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FORMULATION AND EVALUATION OF ETHOSOMAL GEL CONTAINING CLOBETASOL.

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ABSTRACT

The present study involves formulation and characterization of ethosomal gel containing Clobetasol propionate used to treat dermatitis anti psoriasis. The transdermal delivery is one of the most important routes of drug administration. The main factor which limits the application of transdermal route for drug delivery is the permeation of drugs through the skin. One simple and convenient approach is application of drugs in formulation with elastic vesicles or skin enhancers. significant enhanced delivery of Clobetasol propionate through transdermal route could be obtained by using vesicular drug carrier systems - Ethosomes. The current study was aimed to investigate the potential of ethosomes in enhancement of clobetasol transport across the skin, characteristics of ethosomes and their *in-vitro* skin permeation behaviour. The present work also focuses on making the formulation more pharmaceutically acceptable. In this study, the drug and the polymer interaction studies were performed by using FTIR Spectroscopy for its unknown compatibility.

KEY WORLD: Ethosomal gel, clobetasol, psoriasis.

INTRODUCTION

Psoriasis is a long-term (chronic) skin problem that causes skin cells to grow too quickly, resulting in thick, white, silvery, or red patches of skin. Normally, skin cells grow gradually and flake off about every 4 weeks.They most often appear on the knees, elbows, scalp, hands, feet, or lower back.

Usually diagnosis of psoriasis is by looking at the patches on your skin, scalp, or nails. Special tests aren't usually needed. Most cases of psoriasis are mild, and treatment begins with skin care.

Ethosomes

"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.

Different Additives Employed In Formulation of Ethosomes

Class	Example	Uses
Phospholipid	Soya phosphatidyl choline Egg phosphatidyl choline Dipalmityl phosphatidyl choline	Vesicles forming component
Polyglycol	Propylene glycol Transcutol RTM	As a skin penetration enhancer
Alcohol	Ethanol Isopropyl alcohol	For providing the softness for vesicle membrane, As a penetration enhancer
Cholesterol	Cholesterol	For providing the stability to vesicle membrane.
Vehicle	Carbopol Đ934	As a gel former

Mechanism of drug penetration

It is thought that the first part of the mechanism is due to the 'ethanol effect' whereby intercalation of the ethanol into intercellular lipids increasing lipid fluidity and decreases the density of the lipid multilayer. This is followed by the 'ethosome effect', which includes inter lipid penetration and permeation by the opening of new pathways due to the malleability and fusion of ethosomes with skin lipids.

Method of preparation of ethosomal gel

This is the most common and widely used method for the ethosomal preparation. Dissolve phospholipid, drug and other lipid materials in ethanol in a covered vessel at room temperature with vigorous stirring. Add propylene glycol or other polyol during stirring. Heat the mixture up to 30^{0}C in a water bath. Heat the water up to 30^{0}C in a separate vessel and add to the mixture and then stir it for 5 min in a covered vessel. The vesicle size of ethosomal formulation can be decreased to desire extend using sonication or extrusion method.2 % w/w gel base was prepared by dispersing carbopol in distilled water containing 0.2% methyl parabens and 0.2% propyl parabens using magnetic stirrer. Here, carbopol was used as gelling agent and methyl parabens and propyl parabens were used as preservatives.

Evaluation parameter

Percent entrapment

The optimized ethosomal formulation was kept in sealed vials (10 ml) at 5±3ºC and at 25±2ºC for 1, 2 and 3 months to study the effect of different storage conditions on percent entrapment.

Physical Appearance: Optimized gel was kept for 1, 2 and 3 months under 5ºC ± 3ºC as well as 25ºC ± 2 ºC temperature conditions to study the effect of storage conditions on their physical appearance.

Spreadability

Two glass slides of standard dimensions were selected. The ethosomalgel formulation whose spreadability had to be determined was placed over one of the slides. The second slide was placed over the slide in such a way that the formulation was sandwiched between them across a length of 6cm along the slide. 100g of weight was placed upon the upper slide so that the ethosomalgel formulation between the two slides was traced uniformly to form a thin layer.The weight was removed and the excess of ethosomalgel formulation adhering to the slides was scrapped off. The lower slide was fixed on the board of the apparatus and one end of the upper slide was tied to a string to which 20g load could be applied with the help of a simple pulley. The time taken for the upper slide to travel the distance of 6cm and separate away from lower slide under the direction of the weight was noted.

Viscosity

The viscosity was determined by Brookfield viscometer (LV DV- II +Pro). The sufficient quantity of ethosomal gel was filled in wide mouth jar separately. The height of the gel was filled in the wide mouth jar should sufficiently allow to dip the spindle. The RPM of the spindle was adjusted to 2.5 RPM[69]. The viscosities of the formulations were recorded.

In-vitro Drug Release Studies

Estimation Of Drug Content (Assay)

Stability studies for Ethosomal gel

Materials

Clobetasol Propionate, Soya lecithin, Ethanol, Propylene glycol, Carbopol, Methyl parabens, Propyl parabens.

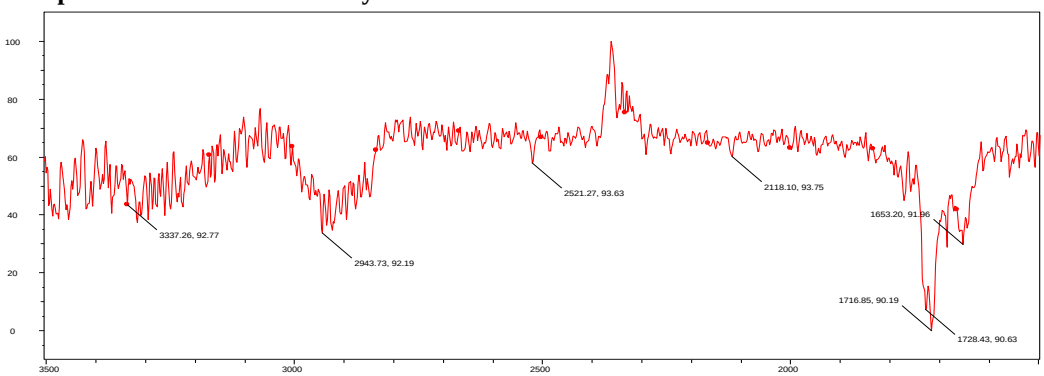
Formulation

Ethosomes Formulation Code	Composition of Ingredients				
	Drug (Clobetasol propionate) (mg)	Soya Lecithin (mg)	Ethanol (ml)	Propylene Glycol (mg)	Double Distilled Water (ml)
F-1	100	300	2.5	600	10
F-2	100	300	3	600	10
F-3	100	300	3.5	600	10
F-4	100	300	4	600	10
F-5	100	300	4.5	600	10

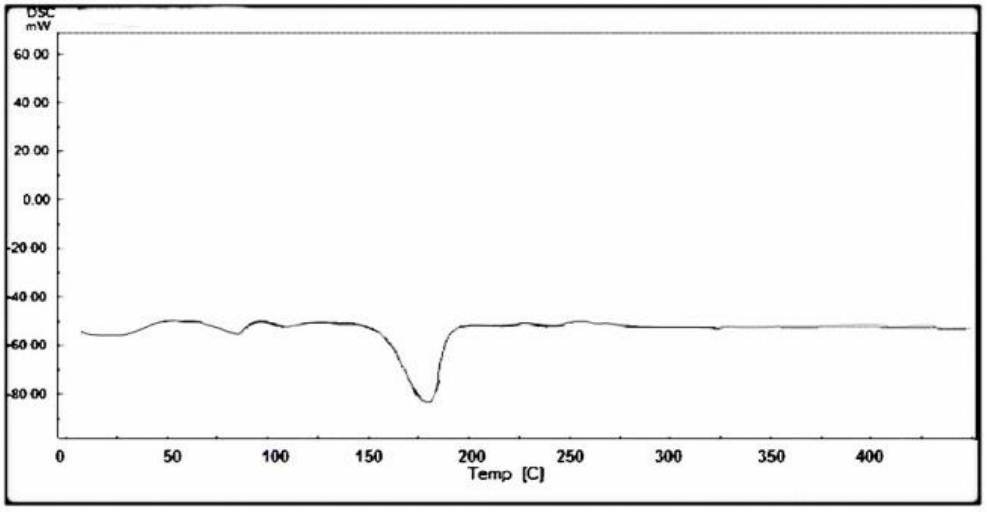
RESULTS AND DISCUSSION

7.1. Pre-formulation studies

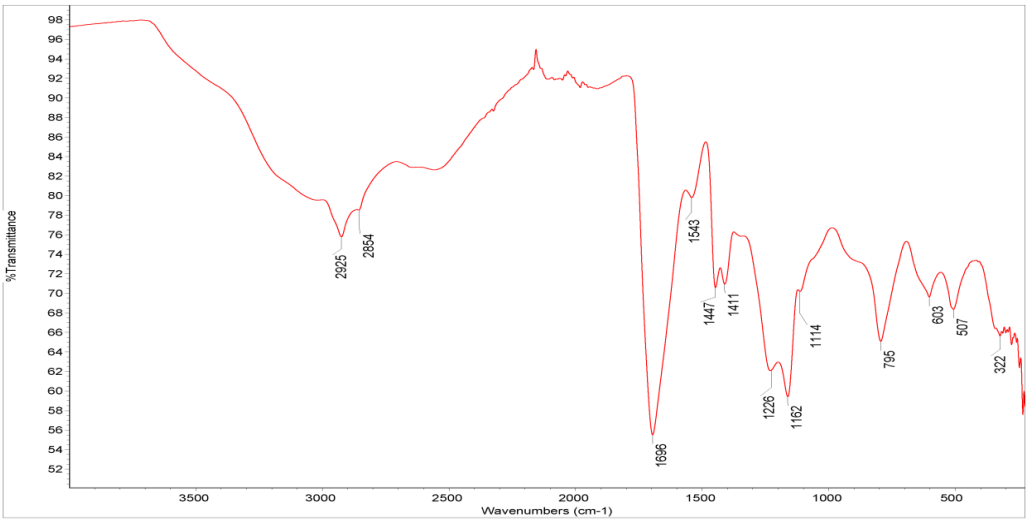
1. Drug – Excipient Interaction Studies by FTIR



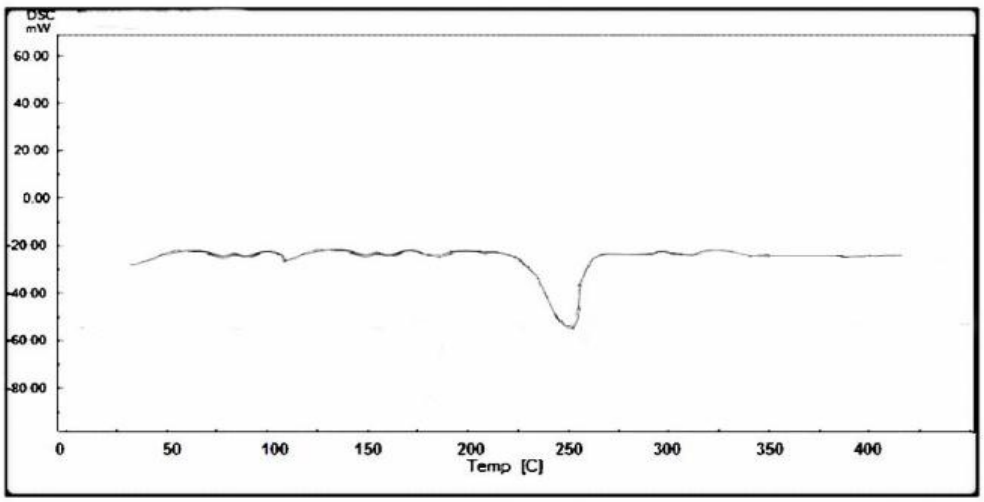
FTIR spectra of Clobetasol (Functional group region)



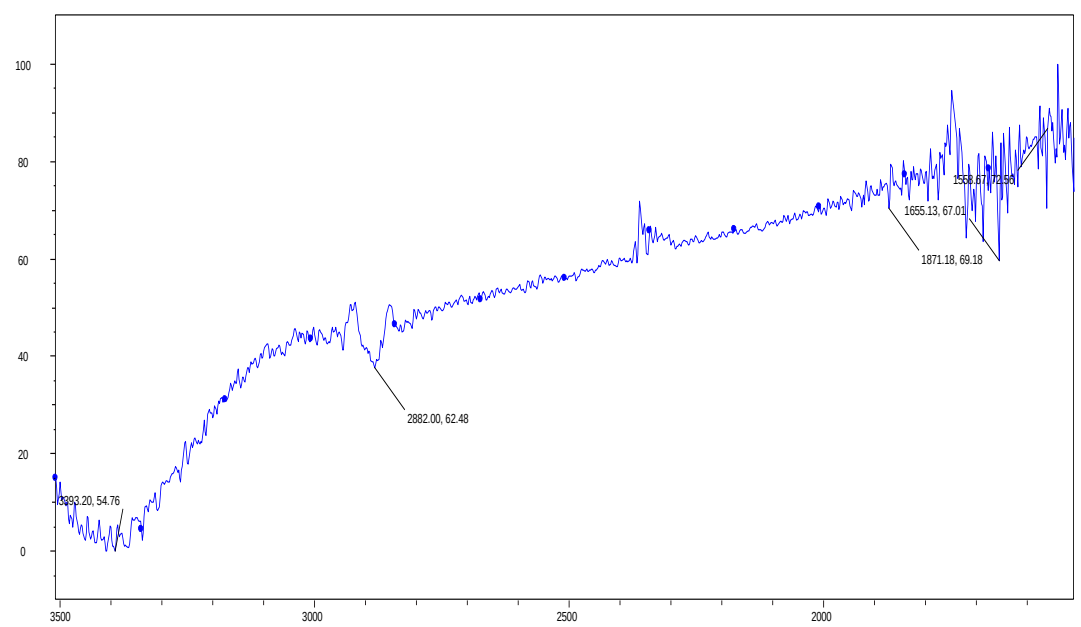
Dsc of Clobetasol



FTIR spectra of Carbopol



Dsc of Carbopol



FTIR spectra of formulation (Functional group region)

Drug – Excipient Interaction Studies by FTIR

S.No	Observed Range Cm^{-1}		Characteristic peak
	Clobetasol	Clobetasol + Excipient's	Functional Groups
1	1450.65 cm^{-1}	1452.57 cm^{-1}	C-H Scissoring and bending 1470-1350 cm^{-1}
2	1471.87 cm^{-1}	1475.72 cm^{-1}	C-H Stretching 2919-1474 cm^{-1}
3	1728.43 cm^{-1}	1725.50 cm^{-1}	C-H_2 Stretching 1750-1680
4.	1716.85 cm^{-1}	1720.32 cm^{-1}	C=O Stretching 1760-1670 cm^{-1}

From the above data, we can conclude that there is no interactions with clobetasol and excipient's and the presence of functional groups are within the range. So this may not affect the formulation stability during its shelf life.

DSC DESRIPTION: From DSC thermo grams, it was evident that the endothermic peak of the physical mixture was found at 194 (of clobetasol), which showed a slight variation when compared with the pure drug 195-197.

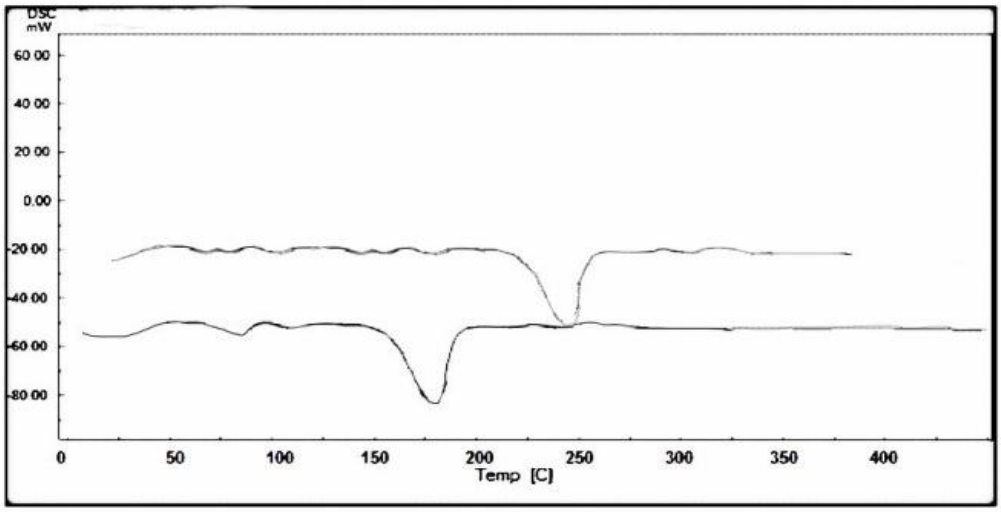


Figure 16: Dsc of formulation

2. **Solubility of Clobetasol propionate:** is having predictable solubility in water (3mg/ml) and freely soluble in alcohol which upon serial dilution using different solvents.

3. **UV spectrum analysis:** The λ max, which represents the maximum absorbance of the procured sample of Clobetasol propionate in the UV absorbtion range (200 nm - 400 nm) was found to be 237 nm.

Characteriztion of Ethosomal gels

Sr. No	Product	Physical Appearance	pH*	Extrudability*	Spreadability* (g.cm/sec)	Viscosity (cps)	% drug content
1.	F1	Creamywhite,smooth on application	6.8	+++	13.51	2,69,000	94.82
2	F2	Creamywhite,smooth on application	6.9	+++	13.42	2,73,000	94.28
3	F3	Creamywhite,smooth on application	6.5	++	13.33	2,98,000	91.28
4	F4	Creamywhite,smooth on application	6.8	+++	13.7	2,68,000	96.18
5	F5	Creamywhite,smooth on application	6.9	+++	13.51	2,72,000	95.64

Each reading is an Average of three determinations
Excellent=+++, Good=++

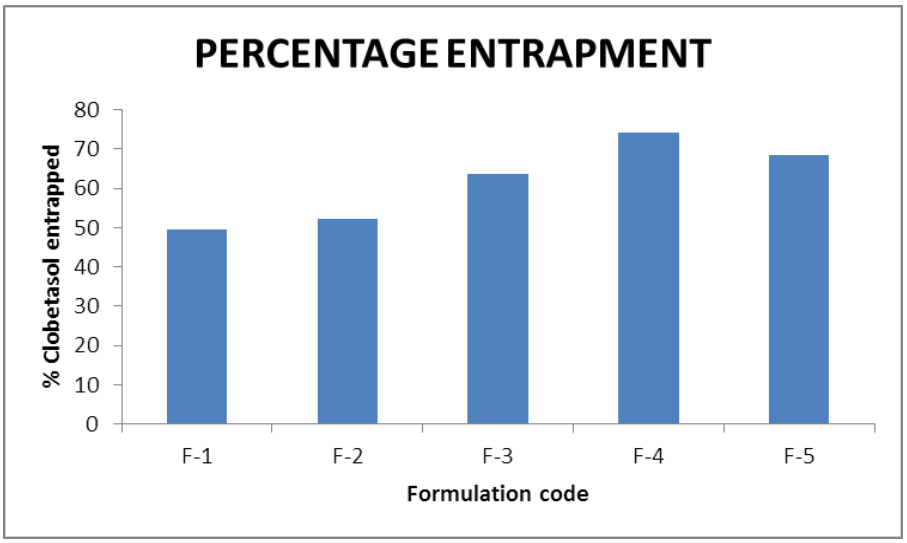
1. Entrapment efficiency

The entrapment efficiency of ethosomes was calculated as percent total drug entrapped within the vesicles. The entrapment efficiency was found to vary with the varying concentration of ethanol and lecithin. Entrapment efficiency was found to increase with ethanol concentration range with different concentration of lecithin. The entrapment was found to be maximum in vesicles of F-4 (74.2%±0.533%) and minimum in F-1 vesicle (49.5%±0.745%), respectively from table.

The relatively high entrapment may be explained by multilamellarity of ethosomal vesicles, however percent entrapment depends upon ethanol concentration.

Percentage Entrapment of Clobetasol

Formulation	Percentage Entrapment
F1	49.5%±0.745%
F2	52.2%±0.423%
F3	63.8%±0.654%
F4	74.2%±0.533%
F5	68.6%±0.562%



2. Skin Irritation study

Table 11: Skin Irritation study

Formulation code	Average score		
	Day-1	Day-4	Day-8
Control	0	0	0
Standard	0	1.8	3.5
Gel Formulation	0	0	1.2

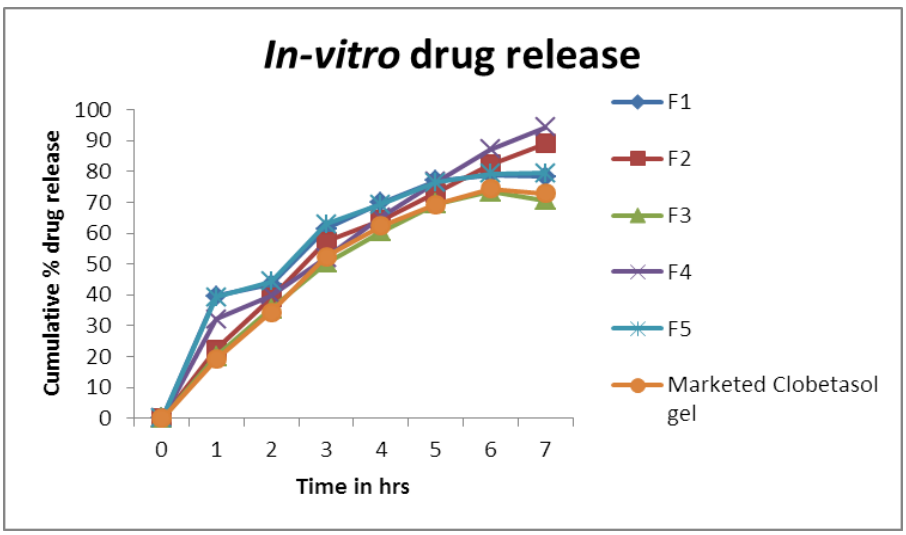
Where,
0 = No irritation,
0.5 = Faint, barely perceptible and slight dryness,
1 = Definite erythema but no eruption or broken skin,
1.5 = Well defined erythema with dryness and epidermal fissuring,
2 = Moderate erythema,
2.5 = Moderate erythema with barely perceptible edema,
3 = Severe erythema,
3.5 = Moderate to severe erythema with eschar formation,
4 = Moderate to severe erythema with eschar formation and edema extending the applied area.

3. *IN-VITRO* DRUG RELEASE STUDIES

***In-vitro* Drug Release data of Formulations from F1 to F5 and marketed formulation**

Time in hours	Cumulative % drug release					
	F1	F2	F3	F4	F5	Marketed Clobetasol gel
30min	39.7±0.73	22.3±0.66	20±0.65	32.1±0.33	39.3±0.14	19.2±0.19
1	43.4±0.54	39±0.85	35.4±0.34	39.8±0.24	44.4±0.23	34.3±0.26
2	61.4±0.35	57.2±0.25	50.4±0.52	52.1±0.65	62.9±0.62	52.4±0.43
3	69.9±0.64	64.3±0.95	60.4±0.16	65.1±0.95	69.3±0.51	62.4±0.65
4	77.1±0.85	73±0.74	69.5±0.27	76.7±0.87	76.4±0.36	69.3±0.59
5	79±0.12	82.3±0.25	73.5±0.26	87.1±0.24	79.3±0.58	74.4±0.26
6	78.4±0.52	89.1±0.26	70.7±0.85	94.3±0.15	79.5±0.26	72.9±0.54

SD = Standard deviation (n=3). The differences in mean of percentage cumulative drug release of all formulations of Clobetasol were significant ($p < 0.0001$)

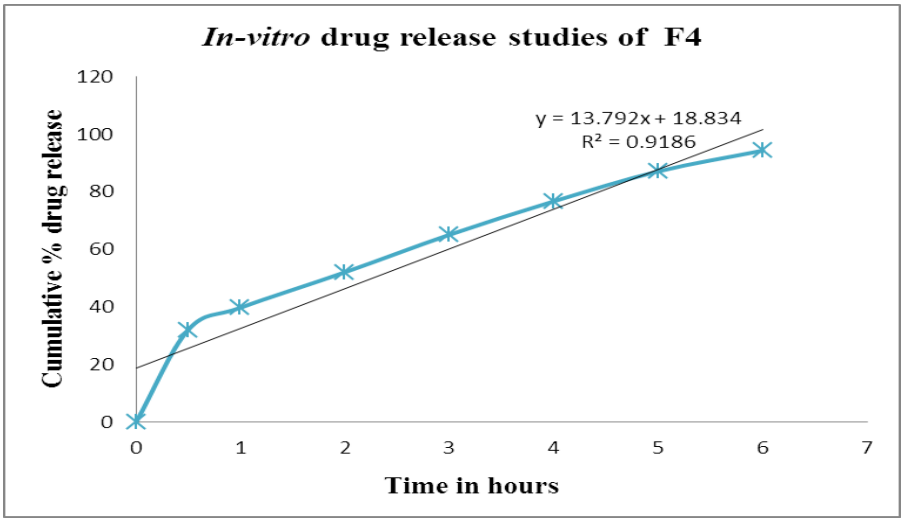


From the above data of *in-vitro* drug release studies, it was clear that all the formulations followed linear kinetics of which F5 showed better immediate release of the drug and F4 showed the maximum release of the drug at 6th hour. The drug release when compared to marketed formulation of clobetasol gel the drug release of the prepared ethosomal gel formulation F4 is found to be better than the marketed clobetasol gel.

4. Release Kinetics

These studies are performed to the formulations F4 because of their better ability to release the drug from the

formulations and also their chemical modification with cross linking agent.



By the release kinetics, it was confirmed that the drug release of F4 follows zero order kinetics and the regression coefficient value supports the above result. This result concludes that the drug release from F4 was not depends upon the concentration of6 drug in the formulation.

5. Stability studies

A) Percentage drug entrapment efficiency

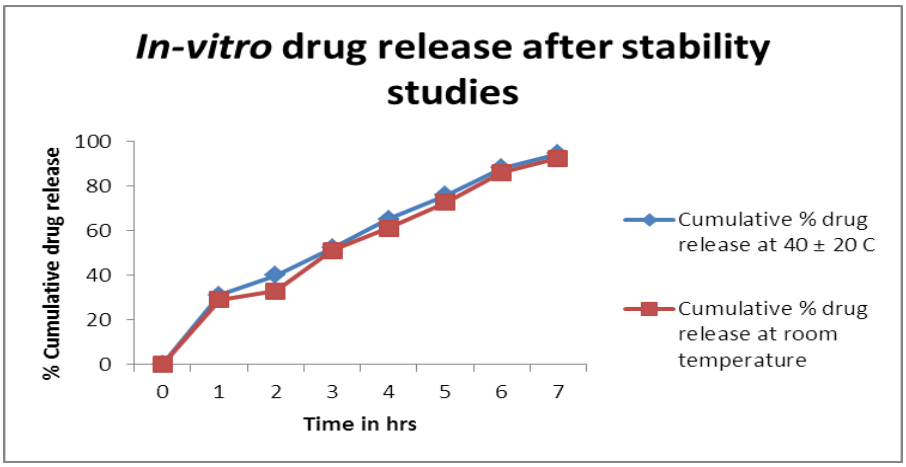
Entrapment efficiency percentage after stability studies

Formulation code	Entrapment efficiency percentage of the optimized formulation after stability studies	
	At $4^0 \pm 2^0$ C	At room temperature
F4	98.04±1.6	96.12±1.72

B) *In-vitro* drug release studies

Percentage cumulative drug release after stability studies

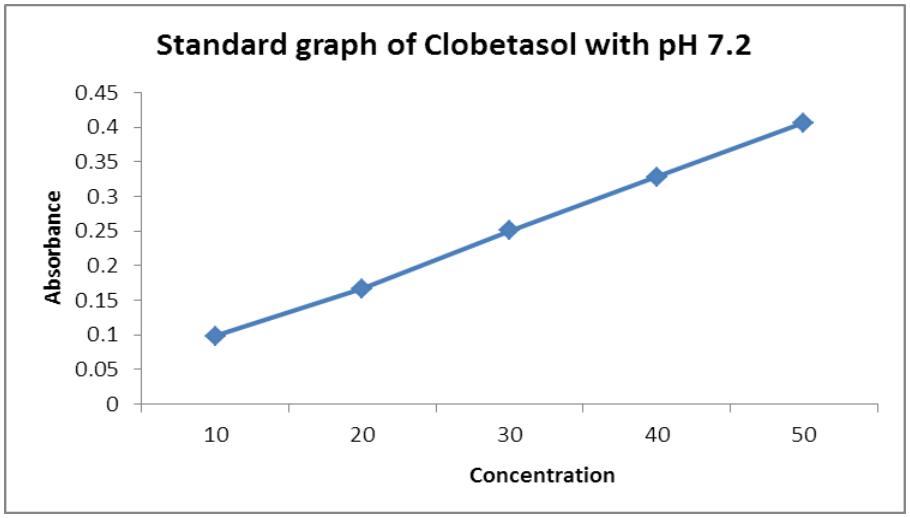
Time in hrs	% cumulative drug release	
	At $4^0 \pm 2^0$ C	At room temperature
30min	31.1±0.33	29.1±0.53
1	39.8±0.24	32.8±0.14
2	52.1±0.65	51.1±0.45
3	65.1±0.95	61.1±0.25
4	75.7±0.87	72.7±0.97
5	88.1±0.24	86.1±0.54
6	94.3±0.15	92.3±0.25



The stability studies of the optimized formulation F4 were performed and the results were given in the table and figure.

Absorbances of various concentrations of standerd solutions prepared with phosphate buffer saline of pH 7.2

S. No	Concentration (µg/ml)	Absorbance (237nm)
1	10	0.098
2	20	0.167
3	30	0.251
4	40	0.328
5	50	0.405



SUMMARY

- The present study involves formulation and characterization of ethosomal gel containing Clobetasol propionate used to treat dermatitis anti psoriasis. The transdermal delivery is one of the most important routes of drug administration. The main factor which limits the application of transdermal route for drug delivery is the permeation of drugs through the skin. One simple and convenient approach is application of drugs in formulation with elastic vesicles or skin enhancers.
- Hence, significant enhanced delivery of Clobetasol propionate through transdermal route could be obtained by using vesicular drug carrier systems - Ethosomes. The current study was aimed to investigate the potential of ethosomes in enhancement of clobetasol transport across the skin, characteristics of ethosomes and their *in-vitro* skin permeation behaviour. The present work also focuses on making the formulation more pharmaceutically acceptable.
- The prepared ethosomal gel formulations were characterized for vesical shape, vesicle size, physical appearance, spreadability, extrudability, viscosity, drug content, skin irritation study and percentage drug entrapment.
- *In-vitro* drug release profiles of prepared ethosomal gels were established.
- Based on *in-vitro* drug release profile it was found that release of medicament from formulated ethosomal gels followed zero order kinetics.

CONCLUSION

The results of present study clearly indicate that ethosomal gel formulations of Clobetasol propionate are possible. From the results obtained, the formulation F4 is concluded as the best formulation with vesicular size of 110 µm, spherical vesicular shape and 74.2% drug entrapment efficiency. The relatively high entrapment may be explained by multilamellarity of ethosomal vesicles From the *in-vitro* drug release studies, it was clear that all the formulations followed linear kinetics of which F5 showed better immediate release of the drug and F4 showed the maximum release of the drug at 6^{th} hour.**The drug release when compared to marketed formulation of clobetasol gel the drug release of the prepared ethosomal gel formulation F4 is found to be better than the marketed clobetasol gel.**In future research, preclinical studies of the optimized formulation are to be performed.

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