Preparation, Evaluation and Optimization of Film Forming Gel of Benzoyl Peroxide

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ABSTRACT – The drug benzoyl peroxide (BPO) is the main drug used for mild to moderate acne due to its effectiveness 8. It is an antimicrobial drug which kills the anaerobic bacteria Propionibacterium acne, the bacteria involved in pathogenesis of disease. It is sebostatic, comedolytic and anti-inflammatory agent. But low water solubility and limited skin permeation upon topical application requires high amount of drug for desired action which leads to many side effects such as redness, skin irritation, dryness oedema and skin peeling 9. These may be due to keratolytic property and high concentration used of benzoyl peroxide. The present study focus on Preparation, Evaluation and Optimization of Film Forming Gel of Benzoyl Peroxide. Benzoyl Peroxide Film Forming Gel of Benzoyl Peroxide was prepared using dispersion method. The main objective of the study is to preparing Benzoyl Peroxide in the form of FFG which may give sustained release action by using Eudragit LR100 and HPMC in combination. Experimental design was obtained using design expert software and by using 3 by 2 factorial design method in which the effect of concentration of Eudragit LR100 and concentration of HPMC 4000 on Film Forming gel was studied. 9 batches of film forming gel of benzoyl peroxide war prepared and evaluated for various parameters. By using Optimization Technique, Batch B8 was selected as the optimize batch in which 12% of Eudragit LR100 and 1 % of HPMC 4000 was used. The optimized batch was evaluated for skin irritation test and stability test. Skin irritation test was carried out and NO presence of erythema or oedema were seen on the animals after 14 days of application of Film Forming Gel. Stability test was also carried out and Film Forming gel was found to be stability for 60 days after preparation.

KEYWORDS – Benzoyl Peroxide, Anti- Microbial Drug, Film Forming Gel (FFG), Sustained Release, Optimization, Skin Irritation test.

1. INTRODUCTION

Topical preparations are used for the localized effects at the site of their application by virtue of drug penetration into the underlying layers of skin or mucous membranes. The main advantage of topical delivery system is to bypass first pass metabolism. Avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption, like pH changes, presence of enzymes, gastric emptying time are other advantage of topical preparations. Semi-solid formulation in all their diversity dominate the system for topical delivery, but foams, spray, medicated powders, solution, and even medicated adhesive systems are in use. The topical drug delivery system is generally used where the others system of drug administration fails or it is mainly used in pain management, contraception, and urinary incontinence. Topical drug delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorders (e.g. acne) or the cutaneous manifestations of general disease (e.g. psoriasis) with the intent of confining the pharmacological or other effect of the drug to the surface of the skin or within the skin. Topical activities may or may not require intra-cutaneous penetration or deposition (1, 2). Topical drug delivery systems include a large variety of

pharmaceutical dosage form like semisolids, liquid preparation, sprays and solid powders. Most widely used semisolid preparation for topical drug delivery includes gels, creams and ointments (3).

The topical dosage forms, i.e. patches, ointments, creams, etc., are associated with several limitations. Patches have various disadvantages, most commonly skin irritation, because of their occlusive properties causing obstruction of sweat ducts, which in turn prevents loss of water vapour from skin surface, difficulty in applying on the curved surfaces, pain while peeling off and poor aesthetic appeal (4). Semisolid preparations like creams and ointments overcome some of these drawbacks but have other limitations. These do not ensure persistent contact with the skin surface and can be easily wiped off by patient's clothes (5). Therefore there is a need for development of a dosage form which permits less frequent dosing by maintaining a close contact with the skin for prolonged time period thereby improving the patient compliance.

Film forming system (FFS) is a novel approach which can be used as an alternative to conventional topical and transdermal formulations. It is defined as non-solid dosage form that produces a film in situ, i.e. after application on the skin or any other body surface. Such compositions can either be liquids or semisolids with a film forming polymer as basic material for the matrix. These systems contain the drug and film forming excipients in a vehicle which, upon contact with the skin, leaves behind a film of excipients along with the drug upon solvent evaporation. The formed film can either be a solid polymeric material that acts as matrix for sustained release of drug to the skin or a residual liquid film which is rapidly absorbed in the stratum corneum (4, 6, 7).

This research work involves the Formulation, Evaluation and Optimization of Film Forming Gel of Benzoyl Peroxide. The drug benzoyl peroxide (BPO) is the main drug used for mild to moderate acne due to its effectiveness (8). It is an antimicrobial drug which kills the anaerobic bacteria Propionibacterium acne, the bacteria involved in pathogenesis of disease. It is sebostatic, comedolytic and anti-inflammatory agent. But low water solubility and limited skin permeation upon topical application requires high amount of drug for desired action which leads to many side effects such as redness, skin irritation, dryness oedema and skin peeling (9). These may be due to keratolytic property and high concentration used of benzoyl peroxide. It is used in the concentration of 2.5-10% in the form of washes, gel and cream. The main objective of the study is to preparing Benzoyl Peroxide in the form of FFG which may give sustained release action by using Eudragit LR100 and HPMC in combination.

2. MATERIAL AND METHOD

2.1. Material - Benzoyl peroxide, Eudragit LR100, HPMC 4000, Isopropyl Myristate, PEG 400, Glycerine, Tween 80, Dioctyl Sodium Sulfoccinate and Ethanol were Obtained from NuLife, Pharmaceuticals, Pimpri-chinchwad. All the ingredient used were of Analytical Grade.

2.2. Interaction studies of the drug and polymer

Presence of any undesirable interaction between the drug and the polymers was assessed using DSC studies. An accurate amount of BPO, polymer and BPO and polymer physical mixture kept under stress conditions of 40°C and 75% RH for 2 months, was weighed and transferred to the aluminum pans and the rate of heating was 10°C per minute from 35°C to 300°C. Any changes in the DSC thermogram were observed (10).

2.3. Method of Preparation of Film Forming Gel

The polymeric solutions of Eudragit LR100 and Hydroxypropyl cellulose were prepared in ethanol using dispersion method. Eudragit LR100 was sprinkled over ethanol containing Isopropyl Myristate. Hydroxypropyl cellulose was sprinkled over ethanol separately. The polymeric solutions were mixed properly with continuous stirring till clear solution is obtained. Glycerine was added to the above solution. Accurately weighed quantity drug (BPO) was dissolved in PEG 400, Tween 80 and Transcutol. The drug solution and polymeric dispersion were mixed properly with continuous stirring and volume was made up to the mark using ethanol (11-18).

2.4. Experimental Design Using Factorial Design

The Experimental Design for formulating Film Forming Gel of BPO was done using Full Factorial design method. The 3^2 Central Composite Factorial design was generated using Design expert stat ease software (Version 13.0). In this design, effect of independent factor on Dependant factor (Response) was studied. Concentration of Eudragit (X1), Concentration of Hydroxypropyl cellulose (X2) were selected as the two independent factor for study. Each factor was evenly set at low, medium, and high levels as shown in the Table no 2.1. % Drug Release (Y1), and Anti-bacterial Activity (Y2) were selected as the Dependent Factors (Response) (19).

Table no 2.1 – Independent Factors with their Level

Independent Factor	Unit	Variable Level		Actual Value
Concentration of Eudragit (X1)	% w/v	Low	-1	6
		Medium	0	12
		High	+1	18
Concentration of Hydroxypropyl cellulose (X2)	% w/v	Low	-1	1
		Medium	0	3
		High	+1	5

2.5. Evaluations

2.5.1. Physical Appearance

The prepared Film forming gel of BPO was inspected visually for clarity, colour and presence of any particulate matter.

2.5.2. pH of the Formulation

pH of the developed gels was measured using digital pH meter. The stable readings were taken after dipping the probe of pH meter into the gel. Likewise, three reading were taken for individual gel formulation and then the average was calculated (20).

2.5.3. Integrity of formulation on skin:

The formulation was applied to the forearm of a volunteer as described for the assessment of the drying time. The dry film was then worn overnight by the test subject. After 24 hours the test area was examined visually for completeness of the film, appearance of cracks or flaking (21, 22).

2.5.4. Viscosity

To determine the viscosity of the gel Prepared, Brookfield Viscometer was used. Halipath spindle no 96 was used. About 25 ml Gel was taken in the cylindrical container. The halipath spindle was dipped in the gel. Viscosity of the gel was determined at 10, 20, 30, 50, 60 and 100 RPM (15, 18).

2.5.5. Spreadability

The spreadability of the formulation was determined by measuring the spreading diameter of 0.5 g of gel formulation in between two horizontal smooth surface glass plates (20 cm × 20 cm). The initial diameter of the spreading of the gel in centimeters formed by placing the gel on the glass plate was noted. Another glass plate with the same dimensions was placed over the gel for 1 min until no more expansion of the gel was observed. The upper plate was gradually removed and diameter of the circle formed after spreading of the gel was measured in centimeters (12, 13, 22).

2.5.6. Drying time:

For the assessment of the drying time the formulation was applied to the inner sides of the forearm of a volunteer, who participated in the study on informed consent basis. After 2 minutes a glass slide was placed on the film without pressure. If no remains of liquid were visible on the glass slide after removal, the film was considered dry. If remains of liquid were visible on the glass slide the experiment was repeated until the film was found to be completely dry (21, 22).

2.5.7. Outward Stickness

The stickiness of the outer surface was tested by pressing cotton wool on the dry film under low pressure. Depending on the quantity of cotton fibres that were retained by the film the stickiness was rated high (dense accumulation of fibres on the film), medium (thin fibre layer on the film) or low (occasional or no adherence of fibres) (14).

2.5.8. Cosmetic Attractiveness

The cosmetic attractiveness of the film was assessed by visual examination of the dry films. Transparent films with a low skin fixation had a high attractiveness as they were almost invisible. Opaque films and films with a medium skin fixation were considered less attractive as they exhibited an increased visibility and a slight wrinkling of the skin. Whitish films and films causing heavy wrinkling of the skin due to strong skin fixation displayed only a low attractiveness (14).

2.5.9. Drug Content

Accurately weighed amount of gel of about 1 g was taken in a 100 ml volumetric flask containing 50 ml of phosphate buffer solution of pH 7.4 and the volume was made up to the mark with phosphate buffer. The volumetric flask containing gel was sonicated for 15 min to ensure complete solubility of the drug. The solution was then filtered through 0.45 µm pore size membrane filter. The absorbance of prepared solution was measured at λmax of 376 nm against phosphate buffer (pH 7.4) as a blank using ultraviolet/visible spectrophotometer (11-13).

2.5.10. *in-vitro* Drug Release (Diffusion study)

Laboratory-assembled apparatus resembling a Franz diffusion cell was used to determine the release profile of drug from film forming gel. The cell consisted of two chambers ie, the donor and the receptor compartment between in 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 5.8. 1 mL of the drug containing film forming gel was placed in the donor compartment over the drug release membrane and was separated from the receptor compartment by the egg membrane. The egg membrane was previously soaked for 24 hours 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 of PBS was placed on a thermostatically controlled magnetic stirrer. It was maintained at 37 ± 0.5 °C and stirred constantly at 50 rpm. Samples of 1 mL were collected at predetermined time intervals and analyzed 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 (24-26).

2.5.11. Anti-Bacterial Activity

An agar diffusion method was used for the determination of anti-bacterial activity of formulations. Standard Petri dishes (7.5 cm diameter) containing medium to a depth of 0.5 cm were used. The sterility of the lots was controlled before use. Inoculum was prepared by suspending *S.aureus* from 24 hr. cultures in agar medium into tubes containing 10 mL of sterile saline. The tubes were diluted with saline. The inoculum (0.5 mL) was spread over the surface of agar and the plates were dried at 35°C for 15 min prior to placing the formulation. Bores of 0.5 cm diameter were prepared and 20µl samples of formulation (1% w/v) were added in the bores. After incubation at 35°C for 2 days, the zone of inhibition around the bores was measured (18, 27).

2.6. Optimization of Film forming Gel of BPO

Optimization of the formulations was studied by 3^2 full factorial design. i.e. 3 level and 2 Factor. The first factor is Concentration of Eudragit (X1) and second factor Concentration hydroxypropyl methyl cellulose (X2) were selected as independent variables and the dependent variables were % drug release and anti-bacterial activity. The data obtained were by using Design expert version 13 software and analysed statistically using analysis of variance (ANOVA). Concentration of Eudragit and hydroxypropyl methyl cellulose's effects on the dependent variables were also investigated using a 3-D response surface methodology on the data (19, 28).

2.7. Evaluation of Optimized Batch

2.7.1. Skin Irritation study

Skin Irritation study was carried out using the Optimized Batch of Film Forming Gels. As per OECD Guidelines 404, animals are divided into groups each containing 6 rats. Approximately 24 hours before test, fur on back of the rats are removed and 2 fields with dimensions 2 cm2 was marked. The formulation should be applied to small area of skin and covered with gauze patch, which is held in place with a non- irritating tape and rats were treated as follows:

1. Group 1: Blank formulation
2. Group 2: Marketed formulation containing BPO
3. Group 3: Optimized film forming gel Formulation (13, 29).

2.7.2. Stability study

Stability study was carried out using the Optimized Batch of Film Forming Gels. The Optimized formulation were evaluated mainly for their physical characteristics at the predetermined intervals of 3 months, 6 months and after 9 months. Physical appearance/clarity, pH, drug content and Anti-Bacterial Activity were evaluated at two different temperatures 40°C and 25°C (13, 30).

2.8. Comparison in-vitro% Drug Release of Optimized Batch, Pure Drug and Marketed BPO Gel

Comparison in-vitro% Drug Release of Optimized Batch, Pure Drug and Marketed BPO Gel (Benzac AC 2.5%, Galderma) was done using the above mention procedure in section no 2.5.10 (24-26).

3. RESULTS AND DISCUSSIONS

3.1. Interaction studies

The DSC studies of the drug and the drug polymer mixture after accelerated stability studies for 2 months were carried out and the results are shown in figure no 3.1 (A – DSC of Drug BPO, B – DSC of Mixture of drug and polymer), BPO drug exhibited an endothermic peak at 106 °C, which is also observed in the drug polymer physical mixture; this indicated that there was no interaction and incompatibility between the drug and the polymer.

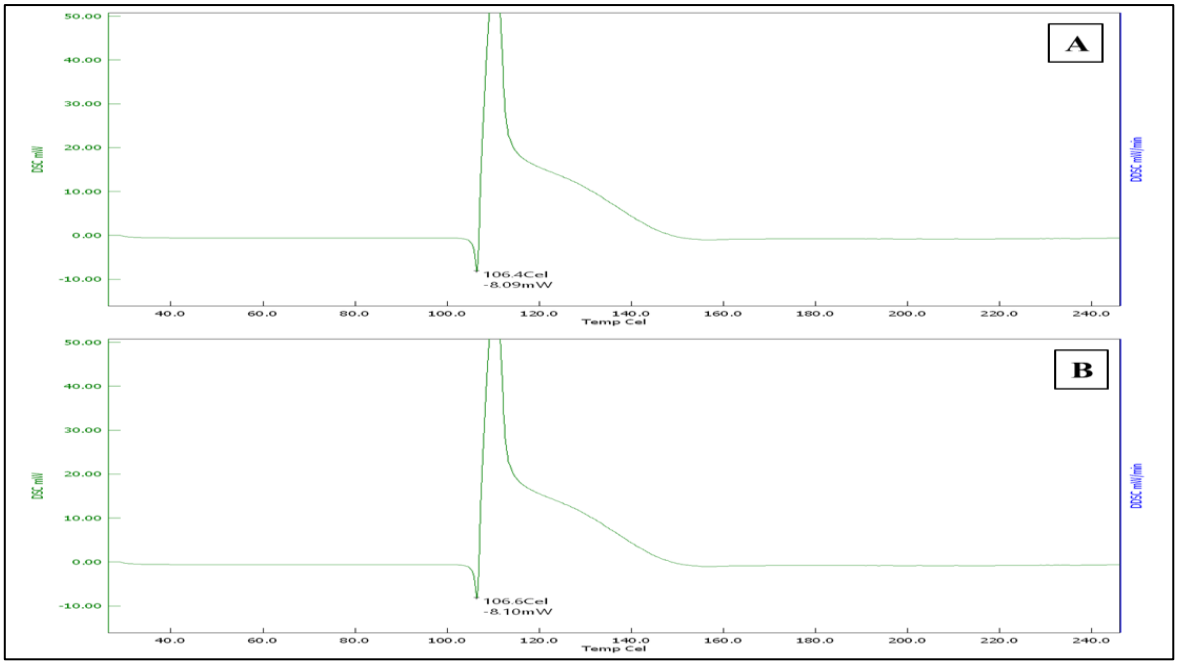

Figure no 3.1 – DSC Thermogram (A – DSC of Drug BPO, B – DSC of Mixture of drug and polymer)

3.2. Experimental Design Using Factorial Design

The Three level Factorial design was run using Design Expert Software and Experimental design Layout was obtained as shown in the Table no 3.1, where X1 is the Concentration of Eudragit (% w/v) and X2 is the Concentration of Concentration of Hydroxypropyl cellulose (% w/v).

Table no 3.1 - Experimental Design Layout

Batch Code	Independent Factor			
	Variable Level		Actual Value	
	X1	X2	X1	X2
B1	+1	+1	18	5
B2	-1	+1	6	5
B3	0	0	12	3
B4	0	+1	12	5
B5	-1	-1	6	1
B6	+1	-1	18	1
B7	+1	0	18	3
B8	0	-1	12	1
B9	-1	0	6	3

Table no 3.2 – Formulation Table

Ingredients	B1	B2	B3	B4	B5	B6	B7	B8	B9
Benzyol peroxide	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Eudragit LR100	18	6	12	12	6	18	18	12	6
HPMC 4000	5	5	3	5	1	1	3	1	3
Isopropyl myristate	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93
Transcutol	12	12	12	12	12	12	12	12	12
PEG 400	4.52	4.52	4.52	4.52	4.52	4.52	4.52	4.52	4.52
Glycerine	8	8	8	8	8	8	8	8	8
Tween 80	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Dioctyl Sodium Sulfoccinate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Ethanol (q.s)	100 ml	100ml	100 ml	100nl	100 ml	100ml	100 ml	100nl	100 ml

3.3. Evaluations

3.3.1. Physical Appearance

Physical Appearance was inspected visually and all the batches of Film Forming Gel of BPO were Clear, Colourless, and free from any particulate matters**.**

Figure no 3.2 – Prepared Film Forming Gel

3.3.2. pH of the Formulation

pH of the developed gels was measured using digital pH meter. The pH of the gels was found to be around 4.6 to 5.2. This can be considered to be the appropriate pH for topical application to the skin. Table no 3.3 Shows pH of all the Batches of Film Forming Gel of BPO.

Table no 3.3 - pH of all the Batches of Film Forming Gel of BPO

Batch no	pH of the formulations
B1	5.2
B2	4.5
B3	4.9
B4	5.0
B5	5.1
B6	4.7
B7	4.9
B8	4.9
B9	4.6

3.3.3. Integrity of formulation on skin:

Integrity of the Formulation on Skin was according to the Procedure mention in the Section 2.5.3. The integrity of formulation on skin was found to be thin, almost invisible film was evaluated for formulation. The result are given below in the Table no 3.4.

Table no 3.4 - Integrity of all the Batches of Film Forming Gel of BPO

Batch no	Integrity of the Formulations on Skin
B1	Thick
B2	Flaky and partly missing
B3	Good
B4	Good
B5	Flaky and partly missing
B6	Thick
B7	Thick
B8	Good
B9	Flaky and partly missing

3.3.4. Viscosity

The viscosity of the gel was determined using Brookfield Viscometer was used. Halipath spindle no 96 was used. The halipath spindle was dipped in the gel. Viscosity of the gel was determined at 10, 20, 30, 50, 60 and 100 RPM. Result are Reported in the Following Table no 3.5.

Table no 3.5 - Viscosity of all the Batches of Film Forming Gel of BPO

RPM of the spindle	Viscosity (in cP)								
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**
10	9915	4103	4375	6356	2693	8014	9235	5489	3461
20	9867	3996	4125	5957	2601	7863	9198	5301	3556
30	9708	3873	3969	5714	2529	7781	9209	5294	3494
50	9672	3746	3731	5697	2445	7625	9184	5369	3316
60	9569	3637	3359	5614	2329	7515	9087	5307	3240
100	9417	3576	2681	5587	2216	7491	8971	5277	3179

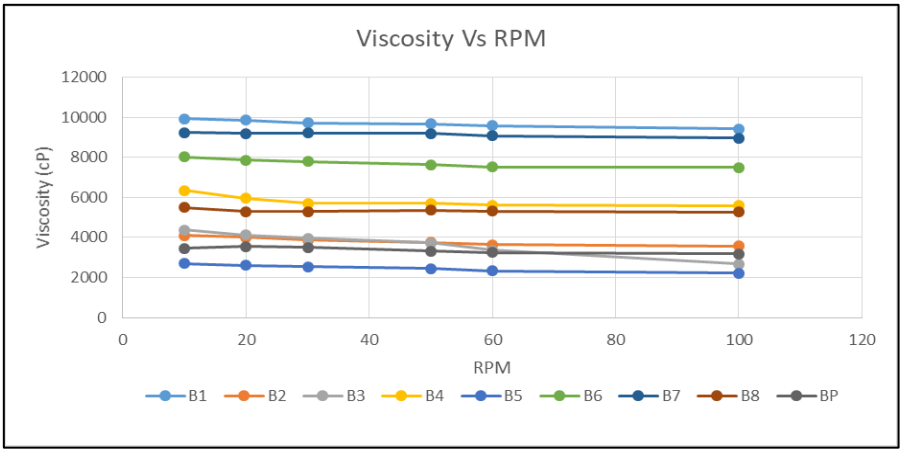

Figure no 3.3 - Viscosity vs. RPM

Viscosity vs. RMP plot for all the formulation shows decrease in viscosity as share rate (RPM) increases concentration of HPMC 4000 and Eudragit LR100 have the major factor affecting viscosity of the formulation exhibited considerable increase in viscosity when the concentration of HPMC and Eudragit increases.

3.3.5. Spreadability

Spreadability was preformed according to the Procedure mention in the section 2.4.5. The diameter of the circle formed after spreading of the gel was measured in centimetres and is reported in the following table no 3.6.

Table no 3.6 - Spreadability of all the Batches of Film Forming Gel of BPO

Batch no	Spreadability of The Formulations (in cm)
B1	4.1
B2	5.3
B3	5.5
B4	5.2
B5	5.2
B6	4.9
B7	4.5
B8	5.6
B9	5.6

3.3.6. Drying time

Drying Time was preformed according to the Procedure mention in the section 2.5.6. Table no 3.7. Shows Drying time of all the batches of Film Forming Gel of BPO.

Table no 3.7 - Drying Time of all the Batches of Film Forming Gel of BPO

Batch no	Drying Time of The Formulations
B1	249
B2	171
B3	184
B4	193
B5	159
B6	209
B7	223

B8	179
B9	163

3.3.7. Outward Stickiness

Outward Stickiness was preformed according to the Procedure mention in the section 2.5.7. The Result for Outward Stickiness of the formulation have been reported in the following table no 3.8.

Table no 3.8 - Outward Stickiness of all the Batches of Film Forming Gel of BPO

Batch no	Outward Stickiness of The Formulations
B1	High
B2	Low
B3	Low
B4	Low
B5	Low
B6	High
B7	High
B8	Low
B9	Low

3.3.8. Cosmetic Attractiveness

The Result for Cosmetic Attractiveness of the formulation formed after drying have been reported in the following Table no 3.9

Table no 3.9 - Cosmetic Attractiveness of all the Batches of Film Forming Gel of BPO

Batch no	Cosmetic Attractiveness of The Formulations
B1	Medium
B2	Low
B3	High
B4	High
B5	Low
B6	Medium
B7	Medium
B8	High
B9	Low

3.3.9. Drug Content

Drug Content was calculated according to the Procedure mention in the section 2.5.9. Table no 3.10 Shows Drug Content of all the batches of Film Forming Gel of BPO.

Table no 3.10 - Drug Content of all the Batches of Film Forming Gel of BPO

Batch no	Drug Content of The Formulations (%)
B1	97.87
B2	96.10
B3	98.10
B4	98.56
B5	96.94
B6	97.08
B7	96.91
B8	98.64
B9	97.76

3.3.10. *in-vitro* Drug Release (Diffusion study)

in-vitro Drug Release (Diffusion study) was carried out using Franz diffusion cell and according to the Procedure mention in the section 2.5.10. Batch B8 shows highest drug release which was 96.29 %. Table no 3.11 Shows Cumulative % Drug Release and Figure no 3.4. Shows Cumulative % Drug Release vs Time Graph of all the batches of Film Forming Gel of BPO.

Table no 3.11 - Cumulative % Drug Release of all the Batches of Film Forming Gel of BPO

Time (in hrs)	Cumulative % Drug Release								
	Batch B1	Batch B2	Batch B3	Batch B4	Batch B5	Batch B6	Batch B7	Batch B8	Batch B9
0 hrs	0	0	0	0	0	0	0	0	0
1 hrs	34.5	29.3	37.15	37.88	23.56	32.49	30.89	41.74	19.99
2 hrs	44.01	42.35	55.51	50.88	38.56	47.49	45.18	56.89	33.41

3 hrs	52.01	47.83	63.21	58.45	46.12	55.59	53.34	64.59	41.49
4 hrs	60.1	55.92	71.52	65.18	54.89	63.53	61.94	72.33	49.12
5 hrs	68.19	65.51	81.57	76.47	62.73	74.23	71.19	82.89	59.89
6 hrs	70.79	67.49	83.11	78.33	64.89	76.91	73.41	84.36	61.07
12 hrs	76.07	73.43	89.36	84.79	70.01	83.91	79.17	90.23	67.83
24 hrs	82.71	79.27	95.63	92.56	76.97	89.01	85.33	96.29	73.69

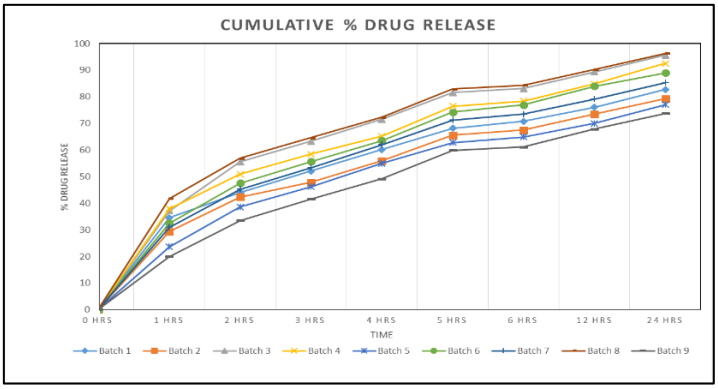

Figure no 3.4 - Cumulative % Drug Release Vs Time Graph

3.3.11. Anti-Bacterial Activity

Anti-Bacterial Activity of all the batches of Film Forming Gel was performed according to the Procedure mention in the section 2.5.11. The standard value of BPO against S.aureus for zone of inhibition is 17 mm. The study indicates that BPO retained its anti-bacterial efficacy when formulated in the form of film forming gel and drug was active against selected bacteria. Table no 3.12. Shows Anti-Bacterial Activity of all the batches of Film Forming Gel of BPO.

Table no 3.12 - Anti-Bacterial Activity of all the Batches of Film Forming Gel of BPO

Batch no	Zone of Inhibition (mm)	% Efficacy (%)
B1	16.9	84.5
B2	16.3	81.5
B3	19.8	99
B4	19.1	95.5
B5	16.1	80.5
B6	18.2	91
B7	17.7	88.5
B8	18.9	94.5
B9	15.5	77.5

3.4. Optimization of Film Forming Gel of BPO

Optimization of the formulations was studied by 3^2 full factorial design. i.e. 3 level and 2 Factor. Concentration of Eudragit (X1) and Concentration of hydroxypropyl methyl cellulose (X2) were selected as independent variables and the dependent variables were % drug release and anti- bacterial activity. The data obtained were by using Design expert version 13 software and analysed statistically using analysis of variance (ANOVA). Concentration of Eudragit and hydroxypropyl methyl cellulose's effects on the dependent variables were also investigated using a 3-D response surface methodology on the data

Table no 3.13 - Experimental Design and Data obtained of Dependent Factor (Response)

Batch No	Conc. of Eudragit LR 100 (% w/v) (X1)	Con of HPMC 4000cps (% w/v) (X2)	Cumulative % Drug Release (%) (Y1)	Anti-bacterial Activity (%) (Y2)
B1	18	5	82.71	84.5
B2	6	5	79.27	81.5
B3	12	3	95.63	99
B4	12	5	92.56	95.5
B5	6	1	76.97	80.5
B6	18	1	89.01	91
B7	18	3	85.33	88.5

B8	12	1	96.29	94.5
B9	6	3	73.69	77.5

3.4.1. Optimization Result

From design expert version 13 Software, Nine batch were found. The batch with Eudragit 13.436 % w/v and HPMC 1 % w/v with desirability 0.968 was found to be optimum. From the actual design formulation B8 were selected as the optimize formulation. The figures below show the effect of concentration of Eudragit and Hydroxypropyl methyl cellulose on drug release and anti-bacterial activity. It is shown that both the independent variables have a significant effect on the dependent variables and drug release and anti-bacterial activity.

1) Analysis of Response Y1 % Drug Release

The Model F-value of 26.15 implies the model is significant. There is only a 1.12% 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, A^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

Table no 3.14 – ANOVA for Quadratic model Response Y1: % Drug Release

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	527.54	5	105.51	26.15	0.0112	**significant**
A-Conc. of Eudragit	122.58	1	122.58	30.39	0.0118	
B-Conc. of HPMC	9.96	1	9.96	2.47	0.2142	
AB	18.49	1	18.49	4.58	0.1218	
A^2	373.37	1	373.37	92.55	0.0024	
B^2	3.13	1	3.13	0.7767	0.4431	
Residual	12.10	3	4.03			
Cor Total	539.64	8				

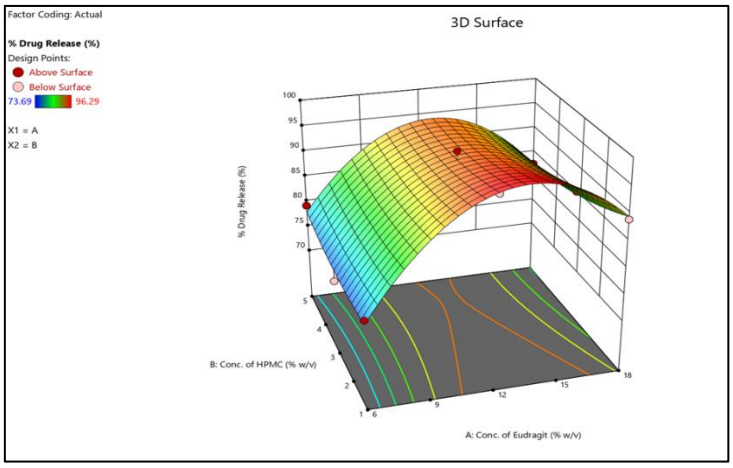

Figure no 3.5 - 3D Surface graph of % Drug Release

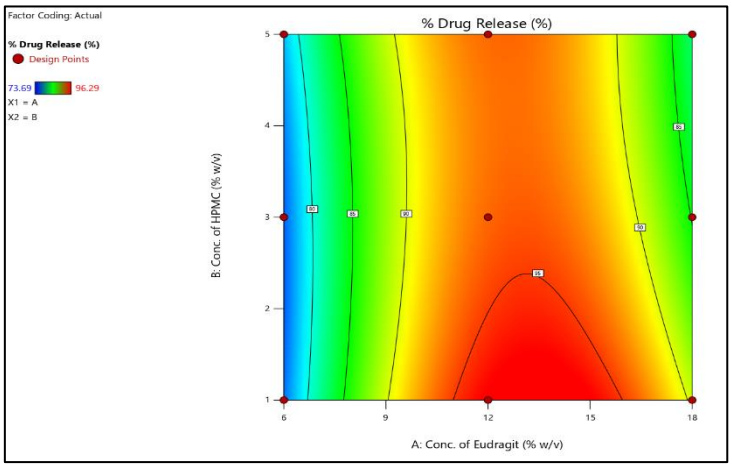

Figure no 3.6 – Contour Graph of % Drug Release

2) Analysis of Response Y2 Anti-Bacterial Activity

The Model F-value of 10.86 implies the model is significant. There is only a 3.88% 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, A^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

Table no 3.15 – ANOVA for Quadratic model Response Y2: Anti-Bacterial Activity

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	426.17	5	85.23	10.86	0.0388	significant
A-Conc. of Eudragit	100.04	1	100.04	12.74	0.0376	
B-Conc. of HPMC	3.38	1	3.38	0.4300	0.5588	
AB	14.06	1	14.06	1.79	0.2731	
A^2	308.35	1	308.35	39.28	0.0082	
B^2	0.3472	1	0.3472	0.0442	0.8469	
Residual	23.55	3	7.85			
Cor Total	449.72	8				

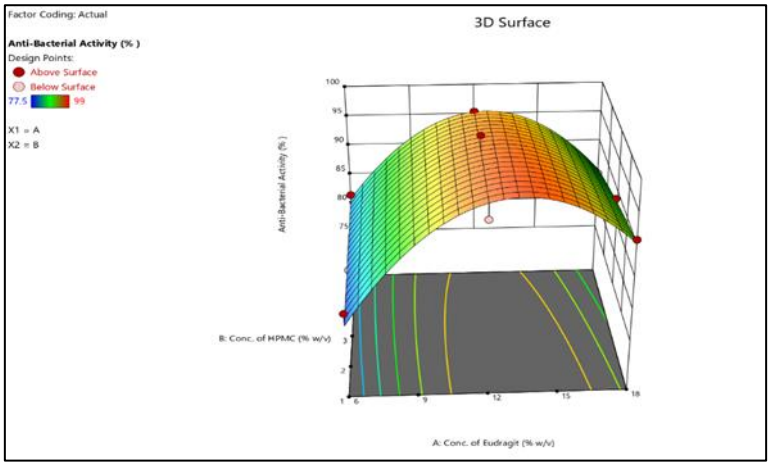

Figure 3.7 - 3D Surface Graph Anti-Bacterial Activity

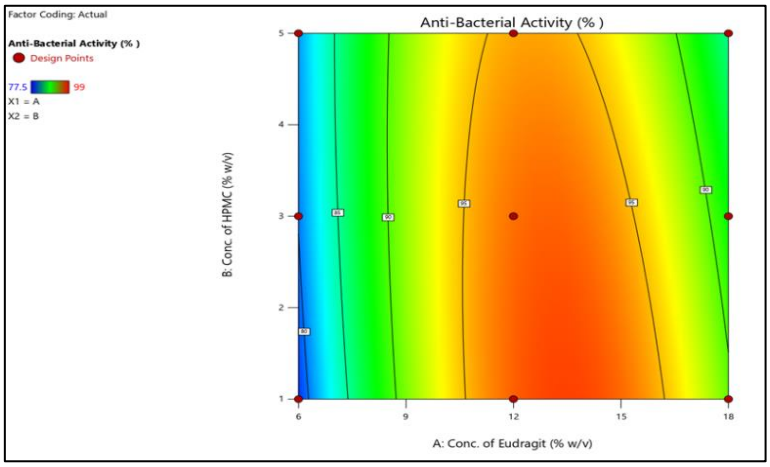

Figure 3.8 - Contour Graph Anti-Bacterial Activity

3.5. Evaluation of Optimized formulation

The Optimize batch were selected by using design expert software and further study were evaluated. B8 was the Final and were selected as optimize batch.

3.5.1. Skin Irritation study

The animals were tested for skin irritation study (Reg. number- 1154/PO/Re/S/08/CPCSEA) by using Optimized Batch B8 .Skin Irritation Study was done according to the Procedure mention in the section 2.7.1. It was concluded that there was NO presence of erythema or oedema were seen on the animals after 14 days of animal study.

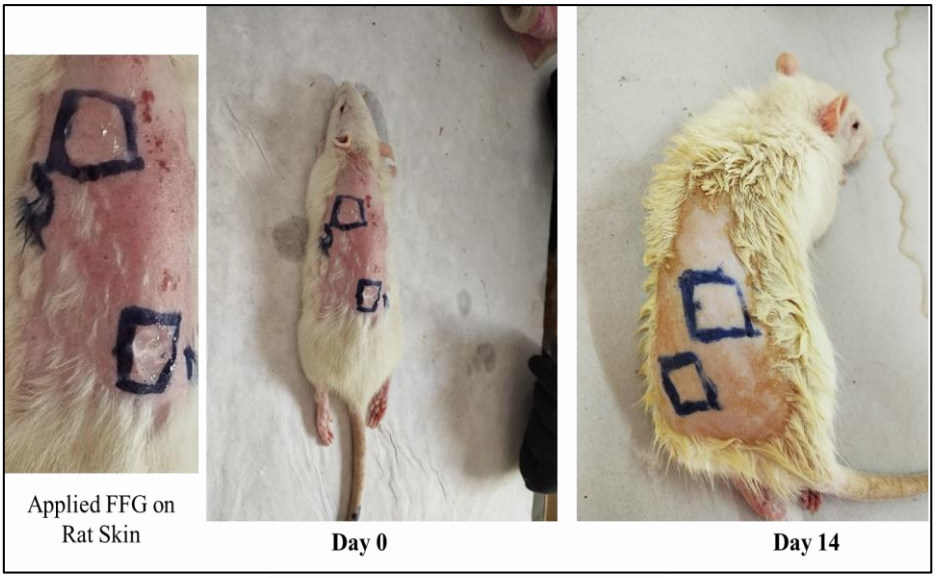

Figure no 3.9 - Skin Irritation Test

3.5.2. Stability study

Stability study was carried out using the Optimized Batch no B8 of Film Forming Gels. The Optimized formulation were evaluated mainly for their physical characteristics at the predetermined intervals of 3 months, 6 months and after 9 months. Physical appearance/clarity, pH, drug content and Anti-Bacterial Activity were evaluated at two different temperatures 40°C and 25°C. The Result for stability is mention in the following Table no 3.15.

Table no 3.15 – Stability Study

Batch	Storage Condition	Duration	Appearance	pH	Drug Content	Anti-bacterial
B8	40 ^{0} C ± 2 ^{0} C at 75% ± 5% RH	30 days	Clear, Transparent	4.9	98.64%	99%
		60 days	Clear, Transparent	4.9	97.98%	98.5%
	25 ^{0} C ± 2 ^{0} C at 60% ± 5% RH	30 days	Clear, Transparent	4.8	98.2%	99%
		60 days	Clear, Transparent	4.9	98.70%	99%

3.6. Comparison Cumulative % Drug Release of Optimized Batch, Pure Drug and Marketed Gel

Comparison in-vitro% Drug Release of Optimized Batch, Pure Drug and Marketed BPO Gel (Benzac AC 2.5%, Galderma) was performed. And the Result are as follows –

Table no 3.16 – Comparison in-vitro% Drug Release of Optimized Batch, Pure Drug & Marketed BPO Gel

Time (in hrs)	Cumulative % Drug Release		
	Pure Drug	Optimized Batch of FFG of BPO	Marketed Product (Gel)
0 hrs	0	0	0
1 hrs	37.26	41.74	19.78
2 hrs	47.52	56.89	41.63
3 hrs	61.79	64.59	63.11
4 hrs	79.64	72.33	78.94
5 hrs	99.73	82.89	85.54
6 hrs	-	84.36	99.83
12 hrs	-	90.23	-
24 hrs	-	96.29	-

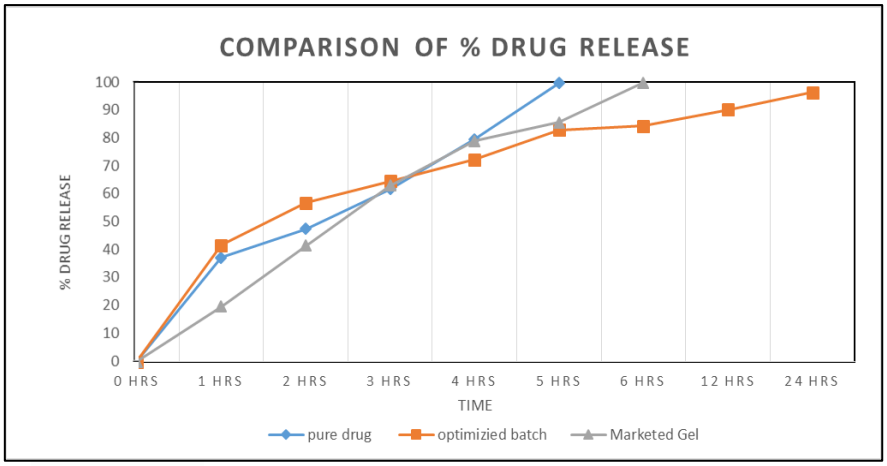

Figure no 3.11-Comparison in-vitro% Drug Release of Optimized Batch, Pure Drug & Marketed BPO Gel

CONCLUSION

The present study focus on Preparation, Evaluation and Optimization of Film Forming Gel of Benzoyl Peroxide. Benzoyl Peroxide Film Forming Gel of Benzoyl Peroxide was prepared using dispersion method. The main objective of the study is to preparing Benzoyl Peroxide in the form of FFG which may give sustained release action by using Eudragit LR100 and HPMC in combination. Experimental design was obtained using design expert software and by using 3^2 factorial design method in which the effect of concentration of Eudragit LR100 and concentration of HPMC 4000 on Film Forming gel was studied. 9 batches of film forming gel of benzoyl peroxide war prepared and evaluated for various parameters. By using Optimization Technique, Batch B8 was selected as the optimize batch in which 12% of Eudragit LR100 and 1 % of HPMC 4000 was used. The optimized batch was evaluated for skin irritation test and stability test. Skin irritation test was carried out and NO presence of erythema or oedema were seen on the animals after 14 days of application of Film Forming Gel. Stability test was also carried out and Film Forming gel was found to be stability for 60 days after preparation.

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CONFLICTS OF INTERESTS

All authors declared no conflicts of interest.

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