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SOLID LIPID NANOPARTICLE EMULSION WITH LOADED CURCUMIN THAT HAS IMPROVED PHYSICOCHEMICAL CHARACTERISTICS

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Abstract

This study goal was to create and describe the characteristics of a curcumin-containing solid lipid nanoparticle (SLN) dispersion. Tripalmitin and lecithin were used as the lipid core of the SLN, which was subsequently synthesised utilising the ultrasonication process and the chitosan (cs) surface coating. The coated SLN (cs-SLN), which had a considerable increase, with a mean particle size of 116.8 ±2.88 nm and an entrapment efficiency of more than 99%. In relation to tripalmitin, the zeta potential increased proportionately and achieved a plateau at 5% CS coating. The Fourier Transform Infrared (FTIR) analysis of SLNs indicated that there was no alteration in the CUR spectrum, suggesting that the lipid and CS components were compatible with CUR. When the coating amount exceeded 2.5% of tripalmitin, the particle size and zeta potential (ZP) remained stable even at 40°C for a period of 90 days. All SLN emulsion exhibited a considerably faster release of CUR compared to pure CUR powder. The release rate at pH 7.0 increased proportionally to the coating amount. Interestingly, at pH 3.0, a CS coating of 5.0% or higher reduced the rate of release. However, the CUR from cs-SLN was notably lower than that from uncoated SLNs.

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Introduction

In the past decade, there has been a growing interest in Solid Lipid Nanoparticle (SLN) emulsion. These colloidal carriers consist of lipids with a high melting point, making them solid particles that are stabilized by surfactants. One of the main advantages of SLNs is their ability to remain in a solid state even at the normal body temperature of humans. This feature allows SLNs to combine the benefits of liposomes and polymeric nanoparticles while eliminating the need for organic solvents in the manufacturing process [1]. Additionally, the release of bioactive compounds trapped within SLNs can be regulated. SLNs offer a solution to the challenges associated with oral delivery of bioactive substances that have low solubility, poor permeability, and are subject to P-glycoprotein-mediated efflux. Furthermore, lipids are far less expensive than synthetic polymers in the production of SLN. To take advantage of these benefits, SLN needs well-designed formulation technology, though, as the bioactives in the SLN may partition during production and/or storage [2]. Numerous studies have shown that flavonoids have anti-inflammatory, antioxidant, and antiplatelet effects. The flavonoid component CUR (Fig. 1), which is present in a variety of fruits, vegetables, leaves, and grains, has similar positive effects to other flavonoids [3]. However, problems with poor absorption and solubility limit its physiological efficacy in the body [4].

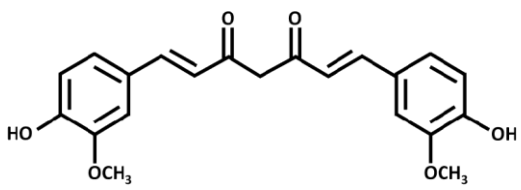

Figure 1. Chemical structure of CUR

The challenges associated with CUR trapped in SLN emulsion can potentially be overcome. However, it is important to address the issue of SLNs being digested in the gastrointestinal environment, as their effectiveness would be compromised. Therefore, it is valuable to modify the surface of SLNs in order to enhance the protection of the lipid core against the harsh conditions in the gut. Chitosan (CS), which is a natural biopolymer, has been extensively studied and has gained recognition as a promising system for drug delivery. CS has been explored in various applications including parenteral delivery, non-viral gene delivery, vaccine delivery, ocular delivery, brain targeting delivery, and tissue engineering. Moreover, its adhesive properties have made it suitable for surface modification of liquid crystalline nanoparticles.

Materials and Methods

Materials

HiMedia Laboratories Ltd in Mumbai, India, provided the curcumin (CUR), tween 80, dialysis membrane, and chitosan (CS) (low molecular weight, Prod. No. 448869). Phosal 50G and Phosal 53MCT were purchased from SRL chemicals, Mumbai, India., while Gatefosse (Bangalore) generously provided Peceol, Transcutol HP, Labrasol, Capmul MCM, Labrafil M 2125, Labrafac PG, and Labrafile WL2609. Lecithin and tripalmitin were bought from Sigma-Aldrich, Mumbai, India. All other compounds were of the analytical variety and were utilised directly.

Solubility Studies

The solubility analysis was conducted using a method previously described, with minor adjustments [5]. Excessive amounts of CUR were added to different oils, namely corn oil, rice bran oil, glycerin, PEG 400, propylene glycol, Phosal 50G, Phosal 53MCT, Peceol, Transcutol HP, Labrasol, Capmul MCM, Labrafil M2125, Labrafac PG, and Labrafile WL 2609. The mixtures were then vigorously mixed using a vortex to ensure proper blending of CUR with the vehicles. Subsequently, the mixtures were allowed to equilibrate at 37 °C in a shaking water bath. After 72 h, the mixtures were subjected to centrifugation at 12,000g for

10 min to separate any undissolved CUR. The resulting solutions were then filtered through a 0.45-µm membrane filter. The filtrates were appropriately diluted with methanol, and the CUR concentration was determined using UV-spectrophotometer (Agilent Cary 60 UV–Vis Spectrophotometer, USA.).

Preparation of SLN and chitosan-Coated SLN (cs-SLN)

The SLN dispersion was prepared using the ultrasonication method, following the procedure described in previous studies [6]. The composition of the dispersion is presented in Table 1. Initially, lecithin was heated to a temperature 10°C higher than its melting point, followed by the dissolution of tripalmitin. CUR, dissolved in PEG 400, was then added to this lipid phase. Simultaneously, an aqueous solution containing Tween 80 was heated to the same temperature as the lipid phase and combined with the lipid mixture. The resulting mixture was sonicated using a probe-type sonicator for 6 min at 60% amplitude, with a pulsing pattern of 2 seconds of sonication (ON) and 2 seconds of resting (OFF). After sonication, the SLN dispersion was cooled in an ice bath and the lipid content in the SLN dispersion was 20 mg/mL.

Table 1. Ingredients of SLN and cs-SLN*

S.No	Preparation code	CMN (mg)	TP (mg)	PEG400 (mg)	LEC (mg)	TW80 (mg)	CS (mg)	Distilled water (g)
1	SLN	5	200	100	30	125	-	10
2	Cs-SLN0.5	5	200	100	30	125	1	10
3	Cs-SLN1.0	5	200	100	30	125	2	10
4	Cs-SLN2.5	5	200	100	30	125	5	10
5	Cs-SLN5	5	200	100	30	125	10	10
6	Cs-SLN7.5	5	200	100	30	125	15	10

CUR-curcumin, CS-chitosan, TP-tripalmitin, LEC-lecithin, TW80-Tween 80, SLN- curcumin solid lipid nanoparticle, cs-SLN-chitosan-coated solid lipid nanoparticle.

SLN's surface was coated using a technique that has been previously reported [7]. CS was dissolved into 1% v/v acetic acid for 24 h using a magnetic stirrer to guarantee total dissolution. CS solutions were then made at various concentrations. SLN dispersion and CS solution were combined using a magnetic stirrer for one hour (1:1 by volume). CS percentage as a percentage of tripalmitin ranged from 0.5 to 7.5% by weight.

Particle Size (PS) Distribution and Zeta Potential (ZP)

The PS distribution of SLN emulsion and their polydispersity index (PDI) were assessed using dynamic light scattering (DLS) with the Zetasizer Nano S90 instrument (Malvern Instruments, UK). The emulsion was appropriately diluted with distilled water with a conductivity of 18.2 MΩ cm^{-1} and measured at 25 °C using disposable cuvettes. Each measurement consisted of at least three sets of 15 runs, and the average size and PDI were calculated based on the results [8]. To determine the ZP, Photal ELSZ-1000 instrument was employed, and three measurements were taken for each sample at 25°C, from which the mean values were determined.

Entrapment Efficiency (EE)

The EE of CUR was determined using the ultrafiltration method with slight modifications based on a previously described procedure [9]. A volume of 0.5 mL of the SLN dispersion was transferred

to the upper chamber of an ultrafiltration tube (Amicon Ultra-4, MWCO 10,000 g/mol, USA) and then subjected to centrifugation at 4000g for 30 min at 4°C. The filtrate, which contained the CUR that was not entrapped within the nanoparticles, was analyzed using UV-Spectrophotometer. The EE was calculated using the following equation:

EE (%) = (D_{total} - $D_{unentrapped}$) / D_{total} × 100, where D_{total} represents the total amount of CUR in the nanoparticle dispersion, and $D_{unentrapped}$ represents the amount of unentrapped CUR.

Fourier Transform Infrared (FTIR) Spectroscopic Analysis

Using a JASCO/FTIR-6300, Japan, the FTIR spectra of CUR, chitosan (CS), freeze-dried SLN, and cs-SLN were obtained [10]. The samples were prepared by combining them with anhydrous KBr powder and pressing them into pellets. The 400 to 4000 cm^{-1} range was covered by the FTIR spectra, which were acquired with a resolution of 1 cm^{-1}.

Scanning Electron Microscopy (SEM)

The surface morphology and shape of SLN and cs-SLN were examined using SEM. To prepare the samples for SEM analysis, the mixture was sparsely dusted on a piece of double-sided tape that was attached to an aluminium stub. Then, using a gold sputter module in a high-vacuum evaporator and an argon atmosphere, the stubs were coated with gold to a thickness of 300 [11]. The coated samples were then randomly scanned on a scanning electron microscope (Carl Zeiss Microscopy Ltd, EVO 18, Germany) and photomicrographs were taken.

***In Vitro* Release Study**

CUR was released from the nanoparticles in vitro in triplicates using 0.5% w/v Tween 80 in PBS buffer (pH 3.0 and pH 7.0). One millilitre of nanoparticle dispersion (Molecular weight cut off of 12- 14 kDa) was placed in a dialysis bag, along with 20 mL of PBS buffer, and gently shaken at a rate of 50 strokes per minute in a water bath at 37°C. At predetermined intervals of 0.5, 1, 2, 3, 4, 5, 6, and 12 h, an aliquot (0.5 mL) of the PBS buffer separate from the dialysis solution was collected and replaced with an equivalent volume of fresh medium. CUR release was assessed using the UV-Spectrophotometric method mentioned above. As a control, CUR dissolved in PBS was utilised.

Statistical Analysis

Using Microsoft Office Excel 2010, the Student's t test was used to analyse all the data. The mean and standard deviation of the data are displayed. Statistical significance was defined as $p < 0.05$.

RESULTS

Solubility of CUR

According to earlier studies, CUR was very poorly soluble in natural oils (0.31 mg/g in maize oil and 0.34 mg/g in rice bran oil) (Fig. 2) (32). PEG 400 produced the maximum solubility (111.2 ± 5.1 mg/g) among the tested oils, followed by Transcutol HP (94.8 ± 3.9 mg/g) and Labrasol (81.2 ± 5.1 mg/g). Based on this finding, PEG400 was chosen as the solvent in this study's formulation of SLNs. Natural bioactives like flavonoids and phytosterols have poor solubility, which is a key concern for their bioavailability. To solve this issue, many drug delivery strategies have been created [12].

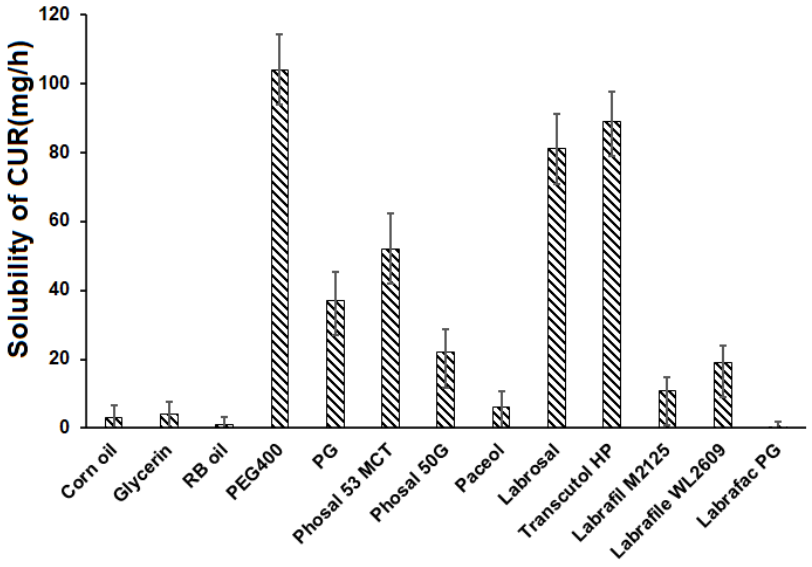

Fig. 2. The solubility of CUR was evaluated in various oils. Each value reported represents the mean ± standard deviation (n = 3).

SLNs offer unique advantages that combine the benefits of polymeric nanoparticles and liposomes. These advantages include controlled release, targeted delivery, and enhanced stability of encapsulated bioactive compounds. The selection of lipid and surfactant components is a crucial factor in achieving an optimal SLN formulation, as it directly influences important physicochemical characteristics such as particle size, in vitro release profile, and long-term stability during storage [13].

Table 2 provides a summary of SLN and cs-SLN characterization. All formulations' PDI values were less than 0.3, which indicates that the particle size is homogeneous. By using dynamic light scattering, the particle size distribution of the various SLNs was determined. The mean particle size of uncoated SLN was 116.8 ±2.88 nm, and it significantly increased following surface modification with CS, indicating that CS had been coated on the surface of the material.

Table 2. Characterization of SLN emulsion

S.No	Preparation code	PS (nm)	PDI	ZP (mV)	EE (%)
1	SLN	116.8 ± 2.88	0.201 ± 0.03	−30.1 ± 2.7	98.18 ± 8.8
2	Cs-SLN0.5	211.2 ± 9.8*	0.219 ± 0.07	+3.11 ± 0.21*	98.18 ± 5.9
3	Cs-SLN1.0	159.6 ± 8.19*	0.271 ± 0.04	+18.11 ± 1.16*	97.19 ± 6. 7
4	Cs-SLN2.5	162.6 ± 7.88*	0.321 ± 0.08	+29.15 ± 3.11*	99.56 ± 8.12
5	Cs-SLN5	156.8 ± 4.39*	0.277 ± 0.090	+57.26 ± 9.11*	98.16 ± 6.5
6	Cs-SLN7.5	160.2 ± 5.6*	0.303 ± 0.033	+52.35 ± 6.48*	98.80 ± 7.7

Each value presented is the mean ± SD (n = 3), with * indicating statistical significance ($p <$ 0.001) when compared to the SLN group.

SEM

The most significant enlargement occurred in cs-SLN 0.5 (211.2 ± 9.8 nm) compared to the other SLNs. The findings from the dynamic light scattering study were supported by the SEM image. The SEM image revealed that the uncoated SLN had a smooth and spherical surface, whereas the coated SLN had a rough surface (Fig. 3). Additionally, the coated SLN exhibited a core-shell structure, indicating successful coating with CS [14].

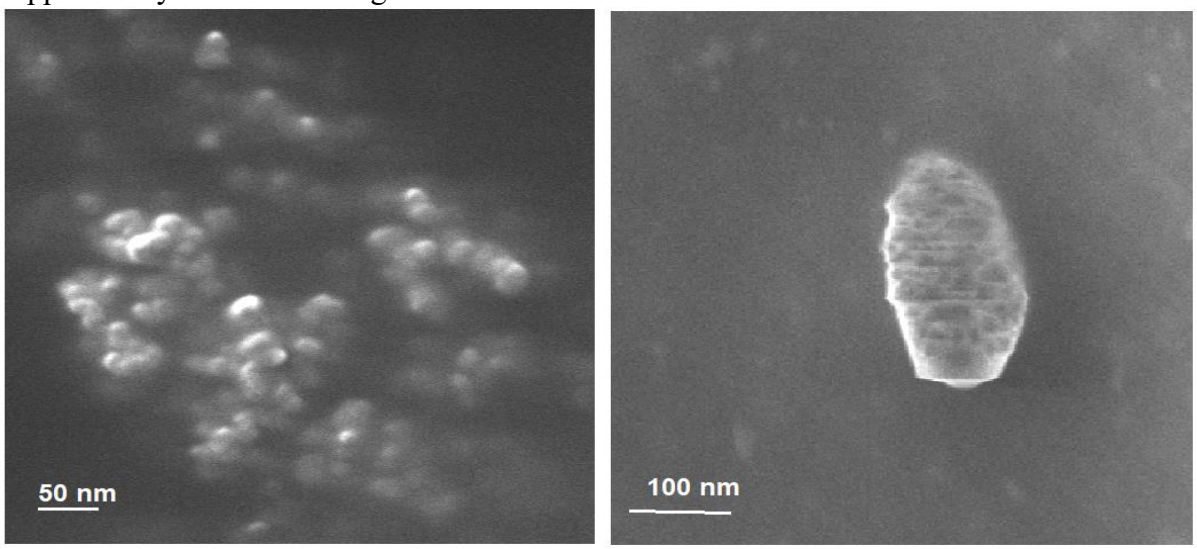

Fig. 3. SEM images of both SLN and cs-SLN were obtained (a) SLN and (b) cs-SLN

The triglyceride of palmitic acid known as tripalmitin has been employed extensively in drug delivery to create a lipid core. Due to their good biocompatibility, lecithin and Tween 80 are also frequently utilised in the SLN formulation as emulsifiers [15]. CS is a naturally occurring polymer that is mucoadhesive, biocompatible, and biodegradable and is utilised in drug delivery to increase gastrointestinal absorption and bioavailability. It creates a gel-like barrier in the stomach environment, allowing bioactives bound in nanoparticles to escape gradually [16].

The ZP exhibited a proportional increase until 5% CS was added to tripalmitin, after which it reached a plateau. Notably, cs-SLN 0.5 showed an almost neutral surface charge, which made it susceptible to nanoparticle aggregation due to the absence of repulsion between the particles. This observation elucidates why the nanoparticle size was largest when coated at 0.5% and decreased as the coating increased. The EE of CUR into the SLN was exceptionally high (>99%) and remained unaffected by the surface coating.

FTIR

CUR's FTIR spectrum is depicted in Fig. 4, where it exhibits several distinguishing bands that correspond to O-H stretching (3700 to 3110 cm^{-1}), C=O stretching (1729 cm^{-1}), C-C stretching (1652 cm^{-1}), C-H bending (1459, 1372, and 840 cm^{-1}), C-O stretching in the ring structure (1269 cm^{-1}), and C-O stretching (1060 to 1090 cm^{-1}). The lack of any peak shift or functional group loss in the FTIR spectra for SLN and cs-SLN suggests that CUR was compatible with the other components utilised to create the SLN formulation. Nevertheless, the FTIR spectrum analysis did not reveal any changes in the position or absence of characteristic peaks associated with the functional groups (such as O-H stretching and C=O stretching) of the CUR molecule. As a result, the ingredients employed in the formulation of SLN in this investigation are deemed to be pharmaceutically compatible with CUR.

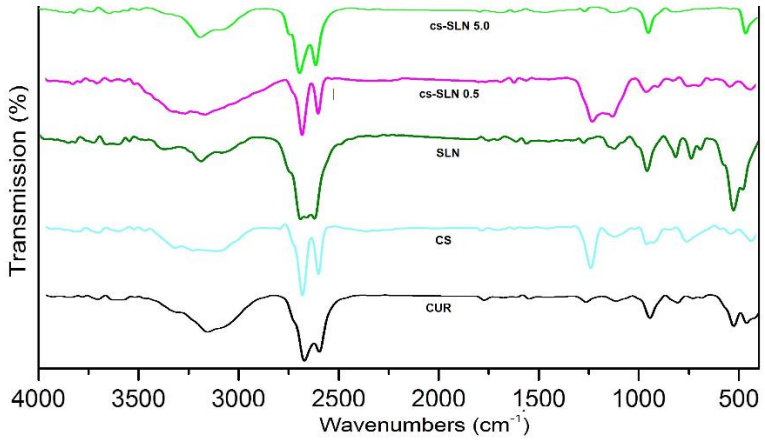

Fig. 4. FTIR spectrum of CUR, CS, SLN, SLN and cs-SLN.

Stability study

For 90 days, the dispersion's SLN particle size progression was observed at 4°C, room temperature, and 40°C (Fig. 5).

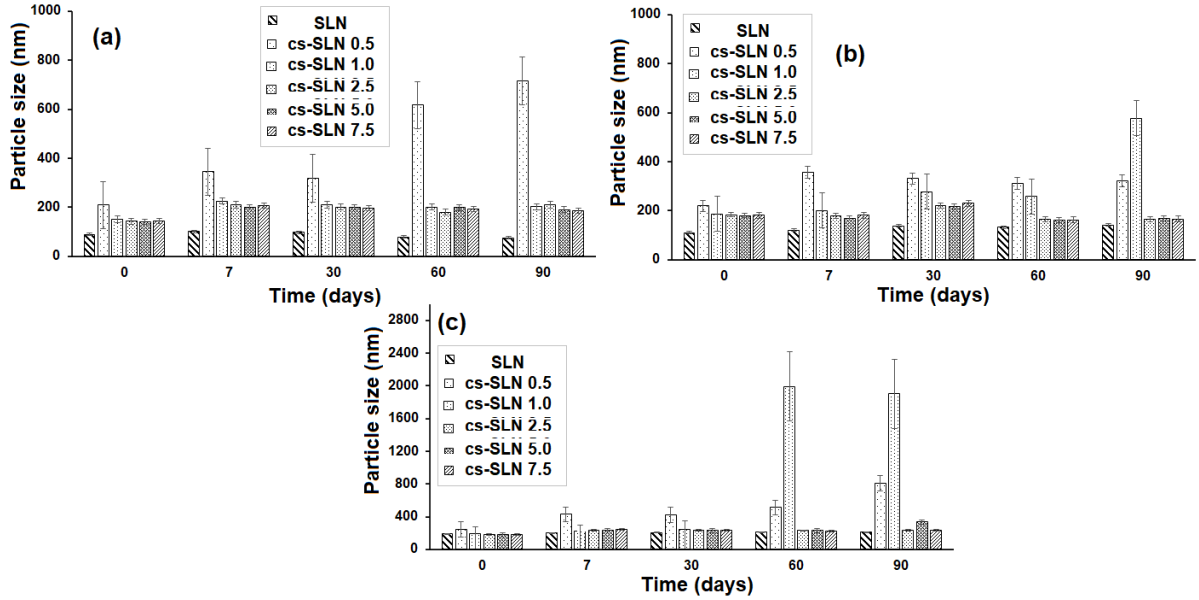

Fig. 5. PS evolution of SLN at at (a) 4 °C (b) room temperature and (c) 40°C on SLN and cs-SLN.

Except for cs-SLN 0.5, none of the SLN emulsion significantly increased at 4°C. A noticeable shift was also seen in cs-SLN 1.0 at 40°C and room temperature. In contrast, the particle sizes of cs-SLN 2.5, 5.0, and 7.5 were extremely consistent over the course of 90 days at every temperature examined. The initial charge of the SLN dispersion grew in proportion to the amount of CS employed up to 5% before plateauing in terms of ZP (Fig. 6).

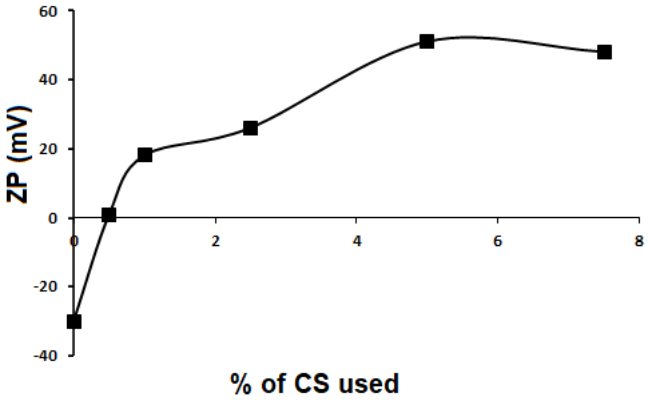

Fig. 6. Initial ZP of SLN as a function of CS used

When CS coating was 2.5% or more, no discernible change of the ZP was seen after 90 days of storage (Fig. 7). cs-SLN 1.0 had a positive starting charge (18.61 ±1.77 mV), but after 90 days at room temperature and 40°C (0.82 ± 0.22 and 4.1 ± 0.38 mV, respectively), it was practically neutral. The initial ZP of cs-SLN 0.5 was nearly neutral (3.01 mV), but following storage at ambient temperature and 40°C, it converted to a negative charge. Notably, after 90 days of storage at ambient temperature and 40°C, respectively, it was 15.11 ± 1.34 and 19.99 ± 1.26 mV. The presence of either a positive or negative ZP on nanoparticles prevents their aggregation in a dispersion, whereas a neutral charge promotes it. This observation might explain why the change in particle size was more noticeable during long-term storage in cs-SLN 1.0 compared to cs-SLN 0.5. The zeta potential of cs-SLN 2.5, 5.0, and 7.5 remained significantly above 20 mV even during long-term storage at 40°C, creating a repulsive force between the nanoparticles.

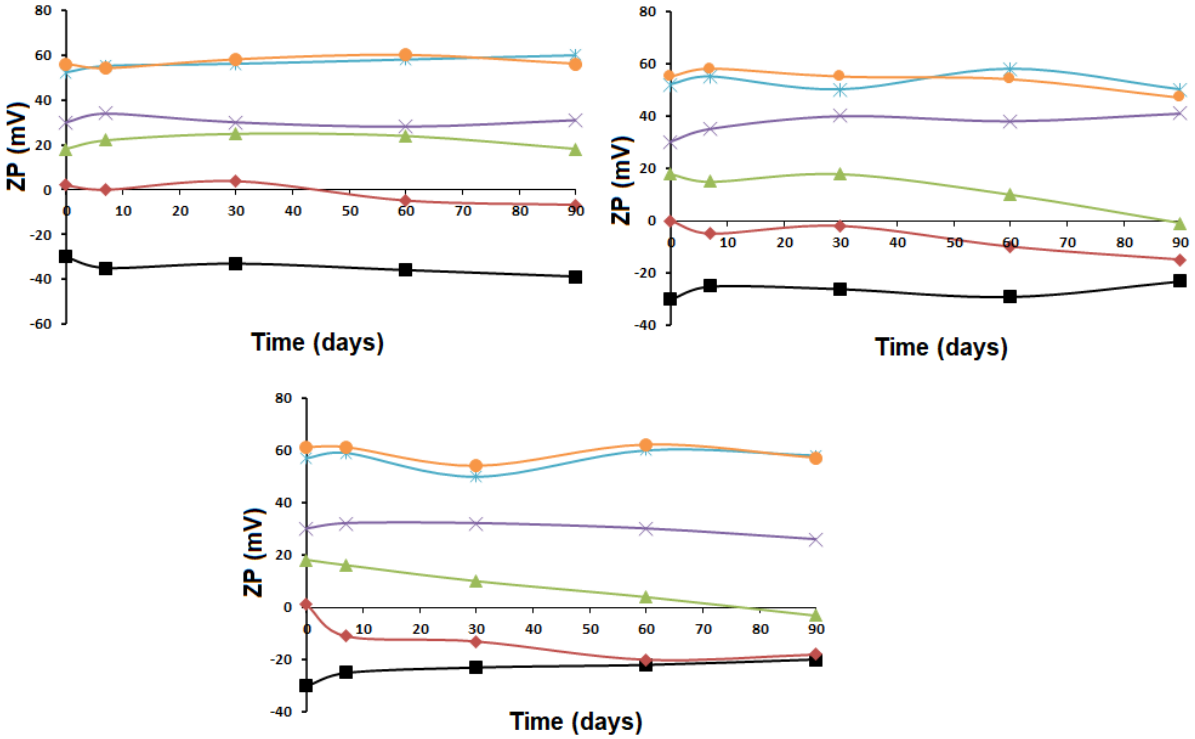

Fig. 7. Evolution of ZP at (a) 4°C, (b) room temperature, and (c) 40°C

Low molecular weight CS was the coating substance employed in this investigation [17]. According to tripalmitin, 0.5% of the CS converted the SLN's negative charge to positive charge (Figs. 6 and 7). The positive charge did not, however, persist more than a week at room temperature or higher since it was barely over zero. Furthermore, during the storage time at all studied temperatures, particle size did increase quite quickly. CS coating of 1% (cs-SLN 1.0) was unable to sustain the charge for the prolonged period of time at the increased temperature.

The release of CUR from the formulations was evaluated in vitro using PBS at pH 3.0 and pH 7.0. In comparison to pure CUR powder (Fig. 8), all SLN emulsion exhibited a consistently faster release profile. The impact of CS coating varied depending on the pH of the release media. Under pH 7.0 conditions, the release rate increased proportionally with the amount of CS used. Interestingly, at pH 3.0, CS coating of 2.5% or lower did not result in any significant change in the release rate, while coating with 5.0% or higher led to a decrease in the rate.

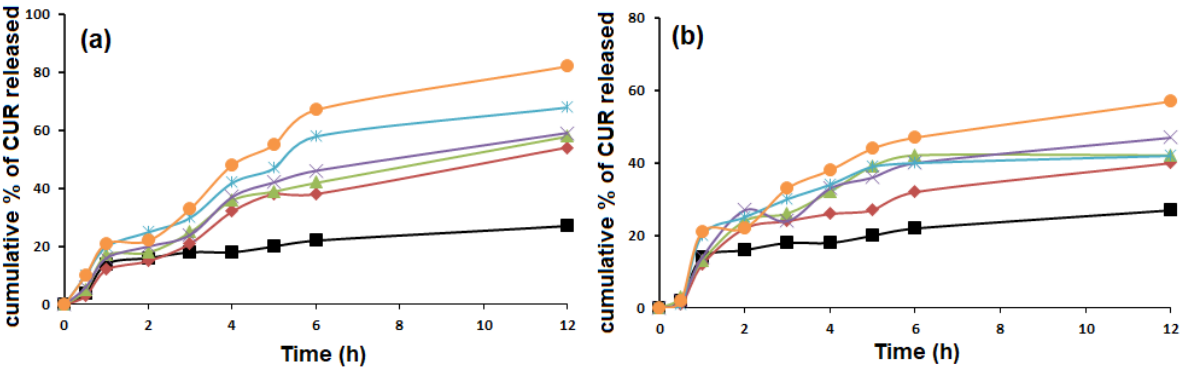

Fig. 8. In vitro release of CUR in PBS buffer at (a) pH 3.0 and (b) pH 7.0. Each value represents the mean ± SD (n = 3), *$p < 0.05$ compared to SLN

Depending on the pH of the release media, the CS coating had a bimodular effect on the *in vitro* release pattern. CS had a little impact in an acidic environment (pH 3.0) up to 2.5% coating, but when the coating reached 5.0% or greater, it significantly reduced the rate of release (Fig. 8). The positively charged amine group ($-NH_3+$) in the CS molecule interacts with the negatively charged phosphatidyl moiety of lecithin to generate a sticky gel, which appears to be the cause of this phenomena. Since the amine group in the CS molecule is non-ionized in an alkaline or neutral environment (pH 7.0), the formation of a sticky gel is prevented. Instead, the CS covering will just swell and hasten the release of the CUR that has been contained.

Conclusion

Successful formulation of CUR into solid lipid nanoparticles (SLN) and chitosan-coated SLN (cs-SLN) emulsion was prepared, employing tripalmitin and lecithin as the lipid core. Characterization of the SLNs demonstrated that CUR was uniformly dispersed at the molecular level within the SLN, and no compatibility issues were observed with the excipients used. When the coating amount exceeded 2.5% of tripalmitin, the particle size and zeta potential remained highly stable, even when subjected to a temperature of 40°C for up to 90 days. The release of CUR from the SLN emulsion was found to be faster compared to the release from pure CUR.

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