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Design of liposomal colloidal systems for ocular delivery of ciprofloxacin

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Abstract   Ophthalmic drug bioavailability is limited due to protective mechanisms of the eye which require the design of a system to enhance ocular delivery. In this study several liposomal formulations containing ciprofloxacin (CPX) have been formulated using reverse phase evaporation technique with final dispersion of pH 7.4. Different types of phospholipids including Phosphatidylcholine, Dipalmitoylphosphatidylcholine and Dimyristoyl-sn-glycero-3-phosphocholine were utilized. The effect of formulation factors such as type of phospholipid, cholesterol content, incorporation of positively charging inducing agents and ultrasonication on the properties of the liposomal vesicles was studied. Bioavailability of selected liposomal formulations in rabbit eye aqueous humor has been investigated and compared with that of commercially available CPX eye drops (Ciprocin®). Pharmacokinetic parameters including Cmax, Tmax, elimination rate constant, $t_{1/2}$, MRT and $AUC_{0-\infty}$, were determined. The investigated formulations showed more than three folds of improvement in CPX ocular bioavailability compared with the commercial product.

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1. Introduction

Ciprofloxacin, a fluoroquinolone antibacterial agent, is active against a broad spectrum of aerobic gram-positive and gram-negative bacteria. It is currently the drug of choice as an anti-infective agent for the eye (Wilhelmus et al., 1993). The antibacterial efficiency of CPX results from inhibiting two enzymes involved in bacterial DNA synthesis leading to rapid bacterial cell death. The interaction between CPX and bacterial pathogen is not located in the blood stream but in peripheral tissues. Thus, effective antimicrobial therapy is dependent upon the delivery of sufficiently high drug concentrations to the target site of infection in order to inhibit bacterial growth

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or/and destroy the invading microorganism (Fisher et al., 1989). Efficacy of the marketed ophthalmic fluoroquinolone products, mostly aqueous solutions, is limited by poor ocular bioavailability, (Ludwig, 2005; Ke et al., 2010). Ciprofloxacin eye drops, which are available presently, need to be administered as 1–2 drops every 15–30 min initially in acute infection and 1–2 drops to be administered 6 times daily or more in severe conditions (Mundada and Shrikhande, 2008). It is known that CPX is the least soluble of the commercially available fluoroquinolones, with particularly low solubility near the pH of tears (~pH 7) (Ross and Riley, 1990). The formation of crystalline corneal deposits indicates a decrease in the concentration of drug dissolved in the tear film and, consequently, a potential decrease in drug bioavailability. Additionally, the presence of deposits in the tear film creates safety concerns (Wilhelmus and Abshire, 2003). Ophthalmic CPX commercial eye drop solutions are the most acidic (pH 4.5), which lead to local burning, irritation, itching and tearing.

Vesicular drug delivery systems such as liposomes have been used in therapeutic approaches for ocular abnormalities. Efficacy of liposomes as a carrier for drugs depends upon various factors such as charge, rigidity, composition of the liposomal membrane, encapsulation efficiency and release rate (Lee et al., 1985). It was reported that positively charged liposomes have a higher binding affinity to the corneal surface than neutral and negatively charged vesicles as a result of interaction of positively charged liposomes with the polyanionic corneal and conjunctival mucoglycoprotiens. It is known that the corneal epithelium is thinly coated with negatively-charged mucin to which the positive surface charge of the liposomes may be absorbed more strongly. This makes the study of positively charged liposomes necessary for optimizing CPX liposomal formulations with prolonged effect and for treating the interior eye tissues (El-Gazayerly and Hikal, 1997).

The aim of this study was to formulate liposomal colloidal dispersions of various compositions. The effect of formulation factors such as type of phospholipid, ratio of cholesterol content, presence of positive charging agent and ultrasonication on the characteristics of the liposomal vesicles was evaluated. Ocular bioavailability of CPX in rabbit eyes has been investigated using selected liposomal formulations and compared with a commercially available CPX eye drops (Ciprocin®). This was achieved by monitoring CPX in rabbit eye aqueous humor. Enhanced ocular bioavailability of CPX, thus, could be useful to improve the treatment of infected eye and prevention of post-surgical infection.

2. Materials and methods

2.1. Materials

Ciprofloxacin (CPX) was obtained from FlukaBiochemika, Buschs, Switzerland. Soybean L-α–Phosphatidylcholine(PC), L-α–Dipalmitoyl-phosphatidylcholine(DPPC), 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC), Stearylamine(SA), Dioctadecyldimethylammonium bromide (DODAB) and Cholesterol (CH) were obtained from Sigma–Aldrich, USA. Chloroform was purchased from BDL laboratory Ltd., Poole, England, UK. All solvents used in chromatographic assay were HPLC-grade and other used chemicals and solvents were

commercially available products of reagent grade and used as received.

2.2. Methods

2.2.1. High performance liquid chromatography (HPLC) assay of ciprofloxacin

A simple and highly sensitive isocratic reversed-phase high-performance liquid chromatographic method (RP-HPLC) proposed by Zotou and Miltiadou (2002) has been modified to be used for the determination of CPX. Shimadzu HPLC system consists of Intelligent HPLC pump, model no. LC-10ADVP and Rheodyne sample injector with 20 μl, model no. LC-SIL 10ADVP, Shimadzu Corporation, Japan have been used in the analysis. The mobile phase consisted of acetonitrile: methanol: acetate buffer (pH 3.6; 0.05 M) (10:20:70 v/v) containing 1% (v/v) acetic acid. The mobile phase was freshly prepared on each day of analysis, filtered through 0.45 μm Millipore filter and degassed. Separation of ciprofloxacin and anthranilic acid (Internal Standard) was achieved on a Kromasil 100, C18, 5 μm (250 × 4.6 mm i.d.) reversed-phase column(phenomenex, USA), using fluorescence detection(Fluorescence detector, model no. RF-10AXL, Shimadzu Corporation, Japan) with $\lambda_{exc} = 300$ nm and $\lambda_{emi} = 458$ nm. The operating temperature was ambient, with a flow rate of 0.8 ml/min.

2.2.2. Preparation of ciprofloxacin liposomes

Liposomal vesicles containing CPX were prepared using the reverse evaporation technique (REV) as described by Szoka and Papahadjopoulos (1978). Table 1 represents the molar ratios of the lipid components used in the different liposomal formulations with total lipid concentration of 66 μmol/ml for each formulation. The lipid components were weighed and dissolved in 15 ml chloroform. The solution was then transferred to a 250 ml round-bottomed flask. An aliquot of 5 ml isotonic phosphate buffer of pH 4.5 containing CPX was added to the chloroform mixture. The chloroform was then removed by rotary evaporation at 40 °C (Rotatory evaporator b̈chirotavapor model no. R210 from Labortechnik AG and vacuum pump model no. V-700 B̈chi, Flawil, Switzerland). The liposomal dispersion was then centrifuged at 30,000 rpm and 4 °C for 45 min (Centrifuge model no. D-7200 from Hitachi Company New Zealand). Supernatant was carefully removed and assayed for its free CPX content while the liposomal pellets were re-dispersed in isotonic phosphate buffer pH 7.4. The liposomal dispersions were then allowed to equilibrate at room temperature and kept overnight at 4 °C (Monem et al., 2000; Li et al., 1998; Gürsoy and Senyücel, 1997).

2.2.3. In-vitro evaluation of liposomal vesicles
2.2.3.1. Particle size and particle size distribution. The mean particle size and polydispersity index for each liposomal formula were determined by photon correlation spectroscopy using 90 Plus particle size analyzer, (Laser nanoparticle size analyzer model no. 90 Plus- software version 3.74, Brookhaven Instruments, Holtsville, New York, USA). The polydispersity index was used as a measure for particle size distribution of the liposomal vesicles' population. Liposomal dispersions were diluted 10 times with isotonic phosphate buffer pH 7.4 before

Table 1 Composition of CPX liposomal formulation (in molar ratio).

Formulations No.	Phospholipid component			CH	SA	DODAB
	PC	DPPC	DMPC			
F 1	1	–	–	–	–	–
F 2	1	–	–	1	–	–
F 3	2	–	–	1	–	–
F 4	7	–	–	1	–	–
F 5	7	–	–	1	2	–
F 6	7	–	–	1	–	2
F 7	–	1	–	–	–	–
F 8	–	1	–	1	–	–
F 9	–	2	–	1	–	–
F 10	–	7	–	1	–	–
F 11	–	7	–	1	2	–
F 12	–	7	–	1	–	2
F 13	–	–	1	–	–	–
F 14	–	–	1	1	–	–
F 15	–	–	2	1	–	–
F 16	–	–	7	1	–	–
F 17	–	–	7	1	2	–
F 18	–	–	7	1	–	2

analysis. Analysis was performed at 25 °C. Each reported value is the average of three measurements.

2.2.3.2. Determination of entrapment efficiency (EE%) of CPX in liposomes. The entrapment efficiency is defined as the ratio of the amount of the CPX encapsulated in liposome to that of the total drug in liposomal dispersion (Guard et al., 2007). The initial liposomal dispersions were centrifuged at 30,000 rpm and 4 °C for 45 min to remove the non-encapsulated drugs. The concentrations of CPX in the supernatant representing the amount of CPX out of the liposomal vesicles were determined using HPLC assay method.

The EE% of CPX was calculated as follows:

$$EE\% = (Mt - Mf/Mt) \times 100$$

where: Mf and Mt represent the free CPX amount and total CPX amount, respectively.

The EE% was determined to evaluate the effect of changing liposomal composition and ultrasonication on the properties of liposomes (Budai et al., 2007).

2.2.3.3. Effect of ultrasonication on the characteristics of liposomal vesicles. Ultrasonic homogenizer SONOPULS HD 2070 (Germany); with titanium flat tip probe of diameter 6 mm and processing frequency 20 kHz with 70 W power has been used. The liposomal sample was held within quartz glass cell. The volume of the sample solution was 3 ml. The quartz cell was fixed to the body of ice bath. The distance between the top of the sonication probe and the bottom of the quartz cell was kept constant (10 mm). The samples were sonicated at 25%, 50%, 75% and 100% of power 70 W for one minute to test the effect of sonication power on liposomal vesicle properties. The effect of sonication time for 0.5, 1, 1.5 and 2 min at 50% of power 70 W was also tested.

2.2.3.4. In vitro CPX release studies. In vitro release of CPX liposomes was performed by applying the dialysis diffusion technique (Avgoustakis et al., 2002), using the dissolution test apparatus USP II (Dissolution apparatus from Erweka DT, 6

Germany). Artificial tears of pH 7.4 (Paulsson et al., 1999) were used as the dissolution medium. The dialysis method was applied using cellulose acetate dialysis membrane of 12,000–14,000 molecular weight cut off (Dialysis sac molecular weight cut-off 12,000–14,000, Spectra Por® Dialysis membrane, Spectrum Medical Industries, Inc., USA). This membrane assures the permeation of the drug with retention of liposomal vesicles. The membrane was soaked in artificial tears for 12 h before use. Four ml of liposomal dispersion was placed in a glass cylinder having a length of 8 cm and diameter of 1 cm and dialysis membrane was fixed to opening of glass cylinder by a thread. Each glass cylinder was attached to the shaft of the dissolution apparatus (USP Dissolution tester) and descended down into a 50 ml beaker containing 10 ml of artificial tears as dissolution medium without touching the bottom surface of the beaker. The beakers were then placed into vessels of dissolution apparatus that contained about 100 ml of water to keep temperature at 34 ± 0.5 °C. The glass cylinders were adjusted to rotate at a constant speed of 20 rpm (Hosny, 2010). One ml of dissolution medium was withdrawn at predetermined time intervals (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8 h).The samples were replaced with fresh dissolution medium to maintain constant volume. Drug concentrations in samples were analyzed using the HPLC method as described previously. The release experiments were carried out in triplicates and the mean ± SD were recorded.

2.3. In-vivo evaluation

2.3.1. Animal

Male New Zealand white Albino rabbits were selected as the model animal for in vivo study. The rabbits (weighed 2–2.5 kg) were free of any gross ocular defects. The rabbits were starved for 24 h before allowed to free access to water. Procedures involving animals were conducted in accordance with US guidelines as found in the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 18–23, 1985). Animals were maintained in accordance with the recom-

mendations in King Saud University Guide for the Care and Use of Laboratory Animals, approved by Animal Care and Use Committee.

2.3.2. Study design

Three liposomal formulations were selected for in vivo evaluation, namely F6, F12 and F13. Bioavailability test was done after sonication of the three formulations (F6, F12 and F13) for one minute using sonication power of 25%. The final particle sizes were 387, 388 and 410 for F6, F12 and F13 respectively. While EE% was 39.6, 37.1 and 36.7 for F6, F12 and F13 respectively. The selection of these formulations based on deferent lipid content, presence of positive charge inducing agent as well as the higher entrapment efficiency with the smallest possible particle size for DMPC. The dose of aliposomal formulation was dropped in the right eye while the commercial 0.3% ciprofloxacin solution Ciprocin® (Riyadh pharma, Riyadh, Saudi Arabia) was dropped in the left eye. The restricted dose volume of 50 µl was placed in the lower conjunctival sac, approximately midway between the inner and outer canthus. For each formulation, the animal was killed after taking a sample from eye aqueous humor at each of the following time intervals: 0, 0.5, 1, 2, 3, 4 and 5 h post installation. The samples were withdrawn, by paracentesis with a 23 gauge 1.25 cm needle attached to 1 ml syringe inserted through the corneal-scleral junction and slightly upward into the anterior chambers. All animals in the study conformed to the guidelines for animal experimentation in the King Saud University. The aqueous humor samples (200 µl each) were immediately centrifuged at 20,000 rpm and 4 °C for 20 min. The supernatant was then collected and frozen at −20 °C pending assay. Samples were suitably diluted with mobile phase and an aliquot of 20 µl of resultant solution was directly injected into the HPLC system.

2.3.3. Pharmacokinetic analysis

The pharmacokinetic parameters were calculated from the data of analysis of CPX level in rabbit aqueous humor samples and presented as mean ± SD. Maximum CPX aqueous humor concentration (C_{max}) and the time to reach this maximum (T_{max}) were determined. The total area under the aqueous humor concentration–time curve (AUC_{0-5}, µg.min/ml) and the area under the first momentum curve ($AUMC_{0-5}$) up to the last time point were estimated based on the trapezoidal rule. The area of the tail was calculated using the aqueous humor concentration at the last time point and the terminal elimination rate constant (k). The value of k from aqueous humor was estimated from the least square regression analysis of the final segment of the curve. The mean residence time (MRT) values were calculated as the ratio of the calculated $AUMC_{0-\infty}$ to $AUC_{0-\infty}$ in each case. The rate of absorption was also calculated using the relation $C_{max}/AUC_{0-\infty}$. Relative bioavailability (RB) was measured by comparing bioavailability of formulation with that of the commercial product (Ciprocin®).

3. Statistical analysis

One- way analysis of variance (ANOVA) with multiple comparisons was performed to test for significance. The pharmaco-

Table 2 Physical characterization of liposomal formulations.

Formulation no.	Particle size (ng)	Polydispersity	Entrapment Efficiency % (EE %)
F1	461 ± 28	0.167	44.7
F 2	512 ± 84	0.203	39.6
F 3	495 ± 21	0.247	40.6
F 4	442 ± 10	0.275	42.5
F 5	528 ± 36	0.247	40.6
F 6	530 ± 25	0.278	41.9
F 7	420 ± 25	0.150	42.2
F 8	788 ± 121	0.165	37.3
F 9	572 ± 33	0.147	39.6
F 10	542 ± 41	0.181	39.9
F 11	576 ± 52	0.205	40.3
F 12	619 ± 71	0.237	39.4
F 13	580 ± 197	0.940	40.2
F 14	1047 ± 41	0.139	36.4
F 15	925 ± 127	0.005	38.4
F 16	748 ± 12	0.128	40.5
F 17	772 ± 103	0.024	39.1
F 18	769 ± 139	0.171	38.2

kinetic parameters were subjected to ANOVA at a significant level of $P \leqslant 0.05$.

4. Results and discussion

4.1. Effect of type of phospholipids on particle size and EE% of CPX Liposomes

The average particle size of liposomal vesicles is an important parameter with respect to physical properties, biological fate and their drug entrapped property. Table 2 shows the effect of type of phospholipids on mean particle size and particle size distribution. Generally, all liposomal preparations showed small particle in the colloidal range. It was clear that DPPC gave the smallest particle size compared with PC and DMPC and this was in agreement with López-Pinto et al. (2005). DMPC phospholipid gave the largest particles which were also characterized by large particle size distribution as indicated from standard deviation and polydispersity index values.

Table II shows also the values of EE% for CPX of liposomes prepared with different types of phospholipids. The results indicated that the highest EE% was obtained for PC compared with DPPC and DMPC formulations. It can be concluded that the EE% was enhanced by increasing the number of carbons in the branched leaving group of phospholipids. Liposomes formulated with PC molecules containing 42 carbon atoms showed 44.7 EE% while, DPPC of 40 carbon atoms showed 42.2 EE% and DMPC of 36 carbon atoms showed the least value of 40.2 EE%. Thus, a correlation between EE% and chain length of phospholipid present in the lipid formulation was observed. The values of EE% were found to be in a descending order as follows: PC (No. of Carbons = 42) > DPPC (No. of carbons = 40) > DMPC (No. of carbons = 36). Similar results were obtained by Ravivarapu and White (1996), McIntosh (1978), Gulati et al. (1998) for both water soluble and insoluble drugs. They observed that entrapment in liposomes increased with an increase in the carbon chain length of PC employed.

4.2. Effect of cholesterol content on particle size and EE% of CPX liposomes

Cholesterol is the most common membrane additives found in vesicular systems. It is important to study the effect of CH on physical properties of liposome since CH influences a number of membrane properties such as ion permeability, aggregation, fusion processes, elasticity, size and shape. Inclusion of CH in membrane increases the rigidity of the bilayer and reduces the leakage of water soluble substances through membranes.

The effect of CH concentration on average particle size of CPX liposome was studied at different phospholipid:CH molar ratios (Tables 1 and 2).The results indicated that, generally, liposome particle size increases linearly with the increase of CH concentration. It is well accepted that incorporation of CH imparts rigidity to the bilayer membranes; thus, improve the physical stability for liposomal systems (Taylor et al., 1990; Essa, 2010).

On the other hand, the encapsulation parameters depend on the fluidity of the phospholipid bilayer. The lower the fluidity, the greater is the encapsulation capacity. The fluidity of bilayers can be controlled by selection and combination of lipids. Increasing EE% in the absence of CH could be due to the fact that CH increases the hydrophobicity of the interfacial region of the bilayers (Abdelbary, 2011) which is not in favor of the entrapment of CPX at the acidic pH during the formation of liposomal vesicles.

It is worthy also to mention that incorporation of CH at low concentrations into bilayer leads to an increase in the trans-membrane permeability, whereas incorporation of higher amounts of CH to some extent can eliminate phase transition and decrease the membrane permeability (Lian and Ho, 2001). These results are also in good agreement with Abdelbary (2011) who studied improvement of the ocular bioavailability of CPX through the preparation of ocular mucoadhesive chitosan coated liposomes. The increase in CH molar concentration resulted in a significant decrease in EE%. This confirms that the presence of high concentrations of CH is not in favor of CPX entrapment (Abdelbary, 2011). Thus, the effect of CH

concentration on EE% of CPX can be explained by conflicting factors which are the increased hydrophobicity and decreased permeability of the bilayer (Mohammed et al., 2004).

4.3. Effect of introducing positive charge on particle size and EE% of CPX liposomes

Charged molecules are additives which are often included in the liposomal bilayer structure with the aim of improving stability via electrostatic means. Using a charge-inducing agent let all liposomes to carry the same electrical charge which produces electrostatic repulsion between liposome vesicles, thus making liposomes to remain discrete and preventing vesicle flocculation, aggregation and fusion. Moreover, preparing charged vesicles was sometimes aimed to improve the therapeutic efficiency of vesicles, as charged liposomes were found to be selectively taken by certain tissues in the body including the eye cornea.

Retention of liposomes on the corneal surface perhaps represents the major challenge for effective ocular drug delivery. Several studies have shown that introducing a positive surface charge on vesicles can prolong precorneal retention time, enhance ocular bioavailability and ultimately augment or increase the duration of pharmacological effect. Liposomes with a positive surface charge may form a more stable adsorption because corneal epithelium is thinly coated with negatively charged mucin. Most studies have used SA to impart a positive surface charge to liposomes (Meisner and Mezei, 1995).

Both SA and DODAB can be used as charge-inducing agents to impart a positive charge to the liposomes' surface. At 7:1:2 M ratio of phospholipid:CH:SA or DODAB, the effect of charge on the particle size of liposome was studied compared with uncharged liposome as control. It was clear that positively charged liposomes formulated from different phospholipids have a mean particle diameter larger than that of neutral liposomes. The effect of inclusion of charged molecules into bilayers with an increase of particle size was in agreement with previous studies (Van Hal et al., 1996). The developed charge could create mutual repulsion between liposome bilay-
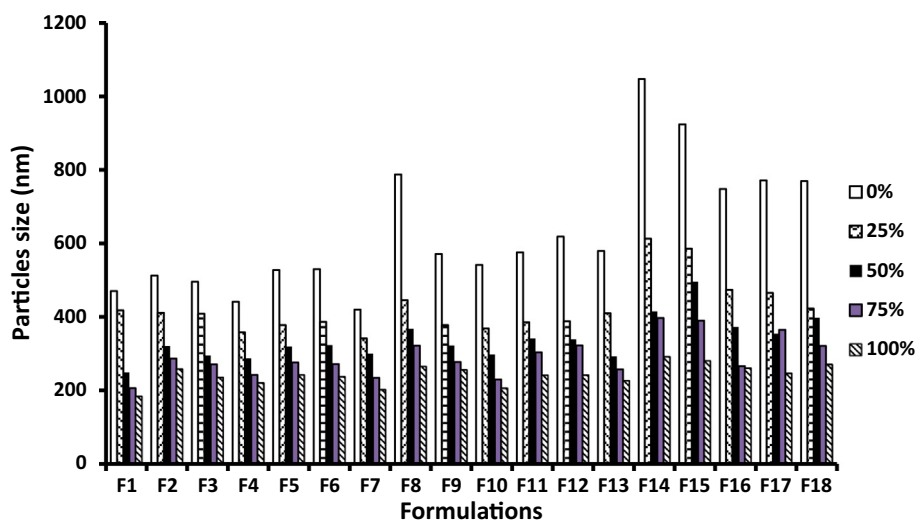

Figure 1 Effect of sonication power (applied for 1 min) on particle size of liposomal formulations.
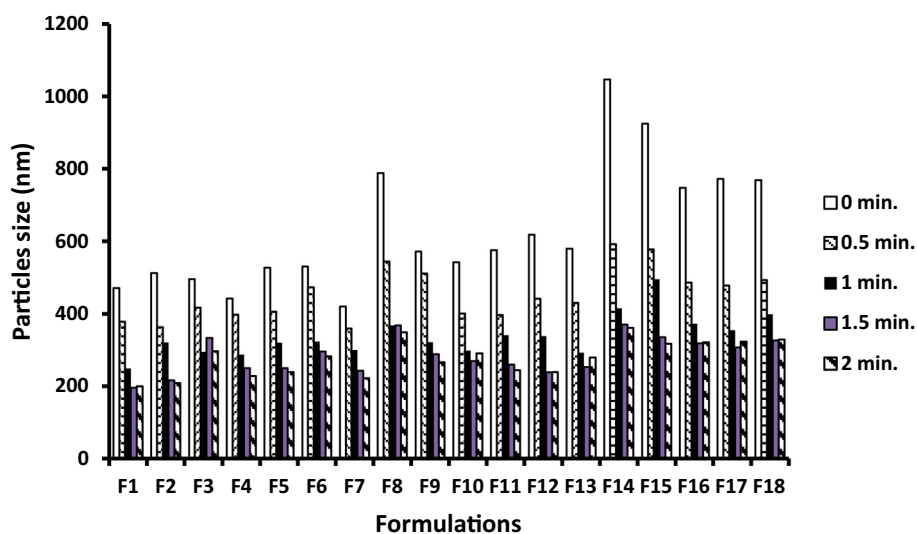
Figure 2 Effect of sonication time (using 50% sonication power) on particle size of liposomal formulations.
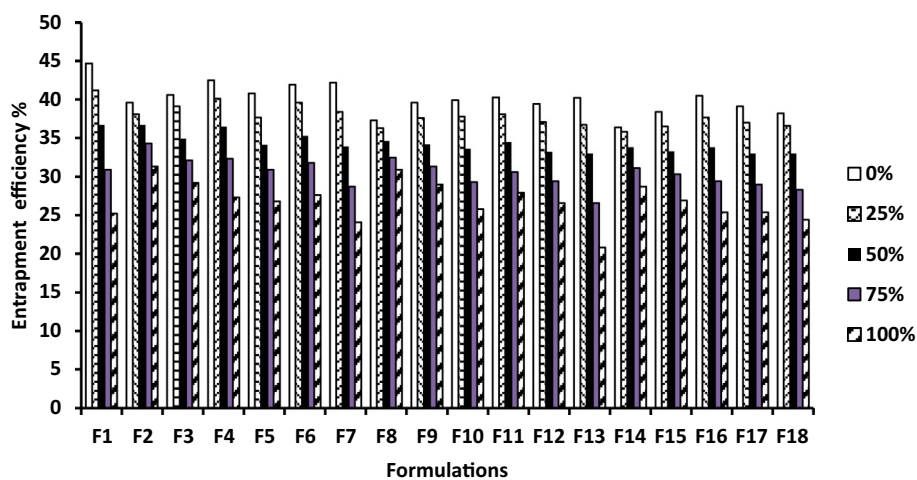

Figure 3 Effect of sonication power (applied for 1 min) on EE% of liposomal formulations.
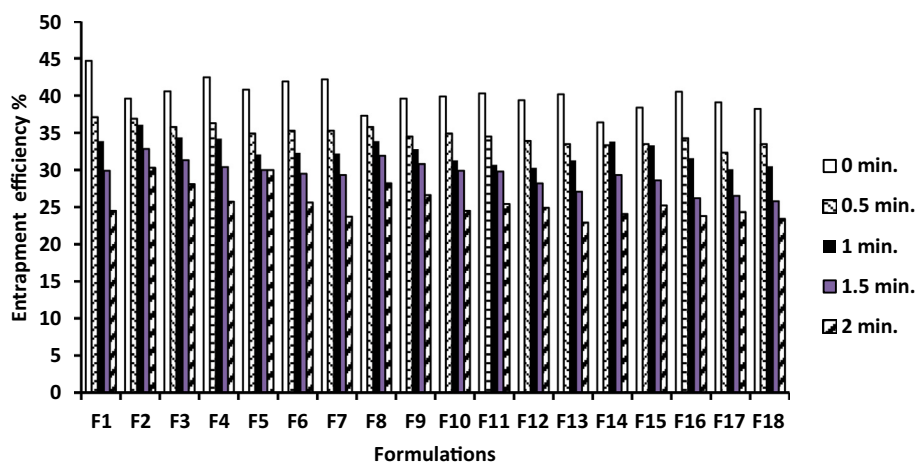

Figure 4 Effect of sonication time (using 50% sonication power) on EE% of liposomal formulations.

ers and hence increases particle size. The nature and molecular structure of the ionized species affected the extent of size enlargement. Generally, DODAB showed a non-significant increase in particle size compared with SA for PC and DPPC and this was not clear for DMPC.

It was also observed that the presence of charge inducing agent decreases to a little extent the leakage of CPX from the liposomes compared with neutral liposomes as indicated from the small decrease in EE% values at the used ratio of the charging agents.

4.4. Effect of sonication power and time on the particle size and EE% of CPX liposomes

Figs. 1 and 2 show the effect of sonication power and sonication time, on CPX liposomal vesicle particle sizes. It was clear that % sonication power (at 70 W) has a great effect on liposomal particle size. Particle sizes were decreased upon ultrasonication and this effect was high after sonication power of 25%. On the other hand, a gradual decrease in particle size was observed with increasing sonication time (at 50% power) up to 1.5 min. It was also noticed that increasing sonication time more than 1.5 min did not show a significant effect on particle size in many cases. In addition, the presence of CH may retard the decrease in particle size. The increase of vesicle rigidity in the presence of CH could be the reason for this observation.

On the other hand, the effect of % of sonication powers applied for 1 min on the encapsulation efficiency is shown in Fig. 3. The results demonstrated that the encapsulation efficiency decreased with increasing sonication power. This could be because sonication can interrupt the packing of liposomal structure and this interruption is great at high sonication power. Also, with the increase of sonication power, the decrease of encapsulation efficiency may be attributed to the high input of energy so that the liposomes from multilamellar vesicles turned to unilamellar vesicles and some amount of CPX was leaked from vesicles (Lasic, 1993).

It was also observed that the high cholesterol content of liposomes can resist CPX leakage with recorded high EE% as shown with formulae F2, F8 and F14. This was also clear even at high sonication power. The high EE% in the presence of CH was attributed to the ability of CH to cement the leaking space in the bilayer membranes, which in turn allowed enhanced core material levels inside the liposomes (Ding et al., 2009).

The values of EE% for CPX in different liposomal formulations after sonication for different times at 50% sonication power are also shown in Fig. 4. It was observed that EE% was decreased with increasing sonication time from 0.5 to 2 min. The presence of CH clearly resists the leakage of CPX from liposomal structure upon sonication for some time. It was also noticed that the presence of charge inducing agent decreases to small extent the leakage of CPX from the liposomes upon sonication compared with neutral liposomes as indicated from EE% values. However, this was not confirmed in all cases.

4.5. In vitro release study

The percent cumulative amounts of CPX released as a function of time from liposomes formulated with different phospholip-

ids are illustrated in Fig. 5 (A–C). The release study was conducted in artificial tears at 34 °C to represent eye surface environment in vivo. All the liposomal formulations of different compositions showed a small initial burst followed by gradual CPX release. The slowest drug release was observed
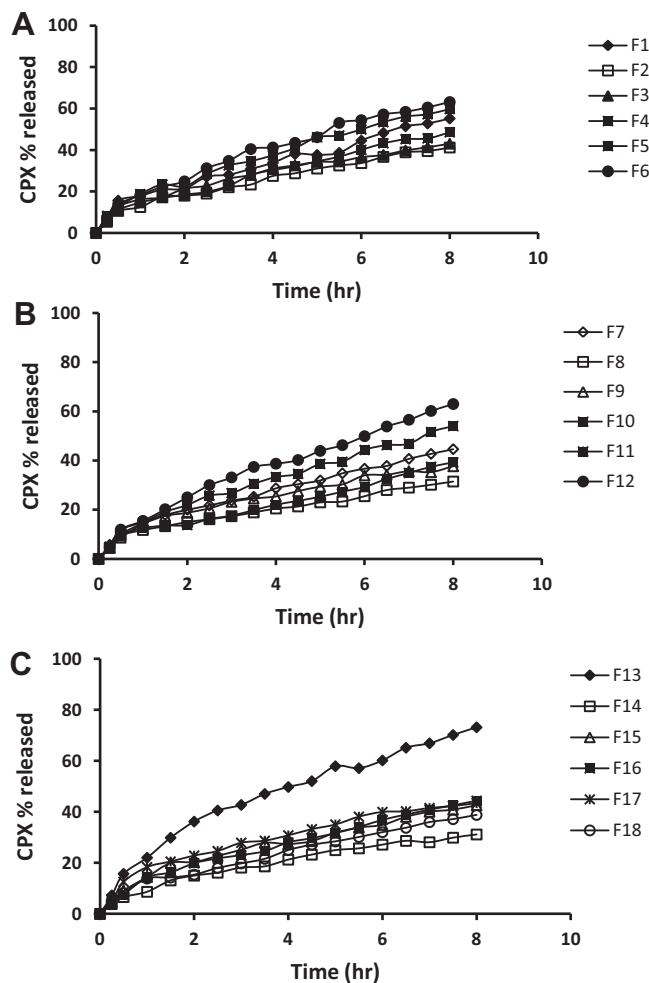

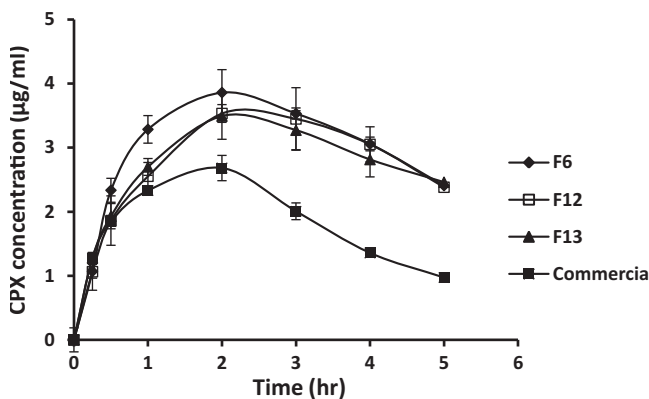**Figure 5** In vitro release profile of CPX from different liposomal formulations.

Figure 6 Concentrations of CPX from different liposomal formulations in rabbit aqueous humor as well as the commercial CPX.

Table 3 Pharmacokinetic parameters of CPX in aqueous humor following topical application of 50 μl of various liposomal formulations and commercial CPX solution.

Parameters	F6	F12	F13	Commercial CPX
Dose (μg)	114.52	107.63	109.9	150
T_{max}(hr)	2	2	2	2
C_{max}(μg/ml)	3.87 ± 0.217	3.66 ± 0.144	3.56 ± 0.177	2.68 ± 0.053
K (h^{-1})	0.192 ± 0.040	0.186 ± 0.068	0.154 ± 0.033	0.36 ± 0.060
$t_{1/2}$	3.77 ± 0.781	4.03 ± 1.120	4.716 ± 1.170	1.96 ± 0.312
MRT (h)	3.69 ± 0.114	3.81 ± 0.225	3.90 ± 0.155	2.92 ± 0.097
$AUC_{0-\infty}$ (μg h/ml)	28.3 ± 2.460	28.27 ± 5.440	30.29 ± 4.270	12.05 ± 0.534
Relative bioavailability	3.1	3.3	3.4	–

for liposomal formulations containing DPPC followed by that containing PC.

It was also noticed that the in vitro CPX release from liposomal formulations is dependent on CH contents. The increase in CH resulted in a decrease in the amount of CPX release in 8 h from the prepared liposomes. The rigidity of liposomal vesicles in the presence of CH could be the reason for the slow release of CPX at high CH concentrations. The same findings were obtained by Abdelbary where, slow release of CPX was obtained at a high content of CH [26]. It seems also that charging agents have no appreciable effect on the CPX release rate from the prepared formulations.

4.6. Bioavailability study

Albino rabbit eye has the property that it simulates an adult human eye with respect to size, shape, physiology, and composition of tears and was chosen as the model animal for the study. Fig. 6 shows CPX aqueous humor concentration versus time curve for the selected formulations compared with the commercial product Ciprocin®. Table 3 shows the pharmacokinetic parameters of liposomal formulations as well as commercial eye drops (Ciprocin®). Maximum aqueous humor concentration (C_{max}) was achieved (3.87 μg/ml) from F6 while C_{max} for F12 and F13 were 3.66 and 3.56 μg/ml, respectively compared with 2.68 μg/ml for Ciprocin®. Time to reach maximum concentration (T_{max}) showed no significant differences between formulations ($p > 0.05$), their values were 2 h in all cases. The values of $AUC_{0-\infty}$ for liposome formulations F13, F6, and F12 were 30.29, 28.30 and 28.27 μg min/ml, respectively, which were significantly higher ($p < 0.05$) than that obtained for commercial eye drops ($AUC_{0-\infty}$ = 12.05 μg min/ml). These values confirmed a superior ocular bioavailability of CPX when applied as drops to the eye in the form of liposomes compared with the commercial eye drops.

Table 3 shows significant differences between the values of C_{max}, $t_{1/2}$, $AUC_{0-\infty}$ and MRT for liposome formulations and commercial product in favor of liposomes. While, there was no significant difference found between the selected formulations regarding C_{max}, $AUC_{0-\infty}$ and MRT. The increase in the values of $AUC_{0-\infty}$ indicates a high extent of drug absorption through the cornea of the eye. The larger value of $t_{1/2}$ for liposomal formulations compared with that of the commercial may indicate longer time for the disappearance of CPX from aqueous humor. This was also supported by the significant high values for MRT which indicates an enhanced residence time of the drug in the eye. The rate of absorption of CPX from all liposomal formulations was significantly higher than that of commercial product ($p < 0.05$) indicating a higher absorption rate through the eye tissues including cornea. It was also clear that the selected liposomal formulations have significant higher bioavailability compared with the commercial products as indicated from the relative bioavailability. The relative bioavailability for the liposomal formulations compared with the commercial formulation (Ciprocin®) can be arranged in a descending order as follows: F13 with relative ocular bioavailability of 3.4 > F12 with relative bioavailability of 3.3 > F6 with relative bioavailability of 3.1. Thus, all the tested liposomal formulations showed improved CPX ocular bioavailability for more than three folds compared with the commercial eye drops.

5. Conclusion

Based on the determined pharmacokinetic parameters for ocular bioavailability of CPX (C_{max}, T_{max}, $t_{1/2}$ and $AUC_{0-\infty}$, rate of absorption and MRT) using aqueous humor CPX assay data, the selected liposomal colloidal formulations showed a significant improvement in ocular bioavailability of CPX compared with the commercial aqueous solution (Ciprocin®). This finding reveals that the application of the selected dosage forms can improve CPX ocular bioavailability due to minimum loss of the applied dose due to minimum lacrimation (pH 7.4), enhanced residence time of the drug in the eye, and more prolonged effect in addition to higher chance for corneal penetration of the drug encapsulated in liposomal carrier. Furthermore, the advantages of less drug to be applied on the eye (small CPX dose) with more chance for drug penetration through the cornea to the inside tissue of the eye should be considered. Thus, the proposed dosage forms have more ability to treat the surface of the eye as well as internal tissues that may be needed in post-operative cases.

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