

Engkabang Fat as a Base in Preparing Encapsulated Titanium Dioxide for Cosmetics Purpose

SITI SALWA ABD GANI¹, MAHIRAN BASRI^{1*}, MOHD BASYARUDDIN ABDUL RAHMAN^{1,2}, ANUAR KASSIM¹,
RAJA NOOR ZALIHA RAJA ABD RAHMAN³, ABU BAKAR SALLEH³ and ZAHARIAH ISMAIL⁴

¹Department of Chemistry, Faculty of Science, University Putra Malaysia, Serdang, 43400 Selangor, Malaysia

²Structural Biology Research Centre, Malaysia Genome Institute, 43600 Bangi, Selangor, Malaysia

³Faculty of Biotechnology and Biomolecular Science, University Putra Malaysia, Serdang, 43400 Selangor, Malaysia

⁴Sime Darby Plantation Sdn. Bhd., Carey Island, 42960 Kuala Langat, Selangor, Malaysia

*Corresponding author: Fax: +60 3 89466997; Tel: +60 3 89467266; E-mail: mahiran@science.upm.edu.my

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The aim of the present study is to encapsulated titanium dioxide using engkabang fat and beeswax in emulsion system in order to enhance the UV attenuation, test the stability of the samples and characterized them. Those formulations were prepared using high shear homogenizer and followed by high pressure homogenizer. Engkabang fat and beeswax were used as a base material in preparation of formulations. Surface charge measurements of formulations comprising values from -30 mV to -36 mV denoted the presence of stable dispersions. The morphological characterization confirmed the encapsulations of titanium dioxide in the formulations F10-2A. The presence of TiO₂ gave higher conductivity values due to the existence of metal material that carried charge. The formulation containing encapsulated of TiO₂ gave higher absorbance compared to the formulation containing non-encapsulated of TiO₂. Thus, it is effective for cosmeceutical industry.

Key Words: Engkabang fat, High pressure, Encapsulation, Titanium dioxide.

INTRODUCTION

Engkabang fat is great in creams, lotions and in body butters such as for massage creams, make-up, sunscreens, lipstick, balm and other stick type applications where a higher melting point is desired. It has wide application in the manufacture of cosmetic and skin care products because of its stable emulsions and its softening effect on the skin. It can also prevent dry skin and the development of wrinkles, reduce degeneration of skin cells and restore skin flexibility and elasticity. Engkabang fat has physical properties like cocoa butter, closely resembling cocoa butter but with a higher melting point. Its slip melting point in range 34 to 38 °C. Engkabang fat has about 50 % stearic-oleic-stearic (SOS) triglycerides and 35 % palmitic-oleic-stearic (POS). Stearic-oleic-stearic material is stearic acid in SN₁ and SN₃ position and oleic acid in SN₂ position. Stearic-oleic-stearic is related to the molecules of triglycerides in cocoa butter. The engkabang fat is principally used in the confectionery industry especially in the manufacture of chocolate¹. The natives, dayaks, have always used engkabang fat for medicinal, food or cosmetic purposes.

The use of sunscreens helps to reduce skin damage produced by the UV radiation from sunlight^{2,3}. In order to increase the efficiency and efficacy of sunscreens, suitable

carrier systems have to be developed⁴. Photoreactivity has been raised as an issue with these materials. Both TiO₂ and ZnO are semiconductors potentially absorbing light and generating reactive species⁵. These effects have been shown *in vitro*⁶. Coating of these materials reduces their photochemical reactivity⁷. To reduce the possibility of photocatalytic activity of TiO₂, it is often coated when used in cosmetic preparations⁸. Coated metallic oxide sunscreens are stable, are very efficient UV attenuators and are nontoxic and safe⁸. Incorporation of sunscreens into the matrix of the particles (encapsulated) leads to a synergistic effect of both sunscreen and the UV scattering by the particles^{9,10}.

EXPERIMENTAL

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 acid¹. Polyoxyethylene (20) sorbitan tri-oleate (Tween 85) were obtained from Merck Schuchardt OHG, Hohenbrunn, Germany. 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: The mixing of compositions was carried out by emulsification method using hot-hot process. The oil which contained engkabang fat and beeswax and water phases which contained deionized water, Tween 85 and glycerol were prepared separately in the required amounts and heated up to 75 °C until all the ingredients are dissolved. To encapsulate TiO₂, it was dispersed in the oil phase and stirred using a magnetic stirrer at 75 °C for 10 min. To evaluate the performance of TiO₂ without oil encapsulated, suspension of TiO₂ were prepared. TiO₂ was dispersed in water phase for 10 min 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 10000 rpm until the temperature of the mixture dropped to 40 °C. At 40 °C, toco-trienol, essential oil and phenonip as a preservative were added into the mixture. Then, the mixture was put inside the high pressure homogenizer at 500 bar for three cycles. The final product was further homogenized using stirrer (IKA®RW 20 Digital, Japan) at 300 rpm, while the temperature dropping to room temperature. Table-1 depicts the compositions of formulations for engkabang fat with encapsulated and non-encapsulated titanium dioxide.

Substances	w/w (%)		
	F10	F10-2 % TiO ₂	
		A-encapsulated	B-non encapsulated
Part A: Oil phase			
Engkabang fat	9	9	9
Beeswax	6	6	6
Part B: Water phase			
Tween85	8	8	8
Glycerin	5	5	5
Deionized water	70.5	68.5	68.5
Part C: Preservative and active ingredient			
Phenonip	0.5	0.5	0.5
Vitamin E	0.5	0.5	0.5
Essential oil	0.5	0.5	0.5
TiO ₂	-	2 (in oil)	2 (in H ₂ O)

Stability and pH value: The assessment of product thermodynamic stability was carried out by the thaw cycles. In this method, the samples were stored in refrigerator at ± 5 °C for 24 h and then thawed at room temperature for 24 h. This step was repeated for three times. Further stability tests were carried out where the samples were stored at room temperature and 45 °C for 3 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 °C, 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: Particle size distribution of samples were measured by diffusion method using a dynamic light scattering (DLS) particle analyzer (Nanophox Sympatec, Germany) with argon laser ($\lambda = 488$ 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 ± 0.5 °C. The results were presented as the volume distribution.

Surface charge: 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 1 mL 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.

Transmission electron microscopy (TEM) analysis: A drop of the sample was dispersed in deionized water in a 10 mL 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 min. The sample on the copper grid was stained using 2 % phosphotungstic acid (PTA) with pH value 7.2 for 10 min. 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.

Thermogravimetric analysis (TGA): Thermogravimetric analysis was conducted using TGA/SDTA851^e (Mettler Toledo, Switzerland). The increasing temperature rate was 10 °C/min at temperature range from 0 to 600 °C. Liquid nitrogen was used as carrier in the system.

UV absorption study: 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 min and put into the cuvette. Measurement of sample absorbance was performed at wavelengths 290 nm to 410 nm at 25 ± 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 of thaw cycle, stability at room temperature (25 °C) for 3 months and stability at 45 °C for 3 months were carried out. All of the formulations were found to be stable at room temperature (25 °C) and at 45 °C for 3 months and also along thaw cycle test. Storage at higher temperatures produce significant acceleration of the changes taking place at normal storage conditions but instability at high temperatures does not necessarily indicate instability at 'normal' temperatures¹¹.

Particle size: The mean particle size of the formulation containing engkabang fat without TiO₂ (F10), formulation containing dispersed TiO₂ without encapsulation (F10-2B) and formulation containing encapsulated TiO₂ (F10-2A) using engkabang fat and beeswax are shown in the Fig. 1. The particle size of the formulation F10 was 154.98 nm and it was quite similar with the formulations loaded with 2 % of TiO₂ which were in the range 100 to 200 nm. Nano-emulsions is emulsions having droplet radius size range of 50-200 nm¹². The particles size of F10-2A and F10-2B were 176.3 nm and 165.08 nm, respectively. It has to be noticed that the particles containing encapsulated pigment were larger as compared to the particles containing non-encapsulated pigment.

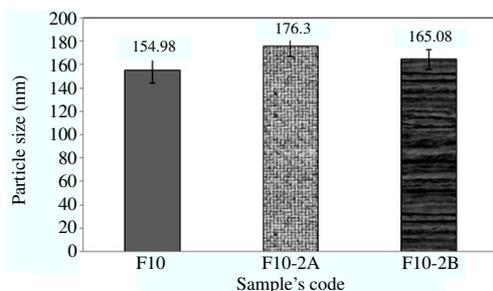


Fig. 1. Particle size distributions containing engkabang fat

Surface charge: Zeta potential is a useful parameter to estimate the stability of dispersed systems⁴. Surface charge measurements of formulations F10, F10-2A and F10-2B comprising values from -30.4 to -36.4 mV denoted the presence of stable dispersions. Zeta potential values of ≤ -21 mV are considered as stable in dispersions, since very little or no agglomeration takes place¹³. The performed measurements of the samples were shown in Fig. 2. All the formulations exhibited negative values. The development of a nett charge at the particle surface effects the distribution of ions in the surrounding interfacial region, resulting in an increased concentration of counter ions (ions of opposite charge to that of the particle) close to the surface. Thus, an electric double layer exists around each particle. 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. The general dividing line between stable and unstable suspensions is generally taken at either +30 or -30 mV. Particles with zeta potential more positive than +30 mV or more negative than -30 mV are normally considered stable¹⁴.

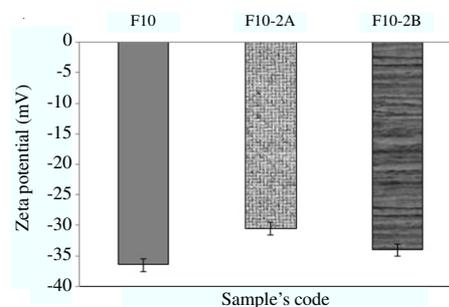


Fig. 2. Surface charge measurements of formulations containing engkabang fat

Transmission electron microscopy (TEM) analysis: The morphological characterization confirmed the information concerning the mean particle size and the encapsulations of particles in the formulations. Typical titanium dioxide (TiO₂) can be visualized in Fig. 3 depicts the particles of TiO₂ that was dispersed in the deionized water. Particles size of TiO₂ exhibit non-specific diameter, which most of them exist in the range of 50 to 200 nm. Fig. 4 depicts the particle distribution of formulation using engkabang fat without TiO₂ or also known as F10 at a magnification 60000x.

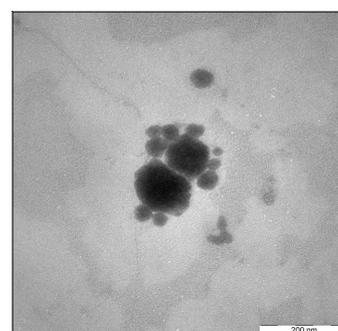


Fig. 3. Titanium dioxide dispersed in deionized water at magnification 150000x

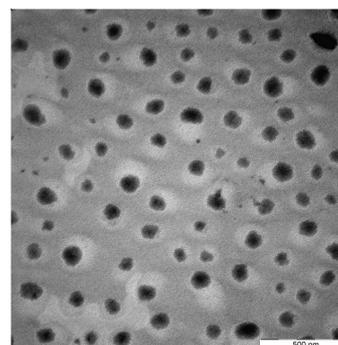


Fig. 4. TEM of formulation using engkabang fat without TiO₂ (F10) at a magnification 60000x

Fig. 5 depicts the particles of the formulation F10-2A, which contained 2 % encapsulated titanium dioxide. From the photomicrographs, some particles of oil droplets contained TiO_2 are observed. It means that the titanium dioxide was successful encapsulated using engkabang fat and beeswax. The darker particles inside the oil droplets were titanium dioxide pigment. Those modified pigments were responsible for the enhancement of phenomena such as UV-scattering, reflection and absorption closely related to the SPF at different grades⁴.

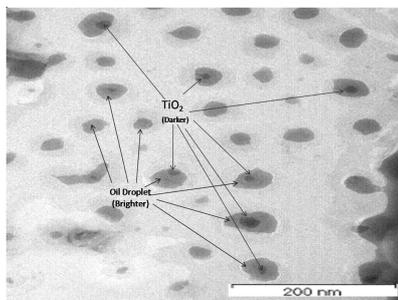


Fig. 5. TEM of formulation using engkabang fat with 2 % encapsulated TiO_2 (F10-2A) at a magnification 60000x

Fig. 6 depicts the particles of the formulation F10-2B, which contained 2 % non-encapsulated titanium dioxide. The photomicrographs show that the particles of oil droplets and TiO_2 dispersed together in the system. As expected for a non-encapsulated process, particle of titanium dioxide and lipid particles were dispersed together in the system without encapsulation.

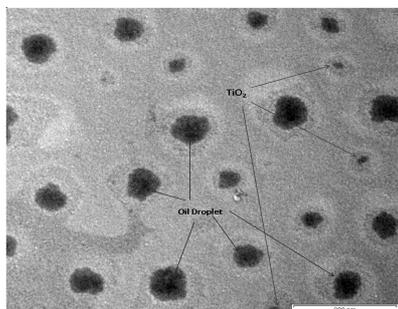


Fig. 6. TEM of formulation using engkabang fat with 2 % non-encapsulated TiO_2 (F10-2B) at a magnification 100000x

pH Measurement: Fig. 7 depicts the pH values of formulations containing engkabang fat. The formulation contained engkabang fat without titanium dioxide (F10), the pH value was 5.59. When 2 % TiO_2 was added in the formulations (F10-2A and F10-2B), the pH values became 4.62 and 4.67, respectively. The addition of titanium dioxide gave the difference

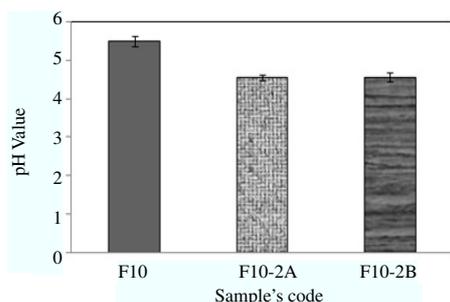


Fig. 7. pH values of formulations containing engkabang fat

values of pH. But the pH values of all the formulations still exist in the range of 4.5 to 6.0, which is the pH of human skin. The pH of skin surface is around 5.5 and often a pH between 4 and 7 is chosen for the aqueous phase of a dermal formulation¹⁵.

Conductivity measurement: Conductivity is the ability of a solution to conduct electricity. Fig. 8 depicts the conductivity measurement of formulations containing engkabang fat. The conductivity of formulation contained engkabang fat without titanium dioxide (F10) was 35.10 $\mu\text{S}/\text{cm}$. When 2 % TiO_2 was added in the formulations F10-2A and F10-2B, the conductivity became 47.27 and 46.00 $\mu\text{S}/\text{cm}$. In the formulations containing engkabang fat, the increasing of titanium dioxide in the samples gave higher conductivity values. A conductivity measurement responds to any and all ions present in a solution¹⁶. Titanium dioxide is a semiconducting material¹⁷. It could give the effect on the conductivity measurement. 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 .

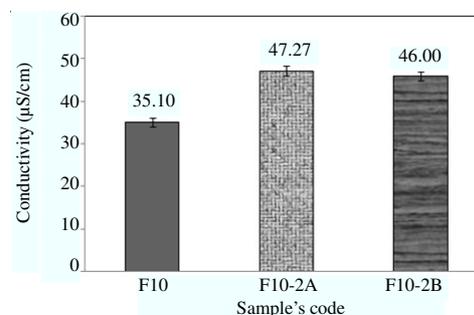


Fig. 8. Conductivity measurement of formulations containing engkabang fat

Thermogravimetric analysis: Thermogravimetric analysis (TGA) is a powerful technique for the characterization of the decomposition or weight loss properties of materials¹⁸. A suitable loss on drying by TGA method for formulations containing engkabang fat with encapsulated TiO_2 (F10-2A) and non-encapsulated TiO_2 (F10-2B) under study were developed. Fig. 9 depicts the TGA thermogram of formulation F10. Three weight losses were observed in the TGA thermogram. The first and second weight losses from room temperature to ca. 160 °C are due to the evaporation of water phase in the samples. The percentages of decomposition at these levels were 20.79

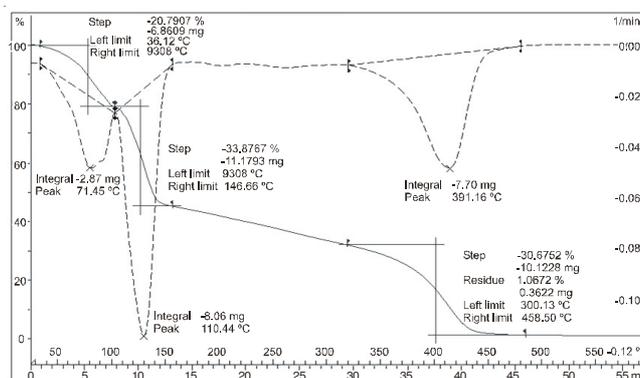


Fig. 9. TGA thermogram of formulation F10

and 33.88 %, respectively. The third weight loss was observed from 330 to 485 °C is due to the decomposition of oil phase of formulation F10. The percentage of the compound decomposed was 30.68 %.

Fig. 10 depicts the TGA thermogram of formulation F10-2A. Three weight losses were observed in the thermogram. The first from room temperature to *ca.* 170 °C is due to the evaporation of water phase (surface water) in the sample. The percentage of decompose at this level was 41.17 %. The second weight loss was observed from 180 to 270 °C is due to the loss of water that is present in the lattice structure. The percentage of compound decomposed was 9.74 %. Then, the weight loss was found again due to the decomposition of oil phase in the sample until 485 °C and the percentage of decomposed was 42.21 %. Most of the compound remained was TiO₂ which has melting point of 1843 °C and boiling point is 2972 °C. The same trend of result was shown in the thermogram of formulation F10-2B. Fig. 11 depicts the TGA thermogram of formulation F10-2B.

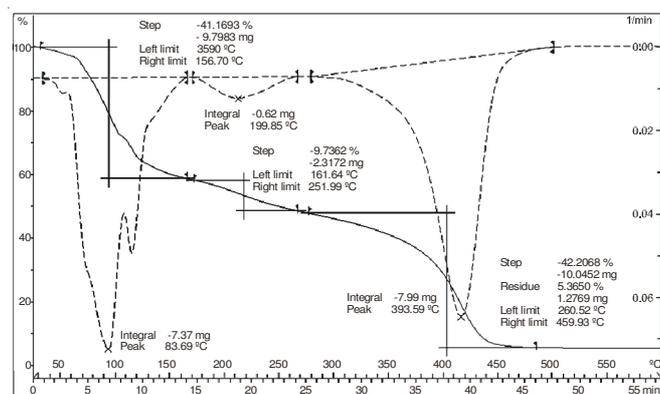


Fig. 10. TGA Thermogram of formulation F10-2A

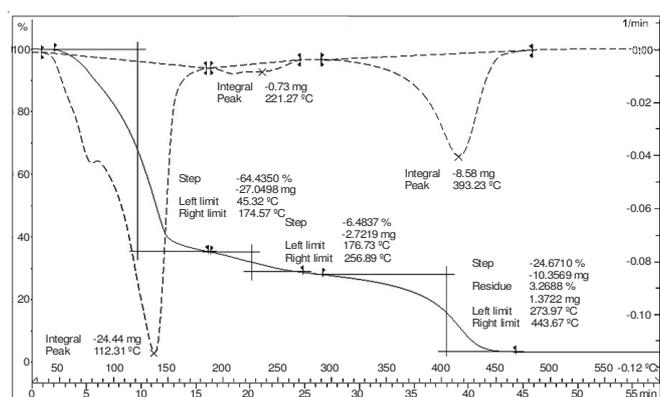


Fig. 11. TGA Thermogram of Formulation F10-2B

UV absorption study: Each of absorption spectra of the formulations using dilute-solution method was shown in Fig. 12. The three spectra illustrated in this figure are for formulations which are primarily considered to be UVA and UVB absorbers or broad spectrum absorber due to the present of TiO₂. Those formulations covered the UVA (290 to 320 nm) and UVB (320 to 400 nm) range. The spectra F10-2A contained TiO₂ to be encapsulated using engkabang fat. Formulation F10-2B which containing non-encapsulated TiO₂

shows lower absorption compared to formulation containing encapsulated TiO₂. The formulations containing encapsulated TiO₂ showed higher absorption compared to formulations containing non-encapsulated TiO₂. Incorporation of molecular sunscreens into the encapsulation leads to a synergistic effect of both molecular sunscreen and the UV scattering by the particles^{9,10}.

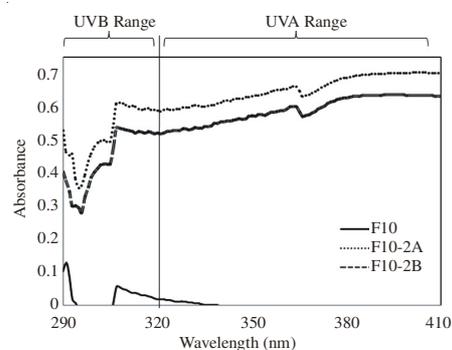


Fig. 12. UV-absorption spectra of the formulations containing engkabang fat

Conclusion

Formulations containing encapsulated TiO₂ using engkabang fat were successfully obtained and characterized. Stable formulations containing encapsulation of TiO₂ enhanced UV absorbance and can be used in preparation of sunscreen formulation.

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