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Original Article

STUDY ON CAUSE–EFFECT RELATIONS AND OPTIMIZATION OF EXEMESTANE-LOADED NANOSTRUCTURED LIPID CARRIERS

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ABSTRACT

Objective: Exemestane is an anti-breast cancer drug, possesses low water solubility and low permeability. This work aimed at the cause-effect relations and optimization of exemestane-loaded nanostructured lipid carriers (EXE-NLCs) for oral delivery.

Methods: Excipient screening was based on exemestane solubilities and the emulsification efficiency of surfactants. A D-optimal design based on three independent variables was applied to evaluate the cause-effect relations and optimise EXE-NLCs formulation. The particle size (PS), polydispersity index (PDI), entrapment efficiency (EE) and drug loading (DL) were investigated with respect to three independent variables including liquid lipid to total lipid ratio (X_1), surfactant concentration (X_2), total lipid concentration (X_3).

Results: EXE-NLCs were prepared by a hot sonication method employing Labrafac CC and Compritol 888ATO as liquid and solid lipids, respectively, and Cremophor RH40 as a surfactant and Lutrol E-400 as a co-surfactant. All investigated factors: liquid lipid to total lipid ratio, surfactant concentration and total lipid concentration showed significant influences on physicochemical characteristics of EXE-NLCs. The optimal EXE-NLC formulation was composed of liquid lipid to total lipid ratio (X_1) of 24 % (w/w), surfactant concentration (X_2) of 4 % (w/v) and total lipid concentration (X_3) of 4 % (w/v). The PS, PDI, EE and DL of the optimized EXE-NLCs were found to be 41.787 nm; 0.11; 97.605 % and 1.935 %, respectively. The optimized formulation was experimentally examined which demonstrated a good agreement between experimental and predicted values.

Conclusion: The cause-effect relations and optimization of EXE-NLCs were investigated and reported for the first time. EXE-NLCs formulation was successfully optimized using D-optimal design and merits further study.

Keywords: Exemestane, Optimization, NLC, BCPharSoft OPT software

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INTRODUCTION

Exemestane (fig. 1) is an anti-breast cancer drug inhibits irreversibly the activity of aromatase, the key enzyme that converts androgens to oestrogens [1]. Exemestane has been approved by the food and drug administration (FDA) for the treatment of breast cancer in postmenopausal women [2].

However, exemestane is a BCS (bio pharmaceutics classification system) class IV drug with a poor water solubility (0.08 mg/ml) and low permeability. Therefore, the bioavailability of exemestane was reported to be low in various animal models at a single dose of 25 mg [3].

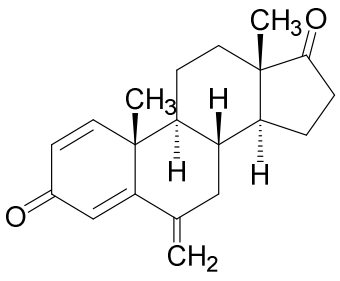

Fig. 1: Structure of exemestane [3]

Different formulation strategies have been employed to overcome the aforementioned biopharmaceutical challenges associated with exemestane such as pro-liposomes [4], polycaprolactone nanoparticles [5], poly (D, L-lactide-co-glycolide)/montmorillonite nanoparticles [6], polymeric nanoparticles [7] and self-emulsifying drug delivery system [3]. However, there are missing studies for lipid nanoparticles.

Lipid nanoparticles, such as solid lipid nanoparticles (SLNs) and the nanostructured lipid carriers (NLCs) are the promising carriers for a lipophilic molecule due to their potential to increase the solubility of the lipophilic drug [8, 9]. NLCs are second-generation of lipid nanoparticles developed using a blend of solid and liquid lipid. NLCs offer many advantages such as good biocompatibility, controlled drug release and the possibility of production on the large industrial scale [10, 11]. Moreover, a high drug loading efficiency can be achieved with the use of NLC [12, 13].

The aim of this study was to investigate the cause-effect relations between independent and dependent variables and develop the formulation of EXE-NLCs using D-optimal design and BCPharSoft OPT intelligent software.

MATERIALS AND METHODS

Materials

Exemestane (98.90 % purity) was purchased from Qilu Pharmaceutical Co., Ltd. (China). Compritol 888ATO (glyceryl dibehenate), Geleol mono and diglycerides NF (Glycerol monostearate 40-55 type 1, GMS), Capryol 90 (propylene glycol monocaprylate), Maisine 35-1 (glyceryl monooleate), Labrasol (caprylocaproyl polyoxylglycerides), Labrafac CC (caprylic/capric triglycerides), and Labrafac lipophile WL 1349 (caprylic/capric triglycerides) were received from Gattefosse (Saint-Priest Cedex, France) via Sapharchem Co., Ltd (Vietnam). Cremophor RH40 (Polyoxyl 40 Hydrogenated Castor Oil), Cremophor EL (polyoxyl 35 castor oil) and Lutrol E-400 (polyethylene glycol) were the gifts from BASF (Germany). Miglyoil 812 (caprylic/capric triglycerides) was received from Sasol GmBH (Germany). Gac oil was a gift from

Vnpofood (Vietnam). All other chemicals were of analytical grade.

Analytical method of exemestane

Exemestane was analyzed by an Azura HPLC system (Knauer, Germany). The separation was performed on a Syncronis C18 column (250 x 4.6 mm; 5 µm) (Thermo Scientific, USA). The mobile phase was acetonitrile: water (75:25, v/v) with a flow rate of 1 ml/min. Detection was performed at a wavelength of 247 nm at 30 °C. The sample injection volume was 20 µl.

Excipient screening

Solubility studies

The saturation solubility of exemestane in various liquid lipids (Miglyol 812, Labrafac CC, Labrafac lipophile WL 1349, Gac oil, Capryol 90 and Maisine 35-1), surfactants (Labrasol, Cremophor RH40, Cremophor EL), co-surfactant (Lutrol E-400) were determined. Excess amounts of exemestane were added into individual tubes containing 1 ml of different liquid lipids, surfactants, co-surfactants and mixed using a vortex mixer (Vortex-Genie 2, Scientific Industries, Inc., New York, USA).

The capped tubes were then continuously shaking to reach equilibrium for 72 h at 25 °C on a Labquake shaker (Barnstead Thermolyne, USA). The equilibrated samples were centrifuged at 10,000 rpm for 10 min. The supernatant was separated and filtered through a 0.22 µm membrane and diluted in ethanol. Exemestane concentrations were analyzed using a validated HPLC method. Each determination was carried out in triplicate and the results were reported as±SD [14].

Miscibility of solid and liquid lipids

The three liquid lipids in which exemestane exhibited maximum solubilities and two solid lipids (Geleol mono and diglycerides NF, Compritol 888ATO) were subjected to a miscibility test. The melted mixtures of solid and liquid lipids in a ratio of 3:1 (w/w) were checked by visual observation to select the binary lipid phase. Each mixture was observed for clarity, uniformity, turbidity, phase separation and left to cool down to room temperature. The mixture which exhibited good miscibility was selected as the binary lipid phase for the NLC design.

Surfactant screening

The binary lipid phase was melted at 85 °C. The homogenous aqueous phase included surfactant, co-surfactant and double distilled water was heated to the same temperature and added drop wise into lipid phase to a liquid lipid/solid lipid/surfactant/co-surfactant ratio of 3:9:9:1 (w/w/w/w). The mixture was then dispersed under magnetic stirring at 600 rpm for 15 min and diluted by cold water (4 °C) to a volume of 50 ml and continued stirring for 5 min. The best surfactant was selected based on its emulsifying potential.

Preparation of EXE-NLCs

EXE-NLCs were prepared by a hot sonication technique [15]. 40 mg exemestane was dissolved in a melted mixture of solid and liquid lipid at 85 °C as a lipid phase. The aqueous phase containing surfactant, co-surfactant and double distilled water was heated to the same temperature and added drop by drop into the lipid phase under continuous magnetic stirring at 600 rpm for 15 min followed by sonication (Sonorex, RK-1028, Bandelin, Japan) at 85 °C to obtain microemulsion. The hot microemulsion was diluted by cold water (4 °C) to a volume of 50 ml and continued stirring for 1 h to cool down to room temperature. The quantities of excipients were varied at different levels (table 1).

Experimental design and data analysis

A D-optimal design with a total of 10 experimental runs was generated by Design Expert software (version 6.0.6, Stat–Ease Inc., Minneapolis, USA) to study the effects of independent variables on dependent variables. Liquid lipid to total lipid ratio (X_1), surfactant concentration (X_2) and total lipid concentration (X_3) were selected as three independent variables whereas particle size (Y_1), poly-dispersity index (Y_2), entrapment efficiency (Y_3) and drug loading (Y_4) were chosen as four dependent variables.

Liquid lipid to total lipid ratio (X_1) and surfactant concentration (X_2) were studied at two levels and total lipid concentration (X_3) was studied at three levels. For each independent variable, the experimental range was selected based on the results of initial trials. The details of the design are listed in table 1. All formulations in these experiments were conducted in triplicate. Data were exhibited as mean±standard deviation [16].

The optimized formulation was performed in triplicate for validation. The observed response data of the optimized formulation were compared with their predicted data created by BCPharSoft OPT software using SPSS version 20.0 (SPSS, Inc., Chicago, IL, USA).

Particle size and polydispersity index

Particle size and a polydispersity index of NLCs were measured at 25 °C by photon correlation spectroscopy using a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK) at a fixed angle of 173 ° in 10 mm diameter cells [17]. Samples were diluted ten-fold with double distilled water to produce a suitable scattering intensity before analysis. All measurements were carried out in triplicate.

Determination of entrapment efficiency and drug loading

The entrapment efficiency (EE) and drug loading (DL) was indirectly determined by measuring the concentration of free exemestane in the lower chamber of centrifugal filter tubes with a molecular weight cut-off of 3500 Da (Amicon Ultra 0.5 ml, Merck Millipore, Germany). About 400 µl of NLCs suspension was placed in the upper chamber of centrifugation tube, followed by centrifugation at 30000 x g (Hermle, Z36HK, Germany) at 20 °C for 15 min [18]. The free exemestane in the filtrates was analyzed by an HPLC analytical method described aforementioned. The studies were performed in triplicate. Entrapment efficiency and drug loading were calculated by the following equations:

Table 1: Variables in experimental design

	Levels		
	Low	**Medium**	**High**
Independent variables			
X_1: Liquid lipid to total lipid ratio (%, w/w)	10	30	
X_2: Surfactant concentration (%, w/v)	2	4	
X_3: Total lipid concentration (%, w/v)	4	6	8
Dependent variables	**Constraints**		
Y_1: Particle size (nm)	Minimum		
Y_2: Polydispersity index	Minimum		
Y_3: Entrapment efficiency (%)	Maximum		
Y_4: Drug loading (%)	Maximum		

The results of experimental design were analyzed using BCPharSoft OPT software (Vietnam). The best fitting model was chosen. In order to achieve a better understanding of the cause-effect relations between the independent and dependent variables, the 3D diagrams of the fitted models were depicted. D-optimal design employed for the study is shown in table 2.

$$\%EE = \frac{total\ amount\ of\ drug - \ amount\ of\ free\ drug}{total\ amount\ of\ drug} \times 100\%$$

$$\%DL - \frac{total\ amount\ of\ drug - \ amount\ of\ free\ drug}{amount\ of\ total\ lipid} \times 100\%$$

RESULTS AND DISCUSSION

Analytical method of exemestane

A well-resolved HPLC chromatogram of exemestane was obtained following the use of an acetonitrile: water mobile phase in a ratio of (75:25, v/v). The total run time was approximately 7 min and the retention time of exemestane was at 5.7 min (fig. 2). In a concentration range of 8–200 µg/ml, a good correlation coefficient was observed between peak areas and concentrations of exemestane standard solutions (r^2=0.9995). Recovery values ranged from 93.06 % to 99.39 %. The coefficient of variation of the within-day precision, expressed as relative standard deviation, was found to be 1.88 %. These results indicated that the method was reliable and reproducible.

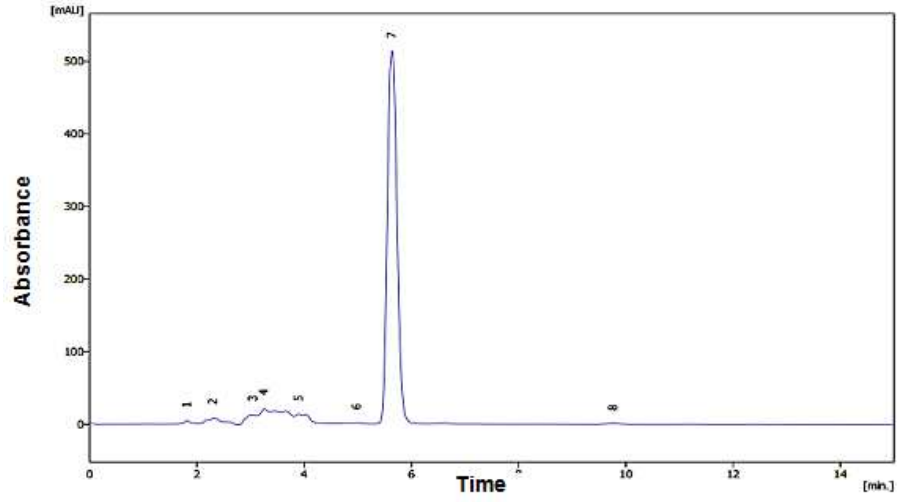

Fig. 2: HPLC chromatograms of exemestane (R_t = 5.7 min)

Excipient screening

For the selection of liquid lipids, the solubility of exemestane is one of the most important factors. According to the results of solubility studies in liquid lipids (fig. 3), Capryol 90 exhibited the highest solubility of 78.36±1.03 mg/ml. Maisine 35-1 and Labrafac CC showed the lower solubilities of 43.32±0.55 mg/ml and 24.29±0.46 mg/ml, respectively.

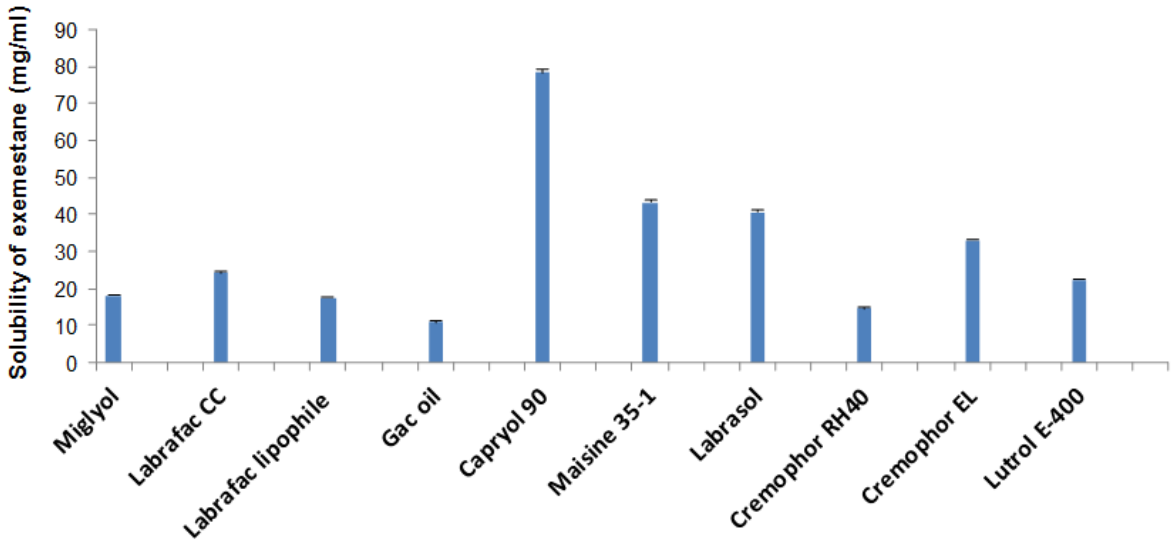

Fig. 3: Solubility of exemestane in liquid excipients at 25 °C. The values represented mean±SD (n=3)

The miscibility tests were carried out to avoid the formation of liquid lipid droplets which could lead to the co-existence of EXE-NLCs and o/w microemulsion. Three liquid lipids with the higher solubilizing capacity (Capryol 90, Maisine 35-1, Labrafac CC) and

two solid liquids (Geleol, Compritol 888ATO) were subjected to miscibility studies. The mixture of Labrafac CC and Compritol 888ATO showed a very good miscibility. Therefore, Labracfac CC and Compritol 888ATO were selected as liquid and solid lipids for NLC formulation.

Surfactant reduces the interfacial tension between the lipid phase and the aqueous phase, therefore, it was important to choose appropriate surfactant to obtain the desired size and the long-term physical stability of NLCs. Among 3 surfactants, the solubility of exemestane in Labrasol (40.66±0.80 mg/ml) was higher than CremophorRH40 (14.70±0.27 mg/ml) and Cremophor EL (33.06±0.44 mg/ml). However, emulsifying capacity for the selected lipid blend (Labrafac CC and Compritol 888ATO) of Labrasol was the lowest among three screened surfactants. The emulsion obtained from the mixture of the lipid blend and Labrasol was found to be white, instable and rapidly delaminated at room temperature while the other emulsions produced by using other surfactants (Cremophor RH40 or Cremophor EL) were clear, transparent and stable. Moreover, it was observed that the mixture of Cremophor RH40 and Lutrol E-400 provided a clearer and more stable emulsion than the mixture of Cremophor EL and Lutrol E-400 at the same ratio. Therefore, Cremophor RH40 was selected as a surfactant for NLC formulation.

Optimization data analysis and validation of optimization model

After the initial screening of excipients, Labrafac CC, Compritol 888ATO, Cremophor RH40 and Lutrol E-400 were chosen as liquid lipid, solid lipid, surfactant, co-surfactant of EXE-NLC formulations. The values of independent variables and their responses of 10 formulations generated by Design Expert software are shown in table 2. The ranges of particle size (Y_1), polydispersity index (Y_2), entrapment efficiency (Y_3) and drug loading (Y_4) were found to be 36–234 nm, 0.112–0.383, 91.24–96.60 % and 0.96–1.94 %, respectively.

Table 2: The independent variables of 10 formulations (F1–F10) and their responses

Formulation	Independent variables			Dependent variables			
	X_1 (%)	X_2 (%)	X_3 (%)	Y_1 (nm)	Y_2	Y_3 (%)	Y_4 (%)
F1	30	2	6	127.85±5.59	0.142±0.007	95.68±0.36	1.24±0.006
F2	10	4	8	102.70±2.97	0.220±0.009	95.36±0.10	0.96±0.006
F3	30	4	8	112.45±0.64	0.200±0.018	96.60±0.03	0.97±0.002
F4	30	2	4	101.16±2.60	0.161±0.002	95.30±0.98	1.94±0.093
F5	10	4	6	52.40±3.73	0.160±0.019	95.10±0.52	1.27±0.009
F6	30	2	8	180.85±1.06	0.184±0.028	96.29±0.07	0.96±0.020
F7	10	4	4	36.26±0.01	0.128±0.006	95.77±0.81	1.89±0.065
F8	10	2	4	112.95±3.75	0.215±0.015	91.24±0.03	1.75±0.022
F9	30	4	4	45.10±0.46	0.112±0.006	96.26±0.10	1.88±0.017
F10	10	2	6	234.25±4.45	0.383±0.006	92.85±0.38	1.19±0.050

Values are expressed as mean±SD; n=3, The data in table 2 were used as inputs for BCPharSoft OPT to study on the cause-effect relations and optimize the EXE-NLC formulation., Training parameters were set at: -Test groups: $Y_1^{(6,9)}$, $Y_2^{(2,7)}$, $Y_3^{(6,8)}$ and $Y_4^{(2,7),}$ -Transfer function: Back Propagation Learning

Table 3: Model statistics from BCPharSoft OPT outputs

Dependent variables	R^2 training	R^2 test
Y_1	0.99	1.00
Y_2	0.95	0.98
Y_3	0.92	0.97
Y_4	1.00	1.00

All R^2 values which were found to be more than 0.9 indicated a very good reliability of the models (table 3). Therefore, these models could be used for multivariate optimization.

The three-dimensional (3D) response surface plots were used to study the interaction effects of two independent variables on the dependent variables at one time when the third variable was kept at a constant level.

Effects of variables on particle size

The average particle size of all formulations (F1-F10) was found to be between 36.26 nm and 234.25 nm (table 2). Particle size analysis demonstrated the positive relationships with liquid lipid to total lipid ratio (X_1), total lipid concentration (X_3) and negative relationship with surfactant concentration (X_2).

Surfactant concentration plays an important role in determining the particle size of EXE-NLCs. It is evident from fig. 4A that particle size (Y_1) decreases with increasing surfactant concentration (X_2). This relation was in accordance with the rule reported previously by Araujo J [19], Chaudhary S [20], Ferreira M [21], Gonzalez-Mira E [22], Jain K [23], Pokharkar VB [24], Pradhan M [18], Phatak AA [25], Shah M [26], Yang CR [27]. Increase in surfactant concentration results in the reduction of interfacial tension between the lipid and aqueous phase, thus, produces the smaller particles. Additionally, the surfactant molecules could stabilize and prevent the coalescence of microemulsion droplets.

As presented in fig. 4B, the particle size (Y_1) increases with raising the total lipid concentration (X_3). Similar results were previously reported by Araujo J [17], Jain K [23], Aslam M [28], Kumbhar DD [29], Mandpe L [30].

This can be explained by the tendency of increasing collisions and aggregation of microemulsion droplets at high concentration. When the total lipid concentration increases, the viscosity increases and leads to higher surface tension and thus larger particle size.

Effects of variables on polydispersity index

Polydispersity index is an indicator of the homogeneity of particle size distribution. The higher the polydispersity index, the lower the uniformity of nanoparticles. The polydispersity index (Y_2) of 10 formulations varied from 0.112±0.006 to 0.383± 0.006.

It is observed that PDI values (Y_2) decrease with decreasing total lipid concentration (X_3) from high to middle values (fig. 5). Similar results were also reported by Araujo J [17]. Nanoparticles tend to accumulate and increase aggregation at high total lipid concentration due to the van der Waal forces of attraction and may lead to an increase in PDI values.

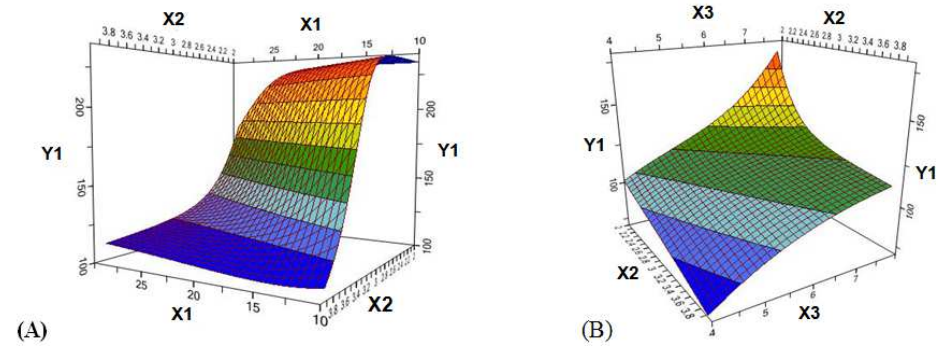

Fig. 4: Response surface plot showing the effect of (A) liquid lipid to total lipid ratio (X_1) and surfactant concentration (X_2) on particle size (Y_1); (B) surfactant concentration (X_2) and total lipid concentration (X_3) on particle size (Y_1)

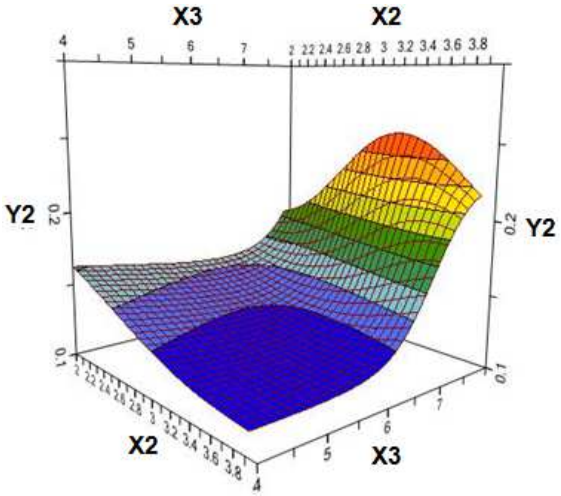

Fig. 5: Response surface plot showing the effect of surfactant concentration (X_2) and total lipid concentration (X_3) on polydispersity index (Y_2)

Effects of variables on entrapment efficiency

The entrapment efficiency of 10 formulations was found to be between 91.24±0.03 % and 96.60±0.03 % as shown in table 2. Entrapment efficiency demonstrates a significant positive relationship with liquid lipid to total lipid ratio (X_1), surfactant concentration (X_2) and total lipid concentration (X_3) (fig. 6).

The entrapment is mainly due to the solubility of exemestane in the solid and liquid lipids and the partition of exemestane between the oil phase and the aqueous phase. Exemestane is a lipophilic compound, therefore, higher exemestane loading could be achieved at a high liquid lipid to total lipid ratio (X_1) and total lipid concentration (X_3) which decreases the exemestane partition in the outer space and leads to higher entrapment efficiency. The incorporation of liquid lipid into solid lipid could lead to a reduction of crystallinity and increase the imperfections in the crystal lattice which helps to accommodate the higher amount of exemestane and results in increasing entrapment efficiency. This rule was found to be in accordance with the rule reported previously by Gonzalez-Mira E [22], Jain K [23], Pradhan M [18], Aslam M [28], Zhang W [17], Zhang X [31].

It is observed from fig. 6B that entrapment efficiency (Y_3) increases when surfactant concentration (X_2) increases. Similar results were also reported by Chaudhary S [20], Gonzalez-Mira E [22], Jain K [23], Pradhan M [18], Phatak AA [25], Shah M [26], Yang CR [27], Aslam M [28], Mandpe L [30]. The positive relationship of entrapment efficiency with the surfactant concentration can be attributed to the ability of the surfactant system to increase the viscosity of aqueous phase with increasing concentration thereby decreasing the diffusion speed of exemestane and increasing the entrapment efficiency. The positive effect of surfactant concentration on entrapment efficiency could also be explained by the increased surface area when smaller particles are formed, where exemestane molecules were adhered or attached. This also could be due to the availability of adequate surfactant which facilitated exemestane to remain within the lipid particles and/or on the surface of the particles results in high entrapment efficiency.

Effects of variables on drug loading

The drug loading of different formulations was found to be between 0.96±0.020 % and 1.94±0.093 % as shown in table 2. Fig. 7 shows that drug loading (Y_4) increases when liquid lipid to total lipid ratio (X_1) increases.

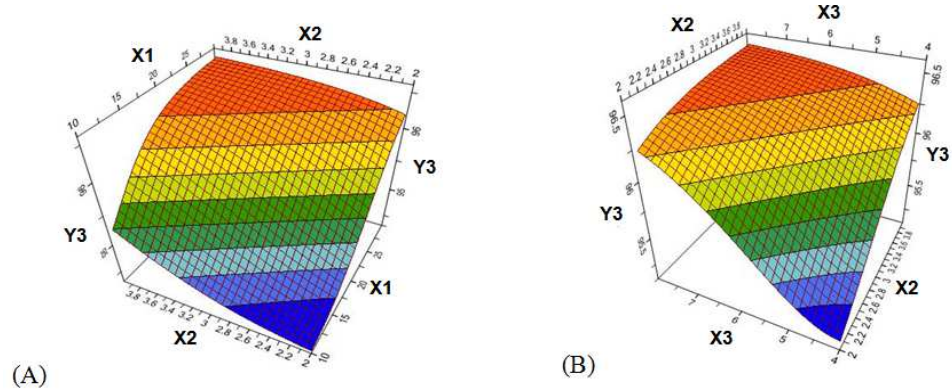

Fig. 6: Response surface plot showing the effect of (A) ratio of liquid lipid to total lipid (X_1) and surfactant concentration (X_2) on entrapment efficiency (Y_3); (B) surfactant concentration (X_2) and total lipid concentration (X_3) on entrapment efficiency (Y_3)

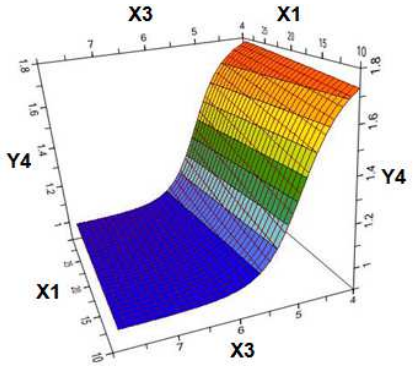

Fig. 7: Response surface plot showing the effect of liquid lipid to total lipid ratio (X_1) and total lipid concentration (X_3) on drug loading (Y_4)

Liquid lipid acts as a solubilizing agent for exemestane at room temperature and provides the additional spaces for exemestane to accommodate and prevents exemestane from diffusing to the external phase, results in increasing drug loading. This relation was found to be in accordance with the rule reported previously by Jain K [23], Yang CR [27], Zhang X [31].

Optimisation of EXE-NLC formulation

The optimized EXE-NLC formulation was achieved with 24 % (w/w) liquid lipid to total lipid ratio, 4 % (w/v) surfactant concentration and 4 % (w/v) total lipid concentration. Three replicated batches of the optimised EXE-NLC formulation were prepared to confirm the validity of the optimization procedure. The EXE-NLCs showed a narrow size distribution (fig. 8). Particle size, polydispersity index, entrapment efficiency and drug loading of the optimized EXE-NLC formulation were found to be at 41.787±1.282 nm, 0.110±0.008, 97.605±0.503 % and 1.935±0.018 %, respectively.

Table 4 demonstrates that the observed values were in good agreement with the predicted values (p>0.05). Therefore, the optimized EXE-NLC formulation was confirmed with 40 mg exemestane, 0.48 g Labrafac CC, 1.52 g Compritol 888ATO, 2.00 g Cremophor RH40, 0.2 g Lutrol E-400 and distilled water qs to 50 ml.

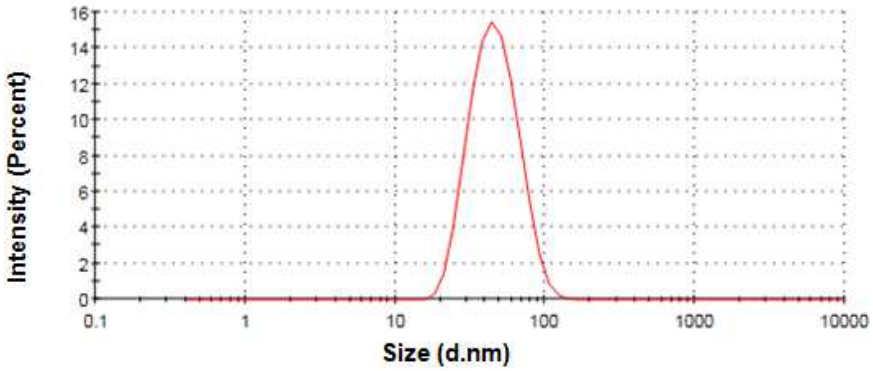

Fig. 8: Size distribution of optimized EXE-NLC formulation. The values are expressed as mean±SD; n=3

Table 4: Comparison of the predicted and observed responses of the optimized EXE-NLC formulation

Responses	Y_1 (nm)	Y_2	Y_3 (%)	Y_4 (%)
Predicted	42.823	0.121	96.396	1.906
Observed	41.787±1.282	0.110±0.008	97.605±0.503	1.935±0.018
P-value	0.371	0.195	0.077	0.148

Values are expressed as mean±SD; n=3

CONCLUSION

NLC preparation of exemestane is possible using a hot sonication technique. Effects of liquid lipid to total lipid ratio, surfactant concentration, and total lipid concentration on the particle size, polydispersity index, entrapment efficiency and drug loading of EXE-NLCs were investigated. The optimized EXE-NLC formulation suggested by the BCPharSoft OPT intelligent software contained 40 mg exemestane, 0.48 g Labrafac CC, 1.52 g Compritol 888ATO, 2.00 g Cremophor RH40, 0.2 g Lutrol E-400 and distilled water qs to 50 ml. Three replicates of the optimized formulation were prepared and the observed responses were in good agreement with the predicted values which confirmed the optimized EXE-NLC formulation.

AUTHOR CONTRIBUTION

There are three authors who contribute to this manuscript. The percentages of the contribution of Le Quoc Thang, Nguyen Duc Hanh and Do Quang Duong are 40%, 45% and 15%, respectively.

CONFLICT OF INTERESTS

All authors declare that this article content has no conflict of interest

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