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Research Article

Novel Sustainable Nanocarrier Systems for Improving Drug Efficacy

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Abstract

The privilege of delivering the drug parenterally in an implantable nanocarrier (IN) system was compared with a previously studied transdermal nanoemulsion (NE) drug delivery system in order to enhance the drug efficacy in terms of dose, frequency and patient compliance over its oral dosage form. These nanocarrier systems were evaluated by studying their drug loading and entrapment efficiency, in vitro release and characterization including particle size and morphology, pH range and viscosity measurements. Both of these systems possessed optimum droplet size, polydispersity and viscosity with a promising release and permeation properties and characterized by their patient friendly nonirritant property due to their compatible pH values with human tissue. Such results shed a beam of light on the opportunity of the parenteral implant polymeric nanocarrier system in providing a more sustainable controlled delivery of the drug for longer periods, over two weeks, in contrast to the transdermal nanoemulsion system that can last just for certain days, noteworthy both systems helped in limiting the inconvenient drawbacks accompanied with the oral dosage form.

Key words: Parenteral route, polymers implant nanoparticles, transdermal nanoemulsion, characterization, sustained prolonged effect.

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1. Introduction

A previous study was done to evaluate the possibility of transdermal (TD) delivery of fluoxetine hydrochloride (Fx HCl) utilizing nanoemulsion (NE) drug delivery as a nanotechnological approach in order to acquire a high permeation and prolonged efficacy [1].

Recent TD formulations have been considered for permeation enhancement by changing the properties of the drug, the vehicle or the skin [2]. NEs were suggested to have a potential of increasing cutaneous drug delivery compared with conventional vehicles [3]. NEs can be considered as ideal liquid vehicles as they possess most of the requirements for achieving this, including thermodynamic stability, ease of formulation, high

flowability, high solubilization capacity and minute droplet size. The latter characteristic provides a better chance for adherence to biological membranes transporting drugs in a controlled manner [4]. The studied Fx HCl transdermal NEs showed the advantages of NEs comprise low cost of preparation, improved bioavailability of drugs, effective vehicles for solubilization of hydrophilic and lipophilic drugs, prolonging the drug release and protection of entrapped drugs from degradation and hydrolysis, thus preventing irritation. They also possess low viscosity, high surface area and very small nano-size with a strong impact on TD permeability [1,4], and thus overcame the inconvenient drawbacks superior to the oral drug delivery.

Nowadays, scientists are interested in novel drug delivery systems which are used to direct drugs to the specific site of action and to achieve a controlled release of drug with desirable release kinetics [5] over a prolonged period of time that can last for months by a single sustained dose regimen. Among the most studied of these systems were the parenteral polymeric implantable nanoparticle drug delivery systems.

Parenteral drug delivery takes the drug directly into the tissue fluid or blood without having to cross the intestinal mucosa thus its action is fast and surer (valuable in emergency). Gastric irritation and vomiting is not provoked. Liver is also bypassed by this route [6]. Thus the limitations of oral route are circumvented. Implant nanocarrier (IN) appears to be a unique approach for overcoming parenteral limitations due to its conventional formulations strategies [7] and its perfect suitability as a convenient painless injection [6] due to its characteristic nano-sized particles below 125 μm that effectively conquer the polymer viscous consistency leading to a sufficiently syringeable system that can be injected by conventional syringe and needle [8-10].

Moreover, these systems are characterized by the absence of any harsh excipients and/or any potentially toxic ingredient [8-10]. Also, they cause obvious improvement in the parentally tolerable dose of the drug, thus leading to a reduction in the cost of the therapy and also an improved therapeutic performance.

These systems have been fabricated using an internal polymer phase (drug, biodegradable polymer, surfactant and water-miscible organic solvent) and an external oil phase (oil and preservative) claiming the implant formation of nanospheres at the injection site as an approach to reduce the unwanted local irritation [11].

The objective of the present work was to evaluate the possibility of formulating these parenteral polymeric INs in order to compare their ability to potentiate the drug effectiveness in the mean of a minimal dose regimen and frequency in contrast to the transdermal NE formulation.

2. Materials and Methods

Materials

2-pyrrolidone (Soluphor®), N-methyl-2-pyrrolidone (NMP), Poly(D,L-lactide-co-caprolactone) (I.V. 0.7-0.9), Polyethylene glycol (PEG 400), Peanut oil, Span 80, Tween 20 and Tween 80, procured from Sigma Aldrich Company, St. Louis, USA, Aluminum-monostearate (obtained from Fluka ChemieAG, Buchs, Swiss, Dimethylsulfoxide (DMSO) (obtained from Merck, Darmstadt,Germany) and Fx HCl B.P., (donated by Misr Medical Products Company , El-Mataria City, Egypt).

Methods

Solubility Study and Screening of Suitable Excipients for INs Preparation

The solubility of drug and polymer was determined in various solvents, viz. Peanut oil, NMP, 2-pyrrolidone, DMSO, Tween 20, Tween 80, PEG 400, 10% ethanolic phosphate buffer (pH 7.4) and phosphate buffer (pH 7.4) by adding an excess amount of drug to 5 ml of each of these solvents in screw capped vials and mixed using a vortex mixer (JULABO Labortechnik, Germany). The vials were then shaken at 37°C in an isothermal shaker (GFL 3032, Germany) for 72h to attain equilibrium. The equilibrated samples were removed from the shaker and left to stand till forming a clear supernatant. The supernatants were filtered through a millipore filter 0.45 μm and the drug concentration in the filtrate was determined spectrophotometrically at the respective λ_{max} after appropriate dilution with absolute ethanol.

Determination of Encapsulation Efficiency in the Prepared Formulations

The drug dose was loaded in about 1gm of completely formed INs, vortexed and sonicated with polymer phase and continuously homogenized with oil phase, and then the IN systems (ND1, ND2, ND3 and ND4) were assayed spectrophotometrically for drug content and loading capacity. Results were presented as percentage drug entrapped and loaded.

Characterization of the Prepared INs

Transmission Electron Microscopy

The morphology of the INs was studied using transmission electron microscopy (TEM), JEOL, JEM-1230, Japan. A combination of bright-field imaging at increasing magnification and of diffraction modes was used to reveal the form of the INs. The samples were diluted with phosphate buffer pH 7.4 followed by sonication then one drop of the diluted sample was deposited on a film- coated 200- mesh copper specimen grid and allowed to stand till complete dryness. The grid was later stained with one drop of 3% freshly prepared phosphotungstic acid and allowed to dry before examination.

Particle Size Determination

The nanoparticle mean size and the polydispersity index (PDI) were assessed by photon correlation spectroscopy using a Zeta-sizer 5000 (Malvern, Worcestershire, England). Dispersion was carried out by mixing the IN samples with phosphate buffer medium pH 7.4, and then sonicated, and subjected for particle size and PDI evaluation. The measurements were performed in triplicate for all batches.

Viscosity Measurement (Rheological Study)

Steady shear measurement was conducted where the rheograms of the prepared formulations was performed at 25 ± 0.1°C using cone and plate programmable viscometer (Brookfield Engineering Laboratories Inc., Model HADV-II, USA). The plate diameter and the cone angle radian and the gap at the cone tip were specified and connected to a digital thermostatically controlled circulating water bath (Polyscience, Model 9101, USA) with spindle 52, the shear rates range from 50 to 400 s^{-1} corresponding to 25 to 200 rpm with 10 s between each two successive speeds in an ascending order. Equilibration of the sample for 5 min was made following loading of the viscometer. Ramp time for each viscosity stage was read after 20 s. All studies were performed in triplicates and the average was taken.

Rheological data was fitted to certain model (Power law) to examine the pattern of flow and the presence of yield value.

• Power law:

$\tau = \eta\gamma^n$

Where τ is the shear stress, η a constant called the apparent viscosity or the consistency index, γ the shear rate and n is the flow index. In case of Newtonian behavior n = 1. The software employed was Graph Pad Prism® version 4.

PH Determination

The pH of the INs was determined by using a Digital pH meter, JENWAY 350, UK. The vials containing the formed nanoparticles were subjected to sonication and the pH measured at room temperature at different time intervals 0, 24 and 168 hrs (a week) after preparation. The results were reported as the mean of three experiments.

In-Vitro Release Study

The preparation was placed in a clean dialysis bag (semipermeable cellulose acetate membrane; MW cut-off 12–14,000 Da). The bag was secured with two clamps at each end and placed into screw capped bottles containing 25 ml ethanolic phosphate buffer (pH 7.4). The buffer was kept at 37±0.5°C and stirred at 50 rpm in a horizontal shaker (HS 501 Digital, IKA-Labortechnik, Staufen, Germany). At 0.25, 0.50, 1, 2, 3, 4, 5, 6, 24, 48, 96, 144, 192, 240, 288 and 336 hrs, 1 ml aliquots of the medium were sampled and replaced with 1 ml fresh medium. The samples were analyzed for drug content spectrophotometrically against ethanolic phosphate buffer (pH7.4) as a blank, in order to cancel any possible interference in absorbance caused by the blank samples. Experiments were conducted in triplicates. The cumulative amount of drug released across the semipermeable membrane was plotted as a function of time. Release efficiency (RE) represents the percent of total area under the release curve (AUC).

3. Results

Solubility Study and Selection of Excipients

It was very important to find out the appropriate excipients to dissolve Fx HCl and the polymer, as the approved compatibility of the drug with other excipients (Figure 1) can allow the formation of a perfectly stable IN system, making solubility properties one of the initial objectives for a pharmaceutical formulation [12].

Drug Entrapment and Drug Loading of INs

In order to assess and compare the different nanocarrier formulations, drug entrapment capacity was investigated. It was found that ND1, ND2, ND3 and ND4 implant nanocarriers, where ND1 has 10% polymer and 50% drug, and ND2 contains 20% polymer and 50% drug and both of them are 1:4 (polymer phase: oil phase ratio), likewise ND3 includes 20% polymer and 50% drug, and ND4 contains 30% polymer and 50% drug and both of them are 1:10 (polymer phase: oil phase ratio), revealed encapsulation efficiencies ranging between 70-90 % and drug loading ranging between 32-47%. Comparing such results with the encapsulation efficiency range (75-98%) showed by the previously studied transdermal NEs [1], it's clear that both systems possessed promising high encapsulation efficiencies.

Characterization of IN Formulae

Transmission Electron Microscopy and Particle Size Study

Photographs of transmission electron microscopy (TEM) shown in figure 2,

demonstrate clearly the spherical form of Fx INs with a well-defined particle size. The average particle diameters of the selected INs by TEM shown in figure 2 perfectly agree with the mean size of INs by Zeta-seizer measurements presented in figure 3, which proved the homogeneity of these INs. All the formulations show particle size in the nano range (56.90–94.83nm), which was reproducible as evident from the acceptable polydispersity index (PI) values (0.33-0.53), below 1, that governs the physical stability of nanoparticles and reveals an adequate uniform distribution. A similar trend was observed in the previously studied transdermal NEs as they revealed spherical outline droplets with a relatively low average diameter ranging between 49.43-93.17 nm and a suitable PI values (below 1) indicating also the uniformity in system distribution [1].

Viscosity Study (Rheological Behavior)

In case of Newtonian behavior, flow index = 1. Based on the values of flow index (n) with a range of (1.01-0.9578) for ND1, ND2, ND3 and ND4 nanocarriers, it is obvious that all these formulations exhibit nearly a typical ideal Newtonian behavior with a flow index = 1 or almost ≈ 1 and a straight line relationship between the shear stress and the shear rate (R^2 >0.99) in all cases, which is depicted in figure 4. It is clearly showed in figure 5 that, ND3 and ND4 nanocarriers showed slightly higher viscosity evaluations in comparison to the other IN systems, where in general, all this study IN systems had proper viscosity results ranging between (91.3 -111.8 mPa.s), that can be applied for smooth easy injection through a wide range of needles without any difficult problems as reported by Cilurzo et al [13]. A similar Newtonian behavior was achieved by the previously studied transdermal NEs and was accompanied with a low viscosity values < 130 mPa.s [1], which is perfectly preferable for such route of administration [14].

PH Study

The pH values of the INs, ND1, ND2, ND3 and ND4, ranges from 5.5 to 5.8. The pH values of the INs reflect a fact that, all INs pH values are quite close and perfectly compatible with the human tissue as well as they fall in the physiologic range accepted for parenteral administrations (2-9) [15,16], and thus these INs will be non-irritant and safely injected to human body. A similar relation was reported in the transdermal NE, as they possessed pH values within the physiologic range of the skin (4.5- 7) [1,17], and thus, are also expected to be safe and non-irritant.

In -Vitro Release Study

The drug release from these IN systems, ND1, ND2, ND3 and ND4, is mainly classified into two main steps; first a relatively faster release during the first 24 hrs called the initial burst step that was very low (1.13-3%) followed by a prolonged controlled release phase through 14 days (Figure 6). Noteworthy, it is noticed that the % of drug released of these INs during 14 days ranges between 5.13-9.74% as shown in figure 6, these results agree with those documented by Jelonek, who found a prolonged controlled drug release that may last from several months to a year [18]. While, lower prolonged sustained release and permeation patterns were achieved by the previously studied Fx HCl transdermal NEs that can just last for around a week [1].

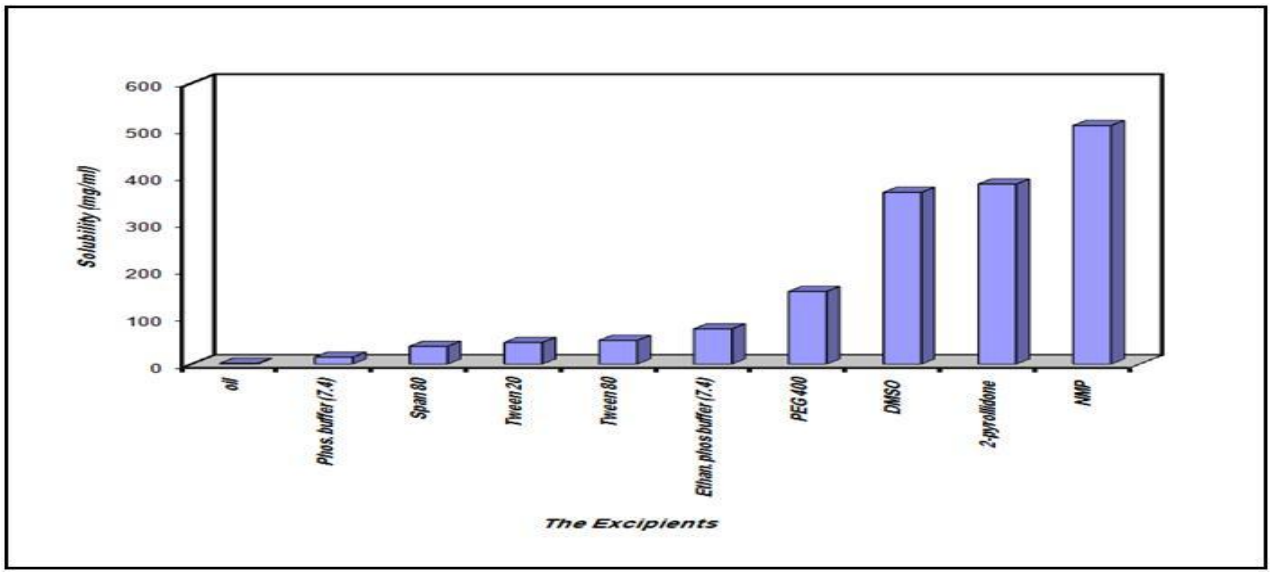

Figure 1. Solubility of Fx HCl in different excipients at 37°C

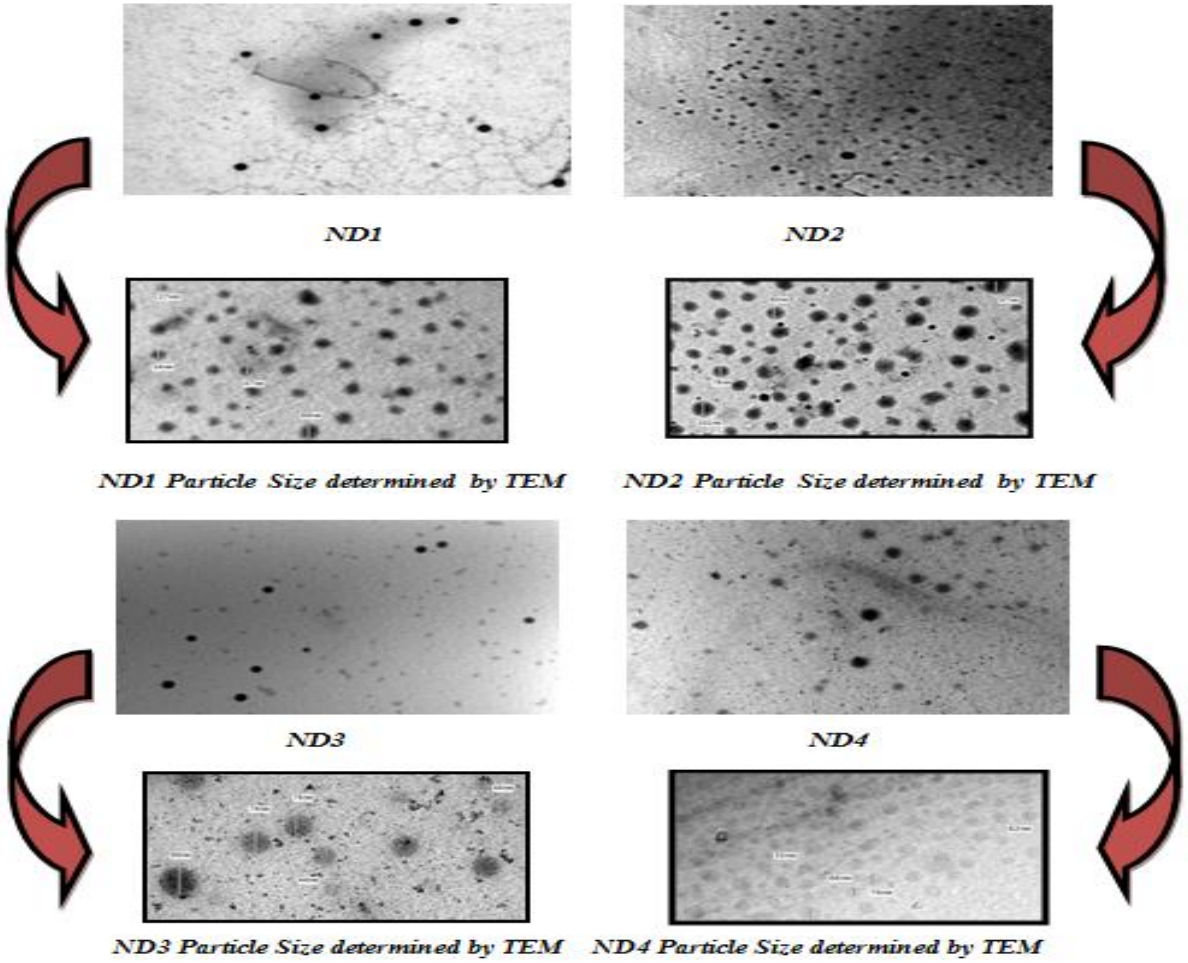

Figure 2.TEMphotographs of the IN formulae

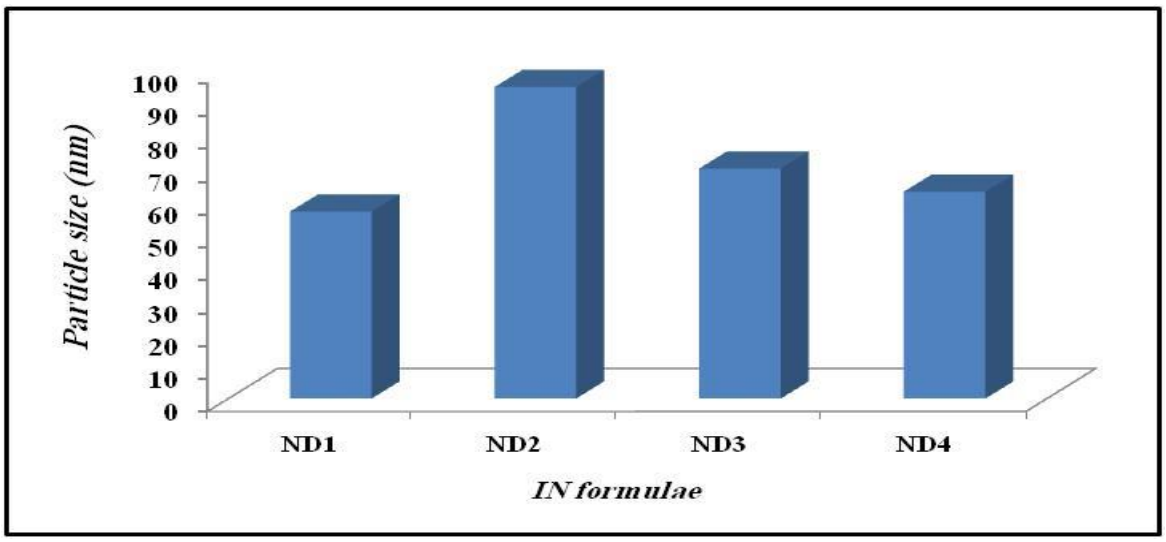

Figure 3. Particle diameter of the IN formulae

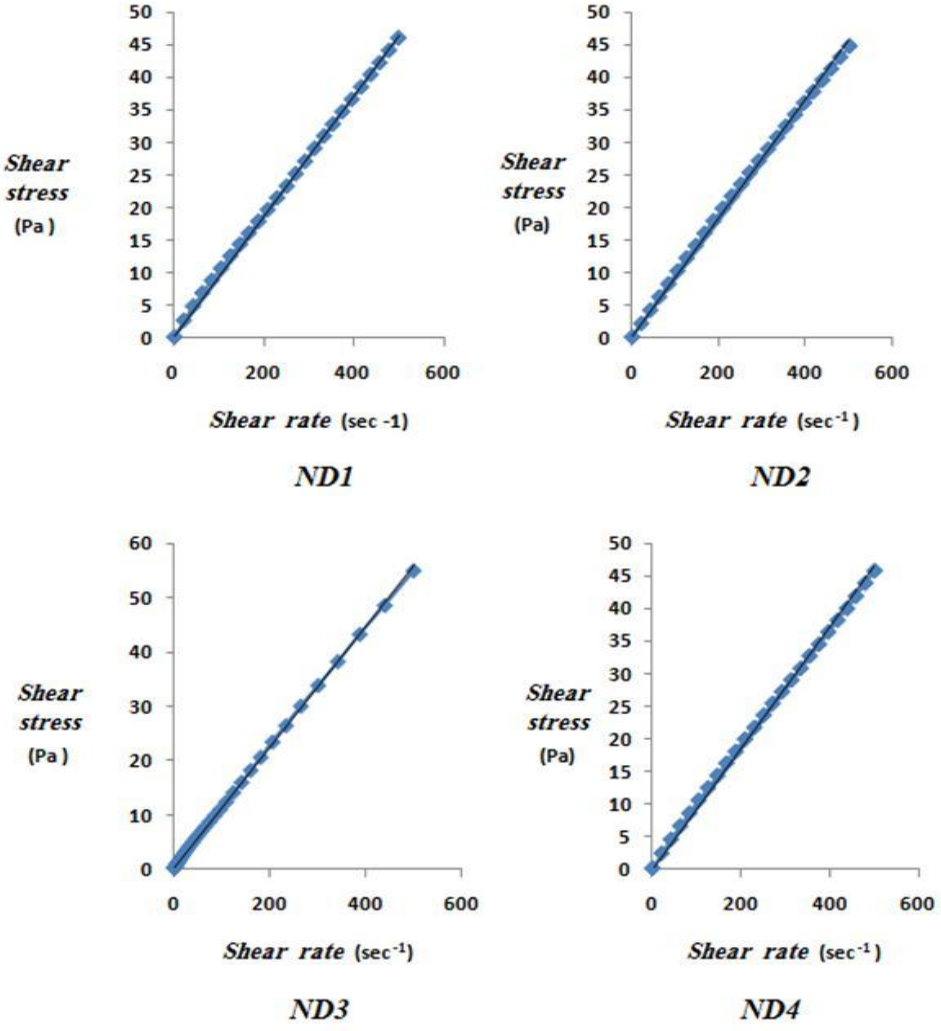

Figure 4. Flow curves of the IN formulae at 25°C

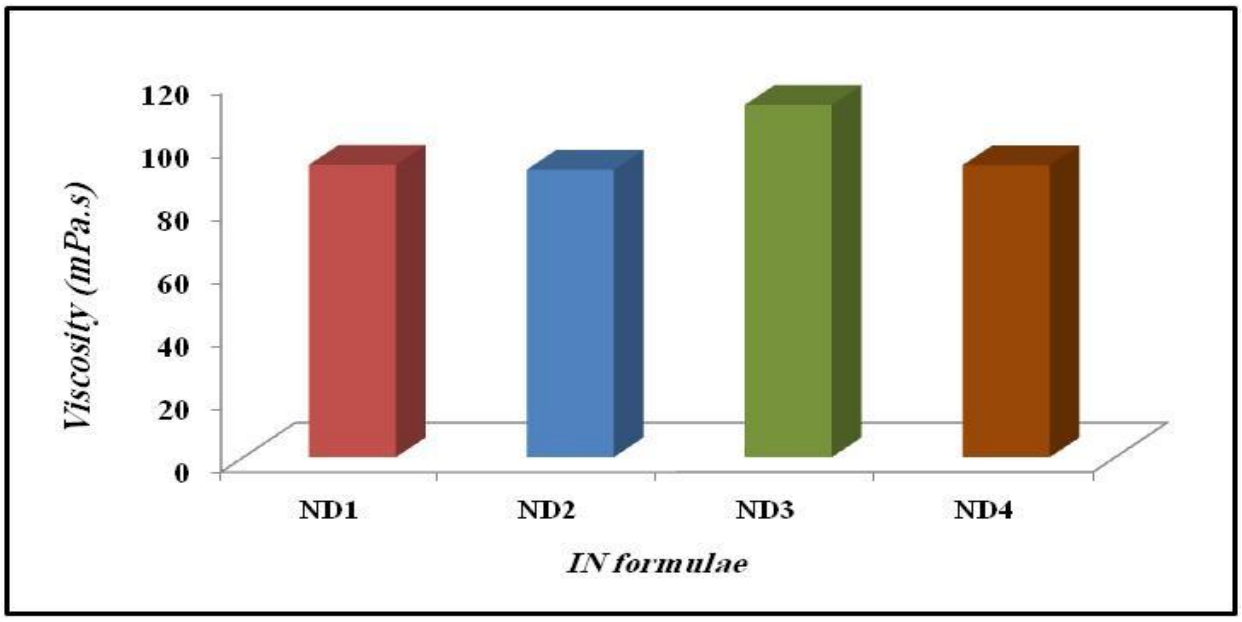

Figure 5. Viscosity of the IN formulae

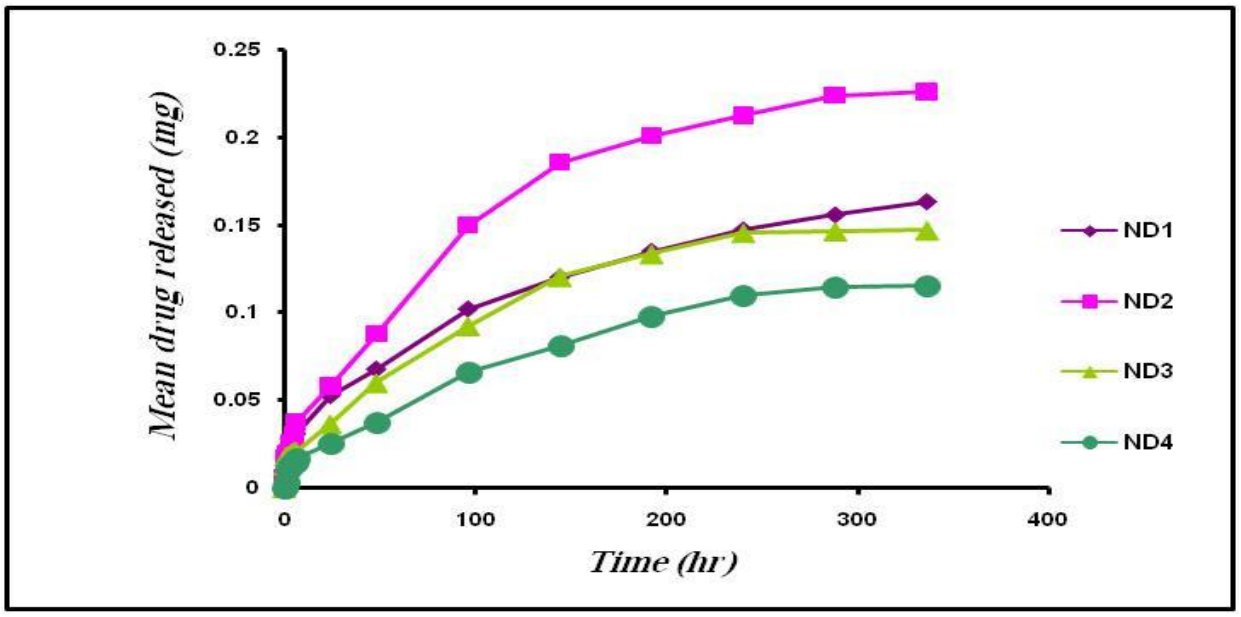

Figure 6. Release profiles of the IN formulae

4. Discussion

The low solubility of the drug in the oil phase (Figure 1) is essential as it causes existence of the drug in the inner polymer phase, thus allows a well enclosure of the drug within the system [19]. Also, polymer solubility in the continuous solvent phase is very important as it is necessary to provide a suitable environment where the polymer can sufficiently encapsulate the drug within stable IN nanoglobules. It was noticed that, 2-pyrrolidone and DMSO were the most suitable solvents for dissolving the polymer in contrast to the other excipients. So, ND1, ND2, ND3 and ND4 are prepared using these excipients.

High drug entrapment of these INs is advantageous since it transports enough

drug at the target site and increases the residence time of the drug, also is their low drug loading, as it causes low initial release with a more prolonged effect [20,21]. The high drug entrapment can be attributed to several factors as the presence of surfactant, Tween 80, as increasing its concentration causes decrease in surface tension of polymer atoms and therefore increases the encapsulating efficiency of nanoparticles [22], also the presence of oil continuous phase in these IN systems impedes the drug loss and leads to increasing in the drug encapsulation efficiency of such systems [23,24].

The small particle size of this study INs can be explained by the presence of the oil continuous phase, as the nanoparticles size depends on the diameter of initial emulsion droplets, which are smaller and more finely dispersed (droplet coalescence is probably less) when a larger oil amount is used in the nanocarrier preparation [25], as in the case of ND3 and ND4 that had lower particle size (70nm, 63nm), compared to ND2 (95nm) as shown in figure 3. In addition, it was reported that the amount of surfactant plays an important role in the protection of the nanoglobules aggregation, and as the amount of the surfactant added increases, it avoids the particles coalescence and thus impedes their tendency to aggregate, resulting in the production of nanoparticles with a smaller particle size [26], as noticed in ND3 and ND4 implant nanocarriers that possess 10% Tween 80 and Span 80 surfactants and smaller particle size compared to bigger particle size ND2 referred to its low surfactant percent (5% Span 80). In general, these IN systems particle size are considered ideal for parenteral route of administration, as reported by Tice and Tabibi [6]. Also, these results was supported by the promising ability of these INs to flow easily even through insulin syringe that was evaluated and proved by a practical test.

The higher viscosity values of ND3 and ND4 can be attributed to their higher oil content and HLB values in comparison to the other IN systems, because as the HLB value of the system increases the viscosity increases [27]. A similar trend was reported in the previously studied transdermal Fx NEs, as the NE system possessing the highest oil content and HLB value revealed higher viscosity and visa versa, which improves and optimizes these NEs penetration properties [1].

The pH values of IN systems ND1, ND2, ND3 and ND4, are in a good agreement with the results of Douglas and Tabrizian who demonstrated that polymeric systems of pH 5–6 generally produce small particle size [28].

The too low initial burst release and the second sustained release phases of these INs are probably caused by the polymer semicrystalline morphology and hydrophobicity that leads to a slow polymer degradation [29-31], a more drug hindering (longer drug encapsulation with the polymer) and thus a prolonged controlled drug release profiles with a minimal initial burst effect that is advantageous as the high undesirable initial release may exhaust the encapsulated drug from nanoparticles too rapidly and even cause toxicity problems [32]. Also, the presence of oil continuous phase and aluminum preservative, that works as a viscosity enhancer, lead to impeding drug loss and so slows the drug release [21,23,24,33]. In addition, the presence of this immiscible oil continuous phase around the polymer-drug nanoglobules protect them by inhibiting the rapid annoying migration of water into the polymer composition that would result in a burst effect and a premature

polymer precipitation, thus this oil phase plays an important role in the formation of the slow sustained release patterns with a low initial effect [23,24]. Also, the conducted research indicated the advantage of preparing such nanocarrier systems with a reasonable viscosity for providing a longer-term release patterns that can last from several weeks to months in contrast to the sustained release and permeation profiles exhibited by the previously studied transdermal NEs of Fx HCl that was reported to last just for almost a week [1].

Conclusion

In summary, it is clear that, ND1, ND2, ND3 and ND4 revealed optimized properties regarding high entrapment efficacy, prolonged controlled release, small nano-sized diameter, in addition to the suitable viscosity and the compatible pH range, which allow these systems to be easily injected with an advantageous patient compliance, paving the way for them to be considered as attractive alternative to conventional nanoparticles as they allow a minimal burst effect with a more prolonged, sustained and steady release of the drug to its site of action in contrast to the drug transdermal NE system, and limiting the oral inconvenient drawbacks.

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