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Formulation and characterization of Liquid Crystalline Hydrogel of Agomelatin: *In vitro* and *Ex vivo* evaluation

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ABSTRACT

Objective: The aim of this investigation was the development and characterization of Agomelatin-loaded liquid crystalline (AM-LC) nanoparticles for improved topical application.
Methods: AM-LC was formulated with the glyceryl monooleate (GMO) and poloxamer 407 as structure forming agent (lipid) and surfactant respectively, by using emulsification of GMO and poloxamer in water using a hydrotrope (Cubosomes) formation method. The obtained dispersion was characterized for particle size, PDI, zeta potential, entrapment efficiency, surface morphology, in vitro studies. Further, conversion optimised formulation in to cubic gel by incorporating 0.5% w/w of carbopol 934P. The prepared gel was characterized by rheological measurements, surface pH and *ex vivo* permeation studies through the rat skin.
Results: The average particle size of formulations was ranging from 187.6±3.97nm to 225.8±7.54nm and ZP from -14.5±4.65 to -23.5±3.86mV. *In vitro* drug release from cubosomes exhibited sustained release profile and the optimized formulation (F2) showed cumulative drug release of 83.96±2.43% during 24h. Transmission electron microscopic photographs confirmed that the formed liquid crystalline nanoparticles were cubic in shape. Results suggested that cubic gel exhibited a retarded release rate (53.5 ± 3.21%) than the control gel (95.33 ± 2.28%) containing 0.1% drug solution.
Conclusion: The obtained results indicated that cubic gel would be a promising carrier for topical delivery of agomelatin into and across the skin.

INTRODUCTION

According to the internal structures of the crystals, lipid-based lyotropic liquid forms mainly categorized into lamellar phase, cubic phase, and hexagonal phase. Most important ones are cubic and hexagonal phases among these and gained much attention due to their highly ordered and flexible internal structures, which have the capability to release the active pharmaceutical ingredients slowly from matrix with different polarities and size (Shah *et al.*, 2001; Borka, 1991). In general, addition of amphiphilic lipids such as Glyceryl mono oleate (GMO) (Kaasgaard and Drummond, 2006) in to an aqueous phase containing stabilizers such as Pluronic copolymers resulted in the formation of cubic and hexagonal liquid crystals. Glyceryl monooleate and phytantriol have been reported as most commonly used lipids to form cubic phase liquid crystals. Lipid used in the present study is monoolein or GMO is biodegradable polar lipid that has no marked toxic effects (Ganem-Quintanar *et al.*, 2000). It also swells in water, forming lipid bilayer and water channels when dispersed in to aqueous phase hence this can be used in formulation of drug delivery systems for both hydrophilic and lipophilic drugs. Hydrophilic drugs are loaded closer to the polar head of lipid or in the water channels, whereas lipophilic drugs are encapsulated in the lipid bilayer (Shah *et al.*, 2001).

Depending on water content, temperature and lipid compositions, several phases can be formed, including cubic and hexagonal phases. The cubic phase of monoolein and water is formed at ambient temperature (figure 1), and has been shown to accommodate and sustain the drug release with varying in physical chemical properties. The reverse hexagonal phase of monoolein and water is obtained only at high temperatures, unless a third nonpolar component (e.g., triglycerides or oleic acid) (Borne *et al.*, 2001) is added to the system.

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Topical/transdermal delivery cubic liquid crystals have been reported early for small molecules such as nicotine, salbutamol, acyclovir and flurbiprofen (Helledi and Schubert, 2001; Shun *et al.,* 2010) Agomelatin (AM), is selected as model drug for this study, it is an antidepressant used for treatment of severe depression, act only on MT1/MT2 melatonergic receptor agonist and 5-HT_2C antagonist. It is well absorbed following oral administration, but absolute bioavailability is relatively low (about 5-10%) (Michele Fornaro *et al.,* 2010) due to its high first-pass metabolism and extensively metabolized by the cytochrome P450 isoforms 1A1, 1A2 and 2C9 to hydroxyl, desmethyl and epoxide metabolites with less activity than the parent drug, with the mean terminal elimination half-life is 2.3 h (Green B, 2011). In the present study, glyceryl monooleate and poloxamer 407 (GMO & P407) liquid crystal (LC) formulations were developed and made it in to hydrogel by dispersing in the gelling agent (0.5% w/w carbopol 934).

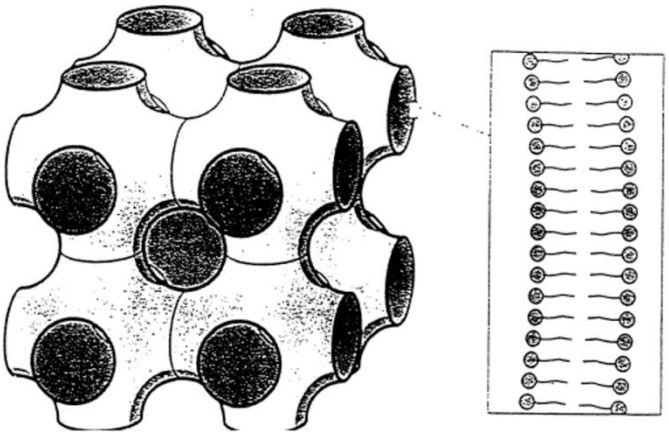

Fig. 1: Structure of three dimensional glyceryl monoleate – water cubic phase (Shah et al., 2001).

Hydrogels are high-water content materials prepared from cross-linked polymers that are able to provide sustained, local delivery of a variety of therapeutic agents, several additive characteristics of hydrogels make them excellent for topical drug delivery vehicle such as bioadhesive nature that enhance residence time of drug.

MATERIALS AND METHODS

Materials

Agomelatin was a kind gifted sample from Symed labs, Hyderabad. Commercial grade Glyceryl monooleate (GMO) was gifted by Mohini Organics Pvt. Limited, Mumbai, India. Poloxamer 407, Carbopol 934 were purchased from Sigma Aldrich, Mumbai. All other chemicals used were of analytical grade.

Preparation of Liquid Crystalline Hydrogel (LCH) Containing Agomelatin

Liquid Crystals or cubosomes were prepared by top down technique i.e., simple emulsification method (Lakshmi *et al.,* 2014), and the compositions were represented in Table 1. Agomelatin was dissolved in ethanol (3mL) and then add to structure forming agent (GMO), the obtained dispersion was finally add to aqueous phase containing poloxamer 407, stirring was continued for 2h till the evaporation of ethanol. Stirring speed of 3000-5000 rpm and temperature of 37°C were kept constant for all the formulations.

Table 1: Formulation composition of agomelatin cubosomes.

Formulation code	Agomelatin (mg)	Glyceryl monooleate (mg)	Pluronic F127 (mg)
F1	20	100	25
F2	20	100	50
F3	20	100	75

The obtained liquid crystal formulations were dispersed slowly with continuous stirring in to 0.5% w/w carbopol 934 dispersion containing triethanol amine (0.1%) as pH modifier to form hydrogel.

Differential Scanning Calorimetry

Thermal behavior of the pure drug and poloxamer 407 and their physical mixture were investigated by a DSC 4000 (Perkin Elmer, USA). Samples of about 8mg were heated in aluminium pans using dry nitrogen as the effluent gas. The analysis was performed within a heating range of 50-200°C and at a heat rate of 20°C/min, indium was used as reference (Narendar and Kishan, 2014)

Measurement of Particle Size, Polydispersity Index (PDI) and Zeta Potential (ZP)

The particle size distribution, PDI, and ZP of cubosomes were measured using a Malvern Zetasizer (NanoZS90, UK) at 25°C and a 90°scattering angle. A 1:100 dilution of the sample was made using double distilled water before the measurements to get 50-200 optimum kilo counts per second (Kcps) for measurements (Thiagarajan *et al.,* 2013).

Analytical methodology for Agomelatin

Entrapment efficiency and assay of cubosomes containing agomelatin were done by using HPLC (Shimadzu) consisted of a Model LC20 AD solvent pump, a rheodyne injector, a 20µL loop, SPD-20A variable wavelength UV detector, The separation was performed by a Lichrospher RP-18 column, A mobile phase of acetonitrile and water 55:45 (flow rate of 1 mL/min) was used, and agomelatin was detected at 231 nm, the retention time was 5.8 min (Janga *et al*., 2012)

Determination of entrapment efficiency (EE) and assay

For EE, 2mL of the dispersions were centrifuged for 30 min at 14,000 rpm to separate the entrapped fraction from the unentrapped. An aliquot of the supernatant was taken and the amount of unentrapped drug was calculated by spiking the supernatant after suitable dilutions with mobile phase in to HPLC (Rizwan *et al.,* 2011). For assay, agomelatin cubosomes (0.1 mL)

were diluted to 1 mL with ethanol and further dilutions are made with mobile phase to determine the drug content using HPLC (Shimadzu, Japan)

Percentage of entrapment efficiency (EE %) = [A total − A unentrapped/ A total] × 100

***In vitro* drug release studies**

In vitro release of agomelatin from cubosomes was studied in pH 6.4 phosphate buffer by dialysis method having a pore size 2.4 nm and molecular weight cut-off between 12 000–14 000 The experimental unit had donor and receptor compartments. Donor compartment consisted of a boiling tube which was cut open at one end and tied with dialysis membrane (Lian *et al.*, 2011) at the other end into which 1 mL of cubosomal dispersion was taken for release study. Receptor compartment consisted of a 250 mL beaker which was filled with 100 mL of release medium and the temperature was maintained at 37 ± 0.5°C. At 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 hour time points, 2 mL samples were withdrawn from receiver compartment and replenished with the same volume of release medium. Samples were suitably diluted and analyzed by UV-Visible Spectrophotometer (SL-159, ELICO) at 231 nm.

Transmission Electron Microscopy

TEM observations were performed to know the morphology of liquid crystals formulations following negative staining with sodium phosphotungstate solution (0.2% w/v). A thin film was made on a carbon-coated copper grid by placing a drop of liquid crystals dispersion (Di *et al.*, 2010). Before the film was dried on the grid, it was negatively stained with phosphotungstic acid by adding a drop of the staining solution to the film, any excess solution was drained off with a filter paper. The grid was allowed to air dry, and samples were viewed under a transmission electron microscope (JEOL-100CX-II, Tokyo, Japan)

Characterization of cubosomal hydrogels

The prepared agomelatin loaded hydrogels were evaluated for pH, viscocity and in-vitro skin permeation studies

Determination of surface pH and viscosity of cubic hydrogel

The pH was determined using digital type pH meter by dipping the glass electrode completely into gel system so as to cover the electrode. The electrode was just brought in contact with the surface of the gel and the measurements were taken.

The viscosity of formulated gel was determined using a Brookfield programmable DV-III Rheometer (cone and plate). The sample holder was filled with the gel, spindle (No.52) was used to measure the viscosity of cubosomal gel at 10 rpm, temperature was maintained at 25°C±1°C. The sample was allowed to settle for 5minutes prior to taking the readings and the results were recorded

Determination of drug content

Drug content was determined by taking 100 mg of the cubic gel and diluting to 5 ml with ethanolic solution then subjected for vortex shaking for 5 min and the volume was made up to 10 ml with phosphate buffer pH6.4 and examined spectrophotometrically for drug content (Nida and Kamla, 2012)

***Ex vivo* permeation Studies**

Ex vivo skin penetration studies were carried out by the Franz diffusion cells using rat skin. The receptor compartment contained phosphate buffer pH 6.4 phosphate buffer (22mL). This solution was stirred with the help of a magnetic bar at 500 rpm and thermo stated at 37±0.5°C.

After placing approximately 100 mg of each gel formulation on the skin surface, the donor compartment was sealed (Elisabetta *et al.*, 2005), diffusion studies were carried out for 24h at intervals (t = 0,1,2,3,4,6, 8,10,12,24h) samples of 3mL were withdrawn and replaced with same volume of fresh solution. The samples were analyzed for agomelatin content by UV spectrophotometry (Elico-SL159, India), plot is drawn between the cumulative amount of drug penetrating the skin against time

RESULTS AND DISCUSSION

Particle size distribution and zeta potential of cubosome formulations

The average particle size of prepared cubosome dispersions was between 187 nm and 225 nm. The evaluated results were shown in Table 2. The PDIs were ranged from 0.208 to 0.283 indicating a relatively narrow size distribution of particles and the zeta potential of all prepared formulations was negative in the range of -14.5 to -23.5mV. Increasing the concentration of poloxamer 407 did not result in a significant reduction in particle size.

Table 2: Physical characterization- size, PDI, ZP, assay and EE of Cubosomes.

Formulation	Size (nm)	PDI	Zeta potential (mV)	EE (%)	Assay (%)
F1	225.8±7.54	0.283±0.04	-14.5±4.65	78.74±1.24	92.73±2.86
F2	187.6±3.97	0.208±0.08	-23.5±3.86	81.79±1.69	94.77±2.17
F3	209.5±5.31	0.257±0.05	-19.3±3.41	83.55±1.03	95.07±1.84

Differential scanning calorimetry (DSC) analysis

The purity of drug and the status of excipients in the AM-LN formulation was determined by DSC study. DSC thermograms of pure drug, pure GMO, pure poloxamer 407 and physical mixture of drug formulation were showed in Fig.2. The DSC thermogram AM showed a sharp melting endotherm peak at 102.6˚C. The peak was observed at its corresponding reported melting point (98-105˚C) which indicated the purity of drug (Wei *et al.*, 2013).

The pure lipid (GMO) and poloxamer 407 showed sharp endothermic peaks at melting points of 32.3˚C and 57.69˚C, respectively. In case of physical mixture, very broader drug endothermic peak at 100.3˚C (slight shift in melting point), lipid at

30.2˚C and poloxamer 407 at 58.1˚C. From these results, there was no interaction between the drug and formulation components were observed.

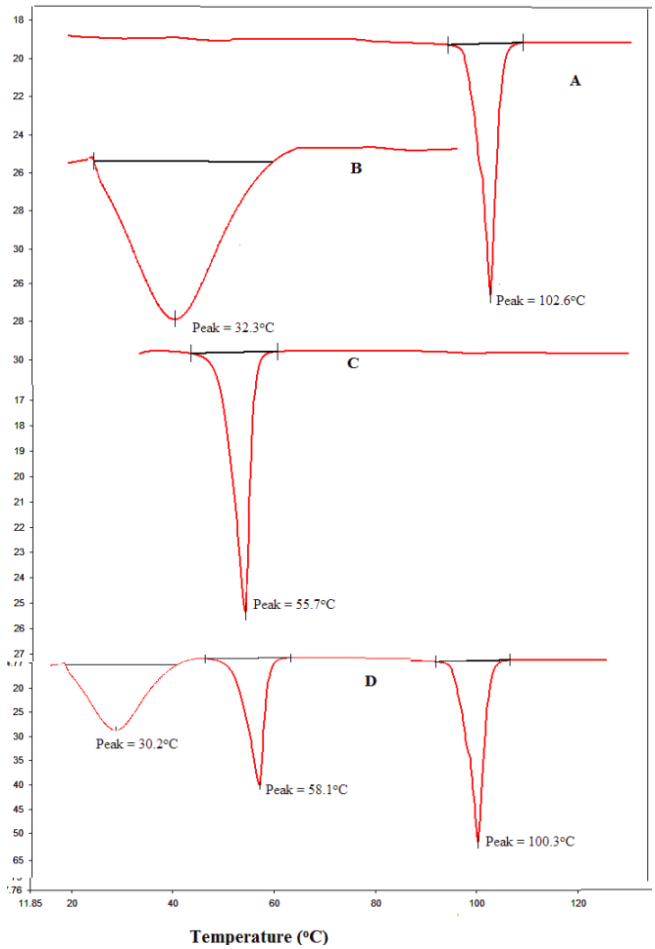

Fig. 2: DSC thermograms of A) Agomelatin, B) GMO, C) Poloxamer 407, and D) Physical mixture of drug, GMO and Poloxamer 407.

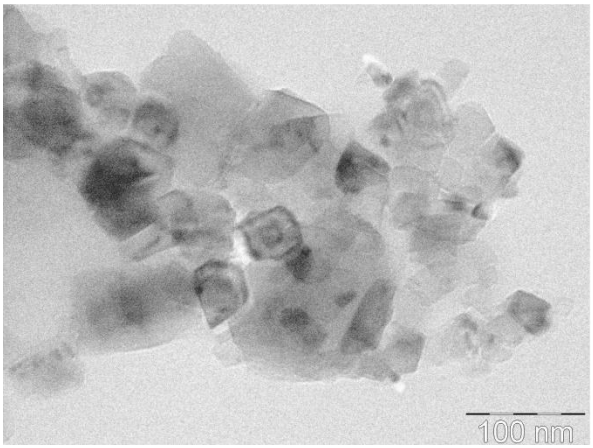

Fig. 3: TEM image of Agomelatin loaded hydrogel.

Transmission electron microscopy (TEM) studies

TEM analysis was performed to investigate the internal structure of the dispersed particles in Glyceryl monooleate based dispersions, cryo TEM analyses have been conducted and morphology of cubosomes was shown in Fig. 3. Cubosomes exhibiting the typical ordered cubic texture can be observed, dimensions have measured by a scale bar, finding that cubosomes with dimensions about 200 nm was reported.

Assay and entrapment efficiency of cubosomes

Entrapment efficiency of all cubosome formulations was in the range of 78.74±1.24 and 83.55±1.03, not much big difference in entrapment efficiency of cubosomes by altering the amount of poloxamer 407. Assay values are between 92.73±2.86 to 95.07±1.84 % and the corresponding results are showed in table 2.

In vitro release studies

In vitro drug release study was performed by dialysis bag diffusion technique and the results were shown in Fig. 4. The optimized formulation (F2) shown nearly 82.12% of the drug was released from the GMO based dispersion over a period of 24h compared to coarse suspension which showed 98 % drug release in 16h, which might be attributed to the sustained release from the matrices of monoolein. The release profiles indicate that agomelatin cubosomes showed a retarded release of the drug from the GMO lipid matrix when compared with plain coarse suspension of agomelatin.

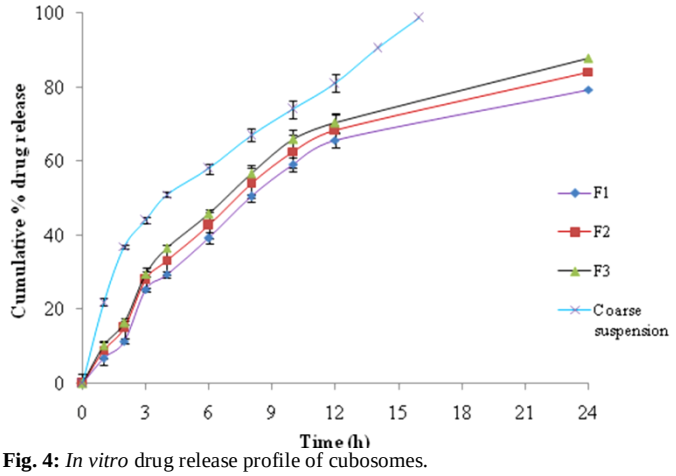

Fig. 4: *In vitro* drug release profile of cubosomes.

Table 3: Characterization of agomelatin loaded cubosome gel.

Formulation	Surface pH	Viscocity (CP)	Drug content
Optimized cubosome hydrogel	5.96	6.35	89.54±3.13
Control	5.61	4.92	91.88±4.58

Characterization cubosomal gel

Based on the particle size, PDI, zeta potential and entrapment efficiency (F2) formulation considered as optimized and converted in to gel by dispersing in the 0.5% w/w of carbopol934p dispersion, obtained gel was characterized for surface pH and rheological characterization results are represented in table 3. The pH of the gel was nearer to the pH of the skin (about 7.38±0.02) and the viscosity was found to be 6.35

centipoises when compared to control gel whose viscosity was 4.92 centipoises.

Ex vivo permeation studies of cubic gel

The ex vivo permeability studies was carried out by franz diffusion cells in pH 6.4 phosphate buffer and the results are shown in Figure 5. After 24 h of diffusion studies, 53.76% and 95.32% of the drug was diffused from the cubic hydrogel and plain gel, respectively. Thus, the amount of the drug diffused through the biological membrane from the cubic gel was in sustained manner as compared to the permeation from plain gel containing 0.1% drug solution.

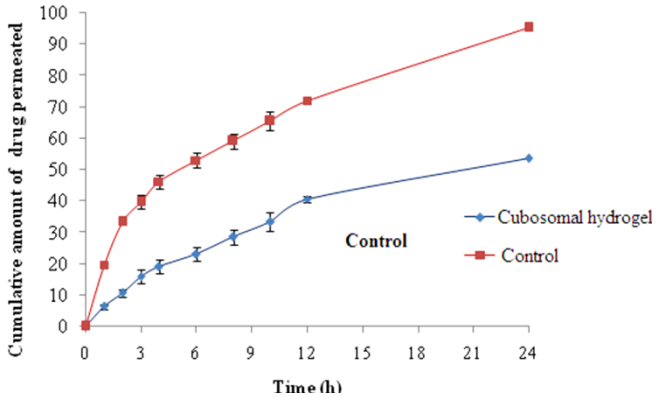

Fig. 5: *Ex vivo* permeation studies of agomelatin cubosome gel through rat skin.

CONCLUSION

The process used for preparation of AM-LN was simple, based on emusification of monooleine and poloxamer 407 in water, leads to the formation of a cubosomes and the optimized F2 formulation was converted in to cubic gel was suitable for the delivery of lipophilic drugs through the skin. Cubosomes loaded with agomelatin showed nanometre size particles with narrow particle size distribution, from the in vitro study, it was concluded that sustain the drug release from cubosomes over a period of 24 h. Ex vivo permeation studies demonstrated that retarded drug release rate through rat skin when compared to control gel was attained.

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