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Screening of factors using Plackett Burman Design in the Formulation and Development of Ivermectin Loaded Ethosomes

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Abstract
Scabies remains a significant public health issue, particularly in resource-limited settings. Effective management relies on early diagnosis, appropriate treatment, and preventive measures to control and eliminate infestations. Ongoing research into new therapeutic agents and vaccines holds promise for future advancements in the control of this pervasive parasitic disease. There are many formulae available in market but not no one have significance effect, where Dose 0.1%Strength 30gm, Odor found Odor less, pH found 06.13±0.20,Viscosity found 9986±51.83, &% Drug content found 93.67.038.
Key Word-Scabies, Formulation, Nanosponges, Ethosomes, Ivermectin, Development of method,Plackett-Burman,ANOVA Method

1 Introduction

Scabies is caused by the Sarcoptesscarbiei mite. It causes a rash so itchy that it interrupts sleep. Scabies is a skin condition caused by the Sarcoptesscabiei var hominis mite. These little bugs make tunnels (burrow) under your skin and cause small red bumps and severe itching. Scabies spreads easily from person to person,especially among people who live close together.

Quality by Design is the modern approach for quality of pharmaceuticals. It describes use of Quality by Design to ensure quality of Pharmaceuticals. In this review, the Quality by Design is described and some of its elements identified. Process parameters and quality attributes are identified for each unit operation. Benefits, opportunities and steps involved in Quality by Design of Pharmaceutical products are described.

“Ethosomes are ethanolic liposomes”. Ethosomes can be defined as noninvasive delivery carriers that enable drugs to reach deep into the skin layers and/or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents. The vesicles have been well known for their importance in cellular communication and particle transportation for many years.

Nanosponge is a novel approach which offers controlled drug delivery for topical use. Nanosponge is an emerging technology for topical drug delivery. Nanosponge drug delivery system is utilized for development of performance of topically applied drugs.

Nanosponges are small sponges with a size of about a virus, which can be loaded with a wide variety of drugs. These tiny sponges can circulate around body until they encounter specific target site and stick on surface and begin to release drug in a controlled and predictable manner.

Ivermectin-Ivermectin is an anti-infective agent with activity against several parasitic nematodes and scabies and is the treatment of choice for onchocerciasis (river blindness). It is typically given as one or two oral doses. Ivermectin therapy has been associated with minor, self-limiting serum aminotransferase elevations and very rare instances of clinically apparent liver injury.

2.Method and formulation-

2.1 Identification and Determination of Wavelength max (λmax) of Ivermectin

Stock solution (1000µg/mL) of Ivermectin in methanol was prepared. This solution was diluted to obtain 100 µg/mL solution. 5 mL was withdrawn and adjusted to 50 µg/mL concentration and scanned between 200 - 400 nm. The U.V spectrum of Ivermectin is shown in Fig. The maximum absorbance was observed at 245 nm which indicates that the λmax of Ivermectin is similar to λmax as per I.P i.e., 245 nm.

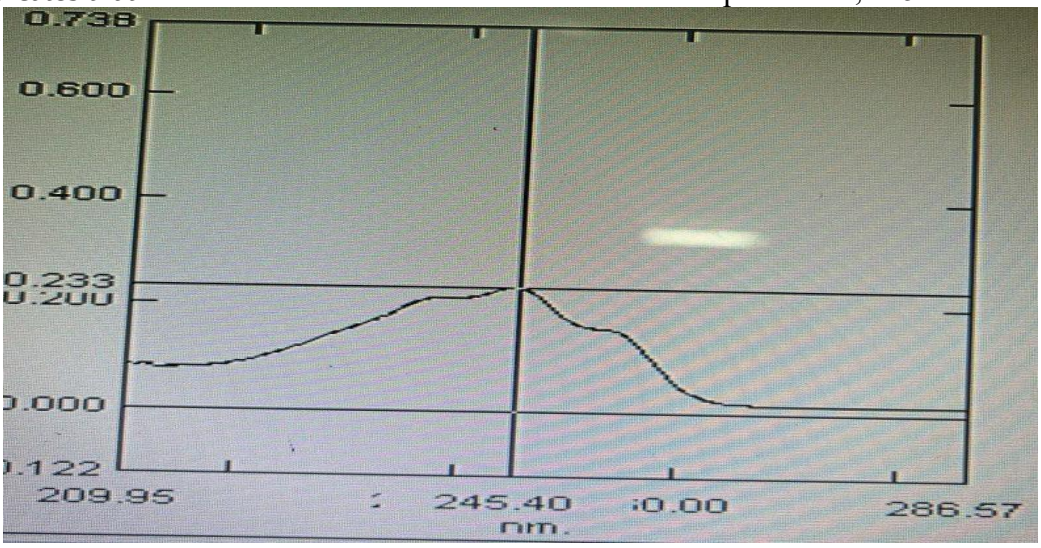


Figure-1Wavelength max (λmax) of Ivermectin

Table -1 Wavelength max (λmax) of Ivermectin

Drug	**Actualλ max**	**Observedλ max**
Ivermectin	245nm	245nm
ComplieswithStandardData ofIP2010		

2.2 Preparation of Calibration Curve of Ivermectin

Table-2 Calibration Curve of Ivermectin

Sr No	**Concentration**	**Absorbance**
1	0	0
2	2	0.19
3	4	0.403
4	6	0.61

5	8	0.8
6	10	0.95

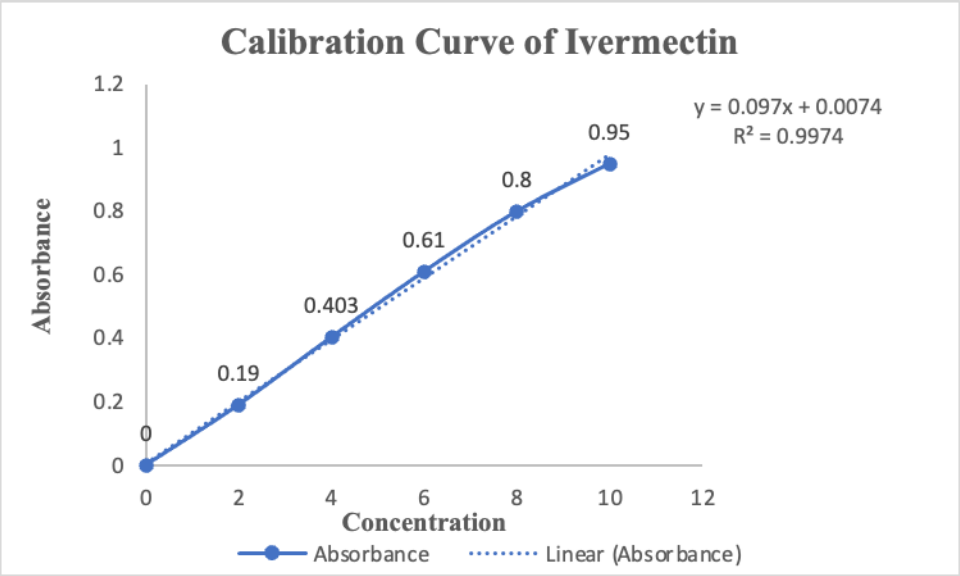


Figure- 2 Calibration CurveofIvermectin

2.3 Particle Size Study- Drug- Ivermectin

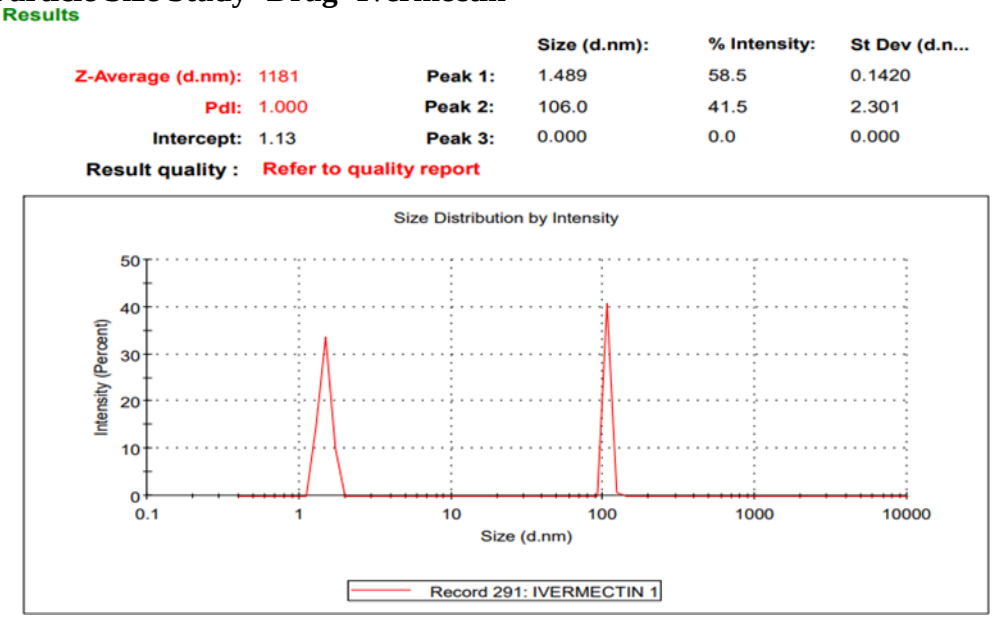


Figure-3 Particle size study of Ivermectin

2.4 RiskIdentification

2.4.1 Screening of significant risk factors using placket-burman design

Plackett-Burman design is an efficient screening method to identify the critical factors using as few experimental runs as possible. The design is used for screening of independent variables: significant (critical) or non-significant (non-critical). It is two-level design i.e. Low (-1) level, High (+1) level.PB design gives 12 runs that may be used for an experiment containing up to 11 factors.

Table 3- 2 -level Plackett-Burman design

Factor Code	**FactorName**	**Level**	
		Low(-1)	**High(+1)**
IndependentFactors			
X1	PhospholipidConc.(%)	2	4
X2	EthanolConc.(%)	20	40
X3	CholesterolConc.(%)	0.1	0.3
X4	OrganicphaseComposition	Ethanol+PG	Ethanol+IPA
X5	StirringSpeed(rpm)	500	700
DependentFactors			
Y1	%EntrapmentEfficiency		
Y2	%CDR		

Table 4- Compositions of Batches in Coded Form

Run	Phospholipid conc.(%)	Ethanol conc.(%)	Cholesterol conc.(%)	Composition	Stirring speed
	X1	X2	X3	X4	X5
1	1	1	-1	Ethanol+IPA	1
2	1	-1	1	Ethanol+IPA	1
3	-1	-1	-1	Ethanol+IPA	-1
4	-1	-1	1	Ethanol+PG	1
5	-1	1	1	Ethanol+PG	1
6	1	1	-1	Ethanol+PG	-1
7	-1	1	1	Ethanol+IPA	-1
8	1	-1	1	Ethanol+IPA	-1
9	1	1	1	Ethanol+PG	-1
10	-1	1	-1	Ethanol+IPA	1
11	-1	-1	-1	Ethanol+PG	-1
12	1	-1	-1	Ethanol+PG	1

Table 5 -Compositions of Batches in Decoded Form

Run	Phospholipid conc.(%)	Ethanolconc. (%)	Cholesterol conc.(%)	Composition	Stirring speed
1	4	40	0.1	Ethanol+IPA	700
2	4	20	0.3	Ethanol+IPA	700

3	2	20	0.1	Ethanol+IPA	500
4	2	20	0.3	Ethanol+PG	700
5	2	40	0.3	Ethanol+PG	700
6	4	40	0.1	Ethanol+PG	500
7	2	40	0.3	Ethanol+IPA	500
8	4	20	0.3	Ethanol+IPA	500
9	4	40	0.3	Ethanol+PG	500
10	2	40	0.1	Ethanol+IPA	700
11	2	20	0.1	Ethanol+PG	500
12	4	20	0.1	Ethanol+PG	700

Table-6Plackett-Burman screening design output matrix with results

RUN	X1: PL (%)	X2: ETH (%)	X3: CHL (%)	X4: Org.Phase	X5:SS (RPM)	Y1: %E.E	Y2: %CDR
1	2	20	0.1	ETHANOL+IPA	500	65.4	78.49
2	2	20	0.1	ETHANOL+PG	500	64.46	77.14
3	2	40	0.3	ETHANOL+IPA	500	71.38	84.86
4	4	20	0.3	ETHANOL+IPA	700	75.86	86.17
5	4	40	0.1	ETHANOL+PG	500	78.27	88.36
6	2	40	0.1	ETHANOL+IPA	700	71.16	83.77
7	2	40	0.3	ETHANOL+PG	700	72.76	83.64
8	4	40	0.1	ETHANOL+IPA	700	79.27	88.23
9	4	40	0.3	ETHANOL+PG	500	80.23	90.35
10	4	20	0.3	ETHANOL+IPA	500	72.49	84.5
11	4	20	0.1	ETHANOL+PG	700	74.48	85.29
12	2	20	0.3	ETHANOL+PG	700	67.16	80.34

2.4.2 Effect analysis of variables on Entrapment Efficiency (Y1)

Table-7 Effect analysis of variables on Entrapment Efficiency (Y1)

ANOVAforselected factorialmodel
Analysisofvariance table[Partialsumofsquares-Type III]

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	296.34	5	59.27	95.61	< 0.0001	significant
A-PhospholipidConc.	194.25	1	194.25	313.35	< 0.0001	
B-EthanolConc.	91.96	1	91.96	148.35	< 0.0001	
C-CholesterolConc.	3.90	1	3.90	6.29	0.0460	
D-Organicphase Composition	0.27	1	0.27	0.44	0.5338	
E-StirringSpeed	5.96	1	5.96	9.62	0.0211	
Residual	3.72	6	0.62			
CorTotal	300.06	11				

ParetoChart

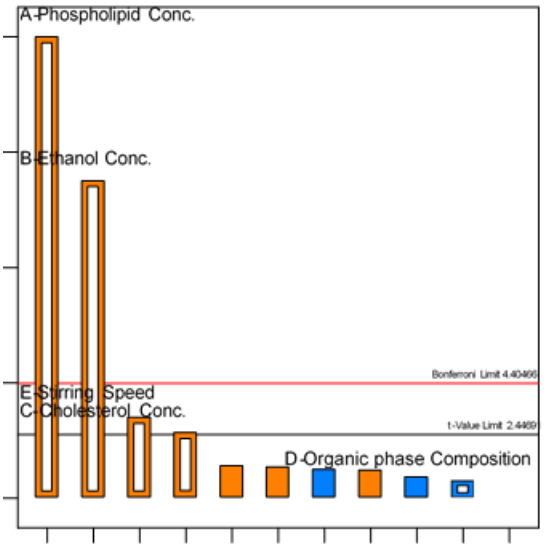


Figure -4 Pareto chart for entrapment efficiency

Results of the % Entrapment Efficiency for all batches of Plackett-Burman screening design are given in Table 6. The analysis of variance (ANOVA) revealed a statistical difference between the batches. A regression coefficient is said to be significant if p-value is less than 0.05. TheR2 value was 0.9843 indicating a good fit. From the result, it is evident that phospholipid conc. (p=<0.0001) and ethanol conc. (p=<0.0001) significantly affect the entrapment efficiency which is again confirmed by Pareto chart (Fig. 5.15). When the higher EE is desired within selected factor range, factor X1and X2have positive coefficients which indicate that increasing factor value increases the response which means that increasing phospholipid and ethanol concentration increases the %EE of ethosomes. Additionally, stirring speed shows least effect on entrapment efficiency with negative co-efficient.

2.4.3 Effect analysis of variables on %CDR (Y2)

Table-8 ANOVA for response entrapment efficiency (Y1)

Response1% Entrapment Efficiency

ANOVAforselected factorialmodel						
Analysisofvariance table[Partialsumofsquares-Type III]						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	296.34	5	59.27	95.61	< 0.0001	significant
A-PhospholipidConc.	194.25	1	194.25	313.35	< 0.0001	
B-EthanolConc.	91.96	1	91.96	148.35	< 0.0001	
C-CholesterolConc.	3.90	1	3.90	6.29	0.0460	

D-Organicphase Composition	0.27	1	0.27	0.44	0.5338	
E-StirringSpeed	5.96	1	5.96	9.62	0.0211	
Residual	3.72	6	0.62			
CorTotal	300.06	11				

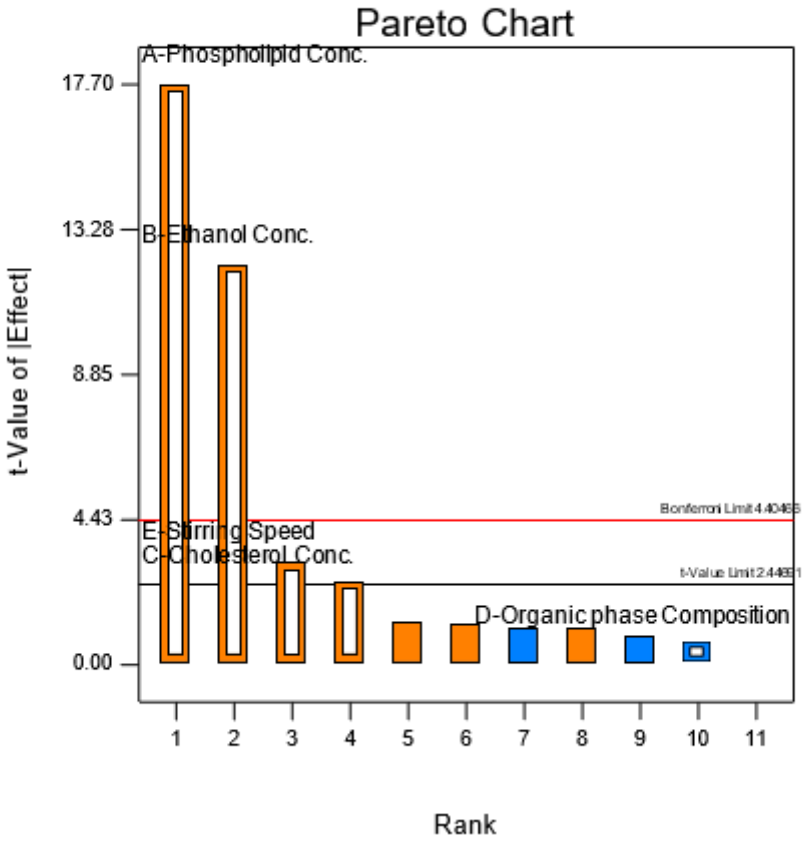


Figure -5 Pareto chart for entrapment efficiency

Results of the% Entrapment Efficiency for all batches of Plackett-Burman screening design are given in Table 6. The analysis of variance (ANOVA) revealed a statistical difference between the batches. A regression coefficient is said to be significant if p-value is less than 0.05. TheR2 value was 0.9843 indicating a good fit. From the result, it is evident that phospholipid conc. (p=<0.0001) and ethanol conc. (p=<0.0001) significantly affect the entrapment efficiency which is again confirmed by Pareto chart (Fig.6). When the higher EE is desired within selected factor range, factor X1and X2have positive coefficients which indicate that increasing factor value increases the response which means that increasing phospholipid and ethanol concentration increases the %EE of ethosomes. Additionally, stirring speed shows least effect on entrapment efficiency with negative co-efficient.

3.0 Formulation and Development of Ivermectin ethosomes by using 32 Factorial Design approach:

A two factor, three level (3^2) factorial design was developed via (RSM) using Design-Expert version 10.0.1 software (Stat-Ease, Minneapolis, MN, USA). Various batches of Ivermectin ethosomes by DoE using QbD approach were prepared according to 3^2factorial designs. Accordingly, phospholipid percentage (X1) and ethanol percentage (X2) were nominated to represent the independent variables that were inspected for their effects on the entrapment efficiency (Y1) and % CDR (Y2) of developed ethosome. Data in Table demonstrated two independent variables that showed their responses on the dependent variables Y1 and Y2 using three different levels (-1, 0, 1).

Table -9 3^2 Factorial Design

Independent variables	**Level**		
	Low (-1)	**Medium (0)**	**High (+1)**
X_1= Phospholipid Conc. (%)	2.5	3	3.5
X_2= Ethanol Conc. (%)	25	30	35
Dependent variables			
Y_1=Entrapment Efficiency (%)			
Y_2=CDR (%)			

Table-10 Formulation of batches from ET1-ET9

BATCHES	**RUN**	**X1: PL (%)**	**X2: ETH (%)**	**%E.E**	**%CDR**
IVE 1	1	3.5	30	81.11	85.73
IVE 2	2	2.5	30	69.22	82.63
IVE 3	3	3.5	25	79.3	83.15
IVE 4	4	3	30	77.35	85.97
IVE 5	5	2.5	25	67.74	78.3
IVE 6	6	3	35	83.77	89.21
IVE 7	7	3.5	35	82.57	88.91
IVE 8	8	3	25	74.53	84.21
IVE 9	9	2.5	35	71.81	82.08

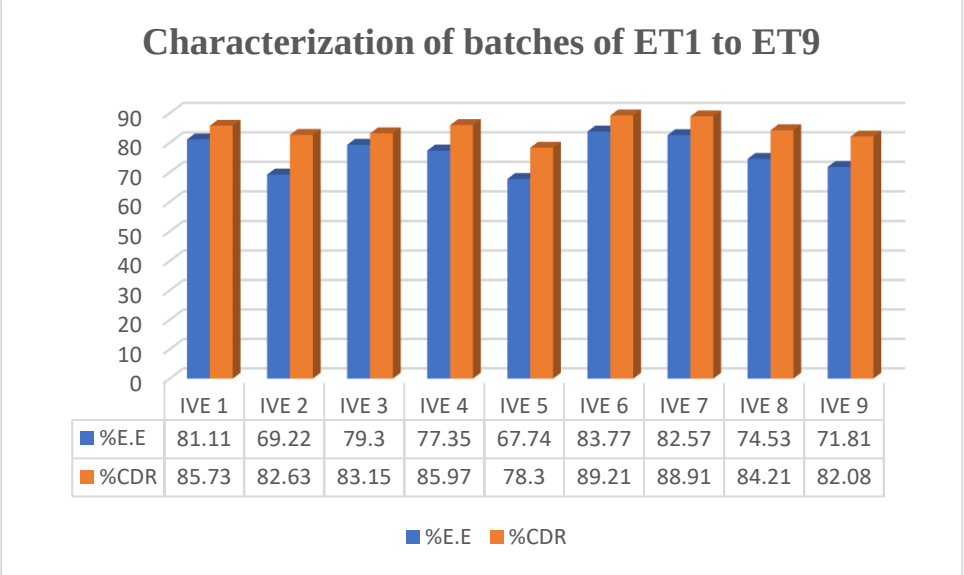


Figure 6-Characterization of batches from ET1-ET9

Statistical analysis:

Design expert version 10.0.1 was used for statistical analysis and produced first order polynomial equations. From preliminary screening results, a 3^2 full factorial design was utilized in which 2 factors was evaluated, separately at 3 levels and possible nine combinations was formulated. Three level factorial studies were carried out using two different variables. In factorial design, amount of phospholipid concentration(X1) and ethanol concentration(X2) was taken as independent variables while % entrapment efficiency (Y1) and % cumulative drug release (Y2) was selected as dependent variables for both factorial designs.

➢ **Effect on entrapment efficiency (Y1) - Surface response study:**

Positive value for the coefficient of X1 in equation indicates increase in entrapment efficiency with concentration of phospholipid. Positive value of coefficient B indicates increase in response of Y1 i.e. entrapment efficiency. It indicates linearity of surface response and contour plot as shown in figure

Final Equation in Terms of Coded Factors:

%E.E = +78.0655 +5.70 * A +2.763* B -0.20* AB - 3.258 *A^2 +0.7267 * B^2

Final Equation in Terms of Actual Factors:

%E.E = -71.06 + 92.0033 * PL CONC. - 0.95134 * ETH - 0.08* PL CONC. * ETH - 13.030 * PL CONC.^2 + 0.029* ETH^2

Table 11 ANOVA TABLE for Response surface Y1

ANOVA for Response Surface Linear model						
Response 1: %E.E						
Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		**Mean**	**F**	**p-value**	
Source	**Squares**	**df**	**Square**	**Value**	**Prob > F**	
Model	263.32	5	52.66	13.54	0.0286	significant
A-PL CONC.	195.05	1	195.05	50.14	0.0058	
B-ETH	45.82	1	45.82	11.78	0.0415	
Residual	0.16	1	0.16	0.041	0.8523	
Cor Total	21.23	1	21.23	5.46	0.1016	

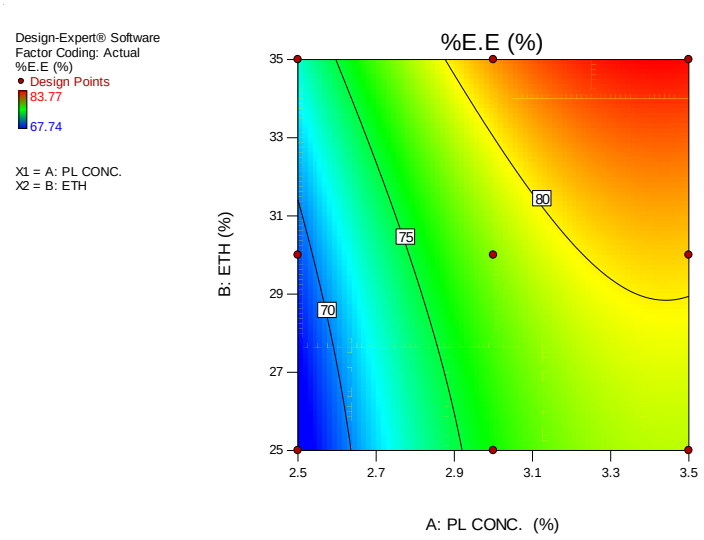


Fig.7 Response surface plot of A phospholipid conc. and B ethanol conc. on entrapment efficiency

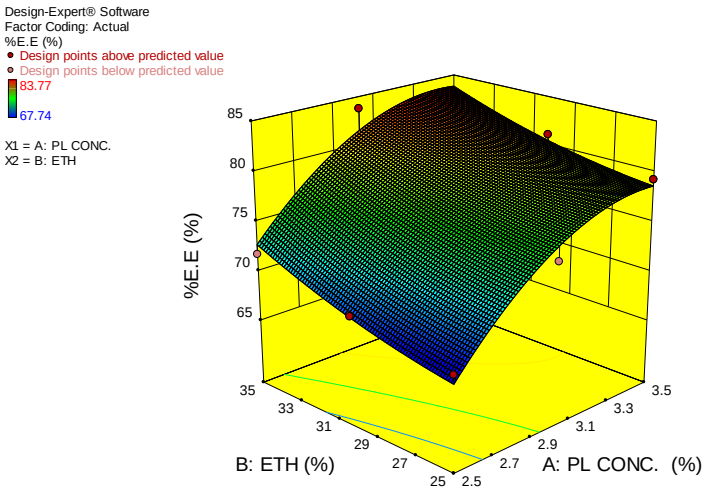


Fig. 8 3D surface plot of A phospholipid conc. and B ethanol conc. on entrapment efficiency

➢ **Effect on %CDR (Y2)-Surface response study:**

Positive value for the coefficient of X1 in the equation indicates increase in %CDR with concentration of phospholipid. Positive value of coefficient B indicates increase in response of Y2 i.e. %CDR. It indicates linearity of surface response and contour plot as show in figure 5.21 and 5.22.

Final Equation in Terms of Coded Factors:

%CDR = +86.77 + 2.464 * A + 2.42 * B + 0.495 * AB - 2.99 * A^2 -0.467 * B^2

Final Equation in Terms of Actual Factors:

%CDR = -49.41 + 70.91 * PL CONC. +1.01 * ETH + 0.198 * PL CONC. * ETH - 11.986 * PL CONC. ^2 - 0.01867 * ETH^2

Table -12 ANOVA table for response surface Y2

ANOVA for Response Surface Linear model

Response 2: %CDR						
Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		**Mean**	**F**	**p-value**	
Source	**Squares**	**df**	**Square**	**Value**	**Prob > F**	
Model	91.02	5	18.20	13.73	0.0280	significant
A-PL CONC.	*36.41*	*1*	*36.41*	*27.47*	*0.0135*	
B-ETH	*35.24*	*1*	*35.24*	*26.58*	*0.0141*	
AB	*0.98*	*1*	*0.98*	*0.74*	*0.4531*	
A^2	*17.96*	*1*	*17.96*	*13.55*	*0.0347*	
B^2	*0.44*	*1*	*0.44*	*0.33*	*0.6066*	
Residual	3.98	3	1.33			
Cor Total	95.00	8				

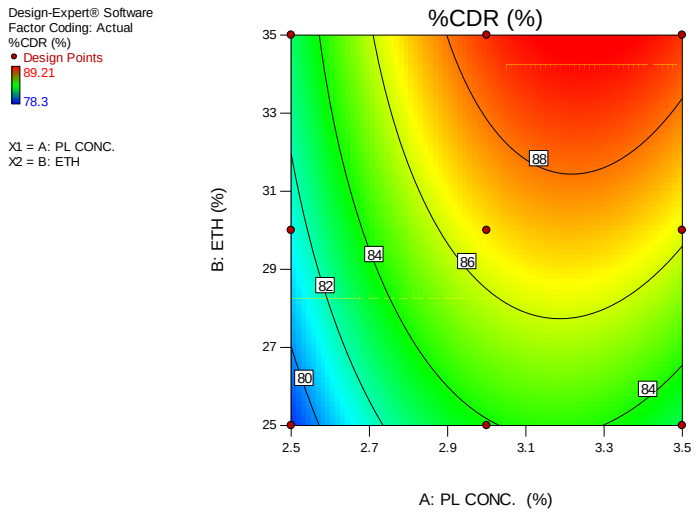


Fig. 9 Response surface plot of A phospholipid conc. and B ethanol conc. on %CDR

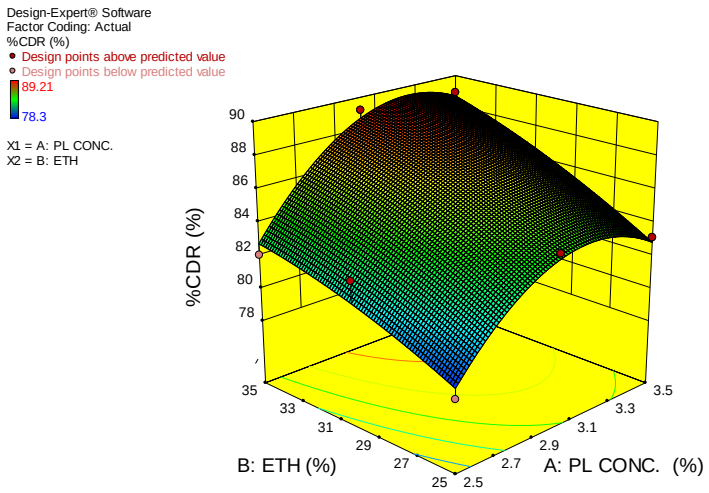


Fig.10 3D surface plot of A phospholipid conc. and B ethanol conc. on %CDR

3.1 Establishing design space and control strategy:

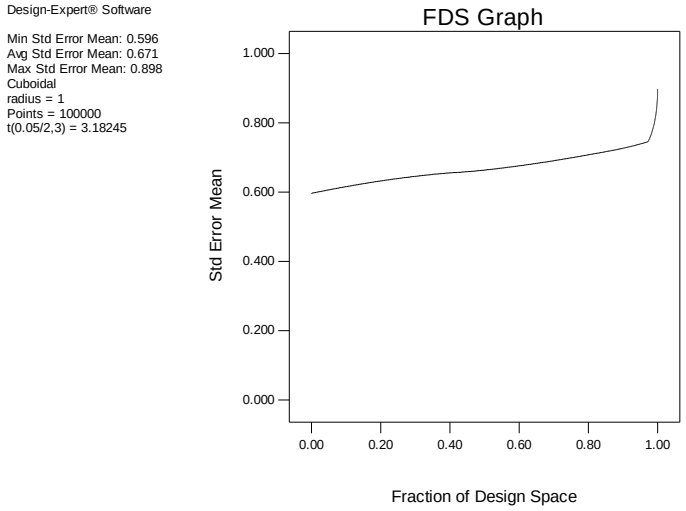
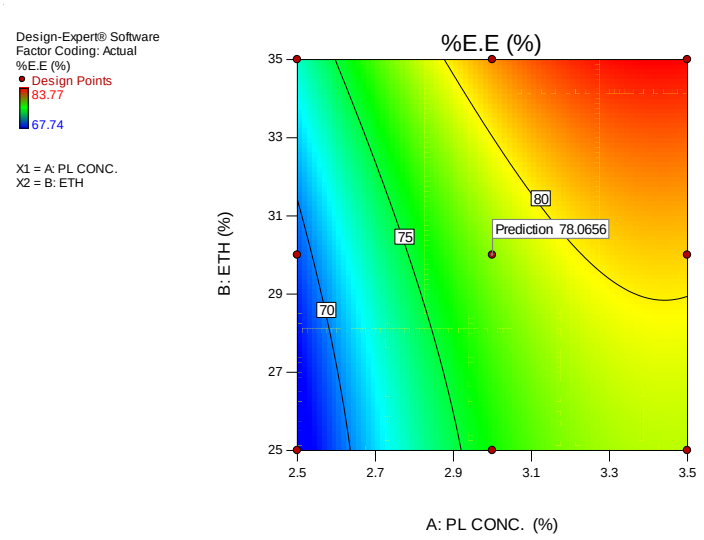


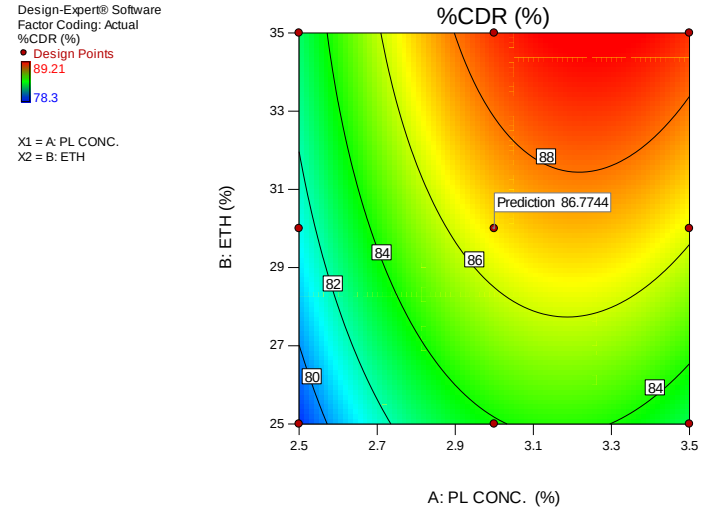
Fig.11 FDS Graph

FDS curve indicates what % fraction of design space has a given prediction error or lower. A good design will have a flatter and curve than a poor design as shown in figure . Flatter means overall prediction error will be constant. Lower means overall prediction error will be smaller. FDS should be least 0.8 or 80% for exploration and 100% for robustness testing. FDS was 0.89 or 89% which indicating robust standard error of prediction related to prediction interval around a prediction response at a given pair of factor level.

4. Validation:

From polynomial equation generated for response, intensive grid and integrated examine was performed over experiment field using design Expert software 12.0.1. During independent variable characterization study, impact of parameter phospholipid concentration (%) and ethanol concentration (%) were assessed. Criteria consideration of response entrapment efficiency (Y1) and %CDR (Y2) is between 66.71- 82.79% and 79.32-90.23% respectively. Design space shown in figure Design Exper 10.0.01also called as overly plot which is shaded region with yellow color indicates that region of successful operating ranges.


Design-Expert® Software
Factor Coding: Actual
%E.E (%)
Design Points
83.77
67.74
X1 = A: PL CONC.
X2 = B: ETH
%E.E (%)
80
75
70
Prediction 78.0656
B: ETH (%)
A: PL CONC. (%)


Design-Expert® Software
Factor Coding: Actual
%CDR (%)
Design Points
89.21
78.3
X1 = A: PL CONC.
X2 = B: ETH
%CDR (%)
88
86
84
82
80
Prediction 86.7744
B: ETH (%)
A: PL CONC. (%)

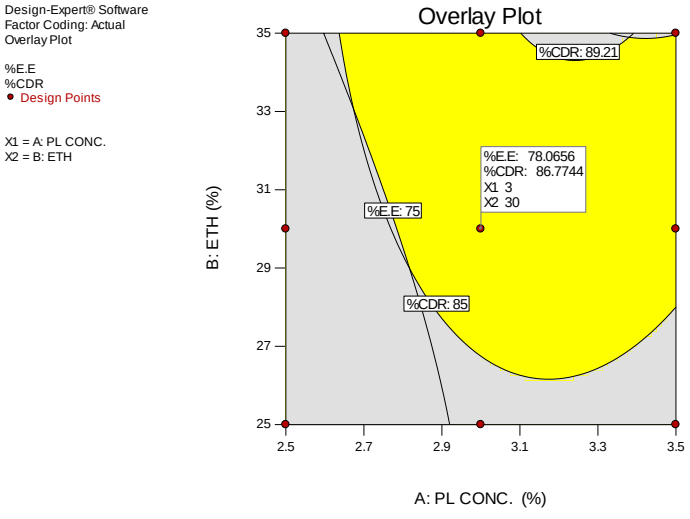
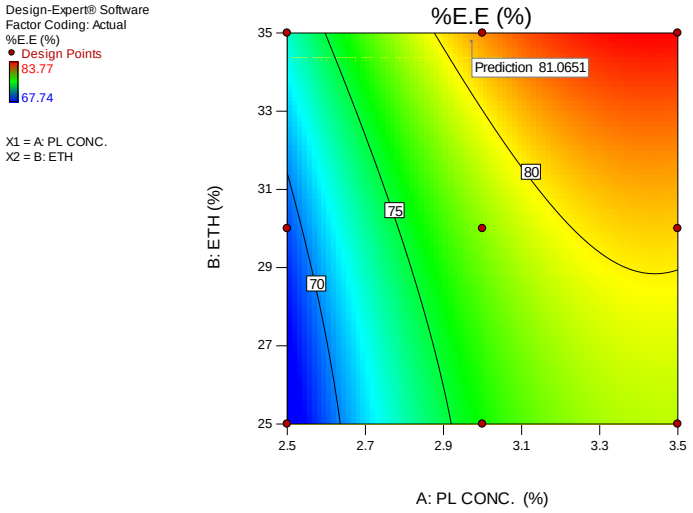
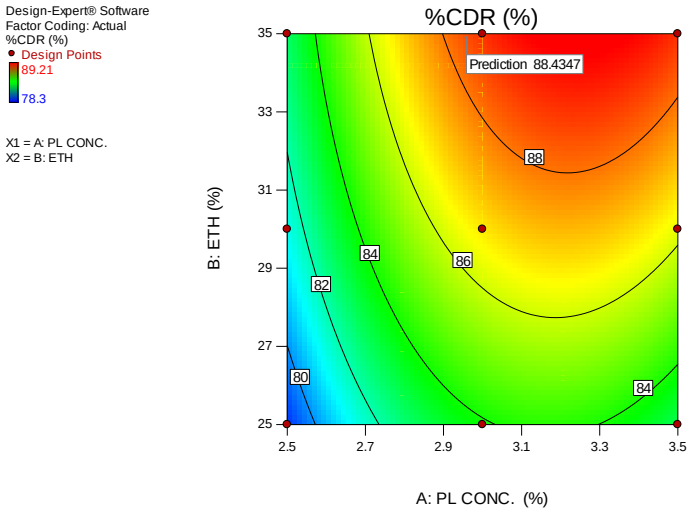

Design-Expert® Software
Factor Coding: Actual
Overlay Plot
%E.E
%CDR
Design Points
X1 = A: PL CONC.
X2 = B: ETH
Overlay Plot
%CDR: 89.21
%E.E: 78.0656
%CDR: 86.7744
X1 3
X2 30
%E.E: 75
%CDR: 85
B: ETH (%)
A: PL CONC. (%)

Fig.12 Overlay plot





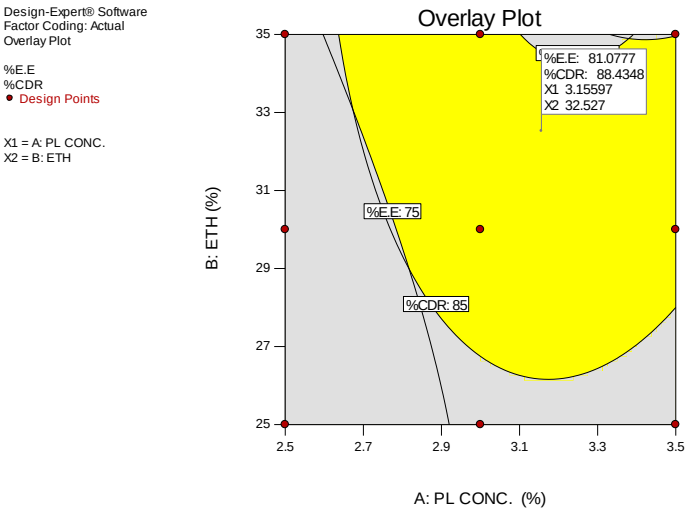
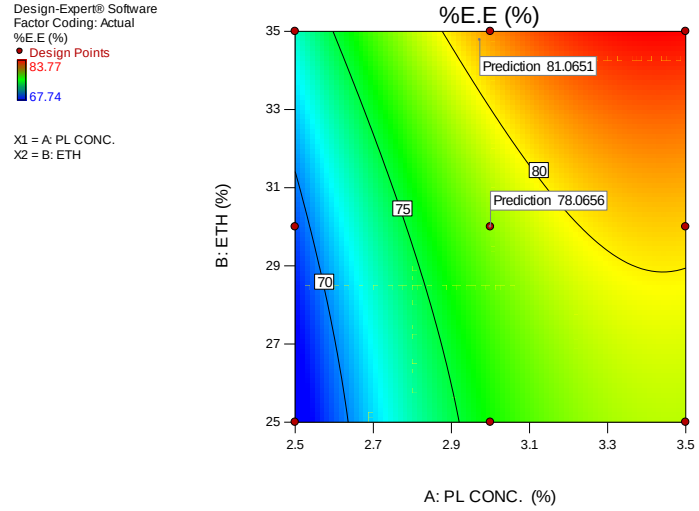
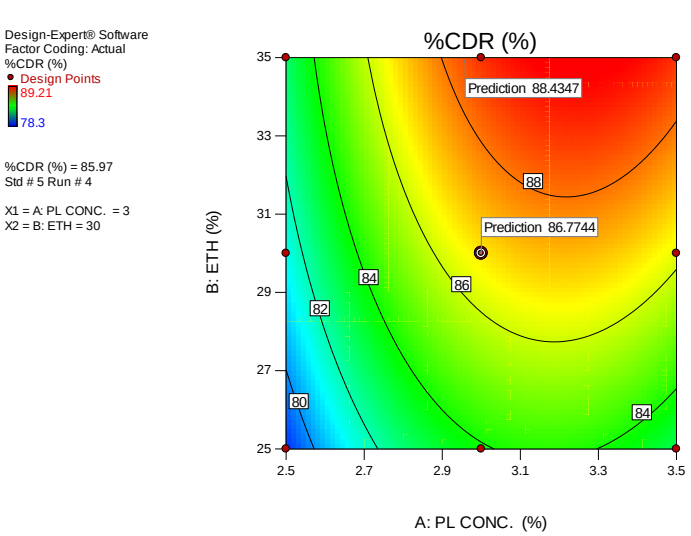


Fig.13 Overlay plot





4.1 Check point analysis of validation batches:

ET11 and ET12 formulation was made for check point analysis and predict and experimental values compared.

Table 13 Validation batch of ET10 and ET11: Prediction response

BATCH	**10th**	**11th**
X1: PL CONC. (%)	**3**	**3.15**
X2: ETH CONC. (%)	**30**	**32.52**
Y1: E.E. (%)	**78.07**	**81.07**
Y2: CDR (%)	**86.77**	**88.43**

Table 14 Validation batch of ET10 and ET11: Actual response

BATCH	**10th**	**11th**
X1: PL CONC. (%)	**3% w/v**	**3.15% w/v**
X2: ETH CONC. (%)	**30% v/v**	**32.52% w/v**
Y1: E.E. (%)	**79.53%**	**82.10%**
Y2: CDR (%)	**87.92%**	**89.27%**

OPTIMIZED BATCH FORMULATION FROM 3^2 FACTORIAL DESIGN

Table 15 Optimized Formulation Batch

INGREDIENTS	**FORMULATION**
Ivermectin	0.1% (30mg)
Phospholipid conc.	3.15% w/v
Ethanol conc.	32.52% w/v
Cholesterol	0.2% w/v
Isopropyl alcohol	10% v/v
Water	q.s

4.2 CHARACTERIZATION OF OPTIMIZED BATCH OF IVERMECTIN ETHOSOMES:

4.2.1 Scanning Electron Microscopy (SEM)

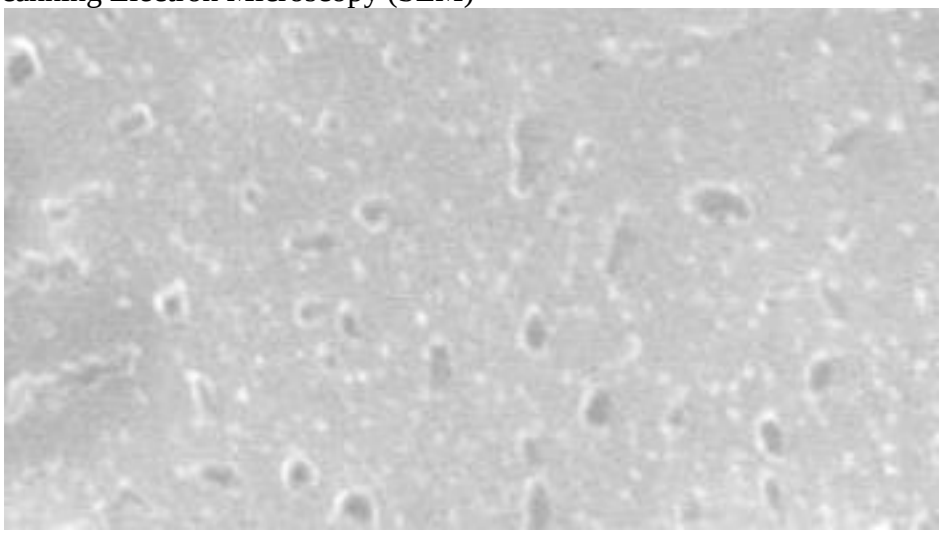


Fig 14 SEM image of optimized batch

The SEM micrograph showed the presence of spherically shaped vesicles in the formulation and somewhat irregular in shape. It also showed that there was no vesicle aggregation in formulation which indicates physical stability.

4.2.2 Particle Size Analysis

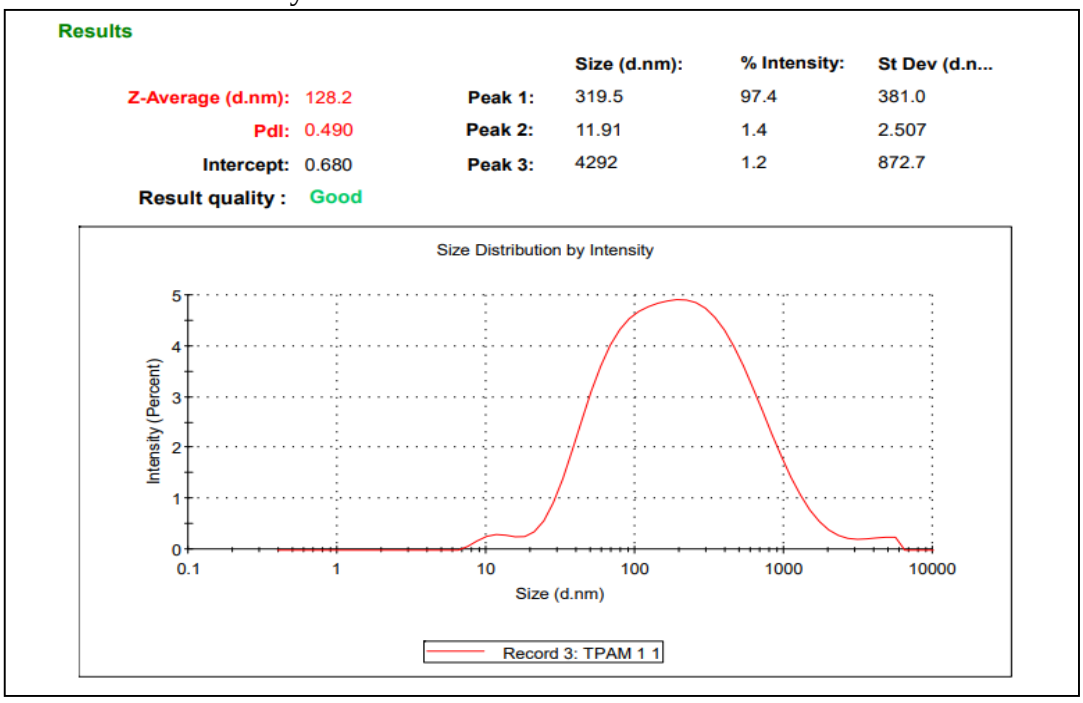


Fig. 15 Particle size analysis of Ivermectin ethosomes

Vesicle size was found to be 128.2 d.nm with PDI ratio of 0.490. Increasing concentrations of ethanol decreases the vesicle charge as it imparts a net negative charge & confers the system some degree of steric stabilization that may finally lead to a decrease in the mean particle size.

4.2.3 Zeta Potential

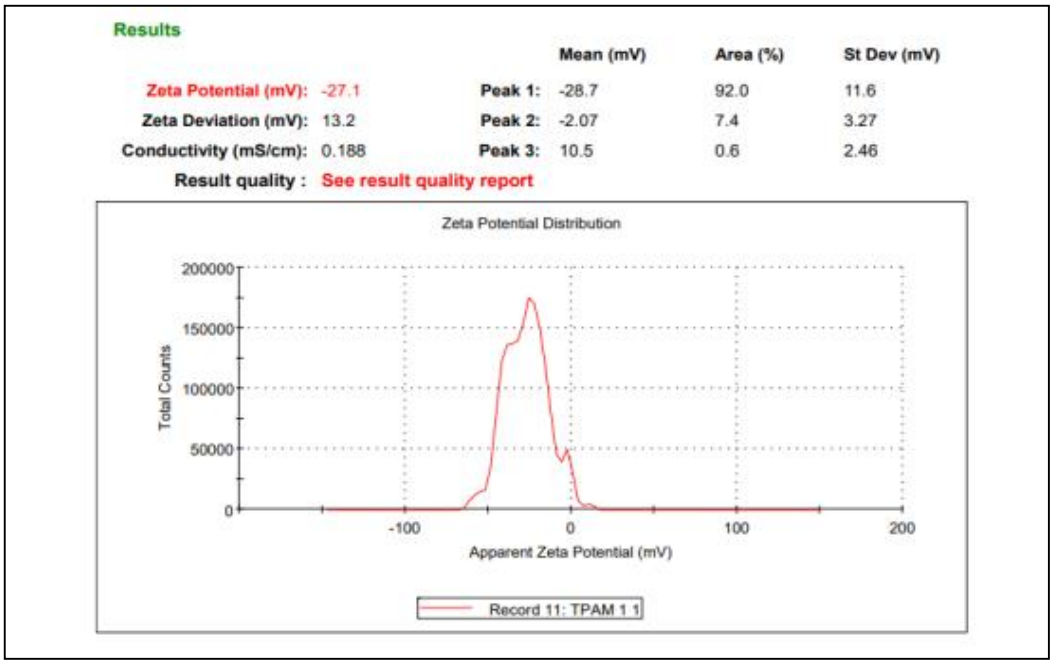


Fig.15 Zeta potential of Ivermectin ethosomes

Zeta-potential of the optimized formulation was -27.1 mV, i.e. negative value. This was due to the presence of ethanol which confers a negative charge to surface of ethosomes. Thus zeta potential value indicates that ethosomal vesicles have good stability.

4.2.4 % Entrapment efficiency

Prepared ethosomes formulation showed good entrapment efficiency of 90.12±0.38.This behavior may be due to the presence of a higher concentration of ethanol which increases AM solubility in ethosomes.

4.2.5 Drug content

Drug content (%) of optimized batch was found to be 96.28±0.75. This indicate good amount of drug in ethosomes.

5. Preparation and characterization of Ivermectin ethosomes loaded topical gel:

5.1 Preliminary Trial batches

Table 16 Formulation Design of Topical Gel Trial Batches

Ingredient	**AEG1**	**AEG2**	**AEG2**
Carbopol 934(%w/v)	**1**	1.5	2
Propylene glycol(mL)	**5**	5	5
Methyl paraben(mL)	**0.1**	0.1	0.1
Propyl paraben(mL)	**0.05**	0.05	0.05
Triethanolamine(mL)	**0.25**	0.25	0.25
Water(mL)	**100**	100	100

Table 17 Result of evaluation of Carbopol gel

Batch code	**Color**	**Odor**	**pH (Mean ± S.D.) (n = 3)**	**Viscosity (cp) Spindle no:62 (Mean ± S.D.) (n = 3)**	**Spreadability (gm.cm/sec) (Mean ± S.D.) (n = 3)**
AEG 1	**Colourless**	**Odourless**	**06.94±00.08**	**10048±446.50**	**11.83±00.46**
AEG 2	Colourless	Odourless	06.90±00.05	13966±800.98	10.96±00.61
AEG 3	Colourless	Odourless	06.83±00.06	16009±473.96	09.03±00.33

AEG 1 Formulation was taken as optimized formulation
The AEG 1 shows good spreadability and viscosity. Therefore, it was taken as optimized formula for further formulation of promising alternative ethosomal loaded gel.

5..2 Dose Calculation of Ivermectin Ethosomes for Topical Gel:
30 gm of marketed Ivermectin Gel contains 0.1% Ivermectin as drug
So, for loading Ivermectin as drug
30 gm of gel = 30 gm Ivermectin i.e. 100% Ivermectin as Drug.
So, (?) gm gel 0.1 % Ivermectin.
30 * 0.1/ 100 = 0.03 gm = 0.03 * 1000 = 30 mg Ivermectin required.
So, marketed Ivermectin gel contains 30 mg Ivermectin as Drug.
Now, 100 mg Ivermectin ethosomes contains 4.3 mg of drug, so, for meeting the requirement of 30 mg i.e. 0.1% ethosomal gel we require 420 mg of ethosomes
4.3 mg drug is present in 100 mg ethosomes
30 mg of drug requires (?) mg of ethosomes
30*100/4.3=697.67 mg ethosomes
≈700 mg of ethosomes.

6.Result and Discussion

Parameter	**Marketed Ivermectin Gel**	**Optimized Ivermectin Ethosomal Gel**
Dose	0.1%	**30 mg**
Strength	30gm	**30 gm**
Clarity	Opaque	**Opaque**
Odor	Odorless	**Odorless**
pH (Mean ± S.D.) (n = 3)	06.13±0.20	**06.79±0.015**
Viscosity (Mean ± S.D.) (n = 3)	9986±51.83	**9820±116.59**

Spreadability (Mean ± S.D.) (n = 3)	12.93±0.08	**11.63±0.61**
% Drug content (Mean ± S.D.) (n = 3)	93.67±0.38	**96.57±0.52**

7.Conclusion

IVM-loaded Ethosomes were successfully prepared by the Cold method. Formulation and process variables were screened by a PB-QbD approach to understand the most important factors influencing the responses of IVM-loaded Ethosomes. The invitro release study of IVM-loaded Ethosomes has showed sustained release pattern. Within the formulation and process factors studied, two formulation factors Phospholipid Concentration&Ethanol Concentrationwere found to have significant effect on Entrapment Efficiency and %CDR. The study concludes that the statistical PB design could be useful to identify influencing significant variables. PB design was proved to be efficient tool to understand the parameters affecting the response variables and to recognize the most influencing factor

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