INTERACTION OF BISOPROLOL OR ENALAPRIL WITH DISTEAROYL PHOSPHATIDYL CHOLIN LIPOSOMES: FTIR AND DSC STUDIES

M.W. SHAFAA

Medical Biophysics Division, Physics Department, Faculty of Science, Helwan University, Cairo, Egypt, e-mail: medhatwi@hotmail.com

Abstract. The biophysical interactions of bisoprolol or enalapril with distearoyl phosphotidylcholine (DSPC) multilamellar liposomes were investigated by using two non-invasive techniques of Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC). DSC showed that the mixtures of DSPC and bisoprolol or enalapril show a single peak, which indicates that they are miscible. The incorporated bisoprolol or enalapril is probably associated and interacted to large extent with the lipid bilayers that perturbed them which results in the strong broadening and shift to lower temperature 69 °C and 61 °C, respectively of the major characteristic endothermic peak of pure DSPC that exists at 102 °C. The pretransition of liposomes was eliminated for all samples containing bisoprolol or enalapril. Analysis of C=O stretching bond in FTIR spectroscopy showed that bisoprolol or enalapril does not make any hydrogen bonds with the interfacial region of DSPC liposomes, instead it induces free carbonyl groups in the system. These results revealed that enalapril or bisoprolol was located in the interfacial region of the membrane. The studies with model membranes could provide a rational approach for drug discovery and development as well as for developing efficient drug delivery systems.

Key words: DSPC liposomes, bisoprolol, enalapril, DSC, FTIR.

INTRODUCTION

Hypertension is simply defined as abnormally high blood pressure and especially arterial blood pressure. High blood pressure is a common condition in which the long-term force of the blood against artery walls is high enough that it may eventually cause health problems, such as heart disease. Arterial hypertension is a major cause of morbidity and mortality because of its association with coronary heart disease, cerebro-vascular disease and renal disease.

Received: February 2018;
in final form March 2018.

ROMANIAN J. BIOPHYS., Vol. 28, No. 3, P. 000–000, BUCHAREST, 2018

Bisoprolol hemifumarate belongs to the group of medicines referred to as beta-blockers used for the management of hypertension and angina pectoris. It is a medicine which works on the heart and blood vessels. It does this by blocking tiny areas (called beta-adrenergic receptors) where messages sent by some nerves are received by heart and blood vessels. As a result, heart beats more slowly and with less force. The pressure of blood within blood vessels is reduced and it is easier for heart to pump blood around body resulting in decrease of arterial hypertension [8, 11].

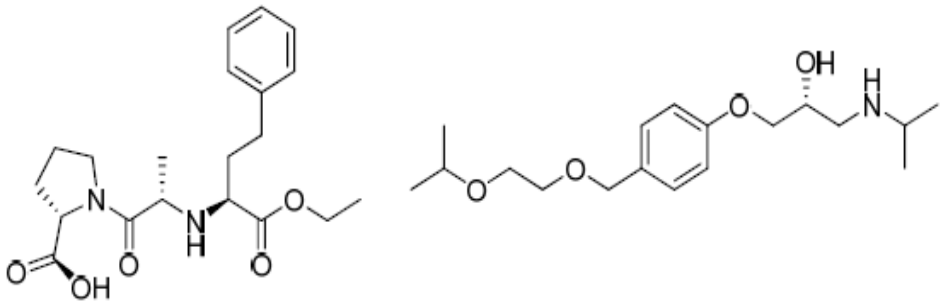
Enalapril is a pro-drug, an ethyl ester of a long-acting inhibitor of angiotensin-converting enzyme (ACE), enalaprilat. After its oral absorption, enalapril is metabolized for hydrolysis by liver yielding enalaprilat [4]. This drug is indicated for the treatment of essential and renovascular hypertension and congestive heart failure [7]. The main mechanism responsible for the antihypertensive effect of the ACE inhibitors is the reduction in the circulating angiotensin II concentration.

Liposomes have been used extensively as biological models for in vitro studies. The resemblance between the liposomal and membrane bilayer core makes liposomes a very useful tool to investigate the significance of the drug-membrane interactions.

Biophysical interaction studies with model membrane could provide a simple yet effective approach to understanding the role of membrane lipids in the transport of drugs and drug delivery systems across biological barriers.

To our knowledge, there were no previous studies on the interaction of bisoprolol or enalapril with phospholipids from the perspective of the thermotropic phase behavior of phospholipids and to observe the changes of acyl chain conformations and characteristic PO_2^- bands in the polar heads of phospholipids. There have been no systematic investigations in relation to bisoprolol or enalapril and phospholipids using differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) spectroscopy. These two techniques are highly effective in probing the interaction between drugs and phospholipids [13].

The main aim of this work is to investigate the impact of bisoprolol or enalapril as antihypertensive drugs on the structural properties of model lipid membranes and to estimate the subtle perturbation of the lipid bilayer structure using two non-invasive techniques such DSC and FTIR. The structure of enalapril and bisoprolol are presented in Fig. 1.



Enalapril Bisoprolol

Fig. 1. Schematic chemical structure of enalapril and bisoprolol.

MATERIALS AND METHODS

CHEMICALS

1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) in powder form and of purity 99% is presented in Fig. 2, with a molecular weight of 790.145 was purchased from Lipoid KG, Ludwigshafen, Germany, Trizma buffer, molecular weight of 121.1 was purchased from Sigma chemicals, Steinheim, Germany. Ethanol was of analytical grade and obtained from Merck, Heliopolis, Cairo, Egypt [3]. Bisoprolol and enalapril, with a molecular weight of 325.443 and 376.447 respectively, were purchased from EIPICO, Egypt. All other reagents and solvents used in this work were of research grade.

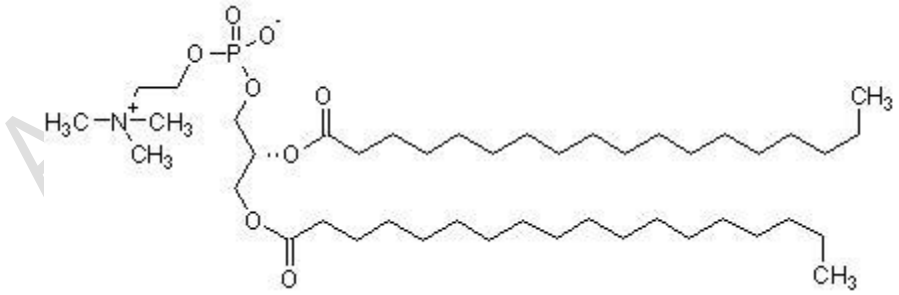


Fig. 2. Schematic chemical structure of DSPC.

PREPARATION OF BISOPROLOL OR ENALAPRIL- DOPED LIPOSOMES

DSPC: Bisoprolol or enalapril molar ratio 7:2 was used to prepare neutral liposomal multilamellar vesicles (MLVs) using the method of Deamer and Uster [2]. In 50 mL round bottom flask, 20 mg of DSPC and 2.4 mg bisoprolol or 2.72 mg enalapril of the drug powder at molar ratio 7:2 were transferred. Then 10 mL of ethanol (EtOH) was added, and the flask was shaken until all lipids dissolved in the EtOH. The solvent was evaporated under vacuum using rotary evaporator until a thin dry film of lipid was formed. 10 mL of buffer (10 mM Trizma at pH = 7) was then added to the flask which was flashed through with nitrogen stream and immediately stoppered. The flask is mechanically shaken for 1h at temperature above the phase transition temperature of lipids (45 °C). The suspension was then centrifuged at 8000 rpm for 20 min and the supernatant was discarded. The liposomes were then re-suspended in 10 mL buffer solution. Control liposomes were prepared following the same classical methods as before using only 20 mg of DSPC.

ENCAPSULATION EFFICIENCY MEASUREMENTS

The entrapment efficiency (EE) of bisoprolol or enalapril incorporated into liposomes was determined using a spectrophotometer (Uvikon 930, Italy). The wavelengths were adjusted at 275 nm and 209 nm, the resonance absorption peaks of bisoprolol and enalapril respectively. The absorption of the supernatant of each sample was compared with the standard curve that relates absorption to the concentration of bisoprolol or enalapril. Entrapment Efficiency (*EE*) was calculated as follows:

$$EE = \frac{Total\ drug\ input(\text{mg}) - Drug\ in\ supernatant(\text{mg})}{Total\ drug\ input(\text{mg})} \times 100 \qquad (1)$$

DSC MEASUREMENTS

Differential scanning calorimetry (DSC) experiments were carried out using (model TA-50 WSI, Schimadzu, Japan) calibrated with indium to investigate the thermal behavior of lyophilized samples of empty and bisoprolol or enalapril-loaded multilamellar liposomes. The analyses were performed on 5 mg samples sealed in standard aluminum pans. The thermogram of each sample covered the 2–200 °C temperature range at a scanning rate of 5 °C/min [10].

FTIR SPECTROSCOPY

FTIR spectra of lyophilized samples of empty DSPC liposomes and DSPC liposomes encapsulated bisoprolol or enalapril deposited in KBr disks were recorded on a NICOLET 6700 FTIR spectrometer (Thermo Scientific, Cambridge, England). Scanning was carried out at room temperature, in the range 400–4,000 cm^{-1} at a speed of 2 mm/s and a resolution of 4 cm^{-1}.

RESULTS AND DISCUSSION

The encapsulation efficiency was found to be increased more than 90% when mixing the drug with the lipid powder before dissolving it in ethanol.

DSC is a fundamental technique for the characterization of membrane behavior, providing all thermodynamic parameters for temperature-induced transitions. The phase transition temperatures studied by DSC can give an idea about the different mechanisms that occur in the liposomal membrane as a model of the biological membrane system.

The most ubiquitous of these is the gel-to-liquid crystalline transition, often referred to as the melting or main transition. This transition is relatively rapid and is highly reversible. It is characterized by the co-operative melting of the hydrocarbon chains and a high enthalpy DSC peak. The liquid crystalline ($L\alpha$) phase has an increased number of gauche conformers and a large increase in membrane fluidity and disorder [12]. The pre-transition from the planar gel ($L\beta'$) to the rippled gel phase ($P\beta'$) has a low enthalpy and is sensitive to sample preparation and the presence of impurities [5]. All of the above phases are strongly affected by changes in lipid structure.

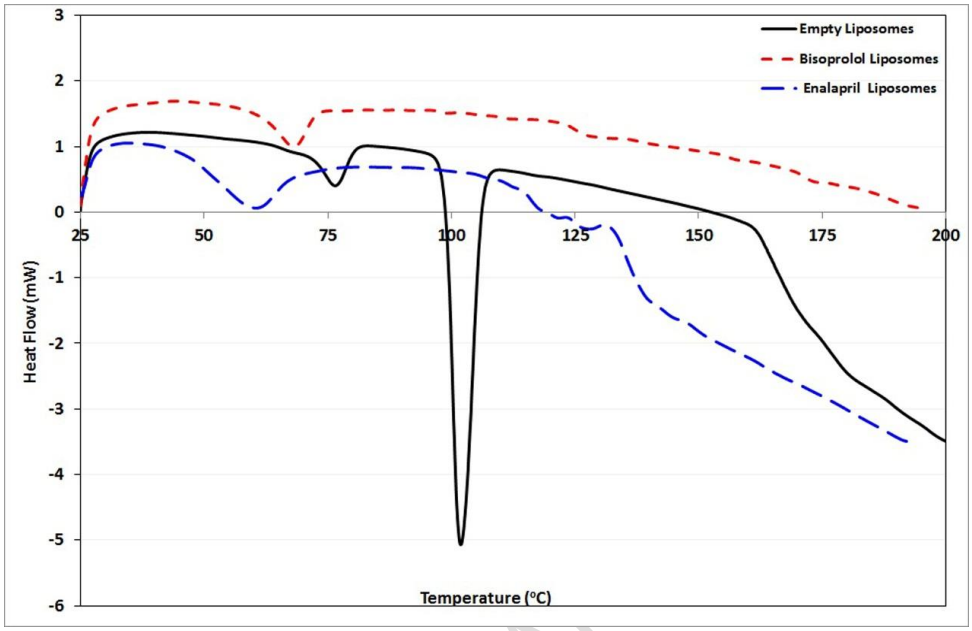


Fig. 3. DSC diagrams of liposomes made of pure DSPC and liposomes doped with bisoprolol or enalapril.

The temperature at which a transition from the gel phase to the rippled phase takes place is called the pre-transition temperature and it is mainly related to the polar region of phospholipids. Subsequently the melting of the bilayer from the rippled phase to the liquid phase occurs at the main transition temperature (T_m). The melting point (T_m) represents the peak temperature of the endotherm for the lipid gel-to fluid phase transition recorded during the heating scan.

The DSPC vesicles were used as model membranes since this phospholipid can mimic many aspects of biological membranes. Pure DSPC vesicles upon dehydration when submitted to DSC analysis, showed a major endothermic peak at 102 °C (Fig. 3), in accordance with [5, 10]. The pre-transition temperature was around 80 °C for pure DSPC liposomes.

The presence of a compound in the DSPC membranes could influence the thermotropic parameters of the vesicle transition. The incorporation of bisoprolol or enalapril into DSPC liposomes exhibited noticeable shift to lower temperature at 69 °C and 61 °C, respectively in a comparison to the main endothermic peak of empty DSPC that exists at 102 °C which suggests that bisoprolol or enalapril had a significant effect on the acyl chains of DSPC bilayers creating a conformational disorder within the acyl chains of phospholipids and decreases the transition cooperatively of lipid acyl chains. The lowered temperature of the main DSPC

transition process indicated that the incorporation of bisoprolol or enalapril is more favorable to the formation of acyl chains in a disordered and loose state. The pre-transition temperature peak for bisoprolol or enalapril liposomes is disappeared, which revealed that bisoprolol or enalapril interacted with the polar head group of phospholipids. The disappearance of the pre-transition is a sensitive for the incorporation of biomolecules into lipid bilayers.

Upon the incorporation into DSPC liposomes, the pronounced effect of bisoprolol or enalapril was observed in comparison with pure DSPC liposomes. The incorporated bisoprolol or enalapril is probably associated and interacted to a large extent with the lipid bilayers that perturbed them which results in the broadening of the major characteristic endothermic peak of pure DSPC that exists at 102 °C. It has been observed using DSC that the mixtures of DSPC and bisoprolol or enalapril show a single peak, which indicates that they are miscible.

Protein and DNA structure, hydration, and binding of biomolecules have been studied using vibrational spectroscopy, as a combined theoretical and experimental approach**.** FTIR spectroscopy is a technique which can provide quantitative chemical composition and identify tissue constituents. FTIR spectroscopy was used to monitor subtle changes in the structure and function of the lipid assemblies by analyzing the frequency and bandwidth changes of different vibrational modes representing the acyl chains, interfacial, and head group region of lipid molecules.

The measuring of some spectral parameters like band frequency, width and intensity change provided information regarding the possible structural interactions and conformational rearrangements taking place.

FTIR spectra of pure lyophilized DSPC liposomes compared with enalapril or bisoprolol/DSPC liposomal sample in the region 4000–400 cm^{-1} are presented in Fig. 4. The spectrum of the DSPC liposomes displayed the main characteristic bands, especially those are due to the symmetric and antisymmetric PO_2^- stretching vibrations at 1,090 and 1,220 cm^{-1}, respectively), the CH_2 bending vibration CH_2 near 1,470 cm^{-1}, the carbonyl stretching vibration C=O at 1,734 cm^{-1}, the OH stretching and bending vibrations at 3,470 and 1,640 cm^{-1}, respectively and The symmetric and antisymmetric stretching vibrations of the CH_2 in the acyl chain around 2,850 and 2,920 cm^{-1}, respectively. These findings are in good accordance with the data reported in the literature [6].

The detailed spectral analyses were performed in three distinct wave number regions, namely 3500–2800 cm^{-1}, 1800–1500 cm^{-1} and 1500–400 cm^{-1}, since identifiable Raman bands were observed mainly in these regions only.

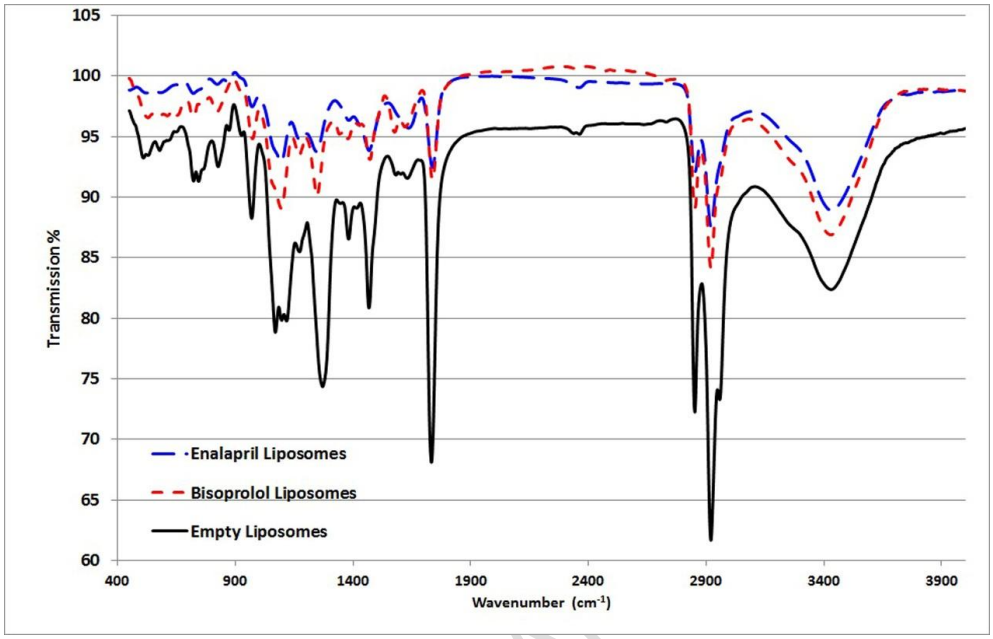


Fig. 4. The full FTIR spectra of empty DSPC and DSPC / bisoprolol or enalapril liposomal samples.

Lipids give rise to number of absorptions in Raman spectra. The most intense of these absorptions are found in the 3500–2800 cm^{-1} region, attributed to asymmetric and symmetric stretching vibrations of CH_3 and CH_2 groups of acyl chains. The most intense band observed at 3500 cm^{-1} in the control and treated has been assigned as presence of hydroxyl compound.

Encapsulation of bisoprolol into the DSPC liposomes exhibited slight change in the frequency of the symmetric CH_2 stretching bands in the acyl chain appeared in Fig. 5, suggesting that bisoprolol create a conformational disorder within the acyl chains of phospholipids. In other words, it had an effect on the order of the membrane. For enalapril /DSPC FTIR spectrum, there was no significant change in the frequency of the CH_2 stretching bands observed in Fig. 5, suggesting that enalapril does not change the number of gauche conformers. In other words, it did not have any significant effect on the order of the membrane.

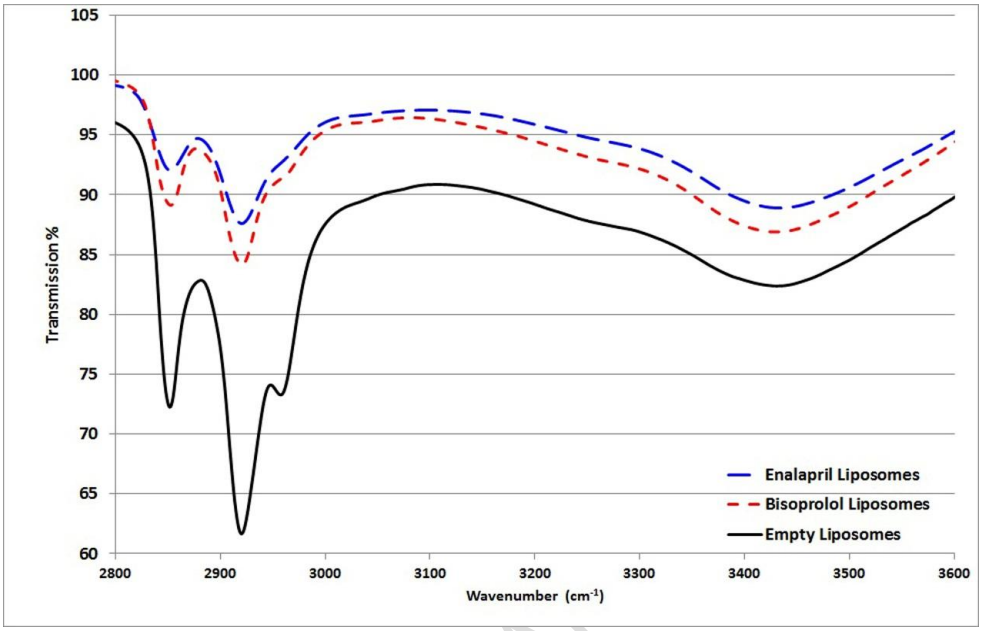


Fig.5. The magnified part (3500–2800 cm^{-1}) of FTIR spectra of empty DSPC and DSPC / bisoprolol or enalapril liposomal samples.

The interaction between enalapril/bisoprolol and the head group of DSPC liposomes was monitored by means of the PO_2^- symmetric stretching band, which is located at 1092 cm^{-1}. Figure 6 shows the PO_2^- symmetric stretching band for DSPC liposomes formulations in the absence and presence of enalapril and bisoprolol. A decrease in the frequency corresponds to the hydrated phosphate group. The frequency of this band determines the presence of hydrogen bonding between phosphate group and hydrogen atoms of water or biological macromolecules. As can be seen from the Fig. 6, the wavenumber was shifted towards higher degrees (1095 cm^{-1} and 1094 cm^{-1}) after the incorporation of enalapril or bisoprolol into DSPC liposomes, respectively. This implied the absence of hydrogen bonding between the liposome head group and enalapril/bisoprolol. In accordance with the empirical rules, decreasing frequency values displays an increase in the strengthening of existing hydrogen bonding or in the formation of new hydrogen bonding between the components [9].

The PO_2^- antisymmetric stretching band, which is located at 1222 cm^{-1} for DSPC liposomes was significantly increased after the encapsulation of enalapril or bisoprolol into DSPC liposomes. This implied the absence of hydrogen bonding between the liposome head group and enalapril/bisoprolol. These results implied that enalapril or bisoprolol was located in the interfacial region of the membrane.

The interesting finding that can be concluded from Fig. 6 is that the CH_2 bending vibration modes which is located at 1467 cm^{-1} is affected by the incorporation of enalapril or bisoprolol into DSPC liposomal preparation. The wavenumber was shifted towards higher values (1468 cm^{-1} and 1471 cm^{-1}) after the encapsulation of enalapril or bisoprolol into DSPC liposomes, respectively. This might assume that the molecules of enalapril and bisoprolol act as small spacers of the polar head group, leading to a slight disorder in the hydrocarbon chains.

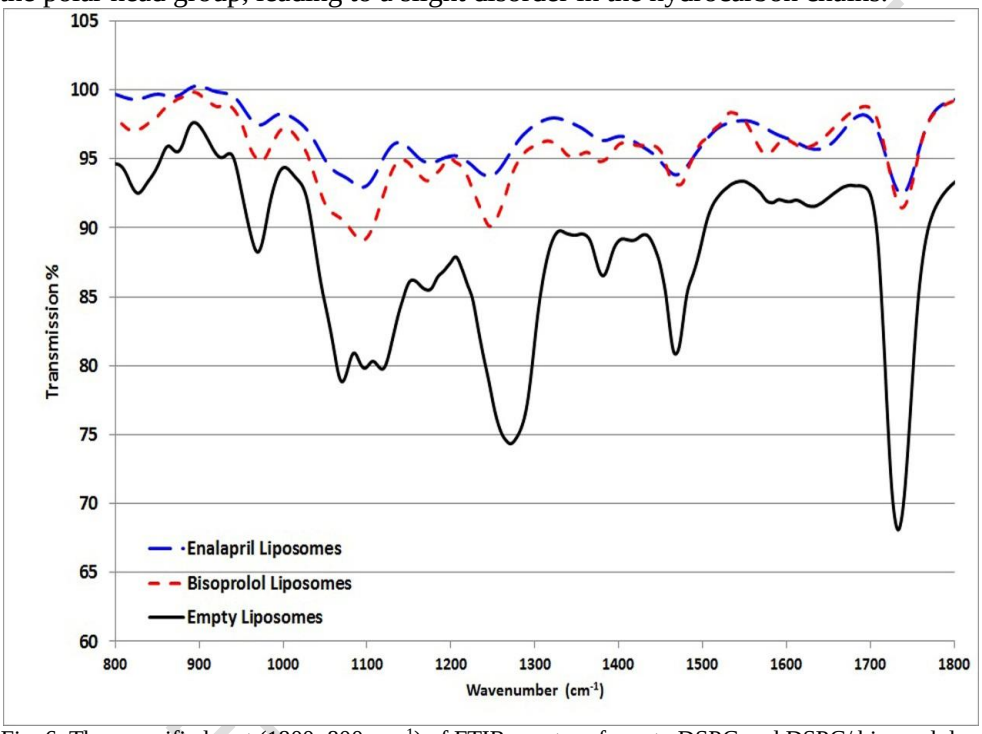


Fig. 6. The magnified part (1800–800 cm^{-1}) of FTIR spectra of empty DSPC and DSPC/ bisoprolol or enalapril liposomal samples.

To examine the interaction of enalapril/bisoprolol with glycerol backbone near the head group of phospholipids in interfacial region, the C=O stretching band was analyzed. The wavenumber variation of this band is shown in Fig. 7. As seen from the Figure 7, the wavenumber value of C=O group was shifted to higher frequencies (from 1732 cm^{-1} 1737 cm^{-1}) for the liposomes sample containing enalapril and bisoprolol respectively, without any evidence of hydrogen bonding formation. The absorption bands of ester C=O are sensitive to changes in the polarity of their local environments and are influenced by hydrogen bonding and other interactions. Therefore, changes in the contours of the ester C=O absorption band can often be interpreted in terms of structural and/or hydration changes of the bilayer polar/apolar

interface [1]. The wavenumber value of C=O group exhibited shift towards higher frequency (from 1732 cm^{-1} to 1737 cm^{-1}) for the liposomes sample containing bisoprolol or enalapril, implying dehydration about these functional groups in the interfacial region of the lipid membranes. Therefore, any effects in the spectra of this region can be attributed to an interaction between enalapril/bisoprolol and the polar/apolar interfacial region of the membrane. enalapril/bisoprolol tends to reduce the forming of hydrogen bonding in the interfacial region of DSPC liposome, implying the existing of the free carbonyl groups in the system. Some H_2O molecules are probably replaced bisoprolol or enalapril from the interfacial region and lead to an increase in the number of free carbonyl groups.

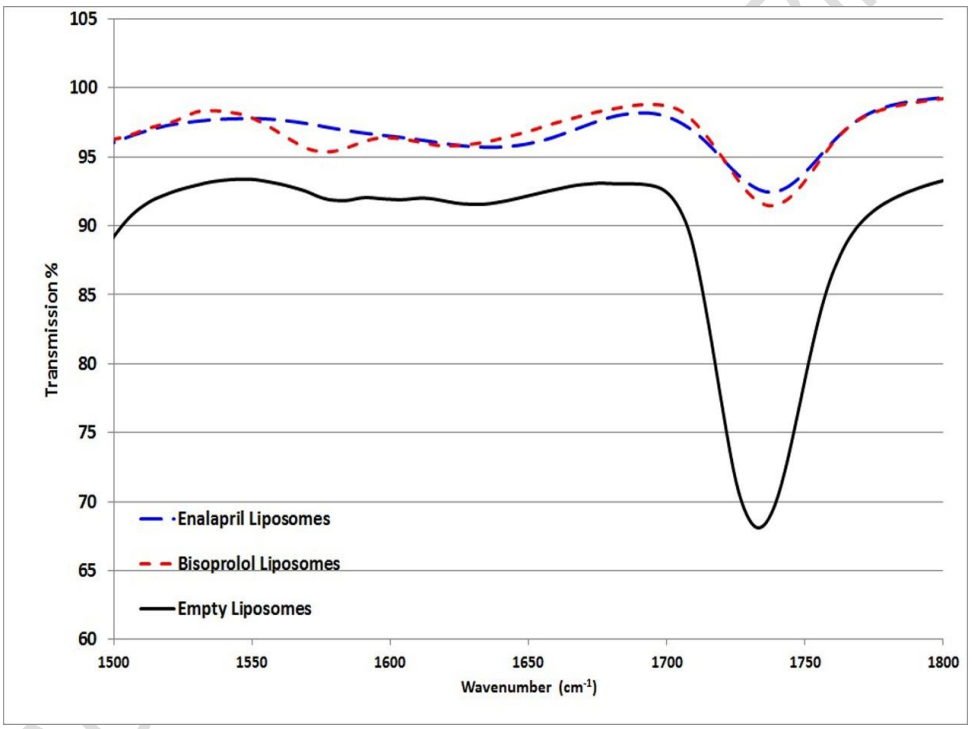


Fig. 7. The magnified part (1500–1800 cm^{-1}) of FTIR spectra of empty DSPC and DSPC/ bisoprolol or enalapril liposomal samples.

Also, the N $(CH_3)_3^+$ antisymmetric stretching band at 970 cm^{-1} is increased for enalapril or bisoprolol /DSPC liposomal sample (Table 1). The existence of a $N(CH_3)_3^+$ group in the polar head of DSPC might prevent the enalapril or bisoprolol NH group from getting close to the PO_2^- group, because of the electrostatic repulsive force between the DSPC $N(CH_3)_3^+$ group and the enalapril or bisoprolol NH group; thus, weakening the interactions between phospholipid PO_2^- and enalapril or

bisoprolol NH group. Table 1 summarizes the chemical shift observed for enalapril or bisoprolol after the incorporation into DSPC liposomes.

Table 1

The chemical shift observed for enalapril or bisoprolol after the incorporation into DSPC liposomes

Peak assignment	Wave number (cm^{-1})	Wave number (cm^{-1})		
		Control	Bisoprolol	Enalapril
Symmetric stretching vibration of CH_2 in acyl chain	2850 (2800–2855)	2852.203	2853.167	2852.203
Antisymmetric stretching vibration of CH_2 in acyl chain	2920 (2920–3000)	2920.663	2920.663	2920.663
OH stretching	3470 (3400–3470)	3430.743	3430.743	3430.743
Carbonyl stretching vibration C=O	1734 (1730–1740)	1732.728	1737.549	1737.549
CH_2 bending vibration	1470 (1456–1470)	1467.563	1471.42	1468.528
Symmetric PO_2^-	1090	1092.477	1094.405	1095.369
Antisymmetric PO_2^-	1220 (1215–1260)	1222.648	1246.754	1244.825
$(CH_3)_3N^+$ symmetric	1405	1415.495	1425.137	–
$(CH_3)_3N^+$ antisymmetric	972	970.0189	972.9116	971.9473

CONCLUSION

The molecular interaction of enalapril or bisoprolol as antihypertensive drugs with DSPC was investigated using two non-invasive techniques FTIR and DSC. Based on these results, we propose that enalapril or bisoprolol can be interacted actively with the lipids and to induce changes in their physical-chemical properties, thus affecting its IR spectra, thermo tropic phase behavior and membrane fluidity. In addition, a possible location of enalapril or bisoprolol in the interfacial region of the membrane has been proposed. These findings facilitate a better understanding of the interactions between enalapril or bisoprolol as antihypertensive drugs and lipid bilayers.

Acknowledgements. We thank Mohamed Nabil and Ismail Mohamed for their valuable technical assistance.

REFERENCES

1. BLUME, A., W. HUEBNER, G. MESSNER, Fourier transform infrared spectroscopy of ^{13}C: O labeled phospholipids. Hydrogen bonding to carbonyl groups, *Biochem*., 1988, **27**, 8239–8249.
2. DEAMER, D.W., P.S. USTER, Preparation of liposomes, In: *Liposomes*, M.J. Ostro, ed., Marcel Dekker, New York, 1983, pp. 27–51.
3. ELAWADY, A.I., M.W. ISMAIL, H.S. KAREEM, Alleviation of cisplatin-induced toxicities by encapsulation into liposomes in the absence or presence Ginkgo Biloba, *J. Am. Sci*., 2011, **7**, 346–356.
4. IP, D.P., G.S. BRENNER, Enalapril maleate, In: *Analytical Profiles of Drug Substances*, K. Florey, ed., Academic Press, London, 1987, pp. 207–243.
5. KOYNOVA, R., M. CAFFREY, Phases and phase transitions of the phosphatidylcholines, *Biochim. Biophys. Acta*, 1998, 1376, 91–145.
6. MADY, M.M., M.W. SHAFAA, E.R. ABBASE, A.H. FAHIUM, Interaction of doxorubicin and dipalmitoylphosphatidyl-choline liposomes, *Cell Biochem. B*iophys., 2012, 62, 481–486.
7. PORTOLES, A., A. TERLEIRA, S. ALMEIDA, M. GARCIA-ARENILLAS, M. CATURLA, A. FILIPE, E. VARGAS, Bioequivalence study of two formulations of enalapril, at a single oral dose of 20 mg (tablets): a randomized, two-way, open-label, crossover study in healthy volunteers, *Curr. Ther. Res*., 2004, 65, 34–46.
8. ROSENBERG, J., F. GUSTAFSSON, Bisoprolol for congestive heart failure, *Expert. Opin. Pharma. Cother.*, 2008, **9**, 293–300.
9. SEVERCAN, F., I. SAHIN, N. KAZANCI, Melatonin strongly interacts with zwitterionic model membranes – evidence from Fourier transform infrared spectroscopy and differential scanning calorimetry, *Biochim. Biophys. Acta*, 2005, **1668**, 215–222.
10. SHAFAA, M.W., N.M. SABRA, R.A. FOUAD, The extended ocular hypotensive effect of positive liposomal cholesterol bound timolol maleate in glaucomatous rabbits, *Biopharm. Drug Dispos*., 2011, **32**, 507–517.
11. SKOTNICKI, M., J.A. AGUILAR, M. PYDA, P. HODGKINSON, Bisoprolol and bisoprolol-valsartan compatibility studied by differential scanning calorimetry, nuclear magnetic resonance and X-ray powder diffractometry, *Pharm. Res*., 2015, **32**, 414–429.
12. SPINK, C.H., Differential scanning calorimetry, *Methods Cell Biol.*, 2008, **84**, 115–141.
13. TOYRAN, N., F. SEVERCAN, Interaction between vitamin D2 and magnesium in liposomes: Differential scanning calorimetry and FTIR spectroscopy studies, *J. Mol. Struc*., 2007, **839**, 19–27.