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Ex-Vivo Absorption Study of a Novel Dabigatran Etexilate Loaded Nanostructured Lipid Carrier Using Non-Everted Intestinal Sac Model

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Abstract

The purpose of the study was to develop Dabigatran Etexilate loaded nanostructured lipid carriers (DE-NLCs) using Glyceryl monostearate and Oleic acid as lipid matrix, and to estimate the potential of the developed delivery system to improve oral absorption of low bioavailability drug, different Oleic acid ratios effect on particle size, zeta potential, entrapment efficiency and loading capacity were studied, the optimized DE-NLCs shows a particle size within the nanorange, the zeta potential (ZP) was 33.81±0.73mV with drug entrapment efficiency (EE%) of 92.42±2.31% and a loading capacity (DL%) of 7.69±0.17%. about 58.5% of drug was released in 8hr in a controlled manner, the ex-vivo intestinal permeation study using the non-everted sac model shows four folds increment in the permeation of DE-NLCs compared to dabigatran etexilate suspension (DE-S).

Key words: Dabigatran Etexilate, NLCs, Ex-vivo intestinal permeation, thrombin inhibitor, Cremophor-EL.

دراسة النفاذية الحيوية خارج الجسم لحاملات الدهون النانوية الجديدة المحملة بالدابيغاتران أيتكزيليت باستخدام نموذج الكيس المعوي غير المقلوب للخارج.

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الخلاصة

ان الهدف من الدراسة الحالية هوه لتطوير حاملات دهون نانوية محملة بعقار الدابيغاتران أيتكزيليت مستخدمين احادي ستيارات الغلسريل و حامض الأوليك كقالب دهني, وكذلك لتقييم امكانية تطوير نظام توصيل لتحسين الامتصاص عن طريق الفم للدواء ذو التوافر الحيوي القليل, نسب مختلفة من حامض الأوليك و تأثيرها على الحجم الحبيبي, جهد زيتا, كفاءة الاحتجاز و قابلية التحميل للدواء قد درست.الصيغة المثالية لحاملات الدهون النانوية المحملة بعقار الدابيغاتران ايتكزيليت قد أظهرت حجم حبيبي ضمن نطاق النانو و جهد زيتا كان ٣٣,٨١±٠,٧٣ ملي فولت مع كفاءة الاحتجاز للدواء ٩٢,٤٢ ± ٢,٣١ % مع قابلية تحميل ٧,٦٩±٠,١٧%. حوالي ٥٨,٥% من الدواء تم تحريرها خلال (٨ ساعة) بطريقة مسيطر عليها, اظهرت دراسة النفاذية المعوية باستخدام نموذج الكيس المعوي غير المقلوب للخارج زيادة النفاذية لصيغة حاملات الدهون النانوية المحملة بالدابيغاتران ايتكزيليت بالمقارنة مع معلق الدواء.

الكلمات المفتاحية: دابيغاتران ايتكزيليت ، حاملات دهون نانوية ، النفاذية المعوية خارج الجسم، مثبط الثرومبين، كريموفور LE.

Introduction

Chronic anti-coagulation therapy is essential for the prophylaxis and treatment of acute venous thromboembolism (VTE) and including the prevention of cardiogenic thromboembolism in patients with atrial fibrillation and myocardial infarction, VTE is common in traumatic patient or those undergoing major surgical operations or with underlying malignancy and also in patients with prolonged immobilization, VTE is an emergency complication with an elevated morbidity and mortality rate, therefore prophylaxis of thrombotic events is considered as standard of care [(1,2)]. Dabigatran Etexilate (DE) is an inactive prodrug of the active compound dabigatran which is a reversible, selective, non-peptide, direct thrombin inhibitor, and by the inhibition of the serine protease it will prevent the conversion of fibrinogen into fibrin, DE has more predicted pharmacodynamic and pharmacokinetic profile and need no antithrombotic monitoring with minimum drug – food and drug-drug interaction compared to traditional antithrombotic agents. DE shows very low oral bioavailability (3 -7 %) and this is related to the pH dependent aqueous solubility of the drug and permeability glycoprotein (P-gp) mediated efflux [(3, 4)].

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Therefore a novel drug delivery system should be designed to improve its oral bioavailability by overcoming these limitations. Nanostructured lipid carriers (NLCs) is a second generation lipid-based nanoparticles was designed to overcome the low entrapment efficiency (EE %) and drug expulsion after storage that associated with solid lipid nanoparticles and this is done because the oil droplets causes several crystal defects in solid lipid matrix and thus imperfections in highly ordered crystal matrix occurs producing adequate space for higher amount of drug to be loaded [5].

The improvement in oral bioavailability that gained by using NLCs as a drug delivery vehicle was related to the fact that NLCs are absorbed mainly through the lymphatic system avoiding the first pas effect, also NLCs mechanism of transportation involve the participation of clathrin and caveolae in their transcytosis and it has a P-gp efflux pump modulation activity and eventually, enhancing the overall permeability of the encapsulated drug [6, 7, 8].

The lipid matrix of NLCs will be converted by the intestinal lipase to mono- , di and triglycerides together with fatty acids that interact with the intestinal bile salts to form a mixed micelle system which has a greater solubilizing capacity of the poorly water soluble drug that delivering it across the stagnant unstirred water layer to the enterocyte and increasing their intestinal permeability [9, 10].

Therefore, the aim of the present work was to evaluate the NLCs as a mean to improve the intestinal permeability and the oral bioavailability of DE thus development of DE-NLCs using a rational mixture of solid and liquid lipids together with surfactant stabilizer, and the prepared formulas were tested for their in-vitro physicochemical properties and also an ex-vivo intestinal permeability study was also conducted.

Materials and Methods

Materials

DE was purchased from Hangzhou Hyper-Chemicals Ltd., China, Oleic acid (OA) was obtained from Riedel De Haen AG Seelze, Honnover, German, Glyceryl monostearate (GMS) was obtained from BDH Chemicals Ltd. Poole, England, Cremophore EL (CR-EL) a Polyoxy 135 Castor oil was obtained from BASF (Ludwigshafen, Germany), PEG400 was purchased from Provizer Pharma, India, HPLC grade methanol and water for HPLC were obtained from Biosolve Chimie SARL, Dieuze, France., ammonium formate was obtained from Merck, Darmstadt, Germany, Potassium dihydrogen phosphate and Disodium hydrogen phosphate were purchased from BDH Chemicals Ltd., India, Ultrafiltration tube 10k MWCO was purchased from PALL Corporation, USA. Microcept™ Advanced Centrifugal Device, all other chemical and reagent obtained are of analytical grade.

Animals: A male Sprague-Dawley (SD) rat weighs 250-300mg were obtained from Animal House, College of Pharmacy-University of Baghdad.

Methods

Formulation of DE-loaded NLCs

DE-NLCs were prepared by hot emulsification-ultrasonication method with slight modification [11]. A 300 mg binary lipid mixture of both GMS as solid lipid and OA as liquid lipid were blended in different ratios and heated in water bath to about 10 ± 0.5°C above the melting point of the solid lipid to prevent lipid memory effect, along with 75mg of DE to form a uniform and clear oil phase. The aqueous phase consisting of surfactant and co-surfactant blend in double distilled water heated to the same temperature of the lipid phase. The melted lipid phase was added drop wise to the hot aqueous surfactant solution under continuous magnetic stirring at 900 rpm to form an oil in water (O/W) pre-emulsion, then the emulsion was sonicated using probe sonicator for 10 minutes at 75% amplitude with 30sec. on, 5sec. off periods, then the formed nanoemulsion was cooled in ice bath were the lipid nano-droplets will solidified and the lipid nanoparticles were formed. The composition of different batches illustrated in table 1.

Characterization of DE-NLCs

Percent entrapment efficiency and drug loading capacity

The entrapment efficiency (EE %) and drug loading capacity (DL %) was determined indirectly by measuring the concentration of free DE in the dispersion medium. The amount of unentrapped free drug was determined using an ultrafiltration technique (12). A 5mL of DE-NLCs solution was placed in the upper chamber of a centrifuge tube matched with an ultrafilter with a molecular cut off size (MWCO) 10 kDa and centrifuged for 30 minutes at 6000 rpm. The ultrafiltrate containing the free drug was diluted with methanol and the concentration of unentrapped DE was determined spectrophotometrically at 315 nm. The EE% and drug loading percent DL% were calculated using the equations (1) and (2) respectively:

$$EE\% = \frac{W_{total\ drug} - W_{free\ drug}}{W_{total\ drug}} \times 100 \quad (1)$$

$$DL\% = \frac{W_{total\ drug} - W_{free\ drug}}{W_{lipid}} \times 100 \quad (2)$$

where,($W_{initail\ drug}$) is the weight of initial drug used,($W_{free\ drug}$) is the weight of free drug detected in the supernatant after ultrafiltration of the aqueous dispersion and (W_{lipid}) is the weight of lipid used [13].

Table 1. Composition of different formulas of DE-loaded NLCs

Formula code	DE (mg)	GMS (mg)	OA. (mg)	CR-EL %w/v	PEG400 %w/v	ddH_2O (ml)
N0	0	270	30	2.5	2	15
N1	75	270	30	2.5	2	15
N2	75	240	60	2.5	2	15
N3	75	210	90	2.5	2	15
N4	75	180	120	2.5	2	15
N5	75	150	150	2.5	2	15
N6	75	300	0	2.5	2	15

-DE, dabigatran etexilate, GMS, glyceryl monostearate, OA, oleic acid, CR-EL, cremophor-EL, PEG400, polyethylene glycol 400, ddH_2O, double distilled water.

Particle size and polydispersity index

Dynamic light scattering was used to determine particle size and polydispersity index (PDI) of the prepared DE-NLCs dispersion after appropriate dilution with double distilled water (1:50) of all formulations was made and placed in 1cm diameter disposable plastic cuvette to yield a suitable scattering intensity at a fixed scattering angle of 90° at room temperature (25°C). From the analysis, the mean particle size (diameter, nm ± standard deviation) and polydispersity index PD of DE-NLCs were calculated using Brookhaven Instruments Corp90 PLUS (ZetaPlus Particle Sizing, NY, Software, Version 5.34) [(14)].

Zeta potential

The zeta potential of DE-NLCs was determined utilizing the NanoBrook ZetaPALS which determines zeta potential using Phase Analysis Light Scattering technique , the NLC dispersions were diluted with double distilled water (1:100) to get a uniform dispersion prior to analysis and the samples were placed in a clean and disposable zeta cells. The conductivity of the diluted samples was measured in order to select the detection model. The whole measurement was carried out at 25°C [(15)].

In-vitro drug release study

In vitro release study of DE-NLCs was performed using a modified dialysis membrane diffusion technique [(16)]. Dialysis membrane (Hi-media, Mumbai, India) with molecular weight cut off between (MWCO 12,000–14,000Da) was previously soaked overnight with dissolution media, 5ml of DE-NLC formulation was placed in the dialysis bag and tied at both ends using silk thread and placed in the dissolution apparatus I of dissolution media, that is 0.1N HCl with pH 1.2 and phosphate buffer solution (PBS) +35% ethanol with pH 6.8 [(17)]. The temperature of the media was maintained at 37 ± 5ºC; the rotation speed was set at 100 rpm. An aliquot of 5ml sample was withdrawn at pre-determined time intervals (0.25, 0.5, 1, 2, 4, 6 and 8 hours), and replenished equivalent volume of fresh dissolution medium. The experiment was performed in 0.1N HCl for the first two hours and transferred to PBS+35% ethanol to the rest time of the study, to provide sink condition. Samples were analyzed spectrophotometrically using Carry win UV, (Varian, Australia) spectrophotometer. A cumulative amount of drug released was calculated.

Analytical method validation of DE loaded NLCs

Instrumentation and chromatography conditions

RP-HPLC system was used for this study, the specifications are given below. A Waters HPLC equipped with SPA- 20A detector, an isocratic chromatographic separation conducted utilizing Symmetry® ODS-C18 (250 × 4.6mm; 5µm) column and Breeze software. Chromatographic conditions: Mobile phase: HPLC grade of Methanol: 0.1M ammonium formate solution in a ratio of 77:23 percent (v/v) was filtered through (0.45µm) Millipore filter. Flow rate of the mobile phase was maintained at 1.0 ml/min the column temperature was 40ºC. Detection was carried out by UV-detector; wave length at 303 nm the running time was 10 min. The volume of the injection loop was 20 µl [(18)].

Preparation of standard stock solution and calibration curve

Prior to injecting the drug solution, the column was equilibrated with the mobile phase flowing through the system for at least 30min.One hundred milligrams of DE was accurately weighed and transferred to a 100 ml volumetric flask. It was dissolved in 50 ml HPLC grade methanol and sonication for about 10 minutes and then made up to the volume with HPLC grade methanol. From this stock solution (1mg/ml) nine serial dilutions (0.5, 1, 2.5, 5, 10, 20, 30, 40, and 50 µg/ml) were prepared. Twenty microliter of each dilution was injected into the column and the corresponding chromatograms were obtained. The peaks areas at a specific retention time were recorded and plotted versus concentrations and the curve linearity was evaluated by linear regression analysis.

Validation of the HPLC method

The method was validated following International Conference of Harmonization (ICH) guidelines [19,20] for analytical procedures validation:

Linearity and range

The linearity of the method was determined by constructing three independent analytical calibration curves. The results were tested statistically and subjected to regression analysis to determine the calibration equation and determination coefficient (R^2).

Specificity and selectivity

Analytical method specificity can be defined as the ability to detect the analyte in the existence of other components such as impurities, matrix compounds and products of degradation. The interference was determined by injection of a sample containing the media without the drug and a sample containing the media with DE at a concentration of about (5µg/mL).

Interday and intraday precision and accuracy

The precision of the method was determined by measuring repeatability and intermediate precision. Repeatability was examined by six replications of the analyte concentration (20µg/ml) on the same day at different intervals, under the same experimental conditions (intraday). The intermediate precision of the method was assessed by carrying out the analysis on three different days (interday), with six replicates being analyzed each day. The percentage relative standard deviation (%RSD) should be less than 2 . The accuracy of the method was determined by the measuring of the percentage recovery of three concentrations of the analyte (2.5, 5 and 10µg/ml) in triplicate, and the accuracy was calculated as the percentage of DE recovered from the injected sample.

Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ were determined as represented by the ICH guidelines [20], making use of a three independent analytical curves mean values, determined via a linear regression model, where the factors of (3.3) for the limits of detection and (10) for the limit of quantitation, were multiplied by the ratio of the *y*-intercept standard deviation to the slope of the regression line.

Robustness: The robustness refers to the ability of an analytical procedure to remain unaffected by small but deliberate variations in the parameters of the analytical method which indicating the reliability of the method for routine analysis. The robustness was determined by analyzing samples of (20µg/mL) under different conditions of the analytical method parameters, such as flow rate (0.9, 1 and 1.1 ml/min), injection volume (19, 20 and 21µl), mobile phase composition (methanol ratio of: 76, 77 and 78%) [21].

Ex-vivo Intestinal Permeation Study

The ex-vivo permeation study of DE-NLCs was carried out using non-averted gut sac method with modification [21, 22], male SD rats, weighing approximately 250–300 g, were fasted overnight with free access to water, anesthetized with ether and a longitudinal abdominal incision was made then the small intestine was removed and the mesentery was stripped manually and washed out carefully with cold normal saline solution using a syringe equipped with blunt end needle. The clean intestine was cut into 10 ± 0.2 cm long sacs having a diameter of 0.25mm. After tying one end, the intestinal sac was filled with (1ml) of selected formula and pure drug suspension containing approximately (7.5mg) of DE, then tying the other end of the sac and placed in a beaker containing Krebs-Ringer solution (pH 7.4) system was maintained at 37 ± 1.0°C in a magnetic stirrer operating at 50 rpm and continuously bubbled with oxygen (50 bubble/min), (5ml) samples were withdrawing at 30, 60, 90, 120, 150, 180, 210 and 240 min. and the sample was analyzed by HPLC, The apparent permeability coefficients were determined using Eq. (3) and Eq. (4) [23]:

$$\boldsymbol{P_{app} = \frac{F}{SA \times C_\circ}} \qquad (3).$$

$$\boldsymbol{SA = 2\pi rh} \qquad (4).$$

The ($\mathbf{P}_{app}$, cm/min) is the apparent permeability, (**F**, µg/min) is the flux, (S**A**) is the area of the intestinal sac in (cm^2) and ($\mathbf{C_0}$) is the initial drug concentration (µg/ml), the slope of the linear portion of the plot was considered as the permeation flux (**F**), (***r***) is the intestinal radius (cm) and (***h***) is the intestinal segment length (cm).

Statistical analysis

The one way analysis of variance (ANOVA) test using SPSS software version 17.0 was used. The level of significance was set at $\alpha = 0.05$, all the results were illustrated as the mean values ±standard deviation (SD) in three replicates (n=3).

Results and Discussion

The results of particle size, PDI, zeta potential, %EE and %DL are shown in table2.

Entrapment efficiency and drug loading capacity

A high %EE was observed in formula N5 (97.62±1.15) and the lowest value was seen in formula N6 (68.87±3.23), and a nonsignificant increase in percent drug entrapped was observed ($P>0.05$) by increasing the oil ratio due to the higher drug solubility in the lipid blend, also the %DL was also increased, a significant reduction in EE% and DL% in formula N6 (without oil) compared to N1 because drug molecules has a higher solubility in oils than in solid lipid matrix and their incorporation will create structural imperfections that increases the amount of drug entrapped within the solid lipid matrix [24].

Table 2. Evaluation parameters of different DE-NLCs

Formula code	Particle size (nm)	PDI	ZP (mV)	EE (%)	DL (%)
N0	18.7±0.87	0.005±0.001	-27.73±3.52	-	-
N1	62.4±5.75	0.286±0.001	-33.81±0.73	92.42±2.31	7.69±0.17
N2	181.8±7.46	0.315±0.018	-12.62±1.66	95.16±3.21	7.97±0.51
N3	500.9±21.9	0.415±0.022	-12.16±2.11	96.31±1.51	8.04±0.38
N4	769±37.2	0.326±0.012	-7.02±0.58	96.65±1.53	8.41±0.13
N5	806±28.76	0.083±0.017	-6.36±0.72	97.62±1.15	9.18±0.10
N6	147.1±7.5	0.358±0.023	-26.32±3.32	68.87±3.23	4.78±0.32

-Data presents mean± standard deviation, (n=3).

-EE, encapsulation efficiency, DL, loading capacity, PDI, polydispersity index, ZP, zeta potential.

Particle size and zeta potential analysis

A significant increase in particle size was seen with increasing oil ratio ($P<0.05$) this is related to the fact that as the oleic acid content exceeds 10%, the particle size increased owing to the swollen core of the oleic acid loaded nanoparticles, a similar observation was obtained by W. Dai et al.[25], formula N6 shows a significant increase in particle size and PDI compared to N1 this is because the oil decrease the system viscosity leading to easier small particle formation [23]. The N1 (blank) shows lower particle size distribution this is expected due to the added mass of drug at a constant stabilizer concentration [26].

All of the prepared formulas have a negative ZP values (Table 2), with the highest value of (-33.81 mV) in formula N1 and the lowest value was (-6.36 mV) in formula N5, and the accepted value to produce a stable nanodispersion should be ≥+30 mV or ≤ -30 mV. However, it is important to notice that this rule applies only to an electrostatically stabilized system [27]. A reduction in ZP value was observed when the oleic acid ratio was increased above (10% w/w) which is associated with increased in particle size leading to reduction in surface charge density on the formed nanoparticles [28].

In-vitro release study

Formulas N1 (NLCs with the lowest particle size together with higher ZP) and N6 (SLNs) was selected for in-vitro release study, they shows a biphasic release profile with initial burst release of (31.76%) and (24.6%) in the first 2hr (obtained from the drug release curve) followed by sustained release of (58.5%) and (45.42%) for the next 6hr for N1and N6 respectively, as seen in figure 1, the initial burst release occur due to the drug exist in the surface of the NLCs and the external phase. Formula N1 shows faster rate of release compared to N6 this is due to the highly ordered crystalline structure of solid lipid matrix that restrict the drug diffusion velocity to the lipid interface before partitioning between the lipid and the aqueous phases [29, 30].

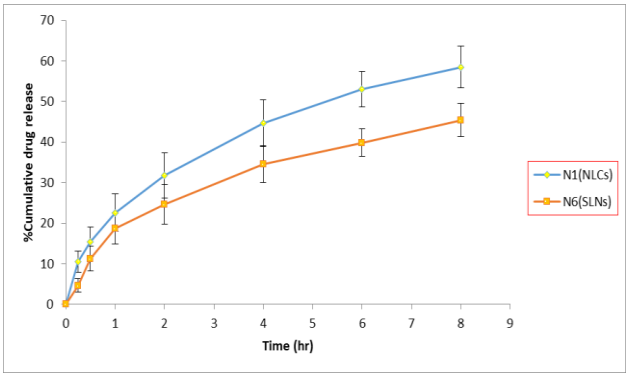

Figure 1. Cumulative release profile of formula N1 (NLCs) and formula N6 (SLNs), mean values± standard deviation

HPLC method validation

Linearity and range

At the concentration range of (1.66-50 µg/ml) the obtained calibration curve was found to be linear with a coefficient of correlation (R^2 = 0.9999) and the regression equation was [y=5.0445x+3.7043] as seen obviously in figure 2.

Specificity and selectivity

The analytical procedure was considered selective and specific because no peak interference from the mobile phase on the drug peak as seen in the HPLC chromatograms of both the drug and that of the blank in figure 3.

Precision and accuracy

The inter- and intraday variation study was conducted to define the analytical procedure precision, the results of method precision and accuracy were shown in table 3 and table 4 respectively, the percentage relative standard deviation was less than 2 which confirm the accuracy and precision of the analytical procedure [19].

LOD and LOQ

The calculated LOD and LOQ were found to be (0.54) and (1.66µg/ml) respectively.

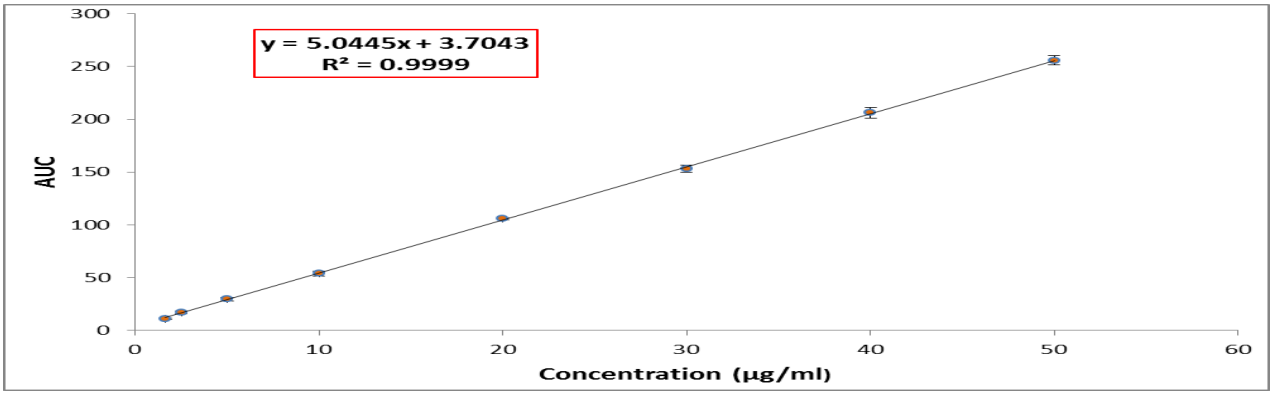

Figure 2. Calibration curve of dabigatran etexilate using HPLC, mean ± SD (n=3)

Table 3. Data of inter- and intraday precision

	Response (Area)		
Response (Area)	**Day (1)**	**Day (2)**	**Day (3)**
Number of replicates			
1	**105.87**	**104.98**	**105.2**
2	**105.2**	**105.43**	**104.8**
3	**105.5**	**105.02**	**105**
4	**104.54**	**104.77**	**104.78**
5	**106.1**	**104.92**	**106.01**
6	**105.71**	**105.76**	**105.91**
Mean	**105.48**	**105.14**	**105.28**
%RSD(n=6)	**0.5280**	**0.3542**	**0.5193**
%RSD (n=18)	**0.4658**		

Table 4. Data of accuracy study

Level of Test	Amount injected (µg/ml)	Amount* recovered (µg/ml)	% recovery	%RSD
1	**2.5**	**2.53±0.00678**	**101.2**	**0.2683**
2	**5**	**5.26±0.05624**	**105.2**	**1.0691**
3	**10**	**10.04±0.09204**	**100.4**	**0.9187**

* Mean values± SD, (n=3)

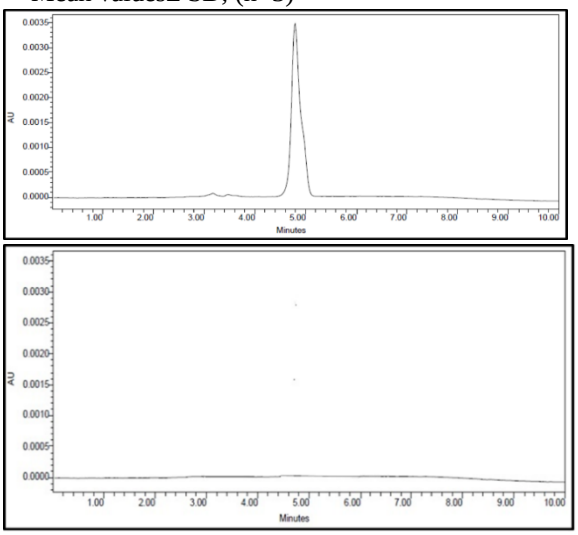

Figure 3. Chromatogram of both blank (lower) and Dabigatran Etexilate standard solution (upper) shows its retention time (chromatogram peak) at a concentration of (5µg/ml).

Robustness

The data obtained during the variation of the method parameters (flow rate, the injection volume and the % methanol in the mobile phase) are shown in figure 5, and the percent relative standard deviation was less than 2% indicating that the analytical procedure was robust [31].

Table 5. Robustness data of DE analytical method validation

variables	Investigated range	Response (Area)			%RSD
		Level (1)	**Level (2)**	**Level (3)**	
Flow rate	**0.9 ml/min**	**106.12**	**105.77**	**105.85**	**0.1731**
	1 ml/min	**104.86**	**105.85**	**106.17**	**0.6465**
	1.1 ml/min	**104.75**	**105.29**	**104.92**	**0.2629**
Methanol%	**76% v/v**	**105.83**	**104.95**	**105.78**	**0.4684**
	77% v/v	**105.87**	**105.80**	**104.91**	**0.5071**
	78% v/v	**104.85**	**105.23**	**104.97**	**0.1849**
Injection volume	**19 µl**	**105.89**	**106.20**	**106.16**	**0.1589**
	20 µl	**105.87**	**104.84**	**106.51**	**0.7968**
	21 µl	**106.01**	**105.89**	**106.11**	**0.1039**

-%RSD, relative standard deviation

Ex-vivo permeability study

Figures 4 and 5 represents the plot of the amount of DE permeate from optimized formula N1 and the pure drug suspension in the two intestinal segments used in the study, duodenum and ileum respectively, The flux (F, µg/min) was obtained from the slop of the linear regression equations and the apparent permeability coefficient (Papp, cm/min) data were calculated utilizing equations (3) and (4) are shown in table 6, data exhibited a significantly higher intestinal permeability compared to the pure drug suspension ($P<0.05$), the apparent permeability coefficient (cm/min) of DE from formula N1 was found to be 13.755±0.12 and 12.139±0.18 $\times 10^{-5}$ from duodenum and ileum segments respectively, and its value for pure drug suspension was 3.181±0.008 and 2.853±0.009 $\times 10^{-5}$ from duodenum and ileum respectively, showing a four folds increase in the drug permeability from DE-NLCs in comparison with pure DE suspension, a similar results obtained by A. Buthiraja et al. and this is may be related to the high adhesion properties of the prepared nanoparticles which is due to the extremely high surface area of these highly small particles leading to high drug diffusion and dissolution may explain the enhanced drug permeation [23]. And the use of permeability enhancing excipients such as Cremophor-EL and oleic acid that inhibits intestinal P-gp efflux pump activity probably improves drug permeability [32].

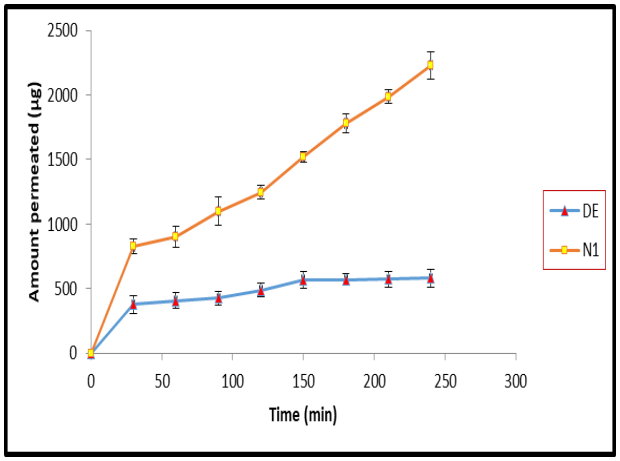

Figure 4. Permeation of dabigatran etexilate from optimized formula N1 and pure drug suspension through non-everted rat duodenum, values of mean ±SD (n=3).

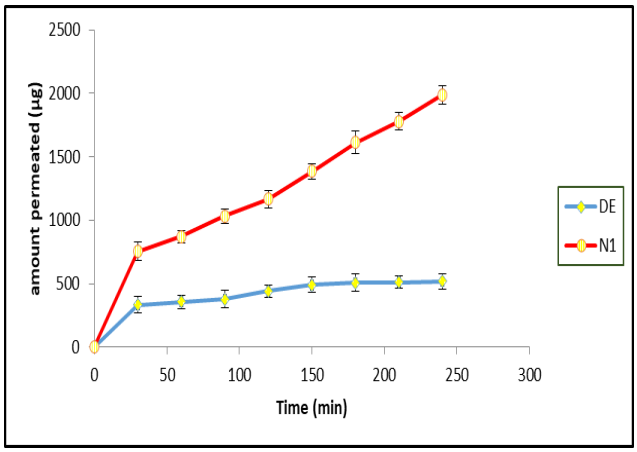

Figure 5. Permeation of dabigatran etexilate from optimized formula N1 and pure drug suspension through non-everted rat ileum, values of mean ±SD (n=3)

Conclusion

In the present study, DE loaded NLCs were successfully prepared utilizing emulsification / ultrasonication method, and the formula N1 was selected due to its preferred particle size distribution, zeta potential, entrapment efficiency and drug release profile and selected to undergo ex-vivo permeability study and the obtained data reveals a four folds increase in drug permeability showing that NLCs is a promising delivery system to increase the DE oral bioavailability.

Table 6. The ex-vivo absorption parameters of dabigatran etexilate from optimized formula N1 and pure drug suspension, results were presented as mean ±SD (n=3)

sample	Duodenum		Ileum	
	F(µg/min)	**Papp×10^{-5} (cm/min)**	**F(µg/min)**	**Papp ×10^{-5} (cm/min)**
N1	**8.0983±0.26**	**13.755±0.12**	**7.1471±0.23**	**12.139±0.18**
DE suspension	**1.8733±0.013**	**3.181±0.008**	**1.6797±0.012**	**2.853±0.009**

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