ผลความเข้มข้นที่มีต่อสเปคตรัมการวาวแสงของสีย้อมในสารละลายน้ำ
Concentration effects on the spectra of fluorescence dyes in aqueous solution

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บทคัดย่อ
วัตถุประสงค์หลักของงานวิจัยนี้คือ เพื่อแสดงให้เห็นว่าผลของความเข้มข้นของสารละลายสีย้อมนั้นมีบทบาทสำคัญต่อการเปลี่ยนแปลงของแถบสเปคตรัมการวาวแสงของสารละลาย โดยอาศัยเทคนิคเลเซอร์อินดิวฟลูออเรสเซนต์ในการศึกษา ทำการเตรียมสารละลายตัวอย่างที่ประกอบด้วยสีย้อมวาวแสงชนิด ฟลูออเรสซีน โบโมฟลูออเรสซีน โรดามีนซิกจี และ โรดามีนบี โดยการเจือจางด้วยน้ำให้มีช่วงความเข้มข้น 10^{-5} ถึง 10^{-3} โมลาร์ จากนั้นทำการกระตุ้นสารละลายตัวอย่างด้วยเลเซอร์ที่ความยาวคลื่น 395 นาโนเมตร และวัดสเปคตรัมของการวาวแสงที่เปล่งออกมาด้วยระบบวัดแสง ซีซีดี ผลที่ได้แสดงให้เห็นว่าเมื่อความเข้มข้นของสารละลายสีย้อมวาวแสงเพิ่มขึ้น แถบสเปคตรัมของการวาวแสงจะเลื่อนไป ยังระดับพลังงานที่ต่ำลง (ความยาวคลื่นสูงมากขึ้น) ซึ่งพฤติกรรมของสเปคตรัมการวาวแสงที่เปลี่ยนแปลงไปเมื่อความเข้มข้นของสารละลายสีย้อมเพิ่มขึ้นน่าจะเกิดจากผลของปรากฏการณ์ “อินเนอร์ฟิลเตอร์” ภายในสารละลายสีย้อมวาวแสง

คำสำคัญ: ผลของความเข้มข้น อินเนอร์ฟิลเตอร์เอฟเฟค ไดเมอร์ รีแอบซอฟชัน

Abstract
In this study, the objective was to show that the concentration of a fluorescent dye solutions plays an important role in changing line spectra of aqueous solutions by using the technique called laser induced fluorescence (LIF). The fluorescent dyes used were Fluorescein, Bromofluorescein, Rhodamine 6G and Rhodamine B and were prepared in solution by diluting with water to obtain concentrations from 10^{-5} to 10^{-3} molar. The sample solutions were stimulated by a laser diode (395 nm), and the fluorescence emission spectra from sample solutions were measured and recorded with a CCD spectrometer system. The results showed that when the concentration of the fluorescent dye solutions increased, the fluorescence spectra shifted to a lower energy (longer wavelength). The behavior of fluorescence spectra changed when the concentration of the fluorescent dye solutions was increased, which may have been caused by an inner-filter effect inside the fluorescent dye solutions.

Keywords: concentration effect, inner-filter effect, dimer, reabsorption

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Introduction

Concentration effect is one of the environmental factors that affects to fluorescence spectra. Changing of concentration will result in changing of fluorescence spectra. There are two forms of change which include the intensity shift and wavelength shift, when the concentration was changed. Fluorescence intensity will shift, which is based on Beer's law. According to the theory, fluorescence intensity that emitted out will depend on number of particle or molecule being able to absorb energy in order to stimulate atoms, which can be expressed as the Equation 1[1,2].

$$F = K'P_o\{2.3\varepsilon bc - \frac{(-2.3\varepsilon bc)^2}{2!} - \frac{(-2.3\varepsilon bc)^3}{3!} - \cdots\} \quad (1)$$

Where F is the fluorescence intensity emitted, K′ is constant dependent on quantum efficiency, P_o are intensities of incident, ε is the molar absorptivity coefficient, b is the optical path length and C is the concentration of the dye solution. The equation above shows that the fluorescence intensity is directly proportion to the concentration of the dye solution. When we plotted both parameters, we will get a straight line but when increasing the concentration of the dye solution the intensity of the fluorescence emitted is reduced[3] as shown in Figure 1.

The wavelength shift occurs at the increasing concentration of the dye solution due to inner-filter effect which is phenomenon that will change fluorescence spectra. It can be explained by two mainly models including incorporation of dimer formation and re-absorption model[4,5]. Dimer occurs when the concentration of the dye solution is increased the molecules that are monomers form together[6]. The molecular structure of the monomer and dimer is different and it results in the absorption spectra and emission spectra changes too.

Re-absorption occurs because molecules of the dye solution can absorb fluorescence emitted. Intensity of fluorescence emitted is reduced by using a graph of Stoke to explain this phenomenon[7]. Figure 2, shows that the overlapping between absorption and fluorescence spectra of some fluorophore species[8]. If overlapping zone between absorption spectra with fluorescence spectra of fluorescent dye sample increases, the re-absorption effect on fluorescent dye solution increases and it damages the shape of fluorescence spectra over another fluorescent dye solution species that has smaller overlapping zone[9].

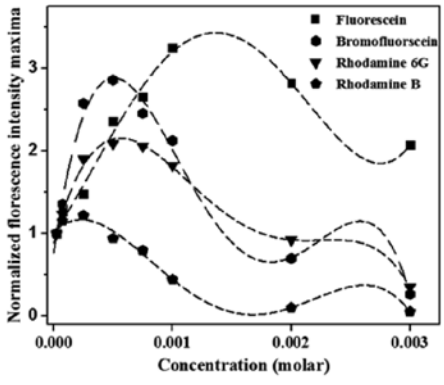

Figure 1 The concentration effect on fluorescence intensity maxima in various dye sample solutions[3].

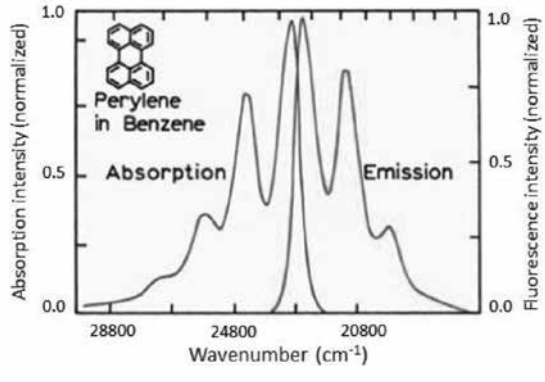

Figure 2 Absorption and fluorescence emission spectra of perylene[8].

In this research we will study the concentration effect on fluorescence spectra of each fluorescent dye such as Fluorescein, Bromofluorescein, Rhodamine B and Rhodamine 6G in water solution by using laser induced fluorescence (LIF) technique. It is also included with the discussion in relation to fluorescence spectra changes as the result of inner-filter effect on fluorescent dye solutions.

Materials and Methods

The experiment was conducted using solute including four types of fluorescent dyes namely Fluorescein, Bromofluorescein, Rhodamine 6G and Rhodamine B. The solutions were prepared with water to obtain the concentrations from 10^{-5} to 10^{-3} molar. The sample solutions were put into the cuvette which is made from quartz cuvette standard size in order to let light pass through almost 100%. The fluorescence dye sample solutions were illuminated with a laser diode (395 nm, 500 μW) as an excitation source. Fluorescence spectra of dye solutions were measured and recorded with the CCD spectrometer system which was set up perpendicularly to the excitation radiation in order to reduce the scattering of light, and the fluorescence spectra data was averaged 10 times so that the data was more accurate. Finally, the fluorescence spectra of each dye solution species were analyzed by peak fit analysis method in order to understand the behavior of fluorescence spectra dependent inner-filter effect in various concentrations.

Results and Discussion

When the molecules of fluorescent dye solutions was excited by 395 nm purple laser, the molecules will change level energy up to excited state. At this state, the molecules are unstable and released energy in the form of fluorescence emission at 500-650 nm. In Figure 3 shows the fluorescence spectra of fluorescent dye solutions including Fluorescein, Bromofluorescein, Rhodamine 6G and Rhodamine B respectively. From it shows that the fluorescence spectra were changed when the concentration was change. The result is similar in all fluorescent dye solution species. The fluorescence spectra changes in two terms including fluorescence intensity maxima shift and wavelength position at fluorescence intensity maxima shift.

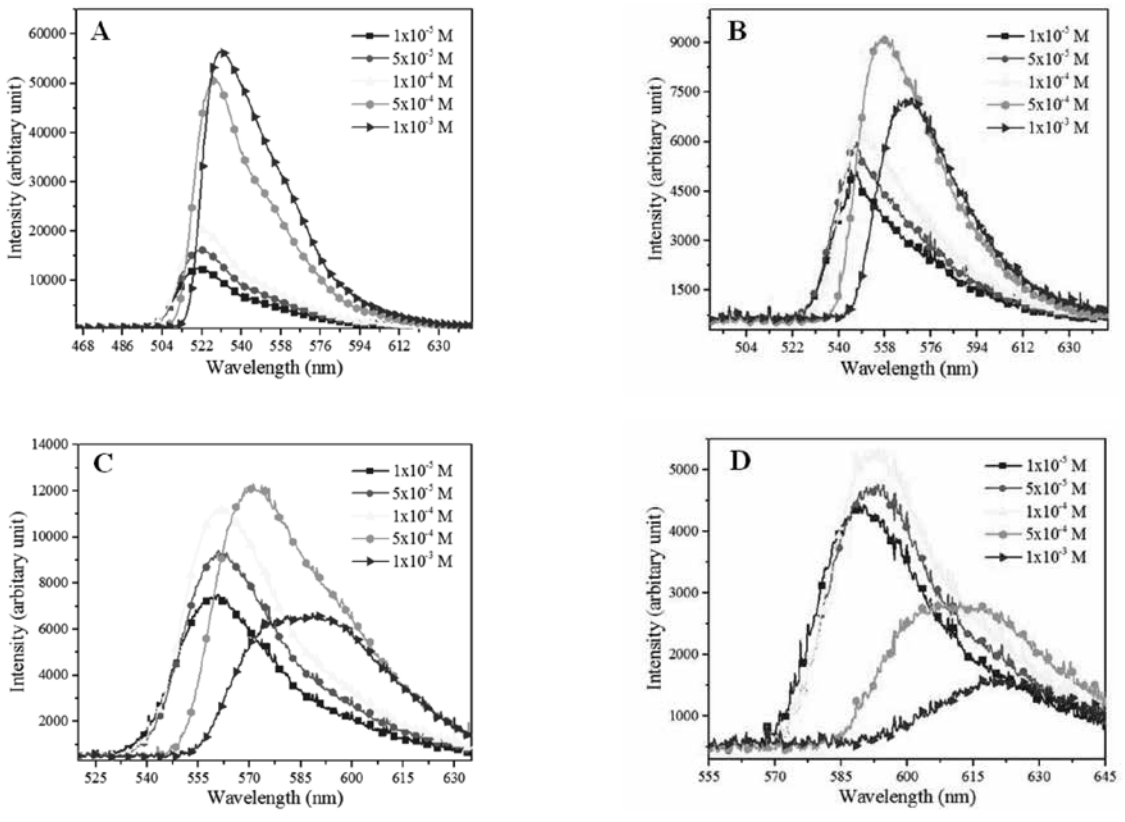

Figure 3 The relationship between the wavelength and fluorescence intensity of each dye solution species including of Fluorescein (A), Bromofluorescein (B), Rhodamine 6G (C) and Rhodamine B (D) at temperature of 25 °C at various concentrations.

The Fluorescence spectra were analyzed by fitting program (Peak functions, R^2 = 0.996-0.999) in order to find the value of fluorescence intensity maxima and wavelength position at fluorescence intensity maxima. The relationship between normalized fluorescence intensity maxima with various concentrations is shown in Figure 4. From Figure 4, the results have a trend to changes in the same way in all fluorescent dye solution species. The value of fluorescence intensity maxima increases linearly in low concentration range (<10^{-4} Molar). However, at high concentration the value of fluorescence intensity maxima decreases which can be explained by Equation 1.

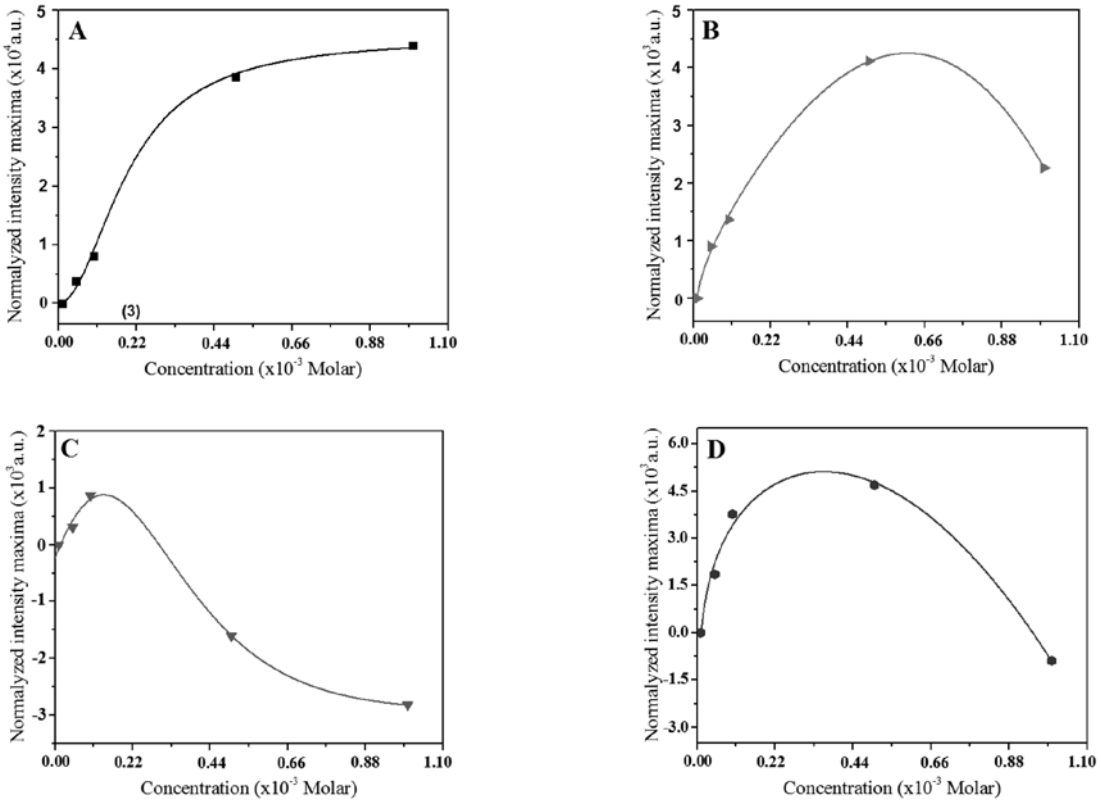

Figure 4 Concentration effect on fluorescence intensity maxima at various fluorescent dye species such as Fluorescein (A), Bromofluorescein (B), Rhodamine B (C) and Rhodamine 6G (D) in water solution.

However, the concentration of fluorescent dye solutions did not only affect the change of fluorescence intensity maxima but still affect the wavelength position at fluorescence intensity maxima. So that the fluorescence spectra data was normalized in order to find the wavelength position of fluorescent dye solution species at different concentrations. Figure 5 shows fluorescence spectra that are normalized of Fluorescein solution which we can see that the wavelength position at fluorescence intensity maxima was shifted into higher wavelength when concentration increases in the same way when the spectra of Bromofluorescein, Rhodamine B and Rhodamine 6G are normalized and the relationship between the wavelength position shift with concentration of each dye solution species is shown in Figure 6.

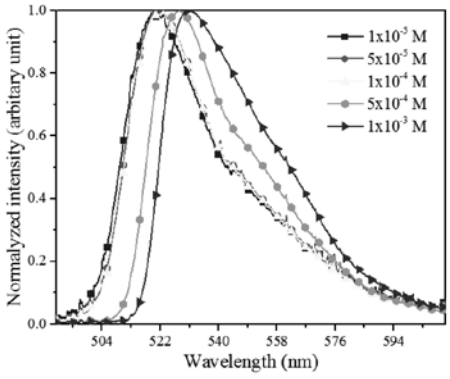

Figure 5 Normalized intensity relationship of Fluorescein solutions at various concentrations.

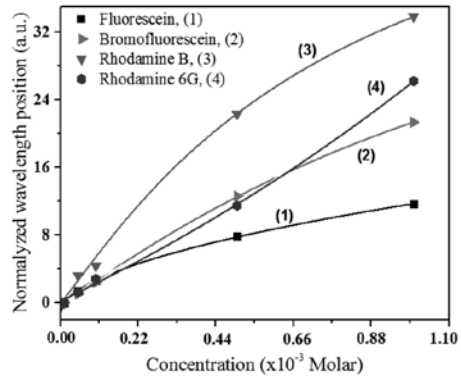

Figure 6 Wavelength-shift behavior in each dye solution species at various concentrations.

Form Figure 6, it can be seen that the value of wavelength position at fluorescence intensity maxima shifted to higher wavelength when concentration was increased. This behavior can be explained by inner filter effect that occurred in solution that has hypothesis in appearance in two factors. The first one is during low concentration we rarely find dimer in other word dimer have no effect on a change of overall spectra but when concentration is higher the amount is higher as well until it has enough effect that it obviously changes fluorescence spectra into higher wavelength. However, we cannot abandon the effect of reabsorption because it is possible that a shift of wavelength position is caused by reabsorption. At low concentration, overlapping of absorption and fluorescence spectra is low so the reabsorption rarely occurred. So fluorescence spectra hardly changed when concentration is higher overlapping increase. The reabsorption of molecules increases as well so the fluorescence spectra shifted clearly.

Conclusion

Effect of concentration has a significant influence on behavior of fluorescence spectra of fluorescent dye solution including Fluorescein, Bromofluorescein, Rhodamine B and Rhodamine 6G. Apparently, the influence of concentration effect on fluorescence spectra changes was classified in two terms. The first is fluorescence intensity maxima was shifted and the second was wavelength position at fluorescence intensity maxima shift. In the case of the wavelength position at fluorescence intensity maxima shift, it can be expanded by the dimer formation effect or reabsorption effect on fluorescent dye molecules, which is possible in both events equally.

Acknowledgment

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