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Development of a Novel Method for Fabrication of Solid Lipid Nanoparticles: using High Shear Homogenization and Ultrasonication

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ABSTRACT

Solid Lipid nanoparticles (SLNs) were introduced at the beginning of 1990s as an alternative to solid nanoparticles, emulsions and liposomes in cosmetic and pharmaceutical preparations. The objective of the present investigation was to develop SLNs of capsaicin by a novel method using modified high shear homogenization and ultrasonication technique. In this method lipid matrix was melted and capsaicin was added to obtain a clear melting solution and this pre-emulsion was ultrasonicated to form nanoemulsion, which was then cooled to obtain the solid lipid nanosuspension. The prepared SLNs were characterized and evaluated for sustained release potential. The SLNs prepared were spherical in shape with the mean particle size of 100 nm with a small polydispersity index, 0.374. DSC studies showed less ordered arrangement of crystals, which was favorable for increasing the drug loading capacity. The entrapment efficiency, drug loading and zeta potential were 90.89%, 5.12% and -48.36 mV respectively. The cumulative amount of drug released in 1 hr from the capsaicin loaded SLNs was 6.5 ± 0.3% compared with 95.8 ± 4.1% from drug solution. Capsaicin release was steady and prolonged to 14 hr with SLNs encapsulation (94.3±3.8%). This study confirmed that the method developed is simple and effective to formulate SLNs of poorly soluble drugs without organic solvents.

Keywords: Solid Lipid Nanoparticles, Zeta potential, DSC, Entrapment efficiency, Capsaicin

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INTRODUCTION

Solid lipid nanoparticles (SLNs) have emerged as an alternative carrier system to traditional carriers, such as polymeric nanoparticles, emulsion and liposomes, and they attract great attention as a novel colloidal drug carrier for topical use [1]. The advantages of the carrier include negligible skin irritation, controlled release, and protection of active substances [2]. Because they are composed of non-irritative and non-toxic lipids, SLNs seem to be well suited for use on inflamed and damaged skin. Moreover, SLNs have distinct occlusive properties due to the formation of an intact film on the skin surface upon drying, which decreases transepidermal water loss and favors the drug penetrating through the stratum corneum [3, 4]. Besides the nonspecific occlusion effect, the enhanced drug penetration might be related with SLNs themselves, the highly specific surface area of nanometer sized SLNs facilitate the contact of encapsulated drug with the stratum corneum[3] The nanometer sized particles can make close contact with superficial junctions of corneocyte clusters and furrows between corneocyte islands, which may favor accumulation for several hours allowing for sustained drug release [5,6]. Other advantages of SLNs are reported to be increased on biotoxicity avoidance of organic solvents, drug stability, high drug payload and incorporation of liophilic and hydrophilic drugs.[1] SLNs have been used to improve the skin/dermal uptake of several drugs such as triptolide, isotretinoin, podophyllotoxin and prednicarbate [7-12], which supports that SLNs can be employed as the carrier for the topical delivery of capsaicin.

Capsaicin (8-methyl N-vanillyl-6 nonenamide), the active compound of hot peppers of the genus Capsicum, exhibits broad bioactivity [13] including antinociception, antihypertension and lipid-lowering activities [14-16]. Capsaicin is also used topically to treat various diseases such as rheumatoid arthritis, osteoarthritis, diabetic neuropathy and post-therapeutic neuralgia [17]. The present work is focused on the preparation, characterization, *in-vitro* release behaviors of capsaicin-loaded SLNs. Topical capsaicin is well absorbed from the skin. Maximal cutaneous concentrations of capsaicin are rapidly achieved when capsaicin is applied topically. These concentrations are greater with isopropyl preparations compared with propylene glycol or mineral oil preparations [18]. In mice, capsaicin is distributed widely to the brain, spinal cord and liver after intravenous administration [19]. Capsaicin is a fat soluble, odorless, pungent tasting, off-white solid with a melting point of 62–65ºC and a molecular weight of 305.4 kDa. As it is not water soluble, alcohols and other organic solvents are used to solubilize capsaicin in topical preparations and sprays [20]. The drug has a poor water solubility, short biological half life and high lipophilicity that made it an excellent candidate for SLNs encapsulation. Topical application circumvents the hepatic metabolism and thus is suitable to develop delivery systems to attain both systemic and local effects for capsaicin. The present investigation was aimed at developing a simple and reliable method for formulating capsaicin loaded SLNs without organic solvent.

MATERIALS AND METHODS

Materials

Capsaicin was obtained *ex-gratis* from Ashian Herbex Ltd., Hyderabad. Glyceryl behnate, sodium collate, soyalecithin were bought from Sigma chemicals (USA). All other chemicals and solvents were of analytical grade.

Preparation of solid lipid nanoparticles

SLNs were prepared by high shear homogenization and ultrasonication. Capsaicin (80 mg) was added to Compritol 888 ATO (4 g) previously melted at 80 °C. Further, this hot lipid phase was dispersed in a surfactant solution (1.5%, w/w), at 8000 rpm, 80 °C for 1 min, using a high-speed stirrer (Ultra Turrax T8, Alliance Analytical Inc., California, USA). The surfactants used were poloxamer and sodium cholate. The obtained pre-emulsion was ultrasonified using a probe sonicator (Vibra cell, Sonics, USA). In order to prevent recrystallization during homogenization, production temperature was kept at least 5 °C above the lipid melting point. The obtained nanoemulsion (O/W) was cooled down in an ice bath to form SLN and finally diluted up to 200 ml with de-ionized water. Nanoparticle dispersions were stored at 4 °C. Three formulation batches of SLNs loaded capsaicin i.e. NS SLN1 (with drug 1.5mg/ml), NS SLN 2 (drug 2mg/ml) and SN SLN3 (drug 2.5mg/ml) were prepared for further studies.

Particle size analysis

The particle size analysis of SLNs was performed by photon correlation spectroscopy (PCS) and laser diffractometry (LD). For PCS measurements, all the samples were diluted with bidistilled water to suitable concentration and analyzed with a Malvern Zetasizer 4 (Malvern Instruments, UK). Prior to particle size analysis by PCS, the semisolid SLN dispersions were diluted with double-distilled water to weak opalescence. All measurements were performed in triplicate.

Zeta potential

The surface charge of SLN was determined by measurement of the zeta potential of the lipid nanoparticles calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility. For the zeta potential measurements a Malvern Zetasizer 4 (Malvern Instruments, UK) was used. The field strength was 20 V/cm on a large bore measuring cell (4 mm). Samples were diluted with bi-distilled water.

The drug entrapment efficiency (ENTRAPMENT EFFICIENCY)

After adding 2ml of nanosuspension solution of known concentration prepared in Sephadex G-25 column, methanol aqueous solution was passed through the column. The collected elute was measured by the UV-detection (Unico, UV-2102PC, USA) at 310 nm. The amount of drug that was not incorporated into the SLNs could be obtained by the UV-detection absorption percent. Entrapment efficiency (EE%) was obtained by the following equation.

$$EE\% = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100\% \qquad (1)$$

Transmission electron microscopy (TEM) and Scanning Electron Microscopy (SEM)

TEM (JEM-100CXII, Japan) is a method of probing the microstructure of rather delicate systems such as micelles, liquid crystalline phases, vesicles, emulsions and also nanoparticles. [13] Without surfactants the lyophilized SLNs were dispersed directly into triple distilled water. Then copper grid coated with carbon film was put into the above solution several times. After being stained by 2% phosphotungstic acid (PTA) solution and dried under room temperature, the sample was ready for the TEM & SEM investigation.

Differential scanning calorimeter (DSC)

Thermal behavior of capsaicin loaded SLNs was analyzed using differential scanning calorimeter DSC-7 (Perkin-Elmer, USA). Approximately 10mg of samples was placed in aluminum crimp cells and heated at the scanning rate of 10 °C/min from 30 to 400 °C in a nitrogen atmosphere. Aluminum oxide was used as the standard reference material to calibrate the temperature and energy scale of the DSC instrument [21,22].

Capsaicin HPLC analysis

The drug content of capsaicin was analyzed by a HPLC system consisting of a Hitachi L-7100 HPLC pump, a Hitachi L-7200 sample processor and a Hitachi L-7480 fluorescence detector. A 25 cm long, 4 mm inner diameter C18 column (LichroCart 250-4, Merck) was used. The mobile phase for capsaicin was 55% citrate–phosphate buffer (pH 4) and 45% acetonitrile at a flow rate of 1.0 ml/min. The column effluent was passed through the fluorescence detector set at an excitation wavelength of 280 nm and an emission wavelength of 310 nm. [23] The detection limit of capsaicin was 20 mg/ml.

In-vitro drug release studies using cellophane membrane

The *in-vitro* release of capsaicin from different capsaicin formulations was studied using locally fabricated Keshry Chein diffusion cell through the cellophane membrane (Molecular weight cut off 12000 to

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140000). Pure capsaicin was dissolved in 1:1 (v/v) ethanol-pH 7.4 citrate–phosphate buffer at a concentration of 20 mg/ml and used as control. The prepared formulation (the same concentration of capsaicin as 20 mg/ml pure drug solution) 2 ml in volume was transferred to a dialysis bag (size cut off = 2.5 nm) immediately. The dialysis bag was placed in a 50 ml-beaker containing 1:1 (v/v) ethanol and citrate-phosphate buffers (pH 7.4). The outer phase was stirred continuously. At predetermined time intervals sample was withdrawn and replenished with same amount of receptor fluid. The drug content in outer phase was analyzed by using HPLC as described earlier.

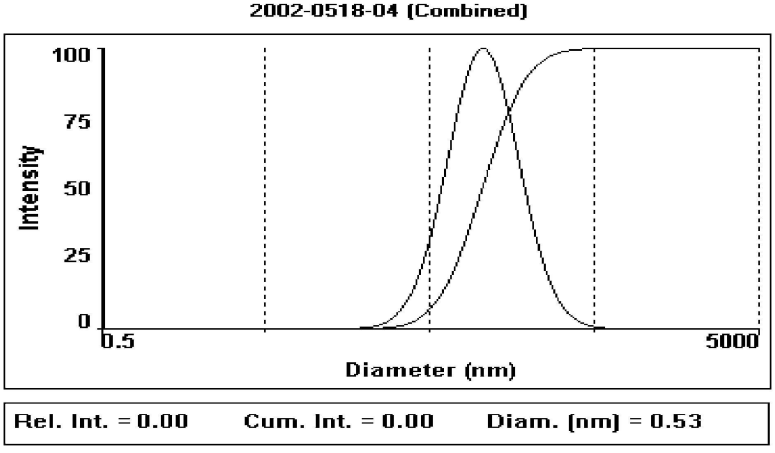
RESULTS AND DISCUSSION

Particle size analysis

In the present study new ultrasonication method is used for preparation of SLNs. Previously solvent evaporation method by precipitation in o/w emulsions [8,9] was used for preparation of SLNs that have the limitation of use of organic solvents that is difficult to remove, time consuming and costly in nature. The results of present study showed that using this method SLNs was prepared and have desired size range (100-110nm), spherical is shape (100-200) and have high entrapment efficacy

A sufficient high-energy input was necessary to break down the droplets into the nanometer range [24]. Finer dispersion could be obtained by inputting higher energy such as the elevated production temperature, higher stirring rate, longer emulsification time, stronger ultrasound power and so on. Besides the production parameters, lipid matrix surfactant blend and viscosity of lipid and aqueous phase influenced the outcome of procedure [25, 26]. In the present study, glycerol behenate was used as lipid matrix as well as but also as surfactant. It was suggested that this surface active partial glyceride facilitated emulsification and formed more rigid surfactant films and therefore improved the long-term stability of SLNs [27]. The mean particle size and polydispersity index for SLNs formulations were observed were 100 nm and 0.374 respectively measured by LD (Mastersizer 2000) [28], Particle size histogram Shown in figure 1, thus the drug loading SLNs showed a narrow distribution width and considerable small particle size. Maximum entrapment was achieved with batch NS SLN2 (Table 4). Therefore the new method in this way had gained a relative high efficiency and good dispersion quality. An outstanding feature of nanoparticles was the increase in saturation solubility and consequently an increase in the dissolution velocity of the compounds. According to the Kelvin and Ostwald-Freundlich equation, for small particles especially the nanometer range, the saturation solubility could be increased significantly [29]. Both the increase of saturation solubility and the enlargement of surface area contributed to the improvement of dissolution velocity by the Noyes- Whitney equation [30]. Drugs in the form of nanoparticles (nanocrystals) had been reported to possess a full range of positive effects and the data on increase in bioavailability was quite impressive [31]. One aim of this experiment was to improve the bioavailability of the poorly soluble lipophilic drug by transforming it into nanoparticles. From the above discussions, it should be presumed that if the nanometer range particles could be obtained, the increase in bioavailability will became available.

Fig 1



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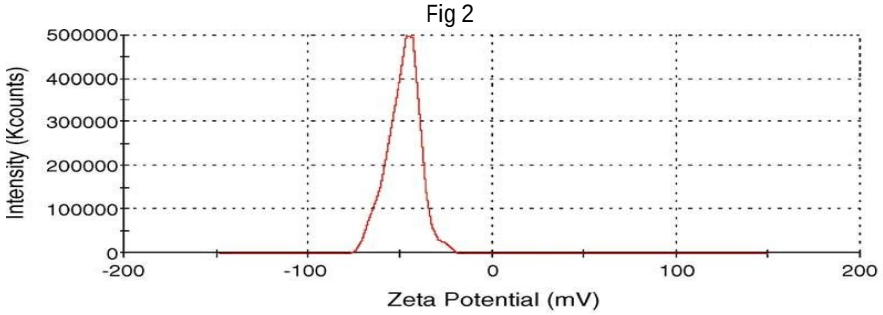
Zeta Potential

The Zeta potential of the ultrafiltrated dispersions was determined by the laser light scattering technique (Brookhaven, New York, USA). Measurements were obtained at an angle of 90°. Scattering intensity data were analyzed by a digital correlator and fitted by the method of inverse Laplace transformation. The dispersions were diluted with 0.005M KNO_3 for zeta-potential determination. The pH of the samples ranged from 6.0 to 6.2. Measurements were made in triplicate for all the batches prepared. The zeta potential of SLN was -48.36 mV. Zeta potential can make a prediction about the stability of colloid dispersions. A high zeta potential (>|30| mV) can provide an electric repulsion to avoid the aggregation of particles [32]. The mean particle sizes and zeta potentials of SLNs with or without capsaicin are presented in Table 1. The incorporation of capsaicin into SLN had no influence on the zeta potentials (Fig 2).

Fig 2



Stability Studies

Vesicular formulations were stored at 4 °C (Table 2) and at 25 °C (Table 1) for 30 days. The stability testing data indicated that vesicular formulations stored at 4 °C were more stable than those stored at 25 °C. Average vesicles size of vesicular formulations was found to increase on storage, which can be attributed to the fusion of vesicles. This effect was least in the case of formulation stored at 4 °C, which indicate fusogenicity to be a temperature dependent process and ideal storage condition being 4 °C present the zeta potential values that were monitored over a period of 1 month. The zeta potential of different vesicular formulation stored at 25 °C were very unstable whereas the zeta potential values of formulations stored at 4 °C were more stable therefore the storage temperature of 4 °C is better than the room temperature in order to maintain favorable zeta potentials (Table1& 2)

SEM and TEM investigation

Figure 3 shows the shape of the nanoparticles entrapping with the drug. It was evident that the particles investigated revealed round and homogeneous shading, the particle size ranging approximately from 100 to 110 nm. SEM was used to examine the submicron size and morphology of the SLNs. The SEM (Figure 4) image revealed that the SLNs were spherical in shape and homogeneously distributed around 100–200 nm in diameter, and that the incorporation of capsaicin did not seem to cause morphological changes.

DSC analysis

DSC was a tool to investigate the melting and recrystallization behavior of crystalline materials like SLNs. Figure 5 shows an overview of the melting process of bulk matrix, lyophilized drug-free placebo and drug loading powder. The heat flow was less than 1 mW. Thermogram of the lyophilized capsaicin loaded SLN did not show the melting peak of crystalline capsaicin, which indicates that capsaicin in SLNs was in amorphous state. For this study, capsaicin and lipid were first dissolved in ethyl acetate. Subsequently, ethyl acetate was evaporated. Therefore capsaicin was dispersed in the lipid homogeneously. There are some similar results revealing that drug in SLN were in amorphous state [33, 34]. The bulk glyceryl behnate melted at 82.28 °C with almost the same melting point at 83 °C.

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Fig. 3

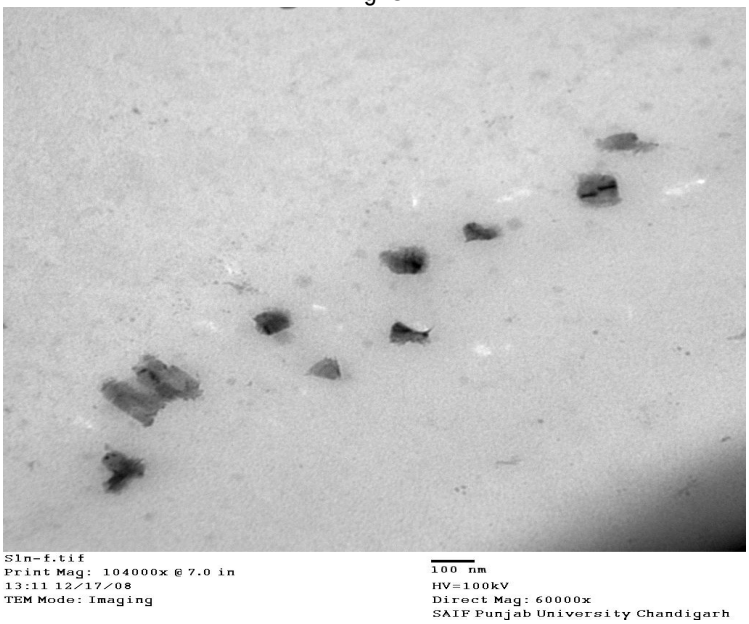

Fig. 4

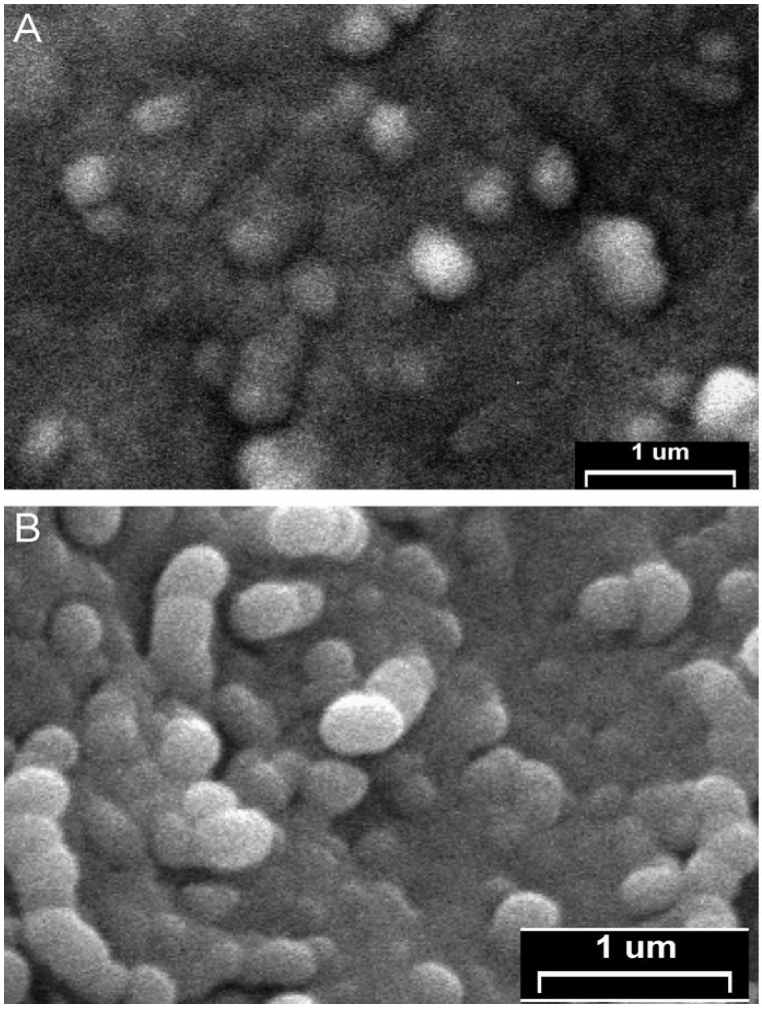

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Fig. 5

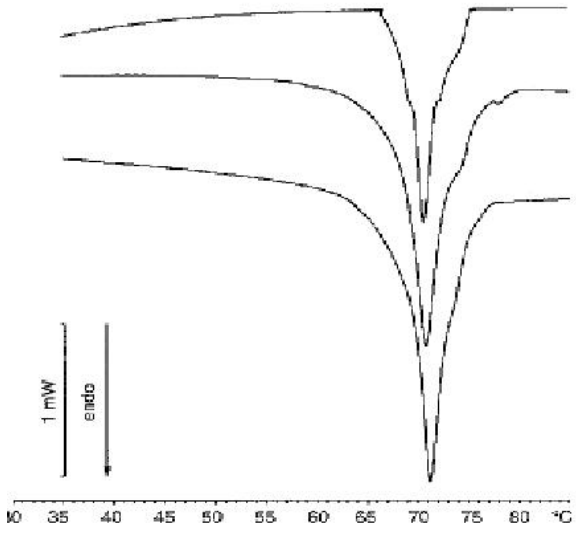
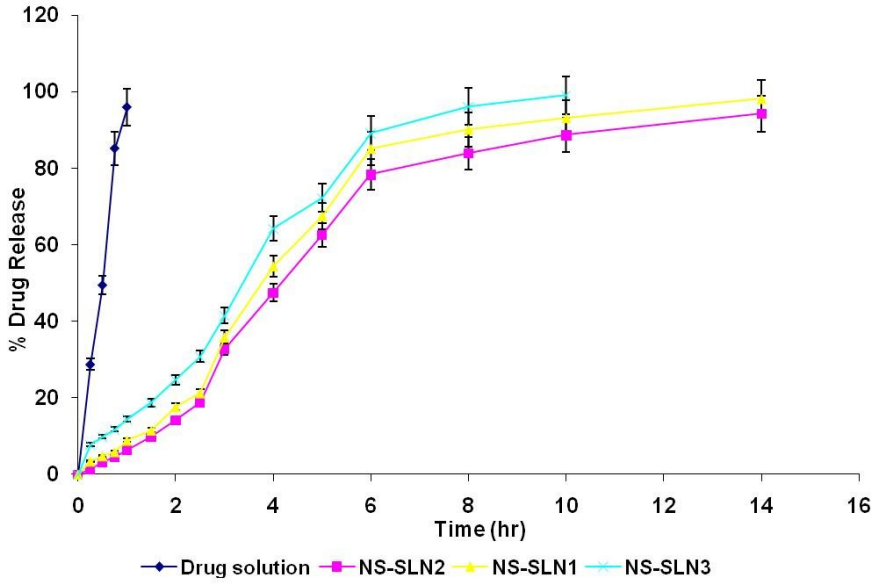


Fig. 6



Drug incorporation and loading capacity

Many different drugs had been incorporated in SLNs [35, 36]. The prerequisite to obtain a sufficient loading capacity was a sufficiently high solubility of the drug in the lipid melt [37]. Relative higher drug EE% was one of the major advantages of SLNs. The lipid crystalline structure related to the chemical nature of the lipid was a key factor to determine whether a drug would be expelled or firmly incorporated into the carrier systems. In the nanoparticle structure, the lipid forming highly crystalline state with a perfect lattice would lead to drug expulsion. On the other hand, the imperfection (lattice defects) of the lipid structure could offer space to accommodate the drugs. As a result, the structure of less ordered arrangement in the nanoparticles would be beneficial to the drug loading capacity like the samples in this study. Table 4 listed the drug EE% of SLNs. The data showed EE% as high as 90.89% for fresh samples. After one-month refrigerated storage the

value only showed little decrease. Therefore, it was revealed that SLNs produced by this modified production method could also achieve higher drug incorporation for lipophilic drugs like capsaicin. Drug loading was found to be 5.12%.

In-vitro drug release studies

The release rate from capsaicin loaded SLNs is an important parameter since a sustained release is necessary in order to decrease the dose dependent side effects of capsaicin and improve its therapeutic index. The drug entrapment and release rates were evaluated by dialysis method using spectrophotometric estimation [38]. Capsaicin loaded SLNs were stable in1:1 (v/v) ethanol-citrate phosphate buffer (pH 7.4) (used as the receptor medium) and a slow release of drug from the complex was found. Table 3 and Figure 4 show the comparative % drug release from different SLNs formulations in comparison with drug solution. Significant prolongation of capsaicin release across the dialysis membrane was achieved with the capsaicin loaded SLNs in comparison with plain drug. The cumulative amount of drug released in 1 hr from the capsaicin loaded solid lipid nanoparticles formulation was 6.5 ± 0.3 % compared with 95.8 ± 4.1% from the control drug solution. Capsaicin release was prolonged to 14 h with encapsulation (94.3±3.8%). From the release studies, NS SLN2 was found to be best batch as release was better sustained for 14 h as compared to NS SLN1 and NS SLN3. Drug release from the capsaicin loaded SLNs were steady and slow and decreased as a function of time as shown in (Table 5 and Figure 6).

Table 1: Mean particle sizes and zeta potentials of the SLNs after 1, 2 and 4 weeks of storage at 4 °C (n=9)

SAMPLE	Average diameter ± S.D. (nm)		
	1 week	2 weeks	4 weeks
NS-SLN2	100.8±9.0	106.7±8.3	109.4±9.4
SLN	108±8.3	109±9.3	110 ±7.3
	Zeta potential (mV)		
NS-SLN2	-48.36 mV	-48.36 mV	-48.36 mV
SLN	-48.36 mV	-48.36 mV	-48.36 mV

Table 2: Mean particle sizes and zeta potentials of the SLNs after 1, 2 and 4 weeks of storage at at 25°C (n=9)

SAMPLE	Average diameter±S.D. (nm)		
	1 week	2 weeks	4 weeks
NS-SLN2	105.8±9.0	106.7±8.3	109±9.4
SLN	108±8.3	109±9.3	113 ±7.3
	Zeta potential (mV)		
NS-SLN2	-48.36 mV	-49.36 mV	-49.36 mV
SLN	-48.36 mV	-48.36 mV	-46.36 mV

Table3: Effect of Storage on the Vesicle Shape

S. No.	Formulation Code	Vesicle shape at 4°C after storage for days			Vesicle shape at 25°C after storage for days		
		7	14	30	7	14	30
1.	NS-SLN2	NC	NC	NC	NC	NC	C
2.	SLN	NC	NC	NC	NC	NC	C

NC = No change C = Change

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Table 4: The initial drug adding (DA) amount and entrapment efficiency (EE%) of SLNs

Sample number	DA (mg/ml)	EE (%)
NS-SLN1	1.5	72.96
NS-SLN2	2.0	90.89
NS-SLN3	2.5	86.05

Table5 Comparative % Drug Release From capsaicin loaded solid lipid Nanoparticles in Comparison to Plain Drug

TIME (in hrs)	% Amount of Drug Release			
	Drug solution	NS-SLN2	NS-SLN1	NS-SLN3
0	0	0	0	0
0.25	28.75±2.2	1.31±0.2	3.42±0.5	7.78±0.5
0.50	49.48±2.8.	3.12±0.5	4.82±0.8	9.82±0.7
0.75	85.2±4.6	4.52±0.7	5.87±1.1	11.8±0.9
1.0	95.97±5.1	6.46±0.9	8.89±1.2	14.5±0.8
1.5		9.846±1.0	11.5±0.7	18.7±1.0
2		14.24±1.8	17.6±0.8	24.7±1.2
2.5		18.91±2.1	21.25±1.0	30.82±1.9
3		32.75±2.7	35.87±1.7	41.52±2.2
4		47.53±3.1	54.45±2.1	64.21±2.5
5		62.62±3.1	67.52±2.4	72.31±2.9
6		78.41±3.2	85.21±3.4	89.2±3.8
8		83.98±4.4	90.21±3.6	96.2±4.1
10		88.72±4.7	93.2±3.5	99.1±4.2
14		94.29±5.2	98.2±4.0	

CONCLUSION

In this paper, the capsaicin-loaded SLNs were prepared by a new method using modified high shear homogenization and ultrasound techniques. Physicochemical characterization revealed that the prepared drug loaded SLNSs were of spherical shape, homogenously distributed and amorphous. The SEM images revealed that the incorporation of capsaicin did not seem to cause morphological changes. The stability testing data indicated that vesicular formulations stored at 4 °C were more stable than those stored at 25 °C. SLNs achieved higher drug incorporation and EE% of SLNs was more than 90 percent and showed relative long-term stability as the leakage was very negligible after being stored for one month. Capsaicin loaded SLNs exhibited sustained release *in-vitro* drug release profile compared to plain drug. Our studies provided evidence that SLNs of lipophillic drugs for topical delivery can be prepared with this novel method.

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