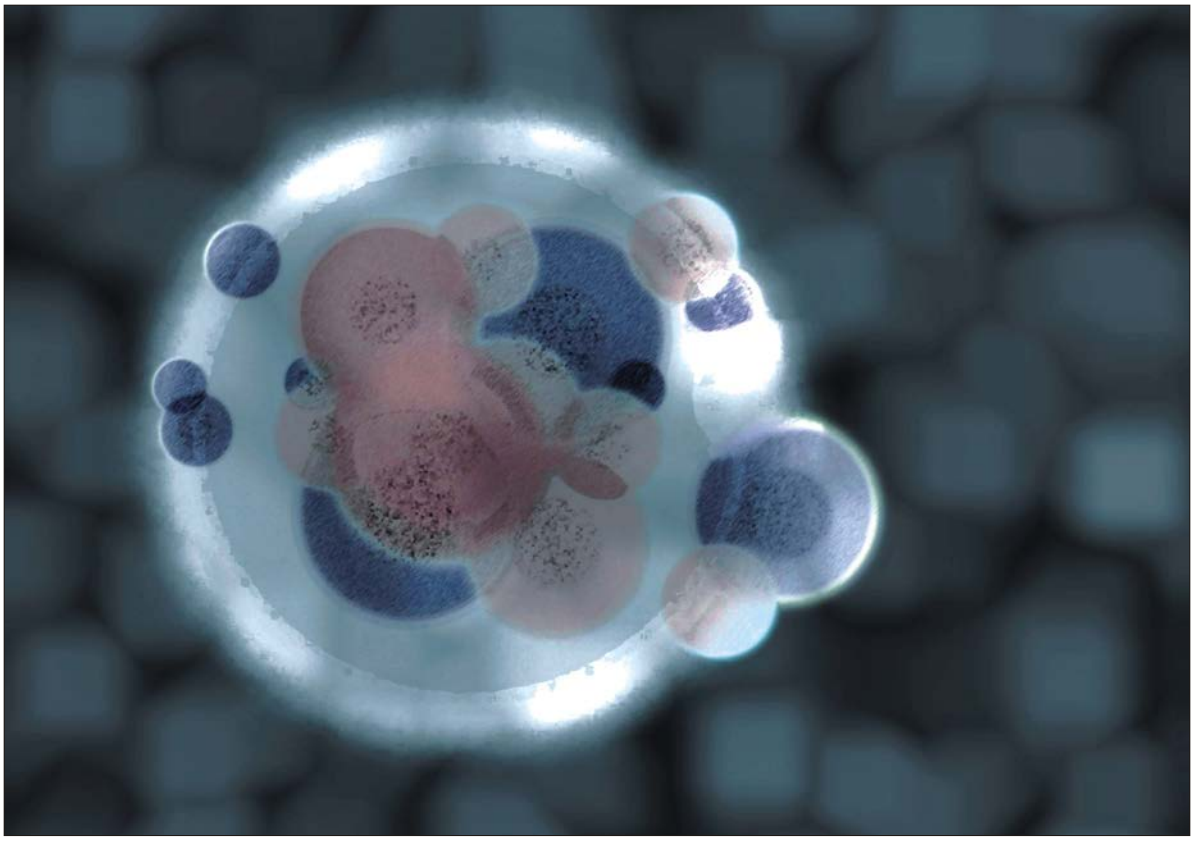

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Research Article
Nanocapsulation of Nitazoxanide in Solid Lipid Nanoparticles as a New Drug Delivery System and *in vitro* Release Study

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Abstract

Background: Colloidal drug delivery system, Solid Lipid Nanoparticles (SLNs), helps to increase the solubility of the drug and its oral bioavailability. The aim of this study was to prepare and characterize physiochemically nitazoxanide (NTX) encapsulated within SLN to evaluate their effectiveness as a new antiparasitic and antiprotozoal delivery system. **Materials and Methods:** Nitazoxanide-loaded SLN with the following compositions: NTX-SLN^a (1:5), NTX-SLN^b (1:10) and NTX-SLN^c (1:20) were prepared. The three formulations were physiochemically characterized by the particle size, polydispersity index, zeta potential, crystallinity and bonding characteristics. **Results:** The particle size of the NTX-loaded SLN obtained ranged from 228-448 nm. The NTX-loaded SLN particles were less ordered in crystalline structure than either bulk lipid or SLN. The NTX-loaded SLNa showed the highest Entrapment Efficiency (EE) (91.81%) and the highest Drug Loading (DL) (18.26%). *In vitro* drug release of NTX-loaded SLN in human plasma and phosphate buffer saline (pH = 7.4) showed that the amount of drug released approached 14.5 and 8.32%, respectively after 8 h of incubation. **Conclusions:** In conclusion, the study suggests that NTX-loaded SLN can be suitable to be used as a new antiparasitic and antiprotozoal drug delivery system.

Key words: Anti-parasite, anti-protozoa solid lipid nanoparticles, nitazoxanide

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nitazoxanide (NTX), as a new antiparasitic and antiprotozoal drug, is recommended in treatment of intestinal protozoan infections, including diarrhea caused by *Giardia lamblia* and *Cryptosporidium* in immune-related diseases such as AIDS or HIV infection[1] and also it had effects on *Entamoeba histolytica*, *Ascaris lumbricoides*, *Hymenolepis nana* and *Taenia solium* and *T. saginata*[2]. According to recent reports, NTX-loaded nanoparticles were prepared by emulsification-solvent diffusion employing poloxamer188 as a stabilizer used as a therapeutic agent in visceral leishmaniasis[3]. In mentioned study, size and morphology of nanoparticles were further evaluated using Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM) and also NTX content and Entrapment Efficiency (EE) were determined by a validated HPLC method. Uptake of nanoparticles *in vitro* was assessed in THP-1 cell line derived macrophages, using flow cytometry. It is reported that the antiprotozoal activity of nitazoxanide is through inhibition of pyruvate ferredoxin oxidoreductase (PFOR), an enzyme with critical role in anaerobic pathway of energy metabolism[2].

Nanoparticles have shown promise in increasing the bioavailability of some chemicals. Nanoparticles incorporated with kaempferol, a natural flavonoid and tested their efficacy in the inhibition of viability of cancerous and normal ovarian cells[4].

Chawla and Amiji[5] used nanoparticulate delivery systems in the form of nanospheres and nanoparticles, in 2003. The basis of this formulation is the attainment of adequate dose of the drug at the lesion site for a known period and the reduction of adverse effects on normal organs. Solid Lipid Nanoparticle (SLN) was recommended for drug delivery systems[6]. The main benefit of SLNs is their lipid matrix composition, which is physiologically tolerable and entails little acute or chronic toxicity. Additional advantages are their widespread application, the scalability of production without the need for organic solvents, their high bioavailability, their ability to protect drugs from degradation agents and their ability to control drug release[7,8].

A useful drug-delivery system should possess high capacity for incorporating drugs between fatty-acid chains or lipid layers or in crystal imperfections. Whether the drug is located within the core of the particles, in the shell, or as a molecular dispersion throughout the matrix depends on the drug-to-lipid ratio and the drug's solubility within the lipid[9]. The mode of drug incorporation influences the drug release, particle size and physical stability and modifying the lipid matrix, surfactant concentration and production parameters can affect the drug-release profile[10].

The aim of this study was to prepare a nitazoxanide-loaded SLN using homogenization for using in the cryptosporidiosis treatment. The authors characterized the nitazoxanide-loaded SLN and determined the optimum drug loading and *in vitro* release profile.

MATERIALS AND METHODS

Hydrogenated palm oil (Softisan 154 or S154) was a gift from Condea. Hydrogenated soybean lecithin (lipoid S100-3, containing 90% phosphatidylcholine, including 12-16% palmitic acid, 83-88% stearic acid, oleic acid and isomers and linoleic acid) was a gift from lipoid. Thimerosal, mercury ((o-carboxyphenyl)thio) ethyl sodium salt and sorbitol, (2S,3R,4R,5R)-hexane-1,2,3,4,5,6-hexol, were purchased from Sigma. Oleyl alcohol (octadecenol or cis-9-octadecen-1-ol) was purchased from Fluka. Ethanol and nitazoxanide, [2-[(5-nitro-1,3-thiazol-2-yl)carbamoyl]phenyl] ethanoate were obtained from sigma-aldrich. Double-distilled water was used in this experiment.

Preparation of nitazoxanide-loaded SLNs: Nitazoxanide-loaded SLN was prepared using the high-pressure homogenization technique[11]. A mixture of S154 and lipoid S100 at a ratio of 70:30 was ground in a ceramic crucible. The mixture was then heated to 65-70°C while being stirred with a PTFE coated magnet until a clear-yellowish Lipid Matrix (LM) solution was obtained. A solution containing 1 mL oleyl alcohol, 0.005 g thimerosal, 4.75 g sorbitol and 89.25 mL bidistilled water (all w/w) at the same temperature was added to 5 g of LM. A pre-emulsion of SLN was obtained using the homogenizer (Ultra Turrax, Ika, Staufen, Germany) at 13,000 rpm for 20 min and high-pressure homogenizer (EmulsiFlex-C50 CSA10, Avestin, Ottawa, Canada) at 1000 bar, 20 cycles and 60°C. The lipophilic drug Nitazoxanide was dissolved in ethanol and mixed with SLN pre-emulsion. This mixture was then incubated overnight at 50-60°C, stirred periodically with a PTFE coated magnet at 500 rpm and finally exposed to air to solidify. Three different types of NTX-loaded SLNs were prepared, which were of the following composition: 1 mg NTX incorporated in 5 mg SLN (NTX-SLN^a), 1 mg NTX incorporated in 10 mg SLN (NTX-SLN^b) and 1 mg NTX incorporated in 20 mg SLN (NTX-SLN^c).

Characterization of nitazoxanide-loaded SLN: The mean particle sizes (i.e., diameter±standard deviation) and size distribution (polydispersity index or PI) of SLNs and nitazoxanide-loaded SLNs were determined using a

high-performance particle sizer (Mal 1033452, Malvern Instruments, UK). Measurements were performed at 25°C in triplicate. Before measurement, each sample was dispersed in filtered bidistilled water by a sonic water bath. The zeta potential (i.e., electrophoretical movement) of the SLN and nitazoxanide-loaded SLN was then measured by an analyzer (Mal 1033452, Malvern Instruments, UK) in triplicate.

Differential scanning calorimetry: The melting points of the bulk lipid and SLN formulation were measured using Differential Scanning Calorimetry (DSC). The DSC analysis was performed using the Mettler Toledo DSC 823e (Switzerland) and thermograms were recorded in the 20-120°C temperature range with a heating rate of 5°C min^{-1}. An empty aluminum sample pan was used as a reference.

Fourier transforms infrared spectroscopy: Fourier transform infrared (FTIR) spectroscopy (Perkin Elmer, Spectrum 65, England) was used to characterize bonding characteristics of the NTX, SLN and NTX-SLN.

Entrapment efficiency and drug loading: A series of nitazoxanide concentrations ranging from 5000-80000 ppm was used to obtain a calibration curve (Y = 0.0783X-0.0011) with linear regression (r = 0.999). To determine Entrapment Efficiency (EE) and Drug Loading (DL), nitazoxanide-loaded SLNs were dispersed in ethanol. The dispersion was centrifuged at 20,000 g for 60 min to remove SLNs. The amount of drug in the supernatant that was not incorporated into the SLNs was analyzed by UV-Vis spectrophotometer (Cecil aquarius 900, UK). Absorbance of the drug was determined at 420 nm[12]. The EE and DL% were obtained[13] using the following Eq. 1 and 2:

$$DL\ (\%) = \frac{W\,\text{initial drug} - W\,\text{fee drug}}{W\,\text{lipid}} \quad (1)$$

$$EE\ (\%) = \frac{W\,\text{initial drug} - W\,\text{fee drug}}{W\,\text{initial drug}} \quad (2)$$

Nitazoxanide release from nanoparticles in human plasma: nitazoxanide release profile from SLN was assayed *in vitro*. Five similar batches of nitazoxanide-loaded SLNa containing 30 µg of nitazoxanide in 2 mL of human plasma were prepared. The experiment was conducted in room temperature with mechanical stirring. At predetermined intervals, each sample was centrifuged at 20,000 g for 60 min and the SLN removed. Plasma proteins in the supernatant were precipitated at a 1:1 ratio with trichloroacetic acid and centrifuged at 1500 g for 15 min. The amount of free drug in the supernatant that was neither incorporated into the SLNs nor linked to albumin was estimated by UV-Vis spectrophotometer (Cecil aquarius 900, UK). Free drug at concentrations ranging from 5000-80000 ppm in methanol (50%) was used to obtain the calibration curve.

Drug release at pH 7.4: Drug release in pH = 7.4 was assayed at 5 prefixed time intervals. For this purpose, 5 batches of nitazoxanide-loaded SLN[a] containing 30 µg of Nitazoxanide in 2 mL of phosphate buffer saline with pH = 7.4 were prepared and kept at room temperature under mechanical stirring. At suitable time intervals, samples were centrifuged at 20,000 g for 60 min at 4°C, to remove the SLNs and analyzed by UV-Vis spectrophotometer (Cecil aquarius 900, UK). Each experiment was carried out in triplicate.

Statistical analysis: The data obtained were subjected to statistical analysis. The differences in means among the groups were expressed as Mean±Standard Deviation. All the data were subjected to one-way analysis of variance (ANOVA) followed by *post hoc* multiple comparison and Duncan test after verification of the normal distribution of the data. The Statistical Package for the Social Sciences (SPSS)[14] version 16.0 was used to perform all statistical tests and p-value less than 0.05 were considered significant.

RESULTS

The authors used high-pressure homogenization to prepare SLN because the matrix lipid composed of palm oil (i.e., a triglyceride mixture of natural, hydrogenated and unbranched fatty-acid chains) was suitable for the incorporation of lipophilic drugs[10], such as nitazoxanide. Soy lecithin was the most useful surfactant in SLN dispersions. The SLN and nitazoxanide-loaded SLN were characterized *in vitro* for particle size, particle-size distribution and zeta potential (Table 1). The diameters of NTX-loaded SLN dispersions were

Table 1: Particle sizes, particle size distribution, specific surface area and zeta potential of NTX-loaded SLN formulations with different amounts of nitazoxanide

Formulation	Particle size (nm)	PI	Zeta potential (mv)
SLN	202±9.40	0.437	-97.0±37.1
NTX-SLN[a]	228±9.00	0.356	-71.7±47.3
NTX-SLN[b]	435±15.4	0.544	-65.5±47.1
NTX-SLN[c]	448±16.4	0.597	-107.0±56.3

NTX-SLN[a], NTX-SLN[b] and NTX-SLN[c] represent 1 mg NTX incorporated in 5, 10 and 20 mg SLN, respectively

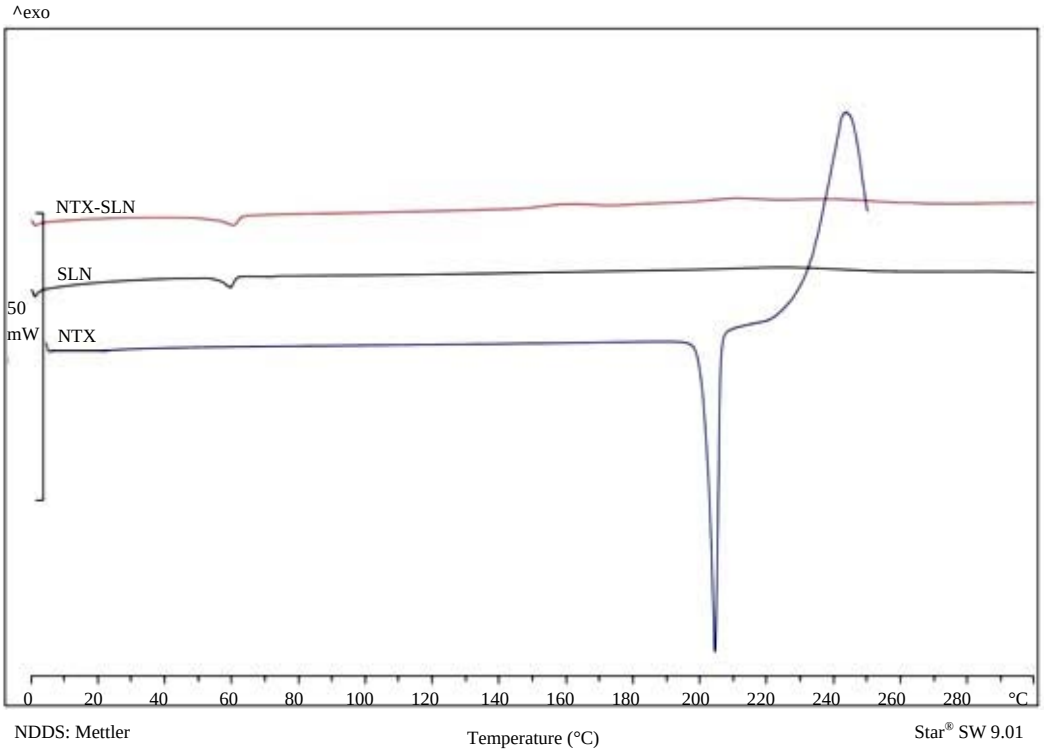

Fig. 1: DSC thermogram and melting point of bulk lipid, Solid Lipid nanoparticle (SLN), nitazoxanide (NTX) and NTX-loaded SLN

between 228.50±9 and 448±16.4 with acceptable particle size distribution (PI). The average sizes of the NTX-loaded SLN were larger than the free SLN. However, in the formulations, the particle size of NTX-loaded SLN was increased with concentration of the drug, although generally the size of the particles showed observable increase in following order: SLN^c>SLN^b>SLN^a. According to the Table 1 the surfaces of nitazoxanide-loaded SLN carried a negative charge.

The DSC thermogram and melting point of SLN, NTX and NTX-loaded SLN are shown in Fig. 1. The melting point of the drug-free SLN and free Nitazoxanide were 71.54 and 201.51°C, respectively. Incorporating Nitazoxanide into the SLN reduced its melting point to 60.28°C.

The FTIR spectra for drug-free SLN, NTX and NTX-loaded SLN are shown in Fig. 2. The specific peaks of SLN were in the following profile: at 3366, 3291.5, 2846.9 and 2918.6 cm^{-1} (relate to the stretching of O-H and C-H bonds) the peaks at 1470 and 1728.1 cm^{-1} (relate to the stretching of C=O and C=C bonds) and at 1079 cm^{-1} single bonds of C-C and C-O were appeared. As it is shown in Fig. 2, the specific peaks of NTX were 3358.73 cm^{-1} (stretching of N-H bond), 3087,94 cm^{-1} (stretching of C-H and O-H bonds), 1772.86 and 1662 cm^{-1} (stretching of C=O, C=C and C=N bonds and bending N-H bond) and 1473.35 and 1364.72 cm^{-1} (stretching and bending of C=O and C-N). The curve is presented in Fig. 2 also revealed a shift from 3358,73 cm^{-1} (related to NTX) to 3354.6 cm^{-1} (related to NTX-SLN) and wider spectra peak at 3354.6 cm^{-1}.

In the present study the three NTX-loaded SLNs ($NTX\text{-}SLN^a$, $NTX\text{-}SLN^b$ and $NTX\text{-}SLN^c$) were prepared and analyzed for Entrapment Efficiency (EE%) and drug loading (DL%). The results of EE (%), ($NTX\text{-}SLN^a$ 91.81±1.5, $NTX\text{-}SLN^b$ 74.54±1.06, $NTX\text{-}SLN^c$ 60.45±2.15) and DL (%), ($NTX\text{-}SLN^a$ 18.26±1.9, $NTX\text{-}SLN^b$ 7.45±1.1, $NTX\text{-}SLN^c$ 3.02±1.6) from fresh samples are as in Fig. 3 and 4, respectively.

The release profile of Nitazoxanide-loaded SLN^a in human plasma is shown in Fig. 5. The release rate remained low for the first 2 h, only increasing by near 1% for that period. Immediately after 2 h there was a sudden burst of drug release approaching 12.57% by 4 h' post-drug incorporation. After 4 h the release rate remained low by the last hour, increasing by 14.5% for that period. Figure 5 shows slowly release profile of NTX from $NTX\text{-}SLN^a$ in PBS solution (pH = 7.4). The NTX-loaded SLN^a showed a 4.7% release during 240 min and increasingly 8.32% release after 480 min.

DISCUSSION

Particle size is an important characteristic for pharmaceutical applications because it significantly affects *in vitro* and *in vivo* studies[15]. The result from this study is

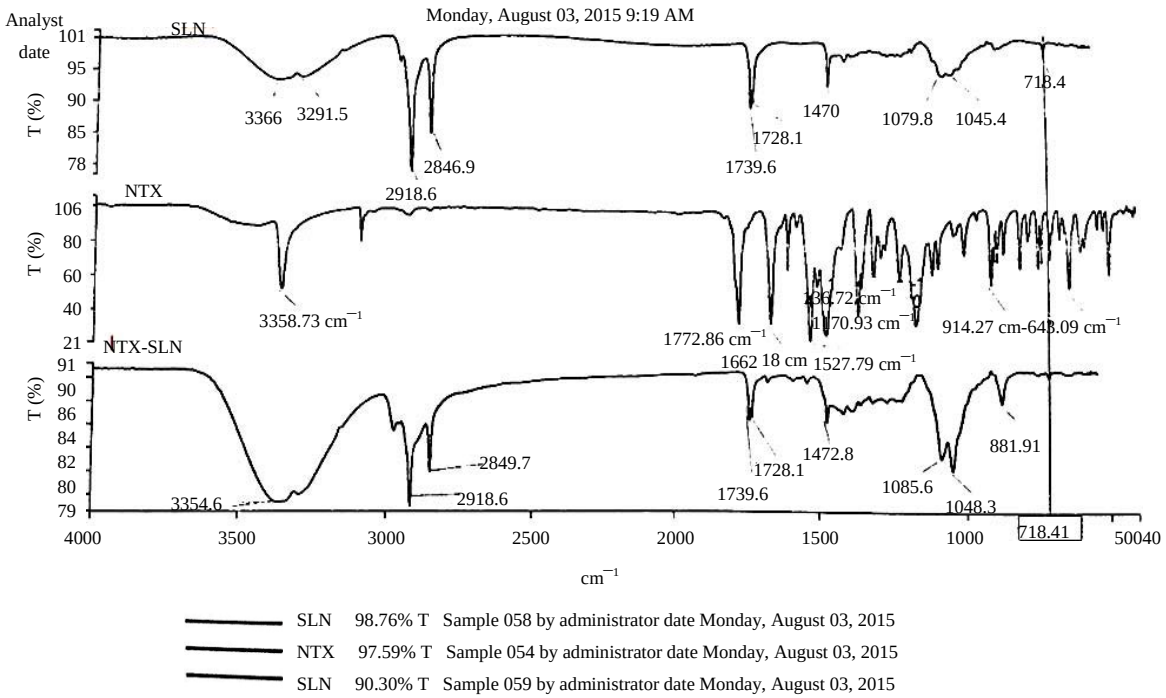

Fig. 2: FTIR spectra of SLN, NTX and NTX-SLN

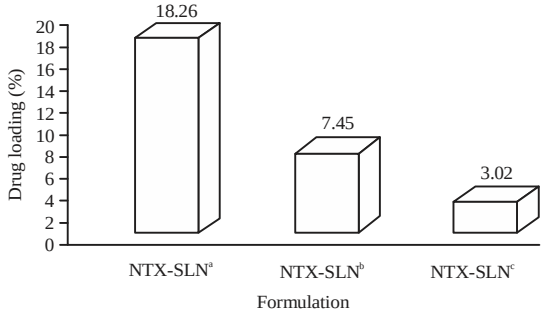

Fig. 3: Drug loading of nitazoxanide-loaded solid lipid nanoparticles. NTX-SLN[a], NTX-SLN[b] and NTX-SLN[c] represent 1 mg NTX incorporated in 5, 10 and 20 mg SLN, respectively. Means with superscripts a-c are significantly different ($p<0.05$), n = 3

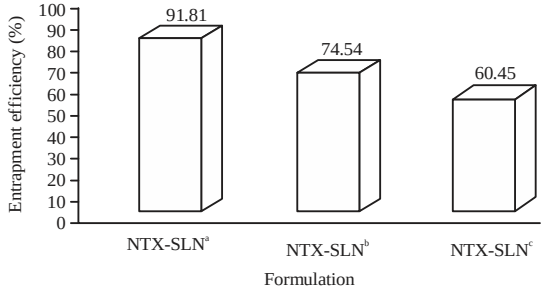

Fig. 4: Entrapment efficiency of nitazoxanide-loaded solid lipid nanoparticles. NTX-SLN[a], NTX-SLN[b] and NTX-SLN[c] represent 1 mg NTX incorporated in 5, 10 and 20 mg SLN, respectively. Means with superscripts a-c are significantly different ($p<0.05$), n = 3

in agreement with that from another study[16] using Ubidecarenone (Q_{10}) as a drug model, which showed increase in the size of the carrier nanoparticle, tripalmitin SLN. This may be assigned to the fact that drug is generally incorporated within the oily core and on the particle surface of the SLN. With NTX incorporation into SLN, the increase in size of particles suggests that the loaded NTX is either adsorbed to particle surface or entangled in aliphatic chains of triglycerides. According to Memisoglu-Bilensoy *et al.*[17] when the size of drug-loaded nanoparticulate system is decreased with respect to blank nanoparticles, the drug generally encapsulated in large amounts within the oily core of the nanoparticulate carrier and when the size increased, the drug probably is accommodating onto the particle surface. When nitazoxanide was incorporated into SLNs, the increase in particle size suggested that loaded nitazoxanide was either adsorbed onto the particle surface or entangled in the aliphatic chains of triglycerides. Drugs incorporated

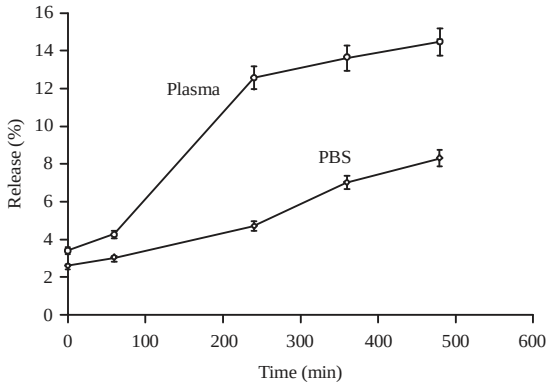

Fig. 5: Phosphate buffer (pH = 7.4) and human plasma concentration profiles of NTX-loaded SLN. Each point represents the Means±standard deviation, (n = 3)

nanoparticles have many useful effects such as increased bioavailability[18]. Incorporation of the poorly soluble lipophilic drugs such as NTX in particles of nanosize will improve its bioavailability.

Zeta potential is also an important factor when evaluating the stability of colloidal systems[19]. In the presence of the drug, it is probable some of the negative charges were neutralized by the complex formation. The positive charge also might be raised by the nitazoxanide amino group and by nitazoxanide localization on the surface of SLNs[13]. Therefore, in our study it seems that NTX is encapsulated in the oily core and some of the drug could also be located on the surface of the SLN.

With regard to the melting point and crystallization behavior of SLNs, incorporating nitazoxanide reduced the melting point of SLN from 71.54-60.28°C. Nitazoxanide thus is probably in an amorphous state and not crystalline. A substance with a less ordered crystal or amorphous state requires less energy to overcome lattice forces and perfect crystalline substances require high melting enthalpy[20]. The shape of the drug-free SLN and nitazoxanide DSC thermogram were a sharp trough, whereas, those of the nitazoxanide-loaded SLNs was broader indicating dispersion[11] and the amorphous state may contribute to drug loading capacity[20]. This study showed that the nitazoxanide was entrapped into the SLN during preparation and decrease in melting temperature could be attributed to the small particle size[21]. Since the thermal behavior of SLN is affected by thermodynamic factors, which depend on quality of the interaction between the components, the presence of guest molecules like NTX would alter the melting point[15]. According to Westesen *et al.*[9] and Radtke *et al.*[22], in drug-free and drug-loaded SLNs the less-ordered crystals and amorphous state will favor high-drug loading. Lipoid S100-3 used in this study is a purified soybean lecithin with >94% phosphatidylcholine (linolic acid, lineolic acid, oleic acid, palmitic acid and stearic acid) content[23]. Therefore, it is expected that the drug becomes incorporated into the crevices or in-between the fatty acid chains[15].

The results obtained from FTIR spectrum confirmed the DSC experiment. The peak at 3354.6 cm^{-1} related to NTX-loaded SLN was wider than corresponded peak in SLN is attributed to the participation of NTX in hydrogen bonding and -NH group interactions[24]. A shift from 3,358.73 cm^{-1} of NTX (N-H stretching) to lower peak at 3,354.6 cm^{-1} can be related to coordination binding between SLN and NTX through amide N-H bond[25]. This result proves successful loading of NTX in SLN and also indicates some interactions between SLN and NTX.

The EE of NTX-loaded SLNs was quite high (i.e., 91.81%). The palm oil used to prepare the SLN dispersion produced the highest entrapment efficiency. Triglycerides with various fatty acids offer relatively better drug solubilization[26]. Wong *et al.*[27] used doxorubicin, verapamil HCl, propranolol HCl and quinidine sulfate in SLNs stabilized with tween 80 and showed that increasing drug concentration led to a significant increase ($p<0.05$) in DL and significant decrease ($p<0.05$) in EE. These effects were a result of reduced SLN dispersions and high solubility of the drug in high lipid concentrations. Other researchers showed a positive correlation between particle size and drug loading[28,29].

High entrapment efficiency is one of the important benefits of using SLNs as carriers[30,31]. The factors that affected loading capacity of drug in nanoparticles are solubility of drug in melted lipid, miscibility of drug and lipid melt, chemical and physical structure of solid lipid matrix and polymorphic state of lipid material. Therefore, high solubility of the drug in the lipid matrix led to adequate loading capacity[9]. The chemical structure and crystalline state of the lipid has an important role in expulsion or incorporation of the drug into the carrier system[20]. A complete lattice is formed by high crystalline state of the lipid would lead to drug expulsion. Therefore, lattice imperfection in the lipid structure of the SLN may provide drugs accommodation. The result of our study showed that as the percentage of SLN increased entrapment efficiency was decreased (ranging from 91.81-60.45%). This may be due to drug expulsion through SLN modification as lipid and surfactant increased.

Among the factors that support fast drug release from SLNs are a large surface area, small molecular size, low matrix viscosity and short diffusion distance of the drug[32]. In the authors' study, however, the release of the drug from SLN, which contains a lipid matrix, was retarded. Characterization

of NTX-loaded SLNs suggested that NTX was either encapsulated within the matrix and membrane of palm oil or entrapped in aliphatic chains. This situation could explain the burst release of the drug from SLNs after 2 h. Considering that SLN is solid at room temperature and that the incorporated drug is released relatively slowly, SLNs have potential as a sustained-release drug carrier. According to Fig. 5 release of NTX from NTX-SLNa in PBS (3%) is considerably lower than plasma (4.27%) within 2 h. It has been suggested this typical kinetics of drug release is due to enzymatic reactions that are involved in degradation of SLN causing drug solubilization in plasma[13-33]. In the present study the profile of drug release in pH = 7.4 may be due to low solubility of NTX in PBS solution and the burst drug release in human plasma is more likely a consequence of rapid surface desorption drug molecules from a high specific surface area provided by particles at nanoscale.

CONCLUSION

This study presented a study on the optimization of parameters for preparation of nitazoxanide (NTX) incorporation into SLN. Of the three types of NTX-loaded SLN studied, the NTX:SLN ratio of 1:5 showed the highest drug loading and entrapment efficiency. Characterization of NTX-loaded SLN indicated that the drug is either encapsulated within the matrix and membrane of palm oil or entrapped in aliphatic chains. In conclusion, SLN can serve as a good drug carrier for sustained-released of NTX.

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REFERENCES

1. Aktar, F., M.R. Kuddus, A. Hossen, M.K. Hossain and M.A. Rashid, 2012. Development and validation of a simple and rapid UV spectrophotometric method for assay of nitazoxanide in pharmaceutical dosage forms. Bangladesh Pharm. J., 15: 47-51.
2. Jadhav, A.S., D.B. Pathare and M.S. Shingare, 2007. A validated stability indicating RP-LC method for nitazoxanide, a new antiparasitic compound. Chromatographia, Vol. 66. 10.1365/s10337-007-0386-4.
3. Pandya, S., L. Dasari, K. Mitra, R. Singh and A. Misra, 2015. Nitazoxanide-loaded PLGA nanoparticles for therapeutic intervention in visceral leishmaniasis. Proceedings of the 50th American Association Pharmaceutical Scientists Conference, June 8-10, 2015, San Francisco.
4. Luo, H., B. Jiang, B. Li, Z. Li, B.H. Jiang and Y.C. Chen, 2012. Kaempferol nanoparticles achieve strong and selective inhibition of ovarian cancer cell viability. Int. J. Nanomed., 7: 3951-3959.
5. Chawla, J.S. and M.M. Amiji, 2002. Biodegradable poly(ε-caprolactone) nanoparticles for tumor-targeted delivery of tamoxifen. Int. J. Pharmaceut., 249: 127-138.
6. Fontana, G., L. Maniscalco, D. Schillaci, G. Cavallaro and G. Gimmona, 2005. Solid lipid nanoparticles containing tamoxifen characterization and *in vitro* antitumoral activity. Drug Deliv., 12: 385-392.
7. Jores, K., A. Haberland, S. Wartewig, K. Mader and W. Mehnert, 2005. Solid Lipid Nanoparticles (SLN) and oil-loaded SLN studied by spectrofluorometry and raman spectroscopy. Pharm. Res., 22: 1887-1897.
8. Yuan, H., L.F. Huang, Y.Z. Du, X.Y. Ying, J. You, F.Q. Hu and S. Zeng, 2008. Solid lipid nanoparticles prepared by solvent diffusion method in a nanoreactor system. Colloids Surfaces B: Biointerf., 61: 132-137.
9. Westesen, K., H. Bunjes and M.H.J. Koch, 1997. Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential. J. Control Release, 48: 223-236.
10. Muhlen, A.Z., C. Schwarz and W. Mehnert, 1998. Solid Lipid Nanoparticles (SLN) for controlled drug delivery-drug release and release mechanism. Eur. J. Pharm. Biopharm., 45: 149-155.
11. Schubert, M.A. and C.C. Muller-Goymann, 2005. Characterisation of surface-modified solid lipid nanoparticles (SLN): Influence of lecithin and nonionic emulsifier. Eur. J. Pharm. Biopharm., 61: 77-86.
12. El-Desoky, H.S., M.M. Ghoneim and M.M. Abdel-Galeil, 2009. A direct spectrophotometric method for determination of antiparasitic drug nitazoxanide in a bulk form and pharmaceutical formulation. Chemia Analityczna, Vol. 54, No. 5.
13. Luo, Y.F., D.W. Chen, L.X. Ren, X.L. Zhao and J. Qin, 2006. Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability. J. Control Release, 114: 53-59.
14. SPSS., 2006. Statistical Package for the Social Science. Version 16.0 SPSS Inc., Chicago, USA.
15. Attama, A.A. and C.C. Muller-Goymann, 2007. Investigation of surface-modified solid lipid nanocontainers formulated with a heterolipid-templated homolipid. Int. J. Pharm., 334: 179-189.

16. Bunjes, H., M. Drechsler, M.H.J. Koch and K. Westesen, 2001. Incorporation of the model drug ubidecarenone into solid lipid nanoparticles. Pharmaceut. Res., 18: 287-293.
17. Memisoglu-Bilensoy, E., I. Vural, A. Bochot, J.M. Renoir, D. Ducheneb and A.A. Hıncal, 2005. Tamoxifen citrate loaded amphiphilic β-cyclodextrin nanoparticles: *In vitro* characterization and cytotoxicity. J. Controlled Release, 104: 489-496.
18. Liversidge, G.C., 1996. Drug nanocrystals for improved drug delivery. Int. Symp. Controlled Release Bioact. Mater., 23: 74-81.
19. Mountasser, I., H. Fessi and A.W. Coleman, 2002. Atomic force microscopy imaging of novel type of polymeric colloidal nanostructures. Eur. J. Pharm. Biopharm., 54: 281-284.
20. Hou, D.Z., C.S. Xie, K.J. Huang and C.H. Zhu, 2003. The production and characteristics of Solid Lipid Nanoparticles (SLNs). Biomaterials, 24: 1781-1785.
21. Siekmann, B. and K. Westesen, 1994. Thermoanalysis of the recrystallization process of melt-homogenized glyceride nanoparticles. Colloids Surfaces B: Biointerf., 3: 159-175.
22. Radtke, M., E.B. Souto and R.H. Muller, 2005. Nanostructured lipid carriers: A novel generation of solid lipid drug carriers. Pharmaceut. Technol. Eur., 17: 45-50.
23. Stuchlik, M. and S. Zak, 2001. Lipid-based vehicle for oral drug delivery. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub., 145: 17-26.
24. Bahreini, E., K. Aghaiypour, R. Abbasalipourkabir, A.R. Mokarram, M.T. Goodarzi and M. Saidijam, 2014. Preparation and nanoencapsulation of L-asparaginase II in chitosan-tripolyphosphate nanoparticles and *in vitro* release study. Nanoscale Res. Lett., Vol. 9. 10.1186/1556-276X-9-340
25. Sarwade, S.S., W.N. Jadhav, B.C. Khade and D. Tayde, 2015. A mononuclear complex of silver (I) with nitazoxanide. Der Chemica Sinica, 6: 1-4.
26. Reddy, L.H., K. Vivek, N. Bakshi and R.S.R. Murthy, 2006. Tamoxifen citrate loaded solid lipid nanoparticles (SLN^{TM}): Preparation, characterization, *in vitro* drug release and pharmacokinetic evaluation. Pharmaceut. Dev. Technol., 11: 167-177.
27. Wong, H.L., R. Bendayan, A.M. Rauth and X.Y. Wu, 2004. Development of solid lipid nanoparticles containing ionically complexed chemotherapeutic drugs and chemosensitizers. J. Pharmaceut. Sci., 93: 1993-2008.
28. Yuan, H., J. Miao, Y.Z. Du, J. You, F.Q. Hu and Z. Su, 2008. Cellular uptake of solid lipid nanoparticles and cytotoxicity of encapsulated paclitaxel in A549 cancer cells. Int. J. Pharmaceut., 348: 137-145.
29. Maschke, A., C. Becker, D. Eyrich, J. Kiermaier, T. Blunka and A. Gopferich, 2007. Development of a spray congealing process for the preparation of insulin-loaded lipid microparticles and characterization thereof. Eur. J. Pharmaceut. Biopharmaceut., 65: 175-187.
30. Jenning, V., A.F. Thunemann and S.H. Gohla, 2000. Characterisation of a novel solid lipid nanoparticle carrier system based on binary mixtures of liquid and solid lipids. Int. J. Pharm., 199: 167-177.
31. Mueller, R.H., R. Dobrucki and A. Radomska, 1999. Solid lipid nanoparticles as a new formulation with retinol. Acta Poloniae Pharmaceut. Drug Res., 56: 117-120.
32. Wissing, S.A., O. Kayser and R.H. Muller, 2004. Solid lipid nanoparticles for parenteral drug delivery. Adv. Drug Deliv. Rev., 56: 1257-1272.
33. Schwarz, C., 1999. Solid Lipid Nanoparticles (SLN) for controlled drug delivery II. Drug incorporation and physicochemical characterization. J. Microencapsulation: Micro Nano Carriers, 16: 205-213.