IJPSR (2020), Volume 11, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 November 2019; received in revised form, 22 December 2019; accepted, 27 December 2019; published 01 January 2020

ISOTRETINOIN ANTI-ACNE GEL FOR THE MANAGEMENT OF *P. ACNE* INFECTION

Shelly Soni and Anjana Bharadwaj *

RKDF College of Pharmacy, SRK University, NH 12 Hoshangabad Road, Misrod, Bhopal - 462033, Madhya Pradesh, India.

Keywords:

Isotretinoin, *Acne vulgaris*, Anti-acne gel, Antibacterial, Neurotoxicity, *Propionibacterium acne*

Correspondence to Author:
Anjana Bharadwaj

Professor,
RKDF College of Pharmacy,
SRK University, NH 12 Hoshangabad Road, Misrod, Bhopal - 462033,
Madhya Pradesh, India.

E-mail: anjanab2210@gmail.com

ABSTRACT: The object of the paper is the development of anti-acne gel of isotretinoin and evaluated for as potential formulation to treat *Acne vulgaris*. The isotretinoin based anti-acne gel was prepared by using carbopol 940 polymers and evaluated for solubility, drug interaction, drug release, pH, viscosity and spreadability. *In-vitro* drug release through Franz diffusion cells, acute skin irritation test and antibacterial test also executed to confirm the potency of the gel formulation. The evaluation test was also compared with marketed formulation Sortet gel. The antibacterial (anti-acne) activity of different formulations was evaluated by the modified agar well diffusion method in the culture of *Propionibacterium acne*. The optimized formulation ISG-7 has shown the highest spreadability (42.422 g/cm^3) with respect to other gel formulations and a high percentage of drug contents (96.42%). *In-vitro* diffusion study suggested that ISG-2, ISG-3, and ISG-7 have shown more diffusion and drug release from all the formulations that is 82.97%, 79.20% and 83.69% as compared to Sotret gel (86.72%). The antibacterial activity was studied on anaerobic microorganism *P. acne*, compared with marketed Sortet gel. The optimized formulation has shown maximum zone of inhibition to *P. acne* and its well below to marketed formulations and standard benzyl peroxide gel. The anti-acne gel of isotretinoin was successfully formulated and evaluated by different parameters. The results indicate that the active component, that is, isotretinoin is more effective when subjected to gel formulations and produces effective anti-acne activity in the management of *Acne vulgaris*.

INTRODUCTION: Acne is a cutaneous disorder of multi-factorial origin. It is a disease whose cause and severity depend on the relationship between hormones, keratinization, sebum, and bacteria. The main aim of acne therapy generally includes controlling acne lesions, preventing scarring and minimizing morbidity [1]. Acne is a common skin disease that nearly affects 80% of the population at some point in their lifetime [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(1).464-73

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(1).464-73

It is associated with sebaceous follicle and starts appearing after the onset of puberty and can extend up to 40-50 years of age [3]. Although acne can sometimes be a cosmetic concern, it can also be disfiguring and scarring.

Propionibacterium acne is thought to be the wrongdoer bacteria contributing to acne. *P. acne* has been described as an obligate anaerobic microorganism. It is implicated in the development of inflammatory *acne* produce free fatty acids that irritate the distended follicular wall, inflammation mediated by the irritant action of sebum, leaking into the dermis as well as the presence of chemotactic factors and pro-inflammatory mediators generated by *P. acne*. This process results in the disruption of the follicle wall leading

to extrusion of *P. acne*, sebum, hair, and cells in the dermis. Leakage of the contents of comedones into the dermis causes inflammatory acne lesions, including papules, pustules, nodules, and cysts. It is implicated in the development of inflammatory acne by its capability to activate complements and by its ability to metabolize sebaceous triglycerides into fatty acids, which chemically attract neutrophils. On the contrary, *S. epidermidis*, anaerobic organism, usually involves in superficial infections within the sebaceous unit. When the chemicals produced by *P. acne* destroy the cellular structure of skin cells, *Staphylococcus aureus*, grows causing acne lesions. These factors provide a potential target for treatment. *P. acne*, S. epidermidis and *S. aureus* are the target sites of anti-acne drugs.

Common first-line treatment for acne is a topical combination of benzoyl peroxide, retinoid, and antibiotic [4]. Topical retinoids such as isotretinoin, adapalene, and tazarotene have proven to be effective as anti-acne agents. These retinoids inhibit the microcomedone formation and reduce the non-inflammatory and inflammatory lesions [5]. Out of these three drugs, isotretinoin is found to be extremely effective which suppresses acne over the long term. It is used for the treatment of severe acne and other dermatological diseases. The irritation of topical isotretinoin therapy is the limiting factor in maximizing treatment because of the high lipophilic (log P=4.20) nature of the drug. It tends to accumulate in the upper stratum corneum, thereby delaying penetration into the lower skin layer [6].

Isotretinoin (ITTN), a derivative of retinoic acid (13-cisretinoic acid), is the most effective compound with the potential to suppress acne over the long term [7, 8]. *Acne vulgaris* is generally characterized by the formation of seborrhea, comedone, inflammatory lesions and presence of bacteria *Propionibacterium acne*, *Staphylococcus epidermidis* and *Staphylococcus aureus* in the follicular canal and sebum production [9].

In the present study, anti-acne gel formulation of isotretinoin has been developed, characterized and evaluated for the antimicrobial and skin non-irritation study and was examined for antimicrobial activities against microorganisms frequently involved in acne inflammation, such as *Propionibacterium acne*.

MATERIALS AND METHODS: Isotretinoin was obtained as a gift sample from Nicholas Piramal India Ltd., Baddi. Ethanol, Isopropyl alcohol, Glycerin, PEG-400, Propyl Paraben sodium and Carbapol 940 were purchased from Sigma Aldrich, New Delhi. Triethanolamine was procured from Central Drug House (P) Ltd., Delhi.

Instrument Used: Ultraviolet (UV)/visible-spectrophotometer (Double beam), Fourier trans-form-infrared spectrophotometer (FT-IR) (Bruker alpha), Homogenizer (Remi Motors, RQ127 A), magnetic stirrer (Remi Motors), Digital pH Meter, Franz diffusion cell, spreadability apparatus, small volume Brookfield viscometer, Sonicator (Single Phase, 230 VAC, D-120/IH), distillation apparatus (Bio Technics, India) and analytical balance.

Determination of λ_{max} of Isotretinoin Spectrum by UV Spectroscopy: Stock solution of Isotretinoin was prepared in a mixture of phosphate buffer (pH 5.8): ethanol (65:35 v/v) solution. The solvent system used for the analysis was the solution of phosphate buffer (pH 5.8): ethanol (65:35 v/v). The concentration of stock solution was 10µg/ml. The scanning of the isotretinoin was performed in a UV spectrophotometer. The maximum absorption of isotretinoin was found at 340 nm [10].

Preparation of Stock Solution of Isotretinoin: Taking 100 mg isotretinoin and mixed with 100 ml of solvent (phosphate buffer pH 5.8: Ethanol (65:35 v/v), concentration was 1000 µg/ml. 10 ml of the above solution was again diluted with 100 ml with solvent; the concentration was 100 µg/ml. Then, 10 ml of the solution of step 2, was diluted to 100 ml with solvent, the final concentration of the solution was 10µg/ml. The stock solution was further diluted and the absorbance of diluted solution was taken using UV spectrophotometer. The procedure was repeated 5 times and the mean was taken for the standard calibration curve. Absorbance was measured at 340 nm against ethanol: Phosphate buffer (pH 5.8) as blank solution [11].

Preformulation Test for Isotretinoin: Pre-formulation studies of API were carried out to study the incompatibility between excipient used.

Drug polymer Interaction study is very essential prior to the development of a formulation of any dosage form. It is very important to check the compatibility of all excipient with the drug, whether the polymer or excipient used in the formulation cannot affect the drug nature or chemical structure. Hence, it was studied by doing the FT-IR study of the drug along with polymer and excipient. In a pre-formulation study, drug and polymer interaction was studied by FT-IR study. FT-IR spectra of the drug molecule, isotretinoin-ethanol, isotretinoin-isopropyl alcohol, isotretinoin glycerin, isotretinoin-Carbopol 940, Isotretinoin-PEG-400 and Isotretinoin-triethanolamine were obtained on FT-IR spectrophotometer (Bruker Alpha Software) [12]. The spectra were scanned over the wavelength region of 400-4000 nm.

Preparation and Composition of Gel: The anti-acne gel of isotretinoin was prepared by taken the required quantity of Carbopol 940. It was taken as 0.4 g, 0.6 g, 0.7 g and 1 g in different batches. The required quantity of Carbopol 940 was accurately weighed on an analytical balance and sprinkled on a specific quantity of water and kept for hydration for 24 h and then stirred slowly using a magnetic stirrer to form uniform mixture [13]. At the same time, in another beaker drug, PEG-400, propylparaben sodium was accurately weighed. The drug was uniformly dispersed in PEG-400 and the respected solvents. Ethanol, isopropyl alcohol, and glycerin were used as solvents. Then, the uniform mixture of Carbopol 940 was neutralized slowly using triethanolamine, without forming an air bubble to form a clear, transparent gel, and then the mixture of drug with solvents and other ingredients was slowly mixed in above-formed gel uniformly using the homogenizer. The PEG-400 was used as a drug solubilizer. All the procedure was carried out by wrapping aluminum foil to glass wares to avoid degradation of drug isotretinoin [14].

TABLE 1: FORMULATION INGREDIENTS OF ISOTRETINOIN GEL

Ingredients	ISG-1	ISG-2	ISG-3	ISG-4	ISG-5	ISG-6	ISG-7	Blank formulation
Isotretinoin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	
Ethanol	14.99	4.9	-	10	5.02		5.03	-
Isopropyl Alcohol	-		5	5	10	10		10
Glycerin	-	10.00	10.00	-		5.04	10	5.04
PEG-400	1	1	1	1	1	1	1	1
Propyl Paraben sodium	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Carbapol 940	0.5	0.5	0.7	0.6	1	0.7	0.4	0.5
Distilled water	81.82	81.82	81.8	81.82	81.82	81.82	81.82	81.82
Triethanolamine	1.6	1.8	1.5	1.5	1.1	1.5	1.7	1.7
Total Weight	100	100	100	100	100	100	100	100`

Evaluation of Anti-acne Gel of Isotretinoin: Physicochemical evaluation of gel formulation includes color, physical appearance, and homogeneity was tested by visual observation. The anti-acne gel formulation was evaluated by the following parameters

pH: The pH of the various formulations was determined using Digital pH Meter (HI 96107). A volume of 1 g of the gel was dissolved in 100 ml of distilled water and stored for 2 h. The measurement of pH was done. The pH of gel formulations was in range 6.15 ± 0.1 to 6.36 ± 0.2, which lies in the normal range of the skin and would not produce any skin irritation [15]. Marketed Sotret gel has shown a pH 6.50 ± 0.1. There was no significant change in pH values as a function of time for all formulations in triplicate and average values were given in **Table 2**.

Spreadability: Spreadability denotes the extent of the area to which the gel readily spreads on application to the skin or affected part. The bioavailability of gel also depends on its spreading value. The spreadability was expressed in terms of time taken in seconds taken by two slides to slip off from the gel, placed in between the slide under certain load [16]. Lesser is the time taken for separation of two slide, better is the spreadability. A volume of 20 g weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 6.0 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated by 3 times and the mean time taken for calculation [17]. Spreadability was calculated using the following formula:

$$S = M \times LT$$

Where, S = Spreadability; M = Weight tied to the upper slide (20 g); L = Length of the glass (6 cm); T = Time taken in seconds.

Drug Content: The procedure was carried out in subdued light. To a quantity of the gel containing 0.5 mg of isotretinoin, 10 ml of dichloromethane was added, shaken until all the gel has dispersed and diluted the solution to 100 ml with 5 ml of 0.1 M hydrochloric acid to 250 ml with ethanol (96%). Measure the absorbance of the solution at about 356 nm by using ethanol hydrochloric acid solution, in the reference cell [18]. The content of $C_{20}H_{28}O_2$ in the gel was calculated taking 1350 as the value of a (1%, 1 cm) at a maximum at about 356 nm using a UV-visible spectrophotometer (double beam spectrophotometer). For the drug, the content sample was taken from the top, middle, and bottom from the container. The experiment was repeated ten times for each batch, four times for the top region, and three times for the middle and bottom region, and then the average value was taken for the drug-content calculation.

Stickiness: Stickiness was evaluated by just applying a small quantity of gel and checking whether there was the presence or absence of stickiness after application of the formulation.

Smell: Evaluation of smell of gel formulation was done by checking the smell of formulation to 4-5 persons, and the observations of these were given as alcoholic, acceptable, or non-acceptable.

Viscosity: The viscosity of gel formulation was determined using a small volume Brookfield viscometer. The determinations were carried out four times at 6, 12, 30, and 60 rpm and that reading were multiplied by the factor and mean of that were taken as final viscosity in centipoises [19]. Brookfield factor finder was used as follows:

Dial reading × factor = Viscosity in centipoise (mpa.s).

***In-vitro* Diffusion Study:** Franz diffusion cell has been the standard system used for the study of the release of semi-solid drug formulations. *In-vitro* diffusion study of the anti-acne gel formulation was done using the Franz diffusion cell. 0.45 µ dialyzing membrane was used. The media used for the *in-vitro* diffusion was a mixture of phosphate buffer pH 5.8: ethanol (65:35) v/v. The dialyzing membrane was soaked in phosphate buffer 24 h before use. The temperature was maintained constant at 32°C. 5 ml sample was withdrawn and replaced with fresh solvent [20, 21]. The time interval was maintained as 15 min, 30 min, 1 h, 1.5 h and up to 8 h. The drug concentration of receptor fluid was determined by UV spectrophotometer at 340 nm. The correlation factor was included in the calculation to account for the drug loss during sampling. Thus, the amounts of drug permeation of all the formulations were calculated.

Determination of Diffusion Kinetics: The *in-vitro* drug diffusion data obtained which were fitted into kinetic models, including (a) Zero-order (b) First-order (c) Higuchi and (d) Korsmeyer–Peppas model to know the pattern of drug permeation [22].

Photostability Study of Isotretinoin in Gel Formulation: The photostability of isotretinoin was assessed by recording its absorption spectra over the wavelength range of 200-400 nm in two matched quartz cells with a 1 cm light path using a double beam UV-visible spectrophotometer at the following conditions: Slow scan speed, time response of 1 s, and a spectral band of 1 nm. The radiant power was adjusted to the lower value in the instrumental scale and the cabinet temperature at 25 °C. These gentle experimental conditions were set because of the high sensitivity of the drug to light, allowing so to obtain more accurate control of the photodegradation process [23]. The methanolic solution of isotretinoin (25µg/mL) was exposed to natural sunlight, and UV spectra of all the samples were recorded just after preparation (t=0) and at time intervals of 15, 30, 60, 90, 120, 150, 180, 210, and 240 min after suitable dilution with methanol.

The formulations were also exposed to light under the same experimental conditions described above for solution, and recording of spectra was done at the same irradiation times. For these formulations, spectrophotometric measurements were performed by diluting it suitably with methanol. Baseline correction was done using a plain gel formulation diluted suitably with methanol to nullify any possible absorption arising from the excipient [24]. Sufficient care was taken to maintain similar experimental conditions for both the samples, *i.e.*, isotretinoin in methanol and isotretinoin in a gel formulation.

The degradation was evaluated on the basis of kinetic photodegradation constant k and half-life time ($t_{1/2}$) with respect to the initial percent absorbance.

***In-vitro* Study:**

Antibacterial Study: The antibacterial activity of different formulations was determined by a modified agar well diffusion method. In this method, nutrient agar plates were seeded with 0.2 ml of 24 h broth culture of *P. acne*. The plates were allowed to dry for 1 h. A sterile 8 mm borer was used to cut four wells of equidistance in each of plates; 1 g of formulations (ISG-1 to ISG-7) and marketed Sotret gel for comparison. Benzoyl peroxide gel was used as a positive control, and distill water was used as a negative control and was introduced into the wells at randomly. The plates were incubated at 37 °C for 24 h. The antibacterial activities were found out by measuring the diameter of zones of inhibition (in mm). This experiment repeated 3 times [25].

Acute Skin Irritation Study: The primary skin irritation test was performed on albino rats and weighing about 150-200 g. The animals were maintained on standard animal feed and had free access to water. The animals were kept under standard laboratory conditions. The total mass was divided into four batches, each batch containing seven animals. Two batches of each were used for control and test. Dorsal hairs at the back of the rats were clipped off 1 day before the commencement of the study. Animals showing normal skin texture were housed individually in cages with copography meshes to avoid contact with the bedding. 50 mg of each formulation of different concentrations was applied over one square centimeter area of intact and abraded skin to different animals. An aqueous solution of 0.8% formalin was applied as a standard irritant. The animals were observed for 7 days for any signs of edema and erythema. The gel was applied to the skin once a day for 7 days and observed for any sensitivity and the reaction if any was graded as A- no reaction, B- slight patchy erythema, C- slight but confluent or moderate but patchy erythema, D- moderate erythema, and E- severe erythema with or without edema. The skin irritation studies showed that anti-acne gel formulations dose not produce any severe irritation, redness of the skin, along with the marketed Sotret gel of isotretinoin, whereas the 0.8% formalin was used as a standard irritant for the comparison (Ethical committee letter number: RKDFCP/IAEC/2019/05).

RESULTS AND DISCUSSION:

Determination of λ_{max} of Isotretinoin Spectrum Scan: The standard calibration was done by taking the average value and the concentrations, and the graph was the plot and the value of the slope, correlation, and regeneration value was calculated and these values are taken as the standard for calculation in *in-vitro* diffusion study. The standard curve of isotretinoin was tabulated in **Table 2**.

TABLE 2: STANDARD CALIBRATION CURVE FOR ISOTRETINOIN IN ETHANOL: PHOSPHATE BUFFER (pH 5.8)

Concentrations µg/ml	I	II	III	IV	V	Avg.	±SD	Standard curve
2	0.056	0.061	0.078	0.074	0.075	0.0688	0.009680	0.072
4	0.134	0.132	0.112	0.129	0.104	0.1222	0.013387	0.1212
6	0.211	0.201	0.143	0.204	0.137	0.1792	0.036031	0.177
8	0.268	0.267	0.189	0.265	0.179	0.2336	0.045429	0.2336
10	0.311	0.312	0.227	0.308	0.238	0.2792	0.042833	0.2792
12	0.378	0.369	0.296	0.363	0.281	0.3374	0.045269	0.3342
14	0.418	0.415	0.337	0.417	0.329	0.3832	0.045926	0.3832
16	0.476	0.479	0.375	0.48	0.384	0.4388	0.054247	0.4388
18	0.502	0.497	0.451	0.511	0.439	0.48	0.032619	0.4848
20	0.571	0.564	0.574	0.578	0.486	0.5546	0.038688	0.5384
Slope	0.027	0.027	0.025	0.027	0.023	0.0258	0.001789	0.024
Intercept	0.03	0.03	0.005	0.029	0.006	0.02	0.013248	0.0249
Correlation	0.99	0.991	0.996	0.996	0.993	0.9932	0.002775	0.9998

Drug Polymer Interaction Study: The major peaks were found in the IR spectra of isotretinoin at wavenumber 3428.77 may be due to the stretching vibration of the OH group and shows a strong peak. Also, at 3075, it gives a weak peak due to aromatic H. Then, it also shows a strong peak at 2927.53 due

to C-H stretch alkene. Strong peak at 1673.04 for the C=O group also gives a strong peak at 1599.32 due to the C=C Aliphatic group, and also C=C Aromatic group gives a strong peak at 1564.81. There were also deformations of OH group take place which gives a broad shallow peak at 1447.90 range and also gives a strong peak at 1249.35 due to C-C aromatic.

IR Spectroscopy of Isotretinoin with all Ingredients: The data of IR spectroscopy of drug, polymer, solvents, and excipient, clearly suggest that drug and other excipient were compatible with each other and there was no chemical reaction among them. The FT-IR spectra of combined mixture shown the peak 3366.88 OH, 1654.79 (C=O), 1249.45 (C-aromatic), 2971.47 (C-H stretch alkene), 951.82 (C-C aliphatic). There were not major interference is occur when these peaks correlated with the spectra of the drug.

Physicochemical Evaluation of Gel Formulation: After physicochemical evaluation, it was clear that all the batches have yellow, transparent, homogenous with good homogeneity, smooth in texture. The physical appearance of gel formulations was transparent fresh lemon color pH ranges between 6.15-6.36. The data was shown in **Table 3**.

TABLE 3: COLOR, PHYSICAL APPEARANCE, HOMOGENEITY, FEEL ON APPLICATION, AND pH (MEAN) OF ANTI-ACNE GEL OF ISOTRETINOIN

Formulation code	Color	Physical appearance	Homogeneity	Feel	pH (mean)
ISG-1	Yellow	Transparent	Homogeneous	Smooth	6.20±0.1
ISG-2	Yellow	Transparent	Homogeneous	Smooth	6.16±0.1
ISG-3	Yellow	Transparent	Homogeneous	Smooth	6.16±0.2
ISG-4	Yellow	Transparent	Homogeneous	Smooth	6.15±0.2
ISG-5	Yellow	Transparent	Homogeneous	Smooth	6.20±0.1
ISG-6	Yellow	Transparent	Homogeneous	Smooth	6.18±0.1
ISG-7	Yellow	Transparent	Homogeneous	Smooth	6.36±0.2
Formulation without drug	Yellow	Transparent	Homogeneous	Smooth	6.23±0.1
Marketed formulation	Yellow	Transparent	Homogeneous	Smooth	6.50±0.1

Spreadability: The spreadability of the formulations was found in between 21.784 and 42.603 g cm/seconds. The affinity of the solvent toward the polymer also affects the structure of the network of the gel. If the solvent has a higher affinity toward polymer then polymer chains get extended, that is, increased entanglement, and thus increases swelling of the polymer thus, increase viscosity of formulation and if the solvent has a low affinity toward solvent, then polymer contracts reduces entanglement.

TABLE 4: AVERAGE SPREADABILITY OF GEL FORMULATIONS

Formulation	Average spreadability (g/cm^3)
ISG-1	29.367
ISG-2	32.603
ISG-3	33.050
ISG-4	28.668
ISG-5	23.508
ISG-6	21.784
ISG-7	42.422
Marketed formulation (Sotret Gel)	49.224

Ethanol has a higher affinity toward water than polymer carbopol 940, that is, it has a low affinity toward carbopol 940, so the gel structure gets contracted, so viscosity was less. Hence, formulation ISG-2, ISG-3, ISG-7 have less viscous as compare to other batches, so it has better spreadability (32.6 g/cm^3) and lower than the marketed product (Sortet gel 49.224g/cm^3). The data of spreadability was shown in **Table 4**.

Drug Content: The result of drug content was listed in table the drug content of the gel formulations was found to be uniform among various formulations prepared and was found to be in range 90.92-96.42%, from the above result, it was clear that the ISG-7 batch shows maximum drug content, that is, 96.42%. The data was shown in **Table 5**.

TABLE 5: PERCENT DRUG CONTENT OF GEL FORMULATIONS (AVG ± SD)

Formulations	Percent of drug content (n=5)	Average	S.D.
ISG-1	90.45	90.462	0.017889
ISG-2	95.22	95.242	0.019235
ISG-3	95.01	95.030	0.015811
ISG-4	91.81	91.828	0.016432
ISG-5	90.92	90.938	0.017889
ISG-6	92.24	92.244	0.016733
ISG-7	96.42	96.428	0.013038
MKP	99.31	99.328	0.013038

Stickiness: The presence and absence of stickiness are the criteria for the evaluation. The result of the evaluation was clearly suggested that anti-acne gel formulation of isotretinoin was free from stickiness after application, and it was freely get spread on the skin and it was also compared with the marketed formulations.

Smell/Odor: The anti-acne gel formulation was prepared by using ethanol, isopropyl alcohol, so evaluated for the odor or smell. The smell of the formulated gel formulations was evaluated by check edit through 4-5 volunteers, and then it was considered as alcoholic, acceptable, and non-acceptable. The data was shown in **Table 6**.

TABLE 6: EVALUATION OF SMELL OF GEL FORMULATION

Formulation	Smell		
	Alcoholic	Acceptable	Non-acceptable
ISG-1	++	+	-
ISG-2	++	+	-
ISG-3	++	+	-
ISG-4	++	+	-
ISG-5	++	+	-
ISG-6	++	+	-
ISG-7	++	+	-

Viscosity: The viscosity of all formulations was evaluated. The viscosity of ISG-2, ISG-3 & ISG-7 batches was shown less as compared to other formulations, ultimately shows more release of the drug. Depending on the concentration of Carbopol 940 and the proportion of solvent, the viscosity changes, which affect the release of the formulation. Glycerin is also played a role in viscosity, higher the concentration of glycerin lower the viscosity of gel formulation (ISG-2, ISG-3 & ISG-7). In the process of neutralization of Carbopol 940, neutralization (ionic repulsion of its charges), polymer concentration increases and repulsion of the chains occurs and thus increases the rigidity of the structure of the gel. The affinity of the solvent toward the polymer also affects the structure of the network of the gel. The data of the viscosity of anti-acne gel formulation was shown in **Table 7**. If a solvent has a higher affinity toward polymer, then polymer chains get extended, that is, increased. Entanglement and increased swelling of polymer thus increase the viscosity of formulation and if solvent has low affinity toward solvent, then polymer contracts reduce entanglement. Ethanol has a higher affinity toward water than polymer Carbopol 940, that is, it has a low affinity toward Carbopol 940, so the gel structure gets contracted, so viscosity was less. Hence gel formulation ISG-2, ISG-3, ISG-4 & ISG-7 had shown less viscosity as compared to other formulation but something higher than marketed Sotret gel formulation.

TABLE 7: VISCOSITIES OF GEL FORMULATIONS

Formulations	Viscosity (cps)
ISG-1	35500
ISG-2	12000
ISG-3	16230
ISG-4	14200
ISG-5	30000
ISG-6	34400
ISG-7	11502
BLANK	29400
MKP	10300

***In-vitro* Diffusion Study:** The *in-vitro* diffusion study shows combined percentage release patterns of the anti-acne gel of isotretinoin (0.05% w/w). Furthermore, there was a comparison made between the marketed formulations of the same drug, that is, isotretinoin Sortet gel 0.05% w/w. It is observed from the result that ISG-2, ISG-3 & ISG-7 showed more diffusion, that is, release from all the formulations, that is, 82.97%, 79.20% & 83.69% after the Sortet gel, because of decrease in viscosity.

The marketed product showed 86.72% release and formulation ISG-6 & ISG-1 showed low release that is, 72.37% & 70.63%, because of higher viscosity. Data was shown in **Fig. 1**.

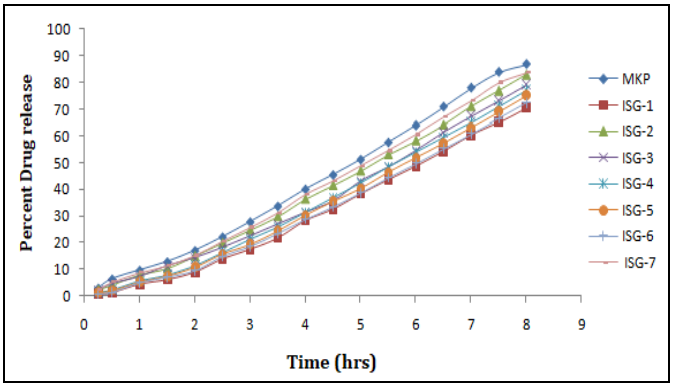

FIG. 1: DRUG DIFFUSION THROUGH THE DIFFERENT GEL FORMULATION WITH MARKETED FORMULATION

Determination of Diffusion Kinetics: Linearity (R^2) and diffusion component (n) value obtained from the kinetic plots of *in-vitro* drug diffusion studies. Linearity (R^2) value by Zero-order model was shown 0.5901, first order was 0.8943, Higuchi

model was 0.9245 and Korsmeyer–Peppas model was shows 0.8965 and diffusion component (n) values from the kinetic plots of the *in-vitro* drug diffusion studies is 0.8462.

Photo-Stability Studies: The photochemical reaction in methanol solution demonstrated a rapid isomerization of isotretinoin followed by a further degradation consisting in the minimization of the absorbance peaks. Therefore, the absorbance values of these maxima were used to evaluate the kinetics of the photo-degradation processes. Photo-stability studies of the formulation demonstrated an increase in isotretinoin half-life to about 13 times in comparison with a methanolic solution under direct sunlight.

Acute Skin Irritation Study: The formulations were non-irritant and did not show any skin toxicity when applied daily for 7 days in albino rats. The skin irritation studies show that anti-acne gel formulations dose not produces any severe irritation, redness of skin, along with the marketed Sotret gel of isotretinoin while the 0.8% formalin was used as a standard irritant for the comparison. Thus, all formulation does not produce any skin irritation and safe to use. The data of skin irritation study was shown in **Table 8**.

TABLE 8: ACUTE SKIN IRRITATION STUDY

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	A	A	A	A	A	A
Standard (0.8% formalin solution)	B	B	B	B	B	B	B
ISG-1 (0.5%)	A	A	A	A	A	A	A
ISG-2	A	A	A	A	A	A	A
ISG-3	A	A	A	A	A	A	A
ISG-4	A	A	A	A	A	A	A
ISG-5	A	A	A	A	A	A	A
ISG-6	A	A	A	A	A	A	A
ISG-7	A	A	A	A	A	A	A
Marketed formulation	A	A	A	A	A	A	A

Antibacterial Study: The zones of inhibitions for the antibacterial activity were compared with the standard benzoyl peroxide gel, marketed preparation of isotretinoin, that is, Sotret gel for *Acne vulgaris*. Formulation ISG-7 has shown comparable zones of inhibitions to that of the marketed preparation. All the formulations have shown greater zones of inhibitions. Zones of inhibitions for benzoyl peroxide were found to be greater than that of all the formulations (ISG-1-ISG-7) as well as marketed preparation. The zone of inhibition of anti-acne gel formulation was shown in **Table 9**. This suggests that the other active ingredients of the formulations containing solvents such as ethanol and isopropyl alcohol may have contributory antibacterial activity. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. *P. acne* was an anaerobic pathogen that employed in the development of inflammatory acne. The formulations having antibacterial agents inhibiting the *P. acne* may also reduce the development of inflammatory acne. Data of the zone of inhibition of gel formulation was shown in **Table 9**. **Fig. 2** show the zone of inhibition of various anti-acne gel formulation of isotretinoin (ISG-1 to ISG-7) with marketed formulation (Sotret gel).

TABLE 9: ZONE OF INHIBITION OF GEL FORMULATION

Formulation	Zone of inhibition in mm			
	1	2	3	Mean ± SD
ISG-1	22.1	22.6	21.8	22.16667±0.404145
ISG-2	28.4	28.8	28.6	28.26667±0.416333
ISG-3	30.4	30.8	30.5	30.56634±0.203456
ISG-4	25.8	26.4	26.3	26.16667±0.321455
ISG-5	24.3	24.6	23.9	24.26667±0.351188
ISG-6	24.2	24.6	23.9	24.23333±0.351188
ISG-7	32.5	32.8	32.2	32.55355±0.301786
Benzyl Peroxide gel (+ control)	40.2	40.2	40.5	40.34565±0.173205
Marketed SOTRET gel	35.5	35.8	35.3	35.53333±0.251661
Distill water (- control)	-	-	-	-

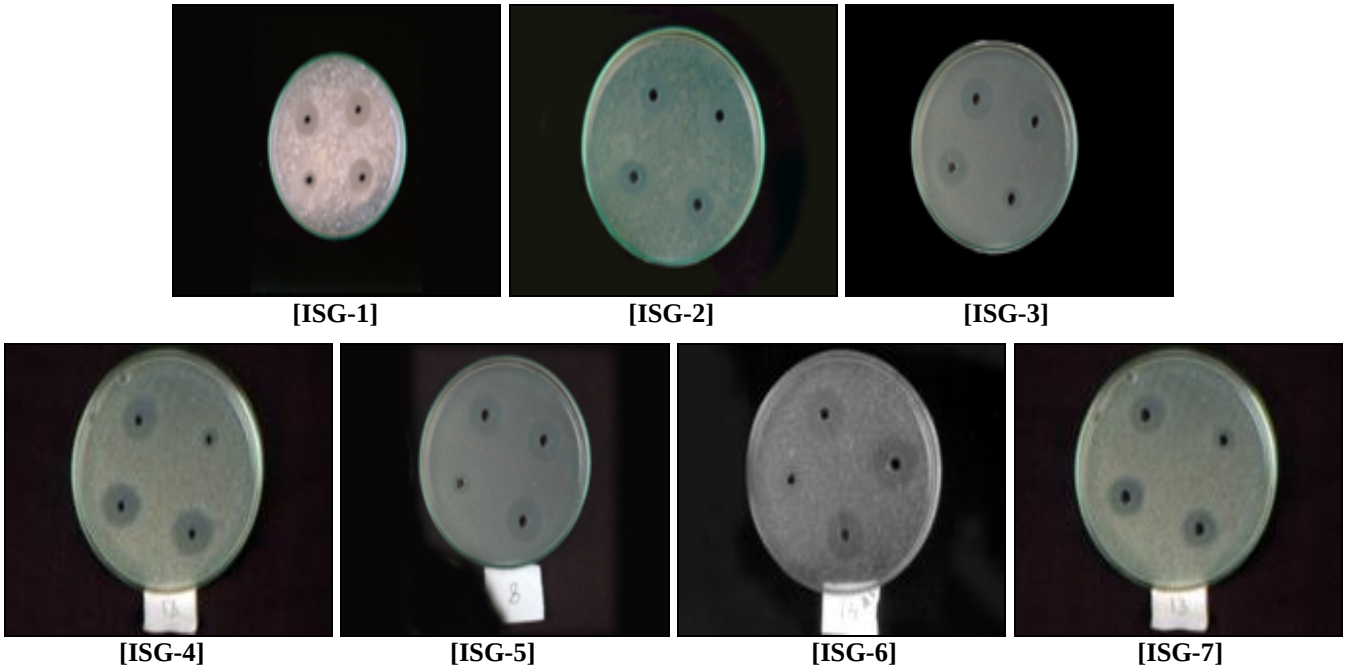

FIG. 2: ZONE OF INHIBITION OF VARIOUS ANTI-ACNE GEL FORMULATION OF ISOTRETINOIN WITH MARKETED FORMULATION

DISCUSSION: The present study was undertaken with an aim to formulate and evaluate the formulation of anti-acne gel of isotretinoin using carbopol-400 polymer. Various formulations (total seven) of isotretinoin gels were prepared by using different ratios of excipient with different proportions by fusion exploiting cold method. The Gels were evaluated for physical characterizations (pH, solubility, viscosity, spreadability, stickiness, UV spectroscopy, FT-IR analysis), *in-vitro* diffusion study, *in-vivo* skin irritation study and *Ex-vivo* antibacterial study on microorganism *P. acne*. The drug content of the gel formulations was found to be uniform among various formulations prepared and was found to be in range 90.92-96.42%. It was clear that the ISG-7 batch shows maximum drug content, that is, 96.42%. The viscosity of ISG-2, ISG-3 & ISG-7 batches was shown less as compared to other formulations, ultimately shows more release of the drug. Glycerin is also played a role in viscosity, higher the concentration of glycerin lower the viscosity of gel formulation (ISG-2, ISG-3 & ISG-7). The *in-vitro* diffusion study shows combined percentage release patterns of the anti-acne gel of isotretinoin (0.05% w/w). It is observed from the result that ISG-2, ISG-3 & ISG-7 showed more diffusion, that is, release from all the formulations, that is, 82.97%, 79.20% & 83.69% after the Sortet gel, because of decrease in viscosity. The marketed product showed 86.72% release and formulation ISG-6 & ISG-1 showed low release that is, 72.37% & 70.63%, because of higher viscosity. The skin irritation studies show that anti-acne gel formulations dose not produces any severe irritation, redness of the skin, along the marketed Sotret gel of isotretinoin. Formulation ISG-7 has shown comparable zones of inhibitions to that of the marketed preparation. All the formulations have shown greater zones of inhibitions.

CONCLUSION: It is therefore concluded that anti-acne gel formulation meets the required specification. The process parameters are recorded and stability observations are also found to meet the specified acceptance criteria and hence stands validated. This formulation is mainly used for acne infection. The topical application of the gel at the affected site would offer the potential advantages of the delivery of the drug directly to the acne site.

ACKNOWLEDGEMENT: The author expresses warmly thanks to Nicholas Piramal India Ltd., Baddi for providing the gift sample of drug isotretinoin. The author thanks IMTECH Chandigarh for providing the antibacterial strains of *P. acne.*

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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How to cite this article:
Soni S and Bharadwaj A: Isotretinoin anti-acne gel for the management of *P. acne* infection. Int J Pharm Sci & Res 2020; 11(1): 464-73. doi: 10.13040/IJPSR.0975-8232.11(1).464-73.