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Niosomes and discomes for ocular delivery of naltrexone hydrochloride: Morphological, rheological, spreading properties and photo-protective effects

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ABSTRACT

Naltrexone hydrochloride (NTX) is a promising treatment for corneal disorders linked to diabetes mellitus (diabetic keratopathy). However, NTX has a major stability problem due to autoxidation, which is likely to hinder its formulation as eye drops for treatment of diabetic keratopathy. In this study, in-house developed NTX non-ionic surfactant vesicles (niosomes and discomes) were evaluated for their spreading, rheological properties and their ability to impede the inevitable autoxidation of NTX in aqueous solutions. The measured contact angles and spreading coefficients for niosomes reflected significantly ($P < 0.05$) better wetting and spreading abilities than the aqueous vehicle. The prepared niosomes were significantly more viscous ($P < 0.05$) than the aqueous solution. The lipid content, size and composition of niosomes are the main factors affecting the viscosity of niosomal dispersions. Exposure of NTX solution to artificial daylight illumination (10,000 lux) can produce extensive degradation of NTX due to oxidation. The prepared formulations were able to significantly ($P < 0.05$) protect the encapsulated NTX from the photo-induced oxidation compared with free NTX solutions. The investigated niosomes lend themselves as a potential ocular delivery modality for NTX.

1. Introduction

A promising and new therapeutic application has emerged for the pure opioid antagonist naltrexone hydrochloride (NTX) in treatment of the diabetic complications affecting the cornea (diabetic keratopathy) (Abdelkader et al., 2011c; McLaughlin et al., 2010). NTX blocks the opioid growth factor receptor (OGR-r) and it subsequently enhances the delayed corneal epithelialisation, restores delayed corneal sensation and reverses the dry eye symptoms (Zagon et al., 2007, 2009). NTX can be considered as a promising ophthalmic pharmaceutical for treatment of diabetic keratopathy (Abdelkader et al., 2011c). Preformulation studies of NTX revealed that it is a hydrophilic agent with a log *P* value of 1.6 at 35 °C and it is susceptible to autoxidation (Abdelkader et al., 2011b). Non-ionic surfactant vesicles (niosomes) were proposed as an ocular drug delivery system for NTX in order to prolong its precorneal residence time and enhance its corneal uptake. Niosomes have the convenience of being easily instilled onto the surface of the eye as eye drops. A non-conventional form of niosomes called discomes formed with incorporation of poly-24-oxyehtylene cholesteryl ether or so called Solulan C24 (Abdelkader et al., 2011a). Discomes are giant (approximately 20 μm in diameter), disc-shaped niosomes that coexisted with conventional spherical niosomes (2–5 μm) (Abdelkader et al., 2011a). Discomes were believed to offer several advantages over conventional niosomes, such as better fit in the cul-de-sac of the eye and improved ocular drug bioavailability due to slower nasolacrimal drainage (Uchegbu et al., 1992; Uchegbu and Vyas, 1998).

The corneal penetration enhancing effect of niosomes and discomes could be attributed to many factors. Disrupting the tight junctions of the corneal epithelium is partly responsible for increasing corneal uptake. Other possible reasons are their better spreading ability on the lipophilic corneal surface and favourable rheological properties. Many reports have shown that increasing the viscosity of ophthalmic solutions increases the precorneal residence time, hence promote ocular drug absorption (Burgalassi et al., 2000; Lang et al., 2002; Ludwig, 2005). However, applying conventional viscous eye formulations (such as gels) onto the eye surface can blur the vision and render the dose adjustment very difficult (Winfield et al., 1990). Increasing ocular bioavailability due to increasing the viscosity is limited to a plateau, where a

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Table 1
Code and composition of the prepared niosomal formulations.

Formulation code	Molar ratio				
	Span 60	Cholesterol	DCP	C24	CH
F-S60	7	3	0	0	0
F-DCP	6.75	2.75	0.5	0	0
F-C24	6.9	2.9	0	0.2	0
F-CH	6.75	2.75	0	0	0.5

further increase in the viscosity produces a slight or no increase in the ocular bioavailability. This plateau effect has been found to be dependent on drug and formulation type (Lang et al., 2002).

It has been previously reported that an increase in the viscosity over 12.5 mPa s using methylcellulose as viscosity imparting agent have resulted in a 3-fold decrease in the drainage rate. This decrease in drainage rate increased the concentration of drug in the precorneal tear film (Chrai and Robinson, 1974). Isopto® plain and Liquifilm® are widely used commercial viscous eye drops comprising 0.5% hydroxypropyl methylcellulose and 1.4% polyvinyl alcohol. The viscosity range for these products are 10–30 mPa s and 4–6 mPa s respectively, and both have the convenience of being available as eye drops (Lang et al., 2002).

We have previously reported on the susceptibility of NTX to autoxidation degradation and photo-induced degradation (Abdelkader et al., 2011b). This report aims at studying the spreading ability of niosomes, rheological properties and influence of vesicular encapsulation on light-induced degradation of NTX.

2. Materials and methods

2.1. Materials

NTX was purchased from Mallinckrodt Inc., St. Louis, MO, USA. Span 60, cholesterol, dicetyl phosphate (DCP) and carboxyfluorescein (CF) were purchased from Sigma–Aldrich, St. Louis, USA. Poly-24-oxyethylene cholestryl ether (C24) was a generous gift from Lubrizol Inc., Cleveland, USA. Sodium cholate (CH) was a generous gift from New Zealand Pharmaceuticals, Palmerstone North, New Zealand. All other solvent and buffer salts were of analytical grade and used as received.

2.2. Preparation of niosomes

2.2.1. Reverse-phase evaporation (REV) method

The REV method used to prepare the niosomes was a modification of that described by (Kirby and Gregoriadis, 1984). A thin film of the lipid was formed, as previously described in the TFH method. The thin film was then dissolved in 12 ml of a mixture of ether: chloroform (1:1, v/v). Four ml of the aqueous NTX solution (4 mg/ml) in PBS was added to the organic phase, such that the organic phase:aqueous phase ratio was 3:1. This mixture was then mixed for 3 min in a sonication bath (Bandelin Snorex, Berlin, Germany), until an opalescent w/o emulsion formed. The formed w/o emulsion was rotary evaporated at 60 °C until a semi-solid gel-like mass or aqueous lipid dispersion (depending on the lipid content) was obtained. The resulting system was purged with a stream of nitrogen gas for 3 min to remove any traces of the organic solvent. The final dispersion was diluted with 4 ml PBS solution and rotary evaporated at a speed of 200 rpm for at least 30 min. Table 1 shows the codes and composition of the prepared niosomes. This method was previously used to generate large unilamellar vesicles (LUV) (Kirby and Gregoriadis, 1984). Percentages of NTX entrapment efficiency (EE%) and average volume diameter (D [4,3]) of the prepared niosomes were determined and presented elsewhere (Abdelkader et al., 2011a).

2.3. Physical properties

2.3.1. Morphological studies by confocal laser scanning microscopy (CLSM)

2.3.1.1. Niosomes preparation. Niosomes were prepared using the REV method, as described in Section 2.2.1. The NTX solution was replaced with a 1.5 mM carboxyfluorescein (CF) solution of PBS pH 7.4 as a fluorescent probe. The free CF was separated from the niosomes by exhaustive dialysis. A cellulose bag containing 2 ml of the prepared niosomes was dialysed against a 500 ml PBS solution at 4 °C. The PBS solution was replaced at least 3 times over 48 h.

2.3.1.2. Niosomes imaging. Three wells were drilled into a microscopic plastic slide by a laser cutter. Each well was 1 cm in diameter and 1.5 mm in depth. The wells were created to hold the samples and avoid squashing of niosomes between the slide and the cover slip. Studies were conducted on a Leica CLSM (Leica DMRXA-2 microscope fitted with a TCS-SP2 scan head, Leica Microsystems, Heidelberg, Germany) using a 40× water immersion lens; a zoom of 1; a pinhole with an Airy disk diameter of 2; and a combination of lasers and emission band pass filters (510–521 nm) to visualise the prepared niosomes. The images were analysed for morphology and size using Leica Confocal Software® (Leica Microsystems, Heidelberg, Germany).

2.3.2. Surface tension measurements

The surface tension (γ) of the prepared niosomes was determined at the ambient conditions using an interfacial tensiometer (Torsion Balance, Malvern Wells, UK). All measurements were performed in triplicate.

2.3.3. Contact angle and spreading coefficient measurements

Contact angle (θ) measurements were performed using a drop shape analyser (goniometer) (KSV-CAM 101, Helsinki, Finland). Goniometry is the analysis of the shape of a drop of test liquid placed on a solid surface. The basic elements of the goniometer include a light source, sample stage, lens and image capture. The contact angle (θ) can be assessed directly by measuring the angle formed between the solid surface (a glass slide) and the tangent to the drop surface.

A Hamilton syringe was filled with each of the tested niosomal formulations. Approximately 20 μl of each formulation were dropped onto a glass slide at ambient conditions. The image of the drop was captured and measured by CAM 101 software (KSV-CAM 101, Helsinki, Finland). The measurements were performed in triplicate and compared with that of an NTX (0.4 mg/ml) aqueous solution in PBS (control).

The conditions required for complete wetting of a solid surface is that the contact angle should be zero. This condition is fulfilled when the forces of attraction between the liquid and the solid surface in contact are equal to or greater than those between liquid molecules (Florence and Attwood, 1998b). The type of wetting in which a liquid spreads over the surface of the solid is referred to as spreading. The tendency for spreading can be quantified using the term spreading coefficient (S). S can be calculated using Eq. (1) (Florence and Attwood, 1998b).

$$S = \gamma_{L/A}(\cos\theta - 1) \quad (1)$$

If θ is larger than 0°, the term $(\cos\theta - 1)$ will be negative, and the value of S as well. Complete or spontaneous wetting is achieved when contact angle has a value of zero.

2.3.4. Viscosity measurements

Viscosity measurements were performed using a Brookfield DV-III programmable cone and plate rheometer (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA). A CP-40 cone

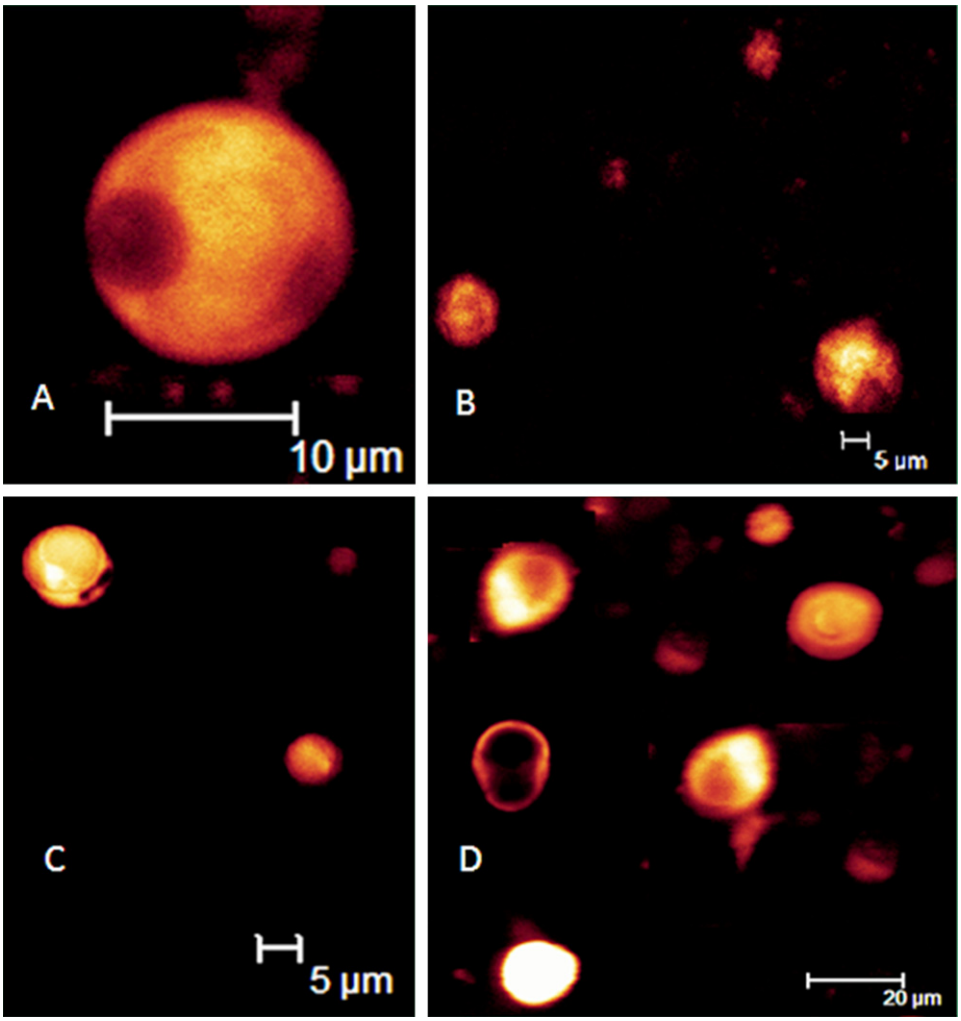

Fig. 1. CLS micrographs of F-S60 (A), F-DCP (B), F-CH (C) and F-C24 (D) niosomes loaded with CF and produced by the REV method.

spindle was used. A water-jacketed sample cup was thermostatically controlled by circulating water pumped using a water pump (Biolab Scientific Limited, Victoria, Australia) maintained at the required temperature ($25 \pm 0.5\,^{\circ}C$ and $35 \pm 0.5\,^{\circ}C$). Brookfield Rheocalc operating software (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA) was employed for data acquisition and analysis. The system was calibrated and found to be accurate within ±2% of the working range. Prior to measuring, the tested niosomes were diluted with PBS to a concentration equivalent to the required therapeutic NTX concentration (0.4 mg/ml). A sample volume of 0.5 ml was used and the measurements were performed in triplicate. Viscosity was calculated using Eq. (2) (Martin et al., 1993).

$$\eta = \frac{F}{G} \tag{2}$$

where G is the shear rate (s^{-1}), F is the shear stress (mPa) and η is the viscosity (Martin et al., 1993).

2.4. Effect of niosomal encapsulation on NTX oxidation

An aqueous NTX solution of 0.4 mg/ml in PBS pH 7.4 or an equivalent concentration of NTX encapsulated in niosomes were exposed to 2% (v/v) hydrogen peroxide (H_2O_2) for 2 h at ambient conditions. Another group of samples were stored in a BINDER KBF 240 series stability chamber (BINDER, Tuttlingen, Germany) and subjected to artificial daylight illumination of 10,000 lux at 40 °C. Samples were withdrawn at a suitable time interval (every 24 h for 5–10 days depending on the degradation rate) and analyzed for NTX content using the previously reported HPLC method (Abdelkader et al., 2011b).

2.5. Statistical analysis

A one-way analysis of variance (ANOVA) followed by Tukey's pairwise test at 5% significance level was used to test for statistical significance between the prepared formulations and control for contact angle, spreading ability, viscosity and protective effects of niosomes. These were performed using GraphPad Software Version 3.05, San Diego, CA, USA.

3. Results and discussion

Fig. 1 shows CLS micrographs of the prepared niosomes loaded with a fluorescent probe (CF). Formulation F-S60 gave rise to conventional, spherically shaped niosomes (Fig. 1 A). F-DCP and F-CH niosomes were also found to be uniform and spherical in shape. These results indicated that the incorporation of DCP and CH into bilayer membranes had no observable effect on the morphology of niosomes (Fig. 1B and C). On the other hand, giant oval vesicles (approx. 20 μm) formed when Solulan C24 was used (F-C24, Fig. 1D). These results were in a good agreement with those obtained from Cryo-SEM micrographs and confirmed the

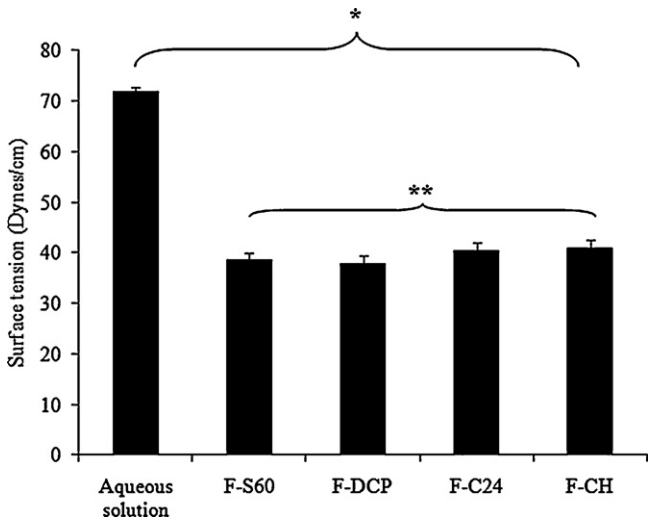

Fig. 2. Surface tension results for the prepared niosomes compared with NTX aqueous solution (0.4 mg/ml) (results are expressed as mean values ± SD, $n = 3$). *Significant difference ($P < 0.05$) and **insignificant difference ($P > 0.05$).

formation of discomes. As previously mentioned, incorporating of such a bulky surfactant (mol. wt. 1443) with a long ploy-oxyethylene chain of C24 is likely to influence the geometry of niosomes, especially at a relatively low level of cholesterol of typically ≤30% mol/mol (Uchegbu et al., 1992, 1996).

In this study, the discomes were prepared in a single step and under relatively mild conditions. They formed at 60 °C, whereas all previous reports on discomes necessitates incubation of the pre-formed spherical niosomes in a solution of C24 for 1 h at 75 °C (Uchegbu et al., 1992; Vyas et al., 1998). Our discomes could therefore be of useful for encapsulating heat sensitive and thermo-labile, drugs.

3.1. Surface tension measurements

Fig. 2 shows the surface tension (γ) measurement results for the prepared niosomes compared with NTX aqueous vehicle (0.4 mg/ml in PBS). The γ values measured for the prepared niosomes ranged from 38 to 41 dynes/cm. Statistical analysis revealed that the γ values for the prepared niosomes were significantly ($P < 0.001$) lower than those for the aqueous solution (72 dynes/cm). However, there was no significant ($P > 0.05$) difference amongst the γ values measured for the prepared niosomes.

It is well accepted that the lower the surface tension, the easier it is for the formulation to wet the hydrophobic surface of the corneal epithelium and the lipid layer of the precorneal tear film (Pawar and Majumdar, 2006; Rathore and Majumdar, 2006). For example, two gatifloxacin eye drops were prepared with distinctly different surface tension properties and their transcorneal permeation was evaluated. A control eye drops (3 mg/ml gatifloxacin isotonic solution) and an optimised formulation comprising 3 mg/ml gatifloxacin isotonic solution containing benzalkonium chloride (BAC) (0.01%) and disodium edetate (EDTA) (0.01%). The amount of gatifloxacin permeated through excised goat corneas from the control and optimised formulations were 29 and 83 μg respectively. The optimised formulation showed 2.9-fold increase in the amount of the drug permeated. This was attributed to the lower surface tension of the optimised formulations (41.2 dynes/cm) compared with that (69.5 dynes/cm) of the control. A better spreading ability and a permeation-enhancing effect were observed with the optimised eye drops (Rathore and Majumdar, 2006). Both BAC (cationic surfactant) and EDTA (chelating agent) are penetration enhancers. Nevertheless, BAC was found to have a more pronounced penetration enhancing effect than EDTA (Rathore and Majumdar, 2006). This in part due to its ability to lower the surface tension compared with EDTA and also due to disruption of corneal epithelium (Rathore and Majumdar, 2006).

Table 2
Contact angle and spreading coefficient values for the prepared niosomes (results are expressed as mean values ± SD, $n = 3$).

Formulation	Contact angle (θ)	Spreading coefficient (dynes/cm)
PBS solution	57.00 ± 1.80*	−32.79 ± 1.90*
F-S60	42.00 ± 1.00	−9.79 ± 0.81
F-DCP	40.00 ± 2.20	−9.00 ± 0.94
F-C24	43.00 ± 1.81	−11.20 ± 0.90
F-CH	44.00 ± 2.60	−11.65 ± 1.26

* The PBS solution was significantly ($P < 0.01$) different to the prepared formulation, while all the niosomes were insignificantly different from each other ($P > 0.05$).

3.2. Contact angle and spreading coefficient measurements

The contact angle is a measure of the spreading or wetting of a solid surface by a liquid, adhesion and biocompatibility (Florence and Attwood, 1998b; Lerk et al., 1977). Low values indicate that the liquid spreads or wets well while high values indicate poor wetting. If the angle is less than 90°, the liquid is said to wet the solid. If it is greater than 90° it is said to be non-wetting. A zero contact angle represents complete wetting (Florence and Attwood, 1998b; Lerk et al., 1977).

Table 2 shows the contact angle (θ) and the spreading coefficient (S) values of the prepared formulations compared with the plain vehicle (PBS). The θ and S values of the prepared formulations ranged from 40 ± 2.20° to 44 ± 1.81°, and −9.00 ± 0.94 to −11.40 ± 0.90 dynes/cm respectively, compared with those for PBS which were 57 ± 1.80° and −32.79 ± 1.90 dynes/cm respectively.

The θ values of all tested niosomes were significantly less ($P < 0.05$) than that of the aqueous vehicle. Additionally, the S values of the prepared formulations were up to 3.5 times higher than that of the aqueous vehicle. These results suggest that spreading of niosomes on the solid surface is more energetically favoured than non-spreading. These findings suggest that the prepared formulations have better wetting properties than the aqueous vehicle. Hence, the prepared formulations could more easily wet, spread and adhere to the hydrophobic surface of the cornea than the aqueous vehicle.

3.3. Viscosity measurements

In this study, the rheological properties of the prepared niosomes were studied at two different temperatures 25 °C (ambient temperature) and 35 °C (ocular temperature). A direct relationship between F and G at a constant viscosity (η) was obtained for all tested niosomes and PBS which indicates shear-independent, Newtonian flow properties (Fig. 3).

Fig. 4 shows the η values of PBS solution and the prepared niosomes at 25 °C and 35 °C. Generally, the η values were higher at 25 °C than at 35 °C. The effect of temperature on the η values was significant ($P < 0.05$) in some instances (Table 3). For example, the η values for F-S60, F-C24 and F-CH were significantly lower ($P < 0.05$) at 35 °C. These results reveal the dependency of viscosity on temperature. Hydrogen bonds, responsible for solvent–solvent or solute–solvent interactions, can be broken by thermal movement at higher temperatures (Martin et al., 1993).

Irrespective of the temperature, all η values for the prepared niosomes were significantly higher ($P < 0.01$) than the aqueous vehicle. The viscosity range for the prepared niosomes was 1.7–8.2 times higher than that for the aqueous vehicle at 25 °C. Similarly,

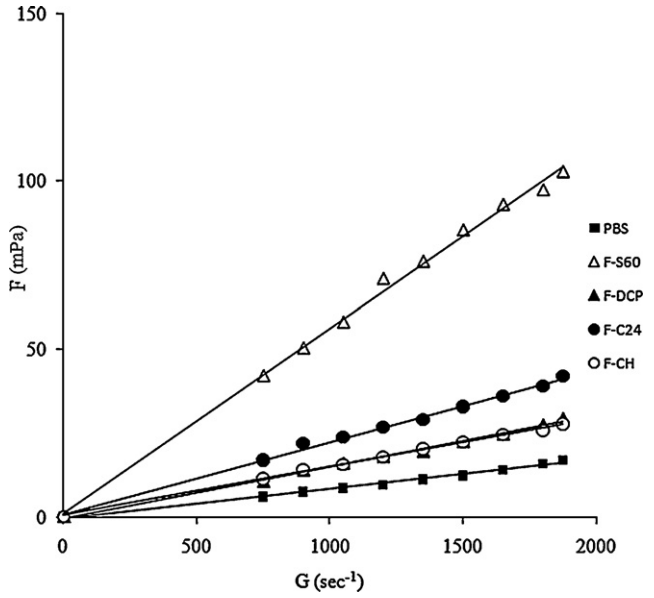

Fig. 3. Representative rheograms for the aqueous vehicle (PBS) and the prepared niosomes at 35 °C (results are expressed as mean values ± SD, $n = 3$).

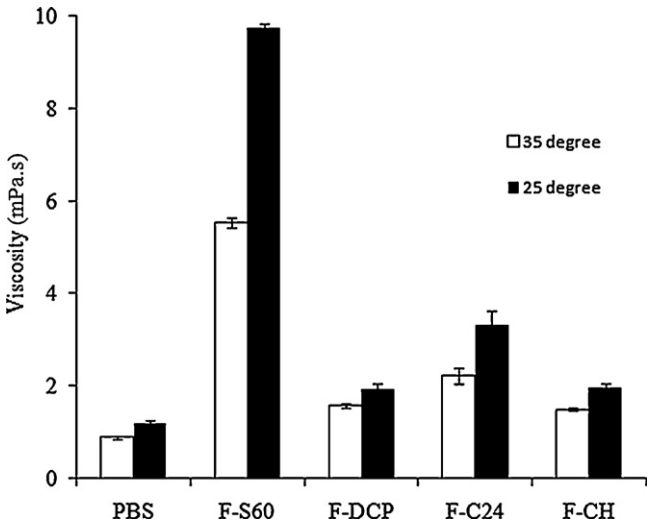

Fig. 4. Viscosity values for the aqueous solution (PBS) compared with the prepared niosomes at two different temperatures 25 °C and 35 °C (results are expressed as mean values ± SD, $n = 3$).

the viscosity of the prepared niosomes was found to be 1.7–6.3 higher than that for the aqueous vehicle at 35 °C (Table 3).

The tested niosomes consisted of different sizes, compositions and lipid contents. The order of the total surfactant/lipid contents of the tested niosomes was as follows F-S60 > F-CH > F-C24 > F-DCP.

Table 3
Tukey's pair wise comparison of viscosity values for the prepared niosomes at 25 °C and 35 °C.

Temperature (°C)	PBS solution 25; 35	F-S60 25; 35	F-DCP 25; 35	F-C24 25; 35	F-CH 25; 35
PBS solution	NS	–	–	–	–
F-S60	S; S	S	S; S	–	–
F-DCP	S; S	S; S	NS	–	–
F-C24	S; S	S; S	S; S	S	–
F-CH	S; S	S; S	NS; NS	S; S	S

S, significant difference ($P < 0.05$); NS, insignificant ($P > 0.05$).

Table 4
Tukey's pair wise comparison of oxidation and photolysis for the prepared niosomes.

	NTX solution Oxid; Phot	F-S60 Oxid; Phot	F-DCP Oxid; Phot	F-C24 Oxid; Phot
F-S60	S; NS	–	–	–
F-DCP	S; S	NS; S	–	–
F-C24	S; S	NS; S	NS; NS	–
F-CH	S; S	S; S	NS; NS	NS; NS

S, significant difference ($P < 0.05$); NS, insignificant difference ($P > 0.05$); Oxid, oxidation due to H_2O_2; Phot, photo-oxidation.

The highest η values (9.7 and 5.5 mPa s) were recorded for F-S60 niosomes at both 25 °C and 35 °C, whereas the lowest η values (2 and 1.5 mPa s) were recorded for F-DCP and F-CH niosomes respectively. F-C24 discomes came in the middle and demonstrated a 2.8-fold increase in the η value compared with that for the aqueous vehicle at 25 °C.

The highest surfactant/lipid content estimated for F-S60 is responsible for the highest η value. Conversely, F-DCP showed the lowest η value, which is mainly due to having the lowest surfactant/lipid concentration. These results suggest that the higher the surfactant/lipid concentration, the more likely the solvent–vehicle interaction to occur; and consequently the greater the viscosity of the final dispersion. Other factors such as size and composition of niosomes could affect the viscosity of niosomal dispersions. For example, the total surfactant/lipid content for F-CH was approximately double that for F-DCP and the η values for F-DCP and F-CH showed insignificant difference ($P > 0.05$) (Fig. 4). F-DCP niosomes had a lower concentration but a larger size compared with F-CH niosomes which suggests that the surfactant/lipid content is not the sole factor determining the viscosity of the niosomal dispersion.

Similarly, the η value for F-C24 discomes was significantly higher ($P < 0.05$) than that for F-CH, although F-CH had a higher content of surfactant/lipid than F-C24.

These results can be interpreted with respect to two factors which are vesicle hydration and vesicle shape (Arunothayanun and Florence, 2000). The hydration of vesicles increases the effective volume fraction compared with less hydrated counterparts. The bilayer membranes of F-C24 were modified by introduction of poly-24-oxyethylne cholesteryl ether (Solulan) resulting in the formation of non-spherical vesicles called discomes. The 24 units of Solulan's head group are highly hydrated. Furthermore, particle asymmetry has a noticeable effect on the viscosity of colloidal dispersions. Disc shaped vesicles interact more favourably with the dispersion medium than spherical vesicles. Similar results have been reported with polyhedral niosomes and conventional spherical ones composed of $C_{16}G_2$:cholesterol:Solulan in the ratios 91:0:9 and 45:24:10 respectively. The relative viscosity for polyhedral niosomes was markedly higher than that for the spherical ones (Arunothayanun and Florence, 2000).

The prepared niosomes showed significantly higher η values than the aqueous vehicle, at the same time they have the convenience of being suitable for use as eye drops. It is well accepted that more viscous vehicles have longer precorneal residence time than less viscous eye drop solutions. This is another reason to consider the prepared niosomes as potential ocular delivery vehicles for NTX.

3.4. Effect of niosomal encapsulation on NTX oxidation

Light, similar to heat, provides the activation energy necessary for oxidation reactions. Daylight is a primary source for creating free radicals and an initiator of the autoxidation propagation (Florence and Attwood, 1998a). One of the genuine properties of an effective drug delivery system is the ability to protect the drug against any possible chemical degradation. Niosomes have been

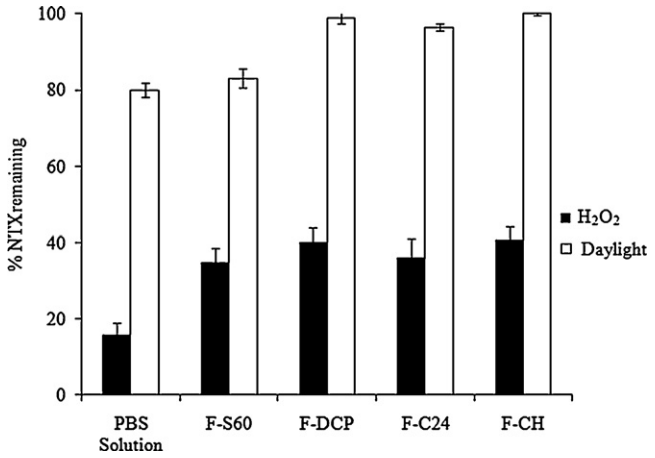

Fig. 5. Effect of niosome encapsulation on the chemical stability of NTX against H_2O_2 oxidation and daylight illumination (results are expressed as mean values ± SD, $n = 3$).

proposed as systems capable of improving the chemical stability of photosensitive drugs such as doxorubicin (Uchegbu and Florence, 1995) and tretinoin (Manconi et al., 2003). The prepared formulations were tested for their ability to protect the encapsulated drug (NTX) from light-induced degradation and the oxidizing agent H_2O_2.

Fig. 5 shows the percentages remaining of NTX after exposure to daylight and H_2O_2 (2%, v/v). The results revealed that 84% and 20% of NTX solution potency was lost after exposure of the free NTX solution to H_2O_2 (2%, v/v) and daylight illumination (10,000 lux) respectively. Also, the potency of NTX loaded niosomes was adversely affected by exposure to H_2O_2 but was significantly ($P < 0.05$) lower than that of the drug solution (Table 4). In terms of protective effects against light-induced degradation (photo-oxidation) and apart from F-S60, NTX molecules encapsulated in the prepared niosomes exhibited 24 h protection against photo-oxidation.

Longer term (10 days) photo-oxidation of NTX in PBS solution and niosomes was studied. Fig. 6 shows plots of %NTX remaining versus time. Linear relationships were obtained ($R^2 > 0.99$) for both the aqueous solution and niosomes indicating first-order photo-oxidation kinetics.

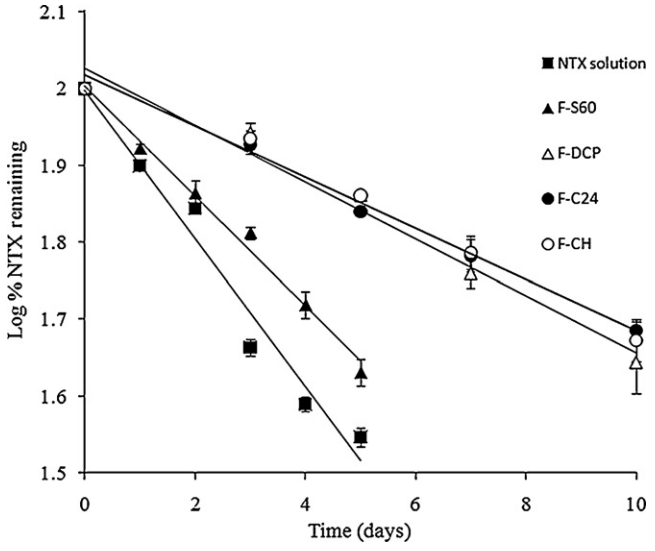

Fig. 6. First-order degradation kinetics for NTX in solution and niosomes under artificial daylight illumination at 40 °C in PBS pH 7.4 (results are expressed as mean values ± SD, $n = 3$).

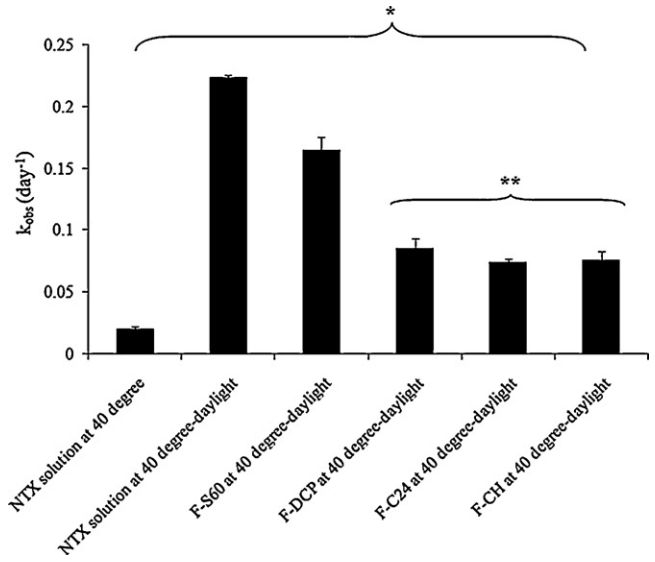

Fig. 7. First-order degradation rate constants for PBS solution of NTX and NTX encapsulated in niosomes under artificial daylight illumination at 40 °C in PBS pH 7.4 (results are expressed a mean values ± SD, $n = 3$). *Significant difference ($P < 0.05$) and **non-significant difference ($P > 0.05$).

Exposure of NTX aqueous solution to artificial daylight obviously accelerated the degradation of NTX. The observed degradation rate constants (k_{obs}) for the aqueous NTX solution stored in the dark and exposed to the daylight illumination were found to be 0.02 and 0.22 day^{-1} respectively. The calculated k_{obs} value for the aqueous NTX solution exposed to the daylight illumination was 11-fold faster than that of the dark stored sample (Fig. 7). This indicates the significance of daylight illumination as a potential initiator for autoxidation reactions.

The calculated k_{obs} values for the photo-oxidised NTX encapsulated in niosomes were significantly smaller ($P < 0.001$) than the PBS solution of NTX. A 1.4–3-fold decrease in k_{obs} was estimated for NTX encapsulated niosomes compared with the NTX solution. These results could be attributed in part to the ability of niosomes to protect the encapsulated NTX from peroxide radicals formed by the daylight illumination by virtue of their lipid bilayer membranes. Also, the bilayer membranes could scavenge the free radicals and prevent them from propagation and as such slowing down autoxidation kinetics (Roda et al., 1998). These findings reveal the protective effect of the prepared niosomes against photolytic and oxidative degradation of NTX. However, the calculated k_{obs} value for NTX F-S60 was significantly higher ($P < 0.001$) than all other niosomes. The lower protective effect for F-S60 could be ascribed to the leakage of NTX molecules from F-S60 due to the presence of a residual gel/liquid transition and its thermo-responsiveness. Abolishment of the gel/liquid transition of the bilayer membranes due to incorporation of the membrane additives would improve the stability of niosomes especially at temperatures higher than the gel/liquid transition temperature (Manconi et al., 2003). These results also suggest that the composition of the bilayer membranes has an influence on its ability to stabilize photo-sensitive molecules.

4. Conclusion

Niosomes and discomes can offer favourable rheological, spreading and wetting properties which are desirable for enhanced corneal uptake of the hydrophilic drug (NTX). More interestingly, the prepared niosomes protected the encapsulated NTX from photo-oxidation compared with conventional NTX aqueous solutions. The investigated niosomes lend themselves as a

potential ocular delivery modality for NTX. Yet, additional protective approaches such as the use of antioxidants and metal chelators should be considered synergistically with selected niosomal formulations. Such combinations are likely to offer extended shelf-life and improved corneal delivery of NTX.

Conflict of interest

None.

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