NONLINEAR OPTICAL SPECTROSCOPY OF POPC LIPOSOMES WITH CHOLESTEROL

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ABSTRACT: The surface potential and charged density of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine liposomes with cholesterol is study by using the second harmonic generation. *The Gouy-Chapman model is applied to data's to find surface potential of samples.* ***The result shows,*** *the surface potential aren't depends to the type of salts but change with the concentration and valence of the electrolyte in solution. The surface potential is changing from 40 to 15 mV with increase of salt concentration from 0 to 5 mM. The charge density was found to be 0.0136 Charge/Å^2, which is consistent with the area of a lipid headgroup. Moreover, the surface potential of POPC/cholesterol is increasing with increase of cholesterol.*

Keywords: Nonlinear Optic, Spectroscopy, Liposome, Nano-structure, Second harmonic generation.
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INTRODUCTION:

The signal of second harmonic generation (SHG) comes from interaction between light and polarized water close to the lipid of liposomal surfaces. The molecule of water is polarized by the liposomal structure. The SHG is good technique to investigating the electrostatic properties of liposome surface because the SHG methods have interaction with water around of liposome and not exactly with structure of it,[1-4]. In general, the size of liposomal structure is change from 5 nm to 1 μm. So, this technique is useful for study the surface potential and charge density of nano-structure **in this range of size**,[5,6].

SHG **befit** electric dipole **permissible** for the bulk molecules, mainly water molecules polarized by the electric field of nanoparticles. The nonlinear polarization of SHG, $P_{2\omega}^{total}$ is equal to the sum **of the different part of polarization that it is** third-order polarization $P_{2\omega}^{(3)}$ and the second-order $P_{2\omega}^{(2)}$ and, [7,8].

$$P_{2\omega}^{total} = P_{2\omega}^{(2)} + P_{2\omega}^{(3)} \quad (1)$$

The polarization is depends to the **susceptibility** so we have, [7,8]

$$P_{2\omega}^{total} = \chi^{(2)} E_\omega E_\omega + \chi^{(3)} E_\omega E_\omega \int_0^\infty E(r)dr \quad (2)$$

Where E(r) is the electric field at a distance r from the charged microparticle surface. The value of $\chi^{(3)}$ is not depend to the distance, but ∫E(r)dr is depend to the r. The ∫E(r)dr is the electrostatic potential at the charged surface. It is well known that the SHG field, (E_{SHG}) is depend to the nonlinear polarization and it has two part, namely $\chi^{(2)}$ and $\chi^{(3)}$ contributions, [7,8].

$$E_{SHG} \propto \chi^{(2)} + \chi^{(3)} \Phi_S \quad (3)$$

That Φ_S is the surface potential. With change the electrolyte concentration of the aqueous phase, the molecules polarized is changing and it can investigate with SHG. The surface potential on the solutions is described with Gouy-Chapman model,[9-11]:

$$\Phi_S = \frac{2kT}{Ze} \sinh^{-1}\left(\sigma \sqrt{\frac{\pi}{2\varepsilon kTc}}\right) \quad (4)$$

That the Boltzmann constant is shows with k, the surface potential (Φ_S) and temperature (T), the valence of the electrolyte (Z), the surface charge density(σ), dielectric constant (ε) and the electrolyte concentration(c) in the solution. The second harmonic field can be expressed as follows:[13-15]

$$E_{SHG} = A + B\frac{2kT}{Ze} \sinh^{-1}\left(\sigma \sqrt{\frac{\pi}{2\varepsilon kTc}}\right) \quad (5)$$

where A and B contain $\chi^{(2)}$ and $\chi^{(3)}$ respectively. Using A,B and σ as the fitting parameters**,[16].**

The charged of headgroup is negative so, the surface of liposomes have negative charge. The liposomes have two interfaces, the interface of the outer layer of the liposome bilayer with the external water solution and the interface of the inner layer of the liposome bilayer with the interior water solution. In the liposomes, the lipid on the opposite surfaces of a bilayer is oppositely oriented. So, $\chi_{in}^{(2)}$ has the opposite phase of $\chi_{out}^{(2)}$, and the second harmonic field from a liposome is the sum of the second harmonic field from the inside and the outside of surfaces,[1,16].

$$E_{SHG} \propto \chi_{out}^{(2)} + \chi_{in}^{(2)} + \chi_{out}^{(3)} \Phi_{out} + \chi_{in}^{(3)} \Phi_{in} \quad (6)$$

We assume the inside of the liposomes don't change with increase of salt concentration. The only term that changes is Φ_{out} .So, it can be written as

$$E_{SHG} = L + M\chi_{out}^{(3)} \Phi_{out}(c) \quad (7)$$

L and M are constant parameters and c is salt concentration that surface potential change with it. **It is to be founded that the electrolyte concentration and the valence of the electrolyte can change the second harmonic.**

Equation 4 can be substituted into eq. 7, leading to the following expression:

$$E_{SHG} = P + Q\frac{2kT}{Ze}\sinh^{-1}(\sigma\sqrt{\frac{\pi}{2\varepsilon kTc_{out}}}) \quad (8)$$

where c_{out} is concentration of the electrolyte in the liposomes. With using the approximation, we can separate the fitting parameters leading to the following equation:

$$E_{SHG} = E_0 + \frac{QkT}{Ze}\ln(c_{out}) \quad (9)$$

where E_0 is a constant in the experiment.

In this work, we studied surface potential and charge density of the 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) liposomal structure with and without cholesterol by using SHG. The surface potential change by adding NaCl, $MgSO_4$, $Fe_2(SO_4)_3$ to the liposomal structure. The structure of POPC liposomal was studied before and this phospholipid shows double layer liposomes,[17-20].

EXPERIMENTS:

1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) was purchased from Avanti polar lipid company and cholesterol (Chol) and chloroform and all type of salts was purchased from Sigma-Aldrich Company, Mainz Germany and methanol from Chemical Labs Company, Iran. Liposomes containing POPC and cholesterol were prepared using lipid film method. Briefly, lipid films consisting of POPC and cholesterol (2:1 M ratio) were prepared in a glass vial by evaporating the chloroform: methanol (2:1, v/v) solution. Traces of organic solvent were removed by keeping the film overnight. The lipid film was then hydrated by adding required amount of distilled water at 55 ^{0}C. The resulting multilamellar vesicles (MLVs) were sonicated in a bath-type sonicator (Decon, England) to form SUVs (small unilamellar vesicles). The composition of the liposomal suspension with different present of lipid and cholesterol to water with and without salts is given by the parameter concentration. The experimental solutions were prepared by adding water to the solution. The POPC concentration in the liposomes after mixing with various solutions of electrolytes was kept constant the mass of POPC to the total mass was 0.2.

The salts of Iron(III) sulfate, sodium chloride and magnesium sulfate were obtained from Aldrich and put in an oven at 80^{o}C overnight before the preparation of the solutions. All the solutions were prepared in double-distilled water.

The experiments were performed using a pulsed an argon ion laser pumped Ti: Sapphire oscillator. After passing through a half-wave plate and a polarizer, the light is gently focused a cylindrical glass sample cell. The scattered light was then collimated with a lens, polarization selected and spectrally dispersed on to into the monechromator. The angle of acceptance was at 90^o. A photomultiplier tube is used to record the signal from monechromator.

In the experiment, the total intensity at frequency 2ω is measured that contains SHG signal from the liposome surface and hyper-Rayleigh scattering from aqueous phase. The hyper-Rayleigh scattering originates from orientation and density fluctuations of water molecules. To obtain the second harmonic field of liposome structure we need to measure the hyper-Rayleigh scattering of water and doing a correction in the equation:

$$E_{SHG} = \sqrt{I_{SHG}} = \sqrt{I^{2\omega} - I_{HR}} \quad (10)$$

That I_{SHG} is the intensity from the surface of liposome's and $I_{2\omega}$ is the hyper-Rayleigh intensity from bulk water.

The sizes and polydispersity of the liposome's were estimated from photon correlation spectroscopy experiment we try to keep constant size of liposome by using sonicator. We used dynamic light scattering (DLS) for find the size of liposomal structure. The DLS measurements were performed on filtered samples using Malvern photon correlation spectroscopy instrument at Ferdowsi University of Mashhad. The light source is a He-Ne laser, operating at a wavelength of 632.8 nm, with vertically polarized light.

3. RESULTS AND DISCUSSION

The Size behavior of liposomal suspension with Salts and Cholesterol was probed with Dynamic light scattering methods. Figure 2, show the correlation function as function delay time for POPC liposomal suspension for pure liposome (down tri-angel) and the liposome with higher concentration of NaCl (up-triangle), $MgSO_4$ (Cross), $Fe_2(SO_4)_3$(Cubic) and Cholesterol (star) at room temperature. The correlation function for all samples showed a single exponential decay at all concentrations. All the correlation functions in this work were fitted by a single stretched exponential function,($g_1(t) = \exp\left(-t/\tau\right)^{\beta}$),[21].

Where the collective diffusion coefficient is calculated from $D_c=1/(q^2\tau)$,[22]. The hydrodynamic radius R_H is related to collective diffusion coefficient D_c by the stokes law in the in the form $D_c = k_B T/(6\pi\eta R_H)$,[23]. Where k_B is the Boltzmann constant, T the absolute temperature and η the liquid viscosity. The results of the fitting data's shows the collective diffusion of liposomal suspension is 1.61×10^{-11} (m^2/S) that hydrodynamic radius R_H from stokes law become 150nm.

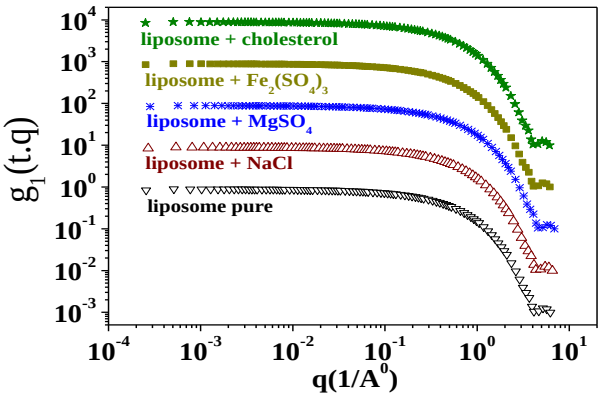

Figure 2. Correlation function as function delay time for POPC liposomal suspension for pure liposome (down tri-angel), liposome with higher concentration of NaCl (up-triangle), $MgSO_4$ (Cross), $Fe_2(SO_4)_3$(Cubic) and Cholesterol (star) at room temperature.

The second harmonic field, E_{SHG}, from the charged surface of POPC liposomes was measured as a function of electrolyte concentration of $Fe_2(SO_4)_3$(star), NaCl (cubic) and $MgSO_4$(circles), shown in Figure 1. The experimental data was fitted to eq 8 using P, Q and σ as fitting parameters, shown as a solid line in Figure 3. The results of the fitting are summarized in Table 1. The results shows the values of P, Q and σ obtained from the independent measurements using three electrolytes $Fe_2(SO_4)_3$, NaCl and $MgSO_4$. The ratio of BkT/Ze of NaCl to that of $Fe_2(SO_4)_3$ is 3 and NaCl to that of $MgSO_4$ is 2. That is consistent with the prediction of the Gouy-Chapman theory because the valence (Z) of $MgSO_4$ is twice that of NaCl. The charge density obtained from the fitting is about 0.0136 charge/A^2. For study the effect of the cholesterol on POPC liposomal structure we used different concentration of cholesterol on liposomal structure. The second harmonic field, E_{SHG}, of the POPC liposomal with different concentration of cholesterol as a function of NaCl concentration is presented in the fig.4. The results of the fitting with Gouy-Chapman theory are summarized in table 2, and it is showing with increase of cholesterol the charge density increase from 0.0135 to 0.0153 Charge/$Å^2$ by adding cholesterol from 0 to 10 percent. The parameter of P and Q is constant with increase of cholesterol.

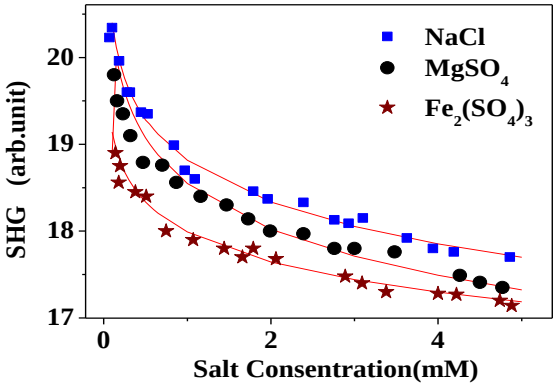

Fig.3. The second harmonic field, E_{SHG}, of POPC liposomal structure as function of salt concentration for NaCl (Cubics), $MgSO_4$ (Circles) and $Fe_2(SO_4)_3$(Stars), the red lines are the Gouy-Chapman Model.

Table 1. Fitting Parameters for the Curves of E_{SHG} versus c of POPC liposomal structure as function of salt concentration, using the Gouy-Chapman Model.

Salt added to liposome	**NaCl**	**$MgSO_4$**	**$Fe_2(SO_4)_3$**
σ (Charge/$Å^2$)	0.0135	0.0136	0.0136
(QKT)/(Ze)	1.6	0.8	0.5
P(arb.Unit)	15.2	15.3	15.4

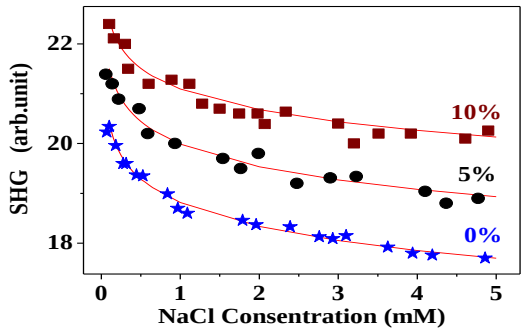

Fig.4. The second harmonic field, E_{SHG}, of POPC/cholesterol with different cholesterol (0% stars – 5% circles – 10% cubic) in lipid as function of NaCl concentration, the red lines are the Gouy-Chapman Model.

Table 2. Fitting Parameters for the Curves of E_{SHG} versus c of POPC/cholesterol with different cholesterol in lipid as function of salt concentration, using the Gouy-Chapman Model.

Cholesterol added to liposome	**0%**	**5%**	**10%**
σ (Charge/$Å^2$)	0.0135	0.014	0.0153
(QKT)/(Ze)	1.6	1.4	1.2
P(arb.Unit)	15.2	15.3	15.5

From eq.4 and the fitting parameters in the table 1, we obtained surface potential of POPC liposomal structure as function of different salt concentrations. The fig.5 shows the surface potentials decrease with increase of salt concentration but don't change with type of salt.

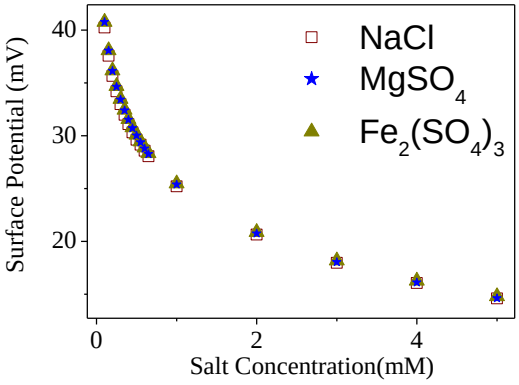

Fig.5. the surface potential of POPC liposomal as function of salt concentration for NaCl (Open cubic), $MgSO_4$ (Stars) and $Fe_2(SO_4)_3$(up-triangle).

The fig.6, is the surface potentials as function of NaCl Concentration for POPC/cholesterol at three different cholesterol concentrations. This result was obtained from eq.4 and table 2. Our results are show with increase cholesterol to the liposomal structure the surface potential is increasing.

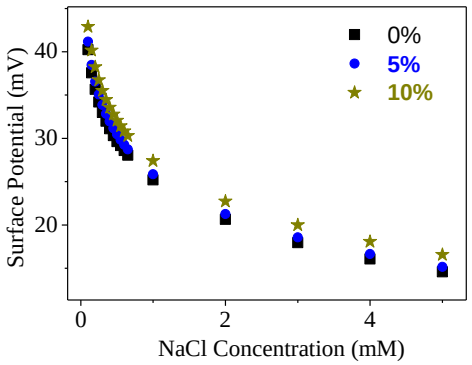

Fig.6. The surface potential of POPC/cholesterol as function of salt concentration for three different cholesterol presents.

CONCLUSIONS:

We have demonstrated that the outer surface potential and surface charge density of the POPC liposomes with and without cholesterol can be obtained by the SHG technique. The experimental data show agreement with the Gouy-Chapman model. The surface potential measured is in the range of 15-40 mV, depending on the valence and concentration of the electrolyte of pure POPC liposomes. The charge density was found to be 0.0136 Charge/Å^2, which is consistent with the area of a lipid headgroup. Moreover, the surface potential and charge density of POPC/cholesterol is increasing with increase of cholesterol.

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