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FORMULATION AND EVALUATION OF TOPICAL PIROXICAM MICROEMULGEL FOR ARTHRITIS

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ABSTRACT

Background: Piroxicam is an anti-inflammatory, analgesic, and antipyretic drug. Piroxicam is widely used in the management of chronic pain. The objective of this work was to develop and analyze topical piroxicam microemulgel to improve drug solubility, enhance permeation, reduce GIT side effects reduce the frequency of the drug. **Method:** The piroxicam microemulgel was prepared by drawing the pseudo ternary phase picture and water titration procedure. Formulation was prepared by using isopropyl myristate as an oil, Tween 80 as an surfactant, n-butanol as Co-surfactant and water. It was converted to gel by using 1% Carbopol 940 and few drops of ethanolamine. The prepared formulation was characterized for thermodynamic stability, pH, droplet size, viscosity, FTIR,DSC, electrical conductivity, dye solubility drug content, and in-vitro release using a Franz diffusion cell. **Result:** Microemulgel formulation was thermodynamically stable on visual inspection after being treated with a freeze-thaw cycle and centrifugation. pH of formulation was 6.5. The mean droplet size for ME gel was 100±0.472nm. The viscosity of the microemulgel was 90.4±0.01 cps which showed Newtonian flow. FTIR and DSC studies showed that microemulgel was compatible with its excipients. Electrical conductivity and dye solubility testing confirmed that the microemulgel was O/W. Piroxicam microemulsion gel showed 89.89% drug content and the release rate of piroxicam was 98±8.63% after 48h. It followed the Korsmeyer Pappas model which means it was a hydrogel-based system. **Conclusion:** Microemulsion gel formulation obtained remarkably inflated skin retention for piroxicam over the piroxicam gel. It might act as a promising vehicle for the topical delivery of poor water-soluble drugs.

Keywords: Microemulgel, Piroxicam, Topical, Permeation.

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INTRODUCTION

Oral route is the preferable choice for drug administration **[1]** but oral and intramuscular injections cause severe toxicity and side effects in patients **[2]**. Some of the noxious effects produced by these routes include GI toxicity, renal failure and at high doses, liver enzymes are also raised **[3]**. To cope these challenges of invasive techniques of delivery of the drugs, a novel transdermal drug delivery system is introduced **[4]**. This delivery system has many advantages over the oral and intramuscular injections. High absorption rates, ease of preparation thermodynamic stability, avoidance of first pass metabolism and ease of application, makes it more beneficial delivery system than conventional type of drug delivery systems. Its non-invasive route and local effectiveness make it more compliant to the patients. Transdermal microemulsion gel, the improved form of emulsion, enhanced the solubility of poorly water-soluble drug.

Piroxicam (prototype drug) is as an NSAID use for the treatment of joint diseases such as osteoarthritis, soft-tissue disorders, rheumatoid arthritis, in post-operative pain and acute gout **[5, 6]**. It is generally use as analgesic, anti-pyretic and anti-inflammatory agent but it shows other different types of activities such as chemosuppressive and chemopreventive

effects in different kinds of carcinoma like lung, breast and colon carcinoma, etc **[7]**. Prolong use of piroxicam worsen the side effects of this drug and it leads to renal failure and cardiotoxicity **[8]**. To deliver this drug through skin is a promising method to diminish its side effects **[9]**.

Therefore, there is a need to develop a transdermal drug delivery system to minimize oral adverse effects and steady state drug levels for longer period of time. Stratum corneum acts as tough skin barrier, to overcome this barrier penetration enhancers and vehicles are used which are expensive and irritating to skin **[10]**. Components of this microemulsion system are act themselves as permeation enhancers **[9]**. Microemulsions offers many advantages over other formulations such as long-term stability, improved drug delivery, high solubilization capacity for both hydrophilic and lipophilic drug, easy preparation, and high permeability with increased bioavailability by creating the concentration gradient towards the skin **[11]**. They are biocompatible, thermodynamically stable, skin-friendly, provide convenient and cost-effective drug carrier systems. This system may overcome the barrier of oral delivery system such as GI degradation or hepatic clearance etc. In microemulsions, the oil phase like IPM interact with the lipids in layer stratum corneum, increase the drug mobility and surfactants increase solubility by increasing the partition coefficient, in result higher solubilization of drug in microemulsion delivery system **[12, 13]**.

We can enhance the drug solubility and skin penetration after making the microemulsion gel of piroxicam. It helps in making the optimized formulation. By converting simple microemulsion of piroxicam into the microemulsion gel form, we can overwhelm the problems of standard topical drug delivery systems by providing a broad interfacial area for the drug absorption.

MATERIALS AND METHODS

Piroxicam USP was gifted by Martin Dow Pharm. Ltd. Lahore. Tween 80, isopropyl myristate (IPM), n-butanol, Carbopol 940 and triethanolamine were also purchased from Sigma-Aldrich Pakistan. All the solvents and materials used were of analytical grade. Distilled water was used in all processes of formulation.

Construction of Microemulsion Phase Diagrams

The oil phase examined contained isopropyl myristate (IPM). Tween 80 was selected as surfactant and n-butanol was used as co-surfactant at S/CoS weight ratios of 1:1, 2:1, and 3:1. Pseudo-ternary phase diagrams of IPMs combined with different S/CoS weight ratios were constructed using the water titration method at 25 °C. **[14]**. The sample was titrated with water and mixed thoroughly until a clear microemulsion phase region appeared **[15]**. Clear regions corresponding to microemulsions were constructed within the triangular phase diagram using Chemix School 3.5 software.

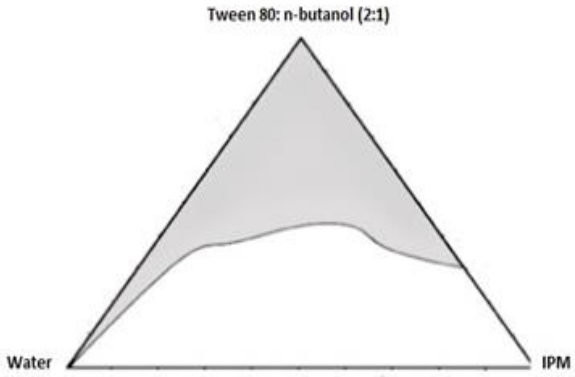

Figure 1: Pseudo-ternary phase diagram for the system IPM: Water/surfactant and cosurfactant (tween80: n-butanol in 2:1) for piroxicam microemulsion.

Preparation of Piroxicam Microemulsion

Isopropyl myristate was used as the oil phase and the surfactant/cosurfactant weight ratios used in the microemulsion were selected from the phase diagram. The percentage composition chosen for the microemulsion system was 5.5 wt% oil, 49.5 wt% water, 45 wt% S/CoS (2:1 ratio). Furthermore, due to the relatively low weight ratio of oil to water, this ratio was expected to give an o/w microemulsion system. This aspect is important because piroxicam is a lipophilic drug and is therefore preferably incorporated into the internal phase of the microemulsion. Drug was accurately weighed and added to the oil phase to represent 1% of the total weight of the formulation **[16]**. IPM oil and co-surfactant were mixed and stirred until the drug was completely dissolved **[17]**. Surfactant was then added and vortexed, and then water was added

dropwise with continued mixing. 1% Carbopol was added to the above microemulsion, vortexed, then 2-3 drops of triethanolamine was added to form a microemulgel.

Preformulation Study

Crystallinity

Solubility of drug was checked in water and in other organic solvents. A pinch of drug sample was placed on the clean glass slide and 2 drops of suitable solvent were added, in which drug was insoluble. A cover slip was placed and observed the slide under the microscope.

Hygroscopicity

0.5 g of sample Was taken on china dish and heated. The difference in weight upon drying was noted and LOD was calculated.

Melting Point

Sample was placed on the chamber of the hot plate of Fisher-johns's melting-point apparatus. Change in temperature was noted and the melting point of the drug was determined.

Angle of Repose

Inverted funnel method was used to calculate angle of repose. Heap of powdered drug was formed on paper and circle is drawn around it and its height and radius were measured to determine angle of repose by formula:

$$\theta = tan - 1 \times h/r$$

Powdered drug was filled in measuring cylinder and tapped by standard tapping procedure to find out the compressibility index and Hausner ratio by Following formulas:

$$Compressibility\ index = 100\ (Tapped\ density - Bulk\ density/Tapped\ density)$$

$$Hausner's\ Ratio = Tapped\ density/\ Bulk\ density$$

Solubility Analysis

Gravimetric Method

0.17g monobasic potassium phosphate was weighed and dissolved in 625ml of distilled water. Similarly 0.22g sodium hydroxide was weighed and dissolved it in 2.8ml of distilled water. The two solutions were mixed and final volume was made upto 25ml to make a buffer of pH 6.8.

Empty test tube was weighed. The drug piroxicam was dissolved in 10ml of pH 6.8 phosphate buffer until it started precipitating. This solution was filtered and the filtrate was heated until it evaporated. After heating test tube was again weighed. Difference between the two weights were calculated and then the solubility of drug in this buffer was determined.

Standard Calibration Curve

Stock solution and a working solution was prepared then dilutions of strength 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml were formulated. These solutions were analyzed on a spectrophotometer at the wavelength of 354nm. The values were noted and a graph was made on excel by comparing these values.

Partition Coefficient

The buffer of pH 6.8 was prepared and 10ml of it was taken in the separating funnel. 10ml of methanol was added to it. 100mg of piroxicam was weighed and added to the separating funnel. The mixture was shaken for 30 minutes and funnel was made to stand for 15 minutes, so the layers separated out. Both layers were extracted and filtered in a separate beaker. Buffer extract was diluted with distilled water and methanol extract was diluted with methanol then it was analyzed on a spectrophotometer.

Evaluation Tests of Piroxicam Microemulsion Gel Preparations

The evaluation tests were performed on piroxicam microemulgel preparations, included viscosity, globule size, pH, and rheological measurements.

Organoleptic test

Organoleptic tests were used to assess the appearance, odor, color, and clarity of the preparations. These studies were done every 2 weeks till 8 weeks.

pH Measurement

The pH of the tested microemulgel formulations was determined by a digital pH meter.

Stability Test

Accelerated stability test was carried out by exposing the drug to a temperature of 40°C, far higher than ambient temperature (25°C) to observe the degradation occurred upon applying stress.

Similarly mechanical stress was applied using centrifuge. Samples taken from microemulsion gel preparation were centrifuged at 3800 rpm for 5 hrs. This treatment is equivalent to the gravitational effect for 1 year. Organoleptic observations were done on the physical condition of the preparation before and after centrifugation. This experiment was performed 3 times.

Particle Size Analysis

The droplet size of the microemulgel was analyzed by using scanning electron microscope. A calibrated micrometer slide containing a drop of microemulsion was observed under eyepiece and droplet size was determined.

Rheological Studies

Brookfield Viscometer (DV-E Brookfield Viscometer Model-LVDVE) was used to determine the viscosity of gel preparation of microemulsion. Brookfield Viscometer comprised of a stationery cup, and a rotating spindle. Rotating spindle was immersed in the sample of microemulsion. Since gel preparation of microemulsion had higher viscosity therefore, small spindle having small diameter and small surface area was used. The spindle was rotated in the gel preparation until a stable reading was obtained on dial of Viscometer. The procedure was repeated three times to obtain reproducible results **[18]**.

Electrical Conductivity

The electrical conductivity test was performed to confirm whether microemulgel was oil in water or water in oil microemulsion. The conductivity (σ) of the formulated sample was measured using a conductivity meter. Conductivity was measured with the help of a probe and a meter. Voltage was applied between two electrodes in a probe immersed in the microemulsion. The drop in voltage caused by the resistance of the microemulsion was used to calculate the conductivity.

DSC (Differential Scanning Calorimeter)

Using a DSC-60 calorimeter (SHIMADZU), samples were heated under liquid nitrogen from ambient to 400 °C at 10 °C/min until approximately 5 mg of sample in a sealed aluminum pan, ran a DSC scan. Pure piroxicam, vehicle, and ME gel were tested separately and the resulting thermograms were compared.

Dye solubility test

10 µl of methylene blue which is a water soluble dye was added to the emulsion to check if dye spreads uniformly or form cluster in order to determine the type of emulsion whether it was O/W or W/O

Fourier transform infrared spectra analysis (FTIR)

Functional groups present in preparation were determined by using FTIR scanning microscope. When IR rays were passed through sample, the absorbed radiations converted into vibrations or stretching (spectrum signals from 4000cm-1 to 400cm-1) that indicated functional groups and the type of bonds present in molecular fingerprint of the sample.

Release Study

Franz diffusion cell with rabbit membrane was used to test release rate of piroxicam from different microemulgel formulations. Abdominal skin of rabbit was shaved and excised. Subcutaneous fat was separated, and examined its integrity. The membrane was placed such that the mucous membrane was upward and epidermis was downward. The membrane was then fixed between the donor and receiver compartments of Franz diffusion cell. It was filled with 9 ml of pH 6.8 phosphate buffer The receptor fluid was stirred continuously by externally driven magnetic bars at 300 rpm throughout the experiment. Piroxicam microemulsion was placed in the donor chamber. After 0.5, 1, 2, 3, 4, 5 upto 48hr time span, 0.5ml sample was taken from receiver chamber for spectrophotometric analysis and replaced instantly with an equal volume of buffer solution. Samples were analyzed by UV visible spectrophotometer at 354nm.

Drug Content

1ml of emulsion were added to 9ml of 6.8 pH buffer and stirred for 30 minutes. Mixture were kept at room temperature for 24 hours. After a day it was again stirred for 30 minutes. Then the solution was centrifuged at 4000 rpm for 30 minutes. A clear supernatant were taken from it.

1ml from supernatant were added to 9 ml buffer solution. Then it was checked on UV spectrophotometer at 354nm and the absorbance was measured. The concentration of piroxicam was obtained by using standard calibrated curve of the drug.

Standard Calibration Curve

50ml of stock solution was formulated by adding 50mg of piroxicam in 10ml ethanol, and making final volume upto 50 ml with buffer of pH 6.8. Then working solution was prepared by taking 5ml of stock solution and adding in 50ml of distilled water (1ml = 0.1mg/100µg) which was further processed to make dilutions of 2µg, 4µg, 6µg, 8µg and 10µg. Spectrophotometer was used to determine the absorbance by each dilution at λ_{max} of 285nm. The

absorbance shown by each dilution was used to draw standard curve.

RESULTS AND DISCUSSION

Preformulation Studies

Table 1: Pre-formulation Tests

Tests	Results
Angle of repose	30
Compressibility index	20
Hausner's ratio	1.2
Partition coefficient	3.09
Melting point	199.7°C
Hygroscopicity	4.85 ± 0.41%
Crystallinity	Monoclinic

The angle of repose, compressibility index, and Hausner's ratio values showed according to USP Pharmacopoeia piroxicam drug has good flow properties.

The partition coefficient value indicated that piroxicam drug was lipophilic and belonged to BSC Class III. The melting point value revealed that it was piroxicam drug, and hygroscopic value indicated that piroxicam was hygroscopic in nature. Crystalinity testing proved that piroxicam was crystalline in nature.

Postformulation Studies

Determination of Solubility

The solubility of Piroxicam microemulgel was checked in different oils (IPM, Propylene glycol, benzyl alcohol, and oleic acid) surfactant (tween 60, tween 80, and span 20), and cosurfactant (n butanol, polyethylene glycol, and diethylene glycol). Solubility of piroxicam was highest in IPM oil, Tween 80 surfactant, and n- butanol co-surfactant as compared to other oils, and surfactants. IPM, Tween 80, and n- butanol form clear and stable microemulsions because of the highest compatibility of these surfactant co- surfactants and oil.

Construction of Microemulsion Phase Diagram

It was observed that the highest concentration area in the phase diagram was with 2:1 of surfactant to Co- surfactant. Micelle formation was optimum with this concentration because of the maximum solubilizing capacity of surfactant and co surfactant with this ratio **[19]**.

Organoleptic Evaluation

The piroxicam microemulsion was transparent with light yellow color and it smelled like alcohol. Piroxicam microemulsion did not undergo phase separation after adding gel in the formulation which showed it was a stable microemulsion [**20]**.

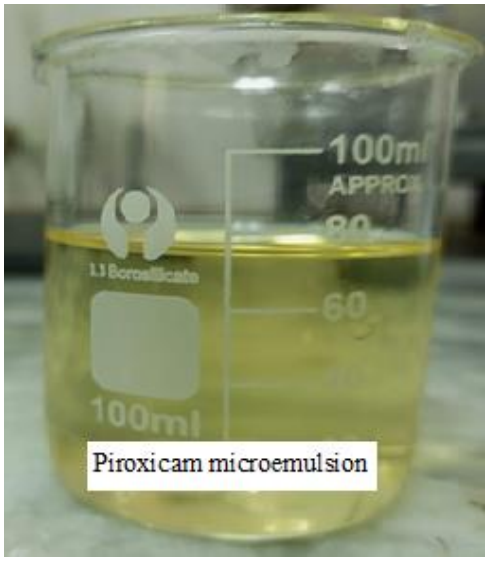

Figure 2: Piroxicam microemulsion.

Measurement of Droplets Size Distribution

The size of the droplets was 100nm. This optimum size of piroxicam microemulsion gel showed that surfactant, co-surfactant, and oil phases were highly compatible with each other.

Measurement of Viscosity of Piroxicam Microemulsion Gel

The viscosity of the piroxicam microemulsion gel was 90.4±0.01 cps. Formulation exhibited Newtonian flow as their viscosity did not show significant variation upon applying some force as stirring. This indicated that microemulsion gel had the capacity to withstand a small amount of stress and its viscosity would not change significantly during handling and storage.

Measurement of pH of Piroxicam Microemulsion Gel

The pH of microemulsion gel was 6.5 which showed that the increased S/CO ratio has increased the pH of the microemulsion.

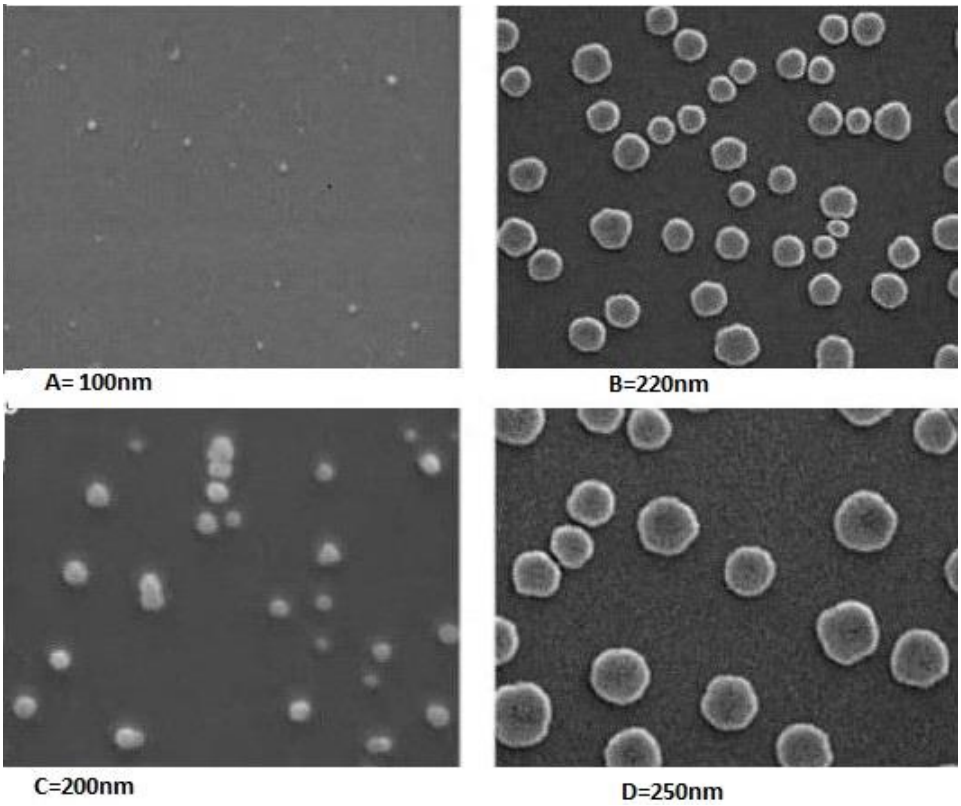

Figure 3: Particle size of piroxicam microemulsion (100nm).

Measurement of Electrical Conductivity of Piroxicam Microemulsion Gel

The conductivity of the piroxicam microemulsion gel was 1Us/cm which showed it was an O\W Microemulsion gel. Pure water conductivity is 0.0 5Us/cm. The higher conductivity value of microemulsion was due to dissolved salts and oils. When there is more salt and oils dissolved in the microemulsion then the conductivity value will be higher. Piroxicam microemulsion gel conductivity was higher because of the high solubility of oil, surfactant, co-surfactant, and water **[18]**.

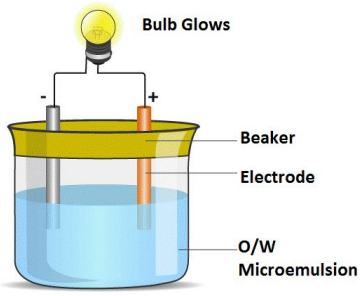

Figure 4: Electrical Conductivity displayed by O/W piroxicam microemulsion.

Dye Solubility Test

Dye spread uniformly in the microemulsion gel so it was concluded that the continuous phase was water. Therefore, the piroxicam microemulsion gel was an o/w type of microemulsion **[18]**.

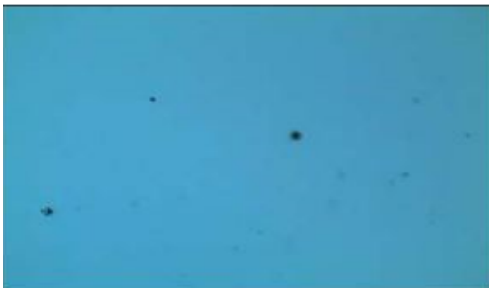

Figure 5: Methylene blue dye distributed showing O/W piroxicam microemulsion

FTIR Studies

The characteristic peaks or FTIR bands of pure piroxicam were at 1632.87 cm^{-1} (C=O) 3338.18 cm^{-1} (NH), 1434.56 cm^{-1} (CN) as shown in the **Fig. 6**. Piroxicam quality peaks were existing in the piroxicam microemulsion and did not represent any auxillary peaks or any noteworthy peak shifts that indicated that no chemical deterioration or

inconsistency between drug and formulation excipients were recognized. The FTIR suggested that the formulation was well constructed and stable.

DSC Studies

As shown, pure piroxicam exhibits a thermogram peak at 200°C. The ME gel thermogram showed the presence of piroxicam with a slight peak shift. A prominent piroxicam peak indicated that the drug was solubilized in the microemulsion-gel system. DSC was used to determine incompatibilities between piroxicam and various excipients. There were no incompatibilities between the excipient and piroxicam as both showed peaks in the ME gel thermograms.

Release Studies

The maximum release of piroxicam microemulgel was 98% at 48 hours which indicated that piroxicam microemulgel has good topical release properties.

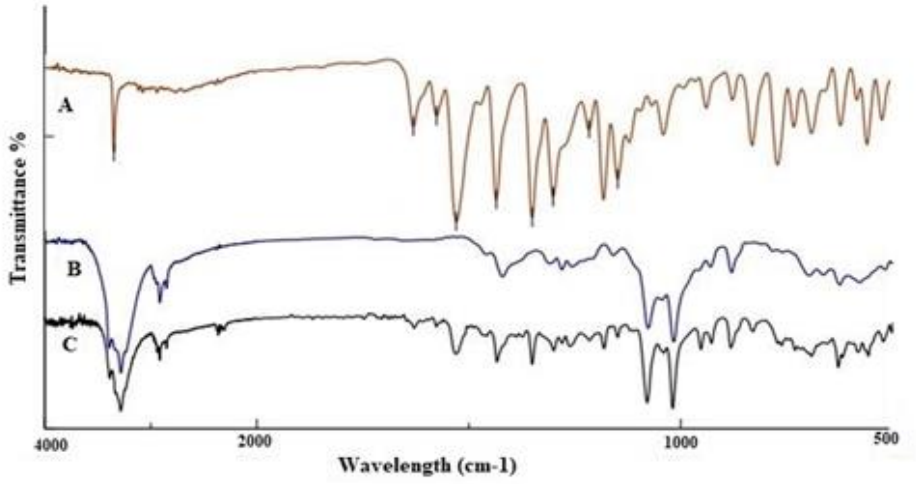

Figure 6: The graph has displayed data from the FTIR measurement of percentage transmittance (%T), corresponding to piroxicam peak at 1526.38 cm^{-1}.

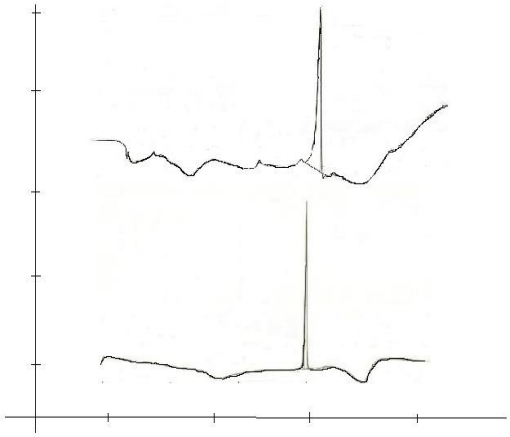

Figure 7: Piroxicam exhibited a sharp endothermic peak at around 201.84 °C indicating its pure crystalline cubic form.

Table 2: Kinetic release from piroxicam microemulsion gel.

Values	Zero order	First order	Higuchi model	Korsmeyer peppas	n value
Rsqr	0.9401	0.9839	0.9079	0.9927	0.749
AIC	79.3411	63.6078	84.4943	50	
MSC	2.6476	3.9587	2.2182	4.5929	

Kinetics Studies

The kinetics studies showed that piroxicam microemulgel followed the first order model as well as it followed the Korsmeyer Peppas model with an n value of 0.749. So, it was a hydrogel-based microemulsion that followed both combined dissolution diffusion control system for the release of drug and due to its dependency on first order, the release of drug was also concentration dependent.

Drug Content

Piroxicam microemulsion gel showed 89.89% drug content. This high drug content demonstrated that drug was highly soluble and compatible with the excipients.

CONCLUSION

The study illustrated that the microemulsion technique can be employed to enhance the solubility and skin permeability of piroxicam. Microemulsion gel was successfully prepared with 1% carbopol 940 as a gelling agent, making it sustained release and increasing its residence time. The permeability of piroxicam achieved from the microemulsion within 36 hours was observed 98% which proved the permeability enhancement of drug with the use of microemulsion-based gel system. The results indicate that this formulation may act as promising vehicle for topical delivery of piroxicam.

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