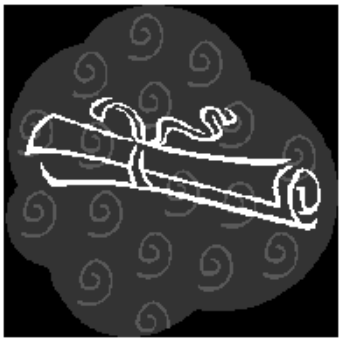
Summary & Conclusion



Chapter 8

Oral administration is the most convenient and preferred mode of drug administration in conscious and co-operating patients, due to convenience, possibility of self-administration, improved patient safety and better compliance. More than 60% of drugs are marketed in the form of oral products. However, more than 40% new chemical entities exhibit poor oral bioavailability due to undesired physicochemical and pharmacokinetic properties.

P-glycoprotein (P-gp) efflux and first-pass metabolism by cytochrome P450 (CYP3A) play critical roles in limiting the absorption and bioavailability of orally administered drugs. Low bioavailability is often associated with oral dosage forms of Biopharmaceutics Classification System (BCS) Class 2 and Class 4 drugs. The poor solubility of these drugs makes their oral delivery very challenging. There are diverse drug delivery approaches which have been explored to improve the aqueous solubility, poor dissolution rate and bioavailability of insoluble drugs. **Lipid based drug delivery system** is one of the potential technique which comprises Self Emulsifying Drug Delivery Systems (SEDDS), Nanoemulsion, Microemulsion, Solid lipid nanoparticles, mixed micelles, liposomes etc. The most important application of lipid based excipients is in bioavailability enhancement via increasing solubility, targeting lymphatic transport, and modulation of enterocyte-based drug transport and disposition. The lipid component enhances the extent of lymphatic transport and increases bioavailability directly or indirectly via reduction of first pass metabolism. Moreover, presence of lipid in the GIT stimulates an increase in Bile Salts (BS) and endogenous biliary lipids like Phospholipid (PL) and cholesterol (CL) leading to formation of BS/PL/CL intestinal mixed micelles and increases solubilization capacity of the GIT. Some surfactants, which are generally a part of these systems like Polysorbates, Cremophor etc., have the ability to minimize the activity of intestinal efflux transporters like p-glycoprotein (P-gp) efflux pump.

Nisoldipine (NISO) and **Dabigatran Etexilate (DE)** are BCS class 2 drugs with absolute bioavailability of about 5% and 6.5 % respectively. The low bioavailability of NISO is due, in part, to pre-systemic metabolism in the gut wall while Pg-p efflux and acid hydrolytic degradation are the main reasons for the low bioavailability of DE. Till date neither NISO nor DE is available in the form of self-micro emulsifying drug delivery system **(SMEDDS) or Nanoemulsion (NE)** in the market. Hence, we formulated and evaluated SMEDDS and

NE, by using P-gp modulator and CYP3 inhibitor type excipients to tackle these issues and elevate the systemic availability of NISO and DE.

8.1. Aim of present study

The present research work was aimed at development of lipid based drug delivery systems such as SMEDDS and NE for oral administration of poorly water soluble drugs- NISO and DE with the objective of enhancing their bioavailability and thereby reducing their therapeutic dose and associated side effects.

8.2. Preformulation

Authentication of both the drugs viz. NISO and DE was done by identification tests like UV Visible spectroscopy, Fourier Transform Infrared spectroscopy (FTIR), Differential scanning calorimetry (DSC) and Melting point determination. For UV spectra, the solutions containing 10µg/mL NISO and DE in 0.1N HCl + 0.5% SLS and 0.01N HCl respectively were scanned and wavelength maxima (λ_{max}) was found to be 238 nm and 325 nm respectively. The observed and reference values of the stretching and bending vibrations in the IR spectra of DE and NISO along with their corresponding functional groups at their respective wave numbers were found similar. DSC thermogram showed sharp endothermic peak at 149.13^{0}C and 127.43^{0}C for NISO and DE respectively. The melting points of NISO and DE were observed to be in the range of 150°C to 151°C (Reported melting point**:**150°C to 155°C) and 127-128^{0}C (Reported melting point**:**128°C to 129°C) respectively. Thus, the drugs were authenticated.

Results of drug-excipients compatibility study using physical observations and FTIR study showed no interactions between the drug and excipients used in the present study.

8.3. Analytical Method Development

8.3.1 UV spectrophotometric methods

UV spectrophotometric methods for estimation of NISO and DE were successfully developed and validated in different media viz. 0.1N HCl + 0.5% SLS, pH 6.8 phosphate buffer + 0.5% SLS, pH 7.4 phosphate buffer + 0.5% SLS, methanol and 0.01N hydrochloric acid, phosphate buffer pH 6.8, phosphate buffer pH 7.4, methanol respectively. DE showed characteristic UV spectra when scanned in the ultraviolet range between 200 nm and 400

nm. The UV-Visible spectrum showed absorption maxima at 325nm, 316nm, 316nm and 315nm for 0.01N HCl, pH 6.8 phosphate buffer, pH 7.4 phosphate buffer and methanol respectively while NISO showed absorption maxima at 238nm, 236nm, 236nm and 236nm in 0.01N hydrochloric acid, phosphate buffer pH 6.8, phosphate buffer pH 7.4 and methanol respectively. Regression analysis was performed on the experimental data. Calibration data showed linearity with a strong linear correlation for all four media for both DE and NISO. The analytical methods for estimation of DE as well as NISO were found to be precise, accurate and stable. The developed UV methods for both the drugs were found to be specific and showed no interference with other formulation components.

8.3.2. HPLC Methods

HPLC analytical method was developed and validated to estimate NISO and DE during cell line studies for the developed formulations (SMEDDS and Nanoemulsion). The chromatographic separation of NISO and DE was carried out on a Synergi^{TM} 4 µm Hydro-RP 80 Å, LC Column (150 x 4.6mm) (Phenomenex, Torrance, USA) column using a reported (slightly modified) mobile phase. In case of NISO, the mobile phase consisted of ACN: Phosphate buffer, pH 3.0. Peak area was recorded using UV detector at 235nm detection wavelength, with the flow rate of 1 mL/min of mobile phase for a run time of 8 min and retention time was found to be 4.0 min for NISO. In case of DE, separation was attained using a mobile phase consisting of acetonitrile and phosphate buffer (pH 5.8) in the ratio of 60:40 (% v/v), pumped at a flow rate of 1 mL/ min. The retention time was found to be 4.60 min at 230 nm of detection for a run time of 10 min. A strong linear correlation with an R^2 = 1 was observed between the concentration of the drugs and peak area over a concentration range of 1 to 10 µg/mL.

Quantitative estimation of NISO and DE in plasma was based on reverse phase chromatography using C18 column and 2cm guard column. Protein precipitation method was employed to separate the drug from plasma. A strong linear correlation with an R^2 = 0.999 was observed between the concentration of the drugs and peak area obtained upon chromatographic extraction over a concentration range of 0.05 to 2.0 µg/mL in plasma. The methods were found to measure the concentrations with significant precision and accuracy.

8.4. Formulation development of SMEDDS for DE and NISO

8.4.1. Preliminary screening of oil, surfactant and cosurfactant

Selection of oil phase was done on the basis of solubility study in different oils while Surfactant and cosurfactant were selected on the basis of their ability to emulsify the oil phase i.e emulsification test and also by solubility studies. Pseudo ternary phase diagrams were developed using the aqueous titration method. Ternary mixtures with varying ratios of surfactant, co-surfactant and oil were prepared. Ratio of 2:1 S_{mix} was selected for DE-SMEDDS while maximum microemulsion region was observed at 1:1 ratio of S_{mix} for NISO SMEDDS.

From solubility studies, Capmul MCM C8 was selected as oil phase in case of DE SMEDDS and Peceol for NISO SMEDDS; from emulsification test, Cremophor EL was selected as surfactant and Transcutol HP as cosurfactant for both DE and NISO SMEDDS.

8.4.2. Optimization using Statistical designs

A three-factor, two-level D-optimal statistical experimental design was used to optimize the formulation variables of DE-SMEDDS. The globule size and % Transmittance were chosen as responses. The GS and %T values for the 16 batches showed a wide variation from 70.2 to 772.1 nm and 30.2 to 99.6%, respectively. The two-dimensional contour plots and three-dimensional response surface plots were plotted.

In case of NISO SMEDDS, factorial design for two factors at three levels was selected to investigate the effect of concentration of oil and mass ratio of surfactant to co-surfactant (Km) on Globule size and % Transmittance. Thirteen different batches were prepared using 3^2 factorial design varying the two independent variables. GS and %T showed a wide variation from 16.7 to 396.1 nm and 52.7 to 99.4 % respectively. The relationship among variables and responses was exemplified by contour plots and response plots for both the responses.

From the preliminary and optimization studies, the final composition for DE and NISO SMEDDS comprised of DE (3.08%), Capmul MCM C8 (9.69%), Cremophor EL (58.15%) and Transcutol HP (29.08%) while NISO SMEDDS comprised of NISO (2.34%), Peceol (9.77%), Cremophor EL (43.95%) and Transcutol HP (43.95%).

8.5.Characterization of optimized DE and NISO-SMEDDS

8.5.1. Robustness to dilution test

All the diluted batches of both the formulations exhibited a globule size of <100 nm and transmittance above 90% irrespective of type and volume of dilution medium. Therefore, the optimized SMEDDS formulations were considered to be robust against dilution as neither precipitation of the drug nor any phase separation was observed even after 24 h.

8.5.2. Thermodynamic stability studies

Neither phase separation nor any precipitation was observed upon different stress tests like heating cooling, centrifugation and freeze-thaw cycle, indicating the stability of the microemulsion thus formed after self-emulsification of both the formulated SMEDDS. Hence, the optimized SMEDDS formulations of DE and NISO were found to be thermodynamically stable in these conditions.

8.5.3. Globule size, polydispersity index (PDI) and zeta-potential

The optimized DE SMEDDS and NISO SMEDDS had globule size of 73.24± 1.10 nm and 16.78± 0.97 respectively. PDI for DE SMEDDS and NISO SMEDDS was 0.085± 0.008 and 0.121± 0.024 respectively. This revealed that both the formulations exhibited uniform globule size distribution in nano range. Zeta potential for the optimized batch of DE-SMEDDS and NISO SMEDDS were found to be -22.4± 0.1mV and -28.6± 1.3mV respectively. The high negative values of the zeta potential indicated that the strong electrostatic repulsion between the globules will prevent their aggregation and thereby stabilize the nanoemulsions.

8.5.4. % Transmittance

The optimized batch of DE SMEDDS and NISO SMEDDS showed % transmittance closer to 100% upon dilution with different media and at different dilution factors indicating isotropic nature and good microemulsification.

8.5.5. Cloud point measurement

The cloud points for DE SMEDDS and NISO SMEDDS were 77.5± 2.9 and 71.4± 2.2 °C respectively which were much higher than body temperature, indicating that they will form stable microemulsion at physiological temperature i.e. *in vivo* and during storage without

any phase separation. Additionally, this also implied good thermal stability of the prepared optimized SMEDDS formulation.

8.5.6. Viscosity

The viscosity of the DE SMEDDS and NISO SMEDDS at 25°C were found to be 124.80± 4.01 and 80.68± 3.44 cps respectively. As the value of viscosity for both the formulations was less than 10,000 cps, it implied that the developed SMEDDS can be filled in capsule shells by commercial liquid filling equipments.

8.5.7. Self emulsification time and precipitation assessment

The time of emulsification of DE SMEDDS in 0.01 N HCl and NISO SMEDDS in 0.1N HCl +0.5% HCl at 37.5°C was found to be 26.0± 2.0 and 19.0± 2.0 sec respectively which indicated the spontaneity of emulsification of the prepared SMEDDS. Moreover, the resultant microemulsions appeared to be clear (transparent or isotropically clear) which indicated desirable emulsification efficiency owing to complete miscibility of lipids in the aqueous phase by micellar solubilization.

8.5.8. Morphological examination using Transmission Electron Microscopy (TEM)

The globule size were found to be in the range of 45-65nm for DE SMEDDS and 15-35 nm for NISO SMEDDS along with uniform size distribution.

8.5.9. Drug content

The drug content of the optimized batch of DE SMEDDS and NISO SMEDDS were found to be 97.78 ± 2.02 and 98.21± 1.68%, indicating uniform dispersion of drug and high entrapment in the oil phase in both the formulations.

8.5.10. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of both the optimized formulation of DE and NISO SMEDDS showed all characteristic peaks of individual pure drugs indicating absence of any form of chemical interaction (incompatibility) with the respective formulation excipients.

8.5.11. Drug release studies

8.5.11.1. *In vitro* dissolution study of DE SMEDDS

The *in vitro* release studies showed increase in drug release for DE SMEDDS (98.74± 3.72% and 70.42± 2.93%) as compared to plain drug suspension (39.86± 2.41% and 7.47± 2.84%) in 0.01N HCl and pH 6.8 phosphate buffer respectively after 60 min.

8.5.11.2. *In vitro* dissolution study of NISO SMEDDS

The *in vitro* release studies showed increase in drug release for NISO SMEDDS (98.90± 2.01% and 97.41± 1.71%) as compared to plain drug suspension (11.43± 3.22% and 10.11± 2.23%) in 0.1N HCl+0.5% SLS and pH 6.8 phosphate buffer + 0.5% SLS respectively after 60 min.

8.5.11.3. *In vitro* diffusion study of DE SMEDDS

Higher amount of the drug was diffused at 300 min from the DE SMEDDS (98.05± 2.09% and 68.84± 2.71%) as compared to the plain drug suspension (26.68± 2.24% and 11.33± 1.28%) in 0.01N HCl and in pH 6.8 phosphate buffer respectively.

8.5.11.4. *In vitro* diffusion study of NISO SMEDDS

The *in vitro* diffusion study showed significant increase in drug release from NE formulations (97.71± 1.30% and 98.84± 2.44%) as compared to plain drug suspension (26.62± 2.91% and 27.54± 3.21%) in 0.1N HCl + 0.5% SLS and pH 6.8 Phosphate buffer + 0.5% SLS respectively.

8.5.11.5. *Ex vivo* release study of DE SMEDDS

The amount of the drug diffused through rat stomach increased when it was given in the form of SMEDDS (98.63± 3.21%) as compared to plain drug suspension (31.38± 2.74%). Intestinal diffusion was relatively slower than stomach i.e. 69.34 ± 2.76% drug was diffused from the DE NE and 10.65± 1.87 % from plain drug suspension which could be attributed to the higher solubility of DE (weak base) in acidic conditions.

8.5.11.6. *Ex vivo* release study of NISO SMEDDS

The drug diffusion from stomach (96.23± 5.87%) as well as intestine (95.14± 4.93%) was significantly higher for the NISO SMEDDS as compared to the plain drug suspension in stomach (14.54± 2.42%) and intestine (11.25± 2.11%). High surface area due to nanosized globules and the lipophilic nature of the formulation enhanced the permeation across the biological membrane.

8.6. Formulation development of NE for DE and NISO

The nanoemulsions prepared in the present study were oil-in-water (O/W) nanoemulsions.

8.6.1. Preliminary screening of oil, surfactant and cosurfactant

Qualitatively, similar oil, surfactant and cosurfactant was used for DE and NISO NE as that of DE and NISO SMEDDS.

8.6.2. Fabrication of DE NE and NISO NE

NE was prepared in two stages- preparation of pre-emulsion followed by fine nanoemulsion. To prepare pre-emulsion, high speed homogenizer was used for both DE and NISO NE.

DE NE: For preparation of oil phase, the required quantity of lipophilic surfactant (Cremophor EL) and drug (DE) were dissolved in oil (Capmul MCM C8) by continuous stirring for 5 min using magnetic stirrer followed by bath sonication for 1 min. The aqueous phase was prepared by dissolving hydrophilic surfactant (Transcutol HP) in required quantity of distilled water under stirring on magnetic stirrer for 5min. To prepare pre-emulsion, the oil phase was added drop wise to the aqueous phase using Ultra-turrax at 10000 rpm for 10 min.

Oil in water nanoemulsion loaded with DE was then prepared by high energy emulsification technique by passing through high pressure homogenizer at 10000 psi pressure for 3 cycles. The vessel used to collect homogenized nanoemulsion was kept in ice-water bath to avoid overheating during run. The o/w nanoemulsion thus formed was transferred to clean transparent glass vial, packed and used for further evaluation. The process and formulation parameters were optimized by full factorial design.

NISO NE: Similar procedure was used to prepare pre-emulsion of NISO except Peceol as oil and NISO as drug. The pre- emulsion was then ultra-sonicated using probe sonication for 9 min at 60% amplitude and 0.6 duty cycles to obtain the nanoemulsion. The o/w nanoemulsion thus formed was transferred to clean amber coloured glass bottle, packed and subjected to characterization. The process and formulation parameters were optimized by full factorial design.

8.6.3. Optimization of pre-emulsion using high speed homogenizer

Formulation components and process parameters for the preparation of pre-emulsion were optimized. For formulation components, S_{mix} concentrations of 10% w/v, 15 % w/v and 20% w/v were tried for both DE and NISO NE. The preparations were evaluated on the basis of physical stability of NE (phase separation and appearance) and globule size over a period of 15 days. For process parameters, effect of homogenization speed and homogenization time were studied on globule size and PDI for both the formulations.

For DE NE, 15% w/v of S_{mix} concentration and 20% w/v of S_{mix} concentration for NISO NE were finalised for the fabrication of pre-emulsion of DE and NE. Homogenization speed of 10000 RPM was considered suitable for obtaining pre-emulsion with desired globule size and uniform PDI. Homogenization time of 10 minutes was considered suitable for obtaining pre-emulsion of both DE and NISO NE.

8.6.4. Optimization of DE NE and NISO NE by full factorial design

Homogenization pressure (psi) and number of homogenization cycles were taken as independent variables in case of DE NE and probe sonication time (min) and amplitude (%) were taken as variables tin case of optimization of NISO NE using 3^2 full factorial design.

Globule size and PDI were the responses selected for the optimization of both DE NE and NISO NE. Thirteen batches of DE NE were prepared varying independent variables and responses were recorded. The GS and PDI values for the 13 batches showed a wide variation from 71.65 to 146.10 nm and 0.109 to 0.486 respectively. In case of NISO NE- Globule size and PDI showed a wide variation from 62.35 to 187.65 nm and 0.108 to 0.636 respectively. The relationship between independent and dependent variables were explicated using contour and response surface plots for both DE and NISO NE's. Check point batches were prepared and the observed value of GS and PDI were found to be in good agreement with predicted values. Desirability plot showed value of 0.976 which is near to 1 which indicated accuracy and suitability of predicted desirability for responses.

From the preliminary and optimization studies, the final composition for DE NE comprised of DE (2.0%), Capmul MCM C8 (10.00%), Cremophor EL (10.00%), Transcutol HP (5.00%), and Water (73.00%) while NISO NE comprised of NISO (1.00%), Peceol (10.00%), Cremophor EL (10.00%), Transcutol HP (10.00%) and Water (69.00%).

8.7. Characterization of optimized DE and NISO NE

8.7.1. Globule size, poly dispersity index (PDI)

The optimized DE NE and NISO NE had globule size of 71.65 ± 1.02 nm and 62.35 ± 2.55 nm respectively. PDI for DE NE and NISO NE was 0.109 ± 0.02 and 0.108 ± 0.01 respectively. This revealed that both the formulations exhibited uniform globule size distribution in nano range.

8.7.2. Measurement of Zeta potential

Zeta potential of DE NE and NISO NE were found to be -19.6 ± 2.1mV and -26.2 ± 3.6mV respectively, indicating strong electrostatic repulsion between the globules which will prevent their aggregation and stabilize the nanoemulsions.

8.7.3. Drug Content

The drug content of DE NE and NISO NE were found to be 98.04± 2.57% and 98.87± 2.12% respectively suggesting uniform distribution of drug in the internal phase of both the nanoemulsions.

8.7.4. pH

The pH of DE NE and Niso NE was found to be 6.2± 0.3 and 6.6± 0.2 respectively demonstrating aptness for oral administration.

8.7.5. Morphological examination using Transmission Electron Microscopy (TEM)

The globules were discrete and spherical with no signs of coalescence and had diameter ranging from 45 to 75 nm and 60 to 90 nm for DE NE and NISO NE respectively.

8.7.6. Conductivity

The conductivity of DE NE and NISO NE was 156.36 and 161.54 μS/cm respectively. Oil-in-water (O/W) NE's are highly conducting since water is in the external phase.

8.7.7. Viscosity

The viscosity of the NISO-NE and DE-NE were found to be 23.69± 1.84 cps and 12.02± 1.01 cps respectively.

8.7.8. Thermodynamic stability assessment

The optimized formulations of the NISO-NE and DE-NE were found to be stable as no phase separation, turbidity, creaming or cracking was observed upon temperature variations and the formulations could withstand high speed centrifugation at 5000 rpm for 20 minutes.

8.7.8. Fourier transform infrared (FTIR) spectroscopy

It was observed that the principal peaks of DE and NISO were retained as such in the spectra of DE NE and NISO NE, thereby indicating the absence of any significant interaction or incompatibility between the drug and excipients used in the formulations.

8.7.9. Drug release studies

8.7.9.1. *In vitro* dissolution study of DE NE

The *in vitro* release studies showed increase in drug release for DE NE (98.33±0.92% and 67.53± 3.11%) as compared to plain drug suspension (35.05±0.44% and 9.23± 3.02%) in 0.01N HCl and pH 6.8 phosphate buffer respectively after 60 min.

8.7.9.2. *In vitro* dissolution study of NISO NE

The *in vitro* release studies showed increase in drug release for NISO NE (99.20± 2.04% and 98.31± 3.17%) as compared to plain drug suspension (15.10± 2.63% and 13.92± 2.88%) in 0.1N HCl+0.5% SLS and pH 6.8 phosphate buffer + 0.5% SLS respectively after 60 min.

8.7.9.3. *In vitro* diffusion study of DE NE

Higher amount of the drug was diffused at 300 min. from the DE NE (98.97± 2.56% and 70.02± 3.12%) as compared to the plain drug suspension (37.14± 1.96 % and 15.87± 2.18%) in 0.01N HCl and in pH 6.8 phosphate buffer respectively.

8.7.9.4. *In vitro* diffusion study of NISO NE

The *in vitro* diffusion study showed significant increase in drug release from NE formulations (98.51± 2.64% and 99.04 ± 3.11%) as compared to plain drug suspension (29.73± 2.15% and 31.76± 2.62%) in 0.1N HCl + 0.5% SLS and pH 6.8 Phosphate buffer + 0.5% SLS respectively.

8.7.9.5. *Ex vivo* diffusion study of DE NE

The amount of the drug diffused through rat stomach increased when it was given in the form of a NE (97.25± 3.61%) as compared to plain drug suspension (28.86± 3.21%). Intestinal diffusion was relatively slower than stomach i.e. 68.64 ± 3.36% drug was diffused from the DE NE and 11.75± 2.11 % from plain drug suspension.

8.7.9.6. *Ex vivo* diffusion study of NISO NE

The drug diffusion from stomach (97.14± 6.21%) as well as intestine (96.36± 5.87%) was significantly higher for the NISO NE as compared to the plain drug suspension in stomach (16.44± 3.98%) and intestine (12.65± 4.23%).

8.8. Cell line studies

Safety of all optimized DE and NISO formulations, their respective suspensions and placebo were assessed by MTT assay through Caco-2 cells. The percent cell viability data for Caco-2 cells was found to be >80% for all formulations, at all the studied concentrations. The cell viability was higher in case of DE SMEDDS, DE NE, NISO SMEDDS, NISO NE than their respective drug suspensions.

Relative extent of uptake of coumarin-6 loaded SMEDDS and NE for both the drugs in comparison to their respective plain dye solutions were analyzed by FACS in Caco-2 cells. FACS uptake studies showed that the fluorescence intensity inside the cells increased with SMEDDS and NE formulations when compared with plain dye solutions. MFI with Coumarin-6 loaded SMEDDS was found to be 1.78 and 1.92 times higher after 1 h and 5.30 and 9.92 times higher after 4 h of plain dye solutions for DE and NISO respectively. MFI with Coumarin-6 loaded NE was found to be 4.39 and 4.64 times higher after 1 h and 7.03 and 13.04 times higher after 4 h than plain dye solution for DE and NISO respectively.

Cellular uptake and distribution of coumarin-6 loaded SMEDDS, coumarin-6 loaded NE and coumarin-6 solution was examined by confocal microscopy using Caco-2 cells. Enhanced fluorescent intensity inside cells for SMEDDS and NE of both the drugs was observed when compared to coumarin-6 solution after 1 h and 4 h incubation period.

Transepithelial permeability of DE SMEDDS, DE NE and NISO SMEDDS, NISO NE was measured at concentration of 100µg/ml, and 80µg/ml respectively as negligible toxicity towards Caco-2 cells was found at these concentrations during MTT assay of the same.

Results showed that there was increase in permeability of the developed SMEDDS and NE formulations as compared to respective drug Suspensions.This was attributed to the higher uptake of SMEDDS and NE by endocytosis in Caco-2 cells.

8.9. *In vivo* studies

8.9.1. Pharmacokinetic study of DE and NISO formulations

The optimized DE SMEDDS, DE NE formulations and DE suspension were evaluated for *in vivo* pharmacokinetic study upon oral administration. C_{max} for drug suspension was 440.13 ng/mL while DE-SMEDDS and DE-NE exhibited increased peak plasma concentration as C_{max}=1002.68 ng/mL and 995.56 ng/mL respectively. The AUC_{last} for DE-SMEDDS and DE NE was found to be 4989.78 ng/ml*h and 4812.95 ng/ml*h respectively which was significantly ($p < 0.05$) higher than drug suspension which showed AUC_{last} of 1935.65 ng/ml*h. Relative bioavailability of DE SMEDDS was enhanced to 2.58 folds and 2.49 folds for DE-NE as compared to drug suspension. DE SMEDDS and DE NE formulations demonstrated 6.03 & 5.94 h of MRT as compared to the drug suspension MRT(4.56 h).

Higher peak plasma drug concentration in case of NISO SMEDDS and NISO NE (C_{max} : 385.46 and 401.22 ng/mL) could be attributed to the faster absorption of drug from its formulation because of lipid based formulation, when compared with drug suspension (C_{max} : 223.97 ng/mL). The AUC_{last} after oral administration of NISO SMEDDS and NISO NE (2460.13 and 2501.85 ng/ml*h) were significantly higher than those obtained from drug suspension (1147.40 ng/mL*h). Relative bioavailability of NISO SMEDDS and NISO NE was enhanced to 2.14 folds and 2.18 folds respectively as compared to drug suspension.

8.9.2. Intestinal Lymphatic transport study of DE and NISO Formulations

Plasma concentration of DE and NISO in CHM treated rats was significantly lower than control group for both the prepared formulations of both the drugs. The experimental outcomes reflect that the peak concentration (C_{max}) of DE SMEDDS and DE NE in plasma after administration of CHM significantly reduced from 1112.34 ng/mL to 417.13 ng/mL and 1087.56 ng/mL to 474.97 ng/mL respectively as compared to saline treated rats. Moreover, a reduction of 62.72% and 58.42% was observed for AUC_{last} values in DE SMEDDS and DE NE respectively when prepared formulations administered in saline

treated rats were compared with CHM treated rats. While, the peak concentration (C_{max}) of NISO SMEDDS and NISO NE in CHM pre treated animals decreased from 379.35 ng/mL to 236.85 ng/mL and 413.48 ng/mL to 253.67 ng/mL respectively. Moreover, the AUC_{last} values reduced by 51.95 % and 51.11% respectively when CHM treated rats compared with saline treated rats ($p>0.05$). Thus, The results of the chylomicron flow-blocking experiments implied that the DE SMEDDS, NISO SMEDDS and NE were absorbed through the lymphatic pathway.

8.9.3. Pharmacodynamic study of DE and NISO formulations

Bleeding-time measurements in animals are used to evaluate the hemorrhagic properties of anticoagulant drug-DE. The bleeding time was found to be 48 ± 4.7 sec, 106 ± 9.3 sec, 162± 8.3 sec and 170± 7.8 sec in case of control, DE suspension treated, DE SMEDDS treated and DE NE treated groups respectively at $p<0.05$. Thus, as compared to control group a ~2-fold elevation in bleeding time for the animals treated with drug suspension was observed. However, DE SMEDDS and DE NE exhibited a ~3-fold elevation in bleeding time which correlated well with pharmacokinetic data.

Fructose-induced hypertension model was used to study antihypertensive activity of NISO. The indirect tail cuff method was used to determine systolic and diastolic blood pressure of rats. The systolic and diastolic blood pressure levels were found to be 126.86±7.89 mm Hg and 91.21±10.03 mm Hg, 287.64±3.76 mm Hg and 174.10±7.38 mm Hg, 201.53±4.95 mm Hg and 139.93±11.74 mm Hg, 154.37±6.44 mm Hg and 120.71±9.80 mm Hg, 147.68±5.46 mm Hg and 109.89±6.10 mm Hg in case of normal control, hypertensive control, NISO suspension, NISO SMEDDS and NISO NE respectively.

Conclusions

In the present study, two lipid based drug delivery systems, SMEDDS and NE were efficiently developed and evaluated for poorly bioavailable drugs, DE and NISO. The physicochemical characterization of all prepared formulations showed globule size below 100 nm with narrow PDI. The safety and efficacy of the developed formulations (SMEDDS and NE) were ascertained by performing in vitro cell lines studies and in vivo pharmacokinetic and pharmacodynamic studies. The gist of current study suggested that the optimized SMEDDS and NE formulations were found to be safe on enterocytes (Caco2 cells) with no significant toxicity. The cellular uptake of DE and NISO from SMEDDS and NE in Caco-2 cells were considerably increased than their respective drug suspension. Pharmacodynamic study displayed better *in vivo* anticoagulation effect for DE and anti hypertensive effect for NISO. These observations led us to the conclusion that SMEDDS and NE seem to be promising drug delivery systems, which can provide an effective and practical solution to the problem associated with drugs with low aqueous solubility and poor systemic bioavailability.