RESEARCH PAPER

Preparation and *in-vitro* evaluation of fluorometholone cubosomes for ocular delivery

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ABSTRACT

Objective(s): In this study, ocular drug delivery systems with a dispersed lipid liquid crystal (cubosomes) containing fluorometholone were used for sustained release and increased permeability to the eye.

Materials and Methods: To obtain the best Cubosomes, 6 formulations (F) were prepared. To prepare the F1, glycerol monooleate (GMO) and water containing fluorometholone were vortexed. After one week, when the liquid crystal gel formed, 0.5 g of the liquid crystal gel was added to 9.5 g of a 1% (w/w%) aqueous solution of Polaxamer F-127, and the mixture were homogenized and sonicated.

Results: The data showed that increasing the weight of gel from 0.5 g to 1.0 g (F2) did not result in a significant increase in drug loading, indicating that increasing the GMO concentration did not affect drug loading. The addition of cyclodextrin to the formulation (F3), along with an increase in cyclodextrin concentration from a molar ratio of 5:1 to 10:1 (F4), did not create a significant alternation in drug loading. Furthermore, the addition of phosphatidyl choline (PC) to the GMO (F5) did not cause a significant change in drug loading. Finally, in formulation F6 (in which GMO, Polaxamer, and the drug was dissolved in ethanol, the ethanol was removed, and the mixture was dispersed in water) the resulting cubosomes showed a higher drug loading efficiency compared to other formulations. Accelerated stability studies of optimal formulation (F6) according to the ICH Q1A(R2) guideline demonstrated no significant changes in physical characterization and *in-vitro* release evaluation, indicating complete formulation stability.

Conclusion: Cubosomes can be used as suitable carriers for fluorometholone delivery to eye.

Keywords: *Fluorometholone, Liquid crystal, Ocular, Stability*

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INTRODUCTION

Fluorometholone is a local corticosteroid and anti-inflammatory agent used for the treatment of keratoconjunctivitis and to improve post-surgical eye inflammation. However, upon entering the eye and passing through the cornea, the drug undergoes metabolic reactions and is converted to dehydrofluorometholone, which reduces its effectiveness. Systemic absorption of these drugs can lead to various side effects, including growth retardation in children [1]. This drug is lipophilic and only slightly soluble in water, hence it is formulated as a 0.01% suspension [2]. Eye drops are generally the simplest method for drug delivery to the anterior part of the eye. However, after 0.5 to 1 min of use, they are rapidly eliminated by tears and ocular clearance mechanisms, resulting in reduced bioavailability of the drug to less than 5%, and requiring multiple applications throughout the day [2]. Novel drug delivery systems can significantly help overcome the limitations of eye drops [3]. Various methods have been tested to increase the solubility and permeability of fluorometholone, including the

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use of nanocrystal forms [4], cyclodextrin solvent assistance [2], and soluplus® polymer [3].

Lipid nanocarriers improve the bioavailability of ophthalmic drugs by increasing their residence time and corneal permeability. The lipid components present in these lipid nanoparticles interact with the tear lipid layer, allowing them to remain in the conjunctival sac for a long time and act as a reservoir for drug release over time. Additionally, lipid nanoparticles can inhibit the drug efflux transporter glycoprotein P and open tight junctions between cells, allowing the drug to penetrate deeper layers of the eye and thus increase the bioavailability of the prescribed drugs through the eye [5]. Furthermore, due to the structural similarity of the two lipid bilayers of these compounds to the cell membrane, they can easily fuse with the membrane and deliver the drug to lower layers. These lipid nanocarriers include liposomes, solid lipid nanoparticles (SLN), nanostructured Lipid Carriers (NLC), and liquid crystalline nanoparticles [6]. Lyotropic lipid- liquid crystals are used to control the release rate of both lipophilic and hydrophilic drugs. The liquid crystal systems are composed of amphiphilic molecules that transform into cubic, hexagonal, and lamellar phases. Their structures are made up of lipid bilayers and aqueous channels with the minimum possible geometric shape. Cubosomess are dispersed colloidal particles of liquid crystalline cubic phases that are typically made using a high-pressure homogenizer with a combination of monoolein (glycerol monooleate (GMO)) or phytantriol, water, and poloxamer 407 as a stabilizer [6]. Due to their strong electrostatic repulsive forces and relatively high lipid bilayer content, cubosomess have greater physical stability than liposomes. Their structure is similar to biological membranes, which allow lipid carriers to be integrated with the lipid layers of the skin or any type of mucosal cell. Therefore, cubosomes are promising systems for ocular drug delivery due to their biocompatibility, ability to penetrate the cornea, suitable surface adhesion, low cost of raw materials, increased solubility of poorly soluble drugs, and high ability for slow drug release [7]. Numerous studies have been conducted on corneal drug delivery using cubosomess for Pilocarpine [8], Ketorolac [9], Dexamethasone [10], Timolol [8], Flurbiprofen [11], and Vancomycin [12] with the help of different lipids, which have shown an increase in drug retention time on the cornea, penetration, and bioavailability compared to eye drops containing the drug alone. The findings on drugs prepared with cubosomes-based carriers have demonstrated their non-toxicity and non-irritating property for ocular formulations. One study on the formulation of liquid crystalline ketorolac has shown a considerable increase in corneal penetration and retention time (about 2 times) compared to simple ketorolac eye drops [9]. The area under the curve (AUC), time to maximum concentration (T_{max}), and mean residence time (MRT) of Fluorbiprofen cubosomes were significantly greater than those of simple Fluorbiprofen eye drops [11]. Additionally, Timolol maleate cubosomes showed longer retention time in the eye and better intraocular pressure-lowering effects than commercial Timolol eye drops [8]. A study on the corneal permeability of Sertaconazole drug in in vitro conditions showed the ability of cubosomes to increase steady-state flux and permeability coefficient compared to sertaconazole suspension [13].

Since the release of flurometholone may be low in the liquid crystalline dispersion system, in this study, the drug-cyclodextrin complex is used to control and increase drug release and solubility. Cyclodextrins are divided into three natural categories, alpha, beta, and gamma, containing 6, 7, and 8 glucopyranose units, respectively, which are connected to each other through a 1,4-alpha bond. Synthetic derivatives of cyclodextrins such as hydroxypropyl beta, sulfobutyl ether beta, and hydroxypropyl gamma have been synthesized to increase their solubility. Studies on various cyclodextrins have shown their ability to increase the solubility of Flurometholone. Among cyclodextrins, the best solubility increase has been observed for sulfobutyl ether beta and hydroxypropyl gamma [2].

In this study, a lipid-liquid crystal dispersion system (Cubosomes) is prepared for flurometholone drug and is expected to have fewer side effects, longer retention time, and greater penetration compared to standard direct Flurometholone eye drops. So far, no studies have been conducted on ocular drug delivery of flurometholone and the flurometholone-cyclodextrin complex of sulfobutyl ether beta using cubosomes systems.

MATERIALS AND METHODS

Materials

Soybean phosphatydile choline (SPC) and Glycerol

monooleate (GMO) were purchased from Dinfen Chemical Technology Company (Qingdao, China). Flurometholone powder (purity 99.99 %) was supplied from Sina Darou Co. (Tehran, Iran). Polaxamer F-127, absolute ethanol and other chemicals and reagents were purchased from Merck (Darmstadt, Germany).

Analysis of flurometholone using HPLC

To draw the standard curve, concentrations of 0.3906, 0.7812, 1.5625, 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL were prepared and the area under the curve (AUC) was determined using an HPLC instrument. The AUC was then used to calculate the linear equation for the area under the curve as a function of concentration, as well as to calculate the limit of detection (LOD) and limit of quantification (LOQ). For the HPLC analysis of the drug, a C18 column (Sepachrom, 4.6×150 mm, 5 µm) was used with a mobile phase consisting of methanol and water in a ratio of 60:40 and a flow rate of 1 mL/min at ambient temperature. The wavelength used was 457 nm, the run time was set at 9 min, and injection volume 20 µL. Using Excel software, the standard error of the line (STEYX) and the slope of the curve relating to the standard concentrations were calculated based on the AUC. The following formulas were then used to calculate the LOD and LOQ:

$$LOD = 3.3 \times (STEYX/Slope) \quad (1)$$

$$LOQ = 10 \times (STEYX/Slope) \quad (2)$$

Preparation of formulation

F1: 350 mg of GMO were transferred to a 2 mL microtube, to which 150 mg of deionized water and 10 mg of fluorometholone were added. The mixture was vortexed at 50 °C until a white-colored solution was obtained. The headspace of the microtube was replaced with nitrogen gas and sealed with parafilm tape. The mixture was then left at room temperature for one week to form a liquid crystal gel. After one week, 0.5 g of the liquid crystal gel was added to 9.5 g of a 1% (w/w%) aqueous solution of Pluronic F127, as a surfactant and stabilizer, and the mixture was placed in an ice bath and homogenized with a homogenizer (4000 rpm for 5 min) and sonicator probe with maximum power for 5 cycles (each cycle for 1 min and 15 s interval between each cycle) to obtain a homogeneous solution.

F2: The effect of increasing the liquid crystal gel from 0.5 g to 1.0 g: This formula involves increasing the GMO amount from 350 mg to 700 mg, as well as raising the water volume for preparing the gel from 150 mg to 300 mg. 1.0 g of the liquid crystal gel was added to 9.0 g of a 1% (w/w%) aqueous solution of Pluronic F127. The remaining steps are analogous to those of F1.

F3: Comparing the effects of adding Cyclodextrin-SBEβ: First, a gel was prepared by adding 350 mg of lipid and 150 mg of water and vortexing at 50 oC for one week. After a week, a solution containing 9.5 g of 1% Pluronic F127, 730 mg of cyclodextrin, and 150 mg of flurometholone with a molar ratio of 5 to 1 was added to the gel. Homogenizer and sonicator probe were employed to achieve homogeneity.

F4: Comparing the effects of increasing Cyclodextrin-SBEβ amount: This formulation is similar to formulation 3, but the molar ratio of cyclodextrin to flurometholone was changed to 10 to 1.

F5: Comparing the effects of adding phosphatidylcholine (PC) to GMO at a 50:50 weight ratio: PC and GMO, each weighing 175 mg, were mixed at 80 oC using a bath sonicator. Next, 150 mg of deionized water and 10 mg of fluormetholone were added to create the initial gel. After a week, 9.5 g of a 1% Pluronic F127 solution was added to the mixture, and a homogenizer and sonicator probe were used to achieve homogeneity.

F6: Comparing the effects of addition of absolute ethanol: 10 mg of fluormetholone along with 2 mL of absolute ethanol (99.5% ethanol) and 95 mg of Pluronic F127 were combined to become completely transparent. After that, 350 mg of GMO was added to the mixture and thoroughly homogenized. Finally, the above solution was placed at a temperature of 80 oC to completely evaporate the alcohol. After one week, 9.5 g of water was added to the mixture and then homogenized and sonicated.

Characterization of formulations

Determination of cubosomes size

During the stability study, we used dynamic light scattering (DLS) with a Malvern Zetasizer ZS instrument (Malvern, UK) to determine the particle sizes, size distributions, and zeta potential of the cubosomes. Measurements were taken at a scattering angle of 90° using a He-Ne laser with an incident beam wavelength of 633 nm.

Evaluation of viscosity

According to the formulation used in the

eye, the prepared formulations should have appropriate viscosity to avoid any issues for the patient during use. Therefore, the viscosity of the prepared solutions was examined using a viscometer. To do this, 3.0 mL of the formulation was transferred to a Brookfield viscometer. The Brookfield viscometer is a type of rotational viscometer that consists of a spindle, which looks like a double cone, and rotates in a cylindrical container called a sample chamber. The spindle is connected to the motor of the device by a screw located at the end of its handle. We attached the spindle to the device, placed it in the sample chamber, and rotated it at predetermined angular velocities. The viscometer allowed us to obtain data on different angular velocities, such as 1, 5, 10, 20, 50, and 100 rpm at a temperature of 25 °C, by adjusting the speed using a screw [14].

Morphology of Cubosomes

Mesophase formed in the structure of a liquid crystal were evaluated using polarized optical microscopy using cross-polarized conditions. A drop of the formulation was placed on a slide and another slide was placed on top of the formed gel, and its edges were sealed with silicone grease to prevent water evaporation at a temperature of 25 °C [15].

Drug Loading and Encapsulation Efficiency

The formulations were centrifuged at 10,000 rpm to remove the unloaded drug, and then the resulting suspension was the final formulation. The unloaded drug was dissolved in ethanol and its amount was determined by HPLC. The encapsulation efficiency (%) and the drug loading (%) inside the formulation were calculated according to the following equations (Eq. 3-4):

$$\text{Encapsulation Efficacy} = \frac{\text{Dtotal} - \text{Dunloaded}}{\text{Dtotal}} \times 100 \quad (3)$$

$$\text{Drug Loading} = \frac{\text{Dtotal} - \text{Dunloaded}}{\text{Ftotal}} \times 100 \quad (4)$$

Where D_{total} is the initial flurometholone, Dunloaded is the precipitated drug, and Ftotal is the mass of the formulation.

For flurometholone analysis by HPLC, a C18 column (Sepachrome 4.6 mm × 15 cm) was used with a mobile phase containing methanol and water in a ratio of 60:40 at a flow rate of 1 mL/min. The wavelength used was 457 nm and the run time was 9 min with a retention time of 5.97 ± 0.25 min.

In-vitro release evaluation

50 mg of the formulation was added to a 5.3 kDa dialysis bag, and after sealing it, it was transferred to 12 mL of Simulated Tear Fluid (STF) (at 37 °C with 90 rpm). The STF contained 6.78 g of NaCl, 2.18 g of $NaHCO_3$, 0.084 g of $CaCl_2 \cdot 2H_2O$, and 1.38 g of KCl, which were dissolved in 1000 mL of deionized water. At time intervals of 20, 40, 60, 90, 120, 150, 180, 240, 300, 360, 420, 480, 540, 600, and 720 min, 1 mL of the release medium was withdrawn and replaced with fresh STF to maintain sink conditions. The withdrawn solution was analyzed by HPLC after centrifugation to ensure the absence of suspended particles. The cumulative amount of flurometholone at different time points was calculated using the following equations derived from the standard curve of HPLC.

$$Mn = Cn\ Vt + \sum Cn\text{-}1\ Vs \quad (Eq.\ 2)$$

$$\%\ \text{Cumulative in-vitro release (w/w \%)} = \frac{Mn}{Mt} \times 100 \quad (Eq.\ 3)$$

Mn = Cumulative value of sample n (µg/mL)
Cn = Apparent concentration of sample n (µg/mL)
Vs = Volume of the withdrawn sample (mL)
Vt = Volume of the release medium (mL)
∑Cn-1 = Sum of the apparent concentrations of samples 1 to n-1 (µg/mL)
Mt = Initial amount of Alendronate in the formulation

Stability study

The ICH Q1A(R2) guideline establishes the minimum requirements for stability studies of new drugs and pharmaceutical products. In this guideline, accelerated stability studies of at least one year were conducted under conditions of 40 °C temperature and 75% humidity. Physical characteristics such as color, morphology, viscosity, drug loading, encapsulation efficiency, Z-average, size distribution, Zeta Potential, and in-vitro release evaluation were assessed at interval 0, 3, and 6 months.

Data analysis

Statistical analysis was performed using Graph Pad Prism 8 software, and one-way ANOVA followed by Tukey's post-test was used to determine significant differences between groups. Results were considered statistically significant at $P<0.05$.

RESULTS & DISCUSSION

Analysis of Flurometholone using HPLC

Fig. 1 shows the HPLC chromatogram of

flurometholone in STF with a concentration of 0.78125 mg/mL (A), in a formulation (B), and in an in-vitro release medium (C). The average residence time of flurometholone in the standard solution, formulation, and release medium was determined to be 5.92 ± 0.31 min, which is an acceptable time for HPLC analysis. Fig. 2 shows the standard curve plotted using data obtained from the HPLC system, with mAU on the vertical axis and concentration (µg/mL) on the horizontal axis. The plotted curve was perfectly linear, with an R^2 value of 0.9925 (Y = 36179 × X + 54097), indicating a direct relationship between mAU and concentration for determining the concentration of unknown samples. The LOD and LOQ of flurometholone were found to be 13.19 ± 1.14 and 39.97 ± 1.78 µg/mL, respectively.

Drug loading and Encapsulation efficiency

Table 1 presents the results for drug loading and encapsulation efficiency. The data showed that increasing the weight of liquid crystal gel from 0.5 g to 1.0 g (formulation F2) did not result in a significant increase in drug loading, indicating that

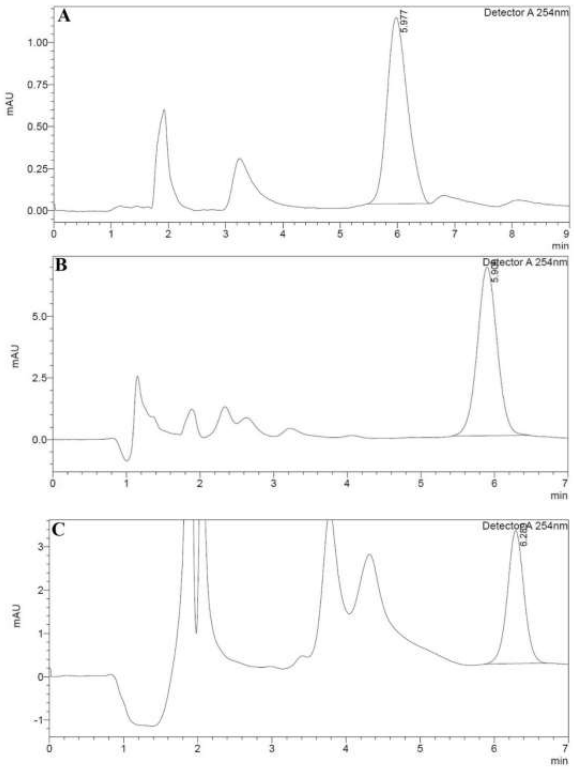

Fig. 1. The HPLC chromatogram of flurometholone in STF with a concentration of 0.78125 mg/ml (A), in a formulation (B), and in an *in-vitro* release medium (STF) (C)

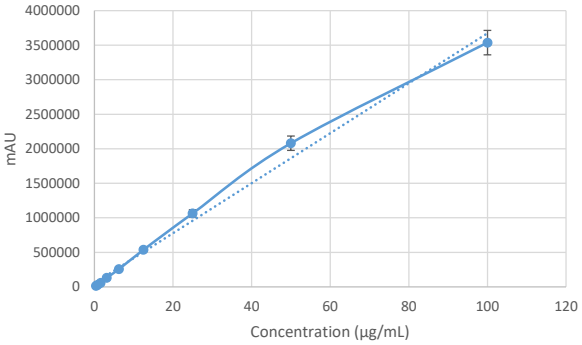

Fig. 2. The standard curve of HPLC for the flurometholone solution in STF

increasing the GMO concentration did not affect drug loading. The addition of cyclodextrin (F3) and increasing cyclodextrin from a molar ratio of 5:1 to 10:1 to the drug (F4) did not lead to a significant change. Formulation F5 demonstrated that adding PC to the GMO did not affect drug loading efficiency. Finally, the formulation F6, in which all the excipients (GMO and Polaxamer) and the drug were dissolved in absolute ethanol, the ethanol was removed, and the mixture was dispersed in water, the resulting cubosomes had a higher drug loading efficiency compared to other formulations.

The primary criterion for selecting the best formulation is the highest encapsulation efficiency, and based on the results, formulation 6 is the preferred choice (Table 1). There are no significant differences between F6 and F1 in terms of Z-average, size distribution, and Zeta potential. Moreover, the stability, particle size distribution, and average particle diameter have not undergone any significant changes with the applied modifications. Additionally, the particle size changed when any of the materials used in the formulation were added, and an increase in the amount of PC resulted in an increase in particle size (F5), while an increase in the amount of cyclodextrin decreased the particle size (F3 and F4).

According to the laboratory findings, it was observed that the particle size of liquid crystal formulations made with the help of lipid (GMO) in all formulations was in the range of 110 to 160 nm (Table 1), and the particle size distribution (PdI) ranged from 0.123 to 0.341, indicating a desirable particle size distribution (Table 1). Comparing with similar studies such as latanoprost drug, the optimal formulation (F6) showed desirable particle size and distribution [6]. For ciprofloxacin, the particle size ranged from 65 to 226 nm with

Table 1. Comparison of different formulations in terms of drug loading (DL), encapsulation efficiency (EE), Z-Average (nm), size distribution (PdI), zeta potential (mV)

Formulations	DL	EE	Z-Average (nm)	Size distribution (PdI)	Zeta Potential (mV)
F1	0.0159 ± 0.0027	31.83 ± 1.98	125.2 ± 1.2	0.268 ± 0.021	-21.8 ± 0.9
F2	0.0163 ± 0.0029	32.54 ± 1.36	131.2 ± 1.1	0.162 ± 0.014	-48.4 ± 1.3
F3	0.0147 ± 0.0019	29.40 ± 2.08	121.6 ± 2.4	0.175 ± 0.012	-20.3 ± 0.8
F4	0.0152 ± 0.0023	30.48 ± 1.73	109.6 ± 1.4	0.123 ± 0.021	-26.8 ± 0.5
F5	0.0163 ± 0.0025	32.64 ± 2.01	159.3 ± 1.6	0.247 ± 0.019	-20.2 ± 0.7
F6	0.0200 ± 0.0032	40.00 ± 2.14	126.8 ± 1.4	0.341 ± 0.028	-23.5 ± 0.4

a distribution of 0.210 [13]. The ophthalmic formulation of sertaconazole showed a particle size between 125.10 to 434.75 nm with a distribution between 0 to 1 [13]. The particle size of diclofenac cubosomes was found to be an average of 480 nanometers with a particle distribution of 0.91 [16]. The study revealed that increasing the lipid content or adding phosphatidylcholine leads to a significant increase in particle size (Table 2). On the other hand, the addition of cyclodextrin led to a decrease in particle size, and as the amount of cyclodextrin was increased (F3-F4), the particle size was decreased. As shown in a study conducted by El-Gendy, the higher was the unsaturated fatty acid content of the lipid, the smaller was the particle size of the nanocrystals due to the bending of their structure [5].

Increasing the lipid content (F5) also led to an increase in particle viscosity, making the process of breaking them down harder and increasing their particle size [13]. In the study on diclofenac, it was observed that the reason for the increase in particle size could be the disruption of the lipid to surfactant (poloxamer) ratio, which should always be 1:1-1:8 (lipid to surfactant (poloxamer) weight ratio) [13]. The addition of surfactants also leads to increased drug penetration and a decrease in particle size, possibly due to a decrease in particle viscosity and surface tension [5]. To evaluate the stability of the formed liquid crystal, the surface charge of the particles was examined. The higher the zeta potential (particle surface charge), the greater the electrostatic repulsion that prevented particle aggregation (Table 1). Based on the obtained information, all compounds were within the range of -18 to -45 mV (Table 1), and thus, the desired solution will be stable, which was about -25 mV in similar studies on latanoprost [6]. For diclofenac, the range was between -17 to -24 [16], and for sertaconazole, it was between -13.30 to -40.98 [13]. Other studies have also shown that increasing the lipid concentration (F5) led to an increase in particle size distribution, which may be due to particle aggregation. Although increasing the amount of poloxamer prevented particle aggregation. It has also been reported that increasing the homogenizer's duration has a positive effect on reducing particle size and distribution [13]. The optimal formulation was evaluated for color, morphology, viscosity, drug loading, encapsulation efficiency, Z-average, size distribution, Zeta Potential, and in-vitro release evaluation after 1, 3, 6, 9, and 12 months of storage according to ICH Q1A(R2) guideline at 30 oC and 65% humidity and no significant difference in particle size was observed, which was confirmed by similar studies on diclofenac [16] and sertaconazole [13] (Table 2). The reason for the

Table 2. Long term stability study of optimal formulation in terms of Color, Drug loading, Encapsulation Efficiency, Z-Average (nm), Size distribution (PdI), and Zeta potential (mV)

Parameter	Time (0 month)	Time (3 month)	Time (6 month)
Drug loading	0.0200 ± 0.0032	0.0197 ± 0.0028	0.0199 ± 0.0031
Encapsulation Efficiency	40.00 ± 2.14	39.47 ± 1.98	39.84 ± 2.01
Z-Average (nm)	126.8 ± 1.4	131.5 ± 1.5	127.4 ± 1.3
Size distribution (PdI)	0.341 ± 0.028	0.328 ± 0.014	0.301 ± 0.018
Zeta Potential (mV)	-23.5 ± 0.4	-22.8 ± 0.3	-24.8 ± 0.9
Color	white	white	white
Viscosity (poise)	0.316 ± 0.032	0.320 ± 0.028	0.331 ± 0.041
Morphology	Cubosome	Cubosome	Cubosome

stability of the formulation could be the presence of a polymer such as Pluronic F127, which covered the surface of the nanoparticles and prevented their aggregation [17].

Morphology

The optimized formulation morphology (F6) is demonstrated in Fig. 3 using polarized light microscopy. Previous studies have shown that cubosomes are isotropic and do not exhibit any color change or produce a completely black image when exposed to polarized light. However, in the presence of hexosomes instead of cubosomes, the hexosomes exhibit an anisotropic state and generate a texture-like colored image when exposed to polarized light. Therefore, this black page indicates the formation of cubosomes. Furthermore, despite being in an accelerated environment (T = 40 °C, humidity = 75 %) for 3 to 6 months, there was no alteration in its morphology and it continued to maintain its cubosomal structure.

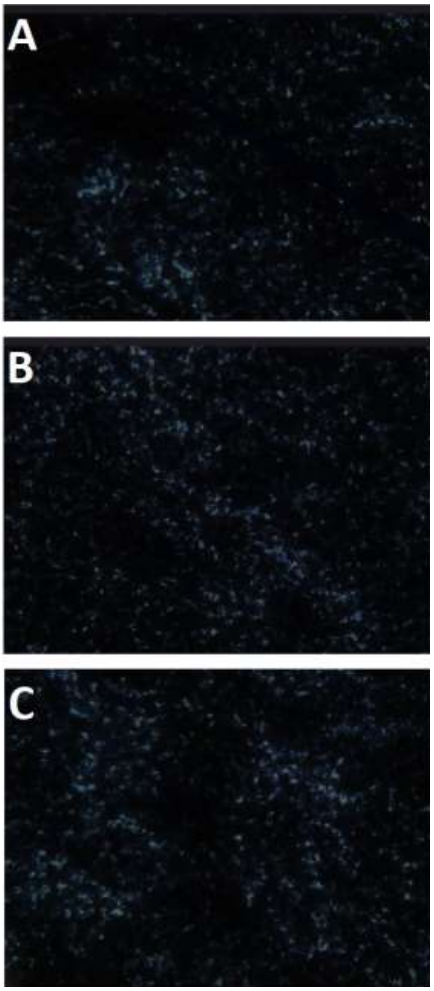

Fig. 3. The morphology of cubosomes using polarized light microscopy (at 40x magnification) for formulation F6 at 0, 3, and 6 month

Viscosity

Fig. 4 illustrated the viscosity of the F6 formulation as 0.316 poise, based on an average shear stress of 49.99 Pa and an average shear rate of 310.91 (1/s). It is crucial to evaluate the rheological behavior (viscosity) of ophthalmic drug delivery systems, as it can impact their dispersibility and duration at the administration site. Studies on viscosity have demonstrated a decrease in the formulation's viscosity with increasing shear force, indicating non-Newtonian behavior (pseudoplastic behavior) of the solution. This behavior is advantageous since it facilitates ease of use during shaking, increases the duration of stay on the cornea, and reduces interference with blinking [13]. Furthermore, as per Table 2, the viscosity of the formulation remained unchanged even when exposed to an accelerated condition.

Cumulative in-vitro release

Based on the cumulative drug release graph (in percentage) over time (in minutes), the pattern of slow drug release over time can be observed (Fig. 5). According to the information on the graph, it has been determined that the drug was almost completely released after 12 hr from the start of the experiment. In similar studies which was conducted on pilocarpine nitrate, it was observed that 87% of the drug was released during the first 48 hr [18]. The sustained-release formulation of latanoprost with a concentration of 0.005% had released less than 1% of the drug after 24 hr and less than 45%

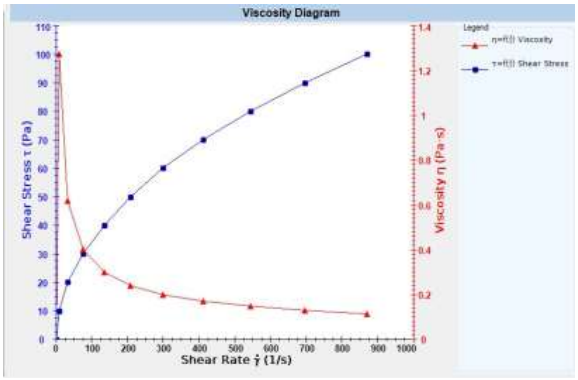

Fig. 4. Shear stress versus shear rate for formulation F6

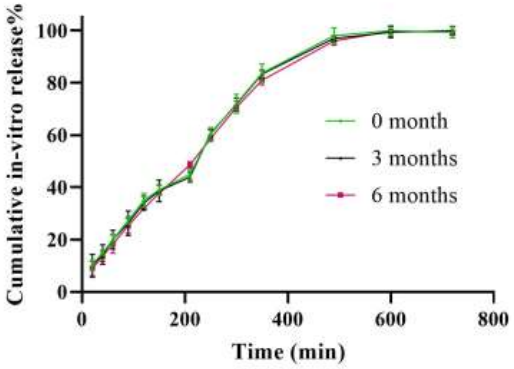

Fig. 5. Cumulative in-vitro release of flurometholone for formulation F6 at 0, 3, and 6 month

after 72 hr, which was slower due to the use of a fatty emulsion and its lower solubility compared to fluorometholone [6]. Additionally, the release of diclofenac in the laboratory environment with the help of Cellophane membrane was approximately 13.6% after 10 hr [19]. Overall, the aim of this study was to increase the time of drug release in order to reduce the topical usage of the drug from multiple times a day to once a day. With the preparation of a liquid crystal formulation, it was observed that the drug was completely released after 12 hr which is desirable in terms of release. However, it is possible that the amount of the released drug, which was only 20% of the loaded cargo, may perform better than the commercial formulation available in the market due to its slower release and greater resistance compared to ocular clearance. In addition, the *in vitro* drug release from the formulation under accelerated stability study conditions at 0, 3, and 6 months demonstrated no significant difference, indicating complete stability of the formulation.

CONCLUSION

The ophthalmic drop of fluorometholone is quickly eliminated by tears and ocular clearance mechanisms within 0.5 to 1 min of use. Additionally, due to the low solubility of fluorometholone in suspension form, its bioavailability is only around 1%, necessitating multiple daily administrations for efficacy. In this study, nanocubosome liquid crystals were utilized to load fluorometholone, resulting in slow release over 12 hr and a reduction in drug administration to twice daily. Furthermore, accelerated stability studies demonstrated no significant changes in color, morphology, viscosity, drug loading, encapsulation efficiency, Z-average, size distribution, Zeta Potential, and in-vitro release evaluation over 0, 3, and 6 months, indicating complete formulation stability.

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CONFLICTS OF INTEREST

The authors report no conflict of interest.

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