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# Formulation development and characterization of curcumin loaded solid lipid nanoparticles for improved aqueous solubility and bioavailability

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**Abstract**
Curcumin is a yellow hydrophobic polyphenol derived from the rhizome of turmeric that is safe and beneficial in several ailments. Low aqueous solubility and poor bioavailability were important of disadvantages of curcumin. The aim of the present study was to formulate the curcumin solid lipid nanoparticles (SLNs) with increased bioavailability profile and improved pharmacological activity. The SLNs were prepared by a hot homogenization coupled with ultrasonication method using tripalmitin, tween 80 and poly vinyl alcohol. The optimized blank SLNs formulations were utilized to entrap curcumin and characterized for particle size, polydispersity index, zeta potential, shape, drug encapsulation efficiency, and *in vitro* drug release. The prepared SLNs were analyzed by FT-IR spectroscopy to confirm the cross-linking reaction between drug, lipid and surfactants. The results demonstrated that the particle size, polydispersivity index, zeta potential, encapsulation efficiency and loading capacity of the SLNs were 214.60± 3.55nm, 0.49±0.03, -29.63±0.50mV, 51.99±4.14% and 5.33±0.34%, respectively. AFM images showed spherical to circular particles with well defined periphery. *In vitro* drug release exhibited biphasic pattern with an initial burst release of 16.5% within 2h followed by sustained release over 96h. FT-IR study suggested that during the process of formulations, lipid and surfactants have not reacted with the drug to give rise to reactant products and it was only physical mixture.

**Keywords:** Curcumin, Solid Lipid Nanoparticles, Hot homogenization *in vitro* release.

## 1. Introduction
Curcumin or diferuloyl methane is a yellow hydrophobic polyphenol derived from the rhizome of turmeric, *Curcuma longa* (*Zingiberaceae*). Effectiveness of curcumin has been established in a wide variety of human and animal diseases [1]. Since the time of Ayurveda (1900 BC) numerous therapeutic activities have been identified in turmeric for a wide variety of diseases and conditions, including those of the skin, pulmonary, and gastrointestinal systems, aches, pains, wounds, sprains, and liver disorders. It had many potential pharmacological effects including anti-inflammatory, antibacterial, antioxidant and anticancer activities. It was also proved against cardiovascular disease, Alzheimer's disease, liver problems, rheumatic arthritis, diabetics, Parkinson's disease and neurological disorders [2].

Despite the therapeutic potential of curcumin, its extremely low aqueous solubility, rapid metabolism, low gastrointestinal absorption, and degradation at alkaline pH limit curcumin bioavailability and clinical efficacy. The delivery of drug molecules through the carrier systems avoid unwanted effects because of controlled biodistribution. Development of efficient drug delivery system for curcumin would be a potential approach to improve its bioavailability and clinical efficacy.

The solid lipid nanoparticles (SLNs) is the most effective lipid based colloidal carriers system have potential in delivering the drugs with poor water solubility and therapeutic efficacy. The use of SLNs as drug carriers made from biocompatible and biodegradable polymers possess good stability, scaling-up feasibility, and the ability to incorporate hydrophilic or hydrophobic drugs. The incorporation of poorly soluble drugs into polymers can enhance gastrointestinal stabilization, absorption and bioavailability of the encapsulated drug [3].

Thus, the nano-sized drug delivery systems of herbal drugs have a potential future for enhancing the activity and overcoming problems associated with plant medicines. Hence, in the present study, it is planned to develop a method for the preparation of curcumin loaded solid lipid nanoparticle with a view to improve its aqueous solubility, bioavailability and clinical efficacy.

## 2. Materials and Methods

### 2.1 Drugs and chemicals

Curcumin, Tripalmitin, tween 80 and polyvinyl alcohol (Sigma Aldrich Chemicals Pvt. Ltd., USA) were utilized for the study. Dialysis membrane procured from Himedia Laboratories Pvt. Ltd., India was used. All other chemicals and solvents were analytical reagent grade and were used without further purification.

### 2.2 Formulation of curcumin SLNs

The formulation method described by Muller *et al.* [5] was followed with modifications. Curcumin, tripalmitin, tween 80 and polyvinyl alcohol were selected in the ratio of 2:5:20:20 based on formulation optimization study. Tripalmitin was heated to its melting point ($70^0$C) and curcumin was dispersed thoroughly in the molten lipid to get organic phase of preparation. Tween 80 and polyvinyl alcohol were mixed (aqueous phase) and heated to the same temperature as the organic phase. The hot aqueous phase was added to the organic phase under magnetic stirring at 1000rpm to form pre-emulsion. The hot pre-emulsion was then homogenised at 10,000psi for 3min using the high pressure homogenizer kept in a water bath maintained at $70^0$C.

The hot emulsion so obtained was ultrasonicated using high-intensity (5/64‘‘2mm tip diameter) microprobe with amplitude 20% for 15min to form nanoemulsion. Then, the nanoemulsion was run under magnetic stirring at 1000rpm for 4h to obtain curcumin loaded tripalmitin SLNs. All the batches were prepared in triplicate and characterized.

### 2.3 Physico-chemical properties of curcumin SLNs

**Surface morphology, particle size, polydispersity index and zeta potential of curcumin SLNs**

The prepared nanoformulation was viewed under Atomic Force Microscopy (PARK XE-100) to analyse the morphology of the particles. Particle size and polydispersity index of curcumin SLNs were measured by Photon Correlation Spectroscopy (PCS) using zetasizer nanoZS with the Malvern PCS software version 6.20. The zeta potential of curcumin nano suspension was measured by electrophoretic light scattering (ELS) mode using using zetasizer nanoZS. The instrument to determine the particle size maintained at $25^0$C. Each value was the average of three measurements.

**Loading capacity and encapsulation efficiency of curcumin SLNs**

Briefly, 0.1mL of freshly prepared nanoemulsion was taken and diluted with 9.9mL chloroform. The obtained suspension was vortexed for 1 h and centrifuged for 45min at 6,000rpm (5804R, Eppendorf, Germany). The supernatant was separated and filtered through 0.2µm filter. The filtrate was diluted using chloroform and analysed at 423nm using UV spectrophotometer (Systronics 2203 Smart, India). The SLNs formulated without curcumin were treated similarly and used as control for the measurements. The assay was repeated 3 times using different preparations. Loading capacity and encapsulation efficiency were calculated as shown below:

$$\text{Loading capacity} = \frac{\text{Weight of curcumin in SLNs}}{\text{Weight of SLN}} \text{X } 100\%$$

$$\text{Encapsulation efficiency} = \frac{\text{Weight of curcumin in SLNs}}{\text{Weight of curcumin added}} \text{ X}100\ \%$$

### 2.4 *In vitro* release of Curcumin SLNs

The release pattern of curcumin from SLNs was carried out using the dialysis membrane method. Curcumin nanosuspension equivalent to 5mg of curcumin was filled in dialysis bag. The bag was then dipped into 100 mL of phosphate buffer (containing tween 80 - 10%) at $37^0$C (pH 7.4) under magnetic stirring at a speed of 100rpm. The drug containing dialysis bag (Molecular weight 12 to 14k.Da, pore size 2.4nm) was dialysed against receiver compartment. To determine the curcumin diffused through the dialysis bag, 2mL samples were withdrawn at regular intervals (0, 5, 10, 20, 30, 45, 60, 90min, and 2, 4, 8, 12, 18, 24, 36, 72, 96 and 120h.) from the receiver solution and same amount of fresh receiver solution was added to maintain the volume constant. Curcumin concentration in the released samples were measured spectrophotometrically at 423nm using a UV spectrophotometer. The release experiments were carried out in triplicate. The control nanoparticles without curcumin were treated similarly and used as blanks for the measurements.

### 2.5 FT-IR Spectroscopic analysis

Fourier Transform-Infra Red (FT-IR) (Thermo Nicolet, Japan) spectral measurement for pure curcumin, tripalmitin, tween 80, polyvinyl alcohol and formulation were analysed separately and then correlated for compatibility.

### 2.6 Statistical analysis

The data obtained on particle size, PDI, zeta potential, loading capacity and encapsulation efficiency were analyzed using a Statistical Package for Social Sciences. All values are expressed as their mean±S.D.

## 3. Results and Discussion

### 3.1 Formulation of curcumin SLNs

Hot homogenization followed by ultrasonication was reported to be an economic, simple, reproducible and most reliable method for the preparation of SLNs. In this method, the preparation of SLNs does not require any organic solvents, which could be difficult to remove after nanoparticle synthesis [6]. By this method, it is possible to scale up to industrial level. Hence, hot homogenization coupled with ultrasonication method was employed in the present study to formulate SLNs.

According to Schwarz *et al.* [10], a sufficient high-energy input was necessary to break down the droplets into the nanometer range. A high energy such as high production temperature, high stirring rate, longer emulsification time and stronger ultrasound power were applied in this study to obtain a finer dispersion of formulation. In the present study, the homogenization pressure 10,000 psi was applied for 3 min and followed by ultrasonication resulted the mean (± SD) particle size of 214.60± 3.55 nm with narrow size distribution. It is in agreement with Reddy *et al.* [11] who found homogenization pressures 10,000 psi resulted the formulation with particle size ranged from 100 to 400nm. The author utilized homogenization at 10,000 psi for 3 cycles at 70°C water bath temperature which resulted in nanoparticles of low mean diameter. The result suggests that the hot homogenization and ultrasonication method was a feasible and compatible method for preparing curcumin loaded tripalmitin SLNs.

### 3.2 Physico-chemical properties of curcumin SLNs

Surface morphology, particle size, polydispersity index and zeta potential

The physical stability, cellular uptake and biodistribution were mainly influenced by the physic-chemical properties of the nanoparticles. As shown in figures 1 & 2, the curcumin SLNs were spherical and circular in shape. The surfaces of the nanoparticle were smooth. The sizes of SLNs measured by AFM were between 171 to 226nm. The particles were well dispersed with good particle size distribution. The measurements of particle sizes of SLNs by AFM and PCS were almost same.

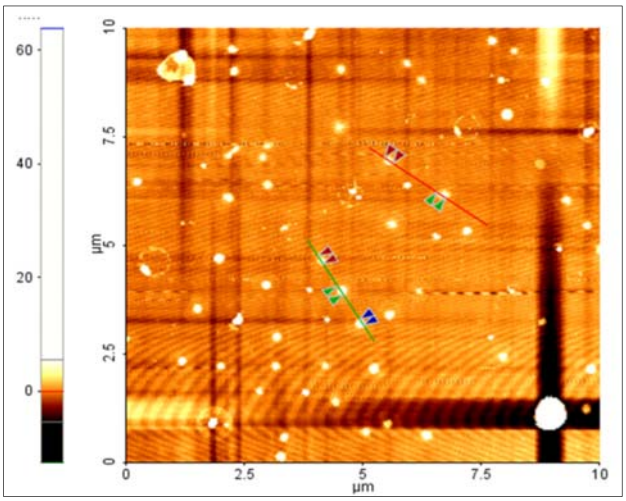


**Fig 1:** Atomic force microscopic image of curcumin SLNs

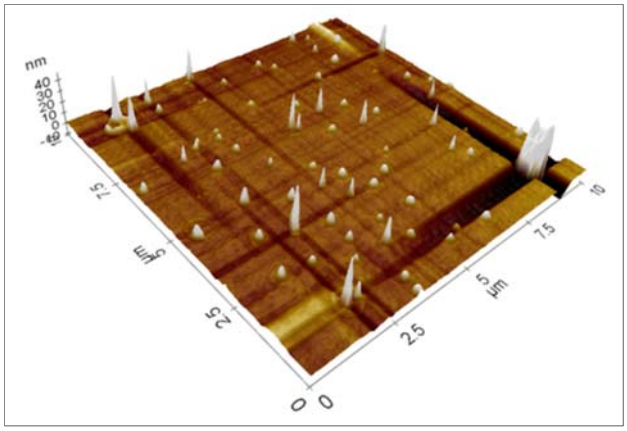


**Fig 2:** Atomic force microscopic 3D image of curcumin SLNs

The mean (±SD) particle size, polydispersity index and zeta potential of the formulations are given in Table 1 and the same are depicted in Fig. 3 and 4. In this study, the mean particle size of curcumin SLNs was 214.60± 3.55nm with narrow size distribution. The loading of drug with the blank SLNs in the present study resulted in a slight increase in the mean (±SE) particle sizes from 208.13±40.07 to 214.60± 3.55nm. These findings are in consistent with Jensen *et al.* [12] who explained that the increase in size of SLNs after incorporation of drug reflected the dissolution of the drug in the lipid phase.

**Table 1:** Mean (±SD) particle size, polydispersity index and zeta potential of curcumin SLNs (n=3)

| Particle size (nm) | PDI | Zeta potential (mV) | EE (%) | LC (%) |
|---|---|---|---|---|
| 214.60± 3.55 | 0.49±0.03 | -29.63±0.50 | 51.99±4.14 | 5.33±0.34 |
| (209.20-221.30) | (0.457-0.554) | (-28.9 to-30.64) | (46.60 to 60.12) | (4.90 to 6.0) |

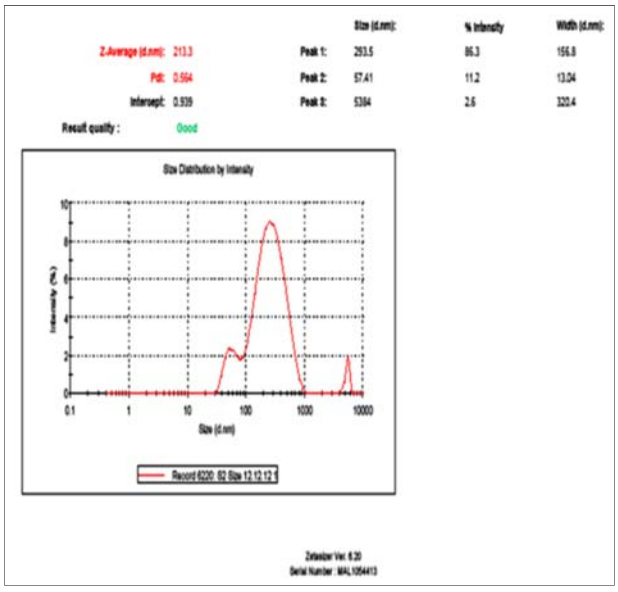


**Fig 3:** Particle size and size distribution of curcumin SLNs

A narrow particle size distribution was an indication of nanoparticles stability and homogeneous dispersion [13]. PDI values ranging from 0 to 0.5 were considered to be monodisperse and homogenous, but those of more than 0.5 indicated nonhomogenity and polydispersity [14, 15]. In the present study, the particle size distribution was monodisperse and homogenous as formulation has less mean (±SE) polydispersity index of 0.49±0.03

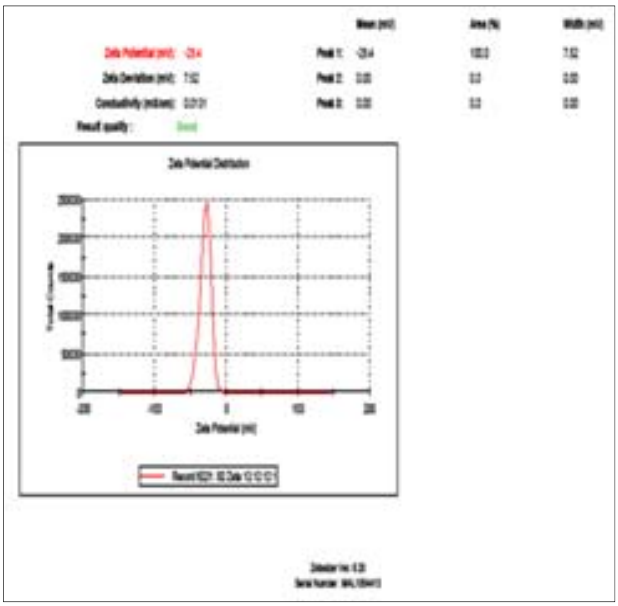


**Fig 4:** Zeta potential of curcumin SLNs

The surface charges of nanoparticles were of interest since it influenced the stability of nano suspension. According to Schwarz and Mehnert [16]; Zimmermann *et al.* [17], the negative charge of zeta potential was conferred by the lipids

used in the SLNs. In agreement with this, the tripalmitin utilized in this study provided negative charge of zeta potential. Nanoparticle with zeta potential values greater than +30 mV or less than -30mV typically have high degrees of stability due to electric repulsion between particles [5]. In this study, the mean (±SD) zeta potential of -29.63±0.50mV, which was enough to make the nanoparticles repel each other, thereby avoiding particle aggregation.

**Drug loading capacity and encapsulation efficiency**

The curcumin loaded SLNs obtained in the present study had relatively medium drug entrapment efficiency (51.99±4.14%) (Table 1). This could be attributed to the physicochemical properties of the drug, most importantly, its lipophilic nature [18] and those of the lipid base. To get sufficient loading capacity, the drug should have sufficiently high solubility in the lipid melt. Typically, the solubility should be higher than required because loading capacity decreases when cooling down the melt and might even be lower in the solid lipid [5]. According to Westesen *et al.* [7], the drug-loading capacity of the lipid carriers was limited owing to the generally low solubilization capacity of the molten lipids for many drugs, thus implying that entrapment efficiency was dependent on the solubility of drug in the lipid portion. The crystallization habits of tripalmitin nanoparticles also varied with the quantity of drug incorporated [19]. In addition to the lipophilicity of drug, its structural parameters were reported to enhance its entrapment in SLNs [20]. Beside the physico-chemical features of the drug, the structure of the lipid influences the drug incorporation capacity of SLNs as drug delivery system [18]. High temperature in production and high surfactant concentration might influence the drug loading and the shape of the loading profile [4].

In the present study, curcumin was having a higher melting point (170-175$^0$C) than the lipid base (67$^0$C). Hence it was expected that lipid phase solidify first upon cooling during the hot homogenization production process with the drug forming a core in the lipid phase [21]. Hence, the formulated SLNs in this study might be drug enriched core model. According to Muller *et al.* [5], drug enriched core is generally formed when the drug precipitates before crystallization of the lipid.

**3.3 *In vitro* release of curcumin SLNs**

*In vitro* release of curcumin from SLNs formulation is illustrated in Fig.5. Since curcumin is a hydrophobic drug, tween 80 was used in the receiver solution. *In vitro* release data obtained under sink conditions indicated biphasic release pattern of curcumin from SLNs with a 16.5% burst release in two hours and sustained release of 50.1% of the drug over a 4 day-study period. The findings are consistent with drug release from different SLNs [22, 4, 5]. The initial fast release (burst effect) could be attributed to the presence of a small fraction of unentrapped drug or drug embedded near the SLNs surface. Other factors contributing to a fast release were large surface area, high diffusion coefficient (small molecular size), low matrix viscosity and short diffusion distance of the drug. The slow release was mainly due to the low diffusion of drug molecules through the lipid matrix of the nanoparticles and hindering effects by surrounding solid lipid shell [4, 5]. Slow drug release contributes to maintaining the effective therapeutic drug concentrations.

The large surface area of SLNs, high diffusion coefficient of the entrapped drug, viscosity of the lipid matrix as well as the presence of surfactants adsorbed and incorporated in the surface during the production process were some important factors affecting the drug release from SLNs [23]. The longer carbon chain length of the fatty acid SLNs had slower release rates *in vitro*, this was attributed to that the enhanced lipophilicity of longer chain fatty acids had better drug retaining capacity. The drug release over 96h obtained in the current study might be due the long chain fatty acid tripalmitin utilized for incorporation. The magnitude of burst release from SLNs depends on various formulation and processing parameters. An increase in burst effect could be promoted by a higher surfactant concentration [24], lower lipid content [25] and higher homogenization temperature [24].

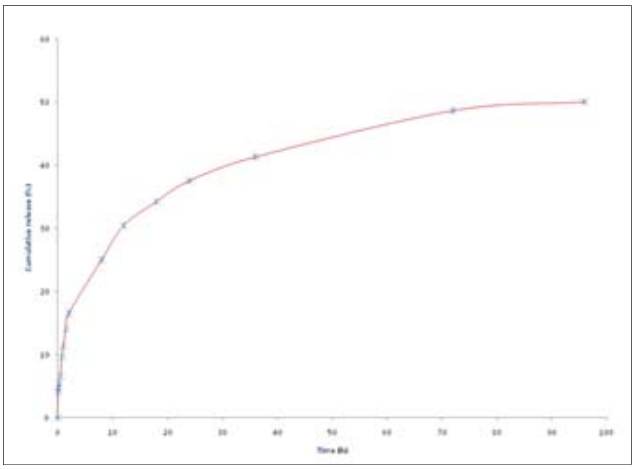

**Fig 5:** *In vitro* release of curcumin SLNs (mean± SD, n=3)

**3.4 Compatibility studies using FT-IR spectroscopic analysis**

The IR spectra of the formulation containing curcumin, tripalmitin, tween 80 and PVA (Fig. 6) showed all characteristics peaks in combination with no significant changes. From the IR spectra, it was clear that functionalities of curcumin have remained unchanged, including intensities of peak [26]. This suggested that during the process of formulations, surfactants, lipid and stabilizer have not reacted with the curcumin to give rise to reactant products. So it was only physical mixture and there was no interaction between them which is on favour to proceed for formulations.

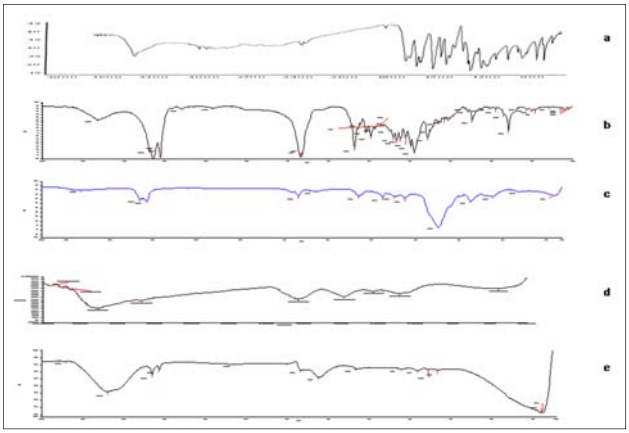


**Fig 6:** FT-IR spectra of curcumin (a), tripalmitin (b), tween 80 (c), PVA (d) and curcumin SLNs formulation (e)

## 4. Conclusion

In the present study, curcumin SLNs were successfully formulated by hot homogenization coupled with ultrasonication method. The formulation with medium encapsulation efficiency had a mean particle size of about 214 nm. An in vitro release experiment indicated that curcumin SLNs exhibited sustained release after an initial burst release. FT-IR study concluded that no interaction occurred between the drug excipients and polymer used in this study. We conclude from these results that SLNs could serve as a promising delivery to enhance the therapeutic efficacy of curcumin.

## 5. Acknowledgement

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