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PREFORMULATION STUDY OF PIROXICAM

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Keywords:

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ABSTRACT: Preformulation testing is the first step in the rational development of dosage forms of a drug substance. The preformulation investigations confirm that there are no significant barriers to the compound's development as a marketed drug. The formulation scientist uses this information to develop an efficacious, stable and safe dosage form. The main objective of the present research work was to do preformulation study of piroxicam drug. Piroxicam (PC) is one of the most potent non-steroidal anti-inflammatory agents that also has anti-pyretic activity and has been used for the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, tendinitis, bursitis, and for the pain that is not related to a musculoskeletal system and traumatic contusions. The Principal advantage of piroxicam is its long half-life, which permits the administration of a single daily dose. As the piroxicam is very effective drug, many researchers use this drug in their research. This research paper helps those people who want to use piroxicam drug for their research.

INTRODUCTION: Preformulation testing is the first step in the rational development of dosage forms of a drug substance. The preformulation study is the process of optimizing the delivery of the drug through the determination of physicochemical properties of the new compound that could affect drug performance and development of an efficacious, stable and safe dosage form. It gives the information needed to define the nature of the drug substance and provide a framework for the drug combination with pharmaceutical excipients in the dosage form.

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Hence, Preformulation studies were performed for the obtained sample of the drug for identification and compatibility studies. Preformulation may be described as a phase of the research and development process where the preformulation scientist characterizes the physical, chemical and mechanical properties of a new drug substance, to develop stable, safe and effective dosage form. The preformulation investigations confirm that there are no significant barriers to the compound's development as a marketed drug. The formulation scientist uses this information to develop dosage forms. Preformulation is a multidisciplinary development of a drug candidate.

Principal Areas of Preformulation:
Bulk Characterization:

i. Crystallinity and polymorphism
ii. Hygroscopicity

iii. Fine particle characterization.
iv. Powder flow.

Solubility Analysis:

i. Ionization constant – pKa.
ii. pH solubility profile.
iii. Common ion effect – K_{SP}.
iv. Thermal effects.
v. Solubilization
vi. Partition coefficient.
vii. Dissolution

Stability Analysis:

i. Stability in toxicology formulation.
ii. Solution stability– pH stability profile.
iii. Solid state stability - Bulk stability, Compatibility.

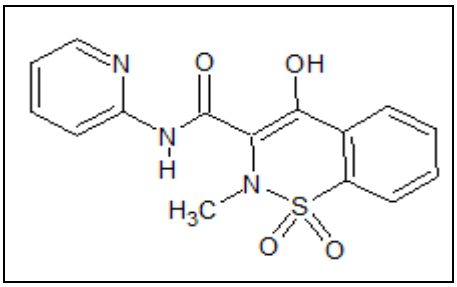

FIG. 1: PIROXICAM

Piroxicam is an oxicam derivative medication belonging to non steroidal anti-inflammatory drugs (NSAIDs) group, used to treat moderate to severe inflammatory diseases such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis (Bechterew's disease), tendinitis, bursitis, and for pain that is not related to musculoskeletal system *e.g.* primary dysmenorrhea and postoperative pain. It reduces pain, joint swelling, morning stiffness, and improves the functionality of the joints during chronic polyarthritis [2].

PC has been classified in the biopharmaceutics Drug Classification system as a Class II drug with low solubility and high permeability. It demonstrates a slow and gradual absorption *via* the oral route and has a long half-life of elimination, rendering a prolonged therapeutic action and a delayed onset of anti-inflammatory and analgesic effect [3] PC is well absorbed following oral administration; however, its use has been limited by a number of side effects, including bleeding and ulceration.

Although as piroxicam have different side effects but, its pharmacological action is more as piroxicam is an effective anti-inflammatory agent; it is an inhibitor of prostaglandin biosynthesis. The Principal advantage of piroxicam is its long half-life, which permits the administration of a single daily dose. Piroxicam is approved in the United States for the treatment of rheumatoid arthritis and osteoarthritis. It also has been used in the treatment of ankylosing spondylitis, acute musculoskeletal disorders, dysmenorrhea, postoperative pain and acute gout [4]. This paper helps to those people who want to use piroxicam drug for their research. As the piroxicam is very effective drug many researchers use this drug some of the research paper is listed below:

1. Formulation and optimization of piroxicam orodispersible tablets by central composite design [4].
2. Development of proniosomal drug delivery with a different type of penetration enhancers [5].
3. Characterization and *in-vitro* evaluation of piroxicam suppositories [6].
4. Novel double loaded transferosomes: evidence of superior anti-inflammatory efficacy- a comparative study [7].
5. Design and evaluation of piroxicam microemulsion [8].
6. Pharmaceutical cocrystal of piroxicam: design, formulation, and evaluation [9].
7. Formulation and characterization of flexible phosphatidylcholine vesicles for systemic delivery of piroxicam [10].
8. Systematic development of transethosomal gel system of piroxicam: formulation optimization, *in-vitro* evaluation and *ex-vivo* assessment [11].
9. Development and validation of a sensitive UV method for piroxicam: application for skin permeation studies [12].

EXPERIMENTAL WORK:

Organoleptic Properties: The drug samples were studied for appearance, color, and odor. The results are shown in **Table 1**.

Melting Point: The melting points of the drugs were determined by an open capillary method using the melting point apparatus. The melting point is shown in **Table 2**.

Ultraviolet Spectroscopy: [13, 14]

Determination of Maximum Wavelength (λ_{max}):

a. In Methanol: Drug (10 mg) was accurately weighed and transferred to 100 ml volumetric flask, volume was made up to the mark with methanol to obtain strength 100 µg/ml. It was used as a standard stock solution. This stock solution was further diluted suitably to give a concentration of 10 µg/ml. The UV spectrums were recorded in the range 200-400 nm by using UV-Visible double beam spectrophotometer (Shimadzu 2450). The wavelength of maximum absorption (λ_{max}) was determined and is shown in **Fig. 2** and **Table 3**.

b. In methanolic HCl: [15] Drug (10 mg) was accurately weighed and transferred to 100 ml volumetric flask, volume was made up to the mark with 0.1 M methanolic HCl to obtain strength 100 µg/ml. It was used as a standard stock solution. This stock solution was further diluted suitably to give a concentration of 10 µg/ml. The UV spectrums were recorded in the range 200-400 nm by using UV-Visible double beam spectro-photometer (Shimadzu 2450). The wavelength of maximum absorption (λ_{max}) was determined and is shown in **Fig. 3** and **Table 3**.

c. In Phosphate Buffer (pH-7.4): 20 mg of drug was accurately weighed, transferred into a 100 ml volumetric flask and dissolved in 15 ml of methanol. The volume was made up to 100 ml using PBS pH 7.4 to get a concentration of 200 µg/ml. From the prepared stock solution, 10 ml solution was withdrawn and transferred to another 100 ml volumetric flask and volume were makeup to 100 ml to get a concentration of 20µg/ml. The UV spectrums were recorded in the range 200-400 nm by using UV-Visible double beam spectrophotometer (Shimadzu 2450). The wavelength of maximum absorption (λ max) was determined and is shown in **Fig. 4** and **Table 3**.

Determination of Beer-Lambert's Plot:

a. In Phosphate Buffer (pH-7.4): 20 mg of drug was accurately weighed, transferred into a 100 ml volumetric flask and dissolved in 15 ml of methanol. The volume was made up to 100 ml using PBS pH 7.4to get a concentration of 200 µg/ml. From the prepared stock solution, 10 ml solution was withdrawn and transferred to another 100 ml volumetric flask and volume was makeup to 100 ml to get a concentration of 20 µg/ml. From the above solution 1, 2, 3, 4, and 5 ml of solutions were separately transferred into 10 ml volumetric flasks respectively, and volume was made up to 10 ml to get a concentration of 2, 4, 6, 8, 10 µg/ml respectively. To scan the wavelength maxima 20 µg/ml solution was taken in a quartz cuvette and scanned on UV-Visible double beam spectro-photometer in range of 200-400 nm. The above-prepared samples were analyzed at 354nm (λ_{max}). Calibration Curve is shown in **Fig. 5**.

b. In methanolic HCl: [15] 10 ml of 100 µg/ml standard stock solution was diluted up to 50ml to obtain a standard working solution of 20 µg/ml concentration which was used for further dilutions of the calibration curve. Aliquots (2.5, 3.7, 5.0, 6.2, 7.5) ml of 20 µg/ml working standard solution corresponding to 2.5-7.5 µg/ml were taken in a series of 20 ml volumetric flask and volume made up with 0.1M methanolic HCl. The absorbance measurements of these solutions were carried out against 0.1M methanolic HCl as blank at 334.5nm. A calibration curve was plotted in **Fig. 6**.

c. In Methanol: 20 mg of drug was accurately weighed, transferred into 100 ml volumetric flask and dissolved in 15 ml of methanol. The volume was made up to 100 ml using methanol get a concentration of 200 µg/ml. From the prepared stock solution, 10 ml solution was withdrawn and transferred to another 100 ml volumetric flask and volume was makeup to 100 ml to get a concentration of 20 µg/ml.

From the above solution 1, 2, 3, 4, and 5 ml of solutions were separately transferred into 10ml volumetric flasks respectively, and volume was made up to 10 ml to get a concentration of 2, 4, 6, 8, 10 µg/ml respectively. To scan the wavelength maxima 20µg/ml solution was taken in a quartz cuvette and scanned on UV-Visible double beam spectrophotometer in range of 200-400 nm. The above-prepared samples were analyzed at 333nm (λ_{max}). Calibration Curve is shown in **Fig. 7**.

Solubility Study of Drug: [15] Solubility studies of the drug were carried out in different types of solvents which are used for further study. Saturated solutions were prepared by adding an excess drug to the vehicles and shaking on the shaker (REMI

DGS-2) for 48 h at 25 ± 0.5 °C under constant vibration. After this period the solutions were filtered, diluted and analyzed by UV spectrophotometer. Three determinations were carried out for each sample to calculate the solubility of the drug. The results are shown in **Table 7**.

Fourier Transform Infrared Spectroscopy of Drug: The infrared spectra of the pure drug were recorded by Shimadzu FT-IR spectrometer. Samples were prepared by KBr disc method (2 mg sample in 100 mg KBr) and examined in the transmission mode. Each spectrum was measured over a frequency range of 4000-400 cm^{-1}. The results are shown in **Table 8** and **Fig. 8**.

Differential Scanning Calorimetry (DSC) Study of Drug: DSC analysis was performed using Shimadzu-Thermal Analyzer DSC 60 on 2-5 mg samples. The sample was heated in an open nitrogen pan at a rate of 10 °C/min conducted over a temperature range of 30 to 230 °C for Piroxicam under a nitrogen flow of 2 bar pressure. Thermogram, as shown in Fig. 9 and inference, showed in **Table 9**.

Partition Coefficient (Kp): [16] The partition coefficient of the drug was determined by shaking equal volumes of oil and the aqueous phase in a separating funnel. A drug solution of 1 mg/ml was prepared in distilled water, and 50 ml of this solution was taken in a separating funnel and shaken with an equal volume of octanol for 10 min and allowed to stand for 24 h with intermittent shaking. Then, the aqueous phase was assayed before and after partitioning using a UV spectrophotometer to get the partition coefficient values which is shown in **Table 10**.

RESULT AND DISCUSSION:

Organoleptic Properties: The Sample of drug received was studied for its organoleptic characters such as color, odor, and appearance as it is one of the first criteria for identification of compound and it shows results/properties which comply with reported literature standards. The result is presented in the following **Table 1**.

TABLE 1: COMPARISON OF THE RESULT OF ORGANOLEPTIC CHARACTERS OF DRUG SAMPLE WITH THE REPORTED STANDARDS

S. no.	Identification Test	Observed Result	Reported Standard [17,18]
1	Appearance	Powder	Off-white to light tan or light yellow powder
2	Colour	White	White / Off-white
3	Odor	Odorless	Odorless

It is complying with the description that is found in the literature.

Melting Point: According to Indian Pharmacopoeia melting range/temperature of a substance is defined as those points of temperature within which / the point at which the substance begins to coalesce and is completely melted except as defined otherwise for certain substances [68]. The melting point of the drug complies with the reported literature values. The melting point of the drug was observed to be in the range of 197 ºC-199 ºC with decomposition, *i.e.* the substance characterize as it starts to melt which is shown in **Table 2**.

TABLE 2: COMPARISON OF THE RESULT OF THE MELTING POINT OF DRUG SAMPLE WITH THE REPORTED STANDARDS

S. no.	Identification Test	Observed Result	Reported Standard[17]
1	Melting Point	197 ºC - 199 ºC	198 ºC - 200 ºC

Ultraviolet Spectroscopy:

Determination of Maximum Wavelength (λ_{max}): Maximum wavelength (λ_{max}) is specific for every drug substances, and it is also one of the identification criteria. The maximum absorbance is for drug taken in methanol, methanolic HCl and phosphate buffer (pH- 7.4). Observed peak and reported standard peak are shown in **Table 3**.

TABLE 3: MAXIMUM WAVELENGTH (λ_{max}) OF THE DRUG IN METHANOLIC HCl, METHANOL AND PHOSPHATE BUFFER

Solvent	λ_{max} (nm)	
	Observed Peak	Reported standard[19]
Methanol	333	333
Methanolic HCl	334.5	334
Phosphate buffer (pH 7.4)	354	354

λ_{max} for the drug in methanol, methanolic HCl, and phosphate buffer (pH-7.4) was found, and it is shown in **Fig. 2**, **Fig. 3**, and **Fig. 4** respectively.

Spectra for the drug in methanol observed in the range of 200 nm to 400 nm which it shows absorption maxima at about 227 nm and 334.5 nm and minimum at about 242 nm which is shown in **Fig. 2**.

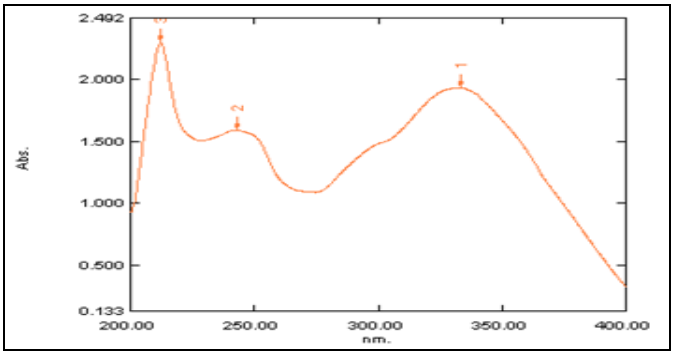

FIG. 2: λ_{max} FOR THE DRUG IN METHANOL

Spectra for the drug in methanolic HCl observed in the range of 220 nm to 400 nm for 0.0007% w/v solution of 0.01M methanolic HCl - absorption maxima at about 242 nm and 334 nm and minimum at about 270 nm which is shown in **Fig. 3**.

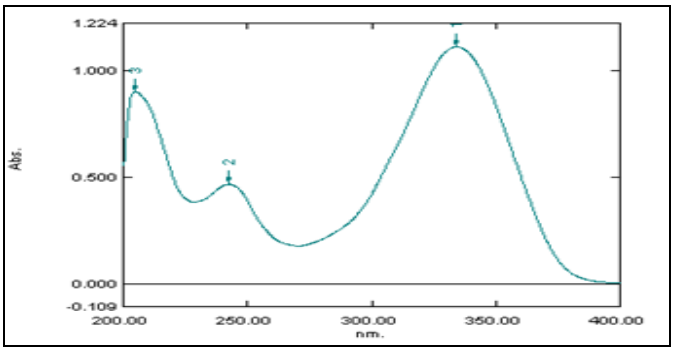

FIG. 3: λ_{max} FOR THE DRUG IN METHANOLIC HCl

Spectra for the drug in phosphate buffer (pH-7.4) observed in the range of 200 nm to 400 nm for phosphate buffer (pH-7.4). This spectrum shows four peak absorption maxima at about 221 nm, 250 nm, and 354 nm and minimum at about 279 nm which is shown in **Fig. 4**.

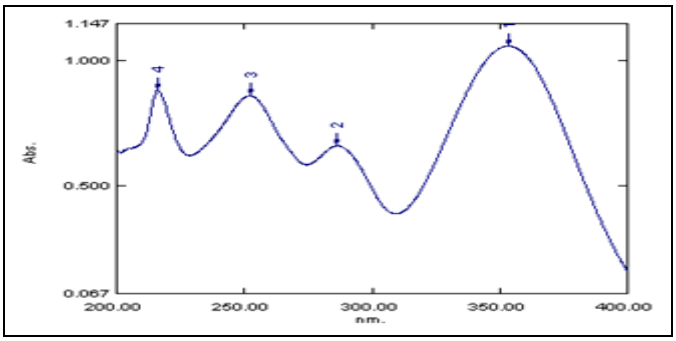

FIG. 4: λ_{max} FOR THE DRUG IN PHOSPHATE BUFFER (pH-7.4)

Preparation of Beer Lambert's Plot:

In Phosphate buffer (pH 7.4): Beer Lambert's plot of the drug was prepared in Phosphate buffer (pH 7.4).

A linear relationship was obtained in between concentration (2-10 µg/ml), and the absorbance of the drug in phosphate buffer (pH 7.4) with an R^2 value of 0.999 at 276 nm is shown in calibration curve shown in **Fig. 5** and line equation, y= 0.072x-0.0038.

TABLE 4: ABSORBANCE VALUE FOR DIFFERENT CONCENTRATIONS OF DRUG IN PHOSPHATE BUFFER (pH 7.4)

S. no.	Concentration (µg/ml)	Absorbance (nm)
1	0	0.000
2	2	0.140
3	4	0.287
4	6	0.429
5	8	0.574
6	10	0.717

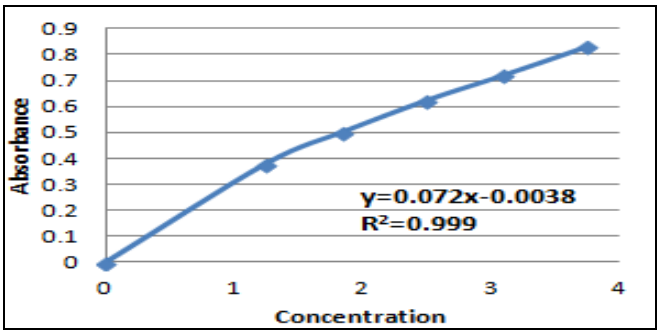

FIG. 5: BEER LAMBERT's PLOT OF THE DRUG IN PHOSPHATE BUFFER (pH 7.4)

In Methanolic HCl: Beer Lambert's plot of drug sample was prepared in methanolic HCl. A linear relationship was obtained in between concentration (1.25-3.75 µg/ml) and the absorbance of the drug in methanolic HCl with an R^2 value of 0.9979 at 276 nm is shown in calibration curve shown in **Fig. 6** and line equation, y= 0.1788x-0.1667.

TABLE 5: ABSORBANCE VALUE FOR DIFFERENT CONCENTRATIONS OF DRUG IN METHANOLIC HCl

S. no.	Concentration (µg/ml)	Absorbance (nm)
1	0.00	0.000
2	1.25	0.381
3	1.85	0.503
4	2.5	0.624
5	3.1	0.721
6	3.75	0.831

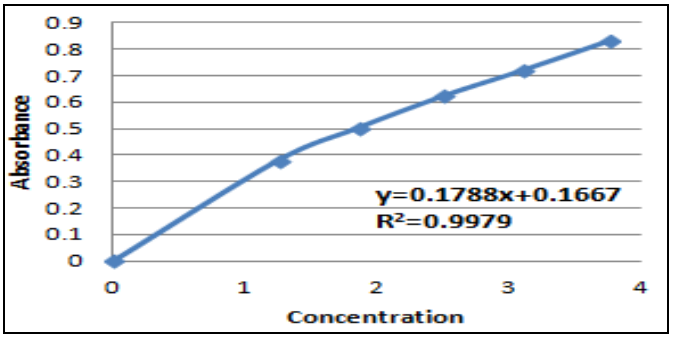

FIG. 6: BEER LAMBERT's PLOT OF THE DRUG IN METHANOLIC HCl

In Methanol: Beer Lambert's plot of the drug was prepared in Methanol. A linear relationship was obtained in between concentration (2-10 µg/ml) and the absorbance of the drug in Methanol with an R^2 value of 0.9999 at 276 nm is shown in calibration curve **Fig. 7** line equation, y=0.0728 x-0.0005.

TABLE 6: ABSORBANCE VALUE FOR DIFFERENT CONCENTRATIONS OF DRUG IN METHANOL

S. no.	Concentration (µg/ml)	Absorbance (nm)
1	0	0.000
2	2	0.144
3	4	0.296
4	6	0.436
5	8	0.582
6	10	0.728

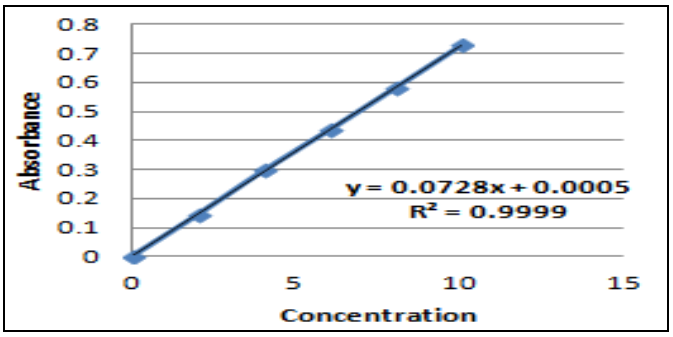

FIG. 7: BEER LAMBERT's PLOT OF THE DRUG IN METHANOL

Solubility Study of Drug: A test for solubility becomes a test for purity only where a special quantitative test is given in the individual monograph and is an official requirement. According to USP 2009, piroxicam is slightly soluble in ethanol and very slightly soluble in water, in dil. acids and most organic solvents [21]. Solubility study of drug sample was studied in different types of solvent and data shows that drug was very slightly soluble in water, soluble in phosphate buffer (pH-7.4) and freely soluble in rest of another solvent which is sown in **Table 7**.

TABLE 7: SOLUBILITY OF DRUG IN A DIFFERENT SOLVENT

S. no.	Solvent	Solubility (mg/ml)
1	Span 80	4.2
2	Lecithin	2.6
3	Isopropyl myristate	2.51
4	Propylene glycol	7.1
5	Oleic acid	5.20
6	DMSO	5.8
7	PEG 400	2.663
8	Methanol	5
9	Phosphate buffer (pH-7.4)	0.592
10	Water	0.0076

Fourier Transform Infrared Spectroscopy of Drug: As we know, the infrared spectroscopy mostly used for the identification of organic compound whose spectra are complex and provides numerous maxima and minima that are useful for comparison purpose. Infrared spectroscopy finds widespread application to qualitative and quantitative analysis as no two compounds (except optical isomers) give similar absorption spectra in the IR region. The powdered mixture of drug sample and KBr was taken in a sampler, and the spectrum was recorded by scanning in the wavelength region of 4000-400^{-1}cm using FTIR spectrophotometer.

The FTIR spectra of piroxicam were taken which is shown in **Fig. 8**. The principal peak for IR of drug sample matched with the standard spectrum for Piroxicam which is shown in **Table 8**.

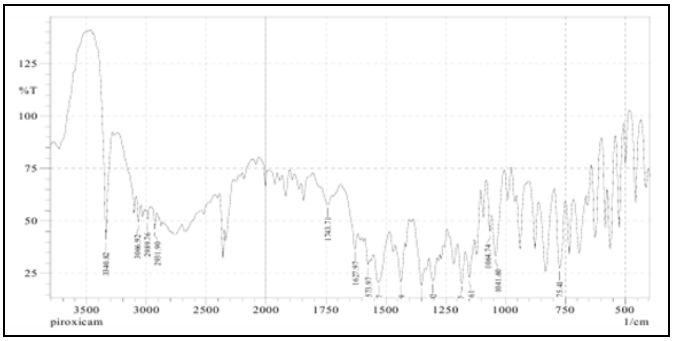

FIG. 8: IR SPECTRA OF DRUG

TABLE 8: INTERPRETATION OF IR OF DRUG

Characteristic functional group	Standard range (cm^{-1})[21]	Reference Peaks (cm^{-1})[22]	Observed peak (cm^{-1})
–OH and –NH streaching	3650-3300	3338cm^{-1}	3340.82
Aromatic -C=C-H	3300-2700	3065	3066.92
C=O streaching	1850-1680	1630	1743.71
Aromatic -C=C-	1680-1450	1574	1627.97
Ar-NH	1360-1250	1351	1350.22
N-CH_3 streaching	1220-1050	1150	1180.47
-SO_2-N= Group	1070-1050	1065.2	1064.74
o-disubstituted phenyl	750	775	775.41

Differential Scanning Calorimetry (D.S.C.) Study of Drug: The endotherm of melting corresponds to the portion of the DSC curve that is far from the baseline and later returns to it. Melting is a physical process that results in the phase transition of a substance from solid to liquid. This occurs when the internal energy of the solid increase, typically by the application of heat which increases the substance's temperature to the melting point. In DSC, as the temperature increases, the sample eventually reaches its melting temperature (Tm). The melting process results in an endothermic peak in the DSC curve.

DSC studies were performed for drug sample. The DSC thermogram of commercial drug sample is presented in **Fig. 9** and interpretation is shown in **Table 9**. Thermogram of DSC of the drug shows melting in the range between 203.45 to 206.11 ºC, and the sharp peak was seen at 201.20 ºC. So, it shows that it is an endothermic reaction. The following figure shows the endothermic peak of the drug with height -25.76 mW.

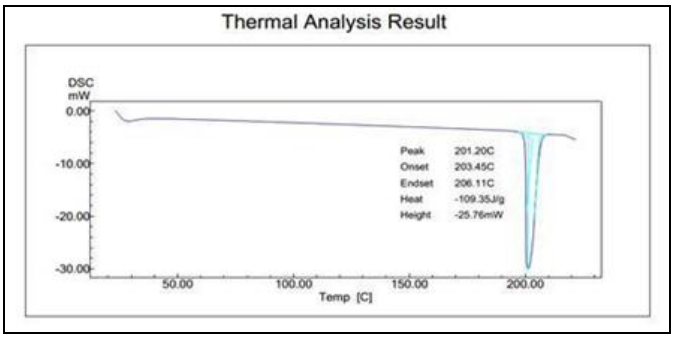

FIG. 9: DSC THERMOGRAM OF DRUG

TABLE 9: INTERPRETATION OF DSC OF DRUG

Name of Substance	Melting Point	Inference
Piroxicam	201.20 ºC	Sharp endothermic peak obtained and it matches with official standard.

Partition Coefficient (Kp): The permeability coefficient was found to be 3.09 which indicate that drug sample is lipophilic and come under high (value 3-4) class and results were shown in **Table 10**. It is worth noting that this is a log *P*=0 means that the compound is equally soluble in water and the partitioning solvent. If the compound has a log *P*=5, then the compound is 100,000 times more soluble in the partitioning solvent. A log *P*=-2 means that the compound is 100 times more soluble in water, *i.e.* it is quite hydrophilic (Kohler *et al*., 1988). Therefore, from obtained result drug have 1000 times more soluble in the partitioning solvent (octanol).

TABLE 10: COMPARISON OF THE RESULT OF PARTITION COEFFICIENT (Kp) DRUG SAMPLE WITH THE REPORTED STANDARDS

S. no.	Observed value	Reported standard[54]
1	3.09	3.06

CONCLUSION: In the present work, the preformulation study of piroxicam drug was done. Preformulation studies have a significant part to play in anticipating formulation problems and identifying a logical path in both liquid and solid dosage form technology. This study shows a satisfactory result for all characterization such as organoleptic properties, calibration curve, DSC, partition coefficient, *etc*. All results matched with the reported standard.

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CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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