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Formulation, Development, and Evaluation of an Emulgel

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ABSTRACT

In recent years, emulgels have been developed as one of the most promising drug delivery systems for hydrophobic medications. Emulgels have emerged both in cosmetics and pharmaceutical preparations. When gel and emulsion are used in the combined form, they are referred to as Emulgel. An emulsion can be made into an emulgel by combining it with gelling agents. Gels' many benefits come with significant drawbacks for the delivery of hydrophobic drugs. The emulsion-based technique is therefore being employed to get around this restriction. Emulgel is an interesting topical drug delivery system as it has a dual-release control release system. i.e. gel and emulsion. The present study aimed to formulate Ketoprofen emulgel using Karanja oil and Nirgundi oil as permeability enhancers. Ketoprofen is a non-steroidal anti-inflammatory drug that has two major problems when administered orally; it is an insoluble drug and has an irritating effect on GIT that leads to ulceration and bleeding. The topical application of this drug prevents these side effects and offers the potential advantage of delivering the drug at the site of action. The rationale of the present study was to increase the penetration of the drug into the skin. The prepared formulations were evaluated for their pH, viscosity, spreadability, content determination, *in-vitro* diffusion, and *in-vitro* anti-inflammatory study. The results of the *in-vitro* diffusion study showed that the oils in the emulgel were shown to increase the permeability of Ketoprofen and its anti-inflammatory activity. The best outcomes have been achieved with F2 (Ketoprofen+Karanja oil) emulgel. The prepared emulgel was shown to be stable at room temperature through a stability study.

1. INTRODUCTION

Drugs have been administered to the human body in a variety of ways throughout the past few decades, including oral, sublingual, rectal, parental, topical, inhalation, etc. to cure illnesses. Localized drug distribution through the skin, vagina, rectal, and ocular cavities is known as topical drug administration.[1] Topical delivery is the application of a drug-containing formulation to the skin to limit the drug's pharmacological or other effects on the skin's surface or deeper layers to treat cutaneous conditions like psoriasis or the cutaneous manifestations of more widespread illnesses like acne.[2] Since the mid-1980s, emulsion gels have been picking up significance in pharmaceutical topical semisolid dosage forms. They were developed as a result of the widespread use of emulsion systems, particularly for dermatological formulas, as a medicinal dosage form.[3]Emulgels are emulsions of the oil-in-water or water-in-oil type that are gelled by mixing with a gelling agent.[4-7] The main aim of the research is to design, develop and evaluate the emulgel containing active pharmaceutical ingredients with a combination of oils and the objectives were to formulate Ketoprofen emulgel with the combination of oils obtained from Karanja(*Pongamia pinnata*) and Nirgundi(*Vitex negundo*) as a permeability enhancer, to evaluate formulated oil based Ketoprofen emulgels, to study comparative *in-vitro* diffusion study, *in-vitro* antiinflammatory activity and stability study of formulated emulgels.

Drug Profile:

Ketoprofen

Ketoprofen, a propionic acid derivative, is a nonsteroidal anti-inflammatory agent (NSAIA) with analgesic and antipyretic properties. Ketoprofen has pharmacologic actions similar to those of other prototypical NSAIDs, which inhibit prostaglandin synthesis.[8-10]

Generic Name: Ketoprofen

Chemical Formula: $C_{16}H_{14}O_3$

Structure:

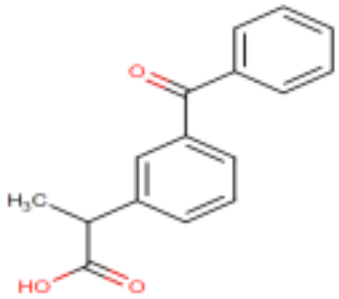


Fig.1: Ketoprofen

Mechanism of action of Ketoprofen:

The anti-inflammatory effects of ketoprofen are believed to be due to the inhibition of cyclooxygenase-2 (COX-2), an enzyme involved in prostaglandin synthesis via the arachidonic acid pathway. This results in decreased levels of prostaglandins that mediate pain, fever, and inflammation. Inhibiting COX-1 is suspected to be the cause of several of ketoprofen's negative effects, including GI distress and ulceration. Ketoprofen is a non-specific cyclooxygenase inhibitor. Ketoprofen is thought to have anti-bradykinin activity, as well as lysosomal membrane-stabilizing action. Antipyretic effects may be due to action on the hypothalamus, resulting in an increased peripheral blood flow, vasodilation, and subsequent heat dissipation.[11-12]

Excipient Profile:

1. Karanja

Scientific name: *Pongamia pinnata*

The malapari or karanja tree, also known as *Pongamia pinnata* (syn. *Millettia pinnata*), has a wide native range in Asia and Australia. Additionally, the species is grown in nations like Africa, the US, and others.[13]

Chemical constituents present in *Pongamia pinnata*:

Karangin, Isopongachromene, Glybanchalcone and Pongal

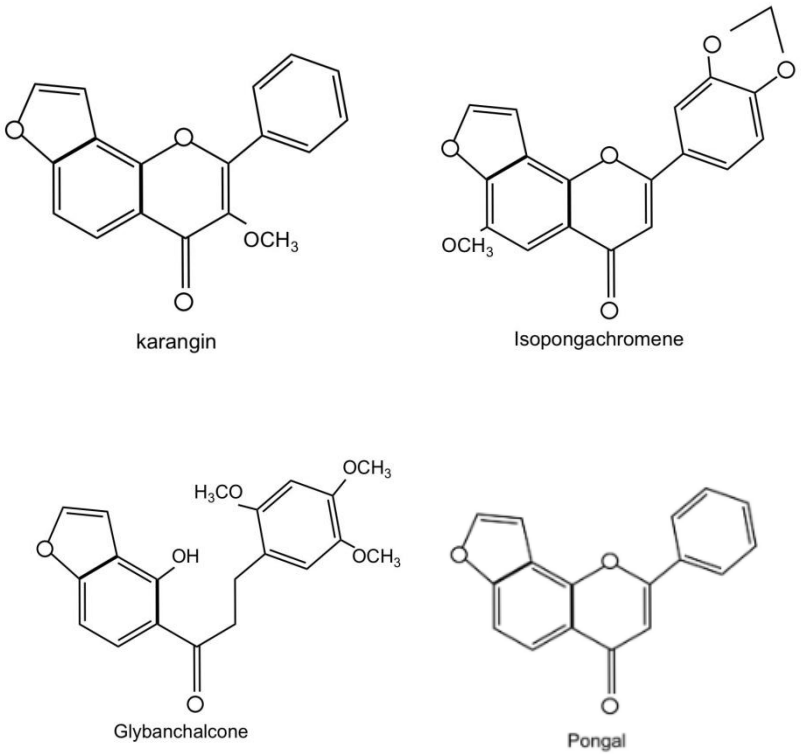


Fig.2: Chemical constituents of *Pongamia pinnata*

Medicinal uses of *Pongamia pinnata*:

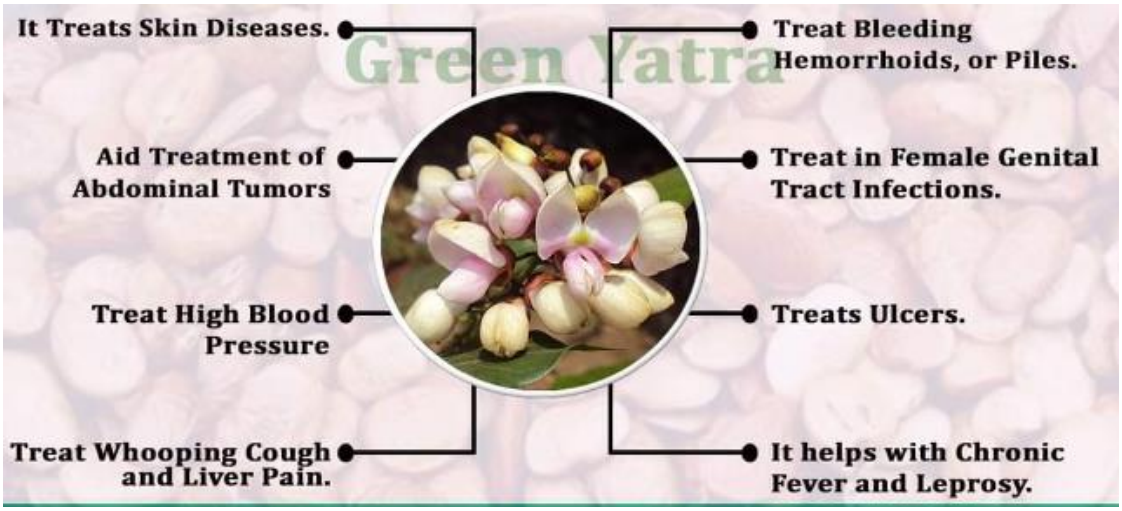


Fig.3: Medicinal uses of Karanja

2. Nirgundi

Scientific name: *Vitex Negundo*

Ayurvedic medication known as Nirgundi is created from the dried leaves of *Vitex negundo* (Family *Verbenaceae*), a huge, fragrant shrub or small tree that may grow to a height of 4.5 meters and is found throughout the nation up to an elevation of 1500 meters in the outer Himalayas.[14]

Chemical constituents present in *Vitex Negundo*:

The principal constituents are Casticin, Isoorientin, Chrysophenol D, Luteolin, p-hydroxybenzoic acid, and D-fructose.

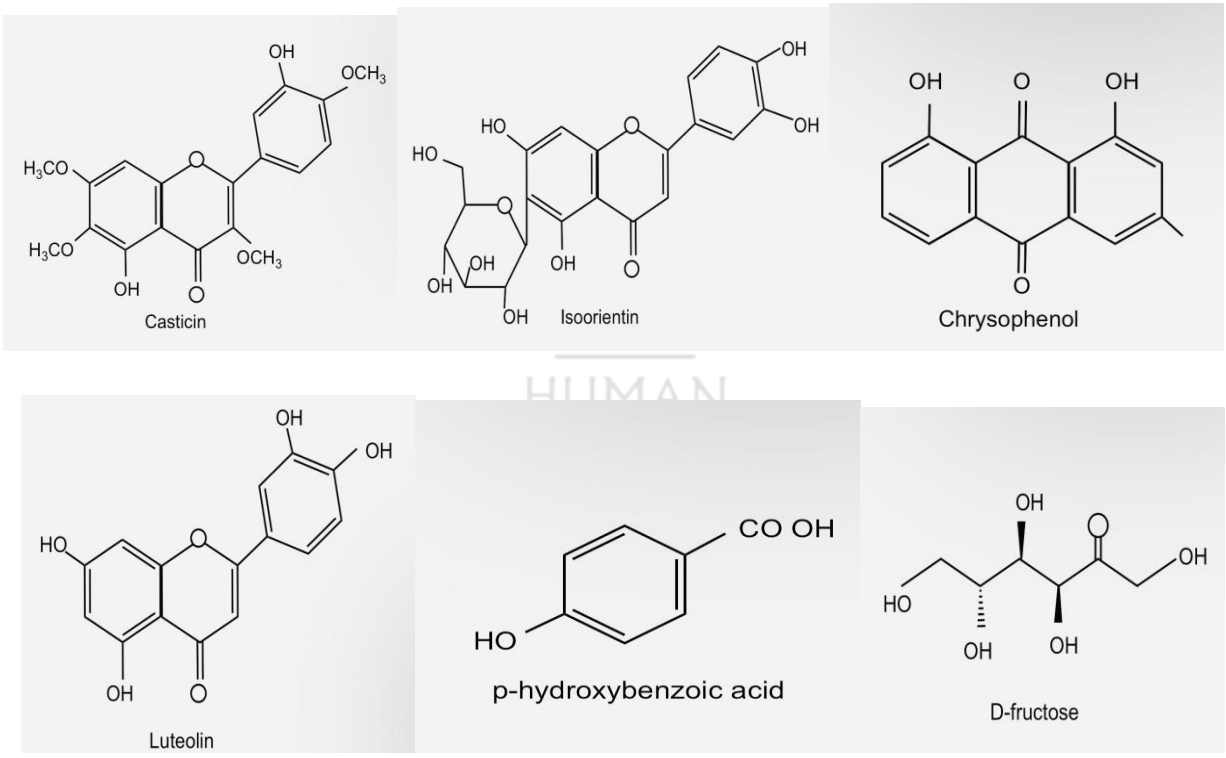


Fig.4: Chemical constituents of Nirgundi

Medicinal uses of *Vitex Negundo*:

Fig.5: Medicinal uses of Nirgundi

2. MATERIALS AND METHODS

Gift samples of Ketoprofen and other excipients were provided by Gopaldas Visram and Co Ltd. (Navi Mumbai, India). Carbopol 940 was obtained from Acofarma. Methyl Paraben, Propyl Paraben, obtained from Sigma Aldrich. Span 80, Tween 80, Liquid Paraffin, Triethanolamine, and Propylene Glycol obtained from Pioneer.

2.1 PREFORMULATION STUDIES OF KETOPROFEN:

A) Determination of Ketoprofen solubility in various solvents

The saturation technique was used to conduct solubility experiments. An excess of the pure drug(50mg) was put in a 50ml volumetric flask with 30 ml of various solvents respectively (Water, Ethanol, Methanol, and Phosphate buffer). The contents were maintained at 37 °C for 24 hours. After 24 hours of equilibrium, 5 ml of supernatant was removed, filtered, and examined using a UV spectrophotometer at 259nm. A solvent showing maximum solubility was selected for further experimental work.

Diluent preparation

Diluent was prepared by using Ethanol and Phosphate buffer pH 7.4 in a 50:50 ratio.

Preparation of phosphate Buffer(pH 7.4)

800 ml of distilled water was prepared in an appropriate container. To this distilled water 8 g of NaCl, 200 mg of KCl, 1.44 g of Na_2HPO_4, and 240 mg of KH_2PO_4 were added. Then, the solution was adjusted to the ideal pH of 7.4. After that, distilled water was added until the volume reached 1 liter.

Preparation of Standard Stock Solution (10 ppm)

25 mg of Ketoprofen was weighed and transferred into 25 ml of a volumetric flask, then added about 10 ml of diluent was to it, sonicated for 10 minutes to dissolve, and the volume was then made up with the same prepared diluent to prepare a drug solution of 1000 ppm, from which 5ml was withdrawn and diluted to 50 ml to make an intermediate standard of 100 ppm drug solution. From this solution, 5 ml was withdrawn and again diluted to 50 ml to obtain a final standard 10 ppm drug solution.

B) Melting point determination of Ketoprofen

The melting point ensures its purity. A Melting point apparatus was used to determine the melting point of the Ketoprofen.

C) Absorption maxima determination

A precisely weighed amount of 25mg Ketoprofen was dissolved in a 25 ml ethanolic phosphate buffer solution (diluent), from which 1 ml was withdrawn and diluted to 10 ml to make a 100 ppm drug solution. From this solution, 1 ml was withdrawn and diluted to 10 ml to obtain a 10 ppm drug solution. The solution obtained was scanned from wavelength 200 nm to 400 nm using a Shimadzu UV 1900 spectrometer.

D) Ketoprofen - Excipient Compatibility

Ketoprofen-excipient compatibility was determined by FTIR spectroscopy.

FTIR spectroscopy is done from Plot No: A-327, T.T.C Ind. Area, M.I.D.C., Mahape, Navi Mumbai: 400 710, India.

Ketoprofen, a physical mixture of ketoprofen and excipients was kept at 40℃ for 14 days, and infrared spectra were recorded as shown in Fig.8- Fig.11.

2.2 FORMULATION OF EMULGEL

A) Preparation of Emulsion

The oil phase of the emulsion was prepared by dissolving Span 80 and light liquid paraffin, and Karanja oil and Nirgundi oil were added, respectively. The aqueous phase was prepared by dissolving Tween 80 in water. Methyl paraben and propyl paraben were dissolved in propylene glycol. Then this solution was added to the aqueous phase. Both the oily and aqueous phases were separately heated to 70 to 80 ℃, then the oily phase was added to the aqueous phase with continuous stirring until it was cooled to room temperature. The emulsion obtained is stored in a well-closed, airtight container. Then the emulsion was evaluated for microscopic observation, pH, and viscosity. The zeta potential of the emulsion was measured by a zeta sizer at 25°C.

B) Preparation of Gel

The quantity of Carbopol 940 was weighed and mixed homogeneously with distilled water at 65-70°C using a magnetic stirrer. The stirring speed was 1000–2000 RPM for 10 minutes to form a smooth dispersion. The preparation is allowed to stand, permitting entrapped air to separate. The pH was then adjusted to 6-6.5 using triethanolamine.

C) Preparation of Ketoprofen solution

The quantity of ketoprofen was weighed, added to an accurate quantity of isopropyl alcohol, and kept aside for rinsing purposes. Then the above solution was added to the gel solution.

D) Preparation of Emulgel

The optimized emulsion was incorporated into the gel base in a 1:1 ratio under continuous mixing with a mechanical stirrer at 5000±6000 RPM for about 10-20 minutes to obtain an emulgel.

Formulation table:

Table 1: Formulation table of Emulgel

Ingredients	F1	F2	F3
Ketoprofen(g)	2.5	2.5	2.5
Karanja Oil(ml)	-	2.5	-
Nirgundi Oil(ml)	-	-	2.5
Carbopol(g)	2.5	2.5	2.5
Methyl Paraben(g)	0.2	0.2	0.2
Propyl Paraben(g)	0.2	0.2	0.2
Span 80(ml)	0.70	0.70	0.70
Tween 80(ml)	0.70	0.70	0.70
Propylene glycol(ml)	5	5	5
Triethanolamine(ml)	QS	QS	QS
Isopropyl Alcohol(ml)	5	5	5
Liquid paraffin(ml)	6.5	6.5	6.5
Water(ml)	QS	QS	QS

2.3 EVALUATION OF EMULGEL FORMULATION

A) Determination of pH

A 1% solution of Ketoprofen, Ketoprofen+Karanja, and Ketoprofen+Nirgundi emulgel was prepared and subjected to pH measurements by a digital pH meter.

B) Viscosity study

The viscosity measurement of the formulations was performed with a Brookfield Viscometer. The formulations were rotated at 1, 2.5, 5, 10, 20, 50, and 100 rotations per minute. At each speed, the corresponding dial reading was noted and viscosity was calculated.

C) Spreadability study

The spreadability of formulations was measured by spreading 1 gm of emulgel on a circle of 2 cm diameter premarked on a glass plate and then a second glass plate was employed. Half a

kilogram of weight was permitted to rest on the upper glass plate for 5 min. The diameter of the circle after spreading the formulation was determined.

D) Globule size and its distribution in emulgel

Globule size and distribution were determined by using an optical microscope. A 1gm sample was dissolved in purified water and agitated to get a homogeneous dispersion. A sample was observed under an optical microscope, and the mean globule diameter and distribution were obtained.

E) Ketoprofen content determination

1000mg of emulgel (equivalent to 25 mg of ketoprofen) was taken in 25 ml of a volumetric flask and 10 ml of diluent was added. Then it was sonicated for 10 minutes on the vortex to disperse and dissolve well. After this, the volume was then made with diluent (1000 ppm). Further withdrawn 5 ml from the prepared solution was into 50 ml of a volumetric flask and again volume was made up with diluent (100 ppm). Further withdrawn 5 ml from the prepared solution into 50 ml of a volumetric flask and again made up the volume with diluent to prepare the final sample solution (10 ppm). Then the final prepared solution was subjected to spectrophotometric analysis for drug content determination.

2.4 *IN-VITRO* DIFFUSION STUDY

An in-vitro diffusion study was carried out using a Franz Diffusion Cell. Emulgel (1 gm) was evenly applied onto the surface of the goat skin. The goat skin was clamped between the donor and the receptor chamber of a diffusion cell-filled pH 7.4 phosphate buffer. The receptor chamber was stirred by a magnetic stirrer. The study was carried out at 37±0.5°C. Samples were withdrawn from the sampling port of the reservoir compartment at regular intervals and absorbance was measured using Shimadzu UV 1900 spectrometer at 259 nm.

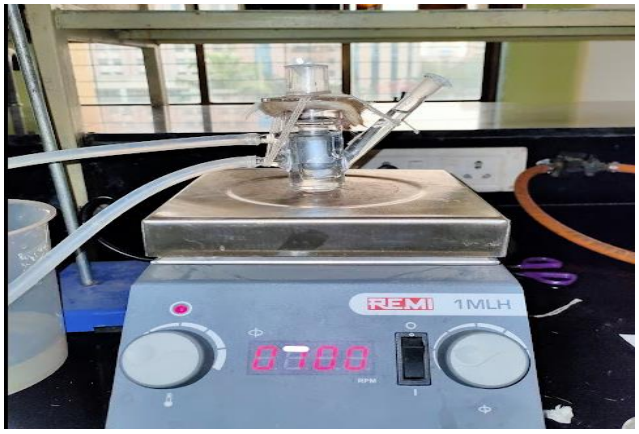


Fig.6: *In-vitro* diffusion study

2.5 *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY STUDY

A) Membrane stabilization

Principle- The inhibition of hypotonicity induced Human red blood cells (HRBC) membrane lysis i.e. stabilization of the HRBC membrane was taken as a measure of the anti-inflammatory activity. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the drug may as well stabilize the lysosomal membrane. Stabilization of the lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophils such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extracellular release.

Preparation of 10% v/v human erythrocyte suspension- Fresh whole human blood(10ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volumes of normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline.

Procedure-The test solution consisted of 2 ml of hypotonic saline (0.25% w/v sodium chloride), 1 ml of 0.15 M sodium phosphate buffer, pH 7.4, 0.5 ml of 10% v/v human erythrocyte suspension, 1 ml of emulgel formulation (Diluted phosphate buffer-ethanolic solution) and final reaction mixtures was made up to 4.5 ml with is saline (0.9% w/v sodium chloride). To determine the anti-inflammatory activity of the human red blood cell membrane stabilization method, the following solutions were used.

Table 2: Solutions used for Membrane stabilization

Sr. No	Reagents	Test solution	Standard solution	Control solution
1.	Hypo saline (0.25%w/v NaCl)	2ml	2ml	2ml
2.	0.15M Phosphate buffer pH 7.4	1ml	1ml	1ml
3.	10% v/v Human erythrocyte suspension	0.5ml	0.5ml	0.5ml
4.	Drug/Formulation/Isosaine	1 ml of emulgel formulation	1 ml of drug	1 ml of is saline
5.	Isosaline (0.9% w/v NaCl)	Volume make up to 4.5ml	Volume make up to 4.5ml	Volume make up to 4.5ml

The reaction mixture was incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant solution was measured spectrophotometrically at 560 nm. Each experiment was carried out in triplicate and an average was taken. The percentage inhibition of hemolysis or membrane stabilization was calculated using the following formula.

$$\% \text{ Inhibition} = A_{Control} - A_{Sample} / A_{Control} \times 100$$

Where $A_{Control}$ is the absorbance of the control sample

A_{Sample} is the absorbance of a test sample or standard drug

B) Heat-induced hemolysis

Procedure- The 2 ml reaction mixture consisted of 1 ml of emulgel formulation solution and 1 ml of 10% RBC suspension. Saline was added to the control test tube. Pure Ketoprofen was taken as a standard drug. To determine the anti-inflammatory activity of heat-induced hemolysis the following solutions were used.

Table 3: Solutions used for Heat-induced hemolysis

Sr. No.	Reagents	Test solution	Standard solution	Control solution
1.	Drug	1 ml of emulgel formulation	1 ml drug	1 ml isosaline
2.	10% RBC suspension	1 ml	1 ml	1 ml

All the centrifuge tubes containing reaction mixtures were incubated in a water bath at 56℃ for 30 min. At the end of the incubation, the tubes were cooled under the running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatant was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percent inhibition of hemolysis was calculated by the formula mentioned below-

$$\% \text{ Inhibition} = A_{Control} - A_{Sample} / A_{Control} \times 100$$

Where $A_{Control}$ is the absorbance of the control sample

A_{Sample} is the absorbance of a test sample or standard drug

C) Inhibition of protein denaturation

Principle- Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent, or heat. Most biological proteins lose their biological function when denatured. The denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, the ability of emulgel formulations to denature the protein was studied.

Procedure- The reaction mixture consisted of a test emulgel formulation and a 1% aqueous mixture of bovine albumin fraction. pH of the reaction mixture was adjusted using a small amount of 1N HCl. Then the sample reaction mixture was incubated at 37℃ for 20 min and then heated to 51℃ for 20 min. After cooling the samples the turbidity was measured spectrophotometrically at 660nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows-

$$\% \text{ Inhibition} = A_{Control} - A_{Sample} / A_{Control} \times 100$$

Where $A_{Control}$ is the absorbance of the control sample

A_{Sample} is the absorbance of a test sample or standard drug

2.6 VALIDATION OF OPTIMISED EMULGEL FORMULATION

The developed 'F2' formulation was validated for parameters such as Specificity, Linearity, Accuracy, Precision, and Robustness as per ICH guidelines.

I. Specificity

The specificity of an analytical method is its ability to accurately measure an analyte in the presence of interference, such as synthetic precursors, excipients, enantiomers, and known(or likely) degradation products that may be expected to be present in the sample matrix. The term specificity is also referring to selectively when several chemical entities may or may not be distinguished from each other.[15]

The spectra of the placebo, sample, blank and standard solution were obtained and interpreted by overlying them for the presence of any component interfering with the absorption maxima of the under-analyzed solution. **(Results in Section 3.6(I))**

II. Linearity

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration(amount) of analyte in samples within a given range.[16]

Preparation of sample stock solution(100 ppm)

1000mg of emulgel (equivalent to 25 mg of ketoprofen) was taken in 25 ml of a volumetric flask and 10 ml of diluent was added. Then it was sonicated for 10 minutes on the vortex to disperse and dissolve well. After this, the volume was then made with diluent (1000 ppm). Further withdrawn 5 ml from the prepared solution was into 50 ml of a volumetric flask and again volume was made up with diluent (100 ppm).

Various aliquots were prepared from the stock solution (100 ppm) ranging from 4-12 µg/ml. The samples were scanned in a UV-VIS spectrophotometer using a prepared diluent as a blank. **(Results in Section 3.6(II))**

The calibration curve was plotted graphically and the standard equation and correlation coefficient were determined.**(Results in Section 3.6(II))**

III. Accuracy

The accuracy of the analytical method is the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value, and the value found.[17]

The accuracy of the method was determined by preparing solutions of different concentrations, i.e., 80%, 100%, and 120%. Three replicates of each solution at the appropriate concentration were made, and the absorbance of each solution was recorded by scanning in a UV-VIS spectrophotometer using a prepared diluent as a blank. **(Results in Section 3.6(III))**

IV. Precision

The closeness of agreement between a series of measurements obtained from successive samplings of the same homogenous sample under the required conditions is the precision of an analytical method.[18]

Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

The test method was performed again by the same analyst after a short duration on the same instrument. Prepared replicas of 10µg/ml solution and recorded the absorbance and calculated the % assay. **(Results in Section 3.6(IV))**

Intermediate Precision: Intermediate precision expresses within-laboratories variation; different days, different analysts, different equipment, etc. Prepared replicas of 10µg/ml solution and recorded the absorbance and calculated the % assay. **(Results in Section 3.6(IV))**

V. Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters and indicates its reliability during normal usage.[19-20]

Following were evaluated as method robustness. **(Results in Section 3.6(V))**

Effect of change in wavelength: Absorption performed at 256 nm and 262 nm

Effect of change in sonication time: Sonication performed for 5 min and 15 min.

Effect of change in diluent ratio: diluent ratios were used in Ethanol: Phosphate buffer pH 7.4 (55:45 % v/v) and Ethanol: Phosphate buffer pH 7.4 (45:55 % v/v).

VI. Filter compatibility study

Filtration is a key sample preparation step in achieving accurate test results. The purpose of filtration is to remove undissolved drugs and excipients from the withdrawn solution. If not removed from the sample solution, particles of the drug will continue to dissolve and which can bias the result. Therefore, filtering the sample is usually necessary. Filtration also removes insoluble excipients that may otherwise interfere with the analytical finish. The selection of the proper filter material is important. Important characteristics to consider when choosing the filter material are type, filter size, and pore size. A filter compatibility study was performed on PVDF 0.45µm and Nylon 0.45µm filters with different discard volumes. The filter that met the acceptance criteria for filtration was then selected for the experiments. **(Results in Section 3.6(VI)**

VII. Solution stability

Solution stability for 24 hours at normal room temperature (37 °C) was established for the standard and sample solutions. Day 1 sample assay and standard area were compared with freshly prepared standards on the next day.**(Results in Section 3.6(VII))**

2.7 STABILITY STUDY AS PER ICH GUIDELINES

Emulgel was packed in aluminum collapsible tubes (5gm) and subjected to stability studies at 5°C, 25°C/60%RH, 30°C/65% RH, and 40°C/75% RH for 3 months. Samples are withdrawn each month as per ICH guidelines and analyzed for physical appearance, pH and drug release profile, etc.

3. RESULT AND DISCUSSION

3.1 PREFORMULATION STUDIES OF KETOPROFEN

A) Determination of Ketoprofen solubility in various solvents

Below are the observations obtained in various solvents:

Water (Not soluble - Turbid)

Water: Methanol (50:50) (Soluble)

Water: Ethanol (50:50) (Soluble)

Phosphate buffer pH 7.4 (Partially insoluble)

Ethanol: Phosphate buffer pH 7.4 (50:50) (Completely soluble)

B) Melting point determination of Ketoprofen

Table 4: Results of melting point determination of Ketoprofen

Drug	Reported melting point	Actual melting point
Ketoprofen	92-95°C	94°C- 96°C

C) Absorption maxima determination

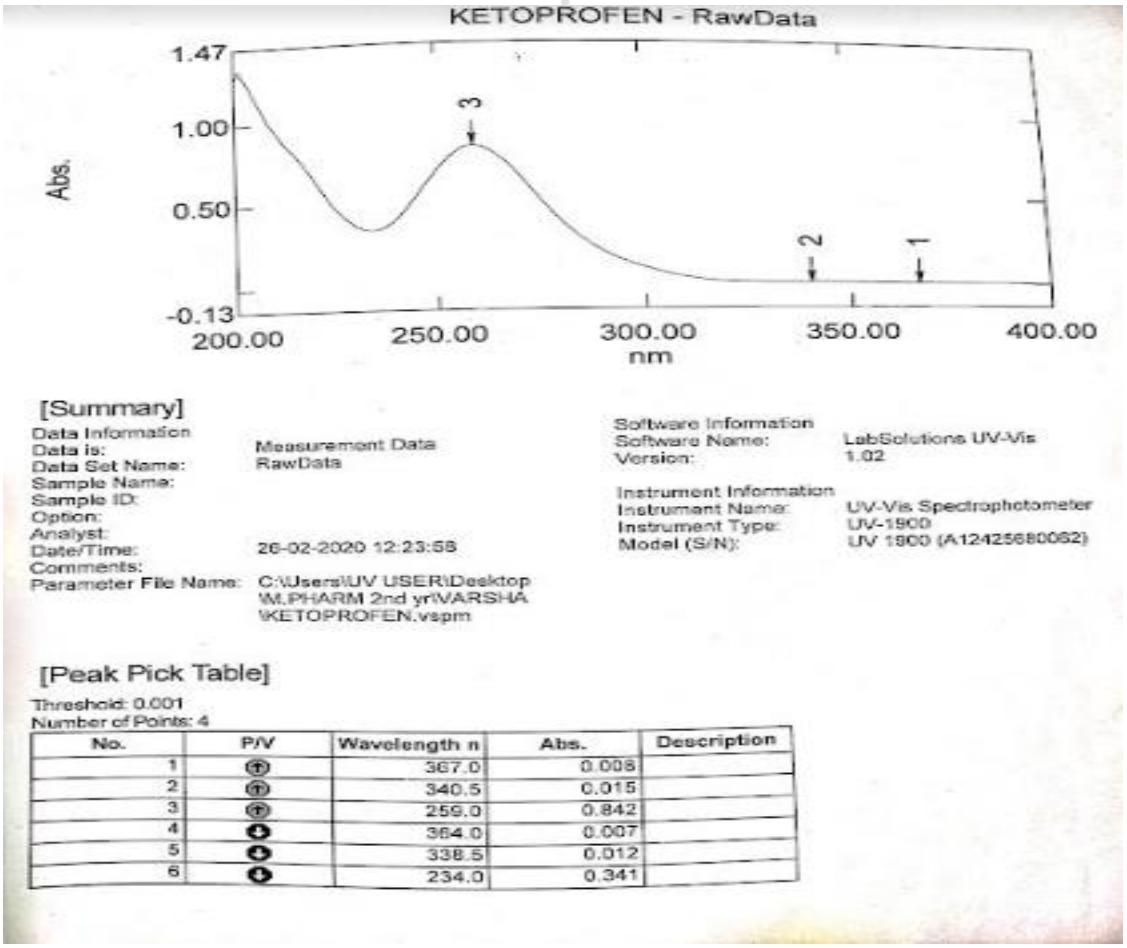


Fig.7: λ max determination of Ketoprofen

Ketoprofen showed maximum absorbance at 259 nm for 10 µg/ml solution and this wavelength was selected for the detection of the drug.

D) Ketoprofen-Excipient Compatibility

FTIR spectrum of Ketoprofen and Ketoprofen-excipients mixture shows absorption bands of O-H stretching vibration of alkoxy group, C=O stretching vibration of carboxylic group, C=O stretching vibration of ketene group and C-H stretching vibration of CH group, appeared at 3327cm^{-1}, 1710 cm^{-1}, 1666 cm^{-1} and 858.30cm^{-1} respectively.

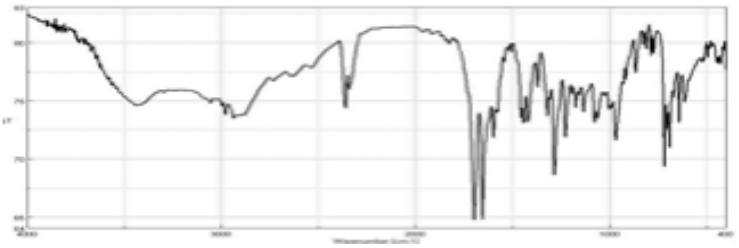


Fig.8: FT-IR of Ketoprofen

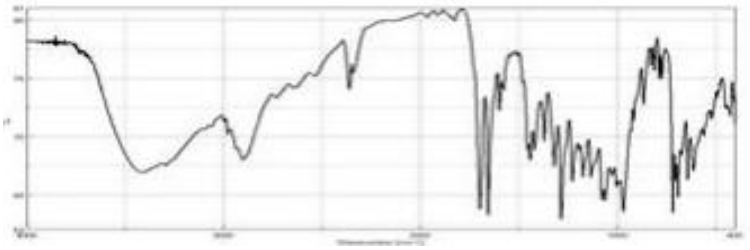


Fig.9: FT-IR of Ketoprofen + Carbopol

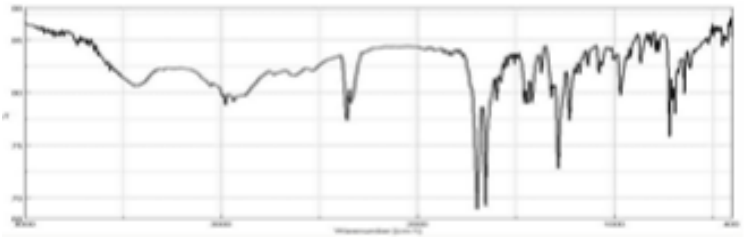


Fig.10: FT-IR of Ketoprofen + Karanja

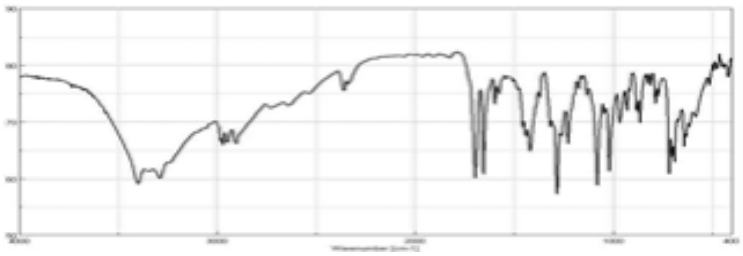


Fig.11: FT-IR of Ketoprofen + Nirgundi

3.2 EVALUATION OF OPTIMISED EMULSIONS

A) Microscopic study

The microscopic observation of all three batches of emulsion was examined by using an optical microscope. The observed emulsions were found to be in oil in water type.

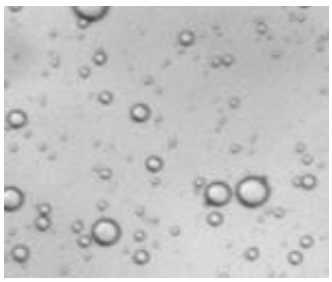


Fig.12 Emulsion A

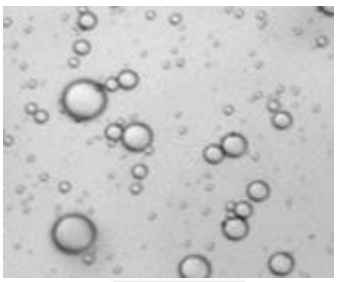


Fig.13 Emulsion B

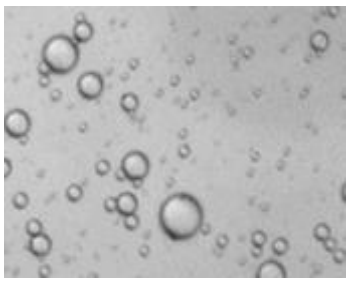


Fig.14 Emulsion C

B) pH determination

The pH of all three prepared emulsions was found to be 6.2, 6.6, and 6.7.

C) Viscosity study

The viscosity of all three prepared emulsions was found to be 580.85 cps, 640.25 cps, and 664.65 cps.

D) Zeta potential

The zeta potential of all three optimized emulsion formulations was found to be -31.2mV, -32.6mV, and -31.7mV which indicated the formulation is stable.

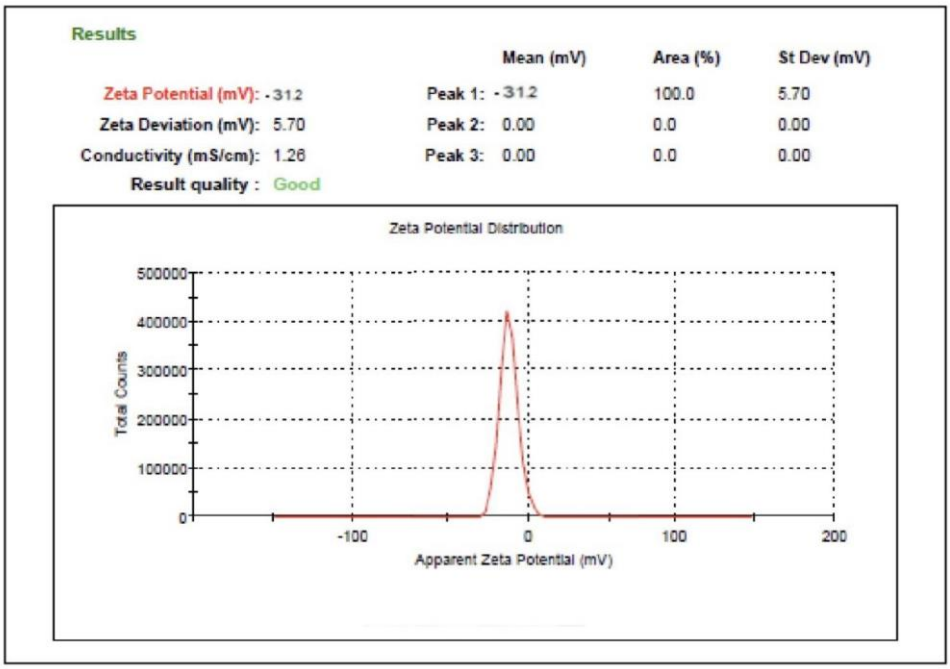


Fig. 15: Zeta potential of Emulsion A

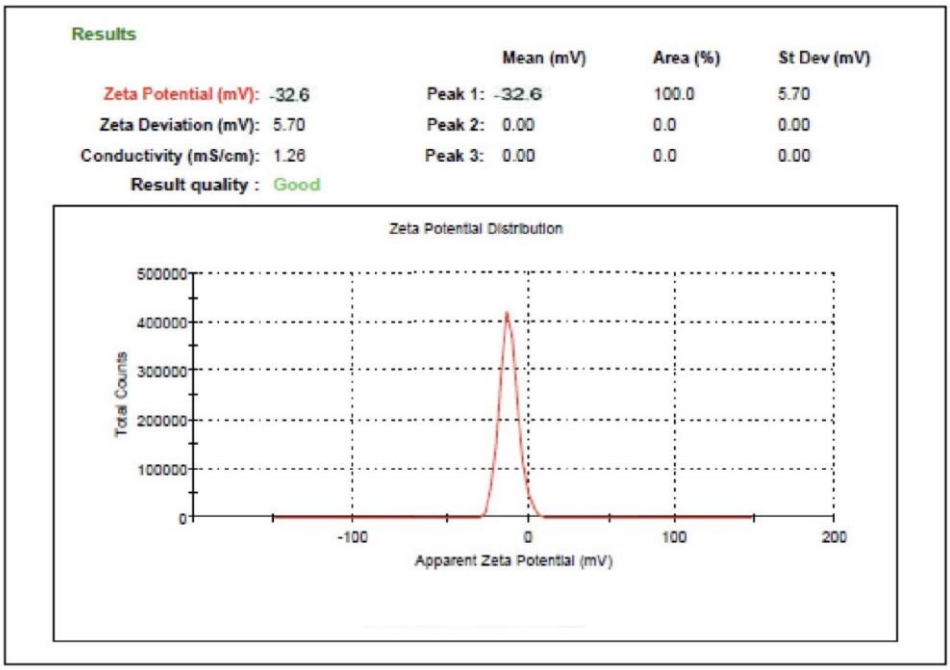


Fig. 16: Zeta potential of Emulsion B

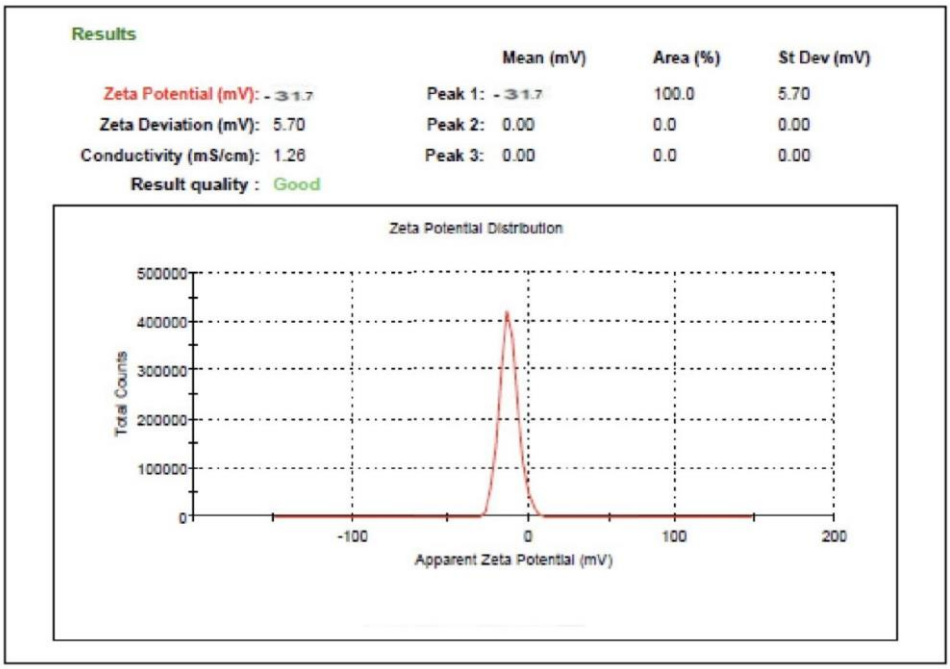


Fig. 17: Zeta potential of Emulsion C

3.3 EVALUATION OF EMULGEL FORMULATION

Fig.18: Prepared Ketoprofen Emulgel Formulation

Fig.19: Prepared Ketoprofen+Karnja Emulgel Formulation

Fig.20: Prepared Ketoprofen+Nirgundi Emulgel Formulation

A) Organoleptic characteristics

Table 5: Organoleptic characteristics of prepared Emulgel formulation

Characteristics	**Ketoprofen Emulgel (KE)**	**Ketoprofen+KaranjaEmulgel(KKE)**	**Ketoprofen+NirgundiEmulgel(KNE)**
Color	White	White	White yellowish
Odor	Odourless	Odourless	Odourless
Appearance	Creamy	Creamy	Creamy
Phase separation	No	No	No
Occlusiveness	Yes	Yes	Yes
Washability	Washable	Washable	Washable

B) Determination of pH

The pH of freshly prepared Ketoprofen, Ketoprofen+Karanja, and Ketoprofen+Nirgundi

emulgel was found to be 6.0, 6.12, and 6.5 respectively.

C) Viscosity study

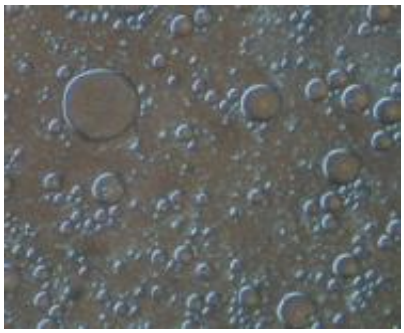
The viscosity of Ketoprofen, Ketoprofen+Karanja, and Ketoprofen+Nirgundi emulgel by Brookfield Viscometer was found to be 2780 cps, 5280 cps, and 11650cps.

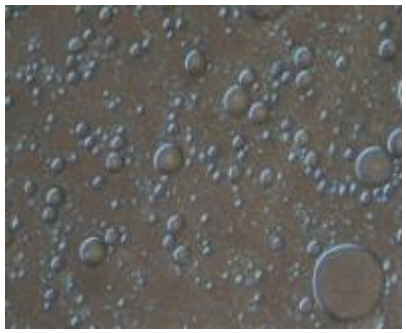
D) Spreadability study

The spreadability of freshly prepared Ketoprofen, Ketoprofen+Karanja and Ketoprofen+Nirgundi emulgel was found to be 30.33±0.2, 32.07±0.6 and 34.0±0.5.

E) Globule size and its distribution in emulgel

The average globule size in all three emulgel was found to be 1µ. About 70-80% of globules had a size range of 1 to 2 µ. It was concluded that the emulsion was uniformly distributed throughout the gel base and formed homogenous emulgel.





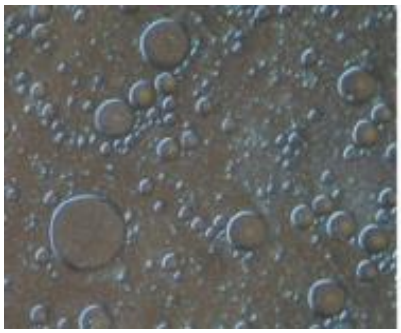


Fig.21 : Ketoprofen Emulgel Fig.22 : Ketoprofen+Karanja Fig.23 : Ketoprofen+Nirgundi

F) Ketoprofen content

%Ketoprofen content of Ketoprofen emulgel, Ketoprofen+Karanja emulgel, and Ketoprofen+Nirgundiemulgel was found to be as shown in the below table.

Table 6: Ketoprofen content of prepared Emulgel formulation

Formulation	Absorbance	% Assay
Ketoprofen emulgel	0.816	96.71
Ketoprofen+Karanja emulgel	0.829	98.26
Ketoprofen+Karanja emulgel	0.821	97.31

3.4 *IN-VITRO* DIFFUSION STUDY

An in-vitro diffusion study was done for formulated all emulgel. Which drug release in the phosphate buffer medium at each interval was calculated by determination of % drug release.

Table 7: *In-vitro* diffusion study

Time (in mins)	Cumulative drug release from formulation (%)			
	Marketed Ketoprofen	**Ketoprofen emulgel**	**Ketoprofen+Karanja emulgel**	**Ketoprofen+Nirgundi emulgel**
15	9.14±1.05	8.10±0.76	12.40±0.38	7.21±0.40
30	12.55±0.88	11.23±0.65	19.88±0.29	10.20±0.28
45	15.55±0.70	14.40±0.40	22.39±0.48	13.66±0.54
60	19.93±0.40	18.88±0.43	29.89±0.35	18.76±0.43
90	22.90±0.44	20.54±0.32	35.67±0.26	21.44±0.47
120	26.62±0.56	27.75±0.19	40.03±0.33	39.56±0.63
150	34.43±0.37	39.99±0.32	48.77±0.28	43.76±0.43
180	49.88±0.67	44.76±0.21	59.34±0.29	58.29±0.43
210	55.54±0.43	54.86±0.20	74.45±0.74	66.37±0.46
240	62.68±0.98	61.97±0.49	85.68±0.54	74.49±0.67

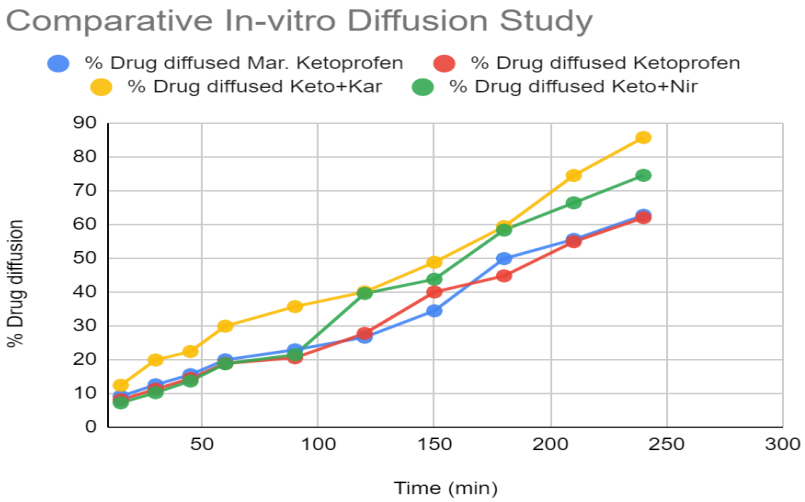


Fig.24: Comparative *In-vitro* diffusion study

3.5 *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY STUDY

The formulations were evaluated for their anti-inflammatory property by the Membrane stabilization method, Heat-induced hemolysis method, and Protein denaturation method.

Table 8: Results of *In-vitro* Anti-inflammatory study

Dose	Membrane stabilization (%Inhibition)			
	Marketed Ketoprofen	Ketoprofen emulgel	Ketoprofen+Karanja emulgel	Ketoprofen+Nirgundi emulgel
100 µg/ml	45.58%	43.45%	54.73%	33.04%
200 µg/ml	49.67%	45.32%	57.34%	35.66%

Dose	Heat-induced haemolysis (%Inhibition)			
	Marketed Ketoprofen	Ketoprofen emulgel	Ketoprofen+Karanja emulgel	Ketoprofen+Nirgundi emulgel
100 µg/ml	55.77%	51.78%	59.87%	36.42%
200 µg/ml	60.99%	55.05%	69.43%	40.44%

Dose	Protein denaturation (%Inhibition)			
	Marketed Ketoprofen	Ketoprofen emulgel	Ketoprofen+Karanja emulgel	Ketoprofen+Nirgundi emulgel
100 µg/ml	48.84%	45.06%	50.43%	39.04%
200 µg/ml	59.07%	49.55%	70.32%	44.53%

3.6 VALIDATION OF OPTIMISED EMULGEL FORMULATION

The developed ‘F2’ formulation was validated for parameters such as Specificity, Linearity, Accuracy, Precision, and Robustness as per ICH guidelines.

I. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Following are the observed UV spectra of blank, placebo, standard, and sample solutions respectively.

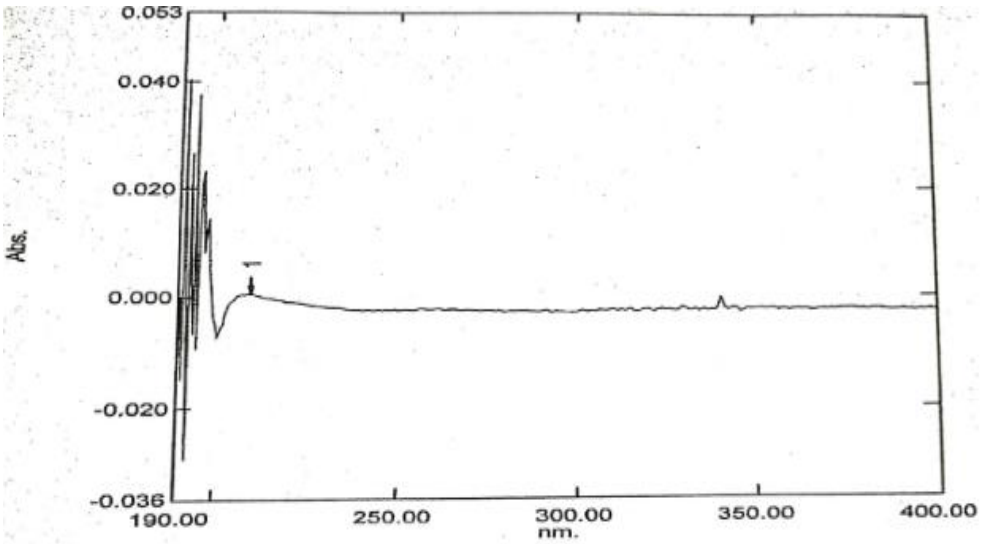


Fig 25: Spectra of Blank

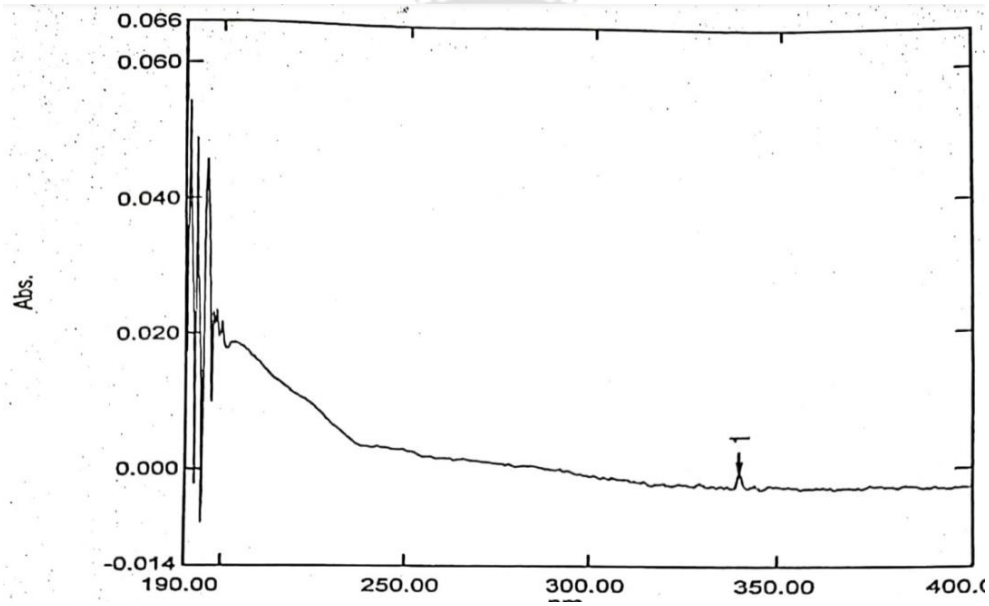


Fig 26: Spectra of Placebo

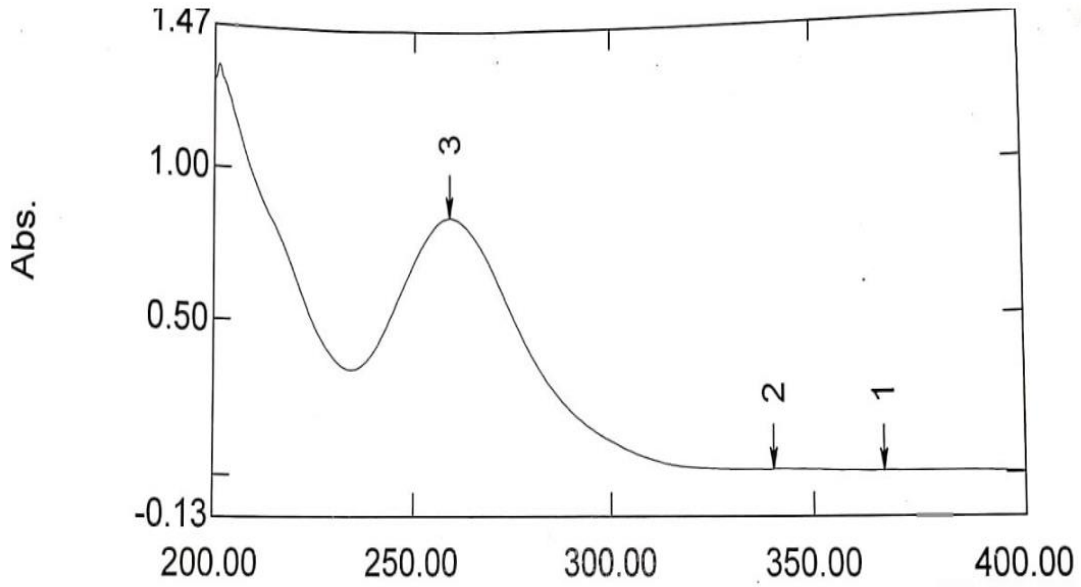


Fig 27: Spectra of Standard(Ketoprofen)

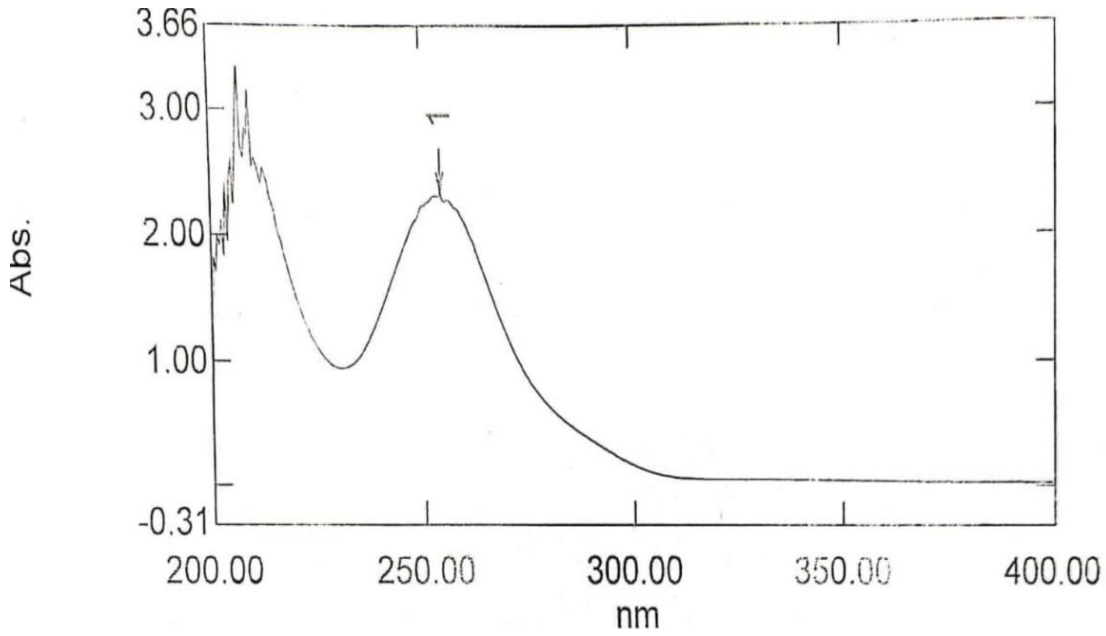


Fig 28: Spectra of sample

Acceptance criteria

There should not be any interference of the placebo with the standard.

There should not be any interference of the placebo with the sample.

There should not be any interference pattern of placebo and blank at the main peak.

Conclusion

The data shows that there is no interference of the placebo with the standard.

The data shows that there is no interference of the placebo with the sample.

The data shows that there is no interference pattern of placebo and blank at the main peak.

II. Linearity

The concentration range in which Beer- Lambert's followed was 4-12 µg/ml and coefficient of correlation was found to be 1.

Table 9: Linearity-result summary

Level in %	Volume from stock 100ppm	Flask volume	Concentration (ppm)	Absorbance
40 %	2 ml	50 ml	4 ppm	0.336
60 %	3 ml	50 ml	6 ppm	0.504
80 %	4 ml	50 ml	8 ppm	0.662
100 %	5 ml	50 ml	10 ppm	0.839
120 %	6 ml	50 ml	12 ppm	0.998

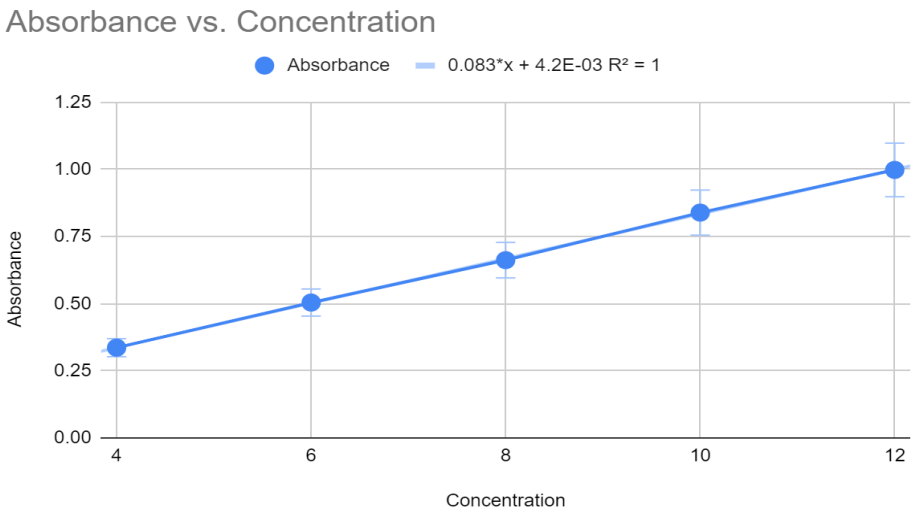


Fig 29: Calibration curve of sample solution

Table 10: Regression analysis data of the developed method

Parameters	Observation values
Beer's range (µg/ml)	4 - 12 µg/ml
Correlation Coefficient (r^2)	1
Regression equation	0.083x + 0.0042
Intercept (a)	0.0042
Slope (b)	0.083

Acceptance criteria:

The response should be linear.

The correlation coefficient (R) should not be less than 0.999.

The limit of the Y-intercept should be within ±2.0%.

Conclusion:

The data shows that the response is linear.

The correlation coefficient is observed to be 1.0.

The data demonstrated that the Y-intercept is 0.0042 which is within ±2.0% acceptance criteria of the corresponding Y-coordinate of the working level and hence the method is linear.

A graph of concentration vs. absorbance is attached as a figure above.

III. Accuracy

The accuracy of the method was studied through recovery experiments. Recovery was checked at three levels, 80%, 100%, and 120%.

Table 11: Accuracy- result summary

Accuracy level	Concentration	Volume from stock 100ppm	Flask	Wt. of placebo	Absorbance	% Recovery	Mean recovery %
80 %__1	8 ppm	4 ml	50	1 gm	0.679	101.04	99.90
80%_2		4 ml	50	1 gm	0.669	99.55	
80%_3		4 ml	50	1 gm	0.666	99.11	
100%_1	10 ppm	5 ml	50	1 gm	0.845	100.16	100.27
100%_2		5 ml	50	1 gm	0.854	101.22	
100%_3		5 ml	50	1 gm	0.839	99.44	
120% _1	12 ppm	6 ml	50	1 gm	0.997	98.67	99.13
120%_2		6 ml	50	1 gm	0.999	98.87	
120%_3		6 ml	50	1 gm	1.009	99.86	

Acceptance criteria

Mean recovery for 80% to 120% should be in the range of 98.0%-102.0% and individual recovery for 80% to 120% should be in the range of 97.0% -103.0%.

Conclusion:

The above results show that the Mean recovery for 80% to 120% is in the range of 98.0%-102.0% and Individual recovery for 80% to 120% is in the range of 97.0% - 103.0%.

IV. Precision

The precision of the method was demonstrated by intraday and interday variation studies.

Interday precision

Table 12: Interday precision study

INTERDAY PRECISION			
Test sample	**Absorbance**	**Avg absorbance**	**% Assay**
1	0.844	0.8425	99.85
	0.841		
2	0.839	0.8415	99.74
	0.844		
3	0.841	0.8435	99.97
	0.846		
4	0.843	0.8410	99.68
	0.839		
5	0.836	0.8390	99.44
	0.842		
6	0.839	0.8375	99.26
	0.836		
Mean	99.656		
SD	0.263		
%RSD	0.264		

Intraday precision

Table 13: Intraday precision study

INTRADAY PRECISION			
Test sample	**Absorbance**	**Avg absorbance**	**% Assay**
1	0.841	0.8430	99.92
	0.845		
2	0.846	0.8465	100.33
	0.847		
3	0.843	0.8460	100.27
	0.849		
4	0.839	0.8440	100.04
	0.849		
5	0.843	0.8415	99.74
	0.840		
6	0.846	0.8455	100.21
	0.845		
Mean	100.085		
SD	0.227		
%RSD	0.227		

Acceptance criteria:

%RSD for peak areas should be within ±2.0%.

Conclusion:

The data shows that the %RSD for peak areas is within the limit.

V. Robustness

By changing the wavelength, sonication time, and dilution ratio, the sample is analyzed and the results were found to be as follows:

Table 14: Results of Robustness

Robustness parameter	**Implementation**	**Absorbance**	**% Assay**
Effect of Change in Wavelength	256 nm	0.838	99.33
	262 nm	0.844	100.04
Effect of change in Sonication time	5 min	0.829	98.26
	15 min	0.841	99.68
Effect of change in Diluent ratio	Ethanol: Phosphate buffer pH 7.4 (55:45 % v/v)	0.831	98.49
	Ethanol: Phosphate buffer pH 7.4 (45:55 % v/v)	0.839	99.44
Mean	99.206		
SD	0.692		
%RSD	0.698		

Acceptance criteria

There should be minimal or no effect (± 2.0% RSD is acceptable) for change in wavelength.

There should be minimal or no effect (± 2.0% RSD is acceptable) for change in sonication time.

There should be minimal or no effect (± 2.0% RSD is acceptable) for change in the diluent ratio.

Conclusion

Effects of changes in wavelength, sonication time, and diluent ratio were found within the specified limit. Thus, the method proved to be robust.

VI. Filter compatibility study

Filter compatibility study was performed on PVDF 0.45µm and Nylon 0.45 filters and the results were found as follows:

Table 15: Filter compatibility of PVDF 0.45µm

	Sample	Absorbance	% Assay	% Difference
PVDF 0.45µm	Centrifuge (As such)	0.846	100.27	-
	Discarded 3ml	0.842	99.80	0.47
	Discarded 5ml	0.836	99.09	1.18
	Discarded 7ml	0.839	99.44	0.83

Table 16: Filter compatibility of Nylon 0.45µm

	Sample	Absorbance	% Assay	% Difference
Nylon 0.45µm	Centrifuge (As such)	0.846	100.27	-
	Discarded 3ml	0.831	98.50	1.77
	Discarded 5ml	0.834	98.85	1.42
	Discarded 7ml	0.837	99.21	1.06

Here, the acceptance criteria for filter compatibility are ±2% of such a centrifuged sample with a discarded one. From the above results, **Nylon 0.45µm was found to be the best.**

VII. Solution stability

Solution stability for 0Hr and 24Hr was studied and the results were found to be as follows:

Table 17: Solution stability study

Solution type	0 Hr Stability	24 Hr Stability
Standard	99.89 %	99.89 %
Sample	99.27 %	99.45%

Here, the acceptance criteria for the stability study are ±2%. Thus, from the above results, the standard and **sample solution was found to be stable at 24 hr.**

3.7 STABILITY STUDY AS PER ICH GUIDELINES

A stability study of prepared emulgel formulations was performed as per ICH guidelines. It can be observed that all three emulgel formulations showed no major alteration in the

appearance, pH, and *in vitro* drug release study. From (Table) it can be concluded that all three prepared emulgel formulations were found to be stable upon storage for 3 months.

Table 18: Stability Study of Ketoprofen Emulgel

Parameters	**Initial**	**1 Month**	**2 Month**	**3 Month**
Appearance	White color	No change in appearance	No change in appearance	No change in appearance
pH	6.0	6.5	6.7	6.9
Drug diffusion at 4 hr(Cumulative drug release from formulation (%)±SD**)**	61.97±0.49	63.97±0.76	64.95±0.54	64.95±0.93

Table 19: Stability study of Ketoprofen+Karanja Emulgel

Parameters	**Initial**	**1 Month**	**2 Month**	**3 Month**
Appearance	White color	No change in appearance	No change in appearance	No change in appearance
pH	6.12	6.2	6.5	6.9
Drug diffusion at 4 hr(Cumulative drug release from formulation (%)±SD**)**	85.68±0.54	87.25±0.54	89.97±0.54	89.28±0.54

Table 20: Stability study of Ketoprofen+Nirgundi Emulgel

Parameters	**Initial**	**1 Month**	**2 Month**	**3 Month**
Appearance	White colour	No change in appearance	No change in appearance	No change in appearance
pH	6.5	6.4	6.6	6.6
Drug diffusion at 4 hr(Cumulative drug release from formulation (%)±SD**)**	74.49±0.67	77.49±0.45	82.42±0.35	86.42±0.95

4. CONCLUSION

The rationale of the present study was to increase the penetration of Ketoprofen into the skin. In the present study, Karanja oil and Nirgundi oil were used as (penetration enhancer) excipients in the making of Ketoprofen emulgel. Ketoprofen was found compatible with Karanja oil and Nirgundi oil by IR spectroscopy. Different formulations of Ketoprofen with Karanja and Nirgundi oils were formulated and evaluated by various evaluation parameters such as pH determination, viscosity study, globule size and distribution, spreadability study, and Ketoprofen content. Also, studied a comparative *in-vitro* diffusion study and an *in-vitro* anti-inflammatory activity study on formulated emulgels. From the diffusion study, it was concluded that oils included in the emulgel were found to boost Ketoprofen's permeability as well as its anti-inflammatory activity. The best outcomes have been achieved with F2 (Ketoprofen+Karanja oil) emulgel. The F2 formulation was validated by using validation parameters, i.e., specificity, linearity, accuracy, precision, and robustness as per ICH guidelines. In a stability study, it was found that the prepared emulgel was stable at room temperature.

5. ACKNOWLEDGEMENT

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