

Design and characterization of ofloxacin niosomes

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Abstract: Niosomes are non ionic surfactant vesicles and potential surrogate for liposomes. The aim of the present investigation was to formulate and evaluate niosomes. The formulated ofloxacin niosomes were evaluated for their particle size, zeta potential, surface morphology, entrapment efficiency, in vitro drug release and *in vivo* pharmacokinetic studies. Niosomes of ofloxacin were prepared by thin film hydration technique using rotary flash evaporator. The formulated ofloxacin niosomes showed a vesicle size of 3.0-3.8 μm and zeta potential of -9 to -13 mV. The in vitro release studies showed 98.79% of ofloxacin release in sustained manner following first order kinetics. The stability study confirmed the stability of Ofloxacin niosomes. Pharmacokinetics studies of ofloxacin niosomes made with Span 60 showed increased C_{max} , AUC, AUMC, $t_{1/2}$ and MRT values compared to marketed intravenous ofloxacin product. The designed ofloxacin niosomes with span 60 showed good physicochemical properties, good stability, improved pharmacokinetic parameters, prolonged action and improved bioavailability than the commercially available conventional dosage form which might be a potential carrier system to improve the patient compliance and reduce the side effects.

Keywords: Niosomes, ofloxacin, non-ionic surfactant, zeta potential, pharmacokinetic studies.

INTRODUCTION

The flexibility and improvement of therapeutic effectiveness by providing controlled and sustained delivery of colloidal carrier system than the conventionally well established drugs have been investigated widely in the recent years. Drug delivery through niosomes was one of the approaches to improve the therapeutic performance of the drug and their distribution in the body. Liposomal formulations gained greater interest by providing increase in bioavailability. On the other hand, the utilization is limited by its own intrinsic chemical instability and higher cost. Niosomes were suggested to be surrogate for liposomes (Baillie *et al.*, 1985). The formation of vesicles on hydration of a mixture of cholesterol and a single alkyl chain non-ionic surfactant was first reported (Handjani-Vila *et al.*, 1979). Number of non-ionic surfactants have been used to prepare vesicles viz., poly glycerol alkyl ethers (Handjani *et al.*, 1979; Baillie *et al.*, 1986), glucosyl dialkyl ethers (Kiwada *et al.*, 1985), crown ethers (Echegoyen *et al.*, 1988), ester linked surfactant (Hunter *et al.*, 1988), polyoxyethylene alkyl ethers (Hofland *et al.*, 1992), Brij (Parthasarathi *et al.*, 1994), series of Spans and Tweens (Chandraprakash *et al.*, 1990; Uchegbu *et al.*, 1997). Spans are the widely used non ionic surfactant in the preparation of niosomes (Biju *et al.*, 2006). Many studies

were performed on liposomal and niosomal vesicles as therapeutic drug carrier systems to reduce the drug toxicity by alternating drug pharmacokinetics or modifying the drug delivery in order to prolong drug action at the target site. The antibiotics-loaded vesicles showed enhanced drug concentrations at the site of action due to targeting of drug to the infected tissues, increase in the intracellular antibiotic concentrations, and reduced the toxicities of potentially toxic antibiotics resulting from the targeting of antimicrobial drugs to the infectious organism (Al-Awadhi *et al.*, 1992). Niosomes systems might be capable of ensuring different pathways of interaction with microbial cells, compared to entering of fluoroquinolones in to cells from other dosage forms. This behavior may be useful in the treatment of infections caused by quinolone-resistant bacteria or by microbes which are, normally, poorly sensitive to this class of drugs (Nassander *et al.*, 1990). So, the entrapment of the fluoroquinolones in niosomes could be of therapeutic interest and could improve the efficacy of these drugs. Ofloxacin, second generation fluoroquinolone antibiotic used for the treatment of bacterial conjunctivitis caused by susceptible organisms has been chosen to encapsulate in the niosomes to improve the efficacy by altering the pharmacokinetics. In the present study we have formulated ofloxacin niosomes by thin film hydration technique using Span 40, Span 60 and Span 80 in different ratios and determined its effect on vesicle size, morphology, entrapment efficiency (EE), *in vitro* drug release, zeta potential and stability.

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Further, the selected niosomes formulation was evaluated and compared with marketed i.v. formulation for their in vivo pharmacokinetics performance in rabbits.

MATERIALS AND METHODS

Materials

Ofloxacin obtained as a gift sample from MMC Healthcare Ltd., Chennai, India. Span 40 (Sorbitan monopalmitate), Span 60 (Sorbitan monostearate) and Span 80 (Sorbitan monooleate) are procured from M/s. Qualigens, Mumbai, India. Cholesterol (CH) and Dicetyl phosphate (DCP) purchased from M/s. Merck Worli, Mumbai, India and Sigma, USA, respectively. Diethyl ether and Triton X-100 were obtained from M/s. Loba chemicals, Mumbai, India and all other chemicals and reagents used were of analytical grade.

Methods

Compatibility studies

The compatibility of physical admixtures of Span 40/ Span 60/ Span 80, cholesterol and DCP with ofloxacin were investigated by fourier-transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC).

In FTIR, the samples were prepared as KBr pellets at high compaction pressure in the ratio of sample to KBr at 2:200. The IR spectrum of samples was recorded using FT-IR (Shimadzu, Japan) in the wavelength 500-4000 cm^{-1} at ambient temperature with resolution of 4 cm^{-1} . The wave number for the characteristic bands of drug and other ingredients in the physical admixtures were compared with wave numbers and relative intensity of absorption band obtained with physical admixtures.

DSC was employed for the thermal analysis of physical admixtures to assess the effect of surfactant composition and inclusion of phase transition temperature (T_m). The prepared samples (2/mg) were accurately weighed, transferred into the aluminium cups and sealed with aluminium caps. Air gas was purged at the rate of 30 ml/min for maintaining an inert atmosphere. The scanning was done under nitrogen gas atmosphere between the temperature range of 35-300°C with a scanning rate of 5°C rise/min. Thermal data analysis was carried out with a HP 9000 series 700 workstation (Hewlett Packard, Palo Alto, CA). The phase transition temperature (T_m) of physical admixtures was obtained from the DSC curve (Wei Hua *et al.*, 2007).

Preparation of niosomes

Ofloxacin niosomes were prepared by using thin film hydration technique (Azmin *et al.*, 1985). An accurately weighed mixture of Span (71.25mg), CH (71.25mg) and DCP (7mg) in the ratio of 47.5:47.5:5 and Span (42mg), CH (21mg) and DCP (7mg) in the ratio 60:30:10 were

dissolved separately in 10ml of diethyl ether (solvent) in 500ml round bottom flask. The solvent was evaporated at 28°C under reduced pressure (260-400mmHg) in a rotary flash evaporator. Excess organic solvent were removed by leaving the flask in desiccator under vacuum condition for overnight. The dried thin film of surfactant deposited on the wall of the flask was hydrated with aqueous 0.1N HCl containing known amount of drug (1mg/ml) at 60±2°C for 30 min to obtain niosomal dispersion. Different batches of ofloxacin niosomes with varying concentrations of surfactants and lipids were prepared as shown in table 1. All the batches were formulated in triplicates and taken for further characterization.

Characterization of ofloxacin niosomes morphology

The morphology of ofloxacin niosomes was examined by using scanning electron microscope (JEOL, JSM-6701F, Japan). Ofloxacin niosomes (30/ μl) were spread over a metal stub and dried under vacuum condition at 25°C on the SEM sample holder and sputtered with platinum to minimize the charging effect by auto sputter fine coater (JFC 1600, JEOL, Japan). The samples were photomicrographed under SEM (Almira *et al.*, 2001).

Vesicle size

The vesicle size of formulated ofloxacin niosomes suspensions was determined by laser diffraction analyzer (Accusizer 780/SIS syringe injection sampler, PSS Nicomp, Particle sizing systems, Santa Barbara, California, USA). Ofloxacin niosomes (100/ μl) were diluted to 10 ml with 0.1N HCl and filtered through 0.45 μm size Millipore filter to remove dust particles and the measurements were carried out in triplicate at 25 ± 1°C with a scattering light angle 90.0° and mean vesicle size were calculated using software (Ahmed *et al.*, 2005).

Entrapment Efficiency

The drug/surfactant ratio represents the drug encapsulating capacity of surfactant. Separation of free drug from ofloxacin niosomes was performed by dialysis method (Thamilarasi *et al.*, 2005). The free drug was removed by placing the resultant niosomes dispersion in dialysis bag (Himedia, India) and exhaustively dialyzed against distilled water. The entrapped ofloxacin in niosomes was determined spectrophotometrically at 294 nm after complete disruption of known amount of dialyzed niosomes using Triton-X-100.

Zeta potential

The zeta potential of ofloxacin niosomes were determined by using Zetasizer (MAL 1004428, DTS Ver. 4.20, Malvern Instruments, UK). Niosomes were diluted 100 times with double-distilled water and voltage was set at 50 or 100 V between the two electrodes for the measurement of zeta potential. The measured values were obtained by the average of triplicate measurements (Gopi *et al.*, 2002).

In vitro release rate studies and kinetics model fitting

In vitro release profile of ofloxacin niosomes was performed in an open ended cylindrical tube with one end tied with dialysis membrane. Ofloxacin niosomes placed in an open ended cylinder and was suspended in a 250 ml of 0.1N HCl receptor medium in a beaker placed on a thermostat magnetic stirrer and constantly stirred at 50 rpm and $37 \pm 1^\circ\text{C}$ temperature. Aliquots (5ml) of samples were withdrawn from the receptor compartment at predetermined time intervals of 0.5, 1, 2, 3, 4 upto 20 hours and after each withdrawal same volume of fresh medium was replenished. The withdrawn samples were made up with 0.1N HCl and ofloxacin content in the withdrawn samples was estimated spectrophotometrically as described earlier. The results were calculated by mean values of three runs. In vitro release of ofloxacin in free drug solution and suitable ratio of ofloxacin niosomes were compared.

The in vitro release data were plotted according to the four different kinetic models, zero order (cumulative percentage drug release vs. time in hours), first order (log cumulative percentage drug remaining vs. time in hours), Higuchi's (cumulative percentage of drug released vs. square root of time) and Korsmeyer-peppas's release model (log cumulative percentage drug released vs. log time) to know the release mechanisms. The order of kinetics and mechanism of the release were confirmed based on linearity of the graphical expression from vitro release data (Costa and Lobo *et al.*, 2001).

Stability study

The stability study of ofloxacin niosomes were carried out as per ICH and WHO guidelines. Best formulations from the each one of the niosomes formulation ratios was packed in amber color vials and stored in a stability chamber (Yorco Scientific Industries, India) at $40^\circ\text{C} \pm 2^\circ\text{C}$ / 75 % RH $\pm$ 5% RH for 3 months to assess their long term stability. The initial drug content and percentage of drug remaining in niosomes at the end of each month was estimated spectrophotometrically as described earlier. The results expressed are the mean values of three runs.

In vivo pharmacokinetic study

The experimental protocol of this study was approved by institutional animal ethics committee (887/AC/05/CPCSEA-JKKNCP/IAEC/13MP03 AUG/2009). Adult male albino rabbits were housed under standard conditions with room temperature/humidity of $21 \pm 2^\circ\text{C}$ / 65% RH and a 12 h light/12 h dark cycle and starved for 18 hours before the experiment with free access to water. The animals weighing about 2-3 kg were grouped into two groups, three animals in each. The best ratio of ofloxacin niosomes was selected for in vivo studies based on in vitro characterization results. Ofloxacin niosomes in 0.1N HCl was centrifuged at 5000 rpm for 15 minutes and the supernatant was removed and the niosomes were re-

suspended in phosphate buffer saline (PH 7.4) for the determination of pharmacokinetics parameters. The results were compared with commercial marketed formulation.

Ofloxacin niosomes formulation and ofloxacin injectable marketed formulation (200 mg / 100 ml of distilled water) at a dose of 5 mg / kg body weight was injected separately into the jugular vein of the rabbits in both groups. Blood samples (3ml) were withdrawn at specified time interval of 5, 30, 60, 120, 240, 360, 540 and 720 minutes. Then the plasma samples were subjected to centrifugation in cooling centrifuge (Sigma, 3K30) at a constant temperature of 4°C at 3000 rpm for 15 minutes and the supernatant plasma was stored at 20°C until analysis. Ofloxacin in plasma samples were analyzed by HPLC (Shimadzu LC 20 AD) method. Pharmacokinetic parameters (AUC, AUMC, MRT, $t_{1/2}$, V_d , V_{dss} , Cl_T , and C_{max}) were calculated by adopting two compartment models (Leandro *et al.*, 2008) using kinetics 5.0 software.

STATISTICAL ANALYSIS

All experimental data were expressed as mean $\pm$ SD (for three independent samples). The statistical significance difference between the mean values was assessed by using student's t-test. Statistical probability (p) values less than 0.05 were considered significantly different.

RESULTS

Compatibility studies of drug with the surfactants, cholesterol, DCP mixtures were determined by IR spectroscopy using Shimadzu FTIR by kbr method. FT-IR spectra revealed that there was no interaction between the drug and excipients used for the preparation of ofloxacin niosomes. Physical admixtures of ofloxacin, cholesterol, DCP were subjected to DSC analysis to assess the phase transition temperature (t_m). The DSC thermogram and phase transition temperature of ofloxacin and its physical admixtures are shown in fig.1 and table 2.

Ofloxacin niosomes formulations were prepared in two different ratios of the selected suitable non-ionic surfactants (Span) having different HLB values, cholesterol and DCP. The compositions of niosomes formulations are shown in table 1. Ofloxacin niosomes was subjected to microscopic examination using scanning electron microscope for characterizing shape and size and of niosomes.

The SEM photomicrographs of ofloxacin multi lamellar niosomes obtained with Span 60 in the two ratios are shown in fig. 2. The vesicle size, zeta potential and entrapment efficiency of ofloxacin niosomes formulations are displayed in table 3. *In vitro* release profile of ofloxacin niosomes formulations was determined in 0.1N

HCL using an open ended cylinder and the release profiles of 47.5:47.5:5 ratio of ofloxacin niosomes and free drug solution are shown in fig. 3 and for the ratio of 60:30:10 ofloxacin niosomes are shown in fig. 4. The in vitro kinetics order and mechanism of release were established for the ofloxacin niosomes as shown in table 4. The stability study of both ratios of ofloxacin niosomes was performed as per ICH and WHO guidelines for three months and the results obtained are shown in the table 5. The drug plasma concentration and the pharmacokinetic parameters of ofloxacin niosomes (OSA 60) and marketed injectable ofloxacin formulation are displayed in fig. 5 and table 6.

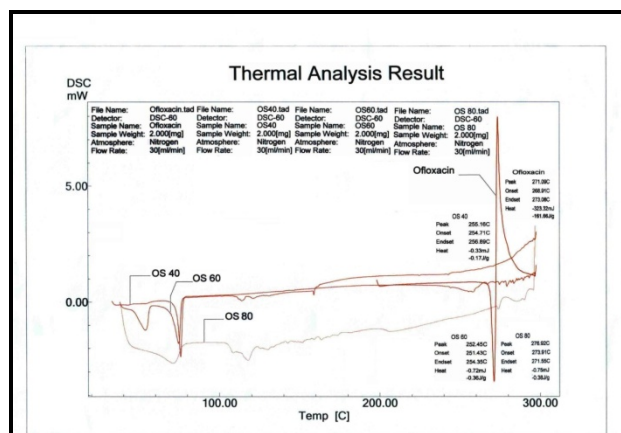


Fig. 1: DSC curve of ofloxacin and physical admixtures of span surfactants with ofloxacin
OS 40-Ofloxacin + Span 40; OS 60-Ofloxacin + Span 60; OS 80-Ofloxacin + Span 80

Table 1: Compositions of ofloxacin niosomes

Formulations Code	Components (in ratios)				
	Span 40	Span 60	Span 80	Cholesterol	DCP
OSA 40	47.5	-	-	47.5	5
OSA 60	-	47.5	-	47.5	5
OSA 80	-	-	47.5	47.5	5
OSB 40	60	-	-	30	10
OSB 60	-	60	-	30	10
OSB 80	-	-	60	30	10

Table 2: Phase transition parameters (T_m) of ofloxacin physical admixtures

Drug and physical admixtures	Temperature scanning cycle	Peak Temp (°C)	Enthalpy (J/g)
Ofloxacin	Endothermic transition	271.09	-161.66
Ofloxacin + Span 40 (OS 40)	Endothermic transition	255.16	-0.17
Ofloxacin + Span 60 (OS 60)	Endothermic transition	252.45	-0.36
Ofloxacin + Span 80 (OS 80)	Endothermic transition	276.92	-0.38

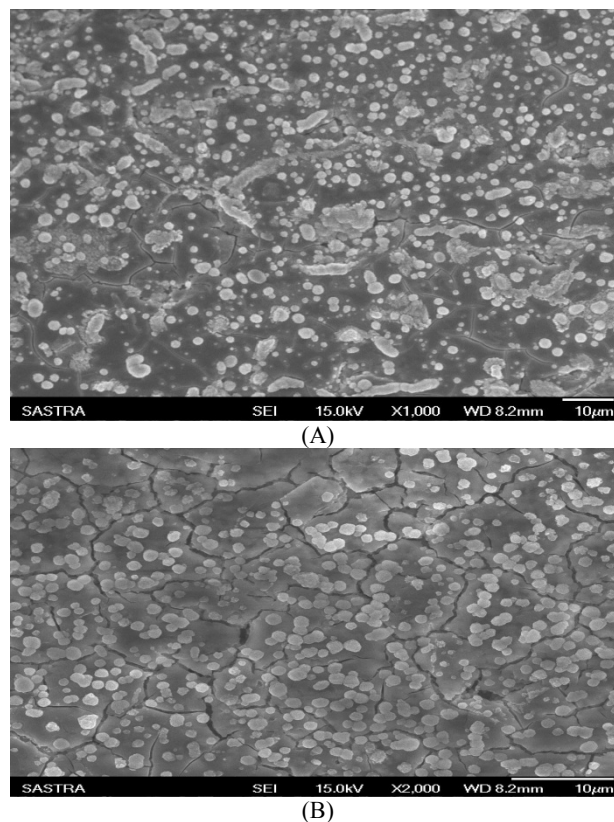


Fig. 2: SEM photomicrograph images of OSA 60 niosomes (A) and OSB 60 niosomes (B).

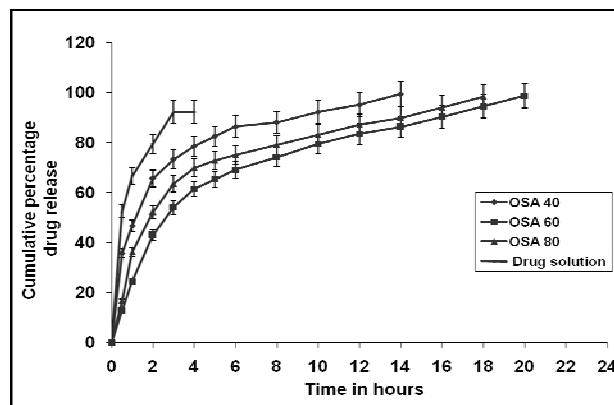


Fig. 3: *In vitro* drug release profile of Ofloxacin niosomes (47.5:47.5:5 ratios) and free drug solution
Each point represents mean $\pm$ SD of three trials (n=3) ($p \leq 0.05$)

DISCUSSION

The FT-IR spectrum of ofloxacin and its physical admixtures with Spans depicts that the characteristics peaks appear in functional group region and finger print region are identical and there is no changes in the peak shape and no shift of peaks indicating no modification or interaction between the drug and carrier. So, the drug is compatible with the Span surfactants in each physical admixture. The results suggest the drug stability during the encapsulation process.

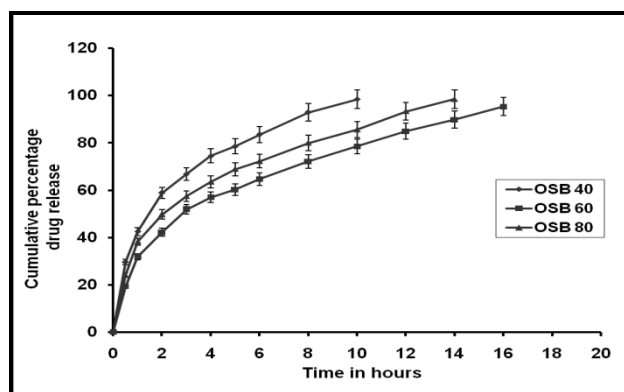


Fig. 4: *In vitro* drug release profile of ofloxacin niosomes (60:30:10 ratios)

Each point represents mean $\pm$ SD of three trials ($n=3$) ($p \leq 0.05$)

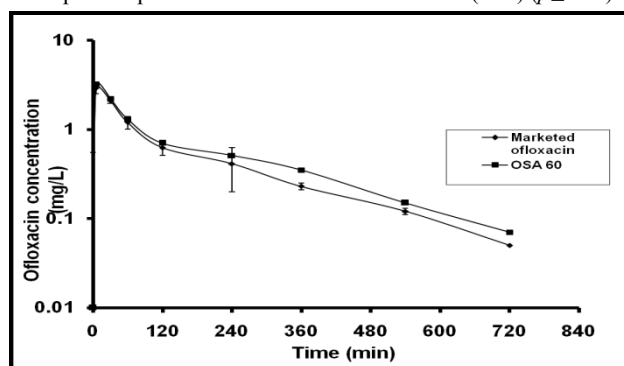


Fig. 5: Plasma concentration-time profile of marketed and niosomes encapsulated ofloxacin.

Each point represents mean $\pm$ SD ($n=3$)

Table 3: Vesicle size, zeta potential and entrapment efficiency data of ofloxacin niosomes formulations

Formulations Code	Mean Vesicle Size (μm)	Entrapment Efficiency ^a (%)	Zeta Potential ^b (mV)
OSA 40	3.30 $\pm$ 0.51	29.01 $\pm$ 0.95 ^a	-9.63 $\pm$ 0.90 ^b
OSA 60	3.79 $\pm$ 0.07	38.98 $\pm$ 1.00 ^a	-10.28 $\pm$ 1.0 ^b
OSA 80	3.60 $\pm$ 0.62	35.23 $\pm$ 0.82 ^a	-13.15 $\pm$ 1.0 ^b
OSB 40	3.02 $\pm$ 0.08	24.65 $\pm$ 0.59 ^a	-9.86 $\pm$ 0.99 ^b
OSB 60	3.14 $\pm$ 0.31	33.13 $\pm$ 0.98 ^a	-10.66 $\pm$ 1.01 ^b
OSB 80	3.06 $\pm$ 0.11	29.94 $\pm$ 0.96 ^a	-13.32 $\pm$ 1.21 ^b

Results are mean $\pm$ SD of three trials ($n=3$), (^a $p \leq 0.001$, ^b $p \leq 0.01$).

DSC measurement is used to prove the effect of heat in the process of temperature-induced niosomes aggregation and phase transformation. DSC thermogram of pure ofloxacin showed a sharp endothermic peak at 271.09°C. DSC thermogram of the ofloxacin loaded physical admixtures exhibited endotherms at 255.16°C (Span 40), 252.45°C (Span 60) and 276.92°C (Span 80) respectively. The onset temperature of the ofloxacin physical admixtures of Span surfactants is determined to be between 254.71°C, 251.43°C and 273.91°C. The melting endotherm of pure cholesterol was found to be 147.45°C. In physical admixtures, the cholesterol endotherms

exhibited a shift from 126.16°C to 170.98°C signifying that all components interact with each other to a great extent while forming the lipid bilayer. The incorporated ofloxacin was expected to get associated and interacted with lipid bilayers.

Sorbitan esters (Span) are most commonly used non-ionic surfactants in niosomes preparations since it has proven track of commercial exploitation and is generally regarded as safe (Gianasi *et al.*, 1997). In this study, a fluoroquinolone drug like ofloxacin was used to formulate niosomes by thin film hydration technique with two different ratios of Spans, CH and DCP (47.5:47.5:5 and 60:30:10) and to study its effect on vesicle size, entrapment efficiency, *in vitro* release and *in vivo* efficacy. The cholesterol was included in the formulation of Ofloxacin niosomes, to alter the properties of niosomes and to markedly decrease efflux-entrapped drug and increase the stability of niosomes dispersion. DCP was added to impart a negative charge on the basis of reports that negatively charged liposomes evince better *in vivo* anti-tumor activity than positively charged or neutral liposomes (Heath *et al.*, 1985). The inclusion of a charged molecule in the bilayer shifts the electrophoretic mobility and the presence of charged moiety (DCP) in lipid formulation also reduces the probability of vesicular aggregation and following the formation of MLVs and to achieve greater protection against flocculation in vesicles suspensions. Span surfactants are different in alkyl chain length (Yoshioka and Florence, 1994) and Span 60 showed the higher transition temperature of 50 $\pm$ 8°C (table 1). Hence the entire vesicle preparations were carried out at 60 $\pm$ 2°C.

Zeta potential is an important parameter for prediction of the stability of colloidal carrier system and fate of vesicles *in vivo*. The zeta potential value of ofloxacin niosomes was found to be in the region of -9 to -13 mV for the two different ratios and these relatively small values indicated that there is little electrostatic repulsion between these vesicles. The near neutral charge is advantageous for *in vivo* use, as large positive or negative charges can lead to rapid blood clearance. It has been documented that positively charged vesicles can cause nonspecific cell sticking (Fujita *et al.*, 1994) while negatively charged vesicles are efficiently taken up by scavenger endothelial cells, or “professional pinocytes” found in the liver (Smedsrod, 2004).

From the SEM morphology images, the prepared niosomes were found to be spherical in shape with smooth surface and vesicles were discrete and separate with no aggregation or agglomeration. The niosomes size measurements revealed that, obtained niosomes are MLVs (3.0-3.8/ μm) with distinct boundaries and all the ofloxacin formulations made with different Span surfactants in different ratios have not shown any significant difference in size.

Table 6: Pharmacokinetic data of ofloxacin niosomes and marketed ofloxacin formulation

Parameters	Ofloxacin niosomes (OSA 60)	Marketed Ofloxacin
AUC ($\mu\text{g h ml}^{-1}$)	6.54 $\pm$ 0.58 ^c	5.28 $\pm$ 0.42
AUMC ($\mu\text{g h}^2 \text{ml}^{-1}$)	23.2 $\pm$ 2.08 ^b	16.34 $\pm$ 1.30
MRT (h)	3.53 $\pm$ 0.32 ^c	2.8 $\pm$ 0.22
t _{1/2} (β)(h)	2.96 $\pm$ 0.26 ^c	2.4 $\pm$ 0.19
V _d (L/kg)	1.33 $\pm$ 0.12 ^c	1.49 $\pm$ 0.45
V _{dss} (L/kg)	2.71 $\pm$ 0.24 ^c	2.93 $\pm$ 0.73
Cl _T (ml h ⁻¹)	762 $\pm$ 68.58 ^c	942 $\pm$ 75.36
C _{max} ($\mu\text{g ml}^{-1}$)	3.79 $\pm$ 0.34 ^c	3.03 $\pm$ 0.24

Results are mean $\pm$ SD of three trials (n=3) (^b $p \leq 0.01$, ^c $p \leq 0.05$)

Note: AUC, Total area under plasma concentration versus time curve; AUMC, Area under the first moment curve; MRT Mean residence time; t_{1/2} (β), Elimination half life; V_d, Apparent volume of distribution; V_{dss}, Steady state volume of distribution; Cl_T, Total body clearance; C_{max}, Maximum concentration

The fate of niosomal system was mainly based on drug entrapment. Thin film hydration technique has been utilized to increase the drug entrapment inside the vesicles. Entrapment efficiency (EE) of Span 80 niosomes were less than those of Span 60 niosomes that might be due to the presence of unsaturated oleate molecule. EE increases in 47.5:47.5:5 formulation ratios with the increasing the CH content in the bilayer than 60:30:10 ratios. The encapsulation of drug is governed by the nature of solute, hydration temperature and method of drug loading. It has been also reported that, EE was found to increase with carbon chain length of lipophilic groups and permeability in lipid vesicles found to increase with the introduction of double bonds into the paraffin chains. These might produce more permeable membrane and be responsible for the lowest EE of Span 80 niosomes. Span 40 exhibited low EE of drug due to its low lipophilic nature, low phase transition temperature and high contact of drug and vehicle than Span 60 (Yongmei *et al.*, 2002).

In vitro release profile of ofloxacin niosomes was determined in an open ended cylinder using 0.1N HCl. *In vitro* release studies showed sustained release of up to 20 hours. The OSA60 niosomes released 98.79% of ofloxacin in 20 hours and OSB60 niosomes released 95.26% of ofloxacin in 16 hours. From the results of an *in-vitro* drug release studies of all batches, ofloxacin niosomes(OSA60) formulated with 47.5:47.5:5 ratio exhibit the best release pattern over the 60:30:10 ratio and it is also concluded that the OSA60 niosomes with 47.5:47.5:5 ratio are the best release in extended period of time in comparison to the other formulations. Hence, ofloxacin niosomes formulation (OSA60-47.5:47.5:5 ratio) has been selected for the comparison study of *in vitro* release study with pure drug solution. Sustained release may be attributed to the fact that MLVs consist of several concentric bilayer separated by aqueous

compartments and therefore the diffusion of entrapped drug in the hydrophobic regions of MLVs would be expected to occur over a prolonged period of time (Ruckmani *et al.*, 2000; Yu H *et al.*, 1998). The prolonged drug release may also due to the presence of CH and DCP in niosomes. Moreover presence of CH in the formulation affects the membrane fluidity by making it more rigid (Gabizon and Papahadjopoulos, 1988).

In order to understand the mechanism and drug release kinetics, *in vitro* drug release data was fitted to four different kinetic models. The *in vitro* drug release results of all formulations produced linear relationship when fitted in first order equation with 'r' value close to unity and higher than correlation coefficient obtained from the zero-order equation, indicating apparent first-order release process. The *in vitro* release profiles of the ofloxacin formulations fitted in Higuchi's equation showed good linearity with regression coefficient values of $r = 0.9024$ to 0.9420 for OSA formulations and $r = 0.9768$ to 0.9903 for OSB formulations. The linearity of the plots indicates that the release process is diffusion-controlled. To confirm diffusion mechanism, the data were fitted into Korsmeyer peppas model and nature of diffusion from the niosomes is explained by the 'n' value of Korsmeyer peppas equation. The plots of ofloxacin formulations showed good linearity (r : 0.9423 to 0.9989) indicating drug release followed the Fickian diffusion (Law *et al.*, 1994; Arica *et al.*, 1995; Glavas-Dodov *et al.*, 2002).

The stability study showed that the niosomes (OSB 60) formulated (60:30:10 ratios) possess less stability than 47.5:47.5:5 (OSA 60) ratios and this may be due to the lower HLB value of Span 60 than other Span formulations. Significant difference ($p \leq 0.05$) was not observed after first, second and third months storage for the niosomes formulations indicating the vesicles stability. It has been reported that, the stability of both the surfactant and the vesicle structure, nature of the encapsulating membrane and encapsulated solute will affect the overall stability of the formulations.

The bioavailability of selected ratio of ofloxacin niosomes was assessed in rabbits and compared with injectable marketed preparation. The plasma samples obtained at different time intervals were analyzed using HPLC. From those results, the pharmacokinetic parameters were calculated using two compartment models. Pharmacokinetics parameters such as C_{max}, AUC, AUMC, T_{1/2} (β) and MRT values of ofloxacin niosomes (OSA60) were higher and the apparent V_d, V_{dss} and Cl_T values were lower when compared to marketed formulation. Ofloxacin niosomes established increase in half life and decrease in clearance. The intravenous administration of Ofloxacin to normal and febrile rabbits as a single dose @ 20 mg/kg body weight showed the higher AUC, AUMC and MRT values in febrile rabbits than normal rabbits. This might be due to the retention of drug in the body of febrile

Table 4: *In vitro* Release Kinetics Data of Ofloxacin Niosomes

Formulations Code	Correlation coefficient (R)/Slope (S)	Zero Order	First Order	Higuchi	Korsmeyer -Peppas	Mechanism of Drug Release
OSA 40	R	0.6761	0.8881	0.9086	0.9423	Fickian
	S	5.1475	-0.1367	19.248	0.2581	
OSA 60	R	0.8041	0.9143	0.9420	0.9426	
	S	4.4566	-0.0754	20.694	0.4081	
OSA 80	R	0.7419	0.9147	0.9024	0.9524	
	S	4.2509	-0.0848	19.599	0.3088	
OSB 40	R	0.8067	0.9590	0.9768	0.9908	
	S	8.2107	-0.1569	27.371	0.3579	
OSB 60	R	0.8641	0.9692	0.9903	0.9971	
	S	4.9511	-0.0698	17.581	0.3854	
OSB 80	R	0.8387	0.9193	0.9848	0.9989	
	S	5.7264	-0.1045	22.982	0.3534	

Table 5: Stability Studies Data of Ofloxacin Niosomes Results are mean $\pm$ SD of three trials (n=3) (^a $p \leq 0.001$, ^b $p \leq 0.01$).

Formulations Code	Percentage Drug			
	Initial (0 month)	1 st month	2 nd month	3 rd month
OSA 40	99.12 $\pm$ 0.59	89.75 $\pm$ 0.96 ^b	87.23 $\pm$ 1.01 ^b	86.54 $\pm$ 0.73 ^b
OSA 60	99.33 $\pm$ 0.58	98.45 $\pm$ 1.04 ^b	96.67 $\pm$ 1.12 ^b	95.32 $\pm$ 1.11 ^b
OSA 80	99.25 $\pm$ 0.56	96.54 $\pm$ 1.05 ^a	92.15 $\pm$ 1.02 ^a	90.25 $\pm$ 1.04 ^a
OSB 40	99.21 $\pm$ 0.10	80.71 $\pm$ 1.18 ^a	69.42 $\pm$ 0.83 ^a	60.92 $\pm$ 0.94 ^a
OSB 60	99.43 $\pm$ 0.48	83.14 $\pm$ 1.07 ^a	73.21 $\pm$ 0.98 ^a	62.92 $\pm$ 1.05 ^a
OSB 80	99.29 $\pm$ 0.36	78.28 $\pm$ 0.99 ^a	63.50 $\pm$ 0.55 ^a	58.85 $\pm$ 1.04 ^a

rabbits for a longer period of time than in normal rabbits. Any significant difference was not observed in mean residence time and total body clearance of ofloxacin the normal and febrile rabbits (Ahmad *et al.*, 2008). The terminal elimination rate constant for ofloxacin from the serum was reported as 2.39 h (Perkins *et al.*, 1995). The formulated ofloxacin niosomes (OSA60) expected to posses several advantages like prolonged drug action, patient compliance and reduced side effects over the conventional form of ofloxacin.

CONCLUSION

Niosomes as drug delivery systems have been utilized to increase the half-life of drug and to achieve better bioavailability. In the present study, ofloxacin niosomes was prepared by thin film hydration technique with different ratios of Span, CH and DCP. The developed niosomes was in the size range of 3.0-3.8 μ m and spherical in shape with smooth surface. Ofloxacin niosomes (OSA60) formulated with 47.5:47.5:5 ratio exhibited higher encapsulation efficiency and showed the drug release in extended period of time in comparison to the other formulations. Further, OSA60 showed significantly higher plasma levels for longer period of time compared to marketed sample. Hence, the developed niosomal formulation may be prospective alternative to currently available drug formulations in the treatment of microbial infection.

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