FABRICATION AND CHARACTERIZATION OF TOPICAL GEL FOR THE TREATMENT OFACNE WITH THE HELP OF SALICYLIC ACID AND NATURAL AGENT (*MOMORDICA CHARANTIA*)

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Abstract

Persistent can evulgarisis characterized by the buildup of dead skin cells and oil in the hair follicles. Acne acne scars ,and uneven skin to near patients with impaired immune systems who reside in warm, humid climates. Many different illnesses can be brought on by them. As a result, novel dosage formulations are being developed to limit disease-causing microorganisms. The Salicylic acid with natural agent (*Momordica charantia*)-loaded gel was characterized using FTIR. When compared to acetate buffer (pH 5.5) and phosphate buffer (pH 9.0), salicylic acid-loaded gels demonstrated high release. The high concentration allowed for high drug permeability across the Franz diffusion cell. As a gelling agent, carbopol-934 was included in the formulation. Acne treatment is more likely to reach the colony of pimples that has formed because of improved diffusion, the gel produced with carbopol-934 had a high zone of inhibition of10.1±0.1mm and 8.7±0.7. As a result, a salicylic acid-loaded gel with a designed formulation has the potential to cure acne vulgaris as well as other skin illnesses.

Keywords: Topical gel, Natural agent, Acne. Drug release study, Stability study

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INTRODUCTION

Acne vulgaris is a common kind of cutaneous inflammation. A staggering eighty percent of teenagers suffer with acne, and many people continue to struggle with it well into adulthood. Diet, medication, pollutants, climatic changes, and life-style factors; have all been associated to an increased prevalence of acne vulgaris. numerous situations change the natural skin barrier and microorganisms, leading to 'hyper-seborrhea, aberrant keratinization of the pilosebaceous duct, inflammation and decreased skin microbial range,and the see xterior disclosure variables are rarely sufficient to create acne. It is thought that seborrhea-acne-prone skin has a higher number of sebaceous lobules per gland than doe's normal skin.A variety of momordicosides, both bitter and sweet, were produced by unripe fruits. The amino acid-rich polypeptide was extracted from the plant's pulp, seeds, and tissue culture. The neurotransmitter 5-hydroxytryptamine and the sterols, cholesterol, lanosterol, and beta-sitosterol were all sourced from fruit. Bittering agents are cucurbitacin glycosides. Human and animal researches have shown that the fruit can lower blood sugar. These findings support claims that the fruit can reduce blood sugar levels. Researchers have warn that the crop extract can lead to erroneous findings in urine tests for the presence of sugar due to its tendency to keep the indicator dye in the glucose oxidase strips and the alkaline copper salts in a reduced condition.

MATERIALS:

The *Momordica charantia* fruits were collected from local market, Kanpur. Pick up some fruits, and then wash it with distill water and then dry them away from the light. Fruits should be stored in an area with plenty of airflow. After Drying of Fruit it is converted into powder. A sturdy jar should hold 250 gm of dried powder of *Momordica charantia* fruits. Salisylic acid, from Himedia Pvt ltd Mumbai, Carbopol from S.D. fine chemical, ethanol. And sodium laryl sulfate from Hi media ltd. Potassium Hydrogen Orthophosphate were purchased from S.D. Fine Chem.Ltd.,Mumbai (Mumbai, India). All other chemicals and reagents used were of analytical grade and were used without further purification.

1.1 Macroscopic characteristics of *Momordica charantia* powder

The macroscopic character was evaluated using compound microscope and color odor taste was evaluated physically.

a. **Determination of ash value:** 2 gram of air-dried crude sample were accurately weighed in a tared silica dish and incinerated at a temperature not exceeding 450 degrees Celsius until carbon-free, then weighed. The proportion of ash relative to the air-dried substance was determined.
b. **Determination of water - soluble ash:** The ash was boiled for five minutes with 25 milliliters of water; the insoluble matter was collected in a Gouch crucible, washed with hot water, and ignited for fifteen minutes at a temperature not exceeding 450^0 Celsius. To find out how much water-soluble ash there is, take the weight of the non-soluble material and subtract it from the weight of the ash. The ratio of water-soluble ash to air-dried material was calculated.
c. **Determination of acid soluble and acid - Insoluble ash:** The ash was boiled with 25 ml of 2M hydrochloric acid for 5 minutes, collected on ashless filter paper, washed with hot water, ignited, cooled in a desiccator, and weighed. It was determined what proportion of acid-soluble and acid-insoluble ash was present in the air-dried substance sample.
d. **Fourier transform infrared analysis:** FTIR spectroscopy was used for drug (*Momordica charantia*) identification and to investigate the chemical interactions between drugs and polymers. A little of the powder was squeezed into a thin, almost see-through pellet. To investigate potential interference, we recorded the infrared spectra of the samples between 500 to 3500 cm^{-1} and compared the results to a reference spectrum. [33]

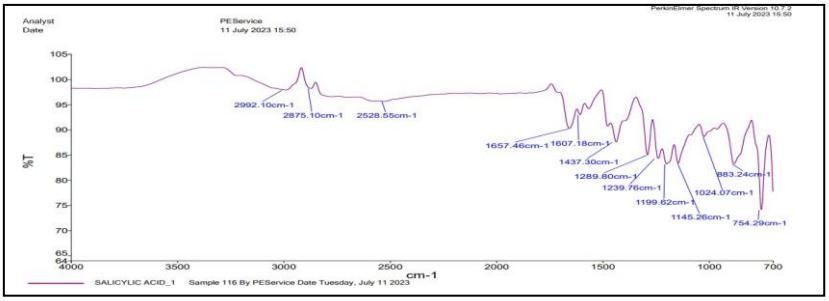

Fig.1 Fourier Transform Infrared spectra of salicylic acid

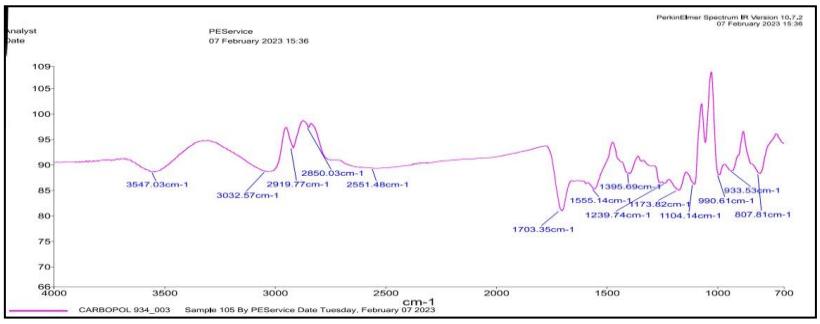

Fig 2 FTIR spectra of Carbopol-934

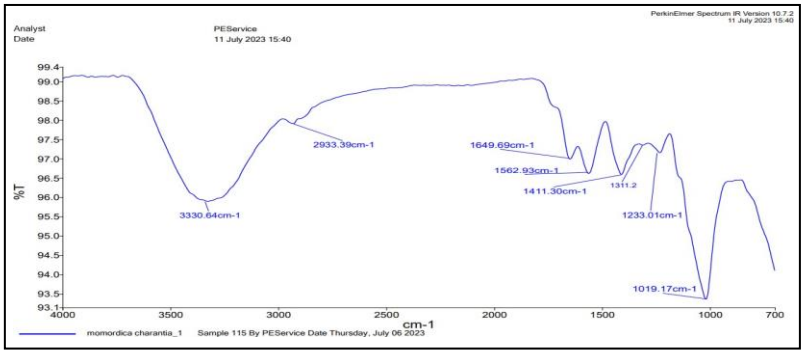

Fig. 3 FTIR spectra of *Momordica Charantia*

e. Scanning Electron Microscope imaging of gel: SEM Analysis

The morphology of control hydrogel samples (without drug loading) was carried out using Scanning Electron Microscope (Tungsten-Electron Microscope(W-SEM) Model/JSM-6010LA;JEOL. For carrying out SEM analysis, gels samples were swollen to equilibrium in the medium of study and then freeze dried. Freeze drying was carried out in vacuum at - 50°C for 24 h using lyophiliser. Prior to .

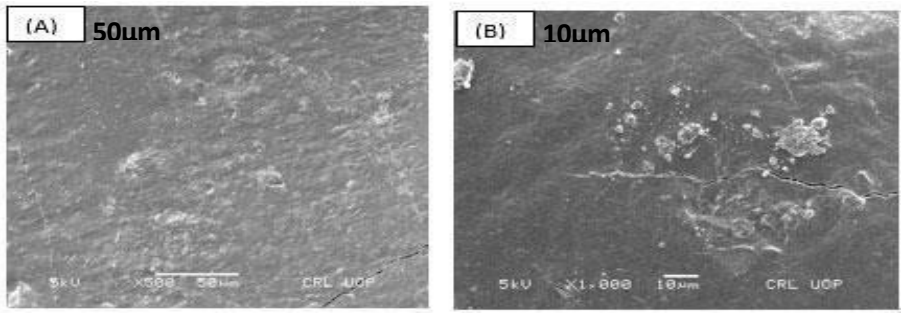

Figure 4 & 5 Scanning Electron Microscope imaging of gel

SEM examination, freeze dried samples were gold-sputter coated to render them electrically conductive

Extraction from *Momordica charantia* powder: *Momordica charantia* fruit powder weighed 254 gram and the sample was extracted using Soxhlet apparatus. It was first placed and extracted in a Soxhlet fora full 18 hours using ethanol as the solvent. A constant 60-70 degrees centigrade was kept in the room. To get at the extract, a rotary flash evaporator was used to evaporate the solvents. The formulation makes use of this extract.

Evaluation of the *Momordica charantia* extract

1.2 Characteristics of *Momordica charantia* extract: The ethanolic extract of the *Momordica charantia* was evaluated used for its physical state colour, odor, and taste**.**

a. Phytochemical testing of *Momordica charantia*- :Primary and secondary metabolites' in bitter melon water constituents were analyzed using phytochemical assays. Secondary metabolites included alkaloids, phenols, flavonoids, saponins, steroids, tannins, and glycosides, whereas primary metabolites included carbohydrates, lipids, and proteins.

Table 1 Phytochemical Screening Tests

S.No	Phyto constituent	Experiment	Inference
1	**Test for Carbohydrate**	**Molish's test:** Sample with 2 to 3 drops of 1% α-naphthol and 2 mL of conc. H_2SO_4 added	Formation of ring
		Fehling's test: Sample and equal vol. of Fehling's solution A and B and heated.	Brick red colour
		Benedicts test: Sample treated with Benedict's reagent	Red precipitate
2	**Test for Alkaloids**	**Dragendorff's test:** Sample with Dragendorff's reagent	Reddish brown Precipitate
		Mayer's test: Sample with Mayer's reagent	Cream colour Precipitate
		Wagner's test: Sample with Wagner's reagent	Reddish brown Precipitate
3	**Test for Terpinoides**	2ml CCl_3 + 5mL plant extract, + 3mL conc. H_2SO_4	Reddish Brown colour Appears
4	**Chemical tests for Steroids (Triterpinoides)**	**Salkowaski test:** Sample with $CHCl_3$& conc. H_2SO_4	Red/Yellow color appears
5	**Chemical test for Flavonoids**	**Shinoda test** Sample is treated with Magnesium ribbon and added concentrated HCL drop wise	Pink scarlet, or occasionally green to blue color
6	**Chemical test for Tannins**	**Ferric chloride test** FeCL3 solution added to the extract drop wise.	Greenish Black colour
7	**Chemical test for starch**	**Iodine test** To the plant extract added few KI solution drop wise	Sample turns blue black colour
8	**Chemical test for protein**	**Biuret test** To the test solution (2ml) add Biuret reagent	Blue colour apperares
9	**Chemical test for Saponins**	**Foam Test:** To the plant fractions, 10-20 ml of water was added and shaking was done for few minutes.	Froth Appeared which persisted for 60-120 seconds
10	**Chemical test for Glycosides**	**Baljet test:**To the plant extract added sodium picrate solution drop wise	Yellow to orange color appeared
11	**Chemical test for Saponins**	**Stain test** Small amount of extract is compressed between two filter papers	Oil stain on I^{st} filter paper
		Saponification test: Add a few droplets of 0.5N alcoholic potassium hydroxide and a drop of Phenolphthalein to small quantities of different extracts, then heat in a water bath for 1 to 2 hours.	Soap production or partial neutralisation of alkali

a. Solubility: The solubility of '*Momordica charantia*' and Salicylic acid was determined in acetone, ethanol, methanol, ethyl acetate, and distilled water.

b. Determination of melting point-

The melting point of Salicylic acid and *Momordica charantia* was determined using melting point apparatus by optical analysis method. The melting point was recorded and compared with the literature value.

c. Spectrophotometric study: A stock solution of salicylic acid (100µg/ml) was prepared in co-solvent (ethanol+ water). The prepared solutions were scanned in a range of 200-400 nm using a UV spectrophotometer in basis spectrum mode. λ max was recorded and compared with the literature value.

d. Preparation of phosphate buffer (pH 9.0): Accurately weigh potassium dihydrogen phosphate 17.4g and dissolve in 800ml of distilled water in a volumetric flask. After then adjust the solution to the final desired pH using potassium hydrogen and 800 ml solution in sufficient distilled water to produce 1000ml phosphate buffer.

f. Preparation of standard plot in phosphate buffer (pH 9.0)

10mg drug in a 100ml pre-dried volumetric flask, salicylic acid was carefully weighed. Add a tiny amount of phosphate buffer (pH 9.0) and agitate

until the medication dissolves, then add 100 ml. The standard stock solution was serially diluted with phosphate buffer (pH 9.0) to get 2, 4, 6, 8, and 10µg/ml salicylic acid solution. At 297.5nm, phosphate buffer (pH 9.0) was used to measure solution absorbance. The calibration curve was plotted against concentration (µg/ml) using absorbance readings.

Table 2 Formulation table of Gel

Sr. No.	Name of Ingredients	Function	F1	F2	F3
1.	Salicylic acid	Active drug	2gm	2gm	2gm
2.	*Momordica charantia*	Natural agent	2ml	2ml	2ml
3.	Carbapol-934	Gelling agent	1gm	1.25gm	1.50gm
4.	Methyl paraben	Preservative	0.06gm	0.06gm	0.06gm
5.	Propyl paraben	Preservative	0.03gm	0.03gm	0.03gm
6.	Propylene glycol	Humectant	2.5ml	2.5ml	2.5ml
7.	Sodium lauryl sulfate	Foaming agent	1.5gm	1gm	0.5gm
8.	Mint oil	Perfuming agent	0.5ml	0.5m	0.5m
9.	Distilled water	Qs to 100	Qs to 100	Qs to 100	Qs to 100

1.3 EVALUATION OF GEL

a. Determination of Colour: Color of the formulation was tested against a white background and found light Green.

b. Determination of odour: The odor of the gel was manually assessed and found mint due to presence of excipient

c. Determination of Consistency: The consistency of the gel was tested by putting it on the skin.

d. Determination of pH: By all outward appearances, the designed face wash gel is a success After 2 minutes after placing the electrode tips into the gel, the pH of the gel was monitored using a pH meter.

f. Determination of viscosity: The viscosity of prepared gels was measured using a Brookfield viscometer with spindle No. 3 and 10 revolutions per minute. The operation was performed at 28^0 Celsius**.**

g. Determination of spreadability: Gel formulation spreadability was measured through using horizontal plate method. A standard weight of 125 gram being set on top of one of the glass plates after placing 1gm of gel in the space between the two horizontal plates. The time it took for the gel on one plate to separate from the gel on another plate was recorded. The formula used to determine the spreadability is as follows:

S =ML/T

Value "**S**" is spreadability, m is the upper slides, "**L**" is the length of a glass slide, and "**T**" is the time taken.

h. **Determination of Extrudability**

After the gel had hardened in the container, the formulation was transferred into the collapsible tube. Weights in grams needed to stretch a 0.5 cm ribbon of gel in 10 seconds were used to assess the extrudability of the formulation.

i. Determination of Homogeneity-

They were visually evaluated for homogeneity after being let to settle in a container. They are assessed based on their appearance and whether or not they have aggregated.

j. **Determination of Grittiness-**Applying the product to the skin allowed us to inspect for any gritty particles

k. **Determination of Foambility:** In a water-filled beaker, a small amount of gel was put. After measuring the starting volume, the beaker was shaken 10 times to obtain the final volume. **Determination of Washability:** The effortlessness and extent of washing with water were individually examined after applying the product to the skin.

1.4STABILITY STUDIES

Stability of the gel formulation were studied at different storage condition (8^0Cand 40^0C) Samples were withdrawn at 7, 15 and 30 days and checked for their physicalcharacteristics like appearance, homogeneity, pH, viscosity ,spreadability, washability and Drug content at various temperature in different intervals of time.

Result and disscusion:

Drug content studies: It was done with two kind of buffer systems.

Determination of Acetate Buffer (pH 5.5)
For the gel formulation, 1g was placed into a 100ml volumetric flask with 100 ml of acetate buffer (pH 5.5), agitated for 30 minutes, and then left to stand for 24 hours. One ml of the aforesaid solution was diluted to fifty ml with acetate buffer pH 5.5 to bring the total volume to one hundred ml. The final product was filtered via a 0.45 m membrane filter. A UV spectrophotometer was used to determine the absorbance of the solution at 297.5 nm.

Determination of Phosphate Buffer (pH 9.0): In the case of the gel formulation, 1g was added to a 100 ml volumetric vessel containing 100 ml of phosphate buffer pH 9.0, stirred for 30 minutes, and then allowed to rest for 24 hours. With the addition of phosphate buffer pH 9.0 to bring the volume to 100 ml, 1 ml of the aforementioned solution was diluted to 50 ml. A 0.45-m membrane filter was used to purify the final product. A UV spectrophotometer was used to measure the absorbance of the solution at 297.5 nm.

***In- vitro* Drug release by franz diffusion cell method:** A cellophane membrane containing 1 gramme of gel was clamped shut and placed into a water bath maintained at 37±0.5°C between the donor and receptor compartments. The receptor cell had a capacity of 50 ml and a permeable surface area of 6.9062cm^2. A 5.5 pH acetate buffer is present in the receptor compartment. By swirling the fluid at 600 rpm with a star head magnet and allowing for diffusion for 10 min, we were able to keep the hydrodynamics of the receptor fluid stable. At 2, 4, 6, 8and 10minute intervals, 5 ml aliquots were taken and replaced with new 5 ml acetate buffer pH 5.5. An identical volume of medium was injected to keep the sink at its original level. Drug release profiles were determined by UV-Visible spectrophotometer analysis of the samples taken at 297.5 nm. Assembly of Drug Release Systems in vitro [Figure3.3] **Klose D, et al. [34]**

Fig. 6 In-vitro drug release using Franz diffusion cell

Microscopic characteristics: Results of microscopic characteristic quantitative analysis and photochemical analysis of *Momordica charantia*

Table 4 Microscopic characteristics of *Momordica charantia* Powder

Characteristics	**Result**
Color of Powder	Light Green
Size of Particle	0.5-1mm

Quantitative analysis
Total crude plant material taken-3kg
After loss on drying Crude Powder obtained = 254gm After Extraction by Soxhlet Apparatus
Plant extract was obtained = 9.8gm

$$\text{Percentage Yield} = \frac{Weight\ of\ plant\ extract}{Weight\ of\ dried\ raw\ materi(powder)} \text{x}100$$

Table 5 Quantitative analysis of *Momordica charantia*

Parameter	Result (in Percentage w/w)
Percentage Yield	3.8
Total Ash Value	13.6
Water soluble ash	2.35
Water insoluble ash	5.2
Acid soluble ash	1.22
Acid insoluble ash	2.4

PHYTOCHEMICAL ANALYSIS: The outcomes of the phytochemical tests have been demonstrated in table no.3.4. The phytochemical studies were showed the presence of carbohydrate, alkaloids, terpinoids, steroids, flavonoids, tannins, saponins and glycosides and the absence of starch and protein.

Table 6 Outcomes of the phytochemical tests

S. No.	Phyto constituent	Experiment	Results
1	Test for Carbohydrate	Molish's test	++
		Fehling's test	++
		Benedicts test	++
2	Test for Alkaloids	Dragendroff's test	++
		Mayer's test	++
		Wagner's test	++
3	Test for Terpinoides	2ml chloroform + 5mL plant extract, + 3mL conc. H2SO4	++
4	Chemical tests for Steroids (Triterpinoides)	Salkowaski test	++
5	Chemical tests for Flavonoids	Shinoda test	++
6	Chemical test for Tannins	Ferric chloride test	++
7	Chemical test for starch	Iodine test	--
8	Chemical test for protein	Biuret test	--
9	Chemical test for Saponins	Foam Test	++
10	Chemical test for Glycosides	Baljet test	++
		Keller killiani test	++
11	Chemical test for Saponins	Stain test	++
		Saponification test	++

++ =Present, -- =Absent

STABILITY STUDY:
Stability study of different formulations were carried out at storage condition of 8^0C and 40^0C for a period of one month. Samples were withdrawn at the time interval of 7,15 and 30 days and the results are tabulated in Table No.3.22. During the study period, all the formulations [kept at 8^0C & 40^0C] were found to be homogenous and free from microbial growth which may attributed to the presence of preservatives. There is a slight change of color in F1 formulation and formulation F3 shows bad smell when stored at 40^0C at 30^{th} day and pH of the gel was also changed in both F1 and F3 formulation.

Drug Content Study after 45 Days of F3 (at different temperature)

Table 8 Percentage drug retained in gel formulation(F3) at various temperature conditions:

Temperature	4^0 C		24^0 C		40^0 C	
Days ↓	% Drug retained	%Drug lost	% Drug retained	%Drug lost	% Drug retained	%Drug lost
0	100	-	100	-	100	-
15	97.81±0.12	2.19	97.27±067	2.73	73.72±0.34	26.28
30	95.07±0.78	4.93	89.94±0.96	10.06	71.56±0.45	28.44
45	93.32±1.58	6.68	81.27±1.54	18.73	59.02±1.67	40.98

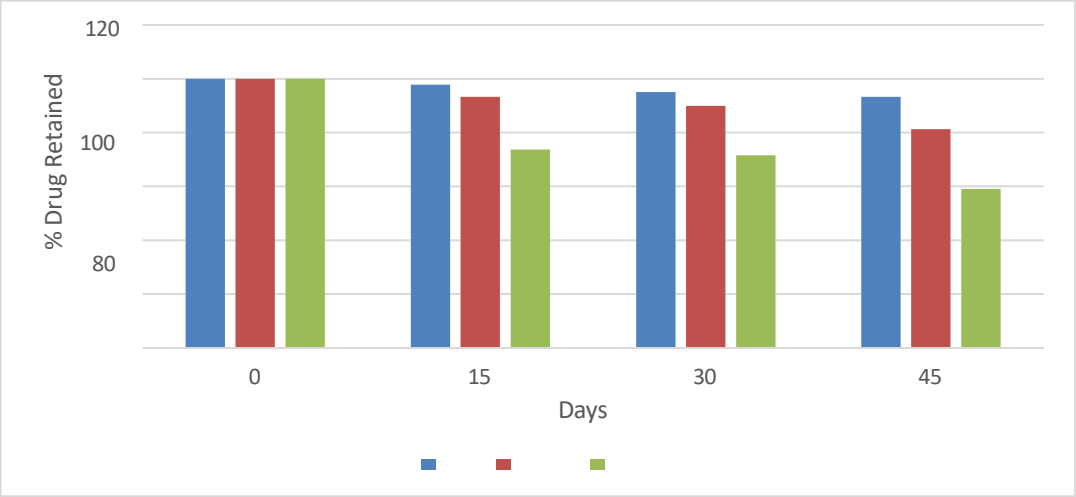

Fig. 8 Graph showing percentage drug retain in gel formulation

***In-vitro* diffusion studies by franz diffusion cell method-:** According to the results of a study on the release of salicylic acid from a gel containing the drug, the drug was released was affected by the polymer's chemical make-up. Extremely thick and sticky solution that inhibited drug diffusion via H-bond formation. The highest release of drugs from of the gel was measured at 2–180 minutes. Permeability testing on drug-loaded gels revealed favorable results. Drug release is improved in the salicylic acid-containing gel.

Table 9 In-vitro drug release studies of gel-

Time (min)	%drug release in acetate buffer (pH 5.5)	%drug release in phosphate buffer (pH 9.0)
0	0	0
2	6.3%	6.8%
4	6.9%	7.2%
6	7.8%	8.4%
15	8.8%	9.3%
30	9.9%	10.7%
60	15.6%	18.2%
120	40.6%	41.8%
180	77.9%	71.2%

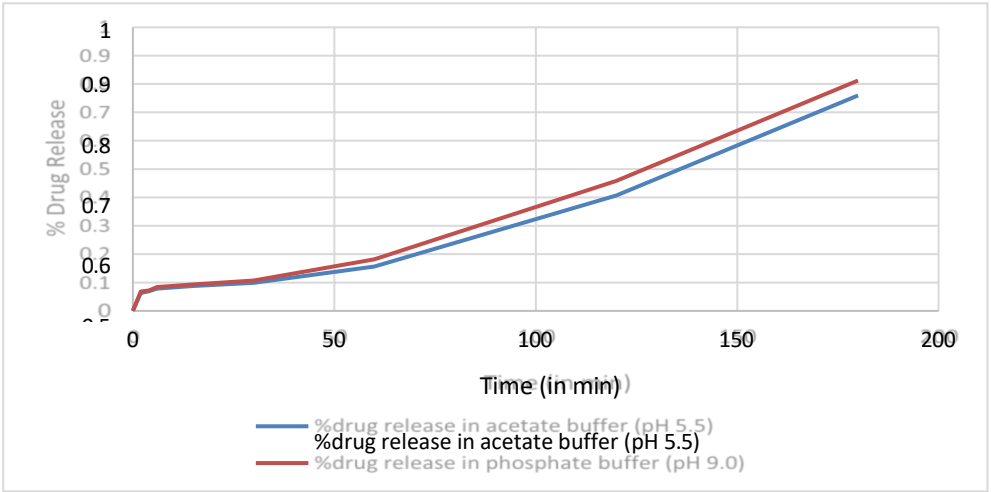

Fig. 9 Comparative diffusion profile of anti acne gel % drug release in acetate buffer (pH 5.5) to % drug release in phosphate buffer (pH 9.0) using Franz diffusion cell method

COMPARISON MARKETED GEL AND F3 FORMULATION

Table 10 Comparison of in vitro release of marketed gel and formulations (F1,F2,F3) after 180 minutes

***In-vitro* release of drug after 3 hours**	
Formulations	**Percentage Drug Release(in percentage)**
Marketed Formulation	97.62 ± 1.56 %
F1 Formulation	82.27 ± 2.11 %
F2 Formulation	87.36 ± 1.20 %
F3 Formulation	90.65 ± 1.32%

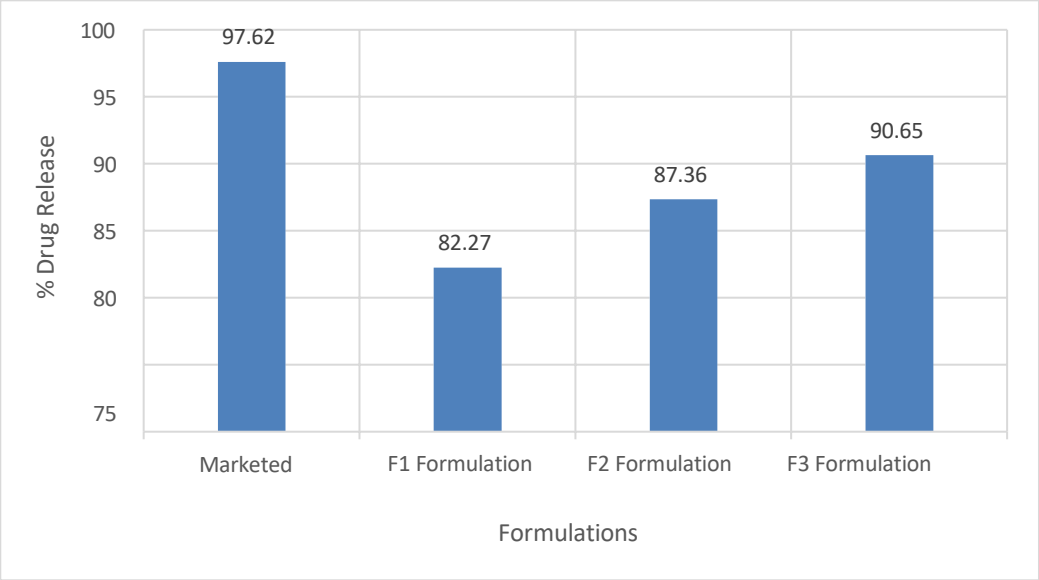

Fig.10 Comparison of different formulation and their % Drug Release

DRUG RELEASE KINETIC MODEL:

In order to describe the kinetics of the release process of drug in all formulations, equations such as zero-order and first- order rate equations were used. Zero order rate equation describes the system where the release rate is independent of the concentrations of the dissolved species. While the first- order equation describes the release from systems where dissolution rate is dependent on the concentration of the dissolving species. It is evident from Table 19, and 19B that the drug release process is not zero order in nature. This indicates that the dissolution rate of the drug is not independent of the amount of drug available for dissolution and diffusion from the matrix. The drug release data of all formulations when fitted in accordance with the first order equation it is evident that a linear relationship was obtained with 'r' (correlation coefficient) value close to unity and higher than 'r' obtained from zero order equation for all formulation (table), showing that the release is an apparent first order process. This indicates that the amount of drug released is dependent on the matrix.

Table: 11 *In-vitro* kinetic release studies of all formulations

FORMULATION	**ZERO ORDER**		**FIRST ORDER**		**HIGHUCHI MODEL**	
	R^2	**K0(h^{-1})**	**R^2**	**K1**	**R^2**	**KH**
CODE						
F1	0.914	1.744	0.9702	-0.0216	0.806	11.64
F2	0.925	1.761	0.9752	-0.0232	0.813	13.068
F3	0.913	1.577	0.9770	-0.0233	0.877	12.42

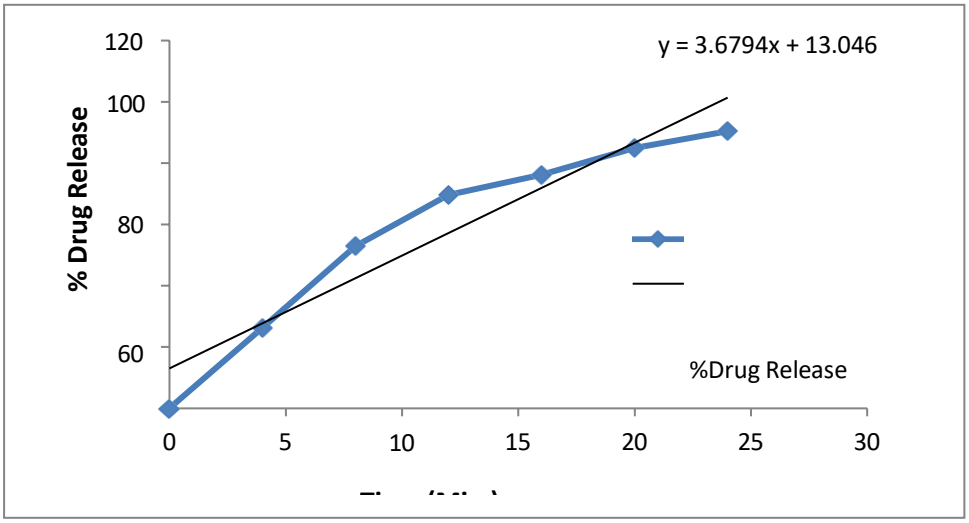

Fig. 11 Zero Order Model

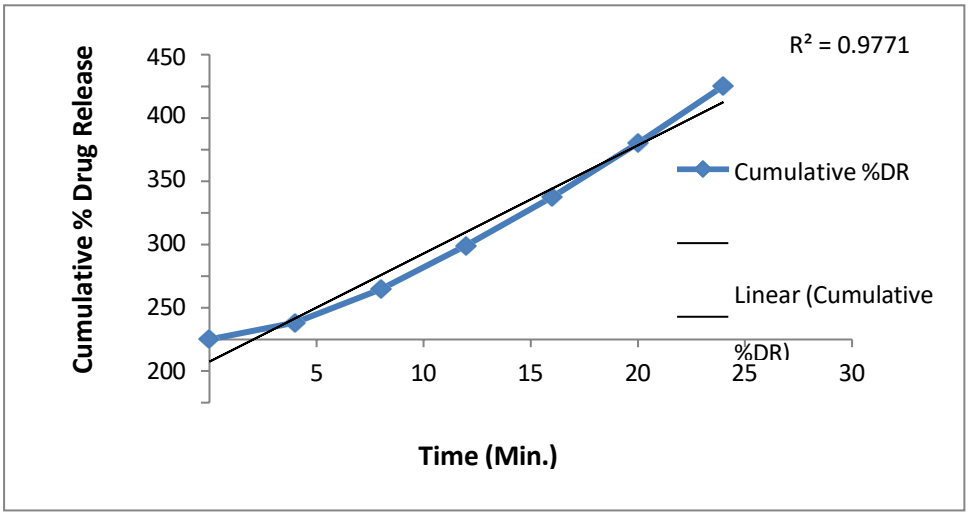

Fig. 12 First order model

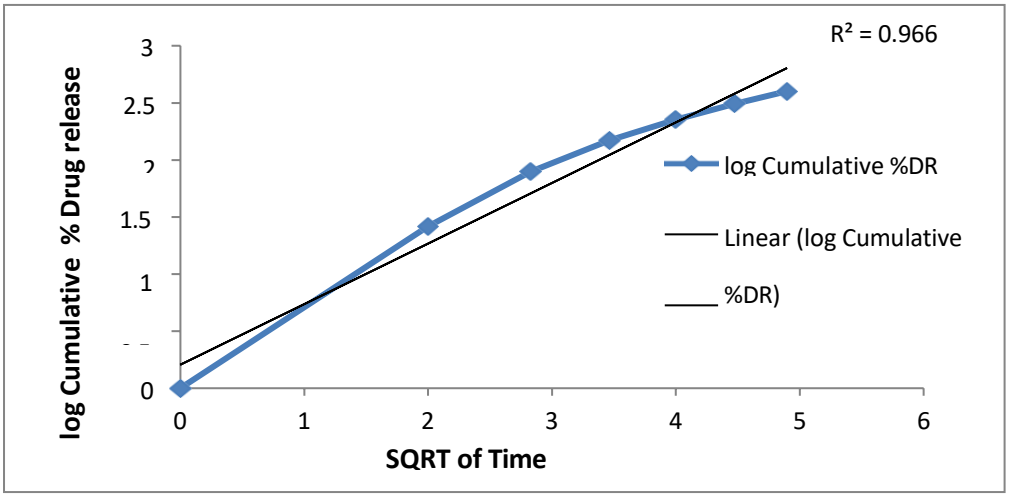

Figure 3.12 Higuchi model

Conclusion:

The reason of this experiment was to develop and test a gel for acne therapy. The manufactured gels are tested for their in-vitro drug release and as well as their FTIR, Zeta potential, drug content, and other physical properties.Bacterial infections have emerged as serious issues for public health in the past two decades. When it comes to opportunistic infections, bacteria are by far the most common culprits among immune-compromised people.

That's why it's so important to find new medicines to combat the microbes that cause illness. Antibiotic chemicals and active principles utilized in medicinal medications are abundant in higher plants, which play a vital role in this context.

In conclusion, salicylic acid with natural agent (*Momordica charantia*) loaded gel was adequately created to control the action of the determined to have promising prospect for prolonged drug release, higher throughput of drug in the skin, suggesting the greater prospects of the delivery system, and thus can be advantageous when used in the treatment of different superficial infectious diseases. Additional advantages include enhanced patient adherence due to decreased application frequency, decreased discomfort, decreased administration frequency, and elimination of systemic adverse effects.

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