การศึกษาผลของตัวทำละลายที่มีต่อสเปคตรัมการวาวแสงในสารละลายสีย้อมวาวแสง

Investigation of Solvatochromic Effect in Fluorescence Dye Solutions

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Received : 12 June 2017

Accepted : 1 September 2017

บทคัดย่อ

ในงานวิจัยนี้ใช้ตัวทำละลายหลากหลายชนิดที่มีค่าสภาพความเป็นขั้วที่ต่างกันเพื่อศึกษาถึงผลของการเปลี่ยนแปลงของสเปกตรัมการวาวแสงของสารละลายสีย้อมวาวแสงได้แก่ฟลูออเรสซีน โบรโมฟลูออเรสซีน โรดามีนซิกจี และโรดามีนบีที่ความเข้มข้นคงที่ (10^{-3} โมลาร์) โดยอาศัยเทคนิคเลเซอร์อินดิวฟลูออเรสเซนต์ ซึ่งประกอบไปด้วยเลเซอร์สีม่วงความยาวคลื่น 395 นาโนเมตรถูกใช้เพื่อกระตุ้นโมเลกุลของสารละลายสีย้อมวาวแสงที่สภาวะอุณหภูมิห้อง โดยที่สเปกตรัมของการวาวแสงจะถูกตรวจวัดด้วยเครื่องสเปกโตรมิเตอร์ ซึ่งอยู่ภายใต้การควบคุมของซอฟแวร์คอมพิวเตอร์อีกที ผลการทดลองชี้ชัดว่าตัวทำละลายแต่ละชนิดนั้นส่งผลถึงสเปกตรัมการวาวแสงของสารละลายสีย้อมวาวแสงในลักษณะที่แตกต่างกันออกไป ซึ่งน่าจะเป็นผลมาจากการเกิดอันตรกิริยากันระหว่างตัวทำลายกับสีย้อมวาวแสงในแต่ละชนิด

คำสำคัญ: ฟลูออเรสเซนต์ ผลของตัวทำละลาย ความเป็นขั้วของตัวทำละลาย โซเวโตโครมิคชิฟ

Abstract

In this research, we used different solvents with different polarity to demonstrate the change of fluorescence spectra of fluorescent dyes including Fluorescein, Bromofluorescein, Rhodamine 6G and Rhodamine B at fixed concentration (10^{-3} molar) by using laser induced fluorescence (LIF) technique. The 395 nm purple laser was used to excite fluorescent molecules at room temperature. The fluorescence spectra were detected by a spectrometer which was performed under a computer interfacing software. It was found from the experiment that each solvent have their own effect on fluorescence spectra which are results from the reaction between solvent and fluorescent dye solution.

Keyword: Fluorescence, Solvent effect, Solvent polarity, Solvatochromic shift

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Introduction

Fluorescent dye is a colorant that can release electromagnetic wave when stimulated by photon energy. There are currently many types of fluorescent dye syntheses depending on applications such as Fluorescein, Rhodamine B, Rhodamine 6G, Acridine Orange, Eosin Yellowish et al. The luminescence from Fluorescent dye will respond to changes in environmental factors such as temperature, pH value, molecular structure and solvent polarity (Yeshchenko *et al.,* 2015; Ghini *et. al.,* 2013; Rauf & Hisaindee, 2013). Hence, the fluorescent dyes are popular for use in environment sensing analysis.

Polarity effect is one of the environmental factors that plays the important role on shifting of fluorescence spectra in dye solutions which is called solvatochromic shift (Chakraborty & Panda, 2011; Nag & Goswami, 2009; Thipperudrappa *et al.,* 2008).It can be explained by the Lippert-Mataga in Equation 1 (Lakowicz, 2006).

$$v_a - v_f = \frac{2}{hc}\left(\frac{\varepsilon-1}{2\varepsilon+1} - \frac{n^2-1}{2n^2+1}\right)\frac{(\mu_E-\mu_G)^2}{a^3} + constant \tag{1}$$

Where h is Planck's constant (6.6256x10^{-27} ergs), c is the speed of light (2.9979x10^{10} cm/s), μ_E and μ_G are excited- state dipole moment and ground-state dipole moment, respectively, a is the radius of the cavity where the fluorophore resides, v_a and v_f are wave number of absorption and emission, respectively, ε is the dielectric constant and n is the refractive index and we name this function $\left(\frac{\varepsilon-1}{2\varepsilon+1} - \frac{n^2-1}{2n^2+1}\right)$ the orientation polarizability $f(\varepsilon, n)$, which depends on two parameters one is dielectric constant and the other is refractive index (Mannekutla *et al.,* 2008; Sharma *et al*., 2015). This $f(\varepsilon, n)$ term tells us how effective solvent affects on red or blue shift phenomenon due to effect of dielectric constant and refractive index of solvent. Each solvent has its own polarity which directly relates to dipole moment.

From the above mentioned, it can be seen that on the basis of the fluorescence changes due to the polarity effects can be developed as a technique in classifying the type of solvent well (Yang et al., 2013; Benchea *et al.,* 2017; Naderi *et al.,* 2014). Another can also calculate the physical parameter values such as n, ε, μ_E and μ_G, where these parameters play an important role in identifying and quantifying the amounts of solvents and solutes. Therefore, this research focused on the investigation of the effective polarity of each solvent (water, acetone, methanol and ethanol) to fluorescence spectra of each fluorescent dye by using laser induce fluorescence technique (LIF). The results of this research are likely to be basic information and can be used to develop the environmental measurement based on fluorescence sensing technique in the future.

Methods

Fluorescence dye solutions were prepared by dissolving fluorescent dyes including Fluorescein, Bromofluorescein, Rhodamine 6G and Rhodamine B into the solvent including water, acetone, methanol and ethanol at fixed concentration of 10^{-3} molar. The required parameters data of solvents as shown in Table 1.

Table 1 The data of solvents parameters

Solvents	Formula	[a]Refractive index n (a.u.)	[a]Dielectric constant ε (a.u.)	[b]Relative polarity (a.u.)
Water	H_2O	1.333	80.10	1.000
Methanol	CH_3OH	1.329	32.66	0.762
Ethanol	CH_3CH_2OH	1.361	24.55	0.654
Acetone	CH_3COCH_3	1.359	20.56	0.355

[a] (Bruno & Svoronos, 2011; Basavaraja et al., 2015), [b] (Reichardt, 2003)

The fluorescence dye sample solutions were put into the cuvette which is made from quartz so that light can pass through without losing its energy. The sample solution was illuminated by laser diode (395 nm, 500 μW). Then, the fluorescence spectra were recorded by CCD spectrometer model BTC-110S (resolution of 0.3 nm) which was positioned perpendicular to the laser source and performed under a computer interfacing software. The experiment set-up is shown in Figure 1. Finally, The fluorescence spectra was analyzed by fitting program in order to investigate the polarity effect on fluorescence spectra of each dye solution species under room temperature.

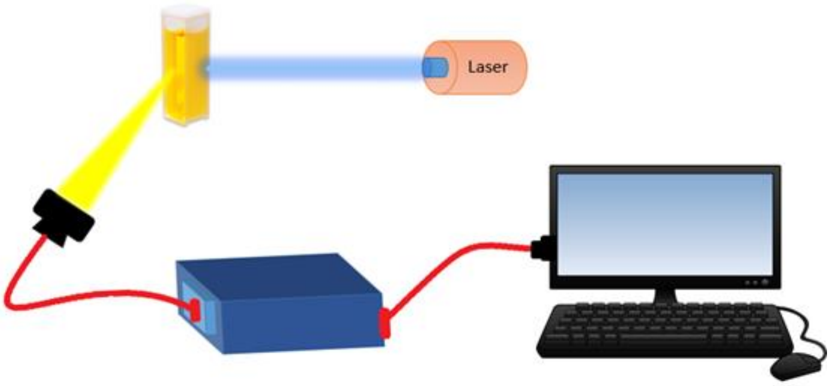

Figure 1 The experiment set-up for investigate of the solvent effect on fluorescence spectra in dye solutions

Results and Discussion

Figure 2 shows the fluorescence spectra of each fluorescent dye solutions, which are dissolved in various solvents (including water, methanol, ethanol and acetone) respectively. The result shows that the fluorescence spectra all of dye solutions were changed in two terms as fluorescence intensity maxima and wavelength position at fluorescence intensity maxima when the solvent species changed.

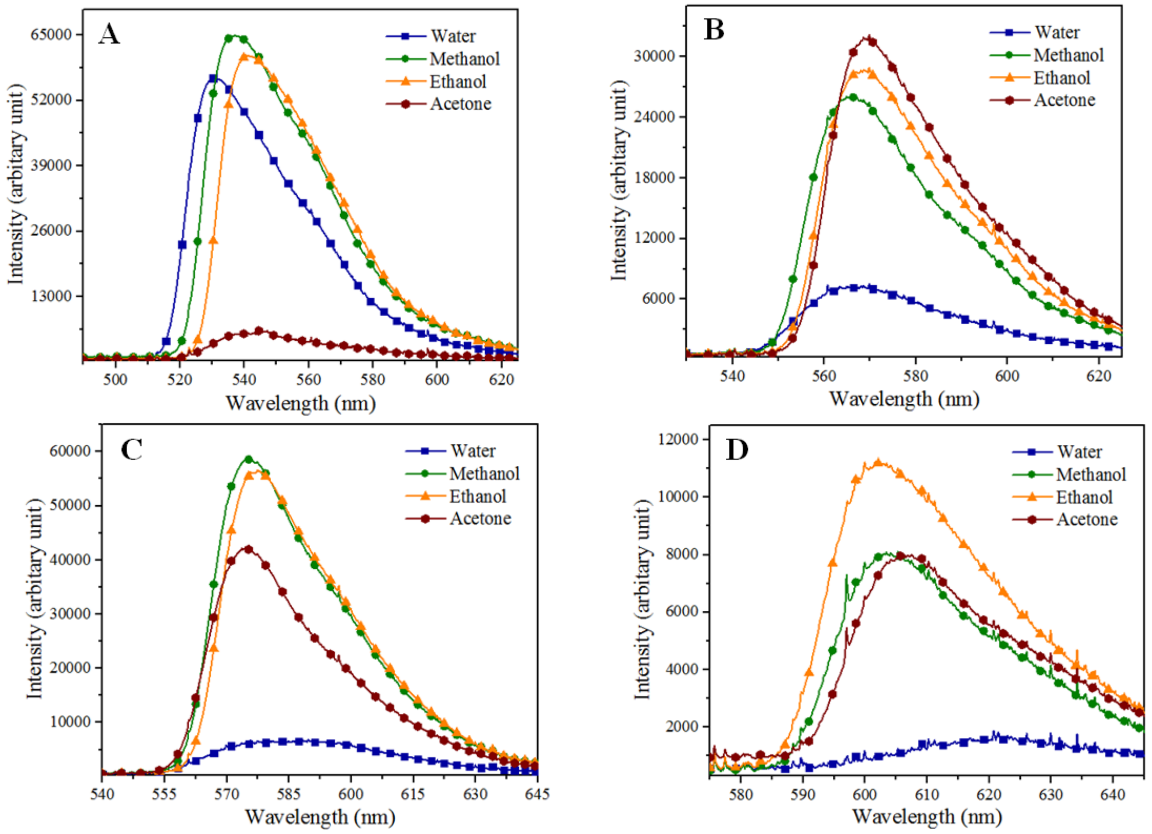

Figure 2 Fluorescence intensity of dye solutions species including of Fluorescein (A), Bromofluorescein (B), Rhodamine 6G (C) and Rhodamine B (D) in various solutions.

In order to understand the solvent behavior that affected the fluorescence spectra, the data of fluorescent spectra in each solvent were analyzed to find the fluorescence intensity maxima and wavelength position at fluorescence intensity maxima. The value of fluorescence intensity maxima was shown in Table 2. When the solvent species changed, the fluorescence intensity maxima were changed in each fluorescent dye solutions. The value of fluorescence intensity maxima is an indicator how effective fluorescent dyes can be dissolved in solvent which related to the number of dye molecules in solution (the fluorescence intensity maxima increases when the number of dye molecules is increased) and the number of molecules in the solution was more or less dependent on the solubility of fluorescence dye samples in solvents (“polar dissolve in polar, nonpolar dissolve in nonpolar”)(Guilbault, 1990; Timberlake, 2004).

Table 2 Peak Fit Analysis data of fluorescence spectra related to solvents specie.

Solvents	Fluorescence intensity maxima at varies Fluorescence dyes (arbitrary unit)			
	Fluorescein	Bromofluorescein	Rhodamine B	Rhodamine 6G
Water	56687	7291	1819	6945
Methanol	64912	26202	8085	58702
Ethanol	61145	28706	11195	56468
Acetone	6375	31878	8025	42371

In Table 2, it was found that the value of fluorescence intensity maxima of dye solution in each solvent species was significantly changed and can be roughly determined the polarity value of the fluorescent dyes. In Fluorescein dye solutions, the fluorescence intensity were highness in aqueous solution and the alcohol group but less valuable in water. Therefore, it can be predicted the polarity value of Fluorescein dye in the range of water and methanol solution (the polarity value of fluorescein dye tend to approach the polarity value of methanol). When using this information accompany the hypothesis of dissolution which relation to the fluorescence intensity. In this assumption, it can be sorted the polarity values the polarity values of fluorescent dye samples based on the solubility by descending order as follows: Fluorescein, Rhodamine 6G, Rhodamine B and Bromofluorescein respectively.

In case of the wavelength position at fluorescence intensity maxima shift was due to the solvent effect. In order to understand the data clearly we normalized the data to find the wavelength position at fluorescence intensity maxima. The results are shown in Figure 3 and the wavelength position at fluorescence intensity maxima data of dye solutions in each solvent species as shown in Table 3.

Table 3 Peak Fit Analysis data of fluorescence spectra related to solvent species.

Solvents	The wavelength position at fluorescence intensity maxima at various Fluorescence dyes (nm)			
	Fluorescein	Bromofluorescein	Rhodamine B	Rhodamine 6G
Water	530	568	621	588
Methanol	537	566	604	575
Ethanol	542	569	602	575
Acetone	546	569	607	575

It was found that the value of wavelength position at intensity maxima was shifted when the solvent species changed as shown in Figure 3. The peak shift can be explained by the changes value of Stokes shift ($\Delta v = v_a - v_f$), which was related to the wavelength position at fluorescence intensity maxima. From equation 1, the dielectric constant (ε) and refractive index (n) of solvents play a great role in a change of

Δv. This causes the wavelength position at fluorescence intensity maxima of each dye solutions to change when dissolved in different solvents.

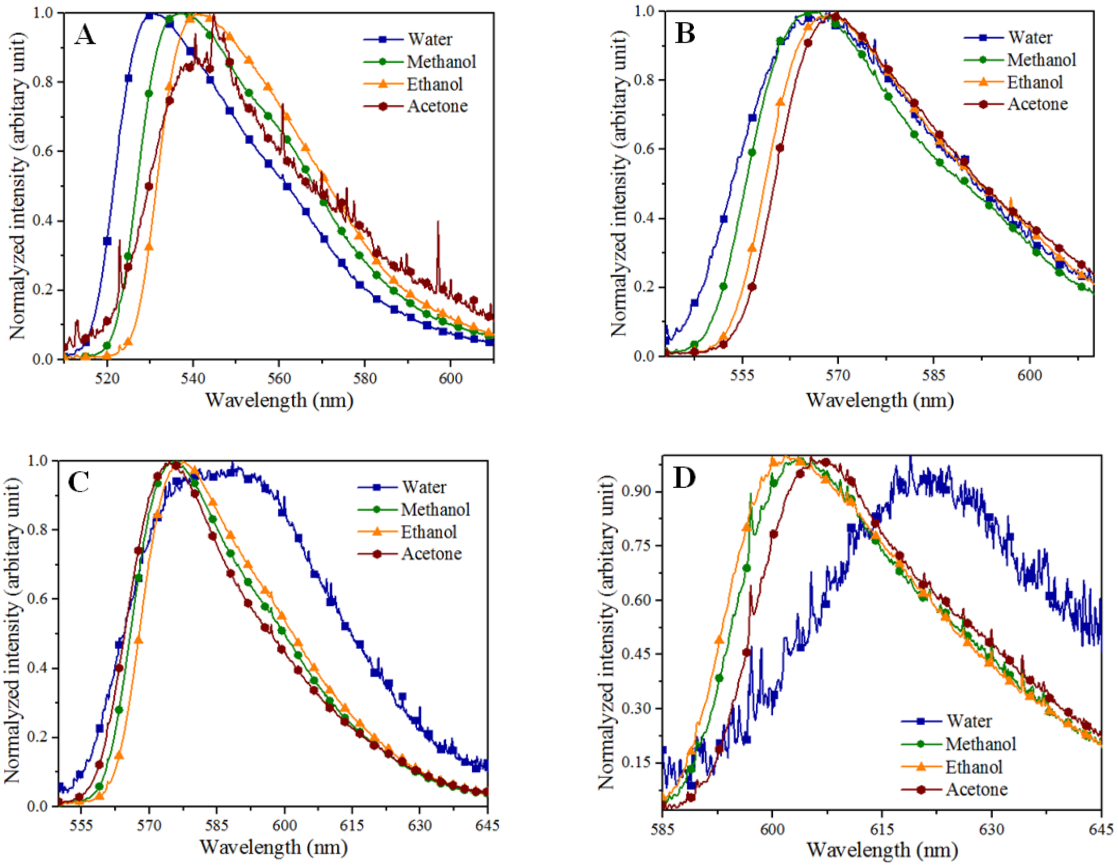

Figure 3 The normalized intensity of dye solution species including of Fluorescein (A), Bromofluorescein (B), Rhodamine 6G (C) and Rhodamine B (D) at various solvents.

It can be seen that the fluorescence spectra in most water solutions are not clustered with the fluorescence spectra in other solutions, this is probably due to the dielectric value of the water (from Table 1) that is more valuable than other types of solvent species (Naderi *et al.,* 2014; Bruno & Svoronos, 2011; Basavaraja *et al.,* 2015). However, all of data shows that the shift of wavelength position at fluorescence intensity maxima all of dye solutions was not significant. This is mainly due to that the fluorescence spectra are disturbed by the inner-filter effect in dye solutions. Inner -filter effect is a phenomenon that causes the fluorescence spectra to be destroyed by the reabsorption fluorescence process from various molecules (monomer, dimer, trimer etc.) within the solution at high concentrations. As a result, the wavelength position at the fluorescence intensity maxima of fluorescent dyes in each solvent species has varied.

Conclusions

This study, the solvent effect which affected behavior of the fluorescence spectra changes was investigated in fluorescent dye solution such as Fluorescein, Bromofluorescein, Rhodamine B and Rhodamine 6G. It was shown that the solvents had their own influence on fluorescence spectra which can be characterized into two categories. The first is the value of fluorescence intensity maximum changes when the solvent species changed in various fluorescence dye species which can be explained by the solubility of each fluorescence dye in various solvent species. In this way, if sufficient data is collected, it is possible to estimate the polarity value of complex molecules structure. And the second is the wavelength position at fluorescence intensity maxima shift as the solvent species changes which can be explained by Lippert-Mataga equation in terms of $f(\varepsilon, n)$. However, the behavior of wavelength position at fluorescence intensity maxima shift was explained from the result of the fluorescence spectra only which was not accurately correct. It is necessary to collect the absorption spectra data of every solvent to analyze to find Stokes shift Δv so that we can give more accurate explanation.

Acknowledgment

The authors are thankful to Faculty of Science at Si Racha, Kasetsart University Si Racha Campus for financial support. This work was supported by Laser and Applied Optics Research Laboratory, Department of Physics and Materials Science, Faculty of Science, CMU.

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