EFFECT OF TITANIUM DIOXIDE NANOPARTICLE ADDITION TO THE SURFACE CHARGE AND STRUCTURE OF DPPC VESICLES

(Kesan Penambahan Nanozarah Titanium Oksida ke atas Cas Permukaan dan Struktur Vesikel DPPC)

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Abstract
Titanium dioxide nanoparticle dispersions interaction with 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) vesicles is reported in this paper. DPPC- TiO$_2$ interactions were investigated using dynamic light scattering (DLS) and transmission electron microscopy (TEM) by focusing on the effect of the interactions on the surface charge, size and localisation of TiO$_2$ in DPPC- TiO$_2$ vesicles. It was observed that surface charge of DPPC- TiO$_2$ vesicles were increased from -26.0 to 12.1 mV as the TiO$_2$ nanoparticle concentrations increased from 0.01 to 100 mg/ml. The binding of positively charged TiO$_2$ nanoparticle to the zwitterionic DPPC vesicles caused the surface charge to become more positive. The size distribution, localisation and positioning of TiO$_2$ nanoparticles in DPPC vesicles were confirmed through TEM analysis.

Keywords: DPPC, vesicles, titanium dioxide, DLS, TEM

Introduction
Vesicles (liposomes) are artificially constructed spherical lipid with controllable size which comprises compartments that can encapsulate or store various molecules such as proteins, DNA and drug molecules [1,2]. However, there is a limitation in manufacturing and storage of lipid vesicles. Fusion occurs when vesicles encounter one another in suspension and leads to aggregation which destabilises vesicles and limiting vesicles as effective drugs carrier. Considering that, several formulation methods have been developed to overcome this problem. Zhang and Granick used carboxyl-modified polystyrene nanoparticles to stabilise liposomes with respect to lipid concentration. They found that the liposomes repel and do not fuse up to 50 days [3]. Currently, there were few works done to understand the interaction between zwitterionic lipid and positively charged nanoparticle. Therefore we intend to investigate the effect of positively charged titanium dioxide (TiO$_2$) nanoparticles addition to the 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) vesicles.
The effect of nanoparticle addition to biological system is a growing interest in relation to the increasing utilization of nanomaterials. TiO$_2$ nanoparticles exhibit many admirable features such as biocompatibility, environmentally benign and used in medical fields [4,5]. Incorporation of titanium dioxide nanoparticles could not only improve lipid vesicles stability but also help to provide multipurpose functionality in single stable vesicle [6]. The important aspect investigated in this work is the effect of TiO$_2$ nanoparticles interaction on the surface charge (zeta potential) and the morphology of DPPC vesicles. As observed in previous works on the similar systems, nanoparticles might have significant effect on the vesicles zeta potential, size and structure as the nanoparticles concentration increases [7].

**Materials and Methods**

**Materials**

1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) supplied from Avanti Polar Lipids (USA) was dissolved in chloroform to get a concentration of 10 mg/ml. TiO$_2$ (P25) nanoparticle was obtained from Sigma Aldrich (USA) with an estimated particle size of 21 nm and phase composition of 80% anatase and 20% rutile. These nanoparticles were dispersed in Milli-Q water.

**Methods**

Lipid vesicles were prepared in deionised water at 10 mg/ml DPPC using the conventional gentle hydration method. The DPPC lipid was dried in an oven overnight at 45°C to form a thin lipid film. The thin film was then prehydrated with deionised water and 0.1M of sucrose and left at room temperature. A bulky white suspension formed in the solution was vesicles and were extruded through 100 nm polycarbonate membranes to obtain homogeneous vesicles. These vesicles were mixed by vortex with TiO$_2$ nanoparticles of various concentrations which varied from 0.01 to 100 mg/ml. The mixed DPPC- TiO$_2$ vesicles were left overnight to reach adsorption equilibrium of TiO$_2$ onto the surface of vesicles.

**Analysis**

The size distribution and zeta potential of both TiO$_2$ nanoparticles (positively charged; +25 mV) and DPPC-TiO$_2$ vesicles were determined by dynamic light scattering (DLS) using Zetasizer (Malvern Instruments, UK). The morphology and structure of TiO$_2$ nanoparticles and DPPC-TiO$_2$ vesicles were characterised using transmission electron microscopy, TEM (FEI Tecnai G2 Spirit Biotwin). Based on Figure 1, the size range of TiO$_2$ nanoparticles dispersion was 15-35 nm in diameter.

(a)
Results and Discussion

Different concentrations of TiO$_2$ nanoparticles dispersion were found to have a significant effect on the zeta potential values of the DPPC vesicles as presented in Figure 2. The zeta potential of DPPC vesicles increases from -26 to 12.1 mV with slight decrease towards negative values at 0.01 till 1 mg/ml TiO2 nanoparticles concentrations. The value decrease may due to the addition of sucrose during the DPPC vesicles preparation which gives a good isotonic medium (in terms of electrostatic stability) for negatively charged nanoparticles. However, for positively charged TiO$_2$ nanoparticles these sucrose additions slightly decrease the DPPC zeta potential to more negative values [8]. Furthermore, adsorption of charged nanoparticles to the neutral (zwitterionic) charged surface of DPPC vesicles causes the movement of the slip plane away from the surface causing a decrease of zeta potential value [9]. It is also known that in the gel phase, positively charged nanoparticles reduce the tilt angle below the angle of 30° to ~65° causing reduce in lipid density [10]. When the zwitterionic headgroup is exposed to positively charged (cationic) nanoparticle, the electrostatic binding are not as strong as the binding between zwitterionic headgroup and negatively charge (anionic) nanoparticle [11]. In agreement with our results, concentration dependent effect in nanoparticle addition to lipid vesicle was also reported by several other studies. Mohanraj et al. used DPPC with silica nanoparticles and reported that lipid size and zeta potential depends on the concentration and charge ratio of vesicles and nanoparticles [7].

Table 1. Composition of DPPC-TiO$_2$ vesicles and the effects on the particle size and zeta potential

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nanoparticle concentration (mg/ml)</th>
<th>Titanium dioxide: lipid mass ratio</th>
<th>Mean particle size (nm)</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoated</td>
<td>0</td>
<td>0</td>
<td>280</td>
<td>-26</td>
</tr>
<tr>
<td>Lipid 1</td>
<td>0.01</td>
<td>1000:1</td>
<td>996</td>
<td>-6.35</td>
</tr>
<tr>
<td>Lipid 2</td>
<td>0.1</td>
<td>100:1</td>
<td>1071</td>
<td>-14.1</td>
</tr>
<tr>
<td>Lipid 3</td>
<td>1</td>
<td>10:1</td>
<td>513</td>
<td>-20.1</td>
</tr>
<tr>
<td>Lipid 4</td>
<td>10</td>
<td>1:1</td>
<td>2384</td>
<td>+6.93</td>
</tr>
<tr>
<td>Lipid 5</td>
<td>50</td>
<td>1:5</td>
<td>2605</td>
<td>+12.1</td>
</tr>
<tr>
<td>Lipid 6</td>
<td>100</td>
<td>1:10</td>
<td>155</td>
<td>+0.392</td>
</tr>
</tbody>
</table>
The presence of nanoparticle caused the diameter of DPPC-TiO$_2$ vesicles to increase from around 280 nm (absence of TiO$_2$ nanoparticles) to >2600 nm at a TiO$_2$ nanoparticles concentration of 50 mg/ml. This indicates formation of large aggregates which is due to an increase in density and a decrease in interparticle repulsion. Meanwhile at high TiO$_2$ nanoparticles concentration (100 mg/ml), vesicle size decreased to 155 nm, suggesting TiO$_2$ fully covered DPPC vesicle surface thereby overcome the attractive forces. Based on TEM images, incorporated TiO$_2$ nanoparticles have been found attached on the vesicle walls resulting in the appearance of dark images surrounding the vesicles (Figure 3b) compare to normal vesicles without nanoparticles (Figure 3a). There is also possibility that the nanoparticle partially embedded inside the lipid vesicles instead of only attached on the vesicles surface.

**Conclusion**

The results obtained based on the zeta potential suggest that the DPPC-TiO$_2$ vesicles formed was facilitated via electrostatic interaction. The interaction/binding strength is determined by the geometrical relation between P$^-$.N$^+$ dipole of the headgroup and the charge of the adsorbing nanoparticle. By dynamic light scattering measurement, it was found that TiO$_2$ nanoparticles increased the zeta potential value of DPPC vesicles with slight decrease at 0.01 till 1 mg/ml TiO$_2$ nanoparticles concentrations. TEM provided confirmation about the localisation of TiO$_2$ nanoparticles on DPPC vesicles and structure of DPPC-TiO$_2$ vesicles. The present findings demonstrate the
importance use of zeta potential value especially in designing drug delivery systems since it allows determining the sign of the charge of particles and to predict the stability of the vesicles conditions. However, new approach and integration with other analysis method such as differential scanning calorimetry might provide further information on nanoparticle embedded lipid vesicle.

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References