lecithin, Poloxamer 188 and Poloxamer 407 were investigated. The

minimum particle size was obtained with Poloxamer 188 and it was chosen as the stabilizer (figure 4). Surfactant concentration was selected as 1% as no significant decrease in size was obtained beyond this concentration.

Figure 2. The effect of lipid concentration on the average particle size of SLN

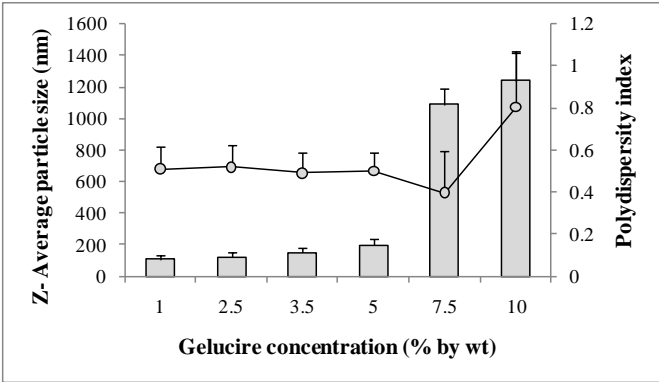


Figure 3. Effect of increasing drug loading on the particle size of SLN

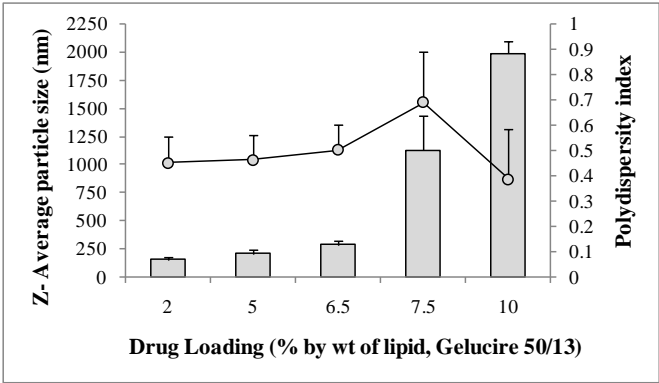
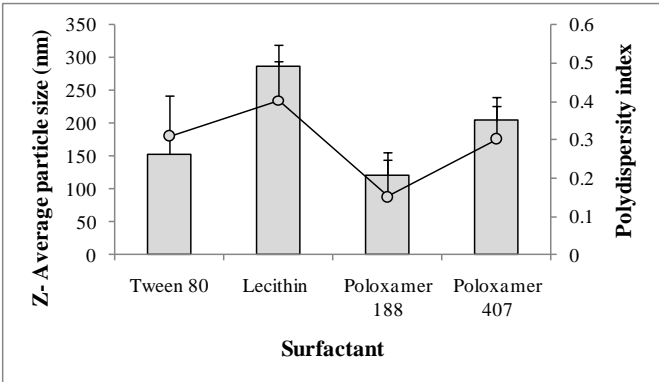


Figure 4. Effect of surfactants on the particle size of SLN

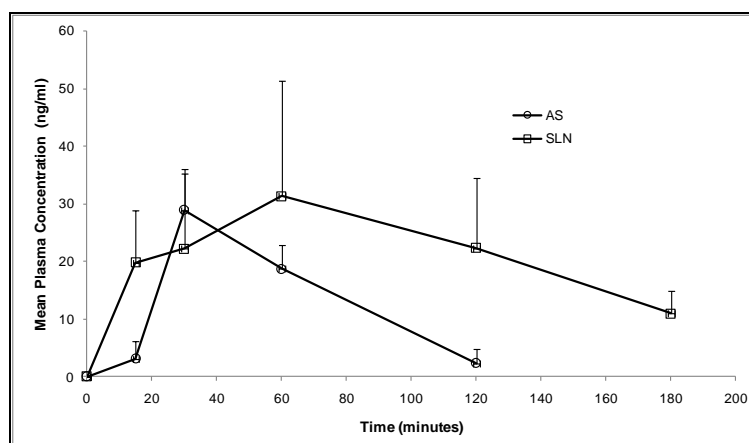


Freeze-drying of SLN requires addition of cryoprotectants for preserving the formulation characteristics. Mannitol at a concentration of 5% showed the least variations in particle size and aesthetically appealing product. Trehalose and sucrose at concentrations of 2.5% and 5.0% were also tried but they failed to form good cake structure. The particle size of the freeze dried CRM loaded SLN, after reconstitution was 375 ± 30 nm with a polydispersity index of 0.198.

Determination of Oral Bioavailability study

The freeze dried SLN were resuspended in ultrapurified water and administered to male SD rats at a dose of 100 mg/kg. Aqueous suspension (AS) was administered at a CRM dose of 250 mg/kg as reference formulation. The mean plasma profiles obtained after oral administration single dose of the two formulations to SD rats are presented in figure 5 and the results of the calculated pharmacokinetic parameters have been tabulated in table 1.

Figure 5. Mean Plasma concentration-time profile after oral administration of SLN of CRM (dose 100 mg/kg body wt) compared with that of aqueous suspension, AS (dose 250 mg/kg body wt) to male SD rats (n=6). The error bars represent standard error of means.



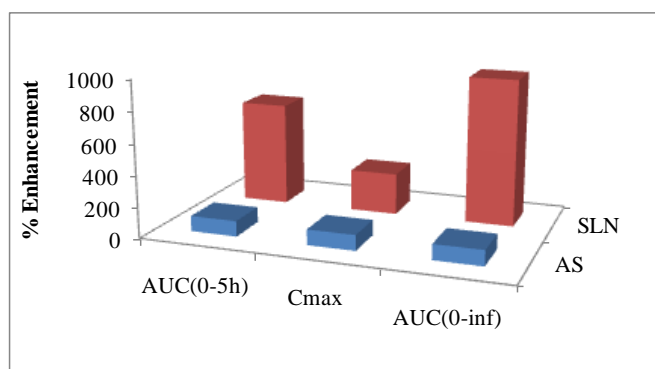
A maximum plasma concentration (C_{max}) of only 28.9 ng/ml was achieved within 0.5 h after the administration of AS of CRM and the plasma levels were reduced to below limit of quantification (BLOQ) within 2 h. The AUC_{0-5h} was found to be 28.0 ngh/ml. The half life ($t_{1/2}$) was found to be only 0.8 h. Rapid clearance and high metabolism have been reported as the major contributing factors for the short $t_{1/2}$ of CRM (Sharma et al., 2007) in the literature. The results obtained in the present study were similar to a study performed by Yang et al (Yang et al., 2007), wherein $t_{1/2}$ and T_{max} of 0.8 h and 0.695 h respectively, were achieved after oral administration of a single dose of CRM in SD rats at a dose of 500 mg/kg.

The AUC_{0-5h} , C_{max} and T_{max} for SLN, were found to be 75.5 ± 19.2 ngh/mL, 31.3 ± 8.4 ng/mL, 1.0 ± 0.4 h. A statistically significant increase ($P= 0.035$, 2 sample unpaired t-test) of 647% was observed after dose normalization in the oral bioavailability of CRM (in terms of $AUC_{(0-5h)}$). The % increase in the C_{max} , AUC_{0-inf} and AUC_{0-5h} is presented in figure 6 taking the results obtained after administration of the aqueous suspension at a CRM dose of 250 mg/kg.

Table1. Comparison of the pharmacokinetic parameters of SLN (without dose normalization) with reference

PK Parameter	Units	Reference	SLN
		(dose= 250 mg/kg)	(dose= 100 mg/kg)
AUC _(0-5h)	ngh/mL	28.0 ±3.7	75.5 ±19.2
C _{max}	ng/mL	28.9 ±7.1	31.3 ±8.4
T _{max}	h	0.5 ±0.1	1.0 ±0.4
AUC _(0-inf)	ngh/mL	28.2	107.0
Ratio	None	1.0	0.7
AUC _{(0-last)/AUC_(0-inf)}	None	1.0	0.7
MRT _(0-5h)	h	0.9 ±0.1	1.6 ±0.28
MRT _(0-inf)	h	0.9	2.2
t _{1/2}	h	0.4	1.0

Figure 6. % Enhancement in the oral bioavailability of curcumin administered in the form of SLN after dose normalization. The values for AS taken as 100%.



SLN also increased the T_{max}, half life (t_{1/2}) and the Mean Residence Time of CRM. The t_{1/2} was increased by 2.5 folds. CRM is rapidly degraded and metabolized in the gastrointestinal tract. Incorporation of CRM in the SLN may protect its degradation and metabolism thus causing an enhancement in the mean residence and t_{1/2} of CRM. Reduction in the particle size is also a key factor for improving the oral bioavailability of poorly soluble drugs. In SLN formulation, the particle size was reduced to less than 400 nm, resulting in an increase in the specific surface area and that may increase the solubility.

3. Experimental Section

Materials

CRM (purity more than 99% by HPLC assay) was extracted from *Curcuma longa* (turmeric). 4-methoxychalcone (4-MC) was purchased from Sigma-Aldrich (Steinheim, Germany). HPLC grade

methanol, ethyl acetate, acetonitrile (ACN) and tetrahydrofuran (THF) were purchased from RFCL Ltd. (New Delhi, India), sodium carboxymethyl cellulose (CMC) from Himedia Laboratories limited (Mumbai, India), Ultra pure water was produced by purification with Ultra pure water system (USF Elga, England), Gelucire® was obtained from Colorcon Ind Pvt Ltd and Poloxamer 188 from BASF (Germany).

Preparation of Aqueous suspension (AS)

The AS was prepared by suspending CRM in citro-phosphate buffer pH 5.0 containing CMC. The buffer was prepared by mixing 48.5 ml of 0.1 M citric acid solution with 0.2 M disodium hydrogen phosphate sufficient to bring the pH to 5.0.

Preparation of SLN

10 g Gelucire® 50/13 was taken and heated in a round bottomed flask in a water bath at 60°C. 650 mg CRM dissolved in 10 ml acetone was added to it under stirring. Acetone was removed by evaporation under. The melted product was rapidly cooled in an ice bath to give CRM loaded lipid. SLNs were then prepared using the CRM loaded lipid via the microemulsion technique proposed by Ugazio et al (Ugazio et al., 2002). 1g CRM loaded lipid was melted under a water bath at 60°C and 10 mL of aqueous solution containing Poloxamer® 188 (1%) acidified with hydrochloric acid and heated to 60°C was added to the oil phase with stirring. The resulting emulsion was dispersed at 24,000 rpm within 5 minutes using a rotor-stator (Ultra-Turrax, IKA T18 basic; Staufen, Germany). The resulting nanoemulsion was allowed to cool to 2°C under stirring at 3000 rpm for the solidification of SLN and freeze dried using VirTis (Wizard 2.0) freeze dryer after addition of 5% Mannitol as cryoprotectant.

Measurement of particle size and zeta potential

The average size and polydispersity of nanoparticles was determined by dynamic light scattering (Nano ZS, Malvern Instruments, Malvern, UK), taking the average of 5 measurements at 25°C.

Entrapment efficiency and drug loading

Entrapment efficiency and drug loading were determined by measuring concentration of free drug, separated from nanoparticles by ultracentrifugation (1 lakh × g), in the aqueous phase using validated reverse-phase high-performance liquid chromatography (HPLC) method on a LiChrospher® C18 column utilizing a mixture of acetonitrile, tetrahydrofuran and 1% w/v Citric acid solution (aq.), pH 3.0 in the ratio 56:14:30 as the mobile phase at a flow rate of 1.0 mLmin⁻¹ and detection wavelength 430 nm.

Determination of oral bioavailability of formulations

In vivo studies were conducted in accordance with Committee for Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines. Male Sprague Dawley rats, weighing 250 ±25 g were randomly assigned to two treatment groups of 6 rats each and kept on fasting for 12 h

before drug administration. Blood samples (approximately 500 μ l) were collected from retro-orbital plexus at 0.25, 0.5, 1.0, 2.0, 3.0 and 5.0 h and plasma was separated immediately by centrifugation.

Plasma samples were analyzed by a validated reversed phase HPLC-UV/Vis method employing 4-methoxychalcone as the internal standard (IS) on a LiChrospher® C₁₈ column (4.6 \times 200 mm, 5 μ m Merck) using a mixture of 1% w/v citric acid monohydrate (pH adjusted to 3.0 \pm 0.05 using 45% w/v potassium hydroxide), ACN and THF (45:35:20) as the mobile phase at a flow rate of 1 ml/min and detection wavelengths of 425 and 319 nm respectively, for CRM and IS.

Pharmacokinetic parameters were calculated using non-compartmental modeling using PCNONLIN version 4.0 Professional Data analysis SCI software (Lexington, KY, USA) and mean plasma concentration profiles were generated. The results were statistically compared by 2-sided unpaired t-test for samples with unequal variance using SigmaStat for Windows Version 2.03 (SPSS Inc.).

4. Conclusions

In our study, a poorly bioavailable molecule, CRM was successfully incorporated into Gelucire® based SLNs by a microemulsion based technique. An oral pharmacokinetic study was conducted in male SD rats and the results indicated significant improvement of oral bioavailability of CRM from SLN.

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