Stabilization of Flavylium Dye by Incorporation into Bentonite Clay

Yoshiumi Kohno*,†, Ryo Hoshino*, Shuji Ikoma**, Masashi Shibata***, Ryoka Matsushima*, Yasumasa Tomita*, Yasuhisa Maeda* and Kenkichiro Kobayashi*

(Received December 24, 2009 : Accepted February 1, 2010)

Abstract

We have enhanced the stability of a flavylium dye, a model compound of a natural anthocyanin dye, by the incorporation into the interlayer of a natural clay, bentonite. The composite of the flavylium and the bentonite shows excellent stability under increased temperature (up to 353 K) or alkaline environment (pH 9) compared to the original flavylium dyes. This material can be candidate of an environmentally friendly coloring material. Increased amount of dye causes a muddy color due to the formation of dye aggregate. Samples with brilliant color can be obtained with the loading amount less than 0.25 mmol dye per g-clay.

Key-words : Anthocyanin, Clay, Stabilization, Intercalation, Environmentally friendly pigment

1. Introduction

Naturally occurring dyes are promising material as a pigment, because of their non-toxicity. Flavylium (FV) is a cationic dye having the 2-phenylbenzopyrylum skeleton, which is the same as those of natural anthocyanin dyes. Therefore, the impact of FV on the environment is expected to be small, compared with other synthetic dyes. In addition, the flavylium derivatives can be synthesized easily by the aldol condensation of salicylaldehydes and acetophenones.

However, the stability of FV is known to be poor; FV easily undergoes transformation to a colorless 2-hydroxychalcone under slightly basic or heated conditions. As shown in Scheme 1, the decoloration process starts by the hydration of FV caused by the attack of water or hydroxyl ion to give a colorless hemiacetal, followed by the conversion to the 2-hydroxychalcone derivatives. If the stability of FV can be enhanced, we expect to be able to use FV as an environmentally friendly coloring material.

We have previously reported that FV can be stabilized by complexation with acidified clay, montmorillonite K10. FV molecules are successfully intercalated into the interlayer of K10. The causes of the stabilization have been thought to be as follows: (1) the electrostatic

\[ \text{flavylium} \xrightarrow{\text{OH}^+} \text{hemiacetal} \]

\[ \text{R} = \text{OMe} \]

\[ \text{trans-2-hydroxychalcone} \xrightarrow{\text{HO}} \text{cis-2-hydroxychalcone} \]

Scheme 1
effect from the charged clay layer, (2) the shielding effect from the external circumstance and (3) the acidity of K10.

The montmorillonite K10 is a modified clay with strong acidity for the usage as a catalyst. From the viewpoint of the materials safety, natural clay is more preferable than the modified one. If the acidity of K10 is the minor reason of the stabilization, non-acidic natural clay may work as a host to stabilize FV. In this study, we have tried to stabilize the FV dye using naturally occurring clay itself, instead of the acidified K10. As natural clay, bentonite (BN) has been used. The main component of BN is montmorillonite, so that the intercalation of FV into the interlayer of BN is expected to be possible. In addition, we have elucidated the state of FV molecules between the clay layers using several spectroscopic methods such as XRD, UV-Vis and fluorescence.

2. Experimental

2.1 Materials

The flavilium dye used in this study was previously synthesized as a perchlorate salt in our laboratory from 4′-methoxyacetophenones and salicylaldehyde by an acid catalyzed aldol condensation. The structure of FV is found in Scheme 1 (R = OMe). As a clay mineral, bentonite and synthetic non-swelling mica (designated as NSM) were obtained from Wako Chemical Co. and used as received. Both BN and NSM have the negatively charged layers. While BN has exchangeable cations in the interlayer, NSM does not show cation exchange property because the K⁺ ions in the interlayer space are strongly bound to the negatively charged layers.

2.2 Sample preparation

The composites of FV and BN were prepared as follows. Firstly, an aqueous solution of FV (0.1 mmol/dm³) was prepared with deionized water. Then, 0.7 g of BN was mixed with given amount of the FV solution, and the mixture was kept in a refrigerator maintained at 277 K to induce the adsorption of FV. The introduced amount of FV was varied from 0.01 to 1.25 mmol/g-BN. After 1 week, the sediment was separated from the solution by filtration, followed by washing with water. Then, the resulting slurry was dried at 353 K in the air for 1 day. The obtained composite samples are denoted as FV (x)/BN, where x indicates the amount of introduced FV (x mmol/g-BN). The same procedure was used to prepare the composite of FV and NSM (FV/NSM).

2.3 Spectrum measurements

XRD patterns were measured with the Shimadzu XD-3 X-ray diffractometer (CuKα, step angle and scan speed 0.02 and 4 /min, 10 mA, 30 kV).

UV-Vis spectra of FV solution were recorded with Hitachi U-3000 spectrophotometer in a conventional transmittance mode. UV-Vis spectra of FV/BN were recorded using JASCO V-550 spectrophotometer equipped with ISV-469 integration-sphere attachment. The spectra of the aqueous dispersion of FV/BN were measured in a diffuse-transmittance mode, whereas the spectra of the powder samples were measured in a diffuse-reflectance mode. The dispersion of FV/BN was prepared by mixing 2 mg of FV/BN and 4 cm³ of deionized water in a plastic cuvette, followed by sonication for 5 minutes. The spectra of FV/NSM were recorded in the same manner as FV/BN.

Fluorescence spectra were recorded with Hitachi F-3010 fluorometer. On measuring spectra, the FV solution was kept in the plastic cuvette, whereas the powdered FV/BN samples were kept in a polyethylene bag. The wavelength of the excitation light was fixed to 470 nm and the emission spectra of each sample were collected.

3. Results and Discussion

3.1 FV/BN composite

Fig. 1 shows the XRD pattern of the FV (0.25)/BN composite, together with that of BN itself. While the peak at around 2θ = 20° resembled each other, the d₀₀₁ reflection peak of FV (0.25)/BN (2θ = 5.74°) was observed at a lower angle than BN. Using Bragg’s law, the interlayer distance of BN was calculated to be 1.28 nm, whereas that of FV (0.25)/BN was 1.54 nm. The expansion of the interlayer distance was due to the intercalation of bulky organic molecule in the interlayer space. Thus, it can be said that FV was successfully intercalated to the BN interlayer.

On the other hand, the d₀₀₁ reflection peak of FV

![Fig. 1 XRD patterns of (a) BN and (b) FV (0.25)/BN.](image-url)
(0.25)/NSM sample was observed at almost the same angle as that of NSM. Therefore, we concluded that FV was not intercalated in NSM but simply adsorbed on the external surface of NSM. The adsorption may be mainly due to the interaction between the negatively charged NSM surface and the cationic FV molecules.

3.2 Stability enhancement of FV

Fig. 2 illustrates the change in the UV-Vis spectra of an FV aqueous solution and FV (0.25)/NSM, FV (0.25)/BN aqueous dispersions maintained at 353 K for 30 minutes.

The \( \lambda_{\text{max}} \) of the FV solution at \( t = 0 \) was 435 nm, whereas that of FV (0.25)/NSM was 442 nm. In FV (0.25)/BN, two peaks were observed at 458 nm and 484 nm. The red shift of \( \lambda_{\text{max}} \) reflects the degree of the electrostatic interaction between FV and clays\(^{14-16} \), as well as of the co-planarization\(^{17} \). Considering the extent of the wavelength shift, the effect of clay hosts on the FV molecule was thought to be stronger in FV/BN than in FV/NSM. This should be due to the difference in the adsorption state of FV. In FV/BN, the FV molecule was intercalated in the interlayer, whereas in FV/NSM the FV molecule was simply adsorbed on the external surface.

When the FV solution was maintained at 353 K, the peak of FV at 435 nm was readily decreased during 30 minutes, in contrast to the growth of the absorption at wavelengths less than 350 nm. This result means that FV was converted by the hydration reaction to 4'-methoxy-2-hydroxychalcone, which exhibits absorption at 340 nm and 320 nm\(^{13,18} \). From this, we can see the fact that the color stability of FV is poor at slightly elevated temperature. To the contrary, in FV/BN the decrease in the absorption of FV was almost negligible. Therefore, it was concluded that the thermal stability of FV was enhanced in FV/BN. The causes of the stabilization have been thought to be as follows: (1) the electrostatic effect from the negatively charged layer of BN, (2) the shielding effect from the external circumstance brought about by the intercalation\(^{10} \). As BN was not acidified clay, it was concluded that the acidity of the inorganic host was not essential to the stabilization of FV.

On the other hand, FV/NSM showed medium stability at 353 K. Considering that the FV molecule of FV/NSM was not intercalated, this result indicates that the stabilization effect due to the electrostatic interaction alone was insufficient. It can be rephrased that the shielding effect by the intercalation of FV was required for the sufficient stabilization of FV. Thus, the attack of hydroxyl ion is inhibited on the FV molecules intercalated between the BN layers, resulting in the resistance for the hydration reaction to the chalcone derivative.

As described above, crude FV is unstable under neutral or slightly basic conditions. Therefore, we compared the chemical stability by maintaining each FV sample in a basic medium. Fig. 3 shows the change in the spectra of FV solution, FV/NSM and FV/BN dispersions after the pH was rapidly raised to 9 by adding 1 drop of 0.008 M NaOH\( \text{aq} \) to 4 ml of the solution or dispersions. After the pH was raised, the spectra were recorded at [A, B] 0, 1, 3, 5, 10, 15, 30 min and [C] 0, 5, 15, 30 min, respectively.

From these results, we concluded that not only the acidified clay (K10) but also the natural one have an ability to stabilize FV by the intercalation.
3.3 Dependency on the amount of FV

Fig. 4 illustrates the dependency of the UV-Vis spectra of FV/BN on the amount of loaded FV. When the amount of FV was changed from 0.13 mmol/g to 1.25 mmol/g, the absorption peak at around 450 nm increased in proportion to the amount of FV. Therefore, it can be said that the quantitative adsorption of FV on BN was achieved. The absorbance reached about 8 in FV \(1.25 \text{ mmol/g}.\)

As for a pigment, high color density is preferable. However, as shown in Fig. 4, the shape of the spectrum was dependent on the amount of FV. In the spectrum of FV \(0.13\)/BN, a single peak was observed at 450 nm. Along with the increase in the amount of FV, the absorption band was broadened. Especially, when the amount of FV exceeded 0.25 mmol/g, the peak broadening was significantly observed and a shoulder peak appeared at longer wavelength of 480 nm. This means that the FV/BN sample lost its brilliant color by increased amount of FV loading. In practice, as the amount of FV increased, the sample looked a little muddy color to the eye. Such an impurity of the color is undesirable for a pigment.

Fig. 5 depicts the luminescence spectra of FV/BN with various FV loadings, together with that of the FV solution. In the spectrum of FV \(0.01\)/BN, an emission peak was observed at 510 nm, which was the same as in the FV solution. Therefore, the emission of FV \(0.01\)/BN at 510 nm was assigned to the FV monomer\(^{19}\). Along with the increase in the amount of FV, the emission intensity decreased, and a new emission peak appeared at 530 to 540 nm. It is generally known that the excited energy is scattered and lost by the intermolecular interaction. Therefore, the suppression of the emission suggests that the intermolecular interaction would take effects even in FV \(0.04\)/BN. On the other hand, in FV \(0.13\)/BN, FV \(0.25\)/BN and FV \(0.50\)/BN, the original peak at 510 nm completely disappeared and the weak emission band at lower energy region \((530 \text{ nm})\) could only be observed.

The emission at around 530 nm appeared when the loading amount of FV was large. The intensity of the emission was rather weak, indicating the existence of the intermolecular interaction. Here, it should be noted that in the UV-Vis spectra, a shoulder peak was clearly observed when the loading amount of FV exceeded 0.25 mmol/g, as shown in Fig. 4. In addition, we observed from the XRD pattern a small but detectable expansion \((0.07 \text{ nm})\) of the \(d_{001}\) spacing in the FV/BN with the loading amount of FV over 0.25 mmol/g (data not shown). Considering these results, it was concluded that both the emission at around 530 nm and the absorption at 480 nm can be assigned to the FV aggregate. In other words, the increase in the amount of FV loading brought about the aggregation of FV between the BN layers.

From these results, it can be said that a large amount of FV can be adsorbed in the interlayer of BN. However, samples showing brilliant color could be obtained with the loading amount less than 0.25 mmol dye per g-clay. In order to obtain the FV/BN composites showing deep and brilliant color, the intermolecular interaction or the aggregation of FV must be inhibited. Modification of the interlayer space by surfactants may help disperse the FV molecules\(^{20-22}\), which is the theme of our future study.

In conclusion, the stability of flavylium dye was
enhanced by the intercalation into the interlayer of the natural clay, bentonite. The bentonite could adsorb the flavylium at least up to 1.25 mmol/g. However, a large amount of adsorption caused the aggregation of the flavylium dye, resulting in the muddy color of the composite. The inhibition of the aggregation may be required for the brilliant color.

References


粘土ベントナイトとの複合化によるフラビリウム色素の安定化

河野芳海*†・星野 壮*・生駒修治**・柴田雅史***・松島良華*・富田靖正*・前田康久*・小林健吉郎*

*靜岡大学工学部物質工学科・静岡県浜松市中区西3-5-1（〒423-8561）
**静岡大学工学部共通講座・静岡県浜松市中区西3-5-1（〒423-8561）
***東京工科大学 東京都八王子市片倉町1404-1（〒192-0982）
† Corresponding Author, E-mail：tykouno@ipc.shizuoka.ac.jp

（2009年12月24日受付；2010年2月1日受理）

要 旨

天然色素アントシアニンのモデル化合物であるフラビリウム色素を、天然粘土であるベントナイト層間に取り込み、その安定性を向上させた。フラビリウム色素とベントナイトの複合体は、353 K程度の加熱下、あるいはpH＝9程度のアルカリ性条件下において、元のフラビリウム色素よりはるかに高い安定性を示した。この材料は環境に優しい色材としての応用が期待される。色素量を増すと、色素収集体形成にともなう色の変化が生じた。鮮やかな色が保存するのは粘土1 g当りの色素添加量が0.25 mmolまではあった。

キーワード：アントシアニン、粘土、安定化、インターカレーション、環境調和型色材