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FORMULATION AND EVALUATION OF CYCLOSPORINE LOADED CUBOSOMES FOR THE MANAGEMENT OF PSORIASIS

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ABSTRACT:
Cubosomes can be considered as novel lipid-based nanosystems similar to well-known vesicular systems such as liposomes and niosomes. Cubosomes have been widely formulated using certain amphiphilic lipids (e.g. glyceryl monooleate and phytantriol) in the presence of a suitable stabilizer. They can represent a novel drug delivery system which could be loaded with hydrophilic, lipophilic and amphiphilic drug molecules. They are widely used for various drug delivery applications such as oral, ocular, transdermal and chemotherapy drug delivery. In this study, preparation and characterization of cyclosporine loaded cubosomes have done for the management of psoriasis. The preformulation study and formulation of cubosomes was performed. Evaluation parameters of the cubosomes were performed and results were reported.

Keywords: Cubosomes, Cyclosporine, Psoriasis, Glyceryl monooleate, Poloxamer 407

1. INTRODUCTION

Psoriasis is a T-cell mediated immune disorder characterized by circumscribed, red, thickened plaques with an overlying silver white scale .The disease relapses after certain period of time. Elbows, scalp and knees are the primary site of psoriatic plaques. In psoriasis, however, the initial clearing is only one aspect of treatment, for recurrence sooner or later after stopping treatment is usual.

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Cyclosporine In 1972, the US Food and Drug Administration (FDA) approved Cyclosporine for treating severe psoriasis. Cyclosporine is very effective for psoriasis. Unfortunately with oral, IM or IV route it may be associated with severe acute and chronic adverse reactions including acute hematologic toxicity and acute and chronic hepatotoxicity. The goal of Cyclosporine therapy in psoriasis is to control the eruption not to completely eliminate it. Careful dosing and monitoring is essential. Baseline laboratory tests should include complete blood count and differential and liver function tests. Most often, a test dose of 2.5 to 5.0 mg is given followed by laboratory testing in 5-7 days. Monitoring is repeated weekly during the period of dosage increases to a maximum of 15-25 mg/week. Because Cyclosporine is cleared by renal excretion, caution must be exercised in patients with suspected renal impairment, especially the Elderly. It is used for moderate-severe psoriasis where other topical treatment is non-responsive.

Cubosomes are nanoparticles whose size ranges from 10-500nm in diameter they appear like dots square shaped, slightly spherical.

• Cubic liquid crystals are transparent and isotropic phases representing unique system for production of pharmaceutical dosage form.

• Cubic phases are more bioadhesive in nature, so that can conveniently used in topical delivery of different drugs.

• High drug payloads due to high internal surface area and cubic crystalline structures.

• Simple preparation method and biodegradability of lipids.

• Ability of encapsulating hydrophobic, hydrophilic and amphiphilic substances.

• Targeting and controlled release of bioactive agents.

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OBJECTIVE OF WORK:

Psoriasis is a T-cell mediated immune disorder characterized by circumscribed, red, thickened plaques with an overlying silver white scale

1. The main objective of the research work was to prepare and evaluate cubasome for the Topical delivery of Cyclosporine for the management of psoriasis.

2. Cyclosporine have serious systemic side effect but Cyclosporine loaded cubasome do not cross the dermis layer and prevent such side effects due to its cubic structure which is similar to structures of human skin.

3. The Topical Delivery of Cyclosporine, deliver drug directly at site and hence side effects will be less

MATERIALS AND METHOD

MATERIALS

Cyclosporin was received as a gift sample from Biocon Company, Banglore India. Excipients were Procured from HiMedia Laboratories and SD Fine Chemicals. All other solvent and reagent are used was of analytical grade.

2. EXPERIEMENTALS

2.1 Identification of Drug

Determination of absorption maximum (λmax): The Ultraviolet absorption maximum was determined by scanning solution of Cyclosporine in absorption media in the range of 200 to 400 nm by Shimadzu-1800 UV/Visible Spectrophotometer.

Determination of absorption maximum (λmax) in PBS 5.5: Cyclosporine 10µg/ml stock solution showed absorption maxima (λ max) at 205nm.

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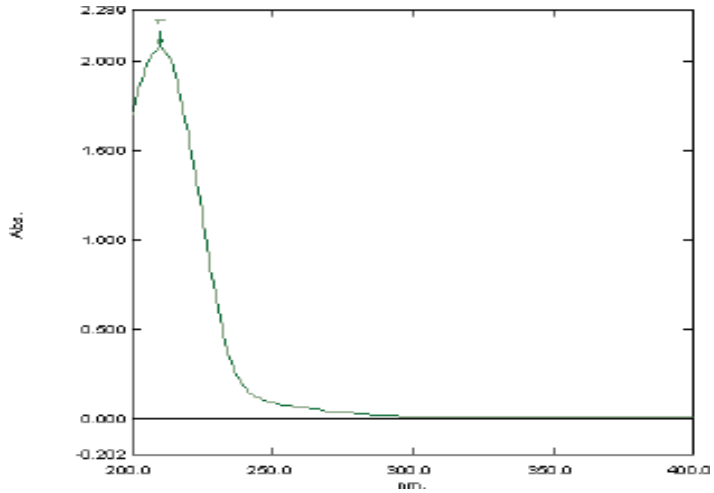

Figure 1: Absorption maximum (λmax) of Cyclosporine in PBS5.5

2.2 By melting point determination

The melting point of drug sample was determined by using melting point apparatus. The melting point was found to be in the range of 145-150^{0}C. The melting point of cyclosporine is shown in the table: 2.

Table 2: Melting Point of cyclosporine

S.No.	Standard	Observed
1.	148-151^{0}C	145-150^{0}C

2.3 Preparation of standard Calibration curve

The standard curve of drug was prepared in PBS 7.4, in the concentration range of 5-25 µg/ml. A straight line with regression coefficient (r^2) = 0.994 was obtained, which indicates that drug follows Beer‟s law.

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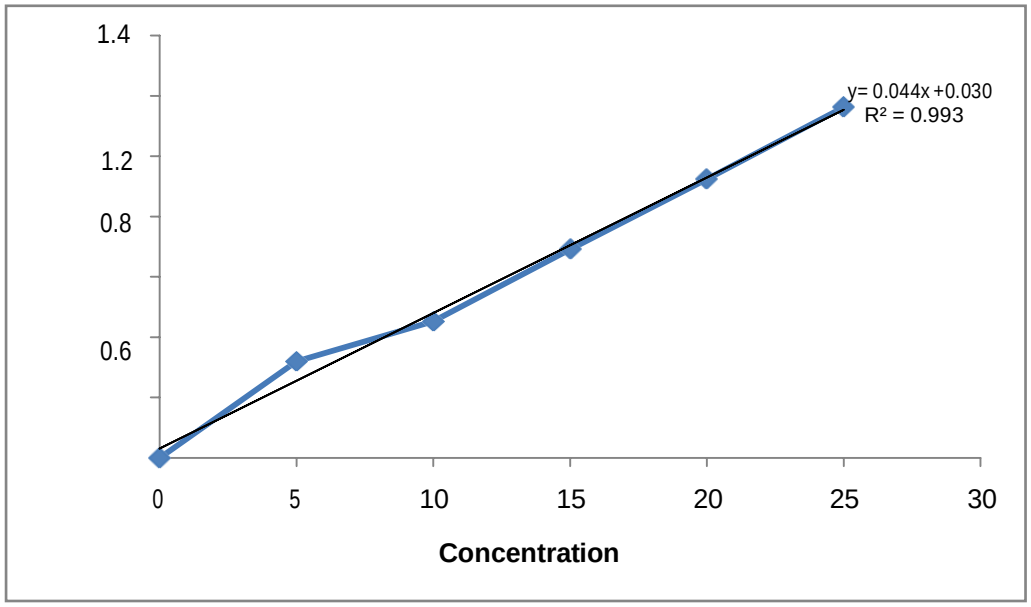

Fig 3 Calibration curve of Cyclosporine

Table 4: Data of standard calibration curve of Cyclosporine in phosphate buffer 7.4

S. No.	Concentration (µg/ml)	Absorbance
1	0	0.0000
2	5	0.3198
3	10	0.4526
4	15	0.6915

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5	20	0.9238
6	25	1.1621

2.4 Solubility studies of drug

Solubility of was determined in different solvents. The drug was found to be insoluble inChloroform, Distilled Water, Ethanol, Ether, and Methanol and partially soluble in PBS 5.5.

Table 5: Quantitative solubility analysis:

S. No.	**Solvents**	**Standard Solubility**	**Observed Solubility**
1	Chloroform	Soluble	Soluble
2	Distilled water	Slightly soluble	Slightly soluble
3	Ethanol	Soluble	Soluble
4	Ether	Soluble	Soluble
5	Methanol	Soluble	Soluble
6	DMF	soluble	Soluble
7	PBS 5.5	-	slightly soluble

2.5 FTIR spectroscopy:
The FTIR spectra of the sample drug (Cyclosporine) showed the principal peaks in wave number region of 1643, 1585, 1515, 1346 and 1253, 1176, 1103 cm^{-1} which is in accordance with the Standard FTIR spectra and proves theauthenticity of the sample drug.

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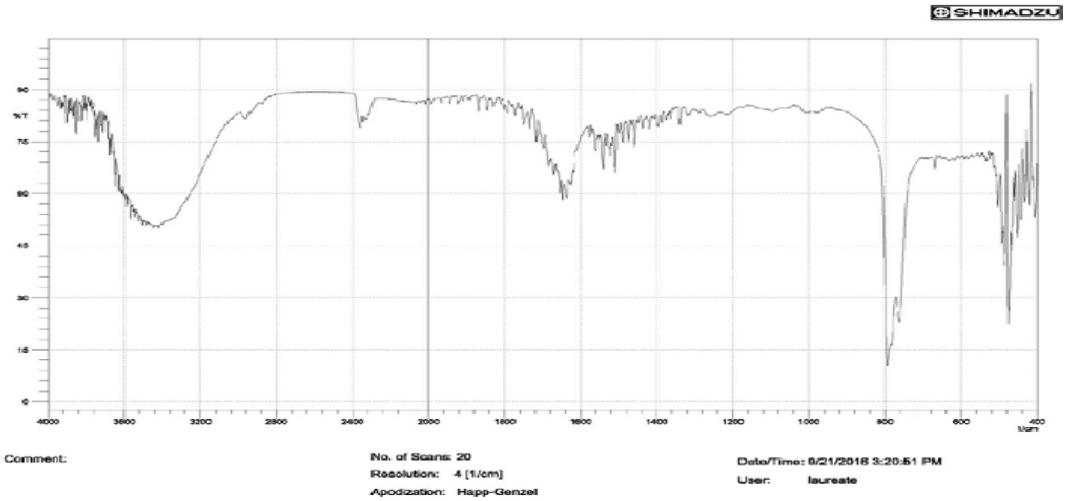

Figure 6: FTIR spectra of Cyclosporine

Table no. 7: FTIR Spectra of Cyclosporin

Functional groups	Wave number (cm^{-1}) Standard	Wave number (cm^{-1}) Sample
C-N Strech (alkyl)	1104.28 cm^{-1}	1103.77 cm^{-1}
C-N Strech (aromatic)	1167.40 cm^{-1}	1176.50 cm^{-1}
C-O	1240.58 cm^{-1}	1253.64cm^{-1}
-C=C Stretching (Aromatics)	1340.52 cm^{-1}	1346.22cm^{-1}
=CH stretching	1440.48 cm^{-1}	1450.37cm^{-1}
–NH Stretching	3300.15 cm^{-1}	3315.94cm^{-1}

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S-S Stretching.	2420.24 cm^{-1}	2400.38cm^{-1}
C=O Strech	1640.28 cm^{-1}	1643.24cm^{-1}

3. Design of Experiment:

A two factor, three level factorial designs (3^2) was selected to study the main effects and interaction of two factors on entrapment efficiency. The independent factors investigated were lipid concentration, surfactant concentration[30]. The three levels of independent factors for experiment domain of each variable are summarized in table (8).

Table 8: Variable Values of (3^2) Factorial Design for Preparation of Cyclosporine Loaded Cubosomes:

Variables	Levels		
	Low	Medium	High
Lipid (Monooleine) (%w/w)	1.75	2.25	2.75
Surfactant (Poloxamer-407) (%w/w)	0.2	0.25	0.3

Table 9: Full Factorial (3^2) Design Layout for Preparation of Cyclosporine LoadedCubosomes with Batch Codes:

S.no.	Batch no.	Lipid concentration(%w/w)	Surfactant concentration(%w/w)
1.	CU-1	1.75	0.20
2.	CU-2	2.25	0.20
3.	CU-3	2.75	0.20
4.	CU-4	1.75	0.25

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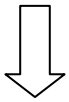
5.	CU-5	2.25	0.25
6.	CU-6	2.75	0.25
7.	CU-7	1.75	0.30
8.	CU-8	2.25	0.30
9.	CU-9	2.75	0.30

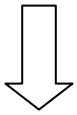
3.1 Preparation of Cubosomes:

Cubosomes was prepared by emulsification method using monooleine as lipid and Poloxamer 407 as surfactant. In this method, Monooleine and Poloxamer 407 were melted in water bath and then mixture were added dropwise in to water at 70° C using mechanical stirring at 1500 rpm. Dispersion maintained under stirring at room temperature for 2 hours and then subjected to homogenization for 1 minute.[32]

Monooliene + Poloxamer407 were melted with drug (cyclosporine) in water bath



Mixture added dropwise in to water at 70ºC under mechanical stirring at 1500rpm



Dispersion maintained under stirring at room temperature for 2 hours

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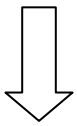
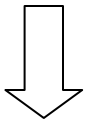


Dispersion was subjected to homogenization for 1 minute



After cooling dispersion were maintained at room temperature in glass vials

Figure 10: Flow diagram forcubosomes preparation

3.2 Formulation of Cyclosporine Loaded Cubosomes Based Carbopol 934p Gel:

Various gelling agent were evaluated for their ability to gel the cubosomal dispersions of Cyclosporine . The suitable gelling agent was selected on the basis of compatibility with nanoparticulate dispersion, feel and ease of spreadibility. For topical application carbopol 934p was found to be suitable for gelling the cyclosporine loaded Cubosomal dispersion.

4. Evaluation of Cyclosporine Loaded Cubosomes

4.1 Determination of Entrapment Efficiency:

Table 11: Entrapment Efficiency of Various Batches of Cubosome Formulation

S.No.	Batch no.	Drug entrapment efficiency (%)
1.	CU-1	35±0.20
2.	CU-2	37±0.50

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3.	CU-3	34±0.52
4.	CU-4	39±0.71
5.	CU-5	30±0.86
6.	CU-6	49±0.64
7.	CU-7	63±0.33
8.	CU-8	66±0.72
9.	CU-9	80±0.76

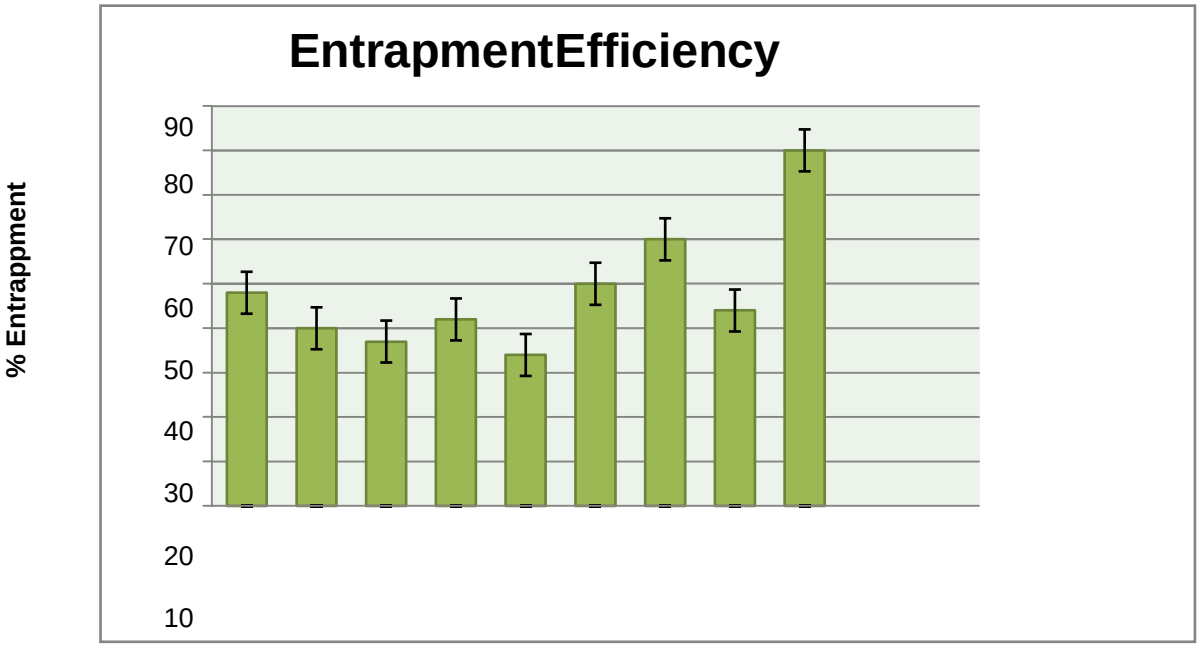

Figure 12: Entrapment efficiency of different Cubosome formulations

The entrapment efficiency of cubosomes was calculated as percent total drug entrapped within the vesicles. The entrapment efficiency was found to vary with the varying concentration of glyceryl monooleate and poloxamer

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407. The % entrapment efficiency of Cyclosporine loadedcubosome formulation was found to be in range of 35% to 80% The entrapment was found to be maximum in vesicles of CU-9 (80%) and and this formulation was used for further work.

4.2 Characterization of Optimized Cubosome Formulation:

Particle Size and Zeta Potential

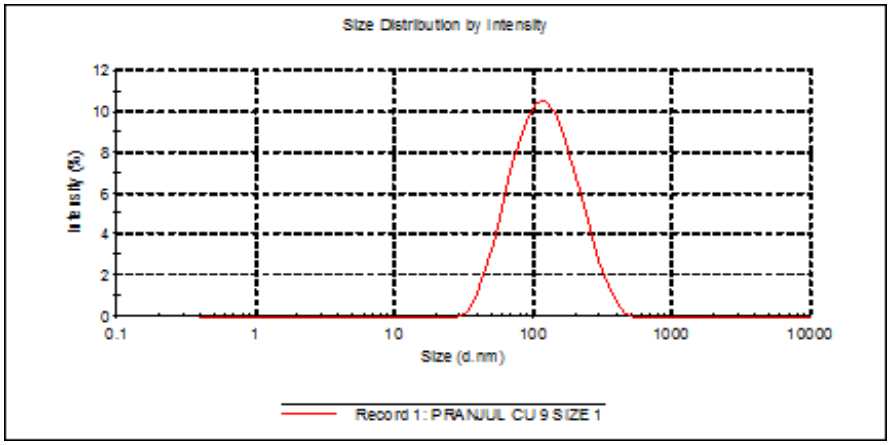

Figure 13: Particle size analysis

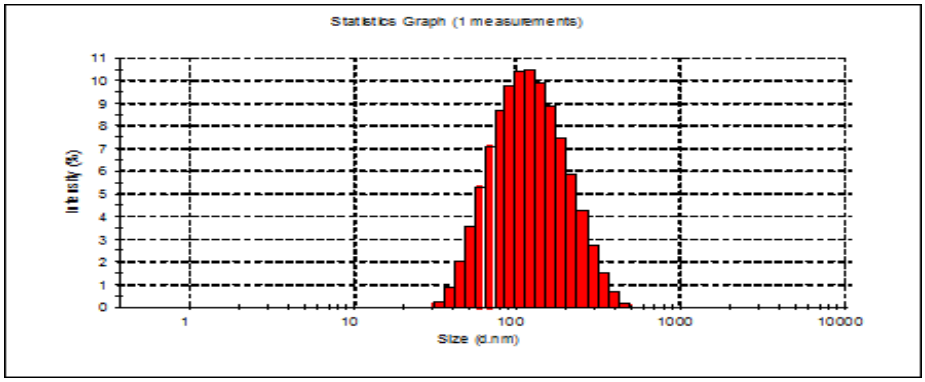

Figure 14: Statistic bar Graph between Percent Intensity Vs Size of Cubosome in Dispersion

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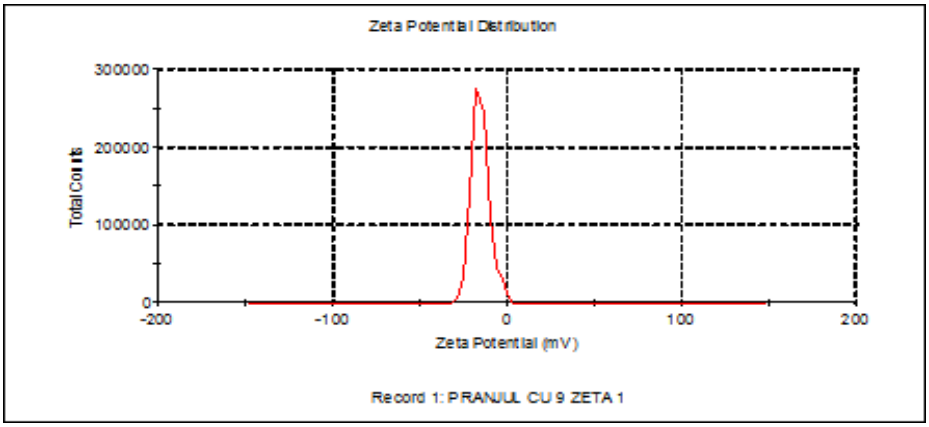

Figure 15: Zeta Potential Analysis

Particle size of optimized CU-9 formulation was found to be 104.8 nm and zetapotential was found to be -15.8 mV. The Poloxamer polymers show a decrease of the zeta potential with increasingmolecular weight from Poloxamer 188 to 407. In addition there is a general tendencyof a further decrease with increasing polymer concentration (especially forPoloxamer407). The decrease in zeta potential confirms the formation of a sterically stabilizingadsorbed polymer layer.

Particle Morphology:

The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample.

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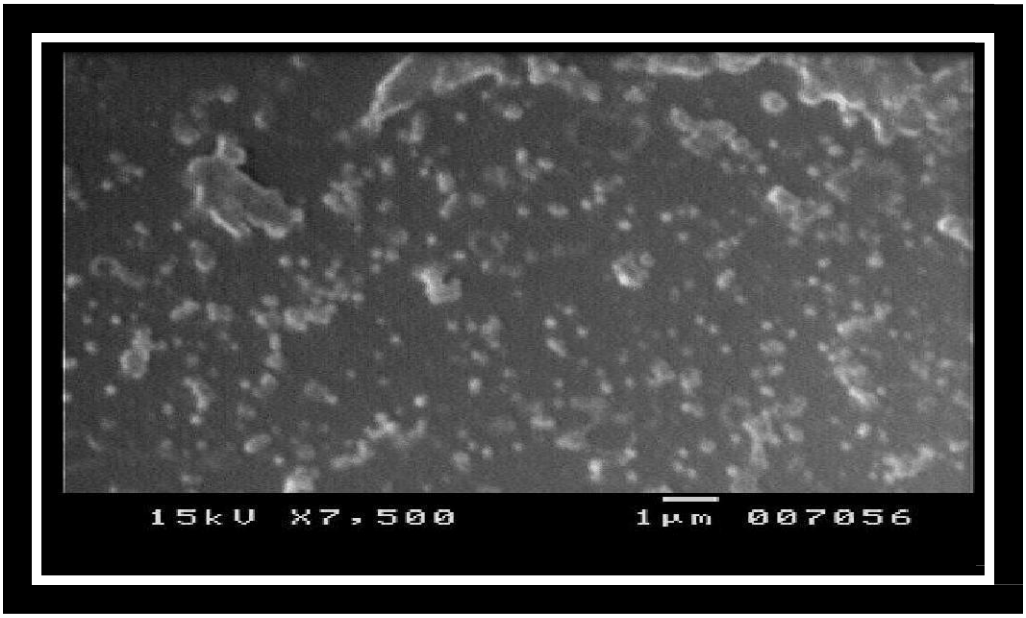

Figure 16: SEM of CU-9 batch

Morphology of the cubosomes was examined by SEM and is shown in figure 9.6.Cyclosporine loaded cubosomes have an almost spherical shape andsmooth surface because these characterstics depend on the lipid.

Physical Examination: Cubosomal Gel was Physically Examined for Color, Homogeneity and Consistency.

Table 17: Physical Examination of Cubosomal and Plain Drug Gel

S.No	Drug	Formulation	Colour	Homogeneity	Consistency
1.	Cyclosporine	Cubosomal Gel	Yellowish Creamy	Good	Good
2.		Plain Drug Gel	Yellow	Good	Good

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pH Determination of Gel:

Table 18: pH Determination of Cubosomal and Plain Drug Gel

S.No.	Drug	Formulation	pH
1.	Cyclosporine	Cubosomal gel	5.0±0.55
2.		Plain drug gel	6.0±0.45

The values are expressed as mean ±SD, n=3

Drug Content:

Table 19: Drug Content of Cubosomal and Plain Drug Gel

S.No.	Formulation	Drug Content
1.	Cubosomal gel	72.5%± 0.66
2.	Plain drug gel	86.2%± 1.56

All the values are expressed as mean ±SD (n=3)

Viscosity:

Table 20: Viscosity Determination of Cubosomal and Plain Drug Gel

S. No.	Formulation	Viscosity(cps) Vmin(20 rpm)	Viscosity(cps) Vmax(100rpm)
1.	Cubosomal gel	745±124	1032±158
2.	Plain drug gel	856±106	1254±102

The values are expressed as mean ±SD, n=3

Gel of Cyclosporine –Cubosome was prepared by using different concentrations of carbopol 934p(0.5-2%) out of which 1% concentration showed good consistency and spreadability. Drug content in gel was 72.5%. The rheological behavior of gel entrapping Cyclosporine – cubosome was found in the range from 745-1032cps at 20 and 100 rpm. The pH of all the

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formulations was between pH 5-6, this lies in thenormal pH range of the skin.

In- Vitro Release Study:

Table 21: In- Vitro Release Study

Time (Hrs)	Drug in Cubosome Dispersion	Drug in Cubosomal Gel
0.5	8.38±0.02	6.15±0.05
1	13.06±0.12	11.58±0.19
1.5	20.16±0.43	15.95±0.51
2	24.15±0.36	21.49±0.43
3	27.05±0.45	23.16±0.55
4	33.55±1.23	30.46±1.29
6	41.05±1.02	37.05±1.10
8	48.48±1.43	41.15±1.54
12	55.48±1.28	47.95±1.34
24	63.73±1.57	55.17±1.64

The values are expressed as mean ±SD, n=3

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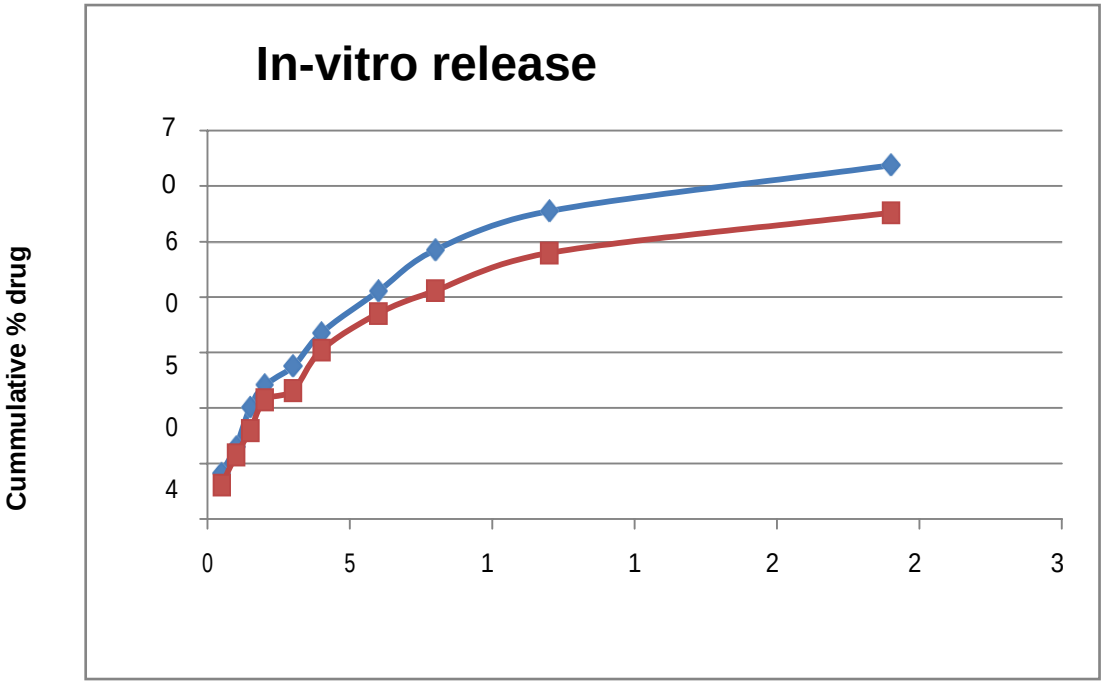

Figure 22: In-Vitro Release Study of Cyclosporine

Figure. 22 shows the in vitro release profile of Cyclosporine from cubosomal dispersion and gel. In the initial 2 hours, the drug release was less than 10%, probably because the slow diffusion of drug from the lipid but it shows faster release as compared to carbopolcubosomal gel. After 2 hours, the drug release rate increased with time until 24 hours following, which the rate declined. The prolonged drug release could be attributed to embedment of drug in the cubosomal matrix. Comparing the drug release from cubosomal dispersion and cubosomes in gel (figure.9.7) the release of Cyclosporine was slower from the gel formulation as compared with cubosomal dispersion. Incorporation of cubosomal dispersion into gels initially decreased the drug release. This result was probably due to the release retarding effect of the polymeric matrix of gelling agents.

In –Vitro Skin Penetration Study

Carbopol gel enriched with Cyclosporin Cubosomes demonstrated higher drug levels in the skin after 24 hours treatment. In this Cyclosporin Cubosomal gel, the drug concentrated more in the skin with only 0.1% of the total drug being detected in the receptor compartment

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and 35% of total drug being detected in the donar compartment after 24 hours. In dermatological treatment, improving the efficacy demands high drug levels in the skin. With Cubosomal dispersion, a greater quantity of drug remained localized in the skin due to their cubicstructure.

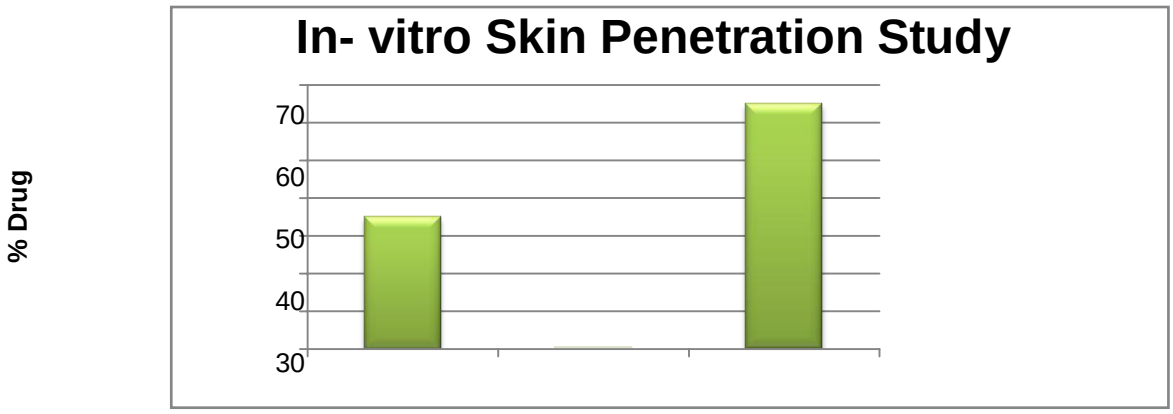

Figure 23: Comparison of the drug levels from In Vitro Skin Penetration study

5. Conclusion

The present work was an attempt to develop Cubosomes of Cyclosporine containing Monooleine and poloxamer 407, to increase the release of drug for prolonged period of time in to the affected part of skin. Cyclosporine was procured from Cipla Ltd. Goa. The melting point of drug was found in the range of 196-206^0 C. IR spectrum of drug showed characteristic absorption of various functional groups of Cyclosporine . The drug was found to be insoluble in chloroform, methanol, ether, slightly soluble in PBS pH 7.4 and 5.5.. For qualitative estimation of drug, λ max scanning was performed. λ max was observed at 302 nm in PBS pH5.5. The partition coefficient of Cyclosporine was observed in noctanol: water system. The partition coefficient of Cyclosporine was found to be -1.86 which depicts its hydrophilic nature. The comparisons of FT- IR spectra of drug and physical mixture of drug and excipients have revealed that, there is no significant interaction between drug and excipients used in formulation. Cubosomes of Cyclosporine were formulated by emulsification method. Two independent variables (lipid concentration and surfactant concentration) and dependent variables (entrapment efficiency) were selected to optimize the formulation using 32 factorial designs. Concentration of lipid and surfactant had significant effects on entrapment efficiency. Entrapment efficiency of Cyclosporine loaded Cubosomes were found to be in the range

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30-80%. The highest entrapment efficiency was obtained with CU-9 formulation. Particle sizes of different formulations were found in the range of 100-300 nm .Particle size was found to be increased with increase in polymer concentration. Based on the collected data, CU-9 formulation was optimized. Characterization of the optimized formulation CU-9 showed particles of nanometer size range and good stability according to zeta potential. FT-IR study showed that the characterstics absorption peak of Cyclosporine was also detected in the Cubosomes indicating the stable nature of Cyclosporine after the encapsulation process. Gel of CYCLOSPORIN-Cubosomes was prepared by using different concentrations of carbopol934p (0.5-2%) out of which 1% concentration showed good consistency and spreadibility. Drug content in gel was 72.5% and also showed good rheological properties. pH of the formulation lies in the normal pH range of the skin between 5.5 and 6.5. In vitro release profile of Cyclosporine from Cubosomal dispersion and its gel showed prolonged release upto 24 hours. In-vitro penetration studies showed higher drug concentration in the skin with Cubosome-enriched gel. Fluorescence microscope study revealed that Cubosomal gel enhances permeation calcein / drug in to the skin and drug remain in the skin for long time. Thus it can be concluded that Cubosome represents a promising particulate carrier having controlled drug release, improving skin hydration, and potential to localize the drug in the skin with possible clinical application for psoriasis treatment by increased penetration of Cyclosporine in to the skin.

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