

Research Article



Preparation and Evaluation of Microwave Generated Nanocomposites for Solubility Enhancement of Ketoprofen

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ABSTRACT

Ketoprofen is non-steroidal anti-inflammatory drug (NSAID) used for treatment of rheumatoid arthritis. Ketoprofen belongs to BCS class II having low solubility which imposes challenge in development of oral dosage form. Nanocomposite formation is novel technique used for solubility enhancement. Microwaves were utilised for formation of nanocomposites. Polymers like HPMC, tragacanth, acacia, avicel were used as carrier for formulation of nanocomposites. From physical characterization of polymers like foaming index, viscosity and swelling index, HPMC and tragacanth were used for further study. 8 different formulations were prepared with varying ratio of drug and carrier and corresponding physical mixtures were also prepared. KTHN (1:4), KTTN (1:4) were the best ratios from different nanocomposites formulations, which showed high solubility. In-vitro drug release showed the drug dissolution of 85.72% in comparison to pure Ketoprofen which showed dissolution of 48.31%. Prepared nanocomposites were investigated for various parameters like FTIR, DSC, SEM. Stability study for 3 months indicated no significant change in appearance, drug content and in-vitro drug release of the nanocomposites. The overall results indicated that microwave assisted formulation of nanocomposites can be used to increase solubility of ketoprofen.

Keywords: Nanocomposites, solubility enhancement, BCS, Microwave, Ketoprofen.

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INTRODUCTION

Oral route is most the promising route of drug administration because of several advantages like ease of manufacturing, easy administration and precise dosing.¹ Once drug is administered through oral route it undergoes either passive or active absorption through cellular membrane of gastrointestinal tract. Solubility and permeability are the two important boundaries which defines drug bioavailability. As per US pharmacopoeia near about 40% drugs are poorly soluble or insoluble in aqueous medium which produces a challenge for oral dosage form development.² Biological classification system classifies drugs in four different classes according to its solubility and permeability. Class I includes drugs with high solubility as well as high permeability. Class II drugs have low solubility but high permeability. High solubility and low permeability drugs come under Class III. Class IV consist of drugs with low solubility and low permeability.³ Drugs belonging to class I does not impose much problem in oral dosage form development but drugs from BCS class II which are having low solubility may impose problem in oral dosage form development. Hence increase solubility of such drugs is major challenge. Various techniques are used for

improvement of solubility like slat formation, solid dispersion, cocrystals, complexation, use of prodrug, use of surfactant and many more.⁴

Formation of nanocomposites is one of the advance techniques used for solubility enhancement. Nanocomposites can be defined as composition of material having one of the phases in nano size. Nanocomposites are composed of two or more different physical components separated by discrete interphase with different physicochemical properties. The material of nanocomposite in which another material is embedded is called as matrix while embedded nanosized filler material is called as nanomaterial.⁵ Nanocomposite formation improves properties of both the components.⁶

Microwave oven uses microwave radiation to break the internal structure of drug particle which causes increase in solubility. The main principle behind the heating in microwave oven is the interaction of charged particle of the reaction material with electromagnetic wavelength of particular frequency. The phenomenon of producing heat by electromagnetic irradiation is ether by collision or by conduction, sometimes by both. All the wave energy changes its polarity from positive to negative with each cycle of the wave. This cause rapid orientation and reorientation of molecule, which cause heating by collision.^{7,8}

Ketoprofen is nonsteroidal anti-inflammatory (NSAID) drug with analgesic and antipyretic effect majorly used in treatment of rheumatoid arthritis. Ketoprofen is aryl carboxylic acid derivative. It was first synthesized in 1967 at the Paris. IUPAC name of ketoprofen is 2-[3-



benzoylphenyl] propionic acid and structure is shown in fig. 1.⁹ Ketoprofen constrains the action of the enzymes cyclo-oxygenase I and II, ensuing in a reduced formation of precursors of prostaglandins and thromboxanes. The resulting reduction in prostaglandin production, by prostaglandin synthase, is accountable for the therapeutic effects of ketoprofen. Ketoprofen also causes a decrease in the formation of thromboxane A₂ synthesis, by thromboxane synthase, thereby inhibiting platelet aggregation.

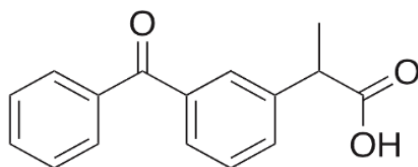


Figure 1: Ketoprofen structure

In this work we are enhancing the solubility and dissolution of BCS class II drug ketoprofen through the formation of bio-nanocomposite by using the biodegradable natural polymer i.e., acacia and tragacanth by microwave diffusion method.

MATERIALS AND METHODS

Material

Ketoprofen was a generous gift sample from BEC chemicals, Roha, Raigad. Hydroxy Propyl Methyl Cellulose (HPMC), tragacanth, acacia, avicel were purchased from Modern science, Nashik. All the material was of analytical grade and used as received without further purification.

Methods

Determination of Solubility

The solubility of Ketoprofen was determined in Distilled water, phosphate buffer pH 6.8. An adding excess amount of drug was added to 10 ml of respective solvent. The contents were stirred continuously for 24 hours at 37 °C and allowed to equilibrate. After 24 hours the sample were withdrawn and filtered through membrane filter and analyzed in UV-visible spectrophotometer (Shimadzu UV-2600).¹⁰

Fourier transforms infrared spectroscopic (FTIR) studies of pure drug

The dry sample of drug was mixed with KBr in the ratio of 1:99. The sample was triturated and finally placed in sample holder and compressed using a motorized pellet press at 15 tones pressure. The pellets were then scanned using an FTIR spectrophotometer (Shimadzu; IR Affinity-1S) over frequency range 4000-400cm⁻¹ in FTIR instrument Shimadzu; IR Affinity-1S. The spectral analysis was done, by standards absorbance range of the functional groups.

Physical characterization of polymer

Swelling Index (SI)

Swelling index of polymer was determined by modified method reported. 1gm of tragacanth and acacia was accurately measured and transferred to 100 ml measuring cylinder. The initial volume occupied by powder was noted. The volume was made up to the 100 ml with distilled water. The open end of cylinder was sealed with aluminium foil and kept aside for 24 hrs. After 24 hrs volume of swelled polymer was noted. The swelling index of polymer was calculated by the following formula.

$$SI = \frac{H_f - H_i}{H_i} \times 100$$

Where, SI- Swelling index of polymer,

H_i - Initial height of powder,

H_f - Final height of powder after 24 hr.

Foaming index

Surfactant property of polymer can be determined by foaming index. Accurately weighed 1 g of powder and transferred in 250 ml measuring cylinder. 100 ml distilled water was added in measuring cylinder to make dispersion. Resultant dispersion was vigorously shaken for 2 minutes. The foaming index of polymer calculated by the following equation,

$$\text{Foaming index} = H_f - H_i$$

Where,

H_f = Height of solution of polymer after shaking

H_i = Height of solution of polymer before shaking.

Viscosity

Viscosity of polymer was calculated by dissolving one gram of each acacia and Tragacanth in 100 ml of water (1% w/v solution). The viscosity of the carrier dispersions of Acacia and Tragacanth were measured by Brookfield viscometer using spindle VI at 200 rpm.

Drug- Excipient compatibility Studies

A compatibility study was carried out in order to establish, that there was no interaction between the drug and excipients used in the formulation. The drug and physical mixtures of drug: polymer (1:1) was filled in vial and sealed. The sealed vials kept at specific temperature (in desiccator) for 1 month. After 1 month mixture was interpreted by IR (Shimadzu: IR Affinity-1S).

Fourier –Transform Infrared Spectroscopy Study

The FTIR spectrum of ketoprofen was recorded from 4500 cm⁻¹. Infrared spectra of pure drug and physical mixture of drug with polymers were obtained by using standard KBr plate method. FTIR spectra is shown in fig. 2.

Preparation of physical mixture²²

Physical mixture of drug with polymer HPMC, Tragacanth was prepared by simple blending of drug with polymer in the ratio 1:1 to 1:4. The quantity of pure drug and respected polymers are showed in Table 1. The physical

mixture of drug with polymer HPMC, Tragacanth was denoted by KTH_P, KTT_P, respectively. The physical mixture

prepared to check the solubility enhancing property of nanocomposites as compared with physical mixture.

Table 1: Ratio of Ketoprofen and Tragacanth For Preparation of Physical Mixture and Nanocomposites

Ratios (for physical mixture)	Quantity (mg)			Ratio (for nanocomposites)	Quantity (mg)		
	KTF	TRG	HPMC		KTF	TRG	HPMC
KTT _P 1:1	500	500	-	KTT _N 1:1	500	500	-
KTT _P 1:2	500	1000	-	KTT _N 1:2	500	1000	-
KTT _P 1:3	500	1500	-	KTT _N 1:3	500	1500	-
KTT _P 1:4	500	2000	-	KTT _N 1:4	500	2000	-
KTH _P 1:1	500	-	500	KTH _N 1:1	500	-	500
KTH _P 1:2	500	-	1000	KTH _N 1:2	500	-	1000
KTH _P 1:3	500	-	1500	KTH _N 1:3	500	-	1500
KTH _P 1:4	500	-	2000	KTH _N 1:4	500	-	2000

KTF: Ketoprofen, **TRG:** Tragacanth, **HPMC:** Hydroxy Propyl Methyl Cellulose

Preparation of physical mixture for nanocomposites¹¹

The nanocomposites were prepared by homogenous mixing of accurately weighed amount of individual drug with individual polymer. In this case the weight to weight (w/w) ratio of drug to polymer was taken from 1:1 to 1:4 keeping amount of mixture constant. The quantity of pure drug and polymer for different ratios were taken as per showed in Table 1. To this mixture (drug and polymer) 4 ml of water was added for each gram of polymer to make homogenous slurry. The fixed amount of slurry was taken in glass round bottom flask and irradiated with microwave radiation at power 556 W (CATA-2R, Catalyst System) with continuous stirring for 5 min. Nanocomposites were grounded in mortar and sieved to achieve the particle size of 80 to 250 μm. The nanocomposites of drug with polymer HPMC and Tragacanth were denoted by KTH_N and KTT_N respectively.

Solubility of formed bio nanocomposites

The solubility of KTH_P, KTT_P and KTH_N, KTT_N was determined in pH 6.8 phosphate buffer. The solubility of drug, physical mixtures and NCs was determined by taking an excess amount of drug (10 mg) and NCs (equivalent to 10 mg of drug) and adding them to 10 ml of solvent (pH 6.8 buffer), in Teflon-facing screw-capped vials. The samples were kept at equilibrium for a period of 24 hrs. in an orbital shaker (Remi Instruments) at 37±0.5°C and 50 rpm. The supernatant fraction collected from the vials was filtered through a 0.45-micron membrane filter and analyzed by UV-visible spectrophotometer (Shimadzu) at a wavelength of 276 nm. Ratio optimization (drug: carrier) was done on the basis of the best solubility results obtained.

Dissolution test

In-vitro powder dissolution test was carried out on Ketoprofen and nanocomposites which was performed by using USP apparatus II (Paddle) method by using 900 ml pH

6.8 phosphate buffers as a dissolution media. Powder that contains accurate dose of drug (or equivalent to 10 mg of Ketoprofen) was added in the dissolution media maintaining temperature at 37± 0.5°C and rotation speed of paddle at 75 rpm. 5 ml of sample were withdrawn at the interval of 0, 5, 10, 15, 20, 25, 30 minutes by replacing 5 ml of pH 6.8 phosphate buffer solution in dissolution media. Samples were filtered by 0.45 μ membrane filter and analyzed spectrophotometrically at wavelength of 276nm.¹²

Drug content analysis

To calculate the amount of drug incorporated into nanocomposites drug content analysis was performed by dissolving nanocomposites mixture in 25 ml of methanol. The resulting solution was filtered through 0.45μ membrane filter and analyzed by UV-visible spectrophotometer at wavelength 276 nm for ketoprofen against methanol as a blank.

Characterization of optimized nanocomposites

From the results obtained by solubility and dissolution studies, the NCs that showed better results were selected for further characterization.

Fourier–Transform Infrared Spectroscopy (FTIR)

FTIR study of optimized ratio of nanocomposites (KTH_N1:4) was carried out. Nanocomposites were mixed with potassium bromide (KBr) of IR grade in a ratio of 1:99 and compressed using a pellet pressed at 15 tones pressure. Then pellets were scanned using an FTIR (Shimadzu; IR Affinity-1S). The FTIR spectra of optimized nanocomposites were compared with that of the pure drug to assess any change in the principal peaks of spectra of optimized nanocomposites ratio shown in fig. 4.



Differential Scanning Calorimetry (DSC)

A DSC study of optimized nanocomposites ratio (KTH_N1:4) was employed to access what changes had actually made when nanocomposites were formulated and by what fact these enhances the solubility of drug. The DSC curves were obtained by Differential Scanning Calorimeter at the heating rate of 10°C/min from 50 to 200 °C in nitrogen atmosphere.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy was used to examine external surface morphology. The morphologies and detailed particle structural characterizations of pure drug and nanocomposites (KTH_N1:4) were observed by scanning electron microscope. NCs that showed the best results in the solubility and dissolution studies were subjected to scanning electron microscopy (SEM) studies to confirm the changes made during the formation of NCs. Samples were prepared by mounting powder onto a brass stub using graphite glue and coated with gold under vacuum before use. Images were recorded at the required magnification at an acceleration voltage of 10 KV using a scanning electron microscope.

Stability study of optimized nanocomposites

Accelerated stability study was carried out as per ICH guidelines. The sample of optimized nanocomposites was placed for 3 months in stability chamber at 40 ±2°C and 75 ±5% RH. Various parameters such as appearance, drug content and in-vitro drug release were measured after 1, 2 and 3 month of stability study.

RESULT AND DISCUSSION

Table 2: Physical Characterization Of Polymers

Polymer	% Swelling	Viscosity (cp)	Foaming Index
Tragacanth	63.91 ± 1.32	2.52 ± 0.13	8.00 ± 0.40
Acacia	68.89± 1.21	2.35 ±0.16	9.20 ±0.70
HPMC	62.41 ± 1.33	2.25 ± 0.13	8.65 ± 0.40
Avicel	65.69± 1.27	2.62 ±0.16	9.40 ±0.70

Solubility study of physical mixture

Solubility studies were performed to analyze the solubility enhancing properties of nanocomposites. Solubility studies provided the basis for selection of the best ratio that was to be forwarded. Pure drug Ketoprofen and physical mixtures of Ketoprofen with individual carriers in varying ratios, as well as nanocomposites of Ketoprofen with individual carrier in varying ratios were analyzed for solubility determination. The solubility of pure drug was found to be 8 mg/ml in methanol and 6.2 mg/ml in phosphate buffer pH 6.8. The results of solubility studies of physical mixture and nanocomposites are shown in following Table number 3. Solubility studies reveals that physical mixtures improves the solubility of ketoprofen significantly compared with pure drug. This may be due to the surfactant and wetting property of acacia and

Solubility of Ketoprofen

Ketoprofen was found to be soluble in methanol and phosphate buffer pH 6.8 and poorly soluble in water. Solubility of Ketoprofen in different solvents was found to be 0.056 mg/ml in water, 0.106 mg/ml in phosphate buffer of pH 6.8 and 0.11 mg/ml in methanol.

Fourier transform infrared spectroscopic studies (FTIR)

The powdered mixture of Ketoprofen and KBr was taken in a sampler and the spectrum was recorded by scanning in the wavelength region of 4000- 400 cm⁻¹ using FTIR spectrophotometer.

FTIR spectrum of Ketoprofen as showed in fig. 3 shown all the peaks corresponding to the functional groups presents in the structure of Ketoprofen.

Physical characterization of carriers

Results of physical characterization are shown in table number 2. From this data, it can be concluded that the swelling characteristics and viscosity of Tragacanth were low and that of Acacia is very high. High viscosity and toughness may limit their application as carriers for dissolution enhancement. Because of less swelling and low solution viscosity, they are more prone to dissolution enhancement. They are less prone to the formation of the tough matrix which will assist rapid liberation of the nanocrystals from the nanocomposites.

On the basis of the physical characterization of the polymer like swelling index, viscosity, foaming index the Tragacanth and HPMC were used for the further processes.

tragacanth. In case of nanocomposites solubility data indicates a tremendous rise in solubility compared with pure drug; this may be due to reduction of crystal size of the drug to a nanocrystalline form.

Solubility studies of physical mixtures and nanocomposites clearly indicated that as the ratio of drug to carrier increases solubility up to specified ratio. It was also found that the high solubility was shown by nanocomposites formulation and nanocomposites prepared by using HPMC (KTH_N) showing best solubility result at 1:4 ratio was considered optimal. The solubility of KTH_N was found to be 1.632 mg/ml. KTH_N (1:4), KTT_N (1:4) are the best ratios from different drug : polymers nanocomposites formulations, which were showing high solubility, hence those were subjected to *in-vitro* drug release study with pure drug and drug content analysis.

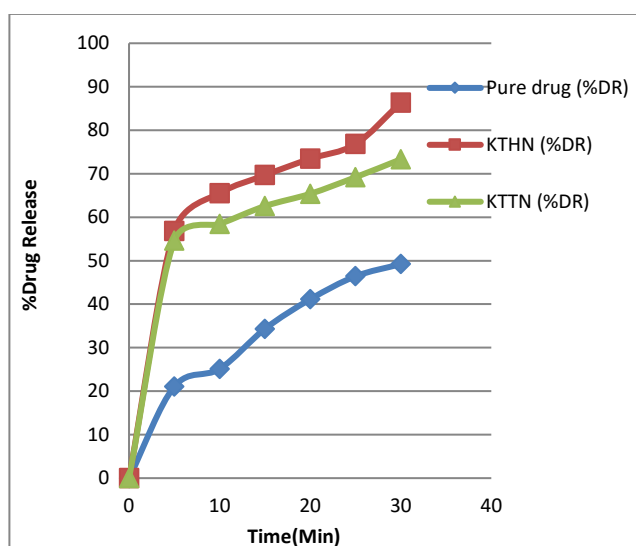


Table 3: Solubility Of Physical Mixture Of Ketoprofen And Polymer

Drug polymer Ratio	KTH _p (mg/ml)	KTH _N (mg/ml)	KTT _N (mg/ml)	KTT _p (mg/ml)
1:1	0.502± 0.01	1.065±0.02	0.727±0.03	0.341± 0.07
1:2	0.629± 0.06	1.398±0.04	1.105±0.08	0.520± 0.09
1:3	0.650± 0.07	1.567±0.04	1.245±0.12	0.617± 0.06
1:4	0.812± 0.04	1.632±0.08	1.210±0.02	0.699± 0.05

In-vitro Drug release

The dissolution profile of pure drug and nanocomposites is shown in fig. 2. From the dissolution profiles of the nanocomposites, there was evidently a remarkable improvement of the dissolution rates in all nanocomposites compared with the pure Ketoprofen. Among all of the nanocomposites, the best result was shown by KTH_N which showed the drug dissolution of 85.72% in comparison to pure Ketoprofen which showed dissolution of 48.31%.

**Figure 2:** Dissolution profile of pure drug, KTH_N and KTT_N

Drug content analysis of nanocomposites

Uniform dispersion of drug in the nanocomposites can be determined by drug content analysis. It was found that 45-92% drug was incorporated in the nanocomposites. KTH_N (1:4) showing uniform dispersion of drug in the nanocomposites. About 91.34 ± 0.52% drug was incorporated in KTH_N and 65.68 ± 0.50 % drug was incorporated in KTT_N.

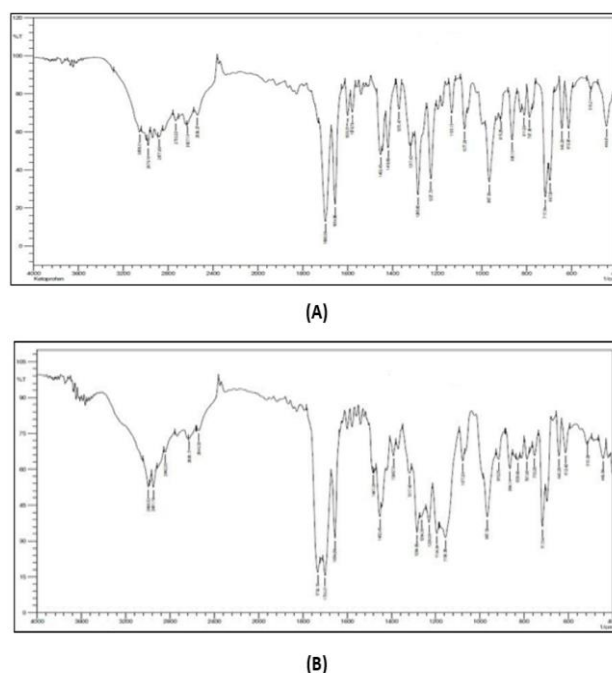
Characterization of optimized nanocomposite

All ratios were subjected to solubility, dissolution and drug content study. From the various batches nanocomposites prepared by using HPMC showed better result and 1:4 ratio is the best optimized ratio. The optimized ratio KTH_N further characterized by following parameters.

Fourier transform infrared spectroscopy (FTIR)

FTIR studies are carried out for characterization of drug and to check the interaction between drug and polymers in the formulation. FTIR study of optimized

nanocomposites KTH_N (1:4) was carried out. FTIR spectra of nanocomposites is shown in the fig. 3. The spectrum of nanocomposites was equivalent to ketoprofen. From this it can be concluded that principal peak values of the drug remain unchanged in the microwave-treated nanocomposites indicating no chemical interaction. Thus, it can be concluded that there is no chemical interaction between the drug and gum carrier.

**Figure 3:** FTIR spectra of optimized Ketoprofen nanocomposites (KTH_N)

Differential Scanning calorimetry (DSC)

DSC was performed to detect the interaction between ketoprofen and polymer. The DSC thermogram of pure drug in fig. 5 showed a sharp endothermic peak corresponding to the melting point of crystalline drug at 90.15 °C. DSC of optimized nanocomposites KTH_N 1:4 shows slight variation in endothermic peak as that of pure drug and intensity of peak is reduced this may be due to the decrease in the crystalline size of the drug. The DSC thermogram of KTH_N shown a broad endothermic peak as shown in fig. 4. The peak broadening indicated that most of the drug is embedded in nanocomposites in the nanocrystalline form. Little shift in melting point was observed due to reduction of drug to the nanocrystalline form. This phenomenon is responsible for the solubility enhancement as the crystallinity has been reduced to the nanocrystalline form solubility gets enhanced.

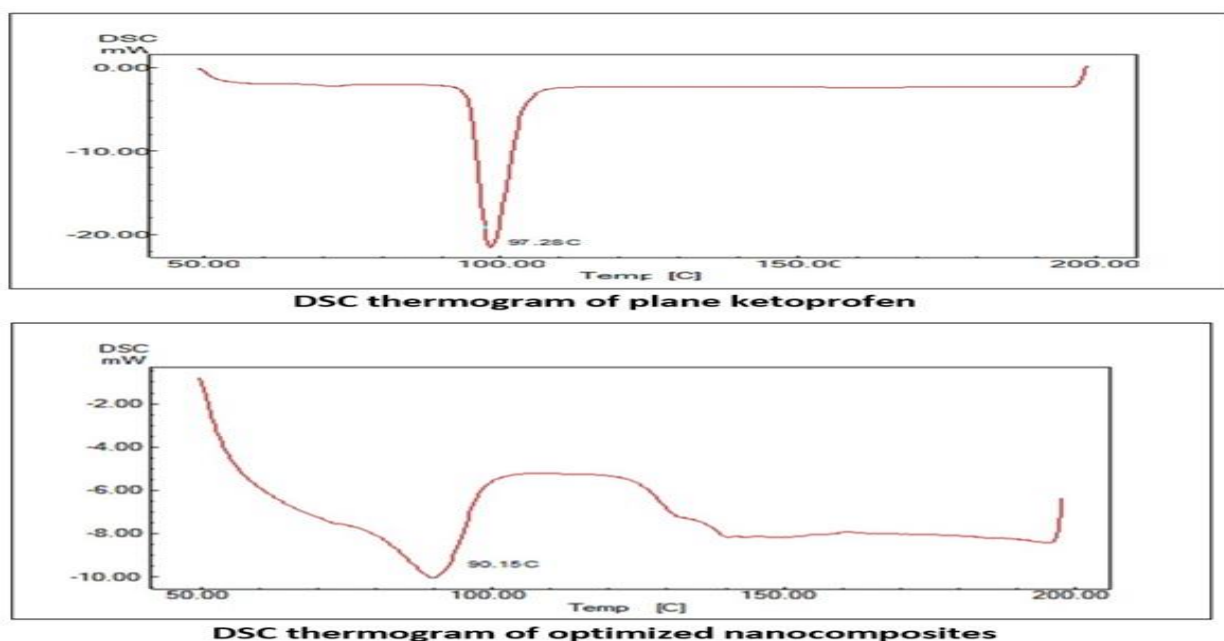


Figure 4: DSC thermogram of pure ketoprofen and optimized nanocomposites

Scanning electron microscopy

SEM studies are usually done to study the surface morphology of drug particles and results are indicated in fig. 5. Optimized nanocomposites of KTH_N 1:4 was

characterized by SEM. From SEM it can be concluded that KTH_N nanocomposites observed were irregular shape and size. Ketoprofen completely changes in nanocomposites. Ketoprofen crystal in the matrix of polymer (HPMC, tragacanth) can be observed.

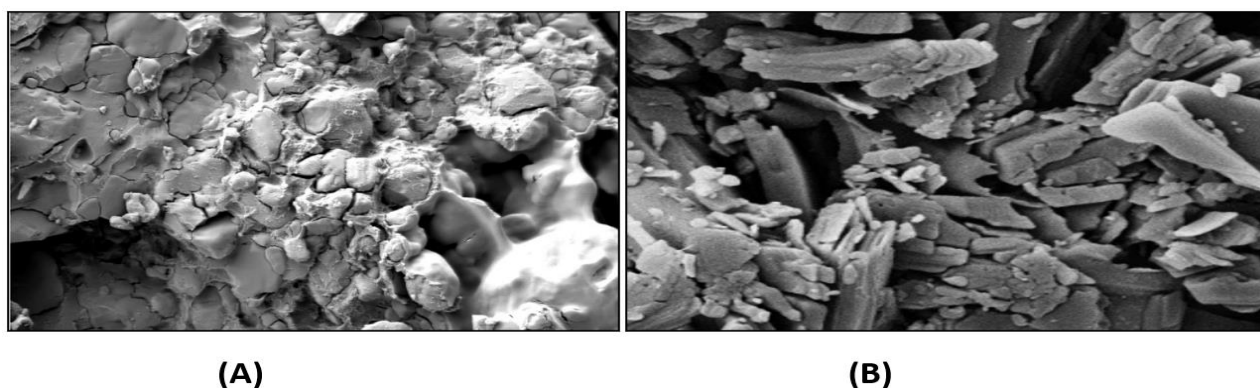


Figure 5: SEM images of (A) Ketoprofen with HPMC (B) Ketoprofen with tragacanth Nanocomposites.

Stability study

Stability study of optimized ratio of powder nanocomposites of ketoprofen was done to see the effect of temperature and humidity on powder nanocomposites during the storage time. Nanocomposites were evaluated periodically 0 and 1, 2, 3 months for appearance, drug content and *in-vitro* drug release. Stability study results indicate that there was no significant change in appearance, drug content and *in-vitro* drug release of the nanocomposites. 83.62 ± 1.56 , 83.11 ± 1.78 , 80.75 ± 1.33 , 80.16 ± 1.12 was observed drug release after 0, 1, 2 and three months respectively.

DISCUSSION

The present study revealed the use of natural carriers such as tragacanth in the microwave-generated nanocomposites for the improvement of solubility and

hence dissolution of drug substance. From the various batches developed, nanocomposites prepared by using tragacanth were found to be applicable on the basis of different evaluation parameters. The solubility of drug was increased several folds along with substantial enhancement in dissolution performance of drug. The important feature of this study comprises the undeviating dispersal of drug in carrier in a nano crystalline form in optimized nanocomposites. The optimized nanocomposites were easy to prepare and sufficiently stable. It can be concluded that, microwave-generated nanocomposites can be effectively used for the improvement of solubility, dissolution and thus bioavailability of BCS class II drugs which are poor water soluble.

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