ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Formulation and In-Vitro Evaluation of Dabigatran Etexilate Loaded Nanostructured Lipid Carriers

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Abstract

The nanostructured lipid carriers (NLCs) is an interesting delivery system that can protect the encapsulated drugs and improve their dissolution, permeability and over all bioavailability. The aim of the present study was to prepare dabigatran etexilate (DAE)-encapsulated NLCs (DAE-NLCs) using glyceryl monostearate (GMS) and oleic acid (OA) as solid and liquid lipid matrix respectively, together with different surfactant types and ratios; DAE-NLCs were prepared using the hot emulsification-ultrasonication technique and the prepared formulations were characterized in terms of their particle size distribution, encapsulation efficiency (EE%), zeta potential, surface morphology and physical state characteristics. The prepared lipid nanoparticles shows a spherical shape with a particle size (62.4±5.75nm), poly dispersity index (PDI) of (0.286±0.001), zeta potential (-33.81±0.001 mV) the EE% was (92.42±2.31) and the drug loading capacity (LC %) was found to be (7.69±0.17). the *in-vitro* drug release study shows a bi-phasic drug release pattern with initial burst followed by a prolonged drug releasing phase with a 92% of the loaded drug was released in 24hr the release kinetics was fitted to Korsmyere-Peppas model with anomalous release mechanism. The solid state characterization depict an amorphous state of entrapped drug within the lipid matrix of the optimized DAE-NLCs. Short term stability study shows no significant change in nanoparticle characterization at refrigerator compared to room temperature. DAE-NLCs could be a potential delivery device for improved drug loading with controlled release properties that will improves oral bioavailability of the drug.

Keywords: *Dabigatran Etexilate, Hot emulsification/ultrasonication, Nanostructured lipid carriers, Cremophor-EL.*

Introduction

Dabigatran etexilate (DAE) is double ester prodrug of dabigatran which is a novel, potent reversible nonpeptide direct thrombin inhibitor, DAE is ethyl 3-[({2-[(4-{N'-[(hexyloxy) carbonyl] carbamimidoyl anilino) methyl]-1-methyl-1H benzimidazol-5-yl} carbonyl) (pyridin-2-yl) amino] propanoate [1], it was found to offers a privilege advantages over other anticoagulants such as warfarin in the treatment and prophylaxis of thromboembolic disorders that it have lower drug-drug interaction potential and no need for frequent coagulation monitoring and produce similar therapeutic efficacy [2].

DAE has a therapeutic indication for the prevention of thromboembolic events in patients with atrial fibrillation (AF) and as a prophylaxis in patients undergoing elective knee-joint or total hip replacement surgery [3], DAE shows a low oral bioavailability (< 7%) and this is related to its pH-dependent solubility with decreased solubility at pH > 4 and also DAE was found to be a substrate to P-gp efflux pump that is responsible for expulsion of drug molecules back to the intestinal lumen [1, 4].

Nanostructured lipid carriers (NLCs) is lipid based nanoparticles with a heterogeneous mixture of solid and liquid lipid (oil) matrix that was designed to improve the drug loading and avoid the expulsion of the incorporated drug during storage that associated with solid lipid nanoparticles (SLNs), because of the many imperfection in the matrix structures of NLCs that occurs after the lipid solidification provides more space to accommodate drug molecules [5, 6].

Higher drug payload capacity and their extended shelf- live storage stability also,

their ability to incorporate both hydrophilic and lipophilic drugs together with sustaining and site specific targeting of drug release makes it a perfect candidate as drug delivery platform compared to other conventional lipid based systems [7, 8].

In addition, NLCs was found to improve oral bioavailability by enhancing drugs solubilization due to their physical state transformation from crystalline to amorphous form [9], also, a mixed micelle formation and lymphatic absorption pathway utilization together with multiple biological barrier penetration capacity making the NLCs a good candidate as an oral bioavailability enhancing delivery system [10]. The aim of our study was to develop a poorly water soluble drug and a P-gp efflux pump substrate DAE loaded NLCs and to evaluate the pharmaceutical and physicochemical properties of this formulation.

Materials and Methods

Materials

DAE was obtained from Hangzhou Hyper-Chemicals Ltd., China, Glyceryl monostearate (GMS), stearic acid (SA), palmitic acid (PA) and stearyl alcohol (STA) were obtained from BDH Chemicals Ltd. Poole, England, oleic acid (OA) was obtained from Riedel De Haen AG Seelze, Hannover, German, Labrafil 1994CS was obtained from Gattefossé (France), sesame oil (SO), eucalyptus oil (EO), cotton seed oil (CO), peppermint oil (PO) and olive oil (OL) was purchased from Loba Chemie Pvt. Ltd.,

Mumbai, India, , cremophor EL (CR-EL) a polyoxy 135 castor oil was obtained from BASF (Ludwigshafen, Germany), Tween 80 and Span 80 were obtained from Hopkin & Williams LTD. Chandwell Heath. Essex. England, polyethylene glycol 400 (PEG400) was obtained from Provizer Pharma, India; mannitol was purchased from BDH Chemicals Ltd., India. Other chemical and reagent obtained were of analytical grade.

Methods

Solid lipid selection: The DE saturated solubility in different solid lipids was determined using the test tube method. A weighed amount of solid lipids (1 g) was placed in a test tube and heated to 5 °C above their melting point with continuous stirring using magnetic stirrer with hot plate at 200 rpm. DE was added in increments of 10 mg until it was completely dissolved in the melted solid lipids and the amount of solid lipids required to solubilize DE was calculated. The experiment was repeated in triplicate and results were represented as mean value (mg/g) ± SD [11].

Screening of Liquid Lipid (Oils): the saturated solubility of DE in different liquid lipids (oils) including (oleic acid, Eucalyptus oil, cotton seed oil, sesame oil, Labrafil 1994CS oil, and olive oil) was determined after addition of an excess amount of DE to five milliliters of different oils in a screw-capped test tubes that were continuously stirred for 72h at 37ºC, using water bath shaker.

At the end of 72hr, the mixture was centrifuged at 5000 rpm for 15 min; the supernatant was carefully separated and filtered using a 0.45μm membrane filter, then the filtrate was appropriately diluted with methanol and UV- absorbance at 315nm were measured using (Carry win UV, Varian, Australia) to determine DE concentration in different oils.

Blank solution was prepared by dissolving respective oil in methanol with same dilution as for the samples [12]. The solubility studies were done in triplicate and results were represented as mean ± standard deviation (SD).

Formulation of DE loaded NLCs

Hot emulsification/ultrasonication method with slight modification [13]. A binary mixture of both solid and liquid lipid were blended and heated to about 10 ± 0.5°C above the melting point of the solid lipid to prevent lipid memory effect, along with 0.75gm 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 stirring at 900 rpm using hot-plate magnetic stirrer /Copley scientific-UK, to form an oil in water (o/w) pre-emulsion, then the emulsion was sonicated using Q500 probe sonicator

Qsonica-USA, 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 compositions of the prepared DAE-NLCs are shown in Table (1).

Table 1: Composition of the prepared DAE-NLCs formulations

Formula code	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12
DAE(mg)	75	75	75	75	75	75	75	75	75	75	75	75
GMS(mg)	270	270	270	270	270	270	270	270	270	270	270	270
OA(mg)	30	30	30	30	30	30	30	30	30	30	30	30
Tween80%w/v	1%	1.5%	2%	2.5%								
Span80%w/v					1%	1.5%	2%	2.5%				
CR-EL %w/v									1%	1.5%	2%	2.5%
PEG400 %w/v	2%	2%	2%	2%	2%	2%	2%	2%	2%	2%	2%	2%

(Zeta Plus Particle Sizing, NY, Software, Version 5.34)and zeta potential (ZP, mV), the sample diluted with double distilled water (1:50) 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) [14], while ZP was determined by the electrophoretic mobility of the sample using NanoBrook Zeta PALS from Brookhaven Instruments Corp., software version (zetapw32.exe:3.57;bi_zetapw32.dll: 3.4; Eclectic:4.4) the sample was placed in a disposable measuring cell after proper dilution and the measurement was conducted at room temperature [15]. Measures were done in triplicate and the results represent the mean ± (SD).

Characterization of Prepared NLCs:

Particle size and zeta potential

The determination of particle size (nm), polydispersity index (PDI) was assessed by dynamic light scattering (DLS) using Brookhaven Instruments Corp. 90PLUS.

Entrapment efficiency (EE %) and drug loading capacity (LC %) of DAE

The amount of unentrapped free DAE was determined using an ultrafiltration technique [16]. Briefly, 5mL of the prepared NLCs solution was placed in the upper chamber of an ultrafiltration tube (Microcept™ Advanced Centrifugal Device PALL Corp., USA) 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 concentrations of unentrapped DE were determined spectrophotometrically at 315 nm. The EE% and drug loading percent DL% were calculated using the equations (1) and (2) respectively:

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

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

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 [17].

Morphology Analysis

Particle morphology was determined using atomic force microscopy (AFM) technique (SPM-AA3000 Angestrom, 2008, USA), a drop of DE-NLCs was deposited onto a small mica disk with a diameter of 1cm and the excess water was removed using a filter paper.

The experiment was conducted under atmospheric pressure, at room temperature, and operated in noncontact mode and scanning frequency of (2 Hz). Also the results were confirmed using scanning electron microscope (TESCAN/ VEGA/SEM, Tescan, Czech Rep.) the lyophilized powder were dusted onto double-sided tape on an aluminum stub and coated with Au/Pd in an argon atmosphere for 10 min by a cold sputter coater in SEM chamber to a 300°A thickness, then photomicrographs were taken by operating at an accelerating voltage of 15Kv electron beam [18, 19].

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry was performed on all formulation constituents and the lyophilized formula using differential scanning calorimeter (DSC-60 plus, Shimadzu, Japan), a 5mg sample were placed in a standard aluminum pans and heated at a constant rate of 10oC/min and scanned between 30-300°C against an empty aluminum pan as a reference under a constant nitrogen purge [20].

Powder X-ray diffraction analysis (PXRD): The study was done using x-ray diffractometer (LabX XRD-6000, Shimadzu, Japan), where CuKα radiation with a wavelength of 1.5405 Å was used as X-ray source. For the measurements, samples were kept in the glass sample holders with a continuous scan range of 2θ = 5 - 80, the operating voltage and current were 40 kV and 30 mA, respectively, the used data were typically collected with a 0.05° step width and a detector resolution in diffraction angle (2θ) between 10°C and 60°C at an ambient temperature, samples of pure DAE, physical mixture and lyophilized DAE-NLCs [21].

Fourier-transform Infrared Spectroscopy (FTIR)

The spectra were recorded for pure drug, Glyceryl monostearate, physical mixtures of drug and lipid and lyophilized DE-NLC, using FTIR Shimadzu (Model No. 8400S). Each sample was weighed accurately and prepared in KBr disk, the spectrum was scanned over the frequency range of 4000–400 cm−1 and 4−1 cm spectral resolution, it was used to study any possible interaction between DAE and other excipients and to confirm drug identity [22].

In-vitro Drug Release

In vitro release study of DE-NLCs was performed using a modified dialysis membrane diffusion technique [23]. 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 and placed in the dissolution apparatus I of dissolution medias, that is 0.1N HCl with pH 1.2 and phosphate buffer solution +35% ethanol with pH 6.8 (Hu et al., 2017).

The temperature of the media was maintained at 37 ± 5ºC; the rotation speed was set at 100 rpm.

A 5ml sample was withdrawn at a pre-determined time intervals (0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours), and replenished with equivalent volume of fresh dissolution medium to maintain sink condition. The experiment was performed in 0.1N HCl for the first two hours and transferred to PBS+35% ethanol to the rest of the experiment to simulate the G.I physiology. Samples were analyzed spectrophotometrically. A cumulative amount of drug released was calculated. All the operations were carried out in triplicate.

Kinetic Drug Release Modeling

Data obtained from in-vitro drug release study were fitted to various kinetic models: Zero order, First order, Higuchi's model and Korsmeyer-Peppas as in equations 2-6, 2-7, 2-8 and 2-9 respectively, in order to find the best fitted line to predict the mechanism of drug release, While n is the release exponent indicating the release mechanism from spherical matrices, when $n < 0.43$, a Fickian diffusion drug release mechanism occurs, while if $0.43 < n < 0.85$ a non-Fickian or anomalous diffusion is predominant [24].

Lyophilization of DAE-NLCs

lyophilization of DAE-NLCs was performed using 5%w/v mannitol as a cryoprotectant then lyophilized for 48h and pressure was 0.4 bar in Heto Drywinner, freeze dryer (Heto-Holten A/S, Denmark) [25].

Short-term Stability Study

To study the short term stability of the liquid DE-NLCs as a function of storage condition, the optimized formula were divided among two tightly closed, amber color glass containers, each container was stored at (2-8º C and 25º C) for 90 days [26]. The average particle size, polydispersity index, zeta potential, and the drug content of the nanoparticles were determined at (0, 45 and 90 days of storage) and results were performed in triplicate.

Statistical Analysis

To evaluate the difference between the results of studied formulations, the one way analysis of variance (ANOVA) test using SPSS software version 17.0 was used.

The level of significance was set at p-value = 0.05, All the results were illustrated as the mean values ±standard deviation (SD) in three replicates (n=3).

Results and Discussion

Solubility in Solid and Liquid Lipids

DAE shows higher solubility in GMS (250±7.84mg/g) compared to its solubility in other lipids being investigated as seen in Figure (1). So GMS was selected as the solid lipid matrix in the formulation of NLCs due to its higher solubilizing capacity, Oleic acid exhibits significantly higher DAE solubility since it possesses the best solubilization capacity than other oils as seen in Figure (2), and this is due to the formation of hydrogen bonding between the carboxylic group of the fatty acids and the drug molecules [27]. Therefore it was chosen as a liquid lipid for the formulation of DAE-NLC.

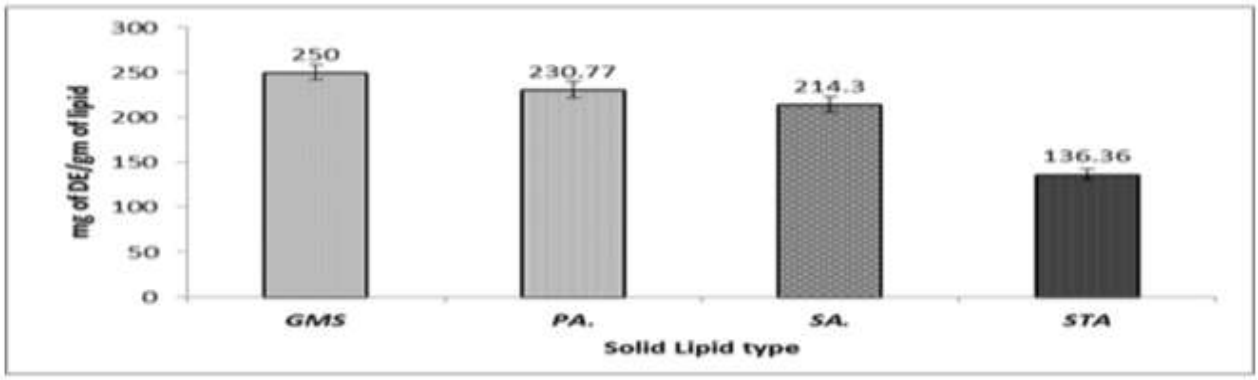

Figure 1: Solubility of DAE in different solid lipids, results represents mean ± SD

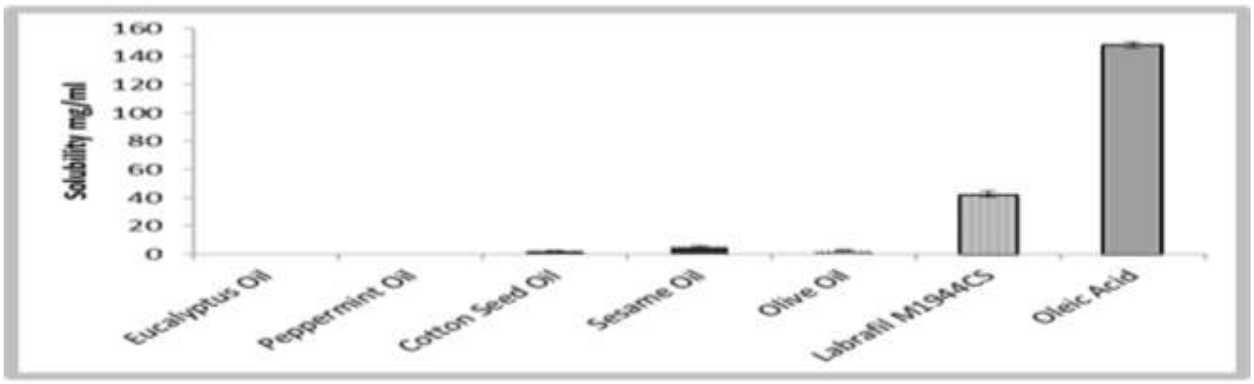

Figure 2: Solubility of DAE in different oils, results mean ± SD (n=3)

Particle Size and Zeta Potential

DAE-NLCs were prepared using the hot emulsification-ultrasonication method, different surfactants type and concentration were optimized in order to obtain nanosize particles with uniform distribution, and Table (2) shows the physicochemical characterization data of the different NLCs formulations, increasing the surfactant concentration from 1% up to 2.5% was associated with a significant ($P<0.05$) decrease in the particle size of the formed DAE-NLCs dispersions this related to the fact that a higher surfactant amount reduces the surface tension of the melted lipid droplets, that will facilitating their breakdown into smaller size and will provide enough surfactant molecules to cover the surfaces of formed lipid nanodroplets, increasing their stability [28].

It was observed that when the concentration of Tween80 increased to 2.5% in formula N4 the particle was also increased and this could be explained by the formation of micellar structures of the excess surfactant molecules in the continuous phase leading to particle aggregation and flocculation by a depletion-flocculation mechanism explained by [29].

A poly dispersity index (PDI) value equal to or less than (0.3) are considered acceptable in lipid based drug delivery systems and the value indicate a homogeneously dispersed system [30], the PDI of most of the prepared DE-NLCs were lower than (0.4) which indicates a relatively narrow size distribution. All formulations in the present study were found to bear negative ZP values table (2) for aqueous dispersion of nanoparticles with the highest value of (-

33.81 mV) in formula N12 and the lowest value was found to be (-11.98 mV) in formula N6, and the accepted value to produce a stable nanodispersion should be equal to/or higher than (+30 mV) or equal to/or lower than (-30 mV). However, it is important to notice that this rule applies only to an electro statically stabilized system [31].

Entrapment Efficiency and Drug Loading Capacity

It was obvious that most of the prepared DAE-NLCs shows %EE higher than 85% as seen in table (2), and this is may be due to the higher solubility of DAE in both solid and liquid lipids utilized in the preparation process and also due to its low aqueous solubility. Therefore, the EE% is mainly depends on the physicochemical properties of both the drug to be encapsulated and the lipid phase used [32].

Particle Morphology

As seen in figure (3) the AFM image shows a spheroidal particle shape with a diameter consistent with results obtained from DLS analysis, and with particle diameter of about 60 nm with no particle aggregation, the SEM image of the lyophilized DAE-NLCs shows that the nanoparticles are spherical in shape as seen in Figure (3).

Table 2: Physicochemical characterization data of DAE-NLCs formulations

Formula code	PSA (nm)	PDI	ZP (mV)	EE%	LC%
N1	351.6±7.13	0.284±0.001	-16.39±0.67	92.31±2.89	9.89±0.24
N2	214.2±6.5	0.227±0.009	-20.87±0.51	92.83±1.53	8.29±0.13
N3	175±8.82	0.313±0.007	-25.48±1.21	93.49±1.15	8.34±0.12
N4	218.7±10.84	0.326±0.012	-17.43±0.98	94.47±3.21	7.33±0.20
N5	331.4±7.12	0.389±0.043	-10.98±0.55	94.51±2.08	9.45±0.12
N6	296±3.75	0.377±0.015	-11.98±0.23	97.16±1.53	8.04±0.07
N7	169.4±8.60	0.317±0.006	-15.28±2.31	98.54±2.52	8.44±0.21
N8	184.9±9.30	0.322±0.005	-12.32±1.91	98.78±1.07	8.02±0.29
N9	433.5±27.15	0.294±0.021	-12.51±0.42	88.28±2.75	9.84±0.22
N10	283.1±13.52	0.005±0.001	-13.91±1.40	89.96±3.06	8.97±0.25
N11	196.3±1.75	0.287±0.009	-27.6±3.21	90.66±2.32	8.38±0.21
N12	62.4±5.75	0.286±0.001	-33.81±0.73	92.42±2.31	7.69±0.17

PSA, particle size, ZP, zeta potential, PDI, polydispersity index, EE, entrapment efficiency, LC, loading capacity.-Results represents mean values± SD (n=3)

Solid State Characterization

The thermo gram of the lyophilized DAE loaded NLCs (N12) did not show drug endothermic peak which indicates that the drug is in the amorphous state and the drug molecules are perfectly entrapped within the lipid matrix (Figure not shown), this could be related to the fact that NLCs prepared by rapid cooling of hot nanoemulsion rendering the drug molecules entrapped unable to crystallized, furthermore the surfactant molecules exists will inhibit the drug crystallization [33]. On the other hand the PXRD diffraction pattern of pure DAE, physical mixture and lyophilized DAE-NLCs displayed in Figure (4) was consistent with that of the DSC thermogram, the XRD pattern of DAE shows numerous diffraction.

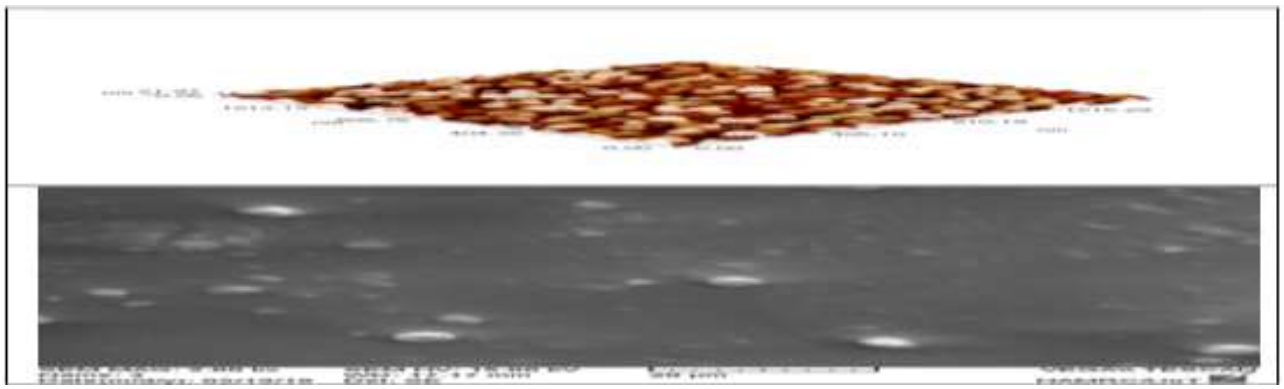

Figure 3: AFM and SEM image of DAE loaded NLCs

Peaks indicating the crystalline structure of the pure drug which was absent in the diffractogram of lyophilized DAE-NLCs indicating the presence of drug in the amorphous state within the lipid matrix [34].

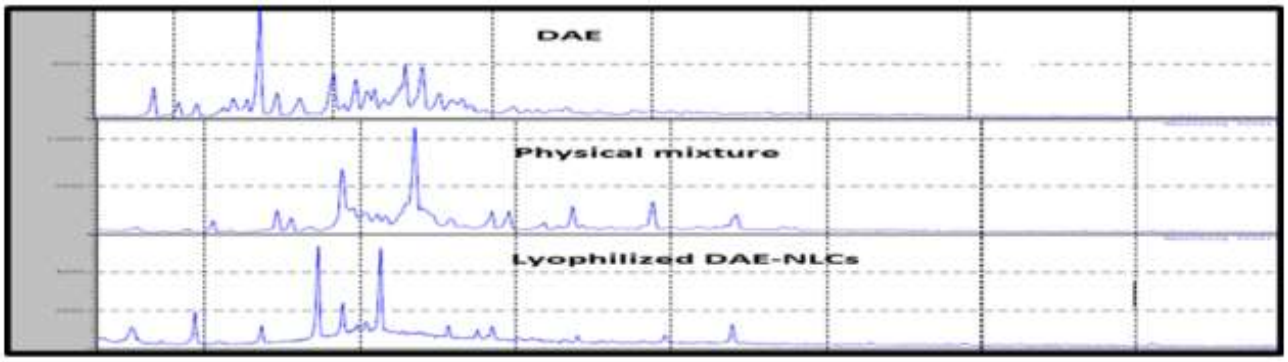

Figure 4: X-ray diffractogram of DAE, physical mixture and lyophilized DAE-NLC

FTIR study was conducted in order to determine if any physicochemical interaction between DAE and other excipients (Figure not shown), the FTIR spectrum of DAE showed a characteristic absorption spectra peaks at 1729.83cm^{-1} which is assigned for carbonyl group (C=O stretching) which is superimposed with the (C=O) stretching of ester or acyl groups, a broad peak at 3378.67 cm^{-1} (N=H vibration) indicate the presence of amine group, 2854.13cm^{-1} and 2931.27cm^{-1} (C-H stretch) of alkane groups [35]. GMS exhibit peaks at three positions that are 2955.3, 2915 and 2848.5 cm^{-1}.These peaks are due to (C-H) stretching of alkane and the carboxyl group (C=O) stretching peak is observed at 1729.6 cm-1[34].

Additionally, the FTIR spectrum of optimized formula (N12) showed fewer peaks of the drug which indicating a higher trapping degree of DAE inside the lipid matrix with no peaks shifting confirming no interaction between drug and the excipient.

In-vitro Drug Release

DAE-NLCs formulas N3, N7 and N12 was tested for drug release study as seen in figure (5), the drug release shows a biphasic pattern with initial burst release phase followed by a sustained releasing phase in a more controlled rate, about 35-45% of entrapped drug were released within the first 4 hours followed by slower rate of drug release for the rest 20 hours of the study, this biphasic mode of drug release could be related to the drug incorporation model in the nanoparticles, when the drug located in the outer most layer (drug-enriched shell model) a burst release would be expected, while in the second stage the drug will be released from inner core of the particle leading to longer diffusion path and a sustained drug release will occurs [37].

The particle size shows a significant effect on cumulative percent of drug release ($P<0.05$), and we can see that in N12 that having a particle size of 62.4nm with a 90.73% of drug released after 24hr, while N3 have a larger particle size of 175nm shows only 55.4% of drug released in the same period, because of higher surface area for drug diffusion to the surrounding media [23].

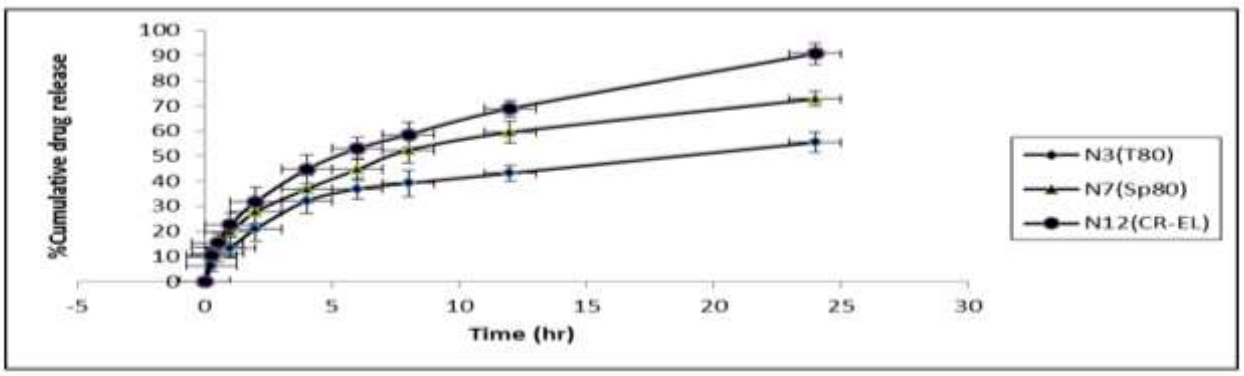

Figure 5: Cumulative percent of drug release from different DAE-NLCs formulations

Kinetic Drug Release Modeling

The obtained data shows best fit to the Korsmeyer-Peppas model with a higher regression coefficient (R2) in the range of (0.9783—0.9981) as seen in table (3), indicating that diffusion, matrix erosion or relaxation contributes to the release mechanism.

Formula N12 shows Korsmeyer (n) values greater than 0.43 (i.e.: $0.43 < n < 0.85$) indicating that an anomalous transport mechanism of drug release which is matrix erosion and relaxation, while in case of formulas N3 and N7 they have an n values less than 0.43 and this mean a Fickian diffusion is predominant release mechanism [38].

Short-term Stability Study

The results of particle size distribution shows a significant increase in particle size and PDI at 25ºC storage temperatures ($P<0.05$) at the end of the study compared to the zero-day measures, and the rate of particle growth was higher at 25ºC temperature compared to 2-8ºC which shows minimum change in particle size and PDI, and a visible flocculation was noticed in the 30th day of storage at 25ºC, a similar results was observed in by other research group [39], and this may be explained by that the higher temperature was associated with an increase in particles collision rate and consequently, a higher possibility of particles aggregation to occur [40]. Table (4) shows the short-term stability data of selected formula N12. Also, ZP and the %EE were decreased to a higher extent at 25ºC. Therefore, DE-NLCs best stored at low temperature (2-8ºC) since it shows good stability indicator with relatively lower particle size growth and high ZP value.

Conclusion

In the present study, DAE was successfully formulated as NLCs drug delivery system utilizing the ultrasonication technique, glyceryl monostearate together with oleic acid were used as lipid matrix in addition to different types and ratios of surfactants, the obtained DAE-NLCs were found to be within the nanometric size range and a negative ZP indicating a good particle stability, the surface morphology study showing a spheroidal particle shape and the solid state characterizations were conducted to prove the drug existence as amorphous state entrapped within the lipid matrix.

The in-vitro release study shows a controlled release profile for 24 hours and the release data were best fitted to Korsmeyer-Peppas kinetic model. In conclusion NLCs prepared an analyzed in our study could be a promising delivery platform to deliver DAE in controlled manner and as a future perspective, further assay to evaluate DAE-NLCs therapeutic potential are needed to be performed.

Conflict of Interest

The authors declare no conflict of interests.

Acknowledgments

Special thanks to the Department of Pharmaceutics/ Collage of Pharmacy-University of Baghdad and the Center of Nanotechnology Research in the University of Technology for providing laboratory facilities to make this work possible.

Table 3: The release kinetic modelling data of DAE-NLCs formulas

Formula Code	Zero order		First order		Higuchi model		Korsmeyer–Peppas model		
	k_o	R^2	k_1	R^2	k_H	R^2	K	R^2	n
F3	3.059	0.3987	0.053	0.7084	12.824	0.9491	16.457	0.9809	0.395
F7	3.632	0.2651	0.076	0.6986	15.389	0.9186	20.643	0.9783	0.392
F12	4.832	0.5353	0.126	0.9320	19.938	0.9844	22.498	0.9978	0.472

Table 4: the short-term storage parameters of optimized formula N12

	Day zero		Day 45th		Day 90th	
	2-8ºC	25ºC	2-8ºC	25ºC	2-8ºC	25ºC
PSA(nm)	62.4±5.75	62.4±5.75	62.4±5.75	63.7±0.8	99.5±1.2	71.2±1.6
PDI	0.286±0.001	0.286±0.001	0.286±0.001	0.291±0.032	0.305±0.041	0.315±0.054
ZP (mV)	-33.81±0.73	-33.81±0.73	-33.81±0.73	-31.82±0.84	-27.6±1.2	-31.62±1.13
EE%	92.42±2.31	92.42±2.31	92.42±2.31	90.31±1.31	88.53±1.12	89.78±0.53
PSA: particle size analysis, PDI: polydispersity index, ZP: zeta potential, %EE: percent entrapment efficiency						

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