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RESEARCH ARTICLE

Preformulation Parameters Characterization towards Design, Development and Formulation of Etodolac Loaded Nanoparticles

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Abstract

The purpose of the present study was to systematically investigate some of the important physicochemical properties of Etodolac loaded nanoparticles. Etodolac is a non-steroidal anti-inflammatory drug, is used to manage rheumatoid arthritis associated symptoms. Almost all drugs are marketed as tablets, capsules or both. Prior to the development of these major dosage forms, it is essential that pertain fundamental physical and chemical properties of the drug molecule and other divided properties of the drug powder are determined. This information decides many of the subsequent events and approaches in formation development. A modified release nanoparticle of Etodolac was prepared by solvent evaporation method using EUTRAGIT RS 100 as coating material. Hence along with selection of excipients, the preformulation study of drug Etodolac is completed for successful formulation of modified release nanoparticles. Preformulation studies included solubility, pKa, dissolution, melting point, assay development, stability in Solution, stability in solid state; microscopy, bulk density, flow properties, excipients compatibility, entrapment efficiency and release profile of nanoparticles were investigated. The experimental values and results of this study will be presented.

Key words: *Nanoparticles, Entrapment efficiency, Etodolac, EUTRAGIT RS 100, Preformulation.*

Introduction

Etodolac (ETD), a non-steroidal anti-inflammatory drug, is used to manage rheumatoid arthritis associated symptoms via inhibition of cyclooxygenase pathways and other inflammatory mediators. ETD is a selective COX-2 inhibitor, which inhibits only cyclooxygenase- 2 mediators. It causes less gastrointestinal complication compared to the majority of other NSAIDs. Conventional delivery systems of ETD were found to engender stomach complications, such as nausea, epigastric pain, heartburn, and indigestion.

Delayed drug release formulation would be a suitable solution especially for chronic patients. In a patent assigned to Michelucci and Sherman, a sustained release dosage form of etodolac was provided in the form of matrix tablets with a release rate modifying agents. Although controlled release medication decreases the frequency of administration and diminishes the sleeping problems, yet the morning complications are not completely exterminated. Thus, a specialized drug delivery device is thought to be helpful in delivering a loading dose in the early morning and a maintenance dose over the day time. Therefore, researches have focussed towards designing a bilayer tablet to include a fast release layer for rapid onset of action, beside a sustained release layer for drug level maintenance [1, 2, 3, 4].

Nevertheless, the rapid drug release in the stomach prevents the success of the system, due to manifested side effects on gastric mucosa. Recently, another sigmoid release profile has attracted many workers interested in the field of pharmaceutical formulation, the so-called pulsatile drug delivery system.

Multiple benefits could be acquired through the new design as the delivery device was capable of releasing the drug in a controlled

programmable strategy after a precisely calculated lag phase [5, 6]. Preformulation commences when a newly synthesized drug shows sufficient pharmacologic promise in animal models to warrants evaluation in man. These studies should focus on those physicochemical properties of the new compound that could affect drug performance and development of an efficacious dosage form.

A thorough understanding of these properties may ultimately provide a rational for formulation design, or support the need for molecular modification [7, 8]. The aim of this study was to determine some of the physicochemical properties such as solubility, pKa, dissolution, melting point, assay development, stability in solution, stability in solid state, microscopy, bulk density, flow properties and excipient compatibility.

Materials and Methods

Etodolac (99.79%) donated by M/s Shasun pharmaceuticals, Puducherry and Eudragit RS 100 was procured from Sigma Aldrich, St. Louis Acetone, tween 80, acetonitrile (ACN) of HPLC grade and dipotassium hydrogen phosphate and phosphoric acid were of analytical-reagent grade supplied by M/S SD Fine chemicals, Mumbai, India. The HPLC grade water was prepared by using Milli-Q Academic, Millipore, Bangalore, India. HPLC of Shimadzu (Tokyo, Japan) model consists of a LC10AD and LC10 ADvp solvent delivery module, UV detector, a Rheodyne injector (model 7125, USA) valve fitted with a 20 µl loop, and UV detector. The system was controlled through a system controller (SCL-10A) and a personal computer using a Shimadzu chromatographic software (LC Solution, Release 1.11SP1) installed on it was used for the assay of Etodolac. All chemicals used in the study were of analytical grade and used without further purification.

Experimental Studies

Determination of Solubility

The Etodolac was evaluated for solubility in water, acetone, methanol, diethyl ether chloroform and ethanol in accordance with the British pharmacopoeia specifications [9, 10].

PH Determination

This was done by shaking a 1% w/v dispersion of the sample in water for 5 min and the pH was determined using a digital pH meter (model 335, Systronics, India) [11]. The data presented here is for triplicate determinations.

True density

True density of Etodolac was determined by liquid displacement method. It is calculated from the volume of intrusion fluid (toluene) displaced in the pycnometer by a given mass of powder [8].

$$D = \left(\frac{M}{Vp - Vi}\right)\left(\frac{M}{Vp - Vi}\right)$$

Where, D is true density, Vp is the total volume of the pycnometer and Vi is the volume of intrusion fluid in the pycnometer containing the mass of powder (M). All the estimations were done in triplicate and average values are reported in Table 1.

Determination of Bulk Density, Bulkiness and Compressibility Index

The bulk density of Etodolac was determined by the three-tap method [12]. 10g of Etodolac powder was carefully introduced into a 100 ml graduated cylinder. The cylinder was dropped onto a hard wood surface 3 times from a height of 1inch at an interval of 2 seconds. The bulk density was obtained by dividing the weight of the sample by volume of the sample contained in the cylinder. Reciprocal of bulk density or the specific bulk volume gives the bulkiness. The percent compressibility index (I) of the Etodolac was calculated using following formula [13] and the results are given in Table 1.

$$I = \left(1 - \frac{V}{Vo}\right)\left(1 - \frac{V}{Vo}\right)_{x100}$$

Angle of repose

The static angle of repose, a, was measured according to the fixed funnel and free standing cone method [14]. A funnel was

clamped with its tip 2cm above a graph paper placed on a flat horizontal surface. The powders were carefully poured through the funnel until the apex of the cone thus formed just reached the tip of the funnel. The mean diameters of the base of the powder cones were determined and the tangent of the angle of repose calculated using the equation:

Tan a = 2h/D

The data presented here were obtained from triplicate determinations.

Determination of Partition Coefficient

10 mg drug was added in 50 ml of n-Octanol (pre saturated with water) and it was shaken well and then 50 ml of distilled water (pre saturated with n- Octanol) was added and mixture was shaken by mechanical shaker for 24 hours. After 24 hour both phases were separated. Absorbance was taken for both the phases and concentration in each phases were calculated [15].

$$\text{Partition Coefficient} = \left(\frac{\text{Drug concentration in Octanol}}{\text{Drug concentration in water}}\right) \text{x } 100$$

Percentage of moisture loss

The Etodolac loaded nanoparticles were evaluated for percentage of moisture loss due to its hydrophilic nature. The nanoparticles weighed initially and kept in desiccator containing calcium chloride at 37 °C for 24 hours. When no further change in weight of sample was observed, the final weight was noted down [16, 17].

$$\text{\% of moisture loss} = \left(\frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}}\right)\left(\frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}}\right) \times 100$$

Dissolution Test

In vitro dissolution studies were carried out using a dissolution apparatus USP (Paddle type) at a paddle speed of 50 rpm. The dissolution medium was 900 ml of phosphate buffer, pH 7.4, which was maintained at 37 ± 0.5°C. 5 ml of dissolution samples was withdrawn and replaced with equal volume fresh phosphate buffer, pH 7.4 at regular intervals. Collected dissolution samples were used for determination of released concentrations of Etodolac by using HPLC method [18, 19].

Drug Polymer Interaction Studies by FT-IR

Drug-polymer interactions were studied by FT-IR spectroscopy using the instrument Shimadzu FT-IR-8400S. The spectra were recorded for Etodolac, Eutragit RS 100, physical mixture of Etodolac: EUTRAGIT RS 100 (1:1) Samples were prepared in KBr disks (2 mg sample in 200 mg KBr) with a hydrostatic press at a force of 5.2 пcm2 for 3 minutes. The scanning range was 400 - 4000 cm2 and the resolution was 4 cm-1 [19, 20, 21].

Differential Scanning Calorimetry

Weigh exactly 2mg of Etodolac and transfer it in to standard aluminium pan cover with aluminium lid and crimp the pan using crimper. EUTRAGIT RS 100 with Etodolac sample prepared by weigh exactly 2mg of mixture of EUTRAGIT RS 100 and Etodolac, transfer it in to standard aluminium pan cover with aluminium lid and crimp the pan using crimper. Formulation excipients with Etodolac sample prepared by excipients mixtures with Etodolac transfer it in to standard aluminium pan cover with aluminium lid and crimp the pan using crimper. Then subject to programmed temperature changes using differential scanning calorimetry [22, 23, 24].

Preparation of Nanoparticles

Nanoparticles were prepared by solvent evaporation method reported by previous authors [25, 26, 27, 28] with some modifications. The method involves preparation of o/w emulsion between organic phase (OP) consisting of Etodolac and Eudragit RS100 in dichloromethane (DCM) and aqueous phase (AP), containing 1% w/v PVA.

Etodolac and Eudragit RS100 was dissolved in dichloromethane by sonication (Ampere 60%, 6 minutes) using probe sonicator (Vibra Cell Sonics, India) and the organic phase was emulsified in aqueous phase containing 1% w/v PVA

The emulsion obtained was stirred overnight (12 to 16 hrs.) at 25±2^{0}C using magnetic stirrer to ensure complete evaporation of dichloromethane. The nanoparticles thus formed were recovered by centrifugation (12,000 rpm, 20 mins, -10^{0}C) using Remi centrifuge and the precipitate was washed repeatedly (at least 3 times) with ice cold MilliQ (MQ) water to ensure complete removal of traces of polyvinyl alcohol (Madhusudhan *et al.* 2010).

Finally, the product was dispersed in cold water and recovered by lyophilisation (Benchtop Pro, SP Scientifics, India).The critical parameters involved are duration of sonication, volume of organic solvent and polymer to drug ratio etc.

Finally, three different batches of Etodolac EudragitRS100 nanoparticles were prepared

Encapsulation Efficiency (EE)

Drug loaded nanoparticles (100 mg) were powdered and suspended in water and then sonicated for about 20 minutes. It was shaken for another 20 minutes for the complete extraction of drug from the nanoparticles. The mixture was filtered through a 0.45 µm membrane filter (MILLIPORE). Drug content was determined by UV- visible spectrophotometer (UV – 160IPC, Shimadzu, Japan) at 232 nm. The percent entrapment was calculated using the following formula [29, 30, 31, 32].The results are given in Table 1.

$$\textbf{Encapsulation efficiency} = \left(\frac{\text{Actual weight of drug in sample}}{\text{Nanoparticles Sample Weight}}\right)\left(\frac{\text{Actual weight of drug in sample}}{\text{Nanoparticles Sample Weight}}\right) \times 100$$

Particle Size Analysis of Nanoparticles

Average particle diameter and size distribution of nanoparticles were determined by laser diffractometry using a Mastersizer 2000 (Malvern Instruments, Malvern, UK). Approximately 10 mg of nanoparticles were dispersed in 2 to 3 ml distilled water containing 0.1% Nonidet P40 for several minutes using an ultrasonic bath.

Then, an aliquot of the nanoparticle suspension was added into the small volume recirculation unit [33, 34, 35, 36], which was subsequently circulated 3500 times per minute. Each sample was measured in triplicate for the analysis. Particle size was expressed as the weighted mean of the volume distribution.

Results and Discussion

Particle size distribution of drug has influence on many bulk properties of pharmaceutical interest such as flow properties, packing, packing densities, compressibility segregation characteristics etc. Hence, it must be the aim of pharmaceutical technologist to study the particle size distribution. The particle size distribution of Etodolac nanoparticles was shown in Figure 1.

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 165	Peak 1:	184.7	100.0	102.3
PdI: 0.218	Peak 2:	0.000	0.0	0.000
Intercept: 0.971	Peak 3:	0.000	0.0	0.000
Result quality : Good				

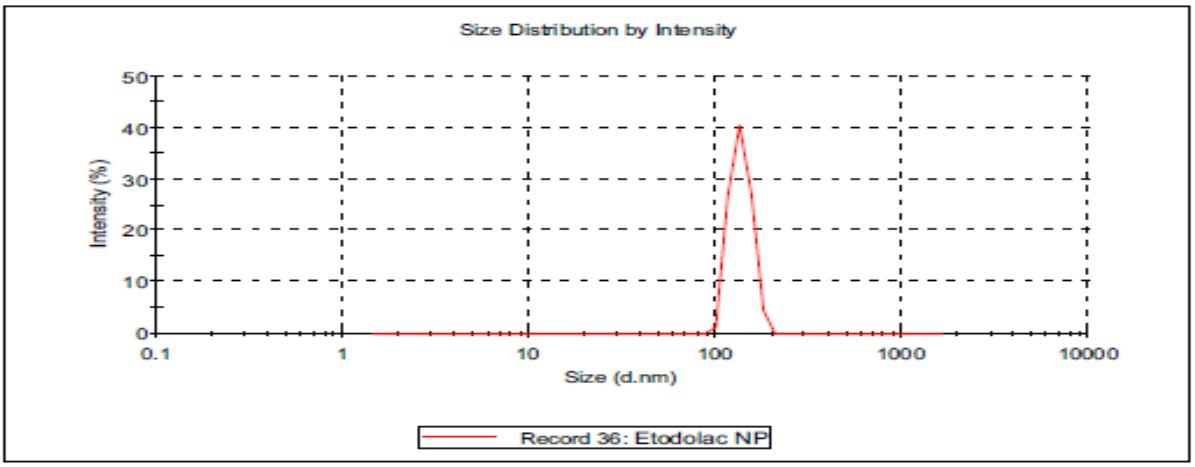

Figure 1: Particle size of Etodolac loaded nanoparticles

The results of solubility, true density, bulk density, compressibility index, angle of repose, moisture content, pH, Partition Coefficient are given in Table 1.

Table 1: physicochemical properties of Etodolac

Parameters	Results
Description	Etodolac occurs as white to off white powder.
Solubility	Insoluble in water but soluble in alcohols, chloroform, dimethyl sulfoxide, and aqueous polyethylene glycol.
pH	7.4+0.29
True density (gm/cc)	1.23+0.57
Bulk density (gm/cc)	0.362+0.037
Compressibility Index (%)	13.92+ 0.40
Angle of repose (°)	25.33° + 0.72
Moisture content (%)	7.92+ 0.50
Partition Coefficient	11.4 at pH 7.4
Melting Point (°C)	145 ° C
Dissolution of Etodolac tablet after 30min	95%

Drug-Excipients accelerated compatibility study based physical observation and assay confirms no colour change was observed. Based on the chemical evaluation it was found that there was no significant change observed indicating that the drug is compatible with the added ingredients. The results of this study were given in Table 2 to 4.

Table 2: Physical characteristics of individual drug and excipients

S. No	Sample ID	Initial description	Final description
1.	Etodolac	White crystalline powder	No change
2.	EUTRAGIT RS 100	Fine white powder	No change
3.	Pluronic F68	Fine white powder	No change

Table 3: Physical characteristics of drug-excipient mixture

S. No	Sample ID	Initial description	Final description
1	Etodolac	White crystalline powder	No change
2	Etodolac+ EUTRAGIT RS 100	White powder	No change
3	Etodolac+ Pluronic F68	Fine white powder	No change

Table 4: Chemical characteristics of drug-excipient mixture

S. No	Sample ID	Initial assay (%)	Final assay (%)
1.	Etodolac	99.87	99.85
2.	Etodolac+ EUTRAGIT RS 100	99.83	99.82
3.	Etodolac+ Pluronic F68	99.84	99.83

DSC is used in pharmaceutical industry to allow evaluation of possible incompatibilities between different components blended in the formulation according to the appearance, shift and disappearance of peaks in the corresponding enthalpies. DSC curves (shown in Figure 1-3) were used to determine the compatibility of ETD with various added excipients. DSC measures the heat loss or gain resulting from physical or chemical changes within a sample as a function of temperature. A sharp symmetric melting endotherm can indicate relative purity, where broad, asymmetric curve suggests impurities or more than one thermal process. The loss of water present in the compound is usually indicated by the endothermic peaks produced in DSC below 120°C. DSC analysis were performed to find out the physical nature of the Etodolac and also to confirm absence of drug-polymer interaction. Individual thermograms of pure drug, polymer and physical mixture were performed and the thermograms of DSC are shown from Figure 2-4.

The thermograms showed the characteristic peeks of the drug at the melting point 144°C. This confirmed there was no interaction between the drug and polymer. The FT-IR

spectra were shown from Figure 5-7. On comparison of the individual spectra of the pure sample with that of physical mixtures, no prominent difference in the spectrums was seen.

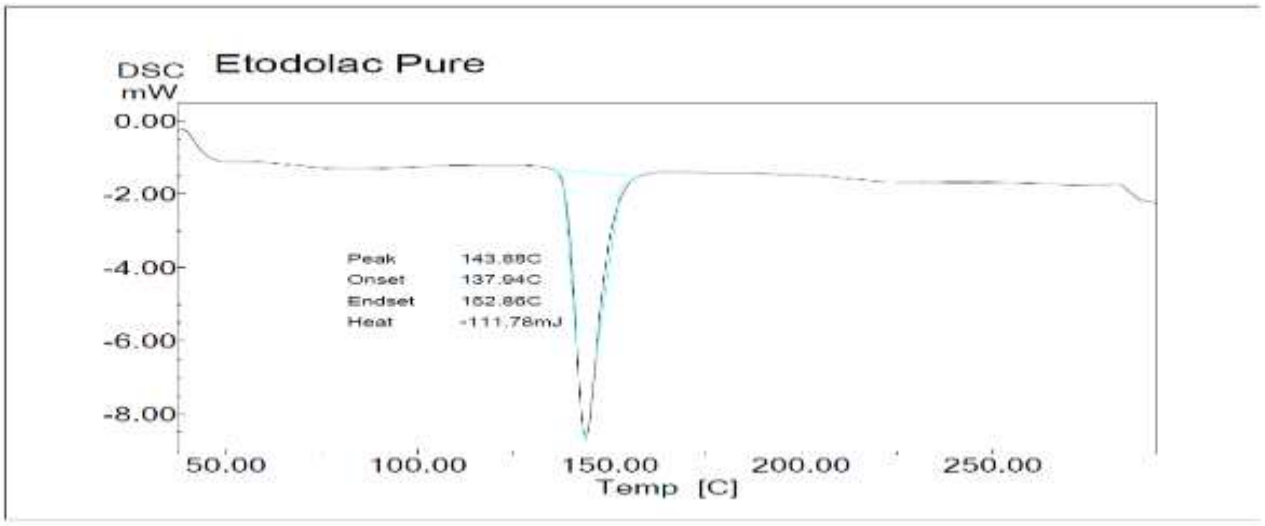

Figure 2: DSC thermo gram of Pure Etodolac

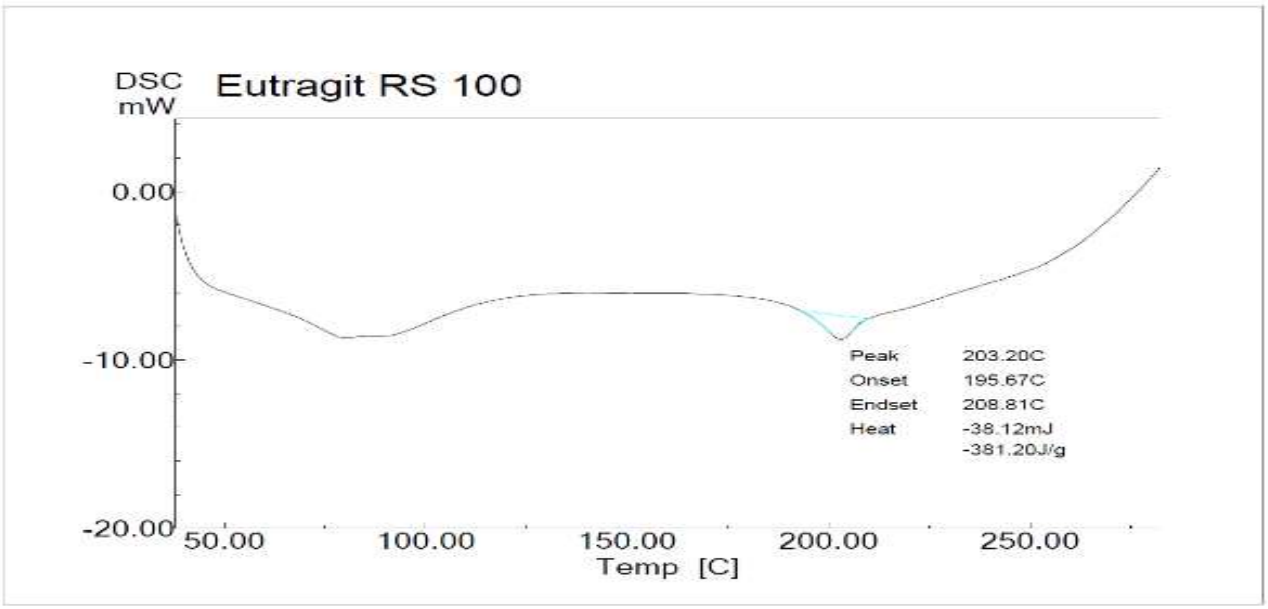

Figure 3: DSC thermogram of Eutragit RS 100

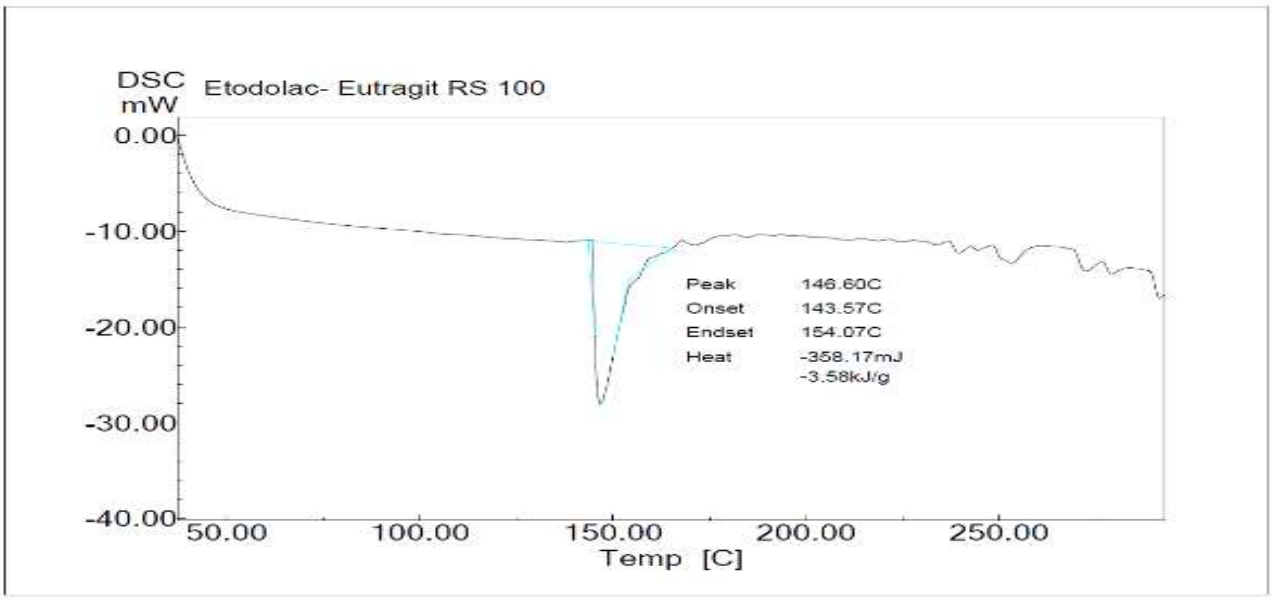

Figure 4: DSC thermogram of Physical mixture Etodolac with Eutragit RS 100

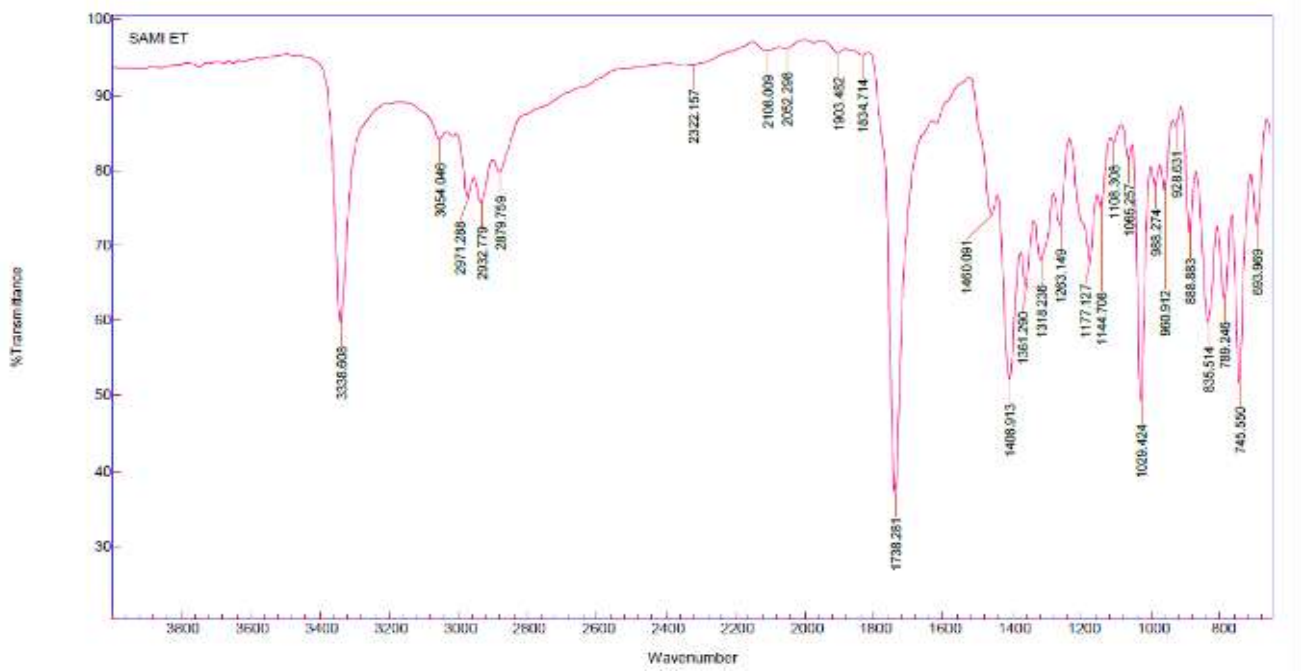

Figure 5: FTIR Spectra of Pure Etodolac

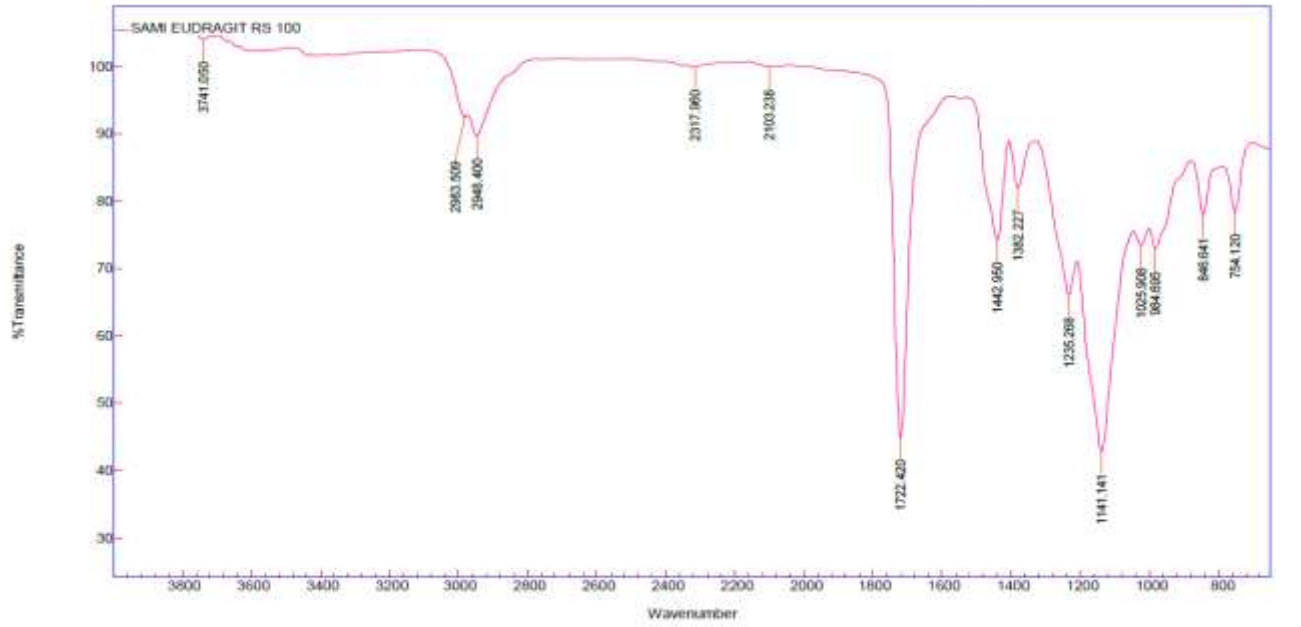

Figure 6: FTIR Spectra of Eutragit RS 100

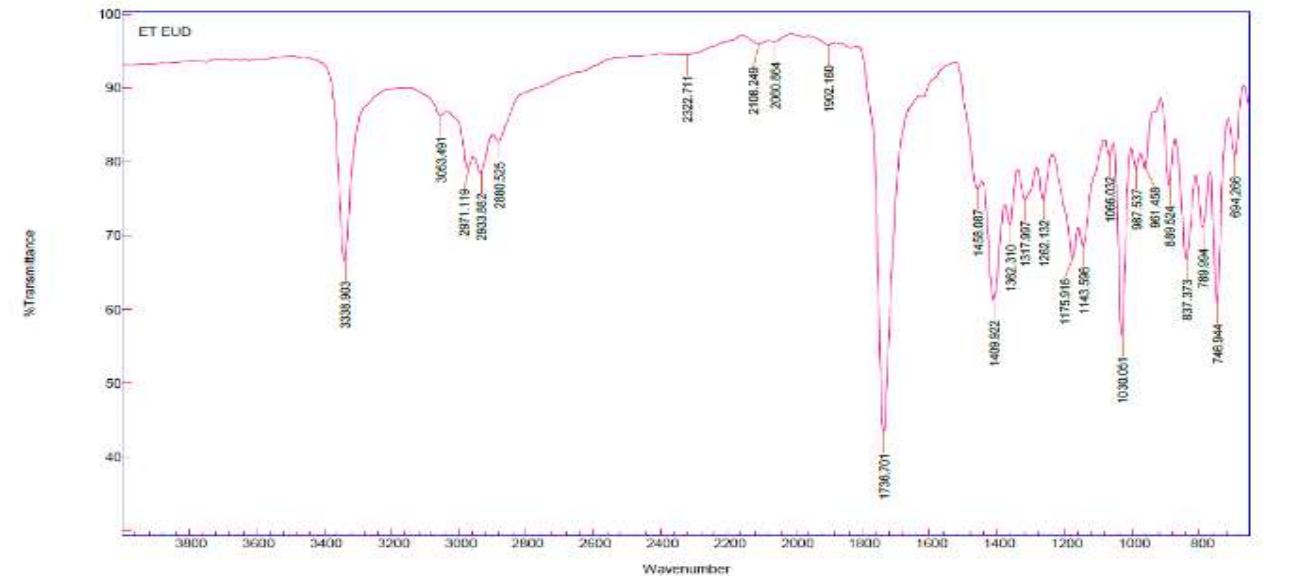

Figure 7: FTIR Spectra of Physical Mixture of Etodolac with Eutragit RS 100

Conclusion

The preformulation phase is a critical phase in establishing the properties of drug that will allow suitable risk assessment for development. Typically it begins during the lead optimization phase, continues through prenomination, and on into the early phases of development. Decisions made on the information generated during this phase can have a profound effect on the subsequent development of those compounds. Therefore, it is imperative that preformulation should be performed as carefully as possible to enable rational decisions to be made.

The quantity and quality of the drugs can affect the data generated as well as the equipment available and the expertise of the personnel conducting the investigations. In this study we successfully completed the physicochemical characterization of Etodolac properties like morphology, size, solubility, pH, partition coefficient, Surface area flow property, drug content and release study. This knowledge obtained may be useful in developing modified release formulations mainly sustained release formulation of Etodolac loaded nanoparticles.

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