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Research Article

ANTI-ACNE GEL OF ISOTRETINOIN: FORMULATION AND EVALUATION

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ABSTRACT

Objective: Isotretinoin is a very effective drug in the treatment of acne vulgaris by topically. The objective of present study was formulation development of anti-acne gel using Isotretinoine and span 80 for topical delivery to cure nodulosystic acne vulgaris. Furthermore, the comparative study of all the evaluation parameters done with marketed formulation of same drug.

Methods: Formulation of anti-acne gel of isotretinoin using Carbopol 940 as a polymer and incorporating isotretinoin in form of topical semi-solid gel using magnetic stirrer, Cremophor RH 40, and butylated hydroxytoluene. Drug was uniformly dispersed in Cremophor RH 40 and the respected solvents. Ethanol, isopropyl alcohol, and glycerin were used as solvents in 15% quantity. Further, the formulation was evaluated for physicochemical evaluation of gel formulations. The prepared gel were optimized statistically and characterized for pH, spredability, drug content, viscosity, *in vitro* diffusion study, acute skin irritation test, and antimicrobial activity. Evaluation test was also compared with marketed formulation of isotretinoin, that is, Sortet gel. The antibacterial and anti-acne activity of different formulations was determined by modified agar well diffusion method on the culture of *Propionibacterium acne* also compared with marketed formulation.

Results: The optimized batch (B10) showed highest spreadability (32.422 g/cm^3) in all formulations and also have high percentage of drug contents (95.60%). The spreadability value was 17.998 g/cm^3 showing good spreadability. The viscosity of optimized batch was observed less as compared to other formulations, ultimately showed releases also more. In the *in vitro* diffusion study, B10 batch release 85.69% of the drug as compared to Sotret gel. The antibacterial activity was studied on anaerobic microorganism *P. acne*, compared with marketed Sortet gel. Optimized batch showed maximum zone of inhibition to *P. acne* below marketed formulations and standard benzyl peroxide gel.

Conclusion: The topical anti-acne gel of isotertinoin was successfully formulated and evaluated for different parameters. The results indicate that the active component, that is, isotertinoin is more effective when subjected in gel formulations and produces effective anti-acne activity in the management of nodulosystic acne vulgaris.

Keywords: Formulation, Isotretinoin, Topical gel, Anti-acne activity, *Propionibacterium acne.*

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INTRODUCTION

Semi-solids constitute a significant proportion of pharmaceutical dosage forms. They serve as carriers for drugs that are topically delivered by way of the skin, cornea, rectal tissue, nasal mucosa, vagina, buccal tissue, urethral membrane, and external ear lining. Because of their peculiar rheological behavior, semi-solids can adhere to the application surface for sufficiently long periods before they are washed off. This property helps prolong drug delivery at the application site**.** Acne vulgaris is a common skin disease, affecting about 70-80% of adolescents and young adults. It is a multifactorial disease of the pilosebaceous unit [1]. The influence of androgens at the onset of adolescence leads to an enlargement of the sebaceous gland and a rise in sebum production. Additional increased proliferation and altered differentiation of the follicular epithelium eventually blocks the pilosebaceous duct, leading to the development of the microcomedo as the primary acne lesion. Concomitantly and subsequently, colonization with *Propionibacterium acnes* increases followed by induction of inflammatory reactions from bacteria, ductal corneocytes, and sebaceous pro-inflammatory agents. Topical retinoid has been used in acne therapy since 1962. The first one was tretinoin, which remains in use today. Due to tretinoin's irritative potential, new formulations with much better tolerability have been developed. Other retinoid-like agents with a different retinoid receptor-binding profile, most importantly adapalene, show much less irritancy and thus better compliance with a comparable clinical effect on comedonic and inflammatory acne lesion. Thus, facts have clearly indicated that a formulation and development of a gel-based topical dosage form for the anti-acne drug will be proved to be worthwhile. Hence, a study on formulation and evaluation of gels for a new anti-acne drug–"isotretinoin" was selected as the principle object of this project work. Topical application of gels overcomes the problems to be associates with other dosage forms are, avoidance of the first-pass metabolism, convenient and easy to apply, avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption, such as pH changes, presence of enzymes, gastric emptying time, achievement of efficacy with lower total daily dosage of drug by continuous drug input, avoids fluctuation in drug levels, inter- and intra-patient variations, and ability to easily terminate the medications, when needed.

A relatively large area of application in comparison with buccal or nasal cavity, ability to deliver drug more selectively to a specific site, and avoidance of gastro-intestinal incompatibility.

Providing utilization of drugs with short biological half-life, narrow therapeutic window. Improving physiological and pharmacological response and patient compliance provides suitability for self- medication [2].

Therefore, the aims of this study were (a) to develop isotretinoin anti-acne gel using Carbopol 940 polymer with different concentrations of solvents such as ethanol, isopropyl alcohol, and glycerin, and drug-solubilizer Cremophor RH 40 and (b) to study the evaluation criteria for it.

MATERIALS AND METHODS

Materials

The drug isotretinoin USP was as gift sample from Cosme Pharma Laboratories Limited, Mumbai. Cremophor RH 40 purchased from Zeel pharmaceuticals, Mumbai. Sotret gel purchased from Ranbaxy Pharmaceuticals. *Propionibacterim acne* culture MTCC 1951 was purchased from the M.T.C.C., Institute of Microbial Technology, Chandigarh (India).

Equipment

Ultraviolet (UV)/visible-spectrophotometer (Systronic AU2701, double beam), Fourier transform-infrared spectrophotometer (FT-IR) (Perkin-Elmer, Shivaji University Kolhapur), Homogenizer (Remi Motors, RQ127 A), magnetic stirrer (Remi Motors), Digital pH Meter (HI96107), 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.

Analytical method development

Determination of λ_{max} of Isotretinoin spectrum scan by UV spectroscopy

Procedure

Stock solution of isotretinoin was prepared in phosphate buffer pH 5.8: Ethanol (65:35 v/v) solution. The concentration of stock solution was 40 µg/mL.

The scanning of the isotretinoin was performed in UV spectrophotometer. The solvent used for the spectrum analysis was mixture of phosphate buffer pH 5.8: Ethanol (65:35) and the scanning was done. The maximum absorption of isotretinoin was found at 340 nm. Standard calibration curve for isotretinoin in ethanol: Phosphate buffer pH 5.8.

Preparation of phosphate buffer pH 5.8

A volume of 50 mL of 0.2 M potassium dihydrogen phosphate were placed in 200 mL of volumetric flask and add 3.6 mL of 0.2 M NaOH. Then, distilled water was added to make volume.

A volume of 100 mL of 0.2M KH_2PO_4 and 7.2 mL NaOH 0.2 M were mixed and volume was made 400 mL with distilled water and pH was checked at 5.8 by digital pH Meter 325 mL of above solution and 175 mL of ethanol then mixed. This was our phosphate buffer: Ethanol mixture for dilution [3].

Procedure

1. 200 mg drug isotretinoin was weighed and mixed with 100 mL solvent, that is, phosphate buffer pH 5.8: Ethanol (65:35 v/v), concentration was 2000 µg/mL.
2. 10 mL of above solution was again diluted with 100 mL with solvent; the concentration was 200 µg/mL.
3. Then, 20 mL of solution of step 2, was diluted to 100 mL with solvent, the final concentration of solution was 40 µg/mL.

Stock solution was further diluted and the absorbance of diluted solution was taken using UV spectrophotometer.

The procedure was repeated 5 times and mean was taken for the standard calibration curve. Absorbance was measured at 340 nm against ethanol: Phosphate buffer as blank solution.

Pre-formulation test for isotretinoin

Pre-formulation studies of API were carried out to study incompatibility between the excipients used.

Drug–polymer interaction study

In pre-formulation study, drug and polymer interaction was studied by FT-IR study. Before 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 drug along with polymer and excipient. FT-IR spectra of drug molecule, isotretinoin-ethanol, isotretinoin-isopropyl alcohol, isotretinoin glycerin, isotretinoin-Carbopol 940, isotretinoin-Cremophor RH40, isotretinoin, butyalated hydroxytoluene, isotretinoin-triethanolamine, and isotretinoin-all excipients were obtained on FT-IR spectrophotometer (PERKIN ELMER). The spectra were scanned over wavelength region of 4000-400 nm.

Preparation and composition of gel

The anti-acne gel of isotretinoin was prepared by first, required quantity of Carbopol 940 was taken. It was taken as 0.5 g, 0.75 g, and 1 g in all 11 batches.

Required quantity of Carbopol 940 was accurately weighed on analytical balance and sprinkled on specific quantity of water and kept for hydration for 24 hrs and then stirred slowly using magnetic stirrer to form uniform mixture. At the same time, in another beaker drug, Cremophor RH 40 [3] butylated hydroxytoluene, was accurately weighed. Drug was uniformly dispersed in Cremophor RH 40 and the respected solvents. Ethanol, isopropyl alcohol, and glycerin were used as solvents in 15% quantity. Then, the uniform mixture of Carbopol 940 was neutralized slowly using trietahnolamine, without forming 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. All the procedure was carried out by wrapping aluminum foil to glass wares to avoid degradation of drug isotretinoin [4].

Evaluation of anti-acne gel of isotretinoin

Physicochemical evaluation of gel formulation

Color, physical appearance, and homogeneity were tested by visual observation.

pH

The pH of the various formulations was determined using Digital pH Meter (HI 96107). A volume of 1 g of 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 5.9 ± 0.1-6.36 ± 0.2, which lies in the normal range of the skin and would not produce any skin irritation. 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 1 [5].

Spreadability

Spreadability denotes the extent of area to which the gel readily spreads on application to the skin or affected part. 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. 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 [5].

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.

Table 1: Formulation ingredients of isotertinoin gel (g)

Ingredients	B1 E	B2 IA	B3 G	B4 E+I	B5 I+G	B6 E+G	B7 E+I+G	B8 E+G	B9 I+G	B10 E+I	B11 (blank E+I+G)
Isotretinoin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	-
Ethanol	14.99	-	-	10.00	-	5.02	5.02	10.00	-	5.03	5.03
Isopropyl alcohol	-	15.01	-	5.03	15.01	-	5.03	-	5.03	10.00	10.00
Glycerin	-	-	15.01	-	-	10.00	5.04	5.04	10.00	-	-
Cremophor RH 40	1	1	1	1	1	1	1	1	1	1	1
BHT	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Carbopol 940	0.5	0.75	01	0.5	0.75	01	0.5	0.75	01	0.5	0.5
Distilled water	75.22	74.75	74.2	75.18	74.7	74.19	75.12	74.72	74.18	74.18	75.18
Triethanolamine	1.6	1.8	2.1	1.6	1.8	2.1	1.6	1.8	2.1	1.6	1.6
Distilled water	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
Total weight	100	100	100	100	100	100	100	100	100	100	100

Table 2: Standard calibration curve for isotretinoin in ethanol:phosphate buffer pH 5.8

Concentrations µg/ml	Cali I	II	III	IV	V	Average	±SD
2		0.061	0.078	0.074	0.075	0.072	0.007528
4	0.99	0.112	0.132	0.129	0.134	0.1212	0.01515
6	0.137	0.143	0.201	0.204	0.2	0.177	0.0338
8	0.179	0.189	0.267	0.265	0.268	0.2336	0.0454
10	0.238	0.227	0.312	0.308	0.311	0.2792	0.0428
12	0.281	0.296	0.369	0.363	0.362	0.3342	0.0421
14	0.329	0.337	0.415	0.417	0.418	0.3832	0.0459
16	0.384	0.375	0.479	0.48	0.476	0.4388	0.0542
18	0.439	0.451	0.514	0.511	0.509	0.4848	0.0366
20	0.486	0.483	0.574	0.578	0.571	0.5384	0.0492
22	0.537	0.549	0.609	0.615	0.612	0.5844	0.038
24	0.584	0.59	0.665	0.663	0.668	0.634	0.0429
26	0.637	0.635	0.718	0.713	0.715	0.6836	0.0434
28	0.689	0.692	0.77	0.769	0.765	0.737	0.0425
30	0.752	0.743	0.82	0.823	0.818	0.7912	0.04
32	0.801	0.814	0.861	0.867	0.869	0.8424	0.0323
34	0.852	0.864	0.912	0.918	0.914	0.892	0.0314
36	-	0.897	0.957	0.953	0.95	0.9392	0.0283
38	-	0.949	0.998	0.991	0.99	0.982	0.0228
40	-	0.988	1.09	1.06	1.08	1.0545	0.046
Slope	0.0252	0.0251	0.0259	0.0257	0.0254	0.02546	0.000251661
Intercept	0.9991	–0.0066	0.05	0.0427	0.0509	0.22722	0.004497036
Correlation	0.0142	0.9985	0.997	0.9972	0.9982	0.80102	0.00064291

Table 3: Absorbance of standard calibration curve of isotretinoin

Concentrations (µg/ml)	Average
2	0.072
4	0.1212
6	0.177
8	0.2336
10	0.2792
12	0.3342
14	0.3832
16	0.4388
18	0.4848
20	0.5384
22	0.5844
24	0.634
26	0.6836
28	0.737
30	0.7912
32	0.8424
34	0.892
36	0.9392
38	0.982
40	1.0545
Slope	0.0255
Intercept	0.0249
Correlation	0.9998

Drug content

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 dilute the solution to 100 ml with 5 ml of 0.1 M hydrochloric acid to 250 ml with ethanol (96%). Measured the absorbance of the solution at maximum at about 356 nm, using ethanolic hydrochloric acid solution in the reference cell. The content of $C_{20}H_{28}O_2$ in gel was calculated taking 1350 as the value of a (1%, 1 cm) at maximum at about 356 nm using UV-visible spectrophotometer (systronic double beam spectrophotometer). For the drug, content sample has taken from top, middle, and bottom from the container. The experiment was repeated for ten times for each batch, four times for top region, and three times for middle and bottom region, and then average value was taken for the drug-content calculation [6].

Stickiness

Stickiness was evaluated by just applying 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 small volume Brookfield viscometer. The determinations were carried out four times at 6, 12, 30, and 60 rpm and that reading was multiplied by the factor and mean of that were taken as final viscosity in centipoises [4].

Brookfield factor finder was used as follows:

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

***In vitro* diffusion study**
In vitro diffusion study of the anti-acne gel formulation 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. 0.45 µ dialyzing membrane was used. The media used for the *in vitro* diffusion was mixture of phosphate buffer pH 5.8: Ethanol (65:35) v/v. The dialyzing membrane was soaked in phosphate buffer 24 hrs before use. The temperature was maintained constant at 32°C. 5 ml sample was withdrawn and replaced with fresh solvent. The time interval was maintained as 15 minutes, 30 minutes, 1 hr, and 1.30 minutes up to 8 hrs. 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 [4,5,9].

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 condition. 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 the each formulation of different concentrations were applied over one square centimeter area of intact and abraded skin to different animals. 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 produces any severe irritation, redness of 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) [5,9].

Antibacterial study
The antibacterial activity of different formulations was determined by modified agar well diffusion method. In this method, nutrient agar plates were seeded with 0.2 ml of 24 hrs broth culture of *P. acnes*. The plates were allowed to dry for 1 hr. A sterile 8 mm borer was used to cut four wells of equidistance in each of plates; 1 g of formulations (B1-B10) and marketed Sotret gel for comparison. Benzoyl peroxide gel was used as a positive control, and distil water was used as negative control were introduced in to the wells at randomly. The plates were incubated at 37°C for 24 hrs. The antibacterial activities were found out by measuring the diameter of zones of inhibition (in mm). This experiment repeated 3 times [8].

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 ploted and the value of slope, correlation, and regration was calculated and these values are taken as standard for calculation in *in vitro* diffusion study.

Pre-formulation test for isotretinoin

Tests	Specifications	Results
Description	Pale yellow to yellow color, microcrystalline powder	Pale-yellow, microcrystalline powder
Solubility	Practically insoluble in water, soluble in chloroform, sparingly soluble in alcohol, in isopropyl alcohol, and in polyethylene glycol 400	Practically, insoluble in water, soluble in, chloroform, sparingly soluble in alcohol, in isopropyl alcohol, and in polyethylene glycol 400
Identification	The infrared spectrum of the sample is concordant with the spectrum obtained with isotretinoin reference standard/working standard The absorptivities at 354 nm do not differ by more than 3.0%	The infrared spectrum of the sample is concordant with the spectrum obtained with isotretinoin reference standard/working standard 5 kol-0.5397 5 g-0.4728
Loss on drying	Not more than 0.5%	0.3%
Residue on ignition	Not more than 0.1%	0.05%
Heavy metals	Not more than 0.002%	<0.002%
Limit of tretinoin	Not more than 0.1%	0.007%
Residual solvents	To comply with USP	Complies
Assay	Contains not less than 98.0% and not more than 102.0% of isotretinoin calculated on dried basis	99.1%
Extraneous matter	Should be nill	Nil

Chemical analysis certificate
Name of product: Isotretinoin USP
Batch No: 20110103
Manufactured date: 2011/01/12
Expiry date: 2016/01/11.

Drug polymer interaction study
The major peaks were found in IR spectra of isotretinoin at wave number 3428.77 may be due to stretching vibration of OH group

Table 4: Test for isotretinoin

Tests	Standards	Results
Description	Yellow crystalline powder	Conforms
Identification	Conforms	Conforms
Organic volatile impurities	Conforms	Conforms
Heavy metals	<20 ppm	<20 ppm
Loss on drying	≤0.5%	0.3%
Residue on ignition	≤0.1%	0.04%
Limit of tretinoin	≤0.1%	0.10%
Assay	98.0-102.0%	99.6%
Conclusion	The result conforms with above standards	

Table 5: IR interpretation of isotretinoin

Groups in structure	Ranges on interpretation	Interpretation
OH	2500-3500	H-bonded
	3200-3400	Stretching, strong peak
C=O	1680-1760	Carbonyl group, strong peak
C=C	1600-1680	Aliphatic-alkene, weak peak
C-C	800-1300	Aliphatic-alkene-weak or medium, stretching
C=C	1450-1600	Aromatic-weak-(bending)
C-C	1650	Weak
	1580	Weak
	Near 1500	Weak
	1450	May be strong
	1200-1400	Aromatic
C-H stretch	2850-2960	Alkane, strong
Aromatic H	3000-3100	Medium

IR: Infrared

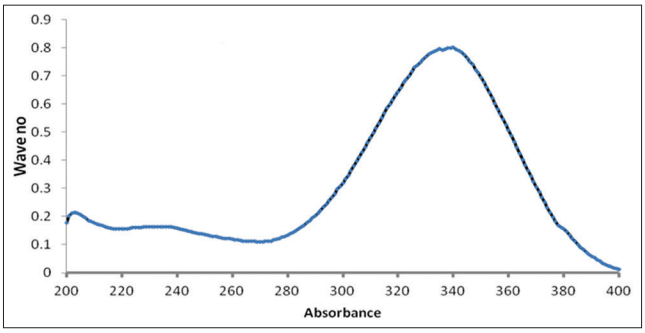

Fig. 1: Ultraviolet spectrum of isotretinoin

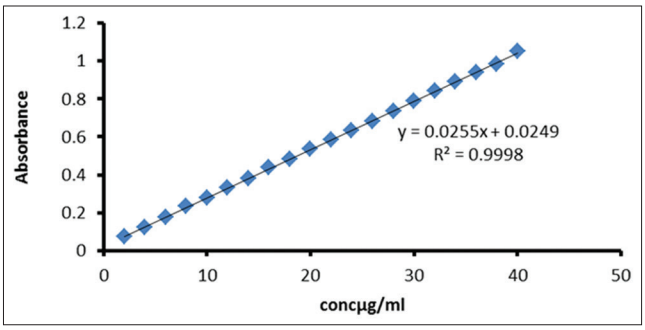

Fig. 2: Standard calibration curve of isotretinoin in buffer:ethanol (65:35 v/v) pH 5.8 by spectrophotometer at 340

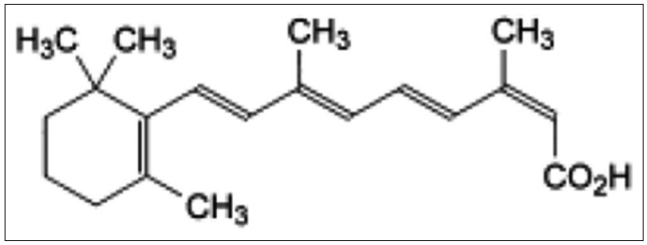

Fig. 3: Structure of isotretinoin

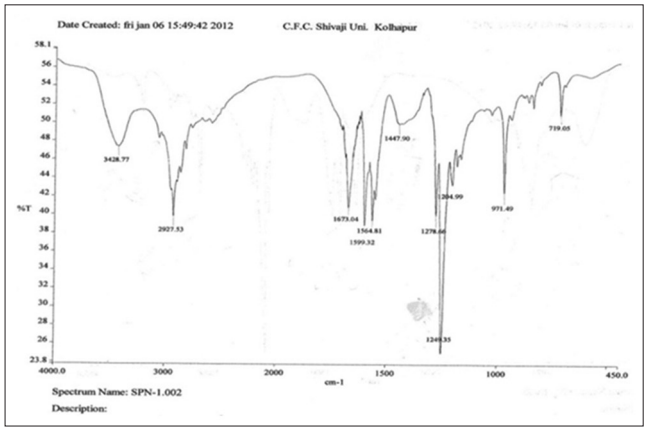

Fig. 4: Infrared of isotretinoin

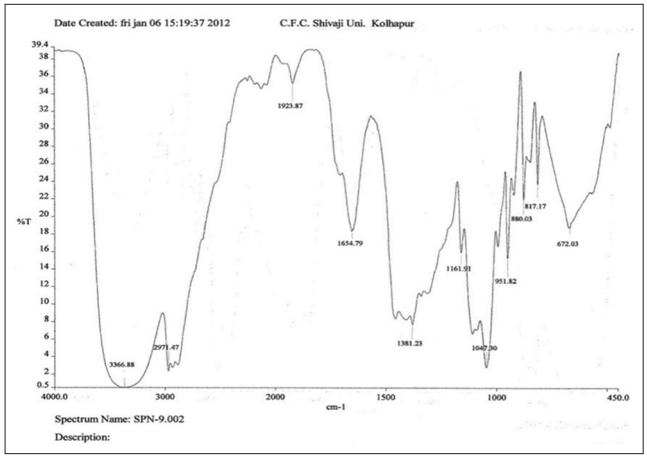

Fig. 5: Infrared spectrum of isotretinoin and mixture of all excipients

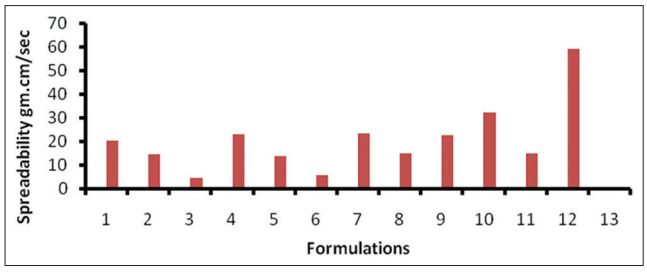

Fig. 6: Spreadability of anti-acne gel of isotretinoin formulations prepared using Carbopol 940 (average ± SD)

and shows strong peak. Also at 3075, it gives weak peak due to aromatic H. Then, also shows strong peak at 2927.53 due to C-H stretch Alkene. Strong peak at 1673.04 for C=O group also gives strong peak at 1599.32 due to C=C Aliphatic group, and also C=C Aromatic group gives strong peak at 1564.81. There were also deformations of OH group take place which gives broad shallow peak at 1447.90 range, and also gives strong peak at 1249.35 due to C-C aromatic.

IR spectroscopy of isotretinoin and all ingredients

From the IR spectroscopy of drug, polymer, and other solvents and excipients, it was observed that the drug and other excipients were compatible with each other and there was no chemical reaction among them.

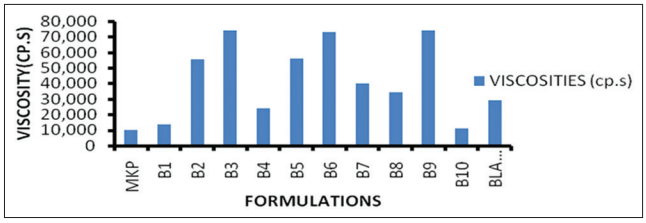

Fig. 7: Viscosity of anti-acne gel of isotretinoin along with marketed product

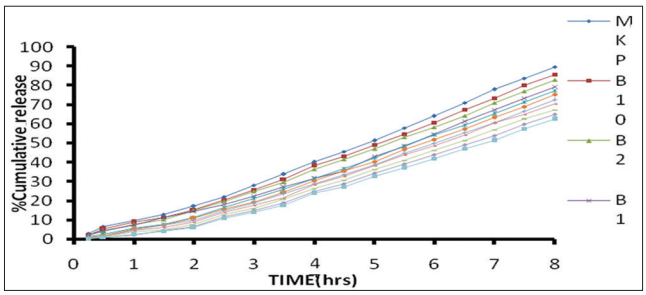

Fig. 8: *In vitro* release data of the anti-acne gel of isotretinoin (0.05%w/w) and Sortet gel

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 between pH ranges 5.9-6.5.

Spreadability

The spreadability of the formulations was found in between 4.654 and 32.422 g cm/seconds. Affinity of solvent toward the polymer

Table 6: Interpretation of isotretinoin carried out by IR

Groups	Ranges	Interpretation
OH	3428.77	Strong peak of carboxyl group
C-H stretch alkene	2927.53	Strong peak
C==C arbonyl	1673.04	Strong peak
C==C aliphatic	1599.32	Strong peak
C=C aromatic	1564.81	Strong peak
OH deformation	1447.90	Broad peak
C-C aromatic	1249.35	Strong peak
C-C aliphatic	971.49	Strong peak

IR: Infrared

Table 7: IR interpretation of isotretinoin, all excipients mixture

Peaks (cm^{-1})	Groups
3366.88-strong	OH
1654.79-strong	C=O
1249.45-strong	C-aromatic
2971.47	C-H stretch alkene
951.82	C-C aliphatic

IR: Infrared

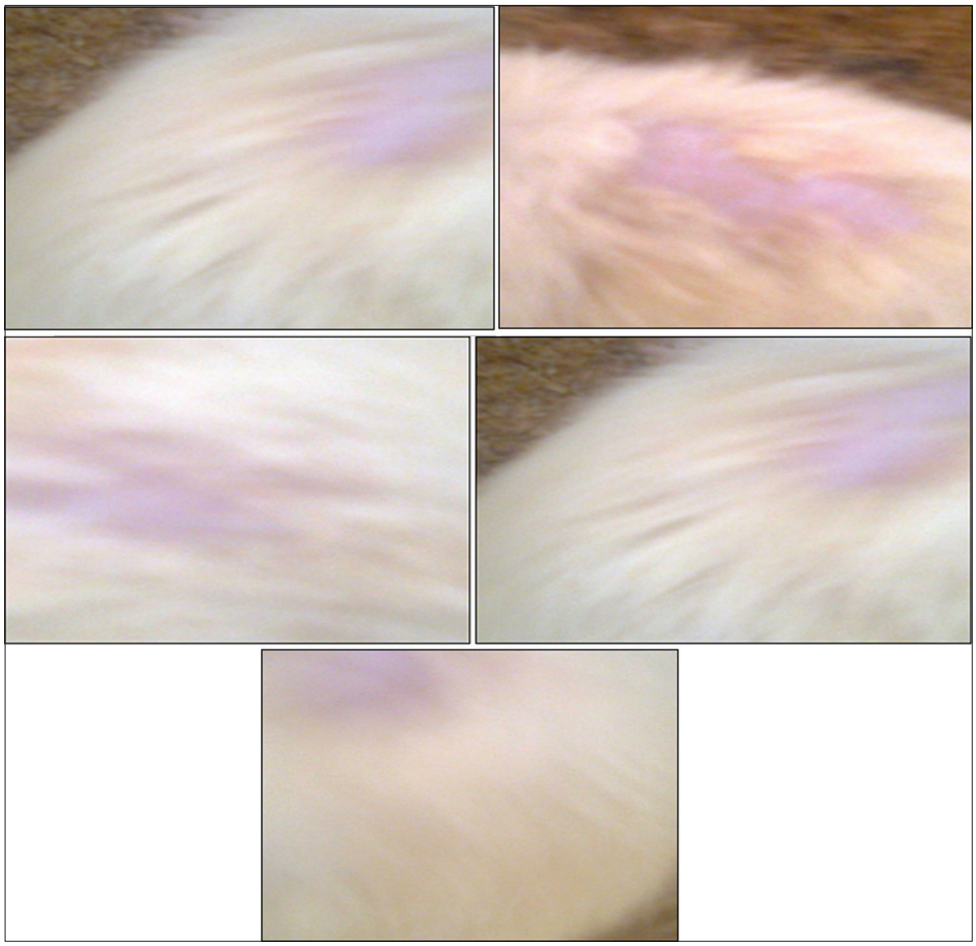

Fig. 9: Photographs of skin irritation study of anti-acne gel of isotretinoin

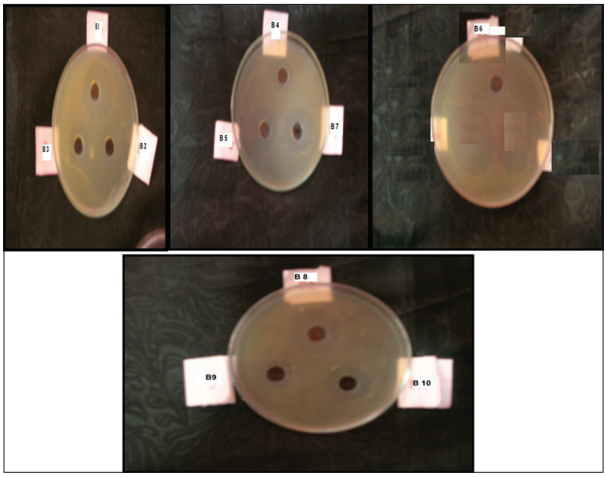

Fig. 10: Photographs of antibacterial study of anti-acne gel of isotretinoin

also affects the structure of network of the gel. If solvent have higher affinity toward polymer then polymer chains get extended, that is, increased entanglement, and thus increases swelling of polymer thus, increase viscosity of formulation. And if solvent has low affinity toward solvent, then polymer contracts reduces entanglement. Ethanol has higher affinity toward water than polymer carbopol 940, that is, it has low affinity toward carbopol 940, so the gel structure get contracted, so viscosity was less. Hence, Batch B10 have less viscous as compare to other batches, so it has better spreadability (32.6 g/cm^3) and lower than marketed product (Sortet gel 59.116 g/cm^3).

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 89.26-95.60%, from the above result, it was clear that the B10 batch shows maximum drug content, that is, 95.96%. Among all formulations of gel contains mixture of ethanol and isopropyl alcohol, containing more quantity of isopropyl alcohol.

Table 8: Color, physical appearance, homogeneity, feel on application, and pH (mean) of anti-acne gel of isotretinoin

Formulation code	Color	Physical appearance	Homogeneity	Feel on application	pH (mean)
MKT PDT	Yellow	Transparent	Homogeneous	Smooth	6.5±0.1
B1	Yellow	Transparent	Homogeneous	Smooth	6±0.1
B2	Yellow	Transparent	Homogeneous	Smooth	6.03±0.1
B3	Yellow	Transparent	Homogeneous	Smooth	6±0.1
B4	Yellow	Transparent	Homogeneous	Smooth	6.16±0.2
B5	Yellow	Transparent	Homogeneous	Smooth	6.06±0.2
B6	Yellow	Transparent	Homogeneous	Smooth	6±0.1
B7	Yellow	Transparent	Homogeneous	Smooth	6.1±0.1
B8	Yellow	Transparent	Homogeneous	Smooth	6.06±0.1
B9	Yellow	Transparent	Homogeneous	Smooth	5.9±0.1
B10	Yellow	Transparent	Homogeneous	Smooth	6.36±0.2
B11 BLANK		Transparent	Homogeneous	Smooth	6.23±0.1

Table 9: Average spread ability of gel formulations

Formulation	Average spreadability, g/cm^3
B1	20.367
B2	14.603
B3	4.654
B4	23.005
B5	13.668
B6	5.851
B7	23.508
B8	15.123
B9	22.784
B10	32.422
B11	14.998
MKP	59.116

Table 10: Spreadability of gel formulations

Formulations	1st	2st	3rd	4th	5th	6th	7th	8th	9th	10th	Average spread ability	±SD
B1	19.7	20.37	22	20	19.8	19.6	20.7	20.33	17.4	23.5	20.367	1.5
B2	14.4	14.38	14.3	15	15.1	14.8	14.4	14.38	14.4	14.7	14.603	0.3
B2	4.66	4.68	4.66	4.7	4.65	4.51	4.67	4.68	4.69	4.65	4.654	0.05
B3	23.1	22.85	22.6	23	23.2	22.9	22.6	23.16	23.1	23.2	23.005	0.2
B5	13.6	13.48	13.7	14	14	13.8	13.7	13.62	13.5	13.5	13.668	0.1
B6	5.87	5.59	5.96	5.9	5.87	5.86	5.88	5.85	5.85	5.87	5.851	0.09
B7	24	21.81	23.8	24	23.6	23.4	23.2	23.85	23.8	23.6	23.508	0.6
B8	15.2	15.03	15.2	15	15	15	15.4	15.2	15.1	15.1	15.123	0.1
B9	22.7	22.68	22.6	23	22.7	22.6	22.4	23.03	23.1	23.2	22.784	0.2
B10	32.6	32	31.5	33	32.9	32.6	32.4	32	31.7	33.3	32.422	0.6
B11	17.8	19.04	18.3	18	17.9	17.7	17.5	18.15	18	17.9	17.998	0.4
MKP	59.1	57.69	59.7	59	59.4	59.7	58.5	59.11	59.7	59.4	59.116	0.6

Table 11: Percent drug content of gel formulations (average±SD)

Formulations	% Drug contents	(±) SD
B1	90.95	0.0019
B2	93.80	0.0031
B3	89.26	0.0022
B4	95.03	0.0024
B5	91.84	0.0036
B6	90.53	0.0038
B7	90.00	0.0038
B8	90.38	0.00113
B9	92.00	0.0029
B10	95.60	0.0038
MKP	99.30	0.00114

Table 12: Stickiness evaluation of gel formulations

Formulations	Presence or absence of stickiness after application
B1	Absence of stickiness
B2	Absence of stickiness
B3	Absence of stickiness
B4	Absence of stickiness
B5	Absence of stickiness
B6	Absence of stickiness
B7	Absence of stickiness
B8	Absence of stickiness
B9	Absence of stickiness
B10	Absence of stickiness
B11	Absence of stickiness
MKP	Absence of stickiness

Stickiness

Evaluation of stickiness was listed in Table 6. From this, it was clear 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.

Smell

The smell of the formulated gel formulations was evaluated by checked it through 4-5 volunteers, and then it was considered as alcoholic, acceptable, and non-acceptable.

Viscosity

The viscosity of all formulations was evaluated. The viscosity of B10 batch was less as compared to other formulations, ultimately shows more release of B10 Batch. Depending on the concentration of Carbopol 940 and proportion of solvent, the viscosity changes, which affect the release of formulation.

In the process of neutralization of Carbopol 940, neutralization means nothing but ionic repulsion of its charges. The polymer concentration increases repulsion of the chains, and thus increases rigidity of structure of the gel. Affinity of solvent toward the polymer also affects the structure of network of the gel. If solvent have higher affinity toward polymer, then polymer chains get extended, that is, increased. Entanglement and increased swelling of polymer thus increases viscosity of formulation. And if solvent have low affinity toward solvent, then polymer contracts reduces entanglement. Ethanol has higher affinity toward water than polymer Carbopol 940, that is, it has low affinity toward Carbopol 940, so the gel structure gets contracted, so viscosity was less. Hence, batch B10, B1, B4, and B8 had low viscosity as compared to marketed gel of isotretinoin, that is, Sotret gel.

***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 batch B10 showed more diffusion, that is, release from all the formulations, that is, 85.69% after the Sortet gel, because of decrease in viscosity. The marketed product showed 89.72% release and the batch B3 showed low release, that is, 62.43%, because of higher viscosity.

Table 13: Evaluation of smell of gel formulations

Formulations	Smell		
	Alcoholic	Acceptable	Non-acceptable
B1	++	+	-
B2	++	+	-
B3	-	+	-
B4	++	+	-
B5	++	+	-
B6	++	+	-
B7	++	+	-
B8	++	+	-
B9	++	+	-
B10	+	+	-
B11	+	+	-
MKP	-	+	-

Symbolised; ++ alcoholic, + Acceptable, -- Non-acceptable

Table 14: Viscosities of gel formulations

Formulations	Viscosity (CP.S)
MKP	10,300
B1	14000
B2	55,500
B3	74,500
B4	24230
B5	56200
B6	73300
B7	40000
B8	34400
B9	74100
B10	11502
BLANK, B11	29400

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.

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 B1 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 (B1-B10) as well as marketed preparation. While the zones of inhibitions for all the formulations are >10.4 mm. 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. acnes*, an anaerobic pathogen, is implicated in the development of inflammatory acne. The formulations having antibacterial agents inhibiting the *P. acnes*, may also reduce the development of inflammatory acne.

CONCLUSION

1. This study has analyzed the formulation of anti-acne gel of isotretinoin and it was found to have significant activity against *P. acne*.

Table 15: Cumulative percentage release of the anti-acne gel of isotretinoin and marketed Sortet gel

Time (hrs)	Cumulative percentage release of the anti-acne gel of isotretinoin and marketed Sortet gel										
	MKP	B10	B1	B4	B8	B7	B2	B5	B6	B9	B3
0.25	3.070018	2.359529	2.162902	2.09736	1.376392	1.179765	0.983137	0.78651	0.589882	0.52434	0.393255
0.5	6.44766	5.189647	4.036209	4.678562	2.437412	1.939398	1.506928	1.336627	1.297412	0.956628	0.996026
1	9.773961	8.752523	7.83566	7.297882	5.686522	5.22868	5.111033	4.521333	3.695608	2.345653	2.555425
1.5	12.94175	11.39634	10.28322	11.38437	7.925882	7.467634	7.218902	6.196915	5.017699	4.685673	4.061621
2	17.14423	15.25859	14.91927	14.40745	11.4617	10.75454	9.627608	9.025569	7.715817	6.662823	6.170222
2.5	22.26303	20.60089	19.84199	18.26878	16.56926	15.77059	14.97192	13.98988	12.89038	11.62805	10.92593
3	27.93085	25.7972	24.72384	22.56167	21.37341	19.36902	18.74102	17.57579	16.17508	15.01806	14.01475
3.5	33.81998	31.28055	29.7749	27.03702	25.87472	24.40865	23.60942	21.85561	21.03208	18.85289	17.77132
4	40.19276	38.19148	36.31927	31.45898	31.55488	30.33828	28.88473	28.41524	26.54304	24.73169	23.91265
4.5	45.60726	43.25232	41.49867	35.68358	36.81417	35.53221	33.56795	32.65255	30.83336	28.79961	27.40395
5	51.34894	48.98128	46.95276	43.22416	42.45273	40.38445	38.82724	38.47558	36.56507	34.29584	32.80886
5.5	57.77163	54.69645	53.08787	48.48162	48.7458	46.53391	44.53135	43.74685	41.29929	39.04369	37.01966
6	64.04925	60.67323	58.22586	54.64306	54.18573	51.97438	49.78881	48.79461	46.387	43.85652	41.93799
6.5	70.87681	67.25242	64.20264	61.45901	59.57281	57.40123	55.36036	54.37942	51.53971	49.10146	47.02607
7	77.92641	73.26659	71.10865	67.48735	65.2611	63.23404	60.81336	60.44863	56.99344	54.04429	51.28768
7.5	83.78408	79.97759	76.97855	73.25425	71.40855	69.15872	66.67286	65.19354	62.46005	59.72115	57.58131
8	89.72165	85.69221	82.97087	79.20544	77.26732	75.20105	72.37459	70.63346	67.46769	65.00421	62.43208

Table 16: 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
B1 (0.5%)	A	A	A	A	A	A	A
B2	A	A	A	A	A	A	A
B3	A	A	A	A	A	A	A
B4	A	A	A	A	A	A	A
B5	A	A	A	A	A	A	A
B6	A	A	A	A	A	A	A
B7	A	A	A	A	A	A	A
B8	A	A	A	A	A	A	A
B9	A	A	A	A	A	A	A
B10	A	A	A	A	A	A	A
MKP	A	A	A	A	A	A	A

Table 17: Zone of inhibition of gel formulation

Formulations	Zone of inhibition in mm			Mean±SD
	1	2	3	
B1	30.4	30.8	30.5	30.566±0.20
B2	20.6	21.2	21.6	21.13333±0.503322
B3	24.3	24.6	23.9	24.26667±0.351188
B4	22.1	22.6	21.8	22.16667±0.404145
B5	25.8	26.4	26.3	26.16667±0.321455
B6	21.4	21.6	21.8	21.6±0.2
B7	28.4	27.8	28.6	28.26667±0.416333
B8	30.5	30.8	30.2	30.5±0.3
B9	18.4	17.9	18.8	18.36667±0.450925
B10	24.2	24.6	23.9	24.23333±0.351188
Benzyl Peroxide gel (+ control)	40.2	40.2	40.5	40.3±0.173205
Marketed SOTRET gel	35.5	35.8	35.3	35.53333±0.251661
Distill water (– control)	-	-	-	-

2. The gel formulation was subjected to physicochemical analysis, spreadability, viscosity, drug content, *in vitro* diffusion study, skin irritation study, and antibacterial study.
3. Further studies are needed to identify exact effect of isotretinoin as anti-acne effect.
4. The study has laid the foundation for discovering an effective topical gel formulation of isotretinoin with possibly lower side effects to treat human nodulosystic acne vulgaris.

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