Original Article

Preparation of Ketoconazole Liposomes with an Ultrasonic and an Injection Method Using Vegetable Oils

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ABSTRACT

Introduction: Ketoconazole as antimycotic drug have a great impact in the treatment of many skin diseases. The toxicity of available ketoconazole in form of tablets, cream and shampoo is overcome by encapsulation in liposome structures which show potential benefits in aspect of biodegradability, increased stability and prolonged drug releasing. **Objectives:** The main objective of this study was to develop new formulations of ketoconazole liposomes characterized with satisfactory encapsulation efficiency and stability. **Methods:** Liposomes were prepared by an ultrasound and an injection method. In the liposome preparation, sunflower and olive were introduced instead of harmful organic solvents. The obtained liposomes were characterized according to the encapsulation efficiency, zeta potential, electrical conductivity, morphological appearance, particle size and stability. The highest encapsulation efficiency of 70.33% and 87.06% was obtained using ketoconazole:cholesterol:L-α-phosphatidylcholine in the ratio of 1:2:1.67 w/w/w and 3.33:1:3.33 w/w/w, respectively, at the ultrasound and the injection method, using water at hydration medium, oil as solvent and centrifugation at 5000 rpm. During one month storage, the stability of obtained liposomes was higher at 4°C compared to 25°C. By application of the ultrasound method small unilamellar ketoconazole liposomes were formed, while large unilamellar and multilamellar by injection method. **Conclusion:** Introducing the sunflower and the olive oil, eco friendly preparation method was established, as well as new formulations of ketoconazole liposomes were developed.

Key words: Ketoconazole liposomes, Vegetable oil, SUV, LUV, MLV.

INTRODUCTION

The application of liposomes has been increased in recent years due to physical and chemical characteristics including biocompatibility, biodegradation and non-toxicity in the cells. Liposomes are vesicles composed of one or more phospholipid bilayers surrounding an aqueous phase.[1] Because of amphiphilic character of phospholipids, hydrophobic and hydrophilic active substances can be encapsulated in the liposomes. They are very important in improving stability and toxicity decreasing of encapsulated active substances. These artificially prepared structures allow to be used for drug delivery via many routes: oral, nasal, topical, ocular, and muscular. Liposomes are considered to be models in the improvement of *in vitro* and *in vivo* activity, also.[2,3] Size, structure, composition, and preparation methods are the main determinants in liposome classification. The liposomes are classified in three main types: small unilamellar vesicles (SUV), large unilamellar vesicles (LUV), and multilamellar

Submission Date: 08-03-2020;
Revision Date: 27-04-2020;
Accepted Date: 13-08-2020

DOI: 10.5530/ijper.54.4.188

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vesicles (MLV). Thin-film hydration method is the most commonly used for obtaining MLV, ultrasound and extrusion methods for SUV, while injection method to prepare LUV. In general, characterization of liposomal vesicles include determination of the encapsulation efficiency, morphology, zeta potential and particle size.[4] The liposome preparations undergo the side reactions including peroxidation, aggregation, hydrolysis, drug permeability and leakage from the formulation during time.[5,6] These problems were overcome by incorporation of compounds such as cholesterol, lipids with short chains, saturated bonds or oil from grape seed, olive, and almond, which positively effect on the stability of liposomal vesicles.[7-9]

One of the actual problems all around the world is the condition of many skin diseases. As antimycotic lipophilic drug, ketoconazole is used in medical treatment of various skin disorders and against fungal infections, available in free form such as tablet, shampoo, and cream.[10-12] The ketoconazole side effects and decreased stability influenced by chemical, thermal, oxidative, and acidic conditions are the main requests leading in development new formulations and structures.[13] Main challenges are vesicular systems, which are able to meet the needs of drugs i.e. achieving a therapeutic index and drug release in a controlled mode. A several aspects were considered in liposome selection such as phospholipid biocompatibility, digestion in the small intestine in the presence of bile and enzymes, encapsulation and delivery of various types of water soluble and insoluble drugs, increased capacity to solubility of insoluble drugs, high effect on stability maintenance, capability to encapsulate small drug amount, as well as in relation to the equipment and preparation methods used.[14-16] MLV ketoconazole prepared with thin-film hydration method,[17] using soya lecithin phospholipid[18] and dichloromethane or chloroform as solvents were reported in the literature.[19,20] In the development and characterization of ketoconazole encapsulated in liposomes by application of ultrasound and injection method, the introducing of the vegetable oils instead of the harmful solvents used was studied.

MATERIALS AND METHODS

Materials

Ketokonazole was obtained as a gift sample from the pharmaceutical company, Replek Farm DOOEL (Republic of North Macedonia). Cholesterol was purchased from Calbiochem (Japan), egg yolk L-*a*-phosphatidylcholine from Sigma (Germany), cedar wood immersion oil from Fluka (Switzerland), analytical grade methanol from Merck (Germany) and sodium chloride from Alkaloid AD (Republic of North Macedonia). Sunflower and olive oil were supplied from the local market in the Republic of North Macedonia.

Methods

Liposome preparation

Liposomes were prepared by application of the ultrasound and the injection method. *Preparation of organic phase.* Ketoconazole (KT), cholesterol (CH) and L-*a*-phosphatidylcholine (PC) were weighed with 0.1 mg accuracy on an analytical balance (Mettler Toledo, Switzerland) as per formulations given in Table 1-3. The organic phase was prepared by dissolving the mixture of KT, CH and PC in 5 mL solvent–SL (methanol–MEOH or mixture of sunflower and olive oil–MSOO in the ratio of 1:1 *v/v*) by stirring on the magnetic stirrer (MM-530, Tehnika Železniki, Slovenia) at 1000 rpm, 40°C, 10 min. *Hydration of organic phase.* In the ultrasound method, the hydration medium–HM (distilled water–H_2O or 0.9% sodium chloride–NaCl) was added in the organic phase, while in the injection medium the organic phase in the water used in the hydration process. The hydration was performed at temperature of 80°C on the magnetic stirrer (1000 rpm, 15 min) by using 5 mL hydration medium. *Solvent evaporation.* On the rotary evaporator (Devarot, Elektromedicina, Slovenia) at 65°C, for 1 h, rotating at 90 rpm, the methanol was removed and thin film of ketoconazole lipid mixture on the inner surface of the rotary flask was formed. *Separation of liposomal dispersion.* After the cooling to 25°C for 2 h, the mixture was kept at 4°C during 24 h to achieve a full hydration. Non-entrapped ketoconazole was removed from the liposomal dispersion by centrifugation for 15 min at 4000 rpm or 5000 rpm using a centrifuge (Pharmachem, Republic of North Macedonia). A liposomal layer was split between the water and oil phase when oil was used as solvent, while in case of methanol due to removing of the solvent, the liposomal upper and water lower phase were formed. The obtained liposomal preparation from *injection method* was stored in refrigerator (4°C) up to evaluation of quality characteristics. In the *ultrasound method*, the resulting liposomal preparation was processed by sonication in the ultrasonic bath (Cole–Parmer 8890, USA) at 25°C, 40 kHz low-frequency for a period of 10 min. After sonication, the liposomal preparation first was kept in desiccator at room temperature during 2 h and then in refrigerator at 4°C, 24 h to complete liposome formation. The formed liposomes were stored at 4°C to characterization.

Evaluation of Ketoconazole Loaded Liposomes

The obtained ketoconazole liposomes were evaluated by encapsulation efficiency, zeta potential, electrical conductivity, morphological appearance, particle size and stability.

Entrapment efficiency: The encapsulation efficiency (EE) of ketoconazole was determined by measuring the absorbance at 296 nm using a double beam UV/Vis spectrophotometer (Varian Cary Scan 50, Switzerland).[21] In quantification of the ketoconazole, absorption maximum of egg-yolk phospholipid (λ_{max} = 240 nm) and cholesterol (λ_{max} = 235 nm) shown no interferences. Encapsulation efficiency was calculated using the following equation: *Encapsulation efficiency* (%) = (C/C_o) x 100, where C is encapsulated ketoconazole quantity, and C_o is the initial quantity of ketoconazole used for liposome preparation. The results for EE were expressed as mean value of three replications (n = 3) ± standard deviation (SD).

Zeta potential and the electrical conductivity: The zeta potential (ZP) and the electrical conductivity (EC) were determined using a zeta potentiometer (Zeta meter 4.0, USA) at constant temperature (22°C) and potential (300 V). The ketoconazole liposomal preparations in distilled water (1:10 *v/v*) prior to measuring were diluted.

Morphology: One drop of ketoconazole liposomal preparation placed on glass slide was observed under the optical microscope (M–100–FL, Konus, Italy) equipped with a digital camera (Vario–Tessar 8x, Sony, China) at magnification of 40x. The mean size of ketoconazole loaded liposome was determined using NCH Software Suite (AU) and ImageJ NIH Software (USA). The measured size of fifteen representative vesicles was expressed as mean value (n = 15) ± standard deviation (SD).

Stability: The stability of ketoconazole liposomes was evaluated by observing the visual appearance and the morphology, as well as by determination of the encapsulation efficiency after one month storage at temperature of 4°C and 25°C.

RESULTS AND DISCUSSION

Ulstrasound Method

The encapsulation efficiency of ketoconazole in liposomes obtained with ultrasound method is presented in Table 1. The ketoconazole entrapment was in the range from 82.26 to 90.03%. In the formulations of KT, CH and PC at ratio of 100:30:100 *w/w/w*, the centrifugation at 5000 rpm resulted in higher encapsulation efficiency (87.06%) in comparison to 4000 rpm where 40.01% ketoconazole in liposomes was entrapped. Regardless to hydration media, the lower quantity of ketoconazole (71.83%) was entrapped in the liposomes with 0.9% NaCl. The higher value for encapsulation efficiency (90.03%) was determined in the liposomes obtained at 1.1:3:0 *w/w/w* ratio of KT, CH and PC, 5000 rpm, using water as hydration medium and mixture of sunflower and olive oil as solvent (1:1 *v/v*). Unilamellar small liposomes were formed differing in size from 50 to 113 nm. Effecting the lipid structure making more incompact, NaCl caused MLV to be formed (330 nm). In the liposomes where the vegetable oil mixture was used as solvent, the higher ZP (-27.63 mV) and EC value (8.34 μS/cm^{-1}) was measured, while using methanol ZP of -27.27 mV and 9.84 μS/cm^{-1} EC was achieved. The suspension is more stable at higher values of the zeta potential, due to the charged particles that repel each other and overcome the natural tendency to aggregate.[22]

Figure 1 (a-e correspond to the composition formulation in Table 1) shows microscopic appearance of the ketoconazole liposomes obtained with the ultrasound method. Unilamellar liposomes were formed when in the hydration water was used. Changing the lipid rigidity was resulted in the bilayer and mostly directly to the lamellar appearance with the use of solution of

Table 1: Encapsulation efficiency of ketoconazole in liposomes obtained with ultrasound method.

No	KT:CH:PC (mg)	SL	HM	CS (rpm)	EE*± SD (%)	PS** ± SD (nm)
a	100:30:100	MeOH	H_2O	5000	82.26 ± 0.52	73 ± 9 (SUV)
b	100:30:100	MSOO	H_2O	5000	87.06 ± 0.97	68 ± 11 (SUV)
c	100:30:100	MSOO	H_2O	4000	40.01 ± 0.78	56 ± 9 (SUV)
d	100:30:100	MSOO	NaCl	5000	71.83 ± 0.62	330 ± 65 (MLV) 50 ± 14 (SUV)
e	1.1:3:0	MSOO	H_2O	5000	90.03 ± 0.64	113 ± 16 (SUV)

*Mean value (n = 3); **Mean value (n = 15).

sodium chloride as hydration medium.[23] In comparison with the thin-film method,[17] with the use of ultrasound bath tended to decrease the diameter and liposome lamellarity.

In Figure 2 are presented the encapsulation efficiency values of ketoconazole determined in the liposomal formulation sample b (Table 1) during stability study at 4°C and 25°C in a period of one month. After a week of the liposomal preparation, the encapsulation efficiency values were 79.71% and 74.67% at 4°C and 25°C, respectively. The encapsulation efficiency rapidly decreased at 25°C where ketoconazole was found in 34.85% less than the initial quantity. At temperatures higher than 25°C, the encapsulation efficiency decreasing resulted from the structural changes in vesicles and drug leaking through the defects of the membrane packing because of fluidity of lipid bilayers and the degradation of phospholipids.[5,6]

Injection Method

The values of ketoconazole encapsulation efficiency using the injection method are presented in Table 2. The EE value of 40.11% was obtained at 4000 rpm, 100:30:100 *w/w/w* ratio of KT, CH and PC, MSOO and water as solvent and hydration medium. In the liposomes obtained at higher CS (5000 rpm), the KT was entrapped in 43.41% and 45.41% from total quantity, using ME and MSOO, respectively. Changing the water as hydration medium with 0.9 % NaCl solution negatively influenced the ketoconazole entrapping in liposomes (34.24%). The liposomes prepared by using vegetable oil were characterized with zeta potential of -30.41 mV. In case of methanol used as solvent the determined zeta potential value was -27.32 mV. MLV, SUV, and LUV were present in ketoconazole liposome preparation by injection method depending on the amount of substance, as well as type of hydration medium and centrifugal speed. The MLV size ranged from 258 to 430 nm. The homogenous narrow distributed liposomes (76÷138 nm) connected to SUV and LUV were calculated.

Microphotographs of the ketoconazole liposomes obtained with the injection method are presented in Figure 3. The microscopic analysis showed the homogenized uniformed appearance of ketoconazole unilamellar vesicles. In the comparison of Figure 3a with the other microphotographs in Figure 3, unilamellar and multilamellar vesicles can be observed where methanol

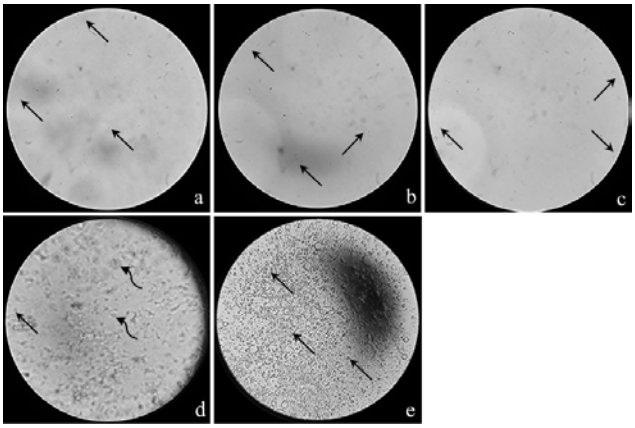

Figure 1: Microphotograph of ketoconazole liposomes obtained with ultrasound method, 40x (a-e correspond to samples given in Table 1), ← SUV; ↖ MLV.

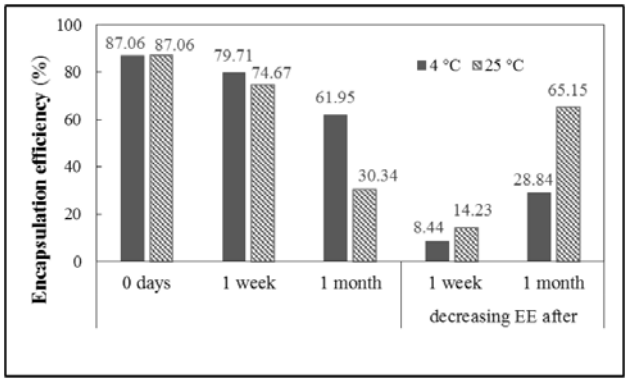

Figure 2: Stability test at 4°C and 25°C of the liposomes obtained with ultrasound method (Sample b, Table 1).

Table 2: Encapsulation efficiency of ketoconazole in liposomes obtained with injection method.

No	KT:CH:PC (mg)	SL	HM	CS (rpm)	EE* ± SD (%)	PS** ± SD (nm)
a	100:30:100	MeOH	H_2O	5000	45.41 ± 0.73	258 ± 38 (MLV) 78 ± 6 (SUV)
b	100:30:100	MSOO	H_2O	5000	43.41 ± 0.82	119 ± 22 (LUV)
c	100:30:100	MSOO	H_2O	4000	40.11 ± 0.48	138 ± 17 (LUV)
d	100:30:100	MSOO	0.9 % NaCl	5000	34.24 ± 0.42	430 ± 38 (MLV) 76 ± 8 (SUV)

*Mean value (n = 3); **Mean value (n = 15).

was used and unilamellar homogeneously distributed liposomes with oil as solvent. The differences in the appearance between the unilamellar liposomes obtained at 4000 rpm and 5000 rpm were insignificant (Figure 3, sample b, c). The sodium chloride solution used in the hydration resulted in not well differentiated vesicles (Figure 3, sample d).

The stability at 4°C and 25°C in the period of one month was evaluated by determination of the EE values (Figure 4) for the formulation b given in Table 2. After one week, the values of the EE of KT decreased to 42.64% and 41.42%, respectively, at 4°C and 25°C. The higher decrease in the ketoconazole encapsulation value (48.21%) was determined at 25°C, after one month of preparation.

In accordance to the higher EE values obtained at 5000 rpm confirmed by the results reported in the literature,[24] the future research was directed to investigate the influence of the ratio of KT, CH and PC on the quality characteristics of the liposomes obtained with the injection method using water as hydration medium and mixture of sun and olive oil as solvent. The values of EE, ZP and EC are given in Table 3.

In the liposomes obtained using 50 mg KT, 30 mg CH, and 100 mg PH (sample A), 46.66% EE was determined. Increasing the CH quantity (60 mg), the EE value of the ketoconazole in liposomes increased to 70.01% (sample B). The higher PC quantity (200 mg) in sample C resulted in obtaining higher EE (50.37%) compared to sample A. The influence of increased CH

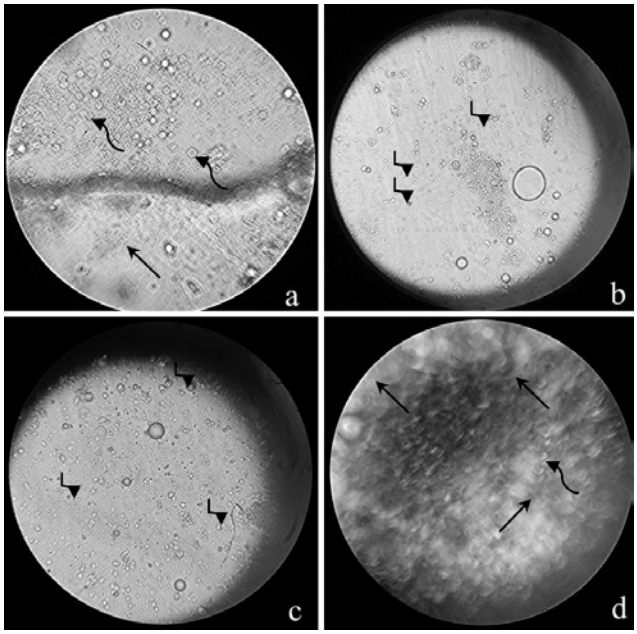

Figure 3: Microphotograph of ketoconazole liposomes obtained with injection method, 40x (a-d correspond to samples given in Table 2), ← SUV; ↳ LUV; ↖ MLV.

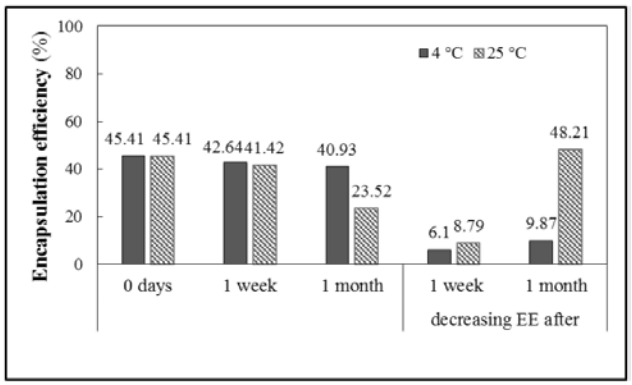

Figure 4: Stability test at 4ºC and 25ºC of the liposomes obtained with injection method (Sample b in Table 2).

Table 3: Characteristics of liposomes obtained with injection method.[1]

No	KT:CH:PC (mg)	EE ± SD (%)	ZP ± SD (mV)	EC* ± SD (µS/cm)	PS** ± SD (nm)
A	50:30:100	46.66 ± 0.85	-18.11 ± 0.03	9.67 ± 0.15	258 ± 33 (LUV)
B	50:60:100	70.01 ± 0.44	-18.79 ± 0.18	9.41 ± 0.05	120 ± 19 (LUV)
C	50:30:200	50.37 ± 0.75	-20.67 ± 0.16	13.51 ± 0.16	424 ± 41 (LUV)
D	50:60:200	51.50 ± 0.71	-24.62 ± 0.17	9.04 ± 0.07	307 ± 27 (LUV)
E	200:30:100	70.33 ± 0.59	-30.22 ± 0.42	9.08 ± 0.05	287 ± 33 (MLV) 93 ± 12 (SUV)
F	200:60:100	28.13 ± 0.41	-24.63 ± 0.37	9.35 ± 0.07	1271 ± 311 (MLV) 420 ± 52 (LUV)
G	200:30:200	53.63 ± 0.76	-18.48 ± 0.04	9.77 ± 0.06	195 ± 20 (MLV) 71 ± 9 (SUV)
H	200:60:200	54.43 ± 0.38	-28.59 ± 0.05	9.35 ± 0.02	151 ± 12 (LUV)

*Mean value (n = 3); **Mean value (n = 15); [1]CS = 5000 rpm, MSOO, H_2O.

quantity to the entrapped ketoconazole in liposomes was insignificant at 200 mg PC (sample D). In the liposomal formulation (sample E) where the KT was used in higher quantity (200 mg) at lower amount of CH (30 mg) and PC (100 mg), the EE reached the highest value (70.33%). By comparing the EE values of the sample C and D with the sample G and H, the insignificant CH influence at 200 mg KT was confirmed, also. The lowest EE value (28.13%) was determined in the sample F6 composed of 200 mg KT, 60 mg CH and 100 mg PC (Table 3). The higher quantity of phospholipid and cholesterol incorporated into the double lipid layer, resulted into increasing the membrane fluidity, as well as in the aqueous phase distribution, thus decrease the encapsulation efficiency of the hydrophobic active substance. The phenomena such as aggregation and fusion occurred influenced on the particle size, also. The cholesterol effects can be explained by competitive action on the lipophilic spaces against to the lipophilic substance, as well as by the double layer permeability decreasing. At the injection method, during the solvent diffusion in the aqueous phase, the dissolved phospholipid in the organic phase was aggregated on the aqueous and organic phase boundary, followed by the formation of the intermediated bilayer phospholipid fragments. Increasing the lipid substance quantity, the phospholipid fragments amount increased, the distance between them reduced and larger vesicles due to the coalescence were formed.[9,25]

Liposomes with the highest ZP value (-30.22 mV) at 200 mg KT, 30 mg CH and 100 mg PH (sample E) were prepared. The ZP value decreased to -28.59 mV using KT, CH, and PC at the highest values (sample G). At the lowest KT quantity, the positive effect of CH and PH resulted in the increased ZP values, and vice versa. Related to the EC values, the influence of the formulation composition is insignificant. In the liposome formulation sample C (50 mg KT, 30 mg CH and 200 mg PC) the highest EC value (13.51 μS/cm) was determined. The EC values in the other liposomes preparations ranged from 9.04 to 9.77 μS/cm (Table 3). The discussed PC and CH effects on the zeta potential and electrical conductivity were confirmed by data given in literature.[22]

Figure 5 presents the microscopic appearance of ketoconazole liposomes obtained with the injection method using ketoconazole, cholesterol, and phospholipid in quantities according to the formulations A-H given in Table 3.

The ketoconazole liposomal vesicles were significant larger obtained by the injection method in the comparison to the thin-film hydration method[17] and ultrasound method (Figure 2). Well-defined spherical vesicles were obtained at the higher PC quantity (Figure 5D), while the smaller spherical vesicles were formed by the formulation F (Figure 5 correlated with Table 3). The negative influence of cholesterol on the appearance and stability of the formed vesicles was shown at higher ketoconazole quantity. The liposomes with undefined spherical appearance with a tendency for their fusion, coalescence, and complete destabilization were obtained using formulation G and H (Figure 5G and Figure 5H). The CH and PC shown the same effects

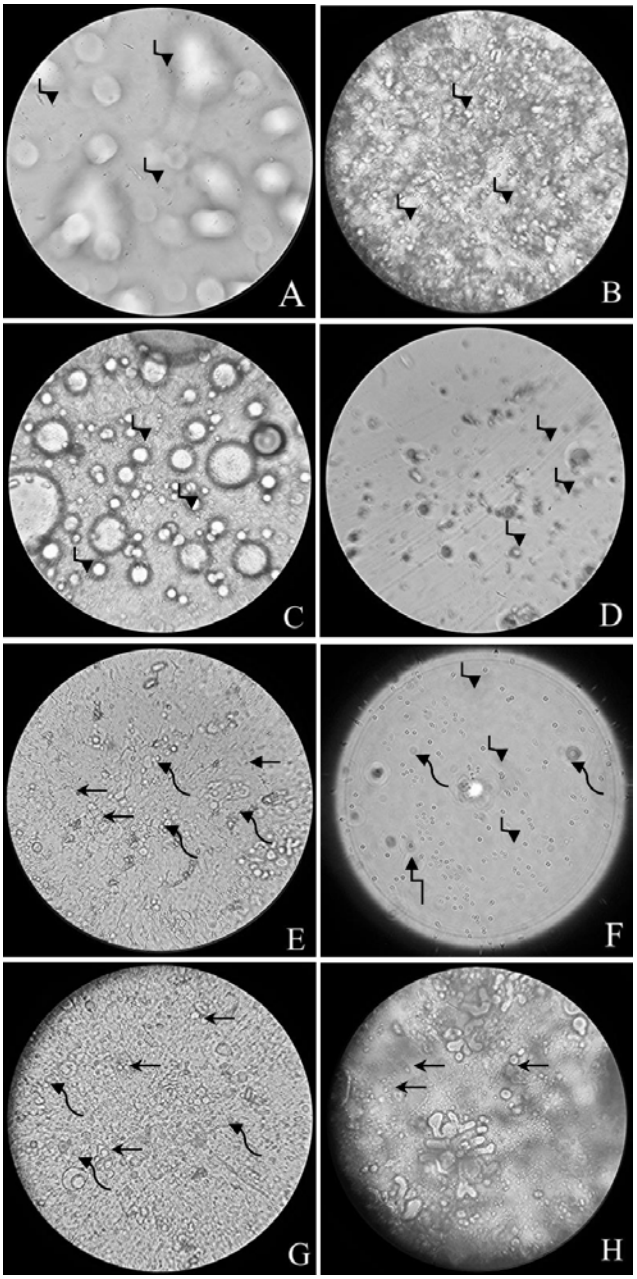

Figure 5: Microphotograph of ketoconazole liposomes obtained with injection method, 40x, (A-H correspond to samples given in Table 3), ← SUV; ↳ LUV; ↰ MLV.

on the encapsulation efficiency (Table 3) and on the microscopic appearance (Figure 5), also. The determined influences of the substances used in the formulation of the ketoconazole liposomes are comparable with those presented in the literature.[22]

CONCLUSION

In the present study, the ultrasound and the injection methods for the ketoconazole entrapping in the liposome structures were developed. The introducing of the vegetable oils, mixture of sunflower and olive oil, in the liposome preparation procedure is high of importance related to the eco-friendly aspect.

The influence of conditions applied during liposome preparation such as composition formulation, type of solvent and hydration medium, and centrifugation speed was evaluated by determination of the physico-chemical properties: encapsulation efficiency, zeta potential, electrical conductivity, microscopic appearance and temperature stability.

The mixture of sunflower and olive oil as solvent, water used in hydration process, centrifugation at 5000 rpm and stability at temperature of 4°C, effected positively on the evaluated characteristics of the ketoconazole liposomes. In aspect of the composition formulation, the higher values of the encapsulation efficiency were determined in the liposomes obtained at lower ketoconazole quantity by increasing the quantity of the cholesterol and phospholipid. The size distribution and homogeneity were depended on the lipid amount and preparation procedure of the liposomes. In terms of size distribution, SUV, LUV and MLV were present.

Although, the ultrasound method involves of one-step more in the preparation procedure, treatment of the liposomal dispersion in ultrasound water bath, the method justification was directed to the higher encapsulation efficiency values of entrapped ketoconazole in the unilamellar consistent liposomes obtained. The advantage in longer period of liposome stability was with the use of injection method including a new approach where lipid mixture consisted of vegetable oils was injected to water.

CONFLICT OF INTEREST

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

ABBREVIATIONS

SUV: Small Unilamellar Vesicles; **LUV:** Large Unilamellar Vesicles; **MLV:** Multilamellar Vesicles; **KT:** Ketoconazole; **CH:** Cholesterol; **PC:** L-*a*-Phosphatidylcholine; **SL:** Solvent; **MEOH:** Methanol; **MSOO:** Mixture of Sun and Olive Oil; **HM:** Hydration Medium; **H_2O:** Distilled water; **NaCl:** Sodium chloride (0.9% solution); **CS:** Centrifugation Speed; **UV/Vis:** Ultraviolet/Visible; **EE:** Encapsulation Efficiency; **SD:** Standard Deviation; **ZP:** Zeta Potential; **EC:** Electrical Conductivity; **PS:** Particle Size.

REFERENCES

1. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo WS, Zarghami N, Hanifehpour Y, Samiei M, Kouhi M, Nejati-Koshki K. Liposome: classification, preparation, and applications. Nanoscale Res Lett. 2013;8:1-9. doi:10.1186/1556-276X-8-102
2. Pattni BS, Chupin VV, Torchilin VP. New developments in liposomal drug delivery. Chem Rev. 2015;115:10938-66.
3. Rahman A, Uahengo V, Likus D. Mini review on emerging methods of preparation of liposome and its application as Liposome drug delivery systems. Open J Pharmacol Pharmacother. 2018;3(1):5-21.
4. Mishra H, Chauhan V, Kumar K, Teotia D. A comprehensive review on Liposomes: A novel drug delivery system. J Drug Deliv Ther. 2018;8(6):400-4.
5. Sułkowski WW, Pentak D, Nowaka K, Sułkowska A. The influence of temperature, cholesterol content and pH on liposome stability. J Mol Struct. 2005;744-747:737-47.
6. Yadav AV, Murthy MS, Shete AS, Sakhare S. Stability aspects of liposomes. Indian J Pharm Educ. 2011;45(4):402-13.
7. Malisiova F, Hatziantoniou S, Dimas K, Kletstas D, Demetzos C. Liposomal formulations from phospholipids of Greek almond oil. Properties and biological Activity. Z Naturforsch C J Biosci. 2004;59(5-6):330-4.
8. Anderson M, Omri A. The effect of different lipid components on the *in vitro* stability and release kinetics of liposome formulations. Drug Deliv. 2004;11:33-9.
9. Sherry M, Charcosset C, Fessi H, Greige-Gerges H. Essential oils encapsulated in liposomes: A review. J Liposome Res. 2013;23:268-75.
10. Choi FD, Juhasz MLW, Atanaskova Mesinkovska N. Topical ketoconazole: a systematic review of current dermatological applications and future developments. J Dermatol Treat. 2019;30:760-71.
11. Khoza S, Moyo I, Ncube D. Comparative hepatotoxicity of fluconazole, ketoconazole, itraconazole, terbinafine, and griseofulvin in rats. J Toxicol. 2017:1-9. doi:10.1155/2017/6746989
12. Sharquie KE, Noaimi AA, Al-Salam WS. The safety of oral ketoconazole in the treatment of skin diseases (single blinded, therapeutic, comparative study). JCDSA 2018;8:264-71.
13. Vojič MP, Popovič GV, Sladič DM, Pfendt LB. Protolytic equilibria in homogeneous and heterogeneous systems of ketoconazole and its direct spectrophotometric determination in tablets, J Serb Chem Soc. 2005;70:67-78.
14. Zylberberg C, Matosevic S. Pharmaceutical liposomal drug delivery: a review of new delivery systems and a look at the regulatory landscape. Drug Deliv. 2016:1-11. doi: 10.1080/10717544.2016.1177136
15. Naeem S, Kiew LV, Chung LY, Ashraf MA, Misran MB. Liposomes as amphiphilic carriers: encapsulation and stability aspects. Sains Malays. 2016;45:71-7.
16. Bulbake U, Doppalapudi S, Kommineni N, Khan W. Liposomal formulations in clinical use: an updated review. Pharmaceutics 2017;9:1-33.
17. Popovska O, Kavrakovski Z, Rafajlovska V. Introducing vegetable oils in a preparation of ketoconazole liposomes using a thin-film hydration method. Farmacia 2019;67:467-71.
18. Wang GT, Wang T. Oxidative stability of egg and soy lecithin as affected by transition metal ions and pH in emulsions. J Agric Food Chem. 2008;56(23):11424-31.
19. Patel RP, Patel H, Baria AH. Formulation and evaluation of liposomes of ketoconazole. Int J Drug Deliv Technol. 2009;1(1):16-23.

20. Sahasrabuddhe SH, Bajpai N, Bais S, Ganesan S. Ketoconazole: liposomal drug delivery system–a boon for cosmetic industry. Int J Advanced Pharm Sci. 2012;3:109-16.
21. Popovska O, Kavrakovski Z, Rafajlovska V. Development and validation of UV spectroscopic method for determination of ketoconazole in pharmaceutical formulations. Int J Pharm. 2014;4:95-101.
22. Surianarayanan R, Shivakumar HG, Vegesna NSKV, Srivastava A. Effect of sample concentration on the characterization of liposomes using dynamic light scattering technique. Pharm Methods 2016;7:70-4.
23. Scott LH, Skinkle A, Kelly GE, Waxham MN, Levental I, Heberle AF. On the Mechanism of Bilayer Separation by Extrusion, or Why Your LUVs Are Not Really Unilamellar. Biophys J. 2019;117(8):1381-6.
24. Cui QY, Yang P, Sun P, Tan DY, Jin YG, Quan SJ. Preparation of PEGylated liposomal ginsenoside; formulation design and *in vitro* evaluation. Indian J Pharm Sci. 2020;82(1):149-56.
25. Yang K, Delaney JT, Ultrich S, Schubert U, Fahr A. Fast high-through put screening of temoprfin-loaded liposomal formulations prepared by ethanol injection method. J Liposome Res. 2012;22:31-41.

PICTORIAL ABSTRACT

Ketoconazole liposome formulations with a mixture of sunflower and olive oil obtained by using ultrasound (A) and injection method (B)

SUMMARY

In the encapsulation of ketoconazole in liposomes by using the ultrasound and the injection method were introduced the vegetable oils, mixture of sunflower and olive oil. The ketoconazole liposomes were evaluated according to the encapsulation efficiency, zeta potential, electrical conductivity, morphological appearance, particle size and stability.

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Cite this article: Popovska O, Simonovska J, Kavrakovski Z, Rafajlovska V. Preparation of Ketoconazole Liposomes with an Ultrasonic and an Injection Method Using Vegetable Oils. Indian J of Pharmaceutical Education and Research. 2020;54(4):946-53.