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Exploration of Skin Permeability Possibility of Cubogels for Keratosis-like Skin Conditions

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ABSTRACT

Actinic keratosis is a chronic skin condition caused primarily by prolonged sun exposure, leading to the development of uneven, scaly patches on sun-exposed areas like the face, chest, and neck. Traditional treatments often encounter challenges such as poor skin permeability and formulation stability. This study investigates the potential of cubosomal hydrogels for enhancing the treatment of AK, focusing on their permeability, and effectiveness. Cubosomes, a type of liquid crystalline nanoparticle, are known for their controlled release and improved skin penetration capabilities. We formulated tretinoin-loaded cubosomes and incorporated them into carbopol-based hydrogels, referred to as cubogels, to address issues related to stability and ease of application. The formulation of tretinoin-loaded cubosomes was optimized, resulting in particles with a size of 69.5 nm, a polydispersity index of 0.312, and an entrapment efficiency of 82.13%. These cubosomes were then dispersed in hydrogels made with varying concentrations of Carbopol 940 (0.5%, 1.0%, and 1.5%). The cubogels were evaluated for physical properties, including appearance, pH, viscosity, spreadability, and drug content uniformity. The hydrogels exhibited good homogeneity and appropriate pH levels, with CG2 (1.0% Carbopol) showing the best spreadability and drug content uniformity. Ex-vivo studies using goat ear pinna demonstrated that CG2 provided the highest steady-state flux and permeability coefficient, indicating superior skin penetration. The cumulative drug permeation over 24 h was also higher for CG2 compared to the other formulations. In conclusion, the incorporation of cubosomes into hydrogels significantly enhances the stability and skin permeability of tretinoin, offering a promising approach for the treatment of actinic keratosis. Future studies should focus on clinical evaluation to confirm these findings and further optimize the formulation for broader therapeutic applications.

Keywords: Cubosomes, Hydrogel, Carbopol 940, Ex-vivo.

INTRODUCTION

Actinic keratosis (AK) is a chronic skin condition that mostly affects sun-exposed portions of the body. Patients with AKs may have uneven, red, scaly papules or plaques on areas exposed to sun frequently such as the face, chest, ears or neck. The possibility exists for AKs to progress into invasive squamous cell carcinoma in the absence of therapy, which emphasises the significance of prior detection and therapeutic plan for its treatment.[1] The appearance of actinic keratoses varies from

person to person. The signs and symptoms include Flat to slightly elevated patch or bump on the outermost layer of skin; Rough, dry, or scaly patch of skin, generally less than 1 inch (2.5 centimetres) in diameter; In rare circumstances, a hard, wart-like surface; Changes in colour, such as pink, red, or brown; Itching, burning, bleeding, or crusting; New lumps or patches on parts of the head, neck, wrists, and forearms exposed to the sun.[2]

Since UV radiation causes early genetic alterations in keratinocytes and encourages the growth of tumour cells, it is a full carcinogen. UV radiation causes the cell to respond defensively, but if the damage and exposure are too great, the cell may undergo apoptosis in order to remove mutant cells from the epidermis. By disrupting the physiological processes necessary for cell development and differentiation, prolonged and excessive sun exposure can cause a variety of pathologic alterations in epidermal keratinocytes. Dysplastic keratinocytes proliferate intra-epidermally as a result of the inflammation and immunosuppression that follow, giving birth to AKs.[3]

Self-assembled nanostructured materials have gained attention recently as a means of regulating the release of integrated chemicals. Viscous lipid-based systems, including bicontinuous cubic liquid crystalline phases, have a great deal of potential for use as drug delivery methods in the pharmaceutical industry. Dispersions of liquid crystalline phases in excess solvent-typically water-are known as liquid crystalline nanoparticles. Cubosomes, also known as cubic-phase nanoparticles, are liquid crystalline nanoparticles that share the distinctive characteristics of the bulk cubic phase[4]. The viscosity of cubosome dispersions is significantly reduced. Glyceryl monooleate's (GMO) bicontinuous cubic phase offers several interesting characteristics. GMOs are lipolytic objects, so the cubic phase breaks down biologically. Within its aqueous and lipid domains, it could solubilise molecules that are amphiphilic, soluble in oil, and soluble in water[5]. The bicontinuous cubic liquid crystal exhibits an appropriate organisation and regulated release of various chemicals, rendering it a viable option for medication administration.[6]

Since hydrogels have a smooth consistency analogous to real tissue and a high-water content, they have found widespread usage in therapeutic applications. The component has high water content which enhances their biocompatibility[7]. After application, the completely hydrated or swollen hydrogels' elastic nature reduces tissue irritation. It was discovered that protein adsorption and cell adhesion are reduced by the hydrogel surface's low interfacial tension with the bodily fluid. Drugs, peptides, and proteins in particular may benefit from the desired protection that hydrogels may offer from the potentially hostile environment around the release point. Cubosomes are nanoparticles with a unique cubical phase structure, offering advantages like controlled release and improved penetration through the skin barrier. When combined with hydrogels, these systems can provide a sustained release of tretinoin, reduce irritation, and enhance patient compliance.[8]

Tretinoin is effective in reducing lesions associated with actinic keratosis, which is characterized by rough, scaly patches of skin caused by long-term sun exposure. Treatment with tretinoin can help in the normalization of the epidermis and prevention of skin cancer. Because of its established safety profile and effectiveness, retinoid remains an integral component in the treatment of actinic keratosis.[9] In the treatment of this illness, investigating novel delivery techniques such cubosomal hydrogels has the potential to enhance patient results and satisfaction.[10]

The formula of cubosomes was already optimized in our previous work but when we conducted the stability studies cubosomes were found to be less stable. In addition, when we further evaluated the developed formulation for permeability studies using goat ear pinna, it was difficult to apply it easily as the cubosomal suspension was in liquid consistency. Therefore, the concept of cubosomes loading into hydrogel came into picture.

The purpose of this work is to prepare and characterise cubosomes loaded with tretinoin, after which the optimized batch of cubosome dispersion will be added to carbopol 940 forming hydrogels (cubogels), may enhance the stability and bioavailability of tretinoin. The produced cubogels will undergo an *in-vitro* permeation studies, and an ex-vivo study will be conducted to determine their effectiveness of the prepared cubogel.

MATERIALS AND METHODS

Materials

Tretinoin was gifted from Akum Drugs and Pharmaceuticals Ltd., Haridwar, India. Poloxamer 407 and (GMO) Glyceryl Mono-oleate, methanol, triethanolamine, and Carbopol 940 were used from the laboratory of Baba Mastnath University, Rohtak, Haryana, India.

Methodology

Preparation of calibration curve

After preparing the tretinoin standard stock solution (100 µg/mL) with methanol, different dilutions were produced from it in concentrations ranging from 10–60 µg/mL. One of the dilutions was scanned to confirm the absorption maxima of tretinoin using a UV-Visible spectrophotometer. Then, the rest of the dilutions were scanned at tretinoin's absorption maxima. The resulting absorbance was recorded, and to derive the regressed equation and regression coefficient, a graph among concentration and absorbance was drawn[11-12].

Formulation of Tretinoin-loaded Cubosomes

Tretinoin-loaded cubosomes were formulated using bottom-up strategy. The formula and the process of formulating cubosomes has already been established in our previous studies. Glyceryl monooleate (350 mg) and poloxamer 407 (110 mg) were utilized as lipid matrix and stabilizer respectively. Firstly, 350 mg of GMO and 110 mg of poloxamer were melted separately at water bath and then mixed uniformly to form lipoidal phase. The temperature of the water bath was maintained up to 60°C. To this molten mixture, 0.025%w/w of tretinoin was added and mixed homogeneously. Separately, distilled water was also heated on the water bath maintained at 60°C. The above-melted lipoidal phase was introduced dropwise into the water phase, using mechanical stirring at 1350 rpm.[13] This mechanical energy will aid to formulate the cubic-structured lipid nanoparticles, which were then loaded into gel for ease of application and enhancing its stability. The developed formulation was evaluated for particle size, polydispersity index, and entrapment efficiency.

Formulation of Tretinoin-loaded cubosomal gel

For the development of cubosomal hydrogels, Carbopol 940 was used as gelling agent at three different concentrations. Firstly, Carbopol 940 was swelled by keeping it for 5 h in distilled water at three different concentrations viz. 0.5%w/v (CG1), 1%w/v (CG2), and 1.5%w/v (CG3) separately. Secondly, cubosomal dispersion (equivalent to 0.025%w/w tretinoin) was added to swelled Carbopol and mixed uniformly. The ratio of swelled Carbopol 940 and cubosomal suspension was 1:2. Later, triethanolamine was added dropwise in all three concentrations, until the pH range between 5.5-6.5 is achieved.[14] Lastly, 0.1%v/v polyethylene glycol was added to developed hydrogels to obtain a smooth consistency. The final formulations were ultrasonicated to remove the air bubbles.

Evaluation of Tretinoin-loaded Cubosomal Gel

Organoleptic Evaluation, pH and gel strength:

The dispersions were visually evaluated for physical appearance (e.g., colour, turbidity, homogeneity, presence of macroscopic particles) about one week after their preparation. 1 g of all formulations was diluted separately with 10 mL of distilled water. Then, with the help of previously calibrated digital pH meter, the pH of all three formulations was measured by submerging the glass electrode in the diluted formulation.[15]

The duration needed for an exact weight (3.5 g) to pass through the gel specimen (5 g) for a distance of 3 cm was noted. A time of 25 to 50 seconds would be adequate.

Rheological Studies

For estimating the viscosity and spreadability of the developed formulations, the Brookfield viscometer and modified petri-plate method was employed. Using spindle number five, 30 g of each developed formulations were evaluated into the sampler tube. The spindle was dropped vertically into the formulation's centre and rotated for ten minutes at a speed of 50 rotation per minute. Every measurement was done three times, and the mean result was noted ± SD.

For the measurement of spreadability, 1 g of developed hydrogels were kept at the centre of inverted petri-plate. Then, another pre-weighed petri-plate was kept over the gel in such a way that the base of both petri-plate faces each other and forms a sandwich in which gel is kept. Later, increasing weight was kept over the upper petri-plate, until the spreading of the gel becomes

constant. Simultaneously, the time was noted down till the formulations achieve constant spreading. The cumulative weight kept, and the maximum diameter obtained were also noted down and spreadability was calculated with the formula given below:

Spreadability = M x L/t

Where: M is the cumulative weight kept over the upper petri-plate

L is the maximum diameter that gel achieves after spreading

T is the total time, gel took to spread

Drug Content Uniformity

To confirm the content uniformity of the drug, a preweighed (0.5 g) amount of developed cubosomal hydrogel was dissolved in 10 mL PBS (pH 6.8). The solution was then sonicated and filtered. The filtrate was then subjected to measurement of absorbance via UV spectrophotometer using PBS (pH 6.8) as a blank. The obtained absorbance was noted down and concentration was calculated by putting absorbance in regressed equation.

The percent drug content was determined using the following formula:

Theoretical Value/Practical Value*100

Ex-vivo permeation studies

An F-D cell with a 3.15 cm^2 diffusing cell area and a fifteen mL receiver volume was used with goat ear membrane was used for ex vivo tests. The goat ear pinna was collected from the local butcher an hour after it was sacrificed. Goat ear skin hair was properly removed using blade razor. The skin of the F-D cell was positioned between the donor and receiver chambers. The dermis was in close touch with PBS (pH 6.8), and the developed formulation was evenly applied to the dermis's uppermost layer. To resemble the skin conditions, the F-D cell was placed atop a magnetic stirrer set at a speed of 50 rpm and a temperature of 37±1°C. The aliquots were taken out at specified times and replaced with a fresh medium at the same time. The withdrawn samples were analysed via UV spectrophotometer and the mean±SD was obtained by taking three readings of the data. In addition, an assessment of skin permeability was performed by the computation of the enhancement ratio, permeability coefficient (Kp), and steady-state flux (Jss).

Results and Discussion

The calibration curve of tretinoin was successfully developed in methanol. The absorption maxima of tretinoin was found to be 347.2nm in methanol which close to the already reported in literatures i.e. 348nm. Subsequently, the calibration curve of tretinoin in methanol was successfully developed.[15] The regressed equation of the calibration curve was found to be as Y=0.103x+0.011 with an r^2 value of 0.999, demonstrating high linearity.

Furthermore, by employing bottom-up approach, the tretinoin-loaded cubosomes were effectively developed. The particle size, PDI, and entrapment efficiency was found to be 69.5nm±2.34, 0.312, and 82.13%±1.02 respectively. The developed cubosomes was then further loaded in hydrogels made up of three different concentrations of Carbopol 940 i.e., 0.5%w/v (CG1), 1%w/v (CG2), and 1.5%w/v (CG3).

Evaluation of Tretinoin cubosomes-loaded hydrogel

The color and homogeneity of all three developed formulation was found to be light pale yellow transparent for CG1, while it was white to light pale yellow transparent and white to light pale yellow slight translucent for CG2, and CG3 respectively. The results of all parameters are compiled in Table 1. There was no grittiness reported in all three formulations. Furthermore, the pH of all three formulations was found to be in the range of 5.35-5.87. Ideally, the pH of the skin lies between 5.0-5.5. The viscosity of the developed formulations CG1, CG2, and CG3 was found to be 21331, 23540, and 29681 cps respectively. CG1 revealed some liquid-like consistency as compared to CG2, and CG3. While CG3 was more viscous and stiffer as compared to CG2. The spreadability of all three gel formulations was found to be 42.5g/cm^2, 47.3 g/cm^2, and 40.6 g/cm^2. CG2 demonstrated more spreadability as compared to CG1, and CG2. The drug content uniformity of the cubosomal gels was found to be 88.41% ±0.21 for CG1, 91.54% ±0.43 for CG2, and 87.65% ±0.17 for CG3. The highest drug content was found in CG2 formulation.

Table 1: Compiled evaluated parameters for cubosomal hydrogels at three different concentrations

Evaluated Parameter	Formulations		
	CG1 (0.5%)	CG2 (1%)	CG3 (1.5%)
Colour and texture	Light pale yellow in colour	White to light pale yellow in colour	White to light pale yellow in colour
pH	5.71	5.35	5.87
Viscosity (cps)	21331	23540	29681
Spreadability (g/cm2)	42.5	47.3	50.6
Drug Content Uniformity (%)	88.41±0.21	91.54±0.43	87.65±0.17

Furthermore, the developed cubosomal gel formulations were evaluated for skin permeation using goat ear pinna. The graph was plotted between cumulative amount of drug permeated (mg) and time (h) as shown in Figure 1.

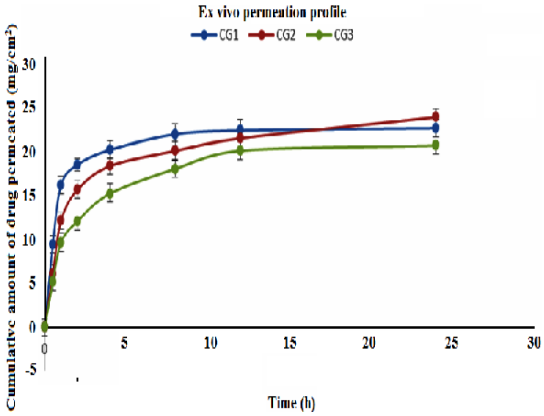

Fig. 1. Ex vivo permeation profile of Tretinoin loaded cubosomal gels

The results of steady-state gradient were found to be 0.142, 0.252, and 0.226 mg/cm^2/h for CG1, CG2, and CG3 respectively. The amount of drug that cumulatively permeated at the end of 24 h was 22.91mg ±1.05, 24.18mg ±0.62, 20.94mg ±0.36 from CG1, CG2, CG3 respectively. The value of permeability coefficients was found to be 3.55 cm/h, 6.3 cm/h, and 5.65 cm/h for CG1, CG2, and CG3 respectively. The results were found to be good for CG2, as compared to CG1, and CG2.

CONCLUSION

In this study, we explored the skin permeability potential of cubosomal hydrogels, specifically prepared for keratosis-like skin conditions. The cubosomal hydrogels demonstrated a promising ability to enhance skin permeability compared to traditional formulations. This is largely attributed to their unique nanostructure, which facilitates deeper penetration into the epidermal layers and improves drug delivery efficiency. Cubosomal hydrogels represent a significant advancement in the formulation of topical treatments for keratosis-like conditions. In conclusion, the incorporation of cubosomes into hydrogels significantly enhances the skin permeability of tretinoin, offering a promising approach for the treatment of actinic keratosis. Further research is needed to fully understand the long-term impacts of these hydrogels on skin health and to optimize their formulation for specific types of keratosis. Additionally, *in vivo* studies are essential to validate these findings and assess the practical benefits of cubosomal hydrogels in real-world scenarios.

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Conflict of interest

The author declare that we have no conflict of interest.

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