RES MILITARIS Social Science Journal

Niosomal Drug Delivery System for Ocular Drug Delivery

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ABSTRACT

The main purpose of the current study was to investigate the feasibility of niosomes as carriers for the ocular controlled delivery of a cholinergic drug; pilocarpine HCl. In the present study, pilocarpine HCl loaded niosomes were prepared using various non-ionic surfactants (span-20, span-60 and span-80), in the presence of cholesterol in different molar ratios by ether injection method. The ability of these vesicles to entrap the studied drug was determined by evaluating the entrapment efficiency after centrifugation and separation of the formed vesicles. Photomicroscopy and transmission electron microscopy as well as vesicle size analysis were used to study the formation, morphology and size of the drug loaded niosomes. The surface properties of prepared niosomes were determined by zeta potential. Fourier transform infrared spectroscopy and differential scanning colorimetry was done to investigate the drug-excipients compatibility and the chemical stability of drug after penetration into the niosomes. TEM analysis confirmed that niosomal formulations were spherical in shape and has a definite internal aqueous space. Results showed a substantial change in the release rate and an alteration in the drug entrapment efficiency from niosomal formulations upon varying type of surfactant and cholesterol content. *In vitro* drug release results confirmed that niosomal formulations have exhibited a high retention of pilocarpine HCl inside the vesicles such that their *in vitro* release was slower and more controlled. A formulation with 1:1 molar ratio of span-60 and cholesterol gave the most advantageous entrapment (93.26±1.75%) and release results after 8 hours (Q8h=66.98±1.87%) as compared to other compositions. These results confirm that niosomes containing formulations may be considered as promising ophthalmic carriers for the topical application of pilocarpine HCl.

KEYWORDS: Niosomes, Controlled release, Ocular delivery, Glaucoma, Ether injection technique.

INTRODUCTION

Glaucoma is a prevalent neurodegenerative disorder of the eye. Increased intraocular pressure (IOP) and subsequent retinal ganglion cell (RGC) death leading to the loss of visual field characterizes the pathology of primary open angle glaucoma (POAG), which is the most common form. The disease affects over 66 million people worldwide, causing bilateral blindness in 6.8 million [1]. Patients with POAG typically exhibit increased resistance to the outflow of aqueous humor through the trabecular meshwork, which can result in an increase in IOP and subsequent cell death from compression of the optic nerve axons [2]. However, IOP is the primary risk factor causing the loss of RGCs; the strategies of treatment mostly involve lowering IOP [3]. Current

treatment options primarily aim at decreasing IOP by utilizing pharmacological agents, laser therapy and surgery. The method of reducing IOP is by enhancing the outflow of humor from the eyes through the use of muscarinic acetylcholine receptor agonists [4, 5].

Pilocarpine hydrochloride is a drug used in the treatment of chronic open-angle glaucoma and acute angle-closure glaucoma for over 100 years [6]. It is a parasympathomimetic alkaloid obtained from the leaves of tropical South American shrubs from the genus *Pilocarpus*. It is a non-selective muscarinic receptor agonist in the parasympathetic nervous system, which acts therapeutically at the muscarinic acetylcholine receptor M_3, found on the iris sphincter muscle, causing the muscle to contract resulting in pupil constriction (miosis). Pilocarpine hydrochloride also acts on the ciliary muscle and causes it to contract. When the ciliary muscle contracts, it opens the trabecular meshwork through increased tension on the scleral spur. This action facilitates the rate that aqueous humor leaves the eye to decrease intraocular pressure [7].

Drug delivery in ocular therapeutics is a challenging problem and is a subject of interest to scientists working in the multi-disciplinary areas pertaining to the eye, including chemical, biochemical, pharmaceutical, medical, clinical, and toxicological sciences [8]. In order to overcome the problems of conventional ocular therapy, such as short residence time, loss of drug through nasolacrimal drainage, impermeability of corneal epithelium and frequent instillation; newer ocular delivery systems are being explored by many researchers [9-11]. It is now common knowledge that topical controlled delivery of ophthalmic drugs improves their ocular bioavailability with respect to traditional eye drops, by decreasing the rate of drug elimination from the precorneal area [12]. The advantage of vesicular systems does not only reside in providing prolonged and controlled action at the corneal surface but also involves providing controlled ocular delivery by preventing the metabolism of the drug from the enzymes present at the tear/corneal epithelial surface. Moreover, vesicles offer a promising avenue to fulfill the need for an ophthalmic drug delivery system that has the convenience of a drop, but will localize and maintain drug activity at its site of action. The penetration of drug molecules into the eye from a topically applied preparation is a complex phenomenon. In vesicular dosage forms, the drug is encapsulated in lipid vesicles, which can cross cell membrane. Vesicles, therefore, can be viewed as drug carriers which can change the rate and extent of absorption as well as the disposition of the drug. Vesicular drug delivery systems used in ophthalmics broadly include liposomes and niosomes.

Niosomes are formed from the self-assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures [13], which can entrap both hydrophilic and lipophilic drugs either in an aqueous layer or in vesicular membrane [14]. Niosomes in topical ocular delivery are preferred over other vesicular systems because of the chemical stability; low toxicity due to their non-ionic nature; handling surfactants with no special precautions or conditions; the ability to improve the performance of the drug via better availability and controlled delivery at a particular site and being biodegradable, biocompatible and non-immunogenic [15]. Various studies have demonstrated the successful use of niosomes as ocular drug delivery carriers where these vesicles significantly improved the ocular bioavailability of drug, with respect to reference buffer solution. No irritation with the niosomal formulation was observed [16]. Some researchers reported that there was approximately a 2.5 times increase in the ocular bioavailability of timolol maleate (a water soluble drug) encapsulated in niosomes as compared to timolol maleate solution [17].

The main purpose of the current study was to prepare pilocarpine HCl encapsulated niosomes possessing a high drug loading capacity in order to be used as ophthalmic carriers for topical ocular infections treatment. In the present study, niosomes were prepared using various non-ionic

surfactants (span-20, span-60 and span-80) in the presence of cholesterol in different molar ratios by ether injection method.

MATERIALS AND METHODS

The pilocarpine HCl was kindly received as a gift sample by M/s Zydus Cadila Health Care Ltd. (Ahmedabad, India). Sorbitan monolaurate (span-20), sorbitan monosterate (span-60), sorbitan monooleate (span-80) and cholesterol were procured from Loba Chemie Pvt. Ltd. (Mumbai, India). Isopropanol, methanol, acetone, chloroform, boric acid, sodium hydroxide, sodium bicarbonate, potassium chloride, glacial acetic acid, magnesium, sodium chloride, calcium chloride dehydrate, potassium dihydrogen phosphate and disodium hydrogen phosphate were purchased from were purchased from S.D Fine chemicals (Mumbai, India). Double distilled water was used throughout the study.

Preparation of pilocarpine hydrochloride loaded niosomes

Niosomes were prepared by ether injection technique using non-ionic surfactants (span 20, span-60 and span-80), cholesterol and pilocarpine hydrochloride in different ratios as shown in Table 1. For each ratio, non-ionic surfactant and cholesterol were weighed accurately and dissolved in 20 ml of diethyl ether. Pilocarpine hydrochloride (40 mg) was then dissolved in this lipid solution. The resulting solution was taken in a syringe and injected slowly through a 16 gauge needle into 10 ml of aqueous phase (phosphate buffer saline pH 7.4) held in a beaker maintained at 60^0C to 65^0C and agitated slowly. As the lipid solution was injected slowly into the aqueous phase, vaporization of diethyl ether resulted in the formation of niosomes. The prepared niosomes were separated by ultracentrifugation (Remi C-24, Mumbai, India) at 4^0C.

Table 1: Formulation table for preparation of niosomes

Formulation code	Surfactant	Amount of surfactant (mg)	Cholesterol (mg)	Drug (mg)	Ratio (Surfactant: Cholesterol)
F1	Span-20	100	100	40	1:1
F2	Span-20	200	100	40	2:1
F3	Span-20	100	200	40	1:2
F4	Span-60	100	100	40	1:1
F5	Span-60	200	100	40	2:1
F6	Span-60	100	200	40	1:2
F7	Span-80	100	100	40	1:1
F8	Span-80	200	100	40	2:1
F9	Span-80	100	200	40	1:2

EVALUATION OF PILOCARPINE HYDROCHLORIDE ENTRAPPED NIOSOMES

Drug entrapment efficiency (% EE)

The proportion of encapsulated Pilocarpine hydrochloride was obtained by ultra-centrifugating 1 ml of the niosomal suspension at 15,000 rpm for 1 h using a cooling centrifuge at 4°C (Remi C-24, Mumbai, India). The niosomes were separated from the supernatant and were washed twice, each time with 1 ml phosphate buffered saline, and recentrifuged again for 1 h. The amount of entrapped Pilocarpine hydrochloride was determined by lysis of the separated vesicles with isopropanol. A 100 µl sample of niosomes was mixed with 1 ml of isopropanol; the volume was

completed to 10 ml with phosphate buffered saline and covered with parafilm to prevent evaporation. The concentration of the drug was determined by UV spectrophotometer (UV 1700 Pharm Spec, Shimadzu, Japan) at 215 nm. Study was done in triplicate and % drug entrapment efficiency can be calculated by using following formula:

$$\% \text{ EE} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \text{ X } 100$$

Vesicle size analysis

After storing for 24 hours, the various ratio of niosomal formulation prepared from all batches were taken for size analysis. Vesicle size was determined by particle size analyzer (Cilas, 1064 L, France). The measurements of different formulation of niosomes were done in triplicate and vesicle size was recorded.

Zeta potential measurements

The change in surface property of the niosomes were evaluated by measuring the zeta potential of the prepared niosomal formulation with a zeta meter (Zetasizer, Malvern, UK). In this technique, a voltage is applied across a pair of electrodes at either end of a cell containing the particle dispersion. Charged particles are attracted to the oppositely charged electrode. The method uses the autocorrelation function of the light scattered in a colloid solution measured by a photon counting system. The particles move in an electric field of known strength in the interference pattern of two laser beams and produce scattered light, which oscillates in time in a way, which depends on the speed of the particles. The light scattered by the particles is collected by the photomultiplier and the measured autocorrelation function is first converted, using a Fourier Transform, into a frequency spectrum. The frequencies are then converted successively to velocities, electrophoretic mobilities, and finally, zeta potentials [18]. The zeta potential of niosomal formulations was measured using 0.1M KCl buffer in demineralized water at 25°C.

Photomicroscopy and transmission electron microscopy (TEM)

The purpose of photomicroscopy is to obtain topographical characteristics, specially the shape and surface morphology of the niosomes. A few sample of niosomal formulation (F1) was deposited onto the glass slide and fixation was done by using a drop of glycerin. This glass slide was mounted on the phase contrast instrument and photomicrographs were taken under triocular microscope (Olympus Model BX 41, Japan) at suitable magnification.

The prepared niosomal formulations were characterized for their shape using transmission electron microscopy (TEM). A drop of the different formulations was placed on different carbon coated copper grids to leave a thin film on the grids. Then, the film was negatively stained with 1% phosphotungstic acid (PTA) by placing a drop of the staining solution on to the film and the excess of the solution was drained off with a filter paper. The grid was allowed to dry thoroughly and formulations were viewed under a transmission electron microscope (JEM-200 CX, JEOL, Tokyo, Japan) at 80 KV, after being stained and TEM micrograph was taken at suitable magnification [19].

Fourier transform infrared (FTIR) spectroscopy

FTIR spectral measurements were performed using FT-IR Spectrometer (FTIR-8400S spectrophotometer, Shimadzu, Japan) to confirm the formation of niosomes, the presence of surfactant and cholesterol, blend formation and also to find the chemical stability of the drug in the niosomes. FT-IR spectra of the blank niosomes and drug loaded niosomes were obtained. Each sample were ground thoroughly with KBr powder in a weight ratio of 1:100 and then pellets

were prepared using a hydraulic pellet press under a hydraulic pressure of 15 tons for a minute. The pellet was placed in the sample holder and spectral scanning were taken in the wavelength region between 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1} and scan speed of 2 mm/sec.

Differential scanning calorimetric (DSC) study

Differential scanning calorimetric analysis were performed on the drug loaded niosomes. Initially, the moisture was removed by heating the samples and then, each sample (about 3-7 mg) was accurately weighed into platinum crucible 40µl aluminum pan in hermetically sealed condition, where alpha alumina powder used as a reference. Thermogram was recorded from 50°C to 300°C at the heating rate of 20°C/min under a constant flow of an inert nitrogen gas atmosphere with the flow rate of 20 ml/min. These analyses were done on Perkin-Elmer instrument (Pyris-1, Osaka, Japan) available at Department of Textile Technology, Indian Institute of Technology, New Delhi, India.

***In vitro* drug release studies**

The *in vitro* release of entrapped drug within niosomes was determined using membrane diffusion technique. The niosomal formulation equivalent to 4 mg of pilocarpine HCl was placed in a glass tube that was previously covered with presoaked cellulose membrane, which acts as a donor compartment. The glass tube was placed in a beaker containing 50 ml of simulated lachrymal fluid (pH 7.4), which acted as receptor compartment. The whole assembly was fixed in such a way that the lower end of the tube containing suspension was just touching (1-2 mm deep) the surface of diffusion medium. The temperature of receptor medium was maintained at 37±100^{0}C and agitated at 100 rpm speed using magnetic stirrer. Aliquots of 5 ml sample were withdrawn periodically and after each withdrawal same volume of medium was replaced. The collected samples were analyzed spectrophotometrically at 215 nm using simulated lachrymal fluid (pH 7.4) as blank.

Stability studies

Stability of the product may be defined as the capability of a particular formulation to remain with the physical, chemical, therapeutic and toxicological specification. Stability study is an important concern in the development of pharmaceutically acceptable product. In the present work, stability studies of prepared formulations loaded with pilocarpine HCl were carried out after storing the formulations at 4±1°C and 37±1°C for 28 days in amber colored glass vials. After every 7 days the formulations were evaluated for particle size, zeta potential and % EE. The initial drug content was considered as 100%.

RESULTS AND DISCUSSION

Drug entrapment efficiency

Effect of surfactant type

The percentage drug entrapment efficiency (% EE) was determined in triplicate and their mean values are shown in Table 2. Percentage entrapment efficiency of pilocarpine HCl in niosomes prepared from span 20, span 60 and span 80 was found to be in the range of 72.33±2.03% to 93.26±1.75% and it has been shown that % EE prepared in 1:1 molar ratio was found to be increase. From the results, it has been revealed that the entrapment efficiency of niosomes composed of span 60 were superior as compared to those prepared from span 20 and Span 80. This can be due to the hydration temperature used to make niosomes should usually be above the gel to liquid phase transition temperature of the system that results in niosomes that are less leaky and have high entrapment efficiency. Span 60 has highest phase transition temperature (50^{0}C) as compared to span 80 and 20 and hence high entrapment efficiency and the length of alkyl chain of

surfactant has a prominent effect on permeability of prepared niosomes as length of surfactant increases entrapment efficiency also increases . Hence, long chain surfactant results in high entrapment. Thus, span 60 has a longer saturated alkyl chain (C16) compared to span 20 (C10), so it produces niosomes with higher entrapment efficiency. The longer alkyl chain influences the HLB value of the surfactant mixture which in turn directly influences the drug entrapment efficiency. The lower the HLB of the surfactant, the higher will be the drug entrapment efficiency and stability as in the case of niosomes prepared using span 60 [20].

Effect of cholesterol weight ratio

Cholesterol is a common component of niosomes providing rigidity to the membrane; stabilizes bilayers, prevents leakiness and controlling permeability [21]. The effect of cholesterol on drug encapsulation efficiency was evaluated by preparing the niosomes at different surfactant: cholesterol weight ratios, namely 1:1, 2:1 and 1:2. The entrapment efficiency for each niosomal formulation was estimated. For span 20 and span 60 niosomes, nonsignificant increase in entrapment efficiency was showed when cholesterol weight ratio increased from 1 to 2 weight ratios. This may be due to the following two factors:

1. With increasing cholesterol, the bilayer hydrophobicity and stability increased [22] and decreased the permeability [23] of the bilayer preventing more entrapment of pilocarpine HCl within the bilayer, as the niosomes form.
2. As the concentration of cholesterol increases, beyond certain limits results in increased viscosity of membrane indicating more rigidity of the bilayers and starts disrupting the regular bilayered linear structure leading to loss of drug entrapment.

It was also concluded from the data in the Table 2 that the entrapment efficiency of niosomes composed of span 60 was higher as compared to those of span 20 and span 80. Formulation F4 shows the maximum % EE among all the formulations.

Vesicle size analysis

Effect of surfactant type on vesicle size

The vesicle size of all niosomal formulations was determined in triplicate and their mean values were shown in Table 2. Vesicle size of all formulation was ranges between 1.44±2.76µm to 7.12±2.80µm. Table 2 shows that the niosomes prepared using span 60 is larger in size than those prepared using span 20 and span 80. Span 60 has a longer saturated alkyl chain compared to span 20 and it was reported that surfactants with longer alkyl chains generally give larger vesicles. This would account for the higher entrapment efficiencies with niosomes prepared from span 60, also the mean size of the niosome increased with progressive increase in the HLB value because surface free energy decreases on increasing hydrophobicity of surfactant. Span 20 has shortest alkyl chain cause smallest particle size [20].

Effect of cholesterol concentration on vesicle size

It was revealed from Table 2 that, in case of span 20 and span 60 niosomes, by increasing cholesterol concentration led to increase in particle size. This is because cholesterol increases the width of bilayer and consequently increases the vesicle size [24]. Also, this occurred in case of span 80 niosomes due to presence of double bond [25].

Zeta Potential Measurements

The zeta potential of all niosomal formulations was determined in triplicate and their mean values are shown in Table 2. It was clearly observed from the data as shown in Table 2 that highest zeta potential was observed with span 20 (-31.04±0.25 mV) whereas lowest zeta potential was in case of span 60 (-15.04±0.45 mV). This might be due to increases in hydrophilicity of surfactant; zeta potential also increases [26]. Zeta potential of all the formulations was found to be negative. This might be due to the presence of free carboxyl groups in cholesterol and surfactant molecule. There

was no statistically significant difference observed between the zeta potential values of different formulations. It implies that equal molarity of non ionic surfactant and cholesterol can make the membrane compact and well organized. The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. If all the particles in suspension have a large negative or positive zeta potential then they will tend to repel each other and there will be no tendency for the particles to come together. However, if the particles have low zeta potential values then there will be no force to prevent the particles coming together and flocculating. It has been established that colloidal system wherein the dispersed particles have surface charge beyond +30 mV or -30 mV are stable systems. The zeta potential given in Table 2 and shown in Figure 1 suggested sufficient kinetic stability of the niosomes.

Table 2: Results of % EE, vesicle size, zeta potential and *in vitro* release (Q8h) of all formulations (F1 to F9)

Formulation Code	% EE (± SD, n=3)	Vesicle size (µm) (± SD, n=3)	Zeta potential (mV) (± SD, n=3)	Q8h (%) (± SD, n=3)
F1	81.43±2.09	1.44±2.76	-31.04±0.25	78.81±4.82
F2	86.17±3.07	1.56±1.59	-29.54±0.72	73.15±6.32
F3	78.56±0.99	2.71±0.57	-28.84±0.27	68.74±0.78
F4	93.26±1.75	6.13±0.31	-15.04±0.45	66.98±1.87
F5	90.66±3.80	5.16±0.27	-22.21±1.28	63.54±4.42
F6	84.06±9.36	7.12±2.80	-24.64±0.53	60.35±3.83
F7	79.11±3.96	3.68±0.69	-30.04±0.72	74.04±2.25
F8	83.47±2.65	3.09±1.32	-28.84±0.47	75.33±0.89
F9	72.33±2.03	4.24±0.41	-27.32±0.34	71.05±0.93

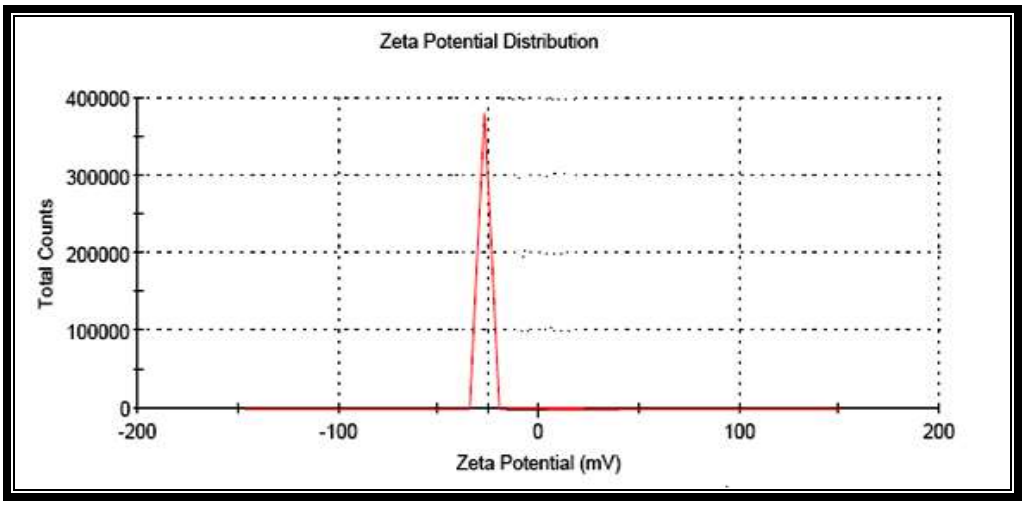

Figure 1: Zeta potential of niosomal formulation F4

Photomicroscopy and transmission electron microscopy

The photomicrographs of pilocarpine HCl entrapped niosomes of formulation F1 was shown in Figure 2. It was observed that formulation F1 composed of span 20: cholesterol (1:1) weight ratio has smaller vesicle size and spherical in shape as compared to the remaining formulations which is coherent to the entrapment efficiency results.

Negative stain transmission electron micrographs of pilocarpine HCl loaded niosomal formulation F1 was shown in Figure 3. It was demonstrated that the vesicles are well identified and present in a nearly perfect sphere like shape with a smooth surface and having a definite internal aqueous space.

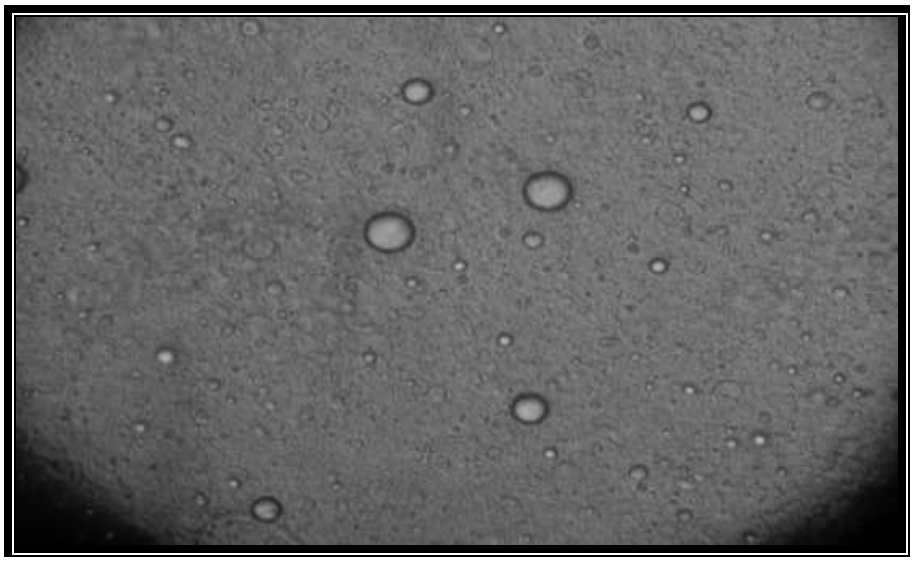

Figure 2: Photomicrographs of pilocarpine loaded niosomal formulation F1

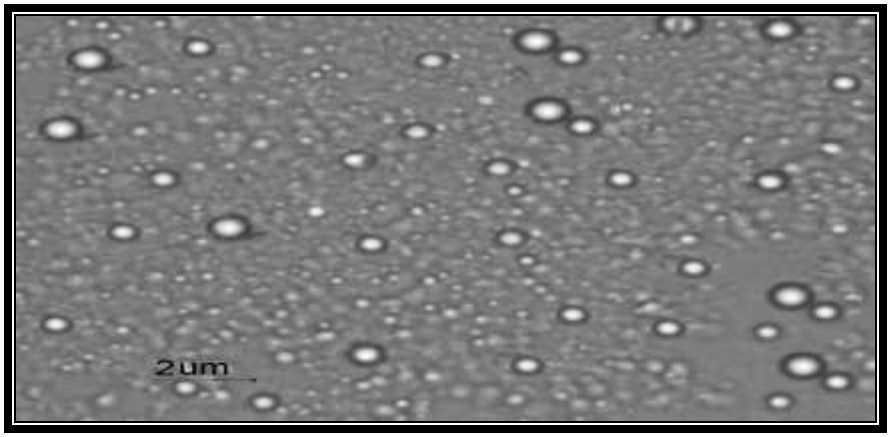

Figure 3: Transmission electron micrograph of pilocarpine loaded niosomal formulation F1

Fourier-transform infrared (FT-IR) spectral analysis

Drug-excipient interaction was studied by FT-IR spectroscopy, to investigate the formation of niosomes and chemical stability of the drug after encapsulation into the formulation. FT-IR

spectral analysis of blank and drug loaded niosomes (F4) was shown in Figure 4 and Figure 5 respectively. Pilocarpine HCl showed that the principle IR peaks at 3401.09 cm^{-1} resulted from N-H stretching, the peak at 1630.38 cm^{-1} resulted from C=O (side chain) stretching, the peak at 2850.50 cm^{-1} resulted from C-H stretching, the peak at 1455.50 cm^{-1} resulted from C-H bending and a broad peak around 3479.34 cm^{-1}, indicating stretching of hydroxyl groups. All the principal peaks of pilocarpine HCl are present in drug loaded niosomes, which confirm the stability of drug in niosomes. In the case of blank niosomes, additive spectra compared to cholesterol and span-20, span-60 and span-80 was observed. FTIR spectra showed minor shifting of some peaks compared with individual excipients i.e. peak at 3390.63 cm^{-1} resulted from O-H stretching of cholesterol while peak at 2935.40 cm^{-1} resulted from C-H stretching of span 60. These shifts was due to the formation of hydrogen bonds seen in the polar groups of excipients which supports the formation of favorable vesicle shape, structure with good stability and sustained drug release.

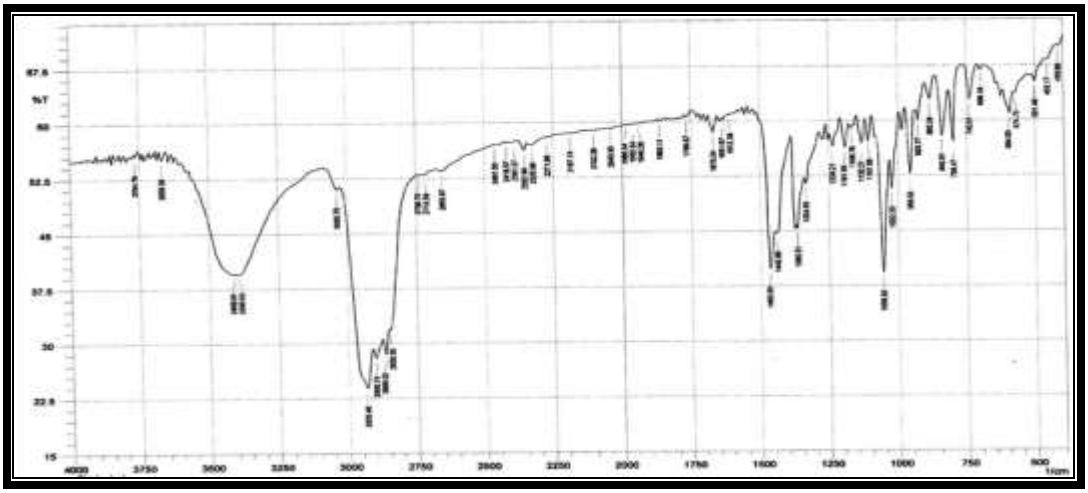

Figure 4: FTIR spectra of blank niosomes

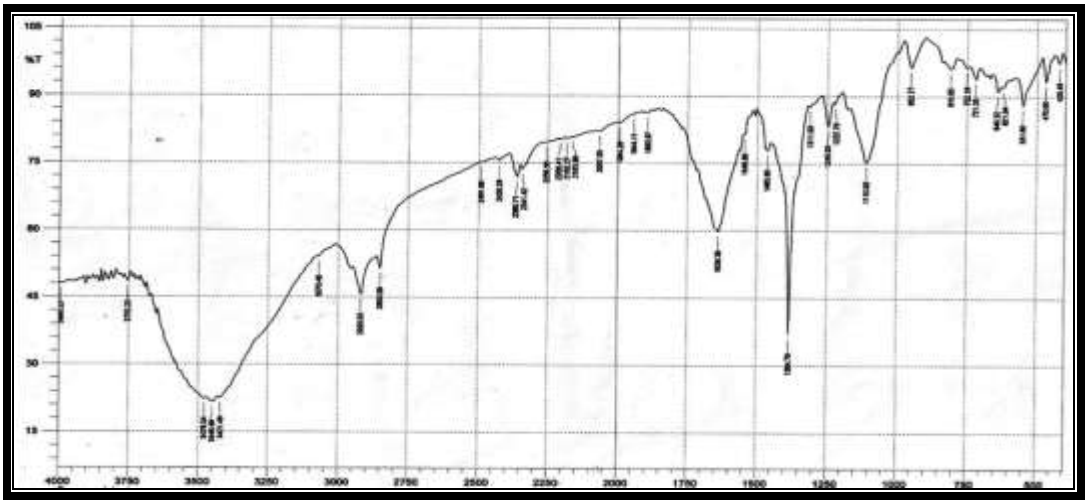

Figure 5: FTIR spectra of drug loaded niosomes F4

Differential scanning calorimetric (DSC) study

DSC thermogram of drug-loaded niosomal formulation (F4) was displayed in Figure 6. In case of drug-loaded niosomes, one sharp exothermic peak was seen at 191.923ºC (area=68.890 mJ, delta

H=22.963 J/g) indicating the peak was in the range of the drug that shows no interaction of the excipients with the drug and there was no significant effect of method of preparation on the stability of niosomes into the cholesterol and non-ionic surfactant matrix. So, it was concluded that there was no sort of interaction between the polymers and the drug and method used in preparation of niosomes.

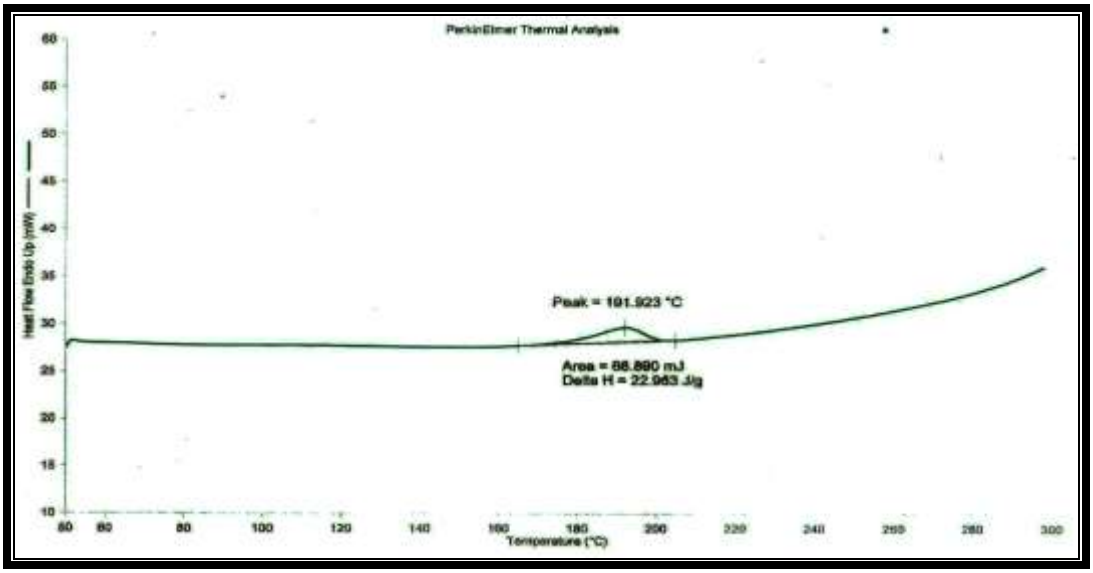

Figure 6: DSC thermogram of drug loaded niosomes F4

***In vitro* release data of prepared niosomes**

Results of an *in vitro* study on the release of pilocarpine HCl entrapped niosomal vesicles prepared using span 20, span 60 and span 80 are shown in Table 2 and Figure 7. The effect of surfactant type on release rate of niosomal formulation was shown in Figure 8. The percentage of drug released after 8 h from the prepared niosomal vesicles at pH 7.4 simulated lachrymal fluid were vary from 60.35±3.83% to 78.81±4.82% as shown in Table 2. By inspection of the data, it could be concluded that niosomal formulations prepared using span 60 yielded a lower rate of release compared to span 20 and span 80 niosomes. This can be explained by the fact that niosomes exhibit an alkyl chain length-dependent release and the higher the chain length, the lower the release rate [27]. By reviewing the data, it has been revealed that release after 8 hrs for the niosomal formulations can be arranged in the following decreasing order: F1 > F8 > F7 > F2 > F 9 > F3 > F4 > F5 > F6 niosomal vesicles. From results shown in the table it is obvious that the increase of cholesterol molar ratio reduced the efflux of the drug from niosomal preparations, which is in accordance with its membrane stabilizing ability [28]. Cholesterol is known to abolish the gel to liquid phase transition of niosome systems, resulting in niosomes that are less leaky. Therefore, the diffusion of pilocarpine HCl entrapped in the hydrophobic regions of the vesicles would be expected to occur over a prolonged period of time [29].

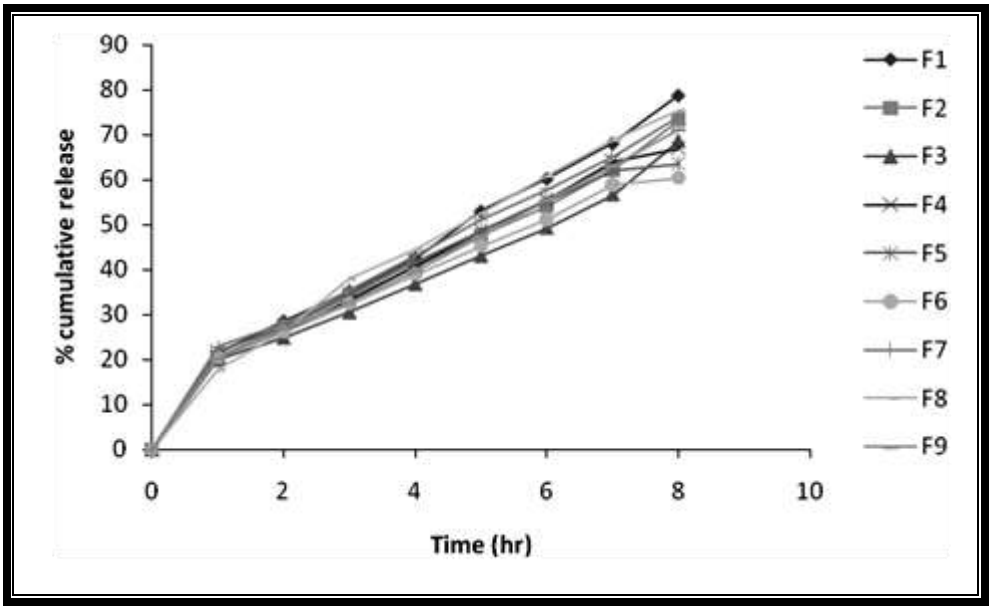

Figure 7: *In vitro* release profile of different niosomal formulations at pH 7.4 media

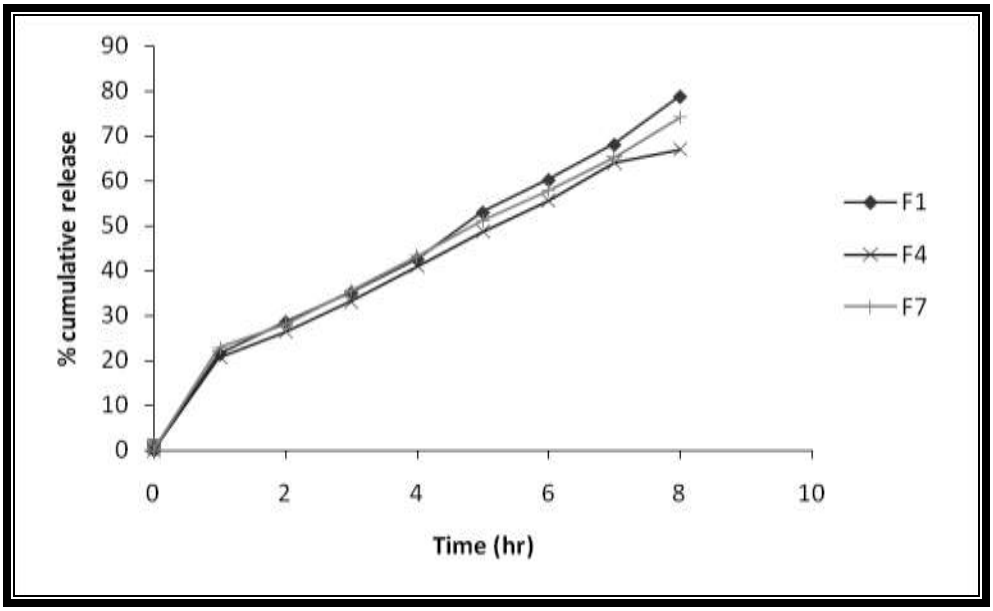

Figure 8: Effect of surfactant type on *in vitro* release profile of niosomal formulation F1, F4, F7 at pH 7.4 media

Stability studies

The Stability Studies of niosomal formulation (F4) was conducted for 28 days and after that changes in their particle size, zeta potential and entrapment efficiency at 4±1°C and 37±1°C was measured which was given in Table 3. It was found that there was an insignificant change in their particle size distribution, % EE and zeta potential after storage of formulation F4 at 4±1°C and 37±1°C for 28 days, indicating that formulation F4 could provide a minimum shelf life of one year.

Table 3: Results of % EE, vesicle size and zeta potential of niosomal formulation F4

Time (Days)	% EE (± SD, n=3)		Vesicle size (µm) (± SD, n=3)		Zeta potential (mV) (± SD, n=3)	
	4 ± 1°C	37 ± 1°C	4 ± 1°C	37 ± 1°C	4 ± 1°C	37 ± 1°C
0	93.32±2.36	93.67±1.45	6.45±1.60	6.78±1.92	-15.34±1.33	-15.92±0.68
7	92.47±2.37	92.86±1.53	7.06±0.39	7.27±3.19	-20.14±0.22	-22.74±2.62
14	91.16±0.79	91.92±2.14	7.81±0.17	7.92±1.17	-24.04±3.07	-25.54±1.26
21	89.26±1.25	89.73±0.35	8.03±0.62	8.19±2.11	-28.62±0.32	-29.96±1.25
28	88.09±4.81	88.21±1.20	8.36±1.25	8.58±1.07	-30.01±1.24	-31.13±2.04

CONCLUSION

The results of this study showed that cholesterol content and type of surfactant altered the % EE, vesicle size and release rate from pilocarpine HCl niosomes. Higher % EE was obtained with niosomes prepared from span 60 and cholesterol in a 1:1 molar ratio. The *in vitro* evaluation of pilocarpine HCl niosomal formulation (F4) showed that niosomes composed of span 60 and cholesterol were the most effective in the prolongation of drug release after 8 hours (Q8h=66.98±1.87%) and more controlled from the ocular delivery system. Niosomes may be considered as promising ophthalmic carriers for the topical application of pilocarpine HCl.

List of abbreviations

IOP, Intraocular pressure; **RGC,** Retinal ganglion cell; **POAG,** Primary open angle glaucoma; **FT-IR,** Fourier transform infrared; T**EM,** Transmission electron microscopy; **UV,** Ultraviolet; **EE,** Entrapment efficiency; **SD,** Standard deviation; **Q8h,** In vitro release after 8 hours; **PTA,** Phosphotungstic acid.

Conflict of interest

The authors report no conflicts of interest.

Acknowledgements

The authors are thankful to the Teerthankar Mahaveer College of Pharmacy, TMU, Moradabad-244102, U.P.,India for their kind support and providing all the necessary facilities and encouragement for successful completion of this work.

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