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Effect of Formulation and Preparation Methods on Naproxen Liposome Performance

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Authors' contributions

This was carried in collaboration between both authors. Author ESG designed this stud, managed the experimental process and the experimental research. Author KCL managed the literature searches, characterization and analysis of the liposome formulations. Both authors wrote and approved the manuscript.

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ABSTRACT

Purpose: The aim of this study was to evaluate the effects of formulation composition and methods of preparation on the physico-chemical properties and drug release of naproxen liposome.
Methods: The ratio of lipid to drug, lipid to lipid, organic to aqueous phase and methods of preparation were varied.
Results: Formulation composed of 0.9 g lecithin:3.6 g cholesterol: 0.9 g cholesteryl hemisuccinate and drug : lipid ratio of 1:3 gave the highest entrapment efficiency (83.6%). Formulations prepared by using different ratios of organic to aqueous phase showed different drug entrapment efficiency depending on the ratio of the volume of the two phases. Also, the method used for preparation of the liposome had an effect on drug entrapment efficiency. The *dr*ug release data indicated that liposome prepared by the heating method and composed of 9 g lecithin:3.6 g cholesterol:0.9 g cholesteryl hemisuccinate using a ratio of 3 lipid:1 drug showed the best control release profile. The percent drug released was 14.9% and 71.5% at 15 minutes and 360 minutes of testing dissolution.

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Conclusion: The physico-chemical properties, drug entrapment and drug release from naproxen liposome were influenced by formulation composition, ratio of aqueous to organic phase and methods of preparation.

Keywords: Naproxen liposome; methods of liposome preparation; drug release from liposome; physico-chemical properties of liposome.

1. INTRODUCTION

Nonsteroidal Anti-inflammatory drugs (NSAID) are non-narcotic drugs with analgesic, antipyretic, and anti-inflammatory effects. NSAIDs are among the most commonly used drugs in the world. In the United States alone, 33 million people regularly take these drugs, spending 4 billion dollars annually [1]. Over 22 million prescriptions are written every year in the United Kingdom and over 70 million in the United States.

Several NSAIDs have been studied with the purpose of transdermal/topical drug delivery, including: dicloflenac, ibuprofen, indomethacin, and ketoprofen, among others [2-6]. However, there is few published studies regarding the use of naproxen liposome as a topical transdermal delivery system.

Liposomes are excellent drug carriers, therefore can be used as an alternative dosage form for the delivery of naproxen sodium. The liposome bilayer forms an effective physical barrier against the diffusion of large molecules and charged or polar small molecules [7]. Depending on the relative solubility in water and lipid, an aqueous soluble drug can pass through the membrane to occupy the internal aqueous volume of the liposome or, if it is less water soluble, it can be incorporated into the membrane, essentially dissolving in the hydrophobic environment. Naturally occurring lipids include phospholipids (lecithin) and cholesterol. Liposome products currently on the market are composed of these types of lipids or modified lipids [8-10].

The drug may be loaded into liposome by several techniques, the simplest one, called passive loading, involves forming vesicles in an aqueous solution of the drug. The entrapped agents are loaded before or during the manufacturing procedure. Many drugs have functional groups such as amines or carboxyls that alter the charge of the molecule in different pH environments. These types of compounds with ionizable groups and those with lipid and water solubility can be introduced into the liposome after the formation of the intact vesicles [11-12].

Moreno et al. [13] investigated the membrane activity of ibuprofen, diclofenac, and naproxen. They carried out their study with lecithin phospholipids and concluded that the interaction process is governed by only tropic reactions at the lipid/water interface. Paavolan et al. [14] in 2010 developed controlled release injectable liposomal gel of ibuprofen using high pressure homogenization. Guar et al. [15] developed rifampicin aerosolized liposome targeting the drug delivery to the lungs. They found that in situ formed liposomes showed more sustained release profile than liposome. Also, they found that upon subjecting to stress condition for 60 days, preformed liposome lost control of drug release. Liposome have been used for targeting many water soluble and water insoluble drugs, anticancer agents and vaccines [16-18].

The hypothesis of this research is that factors such as methods of preparation, drug:lipid ratio, lipid :lipid ratio, and ratio of organic to aqueous phase may affect the physico-chemical properties and the quality of the output product. In this research a novel naproxen sodium liposome is prepared by a heating method using glycerol and compared to liposome prepared by conventional (thin film lipid rehydration) method and or reverse phase evaporation method. The effects of lipid:lipid ratio, formulation composition and organic:aqueous solvent ratio were investigated. The kinetics of drug release from liposome, particle size and surface morphology were also investigated.

2. MATERIALS AND METHODS

2.1 Materials

Naproxen Sodium USP, lot No. M16674, donated by Syntex Puerto Rico Inc. Cholesterol, lot No. 11013BE, cholesteryl hemisuccinate, lot NO. 84H8455, and lecithin, Lot 28H8000 were purchased from Sigma Chemical Co., St. Louis, MO, USA. Glycerol, lot No. 041365, Fisher Scientific, NJ, USA.

2.1.1 Experimental design

Ten liposome formulations were prepared in which the ratio of lipid to drug, lipid to lipid, and organic to aqueous phase were varied (Table 1).

2.1.2 Preparation of liposome with different lipid to drug ratio

Four liposome formulations were prepared by incorporation of different lipid to drug ratio and both drug entrapment efficiency and drug release, were evaluated.

The components of the lipid phase, Lecithin (LEC) 3% w/v, Cholesterol (CH) 1.2% w/v, and Cholesteryl Hemisuccinate (CH-H) 0.3% w/v, were dissolved in 100 mL of chloroform and mixed in round bottle flask with long extension neck. The chloroform was removed under reduced pressure using a rotary evaporator (Buchi, Brinkman, Instruments Inc., N.Y, U.S.A), at 65-75ºC and 200 rpm. and the dried lipid dispersion was produced. The drug was dissolved in 100 mL phosphate buffer pH 7.4 prepared as described in the USP by mixing specific volume of 0.2 M potassium phosphate and 0.2 M NaOH solution. The aqueous phase was slowly added to the lipid film producing a liposome dispersion. The dispersion was stirred for 5 hours using a mixer at 300 rpm Drna Mix, model 143, Fisher Scientific, USA). The dispersion was kept overnight at 4ºC and then the dispersion was centrifuged (centrifuge model J2, Beckman, US) for 1 hour and the liposomes were separated for characterization of the physico-chemical properties.

2.1.3 Preparation of liposome containing different lipid to lipid ratio

Two liposome formulations were prepared by incorporation of different lipid to lipid ratio, lecithin:cholesterol:cholesteryl hemisuccinate at a ratio of 5:1:1.4 and 5:2:1 and the drug entrapment and drug release were evaluated.

The components of the lipid phase, lecithin, cholesterol, and cholesteryl hemisuccinate, at different ratios, were dissolved in 300 mL of chloroform and the same procedure described under preparation of liposome with different lipid to drug ratio was followed.

Table 1. Liposome composition

Experiment number	Lipid :drug ratio	Lipid:lipid ratio	Organic:aqueous ratio	Methods of preparation
1	1:1	9 g lecithin:3.6 g olesterol:0.9g cholesteryl hemisuccinate	3:1	Thin film rehydration (Conventional)
2	2:1	9 g lecithin:3.6 g cholesterol : 0.9 g cholesteryl hemisuccinate (ratio of 10:4:1)	3:1	Thin film rehydration (Conventional)
3	3:1	9 g lecithin:3.6 g cholesterol :0.9 g cholesteryl hemisuccinate (ratio of 10:4:1)	3:1	Thin film rehydration (Conventional)
4	1:3	9 g lecithin:3.6 g cholesterol ::0.9 g cholesteryl hemisuccinate	3:1	Thin film rehydration (Conventional)
5	3:1	9 g lecithin:1.8 g cholesterol:2.6 g cholesteryl hemisuccinate (ratio of 5:1:1.4)	3:1	Thin film rehydration (Conventional)
6	3:1	9 g lecithin:3.6 g cholesterol :1.8 g cholesteryl hemisuccinate (ratio of 5:2:1)	3:1	Thin film rehydration (Conventional)
7	3:1	9 g lecithin:3.6 g cholesterol :0.9 g cholesteryl hemisuccinate (ratio of 10:4:1)	10:1	Thin film rehydration (Conventional)
8	3:1	9 g lecithin:3.6 g cholesterol:0.9 g cholesteryl hemisuccinate (ratio of 10:4:1)	1:1	Thin film rehydration (Comventional0
9	3:1	9 g lecithin:3.6 g cholesterol:0.9 g cholesteryl hemisuccinate (ratio of 10:4:1)	-	Heating
10	3:1	9 g lecithin:3.6 g cholesterol:0.9 g cholesteryl hemisuccinate (ratio of 10:4:1	-	Reverse phase evaporation

2.1.4 Preparation of liposome using different ratio of organic to aqueous phase

As indicated in Table 1, formulations were prepared by incorporation of different organic to aqueous phase ratio at 1:1, 3:1, and 10:1 organic to aqueous phase ratio and were evaluated for drug entrapment efficiency and drug release.

The components of the lipid phase were Lecithin (LEC) 3% w/v, Cholesterol (CH 1.2% w/v, and Cholesteryl Hemisuccinate (CH-H) 0.3% w/v, were dissolved in 100 mL of chloroform. The chloroform was evaporated at 65-75ºC and the procedure described under preparation of liposome with different lipid to drug ratio was followed.

2.1.5 Preparation of liposome using alternative methods

As presented in Table 1, liposome formulations were prepared by different methods, conventional (thin film rehydration), heating, reverse phase evaporation
and the drug entrapment efficiency and release were evaluated.

2.1.6 Heating method

The lipid components, lecithin, cholesterol, and cholesteryl hemisuccinate described in Table 1 under hearing method [19], were hydrated in 100 mL of phosphate buffer pH 7.4 for 1 hour. The hydrated lipids were heated in the presence of glycerol (3% v/v) at 60-70℃ for additional one ho ur. The drug was added to the mixture containing the lipids and the glycerol. The mixture was centrifuged (Centrifuge, model J2, Beckman, USA) for an hour and a half and the liposomes were separated.

2.1.7 Reverse phase evaporation method

The lipid components, lecithin, cholesterol, and cholesteryl hemisuccinate, were dissolved in 25 mL of diethyl ether. The solvent was evaporated in rotary evaporator under vacuum at 30-40℃ and 200 rpm [20], The drug: lipid ratio was 1:3. The drug was mixed with 15 mL of phosphate buffer and added to the lipid film. In order to dissolve the lipid film, 75 ml of ether were added and then mixed in rotary evaporator (Rotary Evaporator, model R-124, Buchi, Brinkmann Instruments Inc., NY, USA) at 5-10℃ and 100 rpm. The solution was left for 30 minutes at room temperature without agitation. The solvent was then evaporated in rotary evaporator at 30-40ºC and 200 rpm and introduced in ultrasonic bath, model Branson 3200-R, Branson Ultrasonic Corporation, CT, USA) for 5 minutes. The resulting lipid dispersion was left over night at 4℃. The mixture was centrifuged for 90 minutes and the liposomes were separated.

2.1.8 Determination of drug entrapment efficiency

Free un-entrapped drug was separated from encapsulated drug liposome by centrifugation for 1 hour. The method used by Panwar et al. [21] was modified and used to determine drug entrapment efficiency. The drug entrapment efficiency was measured by mixing 0.2 g lliposome with 200 ml phosphate buffer pH 7.4.for half an hour at 98-100℃ to lysis the liposome. The phosphate buffer was evaporated until the volume remained constant. Subsequently, the sample was placed in an ice-water bath for half an hour. Filtered samples using Millipore filter (0.22 um) were measured using an UV spectrophotometer (UV spectrophotometer, model DU-520, Beckman Coulter, USA) at maximum wave length of 331 nm using a blank of the same composition but without drug. Three replicates were tested from each formulation and their mean was calculated.

$$\text{Entrapment efficiency percent} = \frac{\text{Total drug} - \text{Free drug}}{Total\ drug} X\ 100$$

2.1.9 Dissolution t testing

The dissolution test was carried out in 900 ml phosphate buffer pH 7.4 at 37.0±0.5℃ using USP/NF rotating basket apparatus (Dissolution apparatus, model 47-200-202, Hanson Research Corporation, CA, USA) at a rotational speed of 50.0±0.5 rpm. A liposome sample (0.20 g) was placed in the basket and tested for dissolution over 6 hours. Filtered samples (10 ml) were withdrawn manually at pre-determined time intervals and replaced with 10 ml phosphate buffer pH 7.4. A spectrophotometer was used to measure the absorbance of the samples at a maximum wave length of 331 nm and the concentration of the drug in each sample was calculated from the slope and the intercept of the standard curve. For each formulation, three replicates were tested and their mean was calculated.

2.1.10 Determination of drug release mechanism

The diffusion model described by Higuchi [22] was used to determine the kinetic model of drug release

$$Q = Kt^{1/2}$$

A plot of Q, percent of drug released; versus square root of time should give a linear relationship and K is constant. Also, zero order kinetic model was evaluated in order to determine if the drug release follows the zero order kinetic model.

2.1.11 Scanning electron microscope (SEM)

The principle aim of the SEM was to analyze the surface morphology and the shape of the different liposome formulations, and not to determine particle size.

In order to study the surface morphology of the various liposome vesicles, scanning electron photomicrographs (Scanning Electron Microscope, Philips, model 515, Philips, Netherland) were taken using an auto scan mode. Samples were mounted in aluminum stubs introduced in vacuum oven for 24 hours to remove all humidity and covered with gold/palladium using a sputter coater (Spurering system, Hummer model 6.2, LADD Research Industries, VT, USA).

The samples were placed in a plate holder and then subjected to vacuum at 60 mtorr in the chamber. Then two flushes of Argon gas were applied to leave the chamber with no air. After the second flush with Argon, it was maintained at constant flow, and then electrical charges was applied inside the chamber to start the ionization of the gas and covering the sample with a layer of gold-palladium. The charges were at 15 mA for a period of 6 minutes. After that, the valve of Argon was closed and the vaccum was turned off and taken out all the layered samples and photomicrographs of liposome were taken.

All sample resolutions were approximately 50X and 200X.

2.1.12 Particle size

The mean particle size of the liposome formulations was determined using the dynamic light scattering technique (Particle size analyzer, Horiba LB-550, Horiba Instruments Inc., CA, USA) which can measure particle size in the range of 1 mm to 6 um and a concentration range from ppm to 40% solids and it provides accurate results for average particle size. The Brownian motion of the particles causes a Doppler shift in the incident light frequency. The amount of frequency shift is related to the frequency of the Brownian motion, which is related to the size of the particles. De-ionized water was the medium for the sample dispersions. A small amount of sample sufficient to fill the cell was measured in a 1 cm cell and stirred using a magnetic bead.

2.1.13 Statistical analysis

The statistical analysis of the data was performed using the software Minitab 15. The one way analysis of variance was used to determine if there were significant differences between compared samples (three samples or more).

The General Linear Model was used to determine if there is statistically significant difference between compared samples (with two independent variables). A P value greater than 0.05 indicates no significant difference exists.

3. RESULTS AND DISCUSSION

Ten liposome formulations were prepared successfully using different lipids (cholesterol, cholesteryl hemisuccinate and lecithin), lipid to lipid ratio, and three alternative methods of preparation. (conventional, heating and reverse phase evaporation). The ratio of lipid : lipid and the ratio of organic to aqueous solvent were also varied. The physico-chemical properties, drug entrapment, drug release and kinetic model of drug release were evaluated. The composition of the different naproxen formulations is shown in Table 1. Drug entrapment in the different formulations is shown in Table 2.

3.1 Entrapment Efficiency

3.1.1 Different ratio of drug to lipid

Four initial formulations as shown in Table 1 were prepared varying the lipid to drug ratio. Formulation with the highest lipid to drug ratio (3:1) entrapped the highest amount of drug (83.62%). These results show that using three times more lipid (lecithin, cholesterol, and cholesteryl hemissucinate) than drug in the

formulation possibly increases the fluidity of the lipid bilayer, and or reduces the permeability of the membrane to water soluble molecules, thus, giving a more stable liposome with a better encapsulation efficiency upon increasing the ratio of lipid compared to drug. The 1 lipid to 1 drug and 2 lipid to 1 drug formulations entrapped similar amounts of drug, 27.28% and 25.40%, respectively. Formulation with the highest amount of drug, 1 lipid to 3 drug ratio, gave the lowest drug entrapment (19.93%). The 3:1 lipid to drug formulation was chosen for further study.

3.1.2 Different lipid to lipid ratio

Three other formulations were prepared with different lipid to lipid ratio. Formulation consists of 9.0 gf Lecithin: 3.6 g Cholesterol: 0.9 g of Cholesteryl hemisuccinate gave the highest drug entrapment (83.62%). Increasing the amount of cholesteryl hemisuccinate decreased the amount of drug entrapped (27.90%). Decreasing the amount of cholesterol and increasing the amount of cholesteryl hemisuccinate decreased the entrapment efficiency of the drug (16.76%). Cholesterol increased the amount of drug entrapped into the liposome and Cholesteryl hemisuccinate improves the stability of the liposome vesicles by preventing their flocculation and helps to increase the encapsulation rate of hydro-soluble substances by increasing the thickness of the aqueous layers between the lipid bilayers. However decreasing the amount of cholesterol decreased liposome resistance to aggregation, affecting the stability of the bilayer, thus increasing leakage of the encapsulated drug. In summary these data indicated that the ratio of lipid to lipid, and type of lipid used affect drug entrapment into the liposome.

3.1.3 Different ratio of organic to aqueous phase

Three formulations were prepared with different ratio of aqueous phase (phosphate buffer 7.4), to organic phase. Formulation with equal amount of organic and aqueous phase, 1:1, entrapped the lowest amount of drug (11.09%). Formulation of 3 organic to 1 aqueous phase ratio entrapped the highest amount of drug (83.62%) while formulation of 10 organic to 1 aqueous phase ratio entrapped 62.37% of drug. These results show that large quantities of aqueous solvent or very small amount such as 300 mL and 30 mL respectively, are not the optimum quantities for the formulations studied, and is not suitable for the amount of drug incorporated (4.5 g). However, the use of 100 ml of aqueous solvent and a ratio of 3: 1 organic to aqueous phase gave the best drug entrapment (83.62%). Increasing too much the organic phase volume decreased drug entrapment efficiency. However using 3 organic phase to 1 aqueois solvent ratio gave high drug entrapment efficiency since the drug has higher affinity and partition to the aqueous phase than the organic phase, more drug will be entrapped in the aqueous phase and consequently the drug entrapment efficiency into the liposome will increase. The use of high volume of organic phase such as 10 organic phase to 1 aqueous phase ratio interfered with the solubility of naproxen sodium and decreased drug entrapment into the liposome.

3.1.4 Alternative methods of preparation

Three formulations of the same composition were prepared, each with a different method of preparation. Formulation prepared by the heating method gave a 35.51% of drug entrapment while formulation of the same composition but prepared by the reverse phase evaporation method entrapped 66.70% of the drug, and formulation prepared by film evaporation rehydration method (conventional method) gave the highest percent of drug entrapment (83.62%). These data indicated that method of preparation has an effect on drug entrapment efficiency.

Table 2 shows the drug entrapment of all liposome formulations.

Table 2. Drug entrapment of all liposome formulations

Formulations	Percent drug entrapment efficiency	Standard deviation
F1	27.28	3.05
F2	25.40	1.81
F3	83.62	9.60
F4	19.93	1.91
F5	16.76	1.08
F6	27.90	3.86
F7	62.37	10.63
F8	11.09	1.44
F9	35.51	9.76
F10	66.70	4.68

F1= 9 g HEC: 3.6 g CH:0.9 g CH-H) prepared by 3 lipid: 1 drug, 3 organic:1 aqueous phase ratio and conventional method; F2= (9 g HEC:3.6 g CH:0.9 g CH-R) prepared by 2 lipid:1 drug, 3 organic:1 aqueous phase ratio and conventional method; F3= (9 g HEC:3.6 g CH:0.9 g CH-H) prepared by 3 lipid:1 drug, 3 organic:1 aqueous phase ratio and conventional method; F4= (3.6

HEC:1.2 g CH:0.3 g CH-H) prepared by 1 lipid:3 drug and conventional method; F5= (9 g LEC:1.8 g CH:2.6 g CH-H) prepared by 3 lipid:1 drug and conventional method; F6= (9 g LEC:3.6 g CH:1.8 g CH-H) prepared by 3 lipid:1 drug and and conventional method; F7= (9 g LEC:3.6 g CH:0.9 g CH-H) prepared by 3 lipid:1 drug, 10 organic:1 aqueous phase ratio and conventional method; F8 (9 g LEC:3.6 g CH:0.9 g CH-H) prepared by 3 lipid:1 drug, 1 organic:1 aqueous phase ratio and conventional method; F9 (9 g LEC:3.6 g CH:0.9 g CH-H) prepared by 3 lipid:1 drug, 3 organic:1 aqueous phase ratio and heating method; F10= (6 g LEC:3.6 g CH:0.9 g CH-H) prepared by 3 lipid:1 drug, 3 organic:1 aqueous phase ratio and rphase evaporation method.

ANOVA one way analysis indicated that there is significant difference in percent drug entrapment between all formulations.

3.2 Dissolution Results

3.2.1 Different dug to lipid ratio

Four initial formulations were prepared varying the lipid to drug ratio as mentioned previously. Formulations with the highest amount of drug to lipid; 1 lipid to 1 drug and 1 lipid to 3 drug, showed immediate drug release and released 74.06% and 83.07% respectively, at 15 minutes of testing dissolution. Formulation containing 3 lipid to 1 drug ratio released 15.06% of drug at 6 hours of testing dissolution while the dissolution profile of formulation prepared with a 2 lipid to 1 drug ratio appears to exhibit a suitable controlled release profile. The percent drug released was 16.73% and 51.90% at 15 and 360 minutes of testing dissolution, as shown in Fig. 1. These results show that formulations with the highest lipid to drug ratio (3:1, 2:1), decreased the drug release from liposome. However, formulation with the same amount of drug and lipid or with more amount of drug than lipid, released 74% of the drug at 15 minutes of testing dissolution. These data indicated that increasing the lipid level, increased the lipid membrane thickness and consequently slow the drug release from the liposome.

3.2.2 Different lipid to lipid ratio

Three formulations of the same composition were prepared. The only difference between these formulations is the lipid to lipid ratio. Formulation consists of 9.0 g lecithin: 3.6 g of cholesterol: 0.9 g Cholesteryl Hemi succinate released the least amount of drug, 15.04%, at 360 minutes (6 hours) of testing dissolution. Increasing the amount of cholesterol hemisuccinate to the double, increased slightly the amount of drug released (29.8%) at 360 minutes of testing dissolution as shown in Fig. 2. These data may be due to the presence of charged lipid as cholesterol hemisuccinate that decreased aggregation, increased repulsion between the vesicles and consequently enhanced liposome stability.

3.2.3 Different organic to aqueous phase ratio

Three formulations were prepared with different ratio of organic to aqueous phase. Formulation prepared with 1 organic to 1 aqueous phase released 64.5% drug at 15 minutes of testing dissolution as shown in Fig. 3. Formulation prepared with large quantity of aqueous solvent gave inconsistent drug release. Formulation prepared with 10 organic to 1 aqueous phase ratio released 43.5% at 15 minutes and 63% at 369 minutes while formulation prepared with 3 organic to 1 aqueous phase ratio released 14% at 360 minutes of testing dissolution as shown in Fig. 3.

These data indicate that the ratio of organic to aqueous phase has an effect on the fluidity and stability of the liposome which consequently affect the physico-chemical properties and the drug release from liposome.

3.2.4 Alternative methods of preparation

Three formulations of the same composition were prepared by different methods. Formulation prepared by phase reverse evaporation released the highest amount of drug. The percent drug released was 66% and 85% at 15 and 360 minutes respectively. Formulation prepared by using heating method released 71.5% of the drug at 360 minutes of testing dissolution. Formulation prepared by film evaporation rehydration (conventional) method gave the least amount of drug release, only 15% of the drug was released at 360 minutes of testing dissolution. As shown in Fig. 4. These data indicated that method of preparation and operating condition clearly affect the drug release from liposome.

The statistical analysis supported the dissolution data and showed significant difference in drug release between different liposome formulations.

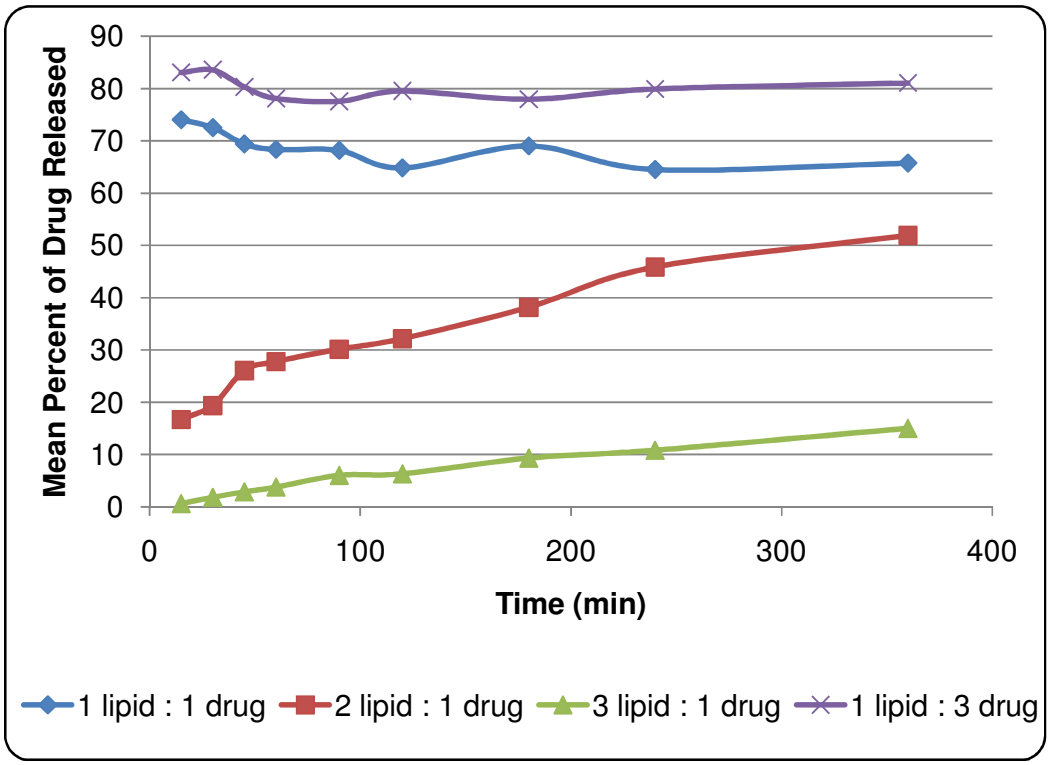

Fig. 1. Dissolution profiles of liposome containing different lipid to drug ratio

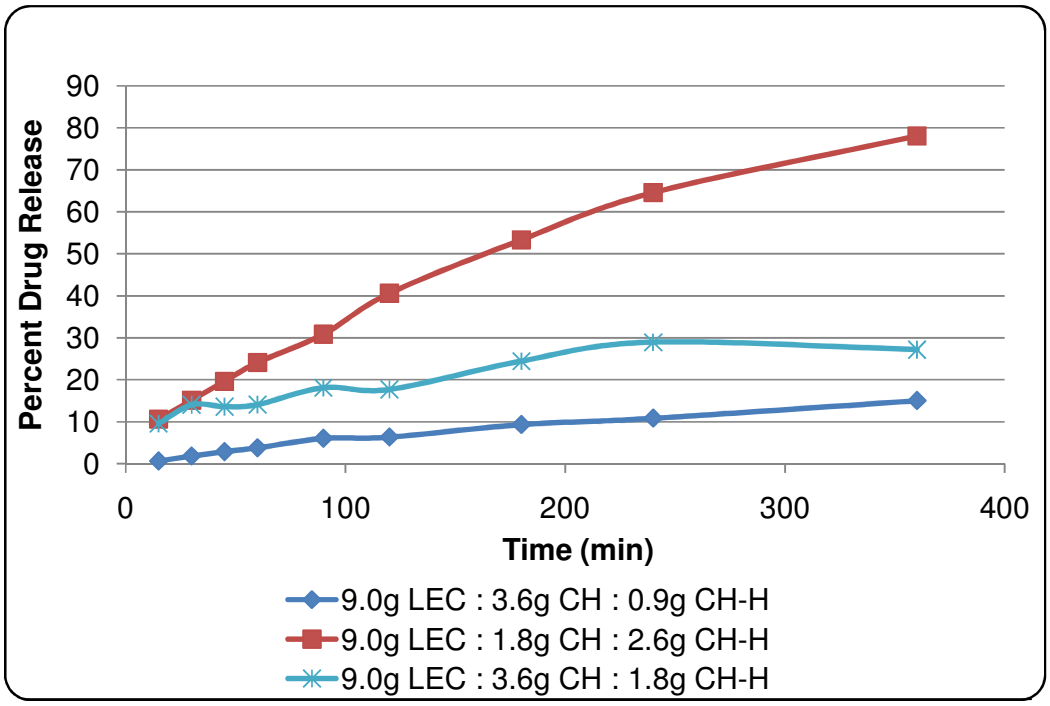

Fig. 2. Dissolution profiles for liposome containing different lipid to lipid ratio

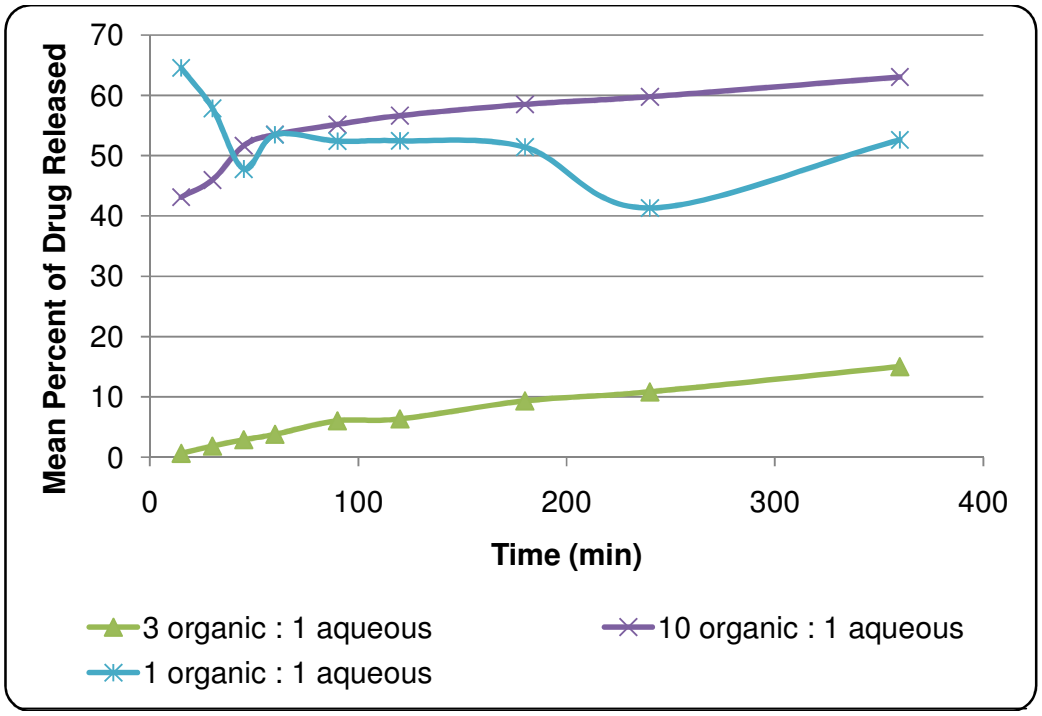

Fig. 3. Dissolution profiles for liposome prepared with different organic to aqueous phase ratio

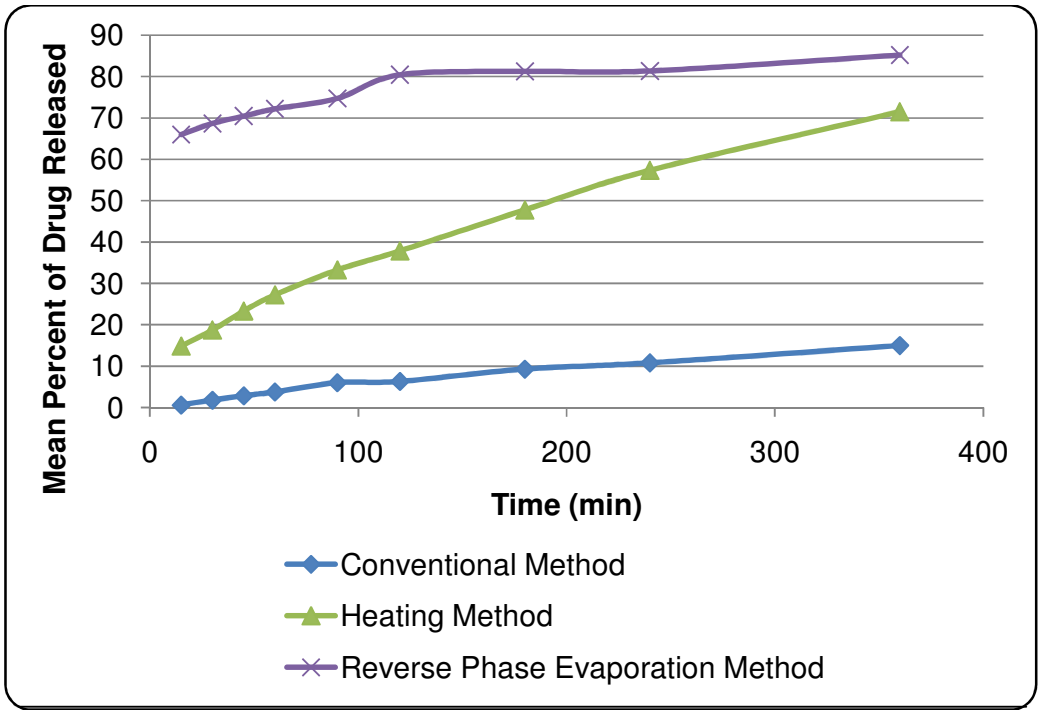

Fig. 4. Dissolution profiles for liposome prepared with different methods

3.3 Mechanism of Drug Release

The mechanism of drug release was found to follow the diffusion model described by Higuchi. A linear relationship was obtained between square root of time and percent drug released as shown in Figs. 5 and 6 for liposome prepared by using 3 lipid to 1 drug ratio and liposome prepared by using heating method respectively.

3.4 Particle Size

The particle size of the different liposome formulations is shown in Table 3. Formulation composed of 9 g lecithin: 1.8 g cholesterol: l2.6 g cholesteryl hemisuccinate and formulation composed of 9 g lecithin: 3.6 g cholesterol: 1.8 g cholesterol hemisuccinate gave the smallest particle size. The particle size was 1.0 um and 0.9726 um respectively.

Formulation prepared with a ratio of 1 lipid to 3 drug gave the largest particle size. The particle size was 3.10 um as shown in Table 3. These data indicated that the ratio of lipid to lipid and method of preparation have an effect on liposome particle size

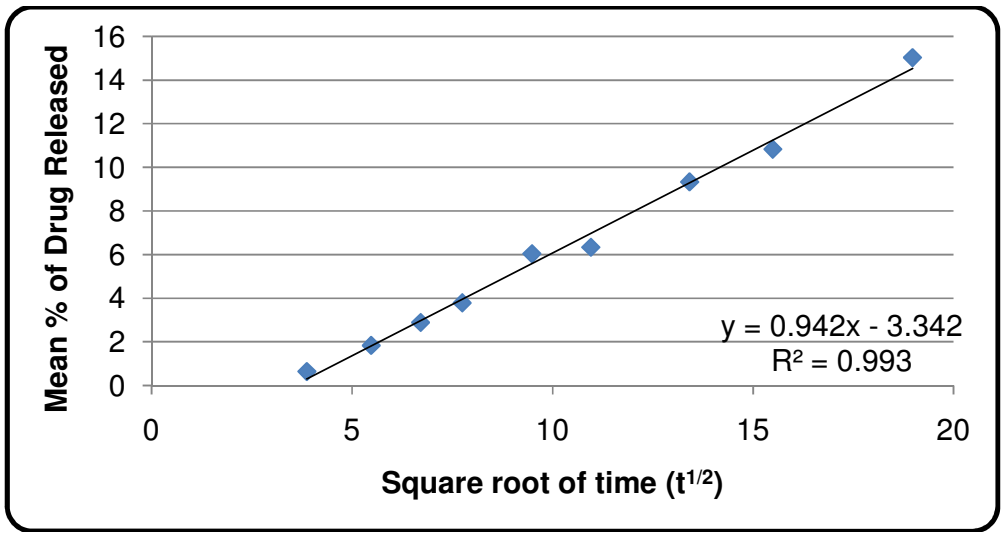

Fig. 5. Square root of time plot for formulation prepared with 3 lipid to 1 drug ratio

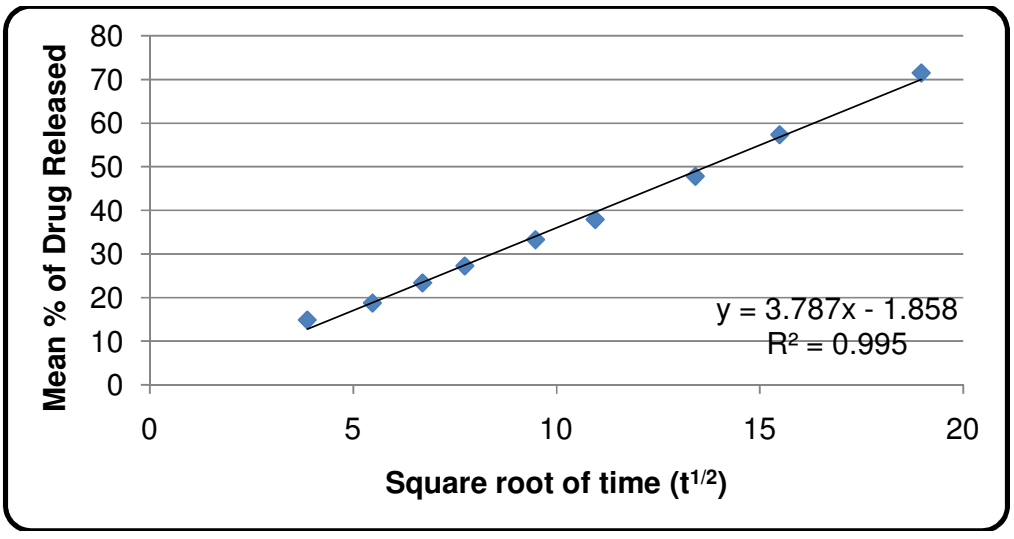

Fig. 6. Square roof of time plot for liposome prepared by heating method

Table 3. Particle size analysis for all liposome formulations

Formulation	Mean(µm)	SD (µm)
F1	1.2079	0.9352
F2	2.1906	1.3129
F3	1.2347	0.6761
F4	3.1704	1.4476
F5	1.0010	0.4129
F6	0.9726	0.3029
F7	2.4598	1.3167
F8	1.3501	1.0090
F9	1.9942	1.1716
F10	1.8801	0.8627

F1= 9 g HEC:3.6 g CH:0.9 g CH-H) prepared by 3 lipid:1 drug, 3 organic :1 aqueous phase ratio and conventional method; F2= (9 g HEC:3.6 g CH:0.9 g CH-R) prepared by 2 lipid:1 drug, 3 organic:1 aqueous phase ratio and conventional method; F3= (9 g HEC:3.6 g CH:0.9 g CH-H) prepared by 3 lipid:1 drug, 3 organic:1 aqueous phase ratio and conventional method; F4= (3.6 HEC:1.2 g CH:0.3 g CH-H) prepared by 1 lipid:3 drug and conventional method; F5= (9 g LEC:1.8 g CH:2.6 g CH-H) prepared by 3 lipid:1 drug and conventional method; F6= (9 g LEC:3.6 g CH:1.8 g CH-H) prepared by 3 lipid:1 drug and and conventional method; F7= (9 g LEC:3.6 g CH:0.9 g CH-H) prepared by 3 lipid:1 drug, 10 organic:1 aqueous phase ratio and conventional method; F8 (9 g LEC:3.6 g CH:0.9 g CH-H) prepared by 3 lipid:1 drug, 1 organic:1 aqueous phase ratio and conventional method; F9 (9 g LEC:3.6 g CH:0.9 g CH-H) prepared by 3 lipid:1 drug, 3 organic:1 aqueous phase ratio and heating method; F10= (6 g LEC:3.6 g CH:0.9 g CH-H) prepared by 3 lipid:1 drug, 3 organic:1 aqueous phase ratio and reverse phase evaporation method.

3.5 Scanning Electron Microscope

These scanning electron microscope data indicated that the ratio of lipid to drug, lipid to lipid, organic to aqueous phase, method and operation technique have effect on morphology and size of liposome Figs. 7 to 10.

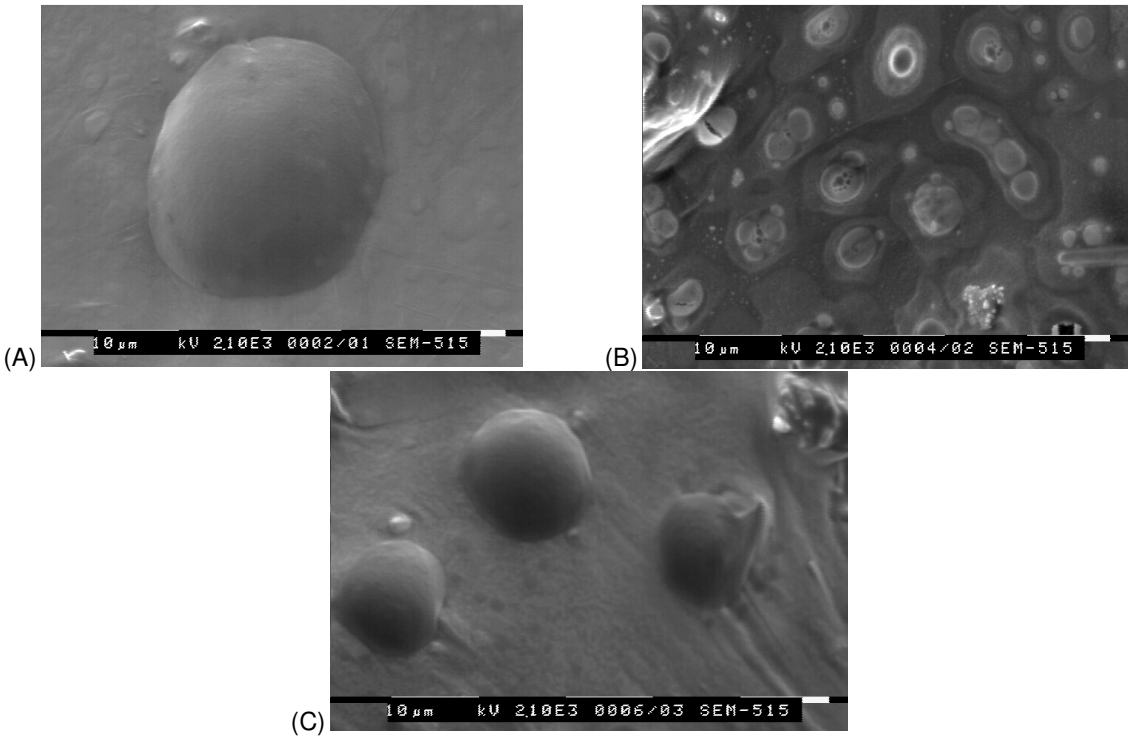

Fig. 7. Scanning electron microscope for liposome composed of different lipid to drug ratio. (A) 1 lipid: 1 drug, (B) 2 lipid: 1 drug, (C) 3 lipid: 1 drug

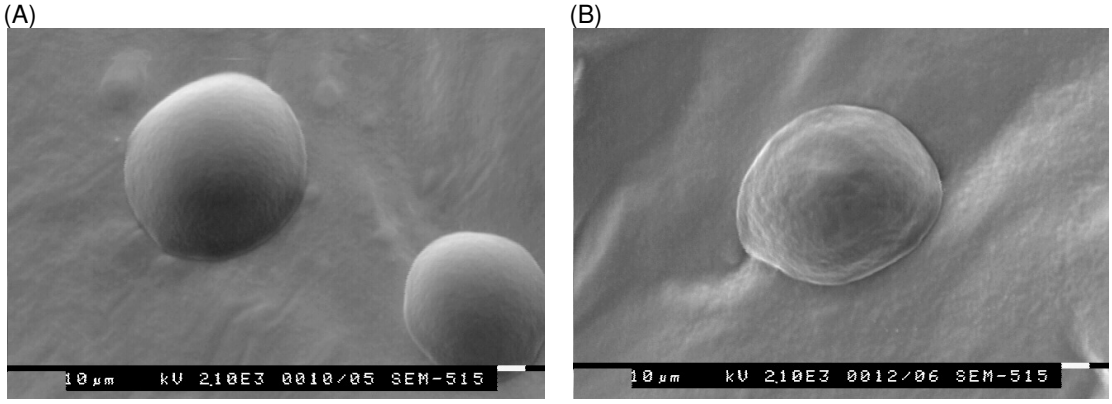

Fig. 8. Scanning eectron mcroscope for lposome composed of dfferent lpid to lpid ratio. (A) 9 g lethicin:1.8 g cholesterol:2.6 g cholesteryl hemisuccinate, (B) 9 g lecithin:3.6 g cholesterol:1.8 g cholesteryl hemisuccinate

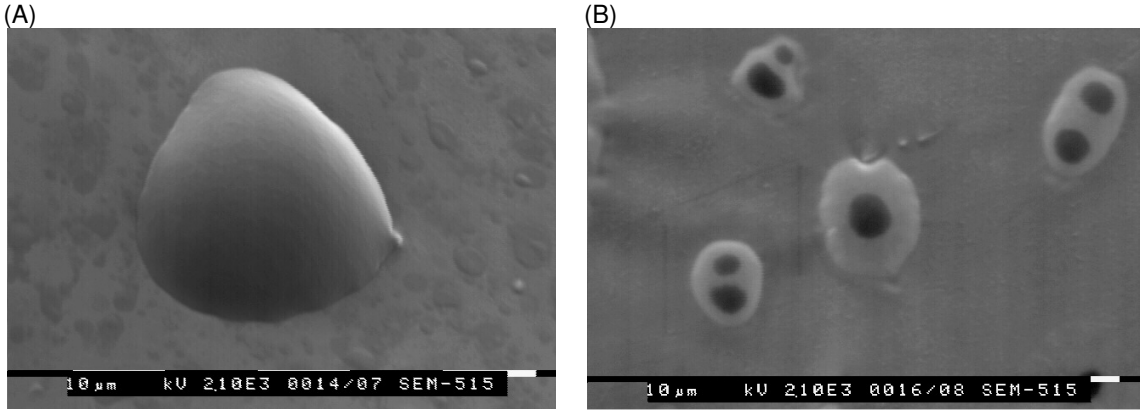

Fig. 9. Scanning electron mcroscope for lposome pepared by dfferent ratio of organic to aqueous phase. (A) 1 organic:1 aqueous phase, (B) 10 organic:1 aqueous phase.

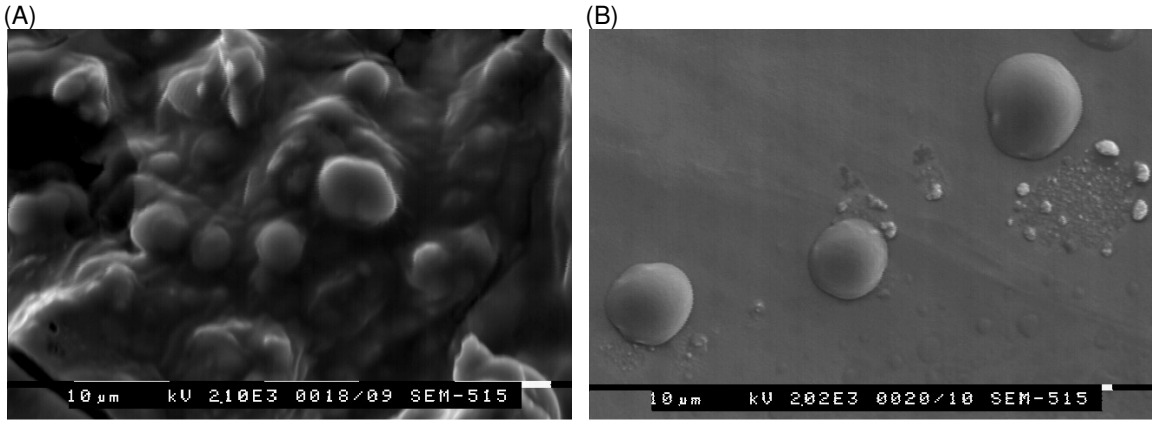

Fig. 10. Scanning electron microscope for liposome prepared by bifferent methods. (A) heating method, (B) reverse phase evaporation

4. CONCLUSION

Naproxen liposomes were successfully designed and prepared. The ratio of lipid to drug, lipid to lipid, organic to aqueous phase and methods of preparation affect the physico-chemical properties of the liposome, drug entrapment efficiency, drug release, particle size and shape of the liposomes. Formulation prepared with a ratio of 3 lipid to 1 drug and 3 organic to 1 aqueous phase using the thin film rehydration (conventional method) gave the highest entrapment efficiency (83.62%) and released only 15% at 360 minutes of testing dissolution. Formulation prepared with 1:1 organic to aqueous phase ratio entrapped only 11.09% drug, Formulation of 3 lipid:1 drug ratio showed 66.7%% drug entrapment, while formulation of the same composition but prepared with heating method gave 35.5% drug entrapment.

Formulation prepared with 3:1 organic to aqueous phase, gave 83.62% and liposome prepared with 10 organic:1 aqueous phase showed 62.37% drug entrapment. As mentioned previously, formulation prepared with 1 organic phase to 1 aqueous phase entrapped only 11.09% drug, As the ratio of lipid to drug increased, the entrapment efficiency increased and drug release decreased. Formulation prepared by heating method released 14.38% and 71.5% at 15 and 360 minutes respectively. The drug release from naproxen liposome followed Higuchi model for diffusion.

Most of the liposome formulations are spherical in shape as shown under the scanning electron microscope. Formulation prepared with 1 lipid to 3 drug gave the largest mean particle size diameter (3.17 um). Formulation composed of 9 g lecithin: 1.8 g cholesterol: 2.6 g g cholesteryl hemisuccinate and formulation composed of 9 g lethicin: 3.6 g cholesterol: 1.8 g cholesteryl hemisuccinate gave the smallest mean particle size, 1.0 and 0.972 ug respectively.

This study demonstrated that liposome is a promising delivery system for naproxen. Adittionaly the use of heating method can be proposed with the aim with the aim of reducing toxicity of residual organic solvent. In the final liposome delivery system.

This research, also demonstrated that the ratio of lipid to drug, organic to aqueous phase ratio, lipid composition and methods of preparation have direct effect on the drug entrapment efficiency, drug release, liposome size and physico-chemifcal properties of the liposome.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Russell RI. Non-steroidal anti-inflammatory drugs and gastrointestinal damage-problems and solutions. Postgrad Med J. 2001;77:82-88.
2. Rainsford KD. Anti-inflammatory drugs in the 21st century. Subcell Biochem. 2007;42:3-27.
3. Mohammed AR, Weston N, Coombes AG, Fitzgerald M, Perrie Y. Liposome formulation of poorly water soluble drugs: optimization of drug loading and ESEM analysis of stability. Int J Pharm. 2004;285:23-34.
4. Schwendener RA, Scott H. Liposome formulation of hydrophobic drugs, Methods Mol Biol. 2010;605:129-138.
5. Samad A, Sultana Y, Aqil M. Liposomal drug delivery systems: An updated review, Curr Drug Deliv. 2007;4:297-305.
6. Puglia C, Trombetta D, Venuti V, Saija A, Bonina F. Evaluation of *In vivo* topical anti-inflammatory activity of indometacin from liposomal vesicles. J Pharm Pharmacol. 2004;56:1225-32.
7. Allison DS. Liposomal drug delivery. J Inf Nurs. 2007;30:89-95.
8. Brown MB, Martin GP, Jones SA, komeah FK. Dermal and transdermal drug delivery systems: Current and future prospects, Drug Deliv. 2006;13:175-87.
9. Maestrelli F, González-Rodríguez ML, Rabasco ML, Mura P. Effect of preparation technique on the properties of liposomes encapsulating ketoprofen-cyclodextrin complexes aimed for transdermal delivery. Curr Drug Deliv. 2006;312:53-60.
10. Choi MJ, Maibach HI. Liposomes and niosomes as topical drug delivery systems. Ksin Pharmacol Physiol. 2005;18:209-219.
11. Singh B, Mehta G, Kumar R, Bjatia A, Ahuja N, Katare OP. Desig, development and optimization of liposomal systems for topical application. Curent Drug Deliv. 2005;2:143-153.
12. Perni Y, Kirby OJ, Mohamed AU, Mcneil SE, Vangala A. Environmental scanning electron microscope imaging of vescicle

system. Methods Mol. Biol. 2010;606:319-331.
13. Morieno MM, Garidel P, Suwalsky M, Howe J, Branderberg K. The membrane activity of ibuprofen, diclfen and naproxen: physico-chemical study with lecithin phospholipid. Biochem. Biophys. Acta. 2009;1788:296-303.
14. Pavola A, Kilpelaimen I, Ylirusi J, Rosenberg P. Controlled Release injectable liposomal gel of ibuprofen for epidural analgesia. Int. J. Pharm. 2000;199:85-93.
15. Gaur PK, Mihra S, Gupta VB, Rathod MS, Purohit S, Savla BA. Targeted drug delivery of rifampicin to the lungs:formulation, characterization, and studies of preformed aerolized liposome nd in situ formed aerolized aerolized liposome. Drug Dev. Ind. Pharm. 2010;36:638-646.
16. Pradhan P, Banerjee R, Bahadur O, Koch C, Mykhayly O, Plank C. Targeted magnetic liposomes loaded with dextrorubicin. Methods Mol. Biol. 2010;605:279-293.
17. Shwendener RA, Ludewig P, Serny A, Engler O. Liposome-based vaccines. Methods Mol. Biol. 2010;605:163-175.
18. Pavlova O, et al. An overview method of preparation and characterization of liposome as drug delivery system, Int. J. Phytopharmacol. Res. 2013;32:182-189.
19. Mozafari MR. Liposome: An overview of manufacturing techniques. Cell. Mol. Biol. LETT. 2005;10:711-719.
20. Szoka F, et al. Procedures for preparation of liposomes with large internal aqueous space and high capture by reverse phase evaporation. Proc. Natl. Acad. Sci. USA. 1978;75:4194-4198.
21. Panwar P, et al. Preparation, characterization, and *In vitro* release study of albendzole encapsulated nano size liposome. Int. J. Nanomedicine. 2010;5:101-108.
22. Higuchi T. Release of drug from homogeneous and granular dosage forms, J. Pharm. Sci. 1961;50:874.

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