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Span 60 Niosomes of Silver Sulfadiazine: A Localized Sustained Release System for Burn Treatment

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ABSTRACT

This investigation deals with the formulation of sustained release niosome based system of silver sulfadiazine for the treatment of burn and thereby decreasing the dosing frequency which is really important for severe burn patient as every reapplication is very painful to them. In this investigation, the effect of different processing variables on entrapment efficiency of drug was evaluated. The vesicle size, photomicroscopy, in *vitro* release, scanning electron microscopy, stability and *in vitro* antimicrobial activity of niosomal vesicles formed were also characterized. Niosomes were developed from span 60 and cholesterol in different molar ratio by ether injection, thin film hydration and non solvent methods. Results indicated that the niosomes manufactured with span 60 and cholesterol in 50:50 molar ratio using 100mg of drug by thin film hydration method gives highest entrapment of 92.11%. The method of preparation and contents of cholesterol as well as drug were found to affect the entrapment. The optimized niosomal formulation exhibited significantly retarded *in vitro* release of 98.04% over 28 hours by a higuchi controlled mechanism. The *in vitro* antimicrobial study using *Staphylococcus aureus* revealed that niosomal formulation of silver sulfadiazine shows better zone of inhibition (18mm) in comparison to conventional dosage form (17mm). In conclusion this study showed that the niosomal formulation can be used as promising sustained release approach for the topical delivery of silver sulfadiazine in the treatment of burn.

Keywords: Silver sulfadiazine; Antimicrobial study; Burn; Entrapment efficiency; Span 60

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INTRODUCTION

Burn is one of the most distressing forms of injury. Morbidity in burn cases may be huge even with small areas of injury. Burn injuries induce problem of immunosuppression which predisposes patients to infectious complications. Burn wound infection is one of the most important and potentially serious complications. Bacteria colonizing the skin wounds after burn injury may originate from the patient's endogenous skin, gastrointestinal and respiratory flora or from environment. Invasion of microorganisms into the tissue layers below the dermis may also result in sepsis, bacteremia and multiple-organ dysfunction [1]. Patients with serious burn injury require immediate specialized care in order to minimize morbidity and mortality.

Several studies have confirmed the role of topical antimicrobial in declining morbidity and mortality in patients with major thermal injuries. Topical antibacterial such as silver sulfadiazine is widely used antibacterial in burn injury [2]. It is used for prevention and treatment of wound sepsis in patients with second and third degree burn [3, 4]. Conventional silver sulfadiazine in 1% water soluble cream is effective against many gram-negative and gram-positive bacteria as well as against yeast and *Candida albicans* but requires two to four daily applications [5].

The conceptual approach behind this study is to reduce dosing frequency by developing sustained drug delivery. Vesicular drug delivery systems like liposomes and niosomes are found to be effective for sustained drug delivery. Lichtenstein and Margalit [5] had developed the liposomal silver sulfadiazine topical formulation for the treatment of infected burn 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. But liposomes are physically and chemically unstable. They are also prone to microbial growth and require specialized storage conditions.

Niosomes are structurally similar to liposomes in having a bilayer, however, the excipients used to manufacture niosomes makes them increasingly stable and thus niosomes offer many more advantages over liposomes. Niosomes behave in-vivo like liposomes, prolonging the circulation of entrapped drug and varying its organ distribution and metabolic stability. Niosomes are favourable because of the low cost of excipients and manufacture, possibility of large-scale production, stability and the resultant convenience of storage. Drug delivery through niosomes is one of the approaches to achieve localized drug action [6,7]. Therefore, the objective of this study is to investigate niosomally encapsulated silver sulfadiazine as an improved sustained release drug depot system for the treatment of burn.

MATERIALS AND METHOD

Materials

Silver sulfadiazine was supplied as a gift sample by Ajanta Pharma Limited, Mumbai, India. Span 60 was purchased from S. D. Fine Chemical Limited, Mumbai, India. Cholesterol was purchased from Himedia Laboratories Privet Limited, Mumbai, India. Monobasic potassium phosphate and sodium hydroxide were supplied by Alkem Laboratory Limited as a gift samples, New Mumbai, India. All solvents used were of analytical grade.

Preparation of niosomes

At start niosomes were developed using span 60 and cholesterol in different molar ratios (Table 1) by different methods like ethanol injection, thin film hydration and non solvent method. A constant quantity of drug (50mg) was added in all of these experiments. The selection of best possible ratio of span 60 and cholesterol as well as best possible method of preparation was done at this stage depending upon the % entrapment efficiency. The ratio and the method which showed the highest entrapment was used for further study to select optimum quantity of drug (10mg, 20mg, 30mg, 40mg, 50mg, 60mg, 70mg, 80mg, 90mg or 100mg) to be incorporated in niosomes. Thus only quantity of drug was changed at this final stage while keeping the other parameters like ratio of span 60 and cholesterol constant. The niosomal formulation which had shown highest entrapment was selected as optimized formulation.

Ethanol injection method

This method is similar to the method reported by Jadon et al. [8] with minor modifications. Briefly, weighed amount of the surface-active agent was mixed with the suitable amount of cholesterol in different molar ratios (Table 1) to make 0.001 mole i.e. 1mmol total concentration. This blend was dissolved in 9ml ethanol in a beaker. This surfactant solution was then mixed with the drug solution which was made by pre-dissolving 50mg silver sulfadiazine with 1 ml of 25 % w/v ammonia solution. This dispersion was then very slowly injected drop by drop through 14 gauge needles into the 10ml of 7.4 pH phosphate buffer maintained at 60°C with slow agitation at 250 rpm using magnetic stirrer (Remi 5MLH DX). Vaporization of organic solvent leads to the formation of single layered vesicles.

Thin film hydration method

Perfectly weighed amount of the surface-active agent was mixed with the suitable amount of cholesterol in different molar ratios to make 0.001 mole i.e. 1mmol total concentration (Table I). This blend was dissolved in 9 ml ethanol, in a round-bottom flask. This surfactant solution was

then mixed with the drug solution which was made by pre-dissolving silver sulfadiazine with 1 ml of 25 % w/v ammonia solution. Then organic solvent was removed with the rotation of 80rpm under vacuum at 60 ℃ leaving a thin layer of solid mixture deposited on the wall of the flask [9, 10]. The vesicles were prepared by rehydrating the dried surfactant film for 30 minutes without vacuum at 80 rpm with the 10 ml phosphate buffer maintained at 60°C to remove the traces of ethanol.

Non-solvent method

This method is similar to ethanol injection method but organic solvent is not used in this process [6]. Quantity of surfactant and cholesterol used was also increased from 0.001 moles to 0.01 moles so that sufficient amount of excipients mixture will be available for injecting. Accurately weighed amount of the surface-active agent was mixed with the appropriate amount of cholesterol in different molar ratios to make 0.01 mole i.e. 10 mmol total concentration. This mixture was then melted at 60°C. This melted surfactant-cholesterol mixture was then tried to slowly inject into the 10 ml aqueous phase maintained at 60°C through 14 needles with the slow agitation at 250 rpm using magnetic stirrer (Remi 5MLH DX). Solution of drug was made by pre-dissolving it with 1 ml of 25 % w/v ammonia solution and then diluting with 9 ml of phosphate buffer.

Separation of unentrapped drug by centrifugation

The drug-loaded niosomes were separated from unentrapped drug by centrifugation (Remi CPR-24) at 15250g relative centrifugal force (rcf) for 30 minutes [11,12]

Evaluation of niosomes

Entrapment efficiency

Entrapment efficiency was determined by using vesicle disruption method. After centrifugation the supernatant was separated and the isolated pellets were again washed by centrifugation twice with 5 ml phosphate buffer. This sample of niosomes was mixed with 5 ml of 25% w/v ammonia solution as drug was soluble in this solvent and covered well with aluminum foil to prevent evaporation. This solution was then sonicated (Pci Analytics JIJ 158) for 15 minutes to obtain a clear solution. This clear solution was diluted up to 100 ml with 7.4 phosphate buffer. Further dilutions were made only if it was necessary and concentration of drug was determined spectrophotometrically at 255nm [11] 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 as optimized batch. Entrapment efficiency was obtained by using following formula [12].

Entrapment efficiency (%) =Amount entrapped /Total amount x 100

Photomicroscopy and vesicle size analysis

Sample of the formed niosomes was spread on a glass slide and photomicrograph was taken by future winjoe projection microscope (MEM 1300) using 10x magnifications power [13]. 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.

In-vitro release study

The release of drug from niosomes was determined by using Franz diffusion cell [14]. The available diffusion area of cell was 3.14 cm^2. The receptor compartment of Franz diffusion cell was filled with 15 ml of pH 7.4 phosphate buffer maintained at 37 °C and stirred by a magnetic bar (Remi 5MLH DX) at 600 rpm. The cellophane membrane with molecular weight cut-off of 8000 was activated in diffusion media by boiling in it followed by keeping in it for overnight [15.] The activated cellophane membrane was then mounted on receptor compartment. After removing unentrapped drug, sample of niosomes equivalent to 1 mg of silver sulfadiazine was suspended in 1 ml of pH 7.4 phosphate buffer and placed on activated cellophane membrane. At appropriate time intervals, 1 ml aliquots of the receptor medium was withdrawn and immediately replaced by an equal volume of fresh receptor solution to maintain sink conditions. These samples were analyzed spectrophotometrically (Jasco V650) at 255nm. All the spectrophotometric analysis was carried out in triplicate and the values were averaged. The mechanism of silver sulfadiazine 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 .

Scanning electron microcopy

The prepared optimized niosomal formulation was also characterized for their morphology using scanning electron microscopy [11]. The lyophilized sample of niosome was sprinkled and fixed on a SEM holder with double sided adhesive tape and coated with a layer of gold of 150 °A for five to six minutes using a sputter coater, working in a vacuum of (3×10^{-1} atm) of argon gas. The sample was then examined using a scanning electron microscope (JEOL 5400, Japan) at 10 kV accelerating voltage.

In vitro antimicrobial study

Zone inhibition study using standard cup plate method was used to compare the *in-vitro* antimicrobial effectiveness of optimized silver sulfadiazine niosomal formulation over conventional cream. *Staphylococcus aureus* (Microbial Type Culture Collection and Gene Bank, Chandigarh, number 3160) was used as test organism as it is frequently implicated as invasive bacteria in burn wound sepsis [16.] Two petri plates of approximately 90 mm internal diameter were filled with 30 ml of previously autoclaved nutrient agar medium. This media was inoculated with 0.1 ml of culture containing overnight inoculums of test organism and after congealing three wells of 12mm diameter were punched using sterile borer in each petri plate [17]. First well was filled with niosomes, next with conventional cream and last was filled with placebo niosomes. The concentration of niosomal preparation added in first petri plate was kept half (0.5%) the concentration of conventional cream (1%) and in second petri plate it was kept same (1%). These petri plates were then kept at place for 4 hours at room temperature as a period of pre-incubation diffusion and then incubated at 37^0c for 24 hours [18]. Zones of inhibition were measured with vernier caliper. Each assay in this experiment was carried out in triplicate.

Stability study

Stability of final selected niosomes was evaluated by keeping the prepared niosomes packed in amber color glass vials up to 6 months, at 5°C ± 3°C and 25°C ± 2°C/60% ± 5% relative humidity (RH) in stability chamber (Classic Scientific CS-03). Samples were withdrawn on 1^{st} day, 30^{th} day, 90^{th} day and 180^{th} day [19]. These samples were then examined for entrapment efficiency and vesicle size after the separation from unentrapped drug by using the methods as described previously.

RESULTS AND DISCUSSION

Entrapment efficiency

At start niosomes were manufactured by different methods using different ratios of span 60 with cholesterol and entrapment efficiency was determined by vesicle disruption method. Quantity of drug (50mg) added was kept constant at this stage. Several factors such as method of preparation and % of cholesterol were found to affect the entrapment at this stage (Table 1). For the further study niosomes were manufactured using 50:50 molar ratio of span 60 with cholesterol by thin film hydration technique as it had shown more entrapment compared to other methods and ratios used. Only quantity of drug used was changed at this stage which was also found to affect entrapment (Table 2).

Table 1 Formulation and evaluation of niosomes composed of different molar ratios of span 60 and cholesterol and 50mg of drug

Molar ratio[a]	Ethanol injection method		Thin film hydration method	
	Mean diameter(µm)	Entrapment efficiency (%)	Mean diameter(µm)	Entrapment efficiency (%)
0.001 : 0.00	Niosomes were not formed in the absence of cholesterol			
0.0009 : 0.0001				
0.0008 : 0.0002	4.57±0.32	27.76±1.32	5.12±0.42	33.48±1.11
0.0007 : 0.0003	3.33±0.24	43.16±1.34	4.09±0.36	51.23±0.65
0.0006 : 0.0004	4.25±0.37	64.32±0.95	4.78±0.34	69.57±0.82
0.0005 : 0.0005	5.02±0.45	72.78±0.87	5.42±0.26	80.66±1.43
0.0004 : 0.0006	1.12±0.21	31.24±1.24	1.46±0.45	38.46±1.23
0.0003 : 0.0007	Niosomes were not formed due to excess of cholesterol and low concentration of surfactant			
0.0002 : 0.0008				

[a] Total concentration of span 60 and cholesterol was 0.001moles

[a] Molecular weight of cholesterol is 386.67 and molecular weight of span 60 is 430.62

[a] Number of moles = weight in gm/molecular wt, so wt in gm= molecular wt X no. of moles

Table 2 Evaluation of niosomes composed of span 60 and cholesterol in the molar ratios of 50: 50 and different quantities of drug

Drug (mg)	Amount entrapped (mg)	Entrapment efficiency (%)	Mean diameter(µm)
10	5.24±0.18	52.40±1.80	4.72±0.24
20	11.83±0.31	59.15±1.55	4.87±0.46
30	19.99±0.50	66.63±1.66	5.05±0.48
40	28.90±0.43	72.25±1.08	5.22±0.35
50	39.89±0.62	79.78±1.24	5.38±0.52
60	50.37±0.47	83.95±0.78	5.57±0.42
70	61.35±0.79	87.64±1.13	5.75±0.28
80	73.70±0.45	92.12±0.56	5.92±0.79
90	82.88±0.86	92.09±0.96	5.92±0.31
100	92.11±0.92	92.11±0.92	5.93±0.63

Effect of method of preparation on entrapment efficiency

Results indicate that (Table 1) there was correlation between the vesicle size and entrapment efficiency as function of the preparation method. Both entrapment efficiency as well as vesicle size was found higher in case of thin film hydration method in comparison with ethanol injection method.

When the development of niosomes was attempted by non-solvent method, the melted lipid mixture got immediately solidified in the injection so it was not possible to inject it into phosphate buffer and hence it was concluded that niosomes preparation is not practically possible using non-solvent method.

Effect of % of cholesterol on entrapment efficiency

Cholesterol is one of the most regular excipient included in vesicular formulations in order to improve their stability, to decrease or prevent leakage of drug and to increase the rigidity of bilayer membrane [10]. Cholesterol was found to affect the entrapment efficiency (Table 1) of niosomes containing different molar ratios of span 60 and cholesterol, manufactured by both ethanol injection and thin film hydration method, in the similar order 80:20<70:30<60:40<50:50>40:60. From the results it was observed that the ratio of surfactant to cholesterol should be optimum to get the maximum entrapment efficiency. Niosomes were not formed in the formulation containing 0 and 10 % cholesterol by both ethanol injection and thin film hydration method. This might be due to the absence of rigidizing effect of cholesterol, owing to its very low concentration. Because of this bilayer membrane remains very much flexible and so it was not possible for surfactant to self assembles in the form of niosomes.

In the formulation containing 20% cholesterol, the niosomes formed were very flexible with different shapes and large size (Figure 1a) but the entrapment efficiency found was lowest because of leaching out of drug from the flexible membrane of these niosomes.

Increase in the cholesterol concentration from 30 to 50% found to increase the size of niosomes (Figure 1b-d). With the increase in size, the encapsulation efficiency also increased. This increase in entrapment efficiency might also be due to increasing cementing effect of cholesterol in the leaking space of bilayer membrane, which was more in the niosomes containing 50% of cholesterol in comparison to niosomes containing 30% of cholesterol. In the formulation containing 60% cholesterol, entrapment efficiency as well as size was found to decrease (Figure1e). The origin of this effect is still uncertain. As the concentration of cholesterol increases beyond 60%, the excess of cholesterol found to disturb the bilayer structure. So niosomes were not formed in the formulation containing 70 and 80 % of cholesterol due to excess of cholesterol and low concentration of surfactant. Thus the entrapment efficiency with the formulation containing 50:50 ratio of surfactant and cholesterol was found maximum in case of both ethanol injection and thin film hydration method. It means that equal molarity of nonionic surfactant and cholesterol can make the membrane compact and well organized [20,21].

Mokhtar et al. [22] had also worked on the effect of cholesterol on entrapment. They got highest entrapment of flurbiprofen at 10% concentration of cholesterol in span60 niosomes. Then they observed decrease in entrapment efficiency continually with increase in cholesterol till 60%. Our results are not in accordance with their study.

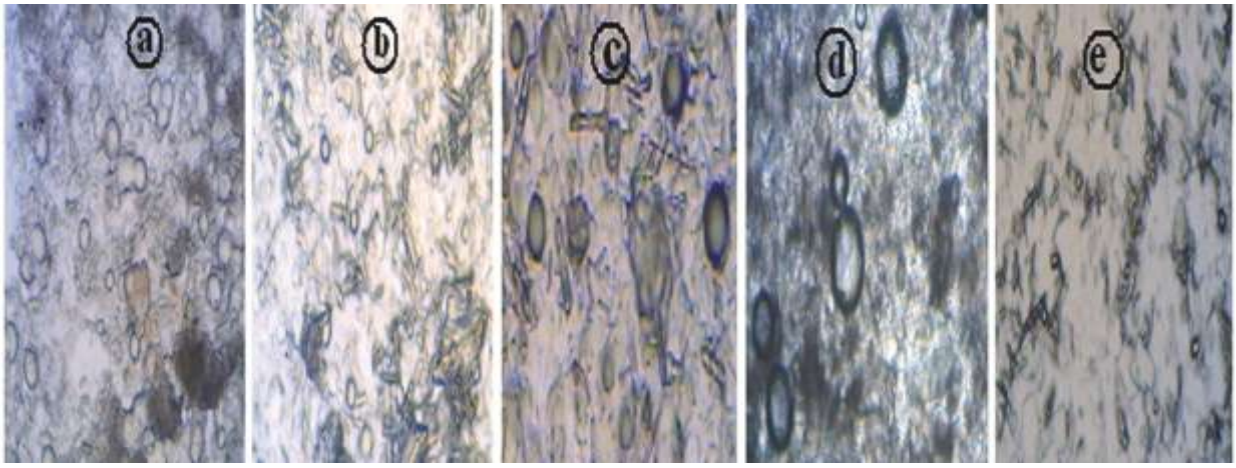

Figure.1. Photomicrograph of niosomes containing (a) 20%, (b) 30%, (c) 40%, (d) 50% and (e) 60% cholesterol.

Effect of quantity of drug on entrapment efficiency

The result of previous study had shown that the cholesterol in 50: 50 molar ratio with span 60 gives maximum entrapment. It was also observed that thin film hydration method shows better entrapment than ethanol injection method. So for the selection of optimum quantity of drug, niosomes containing span 60 and cholesterol in the molar ratio of 50: 50 were developed by thin film hydration method. Only quantity of drug added was changed in this experiment.

The result of this experiment (Table 2) had shown that, with increase in the quantity of drug from 10mg to 80mg, the entrapment efficiency also increased from 52.40 to 92.12. The increased entrapment efficiency of silver sulfadiazine with higher amount of drug used in the formulation could be due to the saturation of the media with silver sulfadiazine that forces the drug to be encapsulated into niosomes [23]. But further increase in drug concentration from 80 to 100 mg does not lead to increase in entrapment but amount of drug entrapped was more with 100mg compared to 80mg.

Thus it was found that niosomes manufactured with span 60 and cholesterol in the molar ratio of 50: 50 by thin film hydration method using 100 mg of drug gives maximum entrapment so it was selected as an optimized formulation.

Similar results were reported by Mokhtar et al. [22] who studied the effect of flurbiprofen concentration on entrapment efficiency of niosomes. When they increased the concentration of flurbiprofen from 25 to 75 mg, entrapment efficiency was increased from 55.99% to 72.25%. Further increase in flurbiprofen concentration to 100mg does not lead to increase in entrapment but precipitation of excess non entrapped drug was observed by them.

Photomicroscopy and vesicle size analysis

Size of niosomes manufactured by using ethanol injection method was always found less than the size of niosomes manufactured by using thin film hydration method at each ratio of span and cholesterol (Table 1). Photomicrographs for all these formulations are shown in Figure. 1a-e. It was also found that with increase in quantity of drug from 10 mg to 100 mg, the vesicle size of niosomes was increased from 4.72 µm to 5.93 µm (Table 2).

In-vitro release study

In case of niosomes manufactured by both ethanol injection and thin film hydration method using different ratios of span 60 with cholesterol and 50 mg of drug, permeation of silver sulfadiazine through cellophane membrane was found greatly affected by the concentration of cholesterol incorporated into niosomes (Figure.2a and 2b). From the obtained results it was observed that drug release was more prolonged from 8 hours to 28 hours with the increase in the concentration of cholesterol from 20% to 50% due to increasing rigidizing effect of cholesterol in the leaking space of bilayer membrane. The faster release of the drug was found form niosomes with low amount of cholesterol. The space-filling action of increasing cholesterol content resulted in a decreasing of membrane permeability and sustained release[24]. The sustained releasing effect with increasing cholesterol concentration can also be explained from the fact that, cholesterol abolishes the gel-liquid phase transition of span 60 resulting in niosomes that are less leaky [13] which can be confirmed from the decrease in intensity of Span 60 peak at 51.6°C in DSC thermogram (SIIO 6300, Japan) of mixture of span 60 in 50: 50 molar ratios with cholesterol in comparison with DSC thermogram of pure span 60 (Figure. 3).

These results are not in accordance with Mokhtar et al. [22] who reported that the release rate increases with increase in the cholesterol concentration. Further increase in the cholesterol concentration above 50 % resulted in faster release within 20 hours due to the increased deformability of the niosomal membranes because of excess of cholesterol.

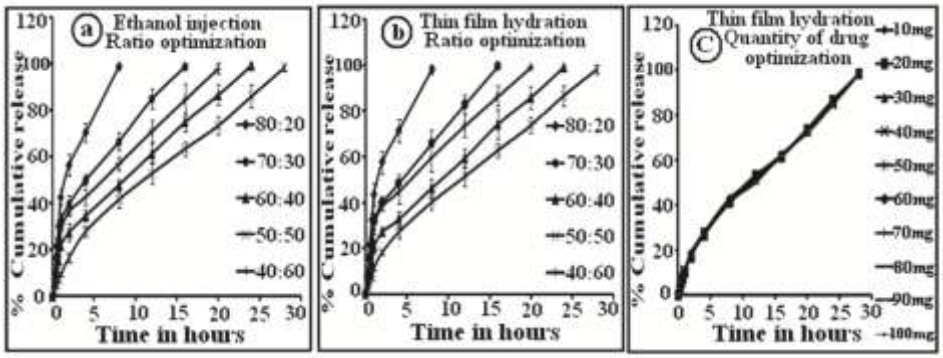

Figure 2. Respective *In vitro* % cumulative release verses time of sample withdrawal graph (zero order) of silver sulfadiazine from niosomes

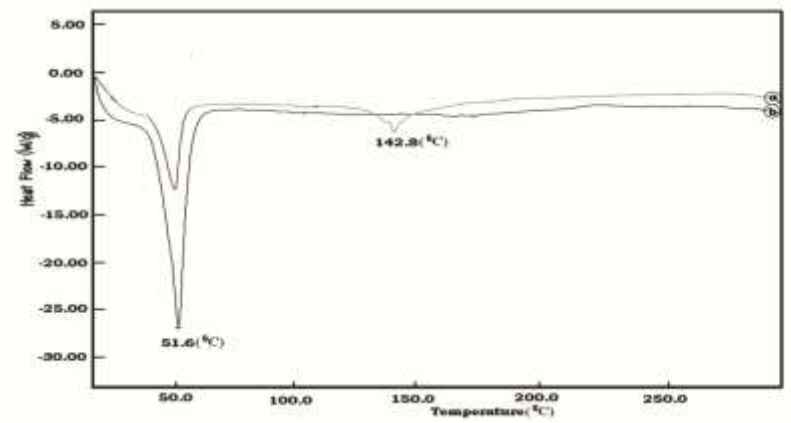

Figure 3. DSC thermograms of (a) span 60 and cholesterol mixture, (b) span 60.

In case of niosomes manufactured by thin film hydration method using 50: 50 molar ratio of span 60 with cholesterol and different quantities of drug (10 mg to 100mg), permeation of silver sulfadiazine through cellophane membrane was found not affected by the concentration of drug incorporated into niosomes. The overall release profiles of silver sulfadiazine from niosomes containing different amount of drug showed little difference. Due to this very little difference in released profile, graphs (Figure. 2c) obtained were found to overlap on each other.

Model fitting analysis

Curve fitting analysis for 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 3. From the results one can conclude that, the drug got released from niosomes by a higuchi controlled diffusion mechanism.

Table 3: **Kinetics model fitting using correlation coefficients (r^2)**

r^2	Ethanol injection method					Thin film hydration method					Optimized
	Span 60: cholesterol					Span 60: cholesterol					
	80:20	70:30	60:40	50:50	40:60	80:20	70:30	60:40	50:50	40:60	50:50
Zero order	0.864	0.913	0.929	0.965	0.981	0.983	0.969	0.917	0.918	0.847	0.982
First order	0.405	0.422	0.492	0.576	0.695	0.684	0.591	0.476	0.467	0.401	0.687
Higuchi	0.978	0.988	0.987	0.990	0.987	0.985	0.987	0.985	0.986	0.972	0.985
Korsmeyer-peppas	0.932	0.937	0.975	0.989	0.954	0.909	0.944	0.969	0.986	0.962	0.983

Scanning electron microcopy

Scanning electron micrograph of optimized silver sulfadiazine niosomes containing 50:50 molar ratio of span 60 to cholesterol and 100 of drug prepared by thin film hydration method is shown in Figure. 4. The vesicles were well identified and were present in a nearly perfect sphere-like shape.

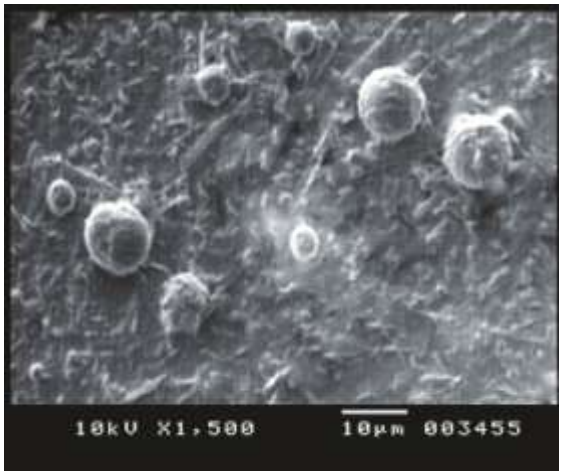

Figure 4.Scanning electron micrograph of optimized silver sulfadiazine niosomes

In vitro antimicrobial study

When 1% and 0.5% niosomal formulation was assayed against the test microorganisms the mean zones of inhibition obtained was 21±1.0 mm and 18±0.5mm respectively, compared to 17±0.5mm obtained with the 1 % conventional cream. The blank niosome had not shown 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.

Stability study

Physical stability study was carried out on optimized niosomal formulation containing 50: 50 molar ratio of span 60 with cholesterol and 100 mg of drug to investigate the effect of different storage condition of temperature and relative humidity for six months on vesicle size and entrapment efficiency. Mean vesicle size was found to increase on storage after 6 months. The increase in vesicle size was more in formulations stored at 25°C ± 2°C/60% ± 5% RH than at 5°C ± 3°C. Results indicate that prepared formulation was relatively stable at 5°C ± 3°C, as compared to 25°C ± 2°C/60% ± 5% RH. The vesicle size of 6.07 µm was recorded after a period of 6 months at storage temperature of 5°C ± 3°C, as compared to initial size of 5.91µm with span 60 niosomal formulation containing 100 mg of drug (Table 4).

Table 4 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)	5.91 ±0.19	5.99 ± 0.23	6.07 ± 0.28	6.07 ± 0.21	6.24 ± 0.39	6.47 ± 0.32	6.98 ± 0.27
Entrapment Efficiency (%)	92.11 ± 0.25	91. 89 ± 0.59	91.67 ± 0.52	91.23 ± 0.37	88.32 ± 0.86	84.21 ±0.79	81. 25 ± 0.82

Physical stability study was also carried out to investigate the leaching of drug from niosomes during storage. At 5°C ± 3°C, there was a minimum loss of drug but marked reduction in the residual drug content was found when formulations were stored at 25°C ± 2°C/60% ± 5% RH for 6 months. At 5°C ± 3°C, a minimum loss of drug was observed, which might be attributed to the regidization of the niosomes at low temperature that reduced the permeability of the drug through the niosomal membrane [8.] After 6 months storage at 5°C ± 3°C, the entrapment efficiency of niosomes composed of span 60 in a 50:50 molar ratio with cholesterol and 100 mg of drug was 91.23 % as compared to initial entrapment of 92.11% (Table 4). Thus niosomes were found more stable at 5°C ± 3°C, as compared to 25°C ± 2°C/60% ± 5% RH.

CONCLUSION

From the results it was concluded that the stable and effective silver sulfadiazine niosomes with highest entrapment efficiency can be manufactured from span 60 in the 50:50 molar ratio with cholesterol by thin film hydration method using 100mg of drug dissolved in organic phase. Different factors like method of preparation, % of cholesterol and quantity of drug were found to affect the entrapment efficiency.

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