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Dissolution Rate Enhancement of Nimesulide Using Electro-spinning and Cogrinding Techniques: A Comparative Study

Shaik Gousiya Begum[1], P. V.Swamy[1]*, SandeepKalepu[1], Prasad Konduri[1], Mohan Varma Manthena[1], SudheerBetha[1]
1. Shri Vishnu college of Pharmacy, Vishnupur, Bhimavaram-534202, W.G. dist., Andhra Pradesh, India.

ABSTRACT

Nimesulide is a selective Cyclooxygenase - 2 enzyme inhibitor useful in inflammatory conditions. It belongs to class 2 of Biopharmaceutical classification system (BCS), which has very low water solubility leading to low oral bioavailability. The objective of present study was to enhance the solubility and dissolution rate of poorly soluble model drug, nimesulide. For the same purpose, two approaches were used. First approach includes Electrospinning technique, and the second approach is co-grinding technique. In Electrospinning technique, the solution containing drug and polymer (PVP K-90) dissolved in an organic solvent was taken and made in to nanofibers of different drug-polymer ratios (1:1, 1:2, 1:3, 1:4, 1:5) using electro-spinning apparatus. In the co-grinding technique, mixtures with different ratios (1:1, 1:3, 1:5, 1:7, 1:9) of drug and carrier were prepared employing potato starch, lactose, microcrystalline cellulose, sodium starch glycolate and treated agar as carriers. Of the two approaches, nanofibers provided a better solubility enhancement when compared to co-grinding mixtures and pure drug. The optimized formulation (nanofibers) was characterized by Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM), Differential scanning calorimetry (DSC) and the *in-vitro* dissolution rate studies. The optimized nimesulide nanofiber formulation displayed nearly 50- fold faster dissolution compared to the pure drug and the optimized co-ground mixture. It has also shown two to three- fold greater anti-inflammatory activity in Wistar albino rats as compared to pure drug. Hence, nanofibers produced by electrospinning technique provided a scope for enhancing the solubility and dissolution rate by encapsulating poorly water soluble drugs within the polymeric matrix leading to potential bioavailability enhancement through the oral or topical routes.

Keywords: Electro-spinning, Nimesulide, Dissolution rate enhancement, nanofibers.

*Corresponding Author Email: vspadavala@svcp.edu.in
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INTRODUCTION

Tissue engineering and drug delivery research has gained a greater importance in the last decade owing to their efficiency in improving human health[1]. Administering drugs via conventional dosage forms (tablets, capsules, parenterals, etc) suffers from drawbacks like poor water solubility and dissolution rate limited absorption into the systemic circulation, rapid metabolism before reaching the target site, local tissue damage due to extravasations and cytotoxicity[2]. In order to circumvent these drawbacks, a suitable drug delivery system that provides a maximum therapeutic action with decreased side effects and improved patient compliance is required. At this juncture, nano- based drug delivery system comes into discussion, due to its distinctive physical, chemical, optical and mechanical properties which differ from those observed in microscopic and macroscopic realms[3]. Among these nano-based drug delivery systems, electro spun nanofibers have gained a greater attention due to its versatility and potential applications in efficient delivery of drugs and bioactives, when compared to conventional dosage forms[4-8]. High specific area, porous structure and a 3 dimensional (3D) reticulate structure mimicking the natural extracellular matrix of these electro spun nanofibers marks their applications in drug delivery and tissue engineering[4]. Electro spun nanofibers can be produced by electrostatic fiber spinning, commonly called as electrospinning technique. The drug release properties from electro spun nanofibers have been first observed by Kenawy *et al*[9]. Various drugs and bioactives could be efficiently delivered to the target site by encapsulating within the polymeric nanofiber. The release rate of drug from the nanofiber can be modulated by modulating the polymer composition, fiber structure and morphology (fiber diameter and porosity)[10-12]. The most widely prescribed medications in the world is occupied by Nonsteroidal anti-inflammatory drugs (NSAIDs) which exhibit analgesic, anti-inflammatory, platelet inhibitory and antipyretic properties[13,14]. Nimesulide (NM) is one of the examples of this class, which has the ability to inhibit the cyclooxygenase enzyme (Cox), selectively Cox-2 enzyme and have less drug related side effects[15]. However, the oral bioavailability of NM varies to a greater extent due to its poor aqueous solubility and wettability. Several attempts have been made to overcome these problems by formulating NM with different techniques like micronization[16], complexation[17], use of co-solvents, surfactants[18], etc. To the best of our knowledge, very little information is available on the enhancement of NM solubility using nanofibers technique. Therefore in the present study, we have developed drug loaded nanofibers and co-ground mixture of a model drug NM. A comparative study was made between these two techniques for the enhancement of drug solubility and release properties. Solid state

characterization was carried out by Fourier-transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC). Phase solubility studies and *in vitro* dissolution experiments were carried out to investigate the interactions in solution. Anti-inflammatory activity of drug loaded nanofibers was investigated by conducting *in vivo* anti-inflammatory studies on Wistar albino rats.

MATERIALS AND METHOD

NM was obtained as a generous gift sample from Dr Reddy's laboratories, Hyderabad. Polyvinylpyrrolidone (PVPK-90) was purchased from Sigma Aldrich, USA, microcrystalline cellulose, potato starch, lactose, sodium starch glycolate and chloroform were purchased from S.D. Fine Chemicals (Mumbai, India). All other chemicals/solvents used were of analytical reagent grade.

Preparation of NM nanofibers by electrospinning

Electro-spinning apparatus (E-SPIN NANO, Physics Equipments Co., Chennai, India) was a used for the preparation of the nanofibers. Different formulations containing varying ratios of drug: polymer (1:1, 1:2, 1:3, 1:4 and 1:5) were formulated. In brief, for the formulation of NM nanofibers containing 1:2 ratio of drug and polymer, 1 g of nimesulide was dissolved in 5 ml of chloroform to obtain a clear solution. This was added to the polymer solution containing 2 g of PVP K-90 in 5 ml of chloroform. The obtained solution of drug and polymer was kept stirring for overnight. The obtained polymer solution containing drug was filled into a syringe and was fixed to position in the electro-spinning apparatus. The process variables for electro-spinning included a 12 cm distance between the tip of the needle and the collector, a voltage of 12 kV and flow rate of 1 ml/hr using a 24 gauge needle. The NM nanofibers were drawn using the fixed process variables. The obtained fibers were stored in desiccators for further use. The drug loaded nanofibers were mixed with the diluent lactose, in a plastic bag. Magnesium stearate, talc, sodium starch glycolate and lactose were passed through # 60 mesh(British standard sieve), mixed and blended with the initial mixture in a plastic container. The blend was filled in the empty hard gelatin capsules of 'zero' size.

Preparation of co-grinding mixtures

Co-ground mixtures (CG-L,CG-PS,CG-TA,CG-SSG,CG-MCC) of drug and excipient were prepared by using four carriers, namely lactose(L), potato starch(PS),sodium starch glycolate (SSG), treated agar(TA) and microcrystalline cellulose (MCC) in five different weight ratios, i.e., 1:1,1:3,1:5, 1:7 and 1:9. For this, required quantity of excipients was passed through # 60 mesh

sieve (British Standard Sieve) to remove lumps and aggregates. To this, weighed quantity of drug was added and the resulting physical mixture was triturated for 20 min in a glass mortar. The obtained powder was sifted through #100 mesh sieve. To ascertain the effect of method, carrier or both on the dissolution rate of pure drug (NM), alone was ground for 20 minutes. All the samples were stored in desiccators at room temperature taking precautions to protect from light.

Preparation of Treated Agar

10 g of agar powder was taken and added to 100 ml of distilled water with continuous stirring at 50 rpm using a mechanical stirrer. It was continued for 24 hrs in order to achieve water absorption and swelling. The obtained liquid was poured in a Petri-dish and dried for 72 hrs in an incubator at 37±1^{o}C. After drying the mass was pulverized and sifted through # 80 mesh sieve[19].

Characterization of Electro-Spun Nanofibers

FTIR Studies

Fourier transform infrared (FTIR) spectra were used to investigate the interaction and establish the compatibility between nimesulide and polymer (PVP K-90). The FTIR spectra of the optimized NM nanofiber formulation, along with pure drug and excipients were studied to confirm the compatibility of the drug with the excipients. The FTIR spectra were obtained by using the FTIR spectrophotometer (Bruker) using the KBr pellet method, which included a scanning range of 400 – 4000 cm^{-1} and a resolution of 1 cm^{-1}.

Differential Scanning Calorimetry (DSC)

The melting point and enthalpy of drug was evaluated by the DSC analysis (DSC Q2000, TA instruments, USA). Previously the equipment was calibrated using Indium and Zinc. The samples were heated at a ramp of 10°C/ min through a range of 20 - 200°C in aluminium pans under nitrogen gas. The DSC study was performed for the pure NM and the optimized drug loaded electro spun nano fibers.

Scanning Electron Microscopy (SEM)

The surface morphology and the diameter of the optimized nanofibers were studied by using a scanning electron microscope (Philips XL 20, Eindhoven, Netherlands), which included an operating voltage of 15 kV. The sample of fiber was placed on a metal stub with double sided adhesive tape. Before observation, the sample was coated with gold under vacuum in an inert gas atmosphere.

Evaluation of Nanofibers

Drug content uniformity

A sample of nanofibers equivalent to 100 mg of drug was accurately weighed and was transferred into a 100 ml volumetric flask. The contents of the flask was dissolved in small quantity of 0.1N sodium hydroxide solution, the volume was made up to the mark with the pH 7.4 phosphate buffer. The contents of the flask were filtered and the drug content was determined spectrophotometrically at 397 nm (Model-UV3000, Lab India Instruments Pvt Ltd, Hyderabad, India) after appropriate dilution. The drug content is calculated from the absorbance obtained with the help of the calibration curve.

Solubility Studies

The solubility studies for the pure drug and the optimized nanofibers (NF4) were carried out by adding an excess drug to 20 ml of distilled water and agitated for 48 h at 200 rpm in a gyratory flask shaker (Remi Laboratory *Instruments*, Mumbai, India). The saturated solution was filtered, diluted and analyzed using the UV-Visible spectrophotometer at 397 nm.

***In-vitro* Dissolution Studies**

Dissolution rate of nimesulide from all the nanofibers/co-ground mixtures was performed using the USP dissolution test apparatus II with the rotating paddle (DS 8000, Labindia Instruments Pvt Ltd, Hyderabad, India). Nanofiber sample equivalent to 100 mg of NM was added to the dissolution medium (900 ml of pH 7.4 buffer), a paddle speed of 50 rpm and a temperature of 37±0.5°C was maintained throughout the study. Samples of dissolution medium (5 ml) were withdrawn at different time intervals (5, 10, 20, 30, 45 and 60 min), filtered through a 0.45 µm membrane filter disc (Millipore) and assayed at 397 nm using the UV-Visible spectrophotometer. The dissolution efficiency (DE) of a pharmaceutical dosage form is defined as the area under the dissolution curve up to the time 't', expressed as the percentage of the area of the rectangle representing 100% dissolution in the same time period[20].

$$DEt = \frac{\int_0^t y \times \ \mathrm{d}\, t}{y100 \ \times t} \times 100$$

Where, y is the percent of drug dissolved at time t.

Stability Studies

Stability studies were performed for the optimized formulation at 25°C/ 60% RH and 40°C/ 75% RH over a period of 3 months. Samples were withdrawn at monthly intervals for the estimation of the drug content. At the end of the three months period, the dissolution test was also performed to determine the drug release profile.

In-vvo Studies (Anti-inflammatory Activity)

Carrageenan-Induced Paw Edema

The batch having optimal fiber diameter and showing satisfactory *in-vitro* drug release was selected for *in-vivo* studies. *In-vivo* studies were carried out to compare the control, API and test formulation. CPCSEA guidelines were followed for care of animals and the experimental protocol was approved by Institutional animal ethics committee (approval number 439/PO/01/a/CPCSEA). The anti-inflammatory activity of test drug from nanofibers against carrageenan-induced paw edema in rats was investigated using the method proposed by Winter *et al.*[21]. Wistar albino rats (150 to 200 gm) of either sex were originally obtained fromM/s Sainath agencies, Hyderabad, India. The access to standard diet was provided to the animals (housed in cages) maintained under environmental conditions in an air conditioned area at 16°C with 10/14 h light/dark cycle. Basing on the pilot experiments carried out using six animals for each group the optimum conditions for experiments were decided. Before starting the experiments, they were kept fasting for 18 hours. Initially, using a plethysmometer, left hind paw volumes to the tibio-tarsal articulation were recorded. Edema was produced by injecting 0.1 ml freshly prepared 1% carrageenan (in sterile saline solution) to the sub-plantar aponeurosis of the left hind limb, after one hour of the drug administration. In order to ensure uniform hydration (i.e. to minimize the variation in edema formation) water was administered to the rats at a dose of 2 ml/100 g body weight. At predetermined intervals of 1, 3 and 24 h, the paw volume was recorded. Results were expressed as percentage increase in paw volume at various intervals of time when compared to the initial values. Eighteen rats were taken in total and divided into three groups having six animals each was used for the study. Sodium carboxymethyl cellulose (sodium CMC) solution (0.5%)was given to the control group and test groups received pure drug (NM) and NM nanofibers orally before 1 hr of carrageenan (1.0% suspension of the powder in 0.5% sodium CMC solution) administration. At '0'hrs of administering 0.1 ml of 1% carrageenan in all the above groups, Plethysmometer was used to measure the volume of rat hind paw up to the ankle joint. At successive intervals of 1, 3 and 24 hrs, the paw volume was further measured. The % difference in the right and left paw volumes of each animal (control, pure drug (standard) and nanofiber treated (test) groups) was calculated. The % edema inhibition by the drug was given by comparing the mean % change in paw volume in control, standard and test animals.

Percent inhibitions were calculated as follows:

$$\% \text{ inhibition} = (1 - V_T/V_C) \times 100$$

Where, V_T and V_C are the mean paw volume of the treated and control groups, respectively, at 1, 3, or 24 hr.

Dose Calculations for Experimental Animal [22]

Conversion Factor = 0.018 per 200 gm of Rat

(Human dose to animal)

Statistical Analysis:Graph pad prism version 6, software was used for statistical analysis. The inhibition of paw edema in rats was described using Histograms and ANOVA.

RESULTS AND DISCUSSION

FTIR Studies

The optimized sample and pure drug were subjected to FTIR studies in order to examine the possible interaction between drug and polymer. Figure1. indicated the FTIR images of drug (a) and nanofibers (b). Observing the spectra of pure drug, major absorption band at 3283 cm^{-1} indicated N-H stretching vibrations. CH_3 stretching can be confirmed by the presence of bands at 2929 cm^{-1} (aliphatic C-H stretching) and 3085 &3065 cm^{-1}(aromatic C-H stretching). The peaks at 1341 cm^{-1} and 1153 cm^{-1} can be attributed to SO_2 stretching. C-O-C ether linkage can be confirmed by the presence of bands at 1217 cm^{-1}. Peaks at 1588 &1248 cm^{-1} can be attributed to asymmetric and symmetric stretching of NO_2. FTIR spectrum of optimized formulation (NF4) indicated the existence of bands at 3420 cm^{-1} and 1654 cm^{-1} attributing the presence of O-H and C=O group respectively of the polymer (PVP K90). The presence of water (O-H) can be confirmed by the appearance of broad endotherm in the DSC thermogram[23]. This also indicated the interaction between the N-H group of drug and N- or C=O groups of pyrrolidone moiety. In nanofibers, the N-H stretching vibration of pure drug (NM) was not observed and a single absorption band at 3420 cm^{-1} was observed. This can be attributed to the presence of intermolecular hydrogen bonding between NM and PVP K-90, leading to the amorphization of nimesulide, thus helping in its increased solubility. Similar type of interaction was reported in the literature, where the authors have confirmed the amorphous state of piroxicam due to the disappearance of N-H or O-H peaks[24]. No other additional peaks related to interacted products were observed. Hence, the undesired drug – polymer interaction could be ruled out by these FTIR studies.

Differential Scanning Calorimetry

The DSC thermograms of (a) pure drug and (b) optimized formulation (NF4) are depicted in Figure 2. The peak at 154.27° C indicated the sharp endothermic crystalline melting point of Nimesulide. The decreased intensity of the peak in thermo gram of nanofiber indicated the reduced

crystallinity or the gradual amorphization of drug in the polymer matrix of nanofibers. This phenomenon implies the loss of crystalline nature of the drug, which in turn increases the free energy with subsequent enhancement of the dissolution. Inhibition of crystallinity can be attributed to the hydrogen bonding between the drug and polymer, entrapment of drug in the polymer matrix or a combination of both.

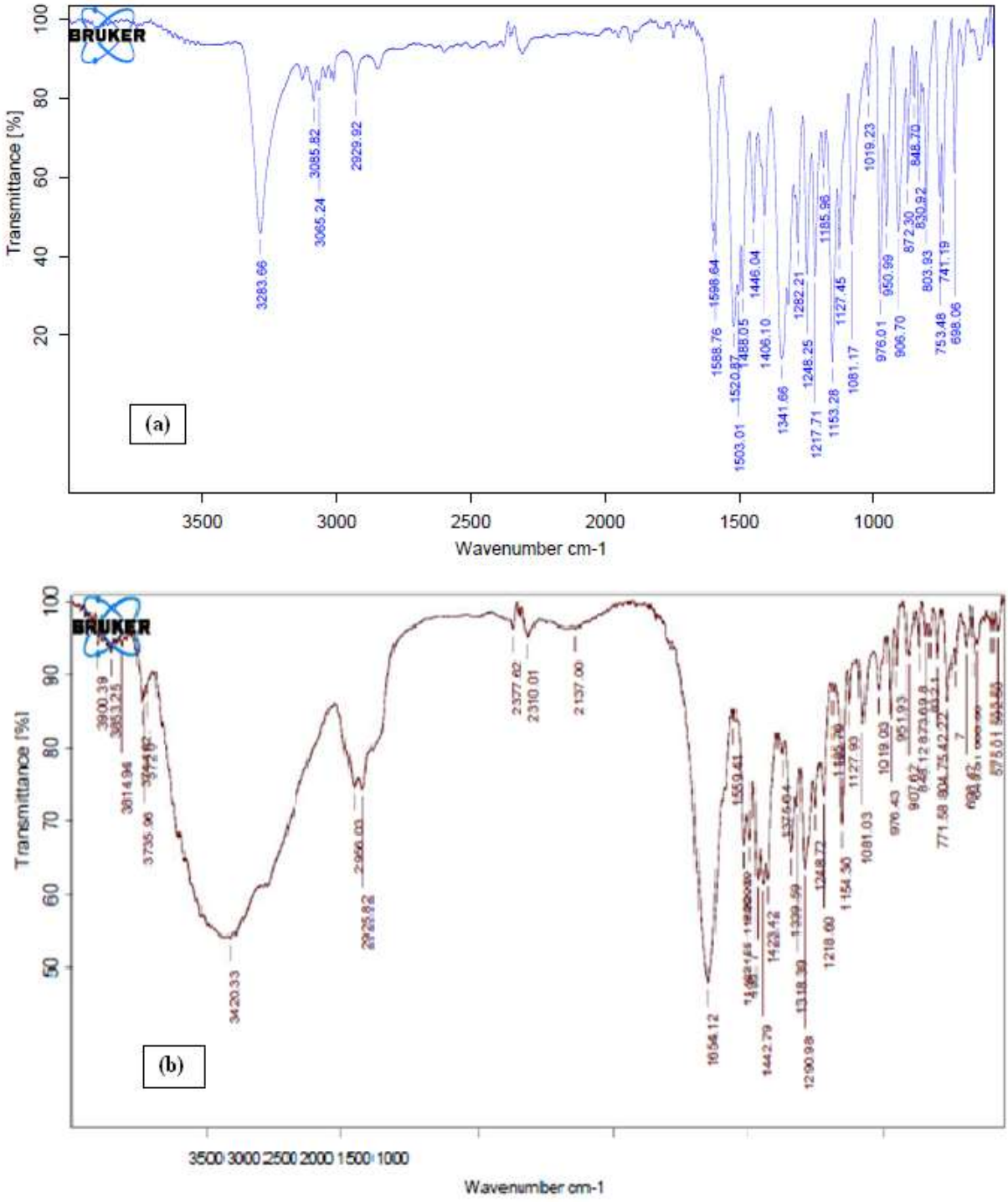

Figure 1: FT-IR spectra of Nimesulide pure drug and nanofibers (NF4)

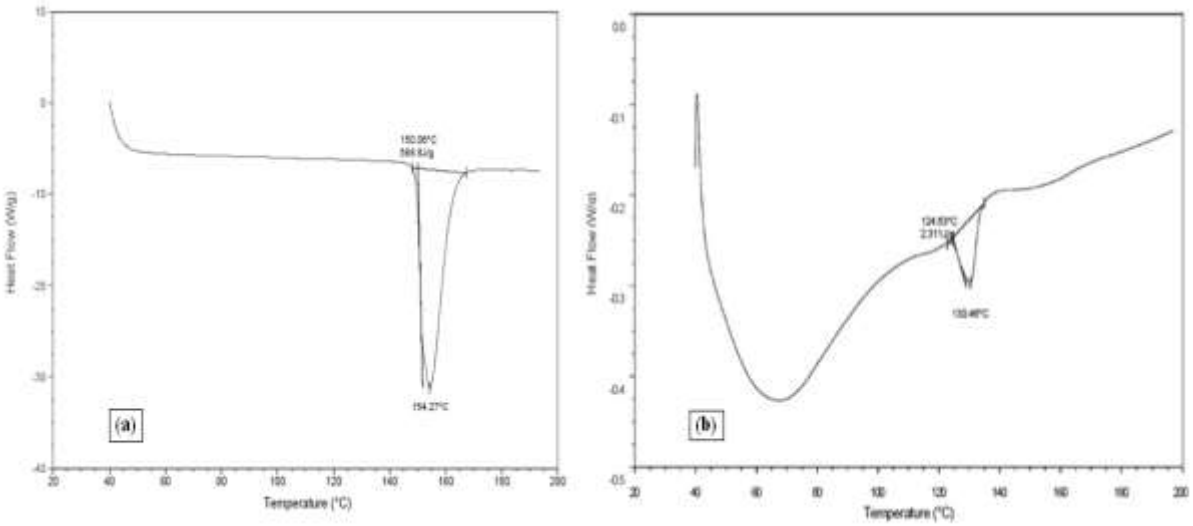

Figure 2: DSC thermograms of Nimesulide pure drug and nanofibers (NF4)

Scanning electron microscopy

The SEM image of the optimized formulation (NF4) is depicted in Figure 3. It is clearly evident from the image that, most of the fibers are of uniform diameter and is well within in the range of 300 and 400 nm. Moreover, the beading of the fibers was negligible. This can be attributed to the optimized process variables which include a voltage of 12 kV, distance of 12 cm between the needle tip to the collector and a flow rate of 1 ml/hr.

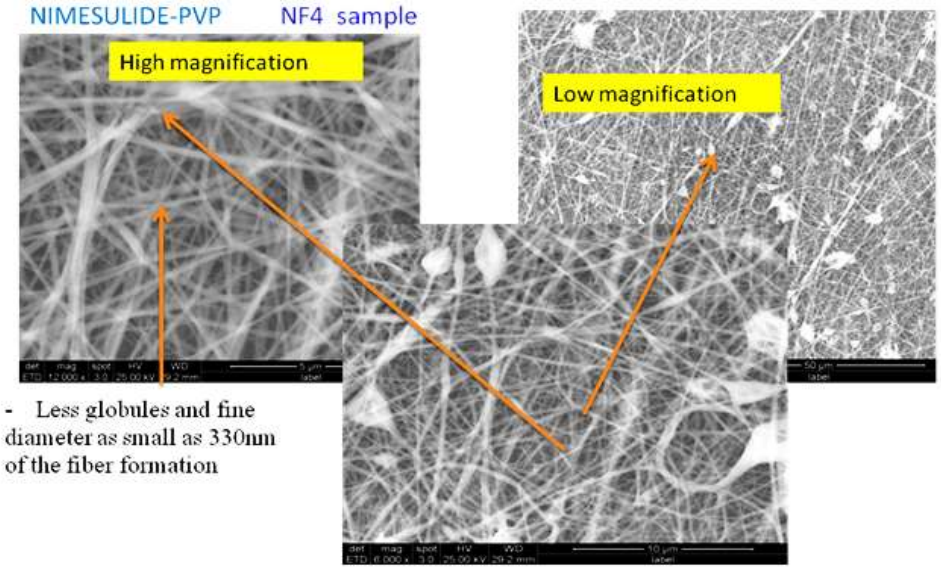

Figure 3: SEM Image of Nimesulide nanofibers (NF4)

Drug content, solubility and dissolution studies

Physical mixtures, co-grinding mixture and nanofibers of nimesulide were formulated with carriers in varied ratios. The formulations were evaluated for drug content, solubility and *in vitro*

dissolution rate. In Electro-spinning technique, the solutions of drug and polymer (PVP-K90) in different ratios (1:1, 1:2, 1:3, 1:4, 1:5), in chloroform, were taken and drawn in to nanofibres using electrospinning apparatus and they can be represented as NF1, NF2, NF3, NF4, NF5. Nanofibers find application in drug delivery, owing to their capability of producing fiber mat with narrow pores, large surface area-to-volume ratio, light weight and small diameter fibers from polymer solutions, which ultimately helps in enhanced drug absorption and bioavailability. In the co-grinding technique, mixtures with different ratios (1:1, 1:3, 1:5, 1:7, 1:9) of drug and carrier were prepared and each one was co-grinded for 20 min in a glass mortar. Carriers employed in this technique include potato starch, lactose, SSG, treated agar, MCC and the formulations were represented as CG-PS, CG-L, CG-SSG, CG-TA and CG-MCC. Similarly, for relative comparison, physical mixtures were also prepared using same carriers as employed for co-grinding mixtures and were represented as PM-PS, PM -L, PM -SSG, PM –TA and PM -MCC. Data indicating the results of drug content (a) and solubility (b) are depicted in Figure 4. The results indicated an enhanced drug solubility of nanofibers, when compared to that of pure drug. This can be attributed to the presence of hydrophilic polymer PVP K-90, which helped in the increased wetting of the particles and consequently an enhanced solubility was observed. Even the presence of particles in smallest (nm) size range leading to larger surface area might have favored enhanced solubility. Similar results of increased solubility were observed with co-ground mixtures. The co-ground mixtures showed improved solubilization when compared to pure drug. This could be attributed to the transformation of large crystals of NM to smaller ones with an increased effective surface area leading to enhanced solubility of the drug. Of all the prepared co-ground mixtures with different carriers, CG-MCC was found to be associated with maximum solubilization. Comparing both nanofibers and co-ground mixtures, highly enhanced solubility was observed with nanofibers. This can be attributed to the increased surface area, due to the size reduction of hydrophobic drug to smallest size range (nm) and moreover the hydrodynamic microenvironment around the particles was changed due to the ability of the hydrophilic polymer to enhance the wettability of the hydrophobic NM particles. Dissolution studies of these formulations were carried out in 900 ml of pH 7.4 phosphate buffer, a speed of 50 rpm and a temperature of 37±0.5°C was used in each test. Samples of dissolution medium (5ml) were withdrawn at different time intervals (5, 10, 20, 30, 45, 60, 90, 120 min), assayed for Nimesulide by measuring the absorbance spectrophotometrically at 397 nm. Among all these co-grinding mixtures prepared by using different hydrophilic carriers, 1:6 ratios of all the co-grinding mixtures (CG-L, CG-PS, CG-SSG, CG-TA, CG-MCC) has shown greater efficiency of drug release; but among all the carriers, co-grinding mixture with MCC with a

drug-carrier ratio 1:6 (CG-MCC) has shown better response. The dissolution profiles of the optimized co-grinding formulations are shown in Figure 6.Ascending order of efficiency of drug release among all carriers: Potato starch < Lactose < SSG < Treated agar < MCC. Hence CG-MCC (drug: excipient ratio 1:6) was considered as optimized co-ground mixture and was selected for further studies. The results of dissolution studies between nanofibers and co-ground mixtures revealed the importance of viscosity of hydrophilic polymer. On comparing the five formulations of nanofibers having a descending order of drug: polymer ratio (1:1, 1:2, 1:3, 1:4 and 1:5), it was observed that, the release rate increased with increasing polymer concentration up to some extent after which the rate was retarded. This behavior can be explained based on the increased viscosity due to increased polymer concentration, which led to a stagnant layer formation around the drug particles, which prevented its release from the inner matrix. Of all the formulations, NF4 (1:4) was selected as the optimized formulation, since it could attain 100% release rate within 2 hrs when compared to other formulations. By observing the results of dissolution studies it was revealed that viscosity of the hydrophilic polymer had a significant effect on the release rate. During the process of dissolution, the drug entrapped polymeric particles come in contact with the dissolution medium, after which wetting of the particle takes place due to the movement of dissolution fluid into the particle. This leads to the formation of stagnant layer (gel state) around the drug entrapped carrier. The dissolved drug within this stagnant layer diffuses out slowly and hence becomes the determining factor for dissolution process. Rapid release of drug can be observed when the drug-carrier particles are less agglomerated, leading to a greater surface area. Dissolution efficiencies of these formulations were calculated and compared with each other and with that of the pure drug. The formulations NF4 and CG-MCC prepared by using electro-spinning technique, and co-grinding technique respectively, have greater solubility in water as compared to pure drug. The optimized formulation of nanofibers (NF4) and co-ground mixture (CG-MCC) has played a better role in the enhancement of drug release. Based on $DE_{30min,}$ DE_{60min}, $T_{50\%}$ and $T_{70\%}$ values, the batch NF4 showed more than 50- fold increase in the dissolution rate and the batch CG-MCC showed more than 6 folds increase in the dissolution rate compared to the pure drug. The study also shows that the dissolution rate of Nimesulide can be enhanced to a great extent by using nanofiber technique rather than co-grinding technique. The dissolution parameters were shown in Table 1 and the dissolution profiles of nanofibers and co-grinding mixtures are depicted in Figure5 and 6 respectively. From the above studies, it can be concluded that the dissolution rate of nimesulide has been increased to a greater extent by employing these techniques. Finally the ascending order

for enhancement of dissolution rate of poorly soluble drug, nimesulide, using different techniques is as follows.

Pure drug <physical mixtures < co-grinding mixtures <nanofibers

Storage of optimized nanofibers (NF4) and co-grinding mixture (CG-MCC) at 25°C / 60% RH and 40°C / 75% RH for 3 months did not affect the stability of the drug. The dissolution profiles of Nimesulide nanofibers and co-ground mixture did not show significant changes in drug content and drug release rate during the entire stability study period ($p < 0.05$).

Table 1: *In-vitro* dissolution parameters of nimesulide loaded nanofibers

Batch codes	Drug: Polymer	[a]$DE_{30\ min}$ (%)	[b]$DE_{60\ min}$(%)	[c]$T_{50\%}$ (min)	[d]$T_{70\%}$ (min)
NM	Pure Drug	2.18±0.85	4.24±0.85	> 120	> 120
NF1	1:1	16.1±0.4	21.80±1.0	>120	>120
NF2	1:2	18.77±4.1	27.92±2.0	59.50±1.7	>120
NF3	1:3	29.06±1.2	49.46±0.8	4.90±3.5	11.5±4.2
NF4	1:4	40.83±0.7	68.00±3.9	2.80±0.6	8.00±2.7
NF5	1:5	30.00±1.0	46.00±0.5	4.80±1.1	60.0±1.9

[a]$DE_{30\ min}$ = dissolution efficiency at 30 min; [b]$DE_{60\ min}$ = dissolution efficiency at 60 min; [c]$T_{50\%}$ = time taken for 50% drug release; [d]$T_{70\%}$ = time taken for 70% drug release.

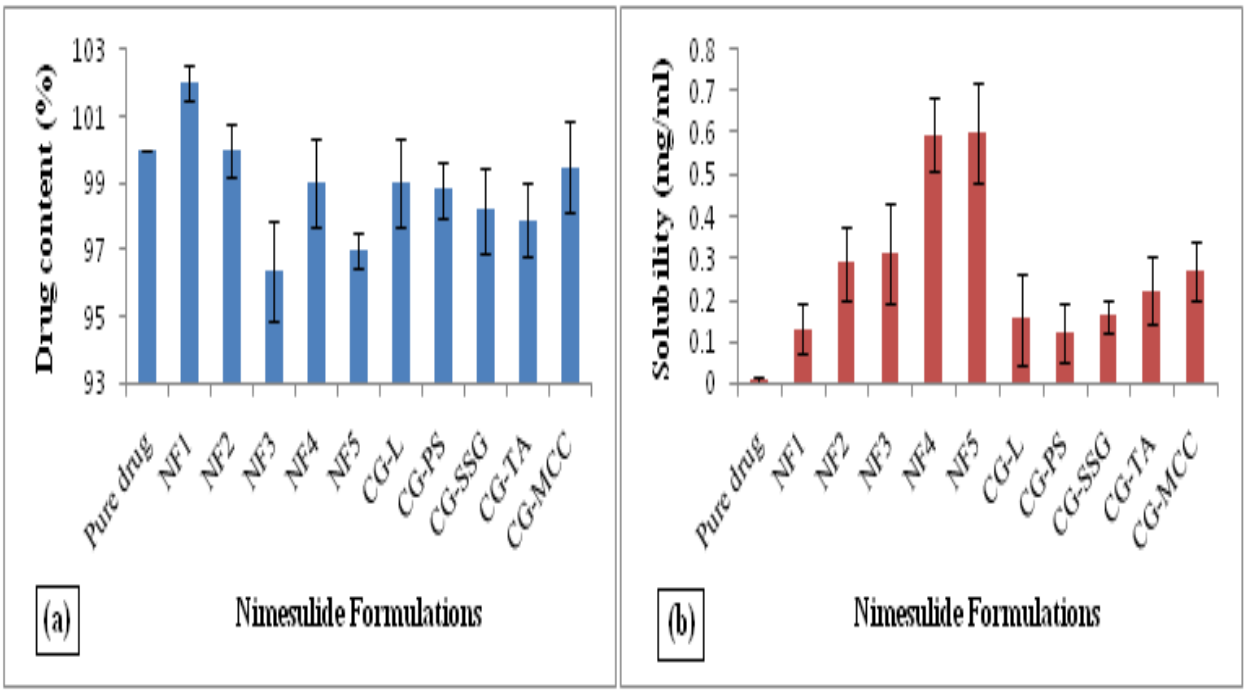

Figure 4: Drug content and solubility results of nimesulide formulations

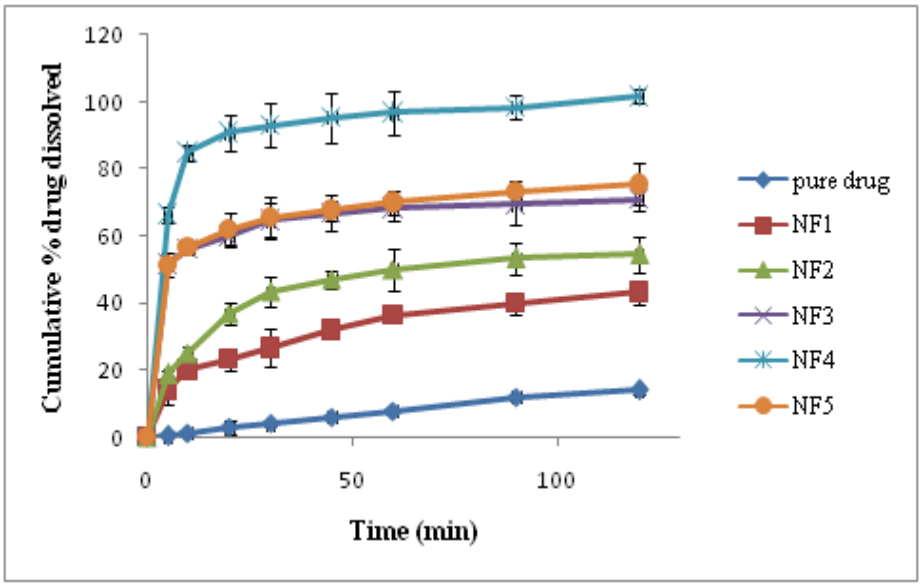

Figure 5: *In vitro* dissolution profile of nimesulide loaded nanofibers

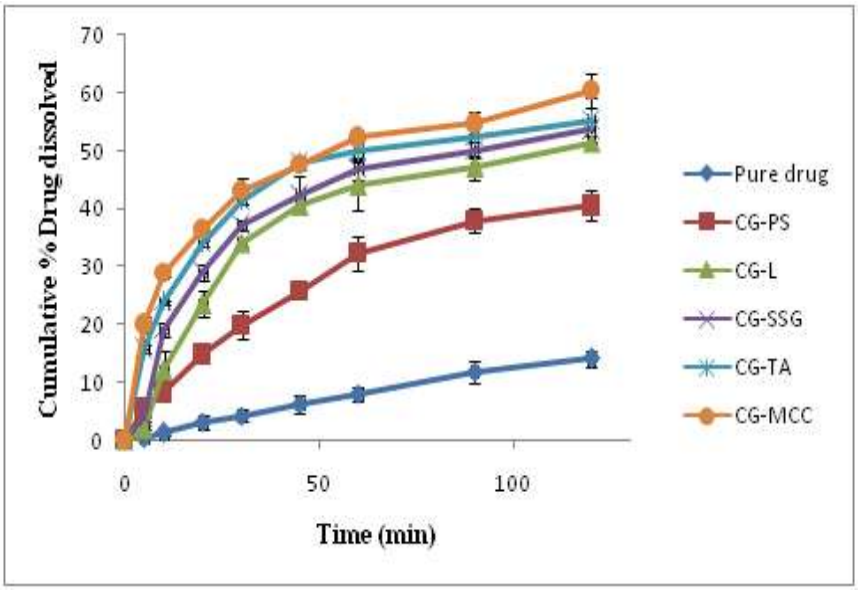

Figure 6: *In vitro* Dissolution profiles of optimized co- grinding mixtures (drug-carrier ratio 1:6) of all the carriers in comparison with pure drug

***In vivo* studies**

The optimized formulation (NF4) was evaluated for the anti-inflammatory activity. Carrageenan was given by subcutaneous route to the left hind paw of female albino rat. The NF4 suspension prepared by using 2ml of 0.5% of sodium CMC solution, 1.35 mg nanofiber formulation was administered orally. After 1hr, injection of 0.1%carrageenan (using saline) was given. The paw odema was measured by Mercury displacement method using Plethysmometer. From the mean values of control, standard, test (NF4) obtained at regular intervals of time, % inhibition was calculated and compared. The NF4 showed 2 to3 fold greater %inhibition of paw volume (depicted

in Figure 7 and Table 2 respectively) as compared to pure drug. It has showed better anti-inflammatory activity compared to the pure drug. All the results were expressed as mean ± SEM. The differences between experimental groups were compared by One-way Analysis of variance (ANOVA) followed by Dunnett's test. Values of $p < 0.05$ indicated statistically significant difference when compared with control.

Table 2: Anti-inflammatory activity of NF4 in albino rats (n = 6)

Groups	Paw Edema volume (ml)								
	1 hr			3 hr			24 hr		
	Mean ±SEM	SD	% inhibi-tion	Mean ±SEM	SD	% inhibi-tion	Mean± SEM	SD	% inhibi-tion
Negative control	0.3			0.3			0.3		
Positive control	0.66± 0.033	0.058	---	0.78 ±0.044	0.076	---	0.50 ±0.032	0.057	---
Pure drug	0.56± 0.057	0.10	15.15	0.51 ±0.016	0.029	34.61	0.45 ±0.044	0.076	10.00
Test (NF4)	0.45± 0.033	0.058	31.81	0.4 ±0.033	0.058	48.71	0.35 ±0.029	0.016	30.00

SEM- standard error of mean: SD- standard deviation

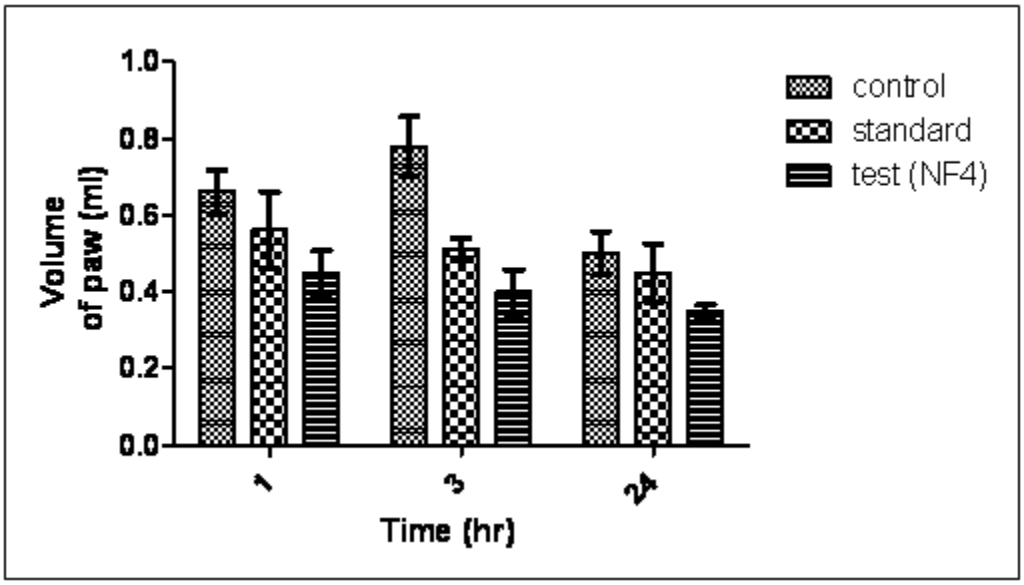

Figure 7: Anti – inflammatory activity of Nimesulide and its nanofiber formulation NF4

CONCLUSION

The results indicated an enhanced solubility and dissolution rate of poorly water soluble drug, Nimesulide by encapsulating within a polymeric nanocarrier (nanofiber) using electrospinning technique. On comparison with pure drug and co-grounded formulations, nanofibers enhanced the dissolution rate of drug, NM much efficiently. Such a rapid release may be beneficial in immediate

relief of inflammatory conditions due to immediate and complete absorption of drug. The *in vivo* (anti-inflammatory) studies also proved the efficiency of the drug when encapsulated in polymeric nanofibrous matrix. Using this technique, there is a possibility to reduce the usual dosage of drug which may lead to decreased toxicity of the drug and moreover reduction in dose can be economically desirable and has a high level of patient compliance. Hence these electrospun nanofibers can be a potential technology to circumvent the insolubility problem of most of the water insoluble drugs and can help in their enhanced bioavailability through the oral or topical routes.

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