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Full Length Research Paper

Photochemical targeted drug delivery of liposomal membrane embedded photoreceptor hypericin

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Hypericin embedded in liposome loaded with oxytetracyclin as a model drug was used as a drug delivery system. Two liposomal systems were prepared, sample (I) and sample (II). In sample (I), liposomes incorporated with hypericin as a photoreceptor and loaded with oxytetracyclin drug, while in sample (II), liposomes loaded with oxytetracyclin drug and incorporated with hypericin as a photoreceptor and alpha tocopherol (vitamin E) as an anti-oxidant. In *vitro* drug release of both samples was examined pre- and post- irradiation to 100 J of 650 nm continuous wave diode laser for 9 min. using optical density spectroscopy. Fourier transform-infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC) were used for sample characterization. From FT-IR, DSC and drug release studies, both irradiated samples (I) and (II) have higher percentage of drug release than unexposed (control) samples. These results indicate that irradiated samples (I) and (II) were less stable than unexposed (control) samples due to the effect of free radicals and singlet oxygen generated from hypericin after irradiation to laser beam, while irradiated sample (II) has less percentage of drug release than irradiated sample (I) which indicates that inclusion of anti-oxidant vitamin E embedded in liposomes bilayer membrane stabilizes the membrane from degradation by free radicals generated from hypericin and hence protects the drug from degradation .

Key words: Liposome, hypericin, oxytetracyclin drug, photoreceptor and alpha tocopherol (vitamin E).

INTRODUCTION

Hypericin (HYP) is a naturally occurring photosensitizing pigment found abundantly in wild flowers from the family hypericum, or Saint John's wort (Sony and Nelson, 1986).

Today, it is still of remarkable interest and is a main topic of discussion concerning its potential scope of clinical applications, antidepressive, antiviral, antiretroviral, antineoplastic, antitumor, photodynamic and photodiagnostic activities of HYP are currently under investigation (Kubin et al., 2005). Because HYP is a hydrophobic molecule, it can be incorporated in phospholipids bilayer of cell membrane (Thomas et al., 1992). Other works have shown that irradiation of HYP with visible lights leads to the production of singlet oxygen and/or free radicals (Weiner and Mazur, 1992).

The aim of this paper is in *vitro* targeting of liposome bilayer embedded the photosensitzer HYP and encapsulated oxytetracyclin as a model drug using laser beam of 650 nm diode laser. We have prepared two samples of liposomes, sample (I) of liposomes embedded with HYP and loaded with oxytetracyclin drug and sample (II) of liposomes embedded with HYP in addition to alpha-tocopherol (vitamin E) as an anti-oxidant and loaded with oxytetracyclin drug. In *vitro* targeting of liposomes will be studied using Fourier transform-infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC) in addition to drug release studies for liposomes of both samples pre- and post- laser irradiation.

MATERIALS AND METHODS

Chemicals

HYP was obtained as a gift from Eye Research Institute (Cairo, Egypt). Oxytetracyclin was supplied as 250 mg capsules from Pfizer International Corporation, Middle East Arab Group (Cairo, Egypt).

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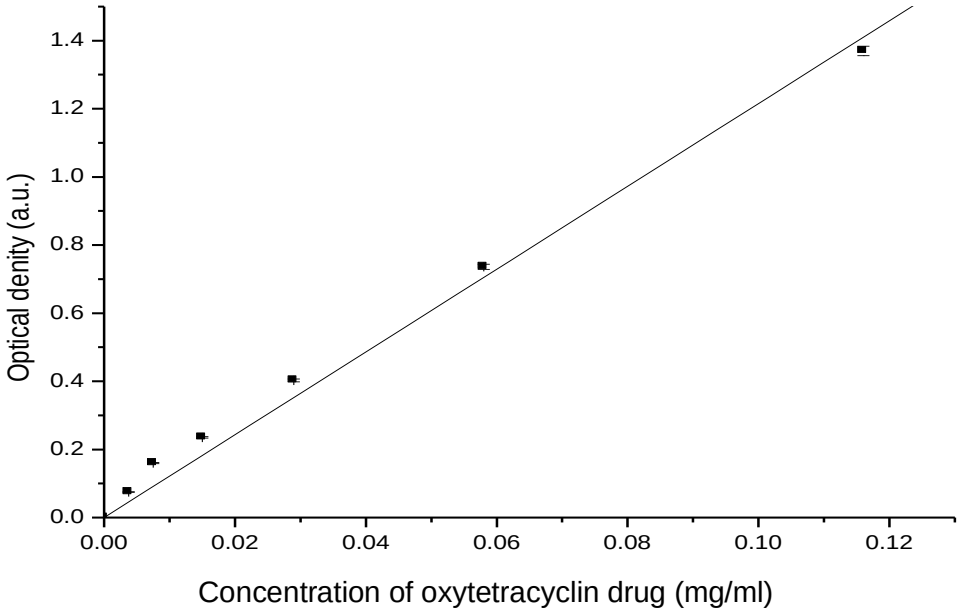

Figure 1. Standard curve of oxytetracyclin drug/at pH=7 and λ_{max}=362.5 nm.

L-α-Egg-yolk phosphatidylcholine (EPC) were supplied by Avanti Polar-Lipids, Inc. (Alabaster, AL, USA). Cholesterol, alpha-tocopherol (Vitamin E), N-(2-hydroxyethyl) piperazine-N-(2-ethane sulphonic acid); 4 (2-hydroxyethyl) piperazine-1- ethane sulphonic acid ($C_8H_{18}N_2O_4S$, Mw =238. (HEPES) buffer (pH =7) and Triton X100 was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Polycarbonate filters (0.2 µm) was purchased from Alkan Chemical Co. (Cairo, Egypt).

Preparation of liposome samples

Sample (I) preparation of liposome

This sample consists of liposomes containing HYP and loaded with oxytetracyclin and prepared as following: Egg-yolk phosphatidylcholin (EPC) liposome containing HYP were prepared at room temperature (EPC, critical temperature -15 to -7°C) following the procedures of Bangham and Huany reported by New (New R.R.C., 1990) in which; 43 mg of EPC/8.6 mg of cholesterol which equal to 20% w/w was added to 3 ml of chloroform/alcohol mixture 1:1 v/v. Then adding 20 µl of HYP (0.5% of alcohol in water) of concentration 0.005 mg/ml (0.03% of EPC + cholesterol) and then the organic solution were evaporated to produce a thin film on the wall of 100 ml rounded-bottom flask of the rotary evaporator. After that 10 ml of oxytetracyclin drug its concentration (50 mg/10 ml) was added to the film forming a suspension of multilamellar vesicles (MLV_s). These MLV_s suspension were sonicated for 10 min. in a sonicator to disperse the lipids. Then the liposomes are extruded through a polycarbonate filter of pore diameter 0.2 µm to get unilamellar vesicles (ULV_s). The unencapsulated oxytetracyclin was removed by centrifuging the sample (I) and sample (II) in a centrifuge (CENTRIKON T-42K by KONTRON instruments) operates at 10000 rpm at 25°C for 30 min. The supernatant was measured for free unencapsulated drug using spectrophotometer (UV-3101 PC, UV-VIS-NIR scanning spectrophotometer, SHIMADZU, Japan). The concentration was estimated from prepared calibration curve of oxytetracyclin in HEPES buffer of pH=7 at wavelength 362.5 nm (Figure 1).The correlation coefficient of the line was $r^2 = 0.999 \pm 0.001$ and the linearity were achieved in range of 0.005 mg/ml - 0.126 mg/ml. The liposomes pellets were re-suspended in fresh 10 ml HEPES buffer at pH=7 for further studies.

Sample (II) preparation of liposome

The above method was repeated but we added 1 ml of alpha-tocopherol (10 mg/ml) during liposome preparation and used as an antioxidant.

Exposure system

The embeddence of hypericin in lipids renders its maximum absorption spectrum shifted to a longer wavelength (Nicola et al., 1997). Therefore to target hypericin in membrane all samples were irradiated with 100 J of 650 nm diode laser type (Intelite, USA) with maximum power 500 mW and of beam size 1 cm^2.

Steady-state measurements

Optical absorption spectra

Optical absorption spectra were measured by spectrophotometer (UV-3101 PC, UV-VIS-NIR scanning spectrophotometer, Shimadzu, Japan) for HYP dissolved in HEPES buffer (pH=7) before and after to exposure to diode laser continuous wave for 9 minutes (100J) were measured (Figures 2 and 3).

Fourier transform infrared spectroscopy (FT-IR) measurements

FT-IR spectra were recorded using a double beam IR spectrometer of type (Jasco-460 FT-IR Plus, Japan).Each spectrum is recorded over wave number range 4000 - 400 cm^{-1}.all samples were mixed with cell contains KBr windows.

FT-IR spectra were recorded to the following samples to detect any change in the chemical stability of liposomes due to photo-sensitization of liposomes bilayer after exposure of HYP-ligand liposomes to continuous wave diode laser 650 nm.

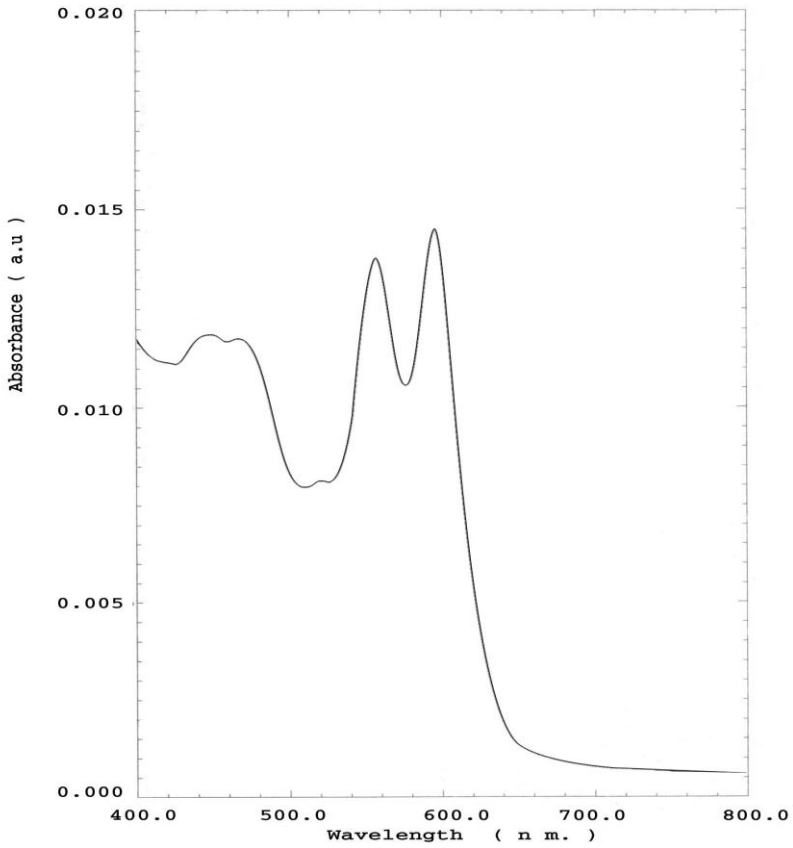

Figure 2. Absorption spectrum of hypericin in 5% alcohol in water before exposure to laser beam.

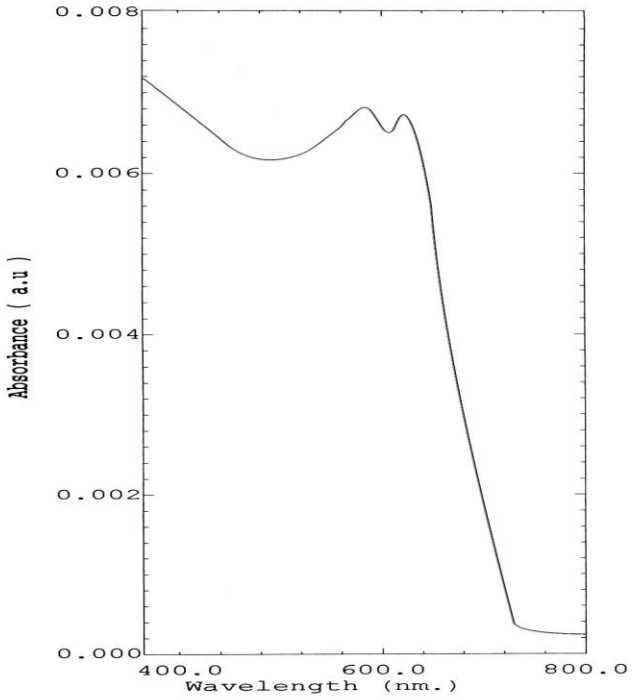

Figure 3. Absorption spectrum of hypericin in 5% alcohol in water after exposure to continuous wave laser beam for 9 min.

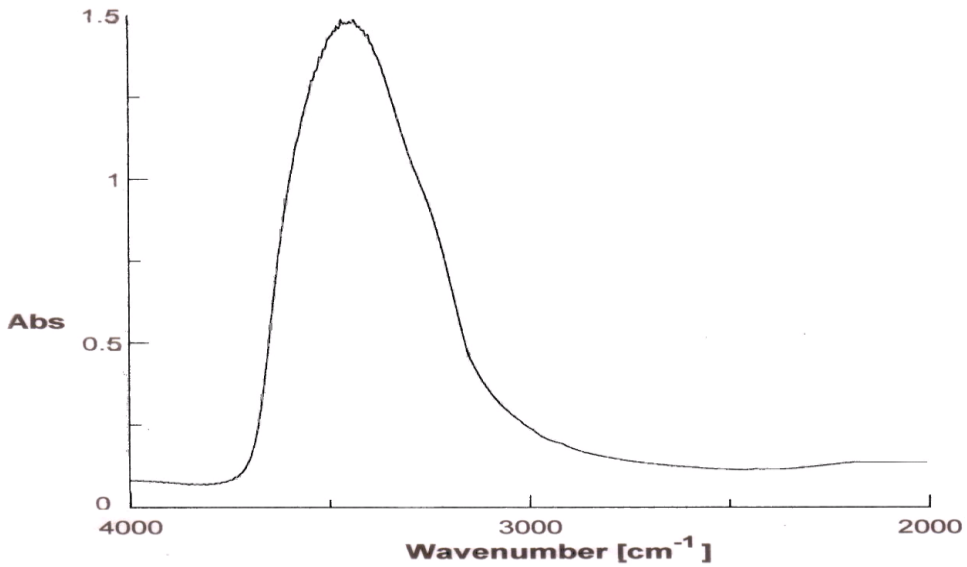

Figure 4. FT-IR absorption spectrum of OH group region of hypericin before exposure to laser beam.

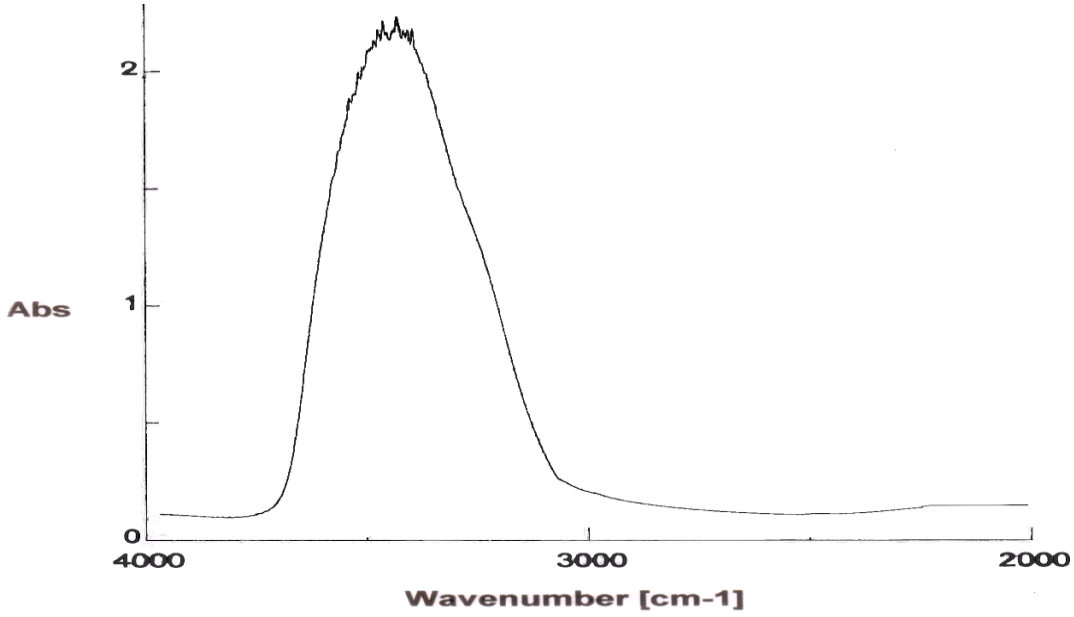

Figure 5. FT-IR absorption spectrum of OH group region of hypericin after exposure to laser beam.

A. FT-IR spectrum for HYP before exposure to laser irradiation (Figure 4).
B. FT-IR spectrum for HYP after exposure to laser irradiation (Figure 5).
C. FT-IR spectrum for control sample (I), before exposure to laser irradiation (Figure 6).
D. FT-IR spectrum for sample (I) (that is, after exposure to laser irradiation) (Figure 7).
E. FT-IR spectrum for control sample (II) (that is, before exposure to laser irradiation) (Figure 8).
F. FT-IR spectrum for sample (II) (that is, after exposure to laser irradiation) (Figure 9).

Differential scanning calorimeter (DSC) measurements

DSC measurements were recorded using (Shimadzu DSC-50, Japan).Each scan is recorded from -50 to +50°C by a scan rate of 5°C /min. in nitrogen as an inert atmosphere using aluminum seal as a cell for the sample and reference.

DSC measurements were recorded to the following samples to detect any change in phase transition temperature of liposomes samples (Enthalpy and Entropy were calculated from the graph): A-DSC thermogram for control sample (I) by weighting 8.630 mg of the sample and using HEPES buffer (pH=7) as a reference (Figure 10) .

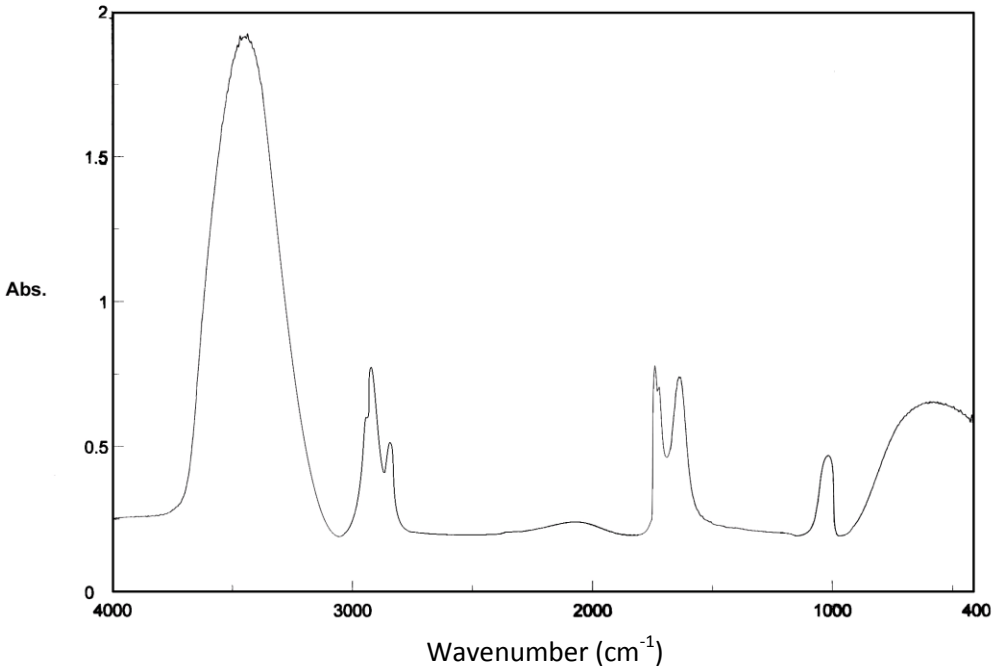

Figure 6. FT-IR absorption spectrum of control sample (I) of liposomes.

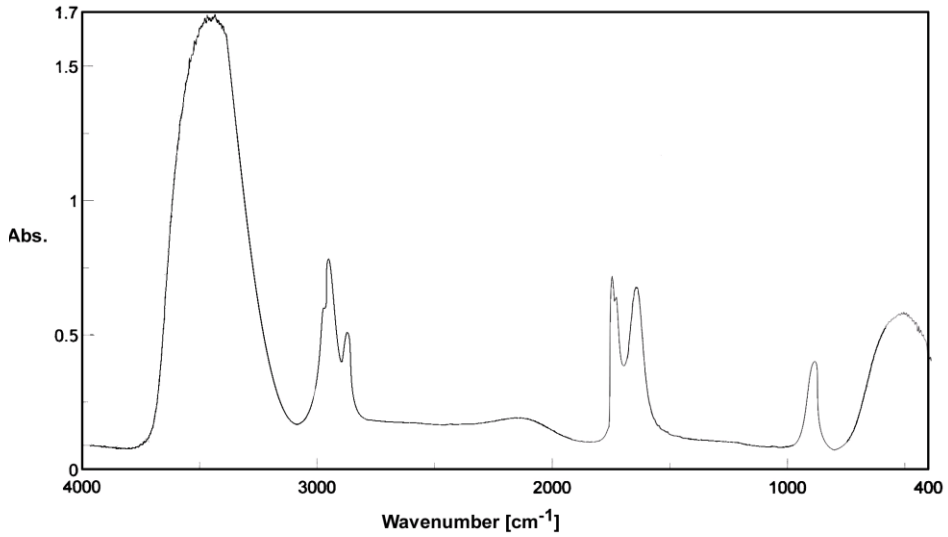

Figure 7. FT-IR absorption spectrum of sample (I) of liposomes after exposure to laser beam.

B-DSC thermogram for sample (I) by weighting 7.190 mg of the sample and using HEPES buffer (pH=7) as a reference (Figure 11). C- DSC thermogram for control sample (II) by weighting 7.260 mg of the sample and using HEPES buffer (pH=7) as a reference (Figure 12). D- DSC thermogram for sample (II) by weighting 7.380 mg of the sample and using HEPES buffer (pH=7) as a reference (Figure 13).

Drug release studies

(i) Drug release studies of unexposed samples (control sample (I) and control sample (II))

1 ml of control sample (I) and control sample (II) were taken separately to study the release of oxytetracyclin drug from liposomes of both samples before exposure to laser irradiation.

Each time we centrifuge the control sample (I) and control sample (II) at 10000 rpm for 15 min. Then the supernatant was taken from both tubes of control sample (I) and control sample (II) for absorption spectra to determine the concentration of the released drug and the percentage (%) of oxyteracyclin drug released for both samples.

The precipitated liposomes were resuspended in 1 ml HEPES

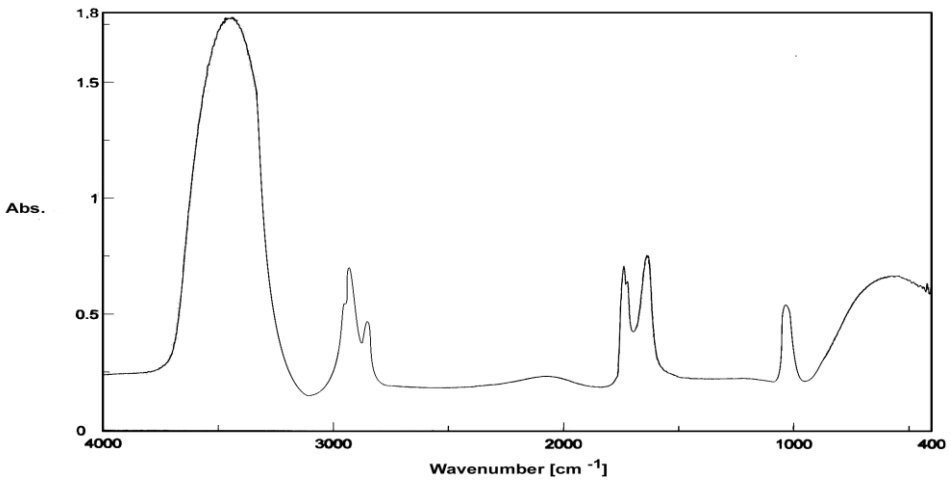

Figure 8. FT-IR absorption spectrum of control sample (II) of liposomes.

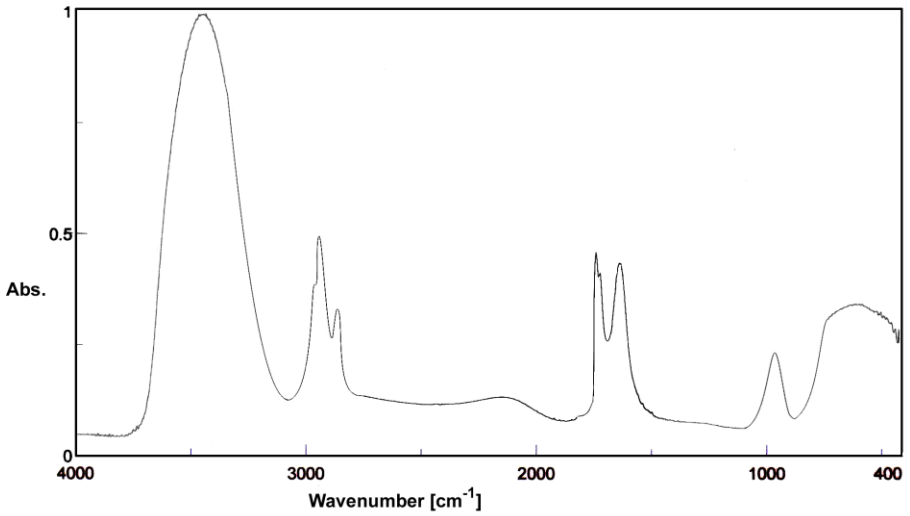

Figure 9. FT-IR absorption spectrum of sample (II) of liposomes after exposure to laser beam.

buffer (pH=7) and kept in a water bath for 30 min. These steps were repeated 6 times for two hours and half.

(ii) Drug release studies of exposed samples (sample (I) and sample (II) after exposure to laser irradiation)

2 ml of sample (I) and sample (II) (exposed to 650 nm continuous wave laser of 1 cm^2 of beam size and 100 J) were taken separately to study the release of oxytetracyclin drug from liposomes of both samples.

Each time we centrifuge the sample (I) and sample (II) at 10000 rpm for 15 min. Then the supernatant was taken from both tubes of sample (I) and sample (II) for absorption spectra to determine the concentration of the released drug and the percentage (%) of oxyteracyclin drug released for both samples.

The precipitated liposomes were resuspended in 1 ml HEPES buffer (pH=7) and kept in a water bath for 30 min. These steps were repeated 6 times for two hours and half.

RESULTS

The results of experimental measurements of the present study about drug releasing classified into two categories of Liposomes. One incorporated with hypericin as a photosensitizer and the other incorporated with hypericin and alpha-tocopherol. Both were loaded by a hydrophilic drug oxytetracyclin as a drug model. The results shown were taken before and after exposure to continuous wave diode laser for 9 min.

Optical absorption spectra

Figures 2 and 3 show the absorption spectra of hypericin

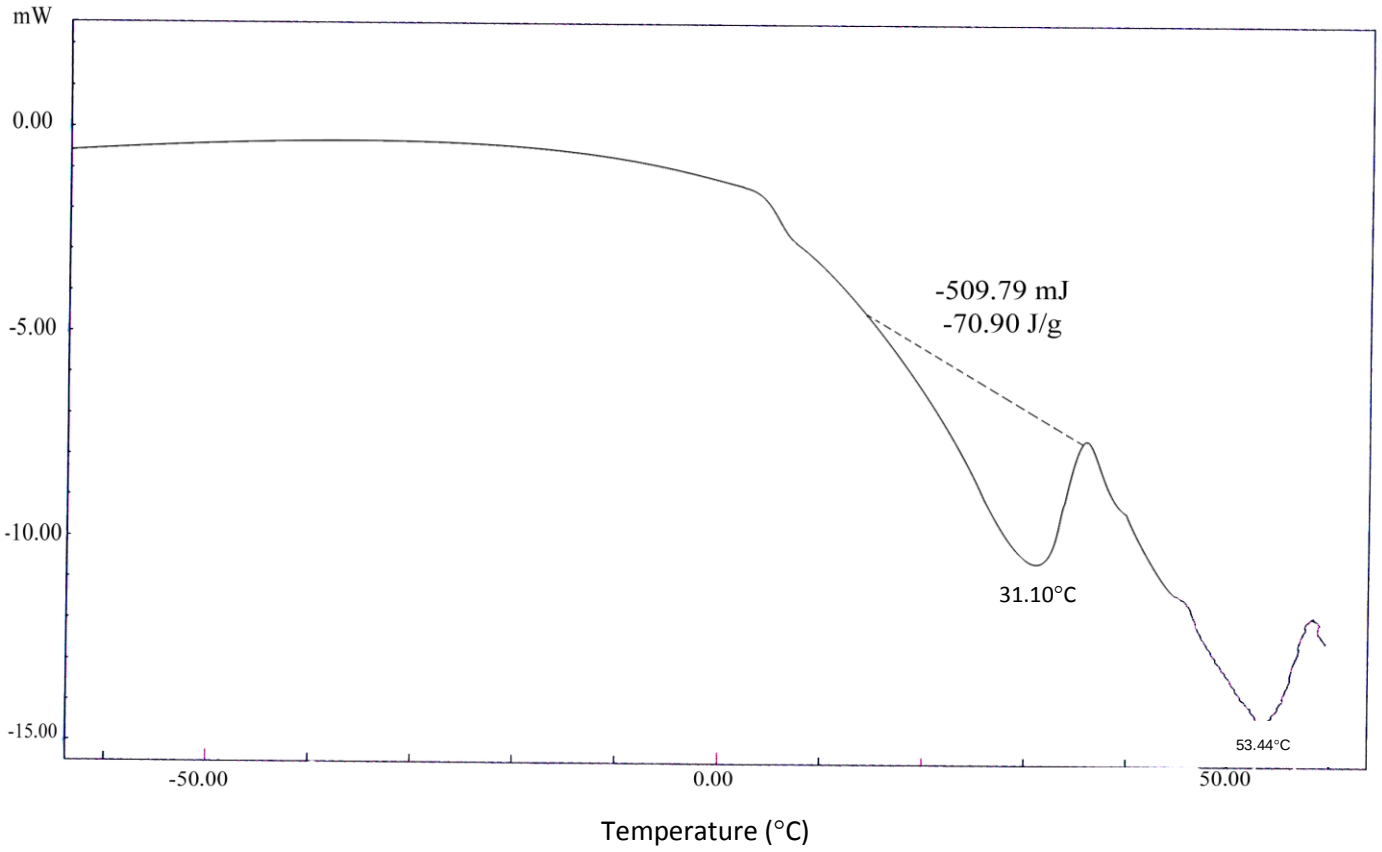

Figure 10. DSC thermogram of control sample (I) of liposomes.

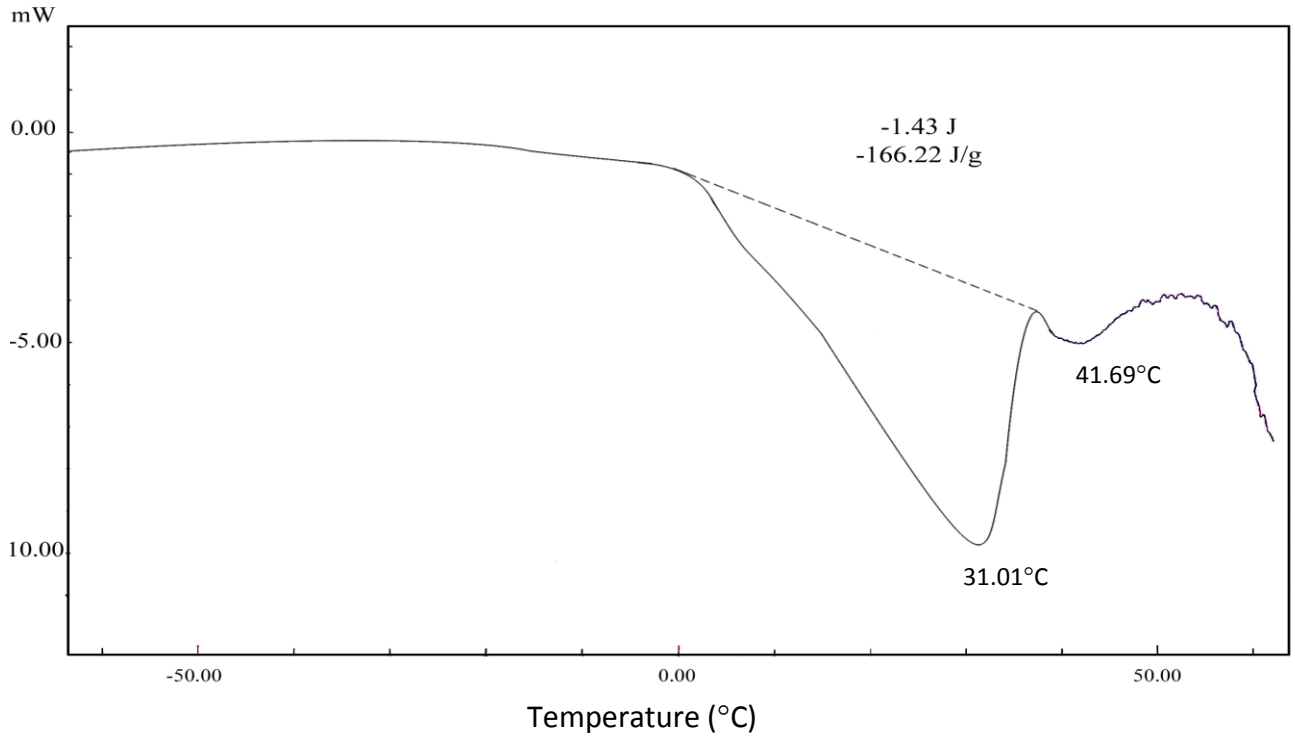

Figure 11. DSC thermogram of sample (I) of liposomes after exposure to laser beam.

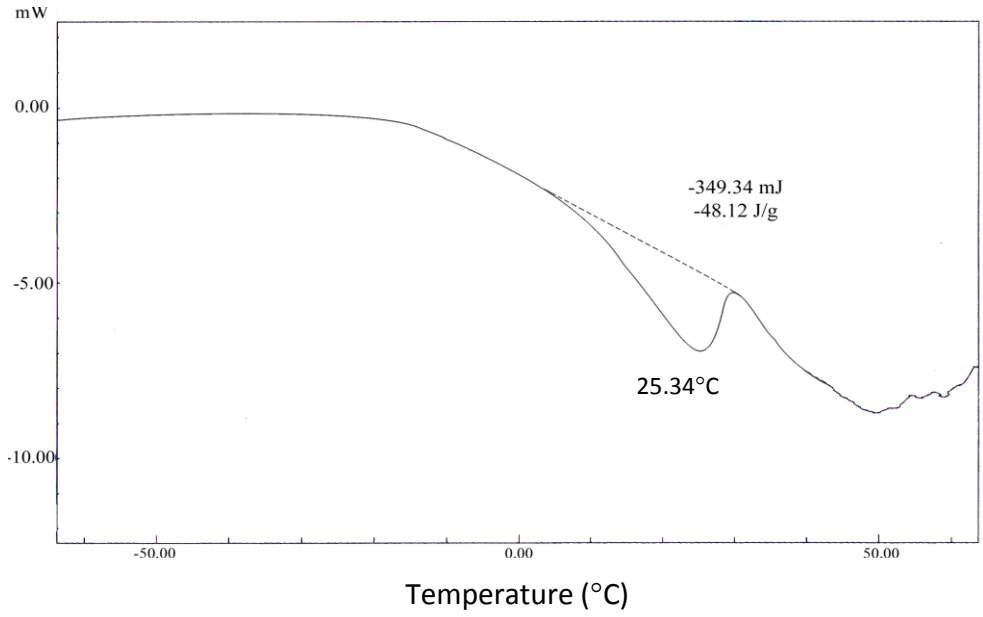

Figure 12. DSC thermogram of control sample (II) of liposomes.

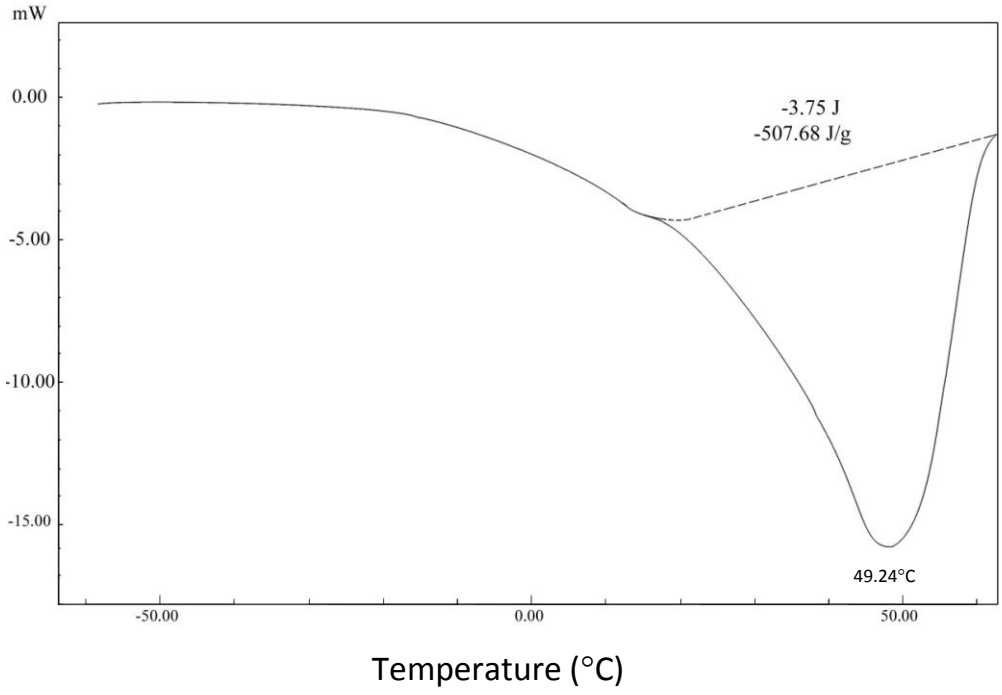

Figure 13. DSC thermogram of sample (II) of liposomes after exposure to laser beam.

before and after exposure to laser dose respectively. In Figure 2, hypericin has two major peaks at 558.00 and 595.00 nm. This result is in good agreement with the spectrum reported by Senthil (1992) with bands at 550 nm and Gad et al. (2007) with bands at 545 and 590 nm these two peaks red shifted to 583 and 637 nm after exposure to laser beam for 9 min. The high base line is a consequence of light scattering.

Fourier transform infrared spectroscopy (FT-IR) measurements FT-IR spectroscopy was used to study the stability of the prepared samples and detect any chemical changes due to exposure to 100 cm^{-2} for 9 min. from

continuous wave laser diode 650 nm and compare it with DSC results and kinetic studies.

FT-IR of hypericin before and after exposure to laser beam

FT-IR spectrum of OH-group region of hypericin dissolved in 0.5% alcohol in water before exposure to laser is shown in Figure 4, while Figure 5 and shows FT-IR spectrum of OH-group region of hypericin after exposure to100 J cm^{-2} (continuous wave laser diode 650 nm) for 9 min.

FT-IR results of control sample (II) and sample (II)

The infrared spectra of lipids have been studied in detail and most bands have been assigned (Feride et al., 1995). Various kinds of information can be derived from these bands. Frequency shifts in different regions (symmetric, antisymmetric stretching or bending, scissoring) or changes in the widths of the corresponding peaks can be used to extract information about various physico-chemical processes taking place. For example, the frequencies of the CH_2 stretching bands of acyl chains depend on the average trans/gauche isomerization in the system. The shifts to higher wave numbers correspond to an increase in number of gauche conformers (Feride et al., 1995). Bandwidth gives dynamic information about the system. As the bandwidth increases, mobility of the phospholipids acyl chains increases. Figures (6, 7, 8 and 9) represent FT-IR absorption spectra of control sample (I), exposed sample (I), control sample (II) and exposed sample (II) respectively.

Differential scanning calorimetry (DSC) results

Differential scanning calorimetry (DSC) was used to measure the phase transition temperature; enthalpy and entropy change of the prepared liposomes and detect any change after exposure to continuous wave laser diode 650 nm 100 cm^{-2} for 9 min.

DSC results of control sample (I) and sample (I) of liposomes

DSC scan of control sample (I) is shown in Figure 10. The EPC liposomes of control sample (I) has a pre- transition temperature at 31.1°C (304.25 K) and an enthalpy change per gram (ΔH) equal -70.90 J/g with an entropy change (ΔS) equal 0.23 (J/g.K) and main phase transition temperature at 53.44°C (326.59 K) with an entropy change (ΔS) equal 0.29 (J/g.K).

Figure 11 represents the DSC thermogram of sample (I) of liposomes after exposure to laser beam. The thermogram shows that, the pre- transition temperature has been shifted from 31.1°C (304.25 K) to 31.01°C (304.16 K) with an enthalpy change per gram (ΔH) equal to –166.22 J/g and an entropy change (ΔS) equal 0.55 J/gm.K and broadening increases and the main phase temperature was shifted into lower temperature from 53.44°C (326.59 K) to 41.69 °C (314.84 K) with an entropy change (ΔS) equal 1.86 (J/gm.K).

DSC results of control sample (II) and sample (II) of liposomes

Figure 12 shows the DSC thermogram of control sample (II) with pre-transition temperature at 25.34°C (298.49 K) with an enthalpy change per gram (ΔH) equal –48.12 J/gm and an entropy change (ΔS) equal 0.16 J/g.K and main phase transition temperature at 51.5°C (324.65 K) with an entropy change (ΔS) equal 0.33 (J/g.K).

Figure 13 shows the DSC thermogram of sample (II) of liposomes after exposure to laser beam. The main phase transition temperature is broadened and shifted to lower temperature at 49.24°C (322.39 K) and an enthalpy change per gram (ΔH) equal –507.68 J/gm and an entropy change (ΔS) equal 1.57 J/g.K while the pre-transition temperature has disappeared.

Drug release studies

The data obtained from drug release studies were used to evaluate the liposomes prepared for drug delivery before and after exposure to 100 Jcm^{-2} continuous wave laser diode 650 nm are shown in Figure 14. The figure also includes, for comparison, the drug release profile obtained for unirradiated samples (I) and (II) compared to irradiated samples (I) and (II). Approximately 62% of the drug is released during the first 90 min for irradiated sample (I) which is less evident in the case of irradiated sample (II), control (unirradiated) sample (I) and control (unirradiated) sample (II). Looking at the drug release and judging by the percent drug released within first 90 minutes for different samples, 61.67 ± 3.1%, 43.9 ± 2.2%, 13.93 ± 0.7% and 11.96 ± 0.6% were released for irradiated sample (I), irradiated samples (II), control sample (II) and control sample (I) respectively.

DISCUSSION

Spectrophotometric analysis, FT-IR and DSC are very useful tools for detecting chemical changes, chemical structures and the thermodynamic of phase transitions and conformational changes in biological systems.

Absorption spectral and FT-IR of HYP

Absorption spectrum of hypericin before exposure to

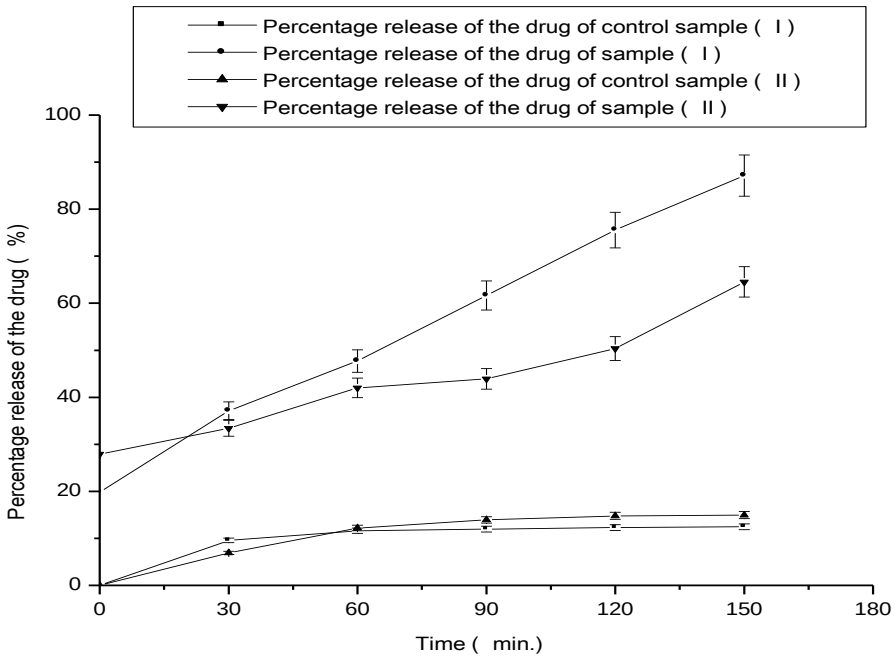

Figure 14. Represents the percentage release of the drug from control sample (I), sample (I), control sample (II) and sample (II).

laser beam has two peaks centered on 558.00 and 595.00 nm respectively as shown in Figure 2. These peaks were shifted into longer wave length (bathochromic shift) at 583.50 and 637.00 nm as shown in Figure 3 with maxima inversion due to aggregate formation after exposure to laser indicating that, heterocyclic structure of hypericin was broken down as expected producing many free radicals. Another evidence of destruction of hypericin molecule after exposure to laser arises from FT-IR results. On the basis of the FT-IR spectra between 2000 and 4000 cm^{-1} where OH-group region of hypericin has two peaks at 3434.6 and 3465.46 cm^{-1} which corresponds to OH-stretching vibration before exposure to laser Figure 4. These peaks were shifter to longer wave number after exposure to laser beam as shown in Figure 5.

Accordingly, the maximum at 3398.92 cm^{-1} was attributed to O-H stretching vibration hypericin in an effective H-bonded structure (network structure) whereas the high wave number shoulders at 3409.53, 3419.17, 3433.64, 3465.46, 3477.03, 3485.70, 3493.42, 3510.77, 3521.38 and 3541.63 cm^{-1} were assigned to free OH radical (disturber hydrogen bonds) (Hans, 2007). These free OH result from destruction of hydrogen bonds of hypericin molecules.

Control samples (I) and irradiated sample (I) of liposomes

Our data provide some insight into the physical and chemical mechanism of liposome membrane stabilized and hence its ability as a drug carrier.

FT-IR analyses were accomplished on C-H, C=O and choline group (N-$(CH_3)_3$) of phospholipids of liposomes and on OH-group and benzene group of hypericin. For OH-group, control sample has broad peak at 3465.46 cm^{-1} and lower wave number shoulder at 3434.60 cm^{-1} Figure 6. These peaks were shifted to longer wave number for sample (I) after exposure to laser Figure 7. These changes indicate that broken down of intra and intermolecular hydrogen bonds between hypericin and hypericin-phospholipids respectively.

The CH_2-stretching (symmetric and antisymmetric) modes at 2841 and 2922 cm^{-1} respectively of control sample (I) Figure 6 have increased after exposure to laser in sample (I) at 2874 and 2955 cm^{-1} respectively Figure 7. These shifts toward higher frequencies indicate on increasing proportion of gauche to trans conformers, consequently, the mobility of the lipid acyl chains would be increased (Constanca and Dirk, 2006).

The C=O band of control sample (I) with narrow band at 1735 was shifted up field (longer wave number) to 1745 cm^{-1} for sample (I) after exposure to laser. This shift is attributed to broken down of hydrogen bonds between C=O of acyl chains of liposomes and OH- of hypericin. Another effect on liposome membrane stabilization, is the interaction of choline group (N-$(CH_3)_3$-) of liposomes with hypericin. Choline group of control sample (I) has asymmetric stretching vibration peak on 1025 cm^{-1} which is higher than of standard value of pure lipid (at 970 cm^{-1}) due to hydrogen bonding between choline group and

hypericin. This peak was shifted down field for sample (I) and liposomes at 863 cm^{-1} due to breaking down this hydrogen bond.

All these changes in FT-IR results between control (I) and sample (I) result in increasing permeability of liposomes to the encapsulated oxytetracyclin drug.

To confirm FT-IR results, DSC were taken for both samples. Figures 10 and 11 show that, the pre-transition temperature control sample (I) was shifted from 31.1 °C (304.25 K) to 31.01°C (304.16 K) after exposure to laser while the entropy change was increased from 0.23 to 0.55 J/g.K indicating that the membrane became more disordered, hence, acyl chains mobility increased. Consequently, membrane permeability to oxytetracyclin drug increased after exposure to laser. These results were confirmed from the decrease in main phase transition temperature from 53.44°C (326.59 K) of control sample (I) to 51.69°C (314.84 K) and increase in entropy change from 0.29 to 1.86 (J/g.K) for sample (I) after laser irradiation and the broadening of the pre-transition temperature. Comparison between control sample (I) and sample (I) in drug releasing is illustrated in Figure 14.

In this figure, percentage of the oxytetracyclin drug released after 150 min. for sample (I) was higher than that is released from control sample (I).

FT-IR, DSC and drug releasing studies of control sample (I) and sample (I) indicate that, hydrogen bonds between hypericin and phospholipids functional groups of liposomes were broken down after exposure to laser beam which result in more space between acyl chains, more disorder in liposome acyl chains and so more drug releasing.

Control sample (II) and (II) of liposomes

Both control sample (II) and sample (II) have incorporated alpha-tocopherol as an antioxidant added to protect encapsulated oxytetracyclin drug from being damaged by free radicals released after exposure to laser by scavenger these radicals. This is observed in our results. By comparing FT-IR spectrum of both control sample (II) and sample (II) Figures 8 and 9 respectively, we found that the stretching vibrations of OH-group remained unchanged at 3434.6 cm^{-1}. This means that, intramolecular hydrogen bond of hypericin not affected by laser and hence no free OH radicals are released or may be captured by alpha-tocopheral forming alpha-tocopheral-hypericin complex. But on the other hand, CH_2- symmetric and antisymmetric vibrations were increased from 2850 and 2930 cm^{-1} respectively for control sample (II) to 2864 cm^{-1} and 2945 cm^{-1} for sample (II) which result in more gauche to transconformers. That is more acyl chains mobility and hence more entropy changes. These results are confirmed by DSC thermograms of both samples as in Figures 12 and 13 respectively. From these figures, sample (II) has pre-transition temperature at 25.34°C (298.49 K) with entropy change equal 0.16 J/g.K. This pre-transition temperature disappeared for sample (II) after laser irradiation indicating that lipid acyl chains became more disordered, also the main phase transition temperature was decreased from 51.5°C (324.65 K) with an entropy change equal 0.33 (J/g.K) for control sample (II) to 49.24°C (322.39 K) with an entropy change equal 1.57 J/g.K for sample (II) after irradiation which result in more gauche conformers, consequently, the lipid acyl chains disorder increased. Hence sample (II) is more permeable to oxytetracyclin drug as shown in Figure 14. In this figure sample (II) has higher percentage of oxytetracyclin drug released after 150 min. than control sample (II).

Another evidence is the interaction between choline group (N-$(CH_3)_3$-) antisymmetric stretching vibration of head group of phospholipids and hypericin OH-group. Choline group has antisymmeteric stretching vibration peak at 1015 cm^{-1} for control sample (II). This peak was shifted downfield (lower wave number) at 948.5 cm^{-1} for sample (II) after exposure to laser beam indication that hydrogen bonds between hypericin and choline group were broken down. This decrease in wave number of choline group between control sample (II) and sample (II) (equal to 66.5 cm^{-1}) was smaller than the decrease for the same peak between control sample (II) and sample (II) (equal to 162 cm^{-1}) indicating that alpha-tocopherol capture the free OH radical of hypericin and/or forms hydrogen bonds with choline group of phospholipids of liposomes so increasing its stability and stability of the encapsulated drug.

Also, there are an increase in symmetric stretching vibration of C=O group from 1735 cm^{-1} of control sample (II) to 1742 cm^{-1} for sample (II). This change to up field is attributed is attributed to the broken down of intermolecular hydrogen bond between C=O of liposomes acyl chains and OH of hypericin. But this increase in C=O wave number was lower than the increase in case of control sample (I) and sample (I). This is due to alpha-tocopherol shifts the frequency of C=O to lower values due to interactions between phenolic-OH of alpha tocopherol and polar group of phospholipids of liposomes (Villalain et al., 1986; Lefevre and Picquart, 1996). This effect causes sample (I) of liposomes more permeable to oxytetracyclin drug than sample (II). This due to alpha-tocopherol stabilize the membrane of liposomes from destruction by free radicals released from hypericin by forming complex with these radicals and/or forming complex with phospholipids membrane. These results are confirmed by comparing DSC thermograms of both samples.

With respect to DSC thermograms of sample (I) and sample (II) Figures 11 and 13 we found that, there is a significant change in phase transition temperature between sample (I) and sample (II) since sample (I) has lower main phase transition temperature than sample (II) but has higher entropy change program (1.86 J/g.K)

than sample (II) (1.57 J/g.K) which is in a good agreement with FT-IR results. On the other hand, control sample (II) has higher percentage of drug releasing than control sample (I) as shown in Figure 14, this is because alpha-tocopherol intercalated between EPC molecules may take them away, in particular at glycerol backbone level increasing their molecular area, and/or changing the conformation in the interfacial region. The distance between acyl chains may then be enhanced, decreasing van der Waals interactions, promoting gauche rotamer formation (Lefevre T. and Picquart M., 1996). This can be an explanation of the increase in entropy change per gram of control sample (II) (0.33 J/g.K) than that of control sample (I) (0.29 J/g.K) at main phase transition temperature for both samples.

Conclusion

From spectroscopic and DSC studies of in the *in vitro* release of hydrophilic drugs, liposomes encapsulated drugs and incorporated with photoreceptor with an antioxidant could be used as a successful and efficienttool as a carrier and photochemical targeting of the drug using an appropriate monochromatic light.

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