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FORMULATION AND EVALUATION OF CUBOSOMAL GEL FOR TOPICAL APPLICATION

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Keywords:

Cubosome, Cubosomalgel, Indomethacin, Top-down technique

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ABSTRACT: The present research aims to develop a transdermal Cubosomal gel formulation of Indomethacin, which would attenuate the gastrointestinal toxicities associated with oral administration and also aimed to encapsulate high drug pay load in Cubosomes for improved therapeutic efficiency. Indomethacin is an NSAID commonly recommended for the relief of pain and inflammation. Indomethacin was formulated as Cubosome to avoid the gastric irritaion. Top- down techniques were used for the preparation of cubosomes. Different formulations (F1-F6) were prepared and optimized for better performance in terms of drug content, SEM analysis, Zeta potential, entrapment efficiency and drug release. Then it is formulated into gel using carbopol as gel base. The physical parameters like appearance, pH, viscosity, spreadability, extrudability, *ex-vivo* drug release and *in-vitro* skin irritation test using HET- CAM were also evaluated. The cubosomal gel formulation (F5) was found to be clear without any aggregate indicating excellent homogeneity. The *ex-vivo* drug release study shows that the formulation (F5) has a good release rate. The kinetic study of the optimized formulation (F5) was also carried out and found that the formulation undergoes zero order kinetics. The mechanism of drug release was found to be Higuchi model.

INTRODUCTION: Cubosomes are discrete, sub-micron, nanostructured particles of the bicontinuous cubic liquid crystalline phases [1, 2]. 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.

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In cubosomes, the cubic phases composed of two separate thermodynamically stable structure consisting of, continuous but non intersecting hydrophilic regions which are separated by a lipid bilayer [3]. The structure of cubosome retains the stability and efficacy of activities like vitamins and proteins.

Cubosomes are thermodynamically stable, long lasting. By the addition of polymers, the colloidal dispersions of cubosomes can be stabilized. It also shows the potential for controlled delivery of drugs, in which diffusion is governed by the passage of the drug through the “regular” channel present in structure of the cubic phase. Cubosomes are liquid crystalline nanostructured particles with the same unique properties of the bulk cubic phase, although

cubosome dispersions have much lower viscosity [4]. Cubosome is a honey-combed structure separating two internal aqueous channels along with large interfacial areas. Cubosomes are Nano sized, more accurately nanostructure particles of a liquid crystalline phase having cubic crystallographic symmetry which is formed by the self assembly of surfactant like molecules [5]. Methods for preparation of cubosomes are high-pressure homogenization, Probe Ultra sonication, Automated Cubosome Preparation; Some Special techniques for The Preparation of Cubosomes are Top-down Technique, Bottom-up Technique [6, 7].

Indomethacin is an NSAID commonly recommended for the relief of pain and inflammation. One of the main side effects of Indomethacin is gastric irritation. To avoid that, Indomethacin was formulated as Cubosome. Compared with oral administration, topical applications lead to relatively high NSAID application are variable but are at least equivalent to that obtained with oral administration. NSAIDs applied topically do reach the synovial fluid, but the extent and mechanism such as topical penetration versus distribution via the systemic circulation remain to be determined. Percutaneous absorption may be strongly influenced by individual skin properties. Topically applied NSAIDs have a high safety profile to oral formulation [8].

MATERIALS AND METHODS: Materials used in this study were Indomethacin, Polaxamer-407, GMO, Carbopol-934, Propylene glycol, Triethanolamine and these materials were purchased from Yarrow chemicals, Mumbai and Spectrum chemicals, Kochi.

Preformulation Study: Preformulation studies include Identification of Drug. FTIR spectrum of the sample was taken using potassium bromide pellet method. The Organoleptic Evaluation, determination of Melting Point, determination of Solubility of Indomethacin, and the analytical Method such as UV spectrophotometer was used in the Determination of Indomethacin, Differential Scanning Calorimetry (DSC) study was carried out using the instrument DSC-60 (Shimadzu).

Identification of Drug: The monograph of Indomethacin signified that the substance under examination was intimately mixed with potassium bromide. The FTIR spectrum of the sample was taken using potassium bromide pellet method. The spectrum of test specimen was recorded over the range from 4000cm-1 to 500cm-1 and compared with the corresponding USP reference standard [9].

FTIR (Fourier Transform Infrared) Study: The IR spectra were recorded using FTIR spectrophotometer. The samples were prepared by mixing the drug and the excipients in 1:1 ratio and the mixtures were stored in closed containers for 1 month. The FTIR spectrum of the samples was taken using potassium bromide pellet method. The physical mixtures of Indomethacin and excipients were scanned in the wavelength region between 4000 and 500 cm^{-1} and compared to check compatibility of drug with excipient.

Formulation of Cubosomal Dispersion: Cubosomal dispersions of indomethacin 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 60°C, to this mixture add indomethacin drug and stir until completely dissolved, then to this solution add drop by drop preheated (up to 70°C) 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 [10].

TABLE 1: FORMULATION DESIGN OF CUBOSOMES

Formulation	Poloxamer- 407 [gm]	Glycerol monooleate [gm]	Indomethacin [mg]	Water [ml]
F1	0.3	2.5	100	50
F2	0.3	2.0	100	50
F3	0.3	1.5	100	50
F4	0.25	2.5	100	50
F5	0.25	2.0	100	50
F6	0.25	1.5	100	50

Evaluation of Cubosomal Dispersions: Formulated cubosomal dispersions were subjected to the preliminary evaluation tests; Physicochemical properties such as optical microscopy, particle size and polydispersity index, yield, drug content, drug entrapment efficiency, Determination of pH of Vesicular Dispersion, In-Vitro Drug Release, Zeta Potential [11].

Scanning Eelectron Microscopy (SEM): The prepared samples of cubosomes are coated with a gold film under vacuum for 2min. The specimens are transferred to an ISI ABT SX-40A scanning electron microscope and digital images captured [12].

Preparation of Cubosomal Gel: The cubosomal gel was obtained by addition of weighted amount of carbomer (2% w/v) in distilled water and kept for half day for getting 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. Then stir for 5 min, the cubosomal gel will be formed [13].

Evaluation of Cubosomal Gel: Various evaluation of cubosomal gel were carried out such as Appearance, pH, Viscosity, Drug content, Spreadability, Extrudability, *Ex-vivo* Skin Permeation Study, *In-vitro* Anti-inflammatory Activity, *In-vitro* Skin Irritation Test were done [14].

***Ex-vivo* Skin Permeation Study:** *Ex-vivo* skin permeation studies were performed using goat ear skin. The skin specimens were then cut into appropriate size after carefully removing subcutaneous fat, washing with normal saline. Skin was then mounted in a modified Franz diffusion cell, which is kept at 37 ºC.

Weighed quantity of cubosomal gel was then spread 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 every one 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 [15].

***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 1N Hydrochloric acid. The samples were incubated for 20 min at 37°C and then heated for 3 min at 57°C. 2.5 ml of phosphate buffer solution was added into each test tube after cooling. Turbidity was measured spectrophoto-metrically at 259 nm. For control tests; 0. 5 ml of distilled water was used instead of cubosomal gel.

***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 at least 300 seconds. The end point is measured by the visual inspections which is Hemorrhage, Lysis, Coagulation

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.

Stability Studies: Accelerated stability studies for optimized gel formulation (D2) were conducted as per ICH guidelines at 30°C ± 2°C/65% ± 5% RH at sampling intervals of 0, 30, 60 and 90 days respectively. The drug content pH and drug release are determined periodically.

RESULTS AND DISCUSSIONS:

Preformulation Study: The organoleptic evaluations of Indomethacin were done. Its color was found to be white or almost yellowish. It was found to be odorless or almost odorless. Its appearance was found to be crystalline powder. The melting point determination of drug was also studied and it was found to be 153ºC by capillary tube method. The solubility studies of drug were also studied.

The drug was found to be very slightly soluble in water and soluble in phosphate buffer pH 7.4, methanol, and chloroform. The analytical methods like absorption maximum, calibration curve of drug and compatibility studies were studied. The absorbance maximum of Indomethacin in Phosphate Buffer pH 7.4 was measured as 259 nm. The standard graph of Indomethacin was plotted between absorbance and concentration (µ g/ml). y intercept and R2 value was found to be 0.073 and 0.995 respectively.

Identification of Drug:

FTIR (Fourier Transform Infrared): FTIR spectrum of Indomethacin exhibited peak signals at 1692 cm^{-1} due to C=O stretching of carboxylic acid, peak signals at 1612 cm^{-1} due to C-n, peak signals at 1687 cm^{-1} due to C-CO-O of aromatic group, peak signals at 1176 cm^{-1} due to O-H stretching of carboxylic acid. The spectrum of Indomethacin and excipients exhibited peak signals at 1694 cm^{-1} due to C=O stretching of carboxylic acid, peak signals at 1652 cm due to C-N peak signals at 1671 cm^{-1} due to C-CO-O of aromatic group, peak signals at 1123 cm^{-1} due to O-H stretching of carboxylic acid. The FTIR spectrum of Indomethacin and excipients was compared with the FTIR spectrum of Indomethacin. It was observed that there were no significant changes in characteristic peaks indicating compatibility between Indomethacin and the excipients.

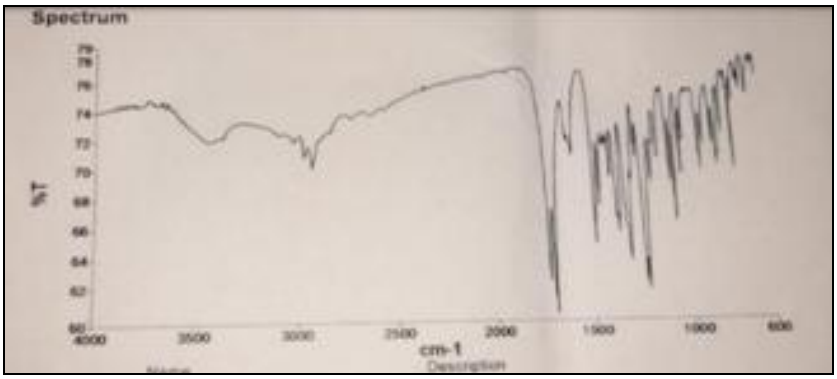

FIG. 1: FTIR SPECTRUM OF INDOMETHACIN (SAMPLE)

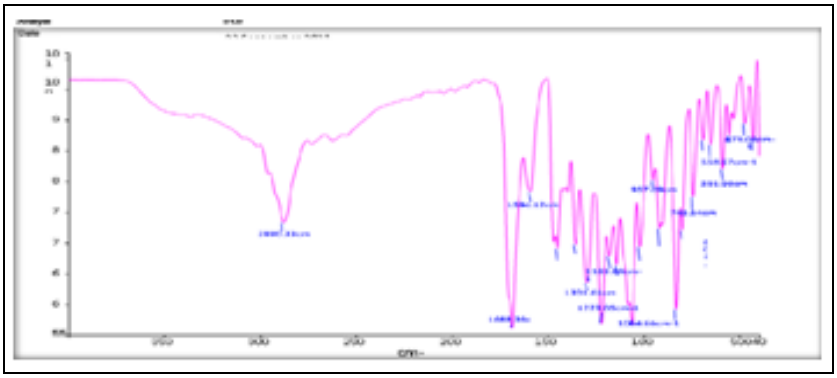

FIG. 2: FTIR SPECTRUM OF INDOMETHACIN+POLOXOMER+GMO+CARBAPOL

TABLE 2: COMPARISON OF FTIR SPECTRA

Sl. no.	Drug	Functional Groups(cm^{-1})			
		C=0	C-N	C-CO-O	O-H
1	Indomethacin	1692	1612	1687	1176
2	Sample+poloxomer+GMO+Carbapol	1695	1511	1671	1123

Evaluation of Indomethacin Cubosomes: The % drug content in various formulations ranged from 90.68-94.03% as given. 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 Indomethacin was uniformly distributed in vesicular dispersions. Drug entrapment efficiency was determined in order to make sure that the added amount of Indomethacin is present in the cubosome

dispersion. The highest entrapment efficiency was found in the batch F5. The pH value of all the prepared cubosomal dispersions ranged from 5.50-6.21. Zeta potential of the cubosomal formulation was determined using Zeta sizer.

TABLE 2: % DRUG CONTENT AND DRUG ENTRAPMENT, PH OF CUBOSOMAL DISPERSION AND ZETA POTENTIAL OF CUBOSOAML FORMULATION

Formulation Code	Percentage drug content (Mean± S.D)*	Percentage drug entrapment (Mean±S.D)*	pH* (Mean± S.D)	Zeta Potential (meV)
F1	92.59±0.583	74.93±0.903	5.50±0.005	-22 ± 1.4
F2	91.27±0.689	78.62±0.304	5.62±0.011	-21 ± 1.8
F3	93.35±0.396	78.50±1.205	5.52±0.011	-19 ± 1.2
F4	93.03±0.846	80.65±0.383	6.11±0.005	-24 ± 1.0
F5	94.68±0.583	88.82±0.238	6.10±0.005	-26 ± 1.1
F6	92.03±0.349	80.06±0.460	6.21±0.005	-23 ± 2.2

*Each reading is an average of 3 determinations.

Scanning Electron Microscopy (SEM): The SEM image of Cubosome using ISI ABT SX-40A scanning electron microscope is shown.

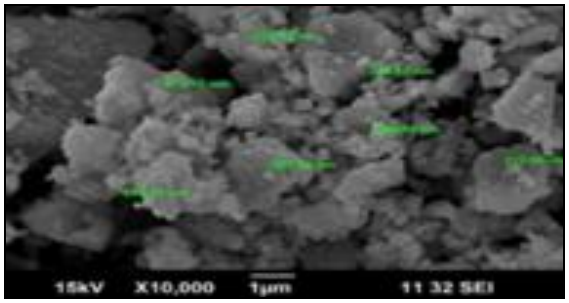

FIG. 3: SEMIMAGE OF CUBOSOM

***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 F5 has higher GMO concentration and lower poloxomer 407 concentrations, and it also showed higher percentage of drug release is, 83.80%

TABLE 3: % CDR OF INDOMETHACIN

Time (hr)	% CDR					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	21.32	31.37	20.63	23.48	26.38	21.97
2	32.34	44.63	31.16	32.45	36.50	34.58
3	41.25	54.19	36.92	40.36	47.45	41.57
4	50.15	63.25	44.12	49.51	53.09	50.29
5	60.47	68.09	53.28	56.17	65.73	58.29
6	66.09	74.23	60.44	61.99	71.47	66.95
7	71.25	77.47	68.60	68.65	79.73	71.34
8	75.00	80.81	75.80	73.23	83.80	79.84

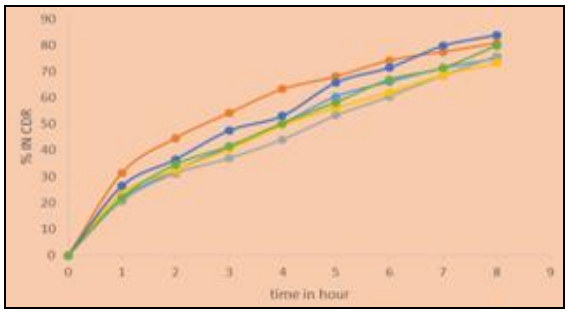

FIG. 4: COMPARISON OF *IN-VITRO* % CDR PROFILE OF INDOMETHACIN CUBOSOME (F1-F6)

Preparation of Cubosomal Gel: From the drug content, drug entrapment and drug release study, it is found that F5 is the best formulation. So, it was selected and formulated to gel.

FIG. 5: INDOMETHACIN LOADED CUBOSOMAL GEL

Evaluation of Indomethacin Cubosomal Gel: The cubosomal gel formulated showed better drug content, pH, viscosity, spreadability, extrudability, *ex-vivo* permeation and *in-vitro* anti inflammatory activity. The drug content was found to be 94±0.583%.

The pH was found to be 5.6. The viscosity was found to be in the range of 4065-7621 cp. The spreadability was found to be 12.33 g cm/sec. The percentage cumulative drug release of cubosomal gel was found to be 83.80% in 8 hrs.

TABLE 4: VISCOSITY, PH, DRUG CONTENT ESTIMATION, SPREADABILITY, EXTRUD ABILITY OF INDOMETHACIN CUBOSOMALGEL

Viscosity	4065-7621 cp
pH	5.6
Drug content estimation of cubosomal gel	94±0.583%.
Spreadability	12.33 g cm/sec.
Extrudability	good

***Ex-vivo* Skin Permeation Study:** The gel prepared using optimized cubosomal dispersion [F5] was used for *ex-vivo* permeation study using goat's ear skin and showed 81.2% permeation through the skin.

TABLE 5: *EX-VIVO* SKIN PERMEATION STUDY OF FORMULATED

Time (hours)	Cumulative % drug release
0	0
1	15.1
2	28.6
3	39.8
4	50.2
5	68.0
6	81.3

***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 cubosomal gel is effective in inhibiting heat induced albumin denaturation. The percentage inhibition was found to be 71.77 %.

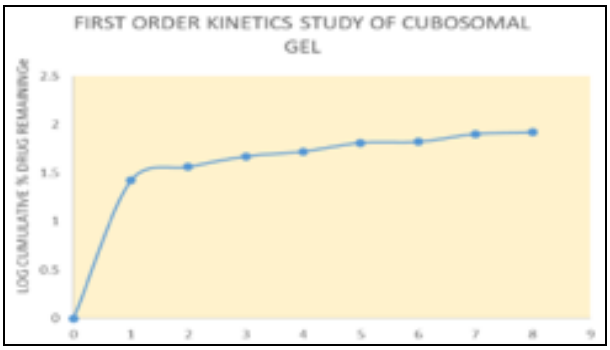

FIG. 6: CDR PROFILE OF INDOMETHACIN CUBOSOMAL GEL

TABLE 6: *IN-VITRO* SKIN IRRITATION STUDY – HET-CAM KINETIC STUDY OF CUBOGEL:

Endpoint	Observation
Hemorrhage	-
Lysis	-
Coagulation	-

***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 are as shown below.

The kinetic models like Zero order model, first order model, Hixson Crowellmodel, Higuchimodel and Korsmeyer Peppasmodel was used to study the drug release kinetics.

The drug release kinetics was found to be zero order kinetics. The mechanism of drug release was found to be Higuchimodel.

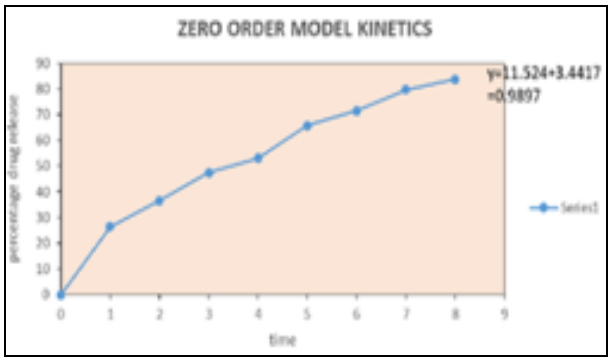

FIG. 7: ZERO ORDER PLOT OF GEL GELCUBOSOMAL

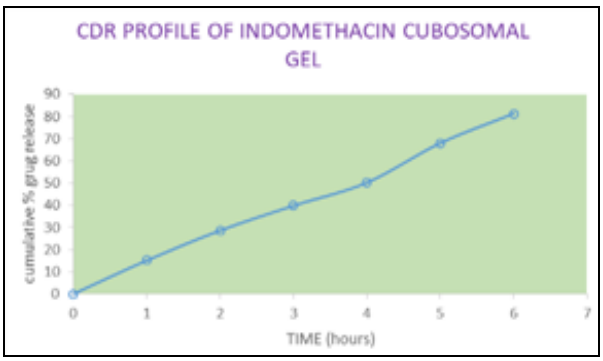

FIG. 8: FIRST ORDER PLOT OF CUBOSOMAL

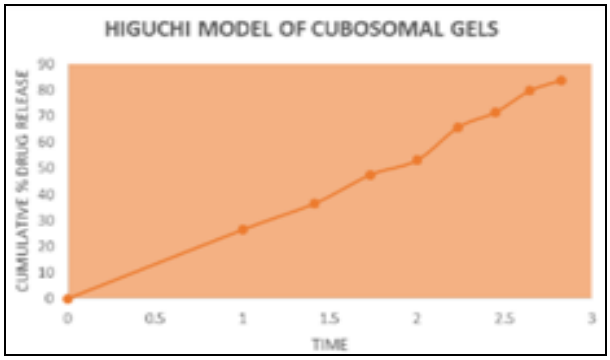

FIG. 9: HIGUCHI PLOT OF CUBOSOAML GEL

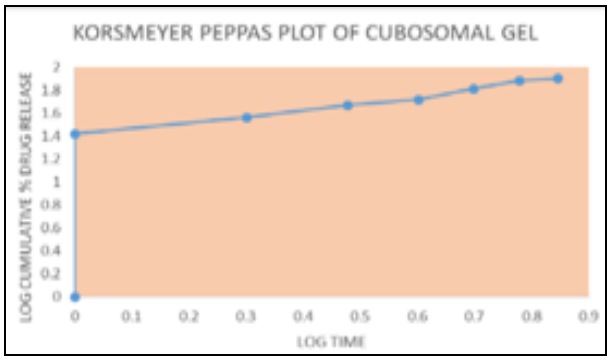

FIG. 10: HIXON CROWELL PLOT OF CUBOGEL

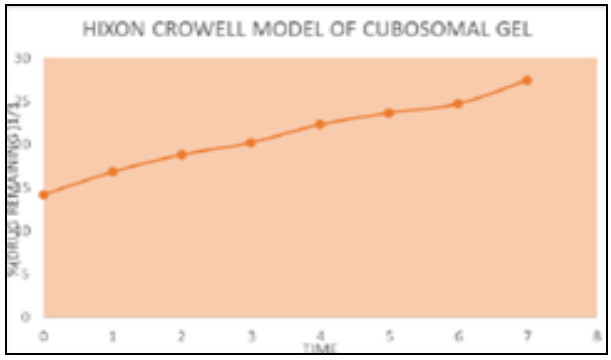

FIG. 11: KORSMEYER PEPPAS PLOT OF CUBOGEL

The accuracy and prediction ability of the models were compared by calculation of R2.

TABLE 7: KINETIC PROFILE OF CUBOSOMAL GEL

Model	Value
Zero Order	r2=0.989
First Order	r2=0.6650
Higuchi	r 2=0.951
Hixon Crowell	r 2=0.988
Korsemeyer-Peppas	r 2=0.708 n =0.883

The model giving R2 close to unity was taken as the best fit model. The value of 'n' indicates the drug release mechanism. The 'n' value is used to characterize different release mechanisms concluding that value n=0.5 indicates fickian diffusion and values of n between 0.5 and 1.0 or n=1.0 indicate non- fickian mechanism.

The release kinetics data indicates that the release of drug from cubosomal gel best fits to zero order release kinetics. R2 values of zero order kinetic equations were found to be close to unity indicating that the release from the films was not dependent on the concentration of drug present in the formulation. The data was fitted with Higuchi equation which gave almost a linear plot with highest R2 indicating the mechanism of drug release was diffusion. The dissolution data was also plotted in accordance with Hixon- crowell cube root law.

To determine whether fickian or non-fickian diffusion existed, data was analyzed using the Korsmeyer Peppas equation. The n value determined lies between0.5 and 1.0 indicates it follows non-fickian diffusion. These observations showed that mechanism of drug release for the formulations was non- fickian diffusion following Higuchi model of drug release.

Stability Study: pH, Drug content and drug release values are analyzed periodically as per ICH guidelines through accelerated stability studies for optimized gel formulation.

TABLE 8: STABILITY STUDY OF CUBOSOMAL GEL

Time	pH	Drug content	Drug release
0	5.7	95	87.20
30	5.5	94	85.38
60	5.4	92	82.56

CONCLUSION: There are many dosage forms like tablets and capsules available in the market for the treatment of inflammation, but still there is a need for new dosage forms which acts effectively. NSAIDs were mainly used for the treatment of inflammation.

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 gout 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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CONFLICTS OF INTEREST: Nil

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