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Formulation and Evaluation of Cubosomal Gel of an Anti-Inflammatory Agent

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Abstract

The present research has been undertaken with the aim to develop a transdermal Cubosomal gel formulation of Ketoprofen, which would attenuate the gastrointestinal toxicities associated with oral administration. This research also aimed to encapsulate high drug pay load in Cubosomes for improved therapeutic efficiency. Cubosomes were prepared by Top- down technique. Different formulations (F1 – F9) were prepared and optimized for better performance in terms of drug content, SEM analysis, Zeta potential, entrapment efficiency and drug release. From F –F9 formulations, studies showed that F7 is better. Then it is formulated into gel using carbopol as gel base. The physical parameters like appearance, pH, viscosity, spredability, extrudability, ex- vivo drug release and in vitro skin irritation test using HET- CAM, were also evaluated. The cubosomal gel formulation (F7) was found to be clear without any aggregate indicating excellent homogenicity. The pH of the formulation was found close to neutral, indicating the absence of skin irritation. In vitro skin irritation study also reveals there is no skin irritation. The ex-vivo drug release study shows that the formulation (F7) has a good release rate when compared to other topical gels (87.2%). The kinetic study of the optimized formulation (F7) was also carried out and found that the formulation undergoes zero order kinetics. The mechanism of drug release was found to be Higuchi model.

Keywords: Cubosome; Cubosomal gel; Ketoprofen; Top-down technique

Introduction

Cubosomes are discrete, sub-micron, nanostructured particles of the bicontinuous cubic liquid crystalline phases. They consist of honeycombed structures separating two internal aqueous channels along with a large interfacial area. They contain similar microstructure as that of the parent with high surface area and their dispersions are less viscous than the parent cubic phases [1]. Almost all cubosomes are composed of polymers, lipids, and a surfactant with polar and non-polar components (amphiphilic). Due to the hydrophobic effect, amphiphilic molecules are driven into the polar solvent to impulsively identify and assemble into a liquid crystalline dispersion of nanometer scale. Thus, cubosomes are bicontinuous cubic liquid phase enclosing two distinct regions of water, divided by surfactant-controlled bilayers [2].

Components of cubosomes

Cubosomes composed of Natural lipids, Cationic and nonionic surfactants, Polymer systems.

Natural lipids

Although the lipid most widely used to construct bicontinuous cubic phases are monoglyceride, monoolein etc.

Monoglycerides: Monoglycerides are spontaneously form bicontinuous cubic phases upon the addition of

water, are relatively insoluble (allowing the formation of colloidal dispersions of cubosomes) and are resistant to changes in temperature [3].

Monoolien: The main precursor of cubosome formation is monoolein. Monoolein or glyceryl monooleate is a mixture of the glycerides of oleic acid and other fatty acids, consisting mainly of the monooleate [1].

Surfactant

Surfactants, which are used in the production of cubosomes, are poloxamer 407 in a concentration range between 0% and 20% w/w with respect to the disperse phase [4].

Polymer system

Polyvinyl alcohol (PVA) used in addition to poloxamer as a stabilizing agent of the dispersion [3].

Advantages of cubosomes

High drug payloads due to high internal surface area and cubic crystalline structures.
Relatively simple method of preparation.
Biodegradability of lipids.
Capability of encapsulating hydrophilic, hydrophobic, and amphiphilic substances.
Targeted release and controlled release of bioactive agents.
The cubic phases of cubosomes can be fractured and dispersed to form particulate dispersions that are colloidally and/or thermodynamically stable for longer time.

Disadvantages of cubosomes

Large scale production is sometimes difficult because of high viscosity [2].

Cubosomal gel

Cubosomal gels are the cubosomal dispersion of hydrogel. The term “Gel” was introduced in the late 1800 to name some semisolid material according to pharmacological, rather than molecular criteria. The U.S.P. defines gels as a semisolid system consisting of dispersion made up of either small inorganic particle or large organic molecule enclosing and interpenetrated by liquid. The inorganic particles form a three-dimensional “house of cards” structure. Gels consist of two-phase system in which inorganic particles are not dissolved but merely dispersed throughout the continuous phase and large organic particles are dissolved in the continuous phase, randomly coiled in the flexible chains [1].

Materials and methods

Materials

Poloxamer-407, Glycerol mono oleate and Ketoprofen were purchased from Yarrow chemicals, Mumbai. Carbopol, Propylene glycol and Triethanol amine were purchased from Spectrum chemicals, Kochi. Carbopol, propylene glycol and triethanol amine were of commercial grade. All other reagents used were of analytical grade.

Table 1: Formulation design of cubosomes.

Formulation	Poloxamer-407 [gm]	Glycerol monooleate [gm]	Ketoprofen [mg]	Water [ml]
F_1	0.3	2.5	100	50
F_2	0.3	2.0	100	50
F_3	0.3	1.5	100	50
F_4	0.25	2.5	100	50
F_5	0.25	2.0	100	50
F_6	0.25	1.5	100	50
F_7	0.2	2.5	100	50
F_8	0.2	2.0	100	50
F_9	0.2	1.5	100	50

Formulation of cubosomal dispersion

Cubosomal dispersions of Ketoprofen were prepared by top-down technique. Accurately weighted quantity of Glyceryl monooleate (GMO) and poloxamer 407 polymer mixed and melted in a water bath at 600C, to this mixture add Ketoprofen drug and stir until completely dissolved, then to this solution add drop by drop preheated (up to 700C) distilled water of suitable quantity by continuous stirring for 2 hours, This whole system is taken into subjected for homogenization at 1500 rpm for 1 minute under at room temperature. Thus, formed liquid dispersion of cubosomes was kept at a room temperature, avoids direct sunlight and which will used for further study [5]. The formulation design was given in table 1.

Evaluation of cubosomal dispersions

Analytical method used in the determination of Ketoprofen

Determination of λmax of Ketoprofen

Absorption maximum of pure Ketoprofen was determined by dissolving Ketoprofen in phosphate buffer saline pH 7.4. A sample of 10µg/ml was prepared and scanned for maximum absorbance using UV Visible spectrophotometer in the range from 200 - 400 nm using phosphate buffer saline pH 7.4 as blank [4].

Preparation of calibration curve of Ketoprofen

10 mg of ketoprofen was accurately weighed and transferred into 100 ml volumetric flask. The drug was dissolved and made up to the volume with phosphate buffer saline pH 7.4. It was further diluted with same buffer to get concentration of 2, 4, 6, 8 and 10µg/ml. The absorbance of solution was measured spectrophotometrically at 259 nm using buffer as blank. The absorbance values were plotted against concentration to obtain the standard graph.

Compatibility studies

Compatibility studies were done using FTIR and DSC [6,7].

Optical microscopy

The cubosomal dispersions prepared were observed under binocular compound microscope at 10X and 40X magnification for studying the shape and surface morphology [4].

Particle size and polydispersity index

The mean particle size and particle size distribution was determined by Malvern nano Zeta sizer instrument. The vesicles after diluted with distilled water were considered for the measurement of size [6].

SEM

The prepared samples of cubosomes are coated with a gold film under vaccum for 2min. The specimens are transferred to an ISI ABT SX-40A scanning electron microscope and digital images captured [5].

Determination of percentage drug content

1ml of dispersion was pipetted from the dispersion and was further diluted with pH 7.4 phosphate buffer saline and the samples were analyzed spectrophotometrically at 259nm [6].

Determination of percentage entrapment efficiency

The %EE of the vesicles was determined using centrifugation technique. The vesicular dispersion was centrifuged for 20 min. Supernatant containing unentrapped drug was withdrawn and measured UV spectrophotometrically at 259nm against phosphate buffer saline pH 7.4. The amount of drug entrapped in liposomes was determined by [7].

$$\%EE = \frac{T\_C}{C} \times 100$$

where, T=Total amount of drug calculated in both supernatant and sediment. C=Drug in supernatant.

In Vitro drug release

In vitro drug release was measured using Franz diffusion cell. 50mg ketoprofen containing cubosomal dispersion was placed on one side of egg membrane in a vertical franz diffusion cell. Other side of membrane was in contact with the dissolution medium phosphate buffer saline of pH 7.4. Entire dissolution assembly was placed on a magnetic stirrer at temperature of 37°C. Aliquots of dissolution medium was withdrawn at different time intervals for 8hr. Drug concentration in the dissolution medium were determined by UV spectrophotometry at 259 nm [8].

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 7. 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 [7].

Evaluation of 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) [9].

pH

pH of all formulations is determined by using digital pH meter by immersing the electrode in gel formulation and pH was measured [7].

Drug content

1g of the prepared gel was mixed with 100ml of methanol. Aliquots of different concentration were

prepared by suitable dilutions after filtering the stock solution and analyzed using UV [10].

Ex- vivo skin permeation study

In vitro skin permeation studies were performed using goat ear skin. The superficial skin was collected from the back of goat ear and the hair on the skin was removed. Skin was then mounted in a modified Franz diffusion cell, which is kept at 37ºC. Weighed quantity of cubosomal gel was then spreaded on the stratum corneum side of skin (donor compartment) and dermis side was facing receptor compartment. Receptor compartment contains 25 ml of pH 7.4 phosphate buffer and after everyone hour 1 ml of sample was taken and replaced with the same volume of phosphate buffer. After 6 hours sampling, absorbance was measured at 259 nm against blank of pH 7.4 phosphate buffer by UV spectrophotometer. And the percentage drug permeated was calculated [11].

In-vitro Anti-inflammatory Activity

The anti-inflammatory activity of cubosomal gel was studied by using inhibition of albumin denaturation technique. The reaction mixture (5 ml) consisted of 4.5 ml of bovine serum albumin (5% aqueous solution) and 0.5 ml of cubosomal gel, pH was adjusted at 6.3 using a small amount of 1N Hydrochloric acid. The samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling the sample, 2.5 ml of phosphate buffer solution was added into each test tube. Turbidity was measured spectrophotometrically at 259 nm. For control tests, 0.5 ml of distilled water was used instead of cubosomal gel. The percentage inhibition of protein denaturation was calculated as follows [12].

Percentage inhibition=(Abs Control –Abs Sample) X 100/Abs control.

In-vitro skin irritation test

HET-CAM (Hen's Egg Test on the Chorioallantoic Membrane) Test.

Incubated eggs of 9 days were collected from hatchery, shells were removed carefully using forceps. Test sample is applied directly to the CAM. Allow the sample for exposure to the CAM for atleast 300 second. The end point is measured by the visual inspections [13].

Release kinetics

Kinetic study was carried out by fitting the in vitro drug release data into Zero order, First order, Higuchi model, Hixon-Crowell Cube Root Law model and Korsmeyer- Peppas models. The best outfit model was confirmed by the value of R2 which is near to 1 [12].

Stability studies

Accelerated stability studies for optimized gel formulation (D2) 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 release are determined periodically [14].

Result and discussion

Analytical method

Determination of λ max

The 10 µg/ ml sample was prepared and scanned between 200 to 400 nm. The drug showed maximum absorption at 259 nm. So, the λ max of Ketoprofen was found to be 259nm.

Calibration Curve

Standard calibration curve data was given in table 2. From figure 1 y intercept and R2 value was found to be 0.021 and 0.995, respectively.

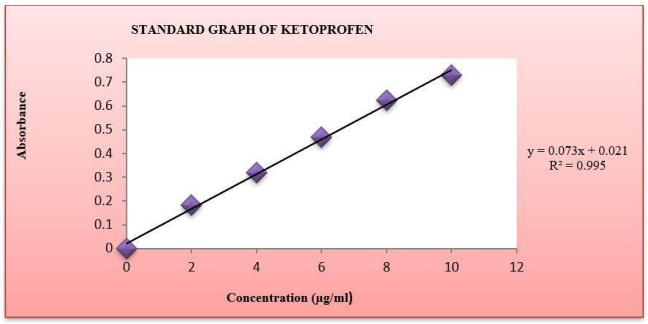

Figure 1: Calibration curve of ketoprofen.

FTIR

There were no significant changes in the frequency of the functional groups of Ketoprofen. So, the drug was compatible with Poloxamer, GMO and Carbopol. Results were shown in table 2 and figure 2.

DSC studies

The melting point remains almost the same, indicated that the drug and excipients are compatible with each other. The result was shown in figure 3.

Table 2: Standard calibration curve data of Ketoprofen.

Sl. No.	Concentration (µg/ml)	Absorbance
1	0	0.00
2	2	0.182
3	4	0.320
4	6	0.468
5	8	0.623
6	10	0.729

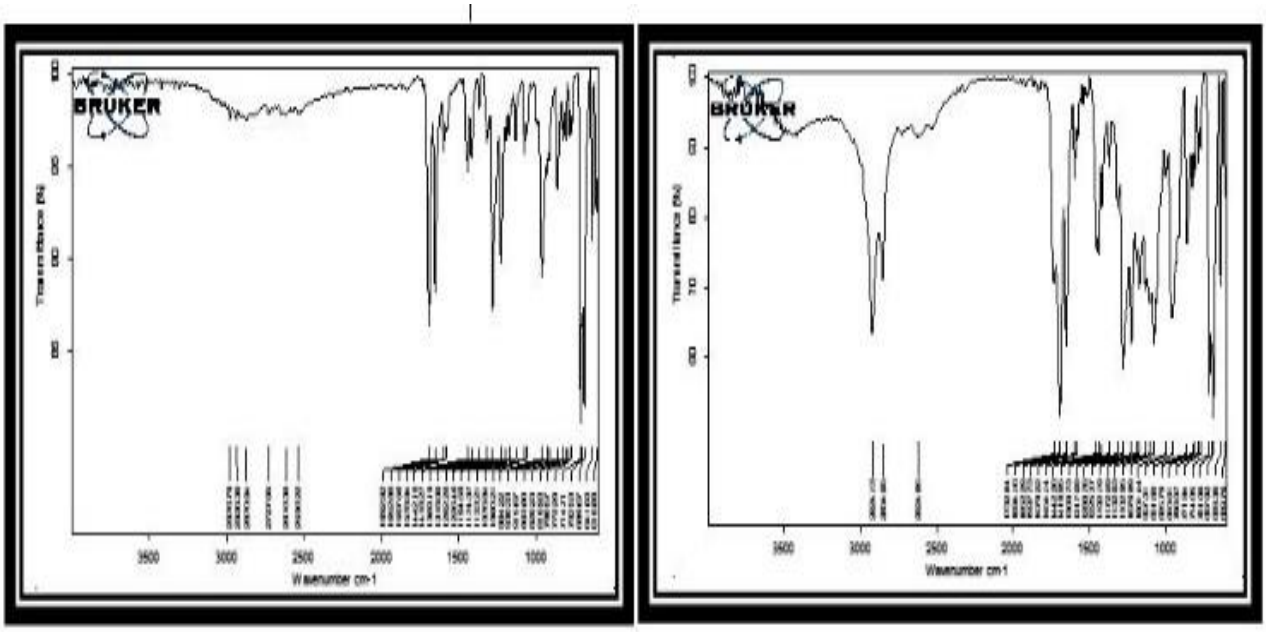

Figure 2: FTIR Spectra of Ketoprofen (sample) and drug mixture.

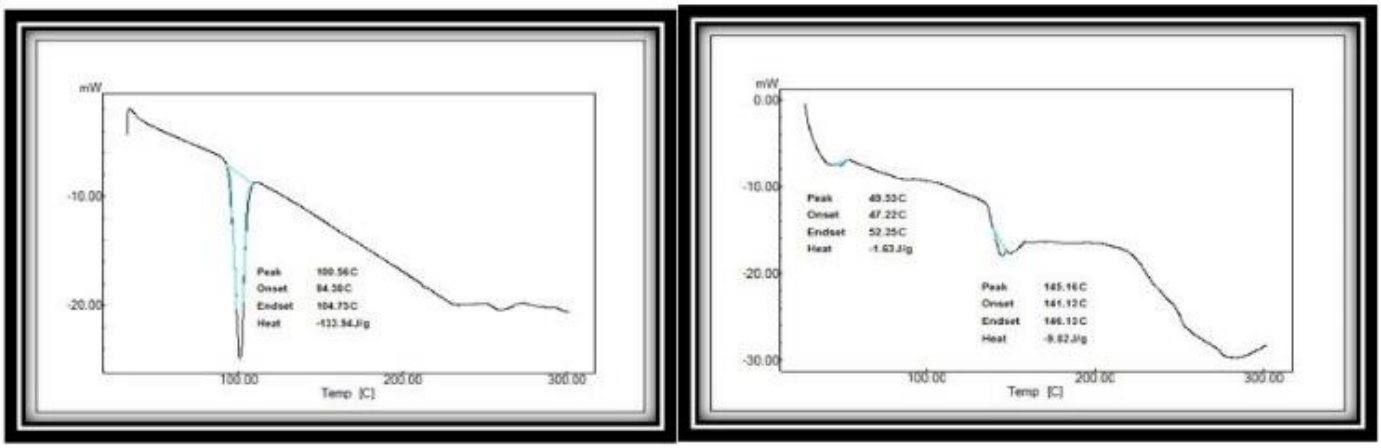

Figure 3: DSC curve of pure Ketoprofen and drug mixture.

Evaluation of ketoprofen cubosomes

Optical Microscopy

Images obtained under an optical microscope confirmed the formation of the crystal structures. Showed in figure 4. It was found that the formed crystals were spherical, and some are in rod shape.

Particle size and polydispersity index

The mean particle size and particle size distributions were determined by Malvern nano Zeta sizer instrument. The result is given in the table 3.

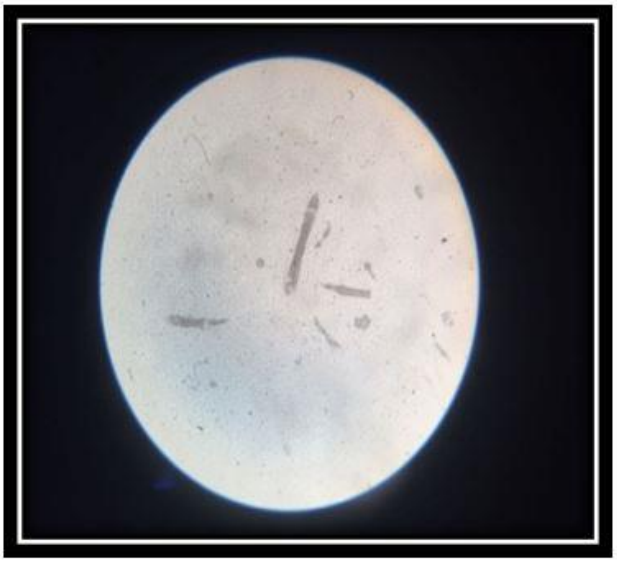

Figure 4: Microscopic view of Cubosome.

Table 3: Comparison of FTIR spectra of ketoprofen and excipients.

Sl. No	Drug and Excipients	Functional Groups (cm^{-1})			
		C=0 stretching of Carboxylic acid	C=O stretching of Ketone	Aromatic C=C	O-H stretching of Carboxylic acid
1.	Ketoprofen	1692	1652	1597	2976
2.	Ketoprofen+Poloxamer	1694	1653	1597	2974
3.	Ketoprofen+GMO	1693	1652	1597	2976
4.	Ketoprofen+ Poloxamer+GMO	1695	1651	1540	2975
5.	Ketoprofen+ Poloxamer+ GMO+ Carbopol	1694	1652	1597	2974

Drug content estimation of cubosome

The % drug content in various formulations ranged from 90.68-95.38%. The drug content data revealed that there was no significant difference in the uniformity of the drug content in the formulations. So, it indicated that Ketoprofen was uniformly distributed in vesicular dispersions.

Drug entrapment studies of cubosome

Drug entrapment efficency was determined to make sure that the added amount of Ketoprofen is present in the cubosome dispersion. The EE of all batches is in the range of 74.93 ± 0.903 - 92.10 ± 0.250. The highest EE was found in the batch F_7, consisted of 2.5 g of GMO, and 0.3g of poloxamer 407. The EE of Ketoprofen into cubic nanoparticles was dependent on the concentration of GMO. The result showed that the EE increased, as the amount of lipid and surfactant increased. Increasing amount of GMO was bound to increase the % of EE because of the increased concentration of mono-, di-, and triglycerides, which act as solubilizing agents for ketoprofen and provide more space to accommodate excessive drugs. This effect may be observed due to the increased viscosity of the medium, because increasing the amount of lipid resulted in faster solidification of the cubosomal nanoparticles, which would prevent drug diffusion to the external phase of the medium. As the percentage of emulsifier increased, part of the ketoprofen was

incorporated in the surfactant layer at the surface of the cubosomes, leading to a high entrapment efficacy.

In Vitro drug release studies of cubosome

The *In vitro* release characteristics of cubosomal dispersions shows that the drug release is directly proportional to the concentration of GMO and inversely proportional to the concentration of P-407 i.e. the cubosomes showed decrease in percent drug release when using of lower concentration of GMO and higher concentration of P-407 polymer. Here F7 has higher GMO concentration and lower Poloxamer 407 concentration, and it also showed higher percemtage of drug release ie, 86.87**%.** Results are shown in figure 5.

Preparation of cubosomal gel

From the drug content, drug entrapment and drug release study, it is found that F7 is the best formulation. So, it was selected and formulated to gel Figure 6.

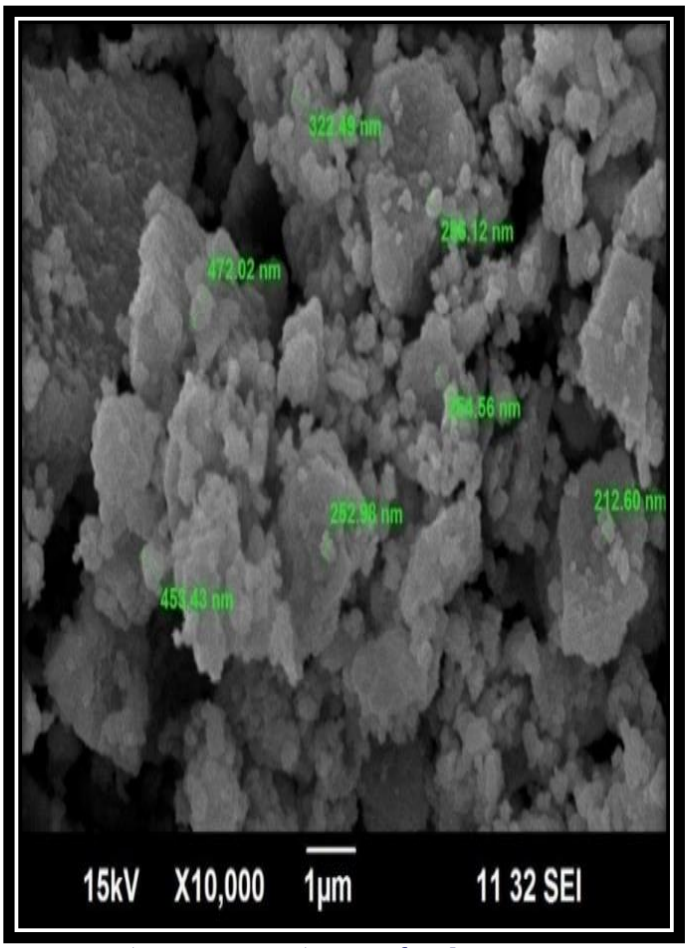

Figure 5: SEM image of Cubosome.

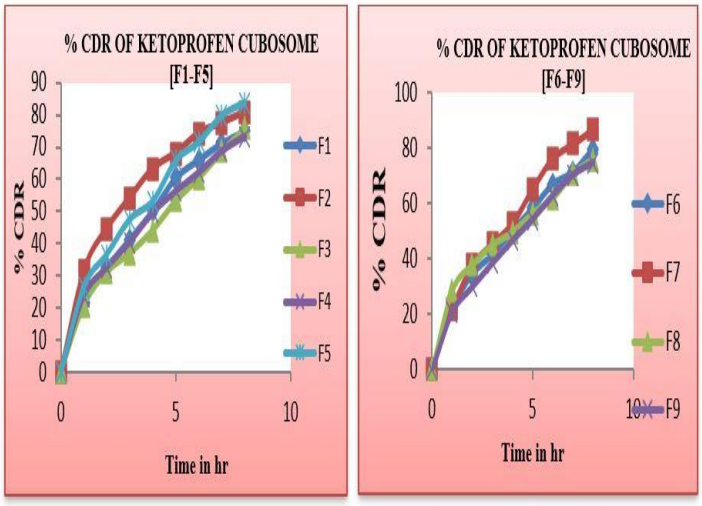

Figure 6: Comparison of in vitro % CDR profile of Ketoprofen Cubosome [F1-F9].

Figure 7: Ketoprofen cubosomal gel.

Appearance

It was determined by visual inspection. All the formulations were found to be homogenous.

pH

The pH was found to be 5.7, which was close to skin pH.

Drug content estimation of cubosomal gel

Drug content of the gel formulations was found to be 95±0.583%. (The reading is an average of 3 determinations).

Ex- vivo skin permeation study

The gel prepared using optimized cubosomal dispersion [F7] was used for *ex-vivo* permeation study using goat's ear skin and showed 87.2% permeation through the skin. It is shown in table 4 and figure 7.

In Vitro anti-inflammatory activity

The cubosomal gel was analyzed for its anti-inflammatory activity. Denaturation of proteins is a well-documented cause of inflammation. From the results of present study, it can be stated that the ketoprofen cubosomal gel is effective in inhibiting heat induced albumin denaturation. The percentage inhibition was found to be 70.77 %.

Table 4: Particle size and polydispersity index of cubosome.

Formulation	Average Particle size (nm)	Polydispersity index (PDI)
F1	488 ± 3.2	3.16 ± 0.1
F2	631 ± 2.4	4.05 ± 0.0
F3	679 ± 2.0	1.00 ± 0.0
F4	740 ± 1.9	1.81 ± 0.1
F5	650 ± 2.2	0.59 ± 0.1
F6	647 ± 3.7	0.91 ± 0.0
F7	453 ± 1.5	0.71 ± 0.0
F8	409 ± 3.0	1.24 ± 0.1
F9	655 ± 2.1	2.03 ± 0.0

In-Vitro skin irritation test

The in-vitro skin irritation test was performed using HET-CAM. The Cubosomal gel formulation was found to be free of irritation and is safe. The observations show absence of haemorrhage, lysis and coagulation. The results are shown in table 5 and figure 8.

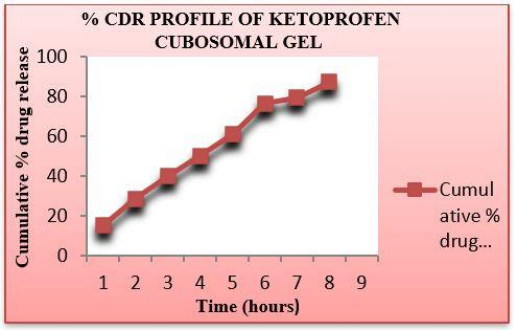

Figure 8: ex-vivo permeation of cubosomal gel.

Table 5: Ex-vivo permeation study of cubosomal gel of optimized formulation.

Time (hours)	Cumulative % drug release
0	0
1	15.1
2	28.6
3	39.8
4	50.2
5	61.0
6	76.3
7	79.2
8	87.2

Kinetic study of ketoprofen cubosomal gel

The kinetic study of the optimized formulation (F7) was also carried out and found that the formulation undergoes zero order kinetics. The mechanism of drug release was found to be Higuchi model. The results are shown in table 6 and figure 9 and figure 10.

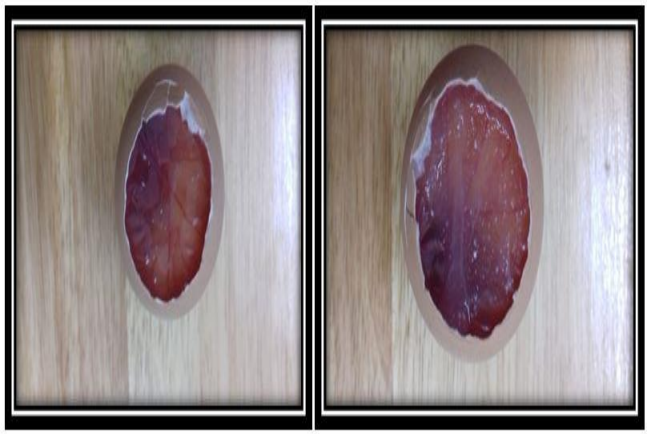

Figure 9: Skin irritation study-Before and after test.

Stability study

pH, Drug content and drug release values are analyzed periodically as per ICH guidelines through accelerated stability studies for optimized gel formulation was shown in table 7.

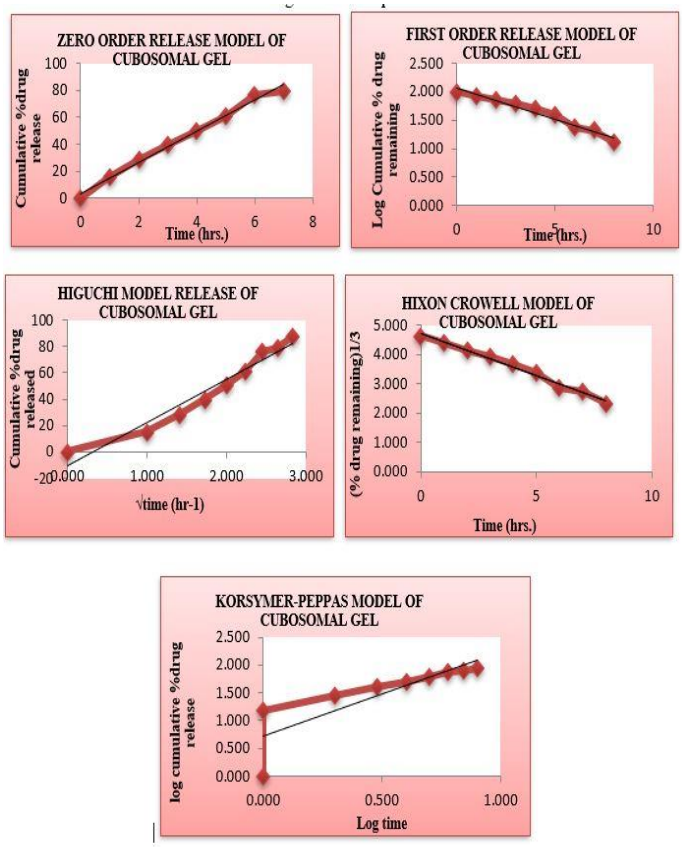

Figure 10: Kinetic plots.

Table 6: In-vitro skin irritation study–HET-CAM.

End point	Observation
Haemorrhage	-
Lysis	-
Coagulation	-

Table 7: Stability study of cubosomal gel.

Time in days	pH	Drug content	Drug release
0	5.7	95	87.20
30	5.5	94	85.38
60	5.4	92	82.56
90	5.3	90	81.27

Conclusion

Cubosomes can be prepared by simple combination of biologically compatible lipids (GMO) and water and are thus more suited for pharmaceutical and body tissue. The ability to form cubosomes during the preparation offers enhanced flexibility for product development. The above research specifies that the cubosomal utility as controlled release drug carrier. Prolonged release is achieved when they are formulated as topical gels on maintaining the cubosome structure. This product can be manufactured in large scale and commercialized for the treatment of arthritic patients, as it provides 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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References

1. Ansel HC, Popovich NG, Allen Loyd V, Pharmaceutical dosage forms and drug delivery systems. 2005; 415-419.
2. Bhosale RR, Osmani RA, Harkare BR et al. Cubosomes: The inimitable nanoparticulate drug carriers. Scholars Academic J Pharm 2013; 2:481-486.
3. Anbarasan B, Shanmuganathn FG. An overview of Cubosomes: Smart drug delivery system. Sri Ramachandra J Med 2015; 8: 215-220.
4. Pawar PD, Arane PM, Saindane HB, et al. Review on pharmaceutical excipients. Am J Pharm health Res 2015; 3: 32-50.
5. Hundekar YR, Saboji JK, Patil SM, et al. Preparation and evaluation of diclofenac sodium cubosomes for percutaneous administration. World J Pharm Pharma Sci 2014; 3: 523-539.
6. Verma N, Deshwal S. Design and *in vitro* evaluation of transdermal patches containing Ketoprofen. World J Pharma Res 2014; 3: 3930-44.
7. Ramani R, Rao CB, Ayanampudi A, et al. Formulation and evaluation of mephenesin topical gel. World J Pharma Res 2013; 2: 1475-1489.
8. El-Saharty YS, Hassan NY, Metwally FH. Simultaneous determination of clotrimazole HCL and triamcinolone acetonide by UV derivative spectrophotometry and spectrodensitometry. J Pharma Biomed Analysis 2002; 28: 569– 580.
9. Ahlin P, Kristl J, Smid-Kobar J. Optimization of procedure parameters and physical stability of solid lipid nanoparticles in dispersions. Acta Pharm 1998; 48: 259-267.
10. Ben JB. Characterisation of drug release from cubosomes using the pressure ultrafiltration method. Int J Pharm 2003; 260: 239-247.
11. Baboota S, Al-azaki A, Kohli K, et al. In vivo assessment of enhanced topical delivery of econazole nitrate to human stratum corneum. J Pharma Sci Technol 2007; 58: 1-10.
12. Bhargavi K, Indira S, Srinivas P. Formulation, and evaluation of aceclofenac loaded Cubosomal topical gel. Int J Pahrma Res Scholar 2015; 4: 27-37.
13. Gulsu A, Ayhan F, Ayhan H. Preparation, and characterization of Ketoprofen loaded albumin microspheres. Turk J Biochem 2012; 37: 120–128.
14. Pattanayek S, Puranik S. Formulation, and evaluation of ketoprofen loaded nanoparticulate gel for topical delivery. Int J Pahrm Pahrma Res 2018; 11: 250-260.