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Formulation and In-vitro Characterization of Metformin Hydrochloride-loaded Liposomes

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Abstract

The aim of study. is to formulate Metformin hydrochloride-loaded novel liposomal vesicles and investigate their physical stability. A new metformin hydrochloride (Met-HCL) liposomal formulation was prepared for topical delivery. Traditionally, the biguanide metformin could be categorized the first line in treatment of diabetes. The prepared metformin hydrochloride-loaded liposomal vesicles were investigated for different in vitro characterisations. Eleven different formulations were developed adopting a thin film hydration method using different molar concentrations of Phospholipon® 90G, cholesterol and metformin hydrochloride. The effect of varying concentrations of Phospholipon® 90G, cholesterol and metformin hydrochloride on entrapment efficiency percent, ex-vivo skin permeation percentage, vesicle size and zeta potential was studied. Metformin-loaded liposome stability over a period of time 90 days was investigated. **Results.** The optimized metformin hydrochloride liposomes, F2, F6 and F11 were selecte. The selected formulations displayed highly efficient permeation percent via the excised mice skin 53±0.09 %, 30±0.4 % and 40±0.02 % respectively. The formulations showed EE % of 80±0.09 %, 28.6±0.02 % and 71.8±0.4 % respectively. Morphology of F2 liposomal surface revealed spherical three-dimensional structure. The stability study revealed about 10-23 percent drug leaching out of the vesicular liposomes (F2) within 90 days. **Conclusion.** Metformin hydrochloride-loaded liposomal vesicles can provide a potentially promising and convenient approach for topical delivery.

Keywords: Metformin HCL; Liposome; ex-vivo permeation; skin; Drug formulations; stability study; parameter.

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1. Introduction

The liposomes are spherical lipid-based artificial vesicular structure consisting of lipid bilayer membrane (phospholipid) and cholesterol surrounding an aqueous core. The main constituent of the lipid bilayer membrane is the phospholipid [1]. These vesicles can capture different pharmaceutical ingredients such as antineoplastic, antimicrobial drugs, chelating agents, steroids, vaccines, and genetic materials [2]. Liposomal vesicles can be modified through various method of preparation to increase their stability such as freeze drying technique [3, 4], surface coating [5] and the surface polymerization [6]. These vesicles have a unique construction with their nano scale, nontoxic, biocompatible, biodegradable and flexibility characters which offer a controlled drug delivery to specific organs [7]. The liposomal vesicle was firstly introduced as skin drug delivery systems by Mezei and Gulasekharam [8]. They have been the first to display the role of the topical liposomal formulations in augmenting the skin deposition of encapsulated medications and depressing their systemic absorption resulting in a diminished toxicity of the entrapped active constituents [9]. The greater role of liposomes in enhancing the drug transport and penetration through the skin may be attributed to their phospholipid components which are structurally similar to the skin lipid layers and their self-assembly properties which permit regaining of closed vesicles when deformed during skin penetration [10]. Metformin Hydrochloride belonging to biguanide classes is considered the first line drug for treatment of diabetic patients that are newly diagnosed with type II diabetes beside a supplement to diet and exercise. Metformin taken orally has a half-life ranging from 1.5 to 1.6 h and daily requirement of 1.5–3 g / day with 50–60% absolute bioavailability [11-13]. Metformin mechanism of action is elucidated through improving glucose tolerance by decreasing both basal and postprandial plasma glucose. The drug improves the body's sensitivity to insulin by increasing peripheral glucose uptake and utilization in peripheral tissues like muscles and fats. It also potentiates insulin action in muscles [14]. Numerous studies have investigated the effectiveness of metformin for management of some diseases[15].

2. Experimental

2.1. Materials

Phosphatidylcholine from Soyabean (Phospholipon® 90G) was a gift from the lipoid company (Ludwigshafen, Germany). Metformin hydrochloride (Met) was a gift from Merck Company (Cairo, Egypt). Cholesterol, Absolute ethanol and Chloroform were purchased from the Sigma Aldrich (St. Louis, MO, USA). Deionized water was provided from the microbiological laboratory for water and food analysis (Minia, Egypt). Sodium hydrogen phosphate and acetic acid were purchased from the ADWIC, El-Nasr Pharmaceutical Co., (Egypt). Mice skin (previously prepared, excised and used within 2 weeks).

2.2. Equipment

Centrifuge (Hermle® Z326 K Centrifuge) (Germany). device of Ion-Sputtering Jeol Fine-Coat JFC 1200E, the Electron Microscope Jeol JSM-5400 LV and X-Ray Diffractometer Jeol, (Tokyo, Japan).Mastersizer 3000E Malvern Instruments, (UK).Milli-DI® Water Purification System (Germany) .Rotary evaporator (Stuart, RE300, Germany).Shaking water bath (PolyScience, model 20 L-M, Niles, IL, USA) . Sonicated water bath BranSonic

220, (Zurich, Switzerland) .UV/VIS spectrophotometer (Spectronic Genesys®, with Winspec Soft-ware, Spectronic, USA). Shaking water bath (PolyScience, model 20 L-M, Niles, IL, USA) Thermostatic stirrer (Germany) .Transmission electron microscope (TEM) JEOL (JM 1400 EX, MA, USA).

2.3. Methods

2.3.1. Analysis and establishing of standard calibration curve for metformin

Spectrophotometric analysis and establishment of standard calibration curve of metformin in deionized water was prepared according to Shankar et.al [16]. Briefly, a stock solution was prepared by dissolving 10 mg of metformin with 100 ml of deionized water. The prepared solution was diluted with deionized water to get a final concentration 0.1 µg/µl. Using the previous stock solution, different serial dilutions were carried out to have concentrations of 20, 40, 60, 80, 100, 120, 140, 160, 180, 200 and 220 µg/ml and their adjuvant absorbance was recorded. The maximum wave-length (λ max) of metformin was observed at 237 nm [17]. The λ max of metformin in the above-mentioned media was obtained at 237 nm by scanning a suitable dilution of the stock using the UV–VIS spectrophotometer[18]. All the previous procedures were done in triplicate and the average values were used. A calibration standard curve of metformin assembled in a phosphate citrate solution buffer (pH=5.5) was prepared by dissolving 10 mg of metformin with 100 ml of deionized water which was diluted with citrate phosphate (PH 5.5) to get a final concentration of 1 µg/ml. The λ max of metformin in the above-mentioned media was obtained at 237 nm by scanning a suitable dilution of the stock using the UV–VIS spectrophotometer [17]. Using the previous stock solution, different standard dilutions were carried out to obtain concentrations of 60, 80, 100, 120, 140, 160, 180, 200 and 220 µg /ml and their adjuvant absorbance was recorded. All the previous procedures were done in triplicate and the average values were used.

2.3.2. Preparation of metformin-loaded liposomal formulations

Thin film hydration (TFH) method is one of the most common methods of preparation of liposomes [19]. Metformin liposome formulations were prepared adopting the thin film hydration technique that reported by Bangham [20].Briefly, a specified amounts of Phospholipon® 90G and cholesterol with different molar ratios (table 1) were dissolved in a least volume of chloroform. The organic solvent has been evaporated slowly under reduced pressure at 65 °C using a rotary evaporator (Stuart, RE 300, Germany) at 110 rpm until a dried thin film from phospholipids and cholesterol on the inner wall of rotating round flask was formed. The lipidic film is re-dissolved and the whole process of organic solvent evaporation was repeated until having uniform and homogenous dried film. The film was kept in desiccator containing anhydrous calcium chloride for 24 hours to ensure removal of any organic solvent residue lipid bilayer. The dried lipid film was then hydrated with a specified amount of metformin hydrochloride dissolved in deionized water and rotated in a water bath at 65 °C and under atmospheric pressure for 2 hours to ensure complete hydration of the lipid film. The resultant liposome was kept at 4 °C overnight to allow the lipid bilayer annealing [21]. The suspension was retained is a fridge for further investigations.

Table 1: Composition of the prepared liposomes using several molar ratios

Formulations	Total lipid molar concentration	Drug to lipid ratio	Molar ratio (lipid: cholesterol)
F1	80 mM	2:1	70:30
F2	80 mM	4:1	70:30
F3	80 mM	1:1	70:30
F4	40 mM	2:1	70:30
F5	40 mM	4:1	70:30
F6	40 mM	6:1	70:30
F7	60 mM	2:1	50:50
F8	60 mM	2:1	40:60
F9	60 mM	2:1	90:10
F10	60 mM	2:1	70:30
F11	60 mM	4:1	70:30

2.3.3. In-vitro characterization of liposomes of metformin HCL

2.3.3.1. Macroscopic examination

The prepared formulations were inspected with naked eye to check homogeneity and sedimentation.

2.3.3.2. Determination of metformin entrapment efficiency (EE %)

The direct method was used to determine the percentage of metformin hydrochloride that encapsulated within the prepared liposomal vesicles. Metformin-loaded liposomal vesicles were separated from the free unentrapped Metformin with centrifugation of 1 ml liposomal suspension at 15,000 rpm and 4 °C for 2 hours. The separated liposomes were washed twice in two separate steps by re-suspending in deionized water to ensure absolute removal of the un-entrapped drug. The washed liposomes were exposed to acetonitrile (liposomes: acetonitrile 1:4) followed by vortexing and sonication until a homogenous suspension was obtained. Then centrifugation the prepared suspension with 15000 rpm for 30 minutes and the supernatant was separated and properly diluted using deionized water. The amount of entrapped metformin was determined by measuring the absorbance spectrophotometrically at 238 nm using a UV spectrophotometer (Spectronic Genesys®, with Winspec Software, Spectronic, USA). The percentage drug entrapped was calculated using the following equation 1:

$$EE\% = \frac{(\text{amount of Metformin entrapped})}{(\text{total amount of Metformin added})} \times 100 \qquad \text{equation 1}$$

Metformin-free liposomal vesicles as a blank were prepared adopting the same procedures. Steps were repeated

in a triplicate manner (n=3) and the mean amount of drug entrapped was considered.

2.3.3.3. Liposomal vesicle Size analysis and zeta potential determination

Laser light diffraction technique was used to determine the vesicular size of the prepared metformin-loaded liposomes and size of distribution. In briefly, the liposomal suspension formula has been diluted by using proper media typically purified distilled water and was analyzed at 25 °C using an Instruments of Mastersizer 3000E Malvern, UK [19]. The procedures were repetitive in a triplicate manner for each preparation then the average values were used. The charges on the metformin liposomal surface have been determined. The prepared liposomes were diluted formerly using Millipore water and the zeta potential was determined for about 60 seconds and the average zeta potentials were determined.

2.3.3.4. Ex-vivo skin permeation study

Ex-vivo permeation via skin of metformin from the prepared systems was studied using an excised abdominal skin of female mice. Briefly, hair was carefully trimmed from the abdominal skin. Mice have been sacrificed via cervical dislocation. The abdominal skin was detached, and the dermal fatty layer was removed using an isopropyl alcohol and a scalpel. The prepared skin was cut into appropriate size, kept frozen and used within two weeks. The skin tissues of the frozen mice were first immersed in a solution of sodium chloride (0.9 %) for one hour before experiment. the mice skin was then fitted at the modified Franz diffusion cell so that the stratum corneum side facing upward (the sample compartment) and the deeper layers of skin facing the reservoir that enclosing the citrate-phosphate buffer solution (pH 5.5) as a receptor media. An appropriate amount of metformin-loaded liposomes was loaded in the sample compartment. The whole cell was kept at 37 ± 0.5 °C as reported by Mostafa and his colleagues [22], and shaken at 50 ± 10 rpm. 2 ml solution samples from the media has been withdrawn at determined time intervals over a period of 6 hours and replaced with fresh media maintained at the same temperature. The amount of metformin permeated through the skin was determined by measuring the absorbance spectrophotometrically at 238 nm. The study was carried out in triplicate and the average values were reported (n=3) [23].

2.3.3.5. Transmission Electron Microscopy (TEM)

The morphology of the metformin-loaded liposomes was investigated via transmission electron microscope (JEM 1400 EX, Peabody, MA, USA) that operated with 80 kV. A droplet of the liposomal suspension was loaded on the surface of carbon-coated copper gride that was left for ten minutes to dry at room temperature before examination.

2.3.3.6. Statistical analysis

All studies were carried out in a triplicate manner and the average value ± SD was calculated. ANOVA test was used to calculate the significance of differences recorded. A (P-value) of less than 0.05 was considered statistically significant. Results are expressed as mean ± Standard deviation.

2.3.3.7. Physical stability study

Physical stability study was performed to investigate the extent of drug leakage from the liposomal formulations and the changing in the liposomal vesicle size during the storage. Three liposomal formulations were selected for this study; F2, F6 and F11, for their higher entrapment efficiencies and optimal release rates. Specific volumes from each batch were sealed in 20 ml glass vials surrounded by aluminium foil for light protection , and stored for 3 months at refrigerator temperature 2–8 ºC [24]. At the predetermined period of time intervals (1, 2 and 3 months of storage), Samples were taken for analysis from each batch and the retained quantity of the trapped drug in the vesicles was determined after the leached drug was removed. The Stability for the formulation was defined in terms of the following equation 2: Metformin HCl retained in liposomes = Amount of metformin HCl entrapped after storage / Amount of metformin HCl entrapped before storage × 100
equation 2. The vesicle size for each sample was analyzed at the start and the end of the storage period and the data was compared to the freshly prepared liposomes as an index of stability.

3. Results

3.1. Calibration curve for metformin

Calibration curve of metformin solution was conducted in two different solvents. The first was deionized water; the second was citrate phosphate buffer pH 5.5. Results indicated that the R2 were 0.998 and 0.997 in both solutions respectively (figure 1).

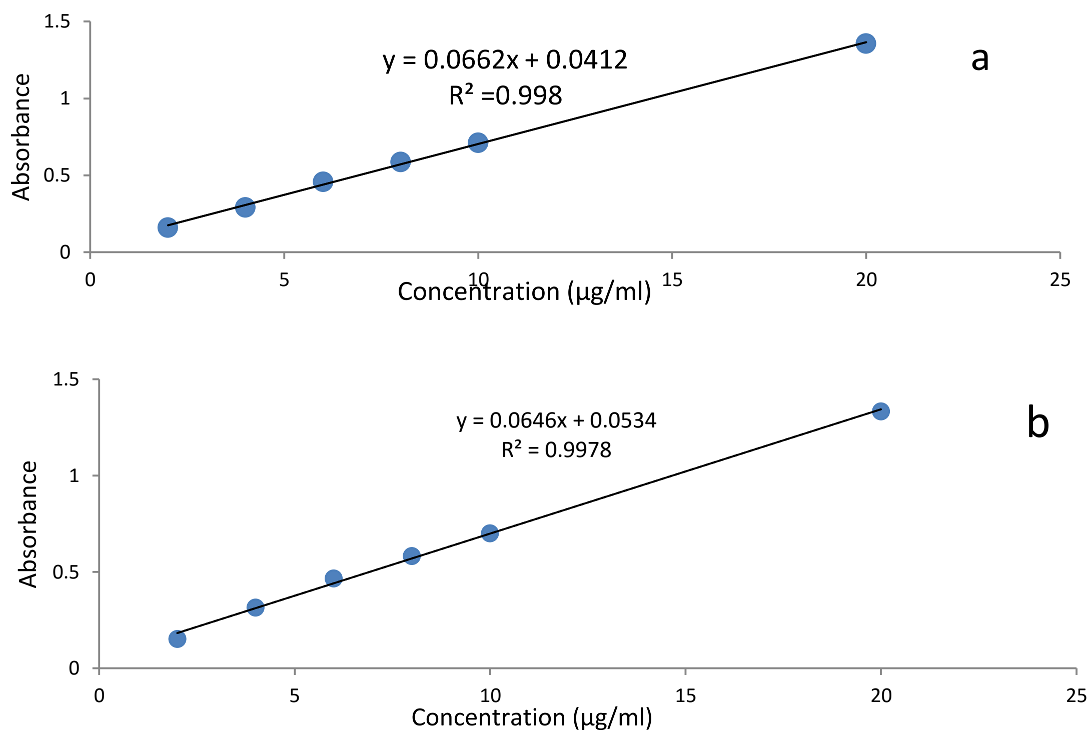

Figure 1: Calibration curve of metformin in (a) Deionized water, (b) citrate phosphate buffer pH 5.5. Data values have been presented the mean of three measurements

3.2. Effect of different parameters on entrapment percentage

As presented in the table (2), the entrapment efficiency (EE %) for the metformin-loaded liposomes ranging from 12.8 % to 80 %. The entrapment percentage of metformin increased from 26 % to 38 % and 80 % at fixed lipid molar concentration 80 mM and cholesterol molar concentration 30% upon increasing ratio of drug to lipid from (1:1) to (2:1) and (4:1). The entrapment percentage also increased from 52.8% to 71.8% and 80% ,upon elevating the total lipid molar concentration from 40 mM to 60 mM and 80 mM on constant 70:30 Molar ratio (lipid: cholesterol).

Table 2: Effect of different formulation parameters on EE % of metformin Liposome

Formulations	Total lipid molar concentration	Drug/lipid ratio	(lipid : cholesterol) Molar ratio	EE%
F1	80 mM	2:1	70:30	38±0.01 %
F2	80 mM	4:1	70:30	80±0.09 %
F3	80 mM	1:1	70:30	26±0.06 %
F4	40 mM	2:1	70:30	25±0.01 %
F5	40 mM	4:1	70:30	52.8±0.04 %
F6	40 mM	6:1	70:30	28.6±0.02 %
F7	60 mM	2:1	50:50	44.5±0.01 %
F8	60 mM	2:1	40:60	36.1±0.01 %
F9	60 mM	2:1	90:10	17.8±0.4 %
F10	60 mM	2:1	70:30	16.2±0.06 %
F11	60 mM	4:1	70:30	71.8±0.4 %

3.3. Effect of different parameters on permeation percentage

Table.3 showed the values of permeation percentage of metformin liposome formulas. Permeation percentage highly significant ($p<0.05$) increased from 30±0.4 % to 40±0.02 % and 53±0.09 % upon increasing the total lipid molar concentration from 40 mM to 60 mM and 80 mM at constant molar lipid to cholesterol ratio (70:30) and drug/lipid ratio (4:1). Also, at constant total lipid molar concentration of 40 mM and lipid to cholesterol ratio of 70:30, the permeation percentage of metformin increased from 13±0.01 % to 30±0.4 %, as the drug/lipid ratio increased from (2:1) to (6:1). On the other hand, a significant decrease in the permeation percentage ($p<0.05$) was observed from 30±0.4 % to 11±0.01 % subsequent to the increase in the lipid to cholesterol ratio from (90:10) to (40:60).

Table 3: Effect of different formulation parameters on P % of metformin Liposome

Formulations	Total lipid molar concentration	Drug/lipid ratio	(lipid : cholesterol) Molar ratio	Permeation %
F1	80 mM	2:1	70:30	35±0.3 %
F2	80 mM	4:1	70:30	53±0.09 %
F3	80 mM	1:1	70:30	31±0.09 %
F4	40 mM	2:1	70:30	13±0.01 %
F5	40 mM	4:1	70:30	15±0.04 %
F6	40 mM	6:1	70:30	30±0.4 %
F7	60 mM	2:1	50:50	13±0.01 %
F8	60 mM	2:1	40:60	11±0.01 %
F9	60 mM	2:1	90:10	30±0.4 %
F10	60 mM	2:1	70:30	19±0.4 %
F11	60 mM	4:1	70:30	40±0.02 %

3.4. Effect of different parameters on liposomal vesicle size

Thin film hydration technique failed to produce homogenous liposomes in terms of size. The vesicle size of prepared metformin liposome ranged between 5.02±2.0 nm and 10.9±3.9 µm (table 4).These values indicated the insignificant effect of the total lipid molar concentration, drug/lipid ratio and the molar ratio of lipid: cholesterol on the vesicular size of metformin-loaded liposomes. Such as in constant Drug/lipid ratio (4:1) and (lipid: cholesterol) Molar ratio (70:30), we observed vesicle size enlarging from 5.99±1.9 µm to 9.09±12.9 µm and 10.9±3.9 µm accompanied with 40 mM, 60 mM and 80 mM Total lipid molar concentration ($p>0.05$).

Table 4: Effect of different formulation parameters on Liposomal size

Formulations	Total lipid molar concentration	Drug/lipid ratio	(lipid : cholesterol) Molar ratio	Vesicle size (µm)
F1	80 mM	2:1	70:30	8.09±10
F2	80 mM	4:1	70:30	10.9±3.9
F3	80 mM	1:1	70:30	10.32±9.5
F4	40 mM	2:1	70:30	5.02±2.0
F5	40 mM	4:1	70:30	5.99±1.9
F6	40 mM	6:1	70:30	6.9±4.1
F7	60 mM	2:1	50:50	9.8±2.3
F8	60 mM	2:1	40:60	7.99±3.8
F9	60 mM	2:1	90:10	8.99±2.9
F10	60 mM	2:1	70:30	7.36±10.6
F11	60 mM	4:1	70:30	9.09±12.9

3.5. Effect of different parameters on zeta potential

The zeta potential of the prepared metformin liposome ranged between -53.44 and -3.2 mV. The effect of various variables on the liposome zeta potential loaded with metformin was readily investigated. Table (5) shows Zeta potential values for all the prepared metformin liposome formulations.

Table 5: Effect of different formulation variables on liposome zeta potential

Formulations	Total lipid molar concentration	Drug/lipid ratio	(lipid : cholesterol) Molar ratio	Zeta potential (mV)
F1	80 mM	2:1	70:30	-40.8 ± 16.0
F2	80 mM	4:1	70:30	-53.44± 17.7
F3	80 mM	1:1	70:30	-10.7 ± 9.2
F4	40 mM	2:1	70:30	-3.2 ± 11.9
F5	40 mM	4:1	70:30	-7.9 ± 22.5
F6	40 mM	6:1	70:30	-16.1± 10.6
F7	60 mM	2:1	50:50	-18.1± 18
F8	60 mM	2:1	40:60	-9.09± 17.7
F9	60 mM	2:1	90:10	-11± 10.2
F10	60 mM	2:1	70:30	-23.4 ± 10.2
F11	60 mM	4:1	70:30	-47.7 ± 17.7

3.6. Selection of the optimized liposomal formulation

The optimized formulations were selected based on the function to have the maximum entrapment percentage, permeation percentage, zeta potential and the possible minimum vesicle size. Thus, F2, F6 and F11 have been selected. Table 6 presented the optimized selected formulations.

Table 6: Different characterizations of the selected optimized formulations

	EE%	P%	Vesicle size (µm)	Zeta potential (mV)
F2	80±0.09 %	53±0.09 %	10.9±3.9	-53.44± 17.7
F6	28.6±0.02 %	30±0.4 %	6.9±4.1	-16.1± 10.6
F11	71.8±0.4 %	40±0.02 %	9.09±12.9	-47.7 ± 17.7

3.7. Transmission Electron Microscopy (TEM)

Figure 2 showed the morphology of metformin hydrochloride-loaded liposomes that concern F2 that has the smaller size which indicated the spherical morphology and three-dimensional structure of liposomal vesicles. This ensures the presence of unilamellar and multilamellar vesicles with wide range of size distribution.

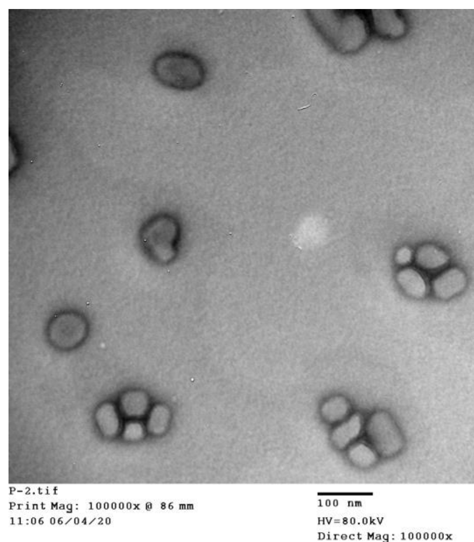

Figure 2: Metformin HCL-loaded liposomes (F2) TEM image

3.8. Physical stability study

The visual observations for all formulations revealed good physical stability with partial sedimentation (without coarse particles aggregation) that could be returned to homogenous form by slight agitation and with no layer separation also with reduced aggregation rate of liposome and fusion during storage. Additionally, was noted that F2 and F11 showed homogenous white color with no color changes [25, 26]. Table 7 represents the percentages of the drug retained in liposomal formulations; F2, F6 and F11 after 30, 60 and 90 days. It is obvious from the results that the percentage of the drug leaching out of the vesicles was in direct relationship with aging. Table 7 also exhibited the vesicle size changes that occurred on metformin HCl liposomes formulations after 90 days of storage compared to their sizes at the initial time of the study. These results showed an insignificant increasing in the vesicle sizes of the stored liposomal formulations ($P<0.05$) where F2, F6 and F11 increased by around 4% than its initial size.

Table 7: Effect of storage at 4 ℃ on the mean drug retained and mean vesicle size of the selected metformin HCl liposomal formulations

Formula	F2		F6		F11	
Time(days)	Drug retained (%)	Mean Vesicle Size(nm)	Drug retained (%)	Mean Vesicle Size(nm)	Drug retained (%)	Mean Vesicle Size(µm)
Initial	100	10.9	100	6.9	100	9.09
30 days	98	___	90	___	90	___
60 Days	96	___	80	___	89.01	___
90 days	90.01	11.6	77	8.5	79	13.39

4. Discussion

The increasing of the entrapment percent of the metformin-loaded liposome might be due to the increase in the total lipid molar concentration as previously reported by Kesisoglou and his colleagues [27]. Increasing the total lipid molar concentration resulted in increasing the core size of the formed liposomes which consequently increased the entrapment of metformin hydrochloride within the aqueous core. These outcomes are in consistence with Xu and his colleagues and Barenholz [28, 29]. Increasing the entrapment percentage due to the increased drug/lipid ratio could be justified by the high aqueous solubility of metformin hydrochloride and saturation of the aqueous media with the drug that augmented by Mokhtar and his colleagues and Pons and his colleagues [30, 31]. The observed increase of metformin permeation through the excised mice skin by the increase in the total lipid molar concentration might be attributed to the increase of the amount drug entrapped. As discussed previous, the enlarged internal size of liposomes consequent to the increase in the total lipid molar concentration would enhance EE% and thus metformin permeation. The increased Permeation % of metformin HCL due to increased drug to lipid ratio was in consistence with Elsayed and his colleagues and Bouwstra and his colleagues [32] who justified that drug permeation increases with the increased amount of the drug accumulated in the vesicle and being available for diffusion. This is in agreement with a previous study that has reported the dependence of the drug permeation on the drug entrapped in the liposomes [33]. On the other hand, increasing the lipid to cholesterol ratio has resulted in decreasing the Permeation % due to decreasing EE% and increasing the rigidity of the lipid bilayer as explained by Jaafar-Maalej and his colleagues [34]. Size of liposome is the most crucial factor for in-vivo behavior which controls penetration and targeting of medication-loaded in liposomes [35]. Different liposome preparation techniques, however, produce a heterogeneous population and may contain organic solvent residues [36]. Liposomal size that larger than 100 nm, are more predisposed to to opsonization and clearance by the reticulo-endothelial system in higher rates than smaller ones [37]. Many methods have been used to regulate the liposomal preparations size and its homogeneity. several studies have been used a Sonication technique which an example of size reduction technique [38] which may destruct the phospholipids due to the energy input and liability for oxidation [39]. Another technique is the extrusion process [40] which involves using expensive, time consuming and sensitive devices [41-43]. We suppose that the main reason for the insignificant vesicle size enlarging and non-homogeneous size distribution with large PDI values more related to the thin film hydration technique[22].Zeta potential of the prepared liposomal formulations was with negative value. The negatively charge observed for all formulations might be attributed to the changes in the liposomal surface structure due to entrapment of different drugs between lipid bilayer molecules [44]. One of the major expected changes is the change in co-ordination of the Phosphatidyl choline head group at the liposomal vesicle surface [45, 46]. The values of zeta potential of the prepared liposomal systems indicated that they had low stability with higher tendency to aggregate. This finding could be attributed to the lipid composition of the liposomal membrane that determines the liposomes' surface charge. The charge type and abundance entirely affect the degree of interaction between the cells and liposomes. Also, the physical stability of the resultant liposomes was significantly reduced by the lack of the surface charge. The neutral charged liposomes are incapable of interacting with cells [47, 48]. The results of physical study could explain the stability of the investigated liposomes and this might be due to slight fusion and aggregation of the liposomes after storage. We draw that the prepared liposomes could better maintain the entrapped drug under

storage conditions [49].

5. Conclusion

Formulations of metformin-loaded liposomes were designed adopting different molar concentrations phospholipid, cholesterol and drug. The results analysis revealed that increasing total lipid molar concentrations and drug/lipid ratio increased EE% and P% while increasing the lipid/cholesterol molar ratio resulted in a decrease in these variables. On the other hand, cholesterol had a negative effect on these variables while liposome vesicle size was not significantly affected by total lipid molar concentrations, lipid/ cholesterol or drug/lipid molar ratio. Thus, these findings show that the formulation of liposomes loaded with metformin HCL is a promising drug delivery system that may be a possible therapeutic option for treating some skin disorders.

6. Recommendation

Further studies are to be attempted to use metformin HCL-loaded liposomes for to investigate their topical efficacy.

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