



## DESIGN DEVELOPMENT AND CHARACTERIZATION OF ETHOSOMES OF BETAMETHASONE VALERATE

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**Abstract**

Ethosomes are the novel lipid carriers composed of ethanol, phospholipids and water. These are soft, malleable vesicles tailored for enhanced delivery of active agents. They are the slight modification of well established drug carrier liposome. The present investigation was to design the Ethosomes containing Betamethasone valerate using different concentration of ethanol and phospholipid (Soya Lecithin). It is a BCS class II drug (low solubility and high permeability). The aim of this work is to improve the bioavailability of Betamethasone by formulating Ethosomes. Ethosomes were prepared according to a Box-behnken design. The selected variables were the amount of soya lecithin, ethanol and stirring speed. In total fifteen batches were prepared. The prepared formulations were characterized for their drug content, particle size, zeta potential and in-vitro permeation study. The optimized formulation had a size of 124.2 nm, zeta potential (-17.0), and entrapment efficiency of 88.5% and showed a sustained release pattern over 12 hrs. Micrographs of the transmission electron microscope confirmed the nanostructure of the optimized formulation.

**Keywords:** Ethosomes, Betamethasone, Box-behnken design, Ethosomal gel, Soya lecithin etc.

### **Introduction**

Ethosomes are the novel lipid carriers composed of ethanol, phospholipids and water. These are soft, malleable vesicles tailored for enhanced delivery of active agents. They are the slight modification of well established drug carrier liposome [1]. The size range of Ethosomes may vary

from tens of nanometers to microns. They are reported to improve the skin delivery of various drugs. The high concentration of ethanol makes the Ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer organization. They permeate through the skin layers sooner and possess significantly higher transdermal flux. The present investigation was to design the Ethosomes containing Betamethasone valerate using different concentration of ethanol and phospholipid (Soya Lecithin). It is steroid ester which is used to help relieve redness, itching, swelling or other discomfort caused by skin conditions. It is a BCS class II drug (low solubility and high permeability) [2].

## **Materials and Methods**

### **Materials**

Betamethasone valerate was gifted from Envee Labs., Nadiad, Gujarat, India. Soya Lecithin, ethanol, propylene glycol, Carbopol 934, acetonitrile, and other solvents were used from the laboratory of Amity University, Noida. All other reagents used were of analytical grade.

### **Methods**

#### **Determination of $\lambda_{\max}$ of Betamethasone valerate**

A sample (10  $\mu\text{g/ml}$ ) was scanned between 200-400 nm to access the  $\lambda_{\max}$  for Betamethasone valerate [3-4].

#### **Preparation of Standard Calibration Curve of Betamethasone valerate**

Serial dilutions were obtained from stock solution in the concentration range of 1-7  $\mu\text{g/ml}$  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. Similarly, the standard curve of Betamethasone valerate was also obtained in pH 7.4 phosphate buffers in the similar composition [5].

#### **Drug- Excipient Compatibility Study**

##### **FTIR Technique**

Drug-excipient compatibility study was carried out by FTIR Spectrophotometry. A fine powder of drug and KBr was compressed into disc was ground into fine powder using mortar pestle and transformed to pellets by 75  $\text{kg/cm}^2$  in a hydraulic pressure, which was scanned 45 time at a resolution of  $2\text{cm}^{-1}$ . The characteristic peaks were recorded [6-8].

### **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).

### **Preparation of Ethosomes of Betamethasone valerate**

Ethosomal formulations were prepared by using the cold method. The ethanolic vesicular system composed of phospholipids (15% to 55% w/v), ethanol (20% to 40% v/v), propylene glycol, drug and distilled water [9].

### **Formulation of Ethosomal Dispersion**

Phospholipids were dissolved along with the drug in ethanol. This mixture was heated to 30 °C and a fine stream of distilled water was added slowly, with constant mixing at 700 rpm with a mechanical stirrer in a closed container. Mixing was continued for an additional 5 min, while maintaining the system at 30°C. The preparation were left to cool at room temperature for 30 min and then it were sonicated at 30°C for five cycles of 3 min each with a minute rest between cycles using probe solicitor. Vesicles start to emerge after 5 min of churning. It is important to keep produced vesicles cold. Fifteen formulations were prepared using different concentration of phospholipid and ethanol among them optimized formulation were selected for characterization and evaluation studies [10].

Box-Behnken design was used to optimize the formulation parameters of Betamethasone Ethosomes to get an optimized composition comprising of good stability and efficacy. In the present study, the Soya lecithin amount, Ethanol amount, and Stirring speed were chosen as critical (independent) factors because soya lecithin was the main matrix component of the Ethosomes , ethanol was used to increase the fluidity of lipid membrane and reduces the density of lipids in the cell membrane, and stirring speed was continues to achieve homogeneous Ethosomes , while the dependent factors were Particle Size and Entrapment Efficiency. At a 95 percent level of confidence, the ANOVA test confirmed the validity of the chosen model ( $p < 0.05$ ). Checkpoint analysis was used to assess the resulting mathematical model's accuracy and precision for the

anticipated values of the dependent variables. The composition of formulations was depicted in table 1.

**Table 1: Formulation Design of Betamethasone valerate by Box–Behnken Design**

Formulation Code	Soya lecithin (X1, mg)	Ethanol (X2, ml)	Stirring Speed (X3, RPM)
F <sub>1</sub>	150	20	500
F <sub>2</sub>	350	20	700
F <sub>3</sub>	350	30	500
F <sub>4</sub>	150	30	300
F <sub>5</sub>	350	20	300
F <sub>6</sub>	350	40	300
F <sub>7</sub>	150	40	500
F <sub>8</sub>	550	20	500
F <sub>9</sub>	350	40	700
F <sub>10</sub>	350	30	500
F <sub>11</sub>	550	30	700
F <sub>12</sub>	550	40	500
F <sub>13</sub>	550	30	300
F <sub>14</sub>	350	30	500
F <sub>15</sub>	150	30	700

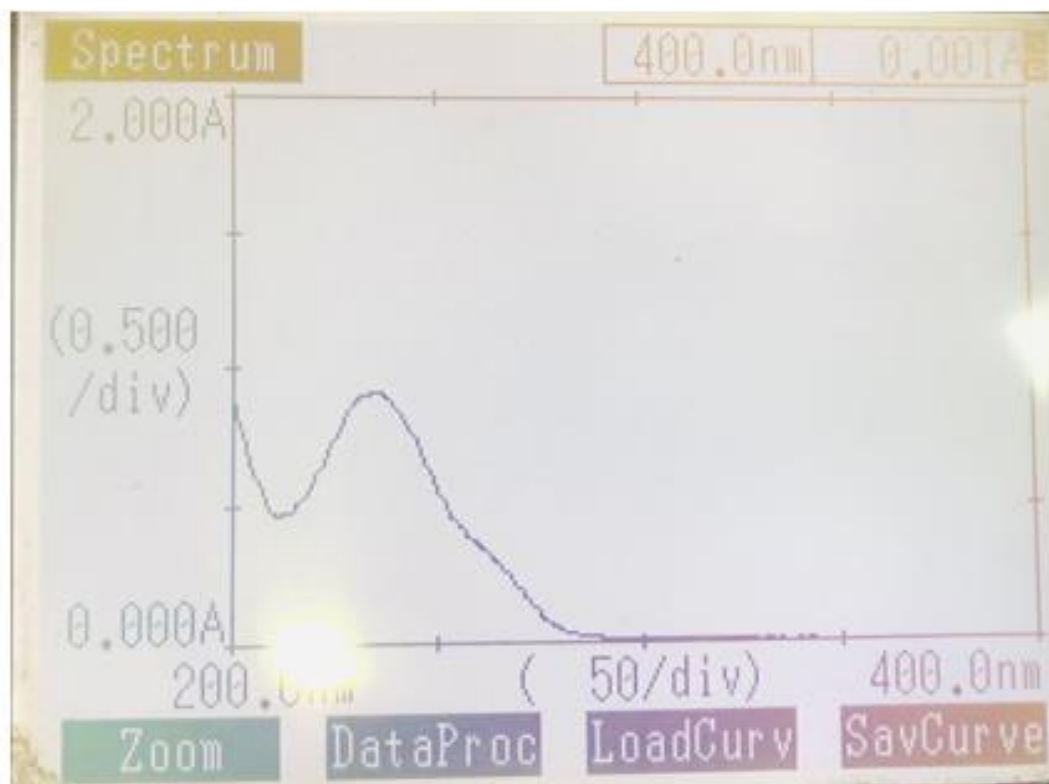
### Particle Size and Zeta Potential Determination

Vesicle properties, particle size diameter and zeta potential were determined at room temperature by Zeta Potential/ Particle Sizer Analyzer. Ethosomes formulation was diluted with water for Zeta potential and particle size determination, respectively. Further, the optimized batch was obtained from results given by Box-behnken design and other evaluation parameters [11].

## Results and Discussion

### Determination of $\lambda_{\max}$ of Betamethasone valerate

Double beam UV-visible spectrophotometer (Shimadzu, UV-1800, Japan) was used to know the  $\lambda_{\max}$  of drug. A 10  $\mu\text{g/ml}$  solution of Betamethasone valerate in acetonitrile was scanned in the range of 200-400 nm. The  $\lambda_{\max}$  of drug was found to be 235 nm (Figure 1).

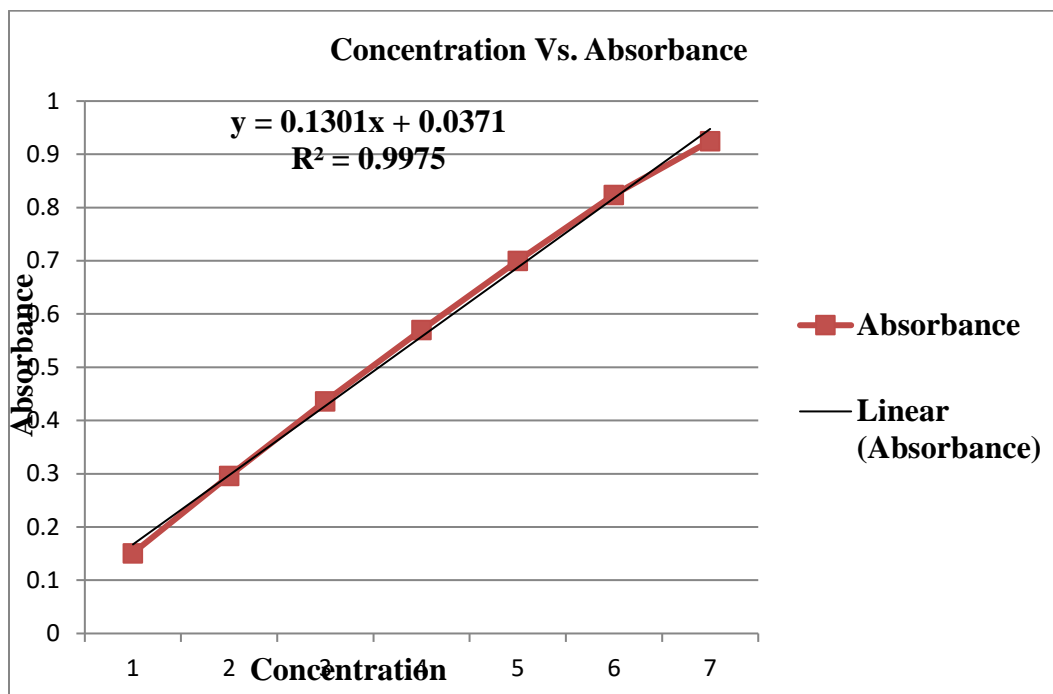


**Figure 1: UV spectrum of Betamethasone valerate in Acetonitrile**

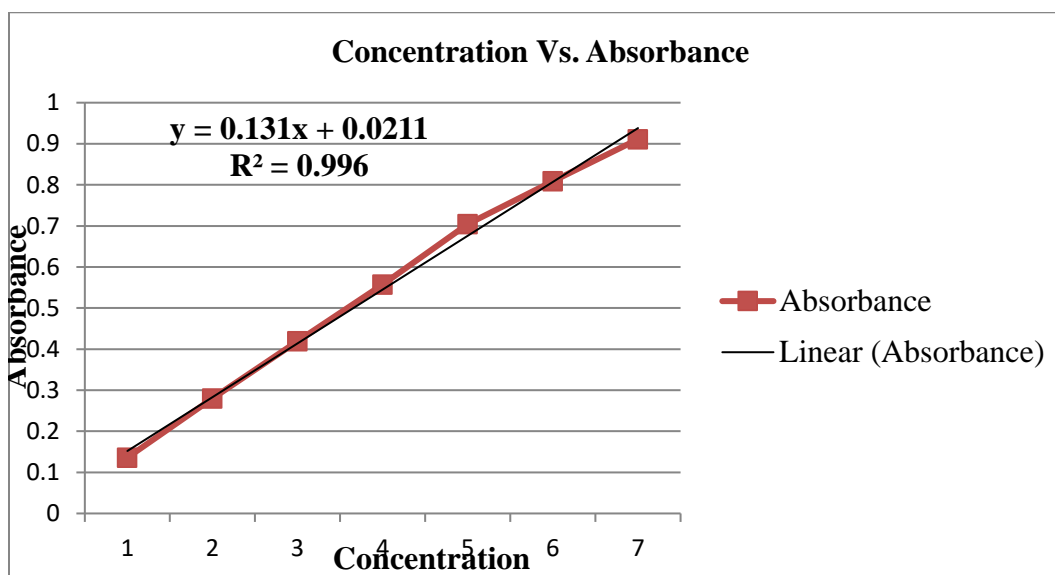
### Preparation of Standard Curve of Betamethasone valerate in acetonitrile

The calibration curve for Betamethasone valerate was obtained by using the 1 to 7  $\mu\text{g/ml}$  concentration of Betamethasone valerate in acetonitrile. The absorbance was measured at 235 nm. The calibration curve of Betamethasone valerate in acetonitrile as shown in graph indicated the regression equation  $Y = 0.130x + 0.037$  and  $R^2$  value 0.997, which shows good linearity as shown in Figure 2. The standard calibration curve of Betamethasone valerate was also prepared in pH 7.4 Phosphate buffer. The calibration curve of Betamethasone valerate in pH 7.4 phosphate buffer as shown in graph indicated the regression equation  $Y = 0.131x + 0.021$  and  $R^2$  value 0.996 which

shows good linearity as shown in Figure 3. Table 2 has shown the statistical parameters and their results of standard calibration curve of Betamethasone valerate in pH 7.4 Phosphate buffer.



**Figure 2: Graph of Standard Calibration Curve of Betamethasone valerate in acetonitrile**



**Figure 3: Graph of Standard Calibration Curve of Betamethasone valerate in pH 7.4 Phosphate Buffer**

**Table 2: Result of Regression Analysis of UV Method for Estimation of Betamethasone valerate**

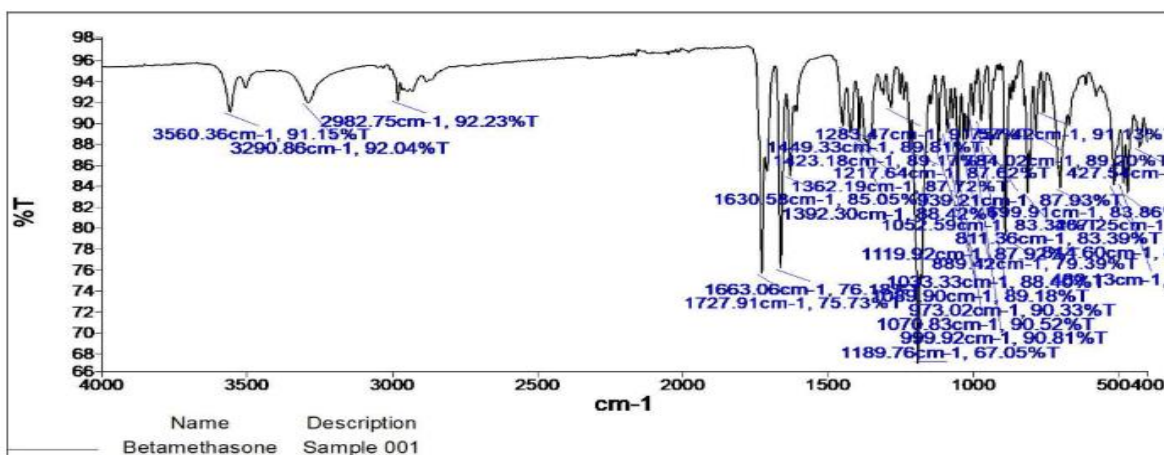
Statistical parameters	Results	
	Acetonitrile	pH 7.4 Phosphate buffer
$\lambda$ max	235 nm	235 nm
Regression equation (Y = mx + C)	Y= 0.130x + 0.037	Y= 0.131x + 0.021
Slope (m)	0.130	0.131
Intercept (C)	0.037	0.021
Correlation coefficient (r <sup>2</sup> )	<b>0.997</b>	<b>0.996</b>

### Compatibility Studies

Compatibility studies were done using FTIR and DSC.

### FTIR Technique

The FTIR spectra of Betamethasone valerate pure drug sample alone and with excipients were constructed and results reflected that Betamethasone valerate was found stable with the excipients selected for formulation of Betamethasone valerate Ethosomes . The FTIR spectra of pure drug (Betamethasone valerate) and different excipients were shown in Figure 4 to 6.

**Figure 4: FT-IR Graph of Betamethasone valerate Drug**

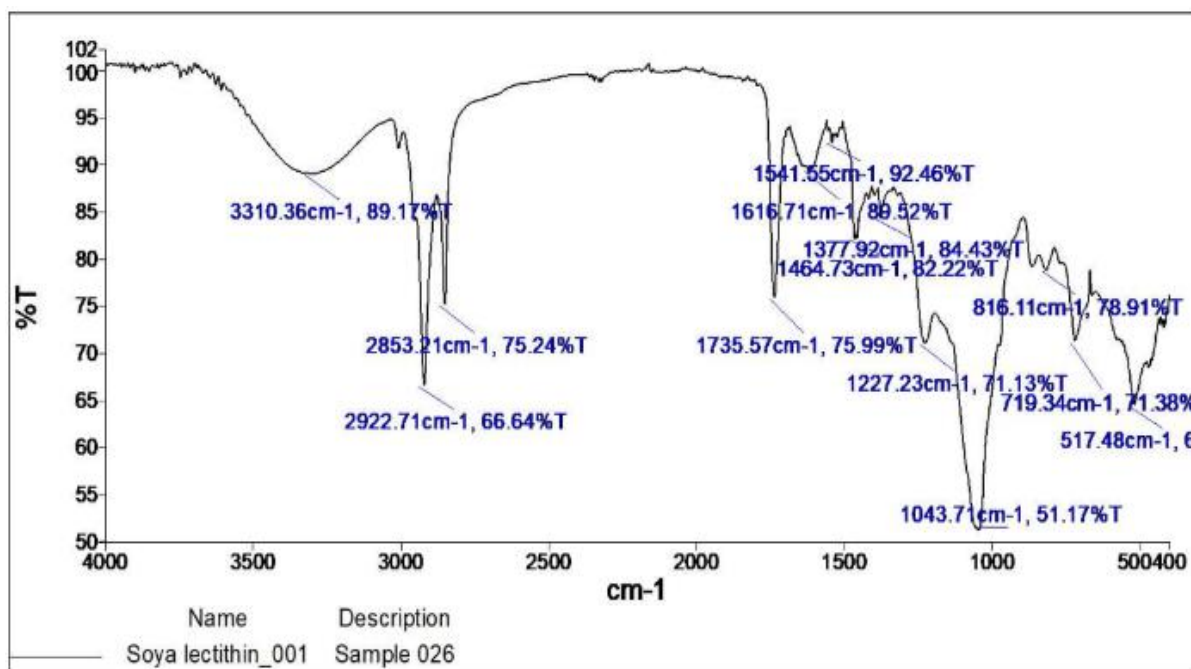


Figure 5: FT-IR Graph of Soya Lecithin

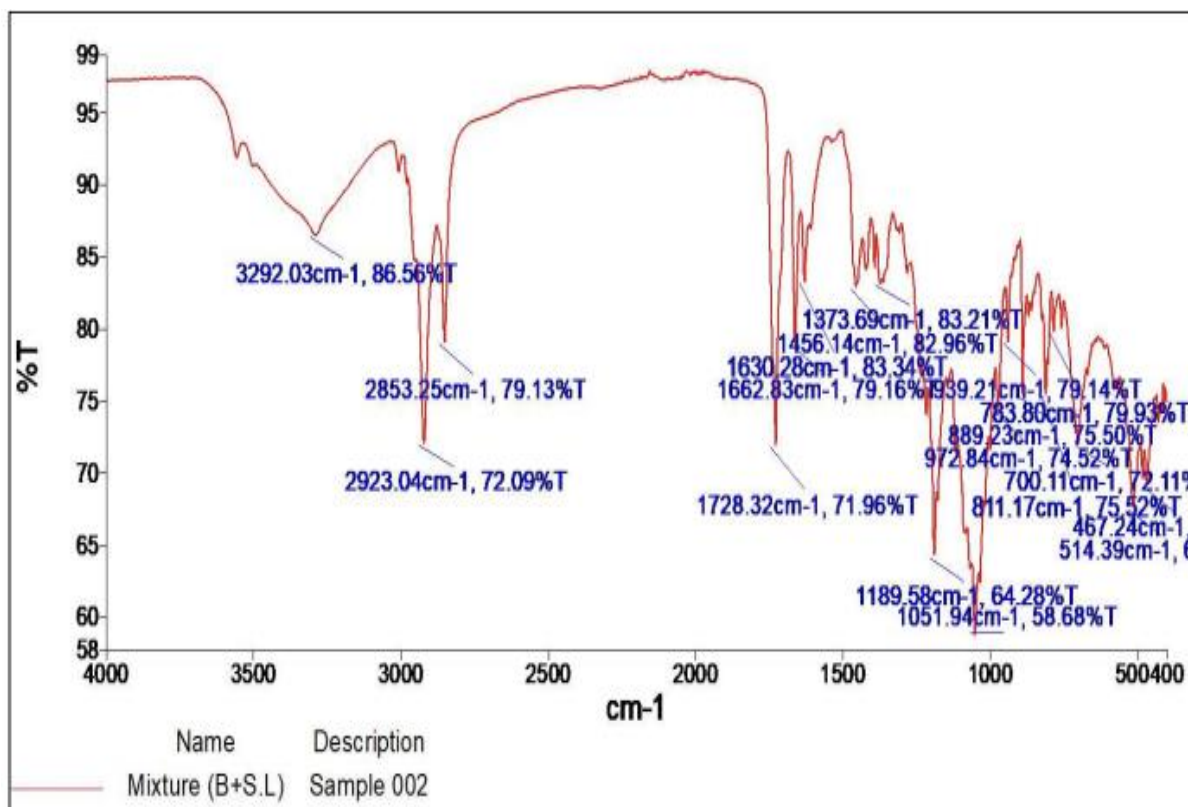


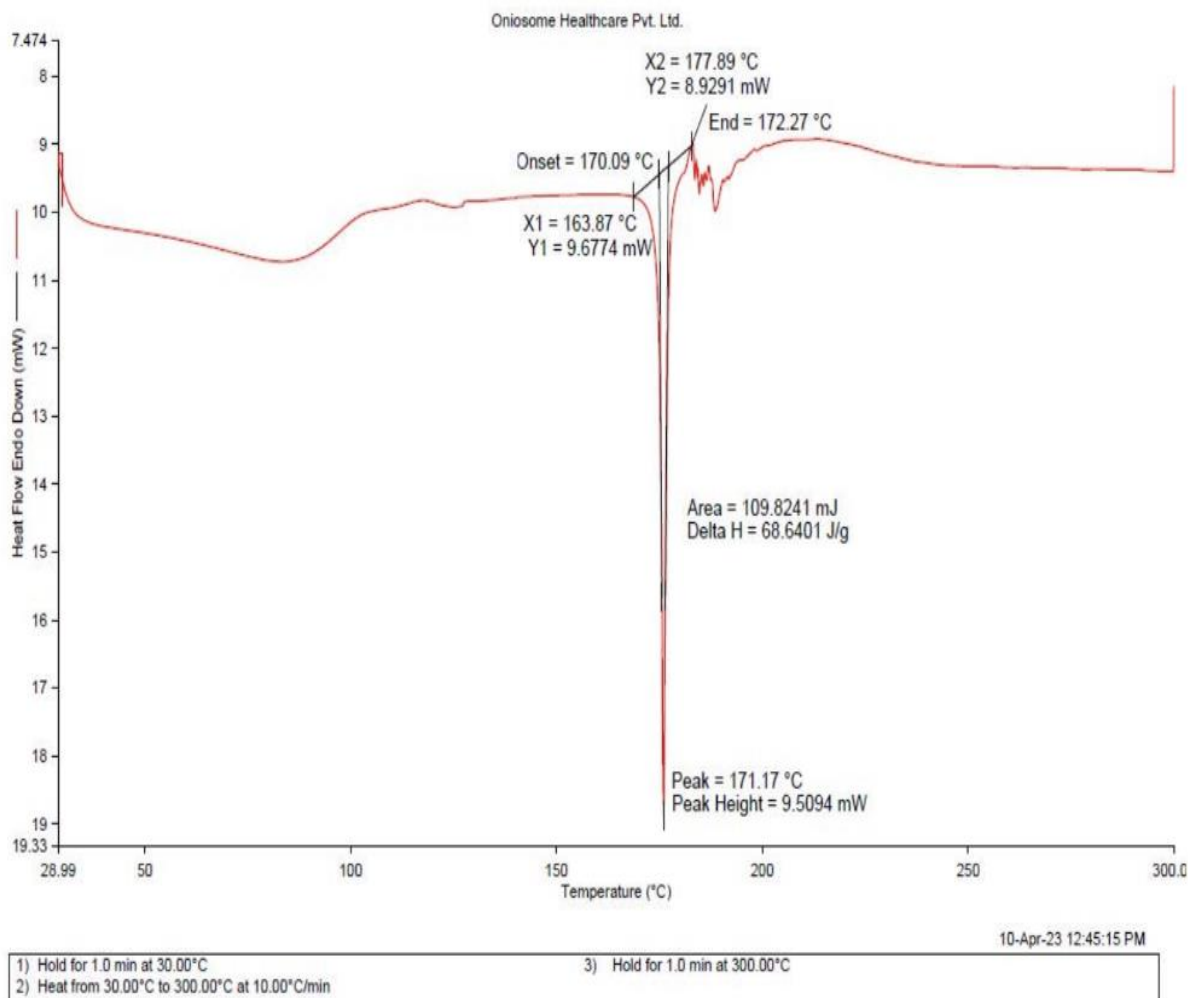
Figure 6: FT-IR Graph of Physical Mixture



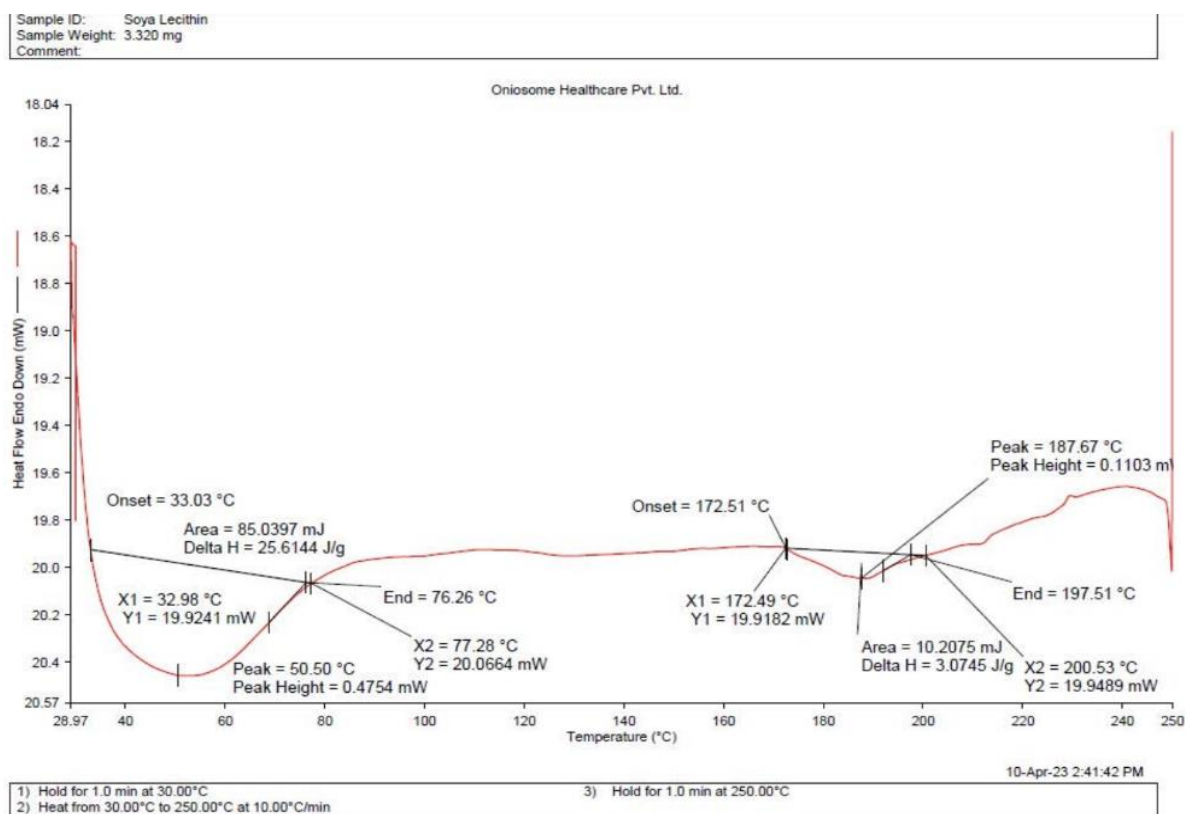
Any possible chemical interaction between the drug and polymer in the solid state was explored using IR spectroscopy (Figure 4 to 6). According to the FT-IR results, the characteristic bonds were observed for the pure Betamethasone valerate powder and Soya Lecithin. Any shift or appearance change in the characteristic bonds was not identified in the physical mixture.

### DSC Analysis

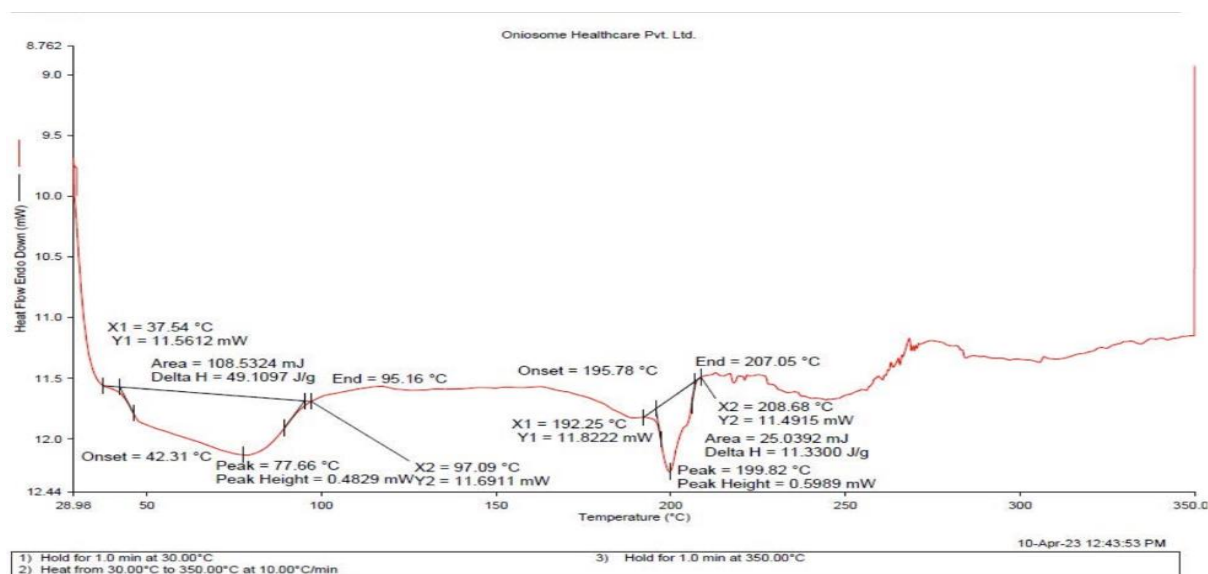
A differential scanning calorimeter was employed to observe the melting and the recrystallization behavior of the drug with the excipients. The samples for the DSC analysis include drug, excipients and physical mixture. The results of DSC study are represented in Figure 7 to 9.



**Figure 7: DSC Thermogram of the Betamethasone valerate Drug**



**Figure 8: DSC Thermogram of the Soya Lecithin**



**Figure 9: DSC Thermogram of the Physical Mixture**

The results of DSC study were in good agreement with the results of FTIR analysis.

## Evaluation of Betamethasone valerate Ethosomes

### Particle Size Analysis

The particle size of Betamethasone valerate Ethosomes formulation batches (F<sub>1</sub>-F<sub>15</sub>) was ranged between 124.2– 555.2 nm. Results are shown in Figure 10.

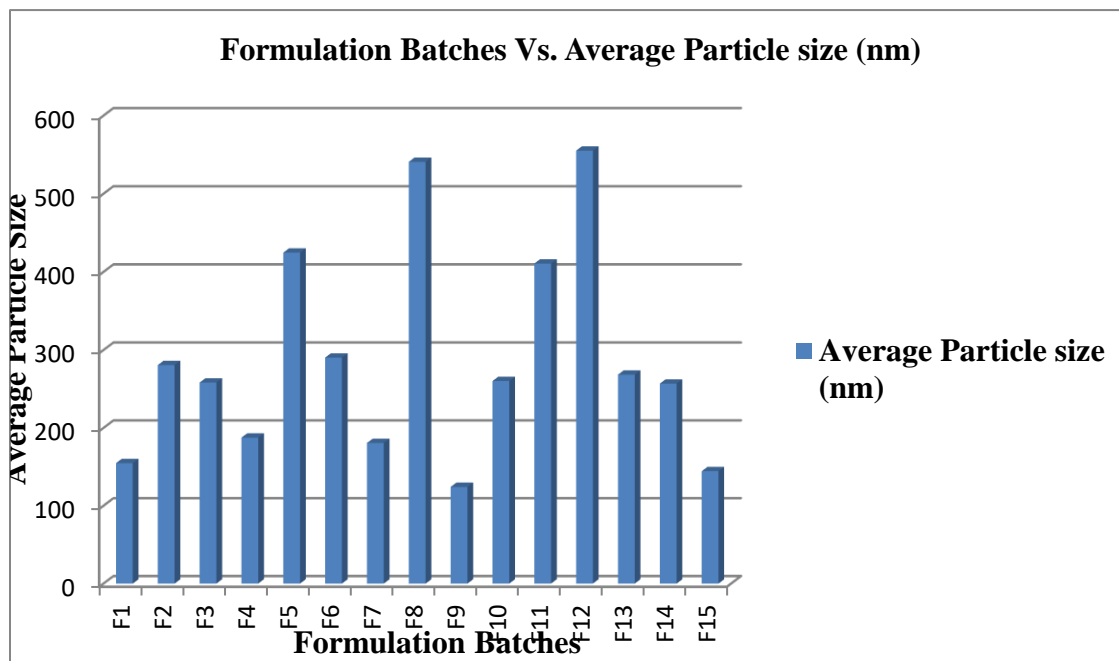


Figure 10: Particle Size of Betamethasone valerate Ethosomes Batches F<sub>1</sub>-F<sub>15</sub>

### ANOVA for Quadratic model

#### Response 1: particle size

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.030E+05	9	22551.46	2.01	0.2283	not significant
A-Soyalecithin	1.535E+05	1	1.535E+05	13.69	0.0140	
B-Ethanol	7862.58	1	7862.58	0.7016	0.4404	
C-Stirring Speed	5554.58	1	5554.58	0.4957	0.5128	
AB	33.64	1	33.64	0.0030	0.9584	

AC	8593.29	1	8593.29	0.7668	0.4213	
BC	123.21	1	123.21	0.0110	0.9206	
A <sup>2</sup>	4802.97	1	4802.97	0.4286	0.5416	
B <sup>2</sup>	14802.46	1	14802.46	1.32	0.3024	
C <sup>2</sup>	6446.20	1	6446.20	0.5752	0.4824	
<b>Residual</b>	56030.87	5	11206.17			
Lack of Fit	56025.38	3	18675.13	6807.46	0.0001	significant
Pure Error	5.49	2	2.74			
<b>Cor Total</b>	2.590E+05	14				

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 2.01 implies the model is not significant relative to the noise. There is a 22.83% 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 is a significant model term. 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 6807.46 implies the Lack of Fit is significant. There is only a 0.01% chance that a Lack of Fit F-value this large could occur due to noise. Significant lack of fit is bad -- we want the model to fit.

#### Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	258.47	1	61.12	101.36	415.58	
A-Soyalecithin	138.50	1	37.43	42.29	234.71	1.0000
B-Ethanol	-31.35	1	37.43	-127.56	64.86	1.0000
C-Stirring Speed	-26.35	1	37.43	-122.56	69.86	1.0000

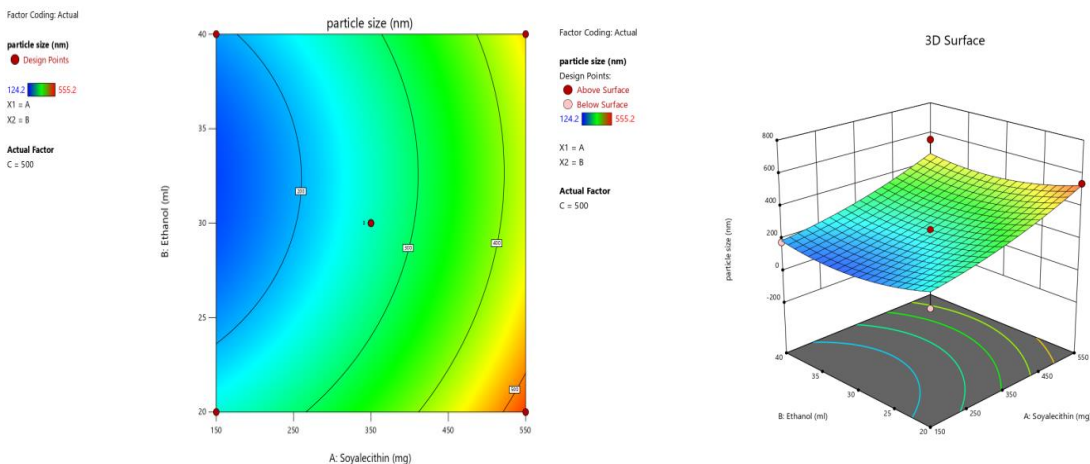
AB	-2.90	1	52.93	-138.96	133.16	1.0000
AC	46.35	1	52.93	-89.71	182.41	1.0000
BC	-5.55	1	52.93	-141.61	130.51	1.0000
A <sup>2</sup>	36.07	1	55.09	-105.55	177.68	1.01
B <sup>2</sup>	63.32	1	55.09	-78.30	204.93	1.01
C <sup>2</sup>	-41.78	1	55.09	-183.40	99.83	1.01

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multicollinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

The particle size of the Betamethasone valerate Ethosomes in the current study varied between 124.2 to 555.2 nm, which may be regarded as a suitable midrange that contributed to reasonable homogeneity and a satisfactory size distribution. A quadratic model was produced by the polynomial analysis of the particle size values from the Betamethasone valerate Ethosomes. The study's design provided evidence for the model's effectiveness in assessing the impact of the Soya Lecithin (A), Ethanol (B), and Stirring Speed (C) on the particle size of the prepared Ethosomes. The one-way ANOVA produced the following equation.

$$\text{Particle Size} = 285.47 + 138.50A - 31.35B - 26.35C - 2.90 AB + 46.35 AC - 5.55 BC + 36.07A^2 + 63.32B^2 - 41.78C^2$$

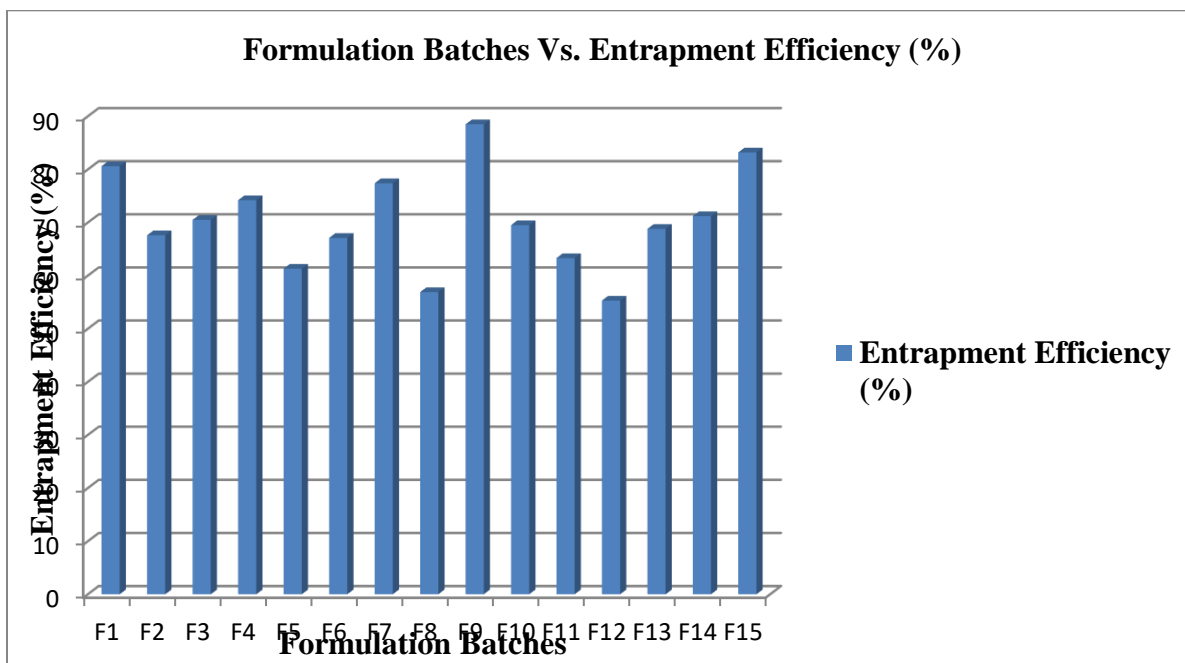
As seen, component A (Soya Lecithin) has the profound effect on the size of Ethosomes with a p-value is 0.0140, while factor B significantly increased the response's antagonistic effect with the same degree of significance. Such results might be useful for creating smaller particles. The capacity to reduce the particle size and promote stability was also correlated with the amount of polymer in the Ethosomes preparation. Figure 11 contour and 3D graphs showing the effect of selected independent factors on response Y1 (particle size).



**Figure 11: Contour graph (left) and 3D graph (right) showing the effect of independent factors (soya lecithin, ethanol, stirring speed) on Y1 (Particle size)**

### Entrapment Efficiency

The Entrapment efficiency is a crucial indicator of Ethosomes size and general stability. As per the results, the Entrapment efficiency (Y2) of the Betamethasone valerate Ethosomes was ranged from 55.3 to 88.5 percent as depicted in Figure 12.



**Figure 12: Entrapment efficiency of Betamethasone valerate Ethosome Batches F<sub>1</sub>-F<sub>15</sub>**

**ANOVA for Quadratic model****Response 2: Entrapment Efficiency**

<b>Source</b>	<b>Sum of Squares</b>	<b>df</b>	<b>Mean Square</b>	<b>F-value</b>	<b>p-value</b>	
<b>Model</b>	973.91	9	108.21	2.15	0.2061	not significant
A-Soyalecithin	631.90	1	631.90	12.58	0.0165	
B-Ethanol	59.95	1	59.95	1.19	0.3245	
C-Stirring Speed	121.68	1	121.68	2.42	0.1804	
AB	0.6400	1	0.6400	0.0127	0.9145	
AC	52.56	1	52.56	1.05	0.3533	
BC	57.00	1	57.00	1.13	0.3355	
A <sup>2</sup>	2.36	1	2.36	0.0470	0.8369	
B <sup>2</sup>	15.52	1	15.52	0.3088	0.6024	
C <sup>2</sup>	28.43	1	28.43	0.5659	0.4858	
<b>Residual</b>	251.23	5	50.25			
Lack of Fit	249.77	3	83.26	114.05	0.0087	significant
Pure Error	1.46	2	0.7300			
<b>Cor Total</b>	1225.14	14				

Factor coding is **Coded**. Sum of squares is **Type III - Partial**

The **Model F-value** of 2.15 implies the model is not significant relative to the noise. There is a 20.61% 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 is a significant model term. 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 114.05 implies the Lack of Fit is significant. There is only a 0.87% chance that a Lack of Fit F-value this large could occur due to noise. Significant lack of fit is bad -- we want the model to fit.

### Coefficients in Terms of Coded Factors

#### Response 2: Entrapment Efficiency

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	70.40	1	4.09	59.88	80.92	
A-Soyalecithin	-8.89	1	2.51	-15.33	-2.45	1.0000
B-Ethanol	2.74	1	2.51	-3.70	9.18	1.0000
C-Stirring Speed	3.90	1	2.51	-2.54	10.34	1.0000
AB	0.4000	1	3.54	-8.71	9.51	1.0000
AC	-3.62	1	3.54	-12.74	5.49	1.0000
BC	3.78	1	3.54	-5.34	12.89	1.0000
A <sup>2</sup>	-0.8000	1	3.69	-10.28	8.68	1.01
B <sup>2</sup>	-2.05	1	3.69	-11.53	7.43	1.01
C <sup>2</sup>	2.78	1	3.69	-6.71	12.26	1.01

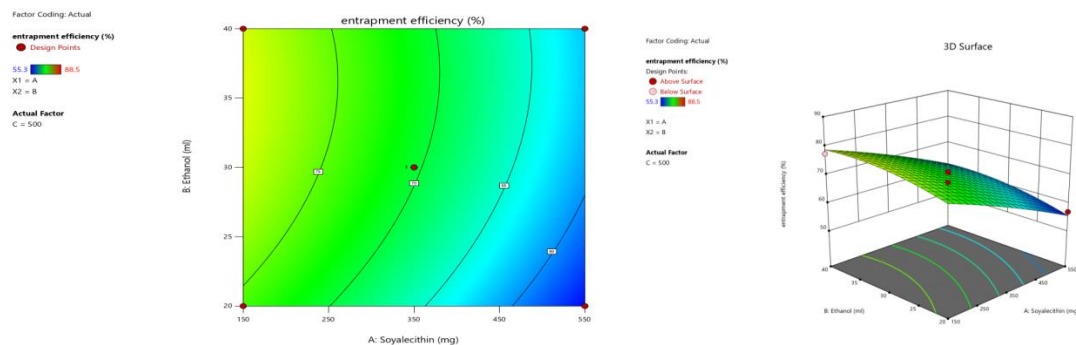
The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multicollinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

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

$$\text{Entrapment Efficiency} = 70.40 - 8.89A + 2.74B + 3.90C + 0.4000 AB - 3.62AC + 3.78 BC - 0.8000A^2 - 2.05B^2 + 2.78C^2$$



The contour and 3D plots in Figures 13 showed how the independent factors affected the response Y2 in each case (Entrapment Efficiency).



**Figure 13: Contour graph (left) and 3D graph (right) showing the effect of independent factors (soya lecithin, ethanol, stirring speed) on Y2 (Entrapment Efficiency)**

All the prepared Betamethasone valerate ethosome batches were also evaluated for Zeta potential and in-vitro release study.

### Zeta Potential Analysis

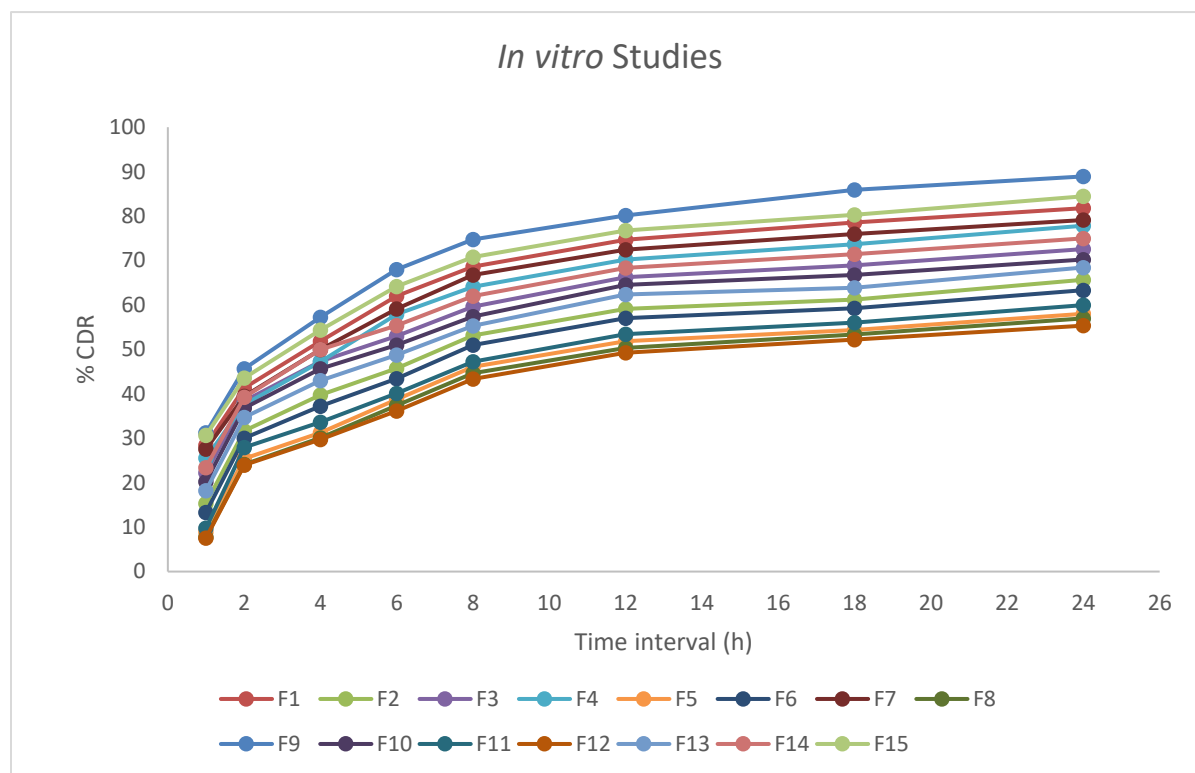
The Malvern zeta sizer conducted the analysis for each of the fifteen batches. The outcomes are displayed in table 3.

**Table 3: Zeta Potential Analysis of Betamethasone valerate Ethosomes Batches (F<sub>1</sub> to F<sub>15</sub>)**

S. No.	Formulation Code	Zeta potential Mean (mV)
1	F <sub>1</sub>	-15.3
2	F <sub>2</sub>	-12.0
3	F <sub>3</sub>	-12.3
4	F <sub>4</sub>	-16.1
5	F <sub>5</sub>	-11.6
6	F <sub>6</sub>	-12.1
7	F <sub>7</sub>	-14.5
8	F <sub>8</sub>	-08.5
9	F <sub>9</sub>	-17.0
10	F <sub>10</sub>	-12.3
11	F <sub>11</sub>	-10.6
12	F <sub>12</sub>	-07.4
13	F <sub>13</sub>	-12.4
14	F <sub>14</sub>	-12.2
15	F <sub>15</sub>	-13.7

### In-vitro Release Studies

The prepared Betamethasone valerate Ethosomes were studied for in-vitro drug release study in pH 7.4 phosphate buffer (figure 14). The percentage (%) drug release was ranged between 55.34-88.92 %. Maximum percentage release was found to be with F9 batch.



**Figure 14: In-vitro Drug Release from Betamethasone valerate Ethosomes**

### Optimization Batch of Betamethasone valerate

A Betamethasone valerate Ethosomes 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. Out of fifteen batches, batch F9 is the optimized batch.

### 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.

Factor Coding: Actual

## All Responses

● Design Points

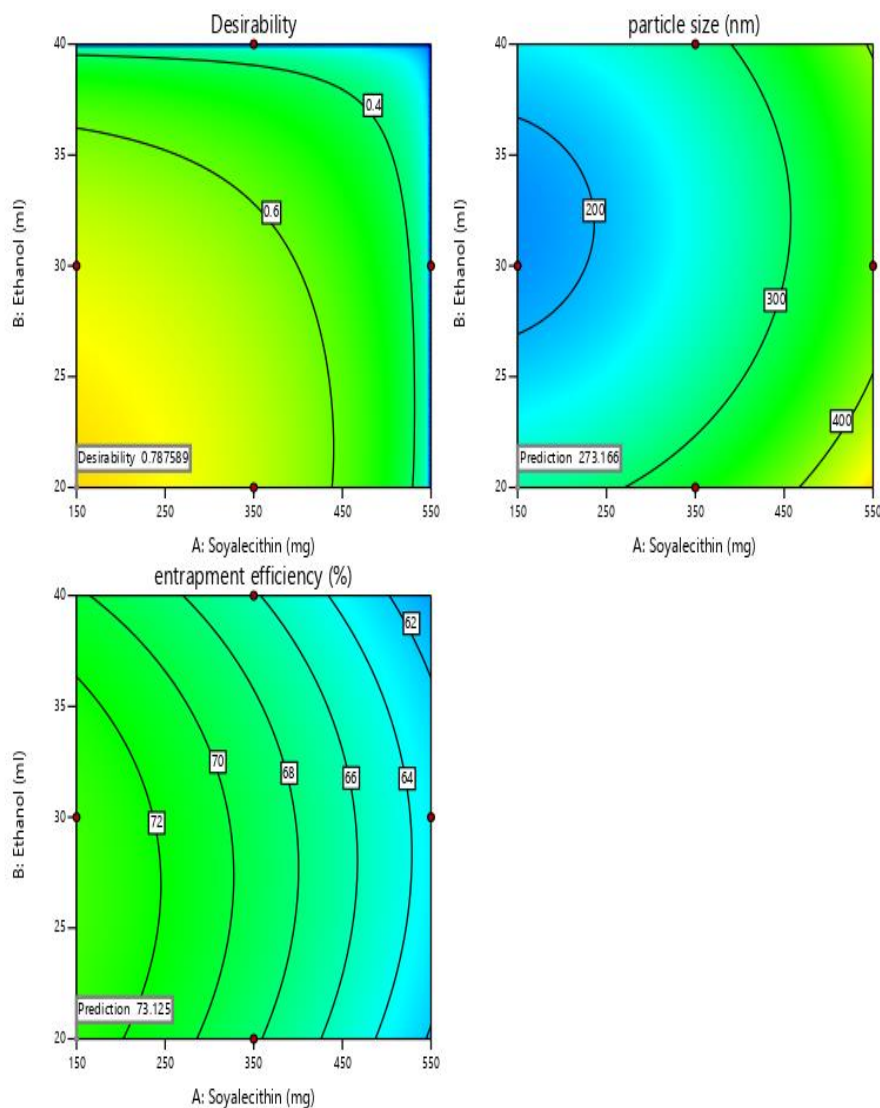
0 1

X1 = A

X2 = B

## Actual Factor

C = 300.001



**Figure 15: 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 Betamethasone valerate Ethosomes

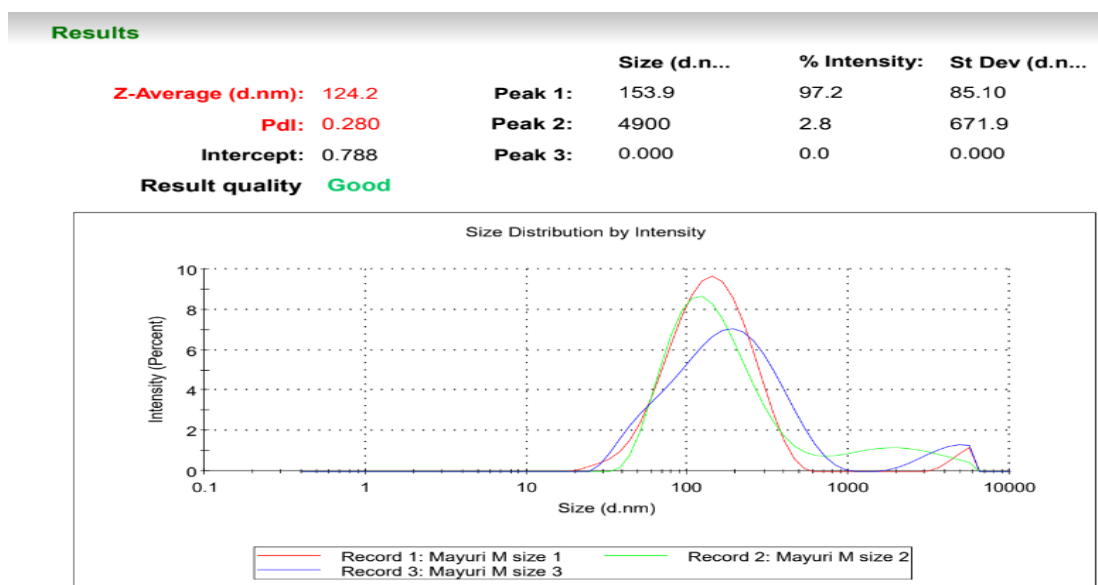
The optimized **Betamethasone valerate Ethosomes** formulation thus prepared was evaluated for particle size, pH, zeta potential, FTIR, TEM and in-vitro release study (Table 4).

**Table 4: Evaluation Parameters for Optimized Betamethasone valerate Ethosomes Formulation**

S. No.	Parameter	Inference
1	Particle size (nm)	124.2 nm
2	pH	5.6 ± 0.03
3	Zeta Potential	-17.0 mV
4	In-vitro permeation study	88.92±0.054% in pH 7.4 Phosphate buffer
5	PDI	0.280

### Particle size Analysis

The Particle size peak of Betamethasone valerate optimized Ethosomes formulation was observed at 124.2 nm (Figure 16).

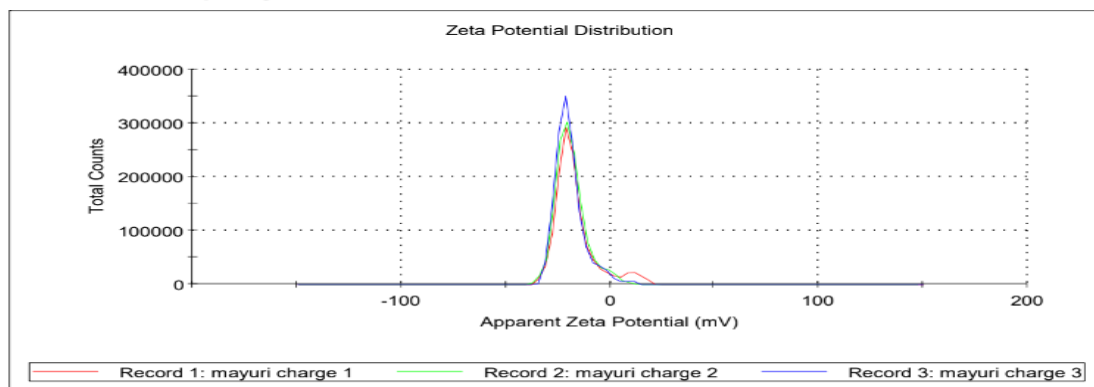
**Figure 16: Particle Size Peak of Optimized Ethosomes Formulation**

### Zeta Potential

Zeta potential of Betamethasone valerate optimized Ethosomes formulation was found to be -17.0 mV. The zeta potential value of optimized formulation was presented in figure 17.

**Results**

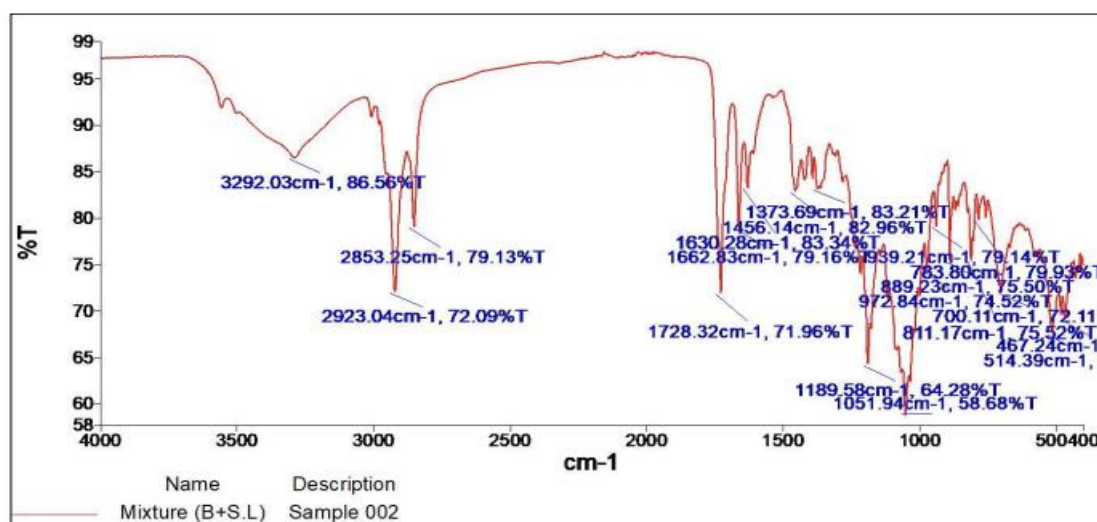
	Mean (mV)	Area (%)	St Dev (mV)
<b>Zeta Potential (mV): -17.0</b>	<b>Peak 1: -18.6</b>	93.6	7.20
<b>Zeta Deviation (mV): 9.93</b>	<b>Peak 2: 11.2</b>	6.4	4.08
<b>Conductivity (mS/cm): 0.268</b>	<b>Peak 3: 0.00</b>	0.0	0.00
<b>Result quality Good</b>			



**Figure 17: Zeta Potential of Optimized Ethosomes Formulation**

**FTIR Analysis**

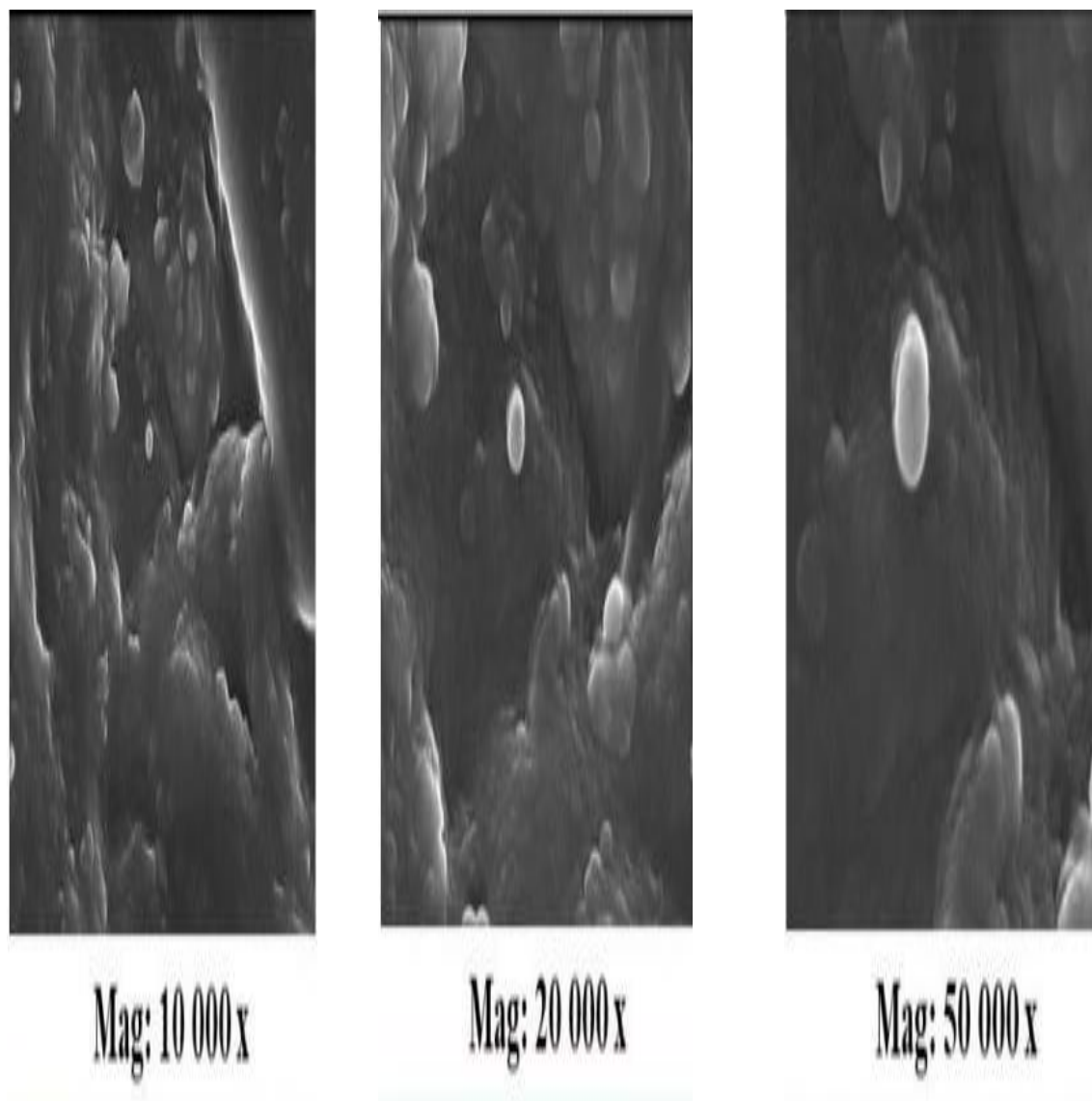
The FTIR spectra of Betamethasone valerate pure drug sample alone and with excipients were constructed and results reflected that Betamethasone valerate was found stable with the excipients selected for formulation of Betamethasone valerate Ethosomes. Any shift or appearance change in the characteristic bonds was not identified in the physical mixture. Figure 18 is presenting the FTIR spectra of optimized Ethosomes formulation.



**Figure 18: FT-IR Graph of Optimized Ethosomes Formulation**

### TEM Analysis

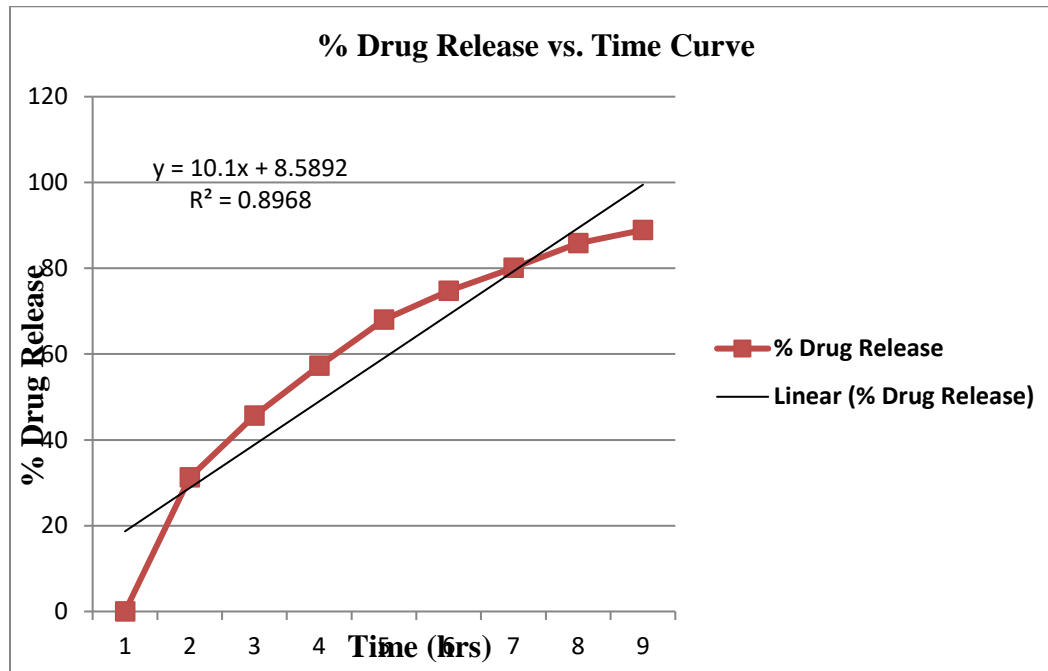
The TEM graph of optimized formulation was depicted in figure 19.



**Figure 19: TEM Graph of Optimized Ethosomes Formulation**

### In-vitro Release Study of Optimized Betamethasone valerate Ethosomes Formulation

In-vitro permeation study of Betamethasone valerate Ethosomes formulation was depicted in figure 20.



**Figure 20: In-vitro Release Study of Optimized Betamethasone valerate Ethosomes Formulation**

### Conclusion

In the present work, the Ethosomes were prepared by utilizing soya lecithin, ethanol and other useful materials. This study reported on the use of a Box–Behnken design in the optimization of Ethosomes dispersion mean diameter for the encapsulation of Ethosomes. The derived polynomial equations proved to be satisfactory in predicting Y1 and Y2 values for the preparation of optimum Ethosomes with desired particle size and entrapment efficiency. The particle size of the optimized batch is 124.2 nm. The zeta potential is -17.0 and PDI is 0.280. The in-vitro release study of the optimized batch is  $88.92 \pm 0.054\%$  in pH 7.4 Phosphate buffer. The entrapment efficiency of the optimized batch is 88.5%.

### Future Perspectives

Prolonged releases will be achieved when they will be formulated as topical gels on maintaining the ethosomal structure. This product can be manufactured in large scale and commercialized for the treatment of skin infections like psoriasis, as it provides controlled delivery of the drug in human via the non-invasive skin route with more sustaining, less frequent dosing and with more bioavailability when compared to oral delivery.

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