Lornoxicam-Loaded Cubosomes: - Preparation and *In vitro* Characterization.

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Abstract

　　　Cubosomes are Nano-sized structures self-assembled materials used for controlling the release of the entrapped drug molecules. Lornoxicam (LXM) is a potent analgesic nonsteroidal anti-inflammatory (NSAID) drug with a short half-life (3-4) hours. The present study aims to prepare LXM-loaded cubosomes with well-defined morphology, small particle size, low PDI, high entrapment efficiency (EE), sustained drug release, and acceptable zeta potential value.

　　　Twelve formulas of LXM-loaded cubosomal dispersions were prepared by a solvent dilution method using Glyceryl monooleate (GMO) as polar lipid with different stabilizers as Pluronic® F127 or tween 80 and different types of hydrotrope as ethanol or propylene glycol. These formulas were evaluated for their particle size analysis and PDI, entrapment efficiency (%EE), and *In vitro* drug release to select a group of the optimum formulas, that further characterized by transmission electron microscopy (TEM) and zeta potential analyzer to select the optimum dispersion.

　　　The obtained results indicated that F3, composed of GMO, Pluronic® F127, ethanol, drug, and phosphate buffer solution pH 7.4 at concentration of 7.28%, 1.82%, 8%, 2%, and 80.9% w/w, respectively, prepared in 20min agitation period, as the optimum formula for its high EE (94.30±0.002%), small particle size (16.3±0.19nm), low PDI　(0.06±0.02), and high zeta potential value (-65.9±0.05mV), and well-defined cubic structure.

　　　This study's conclusion illustrated that LXM-loaded cubosomal dispersion could be considered a promising Nano-carrier for drug delivery.

Key Words: Lornoxicam, Cubosomes, Glyceryl monooleate.

الكوبوسومات المحمّلة باللورانكسكم: تحضيير وتقييم

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الخلاصة

الكوبوسومات هي هياكل نانوية تستخدم للتحكم في تحرير الدواء. اللورانكسكام هو دواء مسكن فعال له عمر نصف قصير(٣-٤) ساعات. تهدف الدراسة إلى تحضير المكعبات المحملة با للورانكسكم.

تم تحضير اثنا عشر صيغة بواسطة طريقة تخفيف المذيب لها من حيث حجم الجسيمات , نسبة تحرر الدواء الفحص المجهري الإلكتروني , جهد زيتا.

اثبتت النتائج ان الصيغة الدوائية الثالثة المكونة من جليسريل مونوليت و بلورونيك ف ١٢٧ و ايثانول و لورانكسكم و بفر فوسفات ذي الاس الهيدروجيني ٧,٤ بنسبة ٧,٢٨٪, ١,٨٢٪, ٨٪, ٢٪ و ٨٩,٩٪ على التوالي هي الافضل حيث اظهرت النتائج الاتية : نسبة كفائة عالية(٩٤,٣٪) حجم الجسيمات الصغيرة (١٦,٣ نانومتر) معامل التشتت (٠,٠٦) قيمة جهد زيتا العالية (- ٦٥,٩ م ف) بالاضافة الى هيكل مكعب واضح

استنتجت الدراسة أن الكوبوسومات المحمّلة بلورانكسكم تعتبر ناقل نانوي واعد لتوصيل الأدوية.

الكلمات المفتاحية: الكوبوسومات , لورانكسكم , كلسرين مونواوليت.

Introduction

　　　Nanoparticles are those particles upon minimizing their size to the nanometer range; tend to show different characteristics from those of the original larger ones. [1, 2] Various types of nano-carriers are used for drug delivery. It offers many advantages over conventional drug delivery systems as modifying the solubility of hydrophobic materials, achieving controlled or sustained release, promoting drugs' stability, and finally targeted therapy to the site of action that increases efficacy and minimizes side effects, nanoparticles (N.P.s) can effectively deliver drugs across the skin due to their unique characteristics. Following topical administration of Nano-particulate formulations, active compounds can enter the skin via intercellular, transcellular or trans-appendageal pathways. Nanoparticles can either remain intact or degrade near the skin surface releasing active substances to penetrate skin layers. [1, 2]

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Cubosomes are nanostructured lyotropic liquid crystalline particles. Their size ranges from 10-500nm in diameter, made of specific amphiphilic lipids (in definite proportions) in an aqueous media where suitable surfactants are used, known as biocompatible carriers in drug delivery. Luzzati and Husson revealed that these structures appeared square in shape while being slightly spherical dots using X-ray scattering. [3]

Lornoxicam (LXM), also known as chlortenoxicam, is a nonsteroidal anti-inflammatory drug (NSAID) of the oxicam class. [4] It has analgesic, anti-inflammatory and antipyretic properties. [5] Lornoxicam is highly bound (99%) to plasma proteins (almost exclusively serum albumin), which results in a low apparent volume of distribution (0.2 L/kg). [6] Lornoxicam undergoes extensive hepatic metabolism in humans, with negligible amounts of unchanged drug being detected in the urine. As with other NSAIDs. [6] The cytochrome P450 (CYP) 2C subgroup of isoenzymes [possibly (CYP) 2C9] appears to play a significant role in the oxidative metabolism of LXM. [7]

The present study aims to prepare cubosomes with well-defined morphology, small particle size, low PDI, high entrapment efficiency, sustained drug release, and high zeta potential value.

Materials

Lornoxicam (Chemshuttle/ USA), ethanol absolute, Triethanolamine (Chem. Lab./ Belgium), KH2PO4 and NaOH (pellet), Glyceryl monooleate and 2% w/v phosphotungstic acid (Sigma-Aldrich/ Germany), Tween 80 (Alpha chemika/ India), Pluronic® F127 (Industrial department actico/Jordan), Deionized water (Janeen for chemical and lab materials/ Iraq), Ortho_phosphoric acid 85% (Merck kga/ Germany), Propylene glycol (Thomas baker/ India).

Preparation of LXM loaded cubosomal dispersion

Lornoxicam loaded cubosomal dispersions were prepared by a solvent dilution method using a vortex. [8] By melting lipid and stabilizer (tween 80 or Pluronic® F127) at 45 ± 2°C using a water bath to produce the oil phase mixture.

The precise amount of LXM (2 gm) was weighed and dissolved in the hydrotrope to prepare a drug solution. This drug solution was then added to a previously prepared oil phase mixture to form a bicontinuous lipid bilayer subjected to vortex at high speed for 3 min to ensure a low-viscosity preparation homogenous melted mixture.

Finally, the low-viscosity homogenous melted mixture was injected into an excess phosphate buffer solution pH 7.4 that was preheated at the same temperature as the homogenous melted mixture. The final mixture was then subjected to vortex at high speed for 20 min to prepare the LXM-loaded cubosomal dispersion, as shown in Table (1), and stored at ambient temperature until required.

Table 1. Composition of LXM-loaded cubosomal dispersions

Formula code	Drug Lornoxicam (gm)	(Oil: stabilizer) mixture	Oil GMO (gm)	Stabilizer Pluronic® F127 (gm)	Stabilizer TWEEN 80 (gm)	Hydrotrope Ethanol (gm)	Hydrotrope Propylene glycol (gm)	Aqueous Phase Phosphate Buffer Solution pH 7.4 (gm)	Agitation time (min.)
F1	2	4:1	7.28	1.82	…	2.5	…	86.4	20
F2	2		7.28	1.82	…	5	…	83.9	20
F3	2		7.28	1.82	…	8	…	80.9	20
F4	2		7.28	1.82	…	…	8	80.9	20
F5	2		7.28	…	1.82	8	…	80.9	20
F6	2		7.28	1.82	…	8	…	80.9	10
F7	2	2:1	6.07	3.03	…	2.5	…	86.4	20
F8	2		6.07	3.03	…	5	…	83.9	20
F9	2		6.07	3.03	…	8	…	80.9	20
F10	2		6.07	3.03	…	…	8	80.9	20
F11	2		6.07	…	3.03	8	…	80.9	20
F12	2		6.07	3.03	…	8	…	80.9	10

Variables affecting formulation

Different variables were studied to investigate their effects on the prepared LXM-loaded cubosomal dispersion properties.

Effect of stabilizer concentration

Two LXM-loaded cubosomal dispersions (F1 and F7), were prepared to assess the effect of the stabilizer concentration on the cubosomal dispersion properties, where Pluronic® F127 was used in the disperse phase in a concentration of 1.82% and 3.03% w/w, with different oil: stabilizer ratio (4:1, and 2:1), respectively

Effect of ethanol concentration

Six LXM-loaded cubosomal dispersions were prepared using different ethanol concentrations as 2.5% w/w (F1 and F7), 5% w/w (F2 and F8) and 8 % w/w(F3 and F9) with different oil: stabilizer ratio (4:1, and 2:1) were used to assess its effect on cubosomal dispersion properties

Effect of hydrotrope types

Two LXM-loaded cubosomal dispersions (F4 and F10) were prepared using 8% w/w propylene glycol with different oil: stabilizer ratios (4:1 and 2:1) instead of ethanol, to assess the effect of hydrotrope type on the cubosomal particle size.

Effect of stabilizer types

Two LXM- loaded cubosomal dispersions (F5 and F11) were prepared using (1.82% and 3.03%) w/w Tween 80, with different oil: stabilizer ratio (4:1, and 2:1), respectively, instead of Pluronic® F127 to assess the effect of stabilizer types on the cubosomal dispersion properties.

Effect of agitation time

Two LXM-loaded cubosomal dispersions (F6 and F12) were prepared by 10 min. agitation using vortex with different oil: stabilizer ratio (4:1 and 2:1), instead of 20min., to assess the effect of decreasing the agitation time on the cubosomal dispersion properties.

Characterization of LXM-Loaded cubosomal dispersion

Particle size and PDI determination

Triplicate measurements of the mean particle size (mean diameter) and polydispersity index (size range of particles) were done using a dynamic light scattering method. The light scattering fluctuations were examined; this fluctuation is due to Brownian motion of dispersion particles. [9]

Drug content

Accurately, one gram of each LXM-loaded cubosomal dispersion was transferred to a volumetric flask of 250 mL; initially, only 200 mL phosphate buffer pH 7.4 was added; then, after sonication for 30 minutes, a clear solution was obtained. Finally, volume was completed with phosphate buffer pH 7.4 to 250 ml. Subsequent dilution was made to determine the percentage of LXM content spectrophotometrically using the UV-Visible spectrophotometer at 375 nm [10].

Entrapment efficiency

For the determination of the loading capacity (entrapment efficiency), each of the LXM-loaded cubosomal dispersions was subjected for 30 minutes to centrifugation at 15000 rpm. The supernatant liquid was collected, diluted appropriately with the phosphate buffer pH 7.4 and estimated using U.V. visible spectrophotometer at 375nm. [11]
The following equation calculated the entrapment efficiency EE %:
% EE = (Total drug - Free drug) / Total drug × 100 …… (Eq.1).

In vitro drug release

The release of LXM from the cubosomal dispersion formulas was done by using a dialysis membrane (MWCO 12-14 KDa). [12]
Rotating paddle dissolution apparatus type II was used to measure the *in vitro* drug release from all prepared formulas. Sink condition was provided throughout the experiments. The sealed dialysis bag containing one gram of the LXM-loaded cubosomal dispersion formula (equivalent to 20 mg of LXM) was sunken in 900 mL phosphate-buffered pH 7.4 (dissolution media) with a speed of 50 rpm, and the temperature of the medium was maintained at 32 ± 0.5 ℃. These conditions were used, since cubosomes may be incorporated in a TDDS in the future work. At predetermined time points of 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 32, and 33h. Five mls were withdrawn and replaced with fresh medium. The withdrawn sample's released drug concentration was determined spectrophotometrically using a UV-Visible spectrophotometer at the selected λ max 375nm. [13]

Selection of the optimum formula

The choice of the best formula was achieved according to the following tests: particle size analysis and PDI, drug content, EE %, and *in vitro* LXM release studies from the LXM-loaded cubosomal dispersions. The selected formulas were exposed to further investigations to select the optimum formulation, such as Zeta potential measurement and morphology determination by (TEM).

Statistical data analysis

The results of the experiments were given as a mean of triplicate samples ± standard deviation. They were analyzed according to the one-way analysis of variance at the level of P- value equal to 0.05 statistically significant is considered at the level of (P-value ≤ 0.05) and non-significant at the level of (p-value > 0.05). [14]

Result and Discussion

The particle sizes and PDI of LXM-Loaded Cubosomal dispersion

The particle size and PDI of the prepared LXM-loaded cubosomal dispersion formulas (**F1-F12)** were characterized by dynamic light scattering (DLS) using the ABT-9000 nanoparticle laser analyzer. [15]

Table (2) shows the particle size of the LXM-loaded cubosomal dispersion formulas. The smallest one was (12.3±0.27 nm), and the highest one was (112.2±0.32 nm). On the other hand, the mean PDI values for the drug-loaded formulations varied in the range of 0.07±2.12 to 0.01±0.00.

Particle size measurement was carried out to confirm that all the dispersion particles were in

the nanometer size range; the small particles might be attributed to many reasons; such as, the bottom-up approach has a unique formation mechanism of cubosomes where the stabilizers are homogenously dispersed onto nanostructured particles' surface and it needs less energy input than the top-down approach because the bottom-up avoid severe fragmentation.

Also, hydrotrope will coat the cubosomal particles and imparts a net negative charge to the cubosomal system. Hence, it confers some degree of electric stabilization that may finally reduce cubosome size.

The index represents the particles' homogeneity and uniformity in the formulations and width of the size distribution. PDI's low value is considered desirable for uniform distribution and high stability of the dispersion. [16]

Table2. The particle size of LXM-loaded cubosomal dispersions (Mean ±SD) n=3.

(Oil: stabilizer)	Formula Code	Particle size in nm	PDI	(Oil: stabilizer)	Formula Code	Particle size in nm	PDI
4:1	F1	35.5±0.45	0.07±0.04	2:1	F7	25.7±0.21	0.07±0.00
	F2	25.6±0.2	0.05±0.02		F8	20.9±0.71	0.01±0.00
	F3	16.3±0.19	0.06±0.02		F9	12.3±0.27	0.06±8.49
	F4	112.2±0.32	0.02±0.00		F10	101.24±0.2	0.01±0.16
	F5	39.3±0.21	0.01±0.00		F11	30.3±0.25	0.01±0.00
	F6	56.4±0.38	0.01±0.00		F12	48.2±0.42	0.01±0.00

Drug content

The mean percent of drug content of the formulations were within the range (97.52±0.085-101.724±0.053%), as shown in Table (3). The results were in inconsistent with the USP requirements [17], indicating high adequacy of the preparation method. [17]

Entrapment efficiency

As shown in Table (3), the entrapment efficiency of the LXM-loaded cubosomal dispersions was within the range (89.41±0.022%-95.08±0.002%), indicating that most of the LXM

was encapsulated in cubosomes. Due to the strong affinity between LXM and the GMO in the cubosomes nanoparticles, the high internal area of cubosomal nanoparticles was 'grabbed' in the liquid crystal structure, and thus, the obtained result matches the result of the entrapment efficiency of the tropicamide-loaded cubosomes for ocular delivery. [18]

The results showed that particle size and entrapment efficiency of cubosomes formulation could be significantly affected by varying the ethanol and lipid concentration and keeping other variables constant.

Table 3. The drug content and entrapment efficiency per cents of LXM-loaded cubosomal dispersions. (Mean ±SD) n=3.

Formula Code	Drug content%	EE %	Formula Code	Drug content%	EE %
F1	99.808±0.012	93.28±0.002	F7	100.1±0.001	93.16±0.002
F2	100.308±0.005	93.52±0.003	F8	100.502±0.002	93.34±0.005
F3	97.856±0.013	94.30±0.002	F9	99.52±0.085	94.05±0.001
F4	99.824±0.07	94.13±0.002	F10	97.74±0.012	93.94±0.001
F5	100.05±0.05	90.39±0.003	F11	100.52±0.025	89.41±0.022
F6	98.524±0.012	93.68±0.001	F12	101.724±0.053	93.61±0.006

Influence of formulation variables on particle size and EE%

Effect stabilizer concentration

Stabilizers used to modify Cubosome's surface properties and hence impart stability. The stabilization is due to shielding of the lipid nanostructure from the surrounding aqueous medium. [19-21]

The results showed that both of the particle size and EE% of cubosomes were indirectly proportional to the increase in Pluronic® F127 concentration. Upon reducing Pluronic® F127 concentration, significantly (p-value<0.05) larger size cubosomes nanoparticles with higher EE% were formed, particle size results may be attributed to the reduced interfacial stability that resulted from an insufficient amount of stabilizer, leading to aggregation of cubosomal nanoparticles. [22] These results were in agreement with the work of Barauskas et al. [23]

While the increased cubosomal EE% may be explained by the hydrophobic nature of the drug provide strong attraction with the hydrophobic domain in the cubic phase bilayer, facilitate a high drug entrapping in the cubic system. Also, reducing Pluronic® F127 concentration combined with increasing the amount of lipid resulted in faster solidification of the cubosomal nanoparticles due to the increased viscosity of the medium. Moreover, this would prevent drug diffusion to the external phase of the medium. In addition ethanol will in the aqueous dispersion medium, assumed to stabilize the formed cubosomal nanoparticles by forming a coat over them. [24]

Effect of ethanol concentration

Ethanol is used to dissolves the lipid and enhance the miscibility between hydrophilic and lipophilic phases that helps bind the two phases. The effect of increasing organic phase volume seems conflicting. Some studies showed that it causes a decrease in the particle size [25] while others showed the opposite phenomenon. [26]

The results showed that the elevation of the ethanol concentration cause reduction of the particle size and elevation in EE% of cubosomal nanoparticles. The particle size of LXM-loaded cubosomal dispersion that contains 2.5% ethanol (F1 and F7) was significantly decreased with increase in the EE% (p-value<0.05) by increasing the concentration of ethanol to 5% w/w as in (F2 and F8) then further significant decreased in particle size and increased EE% (p-value<0.05) by increasing the concentration of ethanol from 5% w/w to 8% w/w as in (F3 and F9).

The results showed the particle size decreased as the total concentration of both stabilizer and hydrotrope increased, which could be explained by the combined effects of the Pluronic® F127 and ethanol in providing a high degree of electric stabilization that may finally lead to a significant decrease in the cubosomes average particle size (p-value<0.05). [27, 9] This might be because ethanol in the aqueous dispersion medium is assumed to stabilize the formed cubosomal nanoparticles by forming a coat over them. The formed coat could retain an excess LXM amount so increasing its entrapment. [18, 9]

Since the LXM-loaded cubosomal dispersions with 8% w/w ethanol showed a smaller particle size than those containing (2.5% or 5%) w/w ethanol, they were used to study the effect of other factors.

Effect of hydrotrope types

The results showed the cubosomal particle size and EE% increased significantly (p-value<0.05) by replacing the ethanol with 8% propylene glycol as in the formulas (F4 and F10). Limayem Blouza et al. investigation showed that an increase in polymer molecular weight generally increases particles' size. [28] The same effect was obtained after using a more viscous organic solvent (propylene glycol). These findings were explained by an increase in the organic phase's viscosity, which hindered solvent diffusion more difficult and thus led to larger nanoparticles' size and an increase in the EE%. [29]

Effect of stabilizer types

The results showed that the mean particle size of the dispersions (F5 and F11), that prepared with Tween 80, was significantly (p-value<0.05) larger than that seen with (F3 and F9) in which Pluronic® F127 was used for stabilizing the cubosomes. [28] While the cubosomal EE % decreased significantly (p-value < 0.05) by replacing the Pluronic® F127 with tween 80. The result may be explained by the increased solubility of LXM in an aqueous solution when tween 80 was used as a stabilizer. [30]

Effect of agitation time

The results showed that by decreasing agitation time from 20 min to 10 min, the cubosomal particle size of the dispersions (F6 and F12) increased significantly (p-value<0.05) in the other side the cubosomal EE % decreased significantly (p<0.05). This result may be because 20min agitation had provided enough time for greater penetration of the oil phase and hydrophobic region of the Pluronic® F127 and, for ethanol to coat the cubosomal particles and confers some degree of electric stabilization that may finally lead to a size reduction of the cubosomes, by reducing particle aggregation risk and enhance the retention of drug in cubosomal particles thus increase the cubosomal EE%. [31-33]

In-vitro drug release

To diffuse the entrapped drug through the dialysis membrane, it should release from the vesicles to the surrounding liquid medium. [34]

The sustained release of a drug from nanoparticles is an essential factor for the successful

development of nanoparticle formulations. As shown in Figure (1), the release of LXM from all cubosomal formulations (F1-F12) had an initial burst release, and then the drug release was sustained for several hours. Initial burst release of all cubosomal dispersions was approximately between (4.778-21.268%) in the first hour after that the drug release was sustained with approximately (62.259-89.95٤%) of the entrapped LXM within 32 hours; these results were in agreement with Zeng et al., who stated that the release pattern of cubosomes

characterized by initial burst release followed by sustained release. [33]

The initial burst release may be explained by the weakly adsorbed drug on the surface of cubosomes or located just at or beneath the nanoparticles' surface. [34] While LXM entrapped inside the nanoparticles contributed to the following sustained release, which can be attributed to the unique structure of the cubosomal nanoparticles. [35, 36]
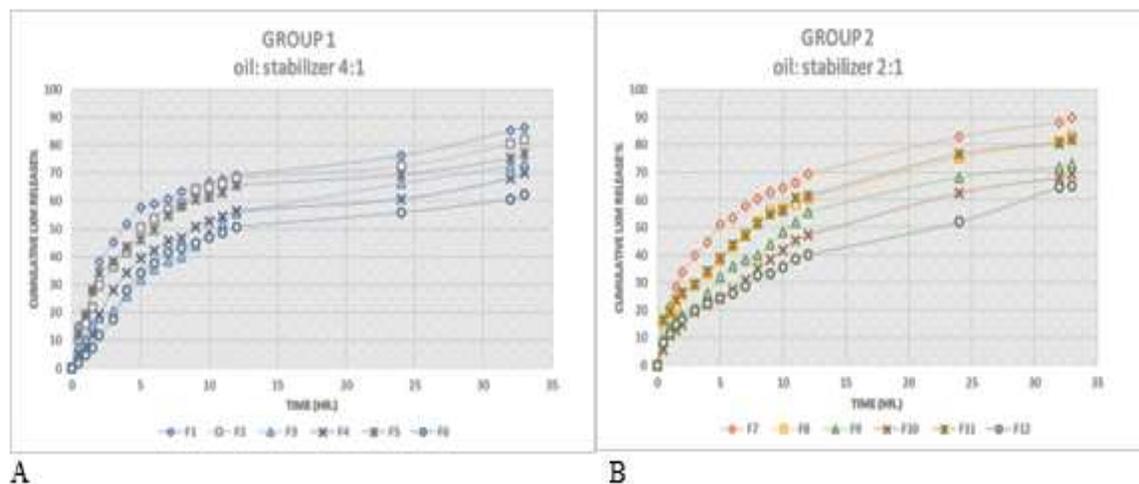
Figure 1.In-vitro release profile of LXM-loaded cubosomal dispersions (F1-F12), divided into two (oil: stabilizer) groups, in phosphate buffer solution pH 7.4 at 32 °C.

As shown in Figure (1), the release comparison of cubosomal dispersion according to stabilizer concentration, where F1 (Pluronic® F127 (١,٨٢%w/w)) released only ٨٦,١٨% of the LXM within 33hr. In contrast F٧ (Pluronic® F127 (3.03%w/w)) released about 89.95 % of the LXM within 33hr. The data shows that the f2 similarity function results of LXM-loaded cubosomal dispersions with different stabilizer concentration > 50 which mean that the in-vitro dissolution profiles were similar.

As shown in Figure (1) after 33 hrs. the released LXM from the cubosomal dispersions containing 2.5% ethanol (F1and F7) was 86.1٨% and 89.95%, respectively, which were the highest among other formulas, while by increasing ethanol concentration to 5% w/w (F2 and F8), only 81.999% and 82.986% of LXM were released, respectively; and by further increasing ethanol concentration to 8% w/w (F3 and F9), only 73.406% and 72.116% of LXM were released, respectively. The data shows that the f2 similarity function results of LXM-loaded cubosomal dispersions with different ethanol concentration were > 50, which mean that the in-vitro dissolution profiles were similar.

In addition the LXM released from the cubosomal dispersions with 8% w/w ethanol (F 3and F9) were 73.40623% and 72.11593%, respectively, while the LXM released from cubosomal

dispersions with 8% w/w propylene glycol (F4 and F10) where only 70.0967% and 69.341%, released respectively. The data shows that the f2 similarity function results of LXM-loaded cubosomal dispersions with different hydrotrope types were >50, meaning the in-vitro dissolution profiles were similar.

In addition, the LXM released from the cubosomal dispersions that contain Pluronic® F127 (F3 and F9) were 73.40623% and 72.11593%, respectively, while the cubosomal dispersions that contain tween 80 (F5 and F11) where 76.964% and 81.883%, released respectively. The data shows that the f2 similarity function results of LXM-loaded cubosomal dispersions with different stabilizer types were >50, which means the in-vitro dissolution profiles were similar.

Furthermore, the LXM was released from the cubosomal dispersion prepared within 20 min. agitation (F3 and F9) were 73.40623% and 72.11593%, respectively, while the cubosomal dispersions prepared within 10 min agitation (F6 and F12) were only 62.259% and 65.181%, released respectively. The data shows that the f2 similarity function results of LXM-loaded cubosomal dispersions with different agitation time were >50, which mean the in-vitro dissolution profiles were similar.

Selection of optimized formulas of LXM-loaded cubosomal dispersion

It was previously reported that Nano-particles with a diameter of 300 nm or less could deliver the drug to some extent to the skin's deep layers. Moreover, if the particles' diameter was decreased to 70 nm or less, this leads to maximum deposition of the drug in the epidermis and the viable dermis. [37-40] that means the cubosomal particle size plays an essential role in drug delivery systems. The results of the study showed that **F9** had the smallest particle while, **F3** showed maximum EE %, and the *in-vitro* release study of (**F3 and F9**) showed the drug's sustained release in both of the formulations. So the selected group of LXM-loaded cubosomal dispersion (F3and F9) exposed to further investigations to select the optimum formula.

Particle surface charge (Zeta potential)

A high zeta potential absolute value indicates a high electric charge on the cubosomal surface, which can cause strong repellent forces among particles and prevent aggregation of the cubosomes in a buffer solution. [41, 42] As shown in Table (4) and Figure (2), cubosomal formulations F3 showed higher zeta potential value and maximum stability than F9; this might be attributed to the higher concentration of the used fatty acid, GMO. [43]

Table 4.Zeta Potential values of LXM-loaded cubosomal dispersions (Mean ±SD) n=3.

Formulas code	(Oil: stabilizer)	Zeta potential value in Mv
F3	4:1	-67.2±0.05
F9	2:1	-63.5±0.11
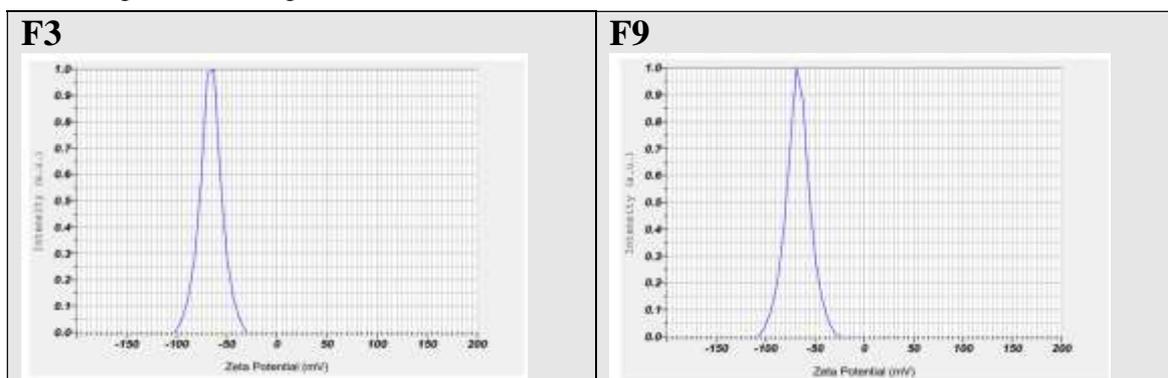
Figure 2. Zeta potential values of LXM-loaded cubosomal dispersions.

Transmission Electron Microscopy

The external morphology images of the selected group of LXM-loaded cubosomal dispersion (**F3 and F9**) were taken using transmission electron microscopy (TEM).
As in Figure (3), the transmission electron micrographs show that the cubosomal dispersion F3 was cubic and well separated from each other and in the Nano-size, which confirms particle size measurement results.

While F9 showed vesicular particles over the formation of the desired particles of cubic structure, which can be explained to be due to high Pluronic® F127 concentrations which resulted in formation of smaller particles, they also promote vesicular formation particles. [44]
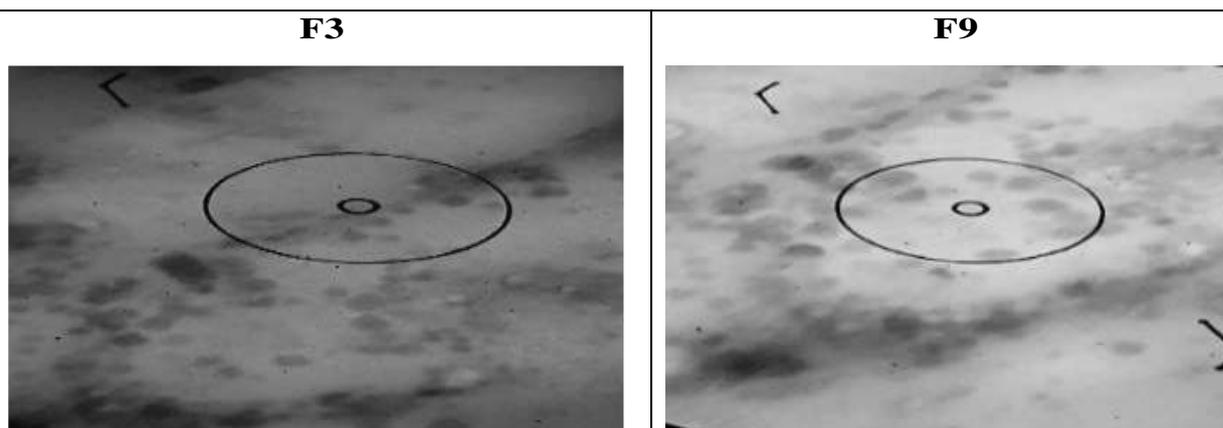
Figure 3. Transmission electron micrographs of LXM-loaded cubosomal dispersions.

Therefore, F3 was selected as the optimum formula for its high EE % (94.30±0.002%), small particle size (16.3±0.19nm), low PDI(0.06±0.02), and high zeta potential value (-67.2±0.05mV), and well-defined cubic structure.

Conclusion

This study illustrated that LXM-loaded cubosomal dispersion could be considered as a promising Nano-carrier for drug delivery.

Future work

The selected formula (F3) shows excellent cubosomal properties that suggested it as a great potential for cubosomal transdermal drug delivery system in the future work.

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