

Solubility and Dissolution Enhancement of Poorly Water-soluble Ketoprofen by Microwave-assisted Bionanocomposites: *In Vitro* and *In Vivo* Study

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Abstract

Aim: Dissolution and diffusion through gastrointestinal membrane are the mechanisms by which drug get absorbed on oral route of administration. The major challenge in case of the majority of drugs is poor water solubility. Hence, the objective of the present study is to develop bionanocomposites (BNCs) by microwave-induced diffusion technique (MIND) to enhance solubility and dissolution of poorly water-soluble drug ketoprofen (KE). **Materials and Methods:** Natural drug carriers, ghatti gum, and acacia were selected for BNCs preparation based on their wetting and surface active agent property. BNCs were prepared by MIND method and characterized by Fourier transform infrared spectroscopy, differential scanning calorimetry, X-ray diffraction studies, scanning electron microscopy, and transmission electron microscopy. The solubility and dissolution enhancing performance of BNCs were assessed by *In vitro* solubility and dissolution studies. Carrageenan-induced rat paw edema model was performed to evaluate *in vivo* presentation of optimized KE formulation. **Results and Discussion:** It was demonstrated that dissolution of KE enhanced with increase in polymer attention. The optimized ratio of drug and polymer for the entire composite was found to be 1:3. The optimized formulation showed a significant reduction in rat paw edema as compared to marketed KE formulation. **Conclusion:** The study showed good *in vitro* and *in vivo* relationship. The MIND technique employed in this study is green and cost-effective method for bionanocomposites arrangement. Enhancement in the solubility might be because of generation of drug dispersion at micro- and nano-scale level. Hence, the development of BNCs is a promising approach to increase solubility and dissolution of poorly water-soluble drug.

Key words: Bionanocomposites, *in vivo*, ketoprofen, microwave assistance, solubility

INTRODUCTION

Solubility of drug is most tricky aspects in the formulation and development.^[1,2] Drug efficiency can be severely limited by poor aqueous solubility, and the majority of the drugs also show side effects due to their poor solubility. Increasing drug solubility increases the effectiveness and reduces side effects of certain drugs.^[3-5] Drugs having poor water solubility are linked with the slow drug absorption and finally inadequate or diverse bioavailability. At present, nearly 40% of newly synthesized drugs have problem of poor water solubility.^[6,7] The reduced aqueous solubility of drug in the gastrointestinal fluid often causes inadequate bioavailability. These drugs require high dose to achieve therapeutic plasma concentration. For oral

administration of drug, it is essential that it should be in an aqueous form at the site of action.^[8] The Biopharmaceutical Classification System (BCS) classified drugs into four categories according to the solubility and permeability. Class II BCS drugs exhibit high permeability and low solubility.^[6,7] Most of the non-steroidal anti-inflammatory drugs (NSAIDs) belong to the BCS Class II. They are

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greatly permeable through biological membrane but have limitation of poor aqueous solubility. The rate of drug absorption and bioavailability of such poorly water-soluble drugs are controlled by rate of dissolution in gastrointestinal fluids.^[8-10] This problem has been tried to resolve by many researchers by various methods in the past to enhance solubility and dissolution ultimately bioavailability.^[11] Ketoprofen (KE) is poorly aqueous soluble (0.5 µg/ml) which results in poor dissolution rate and reduces in its gastrointestinal absorption.

Oral route is the most common way of drug delivery system for drug administration. The majority of sold pharmaceutical products (drugs) are given orally.^[12] A key objective in the development of oral dosage forms is a good understanding of the *in vivo* and *in vitro* performance of the dosage form.^[3,13] The increase in surface area by particle size reduction increases dissolution property. Nanosize means the particle size between 100 to 1000 nm.^[11] BNCs produce improved saturation solubility and therefore shows increased dissolution velocity.^[14] Continuous advancement in the other drug delivery system leads to pay attention toward oral drug delivery system to enhance clinical efficiency and patient compliance. From pharmaceutical point of view, a numerous types of polymer are used to control drug release as of dosage form. The use of natural polymer rather than synthetic polymer is additionally preferred. Natural polymers are mainly used since they are readily available, inexpensive, nonreactive, capable of chemical modification, and potentially well suited and degradable.^[15-17] Due to the development of polymer-based BNCs, these are extensively used in the pharmaceutical manufacturing. Polymer BNCs are the polymer that has been reinforced with small quantities of nanosize particles having high characteristic ratio.^[18]

In this study, we have used novel technique called microwave-induced diffusion (MIND) which is green and cost-effective for the production of BNCs. Microwave heating starts from the direct interaction of electromagnetic force with the material. The extent to which the material is heated by microwave energy is reliant on the range of parameters, but particularly dielectric properties are more significant.^[19] Polar liquids such as water are very readily heated. Microwave heating can confer a number of benefits over conventional heating, which includes rapid heating and cooling, reduced temperature gradients across the sample, lower energy practice, and enhanced reaction rates.^[20] BNC is the process of complex formation between drug and natural polymers (acacia [AC] and ghatti gum [GG]) where microwave energy plays a significant role in reducing particle size of materials. It breaks intermolecular bonding and reduces the particle size. Nowadays, microwaves also applied for reducing particle size of drug material up to nanometer (nm). Reduction in particle size increases efficient surface area of the drug and thereby enhances solubility and dissolution rate. In the present study, KE BNCs formation enhances solubility which ultimately leads to increase in bioavailability of the

drug. Microwave heating is a well-established method for processing and drying.^[21,22]

The natural polymer acacia and gum ghatti are used for the formation of different BNCs. The sources of Acacia and gum ghatti are *Acacia senegal* and *Anogeissus latifolia*, respectively.^[23-24] Gum ghatti is naturally occurring, water soluble, complex polysaccharides obtain from the barks of *A. latifolia*. These are selected on the basis of their good surfactant and wetting property which are associated with enhance in the solubility and dissolution.^[18] KE is an active component of NSAIDs. KE is a 2-(3-benzoylphenyl) propionic acid which is used as analgesic and as well as used in the acute and long-term treatment of rheumatoid arthritis and osteoarthritis. Most of the NSAIDs are Class II drugs according to BCS. They are highly permeable through biological membrane but possess low aqueous solubility.^[9] KE has low bioavailability and fast elimination time which is about 1.5-2 h. Therefore, it is needed to enhance the solubility and dissolution ultimately bioavailability of KE.

MATERIALS AND METHODS

KE drug was obtained as gift sample from Glukem Pharmaceuticals (Hyderabad, Andhra Pradesh, India). AC (100 cps at 20°C) and GG (100 cps at 20°C) were brought from SD Fine Chemicals (Mumbai, Maharashtra, India) and Krystal Colloids Ltd. (Mumbai, Maharashtra, India), respectively. The microcrystalline cellulose, sodium starch glycolate, talc, and magnesium stearate were purchased from the SD Fine Chemicals (Mumbai, Maharashtra, India). Methanol, hydrochloric acid, and sodium dihydrogen phosphate were analytical grade.

Characterization of pure KE

Solubility determination

The solubility of KE drug was determined by taking excess amount of drug into 150 ml of distilled water and kept for 24 h on orbital shaker incubator at room temperature 25°C.^[24,25] The obtained solution was filtered by Whatman filter paper no. 1, and the drug concentrations were determined by taking ultraviolet absorbance using UV-Visible Spectrophotometer (UV-1800; Shimadzu, Tokyo, Japan) at 258 nm wavelength.

Fourier transform infrared spectrophotometric studies (FT-IR)

The detection of KE was done by FT-IR Spectroscopy. The FT-IR spectrum was obtained using FT-IR spectrophotometer (Shimadzu DR-8031, Japan). The wavelength of rang from 400/cm to 4000/cm with a resolution of 4/cm was used. KE gives characteristic peaks at 2983-2930/cm (due to aromatic C-H stretch, carboxylic acid O-H stretch), 1695-1649/cm (due to C=O stretch), 1595/cm (aromatic C=C stretch),

1437/cm (CH-CH deformation), 2891/cm (C-H stretch and O-H deformation), 1690/cm (carboxylic O-H out of plane deformation), and 860-640/cm (C-H out of plane deformation for substituted aromatic).^[26]

Characterization of polymers

Swelling index (SI)

SI of gums was calculated to check the swelling pressure. Accurately weighed 10 g of AC and GG was transferred to 100 ml measuring cylinder. The initial volume engaged by gum was well known. Distilled water was additional in the cylinder up to 100 ml, and the open end of cylinder was sealed with an aluminum foil. Measuring cylinder was kept aside for 24 h and volume of swelled gum was noted.^[27,28] The SI of gum was calculated by the following formula:

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

where SI: Swelling index; H_i : Initial height of powder; H_f : Final height of powder after 24 h.

Viscosity determination

Viscosity of gum was determined by taking 1 g of each AC and GG and dispersed in 100 ml distilled water (1% w/v). The viscosity of resultant dispersion was measured by viscometer (Brookfield DV-E, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) using spindle 3 at 100 rpm.^[29,30]

Foaming index

The foaming index was calculated to check the surfactant property of AC and GG. Accurately weighed 1 g of gum was transferred in 250 ml measuring cylinder containing 100 ml distilled water to make dispersion. Resultant dispersion was vigorously shaken for 2 minutes. The foaming index of gum was calculated by the subsequent equation:

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

where H_f is the height of solution of gum after shaking and H_i is the height of solution of gum before shaking.^[31]

Preparation of bionanocomposites (BNCs)

The BNCs were prepared by adding accurately weighed drug (KE) and carrier (AC and GG) in 1:1 to 1:9 w/w proportion. Homogeneous physical mixture of drug and carrier was prepared using mortar and pestle. Slurry was prepared by adding 5 ml of distilled water in each gram of drug-carrier physical mixture. A fixed amount of slurry (6 g) was placed in a glass beaker and irradiated with microwave radiations at power 640 W (Power Grill 20 Black, ONIDA, Mumbai, Maharashtra, India) with constant stirring.^[12] The temperature was noted using internal temperature measurement probe at the end of the treatment. BNCs were grounded using mortar

and pestle to obtain required size of 80-250 μm .^[11,32] The formulated BNCs of KE drug with carrier (AC and GG) were denoted by KEACNC and KEGGNC.

Drug content analysis of BNCs

The KE drug incorporated into the BNCs (KEACNC and KEGGNC) was calculated by dissolving BNCs mixture in the 25 ml methanol. The resulting solution was filtered by 0.2 μ membrane filter and analyzed by UV-visible spectrophotometer at the wavelength of 258 nm against the methanol as a blank.

Solubility study

The solubility study of BNCs (KEACNC and KEGGNC) was carried out by adding excess amount of pure KE drug (equivalent to 30 mg) and BNCs to 150 ml distilled water in a separate flask. The resultant mixture was stirred for 24 h at 25°C temperature using orbital shaker incubator. The supernatant liquid was collected and filtered through 0.2 μ membrane filter and analyzed by UV-Visible spectrophotometer at 258 nm wavelength.^[33] The solubility of pure KE drug was observed to be 0.13 mg/ml. The drug:carrier ratio was optimized from the result of solubility study.^[34]

Characterization of BNCs

Characterizations of BNCs were carried out by FT-IR, differential scanning calorimetry (DSC), X-ray diffraction (XRD), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) to ensure the compatibility of drug and polymer.

FT-IR spectroscopy (FT-IR)

FT-IR spectra of pure drug (KE), pure polymers (AC and GG), and BNCs of drug with individual polymers (AC and GG) were carried out to check the compatibility of drug with polymer. BNCs of drug with each polymer (KEAC_M and KEGG_M) were mixed with potassium bromide of IR grade in the ratio of 1:100. The pellets were scanned using FT-IR spectrophotometer (Shimadzu DR-8031, Japan). Infra-red spectrum of material gives the information regarding drug-polymer relations. The materials were scanned through a range from 400/cm to 4000/cm with a resolution of 4/cm. Characteristic peaks of the drug, AC and GG were compared with the formulated BNCs to check the compatibility of drug-polymer.^[35]

DSC

DSC studies of pure drug (KE), pure polymer (AC and GG), and BNCs of drug with individual polymer (AC and GG) were performed to access the better solubility of drugs. DSC thermogram could be obtained using DSC (DSC 60; Shimadzu) at heating rate of 10°C/min from temperature 0-250°C. The DSC gives the information related to melting point, type of heating reaction (either endothermic or exothermic), and physical and chemical interaction between drug and polymer.^[36]

XRD studies

XRD study of drug (KE), pure polymers (AC and GG), and BNCs of drug (KE) through individual polymers (AC and GG) were determined to estimate the changes in the crystallinity made when drug was mixed with gums. The crystallinity property is associated with physicochemical properties of material. The XRD patterns of the drug, polymers, and BNCs were recorded using (D8 ADVANCE with DAVINCI, India). The scanning angle ranged from 1 to 40 of 2θ .^[36]

SEM

The surface morphology of KE BNCs is observed by SEM. The samples were mounted unswervingly onto the SEM sample holder using double-sided sticking tape and images were recorded at the required magnification at acceleration voltage 15 kV and working distance of 8 mm on XL30-SFEG Philips (Labexchange, Burladingen, Germany). The mean particle size, standard deviation, and 95% confidence interval were calculated by a written program which randomly selects 100 particles of the SEM images.^[36]

TEM

TEM study was performed to confirm size and shape of drug crystals dispersed in the polymer. The sample for TEM (Hitachi Model H600-3, Tokyo, Japan) was mounted on a carbon-coated copper grid made up of disc type with thinned central area of size 3 mm.

Preparation of immediate release (IR) tablet

The optimized BNCs with orientation to solubility, dissolution, type of polymer, and drug to polymer ratio were selected for formulation of IR tablet. The compositions of IR tablet are shown in Table 1. All ingredients were sieved through #60 sieves. Direct compression method was used to prepare tablet using 10 mm punch (Mini press - II MT, M.S. No.03008290, Karnavati Engineering Ltd, Gujarat).

Evaluation of IR tablet

Pre-compression evaluation

Pre-compression evaluation of IR tablet for angle of repose, Carr's index (compressibility), and Hausner's ratio of tablet mixture were performed according to USP 30 (2007).^[37]

Table 1: Physical characterization of acacia and ghatti gum

Material	% Swelling	Viscosity (cps)	Foaming index
Acacia gum	71.92±2.21	4.16±0.25	18±0.92
Ghatti gum	75.31±1.01	5.67±0.11	16±0.65

All values are represented as means±SD, n=3. SD: Standard deviation

Post-compression evaluation

Post-compression evaluation was carried out for hardness, weight variation, friability, disintegration time, and drug substance according to USP 30 (2007).^[38,39]

In vitro dissolution test

In vitro dissolution test of optimized tablet was performed using USP XXIV apparatus Type II (Electro labTDT-08L, Mumbai, Maharashtra). Phosphate buffer 900 ml of pH 6.8 was used as dissolution media. Tablet containing 100 mg (or equivalent to 100 mg) of KE was added in dissolution media of temperature $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, and rotation speed of paddle was constant at 50 rpm. A 5 ml of sample was taken at 5, 10, 15, 30, 45, and 60 min by replacing 5 ml buffer solution in dissolution media. Samples were filtered through 0.5 μ membrane filter and analyzed spectrophotometrically at the wavelength of 258 nm. Correction factor was notable during each sampling and calculating percentage CDR.^[40,41]

In vivo evaluation

In vivo study was performed according to a protocol submitted and approved by the Institutional Animal Ethical Committee of Nanded Pharmacy College, Nanded, MS, India. Anti-inflammatory activity of F2 tablet was evaluated by carrageenan-induced rat paw edema study as described by 0.1 mL of 1% (w/v) carrageenan suspension in 0.9% (w/v) sterile saline being injected into the plantar tissue of the left hind paw of Albino-Wistar rats. Rats were divided into three groups each containing eight rats. Animals of respective groups ($n = 8$) were administered orally with vehicle (tween 80, 3 mL of 1% solution), indomethacin 10 mg/kg b.w., p.o. and 100 mg/kg b.w., p.o. of F2 tablet before 1 h prior to the carrageenan-induced paw edema. The right paw served as a reference to non-inflamed paw for comparison. The paw volume of all the groups was measured using plethysmograph for next 24 h after carrageenan injection. The percentage inhibition of edema volume by F2 tablet and indomethacin drug-treated groups was compared with control group.^[42,43] The percentage inhibition of edema volume was calculated using the formula:

$$\text{Percentage inhibition} = (1 - V_i/V_c) \times 100$$

where V_i and V_c are the relative changes in the edema of the F2 tablet and control respectively.

RESULTS AND DISCUSSION

Physical characterization of carriers

The percentage swelling, viscosity, and foaming index are shown in Table 1. The swelling property and viscosity of AC and GG was low. Due to the less viscosity of AC and GG, they were considered for solubility and dissolution enhancement of drug.^[8] AC showed low viscosity and high foaming index than the GG. Hence, AC gum was more suitable than GG for increasing solubility and dissolution rate of drug.

Solubility studies

Solubility of KE

The solubility of pure KE drug was observed to be 0.13 mg/ml.

Solubility of physical mixture

The drug:carrier ratio was optimized from the outcome of solubility study. The solubility study showed that AC and GG enhance solubility. Solubility studies of physical mixtures and BNCs clearly indicated that the ratio of drug to polymer increases solubility. No significant increase in solubility was shown after 1:5 ratio of drug to polymer, so 1:5 ratio was optimized [Figure 1a and b]. This optimized ratio was then confirmed with powder dissolution and found to be increased in solubility [Figure 2].^[8,28] Enhancement of solubility with KEACNC was established to be more than KEGGNC; this may be due to more foaming index of AC than GG.

Powder dissolution test

The powder dissolution test was performed to check solubility enhancing properties of the materials. The

dissolution profile of BNCs showed notable improvement in the dissolution rate in KE BNCs when compared with the pure KE. BNCs of KE with AC demonstrated good result. It released 81% in comparison to pure KE which released 61% after 60 min. On the basis of obtained results of solubility and dissolution studies, KEACNC was selected to formulate the tablet. The dissolution profile of KE and KE BNCs is shown in Figure 3. KEACNC powder released 81% of drug in a solution compared to pure drug which was released only 61%. Therefore, it can be concluded that dissolution rate of KE drug has been enhanced with BNCs.

Drug content analysis of BNCs

Uniform dispersion of drug in the BNCs was determined by drug content analysis. It was found that 96-98% drug was incorporated in the BNCs showing consistent dispersion of drug.

FT-IR studies

The infrared spectrum of pure KE was obtained as 2978.5, 2955.3, 2973.3, 1697.6, 1689.3, 1700.1, 1597.3, 1560, and 1560. Pure KE represents principle peak at O-H stretching

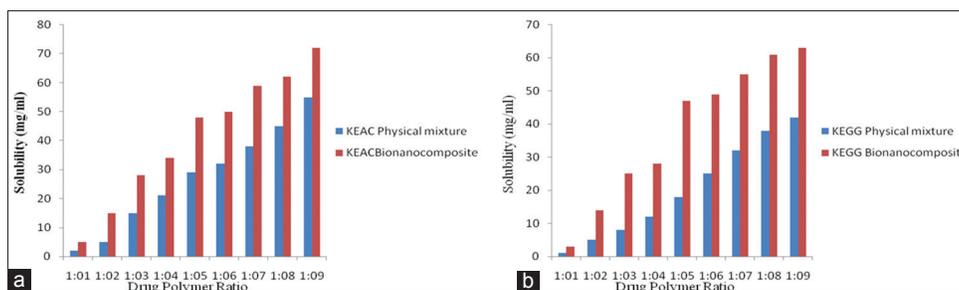


Figure 1: (a) Solubility comparisons between KEAC physical mixture and KEGG. (b) Solubility comparisons between KEGG physical mixture and KEGGNC. Data are mean±SD, n = 3. Values are expressed as percentage of solubility of pure ketoprofen. KE: Ketoprofen, AC: Acacia, GG: Ghatti gum, SD: Standard deviation

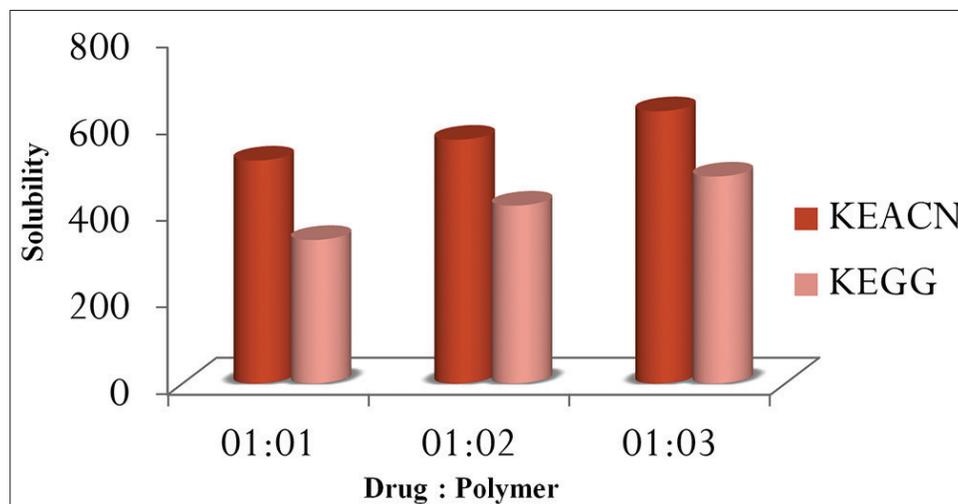


Figure 2: The solubility of ketoprofen and bionanocomposites

(Carboxylic acid group at 2978.5), C=O stretching (1697.6), and C=C bonding (1597.3). All the peaks showed in the pure drug were unchanged in the FT-IR spectra of KEACNC and KEGGNC [Figure 4]. It shows that there is no chemical reaction between drug and polymer after microwave irradiation of KE.

DSC studies

DSC thermograms of pure drugs (KE), polymers (AC and GG), and BNCs of individual drug with individual polymer are shown in Figure 5. DSC of pure KE exhibited sharp

endothermic peak at 94°C indicating melting of KE. DSC of KEACNC and KEGGNC had shown same endothermic peak as that of pure drug but with reduced intensity which might be due to decrease in the crystalline nature of drug. Slight shift in the melting point indicated reduction of drug to nanocrystalline form. Broadening of peak shows that most of the drug converted into nanocrystalline form. No chemical interaction involving drug and polymer was observed. Physical interaction was the mechanism by which drug bound to the polymer. These studies confirmed that as the crystal size of crystalline nanoparticle reduces, its melting point also reduces minutely.

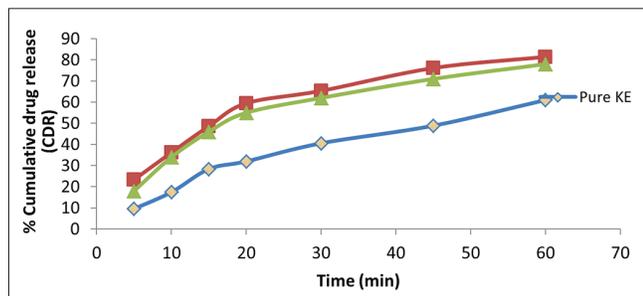


Figure 3: Powder dissolution studies of pure ketoprofen, KEACNC, and KEGGNC powder

XRD studies (XRD)

XRD was performed to check the physical state of drug and its BNCs. XRD pattern of pure drug (KE), pure polymer (ACC and GG), and its BNCs is shown in Figure 6. The XRD pattern of pure KE represents crystalline peak between 100 and 600. It demonstrated characteristic diffraction peaks at 5, 13, 14.50, 17.50, 19, 21, 24, 25.50, 28, 30, 32, and 35 with intense peak at 24 indicating crystalline nature of KE. XRD pattern of KEACNC and KEGGNC showed reduced peak intensity due to decreased crystallinity. Reduced peak

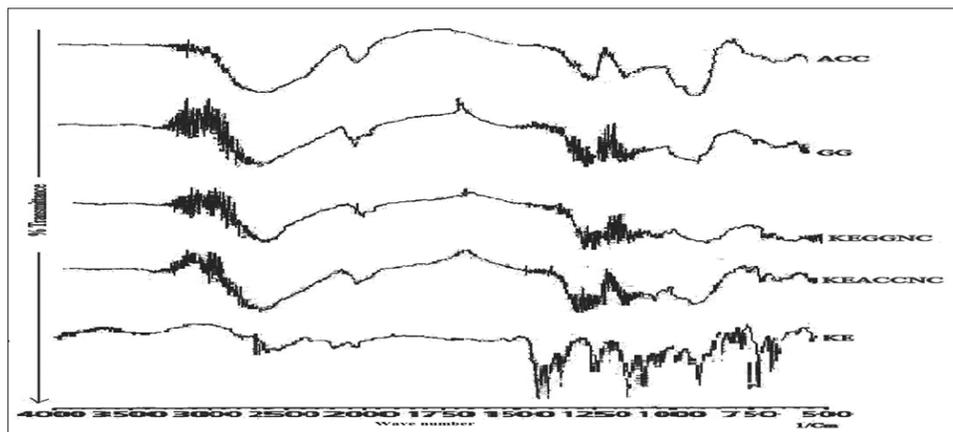


Figure 4: Fourier transform infrared studies of pure acacia, ghatti gum, pure ketoprofen, bionanocomposites (BNCs) of ketoprofen with acacia, and BNCs of ketoprofen with ghatti gum

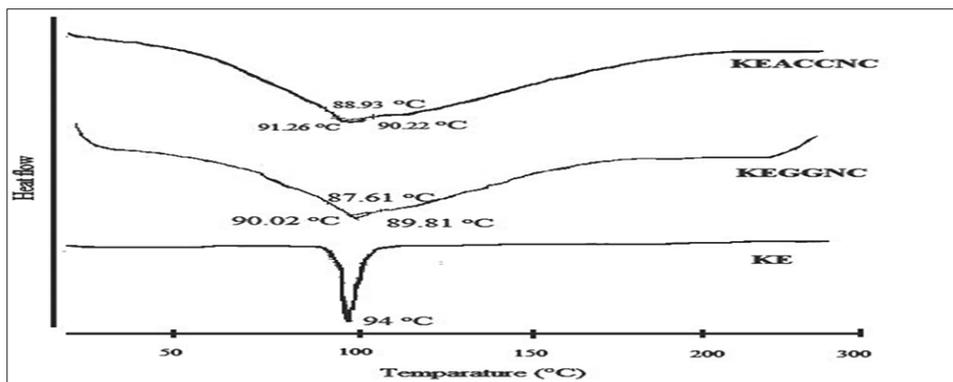


Figure 5: Differential scanning calorimetry studies of ketoprofen and acacia bionanocomposites (BNCs), ketoprofen and ghatti gum BNCs, and pure ketoprofen

intensity of BNCs might be due to reduction in the drug size to the nanolevel.

SEM

The SEM study was done to check surface morphology of the drug particles. The SEM of polymer (ACC) and its BNCs is shown in Figure 6. KE particles were plate-shaped with a smooth surface while KEAC particles were of irregular shape and size. Figure 7 clearly demonstrates that crystal shape of KE was completely changed in KEACNC showing embedded KE crystals in the matrix.

TEM

TEM was carried out to confirm particle size of embedded KE drug particles. The TEM images of pure drug (KE) and bionanocomposites KE with AC (KEACNC) are shown

in Figure 8. The TEM results demonstrated rapid release of KE drug from the BNCs due to loose network structure which enhances solubility and dissolution characteristics of KE drug. It was clear that the KE nanoparticles were rod-shaped morphology having 1 μm (1000 nm) diameter. TEM of KEACNC showed surface dark spots of AC polymer in which the drug has been dispersed. The structure of KE BNCs was looser, smaller size with average diameter of 110nm. This revealed that MIND process was responsible for agglomeration and change in size of the particles which might be due to crosslinking among the different nanoparticles with polymers.

Evaluation of IR tablet

Pre-compression evaluation

The angle of repose, Carr's index, and Hausner's ratio of all formulation were measured [Tables 2 and 3]. The results

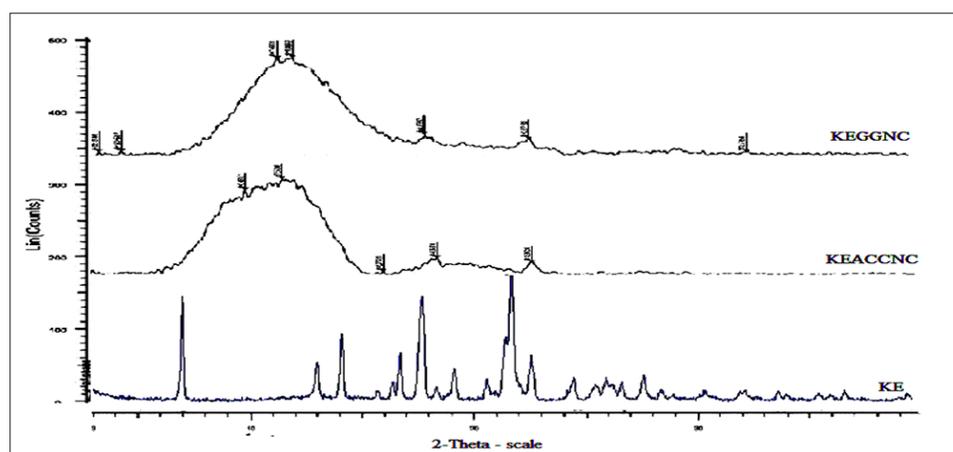


Figure 6: X-ray diffraction studies of bionanocomposites (BNCs) of ketoprofen with ghatti gum, BNCs of ketoprofen with acacia, and pure ketoprofen

Table 2: The solubility of pure ketoprofen drug was observed to be 130 $\mu\text{g/ml}$

Ratio of drug to polymer	Solubility of KEACNBC ($\mu\text{g/ml}$)	Solubility of KEGGNBC ($\mu\text{g/ml}$)	KEACNBC (Fold)	KEGGNBC (Fold)
1:1	514.21 \pm 0.46	331.16 \pm 1.32	10.07	6.49
1:2	562.32 \pm 1.65	410.28 \pm 0.87	11.01	8.03
1:3	627.87 \pm 2.43	477.37 \pm 1.89	12.29	9.35

KEACNBC: Ketoprofen acacia nanobiocomposite, KEGGNBC: Ketoprofen gum ghat nanobiocomposite

Table 3: Formulation batches of IR tablets of KEACNC

Ingredient	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)
KEACNBC	400	400	400	400
Microcrystalline cellulose	50	50	65	65
Sodium starch glycolate	30	40	30	40
Magnesium stearate	3	3	3	3
Talc	3	3	3	3

IR: Immediate release, KEACNBC: Ketoprofen acacia nanobiocomposite, KEACNC: Ketoprofen Acacia Nanobiocomposite, KEAGNC: Ketoprofen Gum Bharti Nanobiocomposite

of pre-compression evaluations of formulation mixtures are represented in Table 4. From the results, it can be concluded that prepared formulation mixture has excellent flow properties, good compressibility, and Hausner's ratio. Mixture can be easily compressible into tablet and does not show any flow problem.

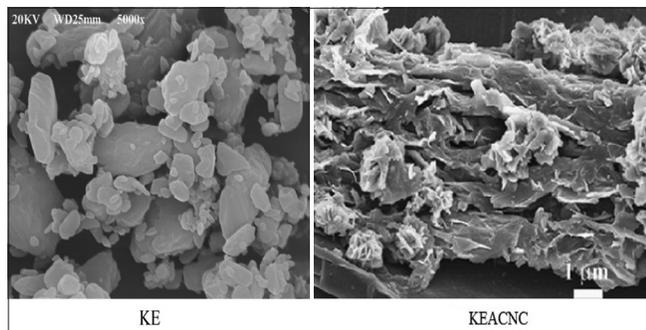


Figure 7: Scanning electron microscopy images of ketoprofen and bionanocomposites of ketoprofen with acacia

Table 4: Pre-compression evaluation of IR tablet

Formulation	Angle of repose	Carr's index	Hausner's ratio
F1	33.69°±2.56	11.11±1.15	1.125±0.01
F2	32.39°±1.48	12.15±0.50	1.136±0.00
F3	32.66°±1.87	13.18±0.61	1.110±0.01
F4	33.82°±2.17	11.21±0.75	1.121±0.02

All values are represented as means±SD, $n=3$. SD: Standard deviation, IR: Immediate release

Table 5: Post-compression evaluation of IR tablet

Formulation	Weight variation (mg)	Hardness (kg)	% friability	Drug content uniformity (%)	Disintegration time (s)
F1	486±2.91	3.81±0.13	0.87	99.2818	69±2.72
F2	498±3.81	3.62±0.21	0.75	100.1813	49±3.15
F3	502±4.29	3.51±0.18	0.69	98.1408	78±2.11
F4	504±4.05	3.38±0.11	0.55	95.1739	60±3.21

All values are represented as means±SD, $n=3$. SD: Standard deviation, IR: Immediate release

Table 6: *In vivo* evaluation of F2 tablet

Time (h)	Increase in paw volume (mL)			% Inhibition	
	F2 tablet (100 mg/kg)	Control	Standard indomethacine (10 mg/kg)	F2 tablet (100 mg/kg)	Standard indomethacine (10 mg/kg)
0 h	0.881±0.140	0.931±0.116	1.241±0.154	NA	NA
30 min	1.156±0.140	1.918±0.118	1.743±0.149	72.41	79.88
1 h	1.06±0.140	2.008±0.118	1.631±0.152	84.51	80.16
2 h	1.98±0.137	2.131±0.116	1.533±0.154	98.14	92.12
3 h	1.938±0.122	2.300±0.112	1.431±0.157	94.72	95.16
4 h	0.876±0.141	2.401±0.096	1.376±0.154	99.57	97.83
24 h	0.851±0.102	1.231±0.117	1.343±0.157	71.79	88.86

Post-compression evaluation

The formulation was subjected to various tests for post-compression evaluations such as hardness, friability, content uniformity, disintegration time, and weight variation [Table 5]. All the results were within the limit as per USP 30.

In vitro dissolution study of IR tablet

Tablet formulation tablet F2 showed least disintegration time, so tablet F2 was selected for further characterization. *In vitro* dissolution test was performed to check drug release from tablet. It was found that $91.45 \pm 1.2\%$ of drug was released after 30 minutes. The results are shown in Figure 9.

In vivo evaluation study of IR tablet

In vivo evaluation of F2 table containing 100 mg/kg KE demonstrated change in paw volume 0.881 ± 0.140 , 1.156 ± 0.140 , 1.06 ± 0.140 , 1.98 ± 0.137 , 1.938 ± 0.122 , 0.876 ± 0.141 , and 0.851 ± 0.102 at 0 h, 30 min, 1, 2, 3, 4, and 24 h, respectively. The effect is same (1.241 ± 0.154 , 1.743 ± 0.149 , 1.631 ± 0.152 , 1.533 ± 0.154 , 1.431 ± 0.157 , 1.376 ± 0.154 , and 1.343 ± 0.157 at 0 h, 30 min, 1, 2, 3, 4, and 24 h, respectively) as shown by standard, indomethacin. The percentage inhibition also increased from 30 min to 4 h. The F2 table exhibited similar type of effect as that of indomethacin [Table 6].

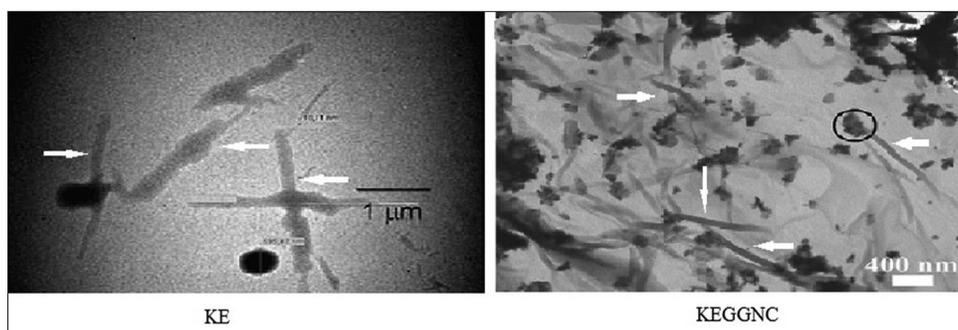


Figure 8: Transmission electron microscopy images of pure ketoprofen and bionanocomposites ketoprofen with acacia

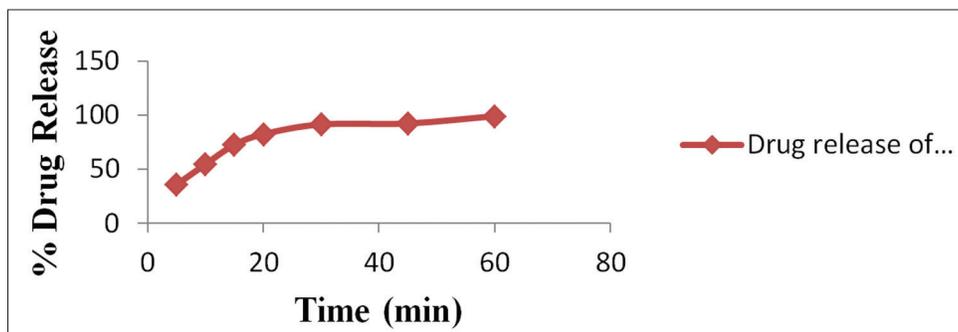


Figure 9: *In vitro* dissolution test of F2 tablet

Table 7: Stability studies of optimized F2 formulation

Time in days	Disintegration time (min)	% drug content	% <i>In vitro</i> drug release
0	48±1.77	98±2.15	99±2.58
30	47±1.22	99±1.98	98±1.36
60	48±1.33	100±1.56	100±1.97
90	49±1.02	99±1.25	98±2.54

All values are represented as means±SD, $n=3$. SD: Standard deviation

Stability studies

Optimized formulation was subjected to stability studies according to ICH guidelines. Different parameters such as disintegration time, drug content, and *in vitro* drug release were considered before and after 0, 30, 60, and 90 days of stability. The results of stability study are depicted in Table 7. No significant change in the disintegration time, drug content, and *in vitro* drug release was observed after stress condition of stability studies. This observation confirms that the prepared formulation is stable and not much affected by stress conditions.

CONCLUSION

The present study revealed the employability of natural carriers such as AC and GG in the microwave-generated BNCs for the enhancement of solubility and therefore

dissolution of drug substance. The solubility of KE was increased 12.2-fold along with significant improvement in dissolution performance of formulation. Result of FT-IR, XRD, DSC, SEM, and TEM shows that KE converted into the BNCs is responsible for the enhancement of solubility and dissolution. It also shows that there is no significant interaction between drug and polymer. *In vitro* and *in vivo* evaluation of optimized formulation confirms the use of BNCs for increasing solubility and dissolution of drug. The *in vivo* evaluation of F2 tablet revealed same actions as that of standard, indomethacin. On the basis of the present study, it can be concluded that microwave-generated BNC is one of the potential approaches to enhance solubility, dissolution, and ultimately bioavailability of drug.

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