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RESEARCH ARTICLE

Formulation and Evaluation of Aceclofenac Loaded Cubosomal Topical Gel

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ABSTRACT

The aim of present work was to formulate and evaluate sustained release formulation of Aceclofenac a Non -steroidal anti-inflammatory drug (NSAID), as cubosomal topical gel to reduce gastro intestinal effects and to improve the bioavailability of the drug. Different formulations of Aceclofenac cubosomes were prepared by Top down approach using GMO as lipid phase vehicle, Poloxamer 407 as stabilizer and distilled water as aqueous phase by varying the concentrations of GMO and Poloxamer 407. Resultant formulations were characterized for particle size, zeta potential, surface morphology, encapsulation efficiency and *in-vitro* drug release. Optimized formulation (F10) showed drug release of 83.25% in 8hours. Aceclofenac cubosomal gel was prepared by using optimized cubosomal formulation (F10), Carbopol 940,Carbopol 934, HPMC K4M and HPMC 15M.Gels were evaluated for pH, viscosity, drug content and in-vitro drug diffusion studies. Among all the preparations, formulation G2 was found to show entrapment efficiency of 96.85 and *in vitro* drug release of 78.5%. *Ex-vivo* permeation of optimized gel formulation (D2) was evaluated across rat epidermis.

KEYWORDS

Aceclofenac, Cubosomes, GMO, Poloxamer 407, Top down approach, *Ex-vivo* permeation

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDS) are considered first line drugs in the symptomatic treatment of Rheumatoid Arthritis, Osteo Arthritis and Ankylosing Spondylitis. Aceclofenac is a Non-steroidal anti-inflammatory drug with high plasma protein binding (90-99%). The oral administration of Aceclofenac causes gastric ulcers and gastro intestinal bleeding with chronic use. Because of gastrointestinal bleeding it also causes anaemia. Using the transdermal routes eliminates these side effects, increases patient compliance and maintain the plasma drug level for a longer period of time.

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Cubosomes are discrete, submicron, nanostructured particles of the bicontinuous cubic liquid crystalline phase[1]. The word 'bicontinuous' refers to the division of the two continuous but non-intersecting aqueous regions by a lipid layer that is contorted into a space filling structure, hydrating a surfactant or polar lipid that forms cubic phase and then dispersing the solid-like phase into smaller particles usually forms cubosomes. Cubosomes size ranges from 10-500nm in diameter, they appear like dots, square shaped, slightly spherical, each dot corresponds to the presence of pore size 5-10nm. Cubosomes are typically produced by high-energy dispersion of bulk cubic phase, followed by colloidal stabilization using polymeric surfactants. After formation of the cubosomes, the dispersion is formulated into a suitable dosage form. Cubosomes have great potential in

formulating nano sized particulate systems for topical delivery owing to their best advantages such as high drug payload due to high internal surface area and, low viscosity and can exist at almost any dilution level.

Structurally cubosomes are lipid vesicles formed from amphiphilic building blocks, which mimics bio membranes that can be used for carrier potential of hydrophilic, lipophilic and amphiphilic drugs as compared to free drug directly to the particular site of action, thus allow drug targeting and the sustained or controlled release of conventional medicines. Cubosomes can be administrated in many ways oral, Percutaneous, intravenous route. Cubic phase is unique and desirable because of its mesoscale structure: a contorted lipid bilayer separating two continuous but nonintersecting water regions. The tortuous structure of bulk cubic phase provides controlled release of solubilized active ingredients, while cubosomes exhibit burst release because of their sub-micron length scales. The purpose of present study was to develop Aceclofenac Cubosomal topical gel to sustain the drug release and to reduce the gastro intestinal side effects.

MATERIAL AND METHODS

Materials

Glyceryl Monooleate (GMO) was a gift sample from Mohini Organics Pvt. Limited, Mumbai, India. Poloxamer 407 was kind gift from Daewoong Pharmaceuticals, Hyderabad. Aceclofenac was a gift sample Symed Labs, Hyderabad. Carbopol 934, Carbopol 940 were gift samples from Loba chemie, Mumbai, India. HPMC K4M and HPMC K15M were of commercial grade. All other reagents used were of analytical grade.

Preparation of Aceclofenac Loaded Cubosomes

The method used for the preparation of cubosomes was top-down method. Varying concentrations of Glceryl Monooleate along with poloxamer 407 as shown in table 1 and 2 was accurately weighed and heated on an electric water bath at a temperature of 40 to 45^0C until Poloxamer 407 completely dissolves in GMO.

Table 1: Formulation of cubosomes using poloxamer407

Form^n code	GMO (%W/V)	Poloxamer 407 (%w/w)	Aceclo-fenac (g)	Water (Up to 100%)
F1	17.5	1	0.3	10
F2	17.5	1	0.3	10
F3	17.5	1	0.3	10
F4	17.5	1	0.3	10
F5	17.5	1	0.3	10

Table 2: Formulation of cubosomes using GMO

Form^n code	GMO (%W/V)	Poloxamer407 (%w/v)	Aceclo-fenac (g)	Water (upto100%)
F6	7.5	1	0.3	10
F7	10	1	0.3	10
F8	12.5	1	0.3	10
F9	15	1	0.3	10
F10	17.5	1	0.3	10
F11	20	1	0.3	10
F12	22.5	1	0.3	10
F13	25	1	0.3	10
F14	27.5	1	0.3	10
F15	30	1	0.3	10
F16	32.5	1	0.3	10

To the above solution drug was added and mixed well. The clear lipid solution obtained was added slowly to distilled water and subjected to probe sonication for 10 minutes. The resultant solution was white opaque dispersion without presence of any aggregates. The prepared dispersions were stored in a closed glass vials at room temperature for 72hrs, protected from light and later evaluation was carried out.

Preparation of Aceclofenac Loaded Cubosomal Gel

Aceclofenac cubosomal topical gels were prepared by dispersion method using optimized cubosomal dispersion and gelling agents such as carbopol 940, carbopol 934, HPMC K4M and HPMC K15M as shown in tables 3.1 and 3.2. Then, Carbopol and HPMC were dispersed in water and kept aside for 4hrs for swelling of polymer. Propylene glycol was added to gel with constant unidirectional mixing. Triethanolamine was subsequently added to adjust the ph of the gel. Then glycerol was added to the gel to balance its viscosity. To this optimized cubosomal dispersion was added and mixed properly. Methyl paraben was added as a preservative. The prepared gels were filled in a glass vials and stored in a refrigerator at a temperature of 4 to 8^0C.

Table 3.1: Formulation of Aceclofenac cubosomal topical gels using carbopol 940 & carbopol 934

Fomu^n code	**Cubosomal dispersion (ml)**	**Carbopol 940(%)**	**Carbopol 934(%)**	**Propylene glycol (%)**	**Triethanolamine (%)**	**Glycerol (%)**	**Methyl paraben (mg)**	**Water (upto 100%)**
D1	5	1	-	30	1.25	2.5	0.75	10
D2	5	1.5	-	30	1.25	2.5	0.75	10
D3	5	2	-	30	1.25	2.5	0.75	10
D4	5	3	-	30	1.25	2.5	0.75	10
D5	5	4	-	30	1.25	2.5	0.75	10
D6	5	-	1	30	1.25	2.5	0.75	10
D7	5	-	1.5	30	1.25	2.5	0.75	10
D8	5	-	2	30	1.25	2.5	0.75	10
D9	5	-	3	30	1.25	2.5	0.75	10
D10	5	-	4	30	1.25	2.5	0.75	10

Table 3.2: Formulation of Aceclofenac cubosomal topical gels using HPMC K15M & HPMC K4M

Formulation code	Cubosomal dispersion (ml)	HPMC K4M (%)	HPMC K15M (%)	Propylene glycol (%)	Triethano-lamine (%)	Glycerol (%)	Methyl paraben (mg)	Water (upto 100%)
D11	5	1	-	30	1.25	2.5	0.75	10
D12	5	1.5	-	30	1.25	2.5	0.75	10
D13	5	2	-	30	1.25	2.5	0.75	10
D14	5	3	-	30	1.25	2.5	0.75	10
D15	5	4	-	30	1.25	2.5	0.75	10
D16	5	-	1	30	1.25	2.5	0.75	10
D17	5	-	1.5	30	1.25	2.5	0.75	10
D18	5	-	2	30	1.25	2.5	0.75	10
D19	5	-	3	30	1.25	2.5	0.75	10
D20	5	-	4	30	1.25	2.5	0.75	10

Evaluation of Aceclofenac Cubosomes

Drug- Excipient Compatibility Studies

FTIR Drug-excipient interaction was studied before developing the formulation by using FTIR spectroscopy, which is one of the most important analyses to investigate the stability of formulation, and molecular interactions between the drug and the excipients used. Fourier-transform infrared spectroscopy (FT-IR) measurements were performed using FTIR spectrophotometer using KBr disc method. The samples were scanned over the range of 4000 to 400cm^{-1}.

Surface Morphology

The morphology of cubosomes was determined using scanning electron microscopy (SEM – Hitachi S 3700N). SEM gives a three dimensional image of the globules. The samples were examined at suitable accelerating voltage 20 kV, at different magnification.

Particle Size Analysis and Zeta Potential

The particle size and zeta potential of cubosomes determined by dynamic light scattering technique using Horiba particle size analyzer.

Samples were diluted in particle-free purified water and measured at 25^0C. Zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion system.

The zeta potential is a key indicator of the stability of dispersions. The magnitude of the zeta potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particles in dispersion

Entrapment Efficiency

For the determination of entrapment efficiency, the cubosomal dispersions were subjected centrifugation at 15000 rpm for 30 minutes. The resulting solution was then separated and supernatant liquid was collected. The collected supernatant was then diluted appropriately and estimated using UV visible spectrophotometer at 273 nm.

The percent of encapsulation efficiency (%EE) was determined by the following equation:

$$\%EE = \frac{[\text{Total drug}] - [\text{free drug}]}{\text{Total drug}} \times 100$$

In Vitro Drug Release

Studies were performed for all the formulations. In vitro drug release studies were carried out using bi chambered donor receiver compartment model (Franz diffusion cell) and this was placed on a magnetic stirrer and temperature was adjusted to 37 ± 0.5^0C.One end of the cylinder was covered with Himedia dialysis membrane (cut-off molecular weight: 12000-14000), which was previously soaked in warm water. The diffusion cell was placed in a 500 ml Borosil beaker that served as the receptor cell. The temperature in the diffusion and receptor cells was maintained at 37^0C, with the help of a thermostat. Phosphate buffer pH 7.4was placed in the receptor cell. Cubosomal formulation was placed on the dialysis membrane, which was in contact with receptor medium. Samples were withdrawn from the receptor cell at specified time intervals of 1, 2, 3, 4, 5, 6, 7 and 8 hours. Each time immediately after the removal of the sample, the medium was compensated with fresh Phosphate buffer (pH 7.4). The samples were analyzed for drug content using a UV spectrophotometer at 273 nm.

Evaluation of Aceclofenac Cubosomal Topical Gels

FTIR

Spectra of drug along with gelling agents, optimized gel formulation is taken and analyzed for the presence of any incompatibility. The samples were scanned over the range of 4000 to 400 cm^{-1}.

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 of all the formulations was determined by using Brookfield (DV Pro-II) viscometer with small sample adaptor, spindle no.64.Speed was increased from 10 rpm to 100 rpm and viscosity was noted in cps.

Drug Content

1g of the prepared gel was mixed with 100ml of suitable solvent. Aliquots of different concentration were prepared by suitable dilutions after filtering the stock solution and analyzed using UV Visible spectrophotometer at 273nm.

Diffusion Studies

Studies were performed for all the gel formulations in pH 7.4 buffer in a manner similar to method used for cubosomal dispersions.

Ex-vivo Studies

After approval of protocol (IAEC/SVCP/2015/001) from Institutional Ethics Committee permission as per ICMR, the study was conducted.

The male albino rats (200-250 g) were sacrificed by aspiration of ethyl ether and the abdominal skin was carefully excised from the underlying connecting tissue using scalpel. The skin was carefully removed and washed after removing subcutaneous fat and other visceral tissue. The ex-vivo skin permeation studies were performed using Franz diffusion cell apparatus. The skin was brought to room temperature and mounted between the donor and receptor compartments of the diffusion cell with the stratum corneum side facing upwards and then the donor chambers were clamped in place. The receptor compartment was filled with phosphate buffer saline (pH 7.4). The receptor fluid was stirred with a magnetic stirrer and the temperature was maintained at 37 ± 1°C. After stabilization of the

skin, optimized gel formulation was placed into the donor compartment. Samples were withdrawn at regular intervals (1, 2, 3, 4, 5, 6, 7, 8) and analyzed using UV Visible spectrophotometer at 273nm. The receptor phase was immediately replaced with equal volume of fresh buffer solution[2].

Kinetic Modelling

The optimized cubosomal gel formulation (D2) was studied for release kinetics. Coefficient of correlation values were calculated for the linear curves obtained by regression analysis of the plots.

Accelerated Stability Studies

Accelerated stability studies for optimized gel formulation (D2) were conducted as per ICH guidelines at 40°C ± 2°C/75% ± 5% RH and at25°C ±2°C/60%±5% RH at sampling intervals of 30, 60 and 90 days respectively. The drug content and pH are determined periodically.

RESULTS AND DISCUSSION

FTIR Studies

The interaction study between the drug and the excipients as well as optimized formulation was evaluated using FTIR spectrophotometer. Aceclofenac has characteristic absorption peaks O-H at 3416cm^{-1}, C=O at 1641cm^{-1} and C-Cl at 663 cm^{-1}. Similar peaks were observed in spectra of different combinations of excipients and in optimized formulation (Cubosomes and Topical gels), along with absence of interfering peaks indicating there is no unwanted reaction between Aceclofenac and other excipients used in the study.

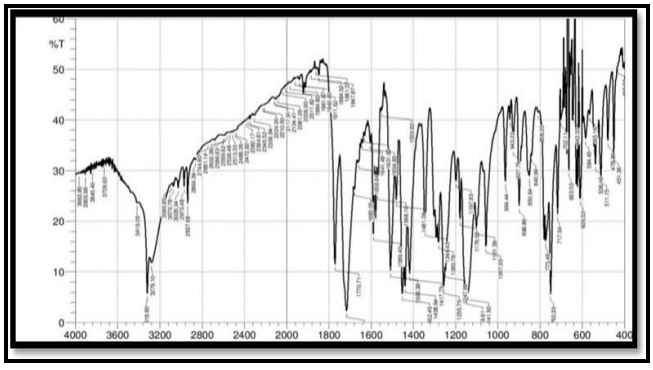

Figure 1: FTIR of Aceclofenac pure drug

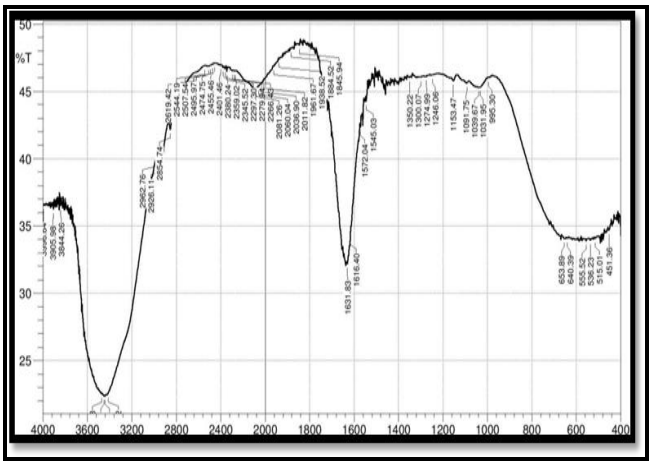

Figure 2: FTIR of Aceclofenac cubosomal gel

Optimization of Formulation Variables

Effect of Poloxamer 407 on Formation of Cubosomes

As the concentration of poloxamer is increased there is a decrease in Entrapment efficiency and drug release from the cubosomes. It was found that 1% poloxamer 407 was the optimum concentration for cubosomes formation and showed entrapment efficiency about 89.2% and drug release about 83.37%.

Effect of Glyceryl Monooleate (GMO) Concentration on Formation of Cubosomes

Cubosomes were obtained using GMO concentration in the range of 7.5% to 32.5%. Cubosomes containing GMO concentration of 17.5% was optimized and showed entrapment efficiency of 93.2% and drug release about 83.25%.

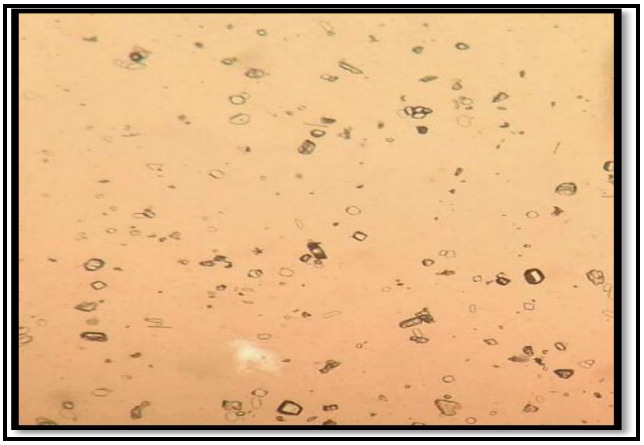

Figure 3: Optical micrograph of cubosomes

Characterization of Cubosomes

Surface Morphology of Cubosomes

The surface morphology of the cubosomes was determined using scanning electron microscopy (SEM). It was observed that the obtained cubosomes have a smooth surface and cubic in shape.

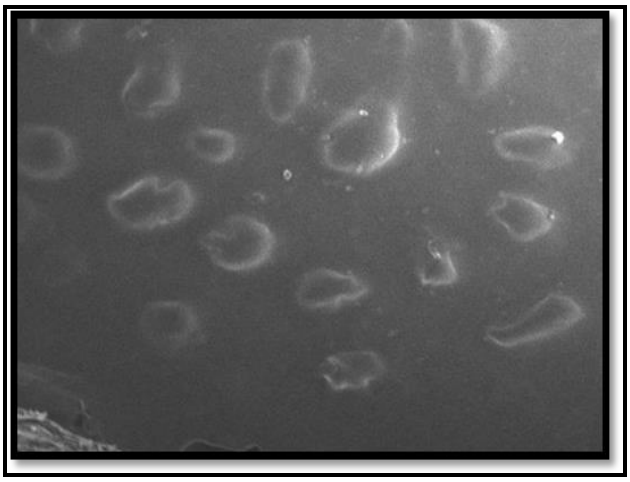

Figure 4: SEM image of cubosomes

Particle Size and Zeta Potential of Cubosomes

The particle size and zeta potential of cubosomes determined by dynamic light scattering technique using Horiba particle size analyzer.

Fromfigure6, it was found that the diameter of cubosomes was found to be in the range of 10 to 500nm and the average particle size was found to be 98.4nm.

From the figure 7, the zeta potential was found to be -22.9mV, which indicates that cubosomes were stable.

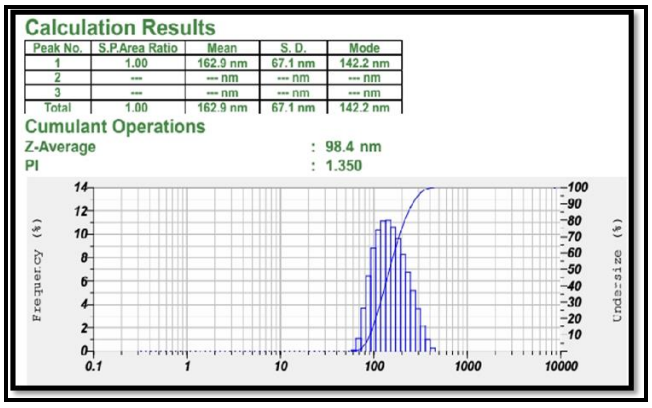

Figure 5: Particle size of cubosomal formulation F10

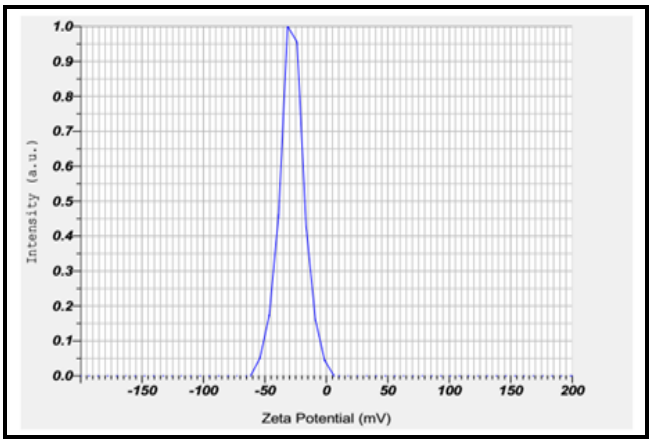

Figure 6: Zeta potential of cubosomal formulation F10

Entrapment Efficiency

The entrapment efficiency of the cubosomal formulations was found to be in the range of 75% to 96%.Entrapment efficiency of the cubosomes was found to increase by increasing GMO concentration 7.5% to 32.5%.so formulation F10 was optimized based on high entrapment and stability. The remaining formulations (F11-F16) were showing phase separation.

Diffusion Studies

Diffusion studies were performed for all formulations and formulation F10 was optimized. Cumulative percentage drug release profile of various formulations was shown in the Figure 7, Figure 8 and Figure 9. Drug release was found to be decreased with increasing poloxamer 407 concentration and increased with increasing the GMO concentration from 7.5 to 32.5%.

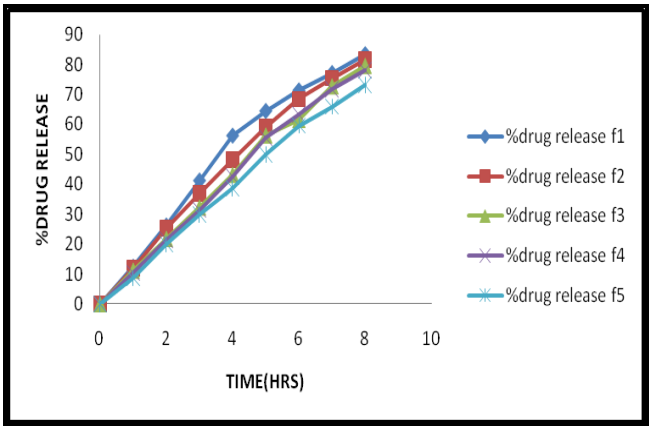

Figure 7*: In vitro* diffusion profile of Aceclofenac cubosomes F1-F5 in pH 7.4 buffer

Formulation F10 was optimized and it was formulated into topical gels because phase separation was observed in the remaining formulations.

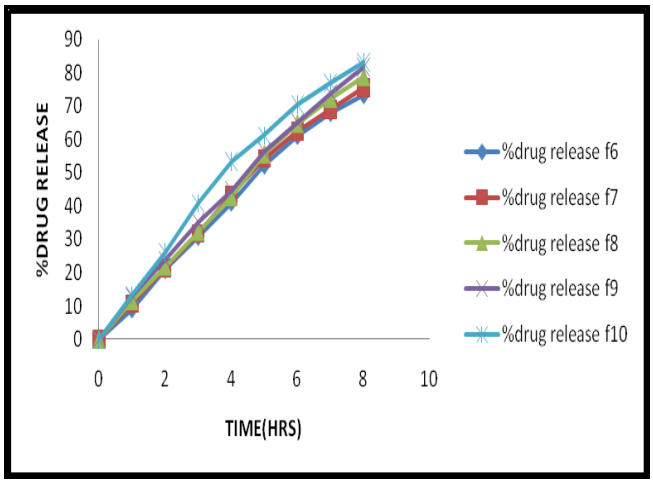

Figure 8: *In vitro* diffusion profile of Aceclofenac cubosomes F6-F10 in pH 7.4 buffer

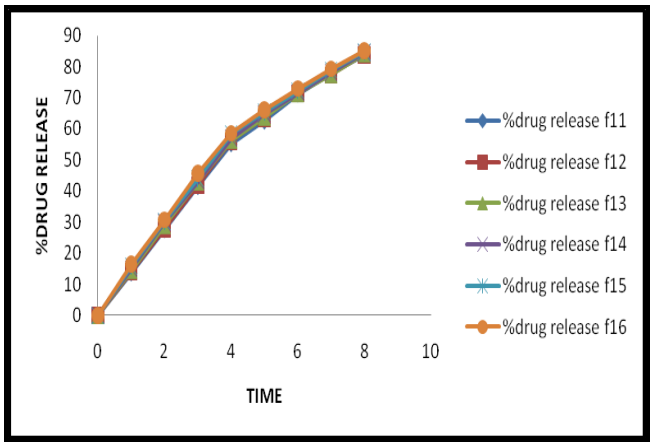

Figure 9: *In vitro* diffusion profile of Aceclofenac cubosomes F11-F16 in pH 7.4 buffer

Evaluation of Cubosomal Topical Gels

Homogeneity

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

pH

The pH was found to be in the range of 5.9 to 7.34, which are close to skin pH.

Viscosity

The viscosity of the optimized cubosomal gel formulation was shown in Table 6. From the figure 10 it was found that there is a decrease in the viscosity as the rpm was increased and pseudo plastic behaviour was noted.

Table 4: Viscosity of optimized cubosomal formulation (D2)

Rpm	Viscosity(cps)
10	7621
20	6300
30	5893
50	5020
100	4065

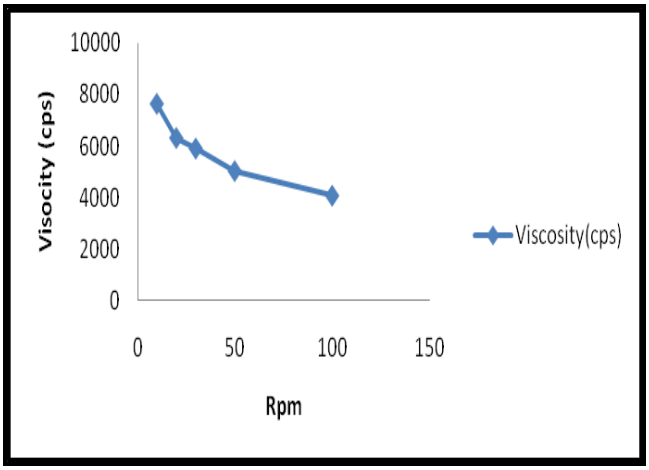

Figure 10: Viscosity of Aceclofenac Cubosomal Topical gel G2

Drug Content

Drug content of the gel formulations was found to be in the range of 90% to 97%.

Diffusion Studies

Cumulative percentage drug release profile of various formulations was shown in the Figure 11, Figure 12, Figure 13 and Figure 14.

Cubosomal gels were prepared using different polymers like Carbopol 940, Carbopol 934, HPMC K4M, and HPMC K15M in the concentration ranges of 1, 1.5, 2, 3 and 4%.

D2 formulation containing 1.5% of Carbopol 940 showed sustained drug release of 78.5% upto

8hrs. Formulation G2 was optimized based on drug content, drug release and stability.

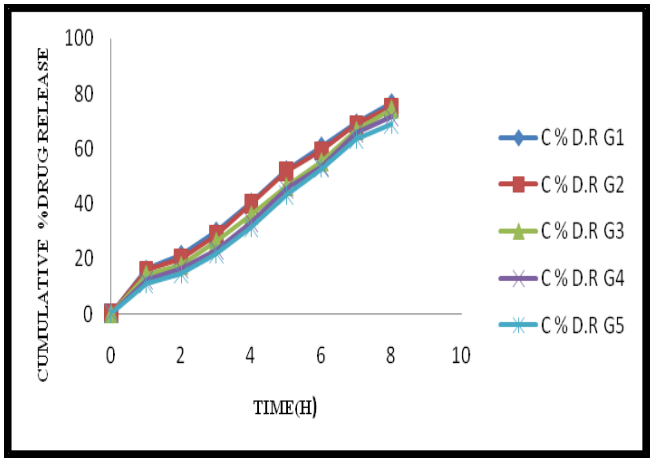

Figure 11: *In vitro* diffusion profile of Aceclofenac cubosomes D1-D5 in pH 7.4 buffer

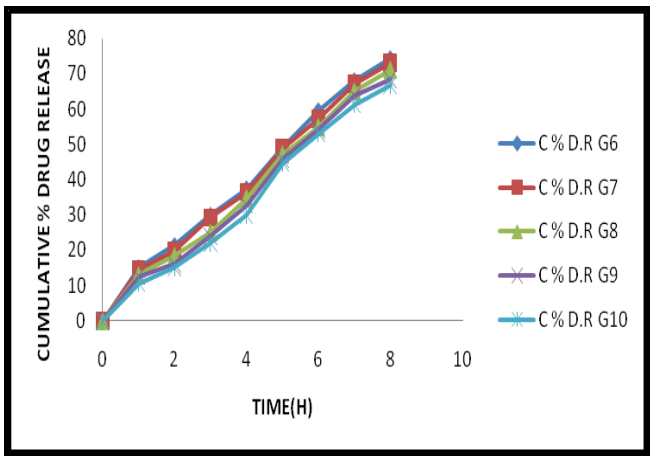

Figure 12: *In vitro* diffusion profile of Aceclofenac cubosomes D6-D10 in pH 7.4 buffer

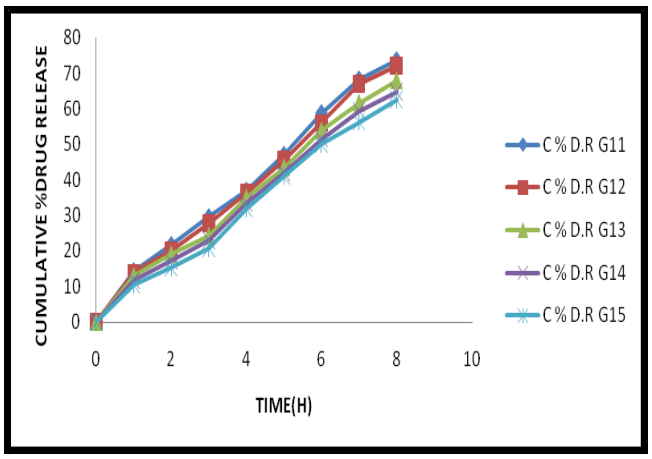

Figure 13: *In vitro* diffusion profile of Aceclofenac cubosomes D11-D15 in pH 7.4 buffer

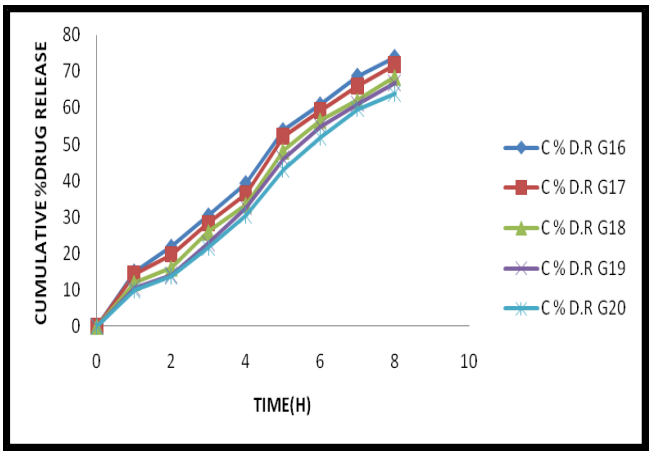

Figure 14: *In vitro* diffusion profile of Aceclofenac cubosomes D16-D20 in pH 7.4 buffer

Ex-vivo Studies

The optimized gel formulation (D2) was selected for ex-vivo permeation studies using excised skin of rat and showed 77.2% permeation through the skin. It is shown in the Table 5.

Table 5: Ex-vivo studies of optimized gel formulation (D2)

Time (H)	Cumulative % drug release
0	0
1	14.1
2	18.6
3	29.8
4	44.2
5	51
6	66.3
7	69.2
8	77.2

Kinetic modeling

The optimized cubosomal formulation was studied for release kinetics as shown in Table 6.It

was found that G2formulation showed zero order drug release.

Table 6: Release kinetics of optimized gel formulation (D2)

Model	r^2 value
Zero order	0.988
First order	0.974
Higuchi	0.919
Korsmeyer-peppas	0.908

Stability Studies

pH, Drug content and drug release values were analyzed periodically as per ICH guidelines for optimized gel formulation D2 as shown in Table 7.

Table 7: Stability studies of optimized gel formulation (D2)

Stability condition	Drug Content			pH		
	1M	2M	3M	1M	2M	3M
40ºC±2 ºC/ 75%±5 %RH	98.6	94.1	91.5	7.3	7.28	7.3

Comparative studies

In vitro diffusion and *ex vivo* permeation studies of optimized cubosomal gel (D2) were compared to the marketed Aceclofenac gel (Hifenac gel manufactured by Intas Pharmaceuticals Ltd.) as shown in the figure no.15.

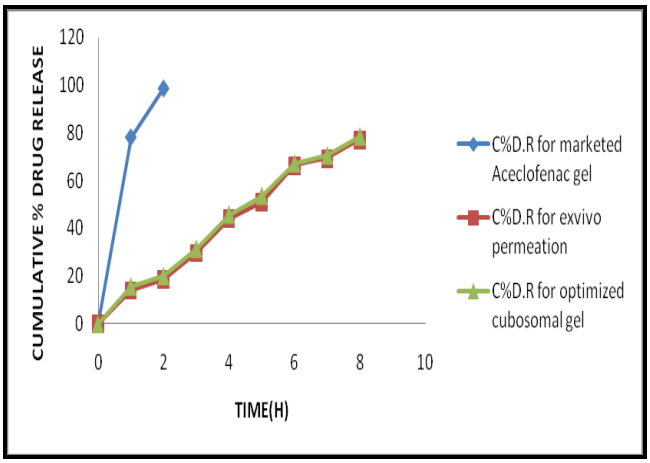

Figure.15: Comparative Graph (cumulative % drug release vs. time) of Marketed

Formulation, *Ex-vivo* Permeation and *In vitro* drug diffusion of Optimized Cubosomal Gel

Optimized cubosomal gel showed sustained drug release when compared to Marketed Aceclofenac gel.

CONCLUSION

A satisfactory attempt was made to develop Aceclofenac cubosomes using GMO monoglyceride and P-407 as polymer and evaluated for compatibility and morphological studies. *In vitro* study revealed that cubosomes formulationF10containing 1% poloxamer 407 and GMO concentration of 17.5% showed better release, good entrapment efficiency and better stability. Optimized cubosomal formulation was incorporated into gels. In vitro drug diffusion and ex-vivo permeation studies of optimized cubosomal gel (D2) revealed effective drug release compared to marketed Aceclofenac gel. It can be concluded that cubosomes are promising vehicle for percutaneous delivery of Aceclofenac.

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