RESEARCH ARTICLE

Exploring solid lipid nanoparticles to enhance the oral bioavailability of curcumin

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Scope: Curcumin, a molecule with pluripharmacological properties, was loaded into solid lipid nanoparticles (SLNs) with a view to improve its oral bioavailability (BA).
Methods and results: Curcumin-loaded solid lipid nanoparticles (C-SLNs) with an average particle size of 134.6 nm and a total drug content of $92.33 \pm 1.63\%$ was produced using a microemulsification technique. The particles were spherical in shape, with high drug entrapment of $81.92 \pm 2.91\%$ at 10% drug loading. The *in vitro* release was predominantly by diffusion phenomenon and was prolonged up to 7 days. No significant variation in particle size and curcumin content of C-SLNs was observed, upon storage, over a period of 12 months at $5 \pm 3^{\circ}C$. *In vivo* pharmacokinetics performed after oral administration of C-SLNs (50, 25, 12.5 and 1 mg/kg dose) and (free) solubilized curcumin (C-S; 50 mg/kg), using a validated LC-MS/MS method in rat plasma revealed significant improvement (at $p < 0.05$) in BA (39 times at 50 mg/kg; 155 times at 1 mg/kg; and, 59 and 32 times at 12.5 and 25 mg/kg, respectively) after administration of C-SLNs at all the doses with respect to C-S.
Conclusions: Enhanced and reliable BA will help in establishing its therapeutic usefulness especially for neurodegenerative and cancerous disorders in humans.

Keywords:
Bioavailability / Curcumin / LC-MS/MS / Liquid–liquid extraction / Solid lipid nanoparticles

1 Introduction

Curcuma longa Linn. is one of several medicinal plants to have attracted the interest of scientists. For centuries it has acted as a remedy for several ailments, offering potential benefits in several chronic illnesses including neurodegenerative, antioxidant, cardiovascular, pulmonary, autoimmune and neoplastic diseases involving inflammation [1]. Despite multiple medicinal benefits, low oral bioavailability (BA) of curcumin continues to be a major concern, irrespective of the route of administration. The latter is attributed to poor absorption, extensive intestinal and hepatic metabolism, rapid elimination and clearance from the body [2, 3].

Formulating curcumin for clinical efficacy has presented many challenges due to its poor physicochemical properties. In spite of the numerous formulation challenges, several strategies such as nanoparticles, liposomes, complexation with phospholipids and cyclodextrins and solid dispersions have been developed to improve the BA of curcumin [4–6]. Solid lipid nanoparticles (SLNs) loaded with curcuminoids for topical application offered no significant improvement in comparison to that of the standard curcumin when tested *in vitro* (70% release in 12 h by SLN *versus* 90% release in 8 h by free curcumin) [6]. The synthesis of curcumin encapsulated polymeric nanoparticles of *N*-isopropylacrylamide, with *N*-vinyl-2-pyrrolidone and poly (ethyleneglycol) monoacrylate has been reported [4]. However, *in vivo* evaluation establishing the superiority of most of the above developed systems is still

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Abbreviations: BA, bioavailability; **bw,** body weight; **C-S,** solubilised curcumin; **C-SLNs,** curcumin-loaded solid lipid nanoparticles; **IS,** internal standard; **SLN,** solid lipid nanoparticles

lacking. Several absorption enhancers have also been used to improve the BA of curcumin. Piperine has been found to enhance the BA of curcumin by 2000% both in preclinical studies and in studies on human volunteers [7]. A recent study reported at least 9-fold increase in the oral BA of curcumin nanoparticles when compared with curcumin administered with piperine as absorption enhancer in rats [8].

SLNs have been reported as an alternative drug delivery system to traditional polymeric nanoparticles [9, 10]. A clear advantage of SLNs over polymeric nanoparticles is the fact that the lipid matrix is made from physiologically tolerated lipid components, which decreases the potential for acute and chronic toxicities [11]. SLNs combine the advantages of polymeric nanoparticles, fat emulsions and liposomes [12]. Sufficient data implicate that the BA of poorly hydrophilic and lipophilic drugs can be improved when these are encapsulated in SLNs. SLNs have been used as drug delivery systems for enhancing the BA of quercitin [13], vinpocetine [14] and topical application of curcuminoid SLNs *in vivo*. There are numerous methods of preparation of SLNs. We report on the preparation of a concentrated SLN dispersion (with a 1:1 dilution factor) by pouring the preoptimized microemulsion into an equal quantity of cold water. In the present study, concentrated curcumin-loaded solid lipid nanoparticles (C-SLNs) were successfully prepared and their physicochemical characteristics were investigated. Furthermore, single-dose pharmacokinetic studies were performed to determine the concentration of curcumin *in vivo*, after an oral administration of four doses of C-SLNs (very high (VH), 50; high (H), 25; medium (M), 12.5 and small (S), 1 mg/kg) and free curcumin (CS (50 mg/kg)), in rat plasma. A highly sensitive and validated LC-MS/MS method was used for determining the concentration of curcumin at various time points during pharmacokinetic profile.

2 Materials and methods

2.1 Materials

Curcumin was a gift sample from Sanat Products, Delhi, India. The sample constituted a mixture of three curcuminoids, namely curcumin (95%), demethoxycurcumin and bisdemethoxycurcumin (latter two constitute the remaining 5%). Nimesulide was used as the internal standard (IS) and was provided by Panacea Biotec (Lalru, India). Soy Lecithin (Hi Media, India); Tween 80 (S.D. Fine Chemicals, India); Compritol 888 ATO® (Glyceryl Behenate, gift sample from Gattefosse, USA) were also used in the study. Sulfatase-free-β glucuronidase (type IX-A from *Escherichia coli*) was purchased from Sigma (St. Louis, MO). HPLC-grade ACN, acetic acid, diethyl ether and other chemicals used in the preparation of buffer were purchased from Merck KGaA, Darmstadt, Germany.

All other chemicals and reagents were of analytical grade and were used without further purification.

2.2 Preparation of C-SLNs

Polysorbate 80 (45.45%), soy lecithin (0.58%) and water were placed together in a beaker and heated to the lipid melting temperature. Lipid (7.27%) was also melted at 82–85°C separately. Curcumin was added to the aqueous phase containing polysorbate 80, following which the hot aqueous emulsifier mix, was dropped at once into the lipid melt, under magnetic stirring to obtain a clear microemulsion. The hot microemulsion thus formed, was transferred into an equivalent amount of cold water (~2°C) under continuous mechanical stirring (5000 rpm) for 1.5 h. In the aqueous medium, SLNs are formed by crystallization of the oil droplets present in the microemulsions [15]. The prepared SLNs were stored in refrigerator until further analysis.

Free curcumin used for comparison was prepared by solubilizing curcumin (C-S) in aqueous Tween 80. Amounts equivalent to those present in the final C-SLNs dispersion were used for solubilization.

2.3 Characterization of SLNs

Total drug content was estimated spectrophotometrically at λ_{max} of 425 nm by disrupting 1 mL of the SLN dispersion using an appropriate volume of chloroform:methanol (1:1, v/v). For determining the entrapment efficiency, SLN dispersion was ultracentrifuged at 90 000 rpm for 2 h at 4°C. The clear supernatant was decanted. The pellet of C-SLNs, was than washed with methanol to remove the unentrapped drug (curcumin being insoluble, the unentrapped drug will also settle down along with the SLNs), and recentrifuged. The two supernatants were combined and the absorbance of both the supernatant and the pellet dissolved in a suitable solvent was recorded after appropriate dilutions. Absorbance value obtained for blank SLNs treated in a similar manner was used as the control value to compensate for any interference of the ingredients. All the determinations were performed in triplicate. Methanol:chloroform (1:1, v/v) was used to dissolve the pellets of C-SLNs. Amount of drug in the pellet gave a direct measure of the extent of drug entrapped.

$$\text{Entrapment efficiency} = \frac{(\text{Amount of drug/mL of SLN dispersion} \times \text{Total volume of dispersion}) \times 100}{\text{Total drug incorporated.}}$$

The mean diameter of SLNs in the dispersion (with appropriate dilutions) was determined by using laser diffraction (Mastersizer 2000, Malvern Instruments, UK). While the morphology of SLNs was examined using an electronic transmission microscope (Hitachi H-100, Japan).

2.4 Stability studies

C-SLNs were stored in vials at $5 \pm 3^{\circ}C$ for 1 year, and the samples were withdrawn at 0, 6 and 12 months as *per* ICH guidelines. The average size, total drug content and the entrapment efficiency were determined at each time point.

2.5 Drug release properties of C-SLNs

Dialysis bag method to study the drug release was performed using a mixture of double-distilled water and ethanol (50:50, v/v) as the dissolution medium [16]. The dialysis bags (12 kDa, Hi Media) were soaked in de-ionized water for 12 h before use. One milliliter of SLN dispersion (3.6 mg/mL) was poured into the dialysis bag. The bag was placed in a beaker containing 200 mL dissolution medium maintained at 37°C and stirred at a rate of 100 rpm. Aliquots of the dissolution medium were withdrawn at different time intervals and were replaced with the same volume of fresh medium to maintain the sink conditions. The samples were suitably diluted and analyzed for curcumin spectrophotometrically. All the operations were carried out in triplicate.

2.6 Study design for *in vivo* pharmacokinetic studies

For *in vivo* pharmacokinetic studies, male Wistar rats weighing 250–300 g were used. The protocol was duly approved by the Institutional Animal Ethics Committee (IAEC) of Panjab University, Chandigarh, India. The animals were divided into five groups ($n = 6$). Group 1 (VH) was administered 50 mg/kg body weight (bw) of C-SLNs; Group 2 (H; 25 mg/kg bw C-SLNs), Group 3 (M; 12.5 mg/kg bw C-SLNs); Group 4 (S; 1.0 mg/kg bw C-SLNs) and Group 5 was administered 50 mg/kg bw free curcumin (C-S; solution of curcumin in 25% Tween 80) *per* orally using an oral dosing cannula. The blood samples (0.5 mL) were withdrawn from sinus under clavicle and, collected into heparinized microcentrifuge tubes (containing 20 μL of 1000 IU heparin/mL of blood) at different time intervals. After each sampling, 1mL of dextrose–normal saline was administered to prevent changes in the central compartment volume and electrolytes. Plasma was separated by centrifuging the blood samples at 4000 rpm for 10 min at 4°C. After centrifugation, the plasma obtained was stored at −20°C until analysis.

2.7 Quantification of curcumin in plasma

The LC-MS/MS analysis was performed using API 4000 mass spectrometer, Applied Biosystems, Sciex Toronto, Canada, with an automatic liquid chromatographic sampler and an autoinjection system hyphenated to a Micromass Quattro Ultima tandem quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an ESI source. The column used was Chromolith rod^{TM} (50 mm × 4.6 mm, 5 μm particle size; Thermo Electron). The column was kept at an ambient temperature. The mobile phase consisted of ACN and ammonium acetate buffer (10 mM, pH 3.5, adjusted with glacial acetic acid) (80:20 v/v). The flow rate was 0.8 mL/min, and the total run time was 3 min.

The volume of injection was 10 μL. For the operation in MS/MS mode, a mass spectrometer with an orthogonal Z-spray electrospray interface (ESI) was used. During analysis, the ESI parameters were set as follows: capillary voltage, 4.5 kV for negative mode; source temperature, 40°C; desolvation temperature, 300°C and desolvation gas flow, 50 L/h. The cone voltage of *m/z* 367 was adjusted to maximize the intensity of the deprotonated molecular ion (precursor) as 65 V and the collision voltage was also adjusted to optimize the product ion signals as 16 eV for curcumin analysis.

The multiple reaction monitoring was used to monitor the transition of the deprotonated molecule *m/z* 367 $[M-H]^-$ to the product ion 217 for curcumin analysis and *m/z* 307/229 for IS analysis. All LC-MS/MS data were processed by the WIN-NONLIN software.

2.8 Data analysis

The pharmacokinetic parameters were calculated based on a non-compartmental model. The area under the concentration–time curve from time zero to time t (AUC_{0-t}) was calculated using the trapezoidal method. Peak concentration (C_{max}) and time of peak concentration (T_{max}) were obtained directly from the individual plasma concentration–time profiles. The area under the total plasma concentration–time curve from time zero to infinity was calculated by Eq. 1:

$$AUC_{0-\infty} = AUC_{0-t} + C_t/K_e \quad (1)$$

where C_t is the curcumin concentration observed at last time, and K_e is the apparent elimination rate constant obtained from the terminal slope of the individual plasma concentration–time curves after logarithmic transformation of the plasma concentration values and application of linear regression. The data obtained from pharmacokinetic parameters were analyzed statistically using the Win-Nonlin software. Statistically significant differences were assumed at $p < 0.05$.

3 Results

3.1 Characterization of C-SLNs

The total drug content and entrapment efficiency of C-SLNs were estimated to be 92.33 ± 1.63 and $81.92 \pm 2.91\%$ ($n = 6$), respectively. High values (approaching 100%) indicate the

efficiency of the method for the preparation of SLNs and that there were insignificant losses during formulation. Average particle of C-SLNs was found to be 134.6 ± 15.4 nm when measured using the laser diffraction (Mastersizer 2000). When observed under TEM, SLNs were found to be spherical in shape (Fig. 1). The size of the nanoparticles observed under TEM however was much smaller in the range of 40–120 nm than the results obtained using Mastersizer. This however may be attributed to the fact, that Mastersizer is based on the principle of laser diffraction, which unfortunately may not detect the particles under 100 nm, due to the brownian movement of the particles.

3.2 Stability study

After 1 year of storage at $5 \pm 3^{\circ}$C the C-SLN were found to be stable in accordance with ICH guidelines for stability with not much increase in the size (Table 1). The entrapment efficiency decreased by about 9% but the total drug content dipped only by 3% indicating the stability of the prepared crystalline SLNs.

3.3 *In vitro* drug release

Solubility of curcumin in water and simulated gastric fluid at room temperature was determined to be 0.003 and 0.0057 μg/mL, respectively in our lab. Thus, to provide sink condition, 50% v/v ethanol in which the solubility of curcumin is 0.693 ± 0.13 mg/mL was chosen as the receptor medium. The drug release from C-SLNs at 37°C is shown in Fig. 2. The release of curcumin from C-SLNs was fitted to a first-order kinetics model and occurs by diffusion. The release was prolonged up to 7 days with 85.90 ± 2.47% being released within this duration (Fig. 2). Almost 47.16 ± 5.97% of the drug was released in the initial two days; however, the release was much delayed in the later stage.

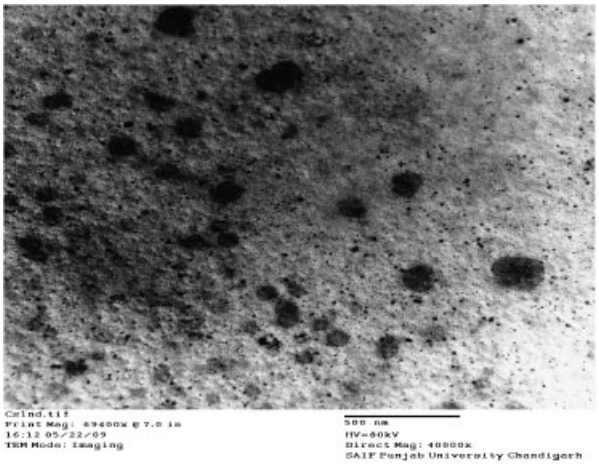

Figure 1. TEM micrograph of C-SLNs.

3.4 *In vivo* pharmacokinetic study

Lower serum and tissue levels of curcumin are observed irrespective of the route of administration due to its poor absorption, extensive intestinal and hepatic metabolism and rapid elimination thus restraining the BA of curcumin [1–3]. Numerous pharmacokinetic studies in humans and rats by different routes of administration have been reported, indicating very low serum and plasma concentrations. An early study by Wahlstrom and Blennow [17] reported that when 1 g/kg curcumin was given orally to rats, 75% of it was excreted in the feces and negligible amounts were found in urine. Pan *et al.* [2] investigated the pharmacokinetic properties of curcumin administered either orally or intraperitoneally [9] in mice. With oral administration of 1.0 g/kg of curcumin, low plasma levels of 0.13 μg/mL appeared in plasma after 15 min, while a maximum plasma level of 0.22 μg/mL was obtained at 1 h; plasma concentrations then declined below the detection limit by 6 h. Entirely different plasma curcumin levels were found after i.p. administration of 0.1 g/kg. Plasma curcumin levels peaked (2.25 μg/mL) within 15 min of administration and declined rapidly within 1 h.

In the present study, C-SLNs were designed to improve the oral BA of curcumin. Plasma levels after oral administration of different concentrations of C-SLNs (50, 25, 12.5 and 1 mg/kg) were compared with 50 mg/kg free curcumin (C-S; using similar amounts of Tween 80, corresponding to that used in the final SLN dispersion) as illustrated in Figs. 3A and B. The mean curcumin concentrations in the plasma after oral administration of C-SLNs and C-S after single dose in Wistar rats was determined using a highly validated and sensitive LC/MS/MS method. The selectivity, precision, accuracy, inter and intraday observations and applicability were found to be adequate for pharmacokinetic studies. Nimesulide was used as IS. The method was linear in the range of 10–2000 ng/mL ($r^2 = 0.999$). The lower limit of quantification was 10.0 ng/mL. The samples were

Table 1. Stability study parameters during storage of C-SLNs at $5 \pm 3^{\circ}$C

Time points	Av. particle size (nm)	Total drug content (%)	Entrapment efficiency (%)
0 month	134.60 ± 15.40	92.33 ± 1.63	84.00 ± 1.63
6 month	132.10 ± 14.90	90.70 ± 4.16	77.30 ± 2.49
12 month	160.40 ± 15.60	89.30 ± 0.58	75.30 ± 3.40

extracted with diethylether using liquid–liquid extraction method and analyzed by LC/MS-MS with an overall recovery of 77.15%. Multiple Reaction Monitoring was used to monitor the transition for curcumin (*m/z*; 367/217 $[M-H]^-$) and IS (*m/z*; 307/229).

The relevant pharmacokinetic parameters including C_{max}, T_{max}, V_d, Cl and $AUC_{0-\infty}$ are listed in the Tables 2 and 3. The studies revealed a significant improvement ($p < 0.05$) in relative BA (39 times at 50 mg/kg; 155 times at 1 mg/kg and 59 and 32 times at 12.5 and 25 mg/kg, respectively) after administration of C-SLNs at all the doses with respect to C-S.

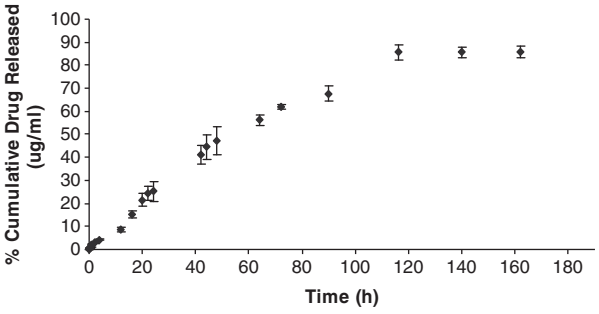

Figure 2. *In vitro* release profile of curcumin, from C-SLNs, by dialysis method. 50% ethanol was used as the release medium. Each data represent the mean ± standard deviation of three tests.

4 Discussion

With increase in life expectancy, majority of people in the older age dwell with multiple disorders, thus creating a need to look upon molecules with multiple targets. Curcumin obtained from the rhizome of *C. longa* Linn. is a lipid-soluble antioxidant compound. Curcumin targets multiple chemotherapeutic and inflammatory pathways and has demonstrated safety and tolerability in humans; however, the clinical literature lacks conclusive evidence supporting its use as a therapeutic agent due to its low BA. The pluri-pharmacology of curcumin compels researchers to play with the limiting biopharmaceutical properties of curcumin and develop it as shotgun for treatment of various ailments. Biologists are enamored with this wonder molecule that has

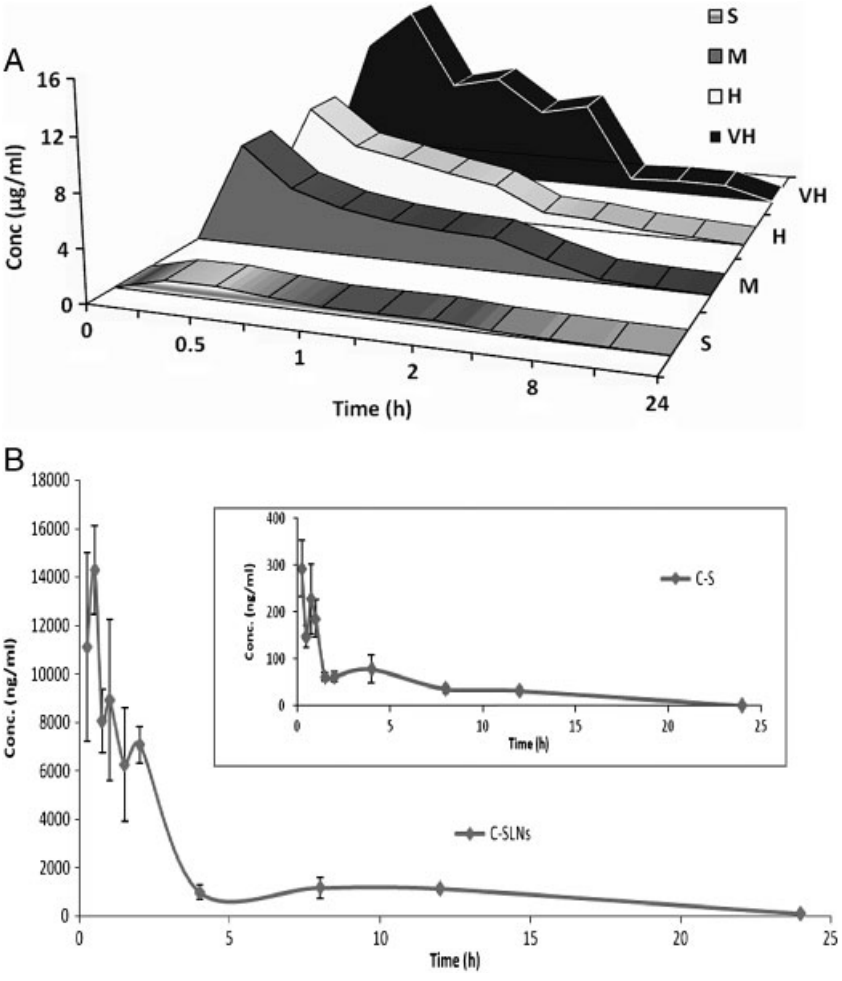

Figure 3. (A) The mean plasma concentration-time curves for different doses of C- SLNs. (B) Concentration *versus* time profile of C-S and C-SLNs after single oral dose administration at 50 mg/kg dose. Each point presents mean ± SEM ($n = 6$).

Table 2. C_{max} of C-SLNs doses and C-S

Formulation	Dose (mg/kg)	C_{max} (μg/mL)
C-S	50	$0.292 \pm 0.06^{a)}$
VH	50	$14.29 \pm 0.15^{b)}$
H	25	$8.00 \pm 1.87^{b)}$
M	12.5	$7.87 \pm 3.02^{b)}$
S	1	$1.00 \pm 0.01^{b)}$

For C-SLNs: very high (VH); high (H); medium (M); and small (S) and free curcumin (C-S).
b) All the values are significantly different from a) at $p < 0.05$.

a history of more than 100 years, while the pharmaceutical scientist is dissuaded by its compromised pharmacokinetics and puts a question mark on its use as a drug.

Considering the potential of SLNs as oral drug delivery system, the present investigation involved development and characterization of SLNs with a view to improve its oral BA. C-SLNs were prepared by microemulsification method. Microemulsions being clear, thermodynamically stable and optically isotropic systems require less energy and are obtained spontaneously by mixing surfactant, co-surfactant, lipid and water [18–20]. In the aqueous medium, SLNs are formed by crystallization of the oil droplets present in the microemulsions [20, 21], consequently, the nanoparticle is affected by the composition of the microemulsion system, particularly by the emulsifier(s) and the co-surfactant used, as well as by experimental parameters [22]. The method of preparation and the formula used were earlier optimized in our laboratory. Compritol 888 ATO® was chosen as the lipid component of the SLN formulations because a preliminary lipid screening had indicated that its use resulted in stable dispersions with smaller particle size [7, 9]. High total drug content and entrapment efficiency achieved confirmed the suitability of formula.

Preparation of SLNs by the described method (1:1 dilution of the microemulsion) overcomes the need to subsequently concentrate the SLN dispersion. This helps in administering the desired dose (which is usually high for natural molecules like curcumin) and does away with the need for lyophilization and redispersion (as it results in an increase in the particle size). The use of polysorbate 80, presently, in the preparation of the microemulsion and the subsequent aqueous dispersion of SLNs in the free polysorbate solution may enhance the solubility of free curcumin and its subsequent BA also [23]. The process of SLN formation was found to be highly reproducible and resulted in particles with a spherical shape as observed by TEM.

The prepared particles were further evaluated for *in vitro* release of curcumin. The choice of dissolution medium and the sink conditions have always been a problem for the low solubility molecules like curcumin. It is reported that for highly lipophilic molecules like Coenzyme Q 10, it is even more difficult to find a release medium providing adequate sink conditions and stability [24]. For such compounds, an aqueous solution containing a surfactant or solvent in which the drug is soluble may be used to enhance its solubility. In the present case, considering the solubility of curcumin in ethanol, a 50% v/v, ethanol was used as the release medium. The release of curcumin from C-SLNs, by diffusion was prolonged up to 7 days (85.9%) befitting the first-order kinetics model. Almost 50% of the drug was released in the initial two days; however, the release was delayed in the later stage. The initial release may be by diffusion from the shell of the SLNs, while the subsequent phase of delayed release may be attributed to the fact that the curcumin dispersed within the core is being released slowly from the solid matrices of lipid through diffusion and dissolution. A series of *in vitro* release experiments conducted on nanoparticles of various drugs by other groups have resulted in varied findings. Zero-order release was observed for estradiol [25] while ellagic acid [26] and curcumin followed Higuchi's release pattern. A prolonged (22 days) release of curcumin from polymeric nanoparticles; with only 43% of the total drug being released in 22 days has been reported recently [8].

The prepared C-SLNs were finally subjected to *in vivo* evaluation in rats. Numerous pharmacokinetic studies in humans and rats report, very low serum and plasma concentrations, irrespective of the route of administration due to its poor absorption, extensive intestinal and hepatic metabolism and rapid elimination [2, 3, 27]. The presented method of preparing C-SLNs resulted in a small particle size, which was one of the major considerations to avoid reticuloendothelial system [28] targeting and thus achieving prolonged circulation times. Since liver is the major site of metabolism of curcumin [16, 17], so RES avoidance of C-SLNs is especially expected to reduce its elimination from the body.

SLNs loaded with curcuminoids for topical application were developed and characterized [6]. C-SLNs having 450 nm size were found to be stable for 6 months at room temperature and gave prolonged *in vitro* release of curcuminoids up to 12 h. Furthermore, the light and oxygen sensitivities of curcuminoids was strongly reduced by incorporating curcuminoids into this unique type of formulation. *In vivo* study with the developed SLNs in healthy volunteers revealed the improved efficiency of a topical application cream containing C-SLNs over that containing free curcuminoids [6]. In a very recent study in healthy volunteers, a 650 mg/kg dose of solid lipid curcumin particles showed plasma levels of 22.43 ng/mL, while free curcumin (95% curcuminoids extract) at the same dose was undetectable [29]. To what degree the enhanced BA is a result of increased absorption or due to reduced conversion of free curcumin to conjugates is still not clear, because in this study the samples were not pretreated with glucuronidase. Various researchers report a maximum of 2–3-fold increase in curcumin absorption by simply dissolving or mixing curcumin in different types of lipids [5, 30] and the

formulations containing lipids and emulsifiers have shown positive effects in an *in vivo* colitis model [28].

In our study, oral administration of C-S resulted in a sharp C_{max} of 0.292 μg/mL within 15 min after which the plasma concentration declined rapidly, indicating a rapid metabolism of curcumin. Whereas, relatively slow increase and sustained plasma concentration of curcumin for a longer time was observed after administration of C-SLNs. A very low volume of distribution (7.72 ± 0.43 L/kg) (5.3 times lower than C-S) and a significantly ($p < 0.05$) high C_{max} of 14.293 μg/mL at 0.5 h for the VH dose with curcumin being detectable at even 24 h (0.012 μg/mL), suggests a sustained effect of the C-SLNs. There was a marked difference in the $AUC_{0-\infty}$ between C-S and C-SLNs at all the doses. The $AUC_{0-\infty}$ for C-SLNs was appreciably higher (39 times) at 50 mg/kg dose of C-SLNs *vis-a-vis* C-S when administered orally to rats. Liu *et al.* [30] reported similar C_{max} values (0.266 μg/mL; similar to C-S); however, these levels were achieved at 100 mg/kg dose which is double the dose used by us (50 mg/kg: C_{max} – 0.292 μg/mL). Yang *et al.* [16] used a 10 times higher dose (500 mg/kg: C_{max} – 0.060 μg/mL) and Pan *et al.* [2] used a 20 times higher dose (1 g/kg: C_{max} – 0.220 μg/mL); while the C_{max} values recorded are either same or lower. It may be concluded that either the method used in the present study is more accurate and sensitive or curcumin does not follow dose dependent kinetics. Furthermore, the use of Tween 80 for solubilizing curcumin (C-S), may also exert a penetration enhancing effect. We wanted to confirm that any BA enhancement observed with C-SLNs is not solely attributable to the use of Tween 80 (in its preparation) that is why, we used C-S as the control for comparison.

Liu *et al.* [30] achieved a C_{max} of 0.6 μg/mL with the prepared phospholipid complexes of curcumin. Yang *et al.* [16] showed that 10 mg/kg of curcumin given i.v. in rats gave a maximum serum curcumin level of 0.36 ± 0.05 μg/mL. Multiplying the dose with a factor of 5 (to have an arbitrary value for a 50 mg/kg dose) would mathematically result in a C_{max} of 1.8 μg/mL. Envisaging the above, our results are highly appreciable, as oral administration of C-SLNs at 50 mg/kg could achieve a C_{max} of 14.20 μg/mL, which has not been achieved even with a similar dose (as discussed above) when given by i.v. route. Furthermore, even a 40 times higher oral dose of 2 g could achieve a C_{max} of only 1.35 ± 0.23 μg/mL in an earlier study [7].

Encouraged by the promising results achieved with the 50 mg/kg dose of C-SLNs, we performed single-dose pharmacokinetics with subsequent subordinate doses of C-SLNs (25, 12.5 and 1 mg/kg). To our astonishment the results were highly encouraging with an increase of 155 times in BA with 1 mg/kg; 59 times with 12.5 and 32 times with 25 mg/kg doses of C-SLNs. This pattern of BA enhancement could possibly be attributed to the fact that the amount of curcumin absorbed (60–66% of the given dose) remained constant regardless of the dose indicating that administration of more curcumin does not result in higher absorption. Similar observation, that is, there is a dose-dependent limitation to BA of curcumin in rats has been reported earlier [31]. Nevertheless, several observations in volunteers and patients also suggest that curcumin might possess biological activity even at low oral dose [27].

BA of the developed SLNs is inveterate by the C_{max} values as illustrated in Table 2. However, the superiority of the developed nanoparticles was also established from the obtainable values of volume of distribution (V_d) and clearance [32]. As it is indicated that higher the V_d, more is the drug distributed to other extravascular (tissues) compartment. The V_d at 50 mg/kg of C-SLNs was 53 times lower and Cl about 39 times lower; while at 1 mg/kg the V_d was 162 times lower and Cl about 155 times lower with respect to C-S. The above results are directly conclusive of prolonged circulation times of the developed SLNs with significantly lower clearance and volume of distribution values.

As the average particle size of nanoparticles was maintained below 200 nm, it helps bypassing the liver first pass metabolism that has been reported to be the major site of curcumin degradation. In addition, use of surfactants such as tween 80 and lecithin, in the preparation of SLNs may contribute toward an increase in the permeability of the intestinal membrane or affinity between lipid particles and intestinal membrane, and may also exhibit bioadhesion to the GI tract wall. Also, by incorporation into SLNs, curcumin is now embedded into a solid lipid matrix thus not only reducing the exposure to enzymatic degradation during the process of absorption, but also offering a long contact time *in vivo*. Finally, C-SLNs could provide curcumin with long

Table 3. Various pharmacokinetic parameters obtained from plasma concentration time data for orally administered C-S and C-SLNs at varying doses

Formulation	Dose (mg/kg)	$AUC_{0-\infty}$ (h*μg/mL)	V_d (L/kg)	T_{max} (h)	Cl_{obs} (L/h/kg)
C-S	50	1.075 ± 0.12[a)]	41.17 ± 0.352[a)]	0.25 ± 0.0	46.50 ± 0.21[a)]
VH	50	41.990 ± 6.18[b)]	7.72 ± 0.43[b)]	0.5 ± 0.02	1.19 ± 0.05[b)]
H	25	17.156 ± 3.24[b)]	8.742 ± 1.26[b)]	0.25 ± 0.04	1.46 ± 0.95[b)]
M	12.5	15.789 ± 2.29[b)]	5.686 ± 0.42[b)]	0.25 ± 0.05	0.79 ± 0.38[b)]
S	1	3.343 ± 1.17[b)]	2.535 ± 0.18[b)]	0.5 ± 0.01	0.30 ± 0.12[b)]

b) All the values are significantly different from a) at $p < 0.05$.

circulation times, and a reduced clearance from systemic circulation resulting in its better BA.

Curcumin with its ability to treat variety of diseases is an interesting molecule of research today. With increasing literature evidence suggesting a linkage between multiple disease conditions in patients, multi-modulatory activity of curcumin can play an important role in curing them. Several clinical trials have determined the potential of curcumin in treating numerous disorders.

This highly bioavailable and stable solid lipid nanoparticulate formulation of curcumin would be a great success in causing a reduction in dose (32–155 times) and improvement in efficacy of curcumin, hence renovating its reflection from just a preventative dietary supplement to a therapeutic agent.

To best of our knowledge this is the first ever reported data on a novel drug system achieving such a high C_{max} value at such a low dose of 1 mg/kg.

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5 References

[1] Aggarwal, B. B., Harikumar, K. B., Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. *Int. J. Biochem. Cell Biol.* 2009, *41*, 40–59.

[2] Pan, M. H., Huang, T. M., Lin, J. K., Biotransformation of curcumin through reduction and glucuronidation in mice. *Drug Metab. Dispos.* 1999, *27*, 486–494.

[3] Anand, P., Kunnumakkara, A. B., Newman, R. A., Aggarwal, B. B., Bioavailability of curcumin: problems and promises. *Mol. Pharm.* 2007, *4*, 807–818.

[4] Bisht, S., Feldmann, G., Soni, S., Ravi, R. *et al.*, Polymeric nanoparticle-encapsulated curcumin (''nanocurcumin''): a novel strategy for human cancer therapy. *J. Nanobiotechnol.* 2007, *5*, 3.

[5] Maiti, K., Mukherjee, K., Gantait, A., Saha, B. P. *et al.*, Curcumin phospholipid complex: preparation, therapeutic evaluation and pharmacokinetic study in rats. *Int. J. Pharm.* 2007, *330*, 155–163.

[6] Tiyaboonchai, W., Tungpradit, W., Plianbangchang, P., Formulation and characterization of curcuminoids loaded solid lipid nanoparticles. *Int. J. Pharm.* 2007, *337*, 299–306.

[7] Shoba, G., Joy, D., Joseph, T., Majeed, M. *et al.*, Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Med.* 1998, 353–356.

[8] Shaikh, J., Ankola, D. D., Beniwal, V., Singha, D. *et al.*, Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with piperine as absorption enhancer. *Eur. J. Pharm. Sci.* 2009, *37*, 223–230.

[9] Kaur, I. P., Bhandari, R., Bhandari, S., Kakkar, V., Potential of solid lipid nanoparticles for brain targeting. *J. Control Release* 2008, *127*, 97–109.

[10] Zur Muhlen, A., Mehnert, W., Solid lipid nanoparticles (SLN) for controlled drug delivery-Drug release and release mechanisms. *Eur. J. Pharm. Biopharm.* 1998, *45*, 149–155.

[11] Mehnert, W., K. M., Solid lipid nanoparticles: production, characterization and applications. *Adv. Drug Deliv. Rev.* 2001, *47*, 165–196.

[12] Schwarz, C., Mehnert, W., Solid lipid nanoparticles (SLN) for controlled drug delivery: II. Drug incorporation and physicochemical characterization. *J. Microencapsul.* 1999, *16*, 205–213.

[13] Li, H. L., Zhao, X. B., Ma, Y. K., Zhai, G. X. *et al.*, Enhancement of gastrointestinal absorption of quercetin by solid lipid nanoparticles. *J. Control. Release* 2009, *133*, 238–244.

[14] Luo, Y. F., Chen, D. W., Ren, L. X., Zhao, X. L. *et al.*, Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability. *J. Control. Release* 2006, *114*, 53–59.

[15] Manjunath, K., Reddy, J. S., Venkateswarlu, V., Solid lipid nanoparticles as drug delivery systems. *Method Find. Exp. Clin.* 2005, *27*, 127.

[16] Yang, K. Y., Lin, L. C., Tseng, T. Y., Wang, *et al.*, Oral bioavailability of curcumin in rat and the herbal analysis from *Curcuma longa* by LC-MS/MS. *J. Chromatogr. B.* 2007, *853*, 183–189.

[17] Wahlstrom, B., Blennow, G., A study on the fate of curcumin in the rat. *Acta Pharmacol. Tox.* 1978, *43*, 86–92.

[18] Utreja, S., Jain, N. K., in: Jain N. K. (Ed.), *Advances in controlled and Novel Drug Delivery*, CBS Publishers and Distributors, New Delhi, India 2001.

[19] Bourrel, M., Schecter, R. S., (Eds.), *Microemulsion and Related Systems*, Marcel Dekker, New York 1988.

[20] Cavalli, R., Caputo, O., Carlotti, M. E., Trotta, M. *et al.*, Sterilization and freeze drying of drug-free and drug-loaded solid lipid nanoparticles. *Int. J. Pharm.* 1997, *148*, 47–54.

[21] Gasco, M. R., Method for producing solid lipid microspheres having a narrow size distribution. *US Patent 5250236,* 1993.

[22] Cavelli, R., Marengo, E., Rodriguez, L., Gasco, M. R., Effects of some experimental factors on the production process of solid lipid nanoparticles. *Eur. J. Pharm. Biopharm.* 1996, *42*, 110–115.

[23] Goppert, T. M., Muller, R. H., Polysorbate-stabilized solid lipid nanoparticles as colloidal carriers for intravenous targeting of drugs to the brain. Comparison of plasma protein adsorption patterns. *J. Drug Target.* 2005, *13*, 179–187.

[24] Ankola, D. D., Viswanad, B., Bhardwaj, V., Rao, P. R., *et al.*, Development of potent oral nanoparticulate formulation of coenzyme Q10 for treatment of hypertension: can the simple nutritional supplements be used as first line therapeutic agents for prophylaxis/therapy? *Eur. J. Pharm. Biopharm.* 2007, *67*, 361–369.

[25] Hariharan, S., Bhardwaj, V., Bala, I., Sitterberg, J. *et al.*, Design of estradiol loaded PLGA nanoparticulate formulations: a potential oral delivery system for hormone therapy. *Pharm. Res.* 2006, *23*, 184–189.

[26] Bala, I., Bhardwaj, V., Hariharan, S., Sitterberg, J. *et al.*, Design of biodegradable nanoparticles: a novel approach to encapsulate poorly soluble phytochemical ellagic acid. *Nanotechnology* 2005, *16*, 2819–2822.

[27] Sharma, R. A., Steward, W. P., Gescher, A. J., Pharmacokinetics and pharmacodynamics of curcumin. *Adv. Exp. Med. Biol.* 2007, *595*, 453–470.

[28] Yadav, V., Suresh, S., Devi, K., Yadav, S., Novel formulation of curcumin for anti-angiogenic and anti-inflammatory activity for optimization of therapy for inflammatory bowel disease. *J. Pharm. Pharmacol.* 2009, *61*, 311–321.

[29] Gota, V. S., Maru, G. B., Soni, T. G., Gandhi, T. R. *et al.*, Safety and pharmacokinetics of a solid lipid curcumin particle formulation in osteosarcoma patients and healthy volunteers. *J. Agric. Food Chem.* 2010, *58*, 2095–2099.

[30] Liu, A., Lou, H. X., Zhao, L., Fan, P., Validated LC/MS/MS assay for curcumin and tetrahydrocurcumin in rat plasma and application to pharmacokinetic study of phospholipid complex of curcumin. *J. Pharm. Biomed.* 2006, *40*, 720–727.

[31] Ravindranath, V., Chandrasekhara, N., Metabolism of curcumin-studies with [3H] curcumin. *Toxicology* 1981, *22*, 337–344.

[32] Orecchioni, A. M., Duclairior, C., Depraetere, P., Osterstock, F. *et al.*, Evaluation of gliadins nanoparticles as drug delivery systems. A study of three different drugs. *Int. J. Pharm.* 2003, *253*, 133–144.

