IJPSR (2020), Volume 11, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 November 2019; received in revised form, 10 January 2020; accepted, 18 January 2020; published 01 February 2020

FORMULATION, CHARACTERIZATION AND EVALUATION OF ISOTRETINOIN GEL FOR TREATMENT OF *ACNE VULGARIS*

Shelly Gupta and Anjana Bharadwaj *

RKDF College of Pharmacy, SRK University, NH-12 Hoshangabad Road, Misrod, Bhopal - 462026, Madhya Pradesh, India.

Keywords:

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

Correspondence to Author:
Anjana Bharadwaj

RKDF College of Pharmacy, SRK University, NH-12 Hoshangabad Road, Misrod, Bhopal - 462026, Madhya Pradesh, India.

E-mail: anjanab2210@gmail.com

ABSTRACT: *Acne vulgaris* is a commonly diagnosed inflammatory skin condition that affects pediatric, young and adult patients. The object of the paper is formulation development and evaluation of isotretinoin gel for the treatment of *Acne vulgaris*. The isotretinoin based anti-acne gel was formulated using different polymers includes carbopol 940, carbopol 934 and carbopol 980. Disodium edetate is used as chelating agents. The anti-acne gel was evaluated for solubility, drug release, pH and drug interaction, *in-vitro* drug release through Franz diffusion cells, acute skin irritation test and antibacterial test. The evaluation test was also compared with the marketed formulation of isotretinoin gel, that is, sortet gel. The antibacterial and anti-acne activity of different formulations was determined by the modified agar well diffusion method in the culture of *Propionibacterium acne.* The optimized formulation (F-IB) showed the highest spreadability (48.623 g/cm^3), in all formulations and also have a high percentage of drug contents (97.2%). *In-vitro* diffusion study suggested that F-IA, F-IC, F-IIA and F-IIB showed more diffusion, and drug release from all the formulations, that is, 94.5%, 96.5%, 92.5% and 92.7% as compared to sotret gel (94.2%). The antibacterial activity was studied on anaerobic microorganism *P. acne,* compared with marketed sortet gel. Formulation batches have shown maximum zone of inhibition to *P. acne* below 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 the most common skin disease worldwide. It is estimated that 80-95% of all adolescents will have acne at some point in their lives, and in some cases, the acne will continue into adulthood. Genetics plays a role in the development of acne and males & females both are equally affected, but males tend to have more severe cases.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(2).873-83

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

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(2).873-83

Teenagers develop acne at a higher rate than any other age group. This is because hormone production during puberty increases the output of the sebaceous glands and the rate of skin-cell turnover within the follicles [1, 2].

A number of factors contribute to the development of acne lesions, includes internal hormones, bacteria, some medications, certain chemicals/ products that come in contact with the skin, local pressure to the skin surface, and stress. While acne cannot be cured, it can be controlled. The goal of treating acne is to reduce the symptoms and to prevent permanent scarring. Acne arises from the interaction of the following factors includes increased sebum production caused by androgenic

stimulation of sebaceous glands in puberty or adulthood, outlet obstruction of the sebaceous follicle arising from an abnormal keratinization process characterized by increased cohesiveness and turnover of follicular epithelial cells, proliferation of *P. acne*, an anaerobic diphtheroid residing in the pilosebaceous follicle [3].

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 [2, 4]. An ongoing trial in patients with antibiotic-resistant *P. acnes* indicates that ITTN is highly effective; treatment of *P. acnes* may well become a new indication of drug [2]. Although, *P. acne*s have a live bacterium in the follicle, it dies when the disruption occurs and acts only to increase the inflammatory process. With the excessive use of antibiotics for long periods has led to the increased resistance in acne-causing bacteria *i.e. P. acne, S. epidermidis* and *S. aureus*. The development of antibiotic resistance is multi-factorial, including the specific nature of the relationship of bacteria to antibiotics, how the antibacterial is used, host characteristics, and environmental factors. In the present study, isotretinoin gel formulation has been developed, characterized and evaluated for the antimicrobial and anti-inflammatory potential 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, Carboxymethyl cellulose and Hydroxypropyl methylcellulose was procured from Colorcon Asia Pvt. Ltd. Goa. Carbopol-934, Carbopol-940 and Carbopol-980 were procured from Noveon Inc., Brussel. Propylene Glycol and PEG-400 were purchased from Sigma Aldrich, New Delhi. Sodium Hydroxide was purchased from Central Drug House (P) Ltd., Delhi. Methyl Paraben and Propyl Paraben were purchased from Clariant (Nipasol).

Instrument Used: Ultraviolet (UV)/visible-spectrophotometer (Double beam), Fourier transform-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.

Preparation of Isotretinoin Gel: The anti-acne gel was prepared by the different concentrations of Carbopol 934, 940 and 980. Isotretinoin gel was prepared by using a cold and hot method by following the procedure in that 84.235 ml of purified water heated to 65 °C and disodium edetate was dissolve in it. Carbopol 940/980/934 (0.6 gm) was sprinkled over the surface of the solution and allowed the dispersion to get uniform and lump free gel. The gel was cooled to 35 °C. Propylene glycol, PEG 400 and Isotretinoin were dissolved at 60 °C underwater bath and add this mixture to the gel with continuous stirring. The preservatives sodium salt of methyl & propylparaben was added to the gel. Check the pH of the gel, where the initial pH was 4.3. The pH was adjusted to 5.8 with a 10% sodium hydroxide solution (USP limit is 4.5 to 6.5). The composition of gel formulation was tabulated in **Table 1**.

TABLE 1: FORMULATION WITH USING CARBOPOL 934, CARBOPOL 940 AND CARBOPOL 980

S. no.	Ingredients	F-IA (%)	F-IB (%)	F-IC (%)	F-IIA (%)	F-IIB (%)	F-IIC (%)	F-IIIA (%)	F-IIIB (%)	F-IIIC (%)
1	Isotretinion	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
2	disodium edetate	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
3	Methyl paraben sod.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
4	Propyl paraben sod.	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
5	PEG 400	5	5	5	5	5	5	5	5	5
6	Propylene glycol	2	2	2	2	2	2	2	2	2
7	Carbopol 934	0.6	0.7	1.2						
8	carbopol 940				0.5	0.6	0.8			
9	carbopol 980							1.2	0.8	1.8
10	Purified water	85.97	85.97	85.97	84.23	84.23	84.23	86.34	86.34	86.34
11	Sodium hydroxide	6.22	6.12	5.62	8.06	8.61	7.76	5.25	6.45	4.65
	Total	100	100	100	100	100	100	100	100	100

Evaluation of Anti-acne Isotretinoin Gel: This Anti-acne Isotretinoin gel has been evaluated for the physicochemical properties [5]. *In-vitro* study and *in-vivo* study also executed for the potential for acne treatment.

Physicochemical Evaluation:

Solubility: [6] The solubility of drug Isotretinoin was determined by using different solvent *i.e.* water, alcohol, ether, propylene glycol, PEG-400, glycerin *etc*. Solubility was indicated by a descriptive phase and intended to apply at 20 °C to 30 °C.

Loss on Drying: Determine on 1g of the formulation by drying in an oven at 100 °C to 105 °C for 3 h. Mix and accurately weigh substances to be tested. Put the sample in a bottle, replace the cover and accurately weigh the bottle and contents by gentle, sidewise shaking, distribute the sample as evenly as practicable to a depth of about 5 mm place the loaded bottle in the drying chamber, dry the sample at the specified temperature for constant weight. Upon opening the chamber, close the bottle promptly and allow it to come to room temperature in desiccators before weighing. The difference between successive weights should not be more than 0.5 mg. Loss on drying is calculated by the formula.

$$\% \text{ LOD} = (W_2 - W_3) \times 100 / (W_2 - W_1)$$

Where, W_1 = Weight of empty weighing bottle, W_2 = Weight of weighing bottle + sample, W_3 = Weight of weighing bottle + dried sample

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 [7].

pH Determination: The pH value conventionally represents the acidity or alkalinity of the solution. In the pharmacopeia standard and limit of pH have been provided for the substance in which pH as a measure of the hydrogen ion activity is important from the standpoint of stability. The pH of the gel was checked by using a digital pH meter at a constant temperature. Before this, the pH meter was calibrated by using a buffer solution of pH 3.99, 7.0 and 9.2 and then the electrode was washed with distilled water. The electrode was then directly dipped into gel formulation and constant reading was noted.

Spreadability: Spreadability denotes the extent of the area to which the gel readily spreads on application to the skin or affected part [8]. 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 a certain load. Lesser is the time taken for separation of two slide, better is the spreadability. A volume of 2 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 the calculation. Spreadability was calculated using the following formula: S = M × 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.

Viscosity: Sample were incubated at 25 °C for at least 16 h in an incubator and then run on Brookfield viscometer using different rotation speed 0.5, 1, 2.5, and 5 rpm. The spindle was chosen to maintain a torque between 10% and 90% of the RV Spindle give viscosity at a single immersion point in sample [5]. The heli path T bar spindle was rotated down and up in the sample giving viscosity at a number of points programmed over the run time two readings taken over a period, the viscosity was calibrated using Brookfield viscosity standard 5000 (100% polydimethyl-siloxane). Brookfield factor finder was used as follows: Dial reading × factor = Viscosity in centipoise (mpa.s)

Drug Content Uniformity: Drug content uniformity was performed as a USP standard for the gel formulation. Uniformity for content was determined by UV analysis method through taken the sample from the filled tube by upper, middle & end portion and analysis by UV. In this case, take 2 gm of anti-acne gel and dissolve in propylene glycol and makeup to volume 100 ml with propylene glycol [9].

Take 2 ml of the sample from the above-prepared solution and make volume up to 10 ml. The above concentration solutions were scanned between 360 nm by using UV spectrophotometer and propylene glycol was used as a blank solution.

Spectroscopy analysis: Calculation

Test abs / Std. abs × Std. weight / 100 ×2 /10 × 100 / Test weight × 10 / 2 × 100 / 100 × 100

Percentage Purity = Test content × 100 / Label claim

Study of Drug-Excipient Interaction: The drug was taken with all the excipient in a 1:1 ratio. These mixtures were kept for one month at the different condition of room temperature (25 °C), 40 °C ± 2 °C/75%, RH ± 5% and 30 °C ± 2 °C/60% RH ± 5%. The mixture was observed visually for any type of change in the color or physical appearance of the drug. The initial observation of the drug was white.

FT-IR Study: FT-IR Spectroscopy was used for the analysis of drug and mixture of drug and excipient. The drug and excipient were passed through the sieve 60. Component and ratio of the drug and excipient were taken as tabulated [10]. IR spectra of the drug in KBr pellets at a moderate scanning speed between 4000-400 cm^{-1} was carried out using FTIR (Perkin Elmer Spectrum one) and obtained were observed for any interaction.

The comparison of the test spectra with reference spectra of the drug shows similar peaks.

UV Spectroscopy Analysis:
Method of Preparation:
Preparation of Standard Solution: Take 50 mg of drug and dissolve in 100 ml of propylene glycol (stock Solution I). Take 2 ml solution and mix with propylene glycol and makeup to volume 10 ml.

Preparation of Test Solution: Take 2 gm of sample and dissolve in propylene glycol and makeup to volume 100 ml with propylene glycol and from this take 2 ml solution and makeup to 10 ml. Prepare different concentrations of 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml.

These concentrations were scanned up to 360 nm by using UV spectrophotometer and propylene glycol was used as a blank solution.

Spectroscopy analysis: Calculation

Test abs / Std. abs × Std Weight / 100 × 2 /10 × 100 / Test weight × 10 / 2 × 100 / 100 × 100

Percentage Purity = Test content × 100 / Label claim

***In-vitro* Drug Release Study:**
Drug Release by Franz diffusion Cells: *In-vitro* diffusion study of the anti-acne gel formulation was done using the Franz diffusion cell **Fig. 1**. Franz diffusion cell has been the standard system used for the study of the release of semi-solid drug formulations. In that, a 0.45µ 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. The 5ml sample was withdrawn and replaced with fresh solvent. The time interval was maintained as 30, 60, 90, 120, up to 180 min. The drug concentration of receptor fluid was determined by UV spectrophotometer at 340 nm [11-16]. 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.

FIG. 1: FRANZ DIFFUSION APPARATUS

***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*s. 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 the plates; 1 g of formulations (F-1A to F-IIIC) and marketed sotret gel for comparison. Benzoyl peroxide gel was used as a positive control, and distilled 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 [17].

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 study [18]. 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 [8]. 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 produces 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: RCP/IAEC/2011-12/P-005).

RESULTS AND DISCUSSION: The topical isotretinoin gel for acne therapy was prepared and evaluated by the following parameter *i.e.* solubility, loss on drying, pH, viscosity, loss on drying, drug content uniformity, drug interaction study, drug-excipient interaction, UV analysis, *In-vitro* drug release, drug content, diffusion study and *In-vivo* skin irritation study.

Physicochemical Evaluation:

Solubility: Gel was soluble in an organic solvent and insoluble in the distilled water. In the solubility analysis gel was completely dissolved in toluene, PEG-400, glycerin, pet-ether, propylene glycol and acetone, partially dissolved in methanol and ethanol and practically insoluble in distilled water.

Loss on Drying: The loss on drying was less than specified limits (not more than 0.5%). The loss on drying is 0.026% for the optimized formulation.

Stickiness: The results were clearly suggested that the formulated gel 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.

pH of the Isotretinoin Gel: The test was performed as a standard procedure and shown in **Table 2**.

TABLE 2: OBSERVATION OF pH

Formulations	Test	Specification	Observation
F-IA	pH at 25°C	5. 0 to 6.0	5.673
F-IB	pH at 25°C	5. 0 to 6.0	5.473
F-IC	pH at 25°C	5. 0 to 6.0	5.667
F-IIA	pH at 25°C	5. 0 to 6.0	5.729
F-IIB	pH at 25°C	5. 0 to 6.0	5.734
F-IIC	pH at 25°C	5. 0 to 6.0	5.765
F-IIIA	pH at 25°C	5. 0 to 6.0	5.742
F-IIIB	pH at 25°C	5. 0 to 6.0	5.568
F-IIIC	pH at 25°C	5. 0 to 6.0	5.566
Marketed Formulation	pH at 25°C	5. 0 to 6.0	5.564

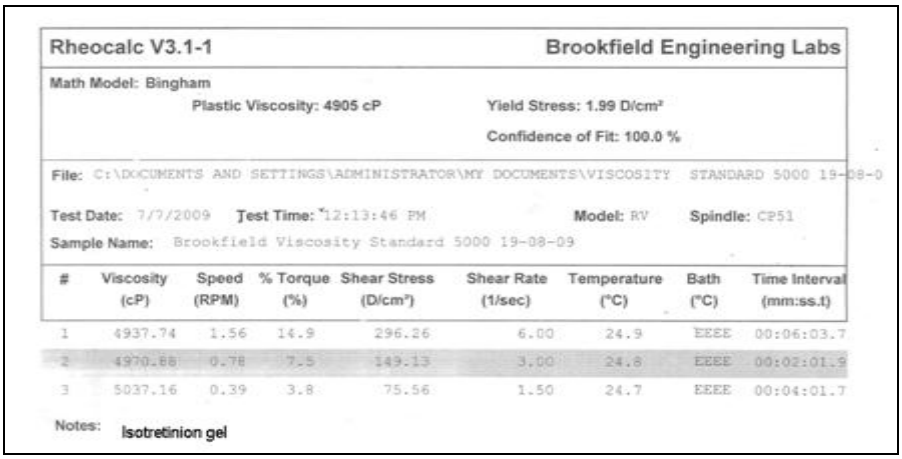


FIG. 2: VISCOSITY OF FORMULATION F-IA BY BROOK-FIELD

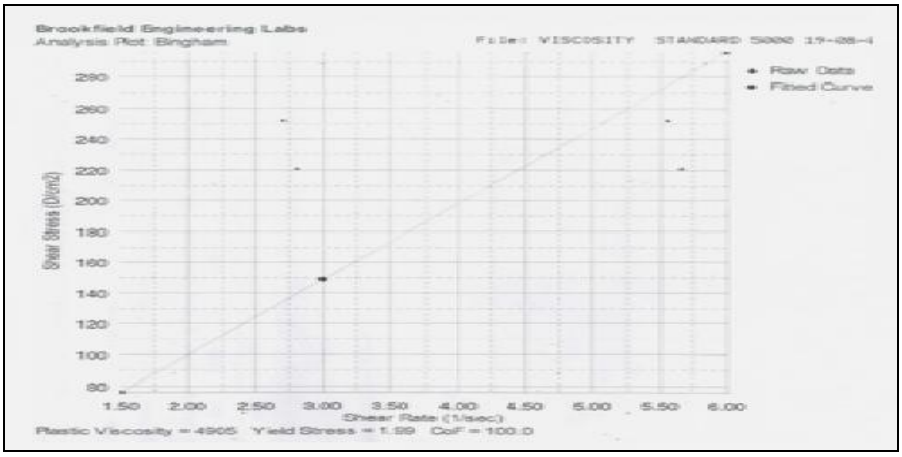


FIG. 3: VISCOSITY OF FORMULATION F-IB BY BROOK-FIELD

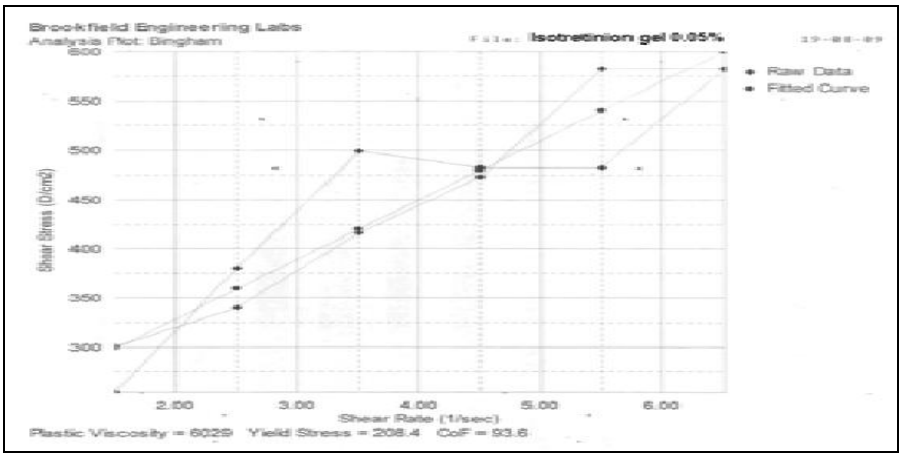


FIG. 4: VISCOSITY OF ISOTRETINOIN GEL- F-IIB BY BROOKFIELD

Viscosity: Viscosity of the isotretinoin gel formulation was determined by the brook-field analog viscometer and the result is illustrated in **Table 3**. The viscosity of formulation F-IA, IB, IIB was shown in **Fig. 2, 3** & **4**.

TABLE 3: VISCOSITY OF FORMULATIONS

Plastic Viscosity of sample (cps)	
Formulation	**Viscosity (cps)**
F-IA	4970
F-IB	4905
F-IC	5037
F-IIA	5074
F-IIB	5029
F-IIC	6083
F-IIIA	6573
F-IIIB	6087
F-IIIC	6733
F (Marketed)	5244

Spreadability: The spreadability of the formulations was found in between 15.508 and 48.623 cm/seconds. Hence, Batch F-IA, IB, I, IIA, IIB & IIC have less viscous as compare to other batches, so it has better spreadability and lower than the marketed product (sortet gel 52.416g/cm^3).

The average value of the spreadability of all formulation was depicted in **Table 4**.

TABLE 4: AVERAGE SPREADABILITY OF GEL FORMULATIONS

Formulation	Average spreadability (g/cm^3)
F-IA	28.904
F-IB	48.623
F-IC	35.763
F-IIA	27.005
F-IIB	45.763
F-IIC	21.851
F-IIIA	15.508
F-IIIB	38.123
F-IIIC	17.784
MKP	52.416

Drug Content Uniformity (Assay): The drug content of the gel formulations was found to be uniform among various formulations prepared and was found to be in range 89.93-96.70%. It was clear that the F-IB, F-IIB, F-IIA batches shown maximum drug content, which is above 95%. The content uniformity of the gel formulation was shown in **Table 5**.

TABLE 5: READING OF CONTENT UNIFORMITY

Final formula	Percent of drug content	Average
F-IA	89.2	
F-IB	97.2	90.53
F-IC	85.2	
F-IIA	96.2	
F-IIB	99.2	96.7
F-IIC	94.7	
F-IIIA	92.7	89.93
F-IIIB	87.3	
F-IIIC	89.5	

Drug Interaction Study: Drug excipient interaction was performed in different conditions (room temperature, 40 °C/75% RH & 30 °C/60% RH) for 4 weeks and shown in **Table 6**.

The rejection and selection of best formulation are based on the texture and color changes. The result indicates that no change in color was seen after four weeks.

TABLE 6: OBSERVATIONS OF DRUG – EXCIPIENT INTERACTION

S. no.	Name of mixture	Observations (Color)					
		Room temperature		40°C/75%RH		30°C /60%RH	
		I week	IV week	I week	IV week	I week	IV week
1	Carbopol 940	No change	No change	No change	No change	No change	No change
2	Disodium edetate	No change	No change	No change	No change	No change	No change
3	Methylparaben	No change	No change	No change	No change	No change	No change
5	Propyl paraben	No change	No change	No change	No change	No change	No change
6	PEG-400	No change	No change	No change	No change	No change	No change
7	Propylene glycol	No change	No change	No change	No change	No change	No change

FT-IR Studies: The FTIR spectra of pure drug, excipient, and polymer were recorded in 400 to 4000 cm^{-1} on IR Bruker Alpha software. No interference peaks are observed with drug **Fig. 5** and other excipients. The different peaks obtained are summarized in **Table 7**.

TABLE 7: FT-IR PEAKS OF ISOTRETINOIN WITH OTHER EXCIPIENT

Peak (cm^{-1})	Characteristics	Other excipient peaks
1460	C-H bending	1452 (C-H bending)
1583	C-C Stretching	1249.45 (C-C aromatic)
1245, 1222	C-O-C asymmetric stretching	3366.88 (OH)
1053, 1033	C-O-C Symmetric stretching	2971.47 (C-H str., alkene)
1647	C = O stretching	1654.79 (C=0)
1087	Characteristic for the chloride substitution on benzene	951.82 (C-C Aliphatic)

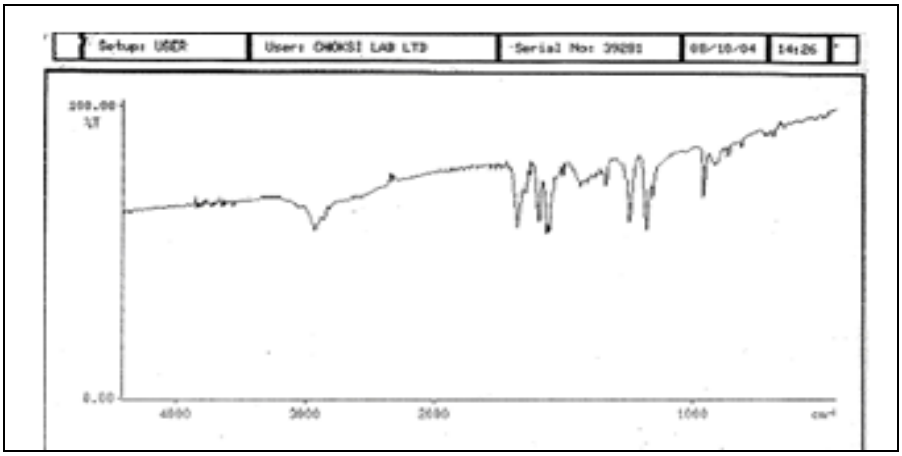


FIG. 5: IR SPECTRUM OF ISOTRETINOIN PURE DRUG

UV Analysis: The calibration curve of Isotretinoin was analyzed by ultra-violet spectroscopy and reading was shown in **Table 8**. The percentage purity of gel formulation was also determined by the UV and shown in **Table 8**. The standard curve of isotretinoin by UV was shown in **Fig. 6**.

TABLE 8: STANDARD CURVE FOR ISOTRETINION

S. no.	Concentration (µg/ml)	Absorbance
1	2	0.097
2	4	0.112
3	6	0.142
4	8	0.168
5	10	0.181

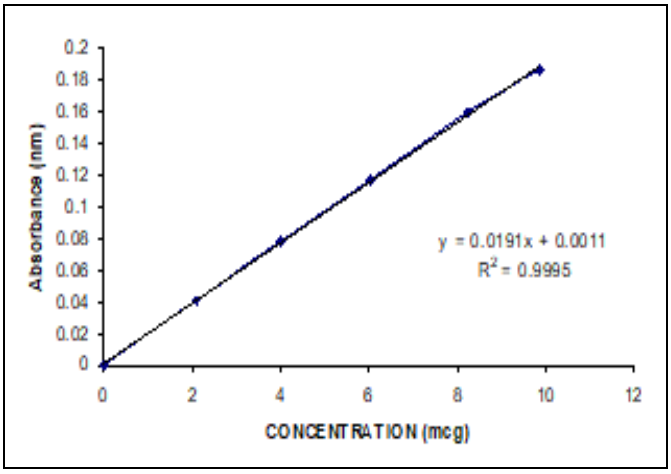


FIG. 6: STANDARD CURVE OF ISOTRETINOIN BY UV

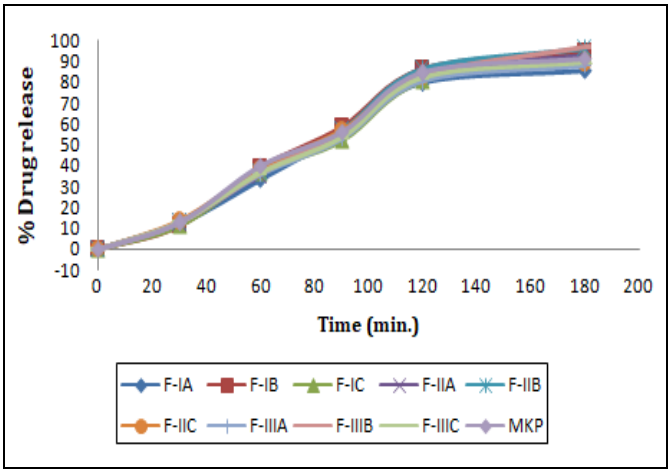


FIG. 7: DRUG RELEASE PROFILE COMPARISON WITH MARKETED PRODUCT

***In-vitro* Drug Release:** *In-vitro* release of the formulation was performed by the Franz-diffusion cell. The *in-vitro* diffusion study shows combined percentage release patterns of the isotretinoin gel (0.05% w/w).

Marketed gel formulation (sortet gel 0.05% w/w) used for the comparison. It is observed from the result that batch F-IA, F-IC, F-IIA and F-IIB showed more diffusion, and drug release from all the formulations, that is, 94.5%, 96.5%, 92.5% and 92.7% after the sortet gel (shows 94.2%), because of decrease in viscosity. The marketed product showed 94.2% release and formulation F-IIIA, F-IIIB, F-IIIC and F-IIC showed low release, that is, 78.3%, 77.2%, 70.2% and 82.7 because of the high viscous formulation. Data was shown in **Table 9**. Drug release profile comparison with the marketed product was depicted in **Fig. 7**.

TABLE 9: DRUG RELEASE STUDY

Time	% Drug Release									Marketed
	F-IA	F-IB	F-IC	F-IIA	F-IIB	F-IIC	F-IIIA	F-IIIB	F-IIIC	
30	11.8	11.5	11.4	12.7	13.2	13.5	12.8	12.5	13.5	12.5
60	33.2	39.2	36.9	35.5	36.5	38.5	35.9	36.5	36.5	40.2
90	55.4	58.3	52.3	56.7	57.4	57.3	52.8	55.6	53.6	56.5
120	79.6	86.5	80.7	84.3	86.6	82.2	65.5	67.2	66.5	84.5
180	85.7	94.5	92.7	92.5	96.5	82.7	78.3	77.2	70.7	94.2

TABLE 10: ACCELERATED STABILITY STUDIES

Parameters	Initial	after one month(4 weeks) (40/75) (°C/RH)
Appearance	Yellowish Translucent	Yellowish Translucent
Feel on application	Smooth	Smooth
pH	5.473-5.765	5.645-6.547
Viscosity	4905-6733	5154-7122
Assay (%)	85.2-99.2	74.5-82.6

Stability Studies: The test was performed as per procedure is given in ICH guidelines for the stability study. A stability study was carried out for the optimized formulation according to ICH guidelines at 40 °C/75% RH for 1 month. The results showed that there was no significant change in the physical and chemical parameter of the gel, hence the formulation F-IB, F-IIB and F-IIIB was found to be stable. Data on stability study were tabulated in **Table 10**.

Acute Skin Irritation Study: The isotretinoin topical gel formulations were evaluated for the skin-irritation study and result clearly denotes that applied formulation is non-irritant and did not show any skin toxicity when applied daily for 7 days in albino rats. The skin irritation studies show that isotretinoin gel formulations dose not produces any harsh irritation, redness of the skin. Marketed formulation of isotretinoin that is sotret gel (Ranbaxy) was used as standard and used for comparison. In this experiment formalin (0.8%) was used as a standard irritant for the comparison. Observation for the skin irritation study was shown in **Table 11**.

TABLE 11: ACUTE SKIN IRRITATION TEST OBSERVATION

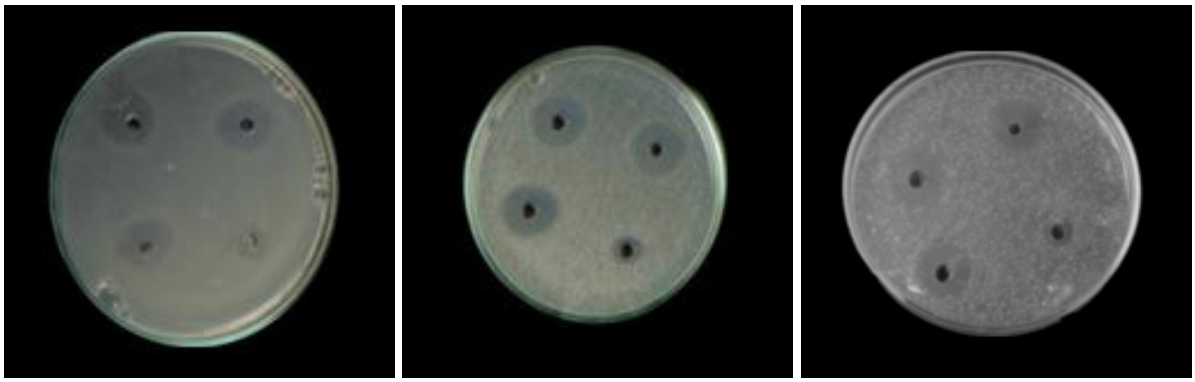
Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	A	A	A	A	A	A
Standard (0.8% formalin solution)	B	B	B	B	B	B	B
F-IA (0.5%)	A	A	A	A	A	A	A
F-IB	A	A	A	A	A	A	A
F-IC	A	A	A	A	A	A	A
F-IIA	A	A	A	A	A	A	A
F-IIB	A	A	A	A	A	A	A
F-IIC	A	A	A	A	A	A	A
F-IIIA	A	A	A	A	A	A	A
F-IIIB	A	A	A	A	A	A	A
F-IIIC	A	A	A	A	A	A	A
MKP	A	A	A	A	A	A	A

***Ex-vivo* Study:**

Antibacterial Study: The zones of inhibitions for the antibacterial activity were compared with the standard benzoyl peroxide gel, marketed preparation of isotretinoin (sotret gel) for *Acne vulgaris*. Formulation F-IA, F-IIB, and F-IIIB have shown comparable zones of inhibitions to that of the marketed preparation. Zones of inhibitions for benzoyl peroxide were found to be greater than that of all the formulations (F-IA to F-IIIC) as well as sotret gel formulation. These results suggested that all formulations having the antibacterial potential to inhibiting the *P. acne* and it may also reduce the development of inflammatory acne. The zone of inhibition of all formulation and the marketed formulation was shown in **Table 12** and **Fig. 8**.

TABLE 12: ZONE OF INHIBITION OF GEL FORMULATION (F-IA TO F-IIIC)

Formulations	Zone of inhibition in mm				
Concentration (0.5%)	1	2	3	Mean	SD
F-IA	22.6	22.2	22.6	22.46667	0.23094
F-IB	32.4	32.6	32.9	32.63333	0.251661
F-IC	28.4	28.5	28.8	28.56667	0.208167
F-IIA	21.4	21.7	21.8	21.63333	0.208167
F-IIB	34.5	34.7	34.2	34.46667	0.251661
F-IIC	28.4	27.8	28.6	28.26667	0.416333
F-IIIA	18.4	17.9	18.8	18.36667	0.450925
F-IIIB	32.4	32.8	32.5	32.56667	0.208167
F-IIIC	25.8	26.4	26.3	26.16667	0.321455
Benzyl Peroxide gel (+ control)	44.3	44.7	44.4	44.46667	0.169967
Marketed SOTRET gel	38.5	38.8	38.3	38.53333	0.20548
Distill water (- control)	-	-	-	-	



[F-IA-IC] [F-IIA-IIC] [F-IIIA-IIIC]

FIG. 8: ZONE OF INHIBITION OF VARIOUS ANTI-ACNE GEL FORMULATION OF ISOTRETINOIN WITH MARKETED FORMULATION

DISCUSSION: The different compositions of carbopol 940, 938 and 980 were utilized for experiments performed. Microorganisms grow well in unpreserved aqueous dispersions so anti-

microbial preservatives are required in the formulation. Propylparaben and methylparaben are widely used as an antimicrobial preservative in parental, topical and oral preparations. The paraben is most effective over a wide pH range. The poor solubility of the parabens, paraben salts, especially sodium salts are frequently used in formulations. The effective combination of paraben is to be 0.18% of methylparaben & 0.2% of propylparaben. Preservative efficacy is also improved by the addition of 2-5 % propylene glycol. Disodium edetate is used as chelating agents *i.e.* chelating agents are as removing ions from solutions, they form stable water-soluble complexes with alkaline earth and heavy metal ions.

SUMMARY AND CONCLUSION: The present study was undertaken with an aim to formulate and evaluate the formulation of isotretinoin gel using different polymers with different concentrations. Preformulation study was carried out initially. Different batches with different polymer ratios were prepared using selected excipient. Various formulations of isotretinoin gels were prepared by using vas polymers Carbopol-940, Carbopol-980, Carbopol-934 in different proportions by fusion and cold method. The isotretinoin gel formulation was optimized on the basis of different physical parameters and mainly with the comparison of formulations on the basis of the *in-vitro* diffusion study. The gels were evaluated for physical characterizations (pH, solubility, viscosity, UV spectroscopy), *in-vitro* dissolution, *in-vivo* skin irritation study and *ex-vivo* antibacterial study on microorganism *P. acne*. It is therefore concluded that the product 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. The formulation is isotretinoin gel would be used as anti-acne. This formulation is mainly used for acne infection. The topical application of the gel at the affected site would offer the potential advantage of the delivery of the drug directly to the acne site. The stability study of the formulation was conducted to store at different temperatures and humidity conditions for one month. Gels were evaluated for appearance, feel on application, pH, viscosity, and assay after one month.

From the above results, it concluded that isotretinoin gel formulation containing carbopol 940 in 0.5% can be taken as an ideal formulation.

ACKNOWLEDGEMENT: Nil

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Russell JJ: Topical therapy for acne. Am Fam Phys 2000; 61: 1-13.
2. Zaenglein A: Topical retinoids in the treatment of *Acne vulgaris*. Semin Cutan Med Surg 2008; 27: 177-82.
3. Atar M, Kausar A and Reheman A: Preparation of new formulation of anti-acne creams and their efficacy. Afr J Pharm Pharmacol 2010; 4(6): 298-03.
4. Patel MR, Patel RB, Parikh JR and Patel BG: HPTLC method for estimation of isotretinoin in topical formulations, equilibrium solubility screening and *in-vitro* permeation study. J Liq Chromatogr Rel Tech 2011; 34: 1783-99.
5. Waghmare N, Waghmare S, Wani S and Yerawar A: Development of isotretinoin gel for the treatment of *Acne vulgaris*. Res J Pharm Biol Chem Sci 2011; 2(1): 220-30.
6. Chavda H, Patel J, Chavada G, Dave S, Patel A and Raval M: Self nano-emulsifying powder of isotretinoin: Preparation and characterization. J Powder Technol 2013; 20(13): 1-9.
7. Nikam S: Anti-acne gel of isotretinoin: formulation and evaluation. Asian J Pharm Clin Res 2017; 10(11): 257-66.
8. Vijayalakshmi A, Tripura A and Ravichandiran V: Development and evaluation of anti-acne products from Terminalia Arjuna bark. Int J Chem Technol Res 2011; 3(1): 320-27.
9. Wani A, Sanghani C and Wani S: Formulation, characterization, and *in-vitro* evaluation of novel microemulsion-based spray for topical delivery of isotretinoin. Asian J Pharm Clin Res 2018; 11(10): 226-32.
10. Patel RB, Patel MR, Bhatt KK and Patel BG: Risperidone loaded mucoadhesive microemulsion for intranasal delivery: formulation, development, physicochemical characterization and *ex-vivo* evaluation. J Drug Del Sci Tech 2013c; 23: 261-67.
11. Patel MR, Patel RB, Parikh JR, Solanki AB and Patel BG: Effect of formulation components on the *in-vitro* permeation of microemulsion drug delivery system of fluconazole. AAPS Pharm Sci Tech. 2009; 10: 917-23
12. Patel MR, Patel RB, Parikh JRand Patel BG: HPTLC method for estimation of isotretinoin in topical formulations, equilibrium solubility screening and *in-vitro* permeation study. J Liq Chromatogr Rel Tech 2011c; 34: 1783-99.
13. Patel MR, Patel RB, Parikh JR, Solanki AB and Patel BG: Investigating effect of microemulsion components: *in-vitro* permeation of ketoconazole. Pharm Dev Tech 2011b; 16: 250-58.
14. Patel RB, Patel MR, Bhatt KK and Patel BG: Formulation consideration and characterization of microemulsion drug delivery system for transnasal administration of Carbamazepine. Bul Fac Pharm Cairo Uni 2013a; 51: 243-53.
15. Patel RB, Patel MR, Bhatt KK and Patel BG: Paliperidone loaded mucoadhesive microemulsion in treatment of

schizophrenia: formulation consideration. J Pharma Inn 2013b; 8: 195-04.
16. Patel RB, Patel MR, Bhatt KK and Patel BG: Risperidone loaded mucoadhesive microemulsion for intranasal delivery: formulation, development, physicochemical characterization and *ex-vivo* evaluation. J Drug Del Sci Tech 2013c; 23: 261-67.
17. Sawarkar H, Khadabadi S, Mankar D, Farooqui I and Jagtap N: Development and biological evaluation of herbal anti-acne gel. Int J Pharmtech Res 2010; 2(3): 2028-31.
18. Jain S and Diwan A: Use of lactic acid and span 80 in the formulation of lipid-based imiquimod vesicles for genital warts. Int J Pharm Pharm Sci 2017; 9(2): 292-01.

How to cite this article:
Gupta S and Bharadwaj A: Formulation, characterization and evaluation of isotretinoin gel for treatment of *Acne vulgaris*. Int J Pharm Sci & Res 2020; 11(2): 873-83. doi: 10.13040/IJPSR.0975-8232.11(2).873-83.