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Formulation and stabilization of norfloxacin in liposomal preparations

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ABSTRACT

A number of liposomal preparations of norfloxacin (NF) containing variable concentrations of phosphatidylcholine (PC) (10.8–16.2 mM) have been formulated and an entrapment of NF to the extent of 41.7–56.2% was achieved. The values of apparent first-order rate constants (k_{obs}) for the photodegradation of NF in liposomes (pH 7.4) lie in the range of 1.05–2.40 × 10^{-3} min^{-1} compared to a value of 8.13 × 10^{-3} min^{-1} for the photodegradation of NF in aqueous solution (pH 7.4). The values of k_{obs} are a linear function of PC concentration indicating an interaction of PC and NF during the reaction. The second-order rate constant for the photochemical interaction of PC and NF has been determined as 8.92 × 10^{-2} M^{-1} min^{-1}. Fluorescence measurements on NF in liposomes indicate a decrease in fluorescence with an increase in PC concentration as a result of formation of NF^{-} species which exhibits poor fluorescence. Dynamic light scattering has shown an increase in the size of NF encapsulated liposomes with an increase in PC concentration. The stabilization of NF in liposomes is achieved by the formation of a charge-transfer complex between NF and PC.

1. Introduction

Liposomal drug delivery systems (LDDSs) are among the most effective vehicles for the entrapment and delivery of drugs to specific sites. These systems have the advantage to stabilize the drug by entrapment externally as well as in the biological system internally. Remarkable progress has been made in the development of LDDSs for pharmaceutical, medical, cosmetic and neutraceutical applications (Gomez-Hens and Fernandez-Romero, 2006; Akbarzadeh et al., 2013; Allen and Cullis, 2013; Cagdas et al., 2014; Garg and Goyal, 2014; Stiufiuc et al., 2015; Daraee et al., 2014). Liposomal preparations are nontoxic, biocompatible and biodegradable. They enhance the stability of the drugs against pH, light and enzymatic degradation. Liposomal drugs possess increased efficacy and therapeutic index with reduced drug toxicity (Gomez-Hens and Fernandez-Romero, 2006; Chang and Yeh, 2012; Slingerland et al., 2012; Allen and Cullis, 2013). Several drugs have been photostabilized in the form of liposomal preparations such as riboflavin (Loukas et al., 1995; Bhowmik and Sil, 2004; Ahmad et al., 2015a), doxorubicin (Bandak et al., 1999), amlodipine (Ragno et al., 2003), tretinoin (Ioele et al., 2005), barnidipine (Ioele et al., 2014), 4-nerolidylcatechol (Gaetil et al., 2015), and nimodipine and felodipine (Brito et al., 2012). In view of the photosensitivity and wide spread use of fluoroquinolones as antibacterial agents for a variety of ailments attempts have been made to stabilize them by entrapment in liposomes (Vazquez et al., 2001; Budai et al., 2008; Isabel and Paula, 2013). This could further be improved by an increase in the entrapment efficacy of fluoroquinolones (Ellbogen et al., 2003; Hosny, 2010). The interaction of fluoroquinolones with phospholipid bilayers in liposomes has also been studied by several workers (Bedard and Bryan, 1989; Maurer et al., 1998; Vazquez et al., 1998, 2001; Grancelli et al., 2002; Bensikaddour et al., 2008). However, no mechanism of these interactions has been reported so far.

Norfloxacin (NF), a fluoroquinolone with a piperazinyl side chain (Fig. 1), is sensitive to light (USP-NF, 2014) and undergoes degradation in the aqueous solution (Nangia et al., 1991; Cordoba–Diaz et al., 1998; Zhang et al., 2012; Chen and Chu, 2012; Babic et al., 2013; Ahmad et al., 2015b) and in the solid state (Vazquez et al., 2001; Budai et al., 2008). The kinetics and pathways of photodegradation reactions of NF in aqueous and organic solvents have been reported (Albini and Monti, 2003; Ahmad et al., 2015b). In the present work the photodegradation of NF in different liposomal preparations has been studied using HPLC and spectrofluorimetry. The physical characteristics of liposomal preparations have been studied by DLS. The effect of compositional variations of phospholipids in liposomes on the photodegradation and stabilization of NF has also been evaluated.

2. Materials and methods

Norfloxacin (≥98%, NF) and cholesterol (99%, CH) were purchased from Sigma-Aldrich (USA). Phosphatidylcholine (PC) was obtained from Avanti Polar Lipids, USA. All solvents and reagents were of HPLC grade form Merck (USA). Deionized water (16.5 MΩ resistance) from milli-Qpore system (Bedford, USA) was used for HPLC work. The solvents and the solutions were filtered using a Millipore filtration unit

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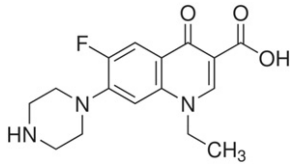

Fig. 1. Chemical structure of NF.

and then degassed before use. The following buffer system was used: Na_2HPO_4–KH_2PO_4–NaCl (0.001 M), pH 7.4. The experimental work was performed in a dark chamber to avoid any photochemical effect.

2.1. Preparation of liposomes

The liposomes were prepared by the reverse evaporation method as described by Szoka and Papahadjopoulos (1978) with a modification involving the hydration of the lipid film (Panwar et al., 2010). Different molar concentrations (10.80–16.20 mM) of the PC and a constant molar concentration of CH (13.50 mM) was used for the preparation of liposomal formulations (Table 1). The lipids were dissolved in a mixture of chloroform and methanol (10:15, v/v) in a 100 ml round bottom flask. Nitrogen gas was purged into the solution to remove oxygen and the organic solvents were removed under reduced pressure using a rotary evaporator. The flask was kept overnight under vacuum. The thin film formed was hydrated by a 5×10^{-5} M solution of NF in 0.001 M phosphate buffer saline (PBS), pH 7.4. The round bottom flask was rotated at a speed of 100–120 rpm at a temperature of ~40 °C. The transparent NF solution was converted into an opalescent viscous liposomal preparation. This preparation was centrifuged at a speed of 14,000 rpm at a temperature of 4 °C. The pellets of liposomes then settled down. The resultant supernatant was removed using a micropipette and the NF entrapped liposomes pellets washed three times with 0.005 M PBS, pH 7.4. The pallets of different liposomal preparations were refrigerated at 4 °C for further use. The physical characteristics of the liposomes were studied by dynamic light scattering.

2.2. pH measurements

The pH of the solutions was measured with an Elmetron LCD display pH meter (Model-CP 501 sensitivity ± 0.01 pH unit, Poland) using a combination pH electrode. The electrode was calibrated using phthalate (pH 4.008) and phosphate (pH 6.865) buffer solutions.

2.3. Spectral measurements

All the spectral measurements have been carried out on a Shimadzu UV-1601 reading UV–Vis spectrophotometer using quartz cells of 10 mm path length.

Table 1
Composition of liposomal formulations.

Formulations	Cholesterol (CH) content (mM)	Phosphatidylcholine (PC) content (mM)
F1	13.500	10.800
F2	13.500	11.475
F3	13.500	12.150
F4	13.500	12.825
F5	13.500	13.500
F6	13.500	14.175
F7	13.500	14.850
F8	13.500	15.525
F9	13.500	16.200

2.4. Fluorescence measurements

The fluorescence measurements on NF solutions and liposomal preparations were carried out at room temperature (~25 °C) with a Spectromax 5 fluorimeter (Molecular Devices, USA) in the end point mode, using 274 nm as the excitation wavelength and 450 nm as the fluorescence emission wavelength (Vilchez et al., 2001; Galaon et al., 2007). The fluorescence was measured in relative fluorescence units using a pure 0.05 mM aqueous solution of NF (pH 7.4) as standard.

2.5. Dynamic light scattering (DLS) analysis

A Laser Spectroscatter-201 system (RiNA GmbH Berlin, Germany) with a He–Ne laser providing a 690 nm light source and an output power in the range of 10–50 mW was used for DLS analysis. An autopiloted run of 50 measurements at every 20 s, with a wait time of 1 s was used at 25 °C for the measurements. The liposomal preparation as such or that on treatment with 5% Triton X-100 was placed into a special quartz SUPRASIL® cell (light path 1.5 mm) for measurement (Hameed et al., 2014). The scattered light was collected at a fix scattering angle of 90°. The autocorrelation functions were analyzed using the CONTIN program to obtain hydrodynamic radius (R_H) distributions. The R_H is related to the diffusion coefficient by the Einstein–Stokes equation. The data were analyzed using XtalConcepts software (XtalConcepts GmbH, Hamburg Germany) provided with the instrument.

2.6. Photodegradation of NF

A ten ml quantity of the control solution and of the liposomal preparations containing equal concentration of NF determined on the basis of entrapment values were placed in petri dishes and immersed in a water bath maintained at 25 ± 1 °C in a dark chamber. The solutions were irradiated using a Philips 30 W TUV tube (50% emission at 274 nm, major absorption maximum of NF). The tube was placed horizontally at a distance of 20 cm from the center of the dishes. Samples were withdrawn at various intervals for the assay of NF in photodegraded solutions.

2.7. Light intensity measurement

Potassium ferrioxalate actinometry (Hatchard and Parker, 1956) was used to determine the intensity of the irradiation source and a value of $5.50 \pm 0.11 \times 10^{18}$ quanta s^{-1} was obtained.

2.8. HPLC assay

The HPLC analysis for the determination of NF in photodegraded solutions was carried out using a Shimadzu (Japan) LC-10ATVP instrument equipped with a UV detector (model SPD-10 AVP) connected to a micro system. The analytical column used was Purospher RP-8 endcapped (5 μM) and the mobile phase consisted of water and acetonitril (50:50 v/v) at pH 3.3 adjusted with H_3PO_4. The analysis was performed at 25 ± 1 °C using isocratic condition. A volume of 25 μl was used for injection. The flow rate was 1.0 ml min^{-1}. All the solutions and mobile phase were sonicated for 25–30 min before use. The detection of NF was carried out at 290 nm. The method was validated under the conditions employed in this study before use (Ahmad et al., 2015b).

2.9. Determination of entrapment efficacy (EE)

Entrapment efficiency has been determined as the percentage of NF entrapment in liposomal preparations in relation to the original concentration of NF used. The concentrations of the total drug, drug in the liposomal preparations and free drug in the supernatant have quantitatively