Nifedipine-loaded polymeric nanoparticles: Preparation and *in vitro* characterization

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Abstract: The purpose of the current study was to prepare nifedipine (NF) loaded-PLGA nanoparticles (NPs) using two different methods (nanoprecipitation method (N-2) and emulsion-solvent evaporation method (N-4)) to achieve the sustained release of NF and to reduce its side effects and also to investigate the *in vitro* characteristics of NPs (surface morphology, particle size and size distribution, encapsulation efficiency and *in vitro* release characteristics). SEM images of nanoparticles revealed their approximate spherical shape. The mean particle sizes of the prepared nanoparticles ranged from 294.27±7.93 to 424.92±4.96 nm with almost neutral zeta potential values (close to 0 mV). The percent encapsulation efficiency values of N-2 and N-4 formulations 13.03±1.82% and 18.96±1.95% ($p=0.05$), respectively. The extents of cumulative drug release from N-2 and N-4 in PB pH 7.4 medium were up to about 100 % in 38 days and 22 days, respectively (when comparing two formulations, $p<0.05$). PLGA nanoparticles are useful systems for the sustained release of NF, and hence for reducing its side-effects and increasing patient compliance.

Keywords: FT-IR, *in vitro* release, nanoparticle, nifedipine, PLGA.

INTRODUCTION

NF, dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl) pyridine-3,5-dicarboxylate, has a molecular weight of 346.3 and is a yellow, crystalline powder and practically insoluble in water (<10 mg/L). It is converted to a nitrosophenylpyridine and nitrophenylpyridine derivatives by exposing to daylight and ultraviolet light, respectively. Nitroso-derivative causes skin photosensitivity and reverses the calcium-channel blocking effect in vitro. Thus, it should be protected from light and its solutions should be prepared in dark (Li *et al*., 2004; Pawar *et al.*, 2012; Sweetman, 2007).

NF inhibits calcium ion entry into cells by primarily blocking the voltage-dependent L-type Ca^{2+} channels in vascular smooth muscle cells and in cardiac muscle (Sousa et al., 2011). Its present indications include Prinzmetal's angina pectoris, hypertension, Raynaud's phenomenon, oesophageal spasm, pulmonary hypertension, haemorrhoids, chronic anal fissure, and also it has been used as a tocolytic agent (Conde-Agudelo *et al.,* 2011; Golfam *et al.,* 2010; Sweetman, 2007). Although it is rapidly and nearly completely absorbed from the gastrointestinal tract, NF undergoes significant hepatic first-pass metabolism. Oral bioavailability of liquid-filled NF capsules is in the range of 45-75% and its half-life is about 2 hours (Sweetman, 2007). When using NF's immediate release dosage forms, serious adverse effects associated with reflex sympathetic nervous system activation such as uncontrolled hypotension, cerebral ischemia, ventricular fibrillation, dizziness, fatigue and tachycardia are observed (Li *et al.,* 2004; Mansoor and Keefer, 2002). Moreover, NF can cause dose-dependent gingival overgrowth (Fu *et al.,* 1998). NF-sustained release dosage forms are mostly preferred to decrease its undesirable side effects and increase its therapeutic activity (Snider *et al.,* 2008).

Particulate drug delivery systems (e.g. microparticles, nanoparticles) have several advantages over the conventional dosage forms. These include higher local drug concentrations, hydrophilic and hydrophobic drug loading, less variation in the gastrointestinal transit times, low variability among individuals, low risk of dose dumping, reduced side effects and possibility of different routes of administration such as oral, inhalation, parenteral (Gelperina *et al.,* 2005). Especially, nanoparticulate drug delivery systems, submicronic (1-1000 nm) colloidal systems, are widely studied for the treatment or diagnosis of different diseases (e.g. neurodegenerative diseases, cancer, cardiovascular disease and hypertension) over the last two decades (Alexis *et al.,* 2010; Spuch *et al.,* 2012; Tang *et al.,* 2012). There are several studies in the literature related to the formulation of NF-loaded nanoparticles using different polymers (chitosan, alginate, PCL, (PLGA) and Eudragit RL/RS) or solid lipid nanoparticles (Barman *et al.,* 2014a; Barman *et al.,* 2014b; Jeong *et al.,* 2004; Kim *et al.,* 1997; Li *et al.,* 2008; Plumley *et al.,* 2009). The main purpose of the current study was to prepare NF-loaded PLGA NPs using two different preparation methods (nano-precipitation method and emulsion-solvent evaporation method) to achieve the sustained release of NF and reduce its side effects, and also to investigate the *in vitro* characteristics of nanoparticles (surface morphology,

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particle size and distribution, encapsulation efficiency, *in vitro* drug release in phosphate buffer (PB) pH 7.4). At the same time, Fourier transform infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC) was used in this study of the characterization of NPs.

MATERIALS AND METHODS

Materials

PLGA (75:25) (RESOMER® RG 756 S, Ave. Mw 76,000-115,000), polyvinyl alcohol (PVA, MW 30,000-70,000 Da), NF, dichloromethane (DCM) and acetone were purchased from Sigma-Aldrich Co. (USA). All other chemicals and reagents used as they received were of analytical grade.

Methods

All experimental studies were carried out under nitrogen atmosphere and in dark.

Preparation of NF-loaded PLGA NPs

The composition of the studied NPs formulations were given in table 1. Briefly, 100 mg of PLGA was dissolved in DCM (for emulsion-solvent evaporation method) or acetone (for nano-precipitation method). The organic phase containing NF (12.5 mg) was introduced drop by drop into PVA aqueous solution prepared using PB pH 7.4 (18 ml; 3% w/v) and homogenized using an ultrasonic probe (with 50% power) (Bandelin Electronics, Sonoplus, HD 2070, Germany) for 10 min. Then, the evaporation of the organic solvent was carried out under the reduced pressure in a rotary evaporator (Heidolph 4001, Heidolph Instruments GmbH & Co., Germany) at 45°C for 15min. After centrifugation, NPs were re-suspended and then lyophilized (at -55°C and 0.021mbar) for 24 hours (Alpha 1-2 LD plus LT, Martin Christ, Germany) and stored in desiccator at -20°C. Nanoparticles were produced at least in triplicate.

Characterization of NPs

The SEM images of lyophilised NPs mounted on metal stubs and spattered with gold were taken for the evaluation of morphological properties of NPs. The mean particle size and zeta potential of the dilute suspensions of NPs in pure water were measured by using a Zetasizer 3000HS (Malvern Inst., UK). Each examination was carried out in triplicate.

Determination of NF Content in the NPs

10 mg of lyophilized NPs in 1.5 ml of dimethylsulfoxide were mixed on a magnetic-stirrer at 600 rpm for 30 min. After mixing, it was placed in ultrasonic bath for 10 minutes at 25°C. To extract NF, 8.5 ml of PB pH 7.4 was added into this mixture and stirred at 600 rpm for 10 minutes. This dispersion was centrifuged at 12000 rpm for 10 min. The NF content of each sample was then measured using a validated UV method at 238 nm.

In vitro release of NF from the NPs

NF release from NPs was investigated by an incubation method. Therefore, 10 mg of lyophilized NPs in amber vials were suspended in PB pH 7.4 (20 ml) and the vials were placed in horizontally shaking water bath at 37±0.5 °C and 50 rpm. At the predetermined time points, samples (3 ml) were withdrawn from the release medium and replaced with 3 ml of fresh buffer. Then, the samples were centrifuged at 12500rpm for 10 min and NF content in the supernatant was measured using the validated UV method at 238 nm. Same procedures were performed for blank NPs.

FT-IR and DSC analysis

FT-IR spectrometer (Bruker, Germany) was used to obtain the IR spectra of the formulations of NPs, NF and PLGA prepared in KBr disks in the region of 4000–400 cm^{-1}.

Thermal analysis was performed using a differential scanning calorimetry (DSC) (Setaram Labsys Evo®, France). Alumina pan was used as reference and the instrument was calibrated using some standards (In, Sn, Pb, Al, Pd, Ni, Au, Zn). All DSC experiments were carried out under 60 mL/min of nitrogen flow and at a temperature range of 20-400°C (10°C/min).

STATISTICAL ANALYSIS

Statistical evaluations were performed using Mann-Whitney U test with SPSS Statistics 20.0 programme (SPSS Inc., Chicago, IL, USA) (p<0.05 shows the statistical significance). All experimental results were expressed as mean ±S.D.

RESULTS

NF-loaded PLGA nanoparticles were prepared by using single emulsion solvent evaporation method and also nanoprecipitation method (table 1). SEM images revealed that the nanoparticles were in approximately spherical morphology with nano-size (fig. 1). The zeta potential and mean particle size values of NPs, as seen in table 2, ranged from -0.541±0.34-0.417±0.19 and 294.27±7.93 to 424.92±4.96 nm respectively. Zeta potential values of all nanoparticle formulations were found to be close to zero (table 2). There was a statistically significant difference between mean sizes of the blank and NF-loaded nanoparticles prepared by both methods ($p<0.05$). However, the zeta potential values between the blank and NF-loaded nanoparticle formulations prepared using both techniques were not statistically different ($p\geq0.05$).

The encapsulation efficiencies of N-2 and N-4 nanoparticle formulations were found to be 13.03±1.82% and 18.96±1.95% ($p=0.05$) (table 2).

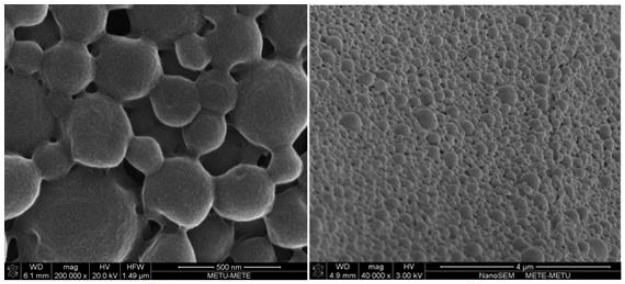

Fig. 1: SEM images of NF-loaded PLGA nanoparticles.

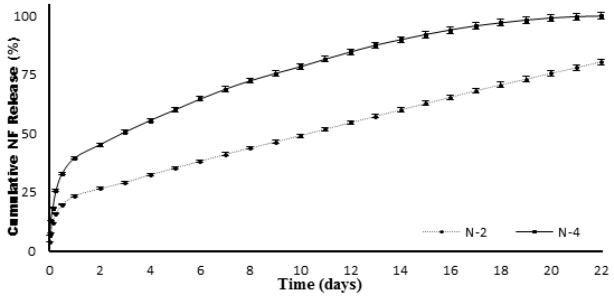

Fig. 2: *In vitro* release profiles of NF-loaded PLGA nanoparticles (mean ±SD; n=3).

The profile of *in vitro* release was given in fig. 2. About 25% of NF was released from N-2 and N-4 formulations within 24 hours and 6 hours, respectively ($p<0.05$). Furthermore, about 80% and 100% of NF was released from N-2 and N-4 formulations, respectively within 22 days ($p<0.05$).

DSC thermograms of NF, PLGA and blank and NF-loaded nanoparticles were showed in fig. 3. The FT-IR spectra of NF, PLGA and nano particle formulations were shown in fig. 4-9.

DISCUSSION

Modified release systems have been developed in order to reduce these drawbacks of the immediate-release dosage forms. Especially, the design of nano-systems is a very attractive subject in the pharmaceutical area. Nanoparticulate drug delivery systems have a significant potential power to control the release rate/the location of drug, to reduce fluctuations in drug plasma concentrations and the side effects of drug, to improve drug stability, to increase the therapeutic efficacy, to reduce the dosing frequency of drug, and to protect the drug from degradation and metabolism (Li *et al.*, 2008; Lin *et al.*, 2013). Poly (lactic-co-glycolic acid) PLG approved by FDA is a copolymer of poly (lactic acid) PLA and poly (glycolic acid) (PGA) and extensively used in the preparation of polymeric nanoparticles for both hydrophilic and hydrophobic drugs. It has predictable biodegradation behaviours, favourable mechanical properties and high biocompatibility. Moreover, PLGA shows the low risk of immunogenicity and toxicity. PLGA nanoparticles have gained notable interest (Muthu *et al.*, 2009). There are very limited number of studies related to NF-loaded PLGA nanoparticles (Kim *et al.*, 1997).

In this study, PLGA nanoparticles were prepared by using single emulsion solvent evaporation method (in this method, the polymer is dissolved in DCM which is a volatile and water immiscible organic solvent) and nanoprecipitation method (in this method, the polymer is dissolved in acetone which is a volatile, semi-polar and water miscible organic solvent. In addition, the nanoprecipitation is based on the interfacial deposition of a polymer after rapid diffusion of the organic solvent into the aqueous medium in the presence or absence of surfactant) (Rao and Geckeler, 2011). SEM is used to observe the image of prepared nanoparticles and the images revealed that the nanoparticles were in approximately spherical morphology with nano-size (fig. 1). The zeta potential and mean particle size values of NPs, as seen in table 2, ranged from -0.541±0.34-0.417±0.19 and 294.27±7.93 to 424.92±4.96 nm respectively. There was a statistically significant difference between mean sizes of the blank and NF-loaded nanoparticles prepared by both methods ($p<0.05$). When the single emulsion-solvent evaporation method was used, smaller NF-loaded nanoparticles were obtained compared to those of nanoparticles using nanoprecipitation ($p<0.05$, table 2). Similar result was previously reported (Alshamsan, 2014; Kalimouttou *et al.*, 2008; Lal *et al.*, 2013). The concentration and viscosity of organic phase were two of the most critical conditions for preparing nanoparticles using nano precipitation method without any bulk precipitation of the raw materials (Kalimouttou *et al.*, 2008). In single emulsion-solvent evaporation technique, particle size was primarily influenced by the speed of homogenization and the type and concentration of stabilizing agent. Thus, ultra sonication/ high-speed homogenization in this technique is often used to obtain smaller particles (Nagawarma *et al.*, 2012). Therefore, the difference between the particle sizes of NF-loaded nanoparticles prepared using both methods can be due to increasing viscosity of the organic phase and reduced the stirring efficiency resulted in the formation of bigger particles.

The zeta potential values of all nanoparticle formulations were found to be close to zero (table 2). According to the results, the preparation method has no effect on the zeta potential values of blank and NF-loaded nanoparticle formulations ($p\geq0.05$). The zeta potential of nanoparticles can affect their pharmacokinetic properties and phagocytosis in the body, thus, it is one of the most important factors for targeting drug delivery (Honary and Zahir, 2013). The zeta potential of NPs close to zero result in reducing phagocytic uptake of the nanoparticles

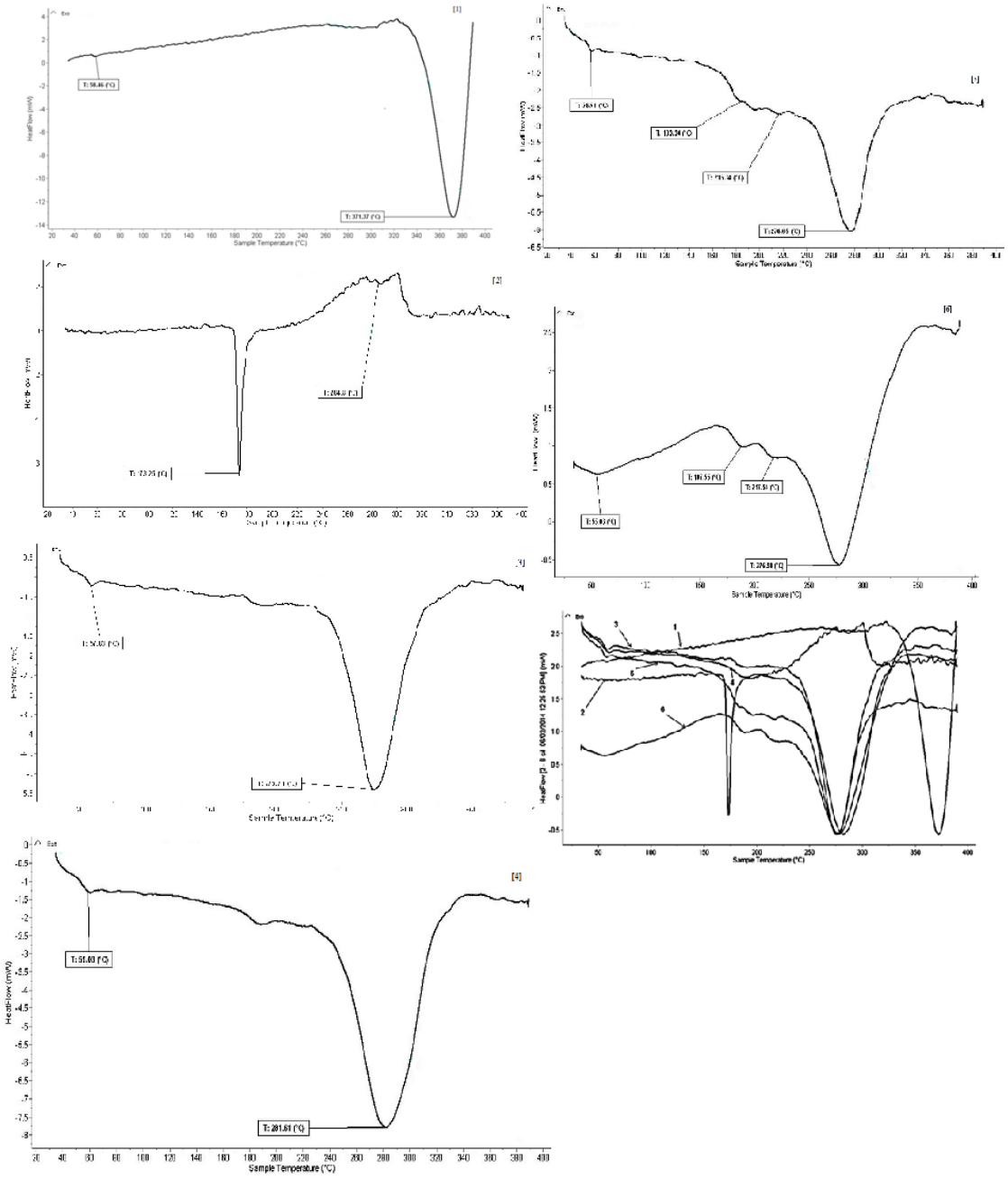

Fig. 3: DSC diagrams (as separately and combined) of NF, PLGA, blank and NF-loaded PLGA nanoparticles. [1]: PLGA (75:25), [2]: NF, [3]: N-1 formulation [4]: N-3 formulation, [5]: N-2 formulation [6]: N-4 formulation.

compared to the charged nanoparticles. When NPs were prepared using the solution of PVA (as a stabilizing agent) at pH 9, the carboxyl groups of PLGA and hydroxyl groups of PVA located near the surface of nanoparticles. Therefore, a high zeta potential value (-24.97 mv) was measured. However, the zeta potential value of nanoparticles dropped to near zero when the PVA solution at pH 7 (Si-Shen and Huang, 2001). The results of zeta potential measurement obtained in this study were similar to the results reported by the previous studies (Mukherjee *et al.*, 2008; Mura *et al.*, 2011). Therefore, in this study, these nanoparticles were lyophilized and stored in desiccator at -20°C in powder form until used for further studies.

The encapsulation efficiencies of N-2 and N-4 nanoparticle formulations were found to be 13.03±1.82% and 18.96±1.95% (p=0.05) (table 2). Under the study conditions, it was determined that the preparation method has no statistically significant effect on the encapsulation

efficiency (p=0.05). Generally, the type and molecular weight of the polymer, the viscosity of organic phase used and drug-polymer ratio and particle size of particles are the critical parameters for drug loading (Song *et al.,* 2008; Sansdrap and Moës 1998). Sansdrap and Moës (Sansdrap and Moës 1998) prepared NF-loaded PLGA microspheres using solvent evaporation method and reported that NF contents in micro spheres with mean particle sizes of 80 µm and 18 µm were 14% and 6%, respectively, and the particle size of microspheres had an important effect on drug loading.

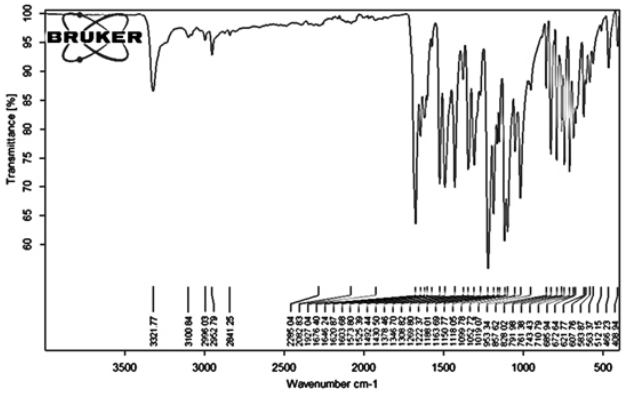

Fig. 4: FT-IR spectrum of NF.

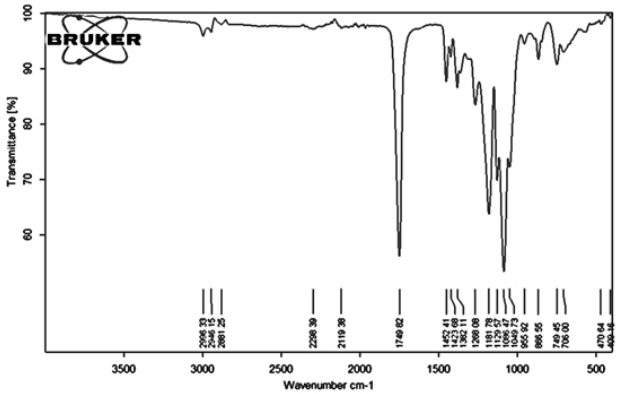

Fig. 5: FT-IR spectrum of PLGA.

The composition and molecular weight of the polymer, the physical properties, size and shape of delivery matrix system, the type and concentration of active substance and the pH of release medium are very important factors affecting the hydrophilicity and rate of degradation of a delivery matrix and the drug release from micro/nano particulate drug delivery systems. High PLA percentage in PLGA copolymer makes it more hydrophobic and thus, PLGA degrades more slowly due to the absorption of less water. In other words, PLGA 50:50 (PLA: PGA ratio) shows faster degradation than PLGA 75:25. The degradation of polymer with higher molecular weight and consequently with longer chains takes more time compared to that of polymer with shorter chain. Besides, drug delivery system with small particles size and thereby high surface area shows a faster drug release. The in vitro hydrolysis of PLGA in alkaline and strongly acidic media occurs faster than the hydrolysis of PLGA in slightly acidic and neutral media (Makadia and Siegel, 2011). In the current study, NF-loaded nanoparticles were prepared using PLGA 75:25 with molecular weight 76000-115000 for developing sustained-release systems for NF and the in vitro release study carried out in buffer media (PB) at pH 7.4. The profile of in vitro release was given in fig. 2. About 25% of NF was released from N-2 and N-4 formulations within 24 hours and 6 hours, respectively ($p<0.05$). Furthermore, about 80% and 100% of NF was released from N-2 and N-4 formulations, respectively within 22 days ($p<0.05$). The release results showed that the NF release from N-4 formulation (with small particle size compared with the particle size of N-2 formulation; $p<0.05$) is faster than the NF release from N-2 formulation ($p<0.05$). Furthermore, biphasic drug release curves for both formulations were obtained and these curves showed an initial burst release (about 15% and 25% of NF were released from N-2 and N-4 within 6 hours, respectively) due to the release of NF adsorbed on the surface of nanoparticles and later, a slow NF release.

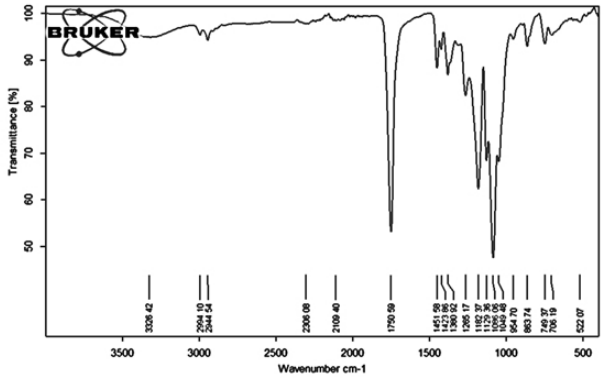

Fig. 6: FT-IR spectrum of N-1.

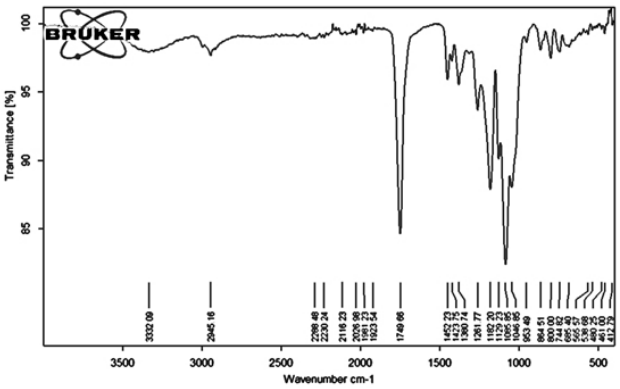

Fig. 7: FT-IR spectrum of N-3.

DSC data can be used to characterize the interactions of possible drug and polymer. DSC thermograms were obtained for NF, PLGA and blank and NF-loaded nanoparticles (fig. 3). In the thermogram of PLGA, there were two peaks at around 50°C (Tpeak: 58.46°C) and around 370°C (Tpeak: 371.37°C) related to the glass transition and thermal decomposition of PLGA, respectively. The glass transition of PLGA 75:25 is in the

range of 49-55°C (Fouad *et al.,* 2013). In a previous study, it is reported that the thermal decomposition of PLGA occurred at 371°C (Fouad *et al.,* 2013). The DSC thermo gram of NF gives rise to an endothermic characteristic peak at about 170°C (Tpeak: 173.25°C), which is near to its melting point (in range of 172-174°C) (fig. 3) (Filho *et al.,* 2008 Lalitha and Lakshmi, 2011). NF thermal decomposition temperatures are in range of 210-390°C (Filho *et al.,* 2008). In DSC thermogram of N-1 and N-3 formulation (fig. 3), the glass transition temperature around 50°C (Tpeak: 57.83 and 59.03°C, respectively) and also endothermic peaks around 280°C (Tpeak: 275.71 and 281.61°C, respectively) were shown. The DSC curves of N-2 and N-4 formulations show the peaks related to melting point of NF at around 180°C and corresponds to its thermal decomposition temperature at about 210°C. Blank and NF-loaded nanoparticles have thermal stability over a lower temperature range compared to pure PLGA, because, the thermal decomposition of more reactive nanoparticles with high surface area occurs faster (Fouad *et al.,* 2013; Mainardes *et al.,* 2006).

The FT-IR spectra of NF, PLGA and nanoparticle formulations were shown in fig. 4-9. The spectrum of NF displayed peaks at 3321.77 cm^{-1} (N-H stretching), 3100.84 cm^{-1} (aromatic C-H stretching), 2952.79cm^{-1} (C-H stretching; $-CH_3$), 1676.40cm^{-1} (C=O stretching), 1525.39cm^{-1} and 1308.82-1378.46cm^{-1} (NO_2 stretching), 1222.37cm^{-1} and 1118.05cm^{-1} (C-O stretching) and 1269.80cm^{-1}'de aromatic C-N stretching (fig. 4) (Gowda et al., 2010). The spectrum of PLGA copolymer showed characteristic peaks at 1749.82cm^{-1} due to -C=O stretching, in range of 1268.08-1181.78 cm^{-1} assigned to symmetric and asymmetric C-C(=O)-O stretching, in range of 2881.25-2996.33 $cm-_1$ associated with -CH stretching, in range of 1181.78-1086.47 cm^{-1} related to C-O stretching and at 1452.41-866.55 cm^{-1} assigned to C-H bending (fig. 5) (Fouad *et al.,* 2013; Singh *et al.,* 2014; [34,39]. The FT-IR spectra of blank nanoparticle formulations (N-1 and N-3) displayed characteristic peaks similar to those of PLGA (fig. 6 and 7). When the spectra of NF-loaded nanoparticle formulations (N-2 and N-4) were compared with spectra of N-1 and N-3 formulations, the additional absorption bands were shown at wave numbers of 1531.66 cm^{-1} and 1308.84 cm^{-1} (NO_2) for N-2 formulation (fig. 8) and also at 3342 cm^{-1} (N-H stretching) and at 2994.05 cm^{-1} (C-H stretching due to -CH_3) for N-4 formulation (fig. 9). The bands confirmed the presence of NF in both NF-loaded nanoparticle formulations.

CONCLUSIONS

Consequently, in the present study, NF-loaded PLGA nanoparticles were prepared using two different methods and in vitro evaluated. The mean particle sizes of prepared nanoparticles ranged from 294.27±7.93 to 424.92±4.96 nm. The encapsulation efficiency values of nanoparticles prepared by using nanoprecipitation method (N-2) and single emulsion-solvent evaporation method (N-4) were 13.03±1.82% and 18.96±1.95% (p=0.05), respectively. The extents of cumulative drug release from N-2 and N-4 in PB pH 7.4 medium were up to about 100% in 38 days and 22 days, respectively (p<0.05). PLGA nanoparticles can be useful systems for the sustained release of NF, and hence for reducing its side-effects and increasing patient compliance.

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