Section A-Research paper
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FORMULATION AND OPTIMIZATION OF CYCLODEXTRIN BASED ADAPALENE AND DAPSONE GEL USING DESIGN OF EXPERIMENT FOR ANTI-ACNE TREATMENT

Rupesh Subhashchandra Kotwal[1*], Vijay Kumar Singh[2], Manmohan Singh Jangdey[3], Shashank Kukreja[4]

[1*,2,3] Shri Rawatpura Sarkar College of Pharmacy, Shri Rawatpura Sarkar University, NH-43, Dhamtari Road, Raipur, Chhattisgarh 492001
***Corresponding Author:** Rupesh Subhashchandra Kotwal
*Shri Rawatpura Sarkar College of Pharmacy, Shri Rawatpura Sarkar University, NH-43, Dhamtari Road, Raipur, Chhattisgarh 492001, Email: rskotwal@gmail.com

Abstract
Dapsone's (Dap) limited solubility in water enforce the formulator to use higher percent of Dapsone to make its formulation effective for treatment of acne vulgaris. However, due to use of higher percent, Dapsone often recrystallize within the formulation leaving the product cosmetically non-elegant and gritty feel.
Cyclodextrins (CDs) are oligosaccharides which forms host-guest inclusion complexes with lipophilic molecules, resulting in enhanced solubility and permeability of such molecules in oral dosage forms. The use of CDs in topical formulations has received much attention from few decades which enhances the drug permeation through skin by modulation of lipidic passage of skin and targeting actives at the site of action by increased permeation. Thus, the present study aimed to formulate the in-situ cyclodextrin (host) based inclusion complex of Dapsone (guest) which causes improvement in solubility and permeation at a concentration effective to treat acne vulgaris and to avoid recrystallization within the formulation. The interaction between Dapsone (Dap) and Hydroxypropyl β-cyclodextrin (Betadex®) (HPβCD) in the solution state was investigated using phase solubility technique. Box-Behnken design was used to optimize the concentration of Dapsone, CDs and solubilizers. To assess the efficacy of all the DoE batches for anti-acne activity, Ex-vivo Antibacterial activity through zone of inhibition studies, Cumulative release of Dapsone and Adapalene through enhancer cell apparatus.
Dapsone showed potent antibacterial activity for *Propionibacterium acnes* strain through the cylinder plate technique from combination gel at concentration much lower than the marketed product due to increased solubility. The responses of all dependent variables were used to generate surface response and contour plots. DoE software "Minitab" was used to derive optimized concentration of variables studied in the research and the optimized formulation has the Dapsone concentration in the range of 2.5 to 3.7%, Cyclodextrin in the range of 1.5 to 2.0% and the solubilizer in the range of 22 to 27% which produced stable gel without recrystallization of Dapsone and have similar or enhanced anti acne activity than marketed products which was studied by ex vivo permeation through rat skin.
The present study demonstrated that the cyclodextrin based combination product of Adapalene and Dapsone gel could be the possible cost effective and cosmetically elegant alternative to the conventional marketed topical formulation for the treatment of acne.
Keywords: Cyclodextrins, Dapsone, Adapalene, Acne, Box-Behnken design, cumulative release, response optimizer.

INTRODUCTION

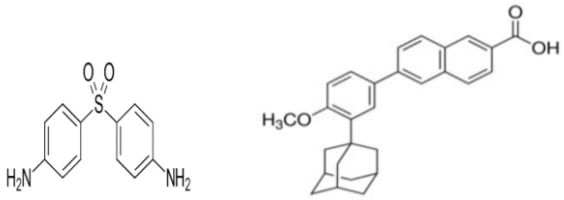
Acne vulgaris is an inflammatory disorder of skin affecting basically the pilosebaceous unit with high prevalence in adolescents and adults [1]. Half of the population experience the symptoms of acne vulgaris in

adulthood [2]. Acne is broadly classified based on the clinical symptoms of lesions, as mild, moderate and severe [1, 2]. Mild acne is characterized by the presence of comedones which could be inflammatory or non-inflammatory lesions on the face. Moderate acne, has increased number of inflammatory papules and pustules which are observed on face and truncal region. However severe acne is characterized by nodules and cysts. Increased production of androgen in adolescent cause abnormal epithelial desquamation and proliferation of Propionibacterium acnes [3]. The chemical mediators released by Propionibacterium acne are responsible for inflammation and development of papules, pustules or cysts [2, 4].

Current treatment of acne includes systemic as well as topical regimens. Mild and moderate acne is treated with topical treatments with retinoids, antibiotics, benzoyl peroxide and their combination products [1]. Severe acne is treated with topical regimens discussed above along with systemic administration of hormones, antibiotics and isotretinoin [5,6]. Retinoids are highly effective in acne treatment but associated with adverse effects of photosensitivity and skin irritation [7]. Systemic use of antibiotics for longer duration is limited by the growing concern of antibiotic resistance.

Combination therapy of retinoid with Antibiotic or benzoyl peroxide for topical application has proved advantageous for its dose duration, dosage regimen and rate of recovery, many marketed combinations are Adapalene with Clindamycin Phosphate, Clindamycin Phosphate with tretinoin, Adapalene with Benzoyl Peroxide and novel combinations like Dapsone (antibiotic) and Adapalene (retinoid) are available in market, however novel combination of Dapsone and Adapalene have few challenges of elegancy due to gritty feel caused by recrystallization of Dapsone within formulation, product cost due to use of higher percent of Dapsone, variability in efficacy, skin irritation and dryness.

Dapsone has broad application in various dermatological disorders due to its unique dual antimicrobial and anti-inflammatory actions. Thus, it has been used topically in alleviating skin rashes, blisters and lesions associated with a number of dermatological conditions including Behcet's disease and systemic lupus erythematosus [15]. Utilization of topical applications provides an efficient strategy both for the alternative treatment of leprosy, where the pathogen and lesions are in the skin, and other dermatological indications, such as acne [16]. Despite its topical therapeutic efficacy, clinical use is limited due to its poor physicochemical properties and is categorized as a Class II agent according to the biopharmaceutical classification system proposed by Amidon et al. [17]. This poor water solubility hinders the production of DPS formulations with adequate bioavailability. Chemically dapsone is 4-[(4- aminobenzene) sulfonyl] aniline with molecular formula $C_{12}H_{12}N_2O_2S$ and molecular weight 248.30 g/mol (**Figure 1 A**).Its logP and pKa values are 0.97 and 2.41 respectively[15]. It shows a mechanism of action like sulfonamides which involve the inhibition of folic acid synthesis for the active site of dihydropteroate synthase [3,4]. It is official in IP, BP, and USP [22,23]. Adapalene is chemically 6-[3-(1Adamantyl)-4-methoxyphenyl] -2-naphthoic acid (**Figure 1 B**) with molecular formula $C_{28}H_{28}O_3$ and molecular weight 412.5 g/mol [7]. Its logP value is 8.6 and pKa is 3.99. It is topical retinoid used in the treatment of acne [8]. It shows a mechanism of action like those of tretinoin and naphthoic acid derivative



(A) **(B)**

Figure 1: Chemical Structure of (A) Dapsone and (B) Adapalene

The field of nanotechnology is being explored for the effective delivery of drug to skin and other tissues [11, 12]. Microspheres, Liposomes, Nonosomes, microemulsions, Solid lipid nanoparticle are the novel drug delivery systems which are used as an alternative to conventional dosage forms, however all these NDDS have their own challenges of stability, scalability, requirement of special equipment etc. Entrapment of higher percent of active is not possible in novel dosage form.

In last few decades the use of cyclodextrins (CDs) to form host – guest inclusion complexes with drugs to improve its solubility, stability and permeability has increased. CDs have been accepted worldwide in

pharmaceutical and cosmetic industries [13]. CDs allow the guest molecule to be retained within its bucket like cavity thus improving its stability and the aqueous solubility is also improved due to its hydrophilic external surface of cyclodextrins. Overall, the use of CDs holds recognized advantages for skin applications related to the improvement of stability, tolerance, apparent solubility, and organoleptic characteristics of the active ingredients, as well as their controlled-release of ingredients in the skin. Cyclodextrins (CDs) are composed of glucose units connected by a1, 4 glycosidic linkages to form a series of oligosaccharide rings. The native CDs comprise 6, 7 and 8 glucose units. CDs are one of host molecules more extensively studied in supramolecular chemistry as they are biocompatible, produced by natural enzymatic degradation of starch, relatively cheap and non-toxic, thus allowing applications in drugs, foods and cosmetics [13,14]. Complexation with CDs has been widely used to enhance the bioavailability of poorly soluble drugs by increasing the drug solubility, dissolution and/or permeability. β-CD is the most widely used natural CD and its used in pharmaceutical applications is limited due to its limited aqueous solubility. Therefore, chemically modified β - CDs have been synthesized to overcome this problem such as hydroxypropyl and methylated β - CDs both much more soluble in water than the native β-CDs [13].
A previous study showed improvement in Dapsone solubility and bioavailability via inclusion complexes in cyclodextrins (HP-β-CD and β-CD) in the presence or absence of polymers (PVP K30 and HPMC) [12].
A combination acne product would provide the benefit of enhanced efficacy compared to the products containing single active agent by taking advantage of the synergistic mechanism of action of the active agents for treatment of acne. The present work is directed to acne products with at least two active compounds and in particular is directed to dapsone and adapalene combination formulations for the use in the treatment of acne.

Material and Methods

Materials

Dapsone and Adapalene were purchased from Sigma Aldrich, different excipients like Dimethyl isosorbide, Carbopol 980, propylene glycol and poloxamer 124 were gifted from Arihant chemicals. Gattefosse gifted Transcutol P, Methylparaben, Sodium hydroxide was purchased from SD Fine as analytical grade. Betadex® (Hydroxypropyl beta-cyclo- dextrin) was kindly supplied by Roquette Frères (Lestrem, France).

Preparation of aqueous gel base and optimization by Box-Behnken design

Excipients and their concentration for Aqueous Gel published in literatures as well as in Handbook of Pharmaceutical formulations uses carbomer homopolymer type A, B or C as gelling agent, along with or without solubilizers for drug solubility, dispersing agents for dispersion of drug, stabilizers like preservatives, chelating agent or antioxidants for product stability, combining these materials the carrier base is formed which carry actives to the site of action on skin surfaces. Marketed formulations studied in this research work are also aqueous gel bases which are formulated using carbomer homopolymers. On the basis of literature survey, formulation and its functions are shown in **Table 1**

Table 1: Component and their percent used for Adapalene and Dapsone gel

Component	Concentration in %w/w	Remark
Adapalene	0.1	Adapalene and Dapsone are used as active ingredients
Dapsone	5.00	
Drug solubilizer	20 to 40	Suitable solubilizer to be used for Solubilization of Dapsone and to avoid recrystallization
Gelling agent	0.8 to 1.0	Polymers which form gel when dispersed in water shall be used to impart viscosity to base
Dispersing agent	0.1 to 0.2	To disperse the active ingredient within the formulation
Cosolvent	5 to 15	Cosolvent also known to enhance solubility of lipophilic actives are used in the formulation
Preservative	0.1 to 0.2	To enhance the product stability and minimize the drug product microbial contamination
pH modifier	q.s. to pH	Carbopol is pH dependent gelling agent hence, pH modifier shall be used to adjust the pH of formulation
Purified water	q.s. to 100	Purified water shall form the gel base.

Manufacturing process used for preparation of DoE batches
Step 1: HPβCD phase preparation: HPβCD was added and dissolved in Purified water under stirring to form clear phase.

Step 2: Dapsone solution preparation: Methylparaben is dissolved in solvent mixture of Propylene glycol and Transcutol P or Dimethyl isosorbide as recommended in DoE trials and mixed till it dissolves completely. Dapsone was further added in the phase and mixed till it dissolved completely to form clear solution.

Step 3: Adapalene dispersion preparation: Poloxamer 124 is added slowly in Purified water and mixed for 10 to 15 minutes, Adapalene is slowly added to this surfactant solution and mixed to form uniform dispersion of Adapalene. Mixing was continued for next 20 to 30 minutes.

Step 4: Dapsone phase addition to step 1: Dapsone Solution of Step 2 is slowly added to HPβCD solution using homogenizer and at controlled rate. This solution was examined for recrystallization of Dapsone by using visual as well as microscopic observation to get the preliminary outcome of the intermediate stage of manufacturing. This solution was kept under stirring for about 60 to 120 minutes to form the Dapsone HPβCD complex within the formulation.

Step 5: Adapalene dispersion addition in step 4 phase: Adapalene dispersion prepared in step 3 was added to step 4 and mixed further for 30 minutes.

Step 6: Carbomer dispersion in step 5 drug phase: Carbomer homopolymer type C was slowly dispersed in parts under high speed vortex mixing in step 5 to form lump free Carbopol dispersion.

Step 7: Sodium hydroxide Solution preparation and addition in gel phase: 10% Sodium Hydroxide solution is prepared in Purified water and added slowly to gel base to get the desired pH and to form viscous gel.

Statistical design
The effects of various formulation variables viz., Dapsone, HP-β-Cyclodextrin and solubilizer system were studied using a statistical design. The solubilizers used are Dimethyl Isosorbide and Diethylene glycol monomethyl ether [Transcutol P] on formulation responses like Dapsone solubilized content within the formulation, antibacterial activity through zone of inhibition and cumulative release of Dapsone and adapalene in vitro diffusion studies from gel formulation. Concentration of other ingredients was kept constant.
Design was performed on Minitab 19 software and Summarized as below
Design Class: Response Surface
Name of Design: Box-Behnken design
Number of Factors (variables): 3
Number of runs: 15
Table 2: Designing of trials for drug content

Factors	**Levels**		
	Level 1	**Level 2**	**Level 3**
Dapsone %	2.50	5.00	7.50
HPβCD%	1.0	2.00	3.00
DMI %	15	NA	NA
DGME %	NA	25	35

Table 3: Randomized experiments designed by software using Box-Behnken

Formulation	**Dapsone**	**HPβCD**	**DGME**	**DMI**
OG 1	2.5	2.0	35	NA
OG 2	5.0	2.0	25	NA

Formulation	Dapsone	HPβCD	DGME	DMI
OG 3	7.5	2.0	35	NA
OG 4	5.0	1.0	NA	15
OG 5	5.0	3.0	35	NA
OG 6	2.5	2.0	NA	15
OG 7	7.5	1.0	25	NA
OG 8	7.5	2.0	NA	15
OG 9	2.5	3.0	25	NA
OG 10	5.0	3.0	NA	15
OG 11	7.5	3.0	25	NA
OG 12	2.5	1.0	25	NA
OG 13	5.0	2.0	25	NA
OG 14	5.0	2.0	25	NA
OG 15	5.0	1.0	35	NA

Table 4: Formulation details of DoE batches as per Box-Behnken design

Ingredients	OG1	OG2	OG3	OG4	OG5	OG6	OG7	OG8	OG9	OG10	OG11	OG12	OG13	OG14	OG15
Dapsone	2.5	5.0	7.5	5.0	5.0	2.5	7.5	7.5	2.5	5.0	7.5	2.5	5.0	5.0	5.0
Adapalene	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
HP β Cyclodextrin	2.0	2.0	2.0	1.0	3.0	2.0	1.0	2.0	3.0	3.0	3.0	1.0	2.0	2.0	1.0
Methylparaben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Carbopol 980	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Poloxamer 124	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
DGME	35.0	25.0	35.0	NA	35.0	NA	25.0	NA	25.0	NA	25.0	25.0	25.0	25.0	35.0
DMI	NA	NA	NA	15	NA	15	NA	15	NA	15	NA	NA	NA	NA	NA
Propylene glycol	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Sodium Hydroxide Solution	q.s. to pH	q.s. to pH	q.s. to pH	q.s. to pH	q.s. to pH	q.s. to pH	q.s. to pH	q.s. to pH	q.s. to pH	q.s. to pH	q.s. to pH	q.s. to pH	q.s. to pH	q.s. to pH	q.s. to pH
Purified water	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100

Table 5: Box-Behnken experimental design with observed responses

Formulation	Dapsone	HPβCD	DGME/ DMI	% dissolved content of Dapsone	Zone of inhibition	Cumulative drug release (Dapsone)	Cumulative drug release (Adapalene)
OG 1	2.5	2.0	35	97.5±3.5	22.7±3.2	88.2	34.0
OG 2	5.0	2.0	25	81.7±3.8	31.7±6.6	75.9	37.6
OG 3	7.5	2.0	35	96.1±3.1	36.9±2.1	76.4	33.9
OG 4	5.0	1.0	15	49.1±4.1	16.6±0.9	37.2	35.1
OG 5	5.0	3.0	35	100.2±0.8	42.2±2.6	69.5	38.1
OG 6	2.5	2.0	15	62.3±3.6	26.9±2.1	36.9	39.9
OG 7	7.5	1.0	25	44.8±4.7	15.5±0.9	67.7	50.5
OG 8	7.5	2.0	15	44.2±4.6	35.1±4.4	39.1	32.8
OG 9	2.5	3.0	25	98.9±0.2	42.7±3.5	50.7	47.1
OG 10	5.0	3.0	15	74.5±6.3	14.7±0.4	42.9	35.7
OG 11	7.5	3.0	25	81.0±4.2	32.8±5.6	76.4	32.1
OG 12	2.5	1.0	25	98.3±2.2	25.3±1.8	70.9	30.9
OG 13	5.0	2.0	25	85.1±3.9	33.1±1.0	67.7	37.1
OG 14	5.0	2.0	25	83.2±5.5	23.4±2.5	60.1	32.0
OG 15	5.0	1.0	35	93.3±4.2	37.3±6.9	67.3	32.9
Adaferin gel#	NA	NA	NA	Not applicable	Not applicable	Not applicable	30.5
Acnedap gel#	NA	NA	NA	49.9±7.4	16.30±0.40	61.1	Not applicable
Acnedap plus gel#	NA	NA	NA	52.7±2.6	21.93±0.25	69.3	35.3

Evaluation of HPβCD based gel formulations

Organoleptic characteristics of hydrogels

Sufficient amount of formulation is taken into clean and dry glass petri plate with spatula, and it is spread uniformly to form thin layer. The spread product is then observed under suitable light to note the description.

Drug Content of Dapsone

Dapsone content was determined by analysis of its concentration in samples from three to four different points of the prepared gel. Samples of the gel were diluted and Dap concentration measured spectrophotometrically at wavelength 293 nm.

Drug Content of adapalene

Adapalene content was determined by analysis of its concentration in samples from three to four different points of the prepared gel. Samples of the gel were diluted and Adapalene concentration measured spectrophotometrically at wavelength 237 nm.

Measurement of pH

pH of formulation is measured by placing the electrode of pH meter in sufficient quantity of product. The pH was checked by using a digital pH meter at constant product temperature at 25ºC.

Viscosity of gel

Instrument: Rheometer MCR 302
Program details:
Speed: 25 RPM
Time: 60 sec
Measuring point: 12
Gap setting: 1 mm
Temperature: 25±1°C
Default program: rotation module for flow curve measurement
Apply the initial setting parameters of instrument on software screen. Take appropriate quantity of sample on the plate of rheometer. Attach the spindle on the instrument and press the lever of spindle to press the sample between spindle and rheometer plate. Remove the excess quantity from the plate by trimmer. Initiate the measurement by pressing the start button. Average of 12-point measurement is reported as viscosity of product [14].

Microscopic evaluation for particle size determination

Take small amount on glass slide. And add cover slip on it. Form a thin layer by pressing coverslip gently and avoid breakage or damage of particles. And analyse through microscope

Ex-Vivo Antibacterial studies of Dapsone

The zones of inhibitions for the antibacterial activity were determined by modified agar well diffusion method for all DoE trial batches to know the activity of Dapsone through formulation and was compared with the standard marketed formulations.

Percent dissolved content of Dapsone in formulation

Known quantity of formulation was placed in centrifuge tube and rotated at a speed of 30000 RPM at controlled temperature for about 120 minutes to separate the liquid part from solid material. The supernatant liquid was used to determine the percent dissolved Dapsone in the formulation.

In-vitro drug release studies through formulations

Manual Enhancer Cell (Immersion cell Apparatus) assembled in a mini vessel will be utilized with a diffusion dosing area of 2 cm2. Membrane selection was done based on the ability of active binding on the

membrane. Nylon membrane (Merck membrane) with 0.45 µ pore size and diameter 25mm selected for IVRT studies. Before initiation of study the membranes are soaked in media for 8 hours and was placed on receptor cell of Immersion apparatus (Type A as per USP<1724>). Approximately 1000 mg (equivalent maximum fill) of formulation was applied on the membrane. Product was applied on membrane and spread uniformly using Teflon spatula. 100ml dissolution chamber with mini paddle rotating at 350 RPM was used. The receptor solution sample of 3ml will be withdrawn at 0.0, 1.0, 2.0, 4.0, 6.0 and 8.0 hours post application in the study. Same amount of receptor media shall be replenished in each apparatus. The study was performed at diffusion cell temperature maintained constant at 32°C±1°C. Cumulative release and release rate (slope) of Dapsone and Adapalene for each cell of Test product and Reference product will be determined. Sample withdrawn shall be directly analysed for concentration of Dapsone and Adapalene at 293nm and 237nm respectively.

Result and Discussion

All the DoE batches were evaluated for physiochemical tests like description, pH, Viscosity and content of Adapalene and Dapsone.

Description

All the formulation was white to off white gel, wherein few formulations are elegant smooth in texture and others showed gritty feel due to recrystallization of Dapsone.

pH

pH was adjusted with Sodium hydroxide solution within the range of 4.8 to 5.8.

Viscosity

Gel formulations contains carbomer homopolymer type b which is pH dependent viscosity modifier, all the DoE batches contains fixed concentration of carbomer and pH was adjusted within desired range hence viscosity for all the formulations were estimated within 9000 to 12200 mPas.

In order to optimize Dapsone, HPβCD and solubilizer concentration, Box-Behnken design, with 3 independent variable and 3 levels, was employed. The effect of all independent variables on responses, dissolved content of Dapsone in formulation, zone of inhibition and cumulative drug release after 8 h was analysed using Minitab 19 software and data is presented in Table 1.

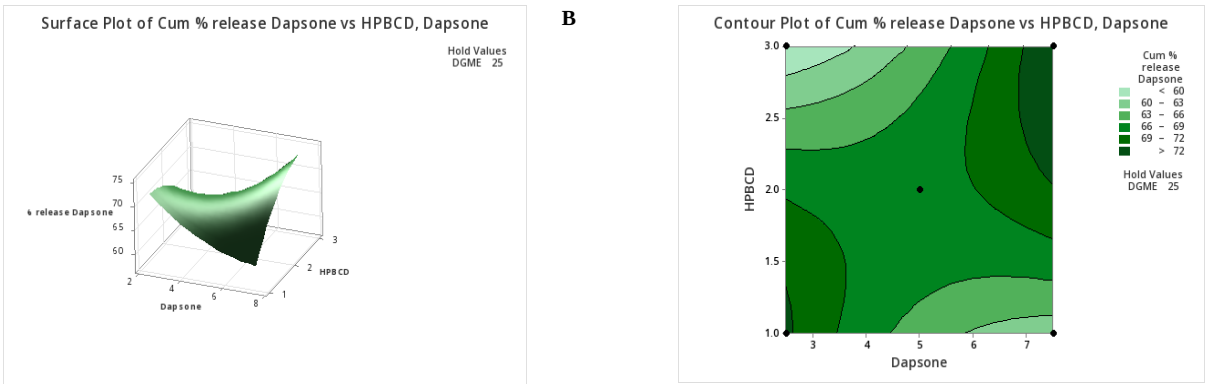


Fig. (1). (A)Surface response plot (B) Contour plot depicting the effect of HPβCD and Dapsone on Cumulative % release of Dapsone

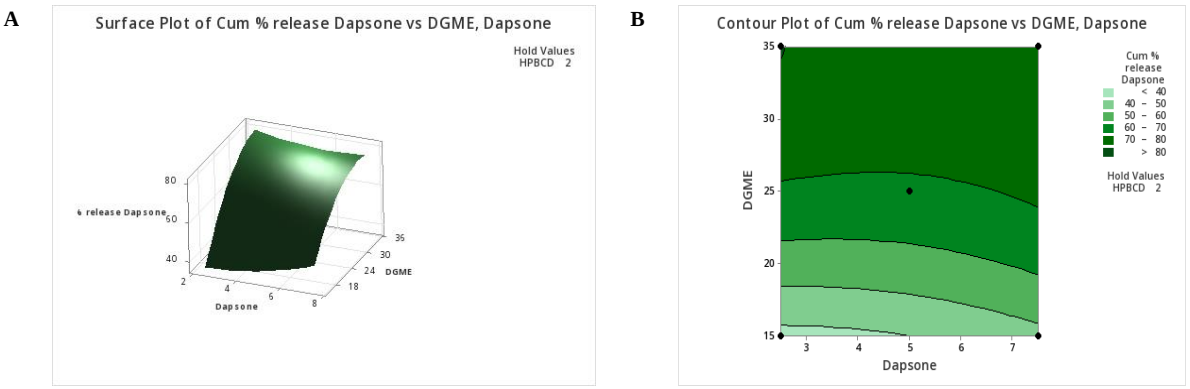


Fig. (2). (A)Surface response plot (B) Contour plot depicting the effect of Dapsone and DGME on Cumulative % release of Dapsone

Fig. (3). (A)Surface response plot (B) Contour plot depicting the effect of DGME and HPβCD on Cumulative % release of Dapsone

Effect on cumulative drug release through formulation

In vitro release studies of Dapsone were carried out to study the effect of independent variables, Dapsone, HPβCD and solubilizer concentration. According to the results obtained, the percentage of drug release of 15 formulations after 8 h of release studies was found to be in the range 37.2% to 88.2%. The effect of independent variables on drug release after 8 h is evident in Figs. (1-3). Cumulative drug release is directly proportional to dissolved content of Dapsone within the formulation. Hence, with the change in Dapsone content from 2.5 to 7.5% within the formulation, drug solubility decreases and release after 8 h was found to be impacted significantly towards negative side. This could be due to recrystallization of Dapsone within the formulation at higher drug percentage resulting the formulation gritty in feel and lower in cumulative release. The formulations containing higher percent of HPβCD showed slower and controlled rate of release of dissolved Dapsone through formulation.

From the response surface plots and contour plots, the negative effect of the dapsone concentration from 2.5 to 7.5 was observed on drug release from gel formulation.

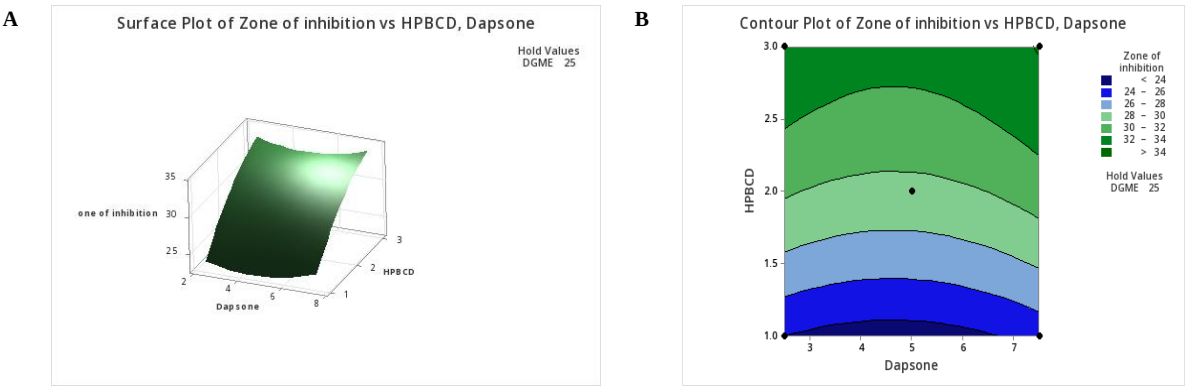


Fig. (4). (A) Surface response plot (B) Contour plot depicting the effect of HPβCD and Dapsone on Zone of Inhibition due to antibacterial activity of Dapsone

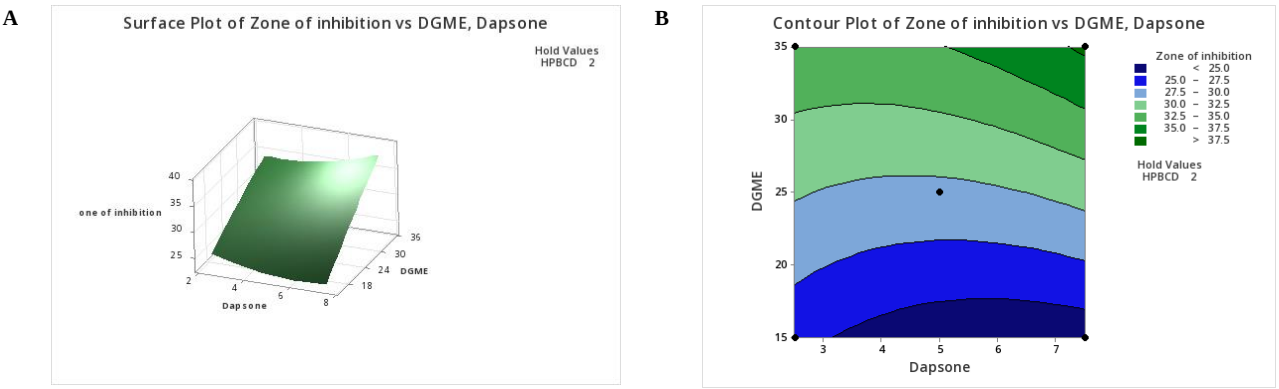


Fig. (5). (A) Surface response plot (B) Contour plot depicting the effect of DGME and Dapsone on Zone of Inhibition due to antibacterial activity of Dapsone

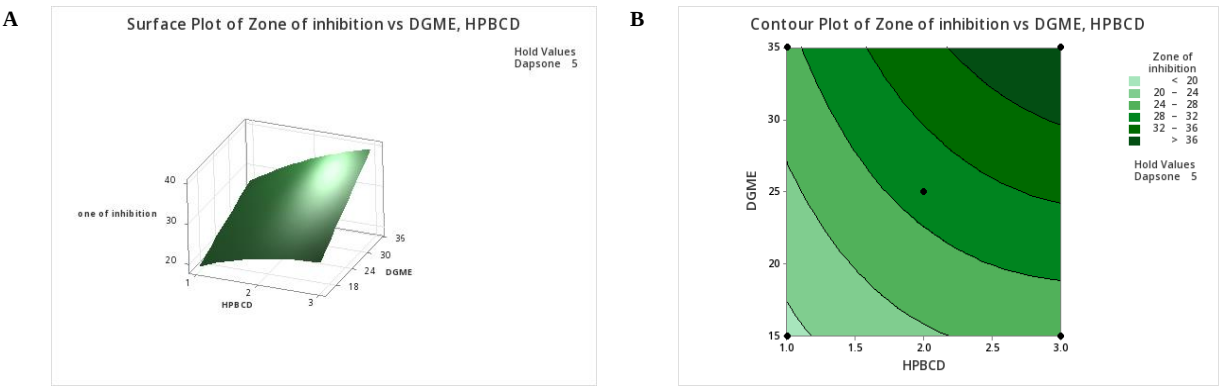


Fig. (6). (A) Surface response plot (B) Contour plot depicting the effect of DGME and HPβCD on Zone of Inhibition due to antibacterial activity of Dapsone

Effect on zone of inhibition

Zone of inhibition for antibacterial activity of Dapsone through formulation were carried out to study the effect of independent variables, Dapsone, HPβCD and solubilizer concentration. According to the results obtained, the zone of inhibition of 15 formulations was found to be in the range 14.7±0.4 to 42.7±3.5%. The effect of independent variables on zone of inhibition was studied and it is evident that the zone increases with increase in concentration of solubilizer and HPβCD. Irrespective of the concentration of Dapsone within the formulation the zone is dependent on the availability of solubilized portion of Dapsone and its migration

through the formulation is dependent on solubilizer concentration. In those formulation where the solubilizer is DMI the zone of inhibition is lesser as compared to formulations containing DGME.
From the response surface plots and contour plots, it is evident that the dapsone concentration does not have any effect on zone of inhibition, however as the concentration of the solubilizer and HPβCD in the formulation increases the formulation shows increased zone of inhibition for those batches.

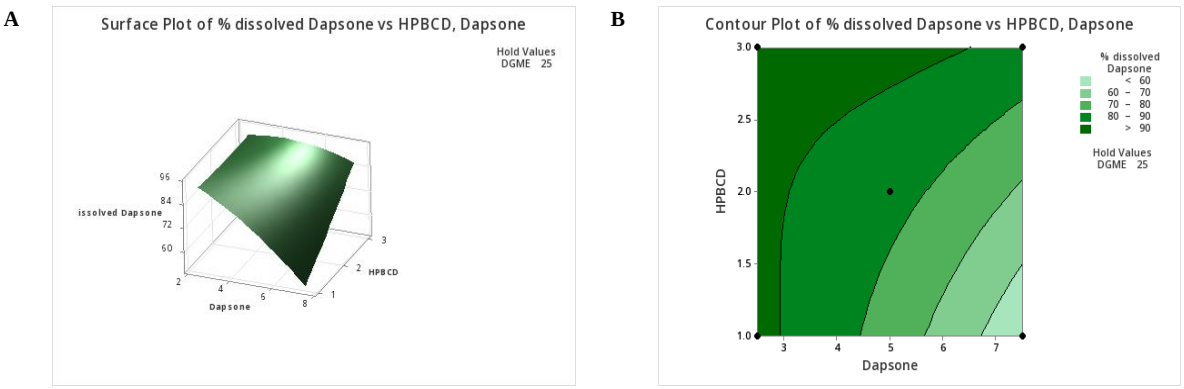


Fig. (7). (A) Surface response plot (B) Contour plot depicting the effect of HPβCD and Dapsone on % dissolved content of Dapsone

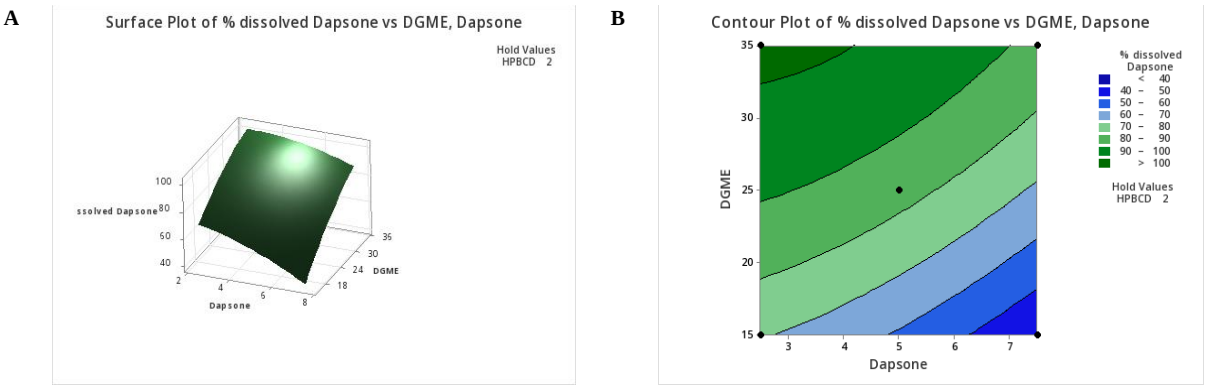


Fig. (8). (A) Surface response plot (B) Contour plot depicting the effect of DGME and Dapsone on % dissolved content of Dapsone

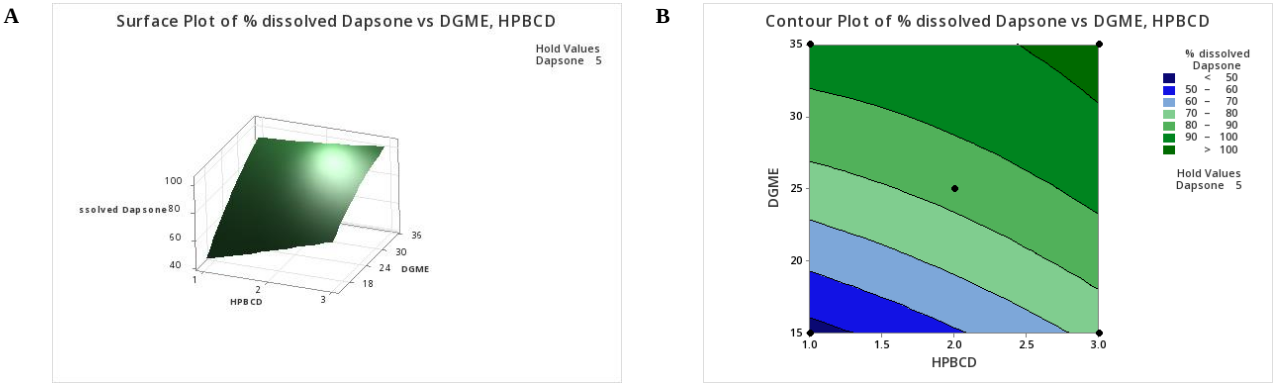


Fig. (9). (A) Surface response plot (B) Contour plot depicting the effect of DGME and HPβCD on % dissolved content of Dapsone

Effect on dissolved content of Dapsone in formulation

The dissolved content of Dapsone within the formulation varied from 44.2±4.6 to 100.2±0.8% (Table 1) depending on the concentration of individual formulation variables. The concentration of HPβCD and solubilizer is directly proportional to the dissolved content of Dapsone, however as the concentration of Dapsone increased from 2.5 to 7.5 the phenomenon of recrystallization is evident and dissolved content gets

reduced. With increase in recrystallization the cosmetic elegance of product gets compromised due to gritty feel.
From the surface response plots and contour plot (Fig 7-9), it is evident that maximum dissolved content of dapsone was observed at the higher levels of solubilizers and HPβCD and lower level of Dapsone in formulation

Response optimizer for estimation of optimized formulation
The data obtained in DoE batches was further used in, the response optimizer using Minitab software to identify the best combination of formulation variables that jointly provide the desirable set of responses. This tool was used to determine the closest possible concentration of Dapsone, HPβCD and Solubilizer.
Accordingly, few formulations were derived for the response as follows

Table 6: Percentage of formulation variables vs responses to achieve desired results

Responses	**Cum % release Dapsone/ Zone of inhibition/ % dissolved Dapsone**	**Cum % release Dapsone/ Zone of inhibition/ % dissolved Dapsone**	**Cum % release Dapsone/ Zone of inhibition/ % dissolved Dapsone**	**Cum % release Dapsone/ Zone of inhibition/ % dissolved Dapsone**
Goal	**60/ 25/ 100**	**70/ 25/ 100**	**88.2/ 42.7/ 100.2**	**36.9/ 14.2/ 100.2**
Formulation variables	**Formula 1**	**Formula 2**	**Formula 3**	**Formula 4**
Dapsone	2.5	3.18	7.5	4.06
HPβCD	1.35	2.04	3.0	3.0
Solubilizer	20.6	24.9	34.79	16.21

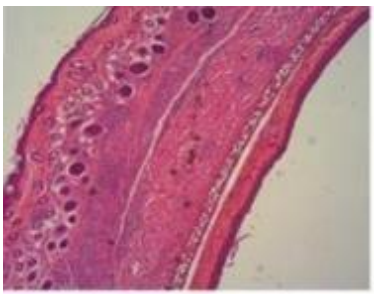
Formula 1 and 2 were evaluated for anti-acne activity using *in vivo* ear thickness model, formula 4 were not evaluated further due to recrystallization of Dapsone after adding in gel base and formula 3 was not used due to issue of skin irritation observed in formulations containing Dapsone 7.5%.

***In-vivo* studies: ear thickness model**
To assess *in-vivo* efficacy of different formulations, rat ear model of Acne was performed. In this study normal immuno-competent Sprague Dawley rats (SD rats) were used to evaluate the *in-vivo* efficacy of HPβCD based gel containing Adapalene and Dapsone. Specific-pathogen-free animals were used for these studies and animals were allowed seven days of acclimatization before commencing experiment. The experimental environment temperature was kept at 25±2°C with a relative humidity of 40-60 %. The rats were provided with sterile water ad libitum and chow food during the whole study period.
Ear thickness of rat was measured after topical application of formulations on ventral aspects of the right ear of the animals of all the groups from day 0 to 14 days. Intradermal inoculation of *P. acne* on day 0 caused a significant increase of ear thickness on day 1 as compared to day 0 just before infection in all the groups of animals, indicating a strong inflammatory response elicited by *P. acne*. The *P. acne* injected ears continued to thicken until day 14. Formulation prepared with formula 1 and formula 2 has shown the significant inhibition in inflammation on day 14 of as compared to un-treated group. These formulations have depicted enhanced efficacy in reducing ear thickness. This superior anti *P. acne* efficacy must be appreciable owing to the substantial anti-inflammatory effect due to enhanced solubility and better permeation of solubilized Dapsone; apart from this due to presence of HPβCD in the formulation the permeation through skin is enhanced which ultimately build-up the absorption of the drug.

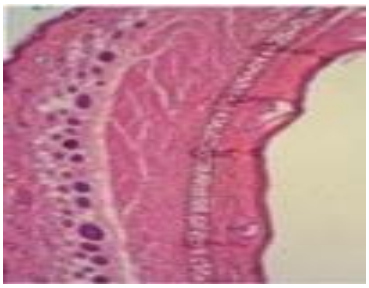
Histopathological study of the rat

After the successful completion of 14 days of anti-acne activity testing, animals from the respective group were sacrificed. The ears were excised and fixed in 10% (v/v) formalin solution before cutting into the fine vertical sections. This step was followed by the staining of sections with haematoxylin and eosin dye. The glass slides with stained ear sections were examined under an optical microscope.

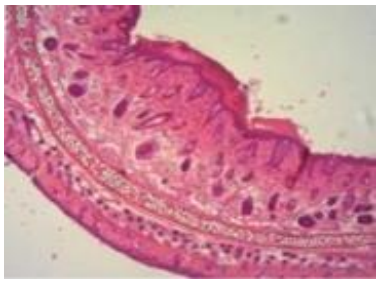
Histological examination of untreated rat showed that the *P. acne* injection induced a significant increase in the number of infiltrated inflammatory cells. The most histological changes occurred in the pilosebaceous follicular structure. However, the test formulation treated SD rat ears showed significantly less inflammatory reactions and infiltrating cells. We observed that although marketed formulation was able to reduce the inflammatory manifestations, but the formulation (Formula 1 and Formula 2) impressively reduced the inflammatory response of *P. acne*. Accordingly, histopathological analysis agreed with the inflammatory changes seen in rat ear thickness model, indicating improved efficacy with (Formula 1 and Formula 2) as compare to the Marketed formulation.



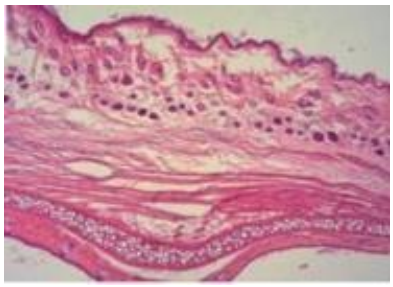
Marketed formulation



Formula 1



Formula 2



Untreated

Figure 10. Histopathology evaluation of rat ear

Conclusion

Depending on the percentage of HPβCD and the type of solubilizer used in the formulation the recrystallization of Dapsone within the aqueous gel is avoided, which increased the dissolved content of Dapsone to 100%. HPβCD performed dual functions in the formulation as solubility enhancer and improvement in cumulative release of Dapsone. Formulation trials where dapsone was not recrystallized showed excellent cumulative release through artificial membrane and better or comparable zone of inhibition than marketed products. Hence, in the present work, a cosmetically elegant formulation was developed by adding the complexing agent HPβCD to enhance the solubility of poorly water-soluble drug, Dapsone. Optimized formulation was a combination product of Adapalene 0.1% and Dapsone 3.2% which showed excellent antiacne activity by ear thickness model using Sprague Dawley rats. The developed formulation was stable, elegant and can be the cost-effective Product than marketed formulations.

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