OPTIMIZATION AND IN VITRO EVALUATION OF THE RELEASE OF CLASS II DRUG FROM ITS NANOCUBOSOMAL DISPERSION

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INTRODUCTION
Development of colloidal carriers as delivery systems for pharmaceutical drugs has stimulated an exponential growth for the encapsulation of bioactive drug molecules into relatively non-toxic and inert carriers for the in vivo delivery constitutes a promising approach for the improvement of their therapeutic index while reducing the side effects [1]. Exceptional success has been made to improve efficacy by developing lipid-based carriers. Cubosomes are structured liquid crystalline particles, composed from certain amphiphilic lipids in definite proportions. It consists of lipid bilayers that are organized in three dimensions as honeycombed structures and divided into two internal aqueous channels which can be used by hydrophobic and amphiphilic substances, and the potential for controlled release through functionalization, cubosomes are regarded as promising vehicles for different routes of administration [2]. Cubosomes are made by the self-assembly of low molecular weight lipids. These lipids are characterized by a maximum continuous interface and high interface to volume ratio, which makes them promising candidates as efficient adsorbents and host-guest applications [4]. The most characterized system of bicontinuous cubic phase forming lipid is the glyceryl monooleate (GMO)/water system. Monoglycerides are non-toxic, biodegradable and biocompatible materials that can self-associate depending on the temperature and aqueous forming content. In this work a model drug (erythromycin) was used which is used to treat bacteria responsible of the skin and upper respiratory tract infections, including Staphylococcus, Streptococcus, Corynebacterium and Haemophilus genera [5]. The aim of this work was to optimize the cubosomal dispersion of the model drug through studying different variables including using different ratios GMO:Smix, type and time of stirring to improve loading capacity and by enhancing penetration of the drug through skin barrier and improving its effectiveness. The release of the drug that may improve patient compliance through its quick relief of different bacterial infections.

MATERIALS AND METHODS

Materials
Erythromycin powder and poloxamer 407 was purchased from Sigma-Aldrich, Chemie GMBH, Germany, Glycerol mono oleate was purchased from Hangzhou Hyper chemicals, China, Potassium phosphate monobasic and Sodium hydroxide were purchased from Himedia, India.

ABSTRACT
Objective: This work involves investigation and evaluation of the factors that affect the preparation and the release of the model class II drug (erythromycin) to optimize the efficiency of its prepared nanocubosomal dispersion to give very fast initial burst effect within the first hour that can continue for further two hours.

Methods: The work involved preparation of ten formulas of cubosomal dispersion by emulsifying different concentrations of glyceryl monooleate (GMO) (lipid content)/surfactant mixtures which were nano-sized and characterized morphologically by Transmission electronic microscopic (TEM), zeta potential, particle size, polydispersity index (pdI), pH, entrapment efficiency, conductivity test, dilution test and in vitro drug release.

Results: The selected nanocubosomal formula (F1) showed pH (7.41), particle size (315.05 nm), pdI (0.194), zeta potential (-30.852), entrapment efficiency (91%) and gave a 70% drug release within the first hour of the in vitro test and continued until it gave 96.3% drug release with further 2 h.

Conclusion: this work succeeded in preparing optimized cubosomal dispersion for erythromycin using different GMO/poloxamer 407 percent. The optimum formula gave an immediate release of the model drug (erythromycin) and it was ready to be incorporated in any suitable dosage form to give fast onset of action.

Keywords: Cubosomes, Erythromycin, GMO, Poloxamer 407, Nanotechnology

Table 1: Composition of the prepared nano cubosomal dispersion formulas

<table>
<thead>
<tr>
<th>Formula code</th>
<th>GMO</th>
<th>Poloxamer 407</th>
<th>Drug</th>
<th>Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>4.4%</td>
<td>0.6%</td>
<td>2%</td>
<td>q.s</td>
</tr>
<tr>
<td>F2</td>
<td>4.2%</td>
<td>0.8%</td>
<td>2%</td>
<td>q.s</td>
</tr>
<tr>
<td>F3</td>
<td>4.0%</td>
<td>1.0%</td>
<td>2%</td>
<td>q.s</td>
</tr>
<tr>
<td>F4</td>
<td>3.8%</td>
<td>1.2%</td>
<td>2%</td>
<td>q.s</td>
</tr>
<tr>
<td>F5</td>
<td>3.6%</td>
<td>1.4%</td>
<td>2%</td>
<td>q.s</td>
</tr>
<tr>
<td>F6</td>
<td>3.4%</td>
<td>1.6%</td>
<td>2%</td>
<td>q.s</td>
</tr>
<tr>
<td>F7</td>
<td>3.2%</td>
<td>1.8%</td>
<td>2%</td>
<td>q.s</td>
</tr>
<tr>
<td>F8</td>
<td>3.0%</td>
<td>2.0%</td>
<td>2%</td>
<td>q.s</td>
</tr>
<tr>
<td>F9</td>
<td>2.8%</td>
<td>2.2%</td>
<td>2%</td>
<td>q.s</td>
</tr>
<tr>
<td>F10</td>
<td>2.6%</td>
<td>2.4%</td>
<td>2%</td>
<td>q.s</td>
</tr>
</tbody>
</table>
Preparation of cubosomal dispersion formulas

Preparation of cubosome dispersions formulas was done by emulsification of Glycerol mono oleate (GMO)/surfactant mixture. Poloxamer 407 was used as the nonionic surfactant in a concentration range between 0% and 16% w/w. The concentration of the GMO/Poloxamer mixture was 5% w/w with respect to the weight of the dispersion (table 1). Erythromycin concentration used was 2%. GMO and Poloxamer 407 were melted on a hot plate and then erythromycin was dispersed in the molten mixture. The molten mixture was then added drop by drop to the aqueous phase (phosphate buffer 7.4) at 70 °C under mechanical stirring at 1500 rpm. The dispersions were maintained under mechanical stirring and they were cooled to room temperature to solidify the lipid droplets. The dispersions were sonicated for 10 min using probe ultrasonicator at 300 w in order to reduce the droplet size to the nanoscale then they were stored in glass bottles at room temperature (25 °C) for further investigations [6, 7].

The physical appearance of the formulas

Evaluation of the physical properties (color and homogeneity) for all the prepared nanocubosomal dispersion formulas (F1-F10) was done visually.

pH determination of the formulas

The pH of the formulations (F1-F10) was determined using a digital pH meter by immersing the electrode of the device in a beaker containing 10 ml of each of the nano cubosomal dispersion formulas and record the results after two minutes.

Zeta potential, particle size and polydispersity index determination

Measurement of the mean particle size (mean diameter), zeta potential (particle surface charge) and polydispersity index (size range of particles) was done by using dynamic light scattering method (ZetaPlus Particle Sizing). By this technique the light scattering fluctuations were examined, this fluctuation is due to the Brownian motion of cubosomal dispersion particles. It measures the size accurately in the range of 0.3 nm to 10 μm. One ml of the diluted cubosomal dispersion was injected into folded capillary zeta cell and monitor the light scattering at 25 °C (190 ° angle). The average particle size, polydispersity index, and zeta potential values for (F1-F10) were recorded [8].

Separation test

This test is very important to check the physical stability of the dispersions. The centrifugation of each nanocubosomal dispersion formula (F1-F10) for 30 min at (10,000 rpm) was applied to check the nanocubosomal dispersions resistance for separation [8].

Drug content

Accurately, one ml of each nano cubosomal dispersion formula (F1-F10) (containing 20 mg/ml erythromycin) was transferred to a volumetric flask (100 ml) and 70 ml methanol was appended, and the clear solution was achieved after sonication for 30 min. The solution volume was diluted with methanol to 100 ml and subjected to centrifugation for 15 min at 3000 rpm then filtered using Millipore filter 0.22 μm. The content of erythromycin was determined spectrophotometrically using the UV-Visible spectrophotometer (Shimadzu 1650 PC-Japan) at λ max 285 nm [9].

Entrainment efficiency

For the determination of loading capacity (entrainment efficiency), each of the cubosomal dispersions (F1-F10) were subjected for centrifugation at 15000 rpm for a period of 30 min, and the supernatant liquid was collected, diluted appropriately and estimated using UV visible spectrophotometer at λ max 285 nm [10]. The percent of entrainment efficiency (%EE) was calculated by the following equation:

\[ \%EE = \frac{\text{total drug} - \text{free drug}}{\text{total drug}} \times 100 \]

Conductivity test

Electrical conductivity measurement was done to determine the nature of the prepared nanocubosomal dispersion formulas (F1-F10). If the external (continuous) phase is aqueous, the nanocubosomal dispersions are o/w (high conduction) but if the internal (dispersal) phase is aqueous, the nanocubosomal dispersions are w/o (no conduction). Measuring of electrical conductivity (σ) was made by the use of a conductometer via immersing the conductometer probe in 10 ml of the prepared dispersion in a beaker at room temperature and the apparatus will register the results in μS/cm [11].

Dilution test

The dilution test was done to check the physical stability of nanocubosomal dispersion formulas (F1-F10). The aqueous dilution test was carried out by diluting 1 ml of each nanocubosomal dispersion formula to 50 ml, 100 ml, and 500 ml with double distilled water at 37 °C with constant stirring at a speed of 50 rpm and observed visually the clarity, turbidity, cracking and phase separation [12].

TEM study

The nano cubosomal dispersions were further characterized by TEM operating at 30 Kilovolts. A drop of the diluted formula was allowed to be deposited on the circular copper film grid of 300 mesh and then stained with formvar and left for drying. The size and shape of the cubosomes were measured after the complete drying of the slide by observing the slide under the microscope [13].

In vitro release

The release of erythromycin from the nano cubosomal dispersion formulas (F1-F10) was done by using dialysis membrane (MWCO 2000 Da). Rotating paddle dissolution apparatus type II was used to measure the in vitro drug release from all prepared formulas. The sealed dialysis bag containing nanocubosomal dispersion formula (equivalent to 120 mg erythromycin) was sunken in 500 ml phosphate buffered (pH 7.4 dissolution media) with a speed of 50 rpm. The temperature of the medium was maintained at 37±0.5 °C. Five ml aliquots outgoing for suitable time periods and immediately making the replacement with fresh dissolution medium. The drug content in the withdrawn sample was determined spectrophotometrically by using a UV-Vis spectrophotometer at the selected λ max 285 [14].

FTIR study

The FTIR spectrum of the dispersions in comparison with the pure drug was recorded by the use of a FT-IR spectrophotometer. They were obtained using liquid cell by dripping several drops of the sample onto NaCl or KBr aperture plate and then sandwiching it under another aperture plate so that no gas bubbles are trapped. The thickness can be adjusted according to the sample absorbance by appropriately tightening the screws or by inserting spacers between the aperture plates [15].

RESULTS AND DISCUSSION

Physical appearance

All the formulas (F1-F10) appeared as a white homogenous milky solution without any aggregates.

pH determination

The pH of the nano cubosomes formulations (F1-F10) was measured using pH meter. The pH values were ranged (7.29-7.42), and this matches the skin requirements for topical preparations to avoid skin irritation and agreed with the results obtained with topical cubosomal gel for ketoconazole [17].

Zeta potential, particle size, and PDI determination

Particle size measurement was carried out to confirm that all particles of the dispersion are in the nanometer size range (fig. 1). The effective diameter of all cubosomal dispersions was in the nanometer range (average particle size values ranged from 315 nm to 416 nm), with a polydispersity index of<1, the low value of polydispersity index 0.08-0.7 is considered to be desirable for uniform distribution and homogeneity of nano-sized particles within the preparation. While pdI value>0.7 to less than 1 is considered to
have a broad distribution of particle size [18]. The zeta potential of all formulas were comparably low, ranging between (-24.786 to -30.852 mV), which could be due to the absence of charge in cubosomal dispersion ingredients, this is mainly because of the use of non-ionic surfactant (poloxamer 407) and the presence of fatty acid (oleic acid oil) which generally made the surface charge of the particle negative [19], the effect of zeta potential on the stability of nanoparticle was explained by rule of thumb. This rule states that values of zeta potential in the range (≤-30 mV) to (≥+30 mV) indicate that there are good stability and values in the range (≤-60 mV) to (≥+60 mV) indicate that there is excellent stability in the formulation [20]. For nonionic surfactant (poloxamer 407) that act by steric stabilization, the value of zeta potential of 20 mV or even lower provided an efficient stabilization due to that the non-ionic surfactant (poloxamer 407) provided good steric stability for maintaining the stability of single layer nanodispersions [21].

Separation test
No sedimentation, creaming or phase separation upon centrifugation for all the prepared nano cubosomal dispersion formulas (F1-F10) was observed, this is a good indication of their high stability [22]. This proved that the thermal motion of the dispersion droplets (the Brownian motion) exceeded the external forces such as centrifugation and/or gravitation [23].

Drug content
The drug content of all nano cubosomal dispersion formulas (F1-F10) results are inconsistent with the requirements of the USP(16), indicating high adequacy of the preparation method and high content uniformity of the prepared formulas, where all the prepared nano cubosomal dispersions have a drug content between (90-98%) and this agreed with the acceptable range according to the USP (85-115%) [24].

Entrapment efficiency
The entrapment efficiency of erythromycin nano cubosomal dispersion formulas is shown in fig. (2). The high entrapment efficiency indicates that the technique applied in the preparation of nanoparticles is applicable and reliable. The entrapment efficiency of (F1-F5) is higher than the entrapment efficiency of (F6-F10) due to the presence of high percent of lipid content (GMO), and as the percent of lipid (GMO) was decreased the entrapment efficiency decreased too, this is mainly due to the high solubility of the drug (erythromycin) in the lipid part of the dispersion. This agreed with Etoposide-loaded nanoparticles made from glyceride lipids [25].

Conductivity test
The electrical conductivity ($\sigma$) of nanocubosomal dispersions defined as a measurement of materials ability to confirm the transport of an electric charge and it was measured to determine the nature or type of the external phase of dispersion and to detect the phase inversion phenomena. Conductivity measurement depends on the higher conductivity of the water compared to the oil and gives
high values in o/w dispersions where water is the external phase. The results indicated that all the prepared nanocubosomal dispersion formulas were o/w type since high conductivity (76-252 μs/cm). The higher conductivity is due to a large percentage of water, which allows higher freedom for mobility of ions [26].

**Dilution test**

The dilution test is very important to define whether the dispersion is o/w or w/o. If the emulsion is o/w type then the addition of more continuous phase (water) will not cause cracking or separation of that emulsion. The results showed that the addition of water to all nanocubosomal dispersion formulas (F1-F10) showed clear nanocubosomal dispersion in less than 1 minute with no cracking or separation adding a further indication that they are all o/w type [27].

**TEM study**

The shape and size of the nano cubosomal dispersions were characterized by the use of transmission electron microscopy (TEM) as in fig. (3). The images showed cubosomes as dark globules with bright surrounding with average droplet size (375 nm) and similar shape was obtained with transdermal cubosomes of Etodolac [28].

**In vitro dissolution test**

The cumulative percentage of erythromycin at different time intervals for each nano cubosomal dispersion is shown in fig. (4). The result of cumulative drug release showed that different GMO content had a profound effect on drug release. The result showed a significant decrease in the initial drug release (after 1 h.) between F1 and F10, 70% and 45% respectively. Also, the release after 3 h was significantly greater for F1 than F10, 96% and 89 % respectively. As the concentration changed from the higher concentration of GMO in F1 (4.4%) to the lower concentration of GMO in F10 (2.6%). In addition to that, the concentration of poloxamer 407 in F10 is much higher than F1 and that caused a significant decrease in the release of the drug from F10 in comparison to F1, since this will be fewer water channels resulting for reinforcement of micellar structure [29, 30]. The result showed that F1 gave 70% drug release within 1 h and the release increasingly continued for the next 2 h (96.3%), which was better than other formulas, and this could be due to the presence of high amount of GMO in F1 (4.4%) [31].

![Fig. 3: (A) TEM picture at zoom 64000 pixels, (B) TEM picture at zoom 180000 pixels](image)

![Fig. 4: (A) In vitro release profile of erythromycin from (F1-F5) formulas (values are mean±SD) (n=3), in phosphate buffer solution (pH 7.4) at 37±1 °C, (B) In vitro release profile of erythromycin from F6-F10 formulas (values are mean±SD) (n=3), in phosphate buffer solution (pH 7.4) at 37±1 °C](image)
FT-IR study
The FT-IR spectrum of the pure drug showed the distinctive peaks values, which are 1639 cm⁻¹ C=O (carbonyl stretching), 3402 cm⁻¹ C-OH (alcohol stretching) and 1085 cm⁻¹ C-O-C (ether stretching). The FTIR spectrum of erthyromycin nanocubosomal dispersion displayed same functional groups band with very slight shifting indicating excellent compatibility of excipients with the drug [32].

CONCLUSION
Depending on the results obtained in this study, this work revealed that slight change in lipid: surfactant ratio as well as using alternative stirring type and time in comparison to related preparation led to improving entrapment efficiency and gave immediate release of the drug from the prepared emulsions as well as the unique structure of the prepared cubosomes that can improve the antibacterial activity of the model drug and can give quick significant action that will improve patient compliance.

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AUTHORS CONTRIBUTIONS
All the authors have contributed equally in the conceptualization and execution of the article

CONFICT OF INTERESTS
The authors declared that they have no conflicts of interest

REFERENCES