

Preparation of Darunavir Cubosomal Gel to Treat HIV Infections

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Abstract

HIV (human immunodeficiency virus) is a virus that attacks the body's system. AIDS could be a chronic potentially life threatening condition caused by HIV. This article provides a summary of studies assessing the pharmacokinetics of antiretroviral drug Darunavir. Darunavir is a BCS class II drug which inhibits the HIV protease enzyme by forming an inhibitor-enzyme complex there by preventing cleavage of the polyproteins. This research work aims to increase the penetration of Darunavir into deeper layers of skin by formulating cubosomes of Darunavir into a genital Gel that helps in enhancing the Bioavailability by providing first pass metabolism. Cubosomes were prepared by the Top- down approach (Emulsification method) employing GMO as a lipid phase vehicle, poloxamer 407 as a stabilizer and distilled water as an aqueous phase. Darunavir is an antiretro viral drug with good bioavailability. The prepared cubosomes were characterized by Visual examination, Entrapment efficiency, Particle size, Zeta potential, In-vitro drug release studies. Optimised formulation (F4) showed good response among all the opposite cubosomal preparation. This (F4) cubosomal preparation was made as Gel using Carbopol 974 BP, and are studied for pH, Drug content, and Diffusion studies. Among all the preparation DARf6 was found for example the utmost drug release. This novel cubosomal genital gel would be promising system for effective drug delivery.

Keywords: Cubosomes, Darunavir, Glyceryl monooleate, poloxamer 407, Carbopol 974 BP, Top-down approach.

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1. INTRODUCTION

There is a huge variety of drug delivery systems that allow drug targeting and therefore the sustained or controlled release of standard medicines. In such a theme cubosomes are part of the drug delivery system or lipid-based mixture system that was discovered in 1980. The term Cubosomes is outlined as nano-structured, separate and sub-micron particles of bicontinuous three-dimensional liquid crystalline phases. The term "bicontinuous" refers to a pair of distinct deliquescent regions separated by the bilayer. Bicontinuous three-dimensional crystalline resources are a dynamic analysis topic as a result of their structure lends itself well to controlled-release applications. The cubosomes are honey-combed in a very structure that is separating the two internal binary compound channels in conjunction with an oversized internal area. Cubosomes are nano-particles having a size vary of 10-500nm. They're showing like Dots, Slightly Spherical. Each single Dot corresponds to the presence of pore

containing binary compound part three-dimensional phases within the lipid-water system in X-ray scattering technique was first known by Luzzati & Husson. An anti-retroviral agent drug Darunavir may be a BCS category II drug that inhibits proteolytic enzyme. This catalyst embrace reduced binding affinity between the matter and therefore the proteolytic enzyme, and causes alterations in catalyst chemical action, that effects on chemical compound stability, alterations in matter binding dynamics, and re-shaping of the situation. Darunavir has restricted solubility and therefore the drug undergoes intensive internal organ metabolism that ends up in poor oral drug bioavailability of the drug and hinders its use for general treatment. The current analysis work aims to increase the penetration of Darunavir into deeper layers of skin formulating cubosomes of Darunavir into a Gel that helps in enhancing the bioavailability by avoiding first pass metabolism.

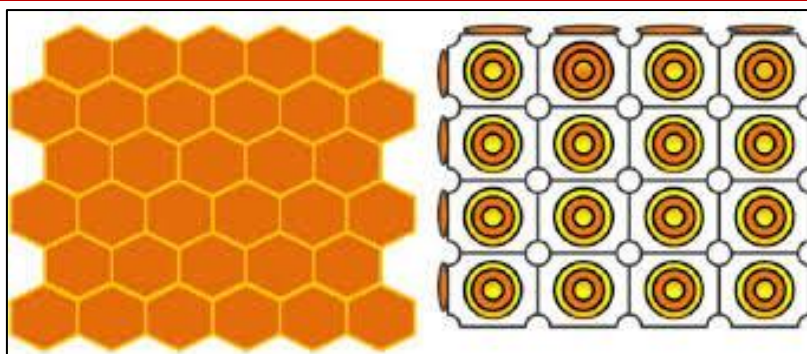


Fig. 1: Structure of cubosomes showing Honey-combed structure

2. MATERIALS AND METHODS

2.1 Materials

Darunavir was a present sample from Hetero labs Pvt Ltd, Hyderabad, Telangana, India. Glyceryl monooleate (GMO) was purchased from Finar Chemicals (LR). Poloxamer 407 was a sort gift from NATCO pharmaceutical company, Hyderabad. Carbopol 974 BP was of economic grade. All different reagents used were of analytical grade.

2.2 Methods

2.2.1 Calibration Curve of Darunavir in Methanol

The activity curve of Darunavir was planned by victimization wood alcohol (Methanol) as a solvent. 10mg of Darunavir was weighed exactly and diluted with wood alcohol in an exceedingly 10ml meter flask and created up to the degree to provide a amount of 1000 μ g/ml. From this reserve answer 'A', 1ml was

taken and diluted to 10ml victimization wood alcohol to provide an amount of 100 μ g/ml. From this reserve answer 'B', 1ml was taken and diluted to 10ml to provides a concentration of 10 μ g/ml, equally a variety of concentrations of one,2,3,4,5 μ g/ml were ready and also the absorbance was measured at 266nm against a blank victimization actinic ray photometer.

Table 1: Linearity curve of Darunavir

Sample ID	Concentration (μ g/ml)	Absorbance in (nm)
1 ppm	1	0.002
2 ppm	2	0.132
3 ppm	3	0.252
4 ppm	4	0.4
5 ppm	5	0.521

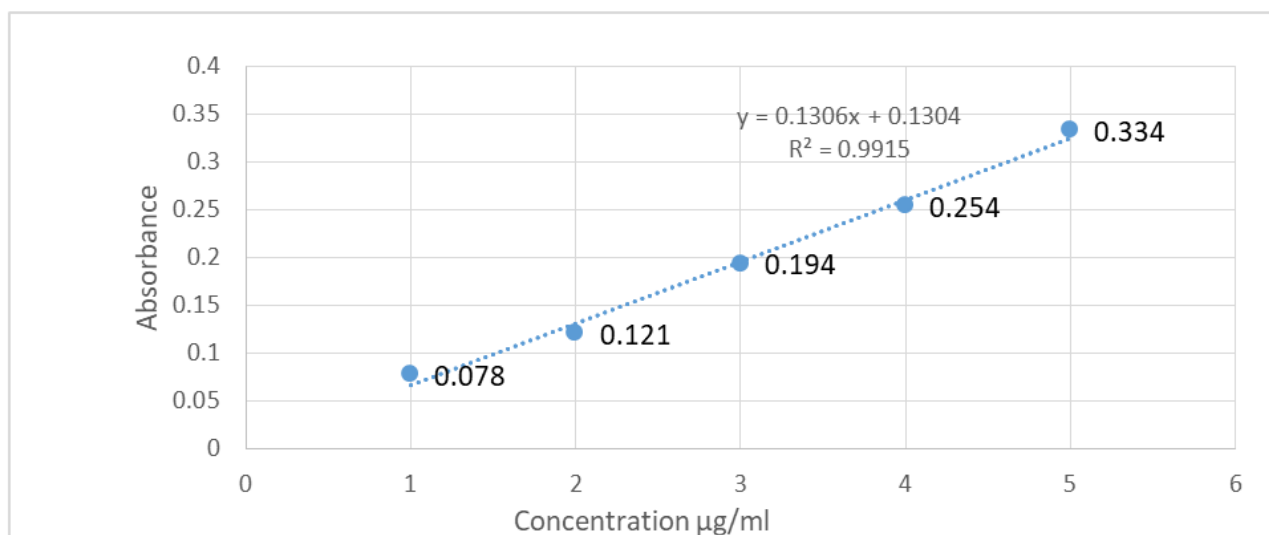


Fig. 2: Linearity plot of Darunavir

2.2.2 FTIR Studies

FTIR spectra of pure drug Darunavir and optimized formulation that's Cubosomal Gel is taken and analyzed for presence of any incompatibility.

3. Formulation of Darunavir Loaded Cubosomal Gel

3.1 Formulation of Darunavir Loaded Cubosomal Dispersion

The technique used for the preparation of cubosomes was the top-down method (Emulsification method). Specifically weighed quantity of glyceryl monooleate and Poloxamer 407 in many ratios was

mixed and fusible throughout a water bath at 60°C until poloxamer 407 absolutely dissolves in GMO upon high resolution Darunavir was mixed well. To the obtained clear supernatant resolution it was further slowly preheated (60°C) and distilled water was added drop by drop of applicable quantity by uninterrupted stirring. Once complete addition of supermolecule section was fully unbroken aside for sooner or later to appreciate stabilization. Formation of a two-phase system and it was fully disturbed by stirring. The whole system was subjected to mix at 1200 rpm below temperature for 2hr. The prepared dispersions were confined in closed glass vials at temperature protected from direct daylight and later analysis was assigned. The resultant was a white opaque dispersion whereas not the presence of any aggregates. Varied formulations were prepared in such associate approach.

3.2 Preperation of Darunavir Loaded Cubosomal Gel

Darunavir cubosomal gel was ready by Cold mechanical methodology victimization Carbopol 974 as a gelling agent. The obligatory live of gelling agent was weighed .The weighed compound was more to the beaker containing water with slow and continuous stirring at 400-600 rpm. The whole mixture was stirred for 60 minutes unendingly till a transparent gel is created .To the formed fashioned clear gel, optimized cubosomal dispersion like a six hundred mg (60 millilitre) Darunavir was more and mixed properly to bring the pH scale neutral Triethanolamine was added. Alcohol was more to balance the consistency. The prepared gel was control in reserve for 24h for complete compound desolvation or swelling.

FORMULATION OF DARUNAVIR CUBOSOMES

Table 2: Formulation of Darunavir Cubosomes

Formulation code	Glycerol monooleate		Distill water		Poloxamer407
	(%v/v) (in ml)		(%v/v) (in ml)		(in mg)
F1	5	0.25	95	4.75	70.5
F2	10	0.5	90	4.5	15
F3	15	0.75	85	4.25	22.5
F4	20	1.00	80	4.00	30
F5	25	1.25	75	3.75	37.5
F6	30	1.50	70	3.50	45
F7	35	1.75	65	3.05	52.5
F8	40	2.00	60	3.00	60
F9	45	2.25	55	2.75	67.5

PREPRATION OF DARUNAVIR CUBOSOMAL GEL

Table 3: Preparation of Darunavir loaded Cubosomal Gel

Formulation code	Cubosomal dispersion (in ml)	Carbopol 974 (%w/v)	Glycerol (ml)	Triethanol amine (ml)	Water (upto 100 %)
DARF1	10	0.5	0.25	0.12	100
DARF2	10	1.0	0.25	0.12	100
DARF3	10	1.5	0.25	0.12	100
DARF4	10	2.0	0.25	0.12	100
DARF5	10	2.5	0.25	0.12	100
DARF6	10	3.0	0.25	0.12	100
DARF7	10	3.5	0.25	0.12	100
DARF8	10	4.0	0.25	0.12	100

4. Characterization of Darunavir Cubosomes

4.1 Visual Examination

The scatterings were outwardly surveyed for visual appearance (e.g., color, turbidity, homogeneity, presence of perceptible particles), around multi week after planning. The visual evaluation was utilized as a fundamental screen to reject exceptionally unfortunate scatterings from the helper concentrate quickly. Very much scattered examples of cubosomes contained no noticeable totals and had a smooth white consistency.

4.2 Assurance of Molecule Size/Molecule Size investigation

The molecule size of cubosomes was unflinching by powerful light dispersing procedure utilizing Zeta sizer Nano-series (Nano ZS, Malvern). Tests were weakened in molecule free cleaned water and estimated at 25°C. Tests of watery weakening were sonicated for 5 min before estimation. Each worth addresses the normal of 3 measurements

4.3 Zeta Potential

Zeta potential estimation uses the Electrophoretic light dissipating technique. Zeta capability of the arranged cubosomal scattering was pondered to decide the surface charge of the nanoparticles which is significant for anticipating the drawn out strength of the colloidal scattering. The high zeta potential qualities give adequate electric repugnance which thusly forestalls molecule conglomeration. Still up in the air by utilizing the Zesizer Nano-series (Nano ZS, Malvern).

4.4 Polydispersity list

PDI gauges the mediocre consistency of a molecule arrangement, and liberally proportioned PDI values compare to a bigger size conveyance in the molecule test. PDI was acquired by combined examination of results from Zeta sizer Nano-series (Nano ZS90, Malvern).

4.5 Entrapment Productivity (EE)

For the assurance of capture productivity, the cubosomes from the weighty scatterings were first isolated by centrifugation. The partition of the (free) un-entangled drug from the captured drug in the cubosome scattering was accomplished by centrifugation at 8000 rpm for 30 minutes. The resulting arrangement was then isolated and the supernatant fluid was collected. The supernatant was gathered, then, at that point, weakened fittingly and assessed utilizing an UV noticeable spectro-photometer at 262 nm. The percent of embodiment productivity not entirely set in stone by the accompanying condition:

$$\% \text{ drug entrapment} = \left\{ \frac{\text{Total measure of medication untrapped drug}}{\text{Aggregate sum of drug}} \right\} \times 100.$$

4.6 In Vitro Medication Delivery Study

In vitro skin penetration studies were performed utilizing a bi-chamber benefactor recipient compartment model (Franz dissemination cell). Reads up were performed for all the details. The detailing was taken in the contributor compartment and phosphate support pH 7.4 was taken in the receptor compartment. The cellophane layer, previously drenched for the time being in the dispersion medium (phosphate cradle pH 7.4) was set between the contributor and receptor compartment. 10ml of Cubosomal detailing was put on the dialysis layer, which is in touch with the receptor medium. The whole framework was put on the thermostatically controlled attractive stirrer with ceaseless blending and the temperature of the medium was kept up with at $37 \pm 0.5^\circ\text{C}$ Examples were removed from the receptor cell at indicated time stretches. Each time promptly after the expulsion of the example, the medium was remunerated with new Phosphate cushion

(pH 7.4). The aggregate measure of medication let out of the cubosomes was determined and plotted against time.

4.7 Light Magnifying Lens

Light magnifying instrument (Fluorescence microscopy) was utilized to notice minutely cubosome scattering at an amplification of 45X.

5. Evaluation of Darunavir Loaded Cubosomal Gel

5.1 Actual Appearance

The arranged cubosomal emulgel were reviewed outwardly for their variety, homogeneity and consistency.

5.2 pH Assurance

The pH of not entirely settled by utilizing a computerized pH meter by drenching the terminal in a gel plan and pH was estimated. The pH of every plan was estimated in sets of three and the normal qualities were determined. The pH meter was aligned with standard support arrangements (pH 4 and 7).

5.3 Lucidity Test (Clarity Test)

The details were outwardly checked for the presence of any naturally visible particles by utilizing a dark foundation.

5.4 Medication Content

1 gm of Darunavir cubosomal gel was moved to a 50 ml volumetric carafe and weakened with methanol. One ml of this arrangement was weakened to 25 ml with ethanol. The medication content was unfaltering by estimating the absorbance at 266 nm utilizing UV-Apparent spectrophotometer. The medication content of the medication stacked plain gel not entirely set in stone in a similar methodology: Drug Content = (Absorbance/Incline) X weakening variable X (1/1000)

5.5 In Vitro Drug Discharge Study

In vitro drug discharge study was led likewise as that of cubosome scattering.

5.6 Motor Demonstrating (Kinetic study)

The advanced plan was seen whether the example of medication discharge follows zero-request/first request/Higuchi/Korse-Meyer Peppas model. Coefficient of connection (r^2) values was determined for the direct bends acquired by relapse examination of the plots.

6. RESULTS & DISCUSSION

6.1 Visual examination

Table 4: Physical Appearance of Darunavir loaded Gel

Formulation	Colour	Consistency	Phase separation
DARF1	White	Good	Yes
DARF2	White	Good	None
DARF3	White	Excellent	None
DARF4	White	Good	None
DARF5	White	Excellent	None
DARF6	White	Excellent	None
DARF7	White	Excellent	None
DARF8	White	Good	Yes

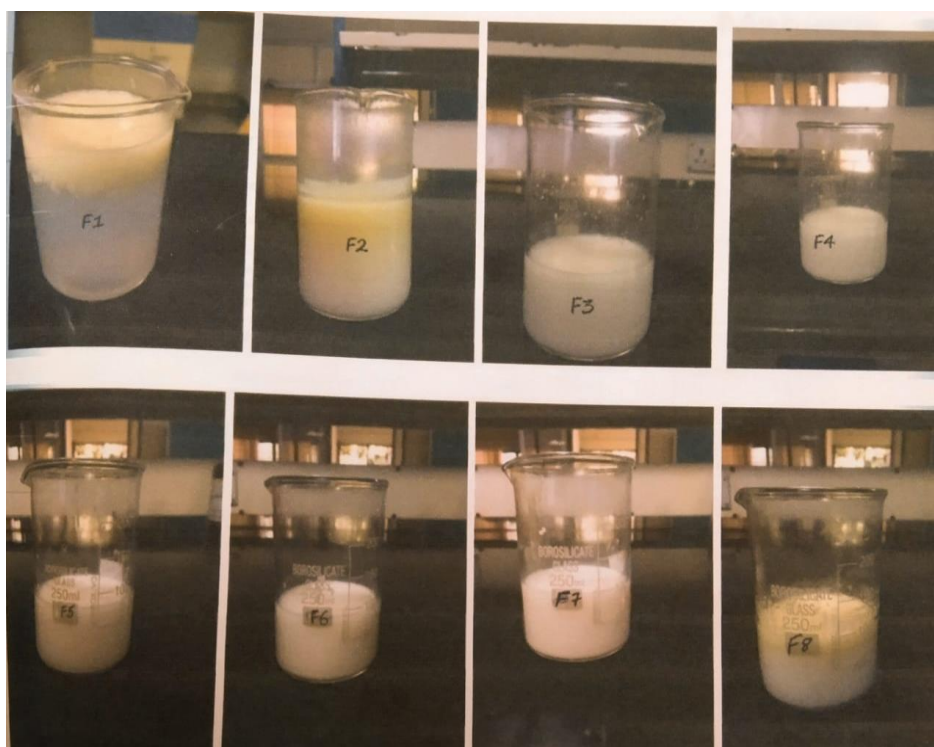


Fig. 3: Darunavir loaded cubosomal dispersion (f1-f8)

6.2 pH and Lucidity Test

The best formulation DARF7 showed excellent pH of 6.93 ± 0.2 and great clarity without any aggregates.

6.3 Drug Content Determination

The percentage drug content of drug loaded plain Carbopol 974 BP gel, as well as cubosome enriched gel was found to be 95.12% and 90.44%

Table 5

Formulation code	Drug content %
DARF7	95.12%
Drug loaded plane gel	90.44%

6.4 Drug Release Kinetics

The optimised formulation DARF7 was equipped in distinct kinetic models i.e. zero order, first order Higuchi and Korsmeyer-Peppas equation for best interpretation.

The optimised formulation DARF7 shows R^2 value 0.980 as the value is near to 1 it was confined that the formulation follows Higuchi release mechanism and

according to this model, the Darunavir cubosomal formulation released the drug throughout non-Fickian super case-II transport ($n > 0.9$).

6.5 FTIR-Drug-Excipient Compatibility Studies

The formulation's FTIR spectra reveal notable peaks of Darunavir showing no interactions between Darunavir and excipients.

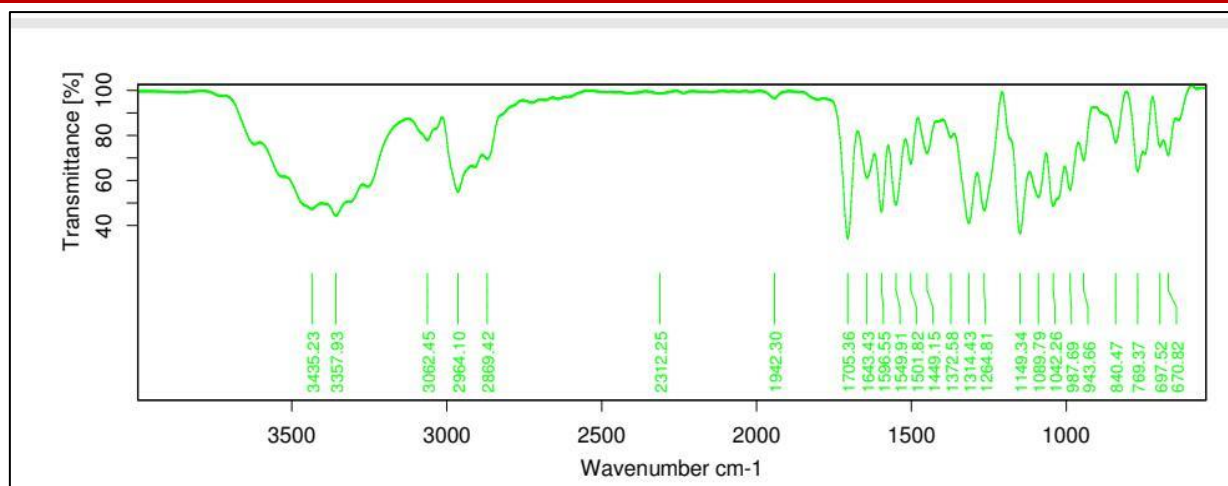


Fig. 4: FTIR Spectra of Darunavir Pure Drug

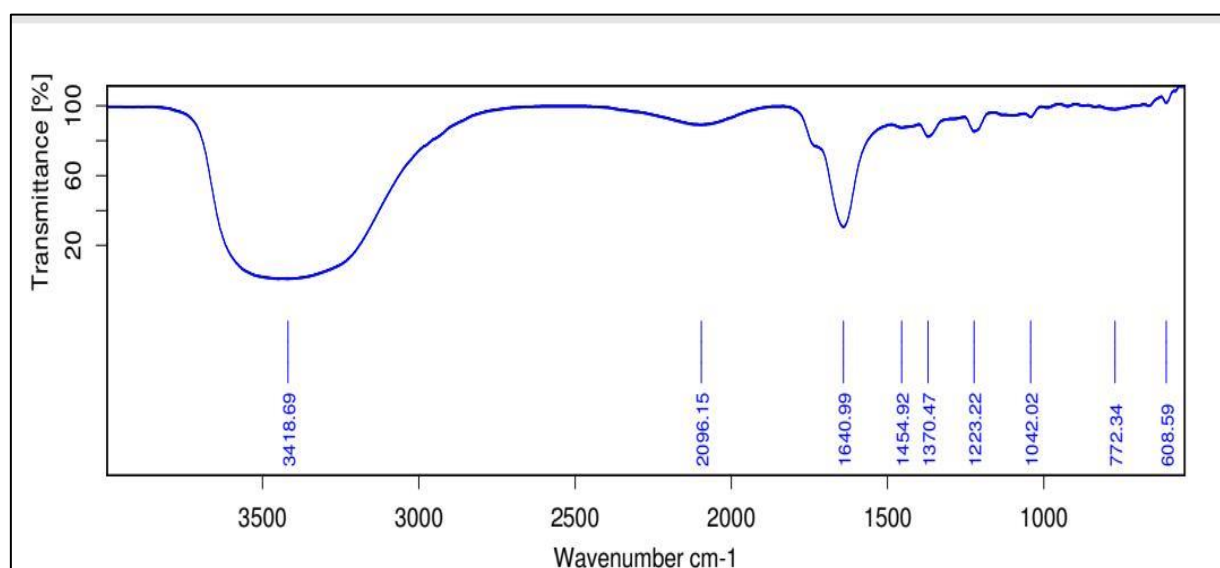


Fig. 5: FTIR Spectra of Darunavir loaded Gel (DARF7)

6.6 In-vitro Drug release Study

Table 6: In-vitro diffusion data of Darunavir loaded gel

Time (hrs)	DAR1	DAR2	DAR3	DAR4	DAR5	DAR6	DAR7	DAR8
0	0	0	0	0	0	0	0	0
1	12.26±0.3	15.26±1.2	14.98±0.1	16.73±0.6	11.72±1.2	16.38±0.1	12.35±0.3	6.7±0.4
2	20.47±1.2	19.52±0.5	20.79±0.8	20.14±1.1	19.06±0.5	20.02±0.7	24.4±0.4	8.91±0.1
3	24.62±0.6	20.99±0.4	25.63±0.6	25.62±1.5	23.16±0.2	20.98±0.2	29.7±0.7	10.22±1.1
4	28.74±1.7	25.39±0.1	30.71±0.2	29.04±1.7	26.03±0.7	23.3±0.2	31.82±1.1	13.4±1.5
5	30.01±0.2	29.04±1.1	36.05±0.3	33.71±0.6	29.58±0.9	26.74±0.4	35.2±1.5	17.38±1.9
6	33.41±0.8	32.67±1.5	40.02±1.1	39.25±0.1	31.02±0.1	28.7±0.9	39.41±1.9	20.65±0.8
7	37.05±0.4	38.91±1.6	45.62±1.8	41.62±0.7	38.5±0.3	32.21±0.3	42.5±0.2	23.5±0.2
8	41.82±1.1	44.23±0.7	49.01±1.7	49.06±0.4	40.30±0.5	39.92±1.1	45.71±0.7	28.2±0.4
9	45.06±1.7	49.07±0.8	50.98±1.1	52.70±0.3	47.23±0.4	43.15±0.2	48.62±1.1	34.45±0.9
10	51.31±0.5	53.72±0.2	57.42±0.1	57.45±0.3	52.63±1.1	50.45±1.5	50.41±1.5	38.1±0.7
12	58.47±0.9	57.45±0.7	61.07±0.3	60.14±0.5	58.17±1.4	55.82±1.7	62.33±1.8	43.5±0.1
14	61.06±0.1	62.03±0.4	68.68±0.7	68.24±0.7	60.02±1.8	60.41±1.6	69.1±1.9	48.36±1.2
16	67.48±1.4	68.91±0.4	73.46±0.4	73.05±1.1	67.04±0.7	64.82±1.1	72.56±0.8	50.7±1.6
18	71.03±0.5	72.38±1.1	78.43±0.4	79.62±1.6	70.01±0.9	69.3±0.2	79.6±0.3	57.2±10.4
20	75.24±1.8	77.81±0.3	81.35±0.6	81.72±1.2	75.81±0.4	71.46±0.8	86.93±0.7	65.8±0.7

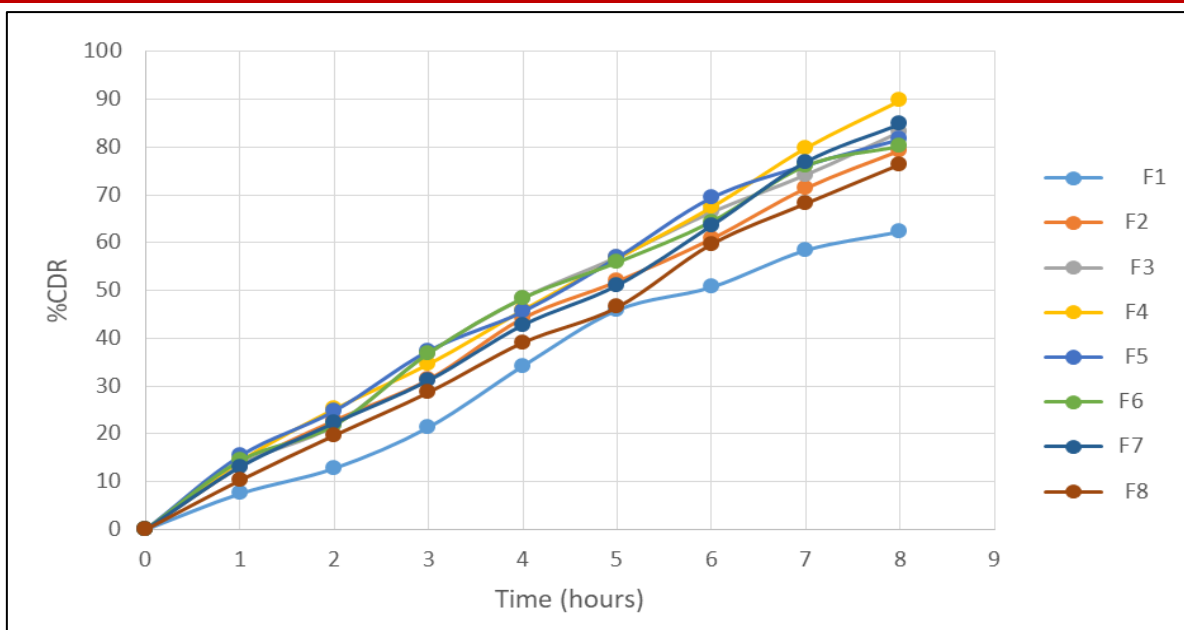


Fig. 6: Comparative Drug release profile of Darunavir Cubosomal Gel using Carbopol 974 BP (DARF1-DARF8)

6.7 Light Microscopy

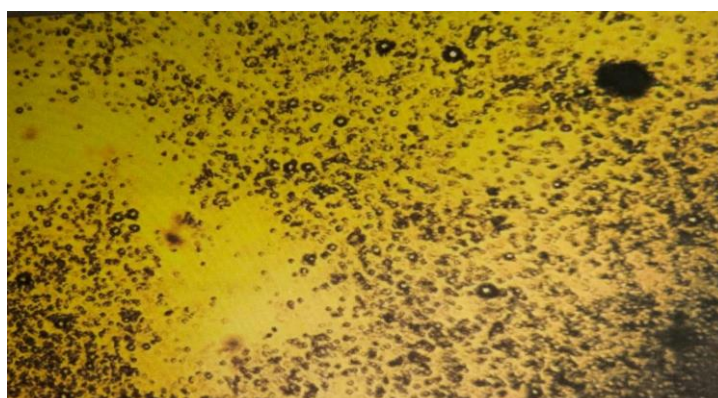


Fig. 7: Cubosome dispersion under Fluorescence microscopy

6.8 Lucidity Test (Clarity Test)

The details were outwardly checked for the presence of any naturally visible particles by utilizing a dark foundation.



Fig. 8: Cubosomal gel examined for clarity test

6.9 Assurance of Molecule Size/Molecule Size Investigation

The molecule size of cubosomes was unfaltering by powerful light dispersing procedure utilizing Zeta sizer Nano-series (Nano ZS, Malvern).

Tests were weakened in molecule free cleaned water and estimated at 25°C. Tests of watery weakening were sonicated for 5 min before estimation. Each worth addresses the normal of 3 measurements.

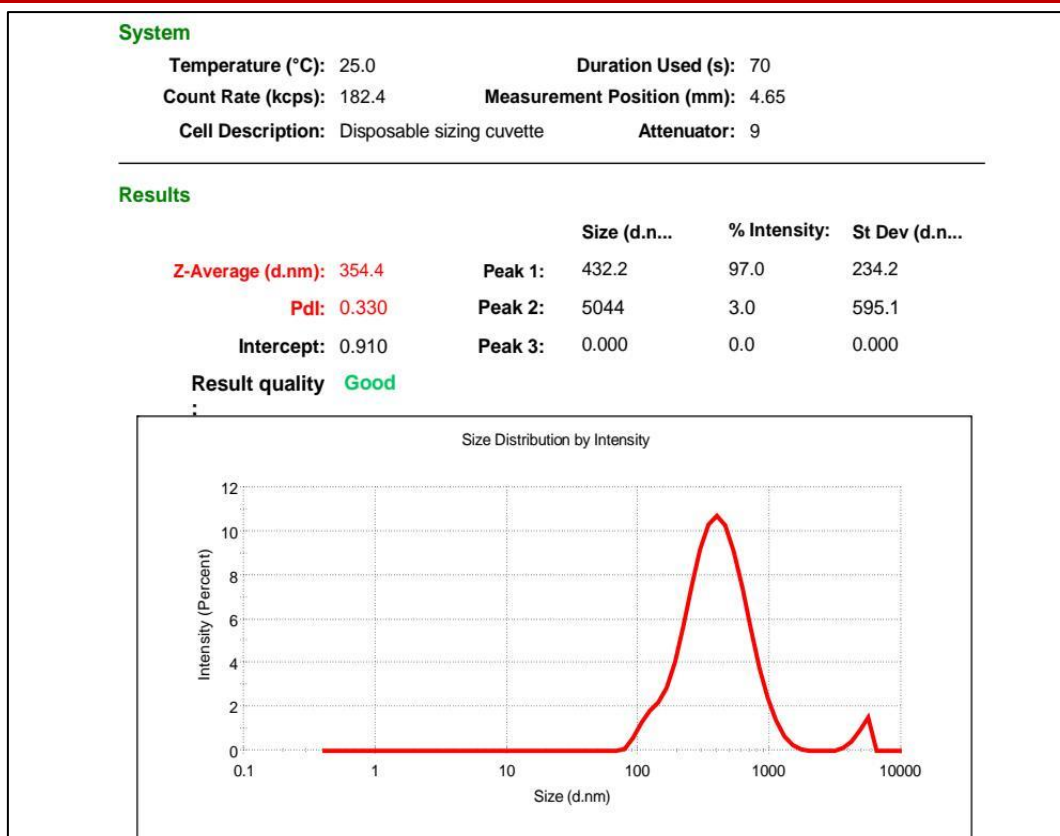


Fig. 9: Avg particle size and PDI (F4 formulation)

6.10 Zeta Potential

Zeta potential estimation uses the Electrophoretic light dissipating technique. Zeta capability of the arranged cubosomal scattering was pondered to decide the surface charge of the nano-particles which is significant for anticipating the drawn

out strength of the colloidal scattering. The high zeta potential qualities give adequate electric repugnance which thusly forestalls molecule conglomeration. Still up in the air by utilizing the Zesizer Nano-series (Nano ZS, Malvern).

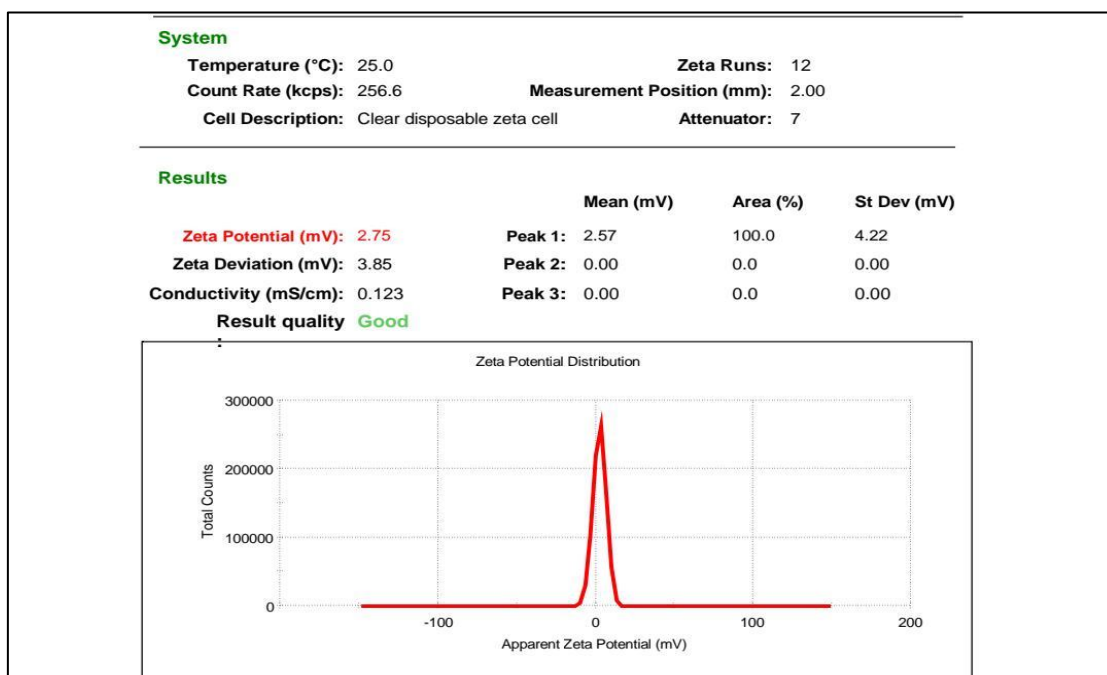


Fig. 10: Zeta Potential of f4 Formulation

CONCLUSION

Cubosomes can be made by a basic mix of organically viable fluids (GMO) and water and in this manner well fitting for drug and body tissues. The capacity to frame cubosomes during make offer unrivaled adaptability for item improvement. The cubosomal skin gels merit thought because of remarkable fluid glasslike design and simplicity of preparation. Cubosomes are novel measurement structures shaped by GMO when added to water. Since a lipid will in general withdraw in the fluid stage Poloxamer 407 is utilized as a stabilizer to keep away from conglomeration.

Darunavir drug has low solvency and formed into cubosomes to support the medication discharge it was planned to skin gels. The urelationship coefficient R2 was viewed as 0.999 in methanol.

Cubosome plan ready (F4) was considered as a best enhanced definition that shows sufficient capture effectiveness (95.40%), and medication discharge (89.76%).As GMO fixation increments ensnarement proficiency and medication discharge are expanded yet the pre-arranged detailing are not steady, thus stage seperation has occured. To supported the medication discharge the improved cubosome definition F4 was formed into Gel utilizing carbopol 974BP and HPMC 15 cps.

The idea of cubosome scattering was noticed infinitesimally. The above work indicates cubosomal viability as a controlled delivery drug carrier. The delayed discharge is accomplished by figuring out as skin gel keeping up with the cubosome structure.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest in this work.

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