Section: Research Paper

DESIGN DEVELOPMENT AND CHARACTERIZATION OF CUBOSOMES LOADED GEL FOR THE MANAGEMENT OF ACTINIC KERATOSIS

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Abstract

Cubosomes are discrete, sub-micron, nanostructured particles of the bicontinuous cubic liquid crystalline phases. The aim of this work is to improve the bioavailability of Tretinoin by formulating cubosomes. Cubosomal formulations were prepared according to a Box-behnken design. The selected variables were the amount of glyceryl monooleate, poloxamer 407 and amount of drug. In total fifteen batches were prepared. The prepared formulations were characterized for their drug content, particle size, zeta potential and in-vitro release study. The optimized formulation had a size of 128.3 nm, zeta potential (−29.2 mV), and entrapment efficiency of 78.852% and showed a sustained release pattern over 12h. Micrographs of the transmission electron microscope confirmed the cubic nanostructure of the optimized formulation. The optimized formulation was further transformed into gel utilizing carboxy methyl cellulose as gelling agent. The prepared gel was evaluated for appearance, pH, viscosity and in-vitro permeation study. The results depicted that the formulation showed uniform appearance, appropriate viscosity and about 91.948±0.011% drug was released in pH 6.8 Phosphate buffer at the end of 12 h in a sustained manner.

Keywords: Cubosomes, Tretinoin, Box-behnken design, Cubosomal gel, Carboxymethylcellulose**.**

Introduction

Cubosomes are square and rounded, discrete sub-micron, nanostructured particles with internal cubic lattice of the bicontinuous cubic liquid crystalline phases [1]. These are thermodynamically stable and consist of honeycombed (cavernous) structures separating two internal aqueous channels and a large interfacial area. These are nanoparticles which are self assembled liquid crystalline particles of certain surfactants with proper ratio of water with microstructure that provides unique properties of practical interest [2]. Hydrating a surfactant or polar lipid that forms cubic phase and then dispersing a solid like phase into smaller particles usually forms a cubosomes [3].

There are various components of cubosomes are used named polymers, lipids (Phytantriol (PHYT), GMO), and a surfactant (Poloxamer 407 (P407)) with polar and non polar components [4]. The lipids used in cubosomes formulation are more stable and offer stability to the formulation during shelf-life. Lipid is used to construct bicontinuous cubic phases are monoglyceride, monoolein etc [5].

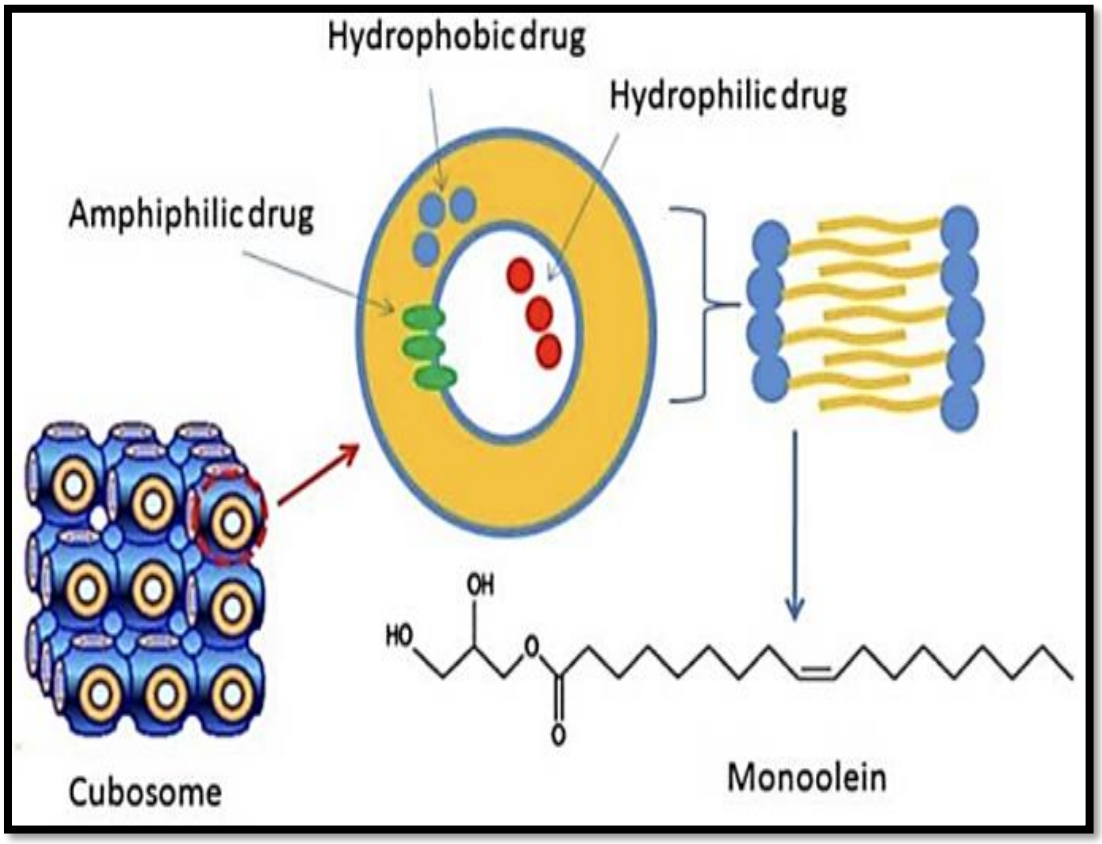

Figure 1.1: Composition of Cubosomes

There are various methods used for the preparation of cubosomes named bottom up technique, top down approach, spray drying method, solvent evaporation method etc. The bottom-up approach first forms the nanostructure building blocks and then assembles them into the final material. It is more recently developed technique of cubosomes formation, allowing cubosomes to form and crystallize from precursors on the molecular length scale. Top-down approach begins with a suitable starting material and then sculpts the functionality from the material. The bulk cubic phase is first produced and then dispersed by high energy processing into cubosomes nanoparticles [6].

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 [7].

Materials and Methods

Materials

Tretinoin was gifted from Akum Drugs and Pharmaceuticals Ltd., Haridwar, India. Poloxamer 407 and (GMO) Glyceryl Mono-oleate, methanol and Carboxy Methyl Cellulose (CMC) were used from the laboratory of Amity University, Noida. All other reagents used were of analytical grade.

Methods

Determination of λ_{max} of Tretinoin

A sample (6 µg/ml) was scanned between 200-400 nm to access the λ_{max} for Tretinoin.

Preparation of Standard Calibration Curve of Tretinoin

Serial dilutions were obtained from stock in the concentration range of 1-6 µg/ml and run-on UV-vis spectrophotometer at maximum wavelength. The respective absorbances were recorded and a graph of concentration v/s absorbance was plotted to obtain the standard calibration curve [8]. Similarly, the standard curve of Tretinoin was also obtained in pH 6.8 phosphate buffer in the similar composition as mentioned above.

Drug- Excipient Compatibility Study

FTIR Technique

Drug-excipient compatibility study was carried out by FTIR Spectrophotometry. A fine power of drug and KBr was compressed into disc was ground into fine power using mortar pestle and transformed to pellets by 75 kg/cm^2 in a hydraulic pressure, which was scanned 45 time at a resolution of $2cm^{-1}$. The characteristic peaks were recorded [9-11].

DSC Analysis

A differential scanning calorimeter was employed to observe the melting and the recrystallization behaviour of the drug with the excipients. The samples for the DSC analysis include drug and physical mixture. Approx. 5 mg of the samples, individually sealed in the aluminium pans, were placed over the sample platform. The reference pan, empty sealed aluminium pan, was placed on the reference platform. The pans were heated from 25 to 300 °C at the rate of 10 °C/min under nitrogen purge (20 mL/min) [12].

Preparation of Cubosomes of Tretinoin

Tretinoin loaded cubosomes were prepared by bottom-up technique, using varying the concentration of lipid and surfactant and drug, a total of fifteen formulations of Tretinoin were developed.

Formulation of Cubosomal Dispersion

Preparation of cubosomes dispersions was based on the emulsification of lipid/surfactant mixtures in water. In particular, the monoglyceride based lipidic phase was glyceryl monooleate (GMO) as used. Poloxamer 407 was used as surfactant. Briefly, GMO and Poloxamer 407 were melted in a water bath at 70ºC. Box-behnken design was used for the optimization of formulation parameters. The independent variables were glyceryl monooleate amount, polymer amount, and drug amount while independent variables were selected as particle size and entrapment efficiency. In the case of Tretinoin containing dispersions, the predetermined amount of the drug was added to the molten disperse phase and solubilized before adding to the aqueous solution. The molten mixture was then added dropwise to the aqueous phase at 70ºC under mechanical stirring at different speeds (i.e., 1500 rpm). Dispersions were maintained under stirring and were cooled to room temperature up to the solidification of lipid droplets (after 2 h). Cooling was conducted using an external ice bath for 10 to 15 min. Dispersions was stored in glass bottles at room temperature for further investigations [13-15].

Cubosomes dispersions were prepared through disrupting a cubic gel phase of GMO and water in the presence of sterically hindering stabilizer with the aid of certain mechanical energy. All the prepared dispersions appeared as uniform opaque white mixtures with no visible signs of aggregate when freshly prepared. An extra effort was done to optimize the formula compositions and ratios seeking for better stability and performance**.**The composition of formulations were depicted in table 1.1.

Table 1.1: Formulation Design of Tretinoin Cubosomes by Box-Behnken Design

Formulation code	**Glyceryl monooleate amount (X1, mg)**	**Polymer amount (X2, mg)**	**Drug-Tretinoin (X3, mg)**
F_1	500	80	2
F_2	500	25	6
F_3	300	25	10
F_4	300	135	2
F_5	500	135	6
F_6	300	25	2
F_7	300	80	6
F_8	500	80	10
F_9	300	80	6
F_{10}	100	80	2
F_{11}	100	80	10
F_{12}	300	80	6
F_{13}	100	25	6
F_{14}	300	135	10
F_{15}	100	135	6

Evaluation of Cubosomes

These preparations were evaluated for particle size, encapsulation efficiency, zeta potential and in-vitro release studies. Further, the optimized batch was obtained from results given by Box-behnken design and other evaluation parameters [16].

Preparation and Evaluation of Cubosomal Gel of Tretinoin

The optimized batch was further converted into gel by utilizing Carboxy methyl cellulose as gelling agent. The prepared gel was evaluated for appearance, pH, viscosity, in-vitro permeation study. Finally, the stability studies of cubosomal gel were also performed.

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 6.8. 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 [13].

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) [15].

pH

pH of all formulations is determined by using digital pH meter by immersing the electrode in gel formulation and pH was measured [13].

Viscosity

Viscosity measurements were performed by Brookfield viscometer (AMETEK Brookfield**,** Germany). The tested formulations were placed in the sampler tube using spindle no. 4. The spindle was lowered vertically into the centre of the formulation and rotate at a speed of 50 rpm for 10 min. All measurements were carried out in triplicate and the mean value was recorded ± SD [7].

***In-vitro* Release Study**

The *in-vitro* release of Tretinoin from the cubosomal gel was performed. Prior to testing, a piece of cellulose membrane (Molecular weight cut off 12,000–14,000 Da, Spectra/Pro, Spectrum Laboratories, Inc., USA) was soaked in pH 6.8 phosphate buffers for about 12 h. Then, the membrane was fixed in position using rubber band to cover one end of a top-cut plastic syringe acting as a dialysis tube of 1.9 cm internal diameter. An accurately weighed quantity of the test preparation (equivalent to 10.0 mg Tretinoin) was placed in the designed release assembly. The

tube enclosing the test sample was then attached to the shaft of a dissolution apparatus I (Hanson Research, California, USA). The dialysis tube was carefully adjusted to a position so that the membrane just touched the surface of the release medium (pH 6.8 Phosphate buffer). A volume of 50 ml of pH 6.8 phosphate buffer (pH 6.8) was used for the study. The temperature was maintained at 37^{o}C ± 0.5^{o}C and the stirring speed was adjusted to 100 rpm. Two ml aliquots of the release medium were withdrawn at 1, 2, 3, 5, 8 and 12 h time intervals, and replaced with 2 ml fresh medium to maintain the volume. The samples were filtered with micropore 0.22 µm syringe filter and analyzed for Tretinoin content using U.V visible spectrophotometer (Nano Drop Model 1000, Thermo Fisher Scientific, DE, USA). The mean cumulative amount of Tretinoin released (n=3, ± SD) was plotted as a function of time [17].

Stability Studies

Accelerated stability studies for optimized gel formulation 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 release was determined periodically [7].

Results and Discussion

Determination of λ_{max} of Tretinoin

Double beam UV-visible spectrophotometer (Shimadzu, UV-1800, Japan) was used to know the λ_{max} of drug. A 6 µg/ml solution ofTretinoin in methanol was scanned in the range of 200-400 nm. The λ_{max} of drug was found to be 346 nm (Figure 2).

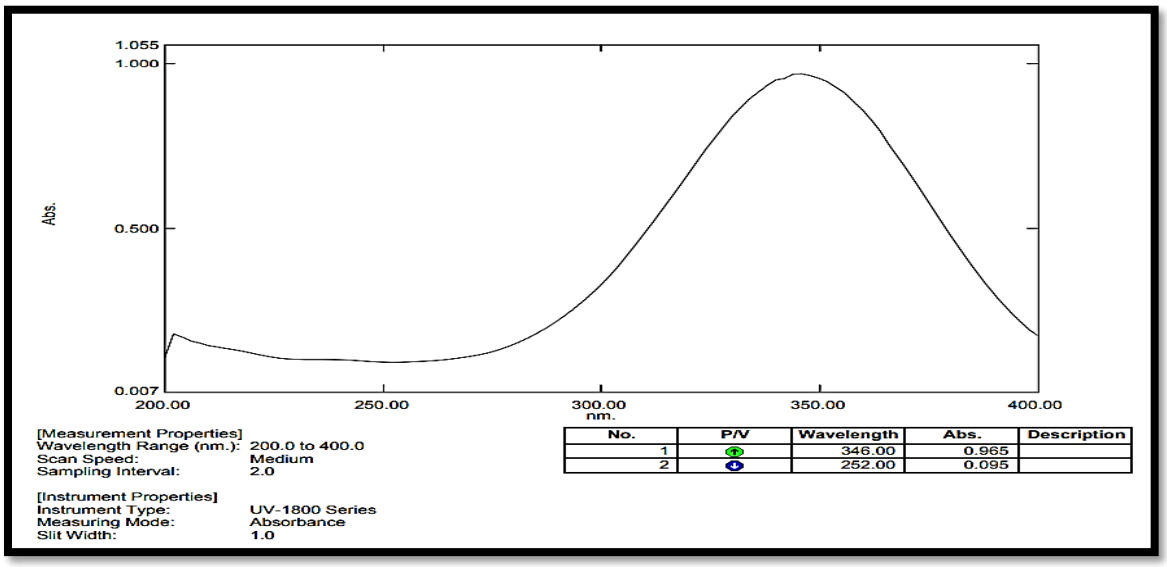

Figure 1.2: UV Spectrum of Tretinoin in Methanol

Preparation of Calibration Curve of Tretinoin

The calibration curve for Tretinoin was obtained by using the 1 to 6µg/ml concentration of Tretinoin in methanol. The absorbance was measured at 346 nm. The calibration curve of Tretinoinin methanol as shows in graph indicated the regression equation Y=0.1444x + 0.0937 and R^2 value 0.9995, which shows good linearity as shown in Figure 1.3a.

Similarly, the standard calibration curve of Tretinoin was also prepared in pH 6.8 Phosphate buffer.The calibration curve of Tretinoin in pH 6.8 phosphate buffer as shows in graph (figure 1.3b) indicated the regression equation Y=0.139x + 0.060 and R^2 value 0.995 which shows good linearity.

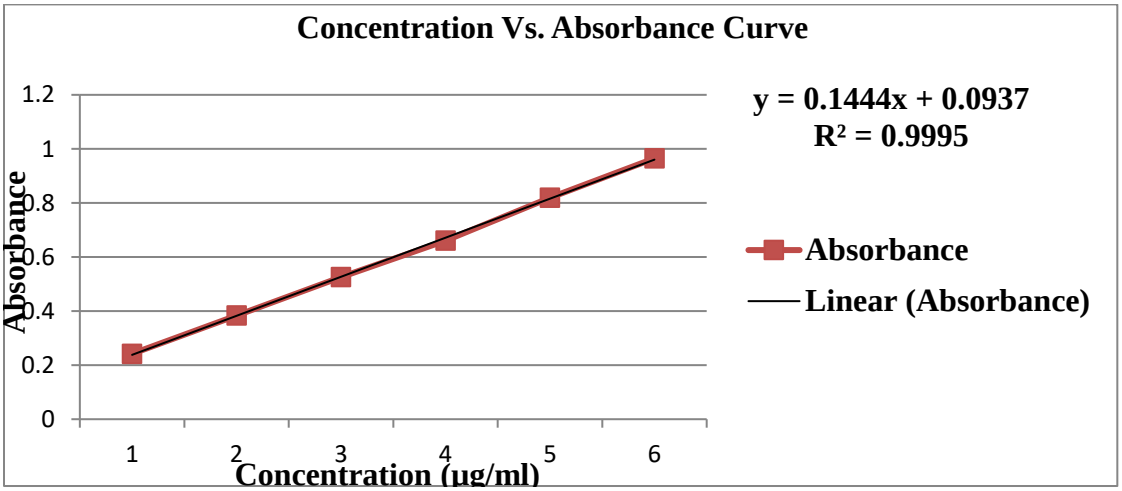

Figure 1.3a: Graph of Standard Calibration Curve of Tretinoin in Methanol

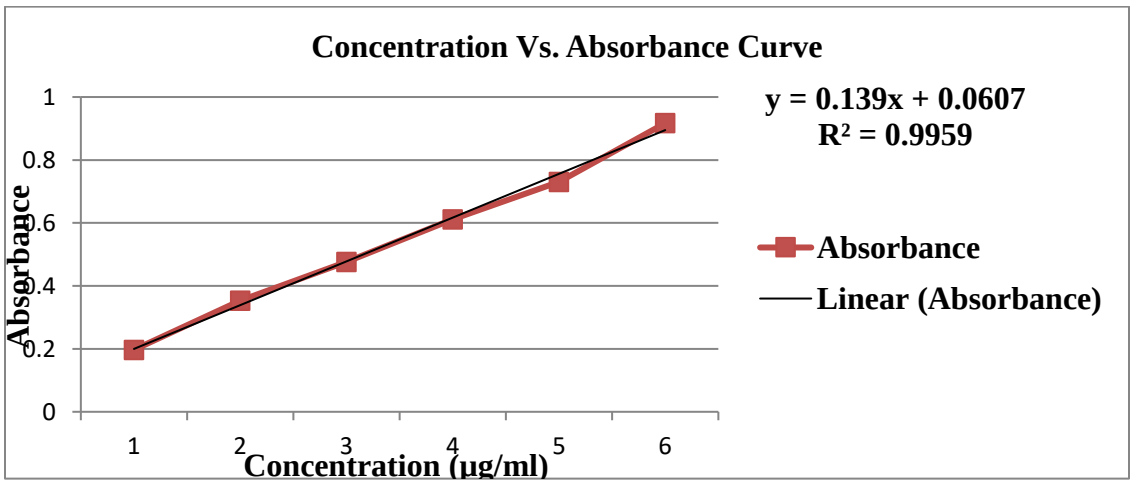

Figure 1.3b: Standard Calibration Curve of Tretinoin in pH 6.8 Phosphate Buffer

Compatibility Studies

Compatibility studies were done using FTIR and DSC.

FTIR Technique

The FTIR spectra of Tretinoin pure drug sample alone and with excipients were constructed and results reflected that the chosen drug was found stable with the excipients selected for formulation of Tretinoin cubosomes. The FTIR spectra of pure drug (Tretinoin) and different excipients were shown in Figure 1.4.

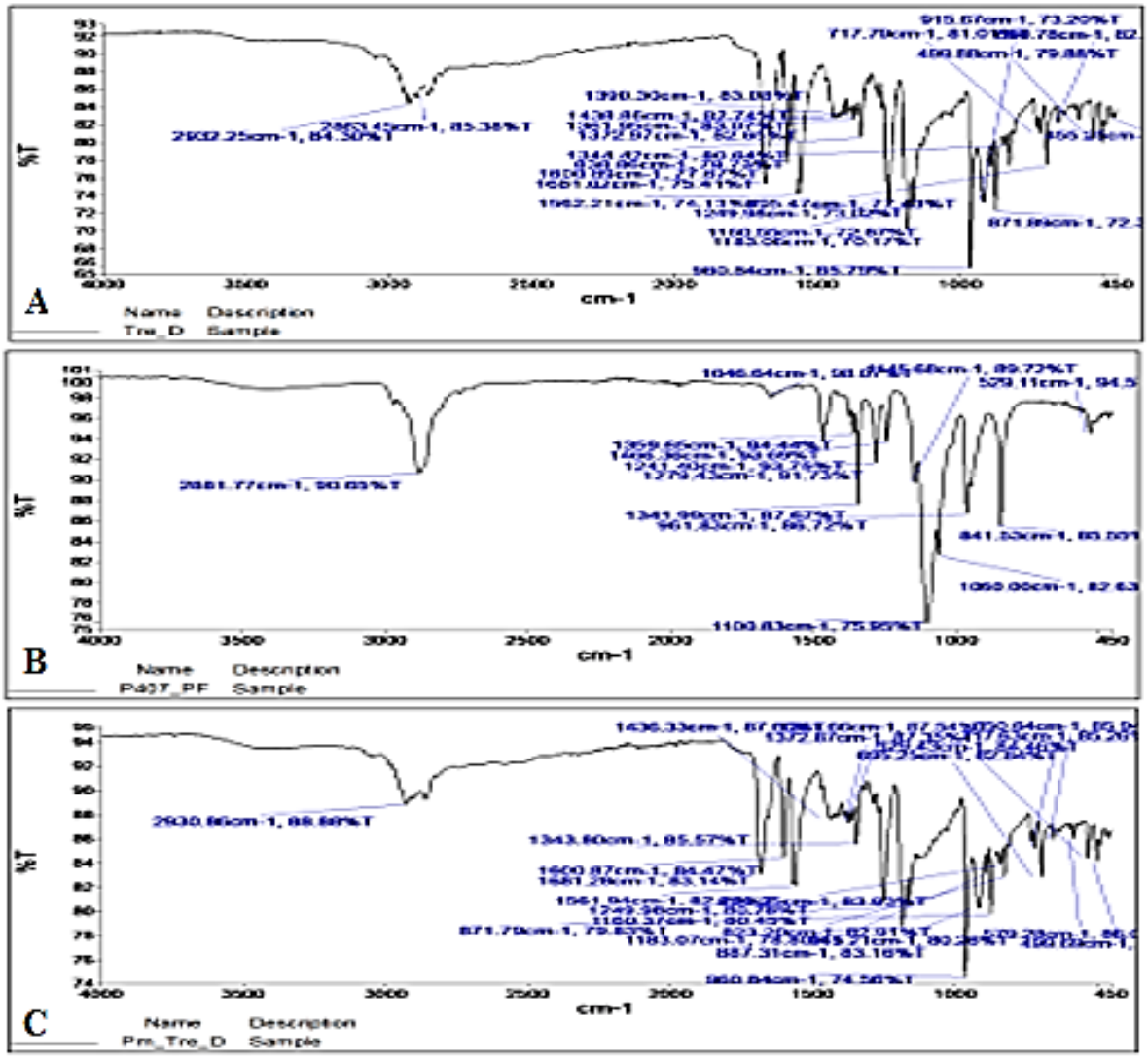

Figure 1.4: FT-IR graph of Tretinoin drug (A), FT-IR graph of Poloxamer 407 (B) and FT-IR graph of Physical Mixture (C)

According to the FT-IR results, the characteristic bonds were observed for the pure Tretinoin powder and Poloxamer 407. Any shift or appearance change in the characteristic bonds was not identified in the physical mixture.

DSC Analysis

A differential scanning calorimeter was employed to observe the melting and the recrystallization behaviour of the drug with the excipients. The samples for the DSC analysis include drug, excipients and physical mixture. The results of DSC study are represented in Figure 1.5.

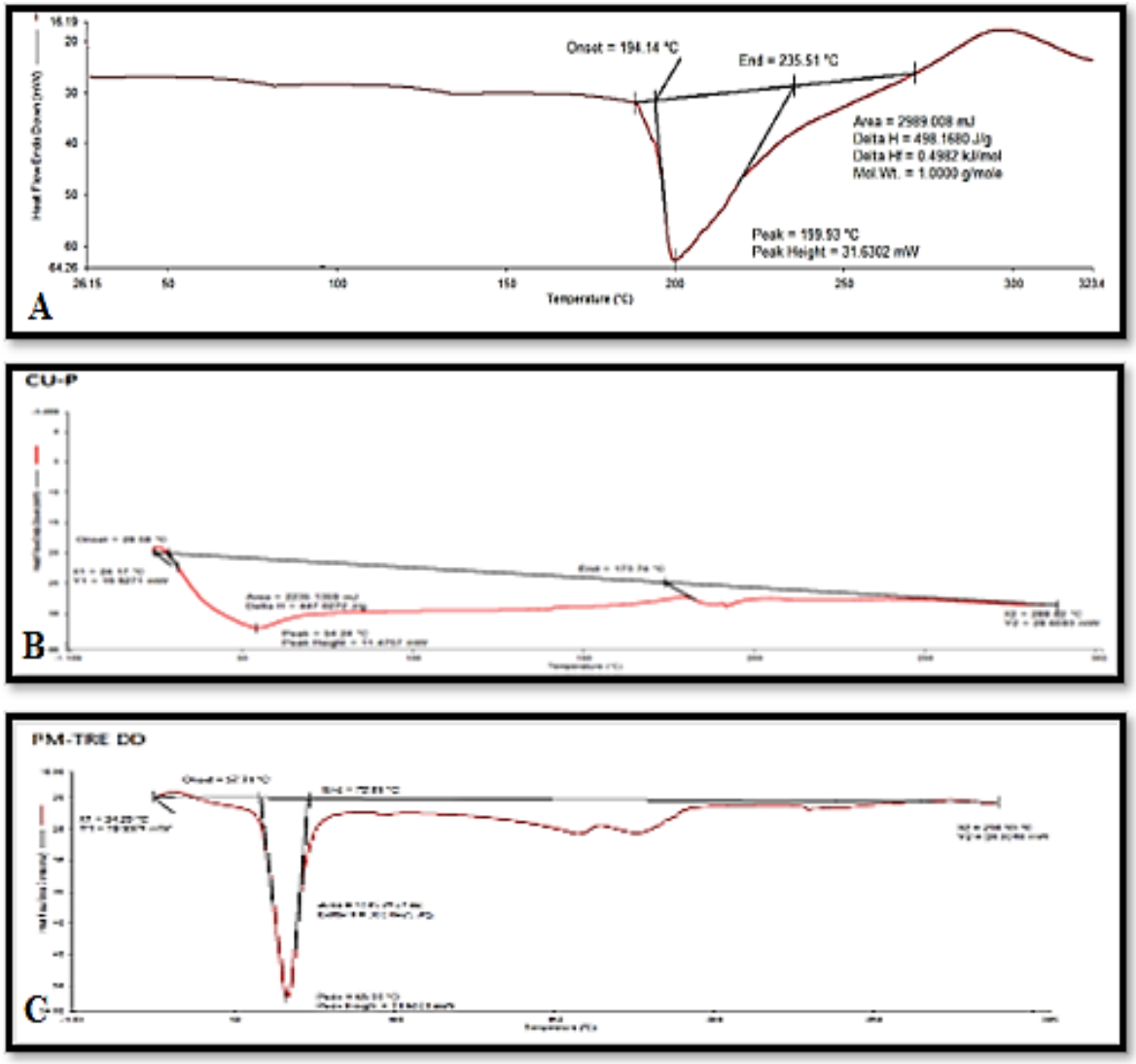

Figure 1.5: DSC Thermogram of the Tretinoin Drug (A), DSC Thermogram of the Glyceryl monooleate (B) and DSC Thermogram of the Physical Mixture (C)

Evaluation of Cubosomes

Particle Size Analysis

The particle size of Tretinoin cubosomes formulation batches (F_1-F_{15}) was ranged between 108.7 -228.3 nm. Results are shown in Figure 1.6.

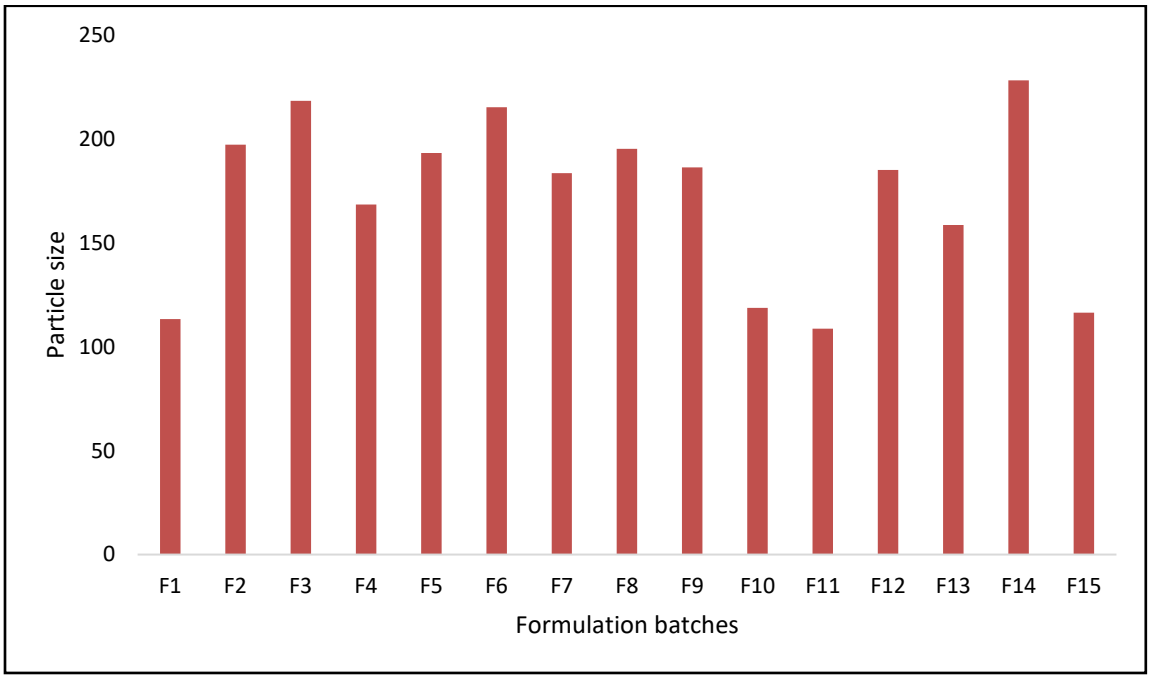

Figure 1.6: Particle size of Tretinoin Cubosomes Batches F1-F15

Optimization Study

Box-Behnken Design was used to optimize the formulation parameters.

ANOVA for Quadratic Model

Response 1: Particle Size

The ANOVA results for Particle size are depicted in table 1.2. The Model F-value of 72.78 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, AC, BC, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.The Lack of Fit F-value of 26.71 implies the Lack of Fit is significant. There is only a 3.63% chance that a Lack of Fit F-value this large could occur due to noise. Coefficients in coded value are represented in table 1.3.The

coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant.

Table 1.2: ANOVA for Response 1 (Particle size)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	22704.93	9	2522.77	72.78	< 0.0001	significant
A-Glyceryl monooleate amount	4831.44	1	4831.44	139.38	< 0.0001	
B-Polymer concentration	863.20	1	863.20	24.90	0.0041	
C-drug concentration	2261.28	1	2261.28	65.23	0.0005	
AB	362.90	1	362.90	10.47	0.0231	
AC	2111.40	1	2111.40	60.91	0.0006	
BC	806.56	1	806.56	23.27	0.0048	
A^2	7846.93	1	7846.93	226.37	< 0.0001	
B^2	2787.23	1	2787.23	80.41	0.0003	
C^2	87.75	1	87.75	2.53	0.1725	
Residual	173.32	5	34.66			
Lack of Fit	169.10	3	56.37	26.71	0.0363	significant
Pure Error	4.22	2	2.11			
Cor Total	22878.25	14				

Factor coding is Coded.Sum of squares is Type III – Partial

Table 1.3: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	185.00	1	3.40	176.26	193.74	
A- Glyceryl monooleate amount	24.57	1	2.08	19.22	29.93	1.0000
B-Polymer concentration	-10.39	1	2.08	-15.74	-5.04	1.0000
C-drug concentration	16.81	1	2.08	11.46	22.16	1.0000
AB	9.53	1	2.94	1.96	17.09	1.0000
AC	22.98	1	2.94	15.41	30.54	1.0000
BC	14.20	1	2.94	6.63	21.77	1.0000
A^2	-46.10	1	3.06	-53.98	-38.22	1.01
B^2	27.48	1	3.06	19.60	35.35	1.01
C^2	-4.88	1	3.06	-12.75	3.00	1.01

The particle size of the Tretinoin cubosomes in the current study varied between 108.7 to 228.3 nm, which may be regarded as a suitable midrange that contributed to reasonable homogeneity and a satisfactory size distribution (Table 1.2). A quadratic model was produced by the polynomial analysis of the particle size values from the Tretinoin cubosomes. The study's design provided evidence for the model's effectiveness in assessing the impact of the GMO amount (A), Polymer amount (B), and amount of drug (C) on the particle size of the prepared cubosomes. (Table 1.3). R^2 values for the proposed mathematical model were 0.9924. The one-way ANOVA produced the following equation.

Particle size=185.00-24.57A-10.39B-16.81C+9.53AB+22.98AC+14.20BC-46.10A^2-27.48B^2-4.88C^2

As seen, component A (GMO) have the profound effect on the size of cubosomes with a p-value of less than <0.0001, while factor B significantly increased the response's antagonistic effect with the same degree of significance. Such results might be useful for creating smaller particles. The

capacity to reduce the particle size and promote stability was also correlated with the amount of polymer in the cubosomes preparation. Additionally, the particle size was negatively impacted by factor C also, as lesser amount of drug can be easily entrapped in the cubosomes lattice (Table 1.2-1.3). Figure 1.7 represented the contour and 3D graphs showing the effect of selected independent factors on response Y1 (particle size).

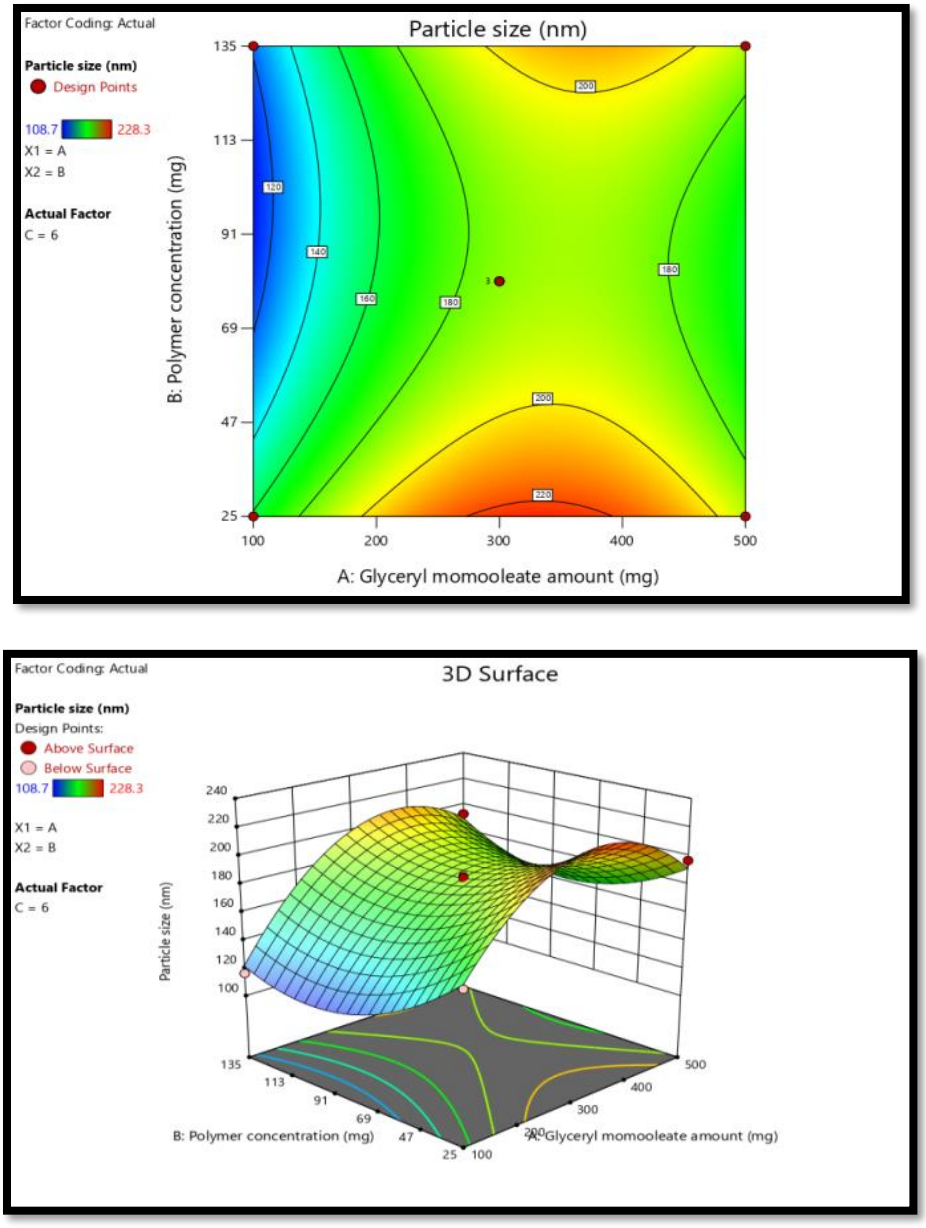

Figure 1.7: Contour Graph (top) and 3D Graph (below) showing the Effect of Independent Factors (GMO, Polymer and Drug) on Y1 (Particle size)

Entrapment Efficiency

The Entrapment efficiency is a crucial indicator of a cubosomes size and general stability. As per the results, the Entrapment efficiency (Y2) of the Tretinoin cubosomes was ranged from 46.5 to 79.9 percent as depicted in Figure 1.8.

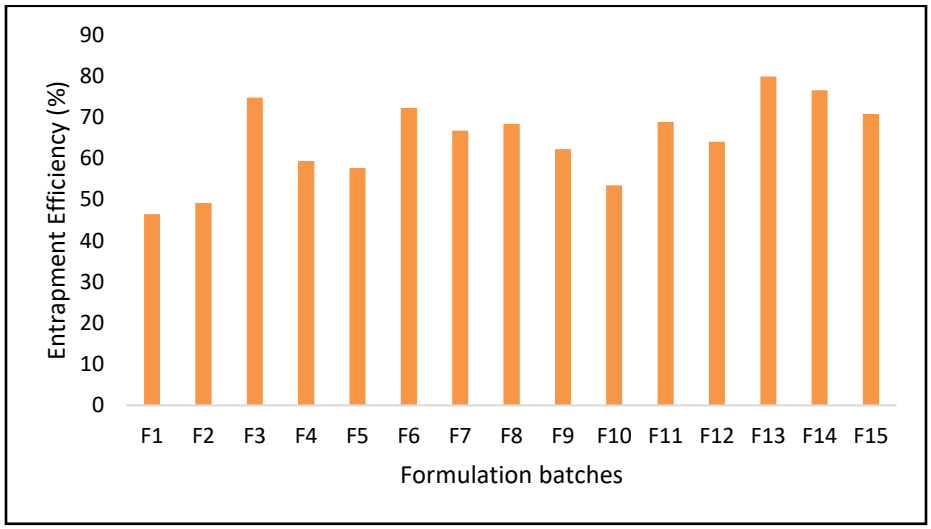

Figure 1.8: Entrapment efficiency of Tretinoin Cubosomes batches F1-F15

ANOVA for Quadratic Model

Response 2: Entrapment Efficiency

The ANOVA results of effect of independent factors on Response 2 (Entrapment Efficiency) were presented in Table 1.4.

Table 1.4: ANOVA for Response 2 (Entrapment Efficiency)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1158.00	9	128.67	2.83	0.0324	significant
A-Glyceryl monooleate amount	328.96	1	328.96	7.23	0.0433	
B-Polymer concentration	17.11	1	17.11	0.3761	0.5665	
C-drug concentration	406.13	1	406.13	8.93	0.0305	

AB	77.44	1	77.44	1.70	0.2488	
AC	10.56	1	10.56	0.2322	0.6503	
BC	54.02	1	54.02	1.19	0.3256	
A^2	121.02	1	121.02	2.66	0.1638	
B^2	121.02	1	121.02	2.66	0.1638	
C^2	1.56	1	1.56	0.0343	0.8604	
Residual	227.47	5	45.49			
Lack of Fit	217.21	3	72.40	14.11	0.0669	not significant
Pure Error	10.26	2	5.13			
Cor Total	1385.48	14				

Factor coding is Coded. Sum of squares is Type III – Partial

The Model F-value of 2.83 implies the model is not significant relative to the noise. There is a 13.24% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, C are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The Lack of Fit F-value of 14.11 implies there is a 6.69% chance that a Lack of Fit F-value this large could occur due to noise.

Coefficients in Terms of Coded Factors

Coefficients in terms of coded factors were presented in Table 1.5.

Table 1.5: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	64.40	1	3.89	54.39	74.41	
A-Glyceryl monooleate amount	-6.41	1	2.38	-12.54	-0.2824	1.0000
B-Polymer concentration	-1.46	1	2.38	-7.59	4.67	1.0000

C-drug concentration	7.13	1	2.38	0.9949	13.26	1.0000
AB	4.40	1	3.37	-4.27	13.07	1.0000
AC	1.63	1	3.37	-7.04	10.29	1.0000
BC	3.67	1	3.37	-4.99	12.34	1.0000
A^2	-5.72	1	3.51	-14.75	3.30	1.01
B^2	5.73	1	3.51	-3.30	14.75	1.01
C^2	0.6500	1	3.51	-8.37	9.67	1.01

The adopted statistical design demonstrates that Y2 followed a linear mathematical model of polynomial analysis considering the findings (Table 1.5). The collected data's ANOVA produced the equation shown below:

Entrapment Efficiency = 64.40 - 6.41A - 1.46B + 7.13C + 4.40AB + 1.63AC + 3.67BC - $5.72A^2$ + $5.73B^2$ + $0.6500C^2$

According to the equation, factor A (antagonistic) and factor C (agonistic) had considerably influenced response Y2 (p-value < 0.0433 & 0.0305).The factor B had shown the antagonistic effect. The amount of GMO& polymer, which is factor A & B, may have had a beneficial impact on response Y2 due to its effect on the size of the globules. Factors A and B reduced the size of the particles, which was expected to improve the formulations' stability. The Entrapment efficiency of the Tretinoin cubosomes was also greatly improved by the quadratic term for component C. (Table 1.4 and 1.5). The perturbation, contour, and 3D plots in Figure 1.9 showed how the independent factors affected the response Y2 in each case (Entrapment Efficiency).

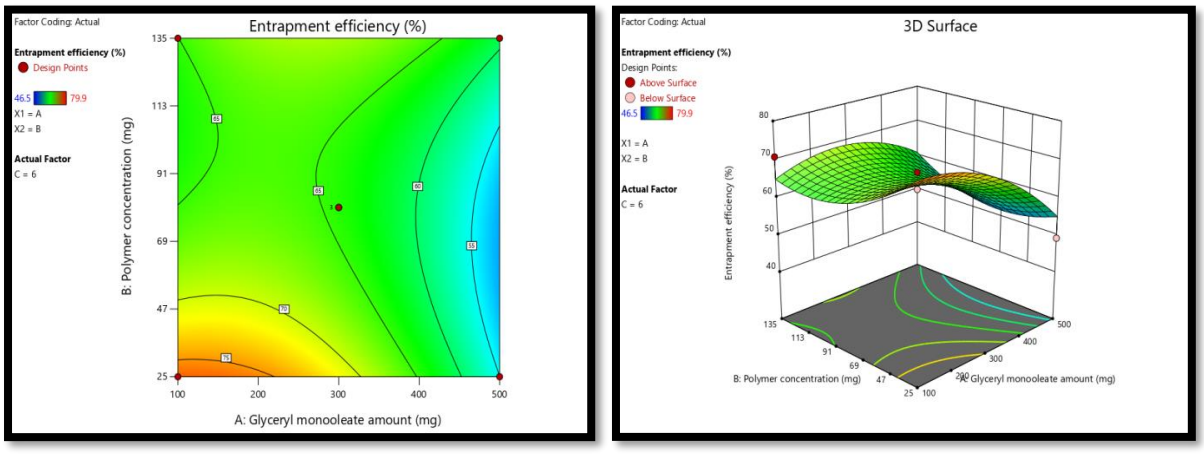

Figure 1.9: Contour Graph (left) and 3D Graph (right) Showing the Effect of Independent Factors (GMO, Polymer and Drug) on Y1 (Entrapment Efficiency)

All the prepared Tretinoin cubosomes batches were also evaluated for Zeta potential and in-vitro permeation study.

Zeta Potential Analysis

The Malvern zeta sizer conducted the analysis for each of the fifteen batches. The outcomes are displayed in table 1.6.

Table 1.6: Zeta Potential Analysis of Tretinoin Cubosomes Batches ($F_1$to F_{15})

S. No.	Formulation Code	Zeta potential mean (mV)
1.	F_1	-21.36
2.	F_2	-19.11
3.	F_3	-14.53
4.	F_4	-22.61
5.	F_5	-26.47
6.	F_6	-19.84
7.	F_7	-23.71
8.	F_8	-17.57
9.	F_9	-33.48
10.	F_{10}	-31.35
11	F_{11}	-26.16
12.	F_{12}	-36.52
13.	F_{13}	-28.95
14.	F_{14}	-25.26
15.	F_{15}	-34.39

In-vitro Permeation Studies

The prepared Tretinoin cubosomes were studied for in-vitro drug permeation study in pH 6.8 phosphate buffer (figure 1.10). The percentage (%) drug release was ranged between 63.75-90.67%. Maximum percentage release was found to be with F13 batch.

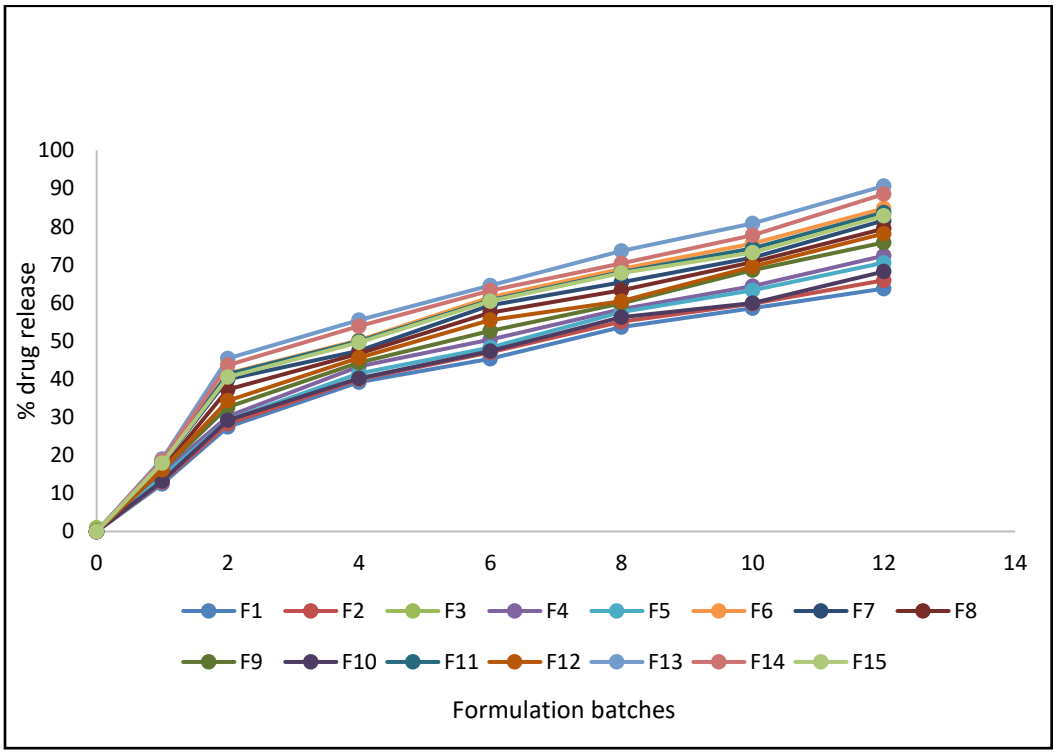

Figure 1.10: In-Vitro Drug Release from Tretinoin Cubosomes

Optimization of Tretinoin Cubosomes Formulation

A Tretinoin cubosomes formulation with the best attributes was created using the data that was gathered. The software provided several recommendations for various combinations of the parameters at various levels. About 100.0 mg of factor A, 33.793 of factor B, and 10.00 mg of factor C were presented in the checkpoint batch. Particle size was 127.479 nm, Entrapment efficiency was 77.116%, and desirability was 0.918 for the optimized cubosomes formulation (Table 1.7). Table 1.7 showed that the model's validity and accuracy were confirmed by the fact that the experimental and proposed values of the responses of the best cubosomes formulation were in agreement without any significant differences ($p< 0.05$). Figure 1.11 is showing the contour graph for desirability.

Table 1.7: Actual and Experimental Values of the Optimized Tretinoin Cubosomes Formulation

Solution	GMO (mg)	Polymer (mg)	Drug (mg)	Particle size (nm)	Entrapment efficiency (%)	Desirability
Predicted	100.0	33.793	10.00	127.479	77.116	0.918
Actual	100.0	33.793	10.00	128.3	78.852	0.918

Checkpoint Analysis

The proposed regression models' superior prediction abilities were supported by the experimental and anticipated R^2 values. Additionally, the ratios of the actual to expected values showed low error rates, and there were acceptable residuals between the projected and experimental results; this shows that the data were not curved and that the model was adequate.

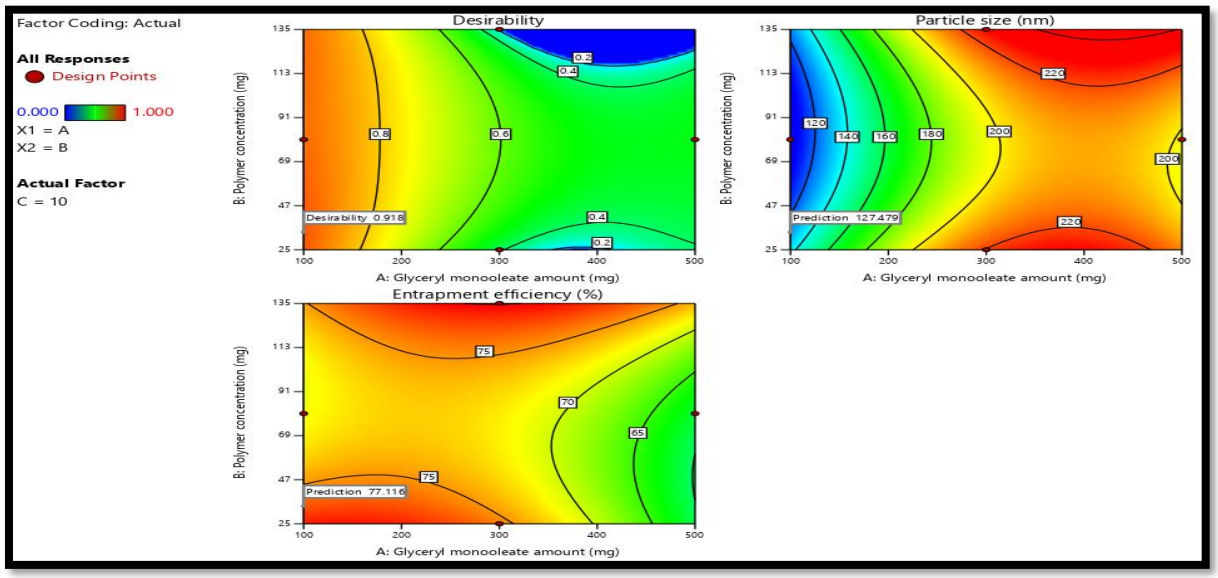

Figure 1.11: Contour Graph of Predicted Responses and Desirability

The optimized batch thus obtained was used for further studies. The results of different parameters of optimized batch were as mentioned below:

Evaluation Parameters for Optimized batch of Tretinoin Cubosomes Formulation (TCu-opt)

The optimized Tretinoin cubosomes formulation (TCu-opt) thus prepared was evaluated for particle size, pH, zeta potential, FTIR, TEM and in-vitro permeation study (Table 1.8).

Table 1.8: Evaluation Parameters for Optimized Tretinoin Cubosomes formulation (TCu-opt)

S. No.	Parameter	Inference
1.	Particle size (nm)	128.3 nm
2.	pH	6.8
3.	Zeta potential	-29.2 mV
4.	In- vitro permeation study	92.855±0.013% in pH 6.8 Phosphate buffer

Particle size Analysis

The Particle size peak of Tretinoin optimizedcubosomes formulation was observed at 128.3 nm (Figure 1.12).

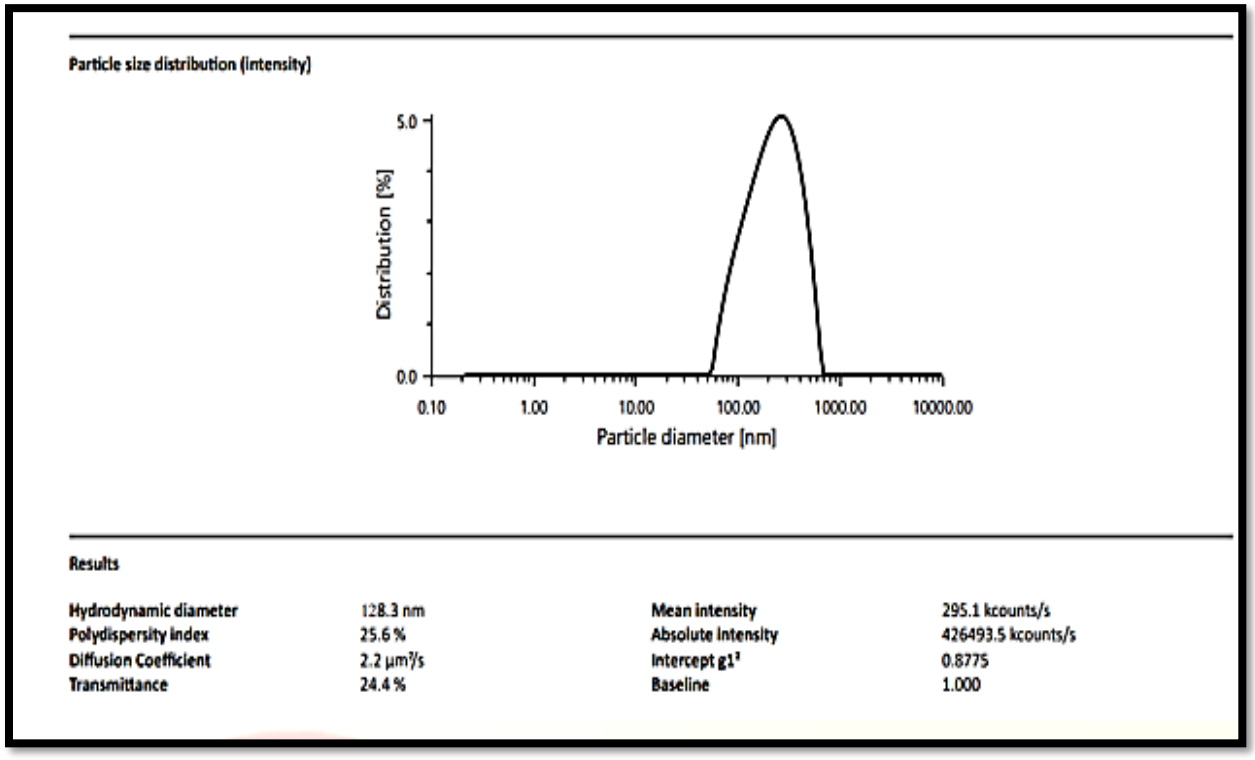

Figure 1.12: Particle size peak of optimized Cubosomes formulation(TCu-opt)

Zeta Potential

Zeta potential of Tretinoin optimized Cubosomes formulation (TCu-opt) was found to be -29.2 mV. The zeta potential value of optimized formulation was presented in figure 1.13.

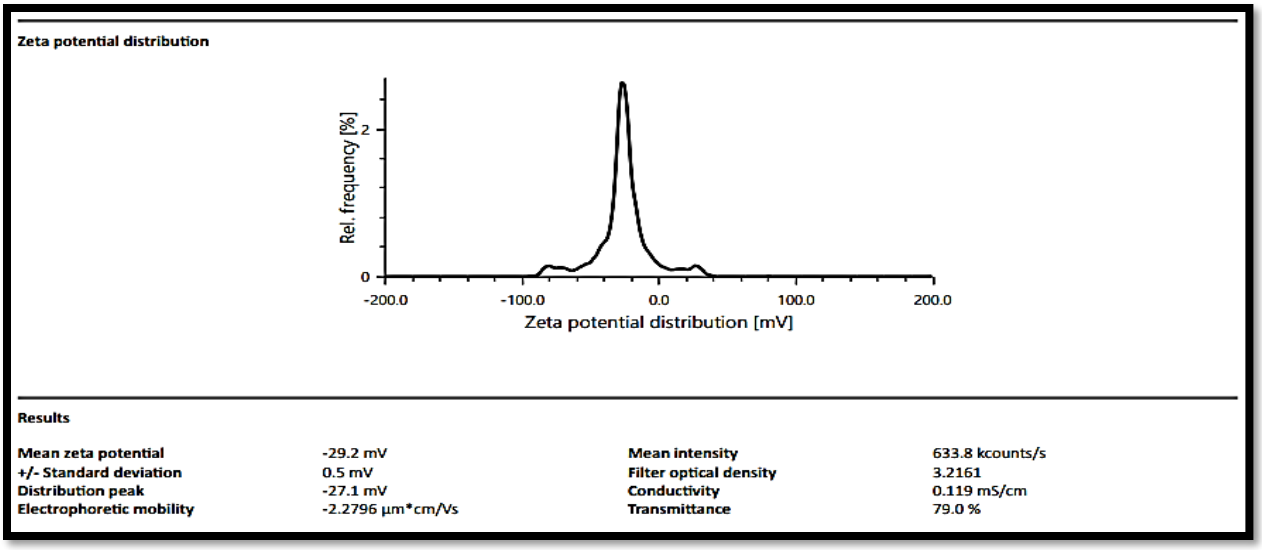

Figure 1.13: Zeta Potential of Optimized Cubosomes Formulation (TCu-opt)

FTIR Analysis

According to the FT-IR results, the characteristic bonds were observed for the pure Tretinoin powder and Poloxamer 407. Any shift or appearance change in the characteristic bonds was not identified in the physical mixture. But during the formulation, drug peaks show less intensity or combine with the ester group because of the presence of lipids. Figure 1.14 is presenting the FTIR spectra of optimized cubosomes formulation.

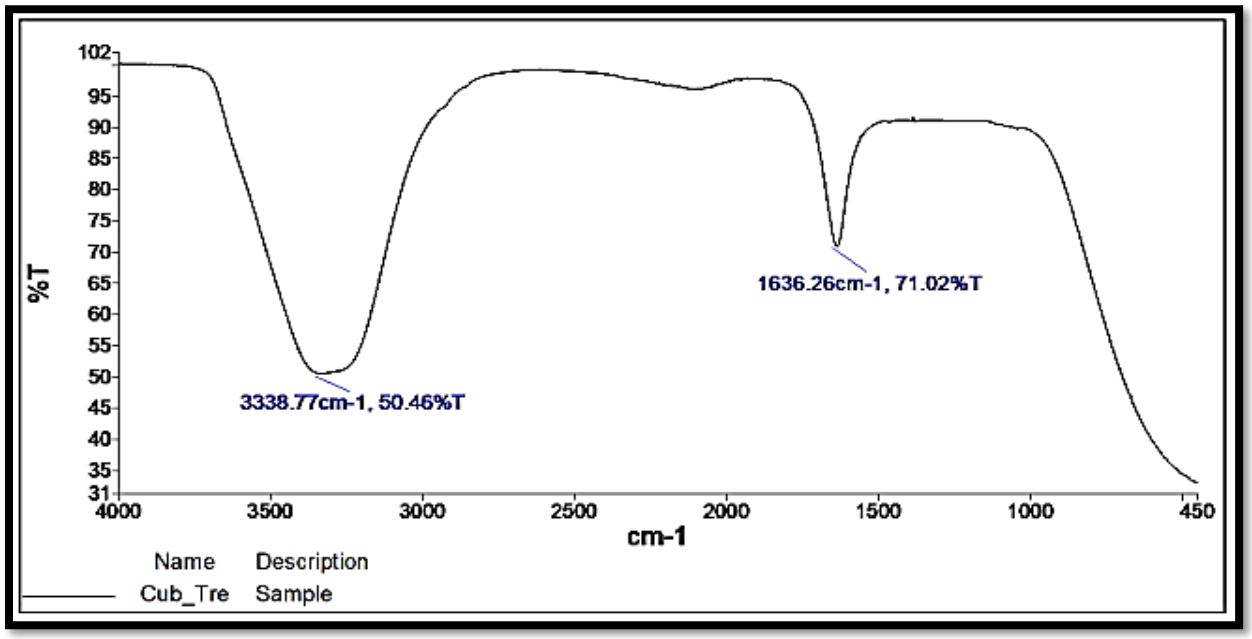

Figure 1.14: FT-IR graph of Optimized Cubosomes Formulation (TCu-opt)

TEM Analysis

The TEM graph of optimized formulation was depicted in figure 1.15.

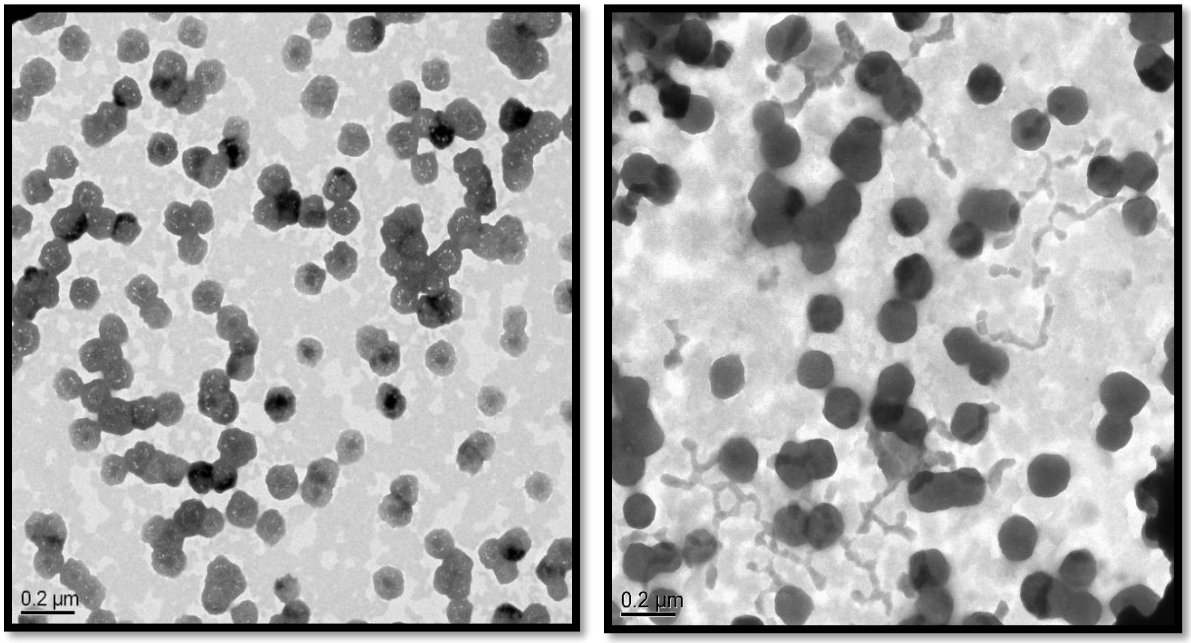

Figure 1.15: TEM Graph of Optimized Cubosomes Formulation (TCu-opt)

***In-Vitro* Permeation Study of Optimized Tretinoin Cubosomes Formulation (TCu-opt)**

In-vitro permeation study of Tretinoin Cubosomes formulation (TCu-opt) was depicted in figure 1.16.

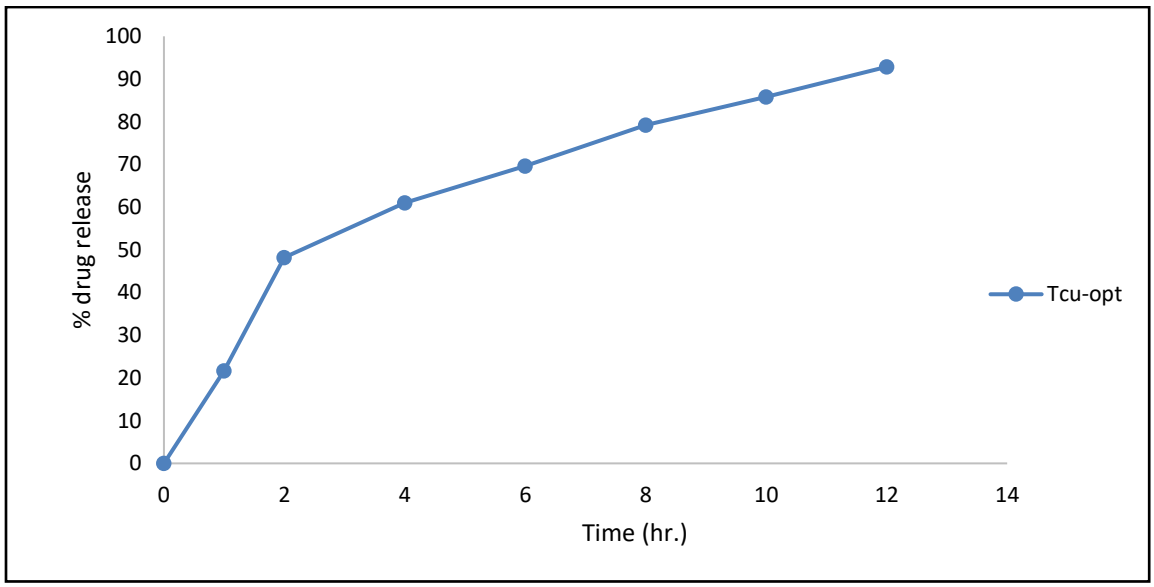

Figure 1.16: *In-Vitro* Permeation Study of Optimized Tretinoin Cubosomes Formulation (TCu-opt)

Preparation of Cubosomes Gel of Tretinoin

The optimized batch was further transformed into the gel formulation utilizing Carboxy methyl Cellulose (CMC).

Evaluation of Tretinoin Cubosomes Gel (TCu-Gel)

The Tretinoin cubosomes gel was checked for appearance, pH, viscosity and in-vitro permeation study. The results of evaluation parameters were depicted in table 1.9.

Table 1.9: Evaluation Parameters for Optimized Tretinoin Cubosomes Gel Formulation (TCu-gel)

S. No.	Parameter	Inference
1.	Appearance	Homogeneous
2.	pH	7.4
3.	Viscosity	145 cP
4.	In- vitro permeation study	91.948±0.011% in pH 6.8 Phosphate buffer

***In-Vitro* Permeation Study of Optimized Tretinoin Cubosomes Gel Formulation (TCu-gel)**

The in-vitro release results are depicted in Figure 1.17.

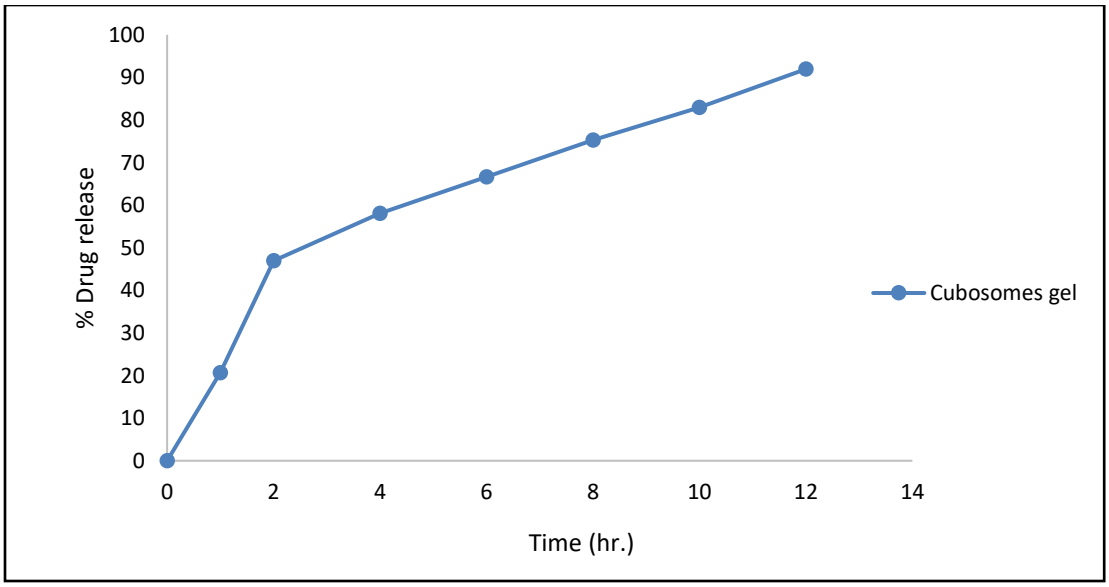

Figure 1.17: In-Vitro Permeation Study of Optimized Tretinoin Cubosomes Gel Formulation (TCu-gel)

Conclusion

In the present work, the cubosomes were prepared by utilizing GMO and polymer. This study reported on the use of a Box–Behnken design in the optimization of cubosome dispersion mean diameter for the encapsulation of Cubosomes. The derived polynomial equations proved to be satisfactory in predicting Y1 and Y2 values for the preparation of optimum cubosomes with desired particle size and entrapment efficiency. The optimal formulation size could be obtained when 100 mg of glyceryl monoolein, 33.793 mg of polymer, and 10 mg of drug as independent variables leading to the formulation of cubosomes with 128.3 nm in mean diameter and 78.852% in encapsulation efficiency. Prolonged release was achieved when they were formulated as topical gels on maintaining the cubosome structure. This product can be manufactured in large scale and commercialized for the treatment of skin infections, as it provided 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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