PREPARATION AND EVALUATION OF ISOLEUCINE MOLECULE BASED GEL FOR WOUND HEALING ACTIVITY

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Abstract

The main purpose of this study was to develop a topical delivery of Isoleucine to increase the therapeutic activity, to improve patient compliance and to avoid the side effects**.** Isoleucine is an aminoacid derivative resulting from reaction of Isoleucine at amino group to control blood sugar Topical gel formulations of Isoleucine were prepared by using carbopol 940 as a gelling agent with different concentrations. Five different formulations were prepared and evaluated with respect to color, spreadability, viscositymeasurement, determination of pH, drug content, Invitro drug release studies, and stability studies. Compatibility study was carried out by FT-IR spectral analysis. FT-IR study revealed that no interaction between drug and polymer. All the prepared formulations show acceptable physical properties. From the above observation it was concluded that this formulation could be a very promising topical substitute for treatment to control blood sugar

Keywords: Isoleucine, Carbopol 940, HPMC,

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Introduction

Topical drug administration is one of the localized drug delivery system given through ophthalmic, vaginal, rectal and skin as topical routes. The main route for topical drug delivery system is through skin and that is the most readily accessible organs on the human body. When it comes to treat local and systemic infections skin is considered as the main route. Topical drug delivery system bypasses the first pass metabolism. Topical gel formulations provide an acceptable delivery system as they are less greasy and easy to remove from the skin[1].

Nowadays, the dermatological problems majorly caused due to fungal infection. The medical practitioner has recommended treatment through following dosage formulations such as solid, liquid and semi-solid. Clear transparent gels are widely used topical formulations in both cosmetics and pharmaceuticals[2].

The drug is applied to the mucous membrane or skin that will enhance the skin basic function and alters its action. Those products are called as topical or dermatological products[3].

We must know the skin anatomy, physiology,and physicochemical properties and it is used for the percutaneous absorptio. Skin is made up of three layers: Dermis, Epidemiand Hypodermis (subcutaneous layer). The thickness of a epidermis is 0.1-1.5 mm and it has five subdivision: stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum. Epidermis secretes melanin from melanocytes.The squamous cell layer is the thickest layer which moves certain substances in and out of the body[4].

Materials and Methods

Materials

Isoleucine was acquired from Sigma Aldrich, Chennai, India. Carbopol was obtained from Sigma Aldrich, Chennai, India. All Other Chemicals used were of the standard Analytical grade.

EXPERIMENTAL WORK

Solubility Analysis

Preformulation solubility analysis has been done to select a suitable solvent system to dissolve the drug and to test the solubility in the dissolution medium to be used[5].

Determination of Melting Point

Melting point determination of the drug sample has been done by open capillary method. The drug which has been taken in the glass capillary whose one end was sealed by flame.

Compatibility Studies by IR-Spectroscopy

The drug, polymer and excipient interactions are studied by using the FTIR method. Generally, drug and excipients must be compatible with each other which producess a stable, safe efficacious product. IR spectral analysis of pure drug and polymers were carried out. The pure drug which produces peaks and patterns were compared with the peaks and patterns with the combination of polymer and drug[6].

Differential Scanning Calorimetry (DSC)

Differential scanning Calorimetry (DSC) was conducted on DSC 131 SETARAM. The samples were equilibrated at 20 ^{o}C for 30 min. Indium standard was used to calibrate the DSC temperature and enthalpy scale[7]. The powder samples were hermetically sealed in aluminium pans and heated at a constant rate of 3 ^{o}C/min, over a temperature range of 20– 170 ^{o}C. Inert atmosphere was maintained by purging nitrogen at the flow rate of 15.8 ml/min, linear velocity35 cm/sec and pressure 24.7 kPa

PREPARATION OF GEL

Topical gel of isoleucine where prepared by using Carbopol 934 and HPMC as gelling agent,propylene glycol and water as dispersed phase. Gels where formed by using two different concentration of various gelling agent carbopol-934 and HPMC by keeping the concentration of Isoleucine and other ingredient constant. The solvent system is made by dissolving water and propylene glycol 80:20 ratio. The required quantity of carbopol-934 is taken and mixed into 100ml of solvent system and stirred it for 3 hours to get a uniform solution. 100mg of Isoleucine was weighed and dissolved in to above Carbopol-934 solution and stirred for 1 hours for uniform distribution[8].

TABLE 1: Formulation of gel (F1 – F5)

INGREDIENTS	F1	F2	F3	F4	F5
Isoleucine (mg)	100	100	100	100	100
Carbopol-934(gm)	1	2	-	-	1
HPMC(gm)	-	-	1	2	1
Propylene glycol(ml)	10	10	10	10	10
Water(ml)	90	90	90	90	90

EVALUATION OF GEL

Measurement of PH

The digital pH meter is used to find out the pH value of a formulated topical gel. The values of developed formulations are between the range of 4-8, which ignores the chance of skin irritation.

Drug content

1 g of gel mixed with 100 ml of suitable solvent stoke solution has been filled. The prepare daliqouers of different concentration by using suitable dilution and absorbance is measured. Linear regression analysis of calibration curve is used to calculated the drug content.

Viscosity study

Brookfield viscometer is used for its study rotate the gels at 0.3, 0.6 and 1.5 Rpm. Corresponding dial reading are been noted at each speed. Viscosity was obtained by dial reading X factor given in the brook field viscometer catalogues[9].

Spreadability

For the evaluation of spread ability, two glass slides were taken and the prepared gel was pressed between the two glass slides to uniform consistency by applying weight and leave for 6 minutes. The value of spreadability is obtained by calculating the time taken for the two glass slides to detach[10].

Extrud ability studies

Before setting inside the container formulations are filled in the collapsible tubes. This is determined in terms of weight in gm required to extrude a 0.5 cm ribbon at gel in 10 second.

Homogeneity

Visual inspection is done to test homogeneity keeping gel in a container and tested for appearance and presence of any aggregated[11]

Grittiness

Light microscope is used formulations were evaluated microscopically to check presence at any visible particulate matter

In-Vitro diffusion studies

It is done by using Franz diffusion cell, for studying dissolution release of gel through a cellophane membrane. 0.5 of gel sample taken in cellophane membrane. Diffusion studies carried out at 37±1o c using 250ml PH buffer (PH7.4) as the dissolution medium[12]

RESULTS AND DISCUSSION

Preformulation study

All the organoleptic characters of Isoleucine were studied and it was found that all the characters comply with USP standards.

Table:2 Organoleptic properties of isoleucine

PROPERTIES	RESULT
Description	Powder
Taste	Bitter
Odour	Strong &unpleasant
Colour	White

Solubility studies

Table 3: Solubility of Isoleucine

S.NO	SOLVENT	DESCRIPTION
1.	Water	Soluble
2.	NH4OH	Soluble
3.	Hcl	Soluble
4.	Hot alcohol	Soluble
5.	Ether	Insoluble

Compatability studies

The FTIR spectroscopic studies were carried out for standard Isoleucine, carbopol 934, HPMC and physical mixture by KBr pellet technique using FTIR spectrophotometer. The FTIR spectrum of standard is compared with that of mixture and found that there is no interference. The results are shown in Fig no: 1 – 3.

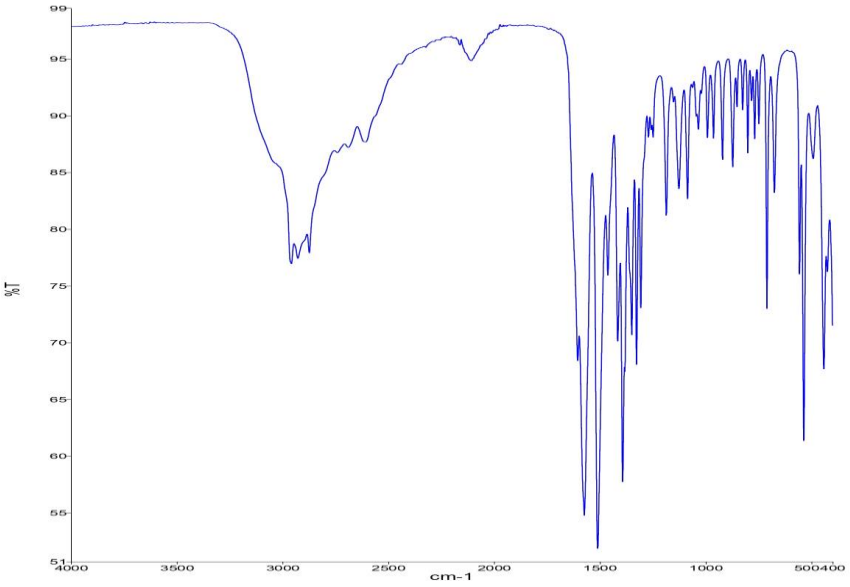

Fig no: 1FTIR spectrum for Isoleucine

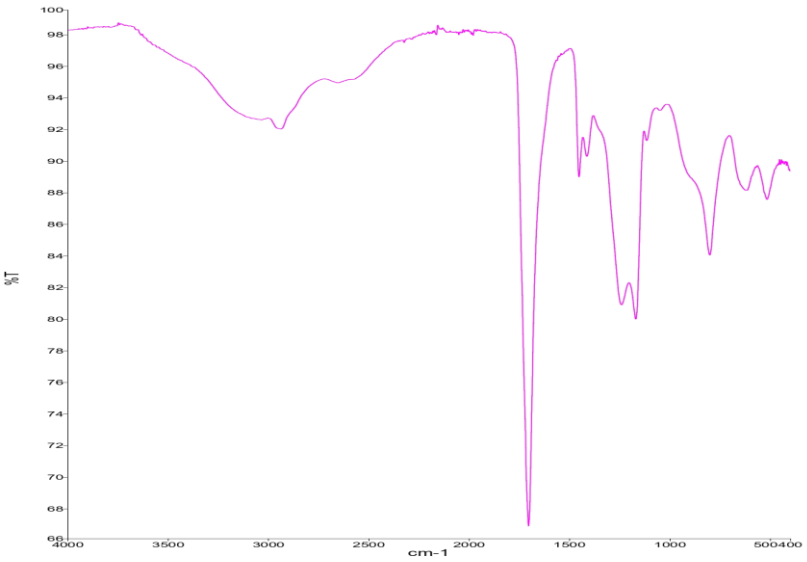

Fig no: 2 FTIR spectrum for carbopol 934

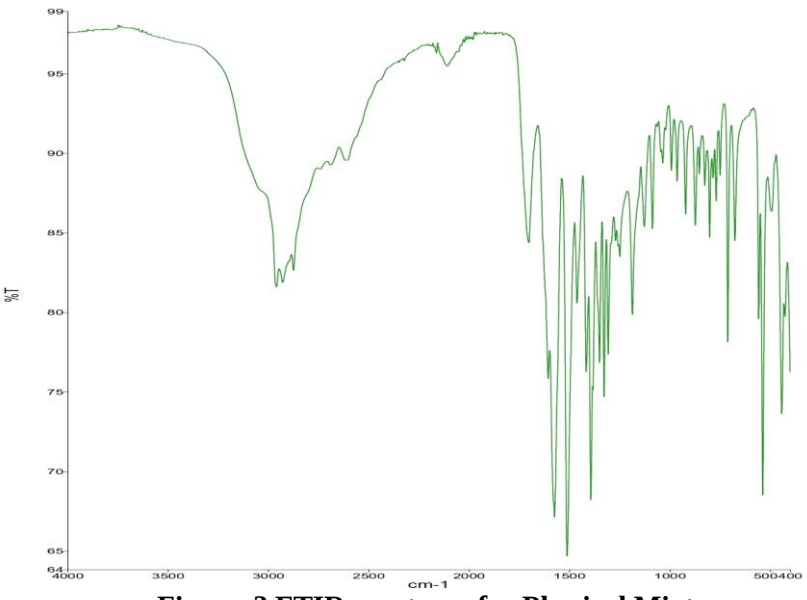

Fig no: 3 FTIR spectrum for Physical Mixture

Differential Scanning Calorimetry (DSC):

The DSC studies were carried out for Isoleucine carbopol934, HPMC and mixtures of Isoleucine-carbopol 934-HPMC by Differential Scanning Calorimeter. It was found that there was no interaction between drug and polymer. The results are shown in Fig no: 4 – 6.

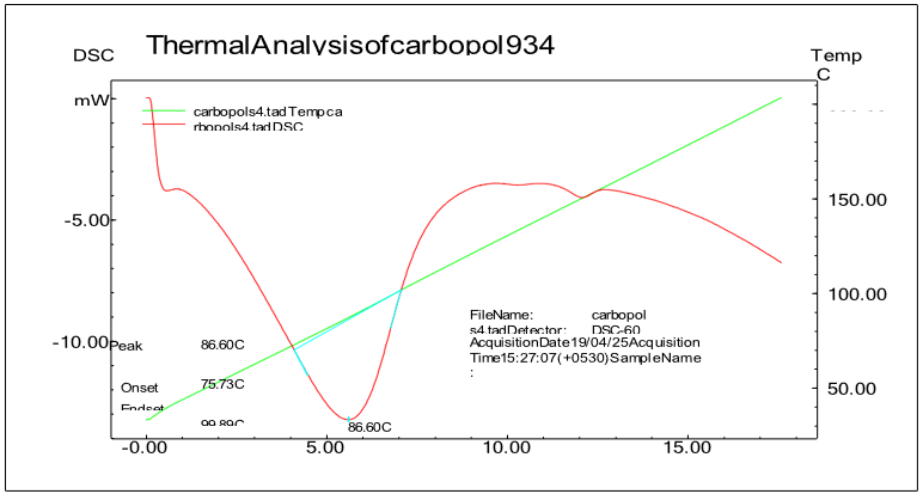

Fig 4: **DSC of carbopol934**

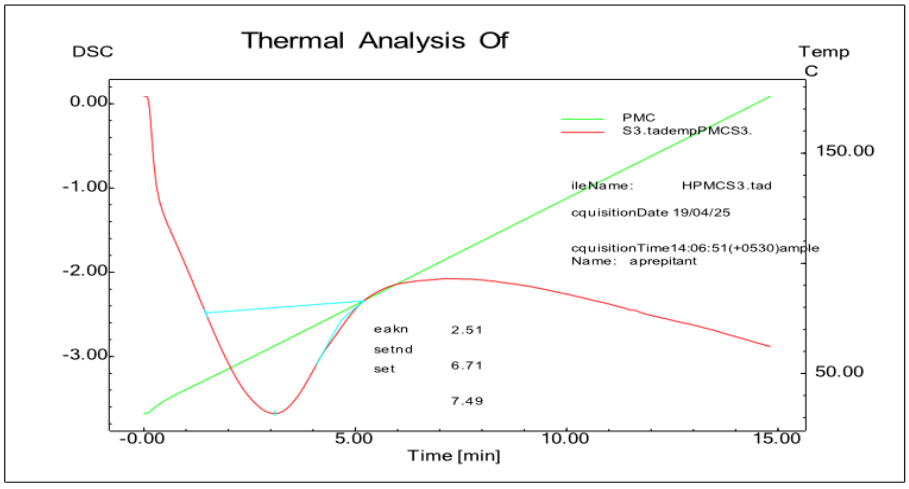

Fig 5: DSC of HPMC

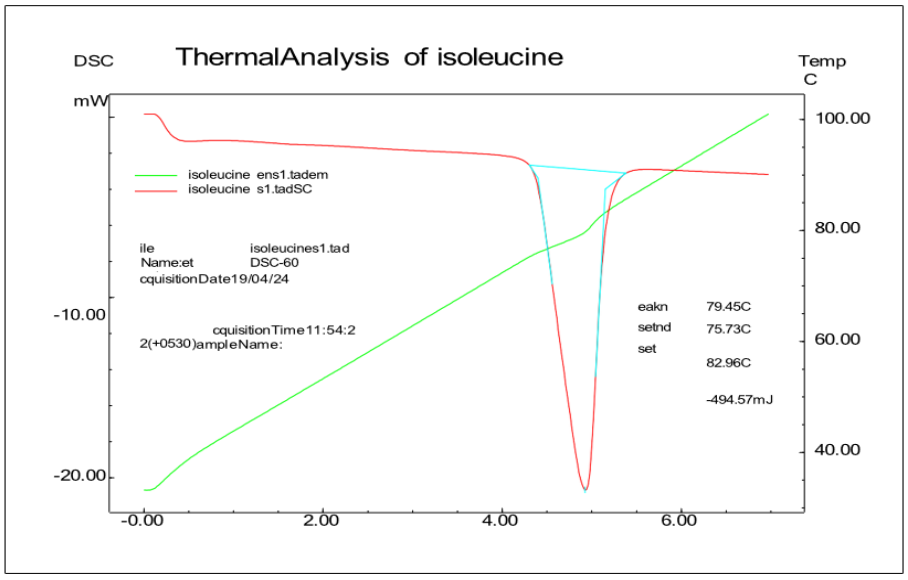

Fig6: **DSC of Isoleucine**

Evaluation of topical gel containing Isoleucine

Topical gels where prepared by 100mg Isoleucine, carbopol 934, HPMC, propylene glycol and water according to the Table 1

Measurement of pH

The pH values of all developed gel was in range of 6.1-7.2.Which is acceptable for applying to skin and avoids the risk of irritation. The results are presented in table 4

Drug Content

The drug content of the formulated gel was estimated. The results are presented in Table 4 . The drug content showed that the drug was distributed uniformly throughout the gel.

Percentage Yield and Viscosity

Percentage yield of topical gel containing Isoleucine was in range of 93.10 – 99.36 % The values are represented in the table 5. It was found that the percentage yield of F5 formulation showed the greater % yield than the other formulations. Generally consistency of formulation depends on the ratio of solid fraction to liquid fraction which produces gel structure.

Spreadability

It is considered as a important factor that shows the gel character which comes out from the tube. The spread ability values are shown in Table4. Spread ability test is carried for all the formulations. Spread ability of the gel formulation decreases with respect to increase in the polymer concentration.

Homogenecity

Visual inspection is done to check the syneresis and colour of the prepared formulation. The preparations must be clear and transparent. At last the formulated gel shows good homogeneity without any lumps and syneresis

Table: 4 pH, Spreadability and Drug content of Formulations (F1-F5)

Formulation code	pH	Spreadability(gm.cm2)	Drug content %
F1	6.3	11.04	95.55
F2	6.2	11.09	96.32
F3	6.8	11.98	97.82
F4	7.2	10.45	93.02
F5	6.1	10.33	98.17

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Fig 7: pH of Various Formulations (F1 – F5)

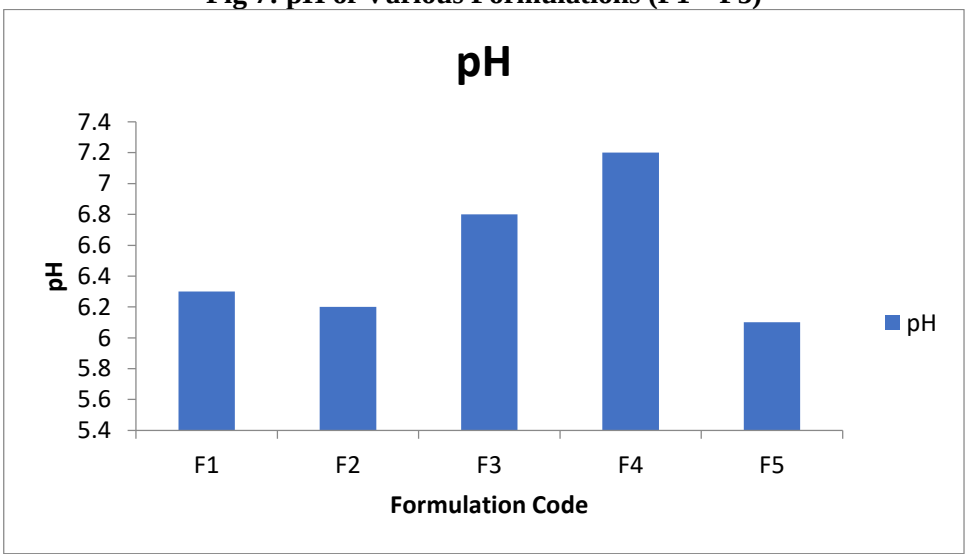


Fig 8: Spread ability of Various Formulations (F1 – F5)

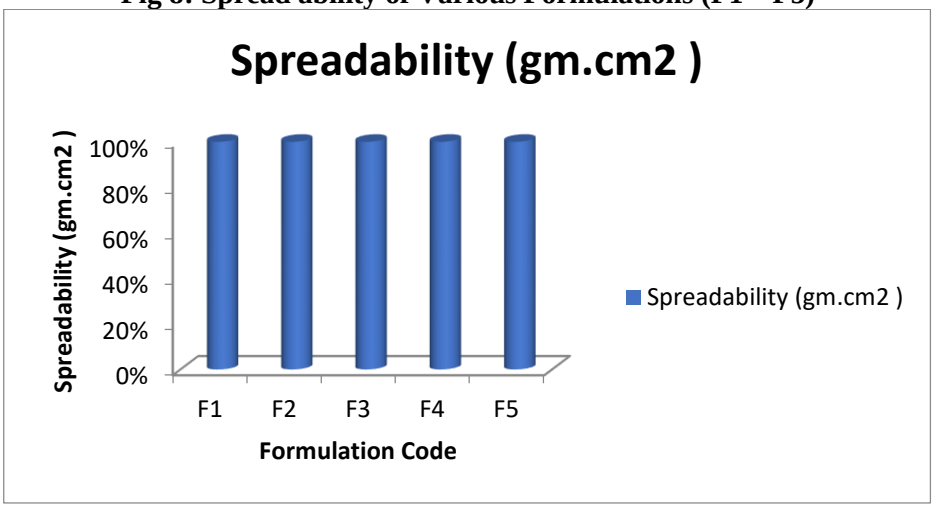


Fig 9: Drug content of Various Formulations (F1 – F5)

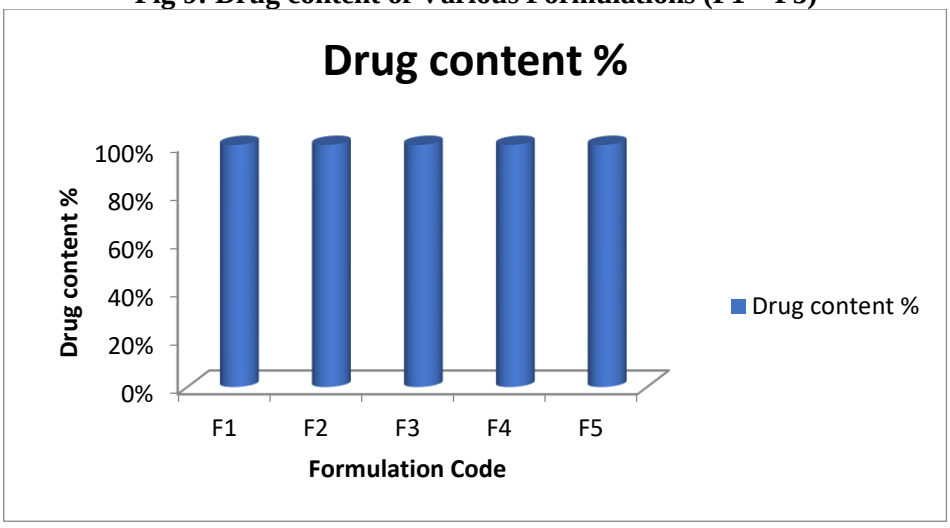


Table: 5 Percentage Yield and Viscosity of different formulations (F1-F5)

Formulation code	Viscosity(centipoises)	Percentage yiels %
F1	4652	96.02
F2	4592	96.89
F3	3684	97.11
F4	2431	93.10
F5	3321	99.36

Fig 10: Viscosity of Various Formulations (F1 – F5)

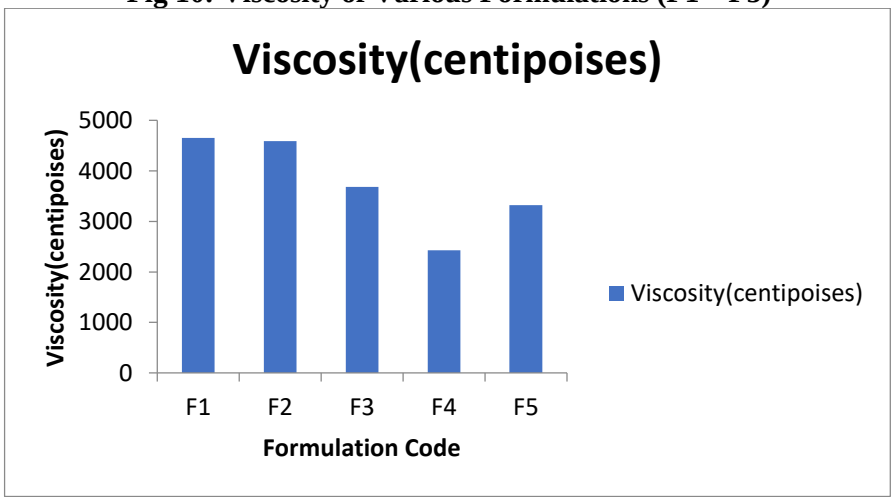
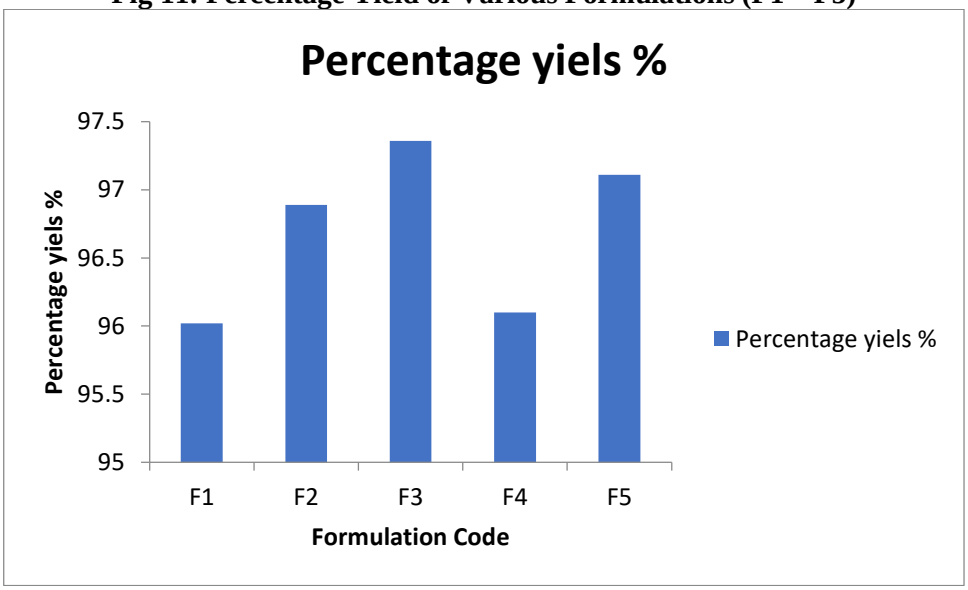


Fig 11: Percentage Yield of Various Formulations (F1 – F5)



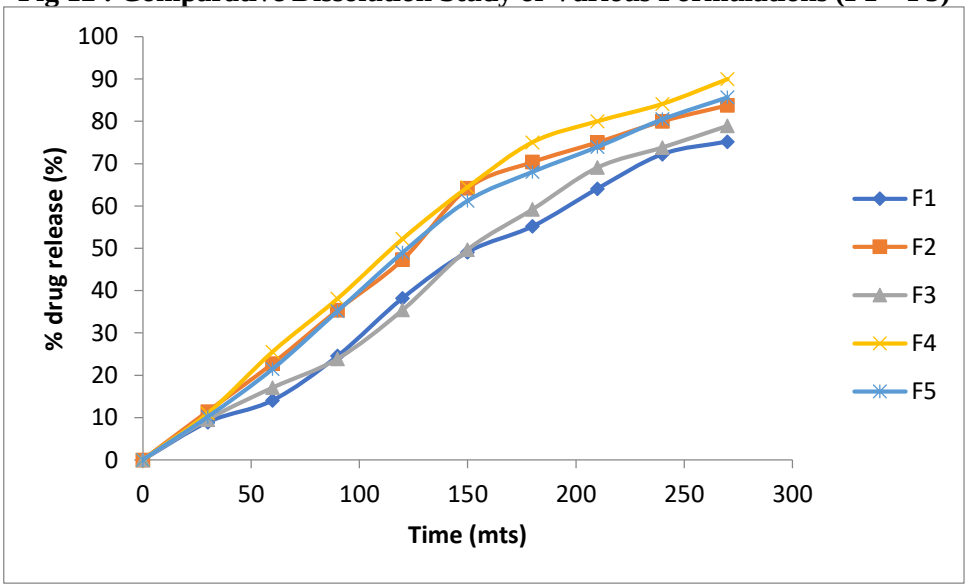
In vitro diffusion

The drug release profile of Isoleucine topical gel formulations was performed by diffusion cell. Results of the in-vitro release studies of all formulations are given in Table 6. The percentage drug release of all formulations after 1 hours using carbopol934, and combination was found to be 80.20% (F1), 83.80% (F2), 78.96% (F3) and 87.98% (F4), 96.70% (F5) respectively. The most important factors in the drug release is the type of polymer and the concentration of polymer

Table 6: Comparative Dissolution Study of Different Formulations (F1 – F5)

S.NO	TIME	%DRUG RELEASE				
		F1	F2	F3	F4	F5
1.	30	8.90	11.45	9.54	10.96	10.15
2.	60	14.06	22.79	17.07	25.56	21.50
3.	90	24.55	35.41	23.91	38.12	35.20
4.	120	38.23	47.33	35.44	52.23	49.02
5.	150	49.10	64.21	49.71	64.45	61.25
6.	180	55.18	70.39	59.23	75.07	68.09
7.	210	64.10	75.06	69.11	80.02	73.99
8.	240	72.24	80.05	73.86	84.14	85.52
9.	270	80.20	83.80	78.96	87.98	96.70

Fig 12 : Comparative Dissolution Study of Various Formulations (F1 – F5)



SUMMARY AND CONCLUSION

Following characterization studies were performed for five formulations i.e, fourier transform spectroscopy (FTIR), differential scanning colorimetery (DSC).All the above gels were evaluated for various physicochemical parameters like pH, viscosity, homogeneity, spredability, visual properties, in – vitro diffusion studies. In the above topical gel formulations F5 shows ideal in- vitro diffusion characterstic 96.70 % in 4.5 hrs. The formulation F5 show good consistency, homogeneity, less viscosity 3321cρ ,97.11% percentage yield, 98.17 drug content and 96.70 % in - vitro diffusion characteristics in 4.5 hrs. Based on the observation it can be concluded that the formulated topical gel containing Isoleucine can be successfully used to increase the bioavailability of drug and to overcome the side effects of drug.

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