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Why do flavylium salts show so various colors in solution? Effect of concentration and water on the flavylium's color changes

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Abstract

When synthesized flavylium salts (FVs) were allowed to stand in an acetonitrile–water mixture, the color of the solutions changed from yellow to red via green. This color change in FV solution has been studied by observing the change in UV–VIS absorption spectra. In particular, change in the color from green to red depends on the concentration of FV and an amount of water. The solution color, however, changed from red to green on dehydration of the solution. It is concluded that water molecules are responsible for the change in solution color from green to red and the change is due to dimerization and/or aggregation of FVs. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Anthocyanin; Three types of flavylium salts; Color change of solution; Dimerization and aggregation

1. Introduction

Various colors of flowers and fruits are due to the compounds of anthocyanin dyes. These anthocyanins have a skeleton base of flavylium salts (2-phenylbenzopyrilium, FVs), and there are a lot of studies in the field of biology [1]. It is well known that the dyes are responsible for various color changes of flowers and fruits. Recently, the variation and stabilization of natural anthocyanins have been successfully rationalized to the molecular stacking phenomena, such as copigmentation and self-association (aggregation), via hydrophobic interaction [2]. The studies have paid attention to photochemical relations between the FVs and 2-hydroxychalcone, which is known to be a precursor during the biosynthesis (Scheme 1) [3–7]. They also have reported the pH dependence of the chemical reaction and color change behavior [3]. In view of photochromic materials (chalcone–flavylium interconversion), these compounds have received attention both by the structural changes similar to natural dyes and their lower toxicity.

We have found for the first time that a color of FV solution changed from yellow to red via green and the color change depends on the amount of water and FV concentration in solution. Though the fact that three types of FV exist in crystal state was already reported at beginning of the 20th century, the cause for the different colors has not been clarified yet [8,9]. These phenomena are particularly interesting in connection with corresponding color changes of the flowers and fruits. In this paper, our attention is focused only on the behavior in solution and we will report the relations between the color change and molecular species in solution. It is concluded that the change of the color from green to red would be attributed to dimerization and/or aggregation of FVs.

2. Experimental

Unsubstituted flavylium perchlorate was synthesized according to a published procedure [10]. The crude product was purified by repeated recrystallizations from a perchloric acid:ethanol = 1:1 mixture. These samples were allowed to stand in a refrigerator under dark condition. The total yield of these products was ca. 50%. FV did not contain any solvent molecule by elementary analysis. (Found: C, 57.59; H, 3.64%. Calculated for $C_{15}H_{11}O_5Cl$: C, 58.74; H, 3.62%.). The little errors of the elementary analysis may be lability of the FVs. Acetonitrile (abbreviate to AN) and *N*,*N*-dimethylformamide (Dojin Chemicals, luminasol grade), ethanol and ethylmethylketone (Wako Chemicals, S grade) were used without further purification. The water

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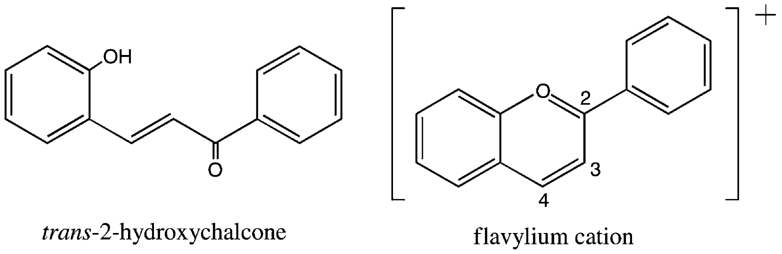


Scheme 1.

was deionized and distilled. Molecular sieves 3A (Wako Chemicals) was washed with water, and then it was treated with heating.

UV–VIS absorption spectra were observed using a Shimadzu UV-2500PC recording spectrophotometer. The cell with 1 cm (solution concentration: 1×10^{-5}, 5×10^{-3} mol dm^{-3} for AN–H_2O systems, and 1×10^{-3} mol dm^{-3} for ethanol, *N*,*N*-dimethylformamide, and ethylmethylketone systems), or 1 mm (2×10^{-2} mol dm^{-3} for AN–H_2O system) path length was used. The obtained spectra were resolved to the individual absorption spectrum by a calculation. Changes in absorption spectra were recorded as a function of time.

3. Results and discussion

Fig. 1(a) shows the absorption spectrum of FV in AN. The spectrum shows a peak at 394 nm and the solution colors yellow. There is no absorption band longer than 450 nm. The spectrum observed just after the preparation is the same as that reported previously [11]. No spectral change in the absorption spectra in pure acetonitrile occurred. This means that the FV is stable in the pure acetonitrile solvent. However, when the acetonitrile–water mixture was used as the solvent and concentration of FV was enough high, the color of FV solution changed from yellow to red via green. Fig. 1 (b) shows the absorption spectra of FV in the AN:H_2O = 100:10, 2.0×10^{-2} mol dm^{-3} solution as a function of time. The spectrum observed just after the preparation is the same as shown in Fig. 1 (a). At several minutes after the preparation of the solution, a broad and structureless band appeared at around 600 nm, on the other hand, the peak intensity at 394 nm decreased and the solution colored green from yellow. As the elapse of time the degree of growing up at 600 nm reduced. It is noted that the absorption intensity at 500 nm was very weak at that time. Further the elapse of time, a shoulder around 500 nm appeared, the tendency of rise-up at 500 nm was markedly accelerated, and the color of the solution changes from green to red. Spectral change of the absorption spectrum of FV in the AN:H_2O = 100:1, 5.0×10^{-3} mol dm^{-3} solution is shown in Fig. 1 (c). It is seen that not only the absorption around 500 nm but also 2-hydroxychalcone was not observed obviously at the low concentration and water content. The result implies that the production of chalcone is not important path in acetonitrile solution. This color change in FV solution has been studied by observing the change in UV–VIS absorption spectra. The spectral features at 600 nm (peak) and 500 nm (shoulder) characterize the absorption of green and red species, respectively. The change in the water-rich solvents occurred

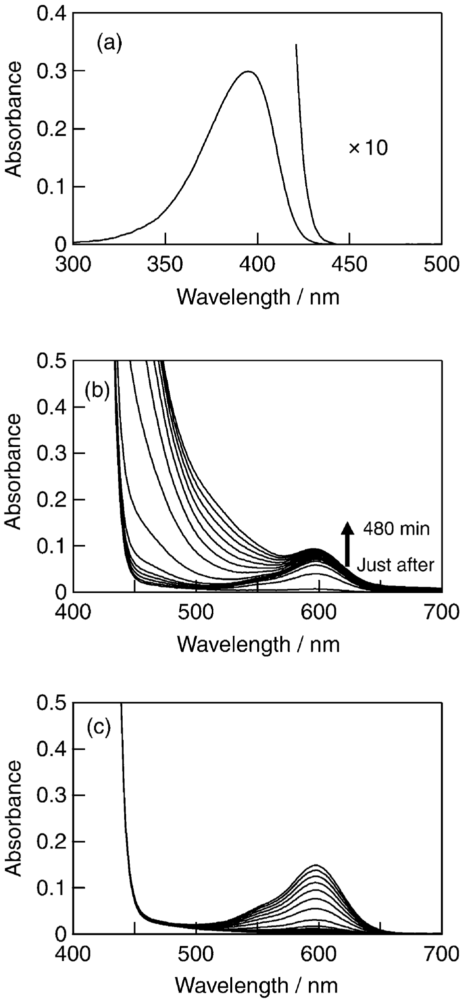


Fig. 1. (a) Absorption spectrum of yellow FV in acetonitrile 1.0×10^{-5} mol dm^{-3}. (b) Changes in absorption spectra of yellow FV in acetonitrile:H_2O = 100:10 as a function of time from just after the preparation to 480 min, 2.0×10^{-2} mol dm^{-3}. (c) Changes in absorption spectra of yellow FV in acetonitrile:H_2O = 100:1 as a function of time from just after the preparation to 480 min, 5.0×10^{-3} mol dm^{-3}.

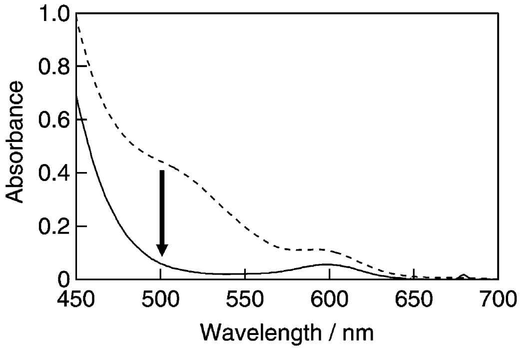


Fig. 2. Changes in absorption spectra of FV by dehydration from 2.0×10^{-2} mol dm^{-3} acetonitrile:H_2O = 100:10 mixture. The dehydration was done by molecular sieves 3A species. Before the dehydration (dashed line: red solution) and after the dehydration (solid line: colored green).

rapidly. This means that the water largely affects the color change of FV in the solution.

In order to examine the effect of dehydration from the solution on the spectral change, molecular sieves 3A was added into the same solution used as shown in Fig. 1 (b) after 1 day. Fig. 2 shows the change in the absorption spectra before and after the dehydration. The absorption around at 500 nm disappeared and the solution color reversed again from red to green by the dehydration. This indicates that the red species returned to green and/or yellow one. It is also indicated that the change from green species to red one is affected clearly by the addition of water molecule.

To make clear the spectral change, we have attempted to resolve the absorption spectra of FV at longer wavelength region into two components by using two absorption species. We assumed that the absorption at low water content and FVs conditions (AN:H_2O = 100:1, 5×10^{-4} mol dm^{-3}) was identified as that of pure green FV peaked at 600 nm, and the absorption of red FV was approximated by the Gaussian distribution peaked at 409 nm. An example of the resolved result is shown in Fig. 3 (AN:H_2O = 100:10, 2×10^{-2} mol dm^{-3}, after 120 min from the preparation). The observed spectra are well reproduced from the resolved spectra. Based on the calculated spectra, we can estimate the relative contribution of green and red FVs into the absorption of FV. The average deviations between the experimental and calculated values at 500 nm (red) and 600 nm (green) are 1.1 and 4.0%, respectively. The maximum deviation is shown as 7.3% at 240 min for 600 nm.

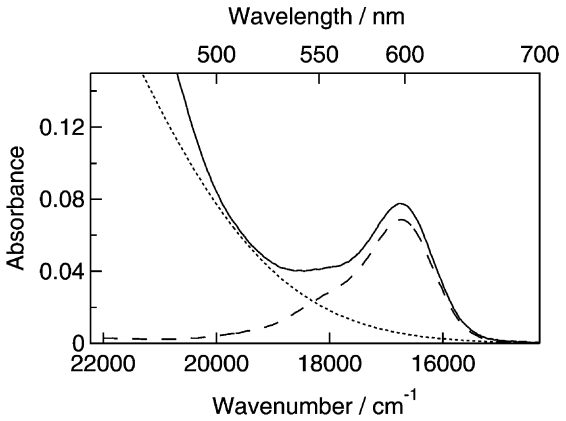


Fig. 3. An example of resolved spectra by assuming green FV (dashed line, drawn from the absorption at low water content and FVs conditions (AN:H_2O = 100:1, 5.0×10^{-3} mol dm^{-3})), and red FV (dotted line, approximated by the Gaussian distribution peaked at 409 nm) for AN:H_2O = 100:10, 2.0×10^{-2} mol dm^{-3}, after 120 min from the preparation.

Fig. 4 shows the dependence of the absorbance at 500 and 600 nm on time, concentration of FV, and water contents of acetonitrile–water mixed solvents (AN–H_2O).

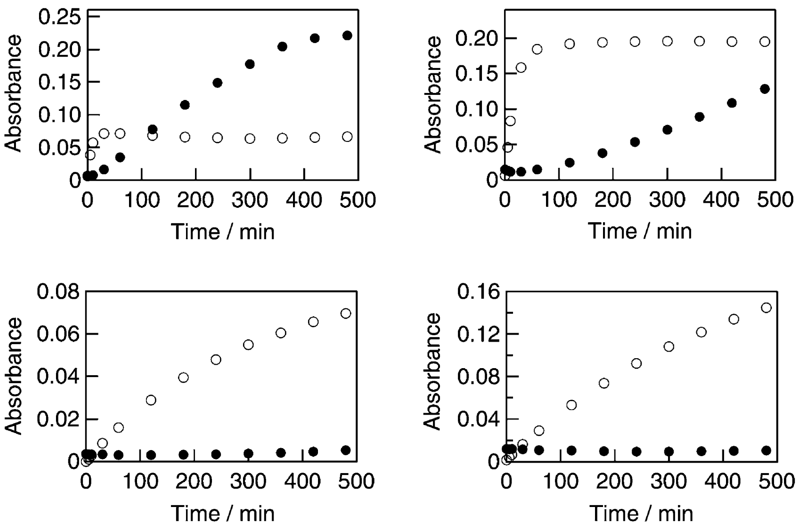


Fig. 4. Time evolution of two peaks at 500 and 600 nm for the acetonitrile and H_2O mixtures. (a) Acetonitrile:H_2O = 100:10 (upper plots). (b) Acetonitrile:H_2O = 100:1 (lower plots). Solution concentration: 2×10^{-2} mol dm^{-3} (left hand), 5×10^{-3} mol dm^{-3} (right hand); absorbance at 600 nm (opened circle) and at 500 nm (filled circle).

The upper and lower plots of Fig. 4 indicate the time profiles of absorbance of FV in the solvents with ratios of AN:H_2O = 100:10 and 100:1 (v/v), respectively. The initial concentrations of FV for samples a-1 and b-1 and samples a-2 and b-2 were 2×10^{-2} and 5×10^{-3} mol dm^{-3}, respectively. The absorption peak at around 500 nm becomes explicitly after standing 1 day at high concentration and high water contents conditions. We have not been able to assign the origin of the absorption band, and the band until 480 min was so trivial that we did not take account of the 500 nm absorption species in the calculation.

For the a-1 system, just after the preparation of the solution, there was no absorption peak in the wavelength region longer than 450 nm; the solution color was yellow. As the elapse of time, a new peak at around 600 nm (indicating the appearance of green FV) appeared, followed by a shoulder at 500 nm (indicating the appearance of red FV). The color change in the solution from yellow to red via green indicated that this change obeys a consecutive reaction.

On the other hand, the absorbance at 500 nm for the a-2 system changed slower than that for the a-1 system. These observations indicate that there is concentration dependence for the color changes in the solution. It is clear that the changes of the absorbance at 500 nm in the b-1 and b-2 systems were slower compared with those of the a-1 and a-2 systems, but rising rates at 600 nm were almost the same both for the b-1 and b-2 systems. The yields of green FV for the b-1 and b-2 systems 8 h after the preparation were nearly the same. This means that concentration dependence of the color change from yellow to green is not obvious.

It is known that dimerization of xanthene dyes is affected not only by concentration but also the amount of water [12]. The dimer formation occurs easily under a high dielectric environment. To dimerize the dye molecules, it needs neutralization of electrostatic repulsion by water molecule(s). A similar phenomenon for dimerization in this FV system would be expected to occur to cause the color change from green to red.

Table 1
The green species peaks (around 600 nm) in various solvents[a]

Solvent	$f'(D,n)$	Peak wavenumber (cm^{-1})
Ethylmethylketone	0.367	17000
N,*N*-Dimethylformamide	0.377	16500
Ethanol	0.380	16700
Acetonitrile–water	0.392[b]	16700

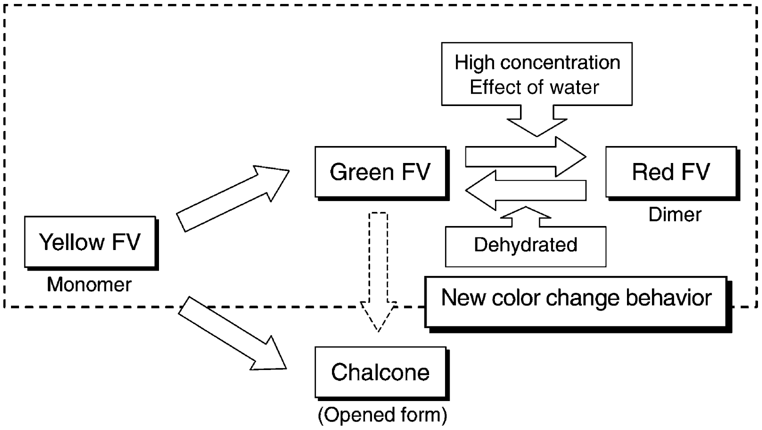
[a] The dielectric constant and reflective index were referred to Ref. [14].

[b] An f' value of pure acetonitrile was used to compare.

The results given in Figs. 1, 2 and 4 indicate that the color change from green to red FVs in the solutions is ascribed to the dimerization and/or aggregation of the FVs. Since green and yellow FVs coexist stably in the dehydrated solvent, the change from yellow FV to green FV mainly depends on the dielectric constant or change of polarity of the solvent. The chalcone peaked at 353 nm [4], although the results are not shown here, appeared markedly at the low concentration and in pure ethanol solution, but it was not observed clearly in pure acetonitrile solution.

Based on the findings in the UV–VIS absorption spectra, we propose Scheme 2 for the color change of FV in the solutions. It is reasonable to identify yellow species with monomer FV. With increase of the solution concentration, the solution colors of FV changed rapidly from yellow to red via green. This behavior indicates that intermolecular interaction is required to the formation of red FV from green FV. Though it is difficult to obtain the experimental information on molecular structure of the green species directly, the longer wavelength absorption at 600 nm suggests that green FV has charge-transfer character from ClO_4^- to FV skeleton. In order to characterize the charge-transfer property, relation between f' values and peak wavenumbers of solvents having absorption band at long-wavelength region is compared in Table 1, where the f' is defined as

$$f'(D,n) = \left[\frac{D-1}{2D+1} - \frac{n^2-1}{2(2n^2+1)}\right] \qquad (1)$$



Scheme 2.

Here, D is the dielectric constant and n the refractive index of the individual solvents. The factor 1/2 in the second term of Eq. (1) is used for the case in which the dipole moment in the equilibrium excited state is much larger than the dipole moment in the ground state, complex, and CT complex [13]. As the f' value becomes larger, the peak wavenumbers shift to the red. This indicates green FV has a charge-transfer character. Green FV is the precursor of a red FV and red species can reasonably correspond to a dimer and/or aggregate of green species.

Since green FV and red FV were unstable in the solution, the equilibrium constants between these species were not clear and we were not able to determine these molar absorption coefficients exactly. In terms of the results in alcoholic solution, in addition to the experimental results shown in the present note, we propose the reaction for Scheme 2. Detail explanation about the scheme will be described in the next paper [15]. In alcoholic solutions we have observed chalcone generated via a ring-opening reaction of FV skeleton, and the color change through the reaction is well known. But, in this note we focus on the unique color change in FV acetonitrile solution without the ring-opening reaction. We will try to establish synthesis method of FV with various counter ions, and to investigate the effect of counter ions on the color change, in particular to get more information of green species character.

4. Conclusion

FV in acetonitrile–water solutions showed color changes from yellow to red via green. It is suggested that yellow, green, and red species of FV are a monomer, a monomer with charge-transfer character, and a dimer and/or aggregate of FV. FV species with the colorful character will be a fascinating molecule with great potential for bringing many applications such as photochromism and photosynthesis in the near future.

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