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PHARMACEUTICAL TECHNOLOGY

FORMULATION AND EVALUATION OF INDOMETHACIN LOADED NANOSPONGES FOR ORAL DELIVERY

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Abstract: Nanosponges (NS) loaded sustained release tablet formulations of a non-steroidal anti-inflammatory drug; Indomethacin was successfully developed and evaluated for their pharmaceutical properties. Twelve nanosponge formulations were fabricated by solvent diffusion method by using different ratios of drug and polymers (ethyl cellulose and polyvinyl alcohol). Particle size of all the formulations was in the nano range of 221 to 625 nm and it was found dependent on the polymer concentration. Drug loading and entrapment efficiency were ranged from in 32.2 to 59.4% and 30.1 to 64.8%, respectively. Formulations with an equal proportion of drug and polymer resulted in higher values of drug loading and entrapment efficiency. Percent yield was also found dependent on the relative drug-polymer ratio with the highest value of 51% was achieved for the formulation having same drug to polymer ratio. SEM results confirmed the formation of spherical and porous structures. Structural analysis by Fourier transform infrared spectroscopy (FTIR), powder x-ray diffraction (PXRD) showed the absence of any interaction between drug and polymer. In comparison to the pure drug, NS formulations showed a linear intrinsic dissolution rate (IDR) profile depicting a controlled release profile. Diffusion studies of NS formulations performed by Franz diffusion cell and dialysis bag methods showed comparable results in terms of precision and linearity of diffusion profile. Tablets prepared from the drug-loaded NS showed acceptable values for hardness, friability and drug content. Release of drug from NS tablets was confirmed as sustained release behavior.

Keywords: nanosponges, indomethacin, emulsion solvent diffusion method, sustained release, Franz diffusion cell

Chronic diseases like arthritis and ankylosing spondylitis (spine arthritis) are associated with severe pain and inflammation and require a long-term oral therapy of analgesics. Chronic therapy with analgesics may result in many side effects including gastric irritation, which may lead to a gastric ulcer. Delivery of active pharmaceutical ingredient (API) in the form of a sustained release dosage forms is the better options to reduce such side effects (1). Various techniques like matrix tablets, osmotic drug delivery system, gastro retentive drug delivery system and ion exchange resin can be employed to alter the release of drug from the dosage form. However these techniques are associated with several limitations, e.g. stability issues with matrix tablets (2), toxicity due to dose dumping in osmotic drug delivery system (3, 4), gastric irritation and instability at acidic pH for gastro retentive drug delivery system (5), high cost and less dose adjustment in ion exchange resin formulations (6). To overcome these problems focus has been drawn towards nanotechnological approaches like nanosponges (NS) and nanocapsules, where the release of drug can be fabricated in more precise and accurate manner.

NS are spherical, porous and small-sized polymeric delivery systems, which release the drug in a more predictable and controlled way (7). Many other advantages like; fewer side effect, flexibility in dosage form, non-irritant behavior and improved elegance to make them an attractive system for sustained release formulations. These encapsulated formulations have compatibility with many ingredients and vehicles so they can carry a variety of drugs. In

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many cases, NS impart stability to the formulation by providing protection against premature deterioration of drug (8). Enhancement of bioavailability, taste masking, and targeted drug delivery systems are other areas of interest for the formulation scientists, where cyclodextrin (CD) based NS has successfully been used to achieve the desired properties of APIs (9, 10).

Variety of polymers such as hyper cross-linked polystyrenes, CD, polyvinyl alcohol (PVA) and ethyl cellulose (EC) can be used to form NS (11). Among these, PVA and EC are most commonly used polymers for sustained release formulations. There are a number of examples in literature e.g. oral and topical dosage forms of artesunate and lansoprazole (12, 13) where these polymers are effectively used for preparing sustained release formulations. EC is a cellulose derivative in which few hydroxyl groups on the glucose are converted to ethyl ether groups. It is soluble in various organic solvents such as methylene chloride and alcohol while insoluble in water (14). PVA is a non-toxic and biodegradable synthetic polymer having aqueous solubility. Surfactant and polymerization properties of PVA make it a good candidate for emulsion solvent diffusion method of NS preparation.

Nanosponges can be prepared by various methods including solvent method, cross-linking of β–cyclodextrins, ultrasound assisted synthesis and emulsion solvent diffusion method. Among these, emulsion solvent diffusion method is an effective, time efficient and economical method for synthesis of NS (15). There are a number of studies presented in literature where this method has successfully been used, e.g. preparation of econazole nitrate loaded NS was carried out by emulsion solvent diffusion method (16). In this study, an NS based hydrogel formulation was prepared that offered more stability and controlled release to the drug. In another study, Jilsha and Viswanad synthesized cephalexin loaded NS with hydroxyl ethyl cellulose and PVA as polymers by using emulsion solvent evaporation technique (17). NS are normally evaluated for their particle size, drug loading, entrapment efficiency, and release profile (18). Structural analysis can be performed by using Fourier transform infrared spectroscopy (FTIR), powder x-ray diffraction (PXRD) and scanning electron microscopy (SEM).

Drug molecules to be incorporated into nanosponges should have certain characteristics for successful entrapment in nanocavities. Drug molecules having a molecular weight between 100 and 400 Da and possessing less than five condensed rings can be easily trapped in the nanocavities and result in high entrapment efficiency. Also, these molecules should have aqueous solubility less than 10 mg/mL and melting point below 250°C (19). In the current study, attempts were made to prepare indomethacin (IND) loaded NS to formulate a sustained release tablet dosage form. IND is a non-steroidal anti-inflammatory drug (NSAID) that is prescribed for the management of arthritis, pain, and inflammation. It inhibits the formation of prostaglandins, molecules that cause swelling, stiffness and pain. Conventional therapy requires multiple dosing for a longer period of time that leads to several side effects such as gastrointestinal irritation, bleeding, perforation and ulcer (20). IND belongs to the BCS (Biopharmaceutical classification system) class II drugs, having an aqueous solubility less than 10 mg/L and molecular weight of 357.79 g/mol, which make it a good candidate for such type of studies (21).

The aim of this study was therefore to prepare and evaluate sustained release formulations of IND loaded NS using EC and PVA as polymers. Prepared formulations were tested for various physicochemical parameters and were assed for the release rate of drug by *in vitro* and intrinsic dissolution methods. Furthermore, diffusion profiles of NS formulations were compared by two different methods, while using modified Franz diffusion cell and dialysis membrane method. Lastly a NS loaded tablet dosage form was prepared and evaluated for formulation properties.

EXPERIMENTAL

Materials

IND (99.5%) was kindly provided by Munawar-Pharma (Pvt) Ltd., Lahore, Pakistan. Polyvinyl alcohol (PVA), ethyl cellulose (EC) and dichloromethane (DCM) were purchased from Sigma-Aldrich, Pakistan. Methanol was of analytical grade and used without further purification. Citric acid and disodium hydrogen phosphate were used to prepare buffer solutions.

Methods

Preparation of IND loaded NS

Emulsion solvent diffusion method was used to prepare IND loaded NS (14). Different proportions of ethyl cellulose (EC) and polyvinyl alcohol (PVA) were used while the quantity of drug was kept constant (i.e. 1 g) (Table 1). Disperse phase was prepared by adding IND and EC to dichloromethane (20 mL), sonicated for ~ 10 min until completely dissolved. Continuous phase was prepared by dissolving PVA in 100 mL of distilled water in a water

bath at 60°C. The disperse phase was added drop wise to the continuous phase under constant stirring of 1000 rpm for 2 h by using overhead stirrer (Heidolph D-91126). The dispersion was filtered (millipore filter with pore size 0.45 µm) and dried for 24 h at 40°C in an oven. After drying the formulations were packed in closed containers and stored in a desiccator until further characterization.

Characterization of IND loaded NS

Prepared formulations were characterized by different physicochemical parameters such as entrapment efficiency, percent yield, drug loading, and particle size. Structural analysis was performed by using Fourier transform infrared spectroscopy (FTIR), powder x-ray diffraction (PXRD) and scanning electron microscopy (SEM).

Determination of percent yield, drug loading, entrapment efficiency and particle size

Percent yield of the NS formulations was determined by calculating the initial weight of all solid materials used and the weight of the dried NS obtained by using equation (Eq. 1) as described by Mathew, Devi et al. (22).

$$\text{Percent yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \quad (1)$$

Percent drug loading and entrapment efficiency were determined by using equations 2 and 3, respectively. Accurately weighed quantity of NS (10 mg) and 5 mL of 0.1 N methanolic HCl was mixed for ~1min using vortex mixer. The volume was made up to 10 mL with 0.1 N methanolic HCl followed by filtration. Absorbance was determined by using a calibrated spectrophotometric method with R^2 value of 0.998 at λ_{max} of 318 nm (23).

$$\text{Drug loading} = \frac{\text{Drug content of NS}}{\text{Weight of NS recovered}} \times 100 \quad (2)$$

$$\text{Entrapment efficiency} = \frac{\text{Drug content of NS}}{\text{Weight of initial drug}} \times 100 \quad (3)$$

Particle size was analyzed by diluting aqueous dispersions up to specified scattering intensity at 25°C and average diameter of NS was determined by dynamic light scattering measurements with zeta sizer (Malvern, ZSP nano) (24).

Structural analysis

Morphological studies of selected NS formulations were carried out by scanning electron microscopy (Hitachi TM-1000) with auto imaging system. Particles were mounted on aluminum stub and coated with gold by using sputter coater (Denton, Desk V HP) operated at 40 mA for 25 seconds under vacuum (25).

Powder samples of plain drug, polymers and selected NS formulations were analyzed using X-ray diffractometer (PAN Analytical) with Cu Kα radiation (λ = 1.54 Å) by a position sensitive detector (PSD). Diffraction data was collected over a 2θ range of 20-80° with a step size of 0.02° (26).

Fourier transform infrared (FTIR) analysis of samples was performed by ATR-FTIR spectrophotometer (Alpha-P Bruker, Germany). The spectra were recorded over the frequency range of 2000 to 600 cm^{-1} (27).

Release studies

The release profiles of pure drug and NS formulations were determined by using two different

Table 1. Composition of different formulations of IND loaded NS.

Formulation code	Drug	Ethyl cellulose	Polyvinyl alcohol	Dichloro-methane	Drug/EC
	(g)	(g)	(% w/v)	(mL)	
F1	1.0	0.5	0.5	20	2 : 1
F2	1.0	1.0	0.5	20	1 : 1
F3	1.0	1.5	0.5	20	2 : 3
F4	1.0	2.0	0.5	20	1 : 2
F5	1.0	0.5	0.75	20	2 : 1
F6	1.0	1.0	0.75	20	1 : 1
F7	1.0	1.5	0.75	20	2 : 3
F8	1.0	2.0	0.75	20	1 : 2
F9	1.0	0.5	1.0	20	2 : 1
F10	1.0	1.0	1.0	20	1 : 1
F11	1.0	1.5	1.0	20	2 : 3
F12	1.0	2.0	1.0	20	1 : 2

methods including *in-vitro* release studies and intrinsic dissolution rate (IDR).

***In-vitro* dissolution**

Dissolution studies on powder samples of pure IND and NS formulations were carried by using USP type II apparatus (Erweka DT 700). Pure drug (100 mg) and NS (equivalent to 100 mg of drug) were accurately weighed and filled in hard gelatin capsules. Dissolution was carried out at 37 ± 0.5°C with paddle speed of 50 rpm using 900 mL phosphate buffer (pH 6.8) as a dissolution medium. Samples (5 mL) were withdrawn at predetermined time intervals and replenished with the same amount of fresh, preheated (37 ± 0.5°C) dissolution medium in order to maintain the sink condition. The samples were filtered (pore size 0.45 µm membrane filter) and were analyzed using a calibration curve with R^2 value of 0.998 at λ_{max} 319 nm (28). All the studies were performed in triplicate. The drug release data was fitted to various kinetic models such as zero order, first order, Higuchi, Korsmeyer-Peppas and Hixon-Crowell model by using DD Solver. Coefficient correlation (R^2) values were used to explain the release kinetics of the drug (29).

Intrinsic dissolution rate

Intrinsic dissolution rates (IDR) of pure IND and NS were determined by rotating disk method using a Wood's apparatus (30, 31). Disks of different samples were prepared by compressing powder samples (200 mg) in a die cavity (surface area 0.785 cm^2) using hydraulic press (Carver, USA) for ~1 min at a pressure of ~3000 psi. The die and holder set was assembled, leaving an exposed surface of the disk for dissolution. The assembly was then attached to a dissolution apparatus (Curio DS 507). Dissolution was carried out at a temperature of 37 ± 0.5°C using 900 mL phosphate buffer (pH 6.8) with a rotation speed of 50 rpm. Aliquots 5 mL were withdrawn at predetermined time intervals and replenished with the same amount of fresh, preheated (37 ± 0.5°C) dissolution medium to maintain the sink conditions. The samples were filtered (pore size 0.45 µm membrane filter) and analyzed in triplicate using calibration curve with R^2 value of 0.998 at λ_{max} 319 nm. Intrinsic dissolution rate was calculated by plotting cumulative amount of drug dissolved per surface area (mg/cm^2) versus time (min) (31).

Diffusion studies

The diffusion profiles of NS were determined by two methods named as method 1 and method 2. The results were compared to assess the better method for diffusion studies of NS.

Method 1

Modified Franz diffusion cell (Fig. 1), in which bottom of receptor chamber was above the base of Franz cell (in order to maintain the uniform temperature in receptor chamber) was used in this method. The receptor chamber was filled with phosphate buffer (6.8 pH). Dialysis membrane (MWCO 12-14000 Daltons, Medicell International Ltd, 239 Liverpool Road, London) was activated by soaking it in phosphate buffer (6.8 pH) for ~24 h and then fitted carefully on receptor compartment to avoid air entrapment. NS powder (equivalent to 10 mg drug) was added and equilibrated with phosphate buffer (6.8 pH) in the donor compartment. Sampling arms and top of donor compartment were sealed with parafilm to prevent evaporation. Diffusion studies were performed at stirring speed of 100 rpm and the temperature was maintained at 37 ± 0.1°C. Samples in triplicate (0.5 mL) were withdrawn at one hour interval for eight hours and replenished with the same quantity of fresh preheated (37 ± 0.5°C) phosphate buffer (6.8 pH). The samples were analyzed using the calibrated spectrophotometric method with $R_{,}$ value of 0.999 at λ_{max} 319 nm (32).

Method 2

Dialysis chamber was prepared by using a dialysis membrane tubing (MWCO 12-14000 Daltons, Medicell International Ltd, 239 Liverpool Road, London) and was soaked for ~24 h in phosphate buffer (6.8 pH) (33). NS powder (equivalent to 80 mg of IND) equilibrated with phosphate buffer (6.8 pH) was added in tube and its ends were tied tightly to make a closed pouch. The pouch was suspended

Figure 1. Modified Franz diffusion cell used in this study

Table 2. Summary of physicochemical parameters of prepared NS formulations.

Formulation code	Particle size (nm)	Zeta potential (mV)	Drug loading (%)	Entrapment efficiency (%)	Percent yield (%)
F1	221.19 ± 2.43	-14.2	47.6 ± 2.59	45.3 ± 3.56	40.0 ± 3.45
F2	282.89 ± 1.83	-19.7	56.7 ± 2.12	64.8 ± 3.91	51.1 ± 2.29
F3	358.67 ± 2.85	-17.7	36.3 ± 1.76	35.3 ± 2.85	32.3 ± 3.28
F4	348.14 ± 2.12	-12.9	36.2 ± 1.92	30.9 ± 4.55	26.0 ± 3.29
F5	381.45 ± 2.22	-10.4	49.8 ± 1.62	43.8 ± 4.83	40.9 ± 2.96
F6	342.46 ± 3.23	-18.5	55.7 ± 1.19	51.2 ± 5.23	41.2 ± 2.71
F7	423.52 ± 3.20	-13.1	37.6 ± 2.66	50.3 ± 2,67	32.0 ± 2.35
F8	423.21 ± 2.75	-16.3	39.1 ± 2.81	30.1 ± 3.23	20.5 ± 2.87
F9	432.35 ± 1.83	-14.3	50.8 ± 1.95	32.1 ± 2.36	21.6 ± 2.76
F10	456.24 ± 1.88	-19.2	59.4 ± 2.22	61.6 ± 1.85	45.1 ± 4.54
F11	532.18 ± 1.96	-15.1	39.0 ± 2.45	44.2 ± 3.41	29.0 ± 4.77
F12	625.08 ± 1.46	-10.3	32.2 ± 2.13	41.9 ± 2.78	32.5 ± 3.71

in a beaker containing phosphate buffer (6.8 pH) (80 mL) withthe temperature maintained at 37 ± 0.5°C and stirring at 100 rpm. Samples (1 mL) were withdrawn at interval of one hour for 8 h and replenished with the same quantity of fresh preheated (37 ± 0.5°C) phosphate buffer (6.8 pH). The samples were analyzed in triplicate using the same procedure as described in method 1.

Preparation of NS tablets

Tablets containing pure drug and drug-loaded NS were formulated by direct compression method. For drug loaded NS tablets, accurately weighed NS (160 mg), microcrystalline cellulose (35 mg) and magnesium stearate (5 mg) powders were mixed geometrically. The powder mixture was then compressed using a 13 mm punch and dies set at a pressure of 1500 psi for ~1min (34). Same excipients were used for the preparation of pure drug tablets.

Evaluation of drug loaded NS Tablets

Tablets were tested for friability, hardness, thickness, drug content and *in vitro* drug release. Friability and hardness were determined by Roche friabilator and Monsanto hardness tester, respectively (35).

Dissolution studies of prepared NS Tablets

Dissolution studies were performed on formulated tablets by using USP type II apparatus (Erweka DT 700) by maintaining the temperature at 37 ± 0.5°C with paddles speed of 50 rpm. The drug-loaded NS tablets were placed at the bottom of a vessel containing a phosphate buffer of pH 6.8 (900 mL). Aliquots (5 mL) were withdrawn at predetermined time intervals and replenished with same amount of fresh, preheated (37 ± 0.5°C) dissolution medium. The samples were analyzed in triplicate using calibrated spectrophotometric method with R^2 value 0.998 at λ_{max} 319 nm (36).

Drug content analysis

Ten tablets were accurately weighed, powdered and mixed properly and a portion of powder (equivalent to 20 mg of IND) was added to the volumetric flask (100 mL) containing 0.1 N methanolic HCl (60 mL). The mixture was sonicated for 10 to 15 min and volume was made up with 0.1 N methanolic HCl. The solution was filtered and analyzed at λ_{max} 319 nm for drug content analysis (37).

RESULTS AND DISCUSSION

Preparation and physicochemical evaluation of NS formulations

NS can be prepared by various methods including solvent, emulsion solvent diffusion and ultrasound assisted methods (15). Selection of the appropriate method depends on the type of drug and polymer used. Among all the methods, emulsion solvent diffusion is a simple and cost-effective technique, which can be employed for the drugs having limited aqueous solubility (38). As the IND belongs to BCS (Biopharmaceutical classification system) class II drugs (39), having a low aqueous solubility, this method was used in this study. The drug was firstly

dissolved in the organic phase followed by dispersion in aqueous medium. Furthermore, the solubility of EC and PVA in organic and aqueous phase respectively makes this method as a choice while using these polymers.

In order to evaluate the effect of polymers on the physicochemical properties of NS, twelve formulations with varying ratios of EC and PVA as shown in Table 1 were prepared. The prepared formulations showed nano-sized particles in the range of 221-625 nm (Table 2). The mean particle size was considerably affected by the polymer concentration. Lower concentrations resulted in relatively smaller particle size where lesser time was required for droplet formation (40). A higher amount of polymers increased the viscosity of the system which creates hindrance in the formation of smaller droplets (41). At lower concentration, diffusion of internal phase (dichloromethane) into external phase (aqueous phase) was improved therefore there was a little time for droplet formation which resulted in smaller particle size (42). With respect to the PVA, smaller particle size was achieved with 0.5% concentration. Higher concentration (0.75% and 1.0%) resulted in larger particle size due to foaming and aggregation. These results can also be attributed to an increase in apparent viscosity at emulsifier (PVA) concentration higher than the critical value. This increased viscosity would result in larger emulsion droplets and finally in greater NS size.

Results of entrapment efficiency and drug loading (Table 2) showed that these parameters are

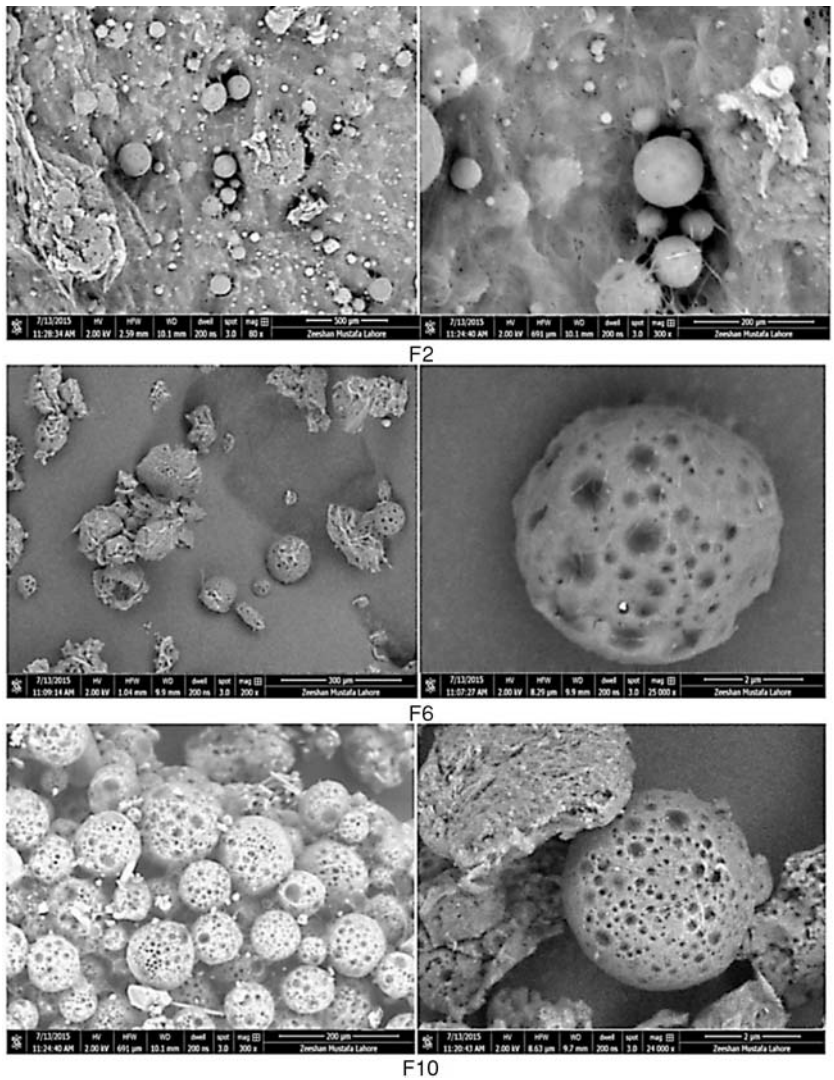

Figure 2. SEM images of NS formulations (F2, F6 and F 10)

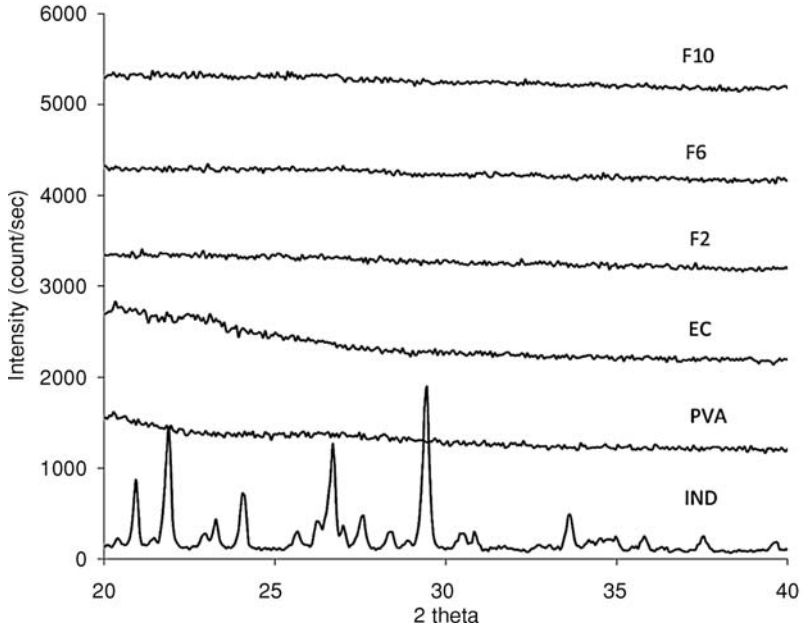

Figure 3. Comparison of PXRD patterns of NS formulations (F2, F6, F10) with IND, PVA and EC

dependent on drug to polymer ratio, formulations with an equal proportion of drug to ethyl cellulose (EC) (i.e. F2, F6 and F10) show higher values for these parameters. This demonstrates that the amount of drug per unit polymer is an important factor in this respect. These results are in contrast to other studies (43), where the higher drug to polymer ratios resulted in increased entrapment efficiency and drug loading. They have demonstrated that this was due reduced diffusion rate of organic phase from concentrated solutions into aqueous phase; however, no simple explanation is apparent. This shows that the parameters (e.g. type of drug, its chemical structure and other physicochemical properties) other than drug-polymer ratio are also involved in loading and entrapment of dug in this type of systems.

Analogous to this, percentage yield of prepared formulations was also found dependent of drug to polymer ratio. The values of this parameter for NS formulations were observed in the range of 20 to 51%. The low percentage yield values are may be due to the adherent nature of the product produced. A Portion of the product was stuck with apparatus due to aggregation and foaming resulted in a loss in percent yield.

Zeta Potential (a measure of the surface charge) is an important factor in determining the nanoparticle stability. Particles with potential values larger than +25 mV or less than -25 mV typically shows a higher degree of stability (44). Our result has shown that all the prepared formulations exhibit values of Zeta potential between -10.4 to 19.7 mV representing stable formulations (Table 2).

Structural analysis

Three formulations (F2, F6 and F10) having an equal drug to polymer ratios, showing a higher value of parameters such as percentage yield, entrapment efficiency and drug loading were selected for structural analysis. Structural analysis was performed by using scanning electron microscopy (SEM), X-ray powder diffraction (XRPD) and Fourier transform infrared spectroscopy (FTIR). Surface morphology of prepared NS by SEM is represented in Figure 2. It is evident that the formed particles are of spherical orange peel like shape with spongy and porous nature. Diffusion of the solvent (dichloromethane) from the surface of the NS is believed to be a reason of this spongy nature (39, 40).

Comparison of PXRD patterns of NS formulations with pure IND, EC and PVA is shown in Figure 3. Major peaks of IND were observed at 2θ values of 21, 22, 27 and 29^{o} representing a crystalline nature of the drug. PXRD pattern of EC and PVA showed an amorphous nature of these polymers as no diffraction peaks were observed. Similarly, PXRD pattern of selected formulations (F2, F6, and F10) showed masking of drug peaks.

This can be explained by the encapsulation of drug in an amorphous core of NS. These results also negate the formation of any chemical interactions between drug and polymer as no new peaks were observed in the PXRD patterns of NS formulations. FTIR spectrum of pure IND, polymers and NS formulations are shown in Figure 4. Pure IND spectrum shows characteristic peaks of amide (C=O of amide) at 1680 cm^{-1}, amine (C-N) at 1221 cm^{-1} and C-O at 1066 cm^{-1} (45). Spectra of EC and PVA (Figs. 4 b and c, respectively) show characteristic peaks of these compounds. Spectra of selected NS formulations show broadening of drug peaks, which can be attributed to the encapsulation of the drug in NS core. An important feature of the spectra is a peak at 1680 cm^{-1}, which is due to the amide group of IND involved in hydrogen bonding. There was no change observed for this particular peak in the spectra of NS, this represents the absence of interaction between drug and polymer.

Dissolution studies on the drug and NS formulation (F10) were performed by two methods, firstly by taking powder samples directly and secondly by intrinsic dissolution method. Comparison of *in-vitro* dissolution profiles obtained from the powder samples of pure drug and NS formulation is shown in Figure 5. Overall, results show that incorporation of drug in NS retarded the release rate as the percent release of drug from NS formulation is much slower than the pure drug. In case of a pure drug, initially, a fast dissolution rate was observed as more than 45% drug was released in first 5 min, then ~ 86.5% in 90 min and reaches to a maximum (99%) in 270 min. In contrast, the NS formulation showed only 17.4%, 37.8% and 45.9% at 5, 60 and 270 min, respectively. In order to predict the behavior of drug release from NS, dissolution data was fitted to various kinetic models. The results show that the drug is released from NS following Korsmeyer-Peppas model with R_{ϵ} value of 0.853 and n = 0.915. This suggests that the release of the drug from NS is governed by diffusion and swelling phenomenon (29). These results are in agreement with the previously published data, where it has been suggested that the thicker matrix wall of polymeric sponges leads to a longer diffusion path that results in slower drug release rate (44).

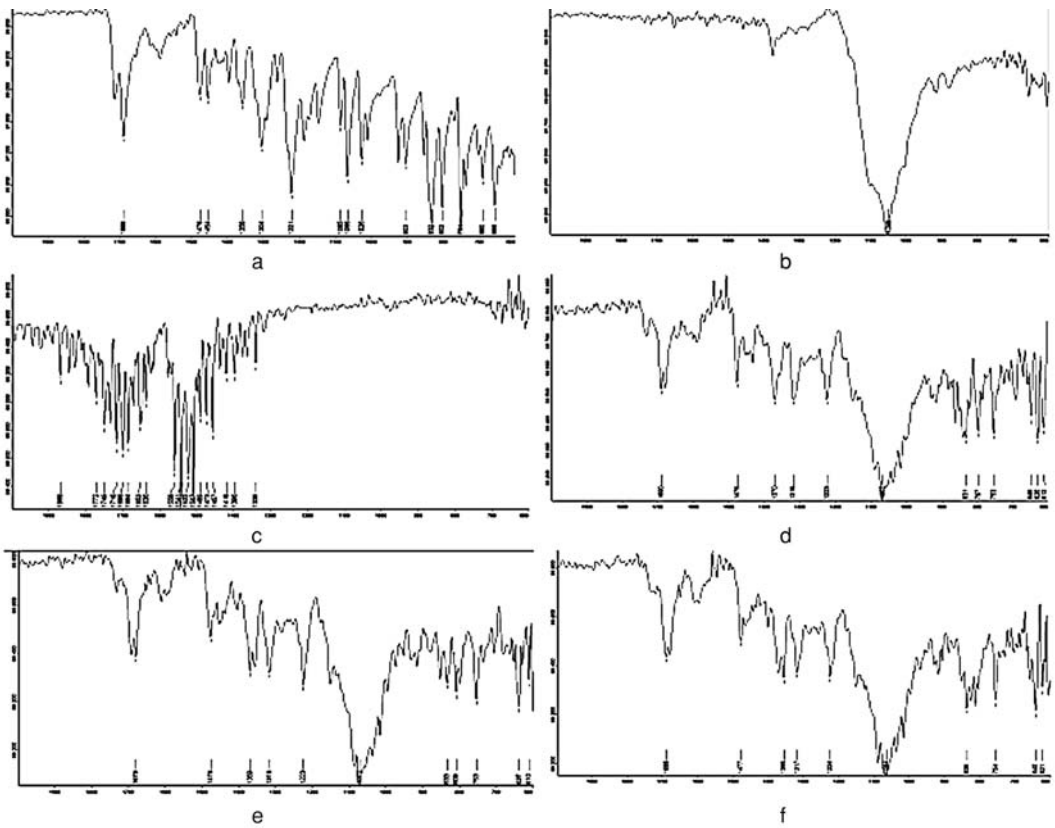

Figure 4. FTIR spectra of IND (a), EC (b), PVA (c), NS formulations F2 (d), F6 (e), F10 (f) *In vitro* release studies

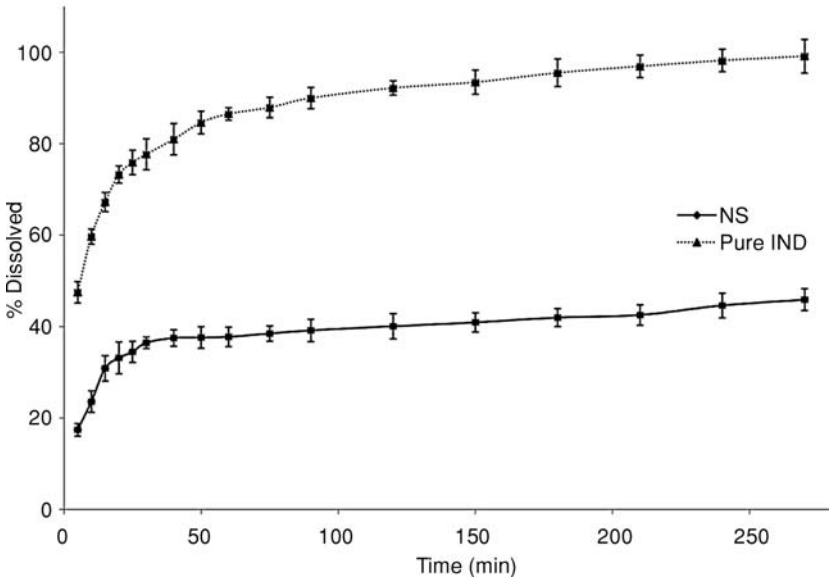

Figure 5. Comparison of *in-vitro* dissolution profile of IND and NS

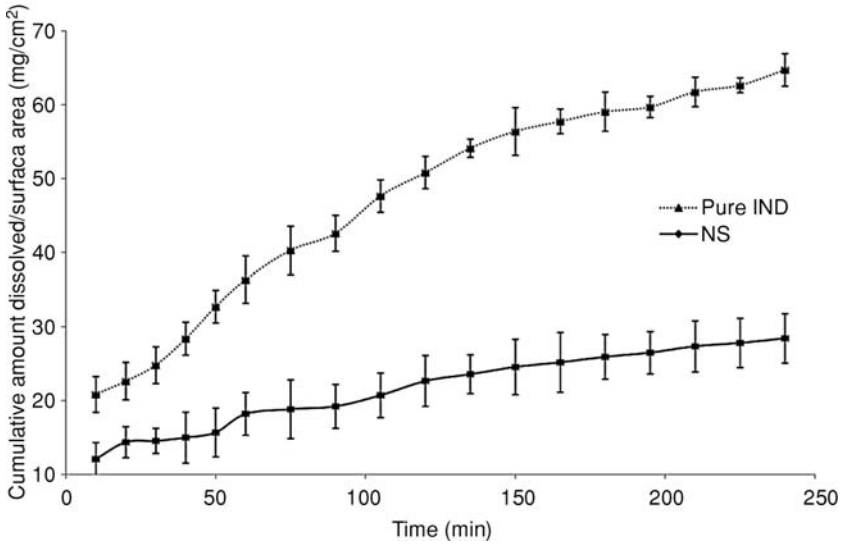

Figure 6. Comparison of intrinsic dissolution profile of IND and NS

Determination of intrinsic dissolution profile is an important parameter to compare the dissolution behavior of drug, alone and in NS formulation because the exposed area of the disk remains constant throughout the test period. This is important as the effect of particle size on the dissolution can be minimized due to constantly exposed surface area of the disk (23). Intrinsic dissolution profile of the pure drug and NS formulation is compared in Figure 6. Linear profiles were observed for both drug and NS formulation. However, cumulative release from NS was much slower than the pure drug. Intrinsic dissolution rate of pure drug and NS was 36.3 mg/cm^{2} and 18.2 mg/cm^{2} at 60 min, 64.7 mg/cm^{2} and 28.4 mg/cm^{2} at 240 min, respectively. Overall, there was ~ 2.5 fold decrease in the intrinsic dissolution rate of NS as compared to the pure drug.

Diffusion studies

In general, the *in vitro* dissolution method (41) provides a direct approach to monitor drug release from the conventional dosage forms. For these formulations agitation of the dosage form and sampling techniques are reasonably simple and straightforward. However, due to the small size of NS, several practical challenges e.g. aggregation of nanoparticles, logging of filters during sampling and difficulty in physical separation have been observed. In addition, poor sink conditions, especially with poorly soluble drugs, cause a problem for these tech-

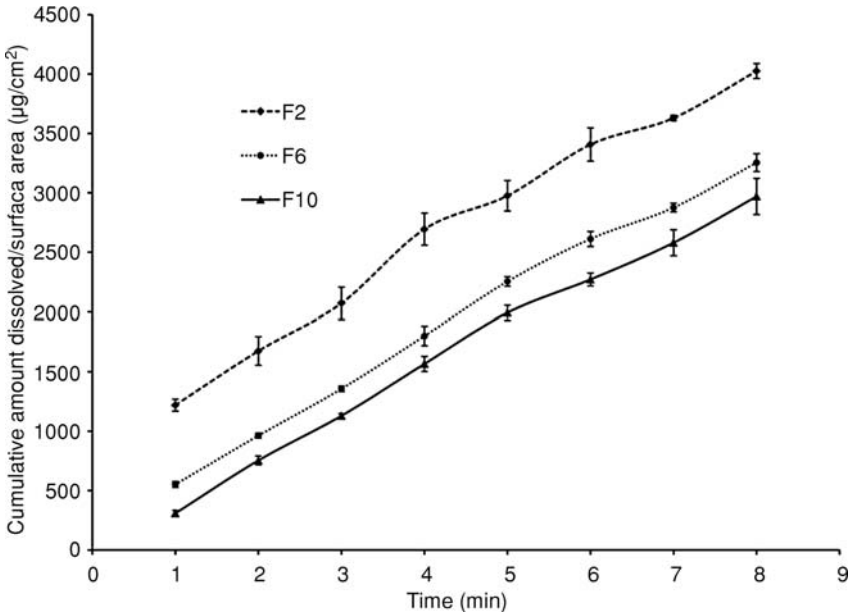

Figure 7. Diffusion studies of NS (F2, F6, and F10) using Franz diffusion cell

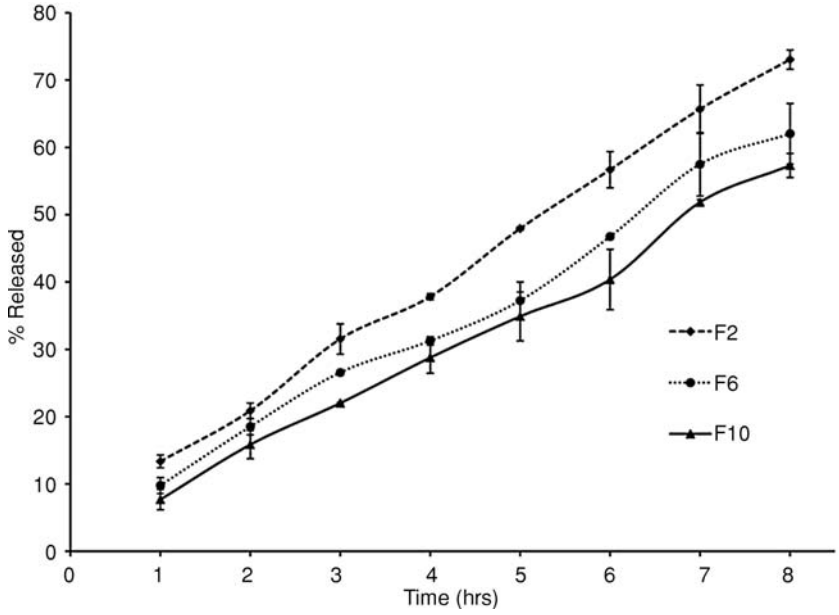

Figure 8. Diffusion studies of NS (F2, F6, and F10) using dialysis bag method

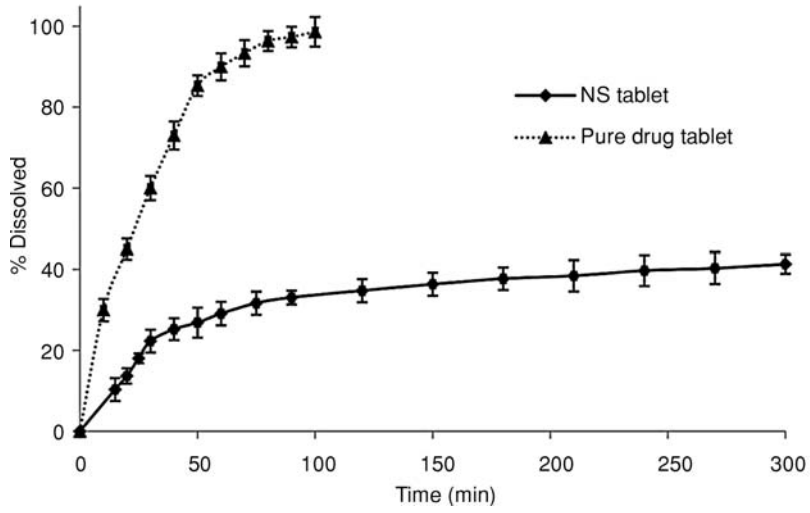

Figure 9. Comparison of dissolution profile of formulated tablets

niques. To overcome these problems, diffusion studies using dialysis membrane bag or Franz cell is being used to assess drug release from nano-sized dosage forms. In these methods, physical separation of the dosage forms is achieved by usage of a dialysis membrane which allows for ease of sampling at periodic intervals (46).

The *in vitro* release profiles of IND from NS formulations F1, F2 and F3, over an 8 h (480 min) time period using Franz diffusion call are presented in Figure 7. The samples of NS were equilibrated with 6.8 pH phosphate buffer in donor compartment separated from receiving chamber by a dialysis membrane. Samples were withdrawn at one hour intervals for eight hours and replenished with the same quantity of fresh preheated (37 ± 0.1°C) phosphate buffer (6.8 pH). The amount of IND released per unit surface area (amount released/ cm^2) was plotted verses time. Using linear regression, the slope, which represents the release rate, was estimated. The release rates (expressed in mg/cm^2 per h) for F1, F2 and F3 were 376.4 (R^2 = 0.995), 389.4 (R^2 = 0.995) and 401.2 (R^2 = 0.989) respectively. These results indicate that the release rate of drug is dependent on the concentration of EC. Formulation prepared from least amount of EC (F1) has shown higher value as compared to the formulations containing higher amounts of EC (F2 and F3). The overall result shows that more persistent and linear response was achieved for all NS formulations.

Figure 8 shows release profiles of NS formulations by using dialysis bag method. In this method, the NS was introduced into a dialysis bag containing release media (inner media/compartment). Tha bag was subsequently sealed and placed in a larger vessel containing release media (outer media/compartment), agitated and samples were collected at regular intervals. Release profile of all formulations showed a continuous release up to 8 h. The percent drug released after 8 h for F2, F6 and F10 was 73.1%, 62.0%, and 57.3%, respectively. These results are comparable with the Franz cell method i.e. higher amount of EC resulted in a lower release rate.

The comparison of the two methods shows that both methods describe almost similar results. However the advantages of Franz cell-like uniformity in temperature, constantly exposed surface area and ease of sampling gave more reliable and better diffusion profile (in terms of linearity) as compared to dialysis bag method (46).

Evaluation of NS tablets

Tablets containing pure IND and IND loaded NS were formulated by direct compression method. These tablets were tested for friability, hardness, thickness, *in vitro* drug release and drug content. All the physical parameters for tablets were within official limit i.e., friability 0.6%, hardness 6 kg/$cm^{,}$ and thickness 3.0 mm. The drug content of pure drug and NS tablets were found to be 98.62 and 98.51%, respectively.

Dissolution studies of prepared NS Tablets

Dissolution testing of the tablets prepared from the pure drug and the drug-loaded NS was conduct-

ing in phosphate buffer (pH 6.8), given that phosphate buffer is a compendia dissolution fluid for such type of preparations as it is close representative of human small intestinal fluid Patterns of drug release from both preparations are shown in Figure 9. For pure drug tablets, there was a fast release during the first hour of testing (represented by a sharp rise in dissolution profile), with 93% drug release and the total drug release reached in less than 1.5 h. However, for tablets loaded with NS, a pattern showing sustained release of drug over the studied period was observed. Initially, as faster release up to ~29% was achieved in the first hour followed by a slow dissolution, only ~41% drug was released in studied period i.e. 5 h. Results from the kinetic modeling of dissolution data obtained from NS tablets showed that the maximum linearity was found for the Korsmeyer-Peppas model with highest 'R^2' i.e. 0.960 and with n = 0.402. Thus it specifies that the drug release mechanism is by Fickian process, which suggests that diffusion process is obeys Fick's law of diffusion which may be controlled by porosity of NS. Initial stability testing based on physical evaluation and drug content analysis after a period of six months have shown that the prepared NS tablets are stable. However through studies are required for complete stability profiling of the dosage form, which will be the subject of our future studies.

CONCLUSION

A sustained release tablet formulation of IND was successfully prepared by incorporating the drug into nano-sized particles called NS. EC and PVA are effectively used as polymers in this study. Emulsion solvent diffusion method is one of the better methods while using these polymers. Drug-polymer ratio is one of the important factors affecting the physicochemical properties of NS formulation. Prepared NS were of spherical shape with porous morphology. Intrinsic dissolution studies showed that NS gave more linear release as compared to the pure drug. The sustained release profile was confirmed by two different methods by using dialysis bag and Franz diffusion cell, where it later was found to be more precise. NS tablets were formulated as final dosage form and the formulation parameters were in official limit. It is concluded that NS based system is more appropriate for developing and formulating sustained release tablets of IND. However, bioavailability studies using animal models could be performed in future in order to predict the pharmacokinetic profile of these specialized systems.

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