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Research Article

SILVER SULFADIAZINE NIOSOMES: A NOVEL SUSTAINED RELEASE ONCE A DAY FORMULATION FOR BURN TREATMENT

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ABSTRACT

Objective: Silver sulfadiazine (SSD) is the topical antibacterial of choice in the treatment of burn. The current conventional marketed formulation of SSD requires to be applied two to four times a day and needs removal prior to each reapplication, which is very painful for the burn patients. Hence, the aim of this study was to develop novel sustained release once a day niosomal formulation of SSD in order to improve the patient compliance.

Method: Niosomes were developed using different nonionic surfactants and cholesterol in the molar ratio of 1:1 by the ethanol injection method. A fixed quantity of drug (50 mg) was added in all the niosomal batches. Effect of different formulation variables like curing time, surfactant structure, HLB and molecular weight of a surfactant, on entrapment efficiency of SSD in niosomes was evaluated.

Results: Results indicated that the niosomes manufactured with span 60 gives highest entrapment. The niosomal formulations exhibited considerably retarded *in vitro* release by a higuchi controlled mechanism. For span 60 niosomes the release was 98.14 % over 28 hours. *In vitro* antimicrobial study using *Pseudomonas aeruginosa* revealed that the niosomal formulation of SSD shows a better zone of inhibition (14 mm) in comparison to conventional dosage form (12 mm) even when used in half the concentration of conventional dosage form.

Conclusion: This study showed that the niosomal formulation of SSD can be used as the promising sustained release approach for the topical delivery of SSD in burn treatment.

INTRODUCTION

A burn occurs when some or all the different layers of cells within the skin are destroyed by a hot liquid (scalds), or a hot solid (contact burns), or a flame (flame burns). Burn is one of the most common and devastating forms of injury. Infection is the major cause of morbidity and mortality in burns [1]. A patient with serious burned injury requires immediate specialized care in order to minimize morbidity and mortality. Therapy with topical antimicrobial remains the most important component of wound care in hospitalized burn patients [2].

Silver sulfadiazine (SSD) is the drug of choice for topical treatment of infected burns [3, 4]. The goal of SSD therapy is to control microbial colonization, thus preventing the development of invasive infections. SSD has been shown to decrease wound-related infections and morbidity in burn wounds when used appropriately [5, 6]. SSD is a sulfa medicine used to treat bacterial and fungal infections in second or third degree burn. At the present SSD is the most frequently used topical prophylactic agent. SSD has good activity against most burn pathogens and it is relatively well tolerated by patients [7, 8]. SSD is practically insoluble in water, in ethanol and in diethyl ether. It is freely soluble in 25 % w/v ammonia solution [9].

In spite of the availability of many conventional topical marketed preparations of SSD, due to the problem of increased doses frequency (two to four times a day), research area has been extremely shifted towards formulation of vesicular dosage forms like liposomes and niosomes. Lichtenstein and Margalit [10] developed the liposomal topical formulation of SSD using soya lecithin and cholesterol in the molar ratio of 1:1. They achieved the entrapment efficiency of 95 % and sustained release with half life up to 24 hours. However, liposomes are found to face stability problems and are expensive. Niosomes are comparatively the most favorable dosage forms. The low costs of ingredients, a possibility of large-scale production, stability and the resultant ease of storage of niosomes have led to the exploitation of these carriers as an alternative to other micro and nano-encapsulation technologies [11, 12]. Therefore, the objective of this study was to investigate niosomally encapsulated SSD as an improved delivery system for the treatment of burn sepsis that could act as a locally targeted sustained-release drug depot.

In this study, for the development of niosomes, different surfactants were used in equimolar ratios with cholesterol while keeping the SSD quantity constant (50 mg) because the formulation of niosomes with an equimolar ratio of surfactant and cholesterol is most advantageous for the efficient encapsulation, and additional cholesterol is unfavorable. It implies that equal molarity of nonionic surfactant and cholesterol can make the membrane compact and well organized [13, 14].

MATERIALS AND METHODS

SSD was kindly supplied as a gift sample by Ajanta Pharma Limited, Mumbai, India. Different nonionic surfactants like tweens and spans were purchased from S. D. Fine Chemicals Ltd, Mumbai, India. Cholesterol was purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. Monobasic potassium phosphate and sodium hydroxide were supplied as a gift sample by Alkem Laboratory Ltd., New Mumbai, India. All solvents used were of analytical grade.

Preparation of niosomes

In the present study, niosomes were prepared by using the method reported by Jadon *et al.* with slight modifications [15]. Briefly, a weighted amount of cholesterol was mixed with the appropriate amount of the surface-active agent (Table 1) in the molar ratio of 1:1 to make 0.001 moles (1mmol) total concentration. This mixture was dissolved in 9ml of ethanol. This surfactant solution was later mixed with a 1ml ammoniacal solution of 50mg of SSD. The organic phase was then very slowly injected drop by drop through 14 gauge needle into 10 ml phosphate buffer (PBS) pH 7.4, maintained at 60 °C with slow agitation at 250 rpm using a magnetic stirrer (Remi 5MLH DX). Vaporization of organic solvent leads to the formation of unilamellar vesicles.

Optimization of SSD niosomes

Niosomes were optimized to get the high level of entrapment of SSD and to check out the effect of different formulation variables on entrapment. In order to study the effect of curing time on entrapment, niosomes of span 60 and tween 60 (Formula F3 and F6; Table 1) were developed by ethanol injection method and kept aside for a particular period of time like 0, 0.5, 1, 1.5 and 2 hours (curing time) before the separation from unentrapped drug. In order to study the effect of surfactant structure, HLB and molecular weight

on entrapment efficiency, formula F1, F2, F4, F5, F7, F8 and F9 (Table 1) were developed using different surfactants.

Entrapment efficiency

The drug-loaded niosomes were separated from the unentrapped drug by centrifugation (Remi CPR-24) at 15250 g relative centrifugal force (rcf) for 30 minutes [16]. Entrapment efficiency was then determined by using vesicle disruption method as described by Elbary *et al* [17]. After centrifugation, the supernatant was separated and the isolated pellets were again washed by centrifugation twice with 5 ml PBS pH 7.4. This sample of niosomes was mixed with 5 ml of 25 % w/v ammonia solution as the drug was not soluble in any other organic solvent except ammonia. This solution was then sonicated (Pci Analytics JIJ 158) for 15 minutes to obtain a clear solution. This solution was diluted up to 100 ml with PBS pH 7.4. Further dilutions were made only if it was necessary and concentration of the drug was determined spectrophotometrically at 255 nm (Jasco V-650, Japan). All the spectrophotometric analysis was carried out in triplicate and the values were averaged. The batch of niosomes which had shown highest entrapment was considered an optimized batch. Entrapment efficiency was obtained by using following formula [18].

$$\text{Entrapment efficiency (\%)} = \frac{\text{Amount entrapped}}{\text{Total amount}} \times 100$$

Photomicroscopy and vesicle size analysis

A sample of the formed niosomes was spread upon a glass slide and photomicrograph was taken by future winjoe projection microscope (MEM 1300, China) using 10x magnification power [19]. Mean diameter of vesicles was also analyzed automatically using the same instrument. A total of about 100 niosomes were observed. This experiment was carried out in triplicate and the results were averaged.

Scanning electron microscopy

The prepared optimized niosomal formulation was also characterized for their morphology using scanning electron microscopy (SEM). The lyophilized samples of niosome was sprinkled and fixed in an SEM holder with double sided adhesive tape and coated by a layer of gold of 150 °A for five to six minutes using a sputter coater, under a vacuum of (3×10^{-1} atm) of argon gas. This sample was then examined with a scanning electron microscope (JEOL 5400, Japan) at 10 kV accelerating voltage.

***In vitro* release study**

The release of drug from niosomes was determined by using franz diffusion cell [20]. The available diffusion area of the cell was 3.14 cm^2. The receptor compartment of the franz diffusion cell was filled with 15 ml of PBS pH 7.4, maintained at 37 °C and stirred by a magnetic bar (Remi 5MLH DX, India) at 600 rpm. The cellophane membrane with molecular weight cutoff of 8000 was activated in diffusion media by boiling in it followed by keeping in it for overnight [21]. The activated cellophane membrane was then mounted on receptor compartment. After removing unentrapped drug, sample of niosomes equivalent to 1 mg of SSD was suspended in 1 ml of PBS pH 7.4 and placed on the activated cellophane membrane. At appropriate time intervals, 1 ml aliquot of the receptor medium was withdrawn and immediately replaced by an equal volume of the fresh receptor solution to maintain sink conditions. These samples were analyzed spectrophotometrically (Jasco V650, Japan) at 255 nm. All the spectrophotometric analysis was carried out in triplicate and the values were averaged.

Model fitting analysis

The mechanism of SSD release from niosomal formulations was determined using the following mathematical models: zero-order kinetics, first-order kinetics, higuchi kinetics and korsmeyer-peppas kinetic by plotting a graph of % cumulative release against time, log % cumulative release against time, % cumulative release against square root of time, and log % cumulative release against log time respectively .

Differential scanning calorimetry analysis (DSC)

The samples of plain drug and optimized niosomal formulation were hermetically sealed in perforated aluminum pans and scanned at the rate of 10 ^{0}C/min over the temperature ranges of 50-400 ^{0}C using differential scanning calorimeter (SIIO 6300, Japan). The reproducibility of the thermograms was determined by repeating the temperature cycle three times for each sample [22].

***In vitro* antimicrobial study**

Zone inhibition study using a standard cup plate method was used to compare the *in-vitro* antimicrobial effectiveness of optimized SSD niosomal formulation over conventional cream. *Pseudomonas aeruginosa* (Microbial Type Culture Collection and Gene Bank, Chandigarh, number 424) was used as test organism as it is frequently implicated as the invasive bacteria in burn wound sepsis [23]. Two petri plates of approximately 90 mm internal diameter were filled with 30 ml of previously autoclaved nutrient agar medium. After congealing, this media was inoculated with 0.1 ml of culture containing overnight inoculum of test organism and then three wells of 10 mm diameter were punched using sterile borer in each petri plate.

First well was filled with niosomes, next with conventional cream and last was filled with placebo niosomes. The concentration of niosomal preparation added to the first petri plate was kept half (0.5 %) the concentration of a conventional cream (1 %) and in the second petri plate; it was kept same (1 %). These petri plates were then kept in place for 4 hours at room temperature as a period of pre-incubation diffusion and then incubated at 37 ^{0}C for 24 hours [24]. A zone of inhibition was measured with zone reader. Each assay in this experiment was carried out in triplicate.

Stability study

Stability of optimized final selected niosomes was evaluated by keeping the prepared niosomes packed in amber color glass vials up to 6 months, at 5 ± 3 °C and 25 ± 2 °C/ 60 ± 5 % relative humidity (RH) in stability chamber (Classic Scientific CS-03). Samples were withdrawn on 1st day, 30th day, 90th day and 180th day. These samples were then analyzed for entrapment efficiency and vesicle size, after separation from unentrapped drug, by the methods as described previously [25].

RESULTS AND DISCUSSION

Entrapment efficiency

Niosomes of SSD were prepared by the ethanol injection method and optimized to obtain the high level of entrapment. Effect of curing time, surfactant structure, HLB and molecular weight on entrapment of SSD was also studied.

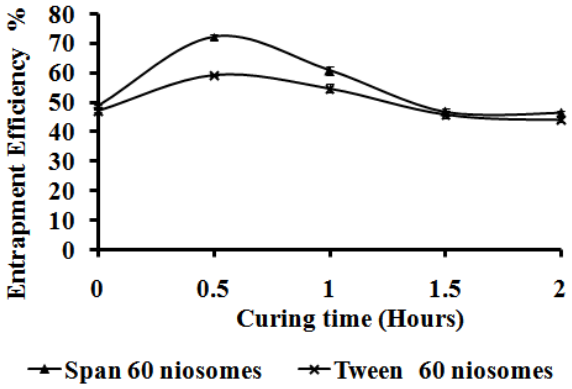

Fig. 1: Effect of curing time on entrapment efficiency of SSD

Effect of curing time on entrapment efficiency

Formula F3 and F6 (Table 1) were prepared using 1:1 molar ratios of cholesterol with span 60 and tween 60 respectively. They were then allowed to stand before the separation from the unentrapped drug for a particular period of curing time in order

to check the effect of curing time on entrapment. The results are shown in Fig. 1. Entrapment efficiency of SSD at curing time of 0.0, 0.5, 1.0, 1.5 and 2.0 hours were 48.68 %, 72.20 %, 60.72 %, 46.74 %, 46.44 % respectively for span 60 niosomes (Formula F3, Table 1) and 47.12 %, 59.28 %, 54.66 %, 45.66 %, 44.14 % respectively for tween 60 niosomes (Formula F6, Table 1). Curing time was found to increase the entrapment up to a certain extent there after entrapment efficiency decreases in cases of niosomes of both spans 60 as well as tween 60. The entrapment efficiency was lowest at the curing time of 0.0 hr and highest at the curing time of 0.5 hr. Then entrapment efficiency found to decrease slowly with the increase in curing time above 0.5 hr for both span 60 niosomes as well as tween 60 niosomes in the following order 0.0 < 0.5 > 1.0 > 1.5 > 2.0. This decrease in entrapment of SSD after 0.5hr may be there because of the leaching out of drug from niosomes due to the effect of a concentration gradient. Thus, curing time of 0.5 hr was found to give the maximum entrapment of the drug.

Table 1: Formulations and evaluation of SSD niosomes

Formula	Nonionic surfactant	Empirical formula	HLB	Molecular weight	Entrapment efficiency (%)	Mean diameter (µm)
F1	Span 65	$C_{60}H_{114}O_8$	2.1	963.54	64.18±1.48	5.89±0.52
F2	Span 80	$C_{24}H_{44}O_6$	4.3	428.61	62.02±1.64	5.07±0.35
F3	Span 60	$C_{24}H_{46}O_6$	4.7	430.62	72.34±0.88	5.03±0.21
F4	Span 40	$C_{22}H_{42}O_6$	6.7	402.58	60.80±1.62	4.62±0.58
F5	Span 20	$C_{18}H_{34}O_6$	8.6	345.64	59.48±0.96	4.36±0.53
F6	Tween 60	$C_{64}H_{126}O_{26}$	14.9	1311.70	59.22±0.78	4.27±0.44
F7	Tween 80	$C_{64}H_{124}O_{26}$	15.0	1309.68	53.82±1.46	4.22±0.68
F8	Tween 40	$C_{62}H_{122}O_{26}$	15.6	1283.65	57.36±1.24	4.07±0.27
F9	Tween 20	$C_{58}H_{114}O_{26}$	16.7	1227.54	54.40±0.82	3.79±0.39

Effect of the structure of the surfactant on entrapment efficiency

The results for this study are shown in Table 1. From the results, it was observed that the length of the alkyl chain of surfactant is a crucial factor. Longer chain surfactant produces high entrapment. Surfactants with long alkyl chains generally give larger vesicles. This might be the reason for the higher entrapment efficiency of vesicles prepared with longer alkyl chain surfactants. In case of niosomes prepared with spans (Formula F1, F2, F3, F4 and F5, Table 1) the entrapment efficiency followed the trend span 20 < span 40 < span 60 > spans 65 > span 80. Span 60 has a longer saturated alkyl chain compared to span 20 and span 40 so it produces niosomes with higher entrapment efficiency. Span 60 and span 80 has the same head group, but span 80 has an unsaturated alkyl chain which results in increased permeability and decreased entrapment. Similar is the reason behind the lower entrapment with span 65. Though span 65 has a longer alkyl chain than span 60, entrapment with span 65 is less because of the presence of unsaturation. In case of niosomes prepared using tweens (Formula F6, F7, F8 and F9, Table 1) entrapment efficiency followed the trend tween 80 < tween 20 < tween 40 < tween 60 which also confirms the effect of chain length and unsaturation. Though tween 80 has a longer alkyl chain than tween 20 and tween 40, it has showed lower entrapment because of the presence of unsaturation. Tween 60 has longest saturated chain in comparison to other tweens so entrapment with tween 60 is more as compared to other tweens. These results are exactly opposite to the results found by Ruckmani and Sankar [26] which indicate that the longer the alkyl chain of the surfactant, less the drug will be entrapped.

Effect of HLB of the surfactant on entrapment efficiency

From the result (Table 1) it was found that the HLB value of the surfactant directly influences the drug entrapment efficiency. The lower the HLB of the surfactant the higher will be the drug entrapment efficiency as in the case of niosomes prepared using span 60. Though span 65 and span 80 has lower HLB than span 60, they had shown less entrapment because of the effect of leaching out as explained previously. Similar is the case with tween 80 in comparison with tween 40 and tween 20. These results are dissimilar to the results obtained by Ruckmani and Sankar [26] which states that lower the HLB value of the surfactant, lesser will be the entrapment efficiency.

Increase in HLB value has resulted in a decrease in entrapment efficiency for the niosomes developed using different tweens and spans (Formula F1, F2, F3, F4, F5, F6, F7, F8 and F9, Table 1). This also explains the lower value of entrapment with all the tweens in comparison with spans as tweens have higher HLB values compared to spans.

Effect of molecular weight of the surfactant on the entrapment efficiency

In addition to surfactant structure and HLB, molecular weight of a nonionic surfactant (Table 1) was also found to directly influence the drug entrapment efficiency. It was observed from the result that with the increase in molecular weight the entrapment efficiency increases for niosomes prepared using saturated surfactants like span 20, span 40, span 60 in case of spans (Formula F5, F4 and F3, Table 1) and tween 20, tween 40 and tween 60 in case of tweens (Formula F9, F8 and F6, Table 1). In comparison to span 60, niosomes manufactured using unsaturated spans like span 80 and span 65 (Formula F1 and F2, Table 1) showed the less entrapment cause of effect of unsaturation as explained previously. Similar is the reason behind lees entrapment shown by tween 80 niosomes (Formula F7, Table 1) though it has more molecular weight in comparison to other tweens.

From the results, it was concluded that niosome manufactured using span 60 and cholesterol (Formula F3, Table1) gives optimum entrapment.

Photomicroscopy and vesicle size analysis

The photomicrograph of optimized SSD niosomes (Formula F3, Table1) is shown in Fig. 2a. Mean diameter of SSD niosomes (Formula F1, F2, F3, F4 and F5, Table 1) prepared using different spans was found in following order span 65 > span 80 > span 60 > span 40 > span 20. The results are shown in Table 1. These results confirmed that, the size of niosomes depends upon the chain length of surfactant. As the length increases the size also increases. Mean diameter of niosomes with all tweens (Formula F6, F7, F8 and F9, Table 1) was found lower than that with spans. Reason behind this is still uncertain. The order of Mean diameter of niosomes in case of tweens was tween 60 > tween 80 > tween 40 > tween 20 which also confirms the effect of chain length on vesicle size.

Scanning electron microscopy

SEM studies were done to get more insight on vesicle formation and morphology of the system. Scanning electron micrograph of optimized SSD niosome (Formula F3, Table1) is shown in Fig. 2b. The vesicles were well identified and were present in a nearly perfect sphere-like shape.

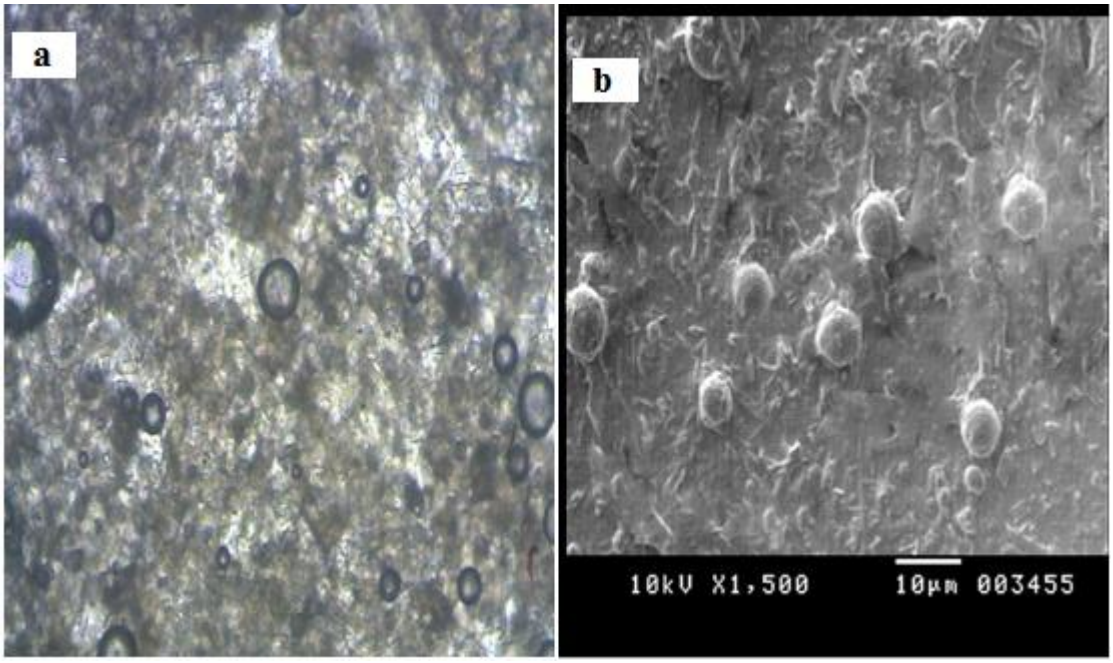

Fig. 2: Optimized SSD niosomes a) photomicrographs and b) scanning electron micrograph

***In-vitro* release study**

Results of *in vitro* release study of SSD from niosomal vesicles indicate that by encapsulation of drug into niosomes, it was possible to sustain the release of the drug for a longer duration. The decreasing order in which different spans (Fig. 3a) sustained the release of SSD from niosomes was span 60 > span 40 > span 20 > span 65 > span 80. The release of SSD from the niosomes of different surfactants was found dependent on the chain length of surfactants. As the chain length increases, the release gets sustained for longer duration. Span 60 sustained the release for 28 hours and span 80 for 8 hours. Though chain length of span 65 and span 80 is longer than other spans the release with them is faster because of the effect of unsaturation and leaking out.

The decreasing order in which different tweens (Fig. 3b) sustained the release of SSD from niosomes was tween 60 > tween 40 > tween 20 > tween 80. This also confirms the effect of chain length and unsaturation. Though tween 80 has a longer alkyl chain than tween 20 and tween 40, it showed the release because of the effect of unsaturation and leaking out.

Apart from chain length, phase transition temperature of surfactants can also affect the release rate of SSD from niosomes. Phase transition temperature of span 60, span 40, span 20, span 65 and span 80 is 53 ^{0}C, 42 ^{0}C, 16 ^{0}C, 14 ^{0}C and 12 ^{0}C respectively [27]. The reduced permeation of SSD from niosomes of span 60 and span 40 can be attributed to their high transition temperatures, which may have made them in a highly ordered gel state at the permeation temperature of 37 °C. On the other hand, the lower transition temperatures of span 20, span 65 and span 80 may have made them in the disordered liquid crystalline state and completely fluid, hence; they were more permeable for the drug at 37 °C. Similar is the effect of phase transition temperature on the release of drug from the niosomes manufactured by using different tweens.

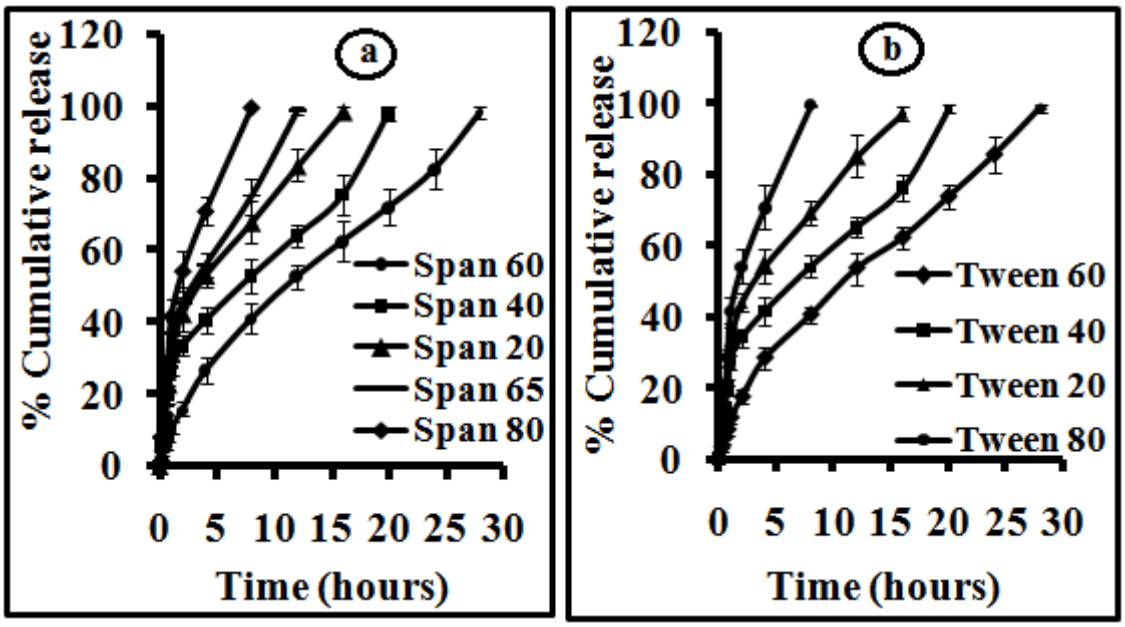

Fig. 3: *In vitro* release of SSD from niosomes of different a) spans and b) tweens

Model fitting analysis

Curve fitting analysis on the release data was done to find out the proper drug release mechanism. Zero, First, Higuchi and Korsmeyer-Peppas equations were applied to all *in vitro* release data and correlation coefficients (r^2) values were determined, which are shown in Table 2. From the results, one can conclude that, the drug got released from niosomes by a higuchi controlled diffusion mechanism.

Differential scanning calorimetry analysis

DSC thermograms of optimized niosome and pure drug are shown in Fig. 4. In the thermograms of pure drug (Fig. 4b) endothermic peak is observed at 275.8 °C due to melting of the drug. In the case of optimized niosomes (Fig. 4a) endothermic peaks are observed at 48.3 °C due to melting of span 60 and at 145.8 °C due to melting of cholesterol, but the intensity of SSD peak at 275.8 °C was found to decrease which indicates that SSD has been encapsulated into niosomes.

Table 2: Correlation coefficient (r^2) data of various kinetic equations

Correlation coefficients (r^2)	Span 60	Span 40	Span 20	Span 65	Span 80	Tween 60	Tween 40	Tween 20	Tween 80
Zero order	0.981	0.930	0.897	0.889	0.877	0.981	0.927	0.884	0.877
First order	0.685	0.498	0.462	0.428	0.425	0.683	0.491	0.445	0.425
Higuchi	0.986	0.974	0.983	0.977	0.979	0.986	0.975	0,981	0.979
Korsmeyer-peppas	0.985	0.932	0.934	0.920	0.929	0.984	0.936	0.932	0.929

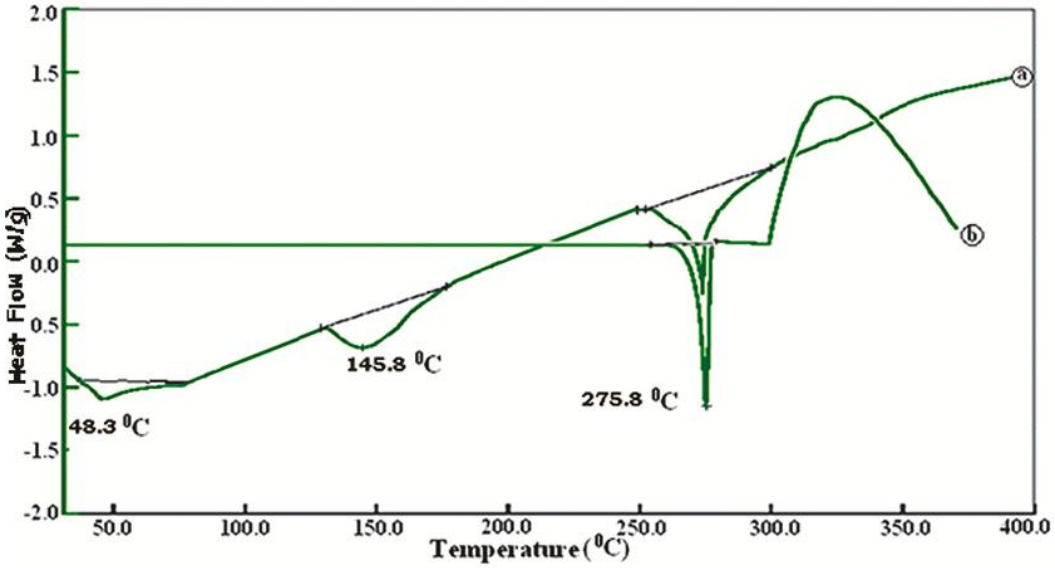

Fig. 4: DSC thermograms of a) optimized niosome and b) SSD

***In vitro* antimicrobial study**

When 1 % and 0.5 % optimized niosomal formulation was assayed against the test microorganisms the mean zones of inhibition (Fig. 5) obtained were 15 mm and 14 mm respectively, compared to 12 mm obtained with the 1 % conventional cream. The blank niosome did not show any zone of inhibition. Since the zone of inhibition of even 0.5 % niosomal formulation was greater than that of the conventional cream, we can infer that the antibacterial activity of niosomal formulation is superior to the conventional formulation.

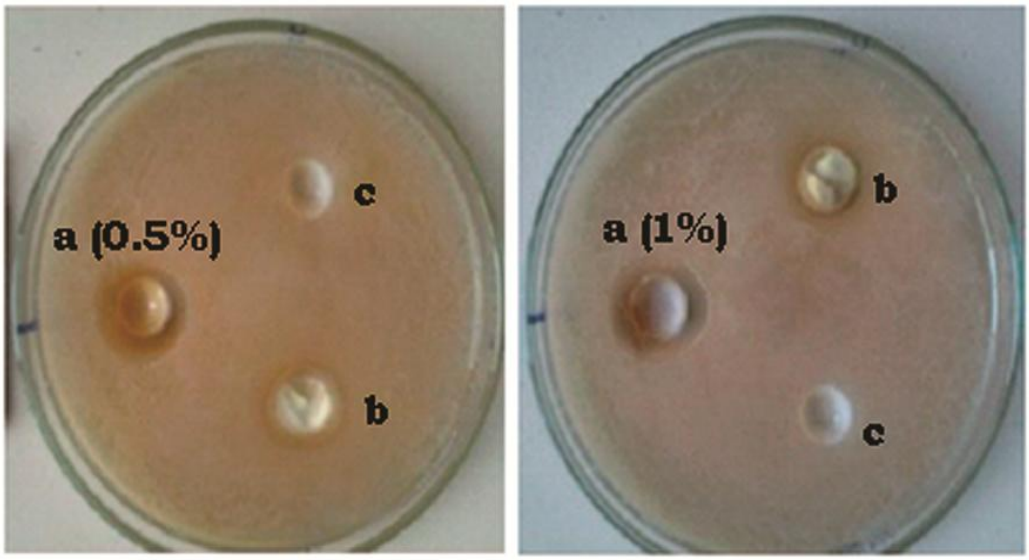

Fig. 5: *In vitro* antimicrobial zone comparison of a) optimized niosomes b) conventional cream c) pseudo niosomes

Stability study

This study was performed on the span 60 niosomes (Formula F3, Table1) which had shown highest entrapment and more sustained release among all the surfactants used. Stable niosomes must exhibit a constant vesicle size and a constant level of entrapped drug after exposing to different conditions of temperature and relative humidity (RH) for six months [28]. Mean vesicle size was found to increase on storage after 6 months. The increase in vesicle size was more in formulations stored at 25 ± 2 °C/60 ± 5 % RH than at 5 ± 3 °C. Results (Table 3) indicate that prepared formulation was relatively stable at 5 ± 3 °C, as compared to 25 ± 2 °C/60 ± 5 % RH. The vesicle size of 5.18 µm was recorded after a period of 6 months at storage temperature of 5 ± 3 °C, as compared to initial size of 5.05 µm with span 60 niosomal formulations.

Physical stability study was also carried out to investigate the leaching of drug from niosomes during storage. At 5 ± 3 °C, there was a minimum loss of drug, but a marked reduction in the residual drug content was found when formulations were stored at 25 ± 2 °C/60 ± 5 % RH for six months. At 5 ± 3 °C, a minimum loss of drug was observed, which might be because of the regidization of the vesicles at low temperatures that reduced the drug permeability through the membrane. After 6 months storage at 5 ± 3 °C, the entrapment efficiency of optimized span 60 niosomes was 71.54 % (Table 3) as compared to initial entrapment of 72.46 %. Thus, niosomes were found more stable at 5 ± 3 °C, as compared to 25 ± 2 °C/60 ± 5 % RH.

Table 3: Effect of stability study on vesicle size and entrapment efficiency

Test	Initial	5°C ± 3°C			25°C ± 2°C/60% ± 5% RH		
		1month	3months	6months	1month	3months	6months
Mean diameter (µm)	5.05 ± 0.34	5.11 ± 0.23	5.17 ± 0.19	5.18 ± 0.35	5.36 ± 0.26	5.57 ± 0.22	6.01 ± 0.17
Entrapment Efficiency (%)	72.46 ± 0.76	71.98 ± 1.18	71.57 ± 1.04	71.54 ± 0.84	67.13 ± 0.66	64.12 ± 0.94	61.42 ± 0.86

CONCLUSION

The results for this study indicate that the niosomal formulation of SSD can be used as a sustained release depot system for the treatment of burn. It was criticized by some authors that, it is not possible to get the high entrapment with the ethanol injection method, but this study showed that if factors like curing time, surfactant structure, HLB and molecular weight are carefully monitored it is possible to get good entrapment with the ethanol injection method. However, the different amounts of the drug and different ratios of span 60 and cholesterol should be tried in order to check out the possibility of further enhancing the entrapment efficiency.

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