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STATISTICAL OPTIMIZATION OF TOPICAL GEL CONTAINING AZELAIC ACID FOR EFFECTIVE TREATMENT OF ACNE

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Keywords:

Azelaic acid,

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ABSTRACT

Acne is a common inflammatory skin disease that mainly affects the face, neck, chest and upper back. Treatment depends on severity. Azelaic acid has anti-keratinizing, antibacterial and anti-inflammatory activity. Bacteriostatic activity which inhibits the growth of bacteria. They mainly act by inhibiting cellular protein synthesis in aerobic and anaerobic bacteria. Azelaic acid is also used as antineoplastic agent. Azelaic acid is used to treat mild to moderate acnes. Azelaic acid works to treat acne by reducing the proliferation of bacteria called *Propionibacterium*, it also lessens inflammation and redness and also it normalize keratinization. The present study was conducted for statistical optimization of topical gel containing azelaic acid for effective treatment of acne. A 3^2 full factorial designs was successfully applied for the optimization. All the formulated gels were evaluated for clarity, homogeneity, pH, viscosity, drug content, spreadibility, extrudability, antibacterial activity and *In-vitro* drug release. *In- vitro* release data were fitted to different models to know exact mechanism of drug release. The antibacterial activity of Azelaic acid against *Staphylococcus aureus (S. aureus)* was investigated using agar plate method and zone of inhibition was measured. Formulation F8 showed the maximum drug content, *in-vitro* drug release and the zone of inhibition among all the formulations.

INTRODUCTION

Acne is a chronic inflammatory disease of the pilosebaceous unit characterized by the formation of both non-inflammatory and inflammatory skin lesions. The condition principally affects the face, chest, and back, which have a high density of sebaceous gland. *Acne vulgaris* is a common skin disease that involves individuals of all ages. Acne is a group of disorders that causes outbreaks of skin lesions commonly called pimples. Factors which contribute to the development of acne include hormonal imbalance, bacterial infection, stress, food, or cosmetic application additionally some species of *Propionibacterium* are found in milk and cheese and some time in other agricultural product. The management of the disease takes into account the severity of the disease, as well as patient factors like age, skin type, lifestyle, menstrual regularity, and so on.[1, 2] Pharmacotherapy of acne includes a number of drugs administered orally or topically. Topical administration of antiacne agents comprises an important part of therapy. Topical delivery is not only devoid of systemic toxicity caused by the drug, but also makes the drug available directly at the site of application.

Azelaic acid is a synthetic dermatologic agent belonging to the dicarboxylic acids and derivatives. It is used for the topical treatment of mild to moderate inflammatory *Acne Vulgaris*.[3] Topical treatment of dermatological disease as well as skin care, a wide variety of formulation ranging from solids to semisolids and liquids preparations are available to clinicians and patients. Topical preparations are formulae which are applied directly to an external body surface by spreading, rubbing, spraying or instillation. Formulations applied on skin such product referred as topicals or dermatologicals.[4]

MATERIALS AND METHODS

Materials:

Azelaic acid was provided by Cadila pharmaceuticals Pvt Ltd, Thane. Carboxymethylcellulose sodium was provided by Reliance cellulose. Propylene glycol, Methyl paraben, Propyl paraben were used of analytical grade.

Methods:

Determination of λmax in PBS pH 6.8: [5]

The UV spectrum of Azelaic acid was obtained using UV-Visible Double Beam Spectrophotometer (V630, Jasco).Accurately weighed 10 mg of the drug was dissolved separately in phosphate buffer solution pH 6.8 and volume was made up to 100 mL by the buffer

solution to obtain a stock solution of final concentration 100 µg/mL. Aliquot (1 mL) of stock solution of Azelaic acid was transferred into a series 10 mL volumetric flask and volume was made up to the mark with the buffer solution to produce the concentration range 10 µg/mL. The resultant solution was scanned from 200 to 400 nm and the spectrum was recorded to obtain the value of maximum wavelength. The λmax was found to be 204 nm.

Drug excipient compatibility study [6]

FTIR:

Compatibility study was carried out by using Fourier transform Infrared spectrophotometer (Shimadzu). FTIR study was carried on pure drug. Physical mixture of drug and polymers were prepared and samples kept for 1 month at 40^0C. The infrared absorption spectrum of physical mixture of drug and polymerswas recorded using KBr disc over the wave number 4000 to 650 cm^{-1}.

Preparation of acne gel of azelaic acid: [7]

Acne gels were prepared by dispersion technique. Preparation of Solution A:Accurately weighed (3 gm) quantity of azelaic acid was dissolved in sufficient quantity of propylene glycol and then parabens were added. Preparation of Solution B: Na CMC was weighed, dispersed in sufficient quantity of water and allowed to hydrate. Solution A was added to solution B with continuous stirring. pH of gel was adjusted using 0.1N NaOH.

Formulation optimization: [8]

3^2full factorial design was applied to the formulation that showed the satisfactory results. To see the effect of concentration of variables Propylene glycol (X1) and Na CMC (X2) on various responses like % drug release and antibacterial activity. For the Propylene glycol lower, middle and higher level were 1.5,3 and 4.5 ml respectively. Similarly for the Na CMC lower, middle and higher level were 0.9, 1.35 and 1.8 g respectively. Composition of batches is shown in table no.1

Formulation code / Ingredient	F1	F2	F3	F4	F5	F6	F7	F8	F9
Azelaic acid (gm)	3	3	3	3	3	3	3	3	3
Propylene glycol (ml)	1.5	3	4.5	1.5	3	4.5	1.5	3	4.5
Na CMC (gm)	0.9	0.9	0.9	1.35	1.35	1.35	1.8	1.8	1.8
Methyl paraben (gm)	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Propyl paraben (gm)	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
NaOH (0.1N)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Distilled water (up to)	30	30	30	30	30	30	30	30	30

Table no.1: composition of formulation

Evaluation of acne gel

1. Clarity: [9]

On careful visual inspection against dark and white background, all the prepared gel formulations were found to be free from any suspended particulate matter. All the formulations were found to be clear.

2. Homogeneity: [10]

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

3. pH: [11]

The pH of the various gel formulations was determined by using digital pH meter.

4.Viscosity Determination: [9]

The viscosity of the prepared hydrogel formulations were measured at room temperature by Brookfield viscometer (DV-II +) attached with spindle 64. The spindle was rotated at varying Rpm and readings were recorded to study the effect of shearing stress on viscosity.

5. Drug content: [11]

The drug content of the gel formulations was determined by dissolving an accurately weighed quantity 1gm of gel in 100ml of solvent phosphate buffer pH 6.8. The volumetric flask containing gel solution was shaken for the specific period of time period until the gel gets completely dissolved. Then the solutions were filtered through suitable filter paper and proper dilutions were made and solutions were subjected to the Spectrophotometric analysis. The drug content was calculated from the linear regression equation obtained from the calibration data.

6. Spreadability: [9]

Ideal gel must have low spreadability values but good consistency. Spreadability of formulations was determined by an apparatus suggested by Multimer et al. which was fabricated itself in laboratory and used for study. The apparatus consist of a wooden block, with a fixed glass slide and movable glass slide with one end tied weight pan rolled on pulley, which was in horizontal level with fixed slide. An excess of gel (2 gm) was placed in between two glass slides and then 100 gm weight was placed on slides for 5 min to compress the sample to a uniform thickness. Weight (25 gm) was added to pan. The time (seconds) required to separate the two slides was taken as a measure of spreadability.

It was calculated using formula:

S = m. l / t

Where, S = spreadability

m = weight tied to upper slide

l= length of glass slide

t = time taken

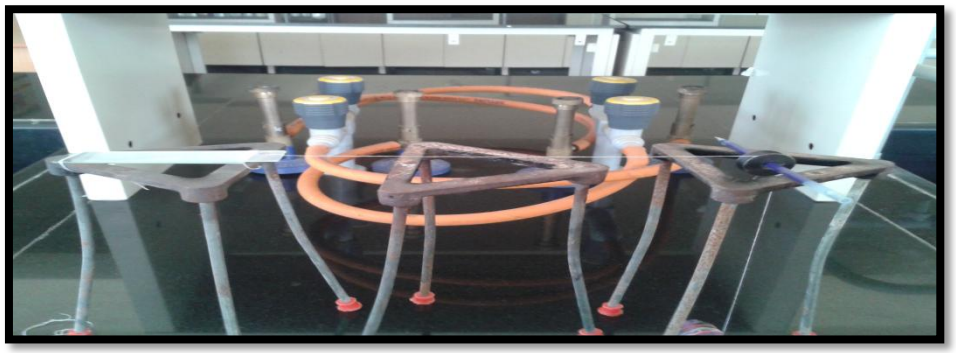

Figure no.1: spreadability assembly

7. Extrudability: [10]

The gel formulation were filled in standard caped collapsible lami-tube and sealed. The tube was weighed and recorded. The tube was placed between two glass slides and was clamped. A 500 g weight was placed over the glass slide and then cap was opened. The amount of gel extruded were collected and weighed. The % of gel extruded was calculated; and grades were allotted (+ + + Good, + + fair, + Poor).

8.Antibacterial Activity: [9, 12, 13, 14]

An Agar diffusion method was used for the determination of antibacterial activity of formulations. Standard Petri dishes (9 cm diameter) containing medium to a depth of 0.5 cm were used. The sterility of the plates and all other glassware was controlled before performing the test, by keeping them in autoclave at 121^0C for 15 mins. Fresh colonies of *S. Aureus* were prepared on Nutrient agar slants by incubating the slants for 24 hrs at 37 ± 0.5^o C. Inoculum were prepared from these freshly prepared colonies of *S. Aureus* by suspending 2-3 colonies in 1 mL of sterile saline solution with the help of nichrome wire loop. The inoculum (0.5 ml) was spread over the surface of nutrient agar plates and the plates were allowed to solidify prior to addition of the formulation. The bores of 0.5 cm diameter were made with the borer and the formulation gel samples were added in the bores. Plates were then kept for incubation at 37 ± 0.5^0C for 24 hrs. After incubation the zone of inhibition (in mm) around the bores was measured.

9.*In-vitro* Drug Release Study: [15, 11, 16, 17]

Figure no. 2Laboratory-assembled apparatus for Diffusion study

Laboratory-assembled apparatus resembling a Franz diffusion cell was used to determine the release profile of drug from topical gel. The cell consisted of two chambers, the donor and the receptor compartment between which a diffusion membrane (egg membrane) was mounted. The donor compartment, with inner diameter 24 mm, was open i.e. exposed to the atmosphere at one end and the receptor compartment was such that it permitted sampling. The diffusion medium used was phosphate buffer solution pH 6.8 (PBS). 1 gm of the drug containing topical gel was placed in the donor compartment separated from the receptor compartment by the egg membrane. The egg membrane was previously soaked for 24 hr. in PBS. The donor and receptor compartments were held together using a clamp. The position of the donor compartment was adjusted so that egg membrane just touches the diffusion medium. The whole assembly was fixed on a magnetic stirrer. The receptor compartment with 100 mL PBS was placed on a thermostatically controlled magnetic stirrer. It was maintained at 37 ± 0.5ºC and stirred constantly at 50 rpm. Samples of 1 mL were collected at predetermined time intervals and analysed for drug content by UV Spectrophotometer at λ_{max} against blank. The receptor phase was replenished with an equal volume of phosphate buffer at each time of sample withdrawal. ($y=0.0025x + 0.0213$, $R^2=0.9878$)

Parameters	
Reference apparatus for test:	Franz Diffusion Cell
Mode of Agitation	Magnetic stirrer (50 rpm)
Mode of Temperature control	Thermostat (37±0.5ºC)
Donor compartment	One side open-ended tube, 24 mm diameter
Receptor compartment	250 mL beaker containing 100 mL phosphate buffer solution pH 6.8
Semi-permeable membrane	Egg membrane

Table no. 2 : Parameters of Laboratory-assembled apparatus for Diffusion study

10. Drug release kinetics: [18]

To examine the drug release kinetics, the release data were fitted to models representing zero order, first order, Higuchi's square root of time kinetics and Korsemeyer Peppas kinetics. The coefficient of determination (r^2) values were calculated from the plots of CDR vs. t for zero order, log %CDR remaining vs. t for first order, %CDR vs. $t^{1/2}$ for Higuchi model and log %CDR vs. log t for Korsemeyer Peppas model, where %CDR is the amount of drug released at time t. The data obtained from study of diffusion kinetics of the optimized formulation was studied to obtain the best fit model. The best fitted model is the one which gives the highest R^2 value and least slope value.

11. Skin irritation test: [19]

Table no. 3: Test conditions for skin irritation test

Test Conditions	
Strain of rat	Wistar albino
Weight of rat	150 g
No. of groups	3
No. of animals per group	3
Dose	1 gm formulation over 2 cm^2 area topically

The protocol was approved by Institutional Animal Ethics Committee with approval no-IAEC/2014-15/061514**.** The rats (n=9) were randomly divided into 3 equal groups for application of standard irritant, optimized formulation or test and negative control (no application). Hairs were removed by hair removal cream (Anne French) from an area (2 cm^2) on the dorsal side of the albino rats to make a hairless area. A 0.8% v/v aqueous solution of formalin was applied as a standard irritant to rats chosen randomly for standard irritant application (n=3) on the following day. The optimized formulation was applied to group 2 of rats (n = 3) for assessing any kind of irritation at specified sites. Formulation was removed after 24 h and skin was examined for any sign of erythema and oedema. The administration sites were assessed for signs of skin irritation, and this test procedure was repeated for another 6 days. The resulting reactions were compared against control group (n=3).

Sr. no.	Score	Rating
1.	0	Nil
2.	0-2	Mild
3.	2-4	Moderate
4.	4-6	Severe
5.	6 and above	Very severe

Table no. 4: Score rating for skin irritation study

12. Accelerated Stability study: [20]

Stability studies were conducted according to ICH guidelines 40°C± 2°C/75%RH ± 5%RH to test physical appearance in terms of clarity, pH, viscosity and drug content were evaluated.

RESULTS AND DISCUSSION

Compatibility study FTIR:

The IR spectra of azelaic acid, polymer and physical mixture were generated. The IR absorption bands observed in the IR spectrum of drug and polymers resembles with that of found in the physical mixture proves compatibility of drug with polymers

Clarity:

On careful visual inspection against dark and white background, all the prepared gel formulations were found to be free from any suspended particulate matter. All the formulations were found to be clear.

Homogeneity:

After the visual inspection all the formulations were found to be free from aggregates, the appearance was clear and gels were homogenous with no lumps or precipitate. Homogeneity test of the gels showed that the drug was completely dissolved in the co-solvent, also all the polymers and the drug solutions were mixed properly.

pH: Table no.6: pH values of formulations

Sr. no.	Formulation code	Observed pH (±SD)
1.	F1	6.72±0.045
2.	F2	6.73±0.044
3.	F3	6.83±0.01
4.	F4	6.89±0.02
5.	F5	6.93±0.037
6.	F6	6.91±0.049
7.	F7	6.80±0.018
8.	F8	6.96±0.032
9.	F9	6.84±0.035

The pH of all the formulations from F1 to F9 was found to be in the range of 6.7 to 6.9.

Viscosity Determination:

The viscosity values of formulations are shown in table no.7

Rpm	Viscosity (cP) at Room Temperature								
	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
1.	59990	64786	48556	53501	54397	54589	49190	46790	42556
2.	53991	57288	46321	52009	53225	50212	46091	35195	39568
3.	52490	46790	42115	51254	52597	45625	37392	25595	37432

Table no.7: Viscosity of formulations

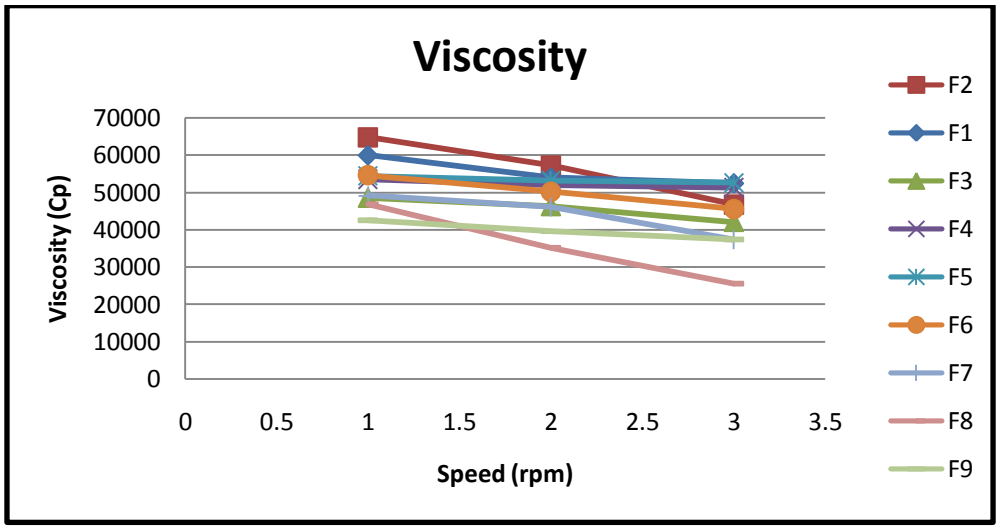

Figure no.3: Viscosity profile of formulations

Viscosity v/s rpm plots for all formulations shows decrease in viscosity as shear rate (rpm) was increased. Concentration of Na CMC and PG was a major factor affecting viscosity of formulations.

Drug content: The Drug content of formulations is shown in table no.8.

Sr. no.	Formulation code	Drug content (%) (±S.D.)
1.	F1	98.28±0.000091
2.	F2	98.62±0.00005
3.	F3	98.2±0.0002
4.	F4	99.08±0.000014
5.	F5	98.52±0.0042
6.	F6	98.08±0.00016
7.	F7	98.2±0.00013
8.	F8	100.16±0.00016
9.	F9	98.24±0.001

Table no.8: Drug content of topical gel

The percentage drug content of all prepared formulations was found to be in the range of 98-102 %.

Spreadability:

Sr.no.	Formulation code	Spreadability (gm.cm/sec)(± S.D)
1.	F1	22.13±0.40
2.	F2	16.25±0.70
3.	F3	15.71±1.08
4.	F4	20.31±0.70
5.	F5	25.63±0.40
6.	F6	16.25±1.73
7.	F7	17.39±1.08
8.	F8	22.13±0.81
9.	F9	15.73±0.40

Table no.9: Spreadability of topical gel

Spreadability of gel is very important in the topical gel formulations. Spreadability shows direct relation with the viscosity of the gel. Formulations with higher viscosities i.e. are very thick in nature are difficult to spread, on the contrary gels having very low viscosities have fluid like appearance, both the extremes are not suitable for any of the topical gel preparation. Hence gel having optimum viscosity provides proper spreadability to the formulations.

Extrudability:

Sr.no.	Formulation code	Extrudability
1.	F1	+
2.	F2	+
3.	F3	++
4.	F4	++
5.	F5	++
6.	F6	++
7.	F7	++
8.	F8	++
9.	F9	+++

Table no.10: Extrudability of topical gel

Antibacterial activity:

Sr.no.	Formulation code	*S. Aureus*	
		Zone Of Inhibition (mm)	% Efficiency
1.	Standard	10	100
2.	F1	8	80
3.	F2	7.3	73
4.	F3	6.3	63
5.	F4	7.2	72
6.	F5	7.9	79
7.	F6	7	70
8.	F7	8	80
9.	F8	9	90
10.	F9	8.6	86
11.	Marketed	10	100

Table no.11: zone of inhibition and % efficiency of topical gel

In the antibacterial studies the bacteria used was *S. aureus*. The studies were carried for the all formulations and zone of inhibition of gel was measured. The results were shown in table no. 11. The result was found satisfactory. These results were compared with marketed product. The F8 formulation showed higher zone of inhibition.

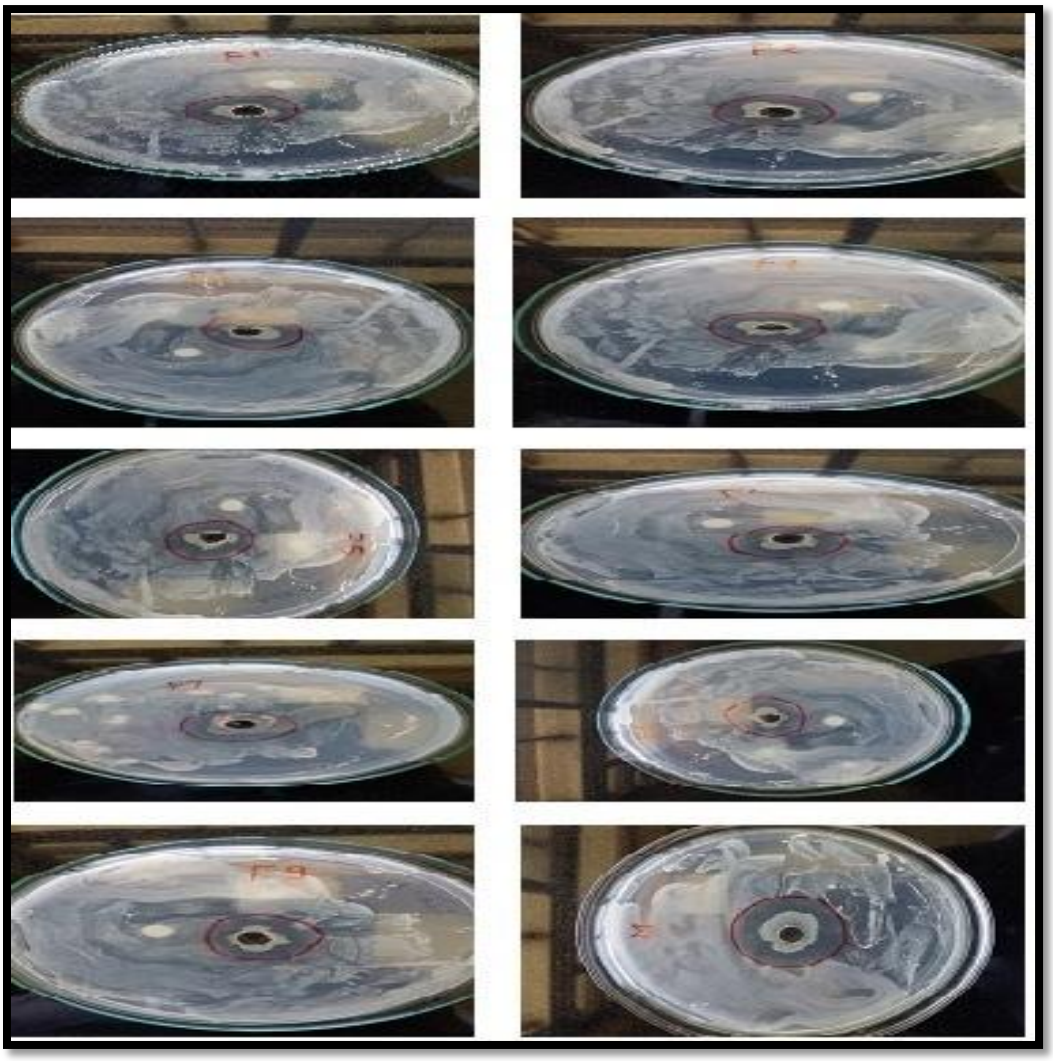

Figure no.4: Antibacterial activity of formulations

The standard value for zone of inhibition of Azelaic acid against *S. Aureus* is 10 mm. The antibacterial study of gel in comparison with marketed formulation indicates that optimized gel formulation of Azelaic acid is capable of eliciting antibacterial activity with better results. Optimized batch F8 showed 9mm zone of inhibition which is the highest among all the batches.

***In-vitro* Drug Release Study:**

Time (hrs)	Cumulative Drug Release (%) (±S.D.)				
	F1	F2	F3	F4	F5
0.	0	0	0	0	0
1.	0.32±0.00011	0.88±0.0001	0.72±0.0003	0.89±0.0002	1.4±0.00011
2.	3.68±0.0002	3.96±0.000093	3.96±0.00014	3.96±0.0002	5.16±0.0002
3.	8.44±0.00026	9.24±0.00026	9.48±0.000072	8.28±0.00026	11.52±0.00014
4.	15.6±0.00026	17.04±0.00011	17.4±0.00011	17.04±0.00026	19.2±0.00026
5.	25.84±0.000086	27.36±0.00015	28.24±0.00014	26.36±0.00014	30.12±0.00026
6.	38.52±0.00013	40.76±0.00015	41.28±0.0001	39.76±0.00022	42.56±0.00011

Time (hrs)	Cumulative Drug Release (%) (±S.D.)				
	F6	F7	F8	F9	Marketed gel
0	0	0	0	0	0
1	0.92±0.000068	0.96±0.00005	1.08±0.00026	0.92±0.00015	5.02±0.0001
2	4±0.000093	4±0.00022	4.92±0.0001	4.12±0.0002	11.31±0.00011
3	9.44±0.00026	9.4±0.00022	11.84±0.00017	9.36±0.0003	18.26±0.00005
4	17.2±0.00026	17.24±0.00014	20.44±0.00017	17.4±0.000068	25.94±0.00015
5	27.76±0.00016	28.28±0.00017	31.32±0.00015	28.24±0.00019	34.94±0.00011
6	40.12±0.000057	42.92±0.00013	44.4±0.000068	41.24±0.00014	44.97±0.00018

Table no.12: Cumulative Drug release of formulations

From the diffusion study it can be said that maximum release is shown by F8 formulation. The data also suggests that gel formulations are capable to produce linear drug release for a longer period of time. The optimized formulation F8 was compared with marketed gel shows 44.4% and 44.97% drug release respectively in 6hrs.

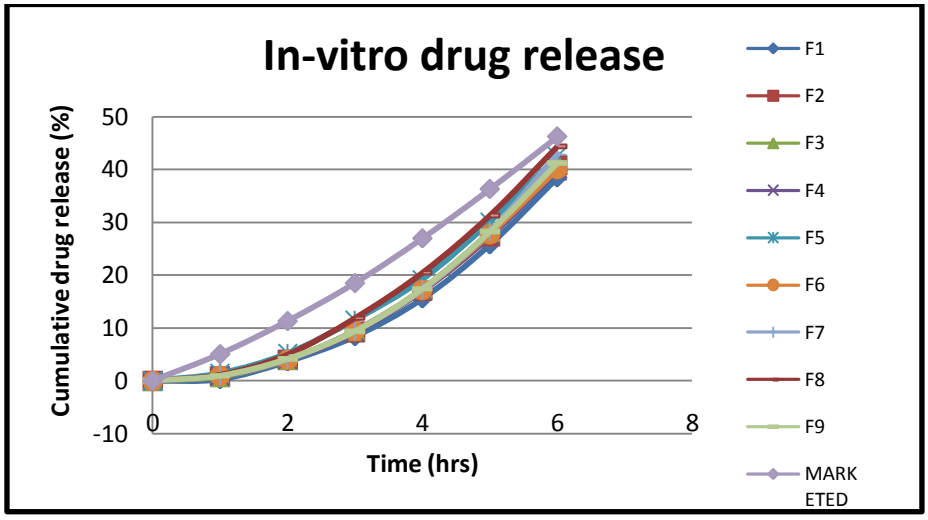

Figure no.5: *In-vitro* drug release profile of formulations F1 to F9

Drug release kinetics:

The classical zero order release curve was found to be linear. The curves plotted according to first order and Higuchi release model were also found to be linear. For the Korsemeyer-Peppas release curves r^2 was found to be ≥ 0.75 for all 9 formulations and n value was found to be ≥ 0.5 which indicates that all the formulations show anomalous (non-Fickian release i.e. swellable matrix).The drug release occurs probably by diffusion and erosion.

Optimization:

In 3^2 Factorial Design X1 and X2 are the amounts of PG and Na CMC respectively, and Y1 and Y2 are % drug release and antibacterial activity in terms of % efficacy respectively. ANOVA for the dependent variables % drug release and antibacterial activity. The values of X_1 and X_2 were found to be significant at $p < 0.05$, hence it can be confirmed that both the variables have a significant effect on the selected responses. From this data optimum concentration of PG was found to be 3 ml and that for Na CMC was found to be 1.8gm. 3D surface response shown in figure no. 6 and 7.

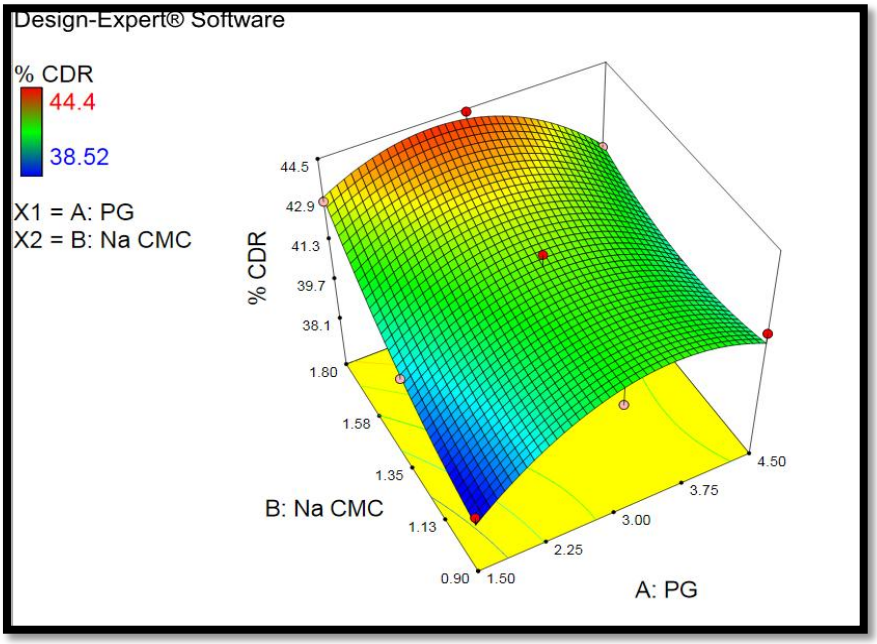

Figure no.6: Surface response plot showing effect of propylene glycol and carboxymethyl cellulose sodium on Drug release

It is shown that both the independent variables have a significant effect on the dependent variable (drug release).

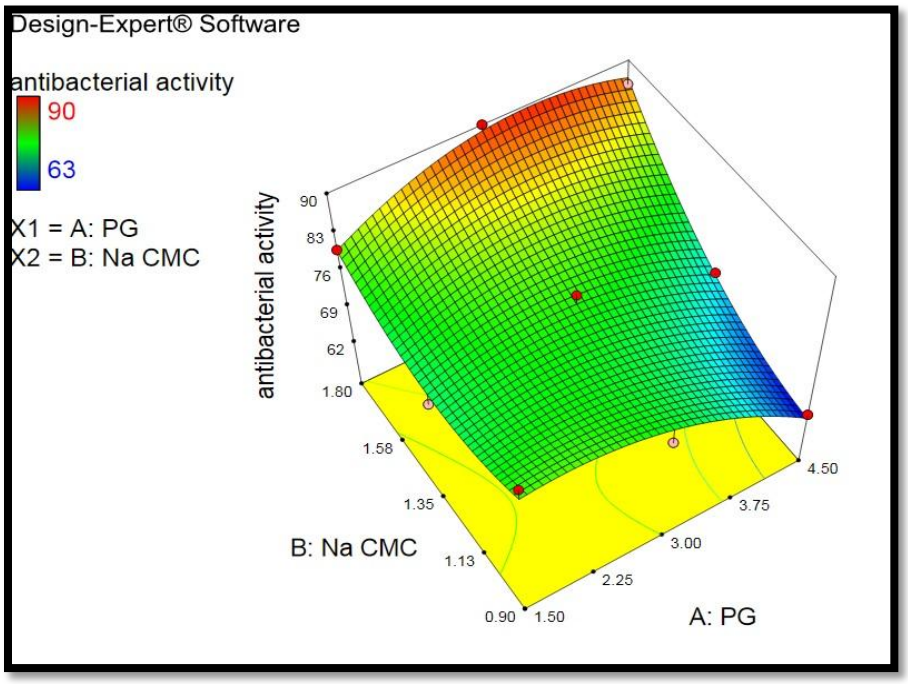

Figure no.7: Surface response plot showing effect of propylene glycol and carboxymethyl cellulose sodium on antibacterial activity

The figures above show the effect of concentration of PG and Na CMC on drug release and antibacterial activity. It is shown that both the independent variables have a significant effect on the dependent variables.

Optimized formula:

After generating model equations relating main effects and responses various gel formulations containing azelaic acid were optimized based on *In-vitro* drug release at 6 hours (Y1) and antibacterial activity (Y2). The optimal values for responses were obtained by numerical analysis based on the criteria of desirability and optimal batch was selected. Optimized batch (F8) having highest drug release and antibacterial activity. This reveals that mathematical model obtained by factorial design to produce optimized responses was well fitted.

Accelerated stability study:

Results of the stability studies showed that there is no change in the physical parameters of the formulation. Drug content of the formulation was found to be same as that before stability testing.

CONCLUSION

The present study was conducted with the view to formulate a topical gel formulation of Azelaic acid for effective treatment of acne. In the present investigation, an attempt was made to develop antiacne topical gel of Azelaic acid using Propylene glycol as co-solvent and penetration enhancer and Na CMC used as gelling agent and bioadhesive polymer which would increase the residence time of applied gel thus prolonging the drug delivery which would thus increase the patient compliance due to reduced frequency of application. The prepared topical gels were characterized by clarity, homogeneity, pH, drug content, spreadability, extrudability, *in-vitro* drug release, antibacterial activity, skin irritation test and stability studies.

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