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Adsorption behavior of natural anthocyanin dye on mesoporous silica

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Abstract

Because of its non-toxicity, naturally occurring anthocyanin is potentially suitable as a colorant for foods and cosmetics. To the wider use of the anthocyanin, the immobilization on the inorganic host for an easy handling as well as the improvement of the stability is required. This study is focused on the adsorption of significant amount of the natural anthocyanin dye onto mesoporous silica, and on the stability enhancement of the anthocyanin by the complexation. The anthocyanin has successfully been adsorbed on the HMS type mesoporous silica containing small amount of aluminum. The amount of the adsorbed anthocyanin has been increased by modifying the pore wall with *n*-propyl group to make the silica surface hydrophobic. The light fastness of the adsorbed anthocyanin has been improved by making the composite with the HMS samples containing aluminum, although the degree of the improvement is not so large.

It has been proposed that incorporation of the anthocyanin molecule deep inside the mesopore is required for the further enhancement of the stability.

KEYWORDS: A. microporous materials, A. organic compounds, D. optical properties, D. surface properties.

1. Introduction

Anthocyanins are one of naturally occurring dyes found in many flower petals and fruits [1, 2]. The structure of the typical anthocyanin is shown in Figure 1. Anthocyanins are non-toxic and used as a colorant of foods and cosmetics [3]. In order to use the anthocyanin more widely, investigations have been made so far in regard to the improvements of the properties of the dye. For the benefit of the ease in handling, and for the increasing affinity to various media, immobilization onto the solid matrices is one of the steps required for the wider practical use of the anthocyanin. In addition, the instability of the anthocyanin is another reason for the limited use: the anthocyanin generally loses its color easily at elevated temperature, neutral to basic pH conditions, and especially under visible-light irradiation [4]. The enhancement of the color stability is essential for practical use of the anthocyanin [5]. The immobilization of the anthocyanin on the solid host materials is also one promising approach to improve the stability [6-8].

In this study, we try to immobilize the anthocyanin on mesoporous silicates. Since the mesoporous silicates are known to have an overwhelming large surface area, they expectedly have potential to adsorb enough amounts of various organic molecules including dye [9-12]. Among many type of mesoporous silicates, an HMS type one is characteristic of rather mild preparation condition that it can be prepared at room temperature in an aqueous solution using neutral alkyl amines as a structure-directing agent [13]. We have previously reported that Al-containing HMS type mesoporous silica adsorbs sufficient amount of the flavylium, a model synthetic dye having the same molecular skeleton as the natural anthocyanin (Figure 1) [14], and that the incorporated flavylium exhibits enhanced stability against visible light irradiation [15, 16]. It has also been reported by other groups that the incorporation into the mesoporous silicate stabilizes other dye molecules [17-19]. In our case, it has been found that the solid acidity caused by the Al^{3+} site on the HMS surface plays an important role in the adsorption and stabilization of the incorporated flavylium.

The present study aims to find the method to let the anthocyanin be adsorbed efficiently on the mesoporous silica without any severe color turn and loss of stability. The effect of the solid acidity on the adsorption behavior and the stability was investigated by adding Al^{3+} to the HMS. In addition, the effect of the hydrophobicity in

1 the pore environment was examined by modifying the inner pore wall of the HMS by
2 alkyl chain groups. Photo-fading of each sample was measured under visible light
3 irradiation to check whether the stability of the anthocyanin was influenced by the
4 modification of the HMS to increase the amount of the adsorbed dye.

2. Experimental

2.1 Materials

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6
7
8 Anthocyanin (AN) was purchased from Kanto Chemical as “Grape Skin Color” and
9 used as received.

10
11 The HMS type mesoporous silica was synthesized following the method described
12 elsewhere [15, 16]. Briefly, tetraethoxysilane and dodecylamine, both supplied from
13 Wako Chemical, were used as a silicon source and a structure-directing agent,
14 respectively. An aqueous ethanolic solution of the dodecylamine (0.91 g in 40 cm³
15 water/5 cm³ ethanol) was mixed with tetraethoxysilane (4.6 g) at 333 K and stirred for 1
16 day. The resulting sediment was collected by filtration and calcined at 903 K for 6 h
17 under air stream. To prepare the Al-containing HMS, 0.12 g of Al(NO₃)·9H₂O supplied
18 from Wako Chemical was blended to the solution of dodecylamine before mixing with
19 tetraethoxysilane. The HMS samples containing aluminum are denoted as Al-HMS. The
20 introduced amount of Al³⁺ was 1.5 mol% to Si⁴⁺. The BET surface area of typical
21 samples was greater than 800 m²/g, and the samples had a narrow pore size distribution
22 with the mean pore diameters in the range between 2.8 and 3.0 nm.

23 Part of the obtained HMS samples was made hydrophobic by modifying the inner
24 pore wall with *n*-propyltriethoxysilane, in a similar manner described in the literature
25 [20]. Thus, 0.5 g of the HMS or Al-HMS was previously dried in an oven at 393 K. The
26 dried HMS or Al-HMS was mixed with 5 cm³ of toluene containing 0.10 g of
27 *n*-propyltriethoxysilane, and then the mixture was refluxed for 6 h under nitrogen
28 stream. The resulting sample was filtered, washed with toluene and acetone, and then
29 dried under reduced pressure for 2 h. Thus obtained hydrophobic HMS and Al-HMS are
30 denoted as HMS(A) and Al-HMS(A), respectively.

2.2 Sample preparation

31
32 The synthesized HMS, Al-HMS, HMS(A) and Al-HMS(A) were all used as the host
33

1 materials for the adsorption of AN. The composite of AN and each host materials was
2 prepared as follows. The aqueous methanolic solution (water: methanol = 7:3 v/v) was
3 used as a solvent of AN. The host material weighed 100 mg was mixed with 100 cm³ of
4 the aqueous methanolic solution containing 20 mg of AN, and then maintained at 278 K
5 in the dark. After 3 days the mixture was filtered and the resulting sediment was dried
6 in the air. The obtained composite sample is denoted as AN/HMS, AN/Al-HMS,
7 AN/HMS(A) or AN/Al-HMS(A).

8 9 **2.3 Measurements**

10 The structure of the synthesized host materials was assured by XRD measurements.
11 The XRD patterns were measured with Rigaku MiniFlex X-ray diffractometer (CuK α ,
12 step angle and scan speed 0.05° and 2°/min, 15 mA, 30kV).

13 The amounts of the adsorbed anthocyanin on each sample were estimated from the
14 optical absorption of the dye in the diffuse reflectance UV-Vis spectra. The UV-Vis
15 spectra were collected with JASCO V-550 spectrophotometer equipped with ISV-469
16 integrating sphere.

17 To investigate the light endurance of the composite samples, the color fading caused
18 by visible light irradiation was evaluated from the decrease in the absorption in the
19 diffuse reflectance spectra. A 100 W halogen lamp (Schott Megalight 100) equipped
20 with a 30 cm length flexible light guide was used as a visible light source. The powder
21 sample was loaded in a plastic cell covered with a thin glass, and placed at the position
22 of 2 cm from the aperture of the light guide.

23 24 **3. Results and discussion**

25 26 **3.1 Structure of the synthesized mesoporous silica**

27 Figure 2 illustrates the XRD patterns of the HMS and Al-HMS samples. A peak at
28 around $2\theta = 2^\circ$ was observed in both samples, indicating that the samples possessed the
29 wormhole type mesopore, as described in the literature [21]. The formation of the
30 uniformed mesopore was thought to be partially obstructed by the addition of
31 heterogeneous atoms (Al³⁺), because the peak intensity of Al-HMS was a little weaker
32 than that of HMS. The loss of the regularity in the mesostructure by the addition of Al³⁺
33 has also been reported on other mesoporous silicas such as MCM-41[22] and

1 FSM-16[23]. However, as the decrease in the peak intensity was small, the extent of the
2 loss of regularity was expected to be negligible in the following experiments.

3 4 **3.2 Adsorption of anthocyanin onto the mesoporous silica**

5 Figure 3 illustrates the diffuse-reflectance UV-Vis spectra of the composite samples.
6 As shown in Figure 3(a), the spectrum of AN/HMS has very low absorption peak at 530
7 nm. This means that the AN/HMS sample only exhibited extremely pale color and that
8 the amount of adsorbed AN was small on the pure HMS. On the other hand, the
9 addition of Al^{3+} to the HMS made the color of the sample strong, as shown in Figure
10 3(b). Therefore, the addition of Al^{3+} was found to be beneficial for the better adsorption
11 of AN on HMS. It has been known that the addition of small amount of Al^{3+} to
12 mesoporous silicas results in the formation of an acidic site, because the shortage of
13 electric charge is caused by the partial substitution of Si^{4+} by Al^{3+} in the silica
14 framework [23]. We have already confirmed the formation of an acidic site by the
15 contamination of Al^{3+} to mesoporous silicas [15]. As the anthocyanin is a cationic and
16 basic natural dye, it seems that the negative charge of the acidic site urged the
17 adsorption of AN on the HMS. In addition, from Figure 3(a) and (b) we can see that the
18 peak shifted to longer wavelength of 548 nm on the AN/Al-HMS. As a consequence of
19 the peak shift, the AN/Al-HMS sample looked a little purplish, but the color change was
20 small to the eye. The bathochromic shift of anthocyanin is generally known to reflect
21 the interaction with metal ions such as Al^{3+} , Fe^{3+} or Mg^{2+} [24-26]. Taking these into
22 consideration, the bathochromic shift seen in the AN/Al-HMS further supports that the
23 enhancement of the AN adsorption was caused by the interaction between the dye and
24 the Al^{3+} site on the HMS surface.

25 The hydrophobization of the mesoporous silicates by modifying the surface with
26 alkyl chains using *n*-propyltriethoxysilane had some effect on the amount of the
27 adsorbed AN. The adsorption of AN on HMS was not prompted by the
28 hydrophobization, since the absorption of AN/HMS(A) was almost the same as that of
29 AN/HMS, as shown in Figure 3(c). On the other hand, Figure 3(b) and (d) show that the
30 amount of adsorbed AN on Al-HMS(A) was quite larger than that on AN/Al-HMS.
31 These results mean that the hydrophobization itself was not effective for the adsorption
32 of AN, whereas the modification with the alkyl chains had a remarkable effect on the
33 adsorption of AN on the Al^{3+} site and helped increase the amount of adsorbed AN. We

1 tentatively think that the coordination of AN to Al^{3+} might be so strong that only limited
2 amount of AN molecule could access to the Al^{3+} site, and that the existence of the alkyl
3 groups around the Al^{3+} site might inhibit an excessive interaction between the Al^{3+} site
4 and the AN molecule. This hypothesis is further suggested by the shift of the absorption
5 peak. As described above, the AN adsorbed on the HMS exhibited its peak at 530 nm,
6 and the peak shifted to 548 nm on AN/Al-HMS. In the spectrum of AN/Al-HMS(A),
7 the absorption peak was found at 542 nm, which was slightly red-shifted compared to
8 AN/Al-HMS. This result implies that the interaction between Al^{3+} site and the AN
9 molecule was weakened by the hydrophobization.

11 **3.3 Photostability of the anthocyanin in the composite samples**

12 The stability of the various AN/HMS samples under visible light irradiation was
13 investigated. Figure 4 illustrates the spectral changes of a typical composite sample,
14 AN/Al-HMS(A) during visible light irradiation from the 100W halogen lamp.
15 Monotonous decrease in the absorbance of the main peak was observed along with the
16 irradiation, indicating that simple degradation of AN occurred. The bleaching of AN
17 under ambient condition is considered to be the destruction of the chromophore ring by
18 the oxidation. The same tendency was also observed in the spectral changes of all other
19 samples than AN/Al-HMS(A) under the irradiation.

20 Figure 5 shows the difference in the stability of AN adsorbed on various host
21 materials. Gradual decrease in the color of AN was observed on all samples under the
22 irradiation. The degree of the fading was smallest on AN/Al-HMS, indicating that the
23 addition of Al^{3+} to HMS had positive effect on the fastness of AN against visible light,
24 although the difference in the stability was not so large from other samples. However,
25 the stabilization effect was perfectly lost by the hydrophobization of Al-HMS, although
26 larger amount of AN was adsorbed on Al-HMS(A) than Al-HMS, as described above.
27 From these results, we concluded that the acidic and electrostatic properties caused by
28 the Al^{3+} contributed to the stabilization of AN on Al-HMS, and that the
29 hydrophobization of Al-HMS hindered the stabilization effect on the AN molecule by
30 the Al^{3+} site. The effect of Al^{3+} in the inorganic host materials on the immobilization
31 and stabilization of natural betalain and another synthetic dye has also been reported [6,
32 27, 28], and the present result was in accordance with the previous reports.

33 The *n*-propyltriethoxysilane has been reported to be immobilized through the surface

1 hydroxyl group of the HMS [29]. On the other hand, the addition of Al^{3+} to silicates
2 causes the formation of the hydroxyl group working as Brønsted acid sites. Accordingly,
3 immobilization of the *n*-propyltriethoxysilane on the Al^{3+} site might preferably take
4 place, resulting in the decrease in the acidic and electrostatic effects caused by the Al^{3+}
5 site, as well as in the steric hindrance of the Al^{3+} site from the AN molecule.

6 7 **3.4 Adsorption behavior of the anthocyanin on HMS**

8 We have reported that the incorporation in the mesoporous silicates has an enormous
9 effect on the stabilization of the adsorbed synthetic flavylum dye, an analogue of the
10 natural anthocyanin [15, 16]. The natural anthocyanin has also been stabilized by the
11 intercalation in the clay interlayer [7]. In those prior researches, the color fading of the
12 incorporated dye has practically been suppressed even under irradiation. We have
13 reached the conclusion that the inhibition of the contact with atmospheric oxygen
14 molecule is one of the reasons of the stabilization of the dye molecules shielded in the
15 inorganic host materials.

16 In the present study, the stabilization effect was indeed seen on AN/Al-HMS,
17 however, the degree of the stabilization was not so large as expected from the previous
18 investigations. As described above, the stabilization of AN on Al-HMS may surely be
19 brought about by the interaction with the Al^{3+} site. Although we do not have a clear
20 answer to the reason why the stabilization effect on AN was not so significant, we think
21 that the inferiority of the stabilization effect of AN to the synthetic flavylum dye may
22 stem from the difficulty in the incorporation of the AN molecules deep inside the
23 mesopore. Since the polarity of the AN molecule is different from that of the flavylum,
24 the affinity of the AN molecule to the environment inside the mesopore may be weaker
25 than that of the flavylum. Steric effect may be another possible reason: as the AN
26 possesses a bulky sugar moiety in its molecular structure, the diffusion of the dye into
27 the inner part of the mesopore is more difficult than the flavylum, having slim
28 molecular structure.

29 The Al^{3+} site of the Al-HMS existed on the outer surface or inside the mesopore near
30 the external surface, as well as the inner pore wall. The electrostatic interaction between
31 the Al^{3+} near the surface and AN might cause the increase in the adsorbed AN, together
32 with the enhancement of the stability, as described in section 3.3. The hydrophobization
33 of the Al-containing HMS by the alkylsilane presented more optimized environment for

1 the adsorption of AN. Thus, the hydrophilic part of the anthocyanin molecule (*i.e.*,
2 hydroxyl groups and sugar moiety) approached to the Al³⁺ site, while the hydrophobic
3 part was protected by the alkyl groups near the Al³⁺ site. Although water-soluble, the
4 AN molecule is reported to have rather hydrophobic character, with the 1-octanol/water
5 partition coefficient (P_{ow}) greater than one [30]. Therefore, it is not surprising that the
6 hydrophobization of HMS contributed to the increase in the adsorption of AN. In the
7 AN/Al-HMS(A), the polar part of the anthocyanin may work as the anchor for the
8 immobilization on the surface of the Al-HMS, whereas the lipophilic property of AN
9 may be avoided by the alkyl chain, resulting in the adsorption of large amount of AN.

10 11 **4. Conclusion**

12
13 Natural anthocyanin can be efficiently adsorbed on HMS type mesoporous silica
14 both by introducing Al³⁺ to the HMS and by making the surface more hydrophobic. The
15 effect of the immobilization of the dye on the stability enhancement against irradiation
16 is limited, perhaps because the dye molecule is not adsorbed in the internal sphere of the
17 mesopore. It is suggested that the adsorption of the dye deep inside the pore may help
18 improving the light fastness of the adsorbed dye molecule.

19 20 21 **Acknowledgment**

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26

1 **Figure Captions**

2
3 **Figure 1.** Typical structure of anthocyanin (Malvidin-3-glucoside). The thick line
4 indicates the flavylum skeleton.

5
6 **Figure 2.** XRD patterns of the (a) HMS and (b) Al-HMS samples.

7
8 **Figure 3.** Diffuse-reflectance UV-Vis spectra of various AN/HMS samples. (a)
9 AN/HMS, (b) AN/Al-HMS, (c) AN/HMS(A) and (d) AN/Al-HMS(A).

10
11 **Figure 4.** Changes in the diffuse-reflectance spectra of the AN/Al-HMS(A) sample
12 during visible light irradiation for 240 min. Irradiation times were 0, 30, 60, 120, 180
13 and 240 min.

14
15 **Figure 5.** Changes in the AN absorption value at λ_{\max} of (a) AN/Al-HMS, (b) AN/HMS,
16 (c) AN/Al-HMS(A) and (d) AN/HMS(A) under continuous irradiation. The stability of
17 AN in the samples is expressed by A/A_0 , where A_0 and A indicate the absorption before
18 irradiation and at the given time after starting irradiation, respectively.

19
20

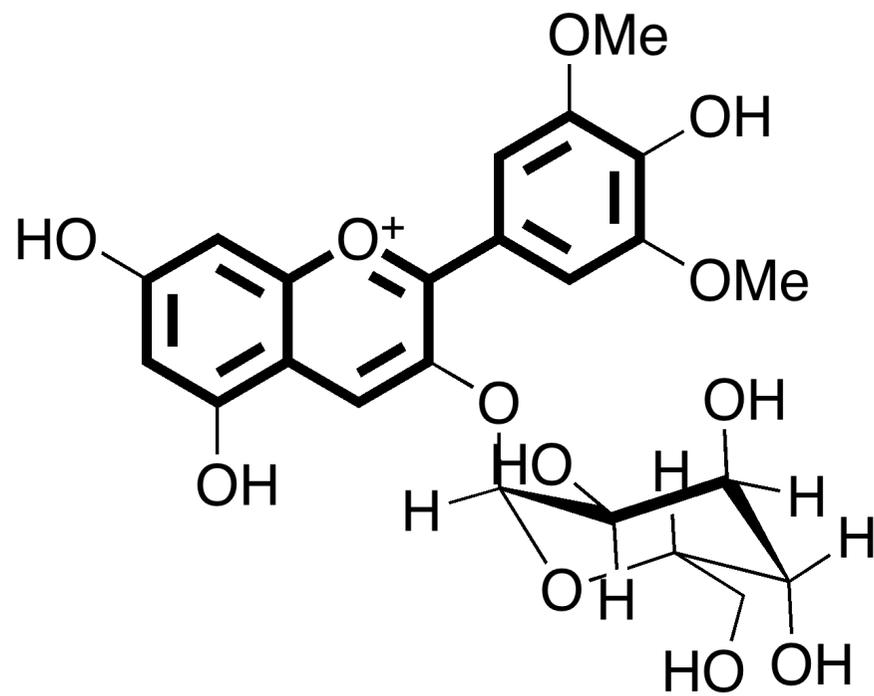


Figure 1

Figure2

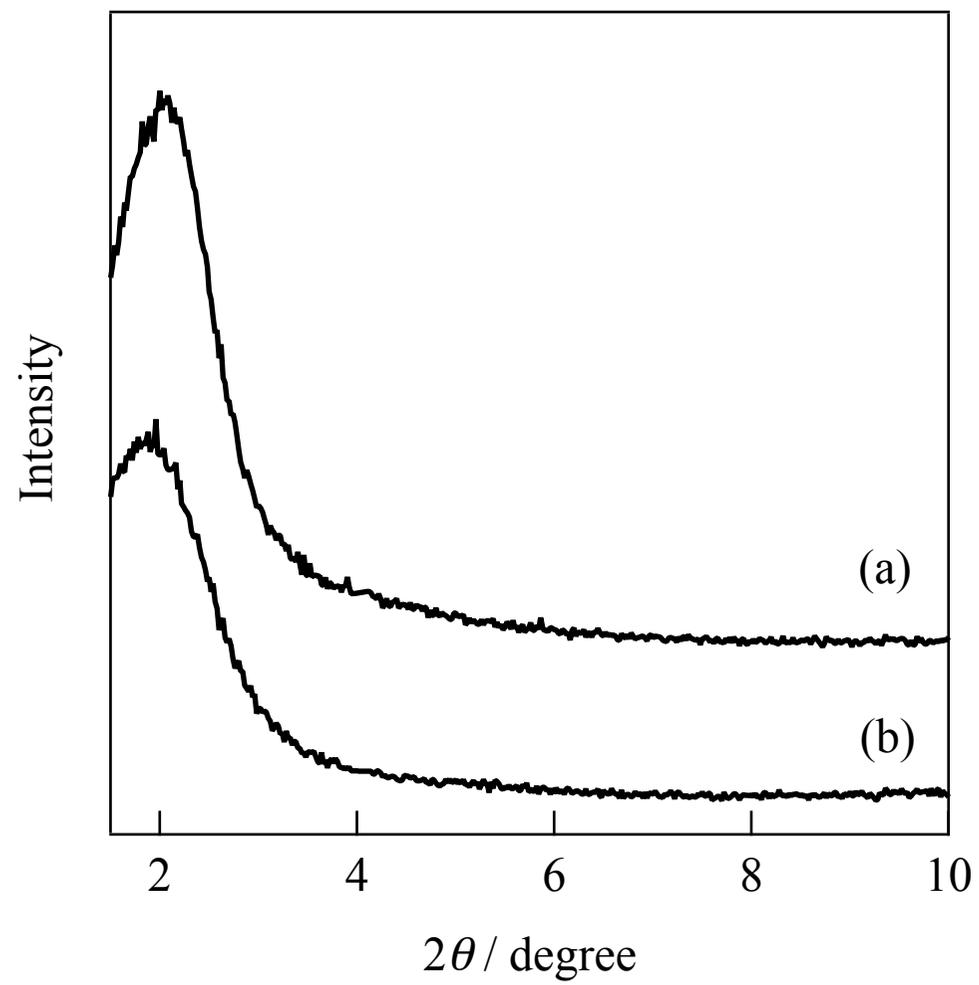


Figure 2

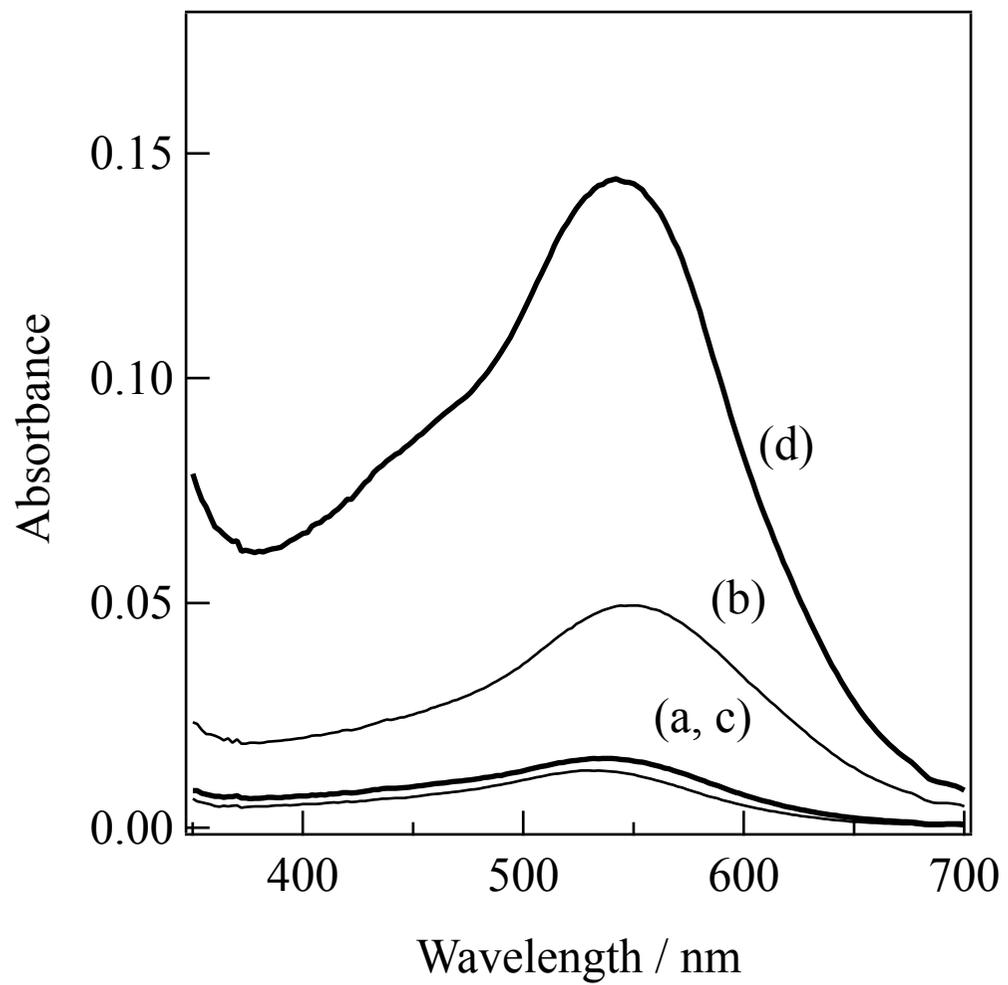


Figure 3

Figure4

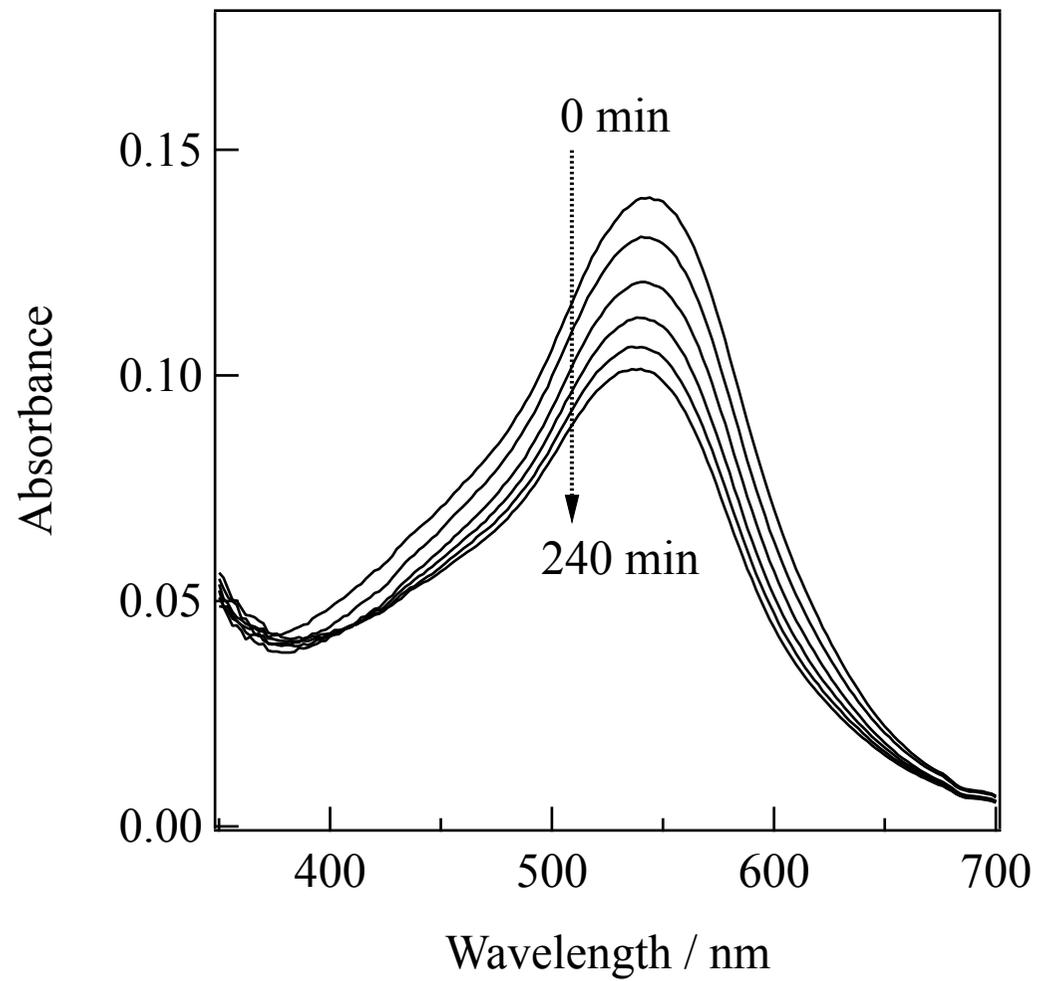


Figure 4

Figure5

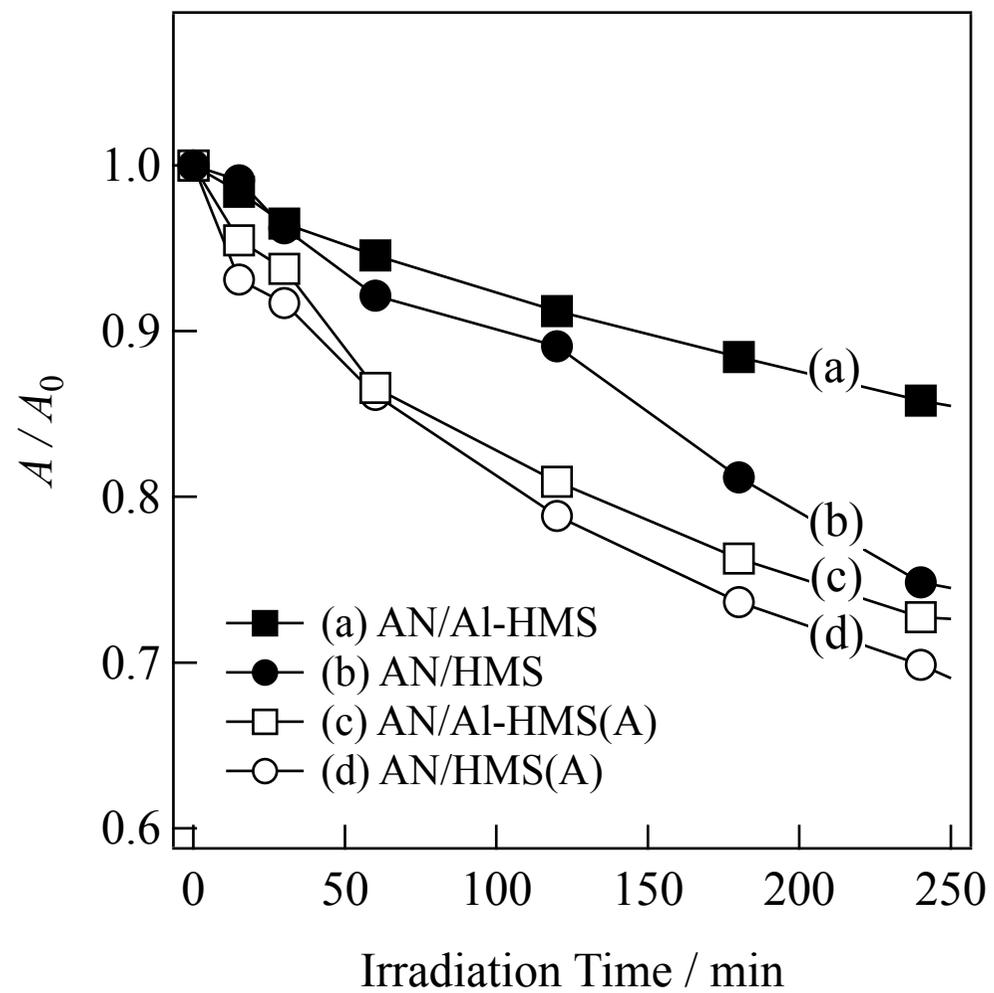


Figure 5