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Research Article

CHARACTERIZATION OF ENCAPSULATED TITANIUM DIOXIDE USING ENGKABANG FAT ESTERS FOR COSMECEUTICAL PURPOSES

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ABSTRACT

The formulations of encapsulated and non-encapsulated titanium dioxide using engkabang fat esters (EFE) and beeswax were produced by emulsification method using high shear homogenizer. All formulations were stable in freeze-thaw cycles test, at room temperature (25 ºC) and 45 ºC for three months. The particle sizes of the formulations were in the range of 80 nm to 406 nm. Surface charge measurements of formulations denoted the presence of stable dispersions. All formulations could be classified as the pseudo plastic materials under a non-Newtonian fluid. The formulations containing encapsulated TiO_2 gave higher absorbance compared to the formulations containing non-encapsulated TiO_2.

Keywords: Engkabang, sunscreen, titanium dioxide, ultraviolet.

INTRODUCTION

The public awareness regarding the harmful effects of UV radiation combined with the problem of the thinning of ozone layer is rising [1]. The ultraviolet radiation from the sun is not fully filtered when the radiation reaches the earth. Therefore, the usage of sunscreen products becomes important to human being to protect ultraviolet radiation from the sun. Ultraviolet radiation from the sun is divided into UVC, UVB and UVA [2]. The use of sunscreens helps to reduce skin damage produced by the UV radiation from sunlight [3-4].

The ultraviolet radiation attenuation of physical sunscreens is known to occur, depending on the nature of the compound, by three mechanisms namely scattering, reflection and absorption [5]. Titanium dioxide (TiO_2) is widely used in a number of commercial products including sunscreens and cosmetics [6]. To reduce the possibility of photocatalytic activity of TiO_2, it is often coated when used in cosmetic preparations [7]. Coated metallic oxide sunscreens are stable, very efficient UV attenuators, nontoxic and safe. Although they are new on the market, they have already found a considerable audience and it appears inevitable that their use will continue to increase [7]. Encapsulation of sunscreen leads to a synergistic effect of both sunscreen and the UV scattering by the particles [8-9]. In order to minimize the photocatalytic reaction pathways, TiO_2 filters recommended for cosmetic

applications are deactivated by encapsulation of TiO_2 [10-11]. The aim of the present study was to encapsulate titanium dioxide using engkabang fat esters and beeswax in emulsion system in order to enhance the UV attenuation, test the stability of the samples and characterize them.

MATERIALS AND METHODS

Materials

Engkabang fat was obtained from Sarawak, Malaysia. Fatty acid compositions of engkabang fat are 43.7% stearic acid, 35.7% oleic acid, 19.9% palmitic acid, 0.4% linolic acid and 0.1% palmitolic acid [12]. Polyoxyethylene(20) sorbitan tri-oleate(Tween85) were obtained from Merck Schuchardt OHG, Hohenbrunn, Germany. Engkabang fat esters (EFE) were synthesized in our laboratory using enzymatic method. Beeswax was obtained from Fluka Chemie GmbH, Tanzania. Glycerol, 99% GC (Glycerin) was obtained from Sigma-Aldrich, Inc., USA. Xanthan gum from Xanthomonas campestris was obtained from Fluka Chemie GmbH, France. Lavender oil from *Lavandula angustifolia L* was obtained from Sigma-Aldrich, France. Cypress oil was obtained from Wellness Original Ingredient, Malaysia. Titanium dioxide was obtained from Sigma-Aldrich, Laborchemikalien GmbH, Seelze. Gold Tri.E™ toco-trienol was obtained from Sime Darby Bioganic Sdn. Bhd., Malaysia and phenonip was obtained from Gattefosse, USA. Deionized water was prepared in our laboratory.

Formulation of Engkabang-Based Emulsions

Compositions were mixed by emulsification method using hot-hot process. The oil which contained EFE and beeswax, and water phases which contained deionized water, xanthan gum, Tween85 and glycerol were prepared separately in the required amounts and heated up to 75 °C until all the ingredients are dissolved. To encapsulate TiO_2, it was dispersed in the oil phase and stirred using a magnetic stirrer at ±75 ºC for 10 minutes. To evaluate the performance of TiO_2 without oil encapsulation, suspension of TiO_2 were prepared. TiO_2 was dispersed in water phase for 10 minutes using magnetic stirrer to give well-fined dispersion. By using high shear homogenizer (Kinematica, Switzerland), the oil phase was added into the water phase. The mixture was homogenized at 10 000rpm until the temperature of the mixture dropped to ±40 ºC. At 40 °C, toco-trienol, essential oil and phenonip as a preservative were added into the mixture. The final product was further homogenized using stirrer (IKA®RW 20 Digital, Japan) at 300rpm, at room temperature. Table 1 depicts the compositions of formulations for engkabang fat esters with encapsulated and non-encapsulated titanium dioxide.

Stability and pH Value

The assessment of product thermodynamic stability was carried out by the freeze-thaw cycles. In this method, the samples were stored in refrigerator at ±5 ºC for 24 hours and then thawed at room temperature for 24 hours. This step was repeated for three times. Further stability tests were carried out where the samples were stored at room temperature and 45ºC for three months. Freshly prepared samples were kept into three containers. They were used in thaw cycles, put at room temperature and in the oven at 45 ºC, respectively. The changes of physical appearance were observed periodically. The pHs of the samples were tested at room temperature using Delta 320 pH meter (Mettler-Toledo, Switzerland) for compatibility with the pH of human skin.

Particle Size Measurement

Particle size distribution of samples were measured by diffusion method using a dynamic light scattering (DLS) particle analyzer (Nanophox Sympatec, Germany) with Argon laser (λ = 488 nm). The measurement was conducted using Photon Crossed Correlation Spectroscopic (PCCS) principle. The samples were diluted into deionized water as dispersing medium in the ratio 1:200(w/w). The measurement was performed at 25 °C ± 0.5 °C. The results were presented as the volume distribution.

Surface Charge Measurement

Measurements of samples were carried out on Zetasizer Nano instrument (Malvern, United Kingdom) using laser Doppler electrophoresis. A sample was prepared in a syringe of at least 1mL capacity. The syringe containing the sample was placed into sample port and slowly injected into the folder capillary cell. If bubbles formed under the sample port, the syringe plunger will be pulled back to draw the bubbles into the syringe body and the sample was re-injected. A stopper was inserted once the sample started to emerge from the second sample port. The syringe was removed and replaced with a second stopper. The cell was tapped lightly to lodge them if necessary. Cell electrodes were made sure to be covered completely. Any liquid that might have spilled onto the electrodes was wiped away. The cell area

lid was opened by pushing the button in front of the lid. The cell was held near the top, away from the lower measurement area and pushed into the cell holder until it stopped. The cell area lid was closed and zeta potential measurement was carried out.

Rheology Study

The rheological properties of the formulations were measured using the Modular Compact Rheometer (Paar Physica 300, Austria). The instrument was warmed-up to allow sufficient time of at least an hour before running the measurement. The instrument was then initialized. During the initialization of the instrument, the vertical reference point and the reference angle of measuring drive system were checked. The measuring system of the instrument was installed. Finally, the rheology and viscosity of the formulations were tested at 25 ºC. The viscosity values of the samples were taken at different range of shear rate from 0 s^{-1} to 500 s^{-1}. Thirty data points were taken in one minute and the whole process took two minutes to complete. Newton's law of viscosity was used to characterize the rheological property of the sample [13].

Transmission Electron Microscope (TEM) Analysis

A drop of the sample was dispersed in deionized water in a 10ml screw-capped test tube. The test tube was shaken for several minutes. Several drops of dispersed sample were dropped on the parafilm. A formvar coated copper grid was put inside the droplet and left open air for 10 minutes. The sample on the copper grid was stained using 2% phosphotungstic acid (PTA) with pH value 7.2 for 10 minutes. The copper grid was dried by evaporation at room temperature. Characterization of sample was conducted by Transmission Electron Microscopy (Hitachi H-7100, Japan).

Conductivity Measurement

The contacting conductivities of the samples were measured using Conductometer (Mettler Toledo, Switzerland). Calibration was carried out before the measurement. Contacting conductivity determination used a sensor (two metal or graphite electrodes) in contact to the electrolyte solution. An AC voltage was applied to the electrodes by conductivity analyzer. The resulting AC current that flowed between the electrodes was used to determine the conductance.

UV Absorption Studies

UV absorption analysis was conducted using UV-vis spectrophotometer, UV-1650PC (Shimadzu, Japan). Sample (0.03 g) was dispersed in 30 mL deionized water. The dispersed sample was shaken and vortexed for 5 minutes and put into the cuvette. Measurement of sample absorbance was performed at wavelengths 290 nm to 410 nm at 25 °C ± 0.5 °C. A spectrum was obtained by scanning the wavelength separator and quantitative measurements were made from it.

RESULTS AND DISCUSSION

Stability Study

Stability tests comprising thaw cycle, stability at room temperature (±25 ºC) for three months and stability at 45 ºC for three months were carried out. All of the formulations were found to be stable without separation occurred at room temperature (±25 ºC) and at 45 ºC for three months and also during the freeze-thaw cycle test. The large molecular structure of xanthan gum supported the encapsulation of TiO_2 using engkabang fat esters without separation. Xanthan gum is used in oil-in-water emulsions to help stabilize the oil droplets against the instability process [14]. It has a double helical structure and undergoes significant hydrogen bonding in solution [15].

Particle Size Measurement

The mean particle size of the formulations, without TiO_2 (E15(1)), with dispersed TiO_2 without encapsulation ((E15(1)-2B, E15(1)-4B and E15(1)-6B)), and encapsulated TiO_2 ((E15(1)-2A, E15(1)-4A and E15(1)-6A)) using engkabang fat esters and beeswax are shown in the Figure 1. The particle sizes of formulation E15(1) was 87 nm. With the addition of 2% TiO_2, the particle size of the formulation E15(1)-2A and E15(1)-2B became 191 nm and 241 nm, respectively. The particle size of formulations E15(1)-4A and E15(1)-4B with 4% of TiO_2 were 195 nm and 268 nm, respectively. Whereas, the particle size of formulations E15(1)-6A and E15(1)-6B with 6% of TiO_2 were 295 nm and 294 nm. The corresponding mean particle sizes of the formulations were increased with the increasing amount of TiO_2 loaded to the samples. The increase in particle sizes can be attributed to the association phenomena between pigment agglomerates and lipid particles [16]. This can be explained through the agglomeration of the compounds undergo either before or during the

manufacturing process, especially when the loads of the compounds are increased [16].

Surface Charge Measurement

The zeta potential of the samples containing engkabang fat esters and beeswax are indicated in the Figure 2. Zeta potential of E15(1), E15(1)-2A, E15(1)-2B, E15(1)-4A, E15(1)-4B, E15(1)-6A and E15(1)-6B were -41 mV, -39 mV, -31 mV, -50 mV, -34 mV, -58 mV and -55 mV, respectively. All the formulations exhibited negative values. Higher zeta potential values were obtained in formulations containing encapsulated TiO_2 than formulations with the non-encapsulated TiO_2. The decrease of zeta potential when TiO_2 was not encapsulated may be due to the decrease of the droplet mobility. The non-encapsulated of TiO_2 could increase the number of particle and droplet in the system due to the distribution of oil droplet and TiO_2 particle. Thus, the available space would become crowded. The separation distance between droplets would also decrease, thus limiting the mobility of the droplets. That may cause a delay in the mobility of the droplets which leads to a decrease in zeta potential.

Zeta potential is a useful parameter to estimate the stability of dispersed systems [16]. The development of a nett charge at the particle surface affects the distribution of ions in the surrounding of interfacial region, resulting in an increased concentration of counter ions (ions of opposite charge to that particle) close to the surface. Thus, an electric double layer exists around each particle. The magnitude of the zeta potential gives an indication of the potential stability of the formulations [17]. Zeta potential values of ≤-21 mV is considered as stable in dispersions, since very little or no agglomeration takes place [18]. On the contrary, the systems showing values higher than -21 mV may have the presence of different grades of agglomeration [18]. If all the particles in suspension have a large negative or positive zeta potential they tend to repel each other and there is no tendency to flocculate. However, if the particles have low zeta potential values then there is no force to prevent the particles from coming together and flocculating.

Transmission Electron Microscope (TEM) Analysis

Figure 3 depicts the particles of TiO_2 that was dispersed in the deionized water. Typical lipid particles containing engkabang fat esters and beeswax can be visualized in Figures 4. Figure 4 depicts the particles of formulation E15(1), which did not contain titanium dioxide. Figure 5 depicts the particles of the formulation E15(1)-2A, which contained 2% encapsulated titanium dioxide. Figure 6 depicts the particles of the formulation E15(1)-2B, which contained 2% non-encapsulated titanium dioxide. The morphological characterization confirms the information concerning encapsulations of particles in the formulations. Particles size of TiO_2 exhibits non-specific diameter, which most of them exist in the range of 50 nm to 200 nm. The oil droplet or particles were seemed to be dispersed evenly in deionized water. The particles size of the droplets was in the range of ±100nm. The droplet can be distinguished, as it has a spherical rough surfaces. In order to determine the changes in the morphology by adding the titanium dioxide pigment, photomicrographs of formulation without titanium dioxide pigments were taken.

Some particles of oil droplets containing TiO_2 were observed in Figures 5(a) and 5(b). In conclusion, the titanium dioxide was successful encapsulated using engkabang fat and beeswax. The dark titanium dioxide pigment was inside the oil droplets. Titanium dioxide pigment was clearly seemed inside the lipid phase. The same results obtained for formulations E15(1)-4A and E15(1)-6A with 4% and 6% encapsulated TiO_2, respectively. Titanium dioxide was particularly favored by this process by virtue of its UV-absorption capacity [16]. Villalobos-Hernández and Müller-Goymann (2005) successfully encapsulated TiO_2 using carnauba wax and decyl oleate in the formulation. TEM micrographs confirmed the presence of encapsulated inorganic particle. The formulation was prepared by dispersing TiO_2 in a lipid phase at 90±5ºC before introducing them into the water phase. High shear dispersion system, Ultraturrax, (IKA, Germany) and high pressure homogenizer (Niro, Italy) were used to prepare the sample [16].

The photomicrographs show that the particles of oil droplets and TiO_2 dispersed together in the system (Figure 6). As expected for a non-encapsulated process, particle dispersed between titanium dioxide clumps and lipid particles could be easily observed. From the previous photomicrographs, the particle size of lipid particles was larger than the particle size of the TiO_2 pigment. In order to minimize the photocatalytic reaction pathways, TiO_2 filters recommended for cosmetic applications are deactivated by microparticle coating (encapsulation) [10-11].

Rheology Study-Flow Behavior

Rheology is well established as the science of the deformation and flow of matter; it is the study of the manner in which materials respond to applied stress or strain [19]. Figures 7 to 9 depict the flow curves of shear stress (τ) and apparent viscosity (η) versus shear rate (γ) for formulations E15(1), E15(1)-2A and E15(1)-2B. The curves of the flow curves showed that at low shear rates, the formulations fluids were more viscous than the Newtonian fluid. At high shear rates, the samples were less viscous. All formulations classified as pseudo-plastic materials under non-Newtonian fluid as the viscosity decreases with increasing velocity gradient. A non–Newtonian fluid is a fluid in which the viscosity changes with the applied shear stress. As a result, non-Newtonian fluids might not have a well-defined viscosity. The addition of TiO_2 and the encapsulation technique did not affect the flow behavior of the formulations. Thus, all formulations could be classified as pseudoplastic materials under a non-Newtonian fluid. Pseudoplastic groups of materials are acceptable in cosmeceutical field. This criterion is important as this sample then could be applied uniformly onto the skin. As shear rate increases, the viscosity of much colloidal dispersion decreases [20].

pH Measurement

Figure 10 depicts the pH values of formulations containing encapsulated and non-encapsulated of TiO_2. The pH value of formulation containing engkabang fat esters without titanium dioxide, E15(1) was 5.38. When 2% TiO_2 was added in the formulations (E15(1)-2A and E15(1)-2B), the pH values became 4.20 and 4.09, respectively. The pH values of formulations with 4% TiO_2 ((E15(1)-4A and E15(1)-4B) were 4.55 and 4.78, respectively. For formulations with 6% TiO_2 ((E15(1)-6A and E15(1)-6B), the pH values were 5.19 and 5.36. The addition of TiO_2 decreased the pH value of the formulations but the increasing of TiO_2 increased the pH values which existed in the range 4 to 6. The pH value of titanium dioxide is in the range of 6.5 to 8.5 and thus gave effect to the pH values of the formulations. Encapsulation of TiO_2 did not affect the pH value of the formulations as exhibited with no significant difference with the non-encapsulated of TiO_2. Coccia, (2009) studied the pH of o/w cosmetic emulsions with high water resistance. The pH of the emulsions ranged from very low values, as in the case of emulsions containing alpha-hydroxyacids (pH 2-3.5), to fairly low values, as in the case of tanning emulsions containing dihydroxyacetone (pH 3-4.5) or only slightly acid, as in the case of sunscreen emulsions or simple skin care emulsions (pH 5-7) [21].

Conductivity Measurement

Conductivity is the ability of a solution to conduct electricity. Conductivity can be used to distinguish O/W from W/O emulsions, since the conductivity is very high when the aqueous phase is continuous and conductivity is very low when oil is the continuous phase [20]. Figure 11 depicts the conductivity values of formulations containing encapsulated and non-encapsulated of TiO_2 using EFE and beeswax. All the formulations show high conductivity values as they were O/W emulsions. Ions are easily formed in aqueous medium and thus make the water rich formulations showing high conductivity values. The conductivity value of the formulation containing engkabang fat esters without titanium dioxide, E15(1) was 270 µS/cm. When 2% TiO_2 was added in the formulations E15(1)-2A and E15(1)-2B, the conductivity values became 181 µS/cm and 162 µS/cm respectively. The conductivity values of formulations with 4% TiO_2 (E15(1)-4A and E15(1)-4B) were 275 µS/cm and 262 µS/cm. For formulations with 6% TiO_2 (E15(1)-6A and E15(1)-6B), the conductivity values were 292 µS/cm and 303µS/cm. Increasing TiO_2 in the formulations gave higher conductivity values due to the existence of metal material that carried charge in both EF and EFE formulations. Titanium dioxide is a semiconducting material [22]. Formulation E15(1) has higher conductivity compared to the formulations of E15(1)-2A and E15(1)-2B with 2% TiO_2 which could be due to the higher water content in the formulation E15(1). Thus, it became less viscous and ions could move more easily. Encapsulation of TiO_2 did not affect the conductivity of the formulations as exhibited by no significant difference with the non-encapsulated of TiO_2.

UV Absorption Study

Each of absorption spectra of the formulations using dilute-solution method was shown in Figure 12. The seven spectra illustrated are for formulations which are primarily considered to be UVA and UVB absorbers or broad spectrum absorber due to the present of TiO_2. Those formulations covered the UVA (290 nm to 315 nm) and UVB (315 nm to 400 nm) range. The spectra E15(1)-2A, E15(1)-4A and E15(1)-6A are those containing TiO_2 which was encapsulated using engkabang fat esters and beeswax. Formulations E15(1)-2B, E15(1)-4B and E15(1)-

6B which contained non-encapsulated TiO_2 showed lower absorption as compared to formulations containing encapsulated TiO_2 when the same percentage of ingredients were used. The formulations containing encapsulated TiO_2 showed higher absorption compared to formulations containing non-encapsulated TiO_2. Encapsulation of TiO_2 leads to a synergistic effect of both molecular sunscreen and the UV scattering by the particles [8-9].

Encapsulation shows a UV-blocking potential where they act as physical sunscreens on their own and can be combined with molecular sunscreens in attempt to achieve improved photoprotection [23]. The increase observed in the UV protection of formulations containing encapsulated TiO_2 as compared to the formulations containing non-encapsulated of TiO_2 might be explained by the applied lipid layers covering fully or partially, the surface of pigments. Those modified pigments were responsible for the enhancement of UV absorption [16]. In this work, formulations containing encapsulated TiO_2 using EFE and beeswax successfully obtained and characterized. Encapsulation of TiO_2 in preparing sunscreen formulation increases the efficiency of sunscreen protection along UVA and UVB spectrum for cosmeceutical industry.

CONCLUSIONS

Formulations containing encapsulated TiO_2 using engkabang fat esters and beeswax successfully obtained and characterized. Encapsulation of TiO_2 in preparing sunscreen formulations increases the efficiency of sunscreen protection along UVA and UVB spectrum for cosmeceutical purposes.

Table 1: Chemical composition of formulations containing engkabang fat esters with encapsulated and non-encapsulated titanium dioxide

Substances	w/w (%)						
	E15(1)	E15(1)-2% TiO2		E15(1)-4% TiO2		E15(1)-6% TiO2	
		A-encapsulated	B-non encapsulated	A-encapsulated	B-non encapsulated	A-encapsulated	B-non encapsulated
Part A: Oil Phase							
Engkabang fat esters	10	10	10	10	10	10	10
Beeswax	5	5	5	5	5	5	5
Part B: Water Phase							
Tween85	8	8	8	8	8	8	8
Glycerin	3	3	3	3	3	3	3
Xanthan gum	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Deionized water	72	70	70	68	68	66	66
Part C: Preservative & active ingredient							
Phenonip	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin E	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Essential oil	0.5	0.5	0.5	0.5	0.5	0.5	0.5
TiO2	-	2(in oil)	2(in H_2O)	4(in oil)	4(in H_2O)	6(in oil)	6(in H_2O)

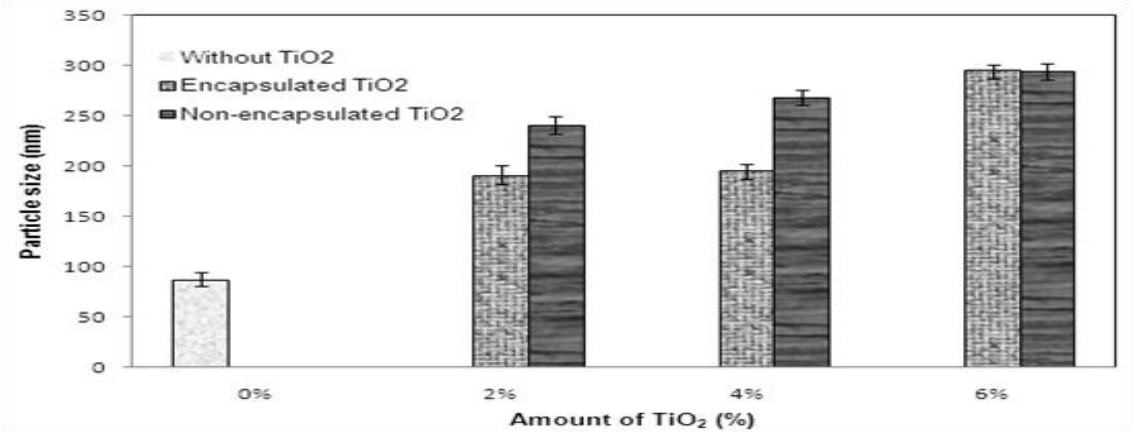

Fig. 1: Particle Size Distribution of Formulations Containing Engkabang Fat Esters

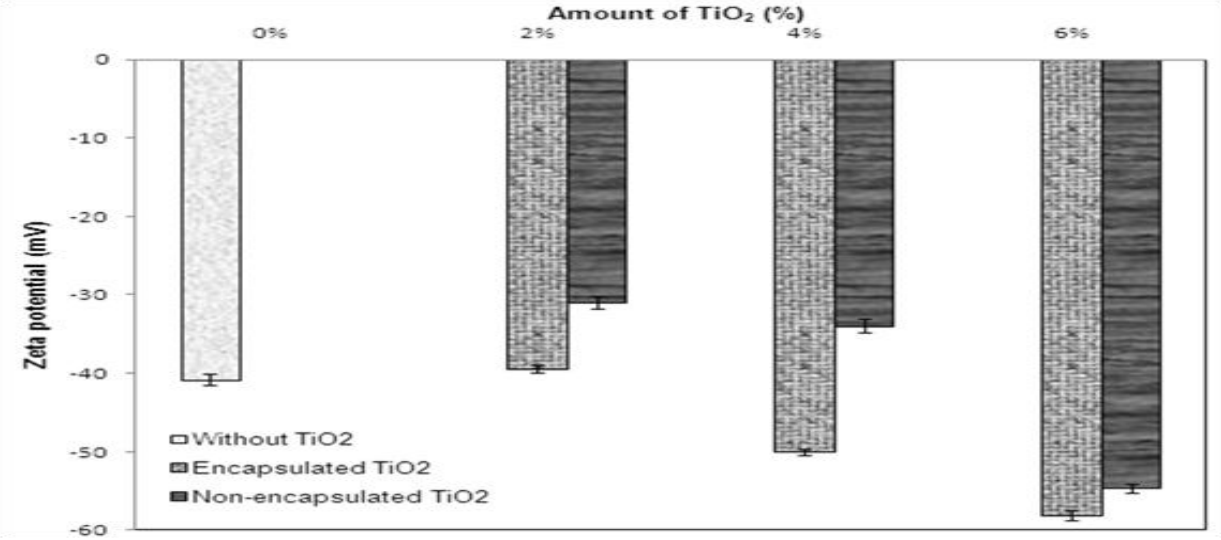

Fig. 2: Surface Charge Measurements of Formulations Containing Engkabang Fat Esters

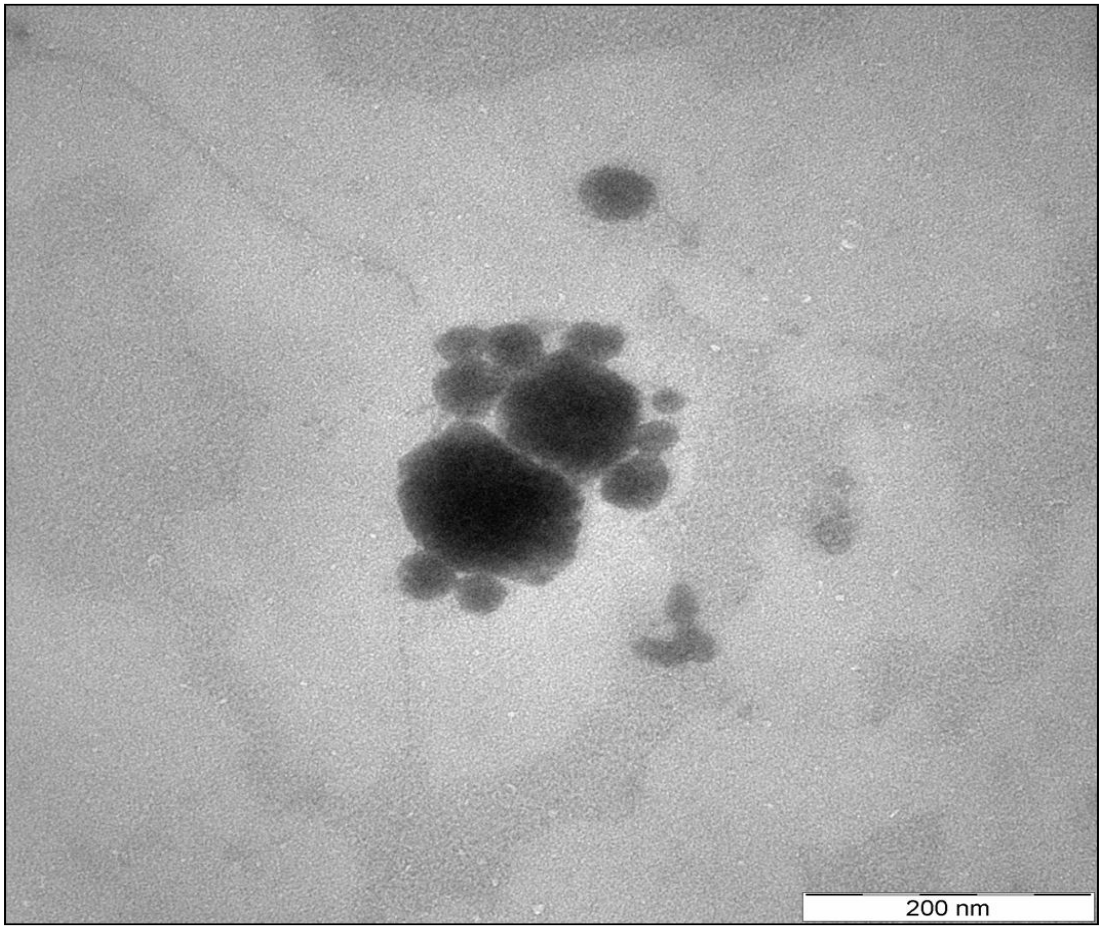

Fig. 3: Titanium Dioxide Dispersed in Deionized Water at Magnification 150 000x

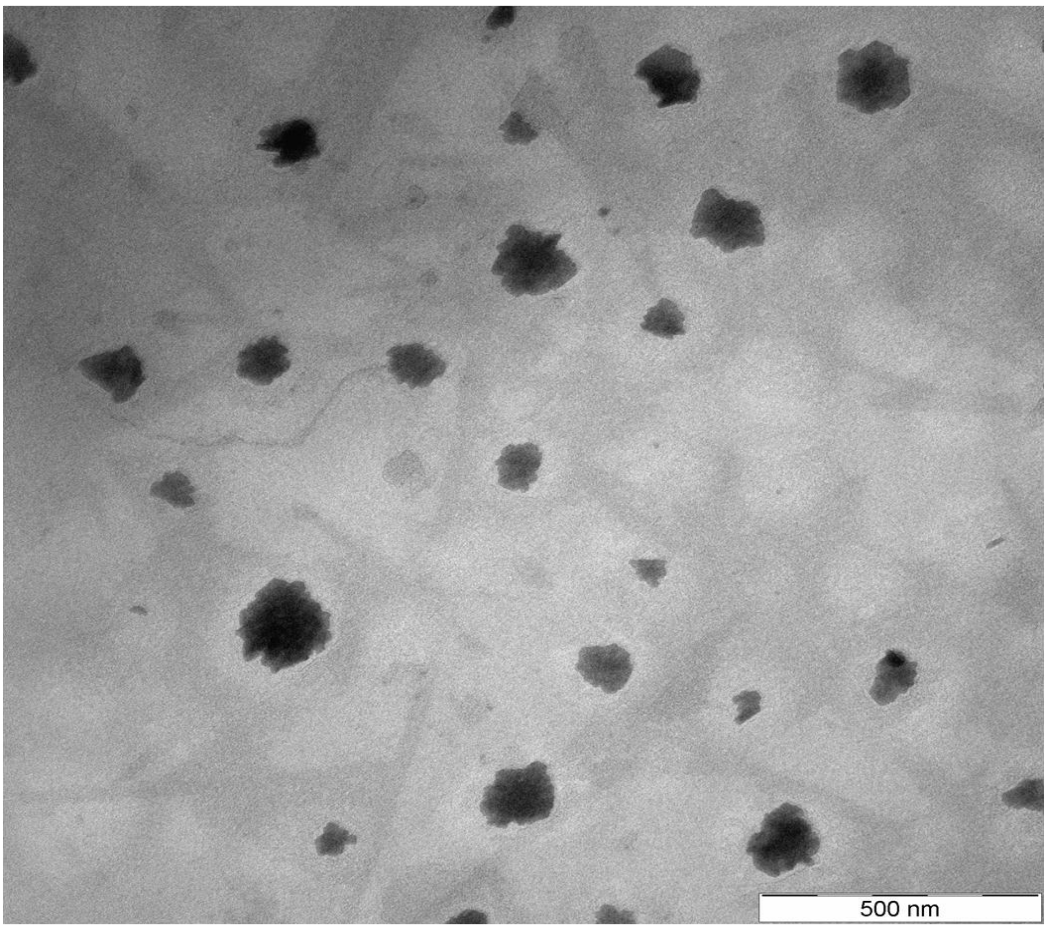

Fig. 4: TEM of Formulation Using Engkabang Fat Esters Without TiO2 (E15(1)) at a Magnification 60 000x

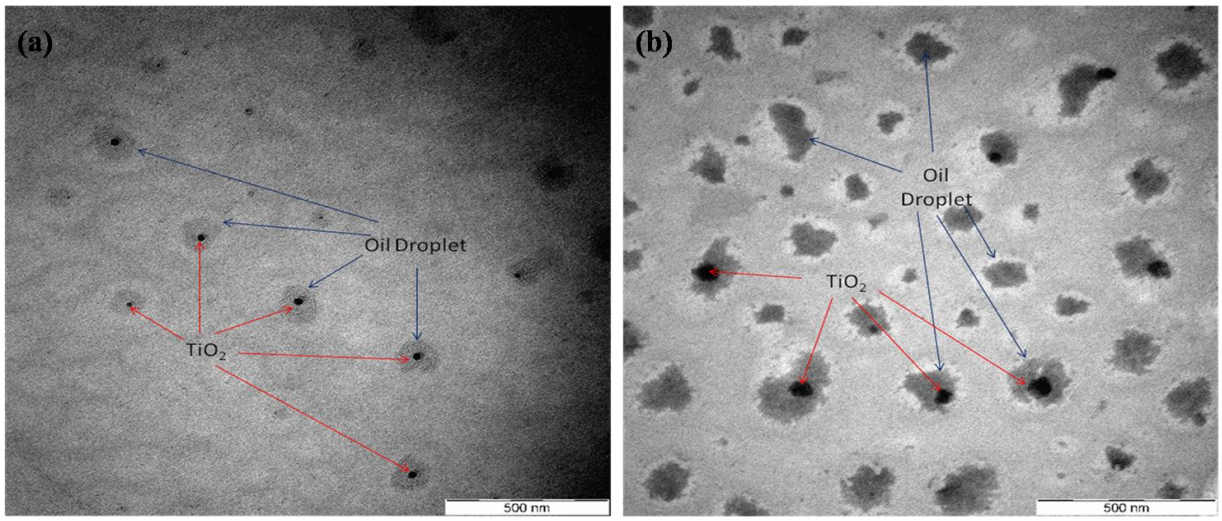

Fig. 5(a): TEM of Formulation Using Engkabang Fat Esters With 2% Encapsulated TiO2 (E15(1)-2A) at a Magnification 60 000x. Figure 5(b): TEM of Formulation Using Engkabang Fat Esters With 6% Encapsulated TiO2 (E15(1)-6A) at a Magnification 70 000x

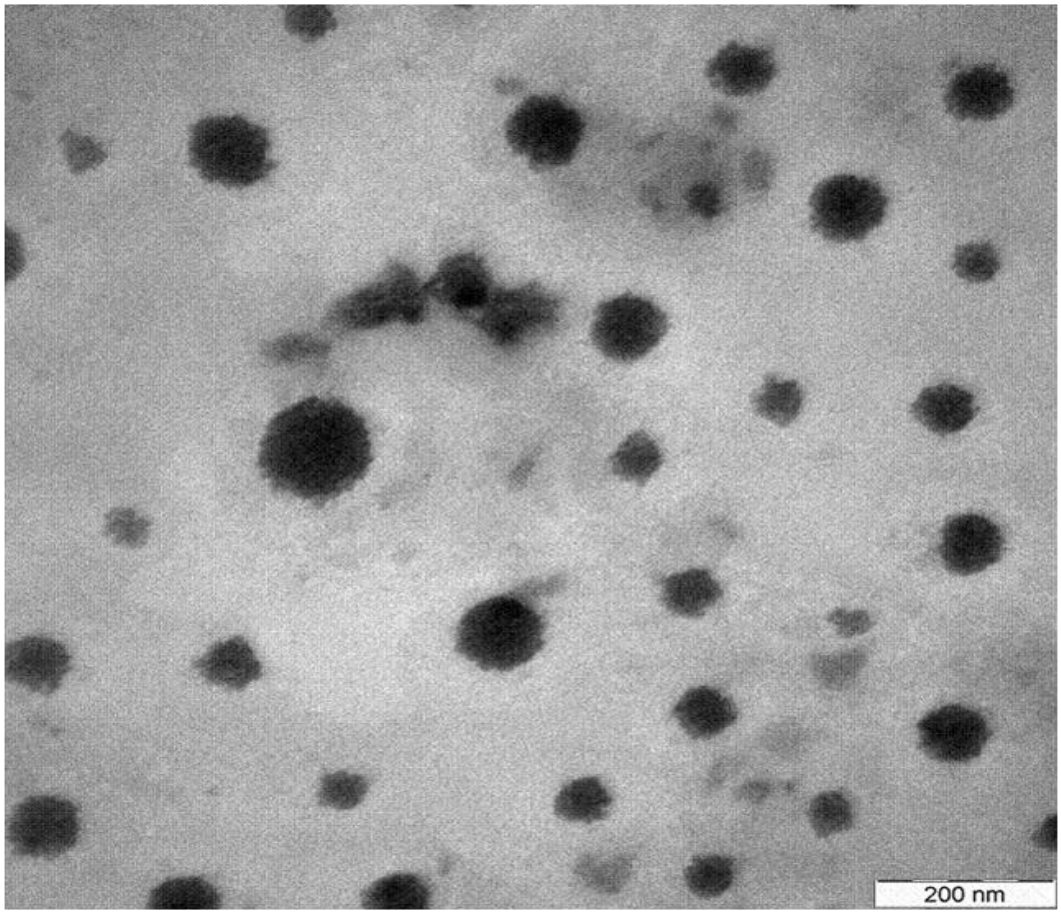

Fig. 6: TEM of Formulation using Engkabang Fat Esters with 2% Non-encapsulated TiO2 (E15(1)-2B) at a Magnification 60 000x

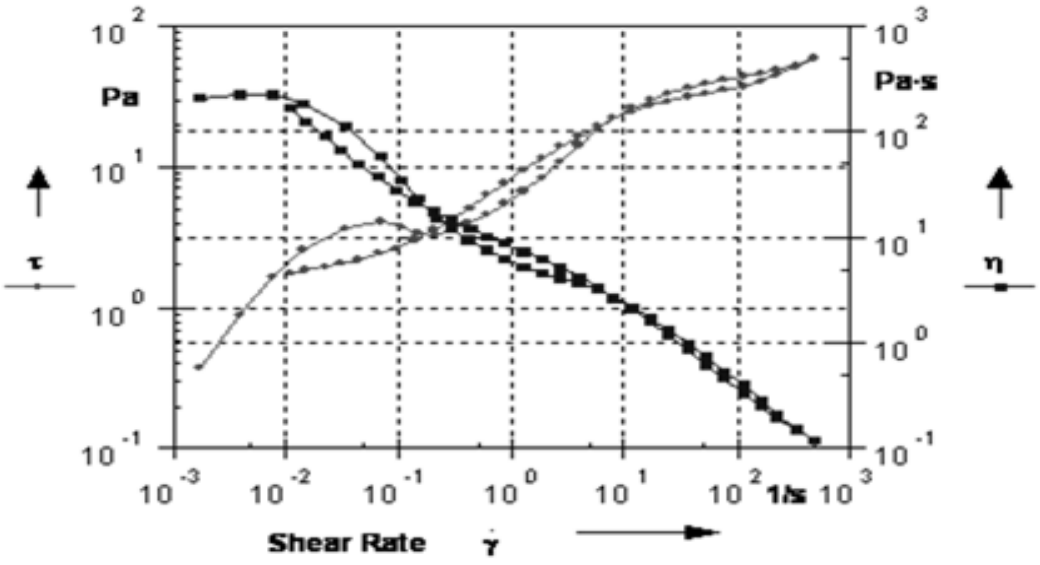

Fig. 7: Graph of Shear Stress (τ) and Apparent Viscosity (η) Versus Shear Rate (γ) for Formulation E15(1

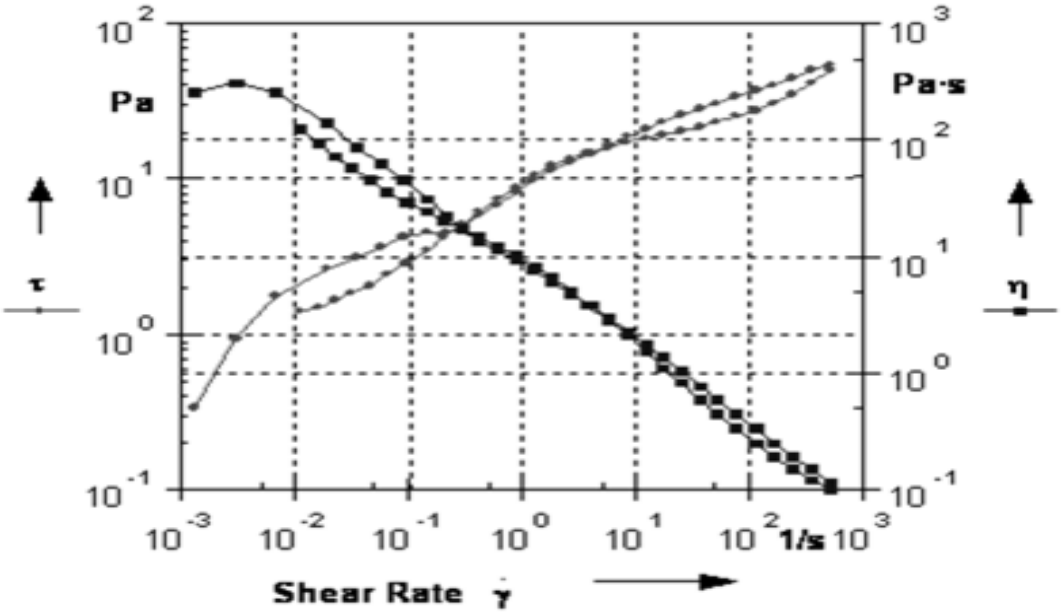

Fig. 8: Graph of Shear Stress (τ) and Apparent Viscosity (η) Versus Shear Rate (γ) for Formulation E15(1)-2A

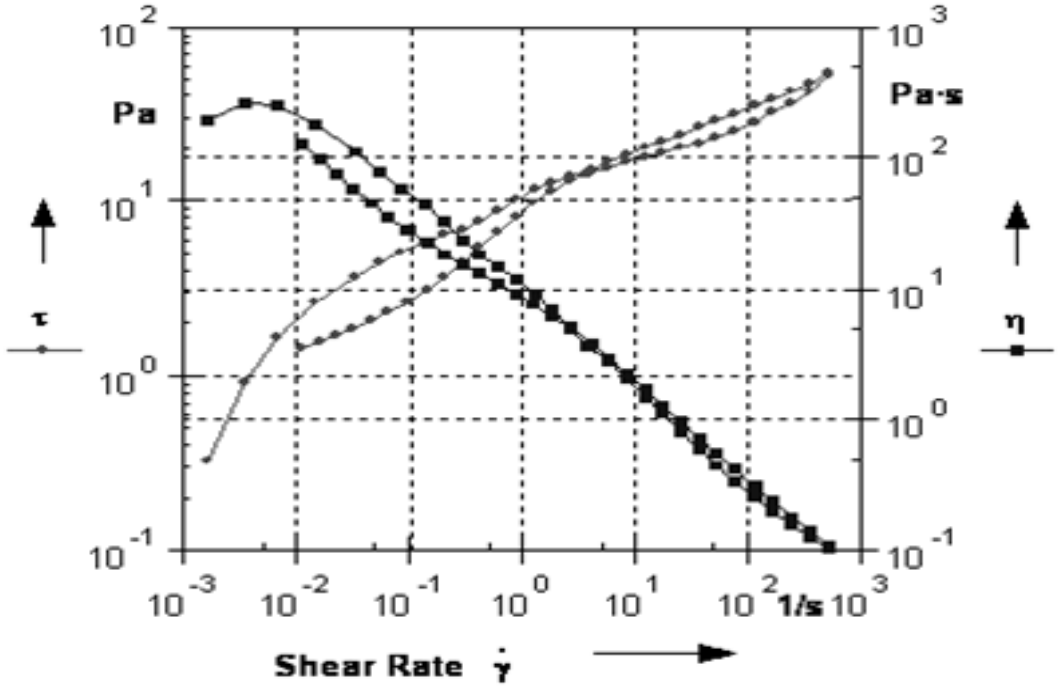

Fig. 9: Graph of Shear Stress (τ) and Apparent Viscosity (η) Versus Shear Rate (γ) for Formulation E15(1)-2B

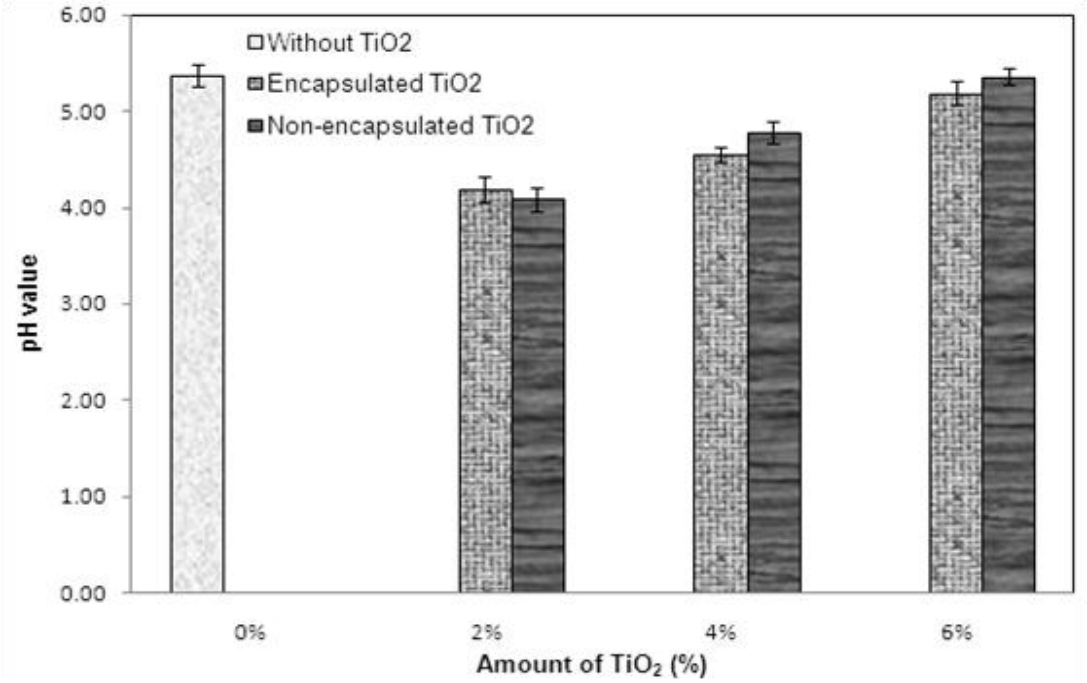

Fig. 10: pH Values of Formulations containing Engkabang Fat Esters

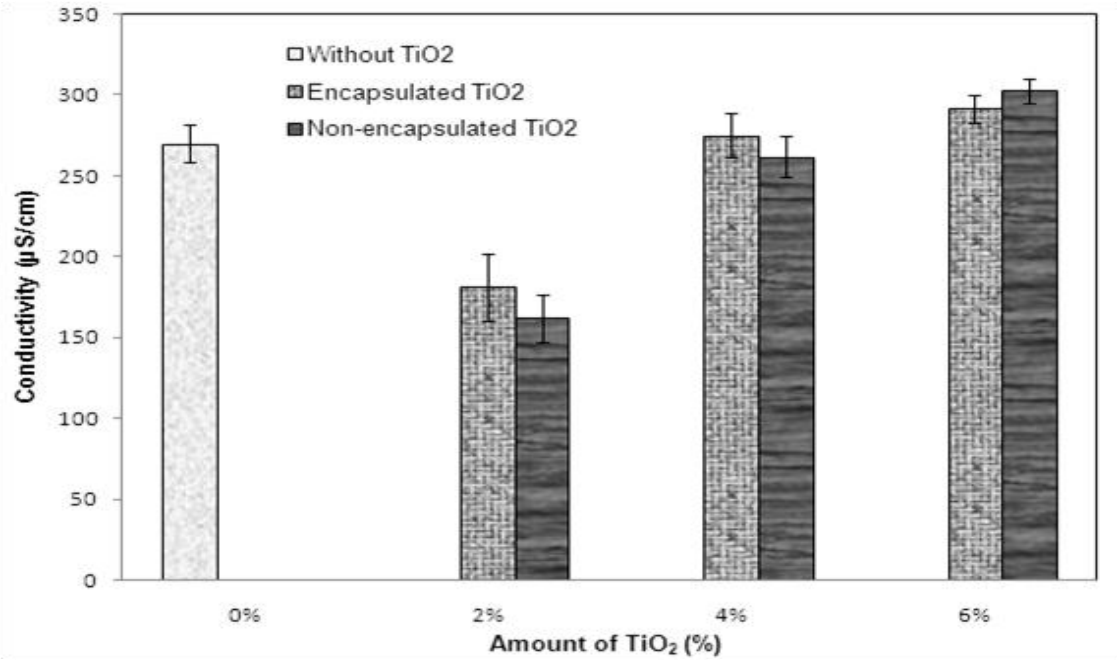

Fig. 11: Conductivity Measurement of Formulations containing Engkabang Fat Esters

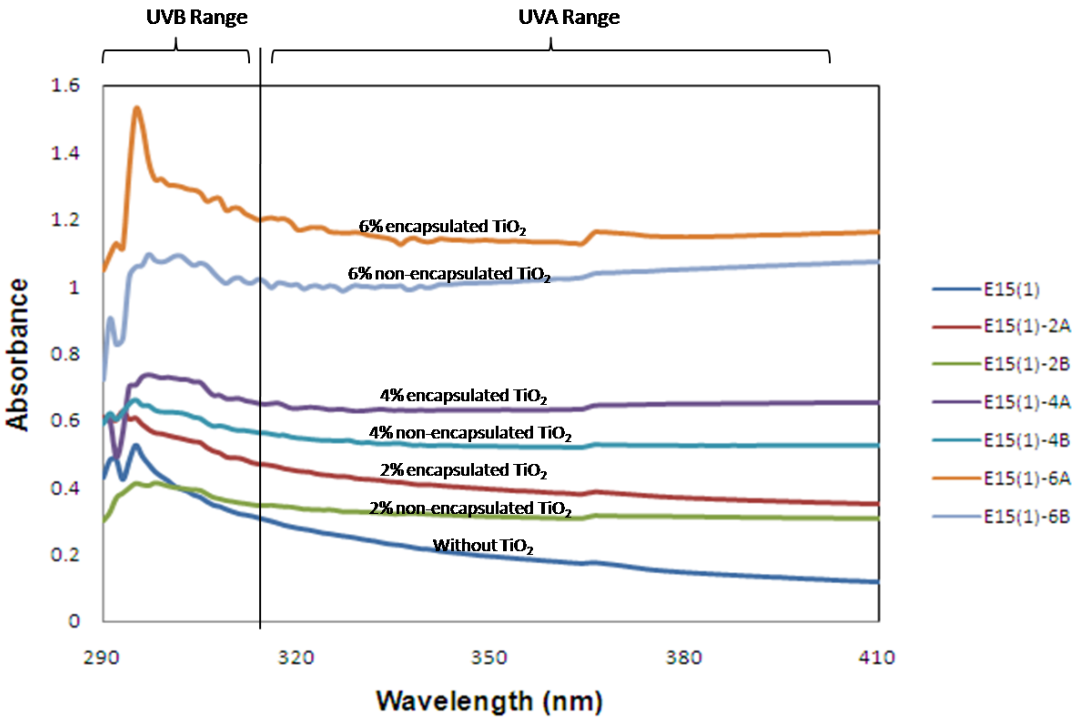

Fig. 12: UV-Absorption Spectra of the Formulations Containing Engkabang Fat Esters

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