QUALITY BY DESIGN APPROACH FOR DEVELOPMENT OF CYCLODEXTRIN BASED TOPICAL GEL FORMULATION OF DAPSONE AND ADAPALENE

Rupesh Subhashchandra Kotwal[1*], Manmohan Singh Jangdey[2], Sonia Singh Thakur[3]

Abstract

The aim of the present study is to formulate the cyclodextrin based topical Gel of Dapsone and Adapalene as antibacterial agent for the treatment of acne. The uniqueness of Dapsone, 4,4′-diaminodiphenylsulfone (DPS) is attributed to its dual antimicrobial and anti-inflammatory effects. Nevertheless, DPS limited solubility in water makes its topical formulation for treatment of dermatological conditions effective at higher percentage. The interaction between DPS and Hydroxypropyl β-cyclodextrin (Betadex®) in the solution state was investigated using phase solubility technique. Dapsone and Adapalene combination gel formulated using different gelling agent and solubilizers. All the formulation was developed by quality by design approach (QBD), which requires a multivariate approach for understanding the multifactorial relationships among formulation parameters. All the formulation was found to be physically stable and efficacious when compared with marketed product. Cyclodextrin used as complexing agent which enhanced the solubility of Dapsone within the formulation thus avoids recrystallization and improves the efficacy with use of optimum percent of Dapsone within the formulation. To assess the efficacy of prepared gel for anti-acne activity, *Ex vivo* Antibacterial studies, Cumulative release studies through vertical diffusion cell. Dapsone and Adapalene gel showed potent antibacterial activity for P. acne strain through the cylinder plate technique.

The Adapalene and Dapsone gel combination product using Cyclodextrin could be the cost-effective product due to use of optimum concentration of Dapsone in solubilized form over the marketed products which have high percent of Dapsone and are gritty in appearance.

Keywords: Cyclodextrins, Dapsone, Acne, Gel, Adapalene.

[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

DOI: - 10.31838/ecb/2023.12.si5.0144

INTRODUCTION

Quality by design (QbD) assemblages of statistical and mathematical models. It is very useful in systemically designing dosage forms with better quality and getting desired better clinical activity. QbD will also help in finding the risks associated with dosage form development, followed by reducing the same [1]. Based on the number of dependent and independent factors, different models are available in QbD to design the products with the utmost quality. Acne is the most common skin disease that affects a large number of adolescents and young adults after they reach puberty. Though not a life-threatening disease, it has serious psychological impact on the patient. Chronic inflammatory acne can also result in permanent scarring of the face.

There are multiple factors that contribute to the pathogenesis of acne, these include:

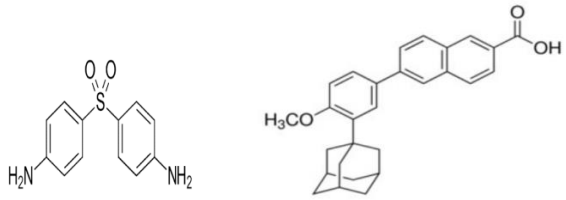
(1) Over activity of sebum production as a result of hormonal changes at puberty.

(2) Colonization of Propionibacterium acnes {P. acnes) in the pilosebaceous unit.

(3) Hyperkeratinization or abnormal desquamation of epithelium of the upper follicle (above the sebaceous gland) that results in blockage of the pilosebaceous canal.

(4) Formation of inflammatory molecules as a result of the action *P. acnes* on sebaceous lipids.

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 [2]. 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 [3]. 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. [4]. 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 log P and pKa values are 0.97 and 2.41 respectively[2]. 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 [5,6]. 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

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. 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 [9,10]. 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 [11]. A previous study showed improvement in DPS 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, Disodium Edetate was purchased from SD Fine as analytical grade. Betadex® (Hydroxypropyl beta-cyclo-dextrin) was kindly supplied by Roquette Frères (Lestrem, France).

Formulation base for the development of gel product

Gel bases published in literatures as well as in Handbook of Pharmaceutical formulations uses carbomer homopolymer type A, B or C or other cellulose based gelling agents which forms the aqueous gel base to carry drug to the site of action on skin surfaces. Marketed formulations are also aqueous gel bases which are formulated using carbomer homopolymers. On the basis of literature survey, we designed formulation as shown in **Table 1**, table also shown the property of each component used in formulation.

Table 1: Component use for formulation of dapsone adapalene gel

Component	Concentration in %w/w	Remark
Adapalene	0.1	Adapalene and Dapsone are used as antiacne treatment
Dapsone	5.00	
Drug solubilizer	20 to 40	Suitable solubilizer to be used for Solubilization of Dapsone and to avoid recrystallization
Gelling agent	0.5 to 4.0	Polymers which form gel when dispersed in water shall be used to impart viscosity to base
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.

Prototype Formulation development of gel base

Traditionally accepted manufacturing process available in literatures and books was followed for preparing the Prototype Gel formulation with below formula shown in **Table 2**. Formula for Prototype Gel Product: **Batch Number: Gel 001**

Table 2: List of components along with quantity for formulation of prototype gel

Component	Qty.(%w/w)	Qty.(mg/gm)	Batch quantity(50g)
Adapalene	0.10	1.00	0.05g
Dapsone	5.00	50.00	2.50g
Diethylene glycol monoethyl ether (Transcutol P)	25.00	250.00	12.50g
Carbopol 980	0.9	9.00	4.50g
Methylparaben	0.2	2.00	0.10g
Sodium Hydroxide 10% Solution	q.s. to pH	q.s. to pH	q.s. to pH
Purified water(RO water)#	q.s. to 100	q.s. to 1g	34.40g#

Manufacturing process

Gel phase preparation: Gelling agent is dispersed in Purified water under stirring to form lump free dispersion and complete hydration.

Drug phase preparation: Methylparaben and Dapsone is dissolved in Transcutol P to form clear solution.

Addition of drug in gel base: Adapalene is slowly added to Gel base and mixed, followed by addition of Dapsone solution. Mixing was continued for next 20 to 30 minutes.

Sodium hydroxide solution is 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.

Evaluation of gels

Organoleptic characteristics of hydrogels

The prepared gel formulations were tested for homogeneity by visual inspection after their insertion in the suitable vials; in addition, occurrence of any aggregates was inspected.

Content uniformity of Dapsone

DPS uniformity 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 DPS concentration measured spectrophotometrically at wavelength 293 nm.

Content uniformity of adapalene

Adapalene uniformity 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 [13].

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].

Result and Discussion

Quality by design (QbD) elements for further optimization of product formula and process

Quality target product profile (QTPP)

The quality target product profile (QTPP) is defined as "A prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product". The QTPP for any dosage forms are those quality attributes that are to be present in the product which is used by customers. Few examples for Topical dosage form specific QTPPs may include dosage form, route of administration, particle size or globule size, rheological behaviour, drug concentration, drug homogeneity, pH, in vitro drug release and permeation, microbial limits, packaging material etc. These can be controlled and optimized within the dosage form by using the modern development concept said as "Quality by Design" further referred as QbD studies, which ensures reproducible and robust formulation which can retain the end-product quality to desired shelf life. Quality Target Product Profile (QTPP) was set for current formulation based on the existing literature and design to be targeted for the research are shown in **Table 3**. QTPP is used to achieve for the drug quality characteristics for the better product quality, safety and efficacy.

Table 3: General Elements of QTPP for a topical cream and gel product

S. N	QTPP Elements		Target	CQAs (Yes/No)	Remark
1.	Dosage form		Aqueous Gel	NA	Skin targeted without systemic side impacts
2.	Route of Administration		Topical	NA	
3.	Dosage strength		Adapalene 0.1% Dapsone 5.0%	NA	Pharmaceutical equivalence as per marketed formulation
4.	Physical parameters of Drug Product	Description	Target shall be decided based on product performance in efficacy studies with the Aim to improve the efficacy of Active in the said indication	Yes	Affect the physiochemical stability
5.		pH			
6.		Recrystallization of Dapsone			Affect the drug permeation
7.		Specific gravity			Affect the product stability
8.		Appearance within Container			Affect the product efficacy
9.	Chemical testing	Assay of Actives			Affect the drug efficacy
10.	IVRT release studies		At least 75% of drug	No	Affect the product performance

		to be released from product		
11.	Efficacy studies	Improved or same as marketed formulations	No	Affect the product performance
12.	Storage Condition	Appropriate for dosage form	No	To maintain product efficacy throughout shelf -life
13.	Stability Studies	15 to 18 months	Yes	Minimum shelf life as applicable for pharmaceutical products available in Indian market
14.	Container closure system	Plastic tubes	Yes	Same as marketed formulation
15.	Dosage regimen	To be in line with marketed formulation or to reduce the dose and frequency to increase the efficacy of Active ingredients		

Critical quality attributes (CQA)

In CQA study, physical, chemical, biological study confirms the safety, and efficacy of drug product. Critical material process parameters can impact the critical quality attributes. CQA parameters are shown in **Table 4.**

Table 4: CQA parameters of drug product

Parameters	**Target**	**Is this CQA Critical?**	**Justification**
Description / Appearance	White to off-white gel	Yes*	Description may not be directly linked to safety and efficacy. Therefore, it is not considered to be critical. However, the target is set to ensure patient acceptability. Process variable may have impact on description so it is discussed in details only in manufacturing process optimization.
Identification	Adapalene and Dapsone	Yes*	Though identification is critical to safety and efficacy, this CQA can be effectively controlled by the quality management system and will be monitored at drug product release.
pH	Between 4.0 to 7.0	Yes	pH shall be monitored in stability to check the trend of pH and its impact on other critical quality attributes. However, pH shall not be discussed further in formulation and process optimization as there are no pH adjusting agents in the formulation. Also, process variables have no impact on pH of finished product.
Viscosity	Suitable to provide efficacious product	Yes	Viscosity shall be monitored during product release and in stability. Viscosity can impact the microstructure of product which can directly impact drug release and efficacy.Formulation and process variable may have impact on viscosity.
Specific gravity	Between 0.85 to 1.05	Yes	Specific gravity shall be monitored during product development and shall be evaluated during process optimization. Formulation variables have no impact on Specific gravity of finished product.
Assay of Adapalene	Between 90% to 110% of label claim	Yes	Assay variability will affect safety and efficacy. Process variables may affect the assay of the drug product. Thus, assay will be evaluated throughout product and process development.
Assay of Dapsone	Between 90.0% - 110.0 % of label claim	Yes	
Recrystallization of Dapsone	Avoid recrystallization of Dapsone	Yes	Particle size of Active ingredient is directly impacting drug release profile and efficacy of product. However, both formulation and process variables may not have direct impact on PSD of Active ingredient and shall be controlled at input material.
In-vitro release studies	Not less than 75%	Yes#	Drug release shall be monitored during product development. Product release profile shall help to decide the formulation which can be effective in delivery of Active ingredients to site of action.Formulation and process variable may have impact on rheological properties.
Microbial limits	Free from microorganisms	Yes*	Non-compliance to microbial limits will impact patient safety due to contamination of Product with micro-organism. The formulation and process variables are unlikely to impact this CQA.

* Formulation and process variables are unlikely to impact the CQA.

Table 5: CQA parameters of excipients used in formulation

Functional Category of Excipients	**Excipients Name**	**CQAs Impacted**	**Remark**
Solvent/ penetration enhancer	Propylene glycol, Diethylene glycol monoethyl ether, Dimethyl Isosorbide	Recrystallization of Dapsone, Invitro release	Solvent will solubilize the drug particles within the formulation, hence will impact the particle size, Globule size and release of drug through the formulation and finally impacts product efficacy
Gelling agent	Carbopol 934, Carbopol 980, Sepineo P 600	pH, Viscosity, rheological properties, Invitro release	Microstructure is formed by gelling agent hence release of drug may affect by gelling agent and finally impacts product efficacy

Critical material attributes (CMA) of Excipients to be used in gel formulation

Critical material attributes for excipients shall be decided based on pharmaceutical function of the excipient in the drug product. Excipients used in topical products are responsible for determining the microstructure of formulation base which decides the release profile of Active ingredient through the formulation (**Table 6**).

Table 6: Critical material attributes for excipients used in formulation

Critical Material Attributes of solvent/ penetration enhancer	**Solvent (penetration enhancer):** Propylene glycol, Diethylene glycol monoethyl ether, Dimethyl Isosorbide								
	Drug product CQA								
	Description/ Appearance	**pH**	**Viscosity**	**Rheological properties**	**Specific Gravity**	**Assay of Adapalene**	**Assay of Dapsone**	**Particle size distribution**	***In-vitro* release studies**
Solubilizer	Low	Low	Low	Low	Low	Low	Low	Medium	High
Quantity of excipient	Low	Low	Low	Low	Low	Low	Low	Medium	High
Critical Material Attributes of gelling agent	**Gelling Agent:** Carbopol 934, Carbopol 980, Sepineo P 600								
	Drug product CQA								
	Description/ Appearance	**pH**	**Viscosity**	**Rheological properties**	**Specific Gravity**	**Assay of Adapalene**	**Assay of Dapsone**	**Particle size distribution**	***In-vitro* release studies**
Type of gelling agent	Low	Medium	High	High	Low	Low	Low	Low	High
Quantity of excipient	Low	Low	High	High	Low	Low	Low	Low	High

Critical process parameters

In manufacturing process of gel formulation, the order of excipient addition is important and can impact product rheological characteristics and drug release profile, hydration of gelling agent in aqueous phase is critical step and will involve process parameters like stirring speed and time (**Table 7**). Drug phase preparation involves stirring/ homogenization time and speed which ensure complete dissolution/ dispersion of active ingredient. Drug phase is added to gel phase either before neutralization or after neutralization Drug phase addition can be through slurry addition port or through powder duction port. Vacuum application at different stages of bulk formation is an important process parameter which removes air pockets formed within the gel base, which may create interference in the drug release profile as well as product elegance.

Table 7: Critical process parameters for aqueous gel formulation

Critical Process Parameters	**Drug product CQA**								
	Description/ Appearance	**pH**	**Viscosity**	**Rheological properties**	**Specific Gravity**	**Assay of Adapalene**	**Assay of Dapsone**	**Particle size distribution**	**Invitro release studies**
Gelling agent dispersing speed	Low	Low	High	High	Low	Low	Low	Low	Low
Hydration time	Low	Low	High	High	Low	Low	Low	Low	Low
Drug Dispersion stirring speed	Low	Low	Low	Low	Low	Low	Low	High	High
Drug dispersion stirring time	Low	Low	Low	Low	Low	Low	Low	High	High
Homogenization speed	Low	Low	High	High	Low	Low	Low	Low	Low
Vacuum	Low	Low	Low	Low	High	Low	Low	Low	High
Bulk mixing time after Drug phase addition	Low	Low	Low	Low	Low	Low	Low	High	Low

Gel formulation optimization

Prepared gel formulation of dapsone adapalene was optimized and shown in **Table 8& 9**.

Design Class: Factorial design

Name of Design: Full factorial

Number of Factors (variables): 2 and levels: 3

Number of runs: 9

Table 8: Optimization of gel formulation

Factors	Levels		
	Level 1	**Level 2**	**Level 3**
Gelling agent	Carbomer homopolymer Type C	Carbomer homopolymer Type B	Sepineo P 600
Solubilizers	Diethylene glycol monoethyl ether	Propylene Glycol	Dimethyl Isosorbide

Table 9: The randomized experiments designed by software using full factorial design with 2 factors and 3 levels

Formulation	Gelling agent	Solubilizer
TG 1	CTC	PG
TG 2	CTB	PG
TG 3	SEP600	DGME
TG 4	CTC	DGME
TG 5	CTB	DGME
TG 6	CTB	DMI
TG 7	SEP600	PG
TG 8	CTC	DMI
TG 9	SEP600	DMI

Formulation of Gel

For formulation of gel different ingredients are used which are shown in **Table 10.**

Table 10: The different grades of excipients used in the experimental design

SN	Ingredients	TG1	TG2	TG3	TG4	TG5	TG6	TG7	TG8	TG9
1.	Dapsone	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
2.	Adapalene	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
3.	Methylparaben	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
4.	Carbomer Type C	0.90			0.90				0.90	
	Carbomer Type B		0.90			0.90	0.90			
	Sepineo P 600			4.00				4.00		4.00
5.	Propylene glycol	30.00	30.00					30.00		
	DGME			25.00	25.00	25.00				
	DMI						15.00		15.00	15.00
6.	Sodium Hydroxide 10% sol	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
7.	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

All the formulation trials were evaluated for Viscosity, Rheology, Microscopy, antibacterial activity and cumulative release through artificial membrane and results are recorded and evaluated for future plan of action.

Discussion on optimized process: After execution of several trials the best possible process derived from process optimization is as follows:

a. It was observed that the rate of addition of Dapsone solution in gel base shows impact on crystal habit and particle size. Hence, Dapsone solution was slowly added to gel base.

b. Addition of Dapsone solution in neutralized thick gel and dispersion of gelling agent before neutralization also showed difference in crystal habit. Hence, Dapsone solution was added to gel base before neutralization under homogenization.

c. Adapalene is added in aqueous phase before addition of gelling agent in powder form under homogenization and gelling agent is added after API dispersion. However, it is recommended to add dispersion of Active ingredient within the formulation to avoid non-uniformity and formation of API aggregates in the product. The same shall be taken in consideration during the manufacturing of optimized final Combination product.

Process optimization improved the product elegancy; however, the gritty appearance of formulation due to recrystallization of Dapsone could not be avoided. The same optimized process was exactly followed for all DoE trials to evaluate the impact of excipients grades on formulation properties. Viscosity of formulation did not change significantly and all formulations found in range of 8000 to 11500mPas for all possible process executed.

The process established produced the Dapsone API in the range of D90: 60 to 90 microns.

Evaluation of physical parameters for gel formulation

All the prepared gel formulation was evaluated for various physical parameters and presented in **Table 11.**

Table 11: Evaluation parameters of prepared gel

Formulation	Description	pH	Viscosity (mPas)	Specific Gravity	Microscopic evaluation	
					Dapsone Particle Shape	Dapsone Particle Size
TG 1	A	6.20	9867	1.09	Spike like	D90: 72µ
TG 2	A	6.15	8007	1.00	Spike like	D90: 94µ
TG 3	B	5.51	11169	0.99	Orthorhombic	D90: 88µ
TG 4	B	6.13	9753	1.08	Orthorhombic	D90: 91µ
TG 5	B	6.21	8091	0.99	Orthorhombic	D90: 69µ
TG 6	A	6.16	7831	1.09	Cubic & monoclinic	D90: 45µ
TG 7	A	5.64	11327	0.99	Spike like	D90: 90µ
TG 8	A	6.25	9916	0.98	Cubic & monoclinic	D90: 81µ
TG 9	A	5.58	11301	1.07	Cubic & monoclinic	D90: 79µ

A: Off white homogenous smooth viscous gel with gritty particles

B: Off white homogenous smooth viscous gel with slight gritty particles

Microscopic images of gel formulation for dapsone particles

Particle size of all gel formulation of cyclodextrin induced dapsone and adapalene was evaluated by microscope and shown in **Figure 2.** Cyclodextrin increase the solubility of dapsone and prepared homogeneous smooth viscus gel.

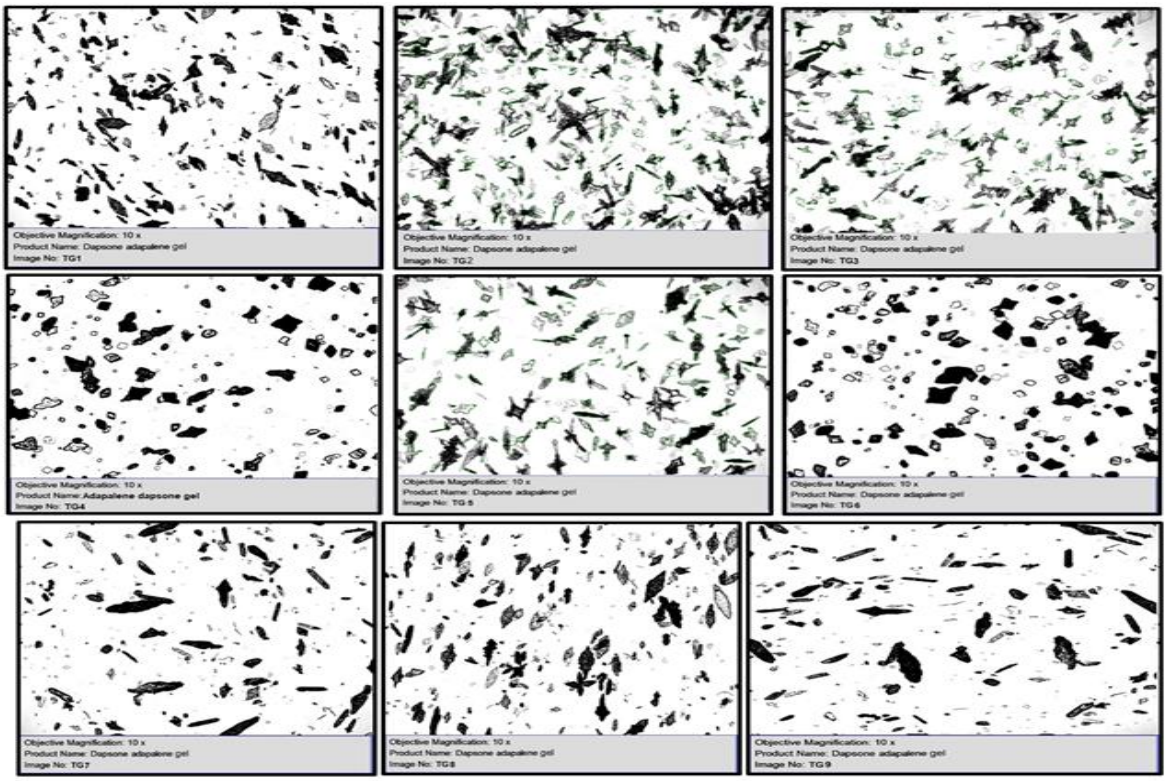


Figure 2: Crystal shapes of recrystallized dapsone in gel formulation at 10X

Optimized process was followed for manufacturing of all gel trials using different grades of excipients to get robust, stable, and efficacious formulation.

pH of gel formulation: Carbopol was used as gelling agent in trial number TG1, TG2, TG4, TG5, TG6 and TG8 hence, pH adjustment was done using 10% solution of Sodium Hydroxide. Formulations TG3, TG7 and TG 9 was manufactured by using Sepineo P 600 as gelling agent which is pH independent viscosity building agent. Hence, formulation with Sepineo P 600 does not contain Sodium Hydroxide as pH modifier.

Viscosity of gel formulation: Viscosity is the rheological characteristics impacting the microstructure of Topical formulation which will directly impact the release characteristics of active ingredient through the formulation. Hence, as per literatures available the concentration of gelling agent was finalized to 0.9% when Carbopol was used and 4% when Sepineo P 600 was used. Viscosity for Sepineo P 600 trials was higher than viscosity of either grade of Carbopol.

Particle size distribution: Micronized Adapalene was dispersed in the gel formulation to form uniform distribution. Microscopic evaluation was done to ensure the uniformity of dispersion. Randomly two formulations were selected for capturing the microscopic images. Both these formulations showed the D90 of Adapalene particles within 5 to 8 µ which was in-line to the input API particle size.

Dapsone was solubilized in solvents and then added in base before formation of viscous gel, however it was clearly observed that after addition of Dapsone solution in gel base, recrystallization of Dapsone happens within the formulation. Microscopic observation of formulation showed that recrystallization was much faster and larger particles are formed in gel formulation. Average particle size of Dapsone in gel formulations was determined to be D90: between 45 to 94 µ. Different crystal habit was obtained by change in the solvent used to prepare Dapsone solution. Crystal shape obtained in formulation containing propylene glycol showed irregular spike shaped structures.

Drug content: DoE trials of gel formulation were evaluated for estimation of Adapalene and Dapsone Drug content using UV visible spectroscopic method. Results were presented in **Table12**.

Table 12: Evaluation of the drug content in formulations of adapalene &dapsone gel

S. No	Formulation code	Dapsone Content	Adapalene content
1.	TG 1	102.04	98.31
2.	TG 2	99.53	99.06
3.	TG 3	100.19	101.50
4.	TG 4	99.95	98.95
5.	TG 5	100.05	102.11
6.	TG 6	99.02	99.05
7.	TG 7	99.11	99.10
8.	TG 8	101.46	100.4
9.	TG 9	101.10	97.90

Ex-vivo studies for antibacterial activity of dapsone through gel formulation

Antibacterial study

Dapsone acts as antibacterial agent against microorganisms responsible for acne vulgaris. Dapsone suppress folate formation in bacteria's deoxyribonucleic acid, Dapsone's antibacterial effect is mediated by competitive suppression of this enzyme. Its mechanism of action resembles sulfonamides to act as antibacterial compounds. Thus, bacteriostatic activity of dapsone against Propionibacterium acnes (P. acnes) suggests the role of Dapsone in acne.

The zones of inhibitions for the antibacterial activity were determined for all DoE trial batches to know the activity of solubilized Dapsone and were compared with the standard marketed formulations. Zone of inhibitions was generated for all the formulation trials (TG1 to TG9) as well as Acnedap gel and Acnedap plus gel and presented in **Table 13**. These results suggested that all formulations have antibacterial potential to inhibiting the P. acne. The zone of inhibition of all formulation and marketed formulation was shown in.

Table 13: Antibacterial activity of all formulation of dapsone adapalene gel in *P. acne*

Formulations	**Zone of inhibition in mm**				
Concentration (Dapsone 5%)	**1**	**2**	**3**	**Mean**	**SD**
TG 1	11.6	11.8	12.1	11.83	0.25
TG 2	11.8	12.1	12.1	12.00	0.17
TG 3	21.7	22.2	21.2	21.70	0.50
TG 4	24.1	24.5	23.9	24.17	0.31
TG 5	26.9	27.0	26.8	26.90	0.10
TG 6	20.9	21.0	21.2	21.03	0.15
TG 7	14.3	14.7	15.0	14.67	0.35
TG 8	23.2	22.9	23.1	23.07	0.15
TG 9	20.9	21.0	21.7	21.20	0.44
Acnedap gel	16.7	15.9	16.3	16.30	0.40
Acnedap plus gel	22.2	21.9	21.7	21.93	0.25
RO water	No inhibition observed				

***In-vitro* diffusion study**

In-vitro diffusion study of combination drug product was done using the Franz diffusion cell. Franz diffusion cell has been the standard system used for the study of release of semi-solid drug formulations. Synthetic membranes commonly used for *in vitro* release studies are cellulose acetate, nylon, polycarbonate having porous

characteristics with pore size of 0.45µ. Tuffryn membranes were used in the study for release of Adapalene and Dapsone. The receptor membrane used to study release characteristics of Adapalene contains 50% tetrahydrofuran, 20% Propylene glycol and 30% phosphate buffer 5.8. Samples were taken at an interval of 0.0, 1.0, 2.0, 4.0, 6.0 and 8.0 hours.

The receptor membrane used to study release characteristics of Dapsone contains 30% tetrahydrofuran, 30% Propylene glycol and 40% phosphate buffer 5.8. Samples were taken at an interval of 0.0, 1.0, 2.0, 4.0, 6.0 and 8.0 hours

Based on Antibacterial studies few formulations were removed from IVRT studies and formulation trials TG3, TG4, TG5, TG6, TG8 and TG9 were evaluated for cumulative release studies and shown in **Table 14 and Figure 3 for Dapsone and** in **Table 15 and Figure 4 for Adapalene**.

Table 14: Release profile of dapsone through gel formulations

Sq. rt of time in minutes	% Drug released of Dapsone from gel							
	TG3	TG4	TG5	TG6	TG8	TG9	Acendap Gel	Acnedap Plus Gel
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7.74	15.2	9.6	18.9	8.0	6.9	7.1	5.8	8.2
10.95	22.1	17.1	28.4	12.8	13.0	16.0	15.7	22.1
15.49	37.6	30.6	40.4	21.9	19.8	29.1	35.8	34.8
18.97	48.6	40.8	59.3	30.9	34.6	38.3	50.2	55.8
21.9	69.9	60.9	77.9	46.7	42.9	50.2	75.1	73.3

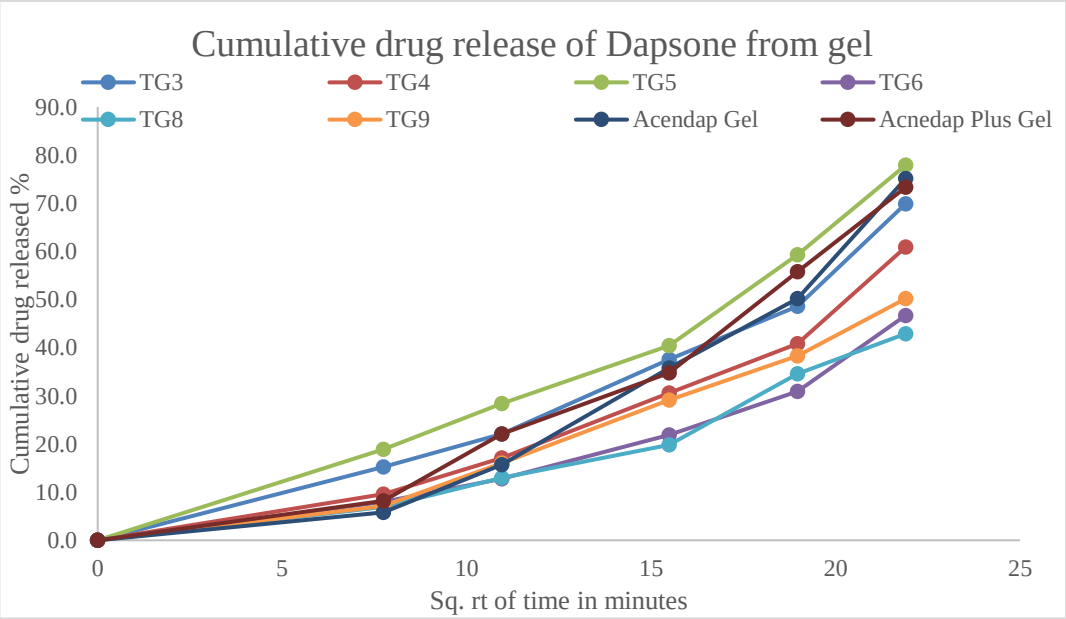


Figure 3: Release profile of Dapsone through gel formulations

Table 15: Release profile of adapalene through gel formulations

Sq. rt of time in minutes	% Drug released of Adapalene from gel							
	TG3	TG4	TG5	TG6	TG8	TG9	Adaferin Gel	Acnedap Plus Gel
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7.74	8.7	8.9	10.1	8.9	11.1	9.2	7.2	10.8
10.95	15.3	14.6	16.6	16.0	19.0	14.9	13.9	17.7
15.49	24.4	20.0	26.2	22.9	27.5	22.1	19.8	25.8
18.97	31.0	26.6	32.3	35.6	37.9	31.2	25.8	30.8
21.9	39.9	35.1	47.9	42.5	50.1	41.0	33.9	45.9

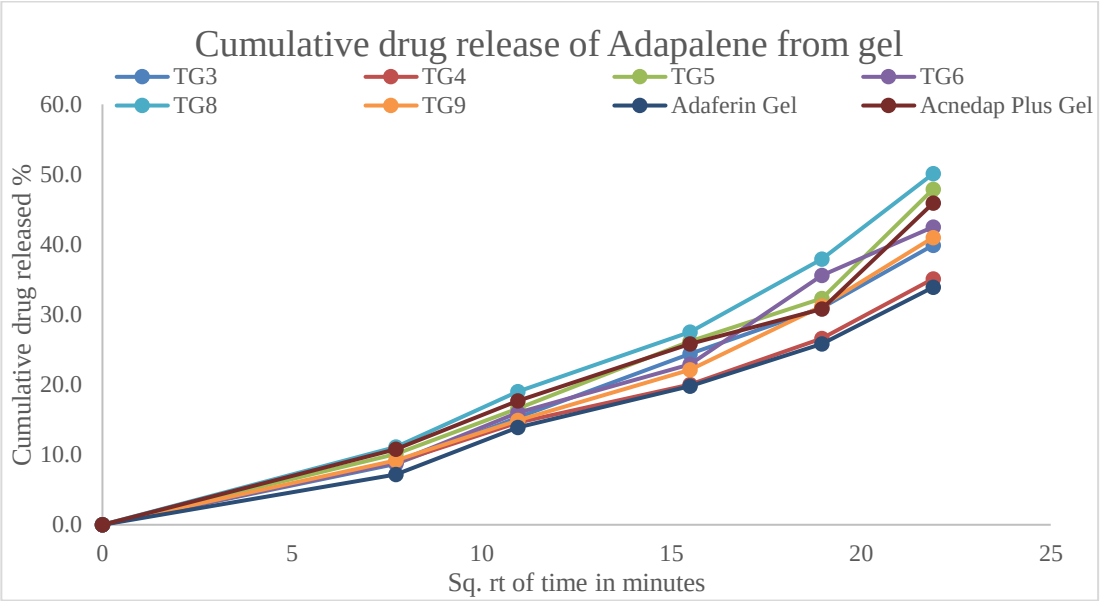


Figure 4: Release profile of adapalene through gel formulations

Conclusion

The combination gel of Dapsone and Adapalene formulated using different gelling agent and solubilizers were found comparable with marketed products and were found to be physically stable and viscous products. However, all the formulation was gritty due to recrystallization of Dapsone within the formulation. The particle size of recrystallized drug was found to be of bigger size when compared to cream formulation. Depending on the solubilizer used the crystal size and shape of Dapsone API within the formulation changed as presented in the microscopic images table. Dapsone & Adapalene gel formulated did not show improvement in antibacterial activity for P. acne strain through the cylinder plate technique, when compared with marketed products. The zone of inhibition was measured using zone reader software Interscience, which directly provides the zone of inhibition for sample. Physicochemical parameters include pH, viscosity, rheology and particle size and shape by microscopy which did not show any significant difference in the *In-vitro* diffusion study and result of all these parameters indicated that trial batches with Dimethyl Isosorbide i.e. trial number TG6 have shown minimum recrystallization of Dapsone and optimum release of Dapsone through the artificial membrane. All the formulations were stable and active against P. acne strain, but the recrystallization of Dapsone was not avoided. Hence, it was concluded that the individual solubilizers didn't avoid recrystallization of Dapsone within the gel formulation. Cyclodextrin was a proved complexing agent capable of enhancing the solubility of lipophilic active in different dosage forms. Very limited research was available towards use of cyclodextrin for topical dosage form. Hence, the research was further extended towards development of Cyclodextrin – Dapsone host guest formation which will avoid the recrystallization and improve efficacy with use of optimum percent of Dapsone within the formulation.

Optimization trials for finalization of drug strength

In order to design and develop an improved combined drug product for treatment of acne vulgaris with cosmetic elegance but without compromising the efficacy, the issue of recrystallization and use of optimum concentration of Dapsone in formulation was utmost important, hence, the study was further extended by using a widely used solubility enhancing agent Cyclodextrins (CDs) which are cyclic oligosaccharides forming inclusion complexes with lipophilic molecules, resulting in improved solubility of such molecules. To know the relationship of host-guest inclusion complexes, several Phase solubility studies were performed according to the method reported by Higuchi and Connors. An excess amount of Dapsone was added to 10 ml of distilled water containing increasing concentration of HP-β-CD solution (0.1-1.0 %) in 10 ml screw capped bottles. The contents were mixed for 2 days at 25°C on a shaker. After 2 days, the samples were filtered through Whatman filter paper No. 42 and absorbance's were recorded. Hence, further trials were conducted on gel formulation by using HP-β-CD as complexing agent and optimization of

Dapsone concentration was performed to get the cost-effective conventional product with better cosmetic elegance and reduced side effects.

Statistical design

A statistical design was created to study the effect of 3 Factors: Dapsone, HP-β-Cyclodextrin and solubilizer system used (Dimethyl Isosorbide or Diethylene glycol monomethyl ether [Transcutol P) on solution stability of Dapsone within the formulation, antibacterial activity through zone of inhibition studies and in vitro cumulative release of Adapalene and Dapsone from gels. Concentration of other ingredients was kept constant. Following is the design Summary

Design Class: Response Surface
Name of Design: Box-Behnken design
Number of Factors (variables): 3
Number of runs: 15
Results are shown in **Table 16, 17**. The amount of different ingredients used in different optimized formulation is showed in **Table 18**.

Table 16: 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 %	NA	15	NA
DGME %	25	NA	35

Table 17: Randomized experiments designed by software using Box-Behnken

Formulation	Dapsone	HPβCD	DGME	DMI
OG 1	5.00	2.0	NA	15
OG 2	5.00	1.0	25	NA
OG 3	5.00	3.0	25	NA
OG 4	7.50	2.0	25	NA
OG 5	2.50	2.0	25	NA
OG 6	2.50	3.0	NA	15
OG 7	7.50	3.0	35	NA
OG 8	5.00	3.0	NA	15
OG 9	2.50	3.0	35	NA
OG 10	7.50	1.0	NA	15
OG 11	5.00	3.0	35	NA
OG 12	7.50	3.0	NA	15
OG 13	5.00	1.0	35	NA
OG 14	2.50	1.0	NA	15
OG 15	5.00	2.0	NA	15

Table 18: Ingredients used in different optimized gel formulation

Ingredients	OG1	OG2	OG3	OG4	OG5	OG6	OG7	OG8	OG9	OG10	OG11	OG12	OG13	OG14	OG15
Dapsone	**5.00**	**5.00**	**5.00**	**7.50**	**2.50**	**2.50**	**7.50**	**5.00**	**2.50**	**7.50**	**5.00**	**7.50**	**5.00**	**2.50**	**5.00**
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**	**1.0**	**3.0**	**2.0**	**2.0**	**3.0**	**3.0**	**3.0**	**3.0**	**1.0**	**3.0**	**3.0**	**1.0**	**1.0**	**2.0**
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		**25.0**	**25.0**	**25.0**	**25.0**		**35.0**		**35.0**		**35.0**		**35.0**		
DMI	**15.0**					**15.0**		**15.0**		**15.0**		**15.0**		**15.0**	**15.0**
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							
Purified water								q.s. to 100							

Manufacturing process used for formulation optimization trials

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 so as 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 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: Carbopol 980 was dispersed 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.

All the DoE trials were manufactured as per the process described above and evaluated for below mentioned test parameters like viscosity, rheology, pH, and microscopy for dapsone recrystallization, antimicrobial activity of dapsone and invitro cumulative drug release. Based on the evaluation the best formulation was identified and evaluated for anti-acne activity through animal studies.

Table 19: Release profile of Dapsone through optimized gel formulations

Sq. rt of time in minutes	OG3	OG5	OG6	OG7	OG9	OG13	Acendap Gel	Acnedap Plus Gel
0	0	0	0	0	0	0	0	0
7.74	11.7	17.6	6.9	12	6.9	21.7	5.8	13.2
10.95	24.6	28.1	12.4	21.2	13	36.1	15.7	22.1
15.49	31.9	37.3	21.4	33.9	19.8	53.1	29.8	34.8
18.97	45.6	50.8	28.3	50.9	34.6	70.3	43.2	51.8
21.9	61.9	69.5	36.9	67.7	42.9	88.2	61.1	69.3

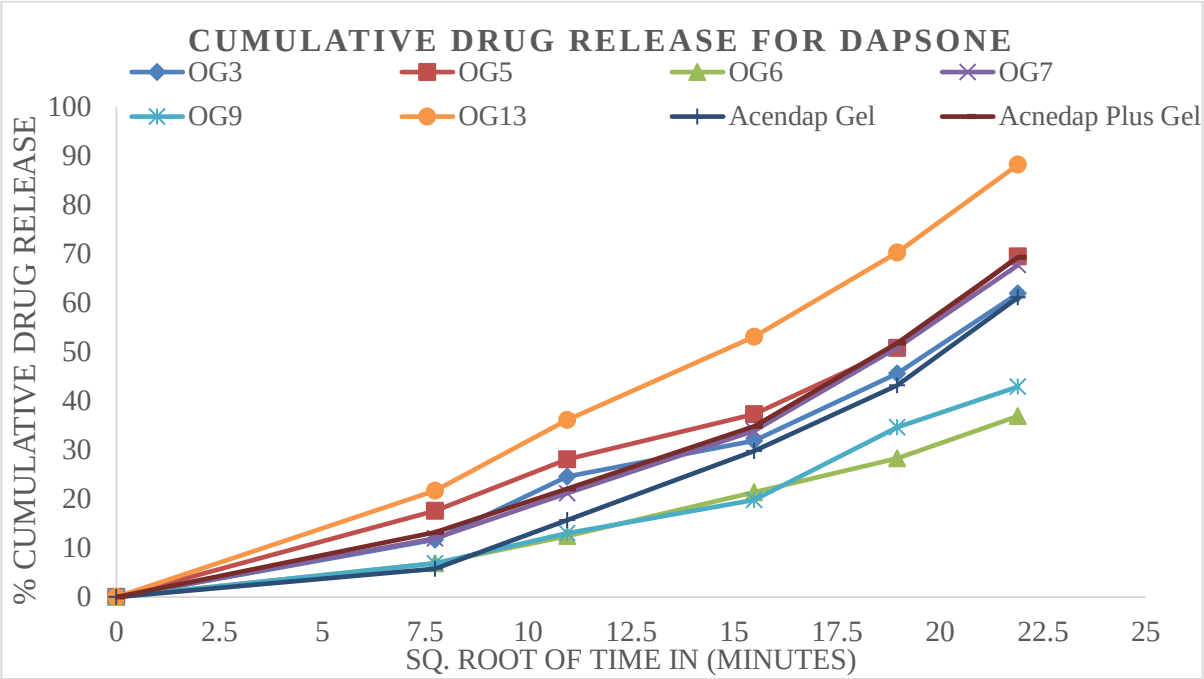


Figure 5: Release profile of Adapalene through gel formulations

Table 20: Release profile of Adapalene through optimized gel formulations

Sq. rt of time in minutes	OG3	OG5	OG6	OG7	OG9	OG13	Adaferin Gel	Acnedap Plus Gel
0	0	0	0	0	0	0	0	0
7.74	5.7	9.1	10.7	17	13.1	9.2	7.2	10.8
10.95	10.3	15	15.6	24.9	23.7	14.9	13.9	17.7
15.49	18.4	22.1	23.2	32.9	30.5	20.1	19.8	25.8
18.97	25.1	29.6	30.3	41.6	39.9	28.2	25.8	30.8
21.9	33.9	38.1	39.9	50.5	47.1	37.1	33.9	45.9

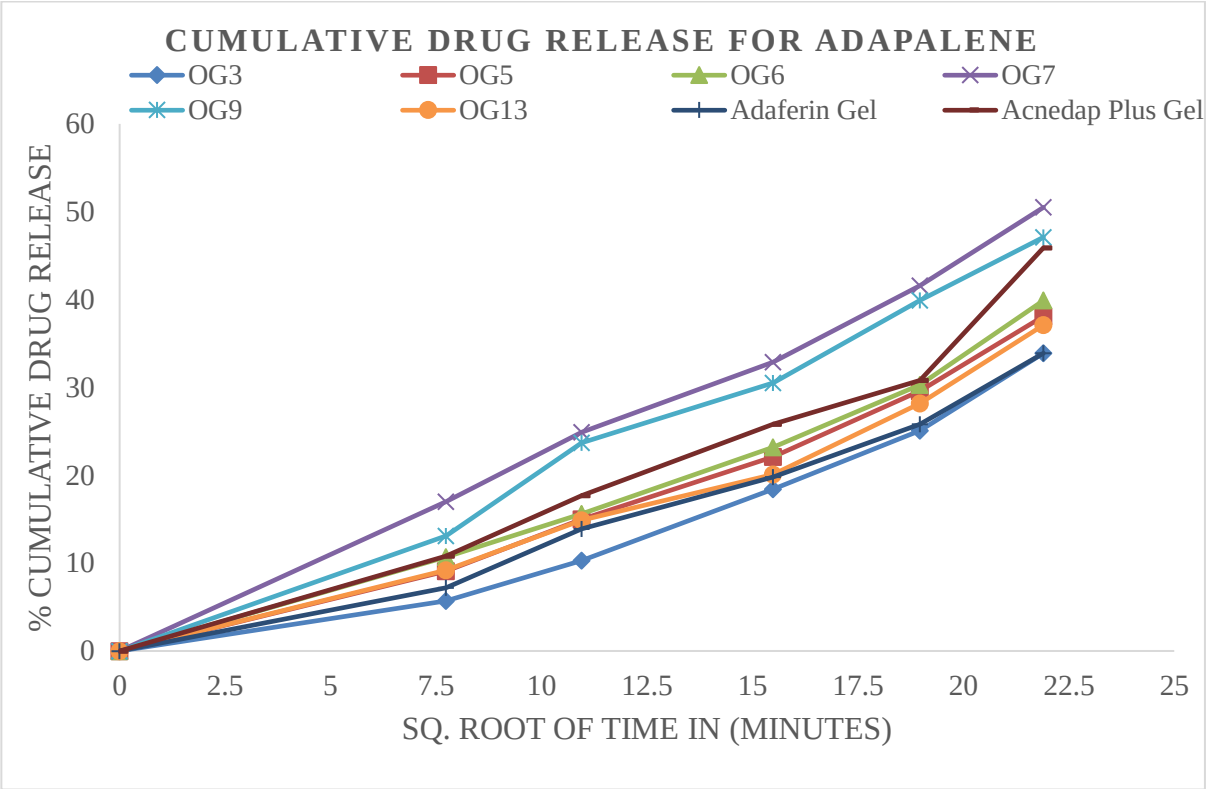


Figure 6: Release profile of adapalene through gel formulations

Table 21: Optimization of Dapsone concentration to improve efficacy with reduced strength

Formulation	Description	pH	Viscosity (mPas)	Zone of inhibition	Cumulative drug release (Dapsone)	Cumulative drug release (Adapalene)
OG 1	White coloured gritty gel obtained	6.05	9870	16.6	Not performed	Not performed
OG 2	White coloured gel with slight gritty feel is obtained	6.12	10030	14.7	Not performed	Not performed
OG 3	White coloured aqueous gel free from gritty particles is observed	6.04	9994	42.2	61.9	33.9
OG 4	White coloured gritty gel observed	6.01	10020	36.9	Not performed	Not performed
OG 5	White coloured aqueous gel free from gritty particles is observed	6.11	10005	25.3	69.5	38.1
OG 6	White coloured aqueous gel free from gritty particles is observed	6.10	9729	26.9	36.9	39.9
OG 7	White coloured aqueous gel free from gritty particles is observed	6.07	9870	42.7	67.7	50.5
OG 8	White coloured gel with slight gritty feel is obtained	6.00	10100	33.1	Not performed	Not performed
OG 9	White coloured aqueous gel free from gritty particles is observed	6.08	11000	22.7	42.9	47.1
OG 10	White coloured gritty gel observed	6.12	9995	15.5	Not performed	Not performed
OG 11	White coloured gel with slight gritty feel is obtained	6.10	10201	37.3	Not performed	Not performed
OG 12	White coloured gritty gel observed	6.06	10027	35.1	Not performed	Not performed
OG 13	White coloured aqueous gel free from gritty particles is observed	6.09	10000	31.7	88.2	37.1
OG 14	White coloured gel with	6.10	9996	13.0	Not performed	Not performed

Formulation	Description	pH	Viscosity (mPas)	Zone of inhibition	Cumulative drug release (Dapsone)	Cumulative drug release (Adapalene)
	slight gritty feel is obtained					
OG 15	White coloured gritty gel obtained	6.11	9771	14.6	Not performed	Not performed
Adaferin gel#	White to off white gel product	4.87	14259	NA	NA	33.9
Acnedap gel#	White to off coloured white gritty gel	5.84	11166	16.3	61.1	Not performed
Acnedap plus gel#	Off white coloured gritty gel	6.12	12154	21.93	69.3	45.9

Conclusion:

Depending on the percentage of HP-βCD and the type of solubilizer used in the formulation the recrystallization of Dapsone within the aqueous gel is avoided in trials OG3, OG5, OG6, OG7, OG9, OG13. 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, it can be concluded that cyclodextrin can be used as complexing agent capable of enhancing the solubility of dapsone to form complex which can reduce dapsone percent within the formulation to get optimized combination of adapalene and dapsone gel to get cost effective and cosmetically elegant Product than marketed products.

References

1. Srikar, G.; Rani, A.P. Study on influence of polymer and surfactant on in vitro performance of biodegradable aqueous-core nanocapsules of tenofovir disoproxil fumarate by response surface methodology. Brazilian. J. Pharm. Sci. 2019, 55, e18736,
2. M. Wakabayashi, et al., Cystic acne successfully treated with diaminodip-henylsulfone, J. Dermatol. 38 (5) (2011) 502–504.
3. V.R. Borges, et al., Nanoemulsion containing dapsone for topical administration: a study of in vitro release and epidermal permeation, Int. J. Nanomed. 8 (2013) 535–544.
4. G.L. Amidon, et al., A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability, Pharm. Res. 12 (3) (1995) 413–420.
5. T.H. Thi, N. Azaroual, M.P. Flament, Characterization and in vitro evaluation of the formoterol/cyclodextrin complex for pulmonary administration by nebulization, Eur. J. Pharm. Biopharm. 72 (1) (2009) 214–218.
6. J. Szejtli, Introduction and general overview of cyclodextrin chemistry, Chem. Rev. (1998) 1743–1754.
7. L. Szente, J. Szeman, T. Sohajda, Analytical characterization of cyclodextrins: history, official methods and recommended new techniques, J. Pharm. Biomed. Anal. 347–365.
8. Ivanna H. Grebogi, et al., Binary and ternary inclusion complexes of dapsone in cyclodextrins and polymers: preparation, characterization and evaluation, J. Inclusion Phenom. Macrocycl. Chem. 73 (1–4) (2012) 467–474.
9. British Pharmacopoeia (2008), volume I: 644
10. USP, The United States Pharmacopoeial Convention (34) (2011) Volume II: 2475.
11. Chowdhury M.R., Chowdhury M.H., Saha P., Rahman M.F., AlKabir M.F., EstiarHaque S.M. (2012). Development and validation of RPHPLC method for the simultaneous determination of Adapalene-Benzoyl Peroxide combination gel. International Journal of Advanced Science, Engineering, Technology & Research.1,5, 1-9.
    https://pubchem.ncbi.nlm.nih.gov/com pound/adapalene(Visited on 4 June 2019)
12. Rehab Nabil Shammaa, Ibtehal Salah Ad-dina, Nourtan F. Abdeltawab. Dapsone- gel as a novel platform for acne treatment: In vitro evaluation and In vivo performance and histopathological studies in acne infected mice. Journal of drug delivey science and technology. 54, 2019.
13. B. Chaudhary, S. Verma, Preparation and evaluation of novel in situ gels containing acyclovir for the treatment of oral herpes simplex virus infections, Scientific World Journal 2014 (2014) 280928.
14. Laura Ferreira, Filipa Mascarenhas-Melo, Sofia Rabaça, Ankita Mathur, Ankur Sharma, Prabhanjan S. Giram, Kiran D. Pawar, Abbas Rahdar, Faisal Raza Francisco Veiga, Priscila Gava Mazzola, Ana Cl´audia Paiva-Santos, Cyclodextrin-based dermatological

formulations: Dermopharmaceutical and cosmetic application, Colloids and Surfaces B: Biointerfaces, Accepted 8 November 2022

15. Hatem Al Sabaa, Fatma M. Mady, Amal K. Hussein, Hossam M. Abdel-Wahab and Maha H. Ragaie, Dapsone in topical niosomes for treatment of acne Vulgaris, African Journal of Pharmacy and Pharmacology, Vol. 12(18), pp. 221-230, 22 May, 2018
16. S.S.Deo, F. Inam and N. P. Karmarkar, Analytical Method Development for Determination of Performance of Adapalene in Adapalene 0.1% Gel Formulation Using Manual Diffusion Cell, Chemical Science Transactions, 2013, 2(1), 251-257
17. Patil J.S., Kadam D.V., Marapur S.C., Kamalapur M.V., Inclusion complex system; a novel technique to improve the solubility and bioavailability of poorly soluble drugs: A Review, International Journal of Pharmaceutical Sciences Review and Research, Volume 2, Issue 2, May – June 2010
18. Amber Vyas, Avinesh Kumar Sonker, and Bina Gidwani, Carrier-Based Drug Delivery System for Treatment of Acne, The Scientific World Journal, Volume 2014
19. D. S. Ghodke et al, Solid State Characterization of Domperidone:Hydroxypropyl- β -Cyclodextrin Inclusion Complex, Indian Journal of Pharmaceutical Sciences, March - April 2010