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Research Article

Formulation and In Vitro Evalaution of Curcumin Loaded Solid Lipid Nanoparticles as Transdermal Drug Delivery

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A B S T R A C T

The current research was to develop Curcumin loaded solid lipid nanoparticles (CUR-SLNs) by nano-emulsion template method using Span 60, Tween 60, Stearyl alcohol and Brij 35. CUR-SLNs were characterized for encapsulation efficiency, particle size and *in vitro* drug release. Further optimal formulation was incorporated into Carbopol gel and studied *ex vivo* permeation and skin irritation tests. The CUR-SLNs shows good encapsulation efficiency and desired particle size with prolonged drug release. The *ex vivo*permeation studies proved that CUR-SLNs considered to be a successful transdermal drug delivery system and provide a sustained release of encapsulated drug for 24 hr. Further, CUR-SLNs loaded carbopol gel considered to be non-irritant and safe to be applied on the skin for the intended period of time.

Keywords: Curcumin, Span 60, Tween 60, Solid lipid nanoparticles, Transdermal

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INTRODUCTION

The transdermal drug delivery system (TDDS) has attracted extensive attention due to its tremendous advantages. On one hand, it iscapable to escape undesirable metabolism both in the gastrointestinaltract and in the liver after traditional oral administration, not only producing a tunable drug release, but also allowing enhanced drug absorption, eventually leading to boost drug bioavailability. On the other hand, TDDS also possesses numerous benefits compared to the parenteral administrations, particularly the painless and easily operated self-administration process with higher patient compliance[1]. However,the efficacy of the transdermal drug delivery in maintaining a therapeutic level depends on the ability of the drug to penetrate the skin inadequate amounts. The barrier nature of the stratum corneum (SC) is amajor challenge that restricts the entry of most drugs via the transdermal route. Many transdermal methods have been tried to achieve greater transdermal permeability in order to overcome the barrier of SC.These techniques are designed for a longer period to deliver the drug, nanotechnology has developed an attractive niche research in transdermal drug delivery[2]. Among all colloidal carriers, Solid Lipid Nanoparticles (SLNs) have emerged as an alternative colloidal carrier due to their advantages such as enhanced physical stability, good tolerability, ease of scale up and growth. SLNs have been widely used for the distribution of skin due to their safe interaction with skinlayers and improved skin permeation[3-5]. Curcumin (CUR), a yellow polyphenolic principal active constituent extracted from the *Curcuma longa L.*(turmeric) rhizome, and has been widely used in the chemical and food industries as a pigment, seasoning, and preservative[6-8]. Literature revealed CUR presents several health promoting benefits has diverse pharmacological activities including anti-oxidant, anti-inflammatory, anti-cancer and antidepressant and wound healing properties[9-11].The use of CUR as a promising new natural chemical for chemoprevention and cancerchemotherapy has been extensively studied over the past several years. However, in spite of the promising antitumor activity of CUR, its poor solubility and stability in aqueous systems,as well as its rapid metabolism and systemic elimination, have limited its clinical application[12]. In an effort to address these limitations,

various CUR delivery systems have been investigated, including solid dispersion[13], liposomes[14], phospholipid complexes[15], polymeric nanoparticles[16] and nanocrystals[17,18]. These systems suffer from several drawbacks, including poor physical stability, drug leakage and the potential toxicity of the excipients. SLNs overcome such drawbacks and prove to be stable. SLNs consist a colloidal solid lipid core matrix which is stabilized and emulsified by a surfactant in an aqueous medium. Small SLNs ensure nanoparticles and are in close contact with the stratum corneum, thereby increasing the number of encapsulated agents that penetrate the via bleskin. Due to the solid lipid matrix, a controlled release of drugs from SLNs could be observed. This becomes an important tool, when the drugproduces irritation in high concentrations, reduces systemic absorption, and when the drug must be supplied for an extended period.The present research was aimed to target transdermal delivery of CUR by loading into SLNsusing novel carriers. The prepared CUR-SLNs were further characterized by particle size, entrapment efficiency, *in vitro* drug release, *ex vivo* permeation and skin irritancy behaviour.

MATERIALS AND METHODS

Materials: Curcumin (CUR)(Mylan Laboratories, Hyderabad), Span 60, Tween 80 (S.D Fine- Chemltd, Mumbai). Brij 35 (Hi Media Laboratories Pvt. Ltd. Mumbai),Stearyl alcohol (Yarrow Chem products, Mumbai), Carpool (Yarrow Chem products, Mumbai). All other solvents and chemicals were used of analytical grade.

Methods

Preparation of CUR-SLNs: CUR-SLNs were prepared by nano-emulsion template method[19] with slight modifications. Concisely, the mixture of CUR/Stearyl alcohol/Brij 35 at different weight proportionswas melted in a water bath at 70 °C (Oily phase).Filtered deionized water was preheated at 70 °C and was added (5 ml) to mixture of Span60/Tween 60(aqueous phase) this aqueous phase was added to oily phase slowly with continuous magnetic stirring at 800 rpm for 2hr to obtain a clear nanoemulsion. The temperature was maintained at 70 °C during the production of nanoemulsion. Subsequently, hot nanoemulsion was rapidly cooled down at 4°C in crushed ice box with continuous stirring at 800 rpm to accomplish solidification of lipid to form CUR-SLNs. The prepared CUR-SLNs were kept at 4 °C for further analysis.The various compositions of CUR-SLNs are summarized in table 1.

Table 1: Formulae of CUR-SLNs

Batches	Curcumin in mg	Span-60 in mg	Tween-60 in mg	Stearyl alcohol in mg	Brij 35 LR in mg
B-1	100	85	430	285	860
B-2	100	430	85	285	860
B-3	100	285	285	285	860
B-4	100	285	285	285	570

Evaluations

FTIR studies: The FTIR spectra for CURandoptimal CUR-SLNs were recorded using BRUKER-FTIR spectrophotometer in the wave number region from 4000cm^{-1} to 500cm^{-1}.

Particle size and size distribution studies: For all the batches of CUR-SLNs size analysis was carried out using Malvern Zetasizer Nano ZS (Malvern Instruments, UK). The freshly prepared CUR-SLNs were dispersed in double distilled water (DDW) and were used to characterize the vesicle size. The homogeneity and stability of CUR-SLNs further assessed byPolydispersity Index (PDI) Zeta potential.

Entrapmentefficiency: The percentage of entrapped CUR in SLNs was determined by exhaustive dialysis method. During the study, dialysis cellophane membrane was mounted between the donor and receptor compartment and the receptor medium was phosphate buffer pH 6.8. 100 mg of CUR-SLNs was placed on dialysis membrane, whole assemble was kept onmagnetic stirrer stirred the receptor medium. After 6 hr the CUR content in receptor compartment was determined by measuring absorbance at 428nm using double beam UV spectrophotometer. The % entrapment efficiency was calculated by using following equation.

$$\% \textbf{ Entrapment efficiency} = \frac{\textbf{Total drug} - \textbf{Free drug}}{\textbf{Total drug}}$$

***In vitro* drug release studies:** The drug release from CUR-SLNs was performed using Franz-diffusion cell. The dialysis cellophane membrane was mounted between the donor and receptor compartment and receptor medium was phosphate buffer pH 6.8. In each case CUR-SLNs equivalent to 10 mg of CUR was placed on one side of the dialysis membrane, the whole assemble was kept on magnetic stirrer. At different intervals of time adequate samples were withdrawn and % CUR release was estimated by UV spectroscopic method. The *in vitro* drug release data was computed and interpreted using dissolution software PCP Disso V3. The same procedure was adapted for in vitro drug release

Among theCUR-SLNs, optimal batch was selected and incorporated into 1% w/w Carbopol gel and assesses the *in vitro* drug release and ex vivo permeation.

Fabrication of CUR-SLNs loaded Carbopol gel: During the fabrication initiallyplain carbopol gel was prepared by dispersing 1% w/w of carbopol in measured amount of distilled water, the mixture was sonicatefor 6-8 min to dissolve carbopol completely and avoid any air drops. Further the mixture is neutralized with triethanolamine to attain the desire pH and gel. In next step, optimal CUR-SLNs

was carefully incorporate intoalready prepared carbopol gel with constant stirring until desired consistency was obtained.

***Ex vivo* permeation study:** *Ex vivo* permeation study was carried out using male Wistar Albino rat skin as reported by Ibrahim et al[19].The *ex vivo* permeation was conducted by using Franz diffusion cell, the rat skin was mounted between the donor and receptor compartment with the stratum corneum facing upper side on the diffusion cell. The efficient diffusion area of the cell was 1.41 cm^2 and 25 ml phosphate buffer pH 6.8 was taken in receptor compartment. The temperature was maintained at 37 ± 1°C. Carbopol gel loaded with optimized CUR-SLNs equivalent to 5 mg of CUR was spread over the rat skin. The receptor compartment content was stirred with the help of magnetic beads. The samples were collected at different time intervals and were immediately replaced with the fresh media. The steady state flux was calculated by analysing samples using UV spectrophotometer.

Skin irritation/sensitivity studies: Albino Wistar rats (200-250g) were selected for the study. All the animals used in the study were caged and maintained according to the guidelines of CCSEA or principles established for care and use of laboratory animals. The rats were divided into two groups (n = 4). The rats were anesthetized and the abdominal area was shaved and wiped with 70% alcohol swab. The plain and optimized CUR-SLNs loaded carbopol gels were applied to the respective groups. The skins were scored according to erythema and oedema scale as 0 = none; 1 = slight; 2 = well defined; 3 = moderate; 4 = scar formation by visual observations as described by Draizeet al[20].

Stability studies: The short stability study was performed according to ICH guidelines. The optimized CUR-SLNs were filled in tightly closed glass vials and subjected to stability testing. The formulations were kept at refrigerated conditions (4±1°C) and at room temperature (25±2°C) and were analyzed for vesicle size and entrapment efficiency after 6 months.

RESULT AND DISCUSSION

FTIR studies:FTIR spectra of CUR and optimized batch of CUR-SLNs was showed in figure 1. The characteristic peak at 2929.76 cm^{-1} (-OH stretching), 1739.88 cm^{-1} (C≡C stretching), 1623.88 cm^{-1} (CH=CH stretching, aromatic), 1593.75 cm^{-1}(CH_2 = CH_2 stretching, cyclohexyl) and 1270.15 cm^{-1} (C=O ester).The characteristic FTIR peaks of CUR were found in optimized batch of CUR-SLNs indicates no interaction.

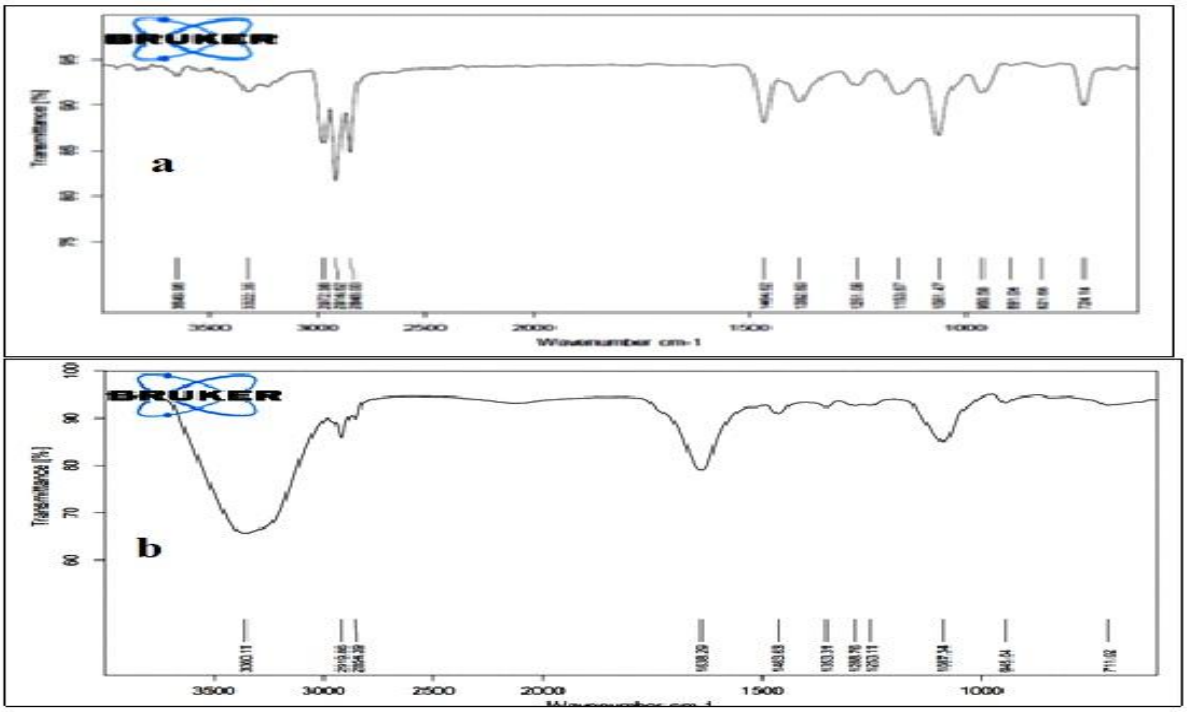

Figure 1: FTIR spectra of(a) CUR and Optimized batch of (b) CUR-SLNs

Particle size: The particles size of CUR-SLNs determined by particle size analyzer and was shown in figure 2. The average particle size was found to be in the range of 320 to 2215 nm for B1 to B4 CUR-SLNs. Among the batches B2 CUR-SLNs shows lowest Particle size (320 nm). Slightly increased in particles size phenomena commonly observed after incorporation of drug in the lipid core, which plays a crucial role in uptake of nanoparticles after transdermal application. CUR-SLNs were uniform in size as evidenced by low PDI (<0.3) is generally accepted optimum value to indicate homogenous distribution of nanoparticles. Zeta potential is the measure of net surface charge and is an important parameter influencing physical stability of colloidal dispersions. High zeta potential values confer stability to colloidal dispersion by preventing aggregation due to electrostatic repulsion between similarly charged particles. CUR-SLNs showed zeta potential in the range of−15.8 Mvto -10.3 mV.

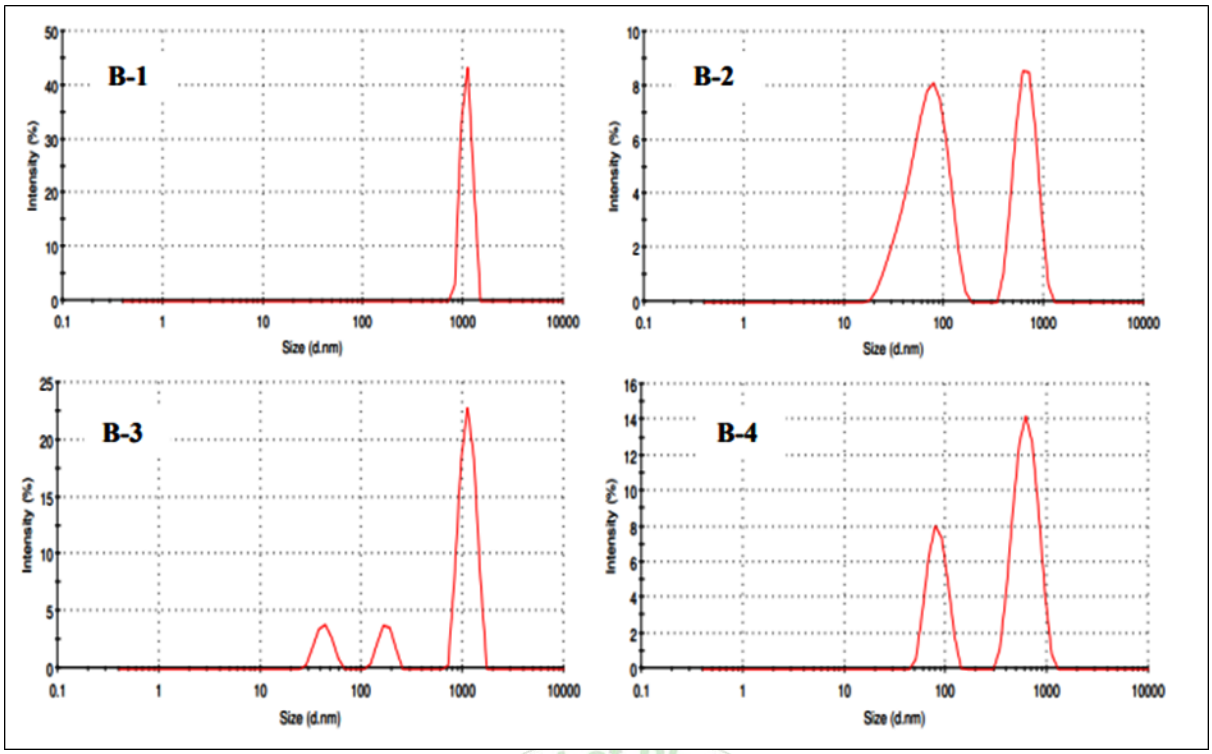

Figure 2: Average particle size of CUR-SLNs

Encapsulation Efficiency (EE): The % EE of CUR in CUR-SLNs was 80.10 ± 0.09539 (B1);84.21 ± 0.03606 (B2);74.55 ± 0.03055 (B3); 72.39 ± 0.02646 (B4). Stearyl alcohol, a 16-C member of saturated fatty alcohols, was used as a solid lipid core of CUR-SLNs stabilized by a surfactant mixture composed of Tween 60 and Span 60. The % EE was directly influence by Stearyl alcohol and surfactants. The results suggest higher the concentrations of Span 60 and Tween 60 shows greater % EE, it is mainly due to interaction of palmitate moiety of non-ionic surfactant with Stearyl alcohol to form miscible surfactant shell. In addition, Brij 35 with long chain polyoxyethylene (polyethylene glycol) moieties was added in the surfactant mixture to function as steric stabilizer to improve the stability of CUR-SLNs by reducing surface interactions between the particles. Solid lipids having melting temperatures above the body temperature possess strong hydrophobic interactions with lipophilic drugs resulting in high encapsulation efficiency and stable lipid core with sustain drug release. The % drug content of CUR in CUR-SLNs was in the range of 98.12 ± 0.08539 (B1); 99.01 ± 0.04606 (B2); 94.55 ± 0.02055 (B3); 97.39 ± 0.01946 (B4). The low SD value indicate the drug content was uniform and adapted method was found to be reproducible.

***In vitro* drug release studies:** *In vitro* drug release studied were conducted for B1 to B4 formulations by using Franz diffusion cell and shown in figure 3. The initial drug release about 10 -13% after 2 hr could be attributed to the presence of a small fraction of unentrapped drug or free drug adhered near the SLNs surface and improperly formed nanoparticles. After 6 hr the drug release was 40 to 43 % could be exhaust of adhered drug particles and improper formed nanoparticles.The steady and slow release of drug was observed up to 12 hr is mainly due to low diffusion of drug molecules through the lipid matrix of the nanoparticles and hindering effects by surrounding solid lipid shell. 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. 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 *in vitro* drug release data for B1 to B4 formulations were fitted into various kinetic equations to find out the order and mechanism of drug release. The B1 formulation follows first order drug release, whereas, remaining all formulations viz., B2, B3 and B4 follows Korsemeyer peppas model, and in all the formulations the release exponent, 'n' was found to be less than 0.5 indicated the drug release followed fickian and release mechanism was indicative of diffusion controlled. *In vitro* release studies are often performed to predict how a delivery system might work in an ideal situation as well as give some indications of its *in vivo* performance since drug release dictates the amount of drug available for absorption.

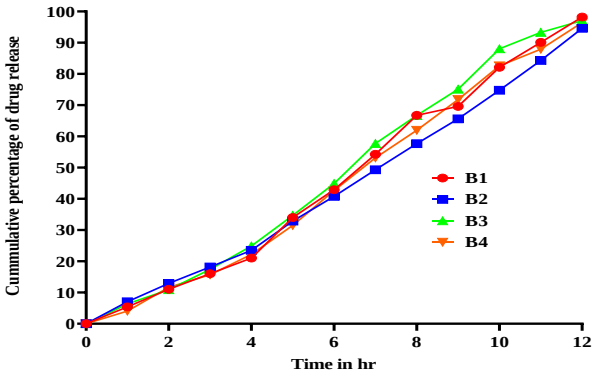

Figure 3: *In vitro* dissolution profile of CUR-SLNs

***Ex vivo* permeation studies:** The amount of CUR permeated across rats' skin after 24 hr, flux was found to be 39.12 ± 1.01 µg/h/cm^2 from plain carbopol gel and 91.91 ± 0.86 µg/h/cm^2 from CUR-SLNs loaded carbopol gel. The low permeability in plain gel was due to lower aqueous solubility CUR, whereas higher skin permeability in SLNs might be the result of one or more of the wing mechanisms i.e. increased solubility of active drug, high association of drugs with vesicle bilayers, increased partitioning of vesicles into the stratum corneum and elasticity of vesicle membrane. The packing nature of unsaturated fatty acids changes the stratum corneum lipid structure upon binding to the keratin filament, hence increase drug permeability across skin.

Skin irritation test: The Albino rats received optimal CUR-SLNs gels as well as CUR loaded plain gel were free from of any irritation and there were no signs of erythema. The results as shown in table 2 suggest that CUR loaded plain carbopol gel and CUR-SLNs loaded carbopol gelconsidered to be non-irritant and formula is safe to be applied on the skin for the intended period of time.

Table 2: Skin irritation data of CUR loaded carbopol gel and CUR-SLNs loaded carbopol gel

Formulation	Application period						
	Erythema scores						
	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6	Day-7
CUR-SLNs gel	0	0	0	0	0	0	0
Plain gel	0	0	0	0	0	0	0

CONCLUSION:

Curcumin loaded SLNs was prepared successfully by nano-emulsion template method. The prepared SLNs exhibited desired entrapment efficiency and Particle size. Among the formulations B2 shows better % EE and drug release and chosen as optimal formulation to incorporate into carbopol gel. The CUR-SLNs loaded transdermal carbopol gel shows good ex vivo permeation data than the plain CUR loaded gel, which concludes CUR-SLNs considered to be a successful topical transdermal drug delivery system and provide a sustained release of encapsulated drug. Furthermore, CUR-SLNs loaded carbopol gel considered to be non-irritant and safe to be applied on the skin for the intended period of time.

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Statement of ethical approval: The University Animal Ethics Committee IAEC of V.L. College of Pharmacy, Karnataka, India (VLCP/IAEC/21-23,557/PO/Re/S/02/CCSEA), approved the study protocol.

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