Formulation and Evaluation of Gatifloxacin Niosomes using Sorbitan Monoesters

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Abstract

Niosomes have been assuring as a cheap and chemically stable alternative to liposomes. In the present study, gatifloxacin niosomes were formulated by thin film hydration technique in two different ratios of Span surfactant, cholesterol and DCP (47.5:47.5:5 and 60:30:10) and evaluated for their particle size, zeta potential, surface morphology, entrapment efficiency, in vitro drug release and in vivo pharmacokinetic studies. The formulated niosomes were shown multi lamellar vesicles in the size range of 3.3-4.1 µm. Zeta potential values of two different ratios of gatifloxacin niosomes were found to be in the region -9 to -13 mV. Surface morphology examinations of the prepared niosomes by scanning electron microscope have shown that niosomes are spherical in shape and uniform in size. The in vitro release studies of gatifloxacin niosomes in the ratio of 47.5:47.5:5 exhibited cumulative drug release of 96.17% in 24 hours. The in vitro release data displayed; the gatifloxacin niosomes is sustained release dosage form and also obey or follow the first order of kinetics. Gatifloxacin niosome were stable, and didn’t show any physicochemical changes for 3 months at 40°C and 75% RH. Pharmacokinetics studies of gatifloxacin niosomes made with Span 60 were shown increased C_max AUC, AUMC, t1/2 and MRT values compared with marketed intravenous gatifloxacin product. The improved pharmacokinetic parameters and stability of formulated gatifloxacin niosomes with Span 60 have exhibited prolonged action and improvement bioavailability over the conventional form which might be to improve the patient compliance and reduce the side effects.

Keywords: Gatifloxacin, Span - 40 - 60 - 80, Niosomes, Entrapment efficiency, Release rate, Pharmacokinetic studies.

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Introduction

Novel drug delivery system provides the delivery of drug at a required rate into the body during the period of treatment as directed by the body. Recently colloidal carrier system like vesicles have been investigated widely due to their flexibility and improvement on therapeutic effectiveness of conventionally well established drugs by providing controlled and sustained delivery. Encapsulation of drugs into the vesicles has increases the drug bioavailability and gained greater interest with the launch of some liposomal formulations. However, their widespread use is still constrained by their own intrinsic chemical instability and higher cost. The alternative to liposomes are suggested is niosomes and it should provide technical advancement and wider scope to study the influence of chemical composition on the biological fate of vesicles (Baillie et al. 1985). Niosomes are non-ionic surfactant vesicles obtained on hydration of synthetic non-ionic surfactants, with or without incorporation of cholesterol (CH) or other lipids, dicetyl phosphate (DCP). 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 (Echequy et al. 1988), ester linked surfactant (Hunter et al. 1988), polyoxyethylene alkyl ethers (Hofland et al. 1991, 1992), Brij (Rajanares et al. 1993, Parthasarathi et al. 1994), series of Spans and Tweens (Chandraprakash et al. 1990, 1992, Uchegbu et al. 1995, 1997), since the first report on formulation of vesicles on hydration of a mixture of cholesterol and a single alkyl chain non-ionic surfactant. Among various non-ionic surfactants used to formulate niosomes, Spans are most suitable for commercial exploitation and are generally regarded as safe but relatively few reports are available on them (Biju et al. 2006). In last two decade number of studies was performed on liposomal and niosomal vesicles as therapeutic drug carrier systems to reduce the drug toxicity by alternating drug pharmacokinetics or modification in drug delivery in order to prolong drug action at the target site. The clinical use of chemo-therapeutic agents is limited in certain clinical conditions due its low therapeutic indices and dose limiting toxic side effects. It has been reported that intravenous administration of conventional liposomes are taken up by RES and hence, they may be used as antibiotic carriers for the treatment of infections involving the RES (Desiderio et al. 1983). Further, antibiotics-loaded vesicles showed enhanced drug concentrations at the site of action due to the following reasons: (i) targeting of drug to the infected tissues (ii) increase the intracellular antibiotic concentrations (enhanced liposome-cell interaction) (iii) reduce the toxicities of potentially toxic antibiotics resulting from the targeting of antimicrobial drugs (biodistribution, away from host cell) 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. Third generation fluoroquinolone antibiotic (Gatifloxacin) approved by US FDA in December 1999 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 gatifloxacin 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. Further, the selected niosome formulation was evaluated and compared with marketed i.v. formulation for their in vivo pharmacokinetics performance in rabbits.
Materials and Methods

Materials

Gatifloxacin received as a gift sample from Syntho Pharmaceuticals Pvt. Ltd., Lucknow, India. Span 40 (Sorbitan monopalmitate), Span 60 (Sorbitan monostearate) and Span 80 (Sorbitan monooleate) are procured from M/s. Qualigens, Mumbai, India. Cholesterol and Dicetyl phosphate (DCP) purchased from M/s. Merck Worrli, Mumbai, India and Sigma, USA, respectively. Diethyl ether and Triton X-100 are procured from M/s. Loba chemicals, Mumbai, India and all other chemicals used were of analytical grade.

Methods

Compatibility studies

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

The interaction between drug substance and excipients were evaluated by comparing the IR spectrum of physical admixtures with the spectrum of individual components. The pellets of samples were prepared as KBr pellets at high compaction pressure in the ratio of sample to KBr are 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 (Tm). The prepared samples (2 mg) were accurately weighed, transferred into the aluminum 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 (Tm) of physical admixtures was obtained from the DSC curve (Wei and Tianqing 2007).

Preparation of niosomes

Gatifloxacin niosomes were prepared by using thin film hydration technique (Azmin et al. 1985). An accurately weighed mixture of Span (71.25 mg), CH (71.25 mg) and DCP (7 mg) in the ratio of 47.5:47.5:5 and Span (42 mg), CH (21 mg) and DCP (7 mg) in the ratio 60:30:10 were dissolved separately in 10 mL of diethyl ether (solvent) in a 500 mL round bottom flask. The solvent was evaporated at an ambient temperature (28°C) under reduced pressure (260-400 mmHg) in a rotary flash evaporator until complete evaporation of solvent was ensured. Excess organic solvent were removed by leaving the flask in desiccator under vacuum condition for overnight period of time. The dried thin film of surfactant deposited on the wall of the flask was hydrated with aqueous phase (0.1N HCl) containing known amount of drug (1 mg/ml) at 60±2°C for 30 min to obtain niosomal dispersion (Jain and Vyas 1995). Different batches of gatifloxacin 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 gatifloxacin niosomes

Morphology

The morphology of gatifloxacin niosomes was examined by using scanning electron microscope (JEOL, JSM-6701F, Japan). Gatifloxacin niosomes (30 µL) were spread over a metal stub and dried under vacuum condition at room temperature (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 with field emission gun in an accelerating voltage 5 to 8 Kv (Jing et al. 2007, Almira and David 2011).

**Vesicle size measurements**

The vesicle size of formulated gatifloxacin niosome suspensions was measured by laser diffraction analyzer (AccuSizer 780/SIS syringe injection sampler, PSS Nicomp, Particle sizing systems, Santa Barbara, California, USA). Gatifloxacin niosomes (100 μL) were diluted to 10 mL with 0.1N HCl and filtered through 0.45 μm size Millipore size filter to remove dust particles. 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. The Limit of detection is 0.5-500 μm (Ahmed et al. 2005)

**Determination of entrapment efficiency**

The drug/surfactant ratio represents the drug encapsulating capacity of surfactant. Separation of free drug (unentrapped) from gatifloxacin niosomes was performed by dialysis method (Vyas and Khar 2002). The free drug was removed by placing the resultant niosomes dispersion in dialysis bag (Himedia, India) and exhaustively dialyzed against distilled water. The entrapped gatifloxacin in niosomes was determined spectrophotometrically at 293 nm (Lakshmi and Muthu 2006) after complete disruption of known amount of dialyzed niosomes using Triton-X-100. The percentage of entrapment efficiency was calculated by the following formula:

\[
\text{Practical drug content} \times 100
\]

\[
\text{Percentage of Entrapment Efficiency} = \frac{\text{Theoretical drug content}}{\text{Practical drug content}} \times X 100
\]

**Zeta potential**

The zeta potential of gatifloxacin niosomes were determined by using Zeta sizer (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 gatifloxacin niosomes was performed in an open ended cylindrical tube, one end is tied with dialysis membrane. A known quantity of gatifloxacin niosomes placed in an open ended cylinder and it was suspended in a receptor medium of 250 ml of 0.1N HCl in a beaker. The beaker that contained the medium was placed on a thermostatic magnetic stirrer and constantly stirred at the speed of 50 rpm and the temperature was maintained at 37±1°C. Aliquots (5 mL) of samples were withdrawn from the receptor compartment periodically at intervals of 0.5, 1, 2, 3, 4 up to 24 hours and after each withdrawal same volume of fresh medium was replenished. To the withdrawn samples 1 mL of ferric chloride reagent was added, shaken well and kept aside for few minutes. It was made up to the volume with 0.1N HCl and gatifloxacin content in the withdrawn samples was estimated spectrophotometrically as described earlier. The cumulative percent of gatifloxacin release was calculated and plotted against time t. The results were calculated by mean values of three runs. In vitro release of gatifloxacin from the free drug solution and suitable ratio of gatifloxacin 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-peppa’s release model (log cumulative percentage drug released vs. log time) to know the release mechanisms (Costa and Lobo 2001). The order of kinetics and mechanism of the release were confirmed based on linearity of the graphical expression of an in vitro release data.
Stability study

The stability study of gatifloxacin niosomes were carried out as per ICH and WHO guidelines. Best formulations from the each one of the niosome formulation ratio was packed separately in amber color vials and kept in a stability chamber (Humidity control oven, Yorco Scientific Industries, India) which was maintained at 40°C±2°C/75% RH±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 intervals was estimated spectrophotometrically as described earlier. The results expressed are the mean values of three runs.

In vivo study

The best ratio of gatifloxacin niosomes was selected for in vivo studies based on in vitro characterization results. Formulated gatifloxacin 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 in rabbits. These results were also compared with injectable commercial marketed formulation. 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±2°C/65% RH and a 12h light/12h dark cycle and starved for 18 hours before the experiment but had free access to water. The animals weighing about 2-3 kg were grouped two in three animals in each (Colino et al. 1998, Raipuria et al. 2006, Hwang et al. 2008, Leandro et al. 2008). Selected gatifloxacin niosome formulation and gatifloxacin injectable marketed formulation (i.v. infusion, 400 mg/200 ml of distilled water) at a dose of 5 mg/kg body weight was injected separately into the jugular vein via the in-dwelling catheter into the first and second group of animals respectively. Blood samples (3 mL) were withdrawn from the ear vein and transferred in to heparinized micro centrifuge tubes using K$_2$EDTA as anticoagulant at specified time interval as 5, 30, 60, 120, 240, 360, 540 and 720 minutes. The rabbit plasma samples in the anticoagulant tube were subjected to centrifugation in laboratory cooling centrifuge (Sigma, 3K30) at a constant temperature of 4°C at 3000 rpm for 15 minutes. Supernatant plasma was collected and transferred into the centrifuge tube and kept at −20°C until being analyzed. Gatifloxacin in plasma samples were analyzed by HPLC (Shimadzu LC 20 AD) using the method described by Tsing Hua (Tsing 2003). Pharmacokinetic parameters (AUC, AUMC, MRT, t$_{1/2}$, V$_d$, V$_{mm}$, CL, and C$_{max}$) were calculated by adopting two compartment models (Leandro et al., 2008) using kinetica 5.0 software and analysis of variance (ANOVA, single factor) was employed in statistical analysis.

Statistical analysis

All experimental data were expressed as mean±S.D (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 gatifloxacin niosomes. Physical admixtures of gatifloxacin, cholesterol, DCP were subjected to DSC analysis to assess the phase transition temperature (Tm). The DSC thermogram and phase transition temperature of gatifloxacin and its physical admixtures are shown in Figure 1 and Table 1.

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Table 1. Phase transition parameters (Tm) of gatifloxacin physical admixtures

<table>
<thead>
<tr>
<th>Drug and Physical Admixtures</th>
<th>Temperature Scanning Cycle</th>
<th>Peak Temp. (°C)</th>
<th>Enthalpy (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gatifloxacin</td>
<td>Endothermic transition</td>
<td>184.07</td>
<td>-97.32</td>
</tr>
<tr>
<td>Gatifloxacin + Span 40</td>
<td>Endothermic transition</td>
<td>198.26</td>
<td>-8.75</td>
</tr>
<tr>
<td>Gatifloxacin + Span 60</td>
<td>Endothermic transition</td>
<td>198.25</td>
<td>-5.7</td>
</tr>
<tr>
<td>Gatifloxacin + Span 80</td>
<td>Endothermic transition</td>
<td>198.77</td>
<td>-0.09</td>
</tr>
</tbody>
</table>

Figure 1. DSC curve of gatifloxacin and physical admixtures of Span surfactants with gatifloxacin (GS 40-Gatifloxacin + Span 40; GS 60-Gatifloxacin + Span 60; GS 80-Gatifloxacin + Span 80)

Gatifloxacin niosome formulations were prepared in two different ratios of the selected suitable non-ionic surfactants (Span) having different HLB values, cholesterol and DCP. The composition of niosome formulations are shown in Table 2: Gatifloxacin niosomes was subjected to microscopic examination using scanning electron microscope for characterizing shape and size and of niosomes. The photomicrographs of gatifloxacin multilamellar niosomes obtained with Span 60 in the two ratios are shown Figure 2. The vesicle size, zeta potential and entrapment efficiency of gatifloxacin niosomes formulations are displayed in Table 3. In vitro release profile of gatifloxacin 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 gatifloxacin niosomes and free drug solution are shown in Figure 3 and for the ratio of 60:30:10 gatifloxacin niosomes are shown in Figure 4. All the niosome formulations exhibited retarded release rate up to 24 hours. The in vitro kinetics order and mechanism of release were established for the gatifloxacin niosomes is given in Table 4. The stability study of both ratios of gatifloxacin niosomes were performed as per ICH and WHO guidelines in every month interval up to three months and the results obtained are shown in the Table 5. The
pharmacokinetic parameters of gatifloxacin niosomes (GSA 60) and marketed injectable gatifloxacin formulation are displayed in Table 6 and Figure 5.

**Table 2. Compositions of gatifloxacin niosomes**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Formulation Code</th>
<th>Span 40</th>
<th>Span 60</th>
<th>Span 80</th>
<th>Cholesterol</th>
<th>DCP</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>GSA 40</td>
<td>47.5</td>
<td>-</td>
<td>-</td>
<td>47.5</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>GSA 60</td>
<td>-</td>
<td>47.5</td>
<td>-</td>
<td>47.5</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>GSA 80</td>
<td>-</td>
<td>-</td>
<td>47.5</td>
<td>47.5</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>GSB 40</td>
<td>60</td>
<td>-</td>
<td>-</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>GSB 60</td>
<td>-</td>
<td>60</td>
<td>-</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>GSB 80</td>
<td>-</td>
<td>-</td>
<td>60</td>
<td>30</td>
<td>10</td>
</tr>
</tbody>
</table>

**Figure 2.** SEM photomicrograph images of GSA 60 niosomes (A) and GSB 60 niosomes (B)
Table 3. Vesicle size, Zeta potential and Entrapment efficiency data of gatifloxacin niosomes formulations

<table>
<thead>
<tr>
<th>Formulation Codes</th>
<th>Mean Vesicle Size (µm)</th>
<th>Entrapment Efficiency (%)</th>
<th>Zeta Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSA 40</td>
<td>3.91±0.37</td>
<td>33.75±1.03</td>
<td>-9.44±0.75</td>
</tr>
<tr>
<td>GSA 60</td>
<td>4.12±0.04</td>
<td>55.77±1.03</td>
<td>-11.06±1.06</td>
</tr>
<tr>
<td>GSA 80</td>
<td>4.10±0.17</td>
<td>48.92±1.20</td>
<td>-13.22±1.08</td>
</tr>
<tr>
<td>GSB 40</td>
<td>3.27±0.08</td>
<td>27.58±1.30</td>
<td>-9.68±1.12</td>
</tr>
<tr>
<td>GSB 60</td>
<td>3.75±0.10</td>
<td>47.40±0.70</td>
<td>-11.48±0.85</td>
</tr>
<tr>
<td>GSB 80</td>
<td>3.68±0.16</td>
<td>38.68±0.96</td>
<td>-13.48±0.76</td>
</tr>
</tbody>
</table>

Results are mean ± SD of three trials (n=3), (p≤0.001, p≤0.01)

Figure 3. In vitro drug release profile of gatifloxacin niosomes (47.5:47.5:5 ratio) and free drug solution
Each point represents mean ± SD of three trials (n=3) (p<0.05)

Figure 4. *In vitro* drug release profile of gatifloxacin niosomes (60:30:10 ratio)

### Table 4. *In vitro* release kinetics data of gatifloxacin niosomes

<table>
<thead>
<tr>
<th>Formulations Code</th>
<th>Correlation coefficient (r)/Slope (s)</th>
<th>Zero Order</th>
<th>First Order</th>
<th>Higuchi</th>
<th>Korsmeyer-Peppas</th>
<th>Mechanism of Drug Release</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSA 40</td>
<td>R 0.8 S 4.16</td>
<td>0.9565</td>
<td>-0.079</td>
<td>0.9398</td>
<td>0.9584</td>
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</tr>
<tr>
<td>GSA 60</td>
<td>R 0.8869 S 3.6125</td>
<td>0.9827</td>
<td>0.9834</td>
<td>0.9841</td>
<td>0.4969</td>
<td></td>
</tr>
<tr>
<td>GSA 80</td>
<td>R 0.8438 S 3.978</td>
<td>0.9363</td>
<td>0.9574</td>
<td>0.9398</td>
<td>0.5132</td>
<td></td>
</tr>
<tr>
<td>GSB 40</td>
<td>R 0.8537 S 5.4831</td>
<td>0.9695</td>
<td>0.972</td>
<td>0.9759</td>
<td>0.4633</td>
<td>Fickian</td>
</tr>
<tr>
<td>GSB 60</td>
<td>R 0.8732 S 4.0337</td>
<td>0.9256</td>
<td>0.976</td>
<td>0.9711</td>
<td>0.509</td>
<td></td>
</tr>
<tr>
<td>GSB 80</td>
<td>R 0.8503 S 4.3582</td>
<td>0.9681</td>
<td>0.9676</td>
<td>0.9706</td>
<td>0.4695</td>
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Table 5. Stability studies data of gatifloxacin niosomes

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Percentage Drug Remaining at</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (0 month)</td>
<td>1st month</td>
</tr>
<tr>
<td>GSA 40</td>
<td>99.29±0.18</td>
<td>92.25±1.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSA 60</td>
<td>99.38±0.49</td>
<td>98.66±0.91&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSA 80</td>
<td>99.25±0.36</td>
<td>94.32±0.86&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSB 40</td>
<td>99.17±0.06</td>
<td>81.34±1.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSB 60</td>
<td>99.39±0.48</td>
<td>85.25±0.97&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSB 80</td>
<td>99.23±0.38</td>
<td>79.40±0.70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are mean ± SD of three trials (n=3) (*)<sup>p</sup>≤0.001, <sup>c</sup><sup>p</sup>≤0.05

Table 6. Pharmacokinetic data of gatifloxacin niosomes and marketed gatifloxacin formulation

<table>
<thead>
<tr>
<th>Parameters (in units)</th>
<th>Gatifloxacin Niosomes (GSA 60)</th>
<th>Marketed Gatifloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (µg h ml&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>7.35±0.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.49±0.21</td>
</tr>
<tr>
<td>AUMC (µg h&lt;sup&gt;2&lt;/sup&gt; ml&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>53.02±4.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.84±1.07</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>7.22±0.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.11±0.30</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>5.71±0.51&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.3±0.25</td>
</tr>
<tr>
<td>V&lt;sub&gt;d&lt;/sub&gt; (L/kg)</td>
<td>2.08±0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.46±0.14</td>
</tr>
<tr>
<td>V&lt;sub&gt;dss&lt;/sub&gt; (L/kg)</td>
<td>4.91±0.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.32±0.44</td>
</tr>
<tr>
<td>Cl&lt;sub&gt;T&lt;/sub&gt; (ml h&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>678±61.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1434±86.04</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (µg ml&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>2.4±0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.04±0.12</td>
</tr>
</tbody>
</table>

Results are mean ± SD of three trials (n=3) (∗<sup>p</sup>≤0.01, <sup>c</sup><sup>p</sup>≤0.05)

AUC, Total area under plasma concentration versus time curve; AUMC, Area under the first moment curve; MRT, Mean residence time; t<sub>1/2</sub> (β), Elimination half life; V<sub>d</sub>, Apparent volume of distribution; V<sub>dss</sub>, Steady state volume of distribution; Cl<sub>T</sub>, Total body clearance; C<sub>max</sub>, Maximum concentration.

![Graph](image)

Each point represents mean ± SD (n=3)

**Figure 5.** Semi logarithmic plot of plasma concentration-time profile of marketed and niosome encapsulated gatifloxacin

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Discussion

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. In this study, a fluoroquinolone drug, gatifloxacin is used to formulate niosomes by thin film hydration technique with two different ratios of Spans, CH and DCP and to study its effect on vesicle size, entrapment efficiency, in vitro release and in vivo efficacy.

The FT-IR spectrum of gatifloxacin 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 and which 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 that drug stability during the encapsulation process.

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 gatifloxacin showed a sharp endothermic peak at 184.07°C. DSC thermogram of the gatifloxacin loaded physical admixtures exhibited endotherms at 198.26°C (Span 40), 198.25°C (Span 60) and 198.77°C (Span 80) respectively. The onset temperature of the gatifloxacin physical admixtures of Span surfactants is determined to be between 186.23°C, 192.37°C and 191.73°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 extend while forming the lipid bilayer. The incorporated gatifloxacin expected to get associated and interacted with lipid bilayers in large extent.

Gatifloxacin niosomes were made with Span surfactants, cholesterol and DCP in two different ratios of 47.5:47.5:5 and 60:30:10. The cholesterol was included in the formulation of gatifloxacin niosomes, to alter the properties of niosomes and to markedly decrease efflux-entrapped drug and increase the stability of niosome dispersion (Gianasi et al. 1997). DCP was added to impart a negative charge on the basis of reports that negatively charged liposomes evince better in vivo anti-tumour 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 also reduces the probability of vesicular aggregation 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±8°C (Table 1), hence the entire vesicle preparations were carried out at 60±2°C.

Zeta potential is an important parameter for prediction of the stability of colloidal carrier system and fate of vesicles in vivo. Zeta potential of >30 mV or <-30 mV are typical for colloids stabilized by electrostatic forces. The zeta potential value of gatifloxacin 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 (Smadsrod 2004).

From the 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 niosome size measurements revealed that, obtained niosomes are MLVs (3.3-4.1 μm) with distinct boundaries and all the gatifloxacin formulations made with different Span surfactants in different ratios have not shown any significant difference in size.

Amount of drug incorporated in the formulation of colloidal drug delivery system is one of the mostly considered parameter and also be the major determinant factor of the fate of any niosomal system. The encapsulation of drug is governed by the nature of solute, hydration temperature and method of drug loading. 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 olate 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. It has been also reported that, EE was found to increase with carbon chain length of lipophilic groups and further the 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 and Wan et al. 1974).

In vitro release profile of gatifloxacin niosomes was determined in an open ended cylinder using 0.1N HCL. In vitro release studies showed sustained release of up to 24 hours. The GSA 60 niosomes released 96.17% of gatifloxacin in 24 hours and GSB 60 niosomes released 98.74% of gatifloxacin in 22 hours. The study of an in vitro release profile of gatifloxacin niosomal formulations made with Span 60 using 60:30:10 ratio showed higher percentage of drug release in definite time intervals than 47.5:47.5:5 ratio exhibit retarded release in extend period of time and it is possible to sustained for a longer duration (Ruckmani et al. 2000). Span 60 niosomal formulations shown very slow release than other Span formulations which may be due to their high EE. Span 40 niosomal formulations showed lowest entrapment and thus required less time for maximum drug release and even though Span 40 was less hydrophobic than Span 60 (De Gier et al. 1968). Lipophilicity of the surfactant might be responsible for the difference in the release rate of drug. And 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 (Yu et al. 1998 and Gabizon and Papahajopoulos 1988).

In order to understand the mechanism and kinetics of drug release, in vitro drug release was plotted according to the four different kinetic models. As shown in results, the difference between correlation coefficients of the four models, first order kinetic is the best fitted method for all formulations. The gatifloxacin niosomes release rates from both ratios is followed Higuchi’s pattern of drug release. The linearity of the plots indicates the release process is diffusion-controlled and follows Fickian diffusion mechanism (Korsmeyer mechanism) and showed good linearity (Law et al. 1994, Arica et al. 1995 and Glavas-Dodov et al. 2002).

The stability study showed that the niosomes formulated in the ratio of 60:30:10 possess less stability efficiency than niosomes formulated in the ratio of 47.5:47.5:5 and this may be due to the lower HLB value of Span 60 than other Span formulations. Significant difference (p≤0.05) was not observed after first, second and third months storage for the niosomes formulations indicated 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 gatifloxacin niosomes was assessed in rabbits and compared with injectable commercial 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 Cmax, AUC, AUMC, T1/2 (β) and MRT values of GSA60 gatifloxacin niosomes were higher and the apparent Vd, Vds and Clr values were lower when compared with marketed formulation. Gatifloxacin niosomes established increase in half life and decrease in clearance. Hence the formulated gatifloxacin niosomes (GSA60) have several advantages like prolonged drug action, patient compliance and reduce the side effects over the conventional form of gatifloxacin.

Conclusion

Niosomes as drug delivery systems have been utilized to increase the half life of drug and to achieve better bioavailability which can be predicted to prolong the existence of the drug in systemic circulation and also reducing the drug toxicity with increased therapeutic activity in various diseases. In the present study, gatifloxacin niosomes was prepared by thin film hydration technique with different ratios of Span, CH and DCP and the developed niosomes shown better stability and improved pharmacokinetic profiles. Hence, niosomal formulation expected to be promising candidate in the treatment of microbial infection.

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References


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