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## Surface charge comparison of liposome and $\text{TiO}_2$

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### ABSTRACT

The effect of an applied magnetic field on suspended particles was investigated in order to understand the particles response towards the applied field. Two types of particles were studied, namely titanium dioxide ( $\text{TiO}_2$ ) sols and caprate-capric acid liposome. The electrophoretic mobility of both types of particles under various conditions was examined through zetasizer which uses principles of laser doppler velocimetry. To elucidate the effects of different temperature, voltages, and the different time lapse, meanwhile the ionic strength medium is fixed at pH 7. Theoretically, higher temperature would increase the kinetic energy of the particles, thus increasing the electrophoretic mobility. Both the electrophoretic mobility of particles demonstrated a proportional relation with temperature. The comparison between  $\text{TiO}_2$  and liposome indicates that liposome elicited a greater electrophoretic mobility at higher temperature but had a lower migration rate as the applied voltage increased. For time lapse more than 10 s between measurements of the surface charge changes on the particles showed that  $\text{TiO}_2$  migrated fastest at the longest time lapse of 120 s which might be due to the aggregation of  $\text{TiO}_2$  in solution. Meanwhile, the greatest zeta potential distribution of liposome was achieved at time lapse of 10s.

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### KEYWORDS

Electrophoretic mobility;  
Zeta potential;  
Colloidal particle;  
Liposome.

### INTRODUCTION

Surface charge study on suspended colloidal particles has been receiving a great attention due to its applications in biotechnology. It has been used as models for biological membranes because some of the colloidal particles properties are similar to biological cells<sup>[1]</sup>. Thus, details of the characteristics of these particles are

in great interest for many researchers. For example, result obtained from conductivity of the particles had increased the efficiency of processing of coating and adhesion on other materials. By having a better understanding on its surface charge, reactions of the colloidal particle in weakening the activities of bacteria can be optimized. Among all colloidal particles, the solid form of  $\text{TiO}_2$  is the most commonly used for industrial appli

cations.

Titanium dioxide ( $\text{TiO}_2$ ) is a white pigment powder which has a high refractive index, leading to a wide usage of  $\text{TiO}_2$  as catalyst support<sup>[2-4]</sup>, semiconductor photocatalyst<sup>[5]</sup>, paints, antimicrobial coating<sup>[6]</sup>, oxygen sensors and as UV filters for cosmetic products<sup>[7]</sup>. The extensive usage of  $\text{TiO}_2$  in consumer products has attracted concerns from many researchers to investigate the effects of  $\text{TiO}_2$  in human body. Moreover, it has been reported that  $\text{TiO}_2$  could damage biological target through oxidative stress<sup>[8]</sup>. Another study had demonstrated that  $\text{TiO}_2$  when used as photocatalyst, generated damaging reactive species in cell structure<sup>[9]</sup>. Hence, the measurement of the electrophoretic mobility of  $\text{TiO}_2$  to study the surface charge in varied conditions would not only help in the discovery of further applications in different fields but also in preventing potential hazards to living beings.

Moving to consider the second particles, liposome is formed from self-assembling phospholipid molecules into bilayer structures that are similar to the basic structure of a biological membrane which consists of phospholipid bilayer matrix<sup>[10]</sup>. This special characteristic of liposome has supported applications in drug deliveries<sup>[11]</sup>. In addition, liposome also helps in improving the medical treatments for various health problems. For instance, it has been observed that cancer cells accumulate electrons, leading to unit cells being high in negative charges<sup>[12]</sup>, thus given the potentialities of liposome. Thus, it is essential to investigate the effects of liposome compositions on the absorption and transport processes based on the cell membrane charges<sup>[13]</sup>. It is worth nothing that the surface charge of liposome has been demonstrated to have a determinant effect on the reaction rate in the electromagnetic field. Hence, a method has been developed to measure the electrophoretic mobility of liposome which can provide estimation of the zeta potential distribution.

The experiment would be conducted with laser doppler velocimetry<sup>[14]</sup>. The electrophoretic mobility ( $\mu$ ) is the migration rate of the charged particles to the electrode when electric field is applied. The electrophoretic mobility is defined as equation 1.

$$\mu = v/E \quad (1)$$

where the  $v$  is the rate of migration (m/s) and  $E$  is external voltage on separation distance (V/m).

The mobility of the charged particles is also influenced by some other factor. For example, the electrolyte with different pH value, ionic strength and conductivity. This has proven in Smoluchowski<sup>[15]</sup> Equation 2,

$$\mu = \frac{\epsilon\epsilon_0\xi}{\eta} = \frac{\sigma\chi}{\eta} \quad (2)$$

which the  $\epsilon$  is dielectric constant,  $\epsilon_0$  is permittivity of the free space,  $\xi$  is zeta potential and  $\eta$  is viscosity of the solution. This is followed by  $\sigma$  is charge density of the surface charge and  $\chi$  is effectiveness of the ionic double layer surrounding the surface.

In this paper, the electrophoretic mobility of  $\text{TiO}_2$  and liposome would be carried out in different combination of conditions such as a range of temperature and voltages were investigated. Results of the real time measurement in the range of 0.5 s measurements would be collected. Besides, by giving higher electric field to the particles would induce a greater attraction force on the charged particles to move towards the electrode. Study was carried out on this portion to achieve better understanding on the particles response at a range of applied voltages from 10V to 100V. On the other hand, investigations were done into the surface charge changes when particles were exposed to the repetitive electric field was conducted. Rest time between the measurements from less than 10s to 120s would be studied. Comparisons between these two colloidal particles in response to different conditions will be discussed later in this paper.

## MATERIALS AND METHODS

### Chemicals

Cyclohexane was purchased from Sigma-Aldrich which was used as an organic solvent and surfactant of Triton X-45 (Sigma-Aldrich) was selected. Tetrapropyl orthotitanate ( $\text{Ti}(\text{OC}_3\text{H}_7)_4$ ) from Fluka and sodium hydroxide (HmbG chemical) were used. Buffer at pH 7 was prepared with Sodium Phosphate Monobasic Dihydrate ( $\text{H}_2\text{NaO}_4\text{P}\cdot 2\text{H}_2\text{O}$ ) from Fluka, assay >>99.0% and Sodium Phosphate Dibasic Dihydrate ( $\text{HNa}_2\text{O}_4\text{P}\cdot 2\text{H}_2\text{O}$ ) with a purity up to 98% was also purchased from Fluka. Liposome was formed with Capric Acid ( $\text{C}_{10}\text{H}_{20}\text{O}_2$ ) (Fluka) and assay is more than 98%. Sodium hydroxide (HmbG) which is insoluble in  $\text{H}_2\text{O}$  was used.

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### 1. Preparation of $\text{TiO}_2$

The calculation of molar ratio ( $w_0 = [\text{H}_2\text{O}]/[\text{Surf}]$ ) which varied the sizes of the synthesized  $\text{TiO}_2$  was determined. Nanosized  $\text{TiO}_2$  particles were prepared by controlled the hydrolysis tetrapropyl orthotitanate (TPOT) with 0.5M of sodium hydroxide in deionized water. Triton X-45 was mixed with cyclohexane as it is dissolvable in organic solvent. The solution of microemulsion was kept stirring for ten minutes and the size of the microemulsion was measured with zetasizer, nano-ZS. On the other hand, the TPOT was mixed in cyclohexane and was added dropwise to the microemulsion. The solution was stirred throughout the process for overnight. Ethanol (95%) was added into the solution in the ratio of 3:2( $\text{Ti}(\text{OH})_4$ : ethanol) and stirred for another 30 minutes to damage the emulsion and solubilize the surfactant. The solution was dried at  $60^\circ\text{C}$  for 24 hours and followed by calcination at  $550^\circ\text{C}$  for 4 hours. Sizes of the dried fine powder of the synthesized  $\text{TiO}_2$  were then examined.

### Preparation of fatty acid liposome

Liposome with final concentration of 20mM at pH 7 was prepared with capric acid and sodium hydroxide in 50mM as stock. 50mL of solution was mixed with approximately ten drops of hydrochloric acid with 1 M. A milky solution was observed, thus liposome was formed. The pH level of the solution was checked and fixed at pH 7 while the remaining volume was filled with deionized water up to 50mL.

### 2. Instrument

The percentages of water content in cyclohexane and Triton X-45 were measured with titrator (Mettler Toledo DL38, Karl Fisher Titrator). The particle size and electrophoretic mobility were determined by using zetasizer series nano-ZS from malvern instrument. Stirrer of HTS-1003 LMS was used. Polarizing microscope from Leica was used to view the image of liposome.

## RESULT AND DISCUSSIONS

The particle size distributions for both  $\text{TiO}_2$  and liposome, with an average size of 255nm and 164nm respectively, are depicted in figure 1. Polydispersity of  $\text{TiO}_2$  particle was much less than liposome whereas the

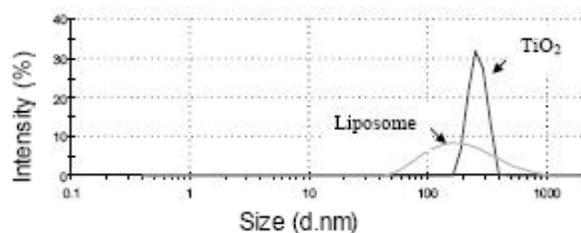


Figure 1: Diameter sizes of the liposome and  $\text{TiO}_2$

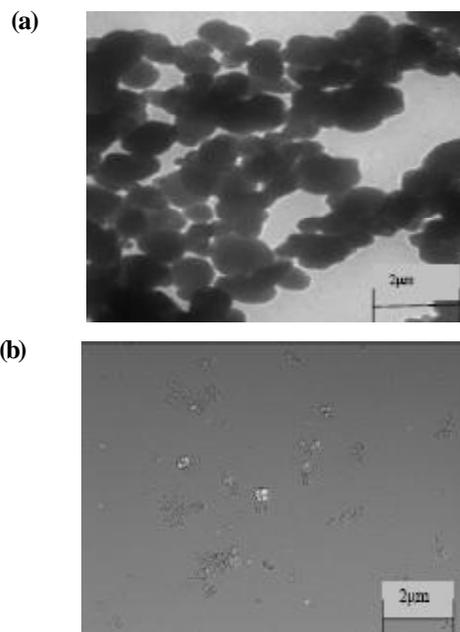


Figure 2 : (a) Micrograph of  $\text{TiO}_2$  (b) Micrograph of caprate-capric acid liposome

size distribution of liposome was wider than  $\text{TiO}_2$ . A micrograph of  $\text{TiO}_2$  was captured with TEM while micrograph of liposome was taken with polarizing microscope.

The changes of electrophoretic mobility of the particles in buffer pH 7 due to the increase of temperature were investigated. As can be seen from figure 3, the electrophoretic mobility of both  $\text{TiO}_2$  and liposome increased linearly. Results provided a 99% best fitting of the practical result to the mean values. The electrophoretic mobility of the particles elevated gradually as the internal energy of the particles increased as a result of the increasing temperature. The high internal energy of particles in the solution had led to a greater kinetic motion of the suspended particles and ions which then causing the double diffusion layers around the particles to be thicken. As a result, screening effects of the ions on the charged particles were reduced, increasing the

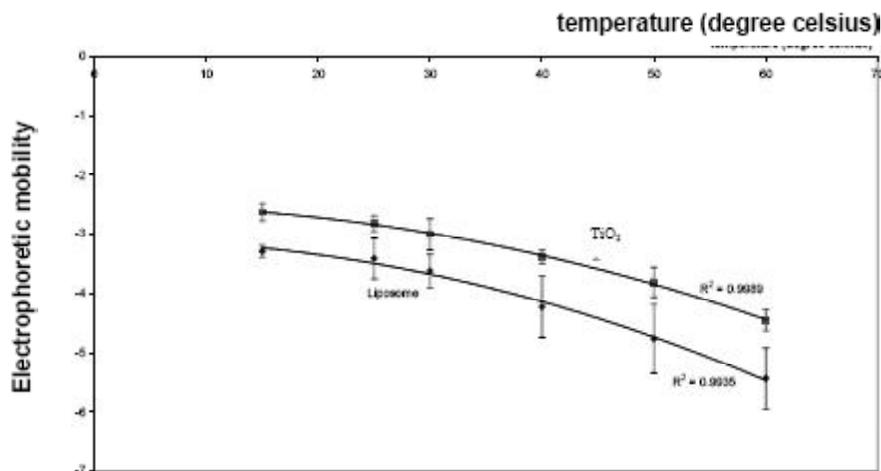


Figure 3 : Electrophoretic mobility of TiO<sub>2</sub> and liposome in varied temperature (15-60°C)

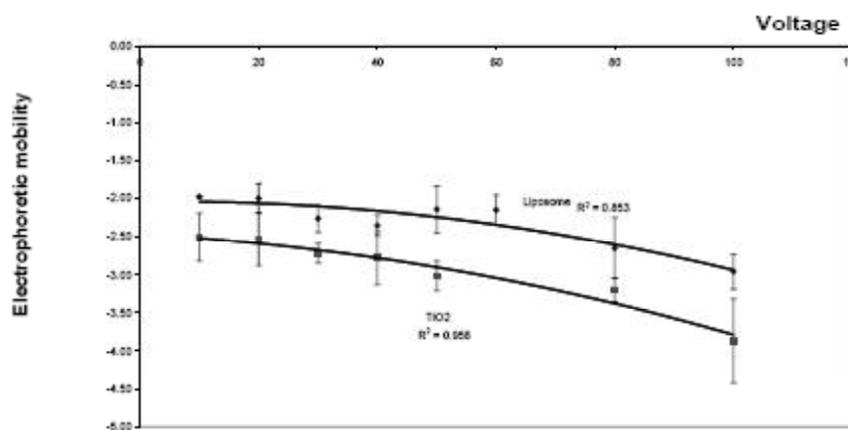


Figure 4 : The electrophoretic mobility of TiO<sub>2</sub> and liposome with different voltages

speed of particles moving towards the electrode. Meanwhile, the heating process had reduced the viscosity of the solution, thus enhancing the electrophoretic mobility of the particles.

Liposome appeared to provide a greater electrophoretic mobility as an overall compared with TiO<sub>2</sub>. It has been demonstrated that the negative surface charge of the liposome is more profound than TiO<sub>2</sub>. In addition, the density of liposome was lower than that of TiO<sub>2</sub> thus requiring a lower amount of heat energy to increase the kinetic energy of the particles migrating towards the electrode as compared to TiO<sub>2</sub>.

The higher the supplied voltage induced a greater attraction force on the charged particles and the counterions to move towards the electrodes. In consequence of the stronger magnetic field, causing the counterions around the particles as the polarizing force increased. The shearing ions could have reduced the

viscous drag of the charged particles which allowed a faster mobility of the particles towards the electrode. Moving to consider the surface charge in conditions that supplied voltages varied at a range increases in electrophoretic mobility was observed for both TiO<sub>2</sub> and liposome. As can be seen from figure 4, TiO<sub>2</sub> the denser particle, TiO<sub>2</sub> demonstrated an obvious linear increase of electrophoretic mobility as compared to liposome. However, the electrophoretic mobility for liposome was erratic along the changes of supplied voltages, but increased linearly again after 80 V. This could be due to the low density in liposome leading to a slower response to the magnetic attraction. The linear increase in electrophoretic mobility after 80V observed in liposome suggests that liposome responses greatly towards higher voltages. The magnetic attraction forces acted on the particle were increased proportionally with the density of the particle. Hence, electrophoretic mobility was

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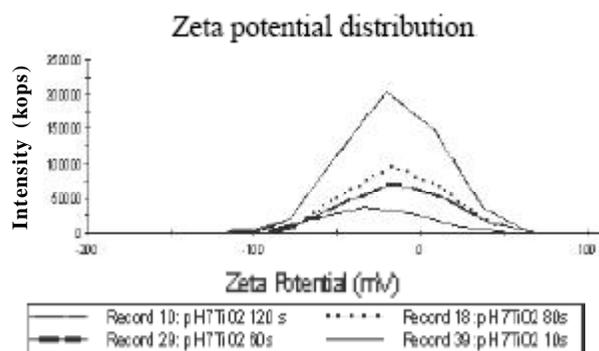


Figure 5 : (a) Zeta potential distribution for different time duration between each measurement for  $\text{TiO}_2$

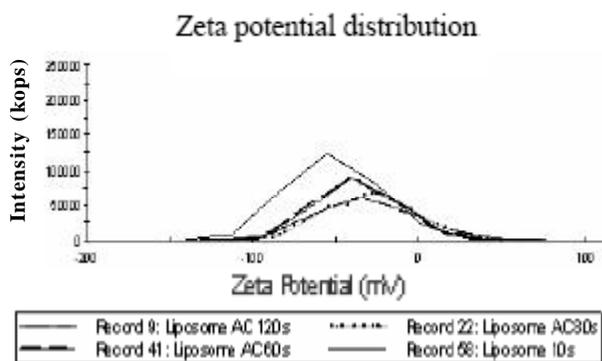


Figure 5 : (b) Zeta potential distribution for different time duration between each measurement for liposome

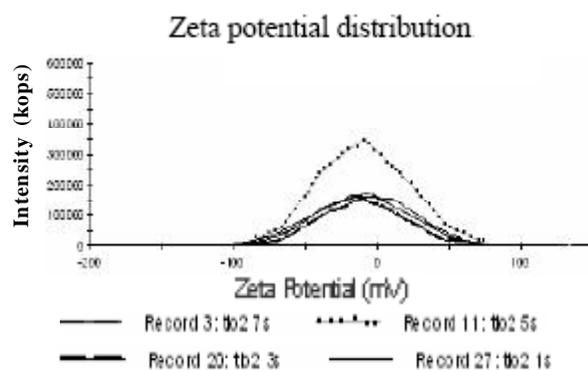


Figure 6 : (a) Zeta potential distribution for different time duration between each measurement for  $\text{TiO}_2$

greater in  $\text{TiO}_2$  compared to liposome for the whole range of voltages.

Zeta potentials for time lapse within measurement for different durations such as 10s, 60s, 80s and 120s are depicted in figure 5(a and b). Measurements were carried out in a duration of more than 10 seconds. The difference in zeta potential distributions between both particles was profound. As can be seen from figure 5(a), zeta potential of  $\text{TiO}_2$  gained the highest intensity at time

lapse of 120s but reduced accordingly as the time duration decreased. The higher count of intensity indicates a greater movement of the particles, thus increasing the strength of the surface charge of these particles. When the supplied voltage cut off after one measurement, the moving particles stopped but still carried a momentum which would keep them swirling and vibrating in the solution for more than 10s. This motion is considered as a retardant force which could have reduced the fast response of particles to the magnetic field when the next measurement starts. The swirl force of the particles appeared to be stronger than the net charge on the surface charge of particles which carried them to the electrode. Thus a lower reading of zeta potential at shorter time lapse was obtained for  $\text{TiO}_2$ . Besides, the heavier and bigger particles of  $\text{TiO}_2$  tended to aggregate and sink due to the gravitational pull as time lapse was prolonged. As a result, majority of the suspending particles were smaller in size. The smaller surface area of the  $\text{TiO}_2$  was denser with counter ion compared to the larger surface area of  $\text{TiO}_2$ , thus higher zeta potential was obtained from the smaller particles.

Results of the zeta potential over the different time lapse for liposome, however appeared to have a pattern that was totally opposite to that of  $\text{TiO}_2$ . As can be seen in figure 5(b), zeta potential of liposome was observed highest at the shortest time lapse of 10s between measurements and lowest time lapse of 120s. Moreover, the peaks of the intensity of liposome shifted to be less negative as the time lapse between measurements was extended. Given that liposome is a semi-solid and less dense particle, the post measurement momentum of liposome was not as strong as that of solid particles. During time lapse of 10s between each running, the negativity characteristic of liposome was strong that it elevated the attraction of positive ions towards the surface of liposome. The oppositely charged ions then screened the electrostatic interaction between the liposome particles, thus reducing the net charges on liposome. This screening effect of liposome was more intense when the duration between measurements was prolonged which was then weakening the repulsion force of liposome. As a result, the distance reduction between particles of liposome would be closer and cohesive when the next measurement started, leading to a lower level of electrophoretic mobility of liposome.

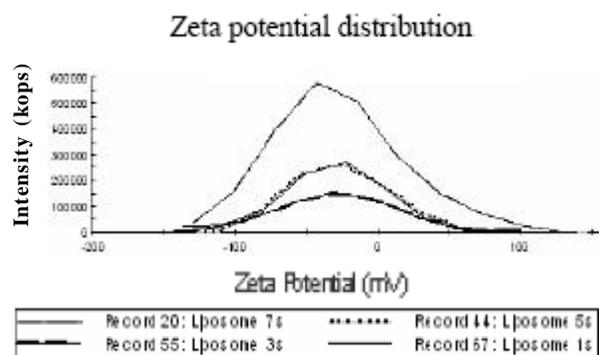


Figure 6: (a) Zeta potential distribution for different time duration between each measurement for  $\text{TiO}_2$

Time lapse between measurements of electrophoretic mobility in less than 10s are shown in figure 6 (a and b). The charged particles would move towards electrode when electric was applied. Results showed that the zeta potential distribution was greatest at 5s for  $\text{TiO}_2$  and 7s for liposome and consistent results were obtained. As for the time lapse less than 5s, the particles appeared to swirl or move with the momentum after the voltage supplied was disconnected intermittently. Thus, the charges on the surface of the particles had not been redistributing evenly.

## CONCLUSION

The electrophoretic mobility of the  $\text{TiO}_2$  and liposome increased almost linearly along with the raise in temperature of the solution which suggesting that an increase in temperature of the solution could raise the internal energy of the ions and the charged particles. In addition, a lower amount of heat energy was needed to increase the kinetic energy for less dense particles such as liposome.  $\text{TiO}_2$  and liposome demonstrated a constant increase in electrophoretic mobility due to the increase in magnetic attraction force and is proportional to the dense particles. The study observed that heavier and bigger particles would settle due to the gravitational force when time lapse between measurements was prolonged, suggesting longer time duration between measurements would reduce the zeta potential of liposome. As for the time lapse less than 5s, the particles still swirled or moved with the post measurement momentum after the voltage supplied was disconnected. Thus, the surface charge on particles may not have been

redistributed evenly, thus a 5s duration between measurements is needed.

## ACKNOWLEDGMENT

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