# INTERACTION BETWEEN EPI-GALLO-CATECHIN POLYPHENOL EXTRACTED FROM GREEN TEA AND LIPOSOMES AS A CELL MEMBRANE MODEL

*A. BAHAA**, S.M. SHERIF*, M.S. TALAAT**, A.M. SALLAM***

* Biophysics and Laser Science Unit, Research Institute of Ophthalmology, Giza, Egypt, sheri_sm@yahoo.com
** Biophysics Branch, Physics department, Faculty of Science, Ain Shams University, Cairo, Egypt

*Abstract.* Tea has been cultivated for centuries, beginning in India and China. Today, tea is the most widely consumed beverage in the world, second only to water. Hundreds of millions of people drink tea, and studies suggest that green tea (*Camellia sinensis*) in particular has many health benefits. Green tea is made from unfermented leaves and reportedly contains the highest concentration of powerful antioxidants called polyphenols. Polyphenols exhibit stronger antioxidant protection for human body than vitamin C and vitamin E. Scavenging effect of lipid free-radicals (one antioxidant property) of polyphenols in green tea extracts can be clearly observed in experiments. Many researches addressed polyphenols from the perspective of its antioxidant and anticarcinogenic effect. Despite that many researches have been carried out using different physical techniques, the exact mechanism of interaction between polyphenols' molecules and membrane lipids is not yet revealed. The aim of work is to investigate, *in vitro*, the mechanism of interaction between epi-gallo-catechin polyphenols molecules and cell membranes represented by liposomes. The approach of our work is based on 2 phases: first is testing the encapsulation efficiency of liposomes to polyphenols. Second, investigating the structural and phase transition temperature changes for lipid bilayer using FTIR and DSC.

*Key words:* Polyphenols, phospholipids, liposomes, encapsulation efficiency, green tea, epi-gallo-catechin, calorimetry, DSC, FTIR.

## INTRODUCTION

There are three main varieties of tea: green, black, and oolong. The difference is in how the teas are processed. Green tea is made from unfermented leaves and reportedly contains the highest concentration of powerful antioxidants called polyphenols. Antioxidants are substances that fight free radicals – damaging compounds in the body that change cells, damage DNA, and even cause cell death. Many scientists believe that free radicals contribute to the aging process as well as

Received: April 2014;
in final form May 2014.

ROMANIAN J. BIOPHYS., Vol. 24, No. 2, P. 89–99, BUCHAREST, 2014

to the development of a number of health problems, including cancer and heart disease. Antioxidants such as polyphenols in green tea can neutralize free radicals and may reduce or even help prevent some of the damage they cause [5].

Green tea has been extensively studied in people, animals, and laboratory experiments. Results from these studies suggest that green tea may help treat many health conditions like atherosclerosis, high cholesterol, cancer, bladder cancer, breast cancer, lung cancer and pancreatic cancer [1, 9]. Many researches addressed polyphenols from the perspective of its antioxidant and anticarcinogenic effect. The anticarcinogenic property makes the green tea extracts a hotspot in recent scientific researches. In many experiments, green tea extracts show inhibitory effects on cancer cells. *In vitro* assays, catechin and caffeine, which are main components in green tea extracts, block the cell cycle of cancer cells (cytotoxicity) and induce programmed cell death; *in vivo*, green tea extracts also inhibit prostatic carcinoma transplanted in nude mice [3].

## MATERIALS AND METHODS

### MATERIALS

Commercially available green tea polyphenol, namely epi-gallo-catechin (EGC) was obtained from SIGMA-ALDRICH through its agent, the Egyptian International Center for Import, Egypt, with HPLC purity more than 98%. L-alpha-lecithin dipalmitoyl (DPPC) serving as the main constituent of the lipid bilayer and tris-buffer (buffer solution for the preparation of the lipid bilayer) was obtained from Cornell Lab for fine chemicals and lab equipment, Egypt. Chloroform and ethanol were obtained from Research Institute of Ophthalmology, Egypt.

### STOCK SOLUTION PREPARATION

EGC polyphenol was dissolved in an appropriate amount of ethanol to prepare a stock solution of 2 mM concentration. Different volumes of 10 μL, 30 μL, and 50 μL were then added to a 10 mg of DPPC and 400 μL of chloroform respectively. Sudden dispense approach was followed using Hamilton syringe of capacity 250 μL. Each preparation was injected in a separate and clean test tube containing tris-buffer and then placed in a water bath sonicator at 50 RPM for 1 hour. Irritation with nitrogen was necessary to avoid oxidation.

### DETERMINATION OF MAXIMUM WAVELENGTH FOR THE DIFFERENT POLYPHENOLS

Using a spectrophotometer to investigate resonance peak and optical density, a volume of 20 μL from the stock was added to a 3 mL of methanol in a 4 mL capacity spectrophotometer cuvette. The cuvette was then placed in the device and

allowed to scan for $\lambda_{max}$ along the electromagnetic spectrum within the range from visible light to ultra violet.

### DETERMINATION OF POLYPHENOL'S OPTICAL DENSITIES

Series of volume stock solution of 20, 40, 60, 80 and 100 μL for the epi-gallo-catechin polyphenol were added separately to 3 mL of methanol in the spectrophotometer's cuvette and the optical density for each concentration was determined and then attributed to its concentration.

### LIPOSOMES PREPARATION

Seeking for high encapsulation efficiency, reverse phase evaporation (REV) was the best way of preparation followed. REV was introduced by Szoka and Papahadjopolous in 1978 [8]. This procedure involves direct addition of aqueous media to three-to-six folder larger volumes of the phospholipids in organic solvent. First, phospholipids are dissolved in organic solvent and then added under control to an aqueous medium with vigorous agitation. Then the organic solvent is removed under reduced pressure. L-alpha-lecithin dipalmitoyl (DPPC), trizma buffer and chloroform were used for liposomal preparation. EGC with concentration of 2mM were included during the preparations using three different volumes of 10, 30, and 50 μL using Hamilton syringe. Samples were then centrifuged at 55 RPM in a bench centrifuge for one hour and the supernatant was used to determine the amount of non-encapsulated polyphenol. UV-visible spectrometer (Shimadzu, UV-240A, Japan) was used to measure the absorbance of these samples at 280 nm.

### STATISTICS AND DATA ANALYSIS

All values are expressed as mean ± SD. Three different concentrations from EGC polyphenol were involved and duplicate aliquots used for the FTIR and DSC characterization. The spectral analysis was performed with OriginPro8 software package (Origin Lab Corporation, Northampton, MA, USA).

## RESULTS

### ENCAPSULATION EFFICIENCY

Although the encapsulation efficiency was determined from the supernatant, the results reflect the amount of EGC polyphenol trapped within the multilamellar liposomes. Interestingly, encapsulation efficiency showed high values for the EGC polyphenol using the reversed phase evaporation method.

FOURIER-TRANSFORM INFRARED ANALYSIS (FT-IR)

**CH stretching region**

CH stretching region FTIR provided significant insight into the location of polyphenols with respect to DPPC molecules in the vesicles.

*Table 1*

The liposomal polyphenol encapsulation efficiency, $CH_2$ symmetric stretching band analysis

| | Encapsulation efficiency (%) | $v$ ($cm^{-1}$)<br>$BW$ ($cm^{-1}$) | | | |
|---|---|---|---|---|---|
| Control DPPC | – | 2850.6±0.3<br>10.61±2.2 | 2875.7±1<br>23.60±3 | 2919.4±1<br>23.85±3 | 2957.8±1<br>18.32±5 |
| DPPC-EGC (10 µL) | 89±4 | 2850.3±0.3<br>†8.41±2.2 | †2858.3±4<br>†16.21±3 | †2920.1±1.3<br>†20.45±3.4 | †2958.7±1.2<br>†19.68±1 |
| DPPC-EGC (30 µL) | 99±8 | 2850.8±0.2<br>†9.33±1.3 | †2860.6±3<br>†18.64±3 | 2920.7±1.3<br>†22.80±1.05 | 2958.2±1<br>†23.39±4 |
| DPPC-EGC (50 µL) | 98±2 | 2850.8±0.2<br>10.11±1.5 | †2867.6±2<br>†24.01±1 | †2920.1±1.3<br>†21.22±2.23 | 2958.3±1<br>†20.47±2 |

†Statistically significant
First line in each cell indicates the vibrational frequency, while second line reflects the bandwidth.

The $CH_2$ stretching region (3000–2800 $cm^{-1}$) for control liposomal preparation, without polyphenol, is shown in Figure 1. Four bands are discernible near 2850, 2920, and 2958 $cm^{-1}$ corresponding to the $CH_2$ symmetric stretch, $CH_2$ asymmetric stretch, and the terminal methyl $CH_3$ asymmetric stretching band, respectively [4].

As shown in Figure 1, an additional band appeared at 2875 $cm^{-1}$, and it was assigned as the $CH_3$ symmetric stretching band of the DPPC. The $CH_2$ symmetric stretching band near 2850 $cm^{-1}$ is of special significance because of sensitivity to changes in the mobility and conformational disorder of the hydrocarbon chains. The frequency of this band has not been significantly affected compared to control DPPC liposomes (Table 1) and it was associated with substantial changes in bandwidth, with the incorporation of EGC polyphenol.

Incorporating EGC polyphenol to control DPPC liposomes appeared to mobilize the $CH_3$ symmetric stretching band of the DPPC as indicated by the changes in the wave number and bandwidth of this band.

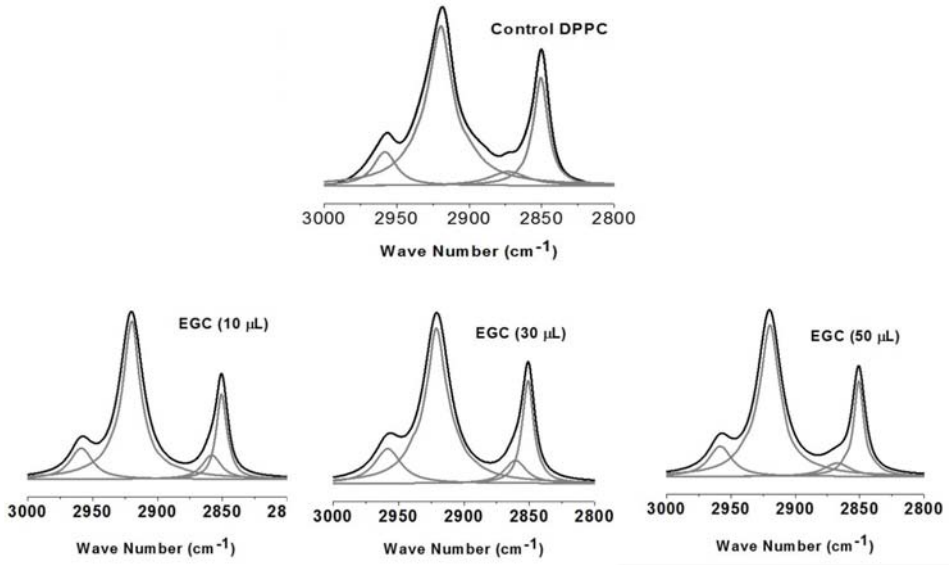


Fig. 1. FTIR spectra for the $CH_2$ stretching region for control liposomal preparation, without polyphenols.

**Interface Region**

The carbonyl band near 1740 $cm^{-1}$ reports on the structure of the bilayer interfacial region. As shown in Table 2, the frequency of this band was centered at 1735 $cm^{-1}$ for control DPPC liposomes and at 1747 $cm^{-1}$ when EGC (30 µL) were included. The carbonyl BW was 5.47 $cm^{-1}$ for control DPPC liposomes and appreciably increased to 10.87 $cm^{-1}$ when EGC was incorporated.

Several structurally interesting features were also apparent in the C=O stretching region of the IR spectra presented in Figure 2. Using a combination of Fourier deconvolution and nonlinear curve fitting, the contour of the carbonyl band of the different preparations was found to be a composite of eleven components with maxima ranging from 1700 to 1793. The estimated number of peaks suggests a heterogeneous structure of liposomes when EGC polyphenol was added (i.e. the carbonyl group is highly affected by the addition of polyphenol DPPC liposomes).

*Table 2*

Analysis of the carbonyl-stretching region of the FTIR spectra of the liposomal preparations

| $\nu$ ($cm^{-1}$)<br>$BW$ ($cm^{-1}$) | **Control DPPC** | **DPPC-EGC (10 µL)** | **DPPC-EGC (30 µL)** | **DPPC-EGC (50 µL)** |
|---|---|---|---|---|
| | 1793.56±0.05<br>4.22±0.11 | 1793.38±0.08<br>†5.19±0.16 | †1700.57±0.15<br>†6.40±0.36 | †1794.55±0.49<br>†17.61±1.68 |
| | †1782.15±0.18<br>†6.96±0.3947 | †1782.51±0.22<br>†6.76±0.61 | †1708.99±0.34<br>4.73±0.82 | †1781.20±0.35<br>†5±0.80 |

*Table 2 (continued)*

| | | | | |
|---|---|---|---|---|
| | †1773.30±0.06<br>†5.27±0.13 | †1772.86±0.08<br>†5.57±0.20 | †1718.66±0.80<br>†7.09±0.72 | †1772.80±0.15<br>†6.27±0.436 |
| | †1761.21±0.25<br>†6.70±0.38 | †1761.09±0.27<br>†7.57±0.63 | †1727.02±1.35<br>†7.86±2.81 | †1760.56±0.91<br>†9.18±1.708 |
| | †1749.61±0.37<br>†8.75±0.62 | †1750.04±0.30<br>†7.11±0.61 | †1734.95±0.49<br>†7.46±2.44 | †1749.07±0.94<br>†8.31±1.73 |
| | †1741.39±0.15<br>†6.59±0.56 | †1741.46±0.21<br>†6.549±0.84 | †1741.44±0.97<br>†6.21±1.49 | †1741.24±0.51<br>†6.77±1.709 |
| | †1735.05±0.14<br>†5.471±0.33 | †1734.73±0.17<br>†5.31±0.45 | †1747.64±1.30<br>†10.87±2.94 | †1734.74±0.46<br>†6.10±1.150 |
| | †1727.45±0.32<br>†10.04±1.22 | †1727.05±0.50<br>†9.51±2.00 | †1760.62±1.87<br>†9.80±2.37 | †1727.32±1.07<br>†9.14±2.73 |
| | †1718.38±0.08<br>†5.11±0.18 | †1718.07±0.12<br>†5.13±0.20 | †1772.99±0.19<br>†6.66±0.55 | †1718.08±0.32<br>†5.98±0.33 |
| | †1707.90±0.12<br>†2.95±0.27 | †1707.27±0.20<br>†3.42±0.42 | †1781.53±0.35<br>†5.42±0.71 | †1707.70±0.33<br>†3.80±0.73 |
| | †1701.43±0.05<br>†3.89±0.1241 | †1701.12±0.06<br>†4.07±0.14 | †1793.88±0.29<br>†12.83±0.71 | †1700.85±0.11<br>†4.75±0.24 |

† Statistically significant

As shown in Table 2, the mean band was centered at 1735 $cm^{-1}$ with BW of 5.471 $cm^{-1}$. The BW was increased to 10.87$cm^{-1}$ when EGC was added in amounts of 30 µL.

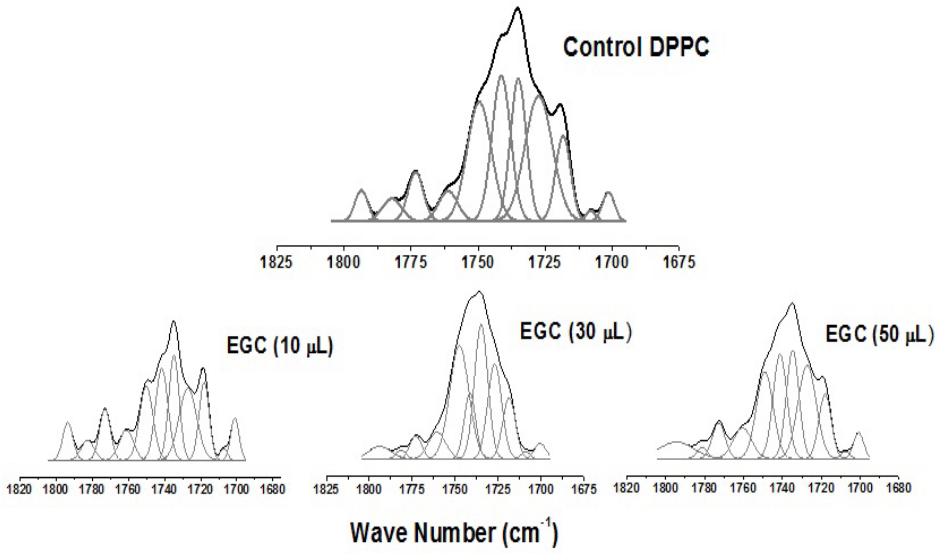


Fig. 2. FTIR spectra of the carbonyl stretching region showing the experimental (dots) and the calculated band after resolution enhancement (solid line). Estimated components are shown under the peaks.

**Fingerprint Region**

Table 3 shows the vibrational frequencies of fingerprint region (1700 – 900 $cm^{-1}$) where distinguished changes can be observed. The band near 1237 $cm^{-1}$ represents the $CH_2$ bending (wagging) vibration. Noticable increase in this band's frequency is observed when EGC polyphenol was added.

Figure 3 illustrates the fingerprint region for control liposomes and different liposomal preparations with polyphenol inside. Eight structural estimated components can be observed. An intersting observation is the appearance of an additional band ($9^{th}$) at 1058.11 and 1056.80 $cm^{-1}$ corresponding to EGC (10 μL) and EGC (50 μL) respectively.

Another observation is the drastic variation in the band width for different liposomal preparations incorporated with polyphenols when compared to control liposomes. As for example the BW was decreased from 285 $cm^{-1}$ to 20 $cm^{-1}$ at frequency of 1231 $cm^{-1}$ when CAT with concentration 10 μL was added compared to control liposome.

*Table 3*

Estimated structural components and their vibrational frequencies of fingerprint region

| $\nu$ ($cm^{-1}$) $BW$ ($cm^{-1}$) | **Control DPPC** | **DPPC-EGC (10μL)** | **DPPC-EGC (30μL)** | **DPPC-EGC (50μL)** |
|---|---|---|---|---|
| | †1522.19±2.63<br>†94.13±5.36 | †1465.13±0.38<br>†22.81±0.91 | †1472.33±0.10<br>†32.27±0.22 | †1467.39±0.09<br>†22.88±0.19 |
| | †1468.96±0.09<br>†27.08±0.27 | †1428.19±0.67<br>†17.38±3.51 | †1421.17±0.73<br>†16.64±1.63 | †1422.24±0.65<br>†22.36±1.35 |
| | †1400.91±1.61<br>†60.77±5.075 | †1387.22±8.61<br>†30.86±26.18 | †1373.79±1.19<br>†46.08±3.12 | †1376.91±0.59<br>†34.14±2.038 |
| | †1231.48±8.34<br>†285.117±17.65 | †1397.12±7.43<br>†110.07±14.55 | †1280.59±7.55<br>†62.37±9.15 | †1338.70±0.91<br>†20.16±2.097 |
| | †1237.75±0.11<br>†52.54±0.38 | †1283.89±2.87<br>†40.57±4.094 | †1235.66±0.68<br>†43.47±1.13 | †1282.75±3.52<br>†50.88±4.27 |
| | †1172.03±0.22<br>†35.15±0.62 | †1236.96±1.14<br>†44.67±2.54 | †1175.38±0.35<br>†39.41±0.64 | †1236.01±0.97<br>†44.19±1.28 |
| | †1093.09±0.26<br>†39.22±0.54 | †1171.01±0.87<br>†46.68±2.40 | †1092.77±0.20<br>†36.90±0.33 | †1173.33±0.38<br>†41.88±0.71 |
| | †1056.47±0.33<br>†30.27±0.37 | †1095.44±1.63<br>†41.14±1.99 | 1057.21±0.24<br>†25.78±0.33 | †1093.23±0.27<br>†38.64±0.42 |
| | – | †1058.11±2.05<br>†38.03±1.63 | – | †1056.80±0.32<br>†28.66±0.37 |

† Statistically significant

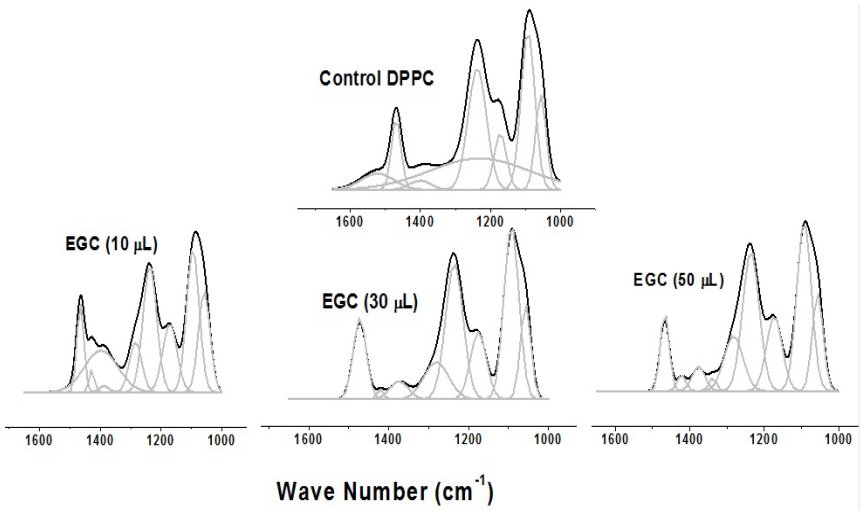


Fig. 3. FTIR spectra of the fingerprint region showing both calculated and resolved peaks.

## DIFFERENTIAL SCANNING CALORIMETRY

DSC was used to investigate the effect of polyphenol on changing the phase transition temperature of liposomes [2]. Liposomal preparations were heated from room temperature to 200 $^{o}$C using DSC calorimeter at the Egyptian National Institute for Standardization. Figure 4 shows the peak analysis of control liposome and different liposomal preparations with polyphenols incorporated inside. For control liposomes, two distinguished peaks can be seen at temperatures 34.7$^{o}$C and 44.17$^{o}$C as an indication of a pre-transition and main transition phases, respectively.

*Table 4*

Endothermic peak analysis for control DPPC and three different concentrations of incorporated EGC

| Center of peak ($^{O}$C)<br>*BW* (cm$^{-1}$) | **Control DPPC** | **DPPC-EGC (10 µL)** | **DPPC-EGC (30 µL)** | **DPPC-EGC (50 µL)** |
|---|---|---|---|---|
| | 34.7±1<br>13.8±3 | 31.25±1<br>†7.2±3 | †37.09±2<br>†19.72±2 | 33.35±0.4<br>†11.55±1 |
| | 41.3±3<br>5.02±2 | †36.34±2<br>†2.83±2 | †54.6±4<br>†15.05±3 | †50.61±3<br>†16.46±4 |
| | – | – | †65.77±3<br>†10.29±3 | †69.43±3<br>†18.70±3 |
| | – | – | †72.02±3<br>†5.76±3 | †85.05±3<br>†13.26±3 |
| | – | – | †74.98±3<br>†2.06±2 | †93.76±4<br>†8.34±2 |
| | – | – | – | †98.44±4<br>†3.30±1 |

† Statistically significant

The effect of different EGC preparations on the thermal characteristics of empty DPPC are shown in Figure 4. Heating thermograms for both empty DPPC, 9 (Figure 4A) showed two main endothermic peaks at 33.14±1 and 42±3 $^{o}$C, respectively. Two component peaks were observed for EGC of concentration 10 µL located at 31.25±1 and 36.34±2$^{o}$C. Additional three partially superimposed components were observed for DPPC liposomes incorporated with 30 µL EGC. By increasing the amount of EGC to 50 µL, fourth component peak appeared at 98.44$^{o}$C. The incorporation of EGC with concentration reduced both the pre-transition and main transition temperatures to 31.25±1 and 36.34±2$^{o}$C respectively. Incorporating EGC with higher amounts (30 µL and 50 µL) resulted in extremely different thermal behavior where the main transition temperature (42.07$^{o}$C) is significantly increased and narrowed when compared to empty DPPC. When the concentration of EGC incorporated is increased, the endothermic pattern was broadened and gave narrow extra resolved peaks for higher range from 74.98 $^{o}$C to 98.44 $^{o}$C and broader peaks for lower range around 69.43 $^{o}$C

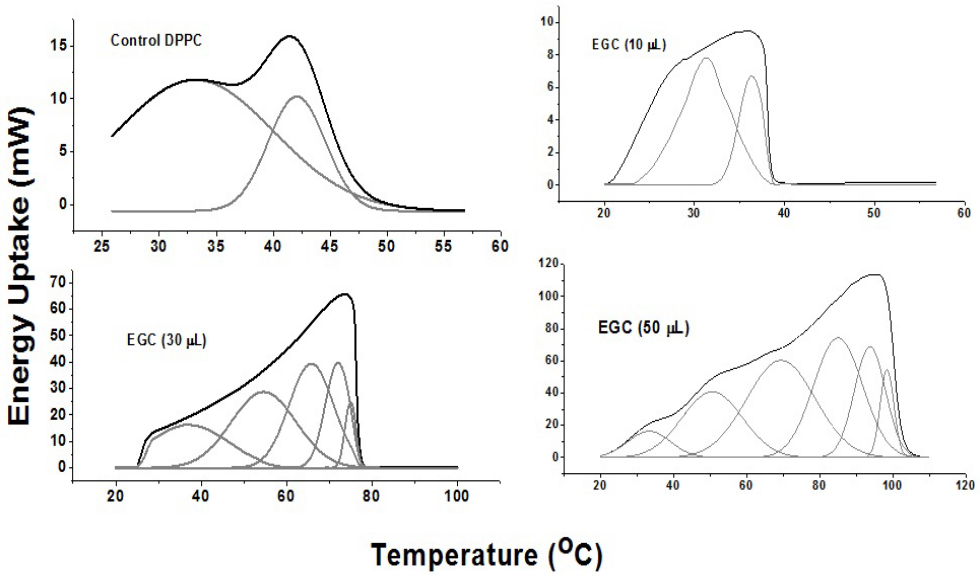


Fig. 4. DSC thermograms for control liposome and liposomal preparations incorporated with different concentrations of EGC.

## DISCUSSION

The plant *Camellia sinensis* yields both green and black tea where the Green tea is produced by lightly steaming the fresh cut leaf. Green tea is very high in polyphenols with potent antioxidant and anti-cancer properties. The chemical composition of green tea varies with climate, season, horticultural practices and

age of the leaf where the major components of interest are the polyphenols. The major polyphenols in green tea are flavonoids (e.g., catechin, epi-catechin, epi-catechin-gallate, epi-gallo-catechin-gallate and proanthocyanidins) [6].

The lipids, which are in the liquid-crystal state, trap aqueous solutes in aqueous compartments between a series of lipid bilayers forming spherules called liposomes, which exhibit many of the properties of natural membrane-bounded structures. Thus liposomes constitute a valuable model system with which to study the properties of biological membranes that may be dependent on their lipid components [7].

Since our study is focusing on the interaction between liposomes as cell-membrane model and EGC, it was necessary to follow experimental method to disperse single-layered liposomes. The reversed phase evaporation (REV) is considered to be the most experimentally preferable method for the preparation of single-layered liposomes if high solute entrapment is desirable.

The results showed that each concentration of EGC had an effect on both the structure and phase-transition temperature of control DPPC liposomes. Optical density investigation reflected strong insertion of EGC polyphenol when high concentrations were used. FTIR analysis for CH stretching region showed that control DPPC suffered little conformational disorder represented by changes in wave number and band width in the range 8–18 $cm^{-1}$, especially for $CH_3$ symmetric stretching band (Table 1) when different concentrations of EGC were incorporated.

Non-linear curve fitting and peak analysis of carbonyl band in the FTIR spectrum for control DPPC liposomes and EGC-containing liposomes showed eleven estimated components with maxima ranging from 1700 to 1793 $cm^{-1}$. Remarkable variations are noticed in both the frequency and band width of estimated components for EGC-containing liposomes (Table 2) when compared to control DPPC liposomes. The estimated number of peaks reflects a heterogeneous structure of DPPC incorporated with EGC. This heterogeneity suggests the existence of heterogeneous microdomains and reflects the changes in the hydrogen-bonding interactions in the interfacial region.

The band near 1468 $cm^{-1}$ (known as scissoring band) represents changes in lipid hydrocarbons. The band width of this band was broader in the control DPPC liposomes which indicate more mobility of the hydrocarbon chains and less lateral interactions. Adding EGC resulted in narrowing the band width of this band as a clear indication on the reduced mobility of the hydrocarbon chains and increased lateral interactions. As evidence on the existence of lipid microdomains is the increase in the number of associated bands, frequencies and band widths of the fingerprint region (Table 3). Two additional bands appeared at frequencies 1058 and 1056 $cm^{-1}$ respectively which are not seen for control DPPC liposomes.

DSC calorimetry was used to investigate the changes in pre-transition and main-transition temperatures of control DPPC when EGC is incorporated with different concentrations. Although pre-transition temperature (33.14$^{o}$C) of control DPPC liposomes was not highly affected by incorporating EGC (Table 4), main transition temperature reached elevated values (above 90$^{o}$C) in case of high

concentrations of EGC. The DSC results support the assumption of lipid microdomains formation as the thermograms for EGC-containing liposomes, with high concentrations, showed four additional endothermic phase transitions when compared to control liposomes. The drastic changes in the main transition temperature suggests that incorporated EGC could have reside on the interface region within the hydrophobic core of the lipid-bilayer, thus giving the liposomes the ability to absorb more energy and become more resilient to phase transition (i.e. phase transition occurs at higher temperature).

## CONCLUSION

Different concentrations of EGC polyphenol affect the physical stability and phase transition temperatures of DPPC liposomes with different levels. High concentrations of EGC resulted in conformational disorder to DPPC liposomes represented by less mobility and increased lateral interactions of hydrocarbon chains of the fingerprint region and lipid microdomains appear to form as reported by FTIR and DSC. Results reflect that high concentrations of EGC have more effect on the physical state of the membrane model. Further studies and experiments are needed to investigate the effect of other polyphenols and/or the combination between them on membrane models.

## REFERENCES

1. ARTS, I.C., P.C. HOLLMAN, Polyphenols and disease risk in epidemiologic studies, *American Journal Clinical Nutrition*, 2005, **81**(1 Suppl), 317S–325S.
2. DEMETZOS, C., Differential scanning calorimetry (DSC): a tool to study the thermal behaviour of lipid bilayers and liposomal stability, *J. Liposome Res*., 2008, **18**(3), 159–173.
3. KUMAZAWA, S., K. KAJIYA, A. NAITO, H. SAITO, S. TUZI, M. TANIO, M. SUZUKI, F. NANJO, E. SUZUKI, T. NAKAYAMA, Direct evidence of interaction of a green tea polyphenol, epi-gallocatechin-gallate, with lipid bilayers by solid-state nuclear magnetic resonance, *Biosci. Biotechnol. Biochem*., 2004, **68**(8), 1743–1747.
4. MAHMOUD, S.S., J.D. GEHMAN, K. AZZOPARDI, R.M. ROBINS-BROWNE, F. SEPAROVIC, Liposomal phospholipid preparations of chloramphenicol for ophthalmic applications, *J. Pharm. Sci.*, 2008, **97**(7), 2691–2701.
5. NAGAO, T., T. HASE, I. TOKIMITSU, A green tea extract high in catechins reduces body fat and cardiovascular risks in humans, *Obesity* (Silver Spring), 2007, **15**(6), 1473–1483.
6. SCALBERT, A., I.T. JOHNSON, M. SALTMARSH, Polyphenols: antioxidants and beyond, *Am. J. Clin. Nutr*., 2005, **81**(1 Suppl), 215S–217S.
7. SESSA, G., G. WEISSMANN, Phospholipid spherules (liposomes) as a model for biological membranes, *J. Lipid Res*., 1968, **9**(3), 310–318.
8. SZOKA, F.C., 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, **60**, 4194–4198.
9. WEISSIG, V., A.Z. GLENDALE, *Liposomes: Methods and Protocols*, Human Press, USA, 2010.