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Cite as: AIP Conference Proceedings **2175**, 020049 (2019); https://doi.org/10.1063/1.5134613
Published Online: 20 November 2019

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Selective Betalain Impregnation from Red Amaranth Extract onto Titanium Dioxide Nanoparticles

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Abstract. In the present work, we reported a selective impregnation of betalain from the red amaranth extract onto titanium dioxide (TiO_2) nanoparticles with the help of (3-chloropropyl)trimethoxysilane (CPTMS) as the organic linker. At first, the red amaranth was extracted using a free-solvent method and encapsulated with maltodextrin. The CPTMS was then used as a linker agent to prepare the composite TiO_2 material containing the red amaranth extract (0.05, 0.10, and 0.20% (w/w)) by an impregnation method in ethanol. After the impregnation, the filtrate color was green while the composite material was obtained as a purplish solid. The diffuse reflectance ultraviolet-visible (DR UV-Vis) spectra of the composite materials showed the absorption peaks at 540 and 665 nm, showing the presence of the betalain pigment. Furthermore, the Fourier-transform infrared (FTIR) spectrum of the composite material confirmed the betalain functional groups, *i.e.* O-H and N-H (3680-2980 cm^{-1}), C-H sp^3 (2924 cm^{-1}), C=O (1632 cm^{-1}), C=C, C=N (1385 cm^{-1}), C-O, C-N (1030-1024 cm^{-1}) and Ti-O-Ti (800-500 cm^{-1}). These results demonstrated that selective impregnation of betalain onto the TiO_2 material could be achieved by using CPTMS as the linker agent.

INTRODUCTION

Red amaranth (*Amaranthus tricolor L.*) is one of the common vegetables that could be easily found, especially in tropical countries. Red amaranth is abundantly available in Indonesia as a tropical country located on the equator line. Generally, red amaranth has two major colors, *i.e.* red and green colors on its leaf and stem parts. These colors come from the existence of natural pigments in red amaranth, such as betalain, chlorophyll, anthocyanin, beta carotene, *etc*. [1]. Many pieces of the research reported the stepwise isolation and purification of red amaranth's natural pigments using the organic solvents. However, these processes were complicated and time-consuming due to the presence of other natural products with similar polarity and chemical properties [2,3].

On the other hand, research on natural dye-sensitized photocatalyst is still attracting and challenging many researchers due to several advantages [4-7]. First, natural sources are usually relative cheap due to their abundant availability in nature. Second, unmodified photocatalyst such as titanium dioxide (TiO_2) is only active in the ultraviolet (UV) region (λ < 390 nm), and thus limiting its application to be used under the visible light region [8]. While impregnation of dye compound onto TiO_2 could be an alternative method to widen the TiO_2 absorption ability

Proceedings of the 5th International Symposium on Applied Chemistry 2019
AIP Conf. Proc. 2175, 020049-1–020049-7; https://doi.org/10.1063/1.5134613
Published by AIP Publishing. 978-0-7354-1922-3/$30.00

to visible light region, the utilization of betalain from natural sources for dye-sensitized photocatalyst is rarely reported. To date, utilization of betalain natural pigment has been limited for dye-sensitized TiO_2 photoelectrode [9] and dye-sensitized solar cell [10].

In the present work, we reported a selective betalain impregnation from red amaranth extract onto the commercial P25 TiO_2 nanoparticles as a promising dye-sensitized photocatalyst candidate. Red amaranth was extracted through a free-solvent method, then betalain natural pigment from red amaranth extract was selectively impregnated onto TiO_2 nanoparticles using (3-chloropropyl)trimethoxysilane as a linker agent. The obtained composite materials were characterized to elucidate and quantify the amount of betalain on the composite materials.

EXPERIMENTAL SECTION

General

Red amaranth plants were obtained from a traditional market in Malang, East Java, Indonesia. The (3-chloropropyl)trimethoxysilane (CPTMS) was purchased from Sigma Aldrich, while aeroxide TiO_2 P25 was purchased from Evonic Industries. Maltodextrin 10-12% was obtained from Yishui Dadi Corn Developing Co. Ltd. and used without any further purification. Ethanol was purchased from E Merck in pro analytical grade.

Procedure

Preparation of red amaranth extract

Red amaranth was extracted in a similar procedure to the one reported previously [11]. Briefly, red amaranth (680 g) was washed with distilled water, dried at room temperature and extracted using a slow juicer without any additional solvents. The obtained viscous extract was encapsulated with maltodextrin in 5% w/w to increase the stability of the red amaranth extract. The mixture was dried using freeze-dry apparatus (Christ, Alpha 1-2 LD plus) for 48 h at 218 K and 0.055 atm to obtain the dried red amaranth extract as a red powder.

Impregnation of betalain from red amaranth extract onto TiO_2 nanoparticles

Preparation of composite material consisting of betalain and TiO_2 nanoparticles was carried out through a simple impregnation method. Three composite materials were prepared by varying red amaranth mass ratio (0.05, 0.10 and 0.20 g) to TiO_2 nanoparticles (1.00 g). The CPTMS (1 mL) and red amaranth extract were added into ethanol (50 mL) and the mixture was stirred at room temperature for 24 h. Afterward, TiO_2 nanoparticles (1.00 g) was added into the mixture and the mixture was stirred at room temperature for 24 h. The mixture was filtered, and the filtrate was characterized by UV-Vis spectrophotometer (Jasco V-760) at 400–800 nm. The residue was washed with ethanol and dried at room temperature to obtain RA-CTPMS/TiO_2 x (x = 0.05, 0.10 and 0.20) composite materials.

Characterizations of RA-CPTMS/TiO_2 Composites

In order to evaluate the successful impregnation of betalain onto the TiO_2, the RA-CPTMS/TiO_2 composites were characterized by diffuse reflectance ultraviolet-visible (DR UV-Vis, JASCO V-760) and Fourier transform infrared (FTIR, JASCO FTIR-6800) spectrophotometers. As for comparisons, the unmodified TiO_2 and the red amaranth extract were also characterized using these instruments.

RESULTS AND DISCUSSION

Preparation of red amaranth extract

Extraction of red amaranth leaves was carried out using a simple procedure [11]. At first, the red amaranth leaves were washed with distilled water to completely remove the soil and dust. The cleaned red amaranth was then dried at room temperature and was further extracted using a slow juicer without any additional solvents to obtain an extract with a high concentration of natural pigments. It is well known that natural pigments are easily degraded by time or by irradiation of light and the presence of oxygen. Therefore, encapsulation of red amaranth extract is necessary to ensure that the pigment would not be degraded during the storage time. Red amaranth extract was encapsulated using maltodextrin at 5% w/w mass ratio to red amaranth extract. The red amaranth extract solution was preconcentrated by freeze-drying method because the natural pigments might be damaged at high temperature. Finally, the red amaranth extract was obtained as a red powder in 4.16% yield.

Impregnation of betalain from red amaranth extract onto TiO_2 nanoparticles

A brief experimental procedure to prepare the RA-CPTMS/TiO_2 composite materials is shown in Fig. 1. The red amaranth extract which amount was in 5, 10, and 20% mass ratio to the TiO_2, as well as CPTMS as the linker agent, were added into ethanol. The mixture was stirred for 24 h to obtain a homogeneous solution (Fig. 1(a)). After additional of TiO_2 nanoparticles, the color of the mixture changed from red to purple (Fig. 1(b)). Furthermore, when the stirring was stopped after 24 h, the mixture was separated into two phases, *i.e.* purplish solid phase and the green filtrate (Fig. 1(c)). The purplish solid was washed with ethanol to remove the adsorbed green pigments from the surface of the composite materials, and the appearance of the obtained composite materials for 5, 10 and 20% mass ratio is shown in Fig. 1(d) from left to right, respectively. While the green filtrates for 5, 10 and 20% mass ratio are shown in Fig. 1(e) from left to right, respectively. This phenomenon is very interesting because two different color substances (purplish solid and green filtrate) could be obtained from a red color mixture.

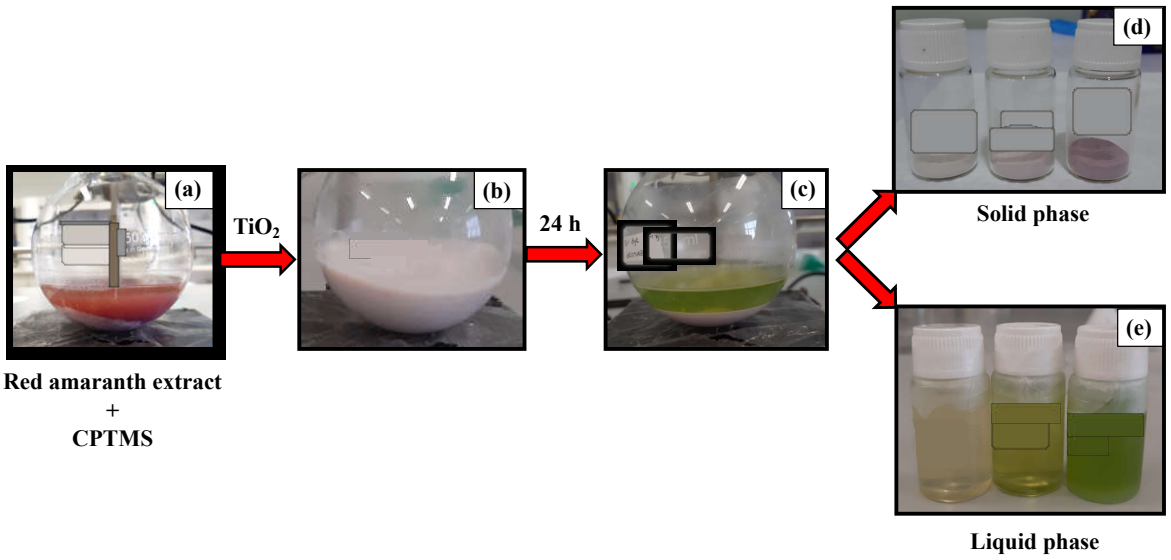

FIGURE 1. Experimental photographs for preparation of RA-CPTMS/TiO_2 composite materials. (a) The reaction mixture of red amaranth extract and CPTMS. (b) The reaction mixture with the addition of TiO_2 nanoparticles. (c) The reaction mixture after the stirring for 24 h was stopped. (d) The obtained solid after filtration. From left to right, the prepared RA-CTPMS/TiO_2 0.05, RA-CTPMS/TiO_2 0.10, and RA-CTPMS/TiO_2 0.2 composites, respectively. (e) The obtained filtrate after filtration of the RA-CTPMS/TiO_2 0.05, RA-CTPMS/TiO_2 0.10, and RA-CTPMS/TiO_2 0.2 composites, respectively.

The green filtrates were checked by UV-Vis spectrophotometer to identify the responsible species exhibiting such green color. The visible spectra of the filtrates obtained after the filtration of the composites are shown in Fig. 2. There are two absorption peaks that could be observed from the visible spectra of the filtrates, *i.e.*, at 434 and 665 nm, which both are the characteristics of chlorophyll pigment [12]. The absorption intensity increased with the increase of the mass ratio of the red dragon fruit extract used to prepare the composites. It is remarkable to be noticed here that the pure chlorophyll pigments could be isolated from the red amaranth extract using this simple procedure.

On the other hand, the DR UV-Vis spectra of the composite materials were recorded to identify which pigment from the red dragon fruit extract that was selectively impregnated on the surface of the TiO_2 nanoparticles. Figures 3 (a)–(c) show the DR UV-Vis spectra of TiO_2 nanoparticles, red amaranth extract, and the obtained composite materials, respectively. The TiO_2 showed a broad absorption peak in the UV region, which maximum was observed at *ca.* 301 nm (Fig. 3(a)), similar to the previously reported spectrum of P25 TiO_2 [13,14]. In contrast, the red amaranth extract showed several absorption peaks in visible region, which were at 322, 439, 554, and 676 nm (Fig. 3(b)). The absorption peak at 322 nm and below could be assigned to the presence of flavonoid [15]. On the other hand, the absorption peaks at 439 and 676 nm would be the characteristics of chlorophyll pigment, which were also found in the visible spectrum of the filtrates, but with a red-shift due to the different environment and solvent effect. In addition to these peaks, the red amaranth extract also showed an absorption peak at 554 nm, which is the characteristic of the betalain pigment [16]. Based on this visible spectrum, it could be suggested that two major natural pigments existed in the obtained red amaranth extract, *i.e.* chlorophyll and betalain pigments.

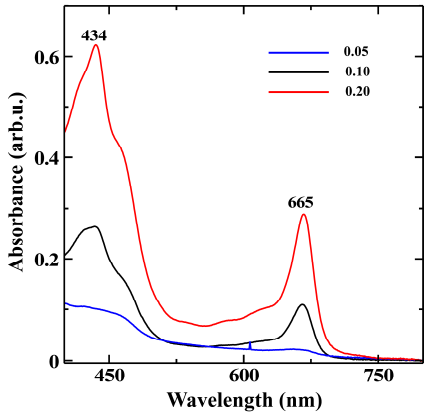

FIGURE 2. Visible spectra of filtrates collected after filtration of RA-CPTMS/TiO_2 composite materials

The DR UV-Vis spectra of the obtained RA-CPTMS/TiO_2 composite materials are shown in Fig. 3(c). There were three major absorption peaks at 248, 301, and 540 nm on all composites. The absorption peaks at 248 and 301 nm were similar to the absorption peaks of the unmodified TiO_2, confirming that the structure of the TiO_2 was not affected and the modification was probably located on the TiO_2 surface. Meanwhile, the presence of an absorption peak at 540 nm confirmed the presence of betalain pigment on the composite material [16]. It could be clearly observed that the natural pigment that was selectively impregnated onto the TiO_2 was the betalain one, not the chlorophyll. The absorption peak intensity increased with the increase of the added red amaranth extract amount on the composite materials during the preparation. Based on the amount of betalain pigment remaining in the filtrate, the amount of the betalain impregnated on the TiO_2 was estimated from the visible spectrum of each filtrate at 540 and 665 nm. It was obtained that the amounts of betalain pigments were 3.44, 8.52 and 17.0% w/w for RA-CPTMS/TiO_2 0.05, RA-CPTMS/TiO_2 0.1, and and RA-CTPMS/TiO_2 0.2 composites, respectively.

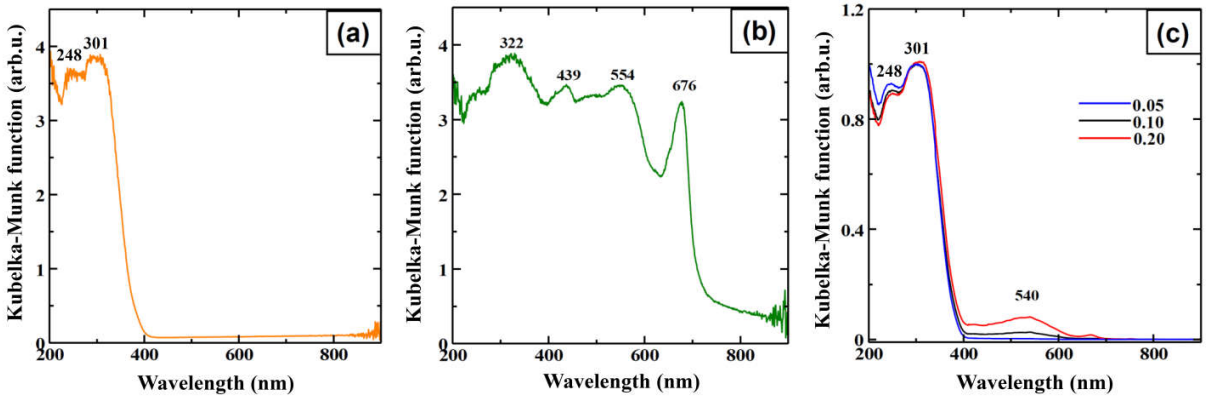

FIGURE 3. DR UV-Vis spectra of (a) TiO_2, (b) red amaranth extract, and (c) RA-CPTMS/TiO_2 composite materials

Further investigation to prove the presence of betalain pigments on the composite materials was carried out by recording the FTIR spectra of the composite materials. Figure 4 shows the FTIR spectra of unmodified TiO_2, red amaranth extract, and the RA-CPTMS/TiO_2 composite materials. The unmodified TiO_2 showed a broad peak at the region of *ca.* 800–400 cm^{-1} due to the presence of Ti-O-Ti functional group. Meanwhile, the red amaranth extract showed several organic functional groups from chlorophyll and betalain compounds, such as O–H and N–H stretching (3670–2985 cm^{-1}), C–H sp^3 stretching (2912 cm^{-1}), C=O is stretching (1630 cm^{-1}), C=C and C=N stretching (1534 and 1381 cm^{-1}), C–O and C–N stretching (1145 and 1013 cm^{-1}). The RA-CPTMS/TiO_2 composite materials showed both characteristic peaks of TiO_2 and the red amaranth extract, indicating the successful impregnation of the red amaranth extract on the TiO_2. Several vibrational peaks from betalain functional groups and TiO_2 could be detected, *i.e.* O–H and N–H (3680–2980 cm^{-1}), C–H sp^3 (2924 cm^{-1}), C=O (1632 cm^{-1}), C=C, C=N (1385 cm^{-1}), C–O, C–N (1030–1024 cm^{-1}), as well as Ti–O–Ti group (800–500 cm^{-1}).

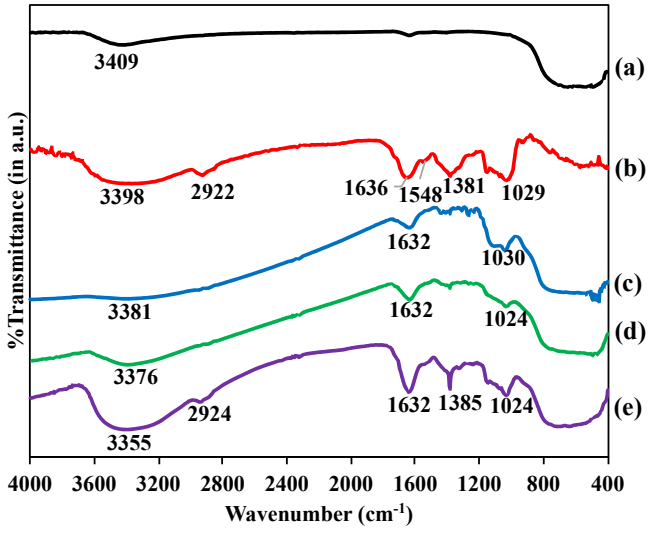

FIGURE 4. FTIR spectra of (a) TiO_2, (b) red amaranth extract, (c) RA-CPTMS/TiO_2 0.05, (d) RA-CPTMS/TiO_2 0.10, and (e) RA-CPTMS/TiO_2 0.2

The interactions between the betalain pigment and TiO_2 in the presence of the CPTMS as the linker agent was proposed and shown in Fig. 5. The most possible interactions would be based on the dipole-dipole interactions between the functional groups of betalain and CPTMS. Fig. 5(a) shows that the interaction between TiO_2-CPTMS

and betalain pigment could happen between the chloro group of the TiO_2-CPTMS and the carboxylic acid group on the tetrahydropyridine heterocyclic ring of the betalain due to less steric repulsion as compared to that of the carboxylic acid group attached in the indoline group. The other possible molecular interaction between TiO_2-CPTMS and betalain is shown in Fig. 5(b), in which the interaction was between the chloro functional groups of the TiO_2-CPTMS and the primary alcohol group of the betalain as the dipole-dipole interaction of primary alcohol would be stronger than secondary alcohol moieties. Therefore, the selective impregnation of betalain pigment from the red amaranth extract was proposed to occur via the molecular interactions between the hydroxyl group of either carboxylic acid or alcoholic parts of the betalain with the chloro group of the CPTMS.

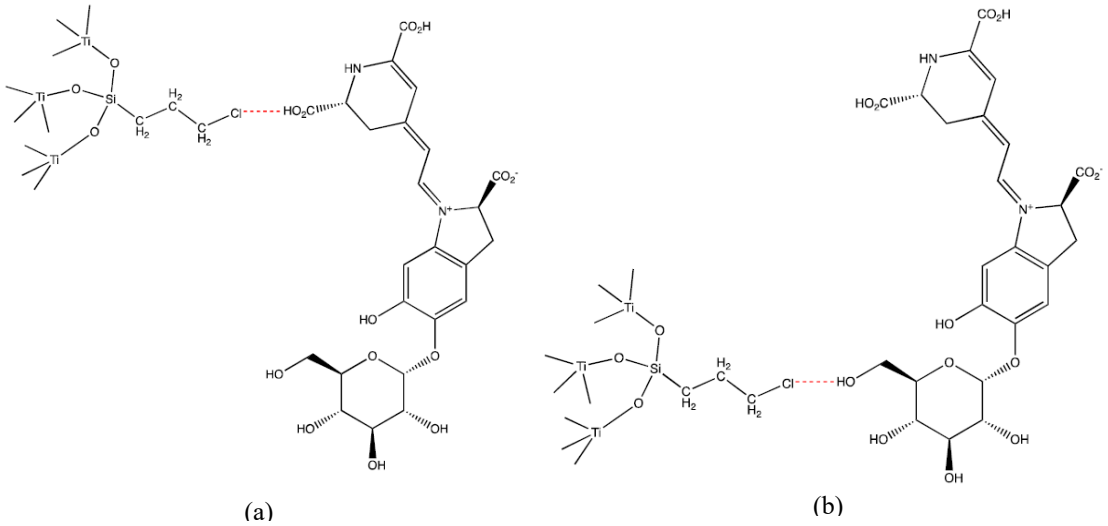

FIGURE 5. Plausible interactions between TiO_2 and betalain pigment using CPTMS as the linker agent via (a) carboxylic acid and (b) hydroxyl group of the betalain and the chloro group of the CPTMS.

CONCLUSIONS

We reported a successful selective impregnation of betalain natural pigment from red amaranth extract containing betalain, chlorophyll, and other natural products onto TiO_2 nanoparticles. The red amaranth was extracted without any additional solvents and freeze-dried to obtain red amaranth extract as a red powder in 4.16% yield. The prepared composite materials using 5, 10 and 20% of red amaranth extract were obtained by a simple stirring method at room temperature using the CPTMS as a linker agent. From the characterizations of the purplish composite materials, it was found that the betalain has been selectively impregnated onto the TiO_2 nanoparticles as evidenced by the appearance of a new peak at 540 nm from the DR UV-Vis spectrum. Moreover, the functional groups of betalain, *i.e.* O-H, N-H, C-H sp^3, C=O, C=C, C=N, C-O, and C-N were detected in the FTIR spectra of the RA-CPTMS/TiO_2 composite materials. These findings are pivotal for simple preparation and development of dye-sensitized materials from natural sources.

ACKNOWLEDGMENTS

Support from Directorate General of Strengthening Research and Development, Ministry of Research, Technology and Higher Education of the Republic of Indonesia via the Higher Education Excellent Applied Research scheme (PTUPT 2019, No. 041/SP2H/LT/MULTI/L7/2019 and No. 005/MACHUNG/LPPM/SP2H-LIT-MULTI/III/2019) is greatly acknowledged.

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Volume 2175

Proceedings of the 5th International Symposium on Applied Chemistry 2019

Tangerang, Indonesia • 23–24 October 2019

Editors • Osi Arutanti, Ahmad Randy and Muhammad Arifuddin Fitriady

AIP Publishing

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Cite as: AIP Conference Proceedings **2175**, 010002 (2019); https://doi.org/10.1063/1.5134564
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Proceedings of the 5th International Symposium on Applied Chemistry 2019
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Conference date: 23-24 October 2019
Location: Tangerang, Indonesia
ISBN: 978-0-7354-1922-3
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Volume number: 2175
Published: Nov 19, 2019

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Fidelis Ayodya Amba, Natasha Vidi Salsabila and Eva Fathul Karamah

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