

Effect of a bioactive curcumin derivative on DPPC membrane: A DSC and Raman spectroscopy study[☆]

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Abstract

Interactions of dimethoxycurcumin (**1**) a lipophilic bioactive curcumin derivative with dipalmitoyl phosphatidylcholine (DPPC) were investigated. The thermodynamic changes caused by (**1**) and its location into DPPC lipid bilayers were monitored by differential scanning calorimetry and Raman spectroscopy. The results reveal that (**1**) influences the thermotropic properties of DPPC lipid membrane causing abolition of the pretransition and broadening of the phase-transition profile and slightly decreases the T_m at increasing concentrations. The Raman height intensity ratios of the peaks $I_{2935/2880}$, $I_{2844/2880}$ and $I_{1090/1130}$ are representative of the interaction of (**1**) with the alkyl chains and furnish information about the ratio between disorder and order that exists in the conformation of the alkyl chain. The intensity changes of the peak at 715 cm^{-1} indicates interaction between the choline head group and (**1**). The Raman spectroscopy results are in agreement with the thermal analysis results. Biologically active lipophilic molecules such as (**1**) should be studied in terms of their interaction with lipid bilayers prior to the development of advanced lipid carrier systems such as liposomes. The results of these studies provide information on the membrane integrity and physicochemical properties that are essential for the rational design lipidic drug delivery systems.

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1. Introduction

Curcuma longa is a ginger-like plant that grows in tropical regions. The yellowish substance, turmeric is found in the root of the plant, which has been used in Ayurvedic and Chinese medicine for centuries [1–4]. Turmeric contains bioactive substances the curcuminoids, such as curcumin, demethoxycurcumin, bisdemethoxycurcumin and dimethoxycurcumin [5]. Much attention has been given lately to curcumin as it demonstrates a plethora of biological activities such as antioxidant, cancer prevention and anti-neovascularization activities [6–8]. These biological activities are derived from the antioxidant prop-

erty of the methoxyphenol group and the action of the aryl group in β -diketone [9,10]. Dimethoxycurcumin (**1**) (Fig. 1) is a lipophilic compound found in turmeric that has been investigated recently against various cancer cells lines and exhibited higher activity than curcumin [5].

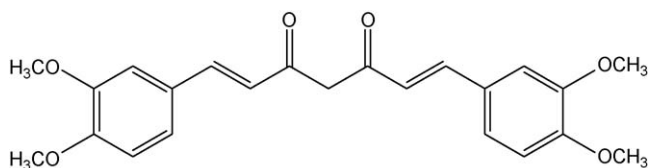
The high water-insolubility of (**1**) makes its in vivo administration a very difficult issue. Fortunately over the last years modern drug carriers, like liposomes, have been proven effective for the delivery of lipophilic molecules [11,12]. The physicochemical properties of liposomes as well as their interaction with the guest molecules is of utmost importance as they define the pharmacokinetic behavior as well as the pharmacological response of the system. Consequently, when designing such a system, the physicochemical properties of the components as well as their interaction with the additives should be thoroughly investigated.

In this study, we investigated the interaction of (**1**) with DPPC model lipid bilayers, using differential scanning calorimetry (DSC) and Raman spectroscopy. DSC measures thermal changes on the lipid bilayers that are caused by (**1**), and it is

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Fig. 1. Dimethoxycurcumin (**1**).

a diagnostic means to investigate the differential effects that result from the incorporation of the guest molecule [13]. Raman spectroscopy is used to investigate the location of the bioactive compound in the DPPC lipid bilayer. These two methods collectively provide information that should be taken into consideration for the rational design of a lipidic carrier system for (**1**).

Our aim is to design a stable liposomal carrier system for the delivery of compound (**1**) to target tissues. Taking into consideration the results from our study, the design of a system with appropriate physicochemical parameters that remain stable after *in vivo* administration is possible.

2. Materials and methods

Compound (**1**) was a gift from Professor P. Pantazis (Foundation for Biomedical Research of the Academy of Athens). DPPC was purchased from Avanti Polar Lipids. Mixtures of DPPC and (**1**) were prepared at molar ratios of 3, 5, 10, 15, 20, and 30%. Subsequently, each mixture was dissolved in pure chloroform and dried by gentle bubbling of nitrogen gas. The residual solvent was removed under vacuum overnight. HPLC grade deionized water filtered through Milipore Filters (pore size 200 nm) was added (1:2 w/w) in the dried mixtures.

2.1. Differential scanning calorimetry

Samples containing mixtures of DPPC and (**1**) were prepared in 40 μ l crucibles hermetically sealed with lid. The crucibles were left for 30 min for DPPC/H₂O equilibration. Prior to DSC scanning, the samples were subjected to quick heating and quick cooling (scanning rate of 10 and 20 °C/min, respectively) to ensure equilibration and exemption of the thermal history of the specimens. All samples were scanned three to four times until identical thermograms were obtained using a scanning rate of 2 °C/min. An empty crucible was used as a reference sample and the temperature scale of the calorimeter was calibrated using indium ($T_m = 156, 60$ °C). A Mettler Toledo DSC822^e heat-flux DSC was used and the evaluation was done by the Mettler-Toledo STAR^e software. A quantity of 4–7 mg (total weight) was used for the DSC measurements.

2.2. Raman spectroscopy

High-frequency Raman spectra were recorded with a Perkin-Elmer GX Fourier transform spectrometer. A diode pumped Nd:YAG laser at 1064 nm was used as excitation source. The spectra were obtained at 4 cm^{-1} resolution from 3500 to 500 cm^{-1} with interval 2 cm^{-1} . The laser power was constant at

400 mW during the experiments. Analysis of the spectra was carried out using GRAMS/32 data analysis software. Quantities of 10–12 mg (total weight) were used for the Raman Spectroscopy. Prior to Raman spectroscopy the hydrated mixture was left in the spectrometric cell for 16–24 h to ensure DPPC/H₂O equilibration. Experiments at all concentrations (3, 5, 10, 15, 20 and 30% molar ratio) were held at 25 °C in order to assess the change of the interaction between DPPC and (**1**) at increasing concentrations while concentrations of 3 and 10% were further investigated from 30 to 50 °C.

3. Results and discussion

3.1. Differential scanning calorimetry

The thermogram of pure DPPC (Fig. 2) shows two characteristic peaks (a): at 36.14 and 41.43 °C. The first peak presents a low enthalpy transition attributed to the mobility of the choline polar head of DPPC, while the sharp enthalpy main transition is attributed to the mobility of the alkyl chains.

Thermal analysis results are based on T_{onset} , T_m , $T_{1/2}$, $\Delta T_{1/2}$ and ΔH (Table 1). The DSC scans for the mixtures of DPPC/(**1**) are shown in Fig. 2. The presence of (**1**) in the fully hydrated DPPC lipid bilayer induces several concentration dependent effects. Pre-transition peak at temperature of 36.14 °C was abolished by the presence of low concentration (3 mol%) of (**1**). The presence of (**1**) caused a broadening of the main transition peak and appearance of an additional peak at 20 and 30% molar concentrations. This new peak, appears after the main transition peak, indicates domain formation and phase separation, and leads to the conclusion that (**1**) at such high concentration induces an heterogeneous system. It seems likely that at concentrations up to 15 mol%, the presence of (**1**) in the DPPC bilayer induces a ‘solution like’ system. This observation is based on the decrease of both the melting temperature (T_m) and ΔH of transition (Table 1). The reduction of ΔH (Table 1) is also greater at 20 and 30 mol% concentrations and this is attributed to the disorganization of the lipid bilayer caused by ‘phase segregation’. This is suggested by the slightly lower T_m , while the $\Delta T_{1/2}$ remains practically unchanged (Table 1) [14].

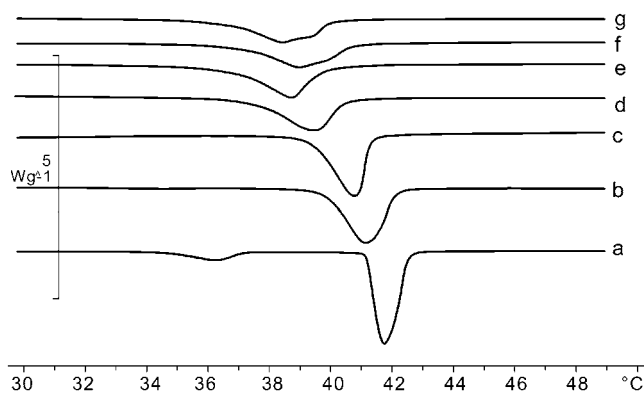


Fig. 2. DSC thermograms of fully hydrated DPPC lipid bilayers with varying amounts. (a) 0%, (b) 3%, (c) 5%, (d) 10%, (e) 15%, (f) 20%, (g) 30% (mol) of (**1**).

Table 1
Interaction of (1) with DPPC lipid bilayers. Calorimetric parameters^a

Sample ($x = \text{mol}\%$)	T_{onset} ($^{\circ}\text{C}$)	T_{m} ($^{\circ}\text{C}$)	$T_{1/2}$ ($^{\circ}\text{C}$)	ΔH (J/g)	$\Delta T_{1/2}$ ($^{\circ}\text{C}$)
DPPC	41.12 \pm 0.01	41.43 \pm 0.02	41.51 \pm 0.02	48.51 \pm 0.07	0.63 \pm 0.04
DPPC/1 ($x=3$)	39.80 \pm 0.00	40.94 \pm 0.02	40.84 \pm 0.04	48.66 \pm 0.06	1.28 \pm 0.02
DPPC/1 ($x=5$)	39.15 \pm 0.18	40.47 \pm 0.15	40.24 \pm 0.14	45.82 \pm 0.43	1.15 \pm 0.11
DPPC/1 ($x=10$)	38.32 \pm 0.14	39.92 \pm 0.17	39.75 \pm 0.14	43.87 \pm 0.33	1.62 \pm 0.16
DPPC/1 ($x=15$)	38.03 \pm 0.13	39.73 \pm 0.22	39.50 \pm 0.17	43.02 \pm 0.55	1.73 \pm 0.09
DPPC/1 ($x=20$)	38.03 \pm 0.13	39.60 \pm 0.07	39.70 \pm 0.07	35.26 \pm 0.12	2.28 \pm 0.14
DPPC/1 ($x=30$)	37.75 \pm 0.62	39.56 \pm 0.34	39.35 \pm 0.30	30.72 \pm 0.15	2.25 \pm 0.14

T_{onset} : temperature at which the thermal effect starts; T_{m} : temperature at which heat capacity (ΔC_p) at constant pressure is maximum; $T_{1/2}$: temperature at which the transition is half completed; ΔH : transition enthalpy, $\Delta T_{1/2}$: width at half-height of the main transition peak ($^{\circ}\text{C}$).

^a Mean of four runs of each experiment.

3.2. Raman spectroscopy

Raman spectroscopy was applied to specify the location of (1) in the lipid bilayer and to confirm the DSC results. The pure DPPC spectrum exhibits three characteristic areas from which useful information can be derived about the conformation of the molecule. These areas are 2800–3000 cm^{-1} (area a), 1000–1200 cm^{-1} (area b), 700–800 cm^{-1} (area c). Area a contains the peaks at 2844 and 2880 cm^{-1} , which correspond to the symmetrical and asymmetrical vibration, respectively, of stretching of the C–H bond of the methylene groups. Area a also contains the peak at 2935 cm^{-1} , which is attributed to the symmetrical vibration of stretching of the C–H bond of the final methyl group of the alkyl chain. Of interest are the height intensity ratios $I_{2935/2880}$ and $I_{2844/2880}$, which indicate the interactions between the alkyl chains, and of their conformation. More specifically, these ratios can be used as an index of the change of the proportion between disorder and order in the conformation of the alkyl chain, over temperature. The area b includes the stretching vibrations of the C–C bonds of the alkyl chains of the phospholipids. The peak at 1130 cm^{-1} is attributed

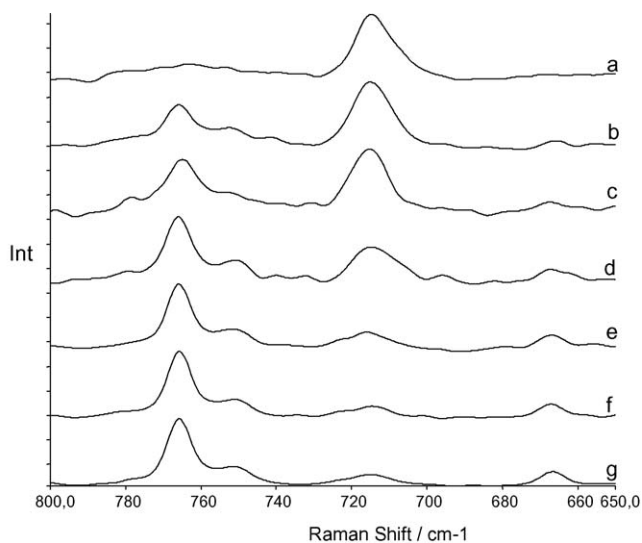


Fig. 3. Raman spectra of the area 650–800 cm^{-1} , including the C–N stretching mode of the choline group at 715 cm^{-1} , at 25 $^{\circ}\text{C}$, of DPPC with varying amounts. (a) 0%, (b) 3%, (c) 5%, (d) 10%, (e) 15%, (f) 20%, (g) 30% (mol) of (1).

Table 2

Interaction of (1) with DPPC lipid membrane. The height intensity ratio $I_{2844/2880}$ corresponds to the bending degree of the alkyl chain of DPPC lipid bilayers at 25 $^{\circ}\text{C}$

Sample ($x = \text{mol}\%$)	$I_{2844/2880}$
DPPC	0.81
DPPC/1 ($x=3$)	0.78
DPPC/1 ($x=5$)	0.82
DPPC/1 ($x=10$)	0.79
DPPC/1 ($x=15$)	0.84
DPPC/1 ($x=20$)	0.85
DPPC/1 ($x=30$)	0.85

to the stretching vibration of the C–C bond for the *trans* conformations of the alkyl chains, while the peak at 1090 cm^{-1} is attributed to the stretching vibration of the C–C bond for the *gauche* conformations of the alkyl chains. The height intensity ratio of these peaks, $I_{1090/1130}$ also provide information about the proportion between disorder and order that exists in the conformation of the alkyl chain. The area c contains the peak at 715 cm^{-1} , which represents the stretching vibration of the C–N bond of the choline group of DPPC (Fig. 3).

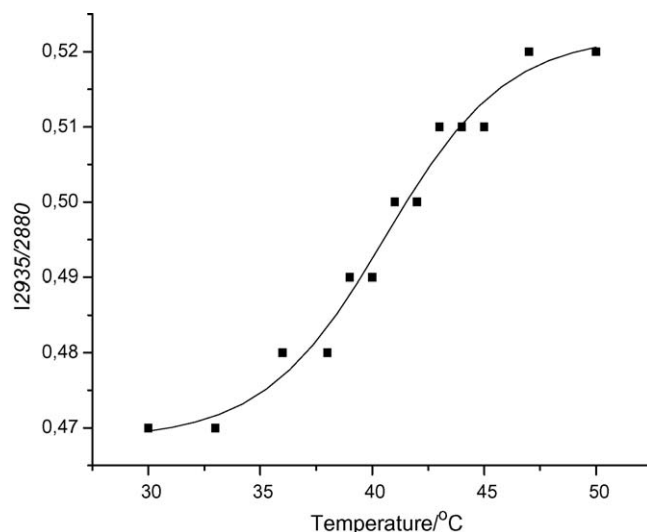


Fig. 4. $I_{2935/2880}$ vs. temperature graph for DPPC incorporating (1) at 3 mol% concentration.

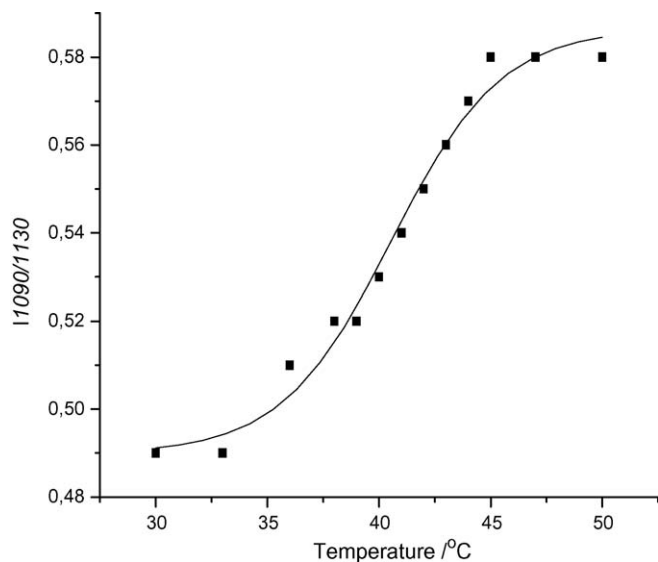


Fig. 5. $I_{1090/1130}$ vs. temperature graph for DPPC incorporating (1) at 3 mol% concentration.

The results of the experiments carried out at 25 $^{\circ}\text{C}$ at increasing concentrations are shown in Table 2. The height intensity ratio $I_{2844/2880}$ was selected as the peaks at 2844 and 2880 cm^{-1} were clearer. These results indicate that there is a slight increase in the intensity ratio at concentrations above 15 mol%, that is, the *gauche* conformation is more dominant at 15 mol% than in lower concentrations. The intensity changes of the peak at 715 cm^{-1} (Fig. 3) indicates significant interaction between the choline head group and compound (1), which is of the same strength, independent of the concentration of (1).

Experiments at 3 and 10% of (1) were done at increasing temperatures: 30, 33, 36, 38, 39, 40, 41, 42, 43, 44, 45, 47, and

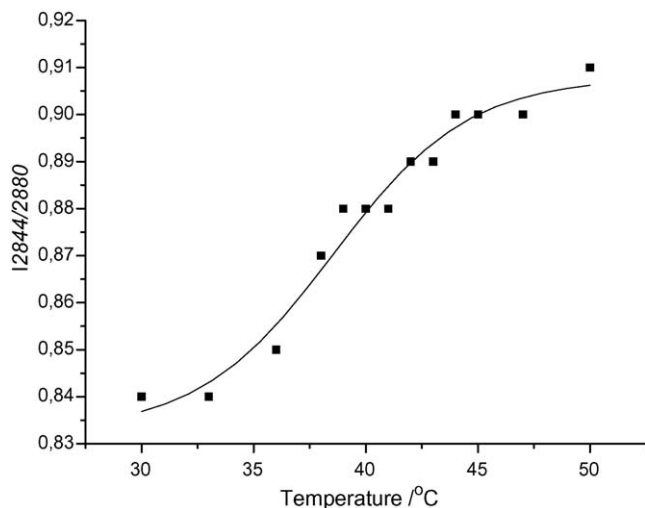


Fig. 6. $I_{2844/2880}$ vs. temperature graph for DPPC incorporating (1) at 10 mol% concentration.

50 $^{\circ}\text{C}$. These experiments were carried out to better understand the interaction between DPPC and (1) at critical concentrations in which the most important thermotropic changes have occurred. Subsequently, the height intensity ratios of $I_{2935/2880}$, $I_{2844/2880}$ and $I_{1090/1130}$ versus temperature were plotted. The incorporation of (1) in DPPC at a molar ratio 3% resulted in the lowering of the transition temperature of DPPC from 41.43 to 40.50 $^{\circ}\text{C}$ (Fig. 4) or 41 $^{\circ}\text{C}$ (Fig. 5). This fact is in accordance with the DSC result (40.94 $^{\circ}\text{C}$). At a molar ratio of 10% the transition of the well-organized gel phase to the liquid-crystalline phase took place at 39–40 $^{\circ}\text{C}$ (Fig. 6), temperature very close to the transition temperature derived from DSC (39.32 $^{\circ}\text{C}$).

4. Conclusions

DSC results showed (a) abolition of the pre-transition peak at concentrations above 5% (b) significant lowering of ΔH at concentrations above 20% and (c) slight decrease of T_m at increasing concentrations. The Raman spectroscopy results agree with the thermal analysis results suggesting (a) interaction of the choline head group with (1) indicated that (1) may be located mainly near the external face of the DPPC lipidic model bilayer; (b) increase of the disorder between the alkyl chains, because of the increase of the stretching of the alkyl chains and of the final methyl group, at concentrations above 15%; (c) transition temperatures close to the ones determined by DSC.

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