LASER INDUCED CONFORMATIONAL CHANGES OF LIPIDS WITHIN MULTILAMELLAR VESICLES

M. MADY[*#]*, MANAL HUSSIEN*[**]

[*]Department of Physics and Astronomy, College of Science, King Saud University, Riyadh 11451, Saudi Arabia, [#]e-mail: mmady@ksu.edu.sa
[**]Photochemistry and Photobiology Unit, National Institute of Laser Enhanced Sciences, Cairo University, Giza, Egypt

Abstract. The biomedical potential of destabilizing liposomes through photoinduction relies on the use of near-infrared light, which offers inherent therapeutic advantages. Researchers have explored the effects of infrared laser light on dipalmitoyl phosphatidylcholine (DPPC) multilamellar vesicles, specifically investigating the interaction between the laser and zwitterionic dipalmitoyl phosphatidylcholine multilamellar vesicles using Fourier transform infrared spectroscopy and differential scanning calorimetry measurements. The results revealed that laser irradiation increased the number of gauche conformers and led to conformational changes within the acyl chains of the phospholipids. The transition temperature of lyophilized dipalmitoyl phosphatidylcholine multilamellar vesicles was also shifted to a lower temperature after laser irradiation, indicating that the laser had a significant effect on the acyl chains in the dipalmitoyl phosphatidylcholine bilayers and decreased the transition cooperativity of lipid acyl chains. These findings could aid in the development of more effective liposomal drug delivery systems by enhancing our understanding of the interaction between laser and lipid bilayers.

Key words: liposomes, DPPC, FTIR, differential scanning calorimetry (DSC), laser, multilamellar vesicles.

INTRODUCTION

According to research, the most commonly used laser for laser-induced thermotherapy is the neodymium-doped yttrium aluminium garnet (Nd: YAG) laser, which emits light at a wavelength of 1064 nm. This therapy involves using laser light to ablate benign or malignant lesions in various organs. Laser light offers a unique advantage in thermotherapy due to its ability to precisely deposit a specific amount of energy in a particular region, making it a preferred method over other techniques.

Received: May 2023;
in final form July 2023.

ROMANIAN J. BIOPHYS., Vol. 33, No. 2, P. 57–65, BUCHAREST, 2023

The application of Nd:YAG lasers in oncology is versatile, with some of its uses including the removal of skin cancers [21], reduction of benign thyroid nodules [33], and destruction of primary and secondary malignant liver lesions [24, 25]. By inducing a local increase in temperature, laser light provides an efficient way to carry out cancer therapy. Vaporization of tumours has been effective in palliative treatment of cancers such as those of the oesophagus, liver, pancreas, and breast. Its primary aim is to concentrate the treatment locally in the tumour region of the body while preserving the original structure of the parenchymal tissue, making it an ideal method for cancer therapy.

Liposomes have been utilized in various diagnostic and therapeutic applications in the medical field for the past five decades. They are versatile and can deliver different substances such as enzymes, antibiotics, antivirals, chemotherapy agents, vaccines, and gene therapy. In addition, liposomes can be engineered to release their contents upon stimulation with heat or laser *in vivo*. This makes them an attractive option for targeted drug delivery [2, 5, 11]. External stimulation of liposomes through heat or laser can lead to the release of their contents *in vivo*. This photoinduced destabilization of liposomes provides a means to combine radiation with reagent delivery, allowing for precise temporal and spatial control over drug release. This approach has significant potential in medical applications [10, 22].

Hyperthermia and thermosensitive liposomes are promising methods to enhance tumour accumulation and increase liposomal drug bioavailability. The use of thermosensitive liposomes for heat-triggered drug delivery and the utilization of tumour vasculature or tumour cell-targeted liposomes are both effective strategies for improving liposomal chemotherapy. The combination of these two strategies can result in targeted thermosensitive liposomes (TTSL), which have the potential to revolutionize tumour drug delivery [9, 13, 26].

The analysis of frequency or bandwidth changes of different vibration modes of lipid molecules using FTIR can detect subtle alterations in the structure and function of lipid assemblies. Information about structural interactions and conformational rearrangements can be obtained by measuring spectral parameters such as band frequency, width, and intensity changes. Different types of information can be obtained from these bands, including the acyl chains, interfacial region, and head group region of lipid molecules [16, 28].

The DSC technique is a non-invasive, inexpensive, and fast thermodynamic method for studying the thermotropic phase behaviour of lipids in both biological and model membrane systems. By analysing the phase transition temperatures, the DSC technique can provide insights into the various mechanisms that take place in the liposomal membrane, which serves as a model for the biological membrane system.

In cancer nanomedicine, photo-triggering is being explored as a method to enhance drug or bioactive molecule delivery. To study the interaction between dipalmitoyl phosphatidylcholine multilamellar vesicles and infrared (IR) laser, FTIR spectroscopy, and DSC measurements were utilized in this study.

MATERIALS AND METHODS

L-α-dipalmitoyl phosphatidylcholine 'DPPC' in powder form of purity 99 % from Avanti polar lipids Inc, Alabama, USA. Chloroform was of analytical grade and obtained from Merck (Heliopolis, Cairo, Egypt). All other reagents and solvents were of analytical grade and were used without further purification.

LIPOSOMAL PREPARATION

Multilamellar vesicles (MLV) of DPPC liposomes were prepared according to the reverse phase evaporation method [30]. The initial step in liposomal preparation involves dissolving the lipids in ethanol. The concentration of lipids in ethanol was 2 mg/mL. Using rotary evaporation, the organic solvent is then eliminated, producing a thin lipid film on the round bottom flask. The lipid film is then hydrated by adding Trizma-buffer (pH 7.4) to the container of dry lipid and agitating it above the phase transition temperature of the lipid. The final lipid concentration of 2 mg/mL results in the formation of multilamellar vesicles (MLV).

LIPOSOMES LASER IRRADIATION

The DPPC multilamellar vesicles liquid samples were irradiated with a pulsed IR laser (three pulses of 8 mJ energy, pulse duration 5 ns, and wavelength of 1064 nm) from Nd/YAG Pulsed Laser (Q-Switched Nd:YAG compact oscillator, Quantel Lasers, Bozeman, MT, USA). Irradiation was performed from above so that the whole sample was irradiated homogeneously. In this way, there will be no loss of the measured incident laser irradiance due to reflections from the cuvette wall.

DIFFERENTIAL SCANNING CALORIMETERY

Differential scanning calorimetry (DSC) was carried out using (SHIMADZU DSC-50, USA) to investigate thermal behaviour of lyophilized DPPC multilamellar vesicles (DPPC) and laser exposed DPPC multilamellar vesicles (DPPC/IR). Lyophilization of multilamellar vesicles was made by Edwards's lyophilizer freeze dryer, Buch and Helma, England. The thermo grams covered the 25–200 ^{o}C temperature range at a 5 ^{o}C/min heating rate.

FTIR SPECTROSCOPY

The interaction between DPPC multilamellar vesicles liquid samples and an infrared (IR) laser was investigated using FTIR spectroscopy. The spectra of DPPC multilamellar vesicles (DPPC) and laser-exposed DPPC multilamellar vesicles (DPPC/IR) were recorded on a NICOLET 6700 FTIR Thermo scientific spectrometer

in England. The scanning was performed from 400–4,000 cm^{-1} at a speed of 2 mm/s and a resolution of 4 cm^{-1} at room temperature. The deposited samples were in KBr disks.

RESULTS AND DISCUSSION

FTIR STUDIES

Figure 1 shows the full FTIR spectrum of the DPPC multilamellar vesicles sample before (DPPC) and after laser irradiation (DPPC/IR). As shown from this figure, the spectrum of DPPC multilamellar vesicles (DPPC) displays the main characteristic bands of DPPC multilamellar vesicles, especially those are due to the symmetric and antisymmetric CH_2 stretching vibrations of the acyl chain (2850 and 2920 cm^{-1}, respectively), OH stretching and bending vibrations at 3470 and 1640 cm^{-1}, respectively, the CH_2 bending vibration CH_2 (1470 cm^{-1}), and the symmetric and antisymmetric PO_2^- stretching vibrations PO_2^- (1090 and 1226 cm^{-1}, respectively). These findings are in good accordance with the data reported in the literature [15, 17, 18, 20, 31].

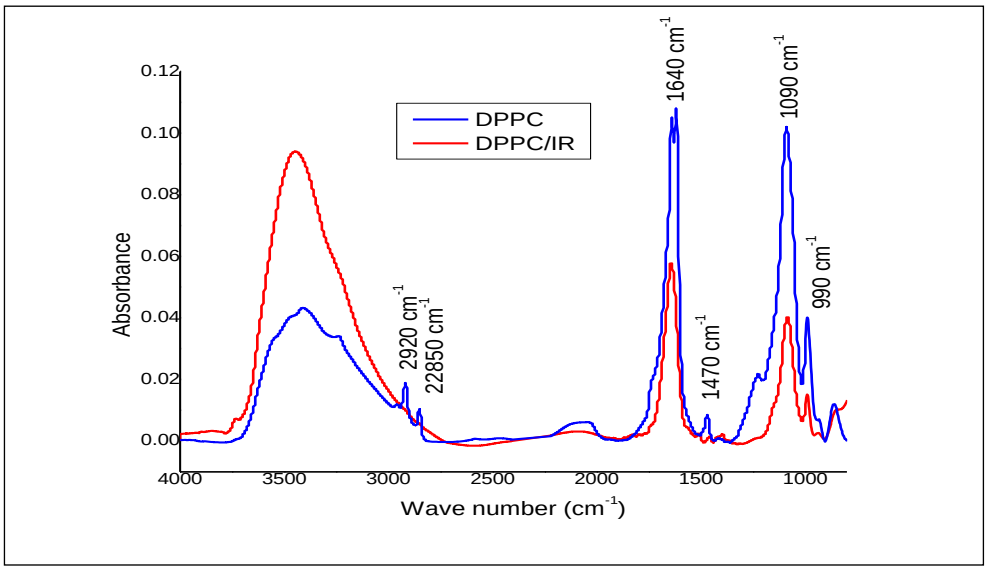

Fig. 1. Full FTIR spectra of DPPC multilamellar vesicles samples before (blue) and after laser irradiation (red).

As can be seen from Fig. 2, the CH_2 asymmetric stretching bands intensities of DPPC were decreased after laser irradiation (DPPC/IR), revealing that IR laser

irradiation caused a conformational disorder of the bilayer or may cause destabilization of the multilamellar vesicles (disruption of the lipidic bilayer) due to the creation/collapse of vapor bubbles. There may be a direct thermo-damage to the lipid molecules by pulsed IR laser, causing a perturbation in the lipid bilayer leading to multilamellar vesicles destabilization.

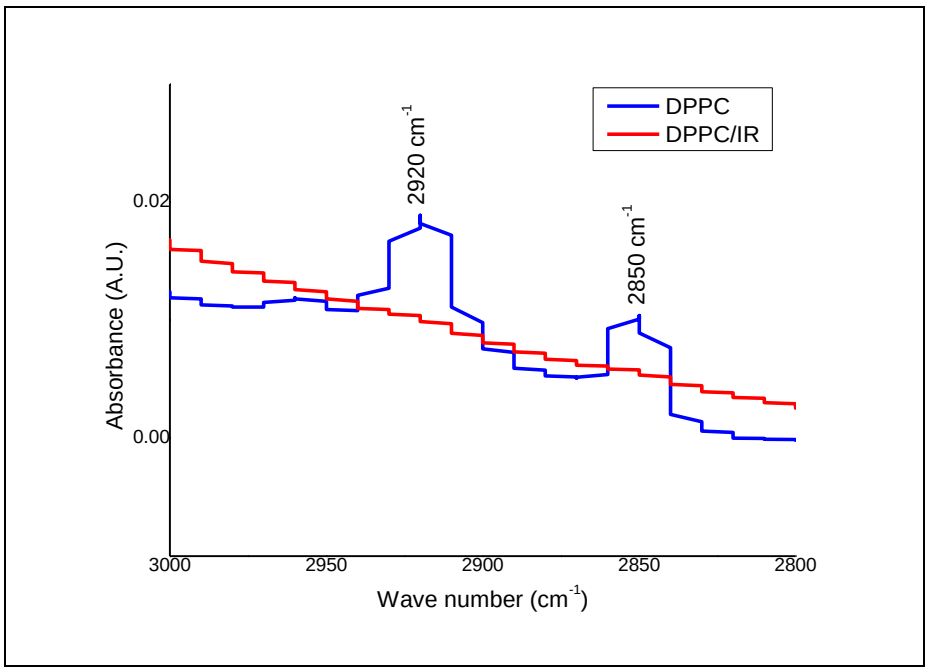

Fig. 2. The magnified part (3000–2800 cm^{-1}) of FTIR spectra of DPPC multilamellar vesicles (blue) and irradiated DPPC multilamellar vesicles (red) samples.

Fig. 3 shows that OH bending vibration at 1640 cm^{-1} for DPPC multilamellar vesicles was shifted to higher frequency (1643.9 cm^{-1}) after laser irradiation (DPPC/IR). The CH_2 bending vibration (1470 cm^{-1}) was shifted to lower value (1458 cm^{-1}) after laser irradiation. As seen from the figure, the frequency the symmetric PO_2^- stretching vibrations PO_2^- (1090 cm^{-1}) was shifted to lower values (1084.6 cm^{-1}), after irradiation of DPPC multilamellar vesicles by IR laser. A decrease in the frequency corresponds to the hydrated phosphate groups [1, 29]. The frequency of this band determines the presence of hydrogen bonding between phosphate group and hydrogen atoms of water or biological macromolecules. These spectral changes may be due to the formation of curved and lipid planes that lead to the lipid lateral diffusion and cause a rotational change of the lipid molecules, these changes may result in an increase in structural disordered of the membrane components.

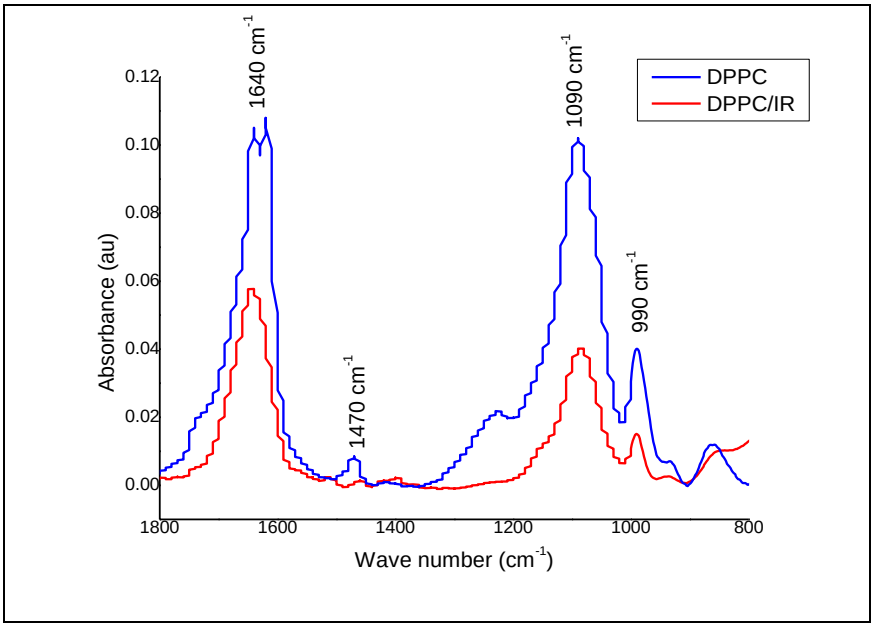

Fig. 3. The magnified part (1800–800 cm^{-1}) of FTIR spectra of DPPC multilamellar vesicles (blue) and irradiated DPPC multilamellar vesicles (red) samples.

THE PHASE BEHAVIOUR OF DPPC MULTILAMELLAR VESICLES

Figure 4 shows the thermal grams of lyophilized DPPC multilamellar vesicles before (DPPC) and after laser exposure (DPPC/IR). The pre-transition temperature was around 60 ºC for pure DPPC multilamellar vesicles, while the main transition temperature of DPPC was 106 °C upon dehydration, in accordance with previous studies [8, 19, 23].

The pre-transition temperature is shifted to a lower temperature (38.5 ºC) for irradiated DPPC multilamellar vesicles which revealed the conformational change in the polar head group of phospholipids. The main transition temperature of DPPC multilamellar vesicles (106 ºC) is shifted to a lower temperature (69.5 ºC) for irradiated DPPC multilamellar vesicles (DPPC/IR), which suggests that IR laser irradiation has a significant effect on the acyl chains of DPPC bilayers, creating a conformational disorder within the acyl chains of phospholipids and decreases the transition cooperatively of lipid acyl chains. The decrease in phase transition temperature after IR laser irradiation was caused by the increase in the spacing between the head groups, which allowed for decreasing van der Waals interactions between the lipid hydrocarbon chains promoting gauche rotamer formation [7, 15, 32]. In this case, the lipid hydrocarbon chains are converted from a relatively rigid, extended, and highly conformation in the gel state to a more oriented disordered state.

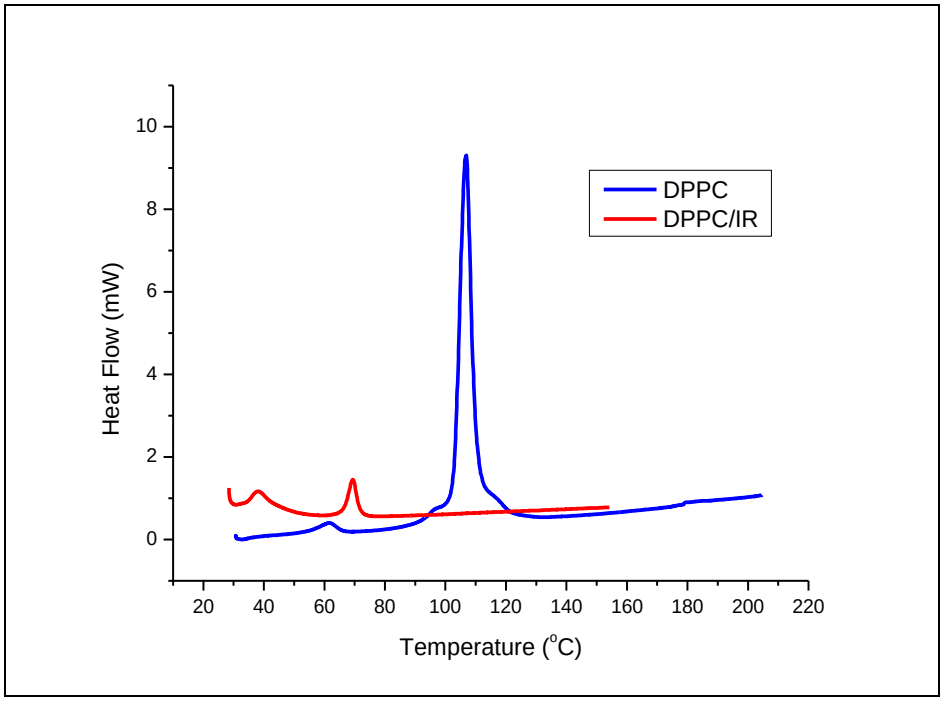

Fig. 4. DSC thermograms of DPPC multilamellar vesicles (blue) and irradiated DPPC multilamellar vesicles (red) samples.

Laser exposure causes some changes in the membrane packing properties, which are reflected on its transition temperature. Consequently, the interaction between laser and multilamellar vesicles may cause a rotational change of the lipid molecules resulting in an increase in structural disordered of the membrane components. This may lead to a change in the membrane packing properties, which is accompanied by an increase in the freedom motion of the hydrocarbon chain and a substantial increase in the partial volume of the bilayer structures [6, 12].

CONCLUSIONS

On the basis of the present findings, we can conclude that such studies of FTIR and DSC of the multilamellar vesicles are very important for getting information about the intermolecular structural change of the membrane bilayer. This information can be used for understanding the interaction mechanism of external radiation with the liposomal membrane bilayer and with the biological cells. The combination between both heating and laser irradiation can be used as a powerful conjunction that may give us the benefit of the therapeutic and radiotherapy treatment. Multilamellar vesicles

may be made photosensitive by the use of uniquely designed lipids that can alter the liposome properties via photoisomerization, photocleavage, or photopolymerization. There is also the possibility of using gold nanoshells, fullerenes, and magnetic nanoparticles for the controlled drug release using infrared laser pulses [3, 4, 14, 27, 34].

REFERENCES

1. ARRONDO, J.R., F.M. GONT, J.M. MACARULLA, IR spectroscopy of phosphatidylcolines in aqueous solutions. A study of the phosphate group, *Biochim. Biophys. Acta*, 1984, **794**, 165–168.
2. BABAI, I., Y. BARENHOLZ, Z. ZAKAY-RONES, E. GREENBAUM, S. SAMIRA, I. HAYON, M. ROCHMAN, E. KEDAR, A novel liposomal influenza vaccine (INFLUSOME-VAC) containing hemagglutinin-neuraminidase and IL-2 or GM-CSF induces protective anti-neuraminidase antibodies cross-reacting with a wide spectrum of influenza a viral strains, *Vaccine*, 2001, **20**, 505–515.
3. BABINCOVÁ, M., P. SOURIVONG, D. CHORVÁT, P. BABINEC, Laser triggered drug release from magnetoliposomes, *J. Magn. Magn. Mater.*, 1999, **194**(1), 163–166.
4. BABINCOVÁ, M., P. SOURIVONG, D. LESZCZYNSKA, P. BABINEC, Photodynamic therapy of pigmented melanoma B16 using sterically stabilized fullerenosomes, *Laser Physics Letters*, 2004, **1**(9), 476–478.
5. BANERJEE, R., Liposomes: applications in medicine, *J. Biomater. Appl.*, 2001, **16**, 3–21.
6. CAMMARATA, F., M. WAUTELET, Medical lasers and laser tissue interactions, *Phys. Education*, 1999, **34**(3), 156–161.
7. CROWE, J.H., J.F. CARPENTER, L.M. CROWE, The role of vitrification in anhydrobiosis, *Annu. Rev. Physiol.*, 1998, **6**, 73–103.
8. CROWE, L., J. CROWE, Trehalose and dry dipalmitoylphosphatidylcholine revisited, *Biochim. Biophys. Acta*, 1988, **946**, 193–201.
9. DICHEVA, B.M., G.A. KONING, Targeted thermosensitive liposomes: An attractive novel approach for increased drug delivery to solid tumors, *Expert Opinion on Drug Delivery*, 2014, **11**, 83–100.
10. EBRAHIM, S., G.A. PEYMAN, P.J. LEE, Applications of liposomes in ophthalmology, *Survey of Ophthalmology*, 2005, **50**, 167–186.
11. FENSKE, D.B., I. MACLACHLAN, P.R. CULLIS, Stabilized plasmid-lipid particles: a systemic gene therapy vector, *Methods Enzymol.*, 2002, **346**, 36–71.
12. HOEBEKE, M., The importance of liposomes as models and tools in the understanding of photosensitization mechanisms, *J. Photochemistry Photobiology B*, 1995, **28**(3), 189–196.
13. JONE, A., Liposomes: A short review, *J. Pharmaceutical Sciences and Research*, 2013, **5**(9), 181–183.
14. KASILI, P.M., T. VO-DINH, Photothermal treatment of human carcinoma cells using liposome-encapsulated gold nanoshells, *Nanobiotechnology*, 2005, **1**(3), 245–252.
15. LEFEVRE, T., M. PICQUART, Vitamin E-phospholipid interactions in model multilayer membranes: A spectroscopic studies, *Biospectroscopy*, 1996, **2,** 391–403.
16. LEWIS, R.N.A.H., R.N. MC ELHANEY, FTIR spectroscopy in the study of hydrated lipids and lipid bilayer membranes, in: H.H. Mantsch, D. Chapman (Eds.), *Infrared Spectroscopy of Biomolecules*, John Wiley & Sons, New York, 1996, pp. 159–202.
17. MADY, M.M., M. DARWISH, S. KHALIL, W. KHALIL, Biophysical studies on chitosan-coated liposomes, *Eur. Biophysics J.*, 2009, **38**, 1127–1133.
18. MADY, M.M., M. FATHY, T. YOUSSEF, W. KHALIL, Biophysical characterization of gold-loaded liposomes, *Physica Medica*, 2012, **28**, 288–295.

19. MADY, M.M., M.W. SHAFAA, E.R. ABBASE, A.H. FAHIUM, Interaction of doxorubicin and dipalmitoylphosphatidyl-choline liposomes, *Cell Biochemistry and Biophysics*, 2012, **62**, 481–486.
20. MADY, M.M., M.A. ALLAM, The influence of low power microwave on the properties of DPPC vesicles, *Bioelectromagnetics, Physica Medica*, 2012, **28**, 48–53.
21. MOSKALIK, K., A KOZLOV, E. DEMIN, E. BOIKO, The efficacy of facial skin cancer treatment with high-energy pulsed neodymium and Nd:YAG lasers, *Photo Medical Laser Surgery*, 2009, **27**(2), 345–349.
22. MUELLER, A., B. BONDURANT, D.F. O'BRIEN, Visible light-stimulated destabilization of peg-liposomes, *Macromolecules*, 2000, **33**, 4799–4804.
23. OHTAKE, S., C. SCHEBOR, S. PALECEK, J.J. DE PABLO, Effect of sugar-phosphate mixtures on the stability of DPPC membranes in dehydrated systems, *Cryobiology*, 2004, **48**, 81–89.
24. PACELLA, C.M., G., RANCICA, F.M. DI LASCIO, V. ARIENTI, E. ANTICO, B. CASPANI, F. MAGNOLFI, A.S. MEGNA, S. PRETOLANI, R. REGINE, M. SPONZA, R. STASI, Long-term outcome of cirrhotic patients with early hepatocellular carcinoma treated with ultrasound-guided percutaneous laser ablation: a retrospective analysis, *Journal of Clinical Oncology*, 2009, **27**(16), 2615–2621.
25. POMPILI, M., C.M. PACELLA, G. FRANCICA, M. ANGELICO, G. TISONE, P. CRABOLEDDA, E. NICOLARDI, G.L. RAPACCINI, G. GASBARRINI, Percutaneous laser ablation of hepatocellular carcinoma in patients with liver cirrhosis awaiting liver transplantation, *European Journal of Radiology*, 2010, **74**(3), e6–e11.
26. PURI, A., Phototriggerable liposomes: Current research and future perspectives, *Pharmaceutics*, 2013, **6**(1), 1–25.
27. RENGAN, A.K., M. JAGTAP, A. DE, R. BANERJEE, R. SRIVASTAVA, Multifunctional gold coated thermo-sensitive liposomes for multimodal imaging and photo-thermal therapy of breast cancer cells, *Nanoscale*, 2014, **6**(2), 916–923.
28. SEVERCAN, F., N. KAZANCI, U. BAYKAL, S. SUZER, IR and turbidity studies of vitamin E-cholestrol-phospholipid membrane interactions, *Bioscience Reports*, 1995, **15**(4), 221–229.
29. STEWART, L.C., M. KATES, Intra-inter molecular hydrogen in diphytanylglycerol phospholipids an IR spectroscopic investigation, *Biochemical Cell Biology*, 1989, **68**, 266–273.
30. SZOKA, F., D. PAPAHADJOPOULOS, Procedure 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.
31. TOYRAN, N., F. SEVERCAN, Infrared spectroscopic studies on the dipalmitoyl phosphatidylcholine bilayer interactions with calcium phosphate: Effect of vitamin D2, *Spectroscopy*, 2002, **16**, 399–408.
32. TOYRAN, N., F. SEVERCAN, Interaction between vitamin D2 and magnesium in liposomes: differential scanning calorimetry and FTIR spectroscopy studies, *J. Molecular Structure*, 2007, **839**, 19–27.
33. VALCAVI, R., F. RIGANTI, A. BERTANI, D. FORMISANO, C.M. PACELLA, Percutaneous laser ablation of cold benign thyroid nodules: A 3-year follow-up study in 122 patients, *Thyroid*, 2010, **20**(11), 1253–1261.
34. VOLODKIN, D.V., A.G. SKIRTACH, H. MÖHWALD, Near-IR remote release from assemblies of liposomes and nanoparticles, *Angewandte Chemie* – International Edition, 2009, **48**(10), 1807–1809.