Potential Use of Niosomal Hydrogel as an Ocular Delivery System for Atenolol

Irhan Ibrahim Abu Hashim,* Marwa Salah El-dahan,† Rehاب Mohammed Yusif,* Abd-ElGawad Helmy Abd-ElGawad,* and Hidetoshi Arima*,b,c

*Department of Pharmaceutics, Faculty of Pharmacy, Mansoura University; Mansoura 35516, Egypt; †Department of Physical Pharmaceutics, Graduate School of Pharmaceutical Sciences, Kumamoto University; and *Program for Leading Graduate Schools “HIGO (Health Life Science: Interdisciplinary and Glocal Oriented) Program,” Kumamoto University; 5–1 Oe-honmachi, Chuo-ku, Kumamoto 862–0973, Japan.

Received September 16, 2013; accepted December 24, 2013

Niosomes have been reported as possible approach to improve the low corneal penetration and bioavailability characteristics for many drugs. The purpose of this study was to prepare and characterize an effective ocular niosomal hydrogel containing 0.5% (w/v) atenolol which is β1 adrenoceptor blocker for treatment of glaucoma. Thin film hydration method was used for the preparation of niosomes using Span 60 and cholesterol at different molar ratios. Niosomes were characterized using laser diffraction particle size analyzer, transmission electron microscopy, and differential scanning calorimetry. The results showed that higher entrapment efficiency (80.7%±1.2) was obtained from niosomes prepared using Span 60/cholesterol at a 2:1 molar ratio. Stability study revealed that a fairly high retention of atenolol inside vesicles (83.1%±2.35) up to a period of 3 months at 4°C. It was found that niosomal hydrogel formulation using carbopol 934P significantly exhibited sustained in vitro release of the drug compared with free drug solution and other polymeric hydrogels. The intraocular pressure (IOP) lowering activity of selected atenolol formulations was determined and compared with that of atenolol solution. It is worth noting that niosomal hydrogel formulation was found to show the most significant prolonged decrease in IOP, suggesting that niosomal hydrogel could be a promising delivery system for atenolol.

Key words atenolol; niosomal hydrogel; carbopol 934P; sustained release; intraocular pressure; glaucoma

Eye is the most important and sensitive organ; in fact, it is the window of our soul. Drug delivery in ocular therapeutics is a challenging problem due to physiological constrains imposed by the unique anatomic structure and efficient protective mechanism of the eyes. The majority of the ophthalmic drugs are administrated topically in the form of conventional eye drops. However, the rapid turnover of lacrimal fluid and extensive nasolacrimal drainage along with eyes blinking reflex rapidly eliminate the administrated eye drops. This causes short pre-corneal residence time, which limits effective transcorneal drug absorption. Thus, frequent instillation of eye drops is required to achieve therapeutic effect. In addition, the topically applied drugs could enter the systemic circulation via conjunctiva and nasal mucosa, which may result in some undesirable side effects. Ophthalmic ointment formulations demonstrated substantially improvement in drug bioavailability when compared with their eye drops counterparts. However, they suffered from some disadvantages that include sticky sensation, blurred vision and induced reflex blinking which also caused patient non-compliance.

To overcome these problems, different approaches such as in situ forming gel, micro and nanocarrier systems, vesicles, and vesicular systems have been adopted. In recent years, vesicular drug delivery systems used in ophthalmics such as liposomes and niosomes help in providing prolonged and controlled action on the corneal surface and preventing the metabolism of the drug by the enzymes present at the tear/corneal epithelial surface. Drug enclosed in the vesicles allows improved partitioning and transport through the cornea. Moreover, vesicles offer a promising avenue to fulfill the need for an ophthalmic drug delivery system that has the convenience of a drop, and at the same time can localize and maintain drug activity at its site of action for longer period of time thus allowing for a sustained action.

From a technical point of view, nonionic surfactant vesicles (niosomes) are considered promising drug carriers as they possess greater stability and lack of many disadvantages associated with phospholipid vesicles (liposomes), such as high cost, stringent storage condition and the oxidative degradation of phospholipids.

Niosomes are formed from the self-assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures which can entrap both hydrophilic and lipophilic drugs either in the aqueous layer or in the vesicular membrane. Moreover, sparingly soluble drugs can be entrapped in the vesicles.

Glaucoma is a disease characterized by higher level of intraocular pressure (IOP) which might progressively hurt visibility. The chronic glaucoma with open angle poses a major problem of public health and it is the second leading cause of blindness in the world. It is estimated that the number of people with glaucoma will be nearly 79.6 million worldwide by 2020. This alarmingly large number of anticipated patients requires urgent improvement in the current therapeutic approaches adopted for the treatment of this disease. Even though, the currently available eye drops for glaucoma treatment reduce its disability. Their long-term effectiveness and efficacy are being questioned due to poor patient compliance.

Atenolol is a β1 adrenoceptor blocker. It is reported to lack intrinsic sympathomimetic activity and membrane-stabilizing properties. This suggests that its action in reducing IOP must in some way be mediated by the inhibi-
tion of the β1-receptors in the eye. It remains to be seen whether this inhibition causes an increase in outflow facility or impairs the production of aqueous humour. Oral administration of atenolol has been shown to reduce the IOP. P-glycoprotein (P-gp) is a 170 kDa membrane protein encoded by the multidrug resistance gene (MDR1) and functions as an energy-dependent efflux pump. P-gp is typically localized at the apical surface of the epithelial cells and has been shown to reduce the transepithelial permeation of diverse drugs. In the eye, P-gp is expressed in the retinal capillary endothelial cells, retinal pigmented epithelial cells, ciliary nonpigmented epithelium, conjunctival epithelial cells, corneal epithelium and iris and ciliary muscle cells. Role of P-gp as a barrier to corneal delivery of drugs has been reported earlier.

Yang et al. investigated the role of P-gp in transepithelial transport and uptake of propanolol in conjunctival epithelial cells cultured on Transwell filters in the presence and absence of P-gp competing substrates. It was found that not only hydrophilic atenolol but also moderately lipophilic metoprolol and highly lipophilic alprenolol failed to affect propanolol uptake. This finding indicated that such β-blockers are non P-gp substrates. In addition, another study examined the effect of elacridar as a P-gp inhibitor on the permeability of atenolol across the blood brain barrier in mice and rats. The authors reported that atenolol, a non P-gp substrate, exhibited poor brain penetration in the presence or absence of elacridar in both species.

In late 1970s, earlier studies have shown that topically applied atenolol eye drops lowered the IOP in patients with ocular hypertension after short duration therapy. Other researchers have attempted to overcome the drawbacks associated with the conventional eye drops through inclusion of atenolol in gel formulations. Still, it is important to develop a suitable long-term ocular delivery system to control the IOP within an appropriate duration, minimizing dosing frequency, reducing systemic absorption and optimizing therapeutic effect. Thus, niosomes could be a useful ocular drug delivery system for the treatment of glaucoma.

The objective of the present study was to prepare and characterize niosomal hydrogels containing atenolol. The factors influencing the encapsulation of atenolol into niosomes were investigated. Characterization of the prepared niosomes regarding physical morphology, particle size, in vitro drug release, and stability study was performed. Moreover, the IOP lowering activity of the selected atenolol niosomal formulations was evaluated.

MATERIALS AND METHODS

Chemicals Atenolol was kindly supplied by European Egyptian Pharm. Ind., Alexandria, Egypt. Sorbitan monostearate (Span 60) and cholesterol (CH) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Sodium alginate (BDH Chemicals, Ltd., Poole, U.K.), hydroxypropylmethylcellulose (Dow Chem. Co., Midland, MI, U.S.A.), carbopol 934P (BF Goodrich, U.S.A.), chitosan (Loba Chemie, Mumbai, India). All other chemicals and solvents were of analytical reagent grade. Spectra/Por dialysis membrane (12000–14000 molecular weight cutoff) was purchased from (Spectrum Laboratories Inc., Rancho Dominguez, Canada).

Methods. Preparation of Atenolol Niosomes Niosomes were prepared by thin film hydration method. Briefly, accurately weighed quantities of Span 60 and CH at different molar ratios, viz. 1:1, 2:1, 3:1 and 1:2 (Table 1), were placed in a 100mL round bottomed flask and dissolved in 10mL chloroform. The organic solvent was evaporated under reduced pressure at 60°C using a rotary evaporator (Buchi Rotavapor, Switzerland) till the formation of a thin lipid film. The excess organic solvent was removed by leaving the flask in a desiccator under vacuum overnight. The lipid film was then hydrated with 10mL of distilled water containing atenolol in a concentration equivalent to 10mg/mL at 55°C, which is above the gel-liquid transition temperature (Tc) of sorbitan monoesters. The resulting niosomal suspension was mechanically shaken for 1h using a horizontal mechanical shaking water bath at 55°C. Then, the vesicle suspension was sonicated for 20min in a bath type sonicator (Ultrasons, Selecta, Barcelona, Spain). The niosomal suspension was left to mature overnight at 4°C and stored at refrigerator temperature (4°C) for further studies.

Characterization of Niosomes. Entrapment Efficiency (EE%) Niosomes containing atenolol were separated from unentrapped drug by cooling centrifugation at 18000rpm for 60min at 4°C. Niosomal pellets were resuspended in distilled water and then centrifuged again. This washing procedure was repeated two times under the same conditions to ensure that the unentrapped drug was no longer present in the void volume between the niosomes. The supernatant was separated each time from niosomal pellets and assayed spectrophotometrically for free atenolol at 274 nm using UV/VIS spectrophotometer (JASCO, V-530, Japan). Amount of entrapped drug was obtained by subtracting amount of unentrapped drug from the total drug incorporated.

\[
\text{entrapment efficiency (EE\%) = } \frac{\text{amount of entrapped drug (mg)}}{\text{total amount of drug (mg)}} \times 100
\]

Determination of Vesicle Size The average size of the prepared niosomes was determined using laser diffraction particle size analyzer (Malvern Instruments Ltd., Worcestershire, U.K.). Before measurement, samples were dispersed in distilled water.

Transmission Electron Microscopy (TEM) The morphology of the prepared niosome formulations was determined by TEM (JEOL 100 CX Transmission electron microscope at 80 KV): a drop of the dispersion was diluted 10-fold using deionized water, then a drop of the diluted dispersion was applied to a carbon-coated 300 mesh copper grid and left for 1 min to allow some of the niosomes to adhere to the carbon substrate. The remaining dispersion was removed by absorb-

| Table 1. Composition and Characterization of Niosomal Formulations |
|-------------|----------------|----------------|----------------|
| Formulation code | Span 60:CH (molar ratio) | Particle size (nm) | Entrapment efficiency (%) |
| F1 | 1:1 | 56.5±6.8 | 60.9±2.1 |
| F2 | 2:1 | 94.2±8.1 | 80.7±1.2 |
| F3 | 3:1 | 155.3±11.6 | 73.6±1.9 |
| F4 | 1:2 | 133.4±10.1 | 32.9±1.5 |

Each value represents the mean±S.D. (n=3).
ing the drop with the corner of a piece of filter paper. After twice rinsing the grid (deionized water for 3–5 s) a drop of 2% aqueous solution of uranyl acetate was applied for 1 s. The remaining solution was removed by absorbing the liquid with the tip of a piece of filter paper and the sample was air dried.45)

**Differential Scanning Calorimetry (DSC)** The thermal properties were analyzed using differential scanning calorimetry (DSC; Shimadzu, DSC-60 with TA-60 WS thermal analyzer, Tokyo, Japan) calibrated with indium. Thermograms were analyzed using Shimadzu TA-60 software. The lyophilized pellets of the niosomal formulations were used for the investigation. Span 60, CH, and atenolol were also investigated. A sample (4 mg) of powder was placed in a hermetically sealed aluminium pan and scanned at a rate of 10°C/min over a temperature range of 20–350°C under nitrogen atmosphere. Alumina powder was used as the reference material in the DSC runs.

**In Vitro Drug Release Studies** In vitro release experiments were assessed for the selected niosomal formulation showing highest drug entrapment level (F2). The in vitro release of atenolol from solution or niosomal dispersion was carried out using dialysis method. Spectra/Por® dialysis membrane (12000–14000 molecular weight cutoff) was washed several times with distilled water and soaked in simulated tear fluid pH 7.4 for 24 h before the experiment. The membrane was stretched over the open end of 3 cm diameter glass tube and was made water tight by a rubber band. A 3 mL sample, either of the freshly purified niosomal dispersion or of free atenolol solution (0.5% w/v equivalent 15 mg atenolol), was placed in the tube. The tube was then immersed upside-down in a beaker containing 50 mL simulated tear fluid pH 7.4 which is preheated and maintained at 37±0.5°C using thermostatically controlled water bath (Hilab, GLF 3202, Germany). The tube height was adjusted, so that the membrane was just below the surface of the release medium. The rotary shaker was adjusted to a rate of 25 strokes/min. At predetermined time intervals of 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 h, aliquots of 1 mL were withdrawn from the release medium and replaced by equivalent volume of the buffer solution. The released amounts of the drug were analyzed spectrophotometrically at 274 nm.

**Preparation and in Vitro Release of Atenolol from Niosomal Hydrogels** Different polymers used as bases for preparing niosomal hydrogels are listed in Table 2. The weighed amount of the polymer was dispersed in distilled water in which 0.01% benzalkonium chloride as a preservative was previously dissolved. The aqueous dispersion was allowed to hydrate for 4–5 h. The selected niosome dispersion (F2) with drug concentration of 0.5% (w/w) was added and gently stirred (Polamed magnetic stirrer, model MM5, Poland) till obtaining a homogenous mixture. The niosomal gel was left overnight at 4°C for air removal. Free atenolol 0.5% (w/w) gel formulations were also prepared following the same procedure. The viscosity of the prepared gels was determined using Rotary viscometer (Hakke Inc., Germany). The release of atenolol from free and niosomal gel preparations was studied using the same method as niosomal dispersion.

**Kinetic Analysis of Drug Release Data** To investigate the mechanism of drug release, the in vitro release data were analyzed mathematically according to the following models: zero-order kinetics (cumulative % drug released vs. time), first-order kinetics (log % drug retained vs. time), Higuchi model (cumulative % drug released vs. square root of time), and Korsmeyer–Peppas equation (log amount of drug released vs. log time). The correlation coefficient (r) values were calculated for the linear curve obtained by regression of the above plots.

**Evaluation of Niosomal Stability** The optimized niosomal formulation (F2) was tested for stability by storing it at 4±1°C and at ambient room temperature. Vesicles size and % atenolol retained were assessed before and after storage for 3 months. Size of the vesicular system was determined by laser diffraction particle size analyzer. In addition, % drug retained was evaluated by taking samples after 3 months and estimated spectrophotometrically at 274 nm.

**In Vivo Studies** Adult male albino normotensive rabbits, each weighing 1.5–2.0 kg were used in the experiments. All animals were healthy and free of clinically observable abnormalities. Animals were housed singly in a standard cages, in a light controlled room (12-h light and 12-h dark cycles) at 20–24°C, with no restriction to food or water.46) The experimental procedures conform to the ethical principles of the scientific committee of the Faculty of Pharmacy, Mansoura University, Egypt for the use of experimental animals. The rabbits were divided into four groups, each consisting of six rabbits: group I received atenolol solution, group II received atenolol niosomal dispersion composed of Span 60:CH in a 2:1 molar ratio, group III received free atenolol/carbopol gel, and group IV received atenolol niosome/carbopol gel. A single dose of 100 mg of ophthalmic drug solution or other investigated formulations (0.5% atenolol) were applied directly into the lower conjunctival sac of the right eye of rabbits while the left eyes served as a control. At certain time intervals, the IOP was measured before and after application of the formulations for both control and tested eyes using Schiotz tonometer (Winters, Eichtabelle, Germany).47) All measurements were done three times at each interval, and the mean values were used to calculate the percentage decrease in IOP.48) The pharmacodynamic parameters taken into consideration were maximum percentage decrease in IOP (E_{max}) time for maximum response (T_{max}), area under percentage decrease in IOP versus time curve (AUC_{0–8h}).

**Statistical Analysis** The data are represented as mean± S.D. Statistical analysis of the data was carried out using one way ANOVA followed by Tukey–Kramer multiple comparisons test at a level of significance of p<0.05 with Instat Graphpad prism software (version 4.00; Graphpad software,

<table>
<thead>
<tr>
<th>Gel formulation code</th>
<th>Polymer Type</th>
<th>Conc. (%w/w)</th>
<th>Atenolol Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 HPMC</td>
<td>2%</td>
<td>Free drug</td>
<td></td>
</tr>
<tr>
<td>G2 HPMC</td>
<td>2%</td>
<td>Niosomes</td>
<td></td>
</tr>
<tr>
<td>G3 Sodium alginate</td>
<td>2%</td>
<td>Free drug</td>
<td></td>
</tr>
<tr>
<td>G4 Sodium alginate</td>
<td>2%</td>
<td>Niosomes</td>
<td></td>
</tr>
<tr>
<td>G5 Chitosan</td>
<td>2%</td>
<td>Free drug</td>
<td></td>
</tr>
<tr>
<td>G6 Chitosan</td>
<td>2%</td>
<td>Niosomes</td>
<td></td>
</tr>
<tr>
<td>G7 Carbopol 934P</td>
<td>1%</td>
<td>Free drug</td>
<td></td>
</tr>
<tr>
<td>G8 Carbopol 934P</td>
<td>1%</td>
<td>Niosomes</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. The Composition of Different Gel Formulations
San Diego, CA, U.S.A.).

RESULTS AND DISCUSSION

Vesicles Preparation and Characterization To obtain the desired vesicle size and the highest encapsulation efficiency, the process variables such as, organic solvent, speed of rotation of flask, hydration medium, hydration time, agitation method, and time of sonication were investigated and optimized.

Entrapment Studies The effect of different molar ratios of Span 60: CH on the entrapment efficiency % (EE%) of atenolol in niosomes is illustrated in Table 1. Significant difference in EE% between all niosomal formulations (p<0.05) was observed. The results revealed that F2 showed the maximum entrapment efficiency (80.7%±1.2) at 2:1 Span 60: CH molar ratio. Span 60 showed the maximum entrapment efficiency at this molar ratio, as it has a long saturated alkyl chain that decreased the amount of CH needed to form niosomes. CH is one of the common additives included in the formulation in order to prepare stable niosomes. It stabilizes bilayers, prevents leakage and retards permeation of solutes enclosed in the aqueous core of these vesicles. Moreover, drug partitioning will occur more easily in highly ordered systems of surfactant and CH. It was observed that further increase in CH content (formula F4) had a significant decrease (p<0.05) in the entrapment efficiency of sorbitan ester niosomes (32.9%±1.5). This could be due to the fact that CH beyond a certain concentration can disrupt the regular bilayered structure of vesicular membranes leading to loss of drug entrapment levels. Nanoparticles less than 200 nm are considered accepted for passive drug targeting and for in vivo study. Vesicle size is an important parameter that influences the biopharmaceutical feature of the carrier. The size of particles in ophthalmic dosage forms apart from influencing bioavailability, plays an important role in the irritation potential of the formulation, hence it is recommended that particles of ophthalmic solution should be less than 10 µm to minimize irritation to the eye.

Differential Scanning Calorimetry (DSC) DSC thermograms of atenolol loaded niosomes composed of Span 60: CH (2:1) molar ratio, plain niosomes, and their individual components are illustrated in Fig. 2. Span 60, CH, and atenolol showed sharp endothermic peaks at 58.16°C, 148.70°C, and 155.61°C, respectively. Characterization by DSC showed that a change in the phase transition temperature of the main constituents is generally observed in the DSC thermograms of plain niosomes. An obvious change in the phase transition temperature of Span 60 from 58.16 to 49.57°C was observed with clear changes in the enthalpy in addition to a considerable peak broadening. A second peak was generally noticed which could represent other components such as CH. DSC thermogram of atenolol loaded niosome interestingly showed disappearance of the melting endothermic peak of atenolol and
the major endothermic peaks of the lipid bilayer components were also shifted with the same manner as observed in plain niosome. This may be due to entrapment of atenolol in the vesicular system.

**In Vitro Release Study** Figure 3 illustrates the drug release profiles from its free solution and niosomal dispersion. Free drug solution appeared to exhibit a significant \((p<0.05)\) higher and faster release in the first 0.5 h than that from the niosomal dispersion. The drug solution showed 40.2% release after 0.5 h, whereas the niosomal drug dispersion showed only 11.2% drug release after 0.5 h. The drug release from the free solution began to plateau after 2 h, whereas the release from the niosomal dispersion was continued for 8 h without reaching plateau. These results pointed to sustained release characteristics with a Higuchi pattern of drug release, where niosomes act as a reservoir system for continuous delivery of the drug. 53) CH reduces the leakage or permeability of encapsulating drug by decreasing the niosomal membrane fluidity. 50) Yoshioka et al. 60) found that the release rate of carboxyfluorescein, a water soluble compound, from niosomes prepared with Span 60 was slower than the release rate from other Span formulations (Span 20, 80, and 85). This result could be due to the fact that at 25°C, the molecules of Span 60 are in the ordered gel state, but those of other Spans are in the disordered liquid crystalline state.

**In Vitro Drug Release from Niosomal Hydrogels** From our experimental data, F2 formulation was selected because of its reasonable size (94.2±8.1 nm), high entrapment efficiency (80.7%±1.2), as well as good release properties. This niosomal formulation was then incorporated in different polymeric gels. As a preliminary study, we investigated the in vitro release profiles of atenolol from different concentrations of polymeric gels compared to its release from niosomal hydrogels. After 8 h, no significant difference was observed on the release of atenolol at various concentrations of the polymers. The same behavior was noticed on the release of the drug from niosomal hydrogels. We found that the release of atenolol from niosomal hydrogels was significantly \((p<0.05)\) sustained compared with its release from free hydrogels (Fig. 4). In addition, increasing the polymer concentration was associated with an increase in the viscosity of the gel (data not shown). This may have some disadvantages including difficulty in topical application to the eye, sticky sensation, blurred vision and reflex blinking. In view of the above mentioned data, we selected the appropriate concentrations of the polymers (2% sodium alginate, 2% hydroxypropyl methylcellulose (HPMC), 2% chitosan and 1% carbopol).

Figure 5 shows the in vitro release profile of atenolol from different polymeric gels compared to its release from the niosomal hydrogels. It is obvious that incorporation of niosomes into a structured gel vehicle resulted in a significant \((p<0.05)\) slower release of the drug compared with free drug gel formulations possibly because of the diffusion restriction imposed by the polymeric network of the gel. 61–63) It is also clear that atenolol release was significantly \((p<0.05)\) sustained and more extended in case of niosomal carbopol gel (47.4%) compared with other niosomal gels (67.0%, 60.8%, 67.0% for HPMC, chitosan, and sodium alginate, respectively) after 8 h. This may be due to the higher viscosity of niosomal carbopol gel, which provides an extra barrier for atenolol release. 64) The viscosity values were found to be 1017, 1035, 1097, and 1220 mPa·s for 2% sodium alginate, 2% HPMC, 2% chitosan, and 1% carbopol, respectively.

**Kinetic Studies of the Release Data** Table 3 summarizes the release kinetic parameters and correlation coefficients \((r^2)\) calculated for the investigated formulations. The in vitro release results showed that the release of atenolol from niosomal gels as well as niosomal dispersion is most fitted to diffusion-controlled mechanism (Higuchi model). 61,65,66) These results pointed to sustained release characteristics with a Higuchi pattern of drug release, where niosomes act as a reservoir system for continuous delivery of the drug. 53) In this study, the Korsmeyer–Peppas equation was utilized to interpret the atenolol release kinetics. It can give more insights on other drug
Fig. 4. *In Vitro* Release Profiles of Atenolol from Free and Niosomal Hydrogels in Simulated Tear Fluid pH 7.4 at Various Concentrations of Polymers

Each point represents the mean ± S.D. (n=3).

Fig. 5. *In Vitro* Release Profiles of Atenolol from Free and Niosomal Hydrogels in Simulated Tear Fluid pH 7.4

Each point represents the mean ± S.D. (n=3).
release mechanisms such as Fickian (diffusion), non-Fickian (anomalous), and erosion-mediated (zero-order) release. Additionally, this equation was successfully used to explain release mechanisms from thin film, cylindrical, disc, and spherical controlled release devices. The $n$ values were in the range between 0.5 and 1 ($0.5 < n < 1$) suggesting the non-Fickian (anomalous) release mechanism for the drug "i.e." both erosion and diffusion.

Evaluation of Niosomal Stability In the present study, the stability of the vesicles was determined by measuring the vesicle size and % drug retained before and after 3 months at 4±1°C and at ambient room temperature. Mean vesicle size was found to increase on storage after 3 months. The increase in vesicle size was more in the formulation stored at room temperature than at 4±1°C. The vesicle size of 128.90±3.85 and 155.33±9.1 nm was recorded at storage temperature of 4±1°C and ambient room temperature, respectively, compared to initial size of 94.2±8.1 nm. At 4±1°C, a minimum loss of the drug was observed, which may be attributed to the regidization of the vesicles at low temperature that reduced the permeability of the drug through the membrane. Thus, it is worth noting that the prepared vesicular systems are more stable at 4±1°C, as compared to storage at room temperature in terms of mean vesicle size and % drug retained.

In Vivo Studies Atenolol niosomal dispersion (ATN) and atenolol niosome/carbopol gel (ATNG) were selected for in vivo studies as they showed slower and sustained in vitro release of the drug. The chosen formulations were then compared with atenolol solution (ATS) and free atenolol/carbopol gel (ATG) in their IOP lowering efficacy. The relevant pharmacodynamic data are listed in Table 4.

![Fig. 6. Percentage Decrease in IOP after Administration of Atenolol Solution and Other Selected Formulations](image)

Each point represents the mean±S.D. ($n=6$).

Table 3. Kinetic Analysis of the Release Data of Atenolol from Niosomal Dispersion and Different Gel Formulations

<table>
<thead>
<tr>
<th>Formula code</th>
<th>Zero order Correlation coefficient ($r^2$)</th>
<th>First order Correlation coefficient ($r^2$)</th>
<th>Higuchi model Correlation coefficient ($r^2$)</th>
<th>Release mechanism</th>
<th>Korsmeyer–Peppas Correlation coefficient ($r^2$)</th>
<th>Release exponent ($n$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2</td>
<td>0.9690</td>
<td>0.9960</td>
<td>0.9970</td>
<td>Diffusion</td>
<td>0.9855</td>
<td>0.6634</td>
</tr>
<tr>
<td>G1</td>
<td>0.8606</td>
<td>0.8448</td>
<td>0.9531</td>
<td>Diffusion</td>
<td>0.9706</td>
<td>0.5074</td>
</tr>
<tr>
<td>G2</td>
<td>0.9495</td>
<td>0.9702</td>
<td>0.9812</td>
<td>Diffusion</td>
<td>0.9919</td>
<td>0.6026</td>
</tr>
<tr>
<td>G3</td>
<td>0.9072</td>
<td>0.9486</td>
<td>0.9783</td>
<td>Diffusion</td>
<td>0.9885</td>
<td>0.5878</td>
</tr>
<tr>
<td>G4</td>
<td>0.9347</td>
<td>0.9712</td>
<td>0.9856</td>
<td>Diffusion</td>
<td>0.9841</td>
<td>0.6234</td>
</tr>
<tr>
<td>G5</td>
<td>0.8880</td>
<td>0.9650</td>
<td>0.9840</td>
<td>Diffusion</td>
<td>0.9872</td>
<td>0.5018</td>
</tr>
<tr>
<td>G6</td>
<td>0.8679</td>
<td>0.9258</td>
<td>0.9796</td>
<td>Diffusion</td>
<td>0.9884</td>
<td>0.5089</td>
</tr>
<tr>
<td>G7</td>
<td>0.8996</td>
<td>0.9445</td>
<td>0.9790</td>
<td>Diffusion</td>
<td>0.9798</td>
<td>0.5330</td>
</tr>
<tr>
<td>G8</td>
<td>0.9301</td>
<td>0.9535</td>
<td>0.9779</td>
<td>Diffusion</td>
<td>0.9750</td>
<td>0.5414</td>
</tr>
</tbody>
</table>
Table 4. In Vivo Pharmacodynamic Parameters after Administration of Atenolol Solution and Other Selected Formulations

<table>
<thead>
<tr>
<th>Formula code</th>
<th>Pharmacodynamic parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_{\text{max}}$ (h)$^a$</td>
</tr>
<tr>
<td>ATS</td>
<td>1.00±0.00</td>
</tr>
<tr>
<td>ATG</td>
<td>2.00±0.00*</td>
</tr>
<tr>
<td>ATN</td>
<td>2.66±0.57*</td>
</tr>
<tr>
<td>ATNG</td>
<td>4.33±0.58*</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.D. ($n=6$). $^a$ p<0.05 versus atenolol solution (ATS). $^b$ a) Time for maximum response. b) Maximum percentage decrease in IOP. c) Area under percentage decrease in IOP versus time curve up to 8 h post-administration.

Gross examination of the rabbit eyes during this study showed no signs of abnormal lachrymation or increased blinking upon instillation of any of atenolol formulations. No irritation with the niosomal formulation was observed. The same finding was reported by other authors.\(^{70}\) Additionally, Guin, et al.\(^{71}\) reported that, no major changes were observed in histological photomicrographs of control corneal tissues and corneal tissues following instillation of multilamellar niosomal formulations composed of Span 60 and CH. This may be due to the fact that the irritation power of surfactants decreases in the following order: cationic>anionic>ampholytic>non-ionic, so the non-ionic surfactants are preferred for ocular delivery.\(^{72}\) Previous studies demonstrated that in vitro cytotoxicity is indicative of irritation potential.\(^{73}\) Cheong, et al.\(^{56}\) compared the in vitro cytotoxicity, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, of eight clinically available β-blockers (propranolol, alprenolol, atenolol, labetalol, metoprolol, pindolol, timolol, and bisoprolol) on human corneal epithelial and retinal pigment epithelial cell lines. Primary and immortalized corneal and retinal cell lines were compared for their susceptibility to the cytotoxic effect of the drugs. The cytotoxicity of β-blockers was also evaluated on human skin keratinocytes and fibroblasts in order to investigate susceptibility differences as a function of the tissue origin. Comparison of the IC\(_{50}\) of the eight β-blockers for different cell lines after 16 h demonstrated that atenolol was the least cytotoxic drug.\(^{74}\) Moreover, the cytotoxic effect of niosomal Ciclopirox olamine composed of Span 60 and CH was evaluated on KB (oral cancer), PC3 (prostate cancer), Siha (cervical cancer) and Vero (kidney epithelial) cell lines using MTT assay. The results indicated that blank niosomes (not containing Ciclopirox olamine) did not show any evidence of cytotoxicity on the cell lines chosen.\(^{75}\) In addition, Zarei, et al.\(^{76}\) studied the cytotoxicity of paclitaxel niosome composed of Span 60 and CH on MCF-7 cell line after 48 h by MTT assay. The authors found that the higher concentration of niosome devoid of drug did not affect the cell line, thereby, it was considered to be safe.\(^{76}\) In this study, no change in IOP was observed in the untreated eye during the course of measurement in any of the formulations, thus indicating that these formulations exerted a local action within the eye and that the observed IOP lowering activity is not because of any systemic absorption.\(^{77,79}\)

After instillation of ATS, the $T_{\text{max}}$ was reached after 1.00 h of instillation, followed by a rapid decline of the percentage decrease in IOP indicating its short duration of action. In case of ATG, $T_{\text{max}}$ was observed at 2.00 h, but the effect was not sustained sufficiently and diminished after 6 h. ATN showed a significant effect which was sustained for up to 7 h. On the other hand, ATNG showed promising results as it significantly decreased the IOP to maximum value (49.80%) after 4.33 h of drug administration, and the effect was sustained and prolonged for the time of the experiment up till 8 h. The $\text{AUC}$ after application of atenolol formulations until 8 h were 2.22, 2.85, 4.58-fold higher than that of atenolol solution for ATG, ATN, and ATNG, respectively.

The better reduction in IOP with niosomes may probably be due to the better partitioning of drug between vesicle and eye corneal surface. Furthermore, the release of drug from niosomes will increase its local concentration at the corneal surface; however, after release from the vesicles, drug molecules rely on passive diffusion to cross the corneal barrier. The corneal penetration enhancing effect of niosomes 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 favorable rheological properties.\(^{80}\) The longer the contact time at the corneal surface, the higher the bioavailability of the drug.\(^{31}\) This will also reduce the amount of drug and the dose frequency necessary for therapeutic effect.\(^{82}\) Thus, niosomes act as drug carriers which change the rate and extent of drug absorption resulting in the reduction of IOP for prolonged period of time.\(^{46}\)

Since, vesicular systems offer a great deal of advantages over the conventional systems, various pharmaceutical approaches can be tried to render their final formulation more effective. The best way to achieve this would be to enhance the precorneal retention. One such approach is the use of combinatorial drug delivery systems which are a promising trend in ophthalmic research, with the great potential of combining the advantages of various systems and overcoming their limitations.\(^{15}\) Mucoadhesive polymers when used in combination with vesicular systems provide vesicles with the necessary site adherence and site retention to achieve carrier and drug targeting in topical ocular therapy and endow them with the ability to be mucoadhesive.\(^{44,56}\) Carboxpols is an important class of ocular bioadhesives.\(^{79}\) Due to the low viscosity of colloidal suspensions of vesicles that does not allow sufficient retention time of the dosage form in the eye upon instillation, different hydrogel matrices such as carbopol have been used to increase the viscosity of topical preparation and to increase the retention time of the formulation at the site of administration due to good bioadhesive properties.\(^{53}\)

Our results have shown that 0.5% (w/v) atenolol niosomal hydrogel extended the duration of action for 8 h with 4.33 fold increase in the $\text{AUC}$ than that of 0.5% (w/v) atenolol solution. The IOP lowering activity of 1% (w/v) atenolol gel formulations was previously studied.\(^{39}\) It was found that, increasing the viscosity of the polymers was associated with the IOP lowering effect. The $\text{AUC}$ after application for the higher concentrations of the polymers were 3.2 and 5.4 fold higher than that of 1% (w/v) atenolol solution with duration of action of 6 and 8 h for 15% sodium alginate and 3% carboxymethyl cellulose, respectively.\(^{39}\) Based on these data, it is clear that, our formulation is significantly better considering that similar effect is obtained at half the concentration of the drug; 0.5% w/v versus 1% w/v reported earlier.\(^{39}\) This will lead to reduc-
ing the amount of the drug and the dose necessary for the therapeutic effect with subsequent limited systemic absorption and side effects. This study indicated that niosomal hydrogel combined two important features, long retention and sustained drug release which is essential and fruitful approach to provide a steady and prolonged release of atenolol into the eye.

CONCLUSION

In the last couple of years, continuous research has been going on for better delivery of anti-glaucoma drugs with the aim of more localized drug delivery and minimization of dosing frequency. An ophthalmic delivery system should preferably release the drug at a controlled rate to prolong the effect in reducing IOP and should be non toxic and comfortable for patient use. Our findings have shown that higher EE% of (80.7±1.2) was obtained from niosomes prepared using Span 60/CH at 2 : 1 molar ratio with particle size diameter of 94±8.1 nm. Niosomal hydrogel formulation using carbopol 934P significantly exhibited sustained in vitro release of atenolol compared with free drug solution and other polymeric hydrogels. In vivo study proved that niosomal hydrogel was found to show the most significant prolonged decrease in IOP compared with other drug formulations of the same concentration. In conclusion, niosomal hydrogel could be a promising delivery system for atenolol with improved ocular bioavailability and prolonged drug release profiles.

Acknowledgments The authors would like to express their heartfelt thanks to Prof. Dr. Osama Abd El-Azeem Soliman, the head of Pharmaceutics Department, Faculty of Pharmacy, Mansoura University, Egypt for his support in measuring the rabbits IOP.

REFERENCES

32) Saha P, Yang JJ, Lee VH. Existence of a P-glycoprotein drug efflux pump in cultured rabbit conjunctival epithelial cells. *Invest. Oph-


