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FORMULATION AND EVALUATION OF ETHOSOMAL GEL OF BETAMETHASONE VALERATE

Mayuri Jain[1], Neha Jain[1], Vinay Pandit[2], Upendra Nagaich[1*]

[1]Amity Institute of Pharmacy, Amity University, Noida, India

[2]Laureate Institute of Pharmacy, Kangra, H.P., India

Corresponding Author

Upendra Nagaich

Professor & Research Coordinator

Email: upendra_nagaich@hotmail.com

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Abstract

Ethosomes are non-invasive delivery vehicles for medications that allow them to penetrate deep into the epidermal layers and/or the systemic circulation. Betamethasone valerate 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). The objective was to prepare Betamethasone valerate ethosomes and then load them into the gel. The optimized ethosomes formulation was selected to be incorporated in the gel system based on the good stability results and the optimized in-vitro study. In the present work, the ethosomes were prepared by utilizing soya lecithin, ethanol and other useful materials. 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. This study reported on the use of a Box–Behnken design in the optimization of ethosomes dispersion mean diameter for the encapsulation of ethosomes. Out of fifteen batches, batch F9 is the optimized batch. The gel was prepared by the use of carbopol 934, triethanolamine and glycerol. Methyl and propyl paraben were used as preservatives. The average particle size of optimized batch is 124.2 nm, PDI is 0.280 and zeta potential is -17.0 mV. The pH of the gel is 5.6 ± 0.03. The in-vitro permeation study is 88.92±0.054% in pH 7.4 Phosphate buffer. The viscosity of the gel is 4980 ± 0.45 cP and the spreadability is 37.88/4 ± 0.05. The gel formulations will be stored at 40˚C and 75% relative humidity for 90 days. Prolonged releases will achieved when they will formulated as topical gels on maintaining the ethosomal structure.

Keywords: Ethosomes, Betamethasone, Ethosomal gel, Carbopol 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 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. Ethosomes are non-invasive delivery vehicles for medications that allow them to penetrate deep into the epidermal layers and/or the systemic circulation. These are soft, pliable vesicles designed to distribute active substances more effectively. 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].

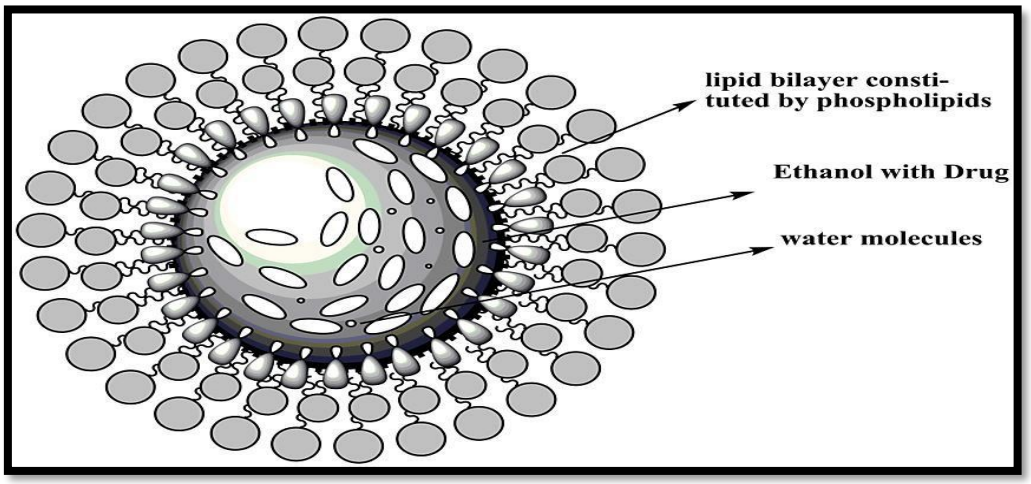

Figure 1: Representation of Ethosomes

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

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 [3].

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 minutes, 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 minutes each with a minute rest between cycles using probe solicitor. Vesicles start to emerge after 5 minutes 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 [4].

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_1	150	20	500
F_2	350	20	700
F_3	350	30	500
F_4	150	30	300
F_5	350	20	300
F_6	350	40	300
F_7	150	40	500
F_8	550	20	500
F_9	350	40	700
F_{10}	350	30	500
F_{11}	550	30	700
F_{12}	550	40	500
F_{13}	550	30	300
F_{14}	350	30	500
F_{15}	150	30	700

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.

Evaluation of Optimized Batch Betamethasone valerate Ethosomes

The optimized batch Betamethasone valerate Ethosomes was evaluated for Particle size, Zeta potential, FT-IR, DSC, TEM and in-vitro permeation study.

Preparation of Betamethasone valerate Ethosomal Gel

The optimized ethosomes formulation was selected to be incorporated in the gel system based on the good stability results and the optimized in-vitro study. The specified amount of carbopol

934 powders is to be slowly added to ultrapure water and kept at 100 °C for 20 min. Triethanolamine were added to it drop wise. Appropriate amount of 1.5% w/w formulation containing drug were incorporated into gel base. Water q.s. were added with other formulation ingredients should be achieved. Gel containing free drug were prepared by similar method using 1.5% Carbopol. [5] 1.5% carbopol gel base was prepared by dispersing 1.5 gm carbopol 934, in 90 ml hot distilled water in which 10 ml glycerol was previously added. Accurately weighed quantity of methyl paraben and propyl paraben was added into it. The mixture was stirred until thickening occurred and then neutralized by the drop wise addition of 50 % w/w triethanolamine to achieve a transparent gel.

Table 2: Composition of Gel base

Ingredients	Concentration
Carbopol 934	1.5 %
Glycerol	5 %
Methyl paraben	0.02 %
Propyl paraben	0.01 %
Distilled water	Upto 100 %

In this gel base ethosomal formulation was slowly added with gentle stirring. Finally the ethosomal gel was mixed using a mechanical stirrer for 5 minutes.

Table 3: Composition of Betamethasone valerate Ethosomal Gel

Formulation code	**Optimized batch Betamethasone valerate ethosome**	**Carbopol 934**
Betamethasone valerate ethosomal gel	Equivalent to 5 mg Betamethasone valerate**)**	1.5% w/w

Evaluation of Betamethasone valerate Ethosomal Gel

Appearance

About 1 week after preparation, the dispersions were visually assessed for optical appearance (e.g., colour, turbidity, homogeneity, presence of macroscopic particles).

pH

1 gm Betamethasone valerate ethosomal gel was mixed in 100 ml distilled water with homogenizer. Then the electrode was immersed in prepared gel solution. The prepared gel formulations were characterized for pH measurements using the pH meter (JENWAY 3510 pH meter, UK).

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 rotates at a speed of 50 rpm for 10 minutes. All measurements were carried out in triplicate and the mean value was recorded ± SD.

Spreadability

Spreadability of gel was determined by modified wooden block and glass slide apparatus. A measured amount of gel should be placed on fixed glass slide; the movable pan with a glass

slide attached to it and can be placed over the fixed glass slide, such that the gel were sandwiched between the two glass slides for 5 minutes. The weight can be continuously removed. Spreadability was determined using the formula.

S = ML/T

Where,

S - Spreadability in g/s

L - Length of glass slide

M - Mass in grams

T - Time in seconds

Drug Content Uniformity

A measured amount of formulated gel were taken and dissolved in 100 ml of buffer pH 7.4. Mechanical shaker were used to shake the gel solution continuously for 2 hrs. The solution thus prepared was to be filtered and analyzed spectrophotometrically at 235 nm using suitable phosphate buffer (pH 7.4) as blank.

Homogeneity

A small quantity of ethosomal gel was pressed between the thumb and the index finger. The consistency of the Ethosomal gel were noticed (whether homogenous or not), if there were any coarse particles appeared on fingers.

***In-vitro* Release Study**

The Betamethasone valerate Ethosomal gel were studied using a modified Keshary-chein diffusion cell. A piece of cellulose membrane (Molecular weight cut off 12,000–14,000 Da, Spectra/Pro, Spectrum Laboratories, Inc., USA) was soaked in pH 7.4 Phosphate buffer for about 2 hrs. 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 5.0 mg Betamethasone valerate) was placed in the designed release assembly. 2 ml aliquots of the release medium were withdrawn at 0.5, 1, 2, 4, 6, 8 and 10 hrs time intervals, and replaced with 2 ml fresh medium to maintain the volume.

Stability Studies

The stability studies for optimized formulation should be conducted in the accelerated conditions as per guidelines of International Conference on Harmonization (ICH). Well closed container was used for the storage of optimized gel formulation. The gel formulations will be stored at 40˚C and 75% relative humidity for 90 days.

Result and Discussion

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.

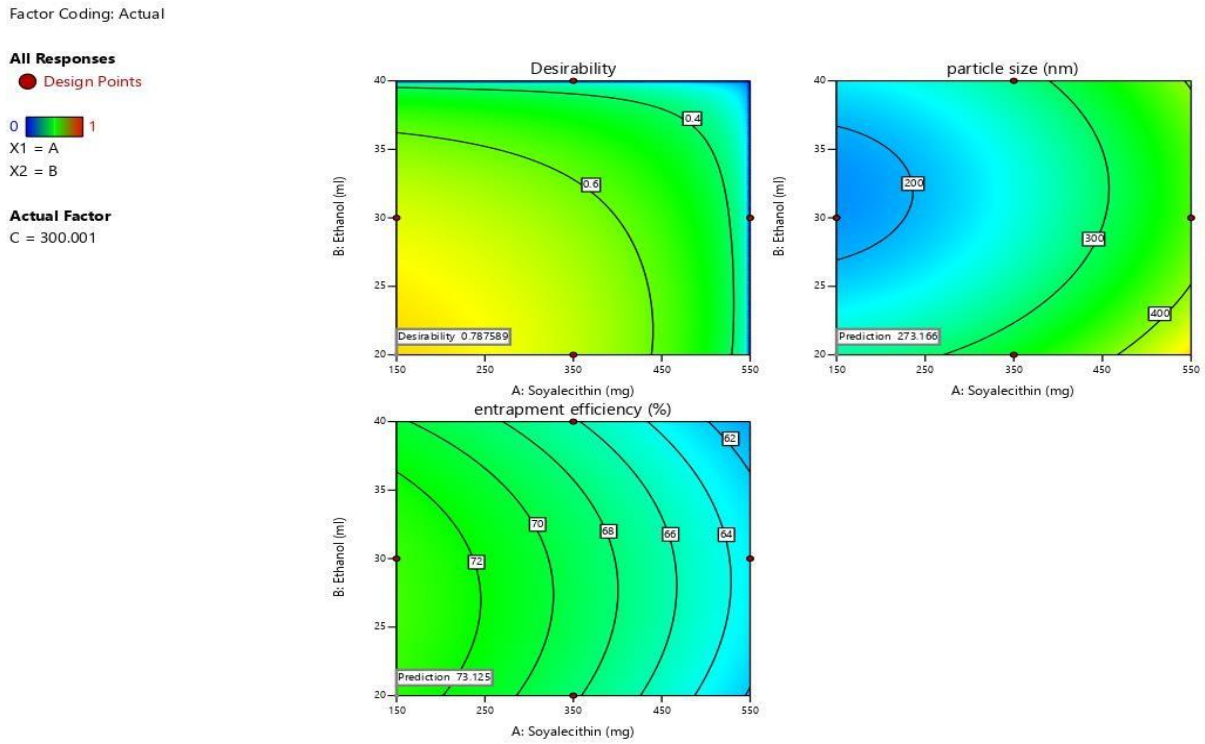

Figure 2: Contour Graph of Predicted Responses and Desirability

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

Evaluation of Betamethasone valerate ethosomal Gel

The Betamethasone valerate ethosomal gel was checked for appearance, pH, viscosity, *in-vitro* release study, spreadability, drug content uniformity, homogenicity etc. The results of evaluation parameters were depicted in table 5.

Table 5: Evaluation Parameters for Optimized Betamethasone valerate Ethosomal Gel Formulation

S. No.	Parameter	Inference
1.	Appearance	White appearance, consistent, no grittiness, no phase separation
2.	pH	5.6 ± 0.03
3.	Viscosity	4980 ± 0.45 cP
4.	Spreadability	37.88/4 ± 0.05

***In-vitro* Release study of optimized Betamethasone valerate Ethosomal Gel Formulation**

The *in-vitro* release results are depicted in table 6 and Figure 3.

Table 6: In-vitro Release Study of Optimized Betamethasone valerate Ethosomal Gel Formulation

S. No.	Time (Hrs)	pH 7.4 PBS
1.	0	0
2.	0.5	31.23
3.	1	45.67
4.	2	57.26
5.	4	67.98
6.	6	74.73
7.	8	80.12
8.	10	85.87
9.	12	88.92

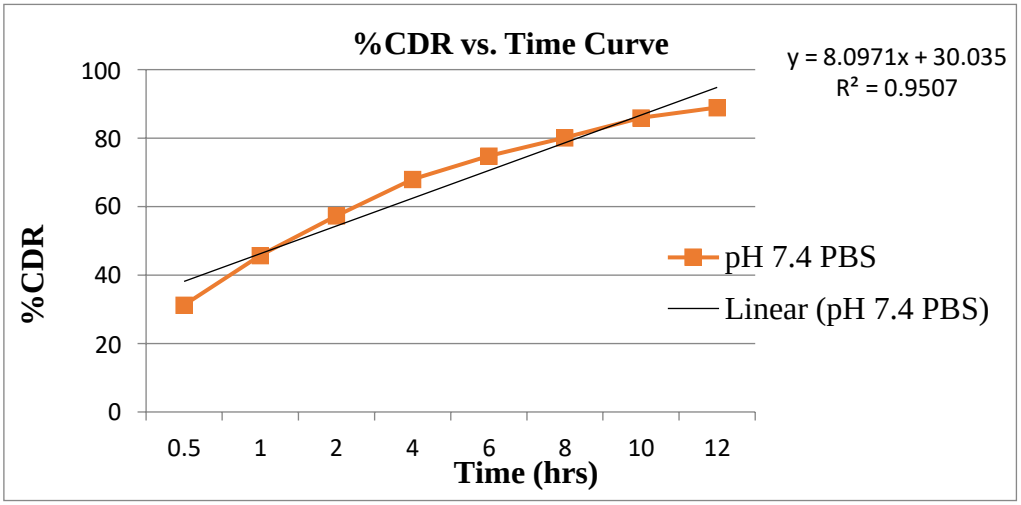

Figure 3: *In-vitro* Release Study of Optimized Betamethasone valerate Ethosomes Formulation

Stability Studies

The stability studies for optimized formulation should be conducted in the accelerated conditions as per guidelines of International Conference on Harmonization (ICH). Well closed container was used for the storage of optimized gel formulation. The gel formulations will be stored at 40˚C and 75% relative humidity for 90 days.

Samples were drawn at a forethought time interval of 30 days, 60 days and 90 days. The gel formulation can be evaluated for their physical properties including appearance, color, and presence of clogs, consistency and phase separation. Gel can also be evaluated for chemical parameters like change in pH and drug content.

Table 7: Different Parameters of Betamethasone valerate Ethosomal Gel Formulations

Formulation Code	Soyalecithin (X1, mg)	Ethanol (X2, ml)	Stirring Speed (X3, mg)	Average Particle size (nm)	PDI	Zeta potential (mV)
F_1	150	20	500	154.7	0.344	-15.3
F_2	350	20	700	280.8	0.453	-12.0
F_3	350	30	500	258.3	0.422	-12.3
F_4	150	30	300	187.5	0.352	-16.1
F_5	350	20	300	424.7	0.564	-11.6
F_6	350	40	300	290.3	0.474	-12.1
F_7	150	40	500	180.6	0.334	-14.5
F_8	550	20	500	540.9	0.713	-08.5
F_9	**350**	**40**	**700**	**124.2**	**0.280**	**-17.0**
F_{10}	350	30	500	260.2	0.423	-12.3
F_{11}	550	30	700	410.7	0.598	-10.6
F_{12}	550	40	500	555.2	0.743	-07.4
F_{13}	550	30	300	268.4	0.428	-12.4
F_{14}	350	30	500	256.9	0.416	-12.2
F_{15}	150	30	700	144.4	0.315	-13.7

Conclusion

In the present work, the ethosomes were prepared by utilizing soya lecithin, ethanol and other useful materials. 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. This study reported on the use of a Box–Behnken design in the optimization of ethosomes dispersion mean diameter for the encapsulation of ethosomes. Out of fifteen batches, batch F9 is the optimized batch. The gel was prepared by the use of carbopol 934, triethanolamine and glycerol. Methyl and propyl paraben were used as preservatives. The average particle size of optimized batch is 124.2 nm, PDI is 0.280 and zeta potential is -17.0 mV. The pH of the gel is 5.6 ± 0.03. The in-vitro permeation study is 88.92±0.054% in pH 7.4 Phosphate buffer. The viscosity of the gel is 4980 ± 0.45 cP and the spreadability is 37.88/4 ± 0.05. Prolonged releases will achieved when they will 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 provide 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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