Int. J. Nano Dimens., 9 (3): 298-313, Summer 2018

ORIGINAL ARTICLE

Synthesis of Metformin Hydrochloride nanoliposomes: Evaluation of physicochemical characteristics and release kinetics

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Received 29 November 2017; revised 07 March 2018; accepted 24 April 2018; available online 25 April 2018

Abstract

Metformin hydrochloride, a drug group of biguanides, with significant efficacy in the treatment of diabetes type II, is decomposed before reaching to the stomach and is wasted in great losses. In this study, the nanoliposomes of metformin hydrochloride were prepared with Bingham method. At first, the single factor test is done. Design variables are: lecithin to cholesterol ratio, organic solvent phase to aqueous solvent phase and formation time. The optimal ratios *in vitro* are obtained 7 : 1, 4 : 1, and 2.20, respectively. Entrapment efficiency is obtained 89.74. Then, by design of expert software, RSM method is used in five areas for evaluating the data. Optimum conditions were 6.97:1, 4.46 : 1, and 151.29, respectively and entrapment efficiency is obtained 90.9. Finally, phosphatidylethanolamine has replaced the lecithin and the entrapment efficiency was obtained as 93.04. So, using of phosphatidylethanolamine has been better than lecithin in pH=7.4. Various physicochemical characteristics of Metformin Hydrochloride nanoliposomes were determined and evaluated. The mean size of metformin hydrochloride nanoliposomes based on phosphatidylethanolamine and lecithin with well-defined spherical shape was 52nm and 83nm, respectively. Based upon the *in vitro* release profiles, Metformin hydrochloride nanoliposomes exhibited a sustained-releasing potential in addition to the release behavior followed the Weibull equation. Release ratio for nanoliposomes based on phosphatidylethanolamine and lecithin are obtained 20% and 36.66%, respectively. The results indicated that the encapsulation of metformin hydrochloride into nanoliposomes proved to be a promising technology for more widespread uses. Encapsulation by phosphatidylethanolamine nanoliposome and release ratio by lecithin nanoliposome has been desired conditions.

Keywords: *Diabetes type II, Encapsulated efficiency, In vitro release, Metformin hydrochloride, Nanoliposome.*

How to cite this article

Niknejad K, Sharifzadeh M, Motallebi Tala Tapeh S. Synthesis of Metformin Hydrochloride nanoliposomes: Evaluation of physicochemical characteristics and release kinetics. Int. J. Nano Dimens. 2018; 9 (3): 298-313.

INTRODUCTION

Diabetes is one of the major causes of heterogeneous endocrine disorders in which hyperglycemia is the manifest feature. Type 1 diabetes is an autoimmune disturbance that results in an insulin deficiency. Type 2 diabetes contains abnormal features including relative insulin deficiency, insulin resistance involving myocytes and adipocytes, and hepatic insulin resistance. Type 3 diabetes is related to circuit of pregnancy that require surveillance intensity [1, 2]. It's showed that hyperglycemia cannot be adequately administrated by diet alone. So, metformin hydrochloride (MH) (as an antidiabetic drug) decreases blood glucose in situations of basal and postprandial-elevated in patients with non-insulin-dependent diabetes mellitus (type 2 diabetes). MH is absorbed in gastrointestinal system imperfectly with the bioavailability of 40-60% [3] and 20-30% of an oral dose is regained in faeces [4, 5]. Out up to half the patient's recipienting MH drug show gastrointestinal

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adverse effects, including diarrhea, nausea, abdominal discomfort, flatulence, dyspepsia, and anorexia [6] are transient within a few days to weeks after the treatment. However, these effects can be lessened by taking MH with food, and/or temporarily lowering the dosage [7]. Due to the short half-life of Plasma (1.5-3 hrs.) and also high aqueous solubility of MH controlled release is required [8]. Since reaching superior therapeutic efficacy and patient expectancy related to the epoch of treatment, new drug delivery systems such as nanoliposomes are developed [9]. In fact, spherical vesicles that accompanies phospholipid bilayer membranes and they used to deliver the drug into a cell, is called nanoliposome [10, 11]. Nanoliposomes are one or bilayer membranes and their size vary between very small (0.025 µm) to large (2.5 µm) vesicles. The amounts of drug encapsulation in the nanoliposomes are affected by the size and the number of bilayers. So nanoliposomes are categorized as multilamellar vesicles (MLV); Oligo lamellar vesicles (OLV), Unilamellar vesicles(UV), small unilamellar vesicles (SUV), Medium sized unilamellar vesicles(MUV), large unilamellar vesicles (LUV), Giant unilamellar vesicles (GUV), and Multivesicular vesicles (MVV) [12]. Moreover, some studies showed that encapsulation of drugs in nanoliposomes have the significant increase in therapeutic efficacy in comparison to non-liposomal formulations. Furthermore, nanoliposomes have attracted great attention due to their significant properties such as biocompatibility, completely biodegradability, non-toxic, flexibility and non-immunogenic properties [13]. Nowadays, micro/Nano carrier technologies supporting targeted drug delivery to tissues are required to improve the therapeutic index of the carried drugs. Recently, some studies have been paid to the encapsulation of MH using various drug delivery systems. In 2006, Patel et al suggested floating drug delivery system such microspheres as metformin hydrochloride (MH) carriers in diabetes therapy [14]. In 2011, Sanjeev Dogra prepared the chitosan-polymer hydrogel bead for MH controlled release oral dosage form [15]. In 2012, Pritam Banerjee et al were prepared Pectin microspheres of metformin Hydrochloride (MH) by the water in oil. emulsion solvent evaporation technique [16]. In 2013, Kumar Sharma et al., [8] formulated metformin hydrochloride loaded microspheres prepared with polysaccharide extracted from natural sources and evaluated in vitro. Polysaccharides extracted from Bora rice, Dillenia indica and Abelmoschus sculentus and metformin hydrochloride loaded microspheres were prepared by the emulsion solvent diffusion method [8]. Shruthi et al. formulated and evaluated of pronanoliposome gel containing metformin hydrochloride using mannitol as a water soluble carrier [17]. In 2018, Marzban et al. Nano-niosome particles as carriers for delivery of deferoxamine were prepared using the reverse phase evaporation method. They were evaluated of morphology, drug release, cytotoxicity, and iron-chelating efficacy to compare with free drug formulation. The unique structure of niosome enables sustained release of the drug over extended periods [18]. In this study, nanoliposome was used as drug delivery system for encapsulation and controlled release of metformin hydrochloride (MH). The metformin hydrochloride nanoliposomes were prepared by thin film hydration technique. Also, effects of various parameters on the metformin hydrochloride loading were estimated and the optimization of encapsulation was performed by DOEs methodology. Furthermore, in order to evaluate the stability and integrity of nanoliposomes, the physicochemical characteristics of metformin hydrochloride nanoliposomes were investigated by entrapment efficiency using spectrophotometer UV-VIS, surface electron microscopy (SEM), determination of permeability, and in vitro release experiment.

EXPERIMENTAL

Materials and apparatus

L--phosphatidylcholine (lecithin), L--phosphatidyl ethanolamine, butylated hydroxyl toluene (BHT) and metformin hydrochloride (MH) were purchased from Sigma-Aldrich, (Co., 3050 Spruce Street, St. Louis, MO 63178 USA.) Cholesterol was purchased from E. Merck, Darmstadt. Ethanol, chloroform and distilled water were used of analytical grades. Scanning electron microscope (SEM) was recorded on the KYKY Co., EM-3200 instrument. UV spectra were recorded at 252 nm on UV1800-DP200A spectrophotometer (Taiwan).

Methods

Preparation of MH nanoliposomes

MH nanoliposomes were prepared by thin film hydration technique. Identically weighed

quantities of phosphatidylcholine and cholesterol were dissolved in the chloroform-ethanol mixture in the constant ratio (2 : 1) in a round-bottom flask. Then BHT, as an antioxidant, was added to the organic phase at the amount of 2% of the total lipids. The resulting mixture was stirred at 6rpm for 24 to 72 h. Then the mixture was evaporated at 50 ^{0}C for about 20 min and 80 rpm using a rotary evaporator under vacuum until thin lipid film was produced. The required amount of MH was dissolved in phosphate buffered saline (PBS) at pH 7.4, this mixture was added into a flask containing lipid film and was stirred at different times to create a homogenous mixture, followed by ultrasound at 30 ^{0}C for 5min and then stored at 4 ^{0}C [12].

Estimation of Entrapment Efficiency (EE) of nanoliposomes

Standard curve of MH: 10 mg of MH was dissolved in PBS (pH 7.4) in a 100-ml volumetric flask. 1, 2.5, 4, 5.5, 7, 8.5 and 10 ml of this solution was pipette into a 50-ml volumetric flask, and brought to volume and formulate into 2, 5, 8, 11, 14, 17 and 20 standard solution. The absorbance of these solutions at 252 nm was recorded with PBS 7.4 as the reference. The regression of absorption on concentration was determined. It exhibited a high quality association:

Y= 0.0016X-0.0018 (R^2= 0.9872) (1)

The absorbance of all clear suspensions was replaced into the regression equation above to estimate the concentration of MH. Each nanoliposome preparation experiment was performed triplicate. The entrapment efficiency (EE) of MH nanoliposomes was determined as follow as [19]:

$$EE\% = \frac{C_1 - C_2}{C_1} \times 100 \quad (2)$$

Where C_1 is the concentration of total MH and C_2 is the concentration of free MH in the structured nanoliposomes system.

Optimization of MH nanoliposomes preparation

The important process parameters are: lecithin-cholesterol ratio, organic phase-aqueous phase ratio and formation time were investigated by single factor experiments. On the basis of the single factor experimental results, major factors were confirmed and a response surface methodology (RSM) and Central Composite Design (CCD) were accomplished for optimization of nanoliposomes preparation process. Also phosphatidylethanolamine is replaced instead of lecithin when the other optimum parameters are fixed. Then EE% is determined again.

Surface Characterization

Scanning electron microscopy (SEM) studies was done to visualize the morphology of the MH nanoliposomes. A mini-drop of the liposomal suspension was paid in part on a lamellar, allowed to dry for several minutes. After air-dried at room temperature, the sample was monitored under the scanning electron microscope (SEM). The SEM studies for formulated nanoliposomes were shown in Fig. 7.

Determination of permeability

The permeability is an impressive index for evaluation of stability of MH nanoliposomes during storage. MH nanoliposomes were stored in a short time after preparation in a refrigerator (4 ^{0}C). The permeability was estimated at usual intervals. The equation is shown as follows [20,21]:

$$P\% = \frac{EE_1 - EE_2}{EE_1} \times 10 \quad (3)$$

where, P is permeability, and EE_1 and EE_2 are the entrapment efficiency before and after a time of storage, regularly.

Fourier Transform Infrared Spectroscopy (FTIR)

An FTIR spectrophotometer has an IR radiation source, interferometer and a detector. It can be used for qualitatively and quantitative purposes. FTIR is used to identify drugs, excipients and polymorphism. The interactions between Metformin hydrochloride and lipid membranes were evaluated by Fourier transform infrared spectroscopy (FT-IR). The FT-IR spectra were recorded with KBr pellets on a Nicolet 380spectrometer between 4000 and 400 cm^{-1}.

In Vitro drug release study

To estimate *in vitro* release of MH, the act of leaking MH from the liposomal suspension was investigated by a dialysis method. 5 ml of MH nanoliposomes suspension was placed in a dialysis bag, and then it was immersed in 100 ml of PBS at pH 7.4 to simulate the intestinal environment, and was incubated in a shaking incubator at 37 ^{0}C with stirring at 80 rpm [12].

Respectively 5 ml of the nanoliposomes

containing MH was sampled at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 4.30 h, at the same time an equal volume of fresh buffer was replenished. The concentration of MH in the samples was determined by calibration equation and UV absorbance. So, the dialysis behavior of MH mixed with PBS was performed using the same method. The cumulative release rate can be calculated by the following formula [22]:

$$Q = \frac{M_t}{M} \quad (4)$$

where, Q is cumulative release rate, and M_t is the amount of drug released from the nanoliposome at the given time, and M is the amount of MH initially encapsulated in the nanoliposomes.

Also, standard equations were used in evaluation of release kinetics is being in Table 1.

RESULTS AND DISCUSSION

Single factor experiments

The effect of three selected parameters on the entrapment efficiency of drug including lecithin-cholesterol ratio, organic phase-aqueous phase ratio, and formation time were investigated in single factor experiments.

Since drug ratio was fixed, the effect of the ratio of membrane material on encapsulation percentage is evaluated. The presence of cholesterol in lipid structure enhances membrane entirety [26]. The effect of different lecithin-cholesterol ratios (2:1, 4:1, 7:1, 10:1, 14:1) on the entrapment efficiency was studied. The other factors were MH (5mg), formation time (2h), organic phase-aqueous phase ratio (5 : 1), PBS at pH 7.4, and constant concentration. The lecithin-cholesterol ratio of 7 : 1 showed maximum entrapment efficiency (Fig.1a).

Cholesterol is used to stabilize the nanoliposome structure. The hydrogen bonding between hydroxyl groups of cholesterol and carbonyl groups of phospholipid molecules decrease the lipid membrane fluidity and inhibit the movement of fatty acid chains [27].

Organic phase-aqueous phase ratio is one of the most important factors affecting entrapment efficiency in thin film hydration method [12]. Different organic phase-aqueous phase ratios of 2:1, 3:1, 4:1, 5:1, and 6:1 were selected, while other preparation conditions were as follows: MH ratio 5mg, lecithin-cholesterol ratio 7:1, ultrasonic time 2h, PBS at pH 7.4.

According to the result (Fig. 1b) an increase of entrapment efficiency could be seen with the increase of ratio from 2:1 to 4:1. The entrapment efficiency reached the peak at the ratio of 4:1, and then reduced. We speculated that when the ratio was lower, the lipid materials could not sufficiently dissolve in organic solvent and thoroughly mixed with MH, so they could not form an initial water-in-oil emulsion. When the ratio was higher, the mixture could not be induced a phase inversion and form an oil-in-water secondary emulsion.

In the preparation process, the creation of clear one-phase dispersion depends on formation action. Formation time plays a key role in the formation of nanoliposomes. Different formation time (1.30, 2.20, 3.10, 4, and 4.30) were chosen, accompanied by MH 5mg, lecithin-cholesterol ratio 7:1, organic phase-aqueous phase ratios 4:1, PBS pH 7.4. The effects of formation time on the entrapment efficiency are shown in Fig. 1c. From 1.30 to 2.20 h, the extension of time promoted higher entrapment efficiency. However, as a result of increased time beyond 2.20h, the entrapment efficiency dropped sharply.

Response surface analysis

In the multivariate analysis, response surface methodology can analyze the regression relationship between dependent variable and independent variables. Response surface methodology (RSM) was used to determine the optimum level of each independent variable by building a mathematical Central Composite Design (CCD) experiment design model, which was possible to obtain the optimal entrapment efficiency.

The software Design Expert can estimate the statistical experimental design. A total of 20 runs were generated by Central Composite Design. In the software, the analysis of variance (ANOVA) established the statistical validation of the polynomial equations, and the responses were fitted to first-order, second-order- and quadratic-models and appraised according to statistically significant coefficients and R^2 values.

According to the results obtained in the single factor experiment, lecithin-cholesterol ratio (A), organic phase-aqueous phase ratio (B), and formation time (C) were confirmed as design variables in the response surface regression analysis because of their great effect on the entrapment efficiency of MH nanoliposomes. The three factors and five levels of response surface

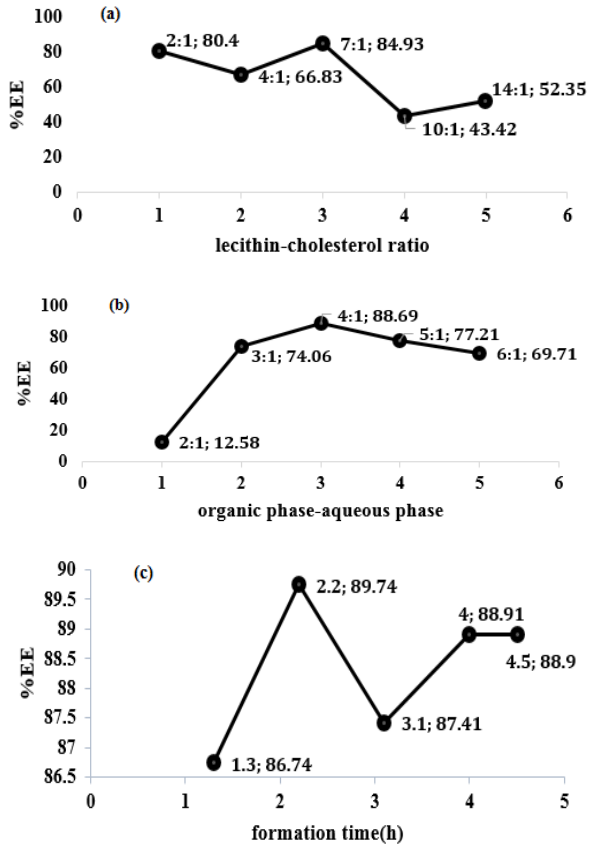

Fig. 1: Effects of different lecithin-cholesterol (a), different organic phase-aqueous phase ratio (b), and different formation time (c). Values are expressed as mean ± standard deviation (n = 3). Constant ratio of MH; 5mg, pH of PBS is 7.4.

Table1: Studied release kinetic equations in research.

Equation's name	formulation	
First-order equation [23]	Ln (1-Q) = - $k_1 t$	
Application: when release of the drug from nanoliposome, exponentially with time change, it is suggested that the drug release follow exactly the first order equation, exactly.		
Higuchi equation [24]	Q = $k_2 t^{\frac{1}{2}}$	
Application: The kinetic equation for the delivery of drugs that have a solubility in water and soluble semi-solid or solid pellets have low solubility.		
Hixson Crowell cube root equation [25]	$(1-Q)^{\frac{1}{3}} = k_3 t$	
Application: The equation used to describe the delivery systems that are associated with changes in surface area and particle diameter.		
Weibull equation [23]	Ln[-ln(1-Q)] = k_4 lnt + a_0	
Conditions & application of Weibull equation		
k_4 < 0.35, Porous environment.	k_4~ 0.35-0.39, The morphology of the substrate is more like penetrating Group.	0.39 < k_4 < 0.69, The main influence on the substrates different groups of penetration.
k_4~0.69-0.75, Sphere of penetration happens in a normal area.	0.75 < k_4 < 1, penetration the normal substrates via distribution.	k_4 = 1, first order kinetics follows from Fick's law.
k_4 > 1 , its show that Complex mechanisms.		

methodology are shown in Table 2. The central composite design, along with experimental data, is shown in Table 3. Design Expert 7.0 software was employed to establish a mathematical model and achieve the optimum conditions of technological process.

The analysis of variance (ANOVA) is applied to test the significance and adequacy of the model. Table 4, shows that "Prob>F" of model was significant and the "Lack of Fit" was not significant, which meant the model terms were precise and applicable [28, 29]. The values of "Prob>F" of A, C, AC, BC, A^2, and C^2 were lower than 0.05, suggesting that they had a significant influence on the entrapment efficiency.

The response surface curves and contour plots reflect the interactive effects of variables on the response value and determine the optimum level of each variable [30]. The 3D response surface plots and contour plots are shown in Fig. 2. The regression equation was

$(EE)^3$= +7.239E+005 + 1.137E+005*A – 28224.94*B +73961.93*C–718.73*A*B+1.135E+005*A*C +94157.98*B*C–50882.04*A^2+30587.62*B^2– 1279.77*C^2

In Fig. 3, the model predicted values against

Table 2: Factors and levels of response surface methodology.

Levels	A	B	C
-1	5:1	2:1	90
-0.5	6:1	3:1	115
0	7:1	4:1	140
+0.5	8:1	5:1	165
1	9:1	6:1	190

Table 3: Central composite design matrix for independent variables and their response values.

	Factor 1	Factor 2	Factor 3	Response
Run	A:lecithin-cholestrol ratio	B:organic phase to aqueous phase ratio	C:formation time (min)	EE (%)
1	7	2	140	98.15
2	8	3	115	83.33
3	7	4	140	89.74
4	7	4	190	87.41
5	6	3	165	68.8
6	8	5	165	95
7	5	4	140	57.34
8	7	4	140	89.74
9	7	4	140	89.74
10	6	5	115	78.35
11	8	3	165	94.02
12	8	5	115	80.9
13	9	4	140	94.54
14	7	4	140	89.74
15	7	4	140	89.74
16	7	4	90	58.72
17	6	3	115	93.92
18	6	5	165	86.89
19	7	6	140	90.44
20	7	4	140	89.74

values obtained from tests showing responses contour charts show the simultaneous impact of two factors on the response. Flat 3D charts show the relationship between two factors simultaneously with the surface response to the level. The factors are A: Lecithin-cholestrol ratio, B: Organic phase-Aqeous phase ratio, C: Agitation Time(min) and EE: Encapsulation Effeciency in these charts.

Fig. 4 showing the correlative effects of lecithin-cholesterol ratio and organic phase-aqueous phase ratio on the entrapment efficiency of MH nanoliposomes in contour plot(Fig. 4a) and 3D plot (Fig. 4b).

In the contour chart, in areas of greater intensity, the effect of the factors on the response is greater and a better result is obtained. On a 3D flattened graph, a factor is kept constant within a specified range, and another factor relationship with the encapsulated percentage is evaluated.

Also, Fig. 5 showing the correlative effects of lecithin-cholesterol ratio and agitation time(min) on the entrapment efficiency of MH nanoliposomes in contour plot(Fig. 5a) and 3D plot(Fig. 5b).

Table 4: Analysis of mean square deviation of regress equation.

Source	Sum of Squares	df	Mean Square	F Value	Prob> F (p-value)	
Model	7.16E+11	9	7.96E+10	6.43	0.0038	significant
A-lecithin-cholesterol ratio	2.07E+11	1	2.07E+11	16.71	0.0022	
B-organic phase to aqueous phase ratio	1.28E+10	1	1.28E+10	1.03	0.3339	
C-formation time	8.75E+10	1	8.75E+10	7.08	0.0239	
AB	4.13E+06	1	4.13E+06	3.34E-04	0.9858	
AC	1.03E+11	1	1.03E+11	8.34	0.0162	
BC	7.09E+10	1	7.09E+10	5.73	0.0376	
A^2	6.51E+10	1	6.51E+10	5.26	0.0447	
B^2	2.35E+10	1	2.35E+10	1.9	0.1979	
C^2	1.28E+11	1	1.28E+11	10.33	0.0093	
Residual	1.24E+11	10	1.24E+10			
Lack of Fit	1.24E+11	5	2.47E+10			
Pure Error	0	5	0			
Cor Total	8.40E+11	19				

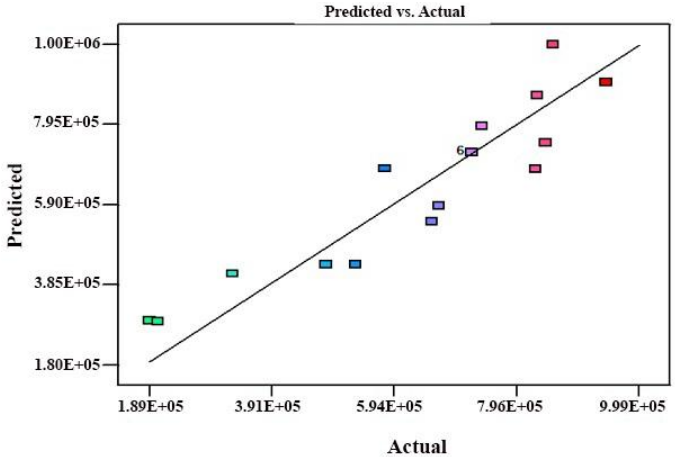

Fig. 2: A comparison between predicted and measured values for metformin hydrochloride encapsulated percent.

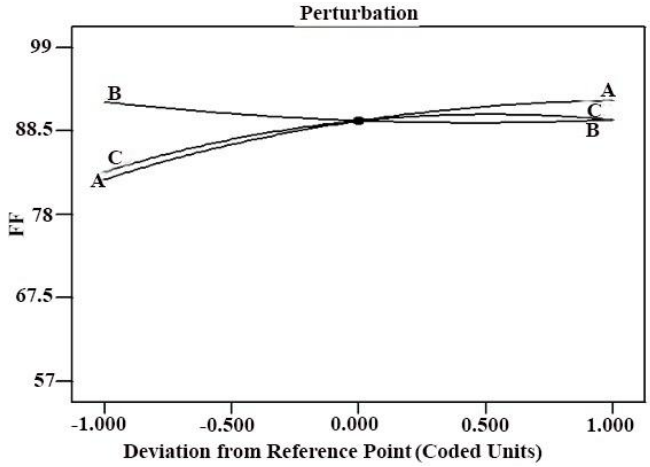

Fig. 3: The effect the general parameteric diagram on metformin hydrochloride encapsulated percentage.

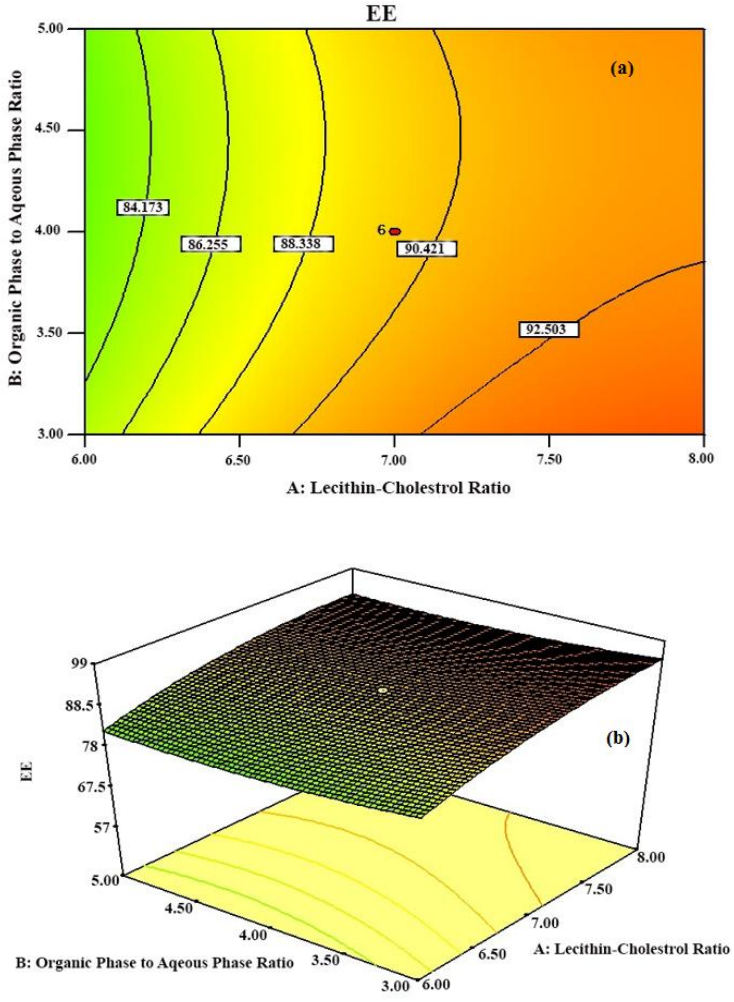

Fig. 4: The contour plot(a) and 3D plot(b) showing the correlative effects of lecithin-cholesterol ratio and organic phase-aqueous phase ratio on the entrapment efficiency of MH nanoliposomes.

In Fig. 6, the contour chart and 3D flat plot for organic phase-aqeous phase ratio and agitation time(min) factors are presented in terms of encapsulated percentages, which can be easily measured for the effects of these factors. The best conditions according to the program were obtained as the rate of lecithin-cholesterol 6.97 : 1, the ratio of organic phase-aqueous phase 4.64 : 1 and formation time 151.29 minutes and predicted maximum response (%EE) determined %90.9. In order to validate the proposed model, same experimental conditions were done and %EE was determined %89.68. Thus, metformin hydrochloride nanoliposome formation optimization response surface method is reliable and practical.

Determination of entrapment efficiency of MH nanoliposomes based-on phosphatidylethanolamine

In the next experiment, lecithin was replaced by phosphatidylethanolamine and the optimum entrapment efficiency of metformin hydrochloride nanoliposome was investigated based on the former optimum condition. At the condition as follows phosphatidylethanolamine-cholesterol ratio 7 : 1, the rate of organic phase-aqueous phase solvent 4 : 1 and the time at 140

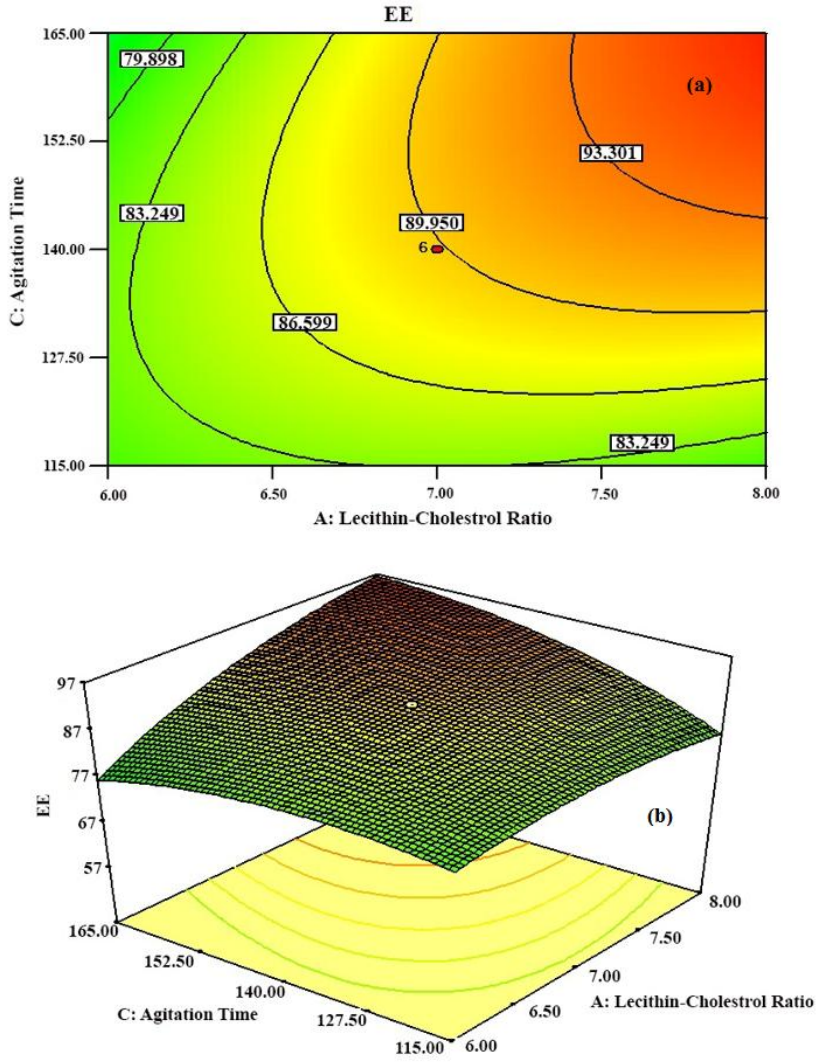

Fig. 5: The contour plot(a) and 3D plot(b) showing the correlative effects of lecithin-cholesterol ratio and formation time on the entrapment efficiency of MH nanoliposomes.

minutes, %EE received to %93.04. It showed that phosphatidylethanolamine has covered metformin hydrochloride more effective than lecithin.

Morphology

Morphological evaluation of MH nanoliposomes was carried out by SEM observation. Fig. 7 depicts the SEM photograph of MH nanoliposomes. The negatively stained nanoliposomes appeared to be gray and white spheres in contrast to the black background. The SEM image in Fig. 7a revealed that the MH nanoliposomes based-on phosphatidylcholine was dispersed as individual with the average size around 52 nm. Fig. 7b indicated MH nanoliposomes based-on phosphatidylethanolamine with the average size around 83 nm.

Permeability and appearance

The MH nanoliposomes based-on lecithin and phosphatidylethanolamine were stored at 4 ^{0}C for 1 to 3 hrs, and the permeability was obtained to assess the stability of nanoliposomes. For MH nanoliposomes based-on lecithin; the permeability of nanoliposomes was only 3.18%, 6.69% and 16.83% after 1h, 2h, and 3h, respectively. The low permeability rate illustrates that the leakage of MH from the liposomal interior was very little

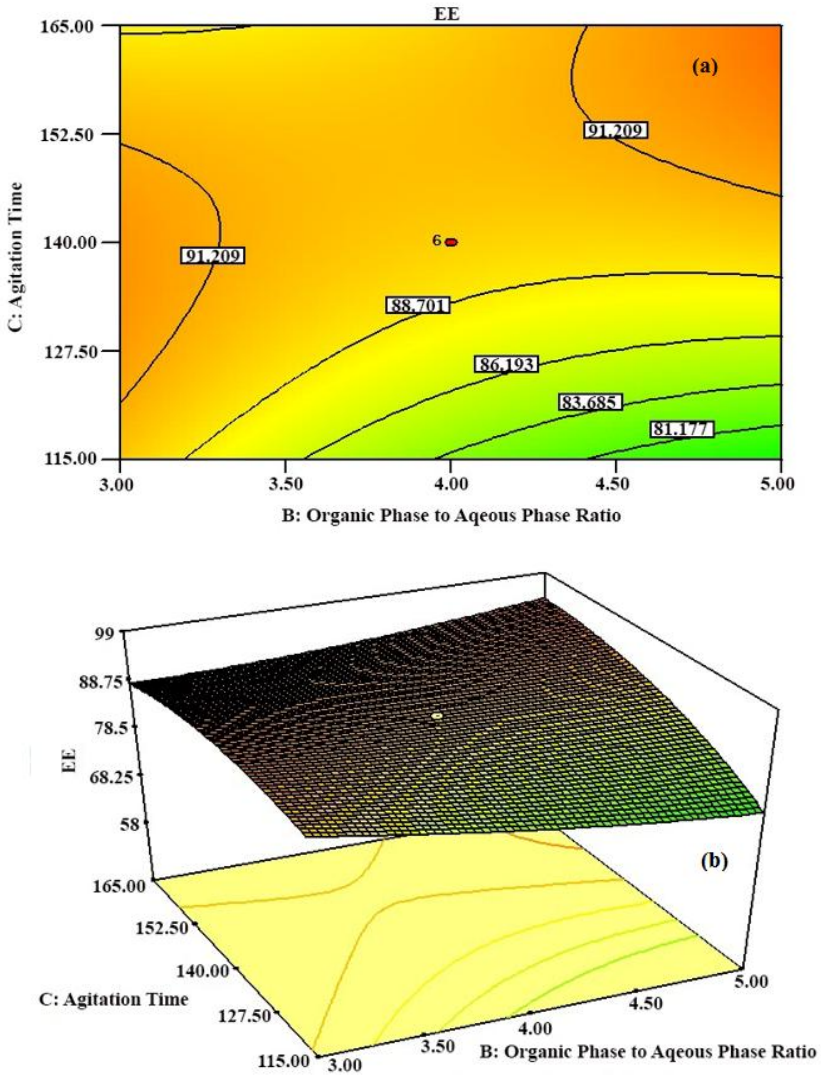

Fig. 6: The contour plot and 3D plot showing the correlative effects of organic phase-aqueous phase ratio and formation time on the entrapment efficiency of MH nanoliposomes.

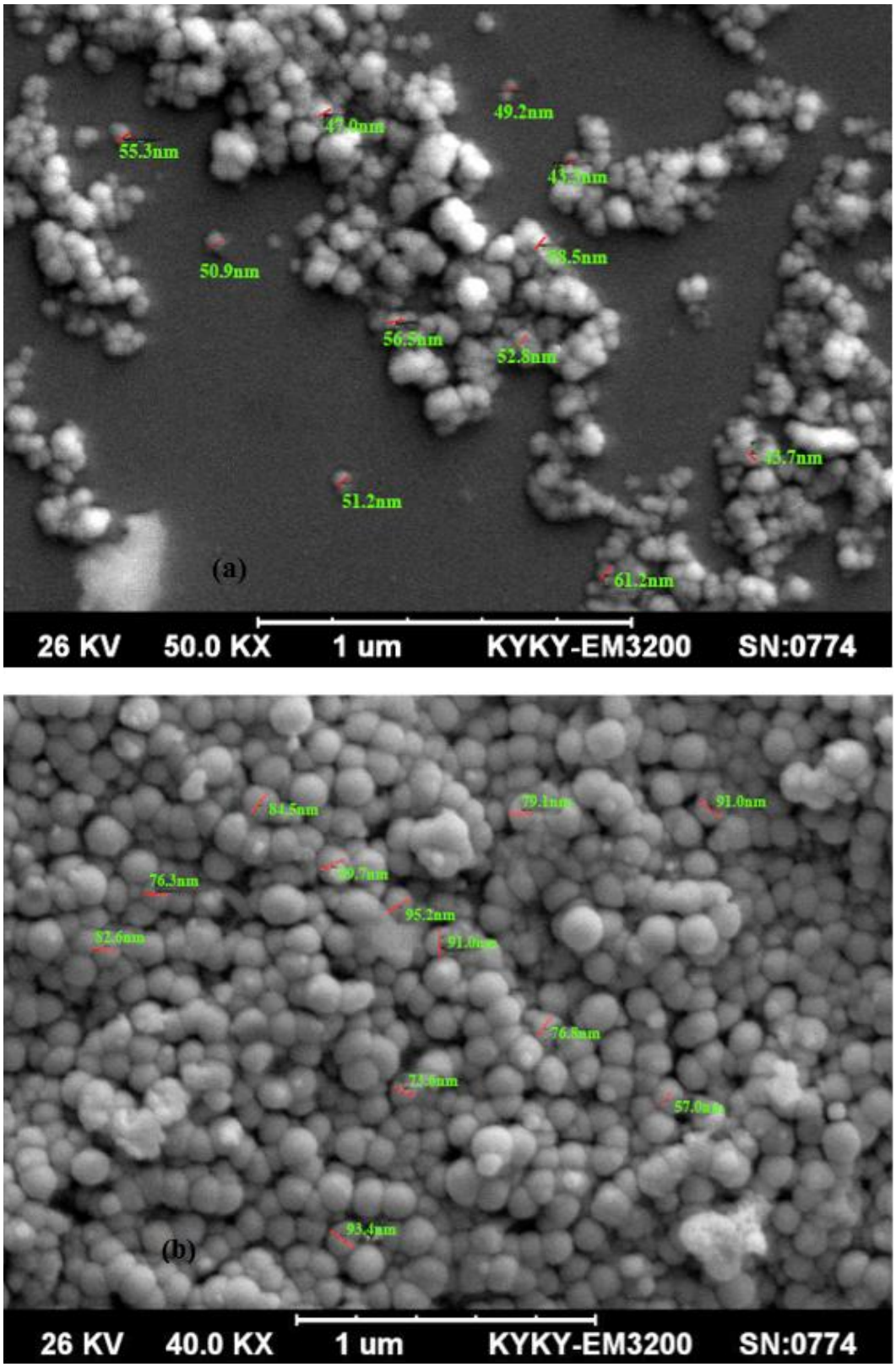

Fig. 7: Metformin hydrochloride liposomal vesicle size distributions (a). Based-on phosphatidylcholine (b). Based-on phosphatidylethanolamine.

when it was preserved in a refrigerator. Also, the permeability of MH nanoliposomes based-on phosphatidylethanolamine after 1h, 2h, and 3h was 2.26%, 17.41%, and 19.75%, respectively.

The gradual reduction of deforestation rate indicates greater stability in the nanoliposome coating. During the three hours, there was no significant change in uniform nanoliposomes phosphatidylethanolamine. But in lecithin nanoliposome suspensions heterogeneous dense halos were seen.

Infrared spectrum analysis

The characteristic peaks of Metformin Hydrochloride based on lecithin appeared at 3406.69, 3180.71, 2922.13, 1630.73, 1571.35, 1476.94, 1079.30, 990.36, 858.55 and 626.29 cm^{-1} (Fig. 8a), and characteristic peaks of Metformin Hydrochloride based on phosphatidylethanolamine appeared at 3400.08, 3177.00, 1629.44, 1571.12, 1488.57, 1080.80, 934.71, 797.94, 611.18 and 539.58 cm^{-1} (Fig. 8b).

According to characteristic peaks of Metformin Hydrochloride Nanoliposomes, No other specific peak was found in the infrared spectrum of MH Nanoliposomes, which confirms that no new chemical bond is formed, further confirms that the MH and wall materials were combined through physical interaction rather than chemical reactions.

In vitro release

The cumulative release rates of MH based-on lecithin and phosphatidylethanolamine were calculated and the in vitro release profiles are shown in Figs. 9a and 9b, respectively. According to Fig. 9a, within 2h and 30 min, the release rate of MH nanoliposomes suspension increased relatively rapidly considering that there was some free MH outside the nanoliposomes

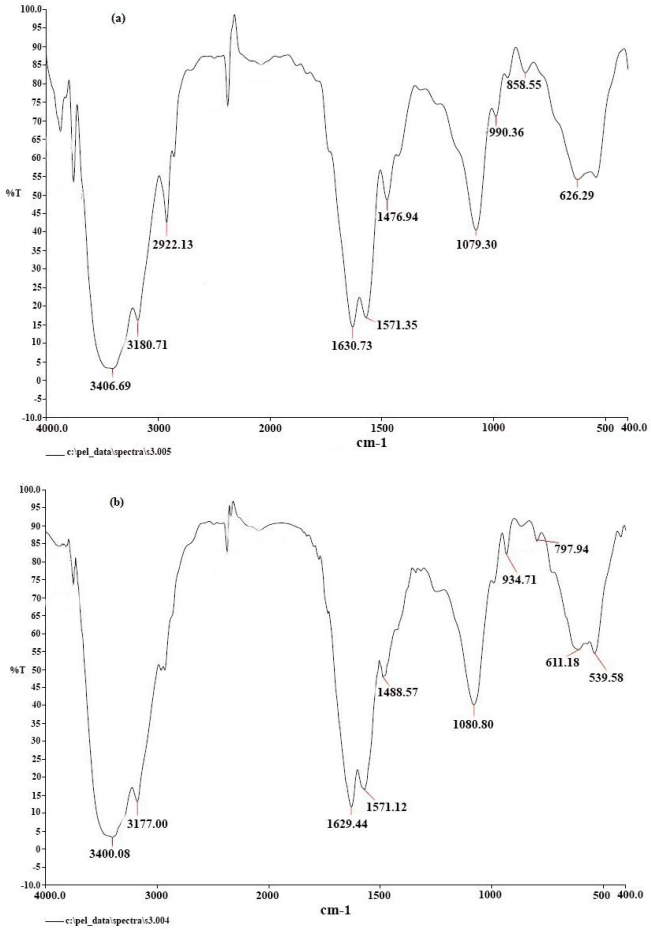

Fig. 8: FT-IR spectra of MH Nanoliposomes based on (a) lecithin, (b) phosphatidylethanolamine.

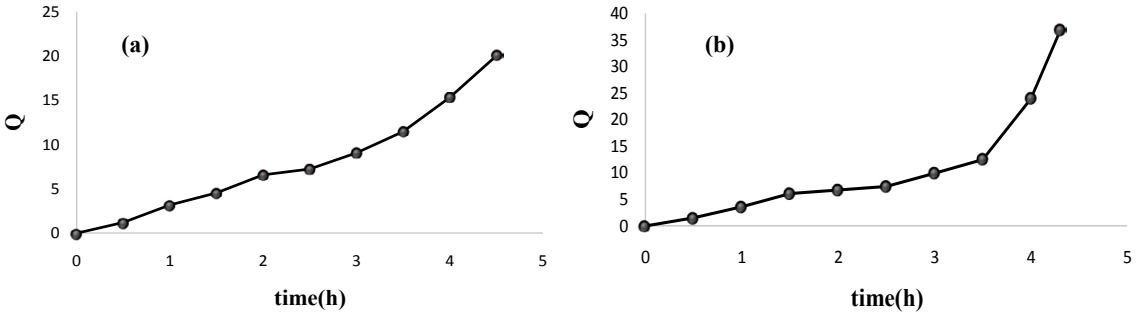

Fig. 9: In vitro release profiles of MH nanoliposomes based-on (a) lecithin and (b) phosphatidylethanolamine.

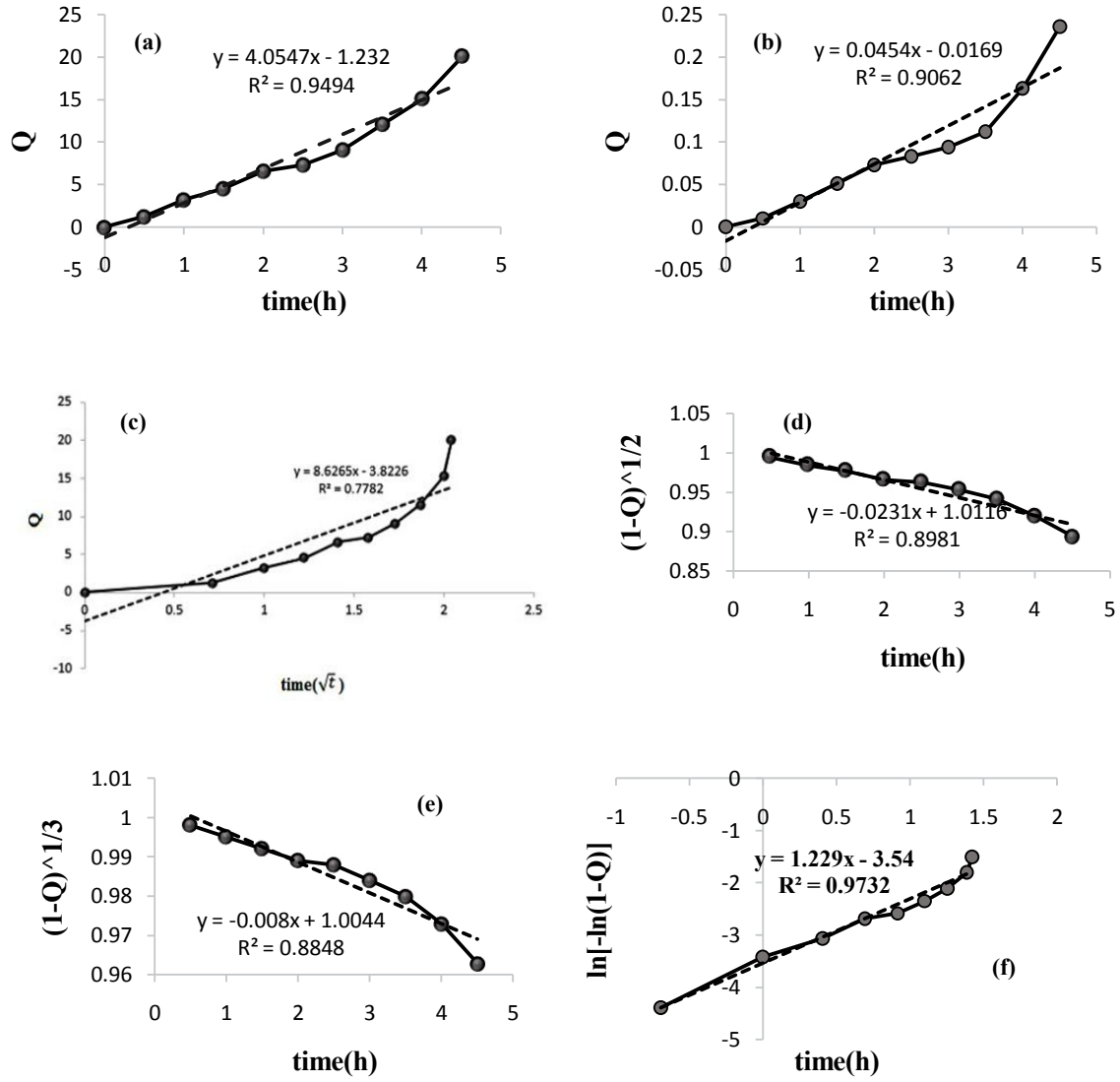

Fig. 10: *In vitro* release kinetic curves of MH nanoliposomes based-on lecithin fitted by the (a) Zero-order; (b) First-order; (c) Higuchi; (d) Neibergal; (e) Hixson Crowell cube root and (f) Weibull equations.

penetrating out from dialysis bag. From 2h and 30 min to 4h and 30 min, the MH encapsulated in nanoliposomes released gradually from the protection of lipid membranes. As seen in Fig. 9b, within 3h, the release rate of MH nanoliposomes suspension released gradually from the protection of liposomal membranes. From 3h to 4h and 25 min, increased release ratio relatively rapidly. Since half-life of metformin hydrochloride is 1.5 to 3 hours in the intestinal environment, then after this time, drug release occurs faster.

As seen in Figs. 10, the release behavior of MH nanoliposomes based-on lecithin was analyzed by kinetic equations including the zero-order equation, the first-order equation, the Higuchi equation, the Hixson Crowell cube root equation and the Weibull equation. Also, release behavior of MH nanoliposomes based-on phosphatidylethanolamine have been shown in Figs. 11. Therefore, the kinetic equation of Weibull is more in agreement with experimental kinetic data, Based on results of 2 sets of kinetic curves. This equation can be applied to forecast the release amount of MH at different times, or calculate the time of the release of a certain amount of MH, which could predict the status of nanoliposomes in practical applications.

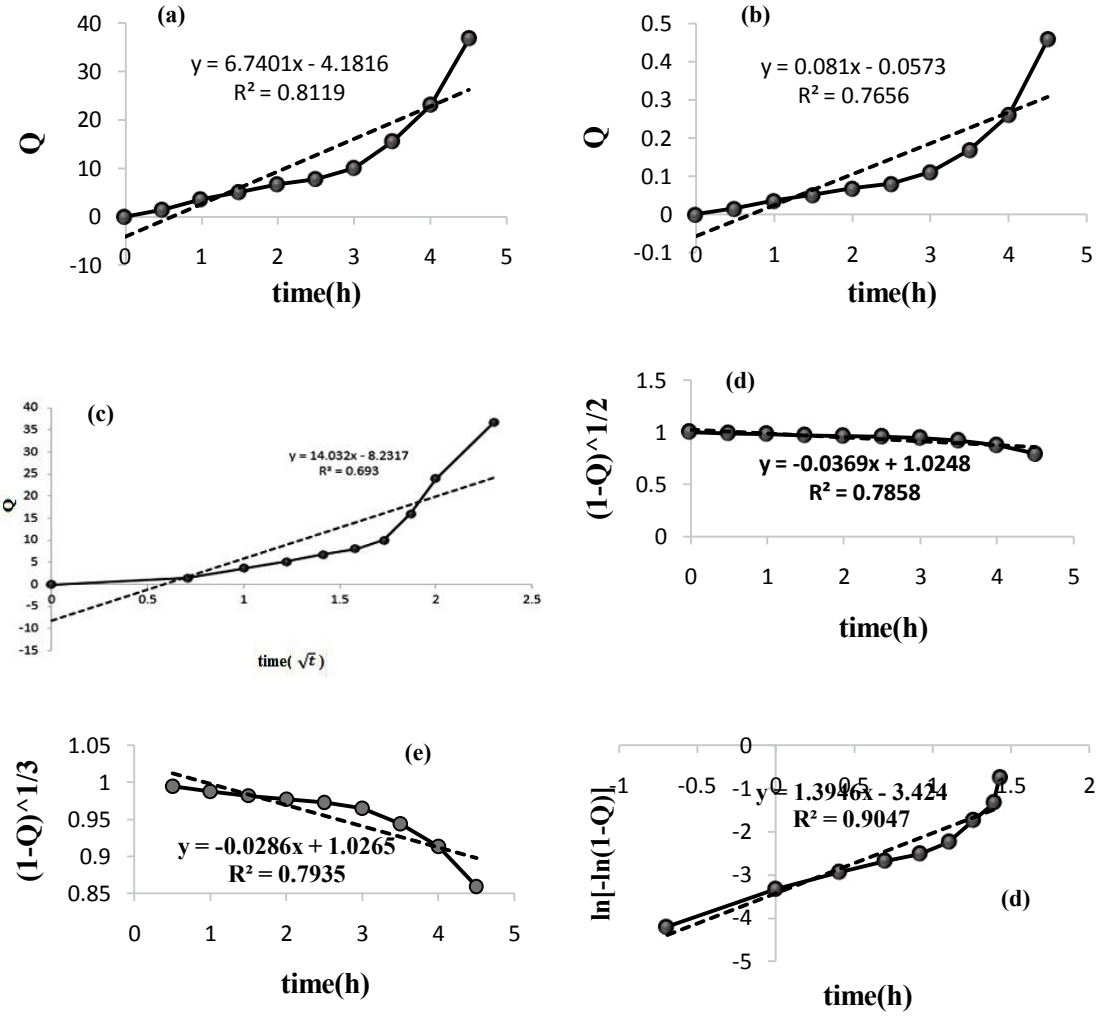

Fig. 11: *In vitro* release kinetic curves of MH nanoliposomes based-on phosphatidylethanolamine fitted by the (a) Zero-order; (b) First-order; (c) Higuchi; (d) Neibergal; (e) Hixson Crowell cube root and (f) Weibull equations.

CONCLUSION

Lecithin-cholesterol ratio, formation time and organic phase-aqueous phase ratio were of great significance to the entrapment efficiency of MH nanoliposomes. The optimal conditions obtained by the preparation process by thin film hydration technique were lecithin-cholesterol ratio 7 : 1, formation time 140 min, organic phase-aqueous phase ratio 4 : 1. The theoretical and practical maximum of entrapment efficiency for nanoliposome of lecithin was 90.9% and 89.74%, respectively. Replacement of lecithin by phosphatidylethanolamine at the same conditions led to %EE 93.04%. So, phosphatidylethanolamine compared to lecithin is more desirable at pH 7.4 for coating.

The mean size of MH nanoliposomes based-on lecithin and phosphatidylethanolamine were 52nm and 83nm, respectively. High stability was exhibited during the period of storage. Based on in vitro release profiles, the release of metformin hydrochloride nanoliposome, after 4.30 hours, based on phosphatidylcholine and phosphatidylethanolamine were 20% and 36.66% respectively. Profile of release in both types of nanoliposomes has a good match with Weibull equation. The results indicate that percentage of encapsulated metformin hydrochloride in nanoliposomes is greater based-on phosphatidylethanolamine than phosphatidylcholine. However, the release profile of nanoliposome based-on lecithin is more stable.MH nanoliposomes in our study have shown high entrapment efficiency and stable properties, which can effectively protect MH from unfavorable conditions, such as poor digestive system conditions, leading to an extension of the shelf life of this product.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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