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DEVELOPMENT AND EVALUATION OF CUBOSOMES LOADED GEL OF TRETINOIN

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Abstract

Cubosomes are square and rounded, discrete sub-micron, nanostructured particles with internal cubic lattice of the bicontinuous cubic liquid crystalline phases. These are thermodynamically stable. Drug and other substance penetration through skin are influenced by the physiochemical properties of the penetrant, the state of the skin, and the nature of the vehicle at relatively slow rates. Antiseptics, antifungals, anti-inflammatory medicines, and skin emollients for protective benefits are among the drugs used topically, primarily for local action. Actinic keratoses (AKs), also referred to as senile keratoses or solar keratoses, are benign intra-epithelial neoplasms which is often associated with chronic sun exposure, individuals with AKs may present with irregular, red, scaly papules or plaques on sun-exposed regions of the body. If left untreated, AKs may evolve into invasive squamous cell carcinoma, which underscores the importance of early detection and development of a treatment plan. Actinic keratosis (AK) is a chronic disease which is mainly located across areas of sun-exposed skin. In the present work, the cubosomes were prepared by utilizing GMO and polymer. The optimal formulation size could be obtained when 100 mg of glyceryl monoolein, 33.793 mg of polymer, and 10 mg of drug as independent variables leading to the formulation of cubosomes with 128.3 nm in mean diameter and 78.852% in encapsulation efficiency. Prolonged release was achieved when they were formulated as topical gels on maintaining the cubosome structure.

Keywords: Tretinoin, Cubosomes, Actinic Keratoses, glyceryl monoolein, Poloxamer 407 etc.

Introduction

Cubosomes are square and rounded, discrete sub-micron, nanostructured particles with internal cubic lattice of the bicontinuous cubic liquid crystalline phases [1]. These are thermodynamically stable and consist of honeycombed (cavernous) structures separating two internal aqueous channels and a large interfacial area. These are nanoparticles which are self assembled liquid crystalline particles of certain surfactants with proper ratio of water with microstructure that provides unique properties of practical interest [2]. Hydrating a surfactant or polar lipid that forms cubic phase and then dispersing a solid like phase into smaller particles usually forms a cubosomes [3].

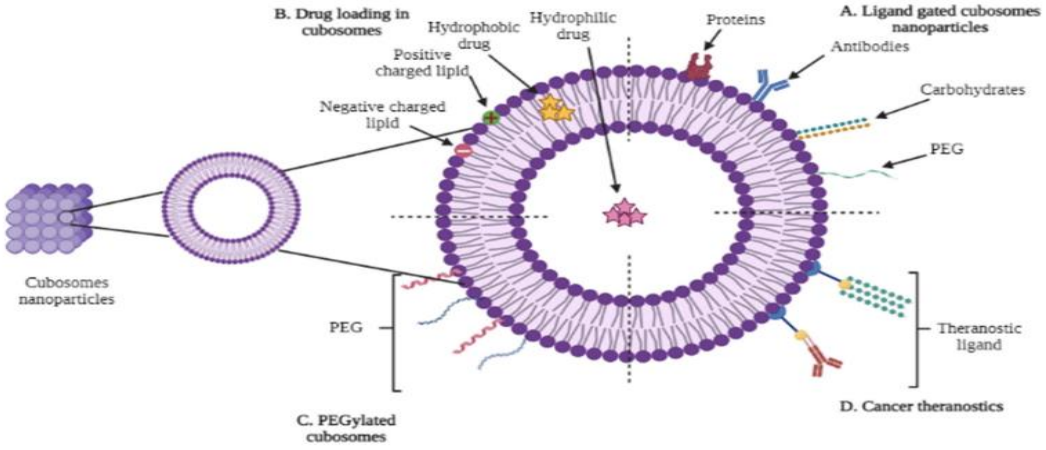

Figure 1.1: Cubosomes Exhibiting Internal and Cubic Structures with Potential of Drug Delivery

Mechanism of Drug Release from Cubosomes

It is based on the drug diffusion and drug concentration gradient across the cubosomes is the driving force. Therefore, drug release from the cubosomes coincide with Higuchi or Fick diffusion equation.

Topical Route of Drug Administration

Although the intact skin is much less permeable than other tissues, many substances do penetrate the skin to some extent. The skin controls the entry and exit of many chemicals, preventing moisture loss and controlling body temperature to preserve balance as homeostasis within the body [4]. Drug and other substance penetration through skin are influenced by the physiochemical properties of the penetrant, the state of the skin, and the nature of the vehicle at relatively slow rates. Antiseptics, antifungals, anti-inflammatory medicines, and skin emollients for protective benefits are among the drugs used topically, primarily for local action. This method can also be utilised to deliver systemic drugs. Hair follicles, sweat glands, and sebaceous glands may disperse a topically administered medicine through the skin, but permeation through the stratum corneum's many lipid bilayers is the most common route, and the pace is very slow [5]. The main route leading to living layers of the skin is winding and highly hydrophobic. Therefore, drugs that successfully diffuse across the stratum corneum should be relatively smaller in size, lipophilic or amphiphilic in nature, and non-irritating. However, many potentially valuable drug and cosmetic compounds have properties that do not meet these requirements [6]. To overcome these obstacles, attention has been focused not only

on the active ingredient but also on the form and composition of the entire formulation of a delivery system. These benefits entice pharmaceutical firms to create topical treatments for skin conditions.

Actinic Keratoses

Actinic keratoses (AKs), also referred to as senile keratoses or solar keratoses, are benign intra-epithelial neoplasms which is often associated with chronic sun exposure, individuals with AKs may present with irregular, red, scaly papules or plaques on sun-exposed regions of the body. If left untreated, AKs may evolve into invasive squamous cell carcinoma, which underscores the importance of early detection and development of a treatment plan. Actinic keratosis (AK) is a chronic disease which is mainly located across areas of sun-exposed skin [7]. UV radiation is a complete carcinogen in that it both induces the initial genetic mutations in keratinocytes and promotes tumor cell expansion. UV exposure induces protective responses in the cell, but when the burden of exposure and damage becomes excessive, the cell may undergo apoptosis to eliminate mutant cells from the epidermis. [8,9]

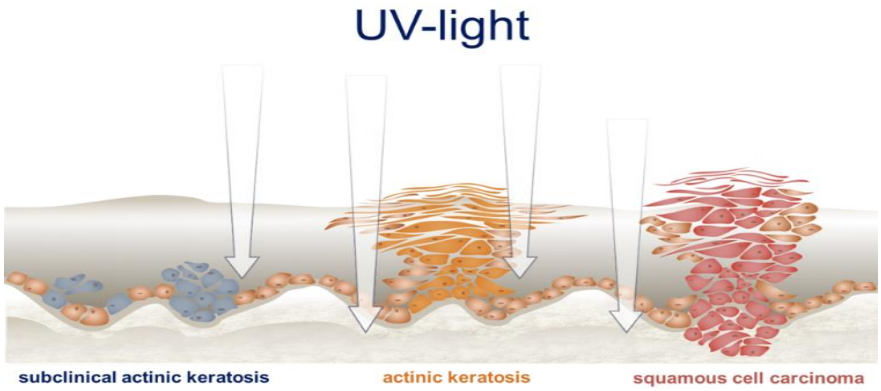

Figure 1.2: UV-light damages skin cells, over time these lesions grow into actinic keratosis (orange) and if ignored, may progress to squamous cell carcinoma.

Materials and Methods

Materials

Tretinoin was gifted from Akum Drugs and Pharmaceuticals Ltd., Haridwar, India. Poloxamer 407 and (GMO) Glyceryl Mono-oleate, methanol and Carboxy Methyl Cellulose (CMC) were used from the laboratory of Amity University, Noida. All other reagents used were of analytical grade.

Preparation of Cubosomes of Tretinoin

Tretinoin loaded cubosomes were prepared by bottom-up technique, using varying the concentration of lipid and surfactant and drug, a total of fifteen formulations of Tretinoin were developed.

Formulation of Cubosomal Dispersion

Preparation of cubosomes dispersions was based on the emulsification of Lipid/surfactant mixtures in water. In particular, the monoglyceride based lipidic phase was glyceryl monooleate (GMO) as used. Poloxamer 407 was used as surfactant. Briefly, GMO and Poloxamer 407 were melted in a water bath at 70ºC. Box-behnken design was used for the optimization of formulation parameters. The independent variables were Glyceryl monooleate amount, polymer amount, and drug amount while independent variables were selected as particle size and entrapment efficiency. In the case of Tretinoin containing dispersions, the

predetermined amount of the drug was added to the molten disperse phase and solubilized before adding to the aqueous solution. The molten mixture was then added dropwise to the aqueous phase at 70ºC under mechanical stirring at different speeds (i.e., 1500 rpm). Dispersions were maintained under stirring and were cooled to room temperature up to the solidification of lipid droplets (after 2 hours). Cooling was conducted using an external ice bath for 10 to 15 minutes. Dispersions were stored in glass bottles at room temperature for further investigations [10-12].

Checkpoint Analysis

After deleting irrelevant variables and/or interactions from the initial equation, a checkpoint analysis was performed to make more sense of the analysis of secondary (reduced) equation, which was very useful in optimizing and predicting the responses. Out of 15 runs of experiments and based on the predicted response given by the Box–Behnken design, a checkpoint batch was selected.

Formulation of Optimized Batch of Tretinoin Cubosomes (TCu-opt)

The checkpoint batch obtained after optimization study was formulated. The composition of optimized batch is as follows (Table 1.1).

Table 1.1: Composition of Optimized Batch of Tretinoin Cubosomes (TCu-opt)

Formulation Code	GMO (X1, mg)	Polymer (X2, mg)	Drug- Tretinoin (X3, mg)
TCu-opt	100.001	60.410	10.00

Preparation and Evaluation of Cubosomal Gel of Tretinoin

The optimized batch was further converted into gel by utilizing Carboxy methyl cellulose as gelling agent. The prepared gel was evaluated for appearance, pH, viscosity, in-vitro permeation study. Finally, the stability studies of cubosomes gel were also performed.

Preparation of Cubosomal Gel

The cubosomal gel was obtained by addition of weighted amount of carbomer (1% w/w) in distilled water and kept for half day forgetting to swell of carbomer and then add triethanolamine drop by drop up to pH 6.8. Propylene glycol is added to adjust the consistency. The obtained gel was then diluted with an appropriate amount of cubosomes dispersion in the ratio between the dispersion and the gel was 2:1 w/w.

Table 1.2: Composition of Tretinoin Cubosomal Gel (TCu-Gel) (1%)

Formulation Code	**Optimized batch Tretinoin Cubosomes**	**Gelling Agent (CMC)**
TCu-Gel	Equivalent to 10 mg	1% w/v

Evaluation of Tretinoin Cubosomal Gel

Appearance

About 1 week after preparation, the dispersions were visually assessed for optical appearance (e.g., colour, turbidity, homogeneity, presence of macroscopic particles) [12]

pH

pH of all formulations is determined by using digital pH meter by immersing the electrode in gel formulation and pH was measured.

Viscosity

Viscosity measurements were performed by Brookfield viscometer (AMETEK Brookfield**,** Germany)**.** The tested formulations were placed in the sampler tube using spindle no. 4. The

spindle was lowered vertically into the centre of the formulation and rotates at a speed of 50 rpm for 10 min. All measurements were carried out in triplicate and the mean value was recorded ± SD [13].

Stability Studies

Accelerated stability studies for optimized gel formulation (TCu-Gel) were conducted as per ICH guidelines at 40°C ± 2°C/75% ± 5% RH at sampling intervals of 0, 30, 60 and 90 days, respectively. The drug content pH and drug permeability were determined periodically.

Results and Discussion

Optimization of Tretinoin Cubosomes formulation

A Tretinoin cubosomes formulation with the best attributes was created using the data that was gathered. About 100.0 mg of factor A, 33.793 of factor B, and 10.00 mg of factor C were presented in the checkpoint batch. Particle size was 127.479 nm, Entrapment efficiency was 77.116%, and desirability was 0.918 for the optimized cubosomes formulation (Table 1.3). Table 1.3 showed that the model's validity and accuracy were confirmed by the fact that the experimental and proposed values of the responses of the best cubosomes formulation were in agreement without any significant differences ($p< 0.05$). Figure 1.3 is showing the contour graph for desirability.

Table 1.3: Actual and Experimental Values of the Optimized Tretinoin cubosomes formulation

Solution	GMO (mg)	Polymer (mg)	Drug (mg)	Particle size (nm)	Entrapment efficiency (%)	Desirability
Predicted	100.0	33.793	10.00	127.479	77.116	0.918
Actual	100.0	33.793	10.00	128.3	78.852	0.918

Checkpoint Analysis

The proposed regression models' superior prediction abilities were supported by the experimental and anticipated R^2 values. Additionally, the ratios of the actual to expected values showed low error rates, and there were acceptable residuals between the projected and experimental results; this shows that the data were not curved and that the model was adequate.

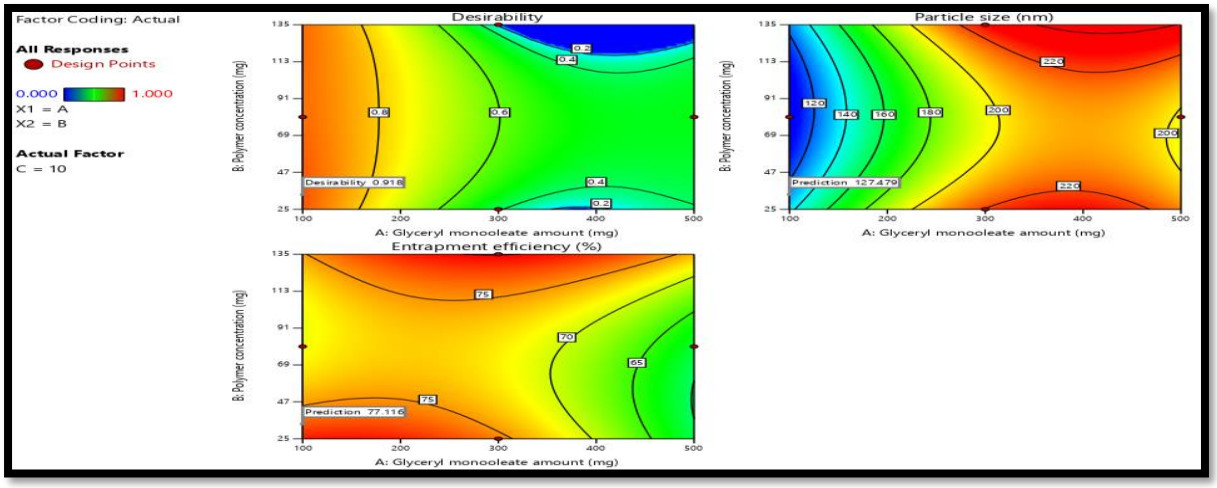

Figure 1.3: Contour graph of predicted responses and desirability

The optimized batch thus obtained was used for further studies. The results of different parameters of optimized batch were as mentioned below:

Evaluation Parameters for Optimized batch of Tretinoin cubosomes formulation (TCu-opt)

The optimized Tretinoin cubosomes formulation (TCu-opt) thus prepared was evaluated for particle size, pH, zeta potential, FTIR, TEM and in-vitro permeation study (Table 1.4).

Table 1.4: Evaluation Parameters for Optimized Tretinoin Cubosomes formulation (TCu-opt)

S. No.	Parameter	Inference
1.	Particle size (nm)	128.3 nm
2.	pH	6.8
3.	Zeta potential	-29.2 mV
4.	In- vitro permeation study	92.855 ± 0.013% in pH 6.8 Phosphate buffer

Evaluation of Tretinoin Cubosomes-Gel (TCu-Gel)

The Tretinoin cubosomes gel was checked for appearance, pH, viscosity and in-vitro permeation study. The results of evaluation parameters were depicted in table 1.5.

Table 1.5: Evaluation Parameters for Optimized Tretinoin Cubosomes gel formulation (TCu-gel)

S. No.	Parameter	Inference
1.	Appearance	Homogeneous
2.	pH	7.4
3.	Viscosity	145 cP
4.	In- vitro permeation study	91.948±0.011% in pH 6.8 Phosphate buffer

In-vitro permeation study of optimized Tretinoin Cubosomes gel formulation (TCu-gel)

The in-vitro release results are depicted in Figure 1.4 and Table 1.6.

Table 1.6: In-vitro Release Study of Optimized Tretinoin Cubosomes gel Formulation

S. No.	Time (Hrs)	pH 6.8 PBS
1.	0	0.00±0.004
2.	1	20.561±0.011
3.	2	46.986±0.018
4.	4	58.064±0.021
5.	6	66.715±0.017
6.	8	75.218±0.005
7.	10	82.427±0.013
8.	12	91.948±0.011

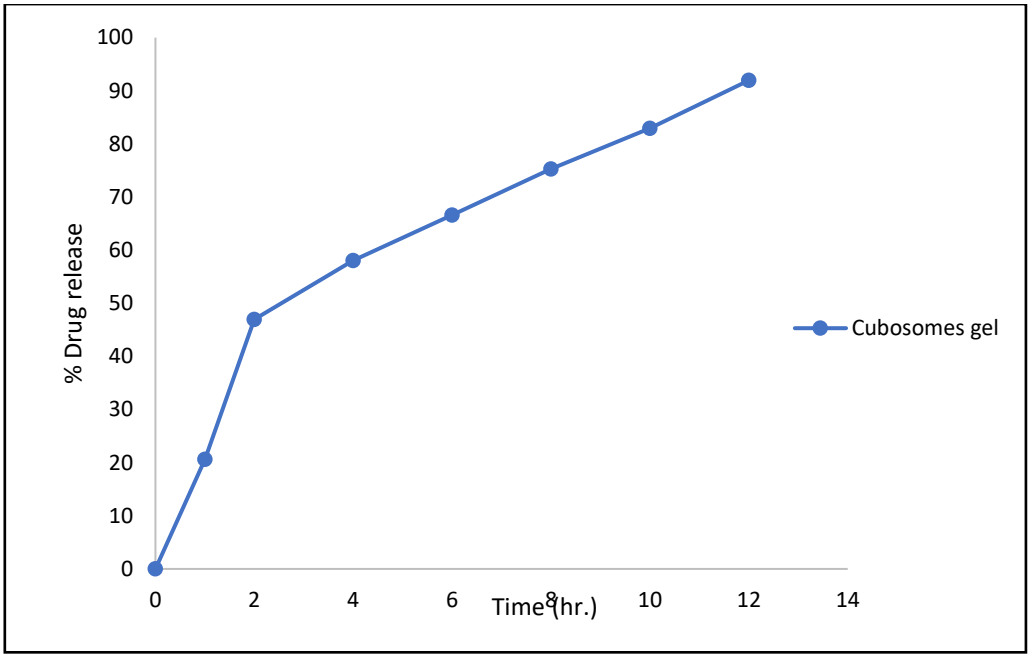

Figure 1.4: In-vitro permeation study of optimized Tretinoin Cubosomes gel formulation (TCu-gel)

Stability data

The gel formulation (TCu-gel) was kept under stressful conditions and found stable. Results were depicted in Figure 1.5.

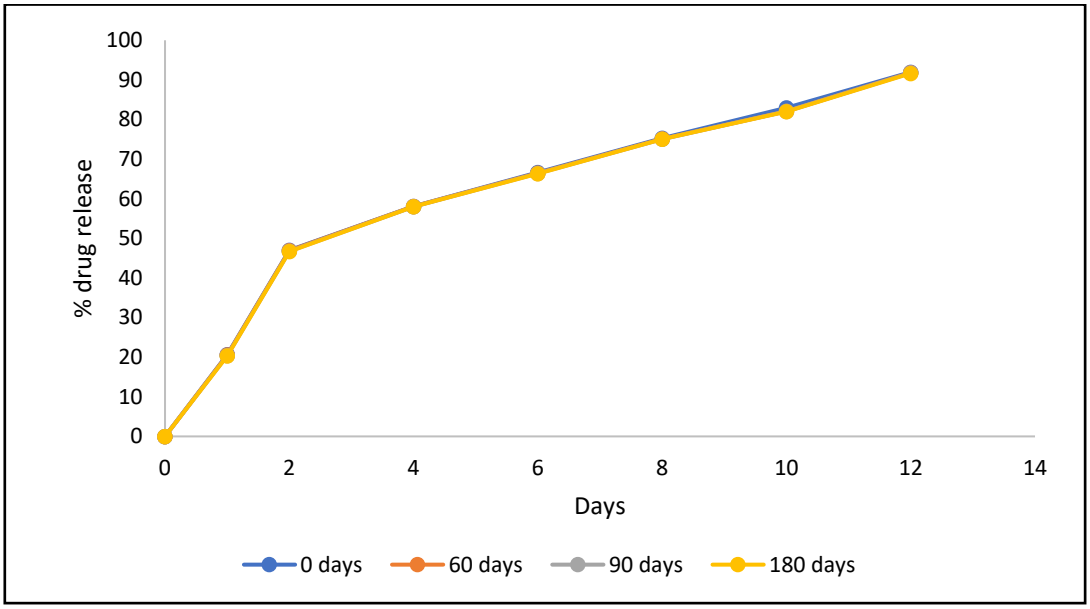

Figure 1.5: Effect of stability on the release of drug from Cubosomes gel formulation (TCu-gel)

Conclusion

In the present work, the cubosomes were prepared by utilizing GMO and polymer. The optimal formulation size could be obtained when 100 mg of glyceryl monoolein, 33.793 mg of polymer, and 10 mg of drug as independent variables leading to the formulation of cubosomes with 128.3 nm in mean diameter and 78.852% in encapsulation efficiency. Prolonged release was achieved

when they were formulated as topical gels on maintaining the cubosome structure. This product can be manufactured in large scale and commercialized for the treatment of skin infections, as it provided controlled delivery of the drug in human via the non-invasive skin route with more sustaining, less frequent dosing and with more bioavailability when compared to oral delivery.

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