Biophysical Studies on the Effect of Gamma Rays on Liposomes

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Liposomes are vesicular structures made of lipids that are formed in aqueous solutions, which can be used as models to study the cell membrane. In the present study the effects of gamma (γ) rays on dipalmitoyl-phosphatidylcholine (DPPC) liposomes were studied by transmission electron microscopy (TEM), Fourier transform infrared (FTIR) spectroscopy, as well as dynamic light scattering (DLS) and viscosity measurements. The DPPC liposomes were exposed to three different doses 40, 80, and 120Gy which emitted from 60Co gamma rays source with a dose rate of 9 kGy/h. The DLS measurements confirmed the mono-dispersity of all samples. TEM results revealed that there is a change in morphology and size of liposomes, which is in a good agreement with the increase in viscosity measurements. FTIR measurements showed significant changes in the characteristics bands of DPPC liposomes confirming the effect of γ-rays on the main groups such as CH2 bending vibrations and the symmetric and antisymmetric PO2- stretching vibrations at 1090 and 1220 cm⁻¹ respectively. In addition to the shifting of the OH stretching vibrations from 3439 cm⁻¹ to 3453 cm⁻¹ due to the 120 Gy exposure. The spectral changes seem to be due to some sort of water loss and molecular conformational changes due to ionization and formation of free radicals which affect the head groups of the DPPC liposomes leading to lipid lateral diffusion enhancing the fusion of small vesicles to form larger structures.

Keywords: Liposomes, DPPC, Gamma rays, Characterization.
peroxidation of unsaturated lipids dispersed as liposomes \(^{(11,12)}\).

In the present study, the effect of different doses of gamma rays on DPPC liposomes was studied by measuring FTIR, size distribution by dynamic light scattering (DLS), transmission electron microscopy (TEM) and viscosity to detect different changes on the molecular level of DPPC liposomes.

**Material and Methods**

**Materials**

L-α-Dipalmitoyl phosphatidylcholine (DPPC) specified 99\% pure was purchased from Sigma (St. Louis, MO, USA). Organic solvents (chloroform and ethanol) were of analytical grade and obtained from Merck and were without surface impurities and used without further purification. Tris buffer, molecular weight (121.14) was purchased from BDH limited poole (England). Water was triple distilled and then ultra-purified by a Millipore system (Mill-Q system).

**Preparation of liposomes**

Liposomes were prepared by the thin film method\(^{(13)}\). DPPC (50 mg) was dissolved in chloroform to ensure a homogeneous mixture of lipids in a rounded bottom flask. The organic solvent (chloroform) was evaporated gradually by rotary evaporation to obtain a thin film of lipids on the sides of the flask. The lipid film is thoroughly dried to remove residual organic solvent by placing the flask on a vacuum pump. Multilamellar vesicles were formed by adding an aqueous solution of 10 mM Tris buffer and NaCl (145 mM) (pH 7.4) to the flask and with vigorous shaking at a temperature 45°C above the phase transition temperature of the lipid.

**Irradiation setup**

The DPPC liposomes in 4 ml glass vials were exposed at ambient temperature, to 40, 80, and 120Gy emitted from \(^{60}\)Co gamma-ray source using a gamma-ray cell at dose rate of 9 kGy/h. The DPPC liposomes, before gamma-ray exposure, was used as a control material.

**Particle size distribution**

The mean diameter of DPPC liposomes, as a function of volume at 25°C, was unimodal and relatively narrow which is shown in Fig.1. The calculated mean diameter for the control was 805.4 nm at 55\% of volume and 55.12 nm at 45\%. The calculated mean diameter at doses 40, 80, and 120 Gy were 887.6, 656.7, and 3087 nm, respectively. The mean value of polydispersity (% Pd) of the 4 samples of DPPC liposome’s dispersions was 0.531\%. Therefore as a rule of thumb, samples with % Pd ≤ 20\% are considered monodisperse\(^{(13)}\).

**FTIR spectroscopy**

FTIR spectra of samples of DPPC liposomes deposited in KBr disks were recorded on a Jasco FT/IR 460 plus (Japan) spectrometer. The scanning was done in the range 400–4,000 cm\(^{-1}\) with speed of 2 mm/s at a resolution of 4 cm\(^{-1}\). The band width was measured at 50\% of height of the peaks.

**Result and Discussion**

**Particle size distribution**

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Transmission Electron Microscopy

Surface morphological studies on the shape of prepared samples using transmission electron microscopy indicated that the control sample has a regular spherical shape while the irradiated ones lose such regularity gradually as the dose increases and the smaller vesicles started to aggregate and fuse together to form larger vesicles at the final dose of 120 Gy, as shown in Fig. 2.

FTIR spectroscopy

FTIR spectroscopy is primarily well-suited for studying conformational order in phospholipids’ acyl chains\(^\text{(14,15)}\). With this technique, it is possible to monitor subtle changes in the structure of the lipid assemblies either in acyl chains or in head-groups region by analyzing the frequency and the band-width changes of the vibrational modes of the different functional groups. These changes can be used to extract information about various physicochemical processes taking place in the systems\(^\text{(16,17)}\).

The FTIR scans were performed separately for the four different liposome batches (control and the exposed samples, 40, 80, and 120 Gy).

Fig. 3. shows the full FTIR spectrum of DPPC liposomal samples. As shown from the figure, the spectrum displays the main characteristic bands of DPPC vesicles \(^\text{(19)}\).

The characteristic bands of DPPC such as C=O and C–O were found to be overlapped by the strong absorption bands of OH groups of the hydrated DPPC, where the OH stretching and bending vibrations at 3470 and 1640 cm\(^{-1}\), respectively. In addition to the symmetric and antisymmetric PO\(_2\)– stretching vibrations at 1090 and 1220 cm\(^{-1}\), respectively, were apparent. These findings are in good accordance with the data reported in the literature \(^\text{(18,19)}\).

Comparing the FTIR scans of irradiated samples with the control, one notices that as the dose of gamma rays increases there is a change in the liposome bands that disappears of others: as in CH\(_2\) bending vibrations and the symmetric and antisymmetric PO\(_2\)– stretching vibrations at 1090 and 1220 cm\(^{-1}\), respectively. In addition to the shifting of the OH stretching vibrations from 3439 cm\(^{-1}\) to 3453 cm\(^{-1}\) of the control and 120 Gy irradiated liposomal sample respectively, as shown in Fig. 3.
Fig. 2. TEM images revealing shape, structure, and sizes of DPPC liposomes at different radiation doses.

Fig. 3. FTIR transmission spectra of DPPC liposomes at different doses.
The spectral changes found as a result of gamma rays irradiation may be due to some sort of water loss and molecular conformational changes due to ionization and formation of free radicals which affects the head groups of the DPPC liposomes as indicated from the change occurred for the symmetric and antisymmetric PO\textsubscript{2}\textsuperscript{-} vibrations and CH\textsubscript{2} bending vibrations. Such molecular conformational changes may affect the lipid molecules that lead to the lipid lateral diffusion which in turn enhances the fusion of the small liposomal vesicles to form larger structures (20).

**Viscosity measurements**

The rheological properties of liposomes were measured to study the effect of gamma ray on DPPC liposome at different doses of gamma rays. Fig. 4 shows the flow curves for DPPC liposomes at different doses (0, 40, 80, and 120 Gy).

![Fig. 4. Flow curves for DPPC liposomes at different doses (0, 40, 80, and 120 Gy).](image)

Curve fitting the data of the different irradiated liposomal samples to the power law model yields the rheological parameters as shown in Table 1:

The consistency index \(k\) is an indication of the viscous nature of liposomes, and the flow behavior index \(n\) is a measure of departure from Newtonian flow (21).

**TABLE 1. Rheological Parameters of DPPC liposomes at different doses of gamma rays.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Plastic viscosity (cP)</th>
<th>Consistency index, (k) (Pa.s)</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>04.08</td>
<td>0.078±0.08</td>
<td>0.504±0.01</td>
</tr>
<tr>
<td>40 Gy</td>
<td>04.03</td>
<td>0.316±0.04</td>
<td>0.309±0.01</td>
</tr>
<tr>
<td>80 Gy</td>
<td>10.98</td>
<td>1.518±0.15</td>
<td>1.363±0.033</td>
</tr>
<tr>
<td>120 Gy</td>
<td>05.50</td>
<td>0.067±0.07</td>
<td>0.577±0.017</td>
</tr>
</tbody>
</table>

For the control and the 40 and 120 Gy irradiated samples, the flow index, \(n\) were calculated to be 0.5, 0.31, and 0.57 and the consistency index, \(k\) values were 0.078, 0.316, and 0.067 Pa.s., respectively. It is clear that liposome suspensions exhibited a pseudo-plastic behavior because the values of the flow index \(n\) <1. However, at 80 Gy, \(n\) and \(k\) values were 1.363 and 1.518 Pa.s., respectively. A flow behavior index equal to 1.363, a value significantly greater than 1.0, is a numerical indication of a large shear-thickening effect and hence an increase in the sample viscosity.
Figure 5 shows the plastic viscosity of DPPC liposomes that increased after gamma irradiation, where it is increased from 4.08 to 10.98 cP at 80 Gy then decreased again to 5.5 cP at 120 Gy.

Based on the present results of liposomal rheological properties, it can be concluded that gamma irradiation has a great effect on the phospholipids molecules whereas the gamma ray dose increases up to 80 Gy the liposomes plastic viscosity increases but it decreased again when the gamma ray dose increased more as in the case of 120 Gy. There is a direct relationship between the particle size of liposomes and the measured viscosity. The interaction of gamma ray with liposomes enhances molecular conformational changes due to ionization and formation of free radicals which affects the head groups of the DPPC liposomes which in turn leads to lipid lateral diffusion enhancing the fusion of small vesicles to form larger structures.

**Conclusion**

The effect of ionizing radiation on DPPC liposomes was confirmed by transmission electron microscope (TEM) images, FTIR spectroscopy, and dynamic light scattering (DLS). The gamma rays affect the particle size distribution whereas the dose increases there is a disruption for the liposomal vesicles to form smaller monodisperse vesicles as shown from dynamic light scattering results (DLS) which in turn affects directly the viscosity measurements whereas the particle size decreases the viscosity increased from 4.08 cP at 0 Gy to 10.98 cP at 80 Gy and then decreased again to 5.5 cP at 120 Gy due to the adhesion of small vesicles to form larger structure and hence the viscosity decreases. Finally, it could be concluded that gamma rays induced structural changes in liposomes as a membrane model system.

**References**


(Received 26/3/ 2018; accepted29 / 4/ 2018)