A Simple Ratiometric Probe System for the Determination of Water Content in Organic Solvents

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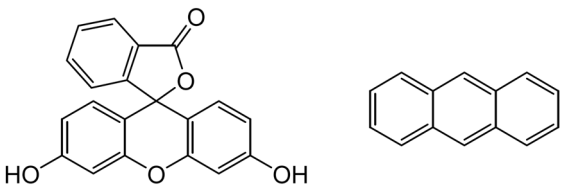
Determination of water content in organic solvents is very important in many of chemical processes and industrial applications.[1,2] Chromatography and Karl Fischer titration are one of the most widely used techniques for this purpose.[3] However, chemosensors are more convenient and simple to use in routine laboratory manipulations.[4] Among the chemosensing systems, chromogenic or fluorogenic sensors are particularly attractive due to their easy of signaling and signal transducing method. Many interesting sensing systems have been reported including merocyanine dyes,[5,6] chromone,[7] flavone derivatives,[8] membrane[9] and optical-fiber[10] sensor employing acridine dyes, and metal complex of $Ru(bpy)(CN)_4^{2-}$,[11] and are well reviewed in recent report.[2a]

Fluorescein and its related compounds are known to have an interesting fluorescence signaling behavior in many biological systems.[12] The spectral properties of fluorescein derivatives are strongly affected by the microenvironments and seems to be promising as molecular probes for the assessment of polarity of the biological systems. Particularly, it is well-known that the fluorescence of some fluorescein derivatives is strongly dependent on the media and the presence of a specific chemical stimulus.[13] Based on this, we tried to elucidate the possibility of the fluorescein for the signaling of water content in common organic solvents.

In preliminary studies, the UV-vis and fluorescence spectra of fluorescein **1** were found to be significantly affected in response to the increase in water content in common organic solvents. The significant fluorescence signaling behavior of the fluorescein was ratiometrically analyzed in reference to the relatively unaffected fluorescence of anthracene as an internal standard. In fact, the devised system exhibited a sensitive ratiometric fluorogenic behavior in response to the changes in water content of acetone and acetonitrile.



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Large fluorescence change in response to the changes in water content

Minor fluorescence change

First, UV-vis spectral behavior of the fluorescein **1** was studied in aqueous acetonitrile (Figure 1). In 100% acetonitrile solution, compound **1** exhibited no significant absorption band above 400 nm, which means that the fluorescein exists mainly in lactone form. As the water content increased, a strong absorption band around 452 and 488 nm emerged and steadily increased. That might be due to the shift in equilibrium of **1** toward ring-opened form over spectroscopically inactive lactone form. The changes in absorbance at 488 nm were not so pronounced up to 5% water then a significant enhancement was observed with further increasing water content in acetonitrile.

Next, the changes in fluorescence of fluorescein in response to the water content in aqueous acetonitrile were measured. As discussed earlier, the fluorescence of **1** was strongly dependent on the water content in aqueous organic solvents. In 100% acetonitrile, compound **1** exhibited a very weak fluorescence around 541 nm, which is due to the fact that the fluorescein exists predominantly in lactone form in organic solvents. As the water content increased up to around 2%, the emission of **1** was significantly enhanced (20-fold) with some blue shift to 529 nm. In response to the further increase in water content from 2 to 50%, further blue shift in absorption maximum from 529 to 521 nm with another large (5.5-fold) fluorescence enhancement was observed.

The chemosensing behavior of fluorescein was found to be efficient and a sensitive signaling of water content in aqueous acetonitrile seems to be realizable. However, the

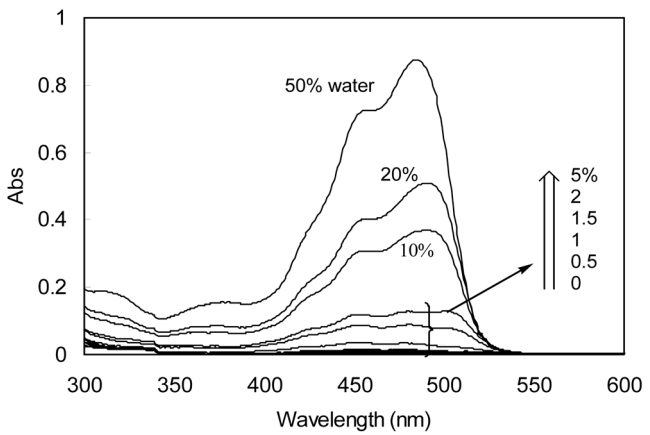


Figure 1. UV-vis spectra of fluorescein **1** in aqueous acetonitrile. [**1**] = 5.0×10^{-5} M.

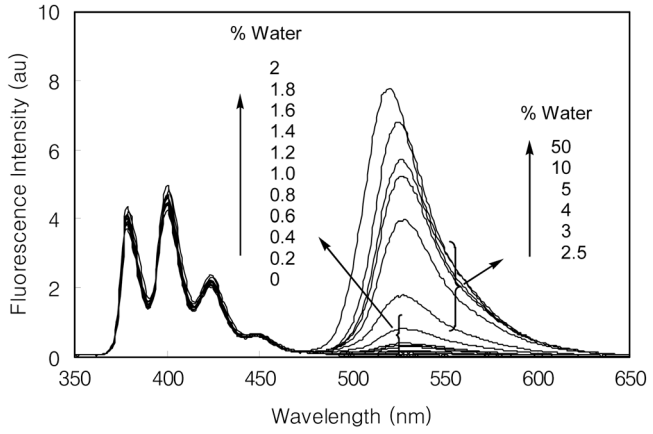


Figure 2. Fluorescence spectra of the chemosensor system (**An-Fl**) as a function of water content in aqueous acetonitrile. [**1**] = [**2**] = 2.5×10^{-6} M, λ_{ex} = 340 nm.

efficient reporting behavior of **1** itself is subject to error, especially in lower water content region (<2%) where relatively minor fluorescence changes were observed. Therefore, we have tried to use anthracene, whose fluorescence was not so significantly affected by the changes in water content in acetonitrile under the measurement conditions, as an internal reference. The fluorescence changes of a mixture of anthracene and fluorescein (**An-Fl**) as a function of water content were measured (Figure 2). In 100% acetonitrile, the system revealed strong emission bands at 379, 400, and 423 nm characteristic of anthracene with very weak emission around 529 nm of fluorescein. As the water content increased, as expectedly, the emission of the fluorescein was significantly enhanced while those of the anthracene were not considerably affected.

To have more insight into the chemosensing behaviors of the **An-Fl** system, the fluorescence intensity ratio of 400 and 530 nm (I_{400}/I_{530}), which are characteristic bands of anthracene and fluorescein, respectively, was plotted as a function of water content in aqueous acetonitrile. A nice profile was observed up to 3% of water and then the trend was leveled off in the range of greater than 5% water content (Figure 3).

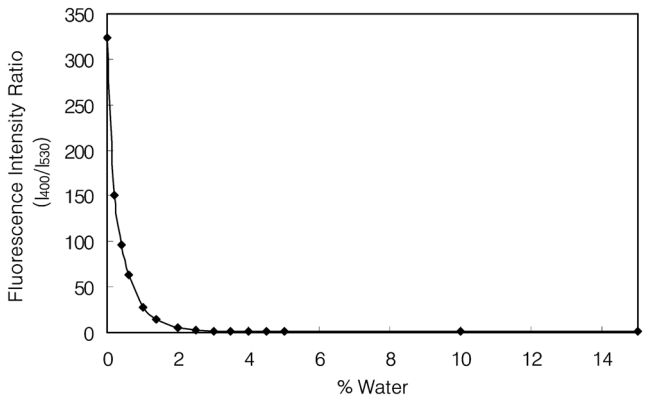


Figure 3. Ratiometric behavior (I_{400}/I_{530}) of the **An-Fl** system in response to the water content in aqueous acetonitrile. [**1**] = [**2**] = 2.5×10^{-6} M, λ_{ex} = 340 nm.

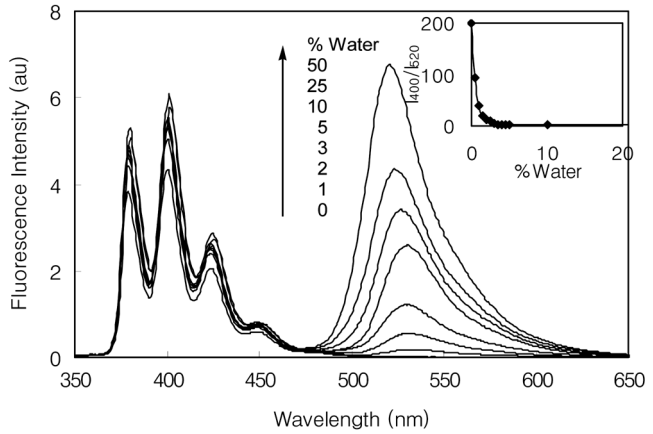


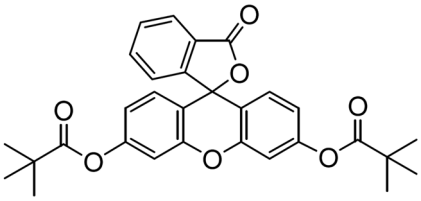
Figure 4. Fluorescence spectra of the chemosensor system (**An-Fl**) as a function of water content in aqueous acetone. [**1**] = [**2**] = 2.5×10^{-6} M, λ_{ex} = 340 nm. Inset shows the variation of fluorescence intensity (I_{400}/I_{520}) as a function of water content.

The ratio (I_{400}/I_{530}) changed over a large span from 320 to almost zero (0.02) in response to the changes in water content from 0% to 5%. As can be seen from Figure 3, the ratiometric changes were particularly prominent in region of less than 2% water. Interesting thing to note is that the solution color changed from colorless to green as the water content increased, which can be discernible even with naked-eye. The detection limit of the **An-Fl** system for the analysis of water content in acetonitrile was found as 0.035%.[14]

The possibility of utilization of the **An-Fl** system's chemosensing behavior for the determination of other organic solvents was tested in acetone. The **An-Fl** system in acetone also exhibited a quite similar spectroscopic behavior (Figure 4) comparable with acetonitrile. As the water content increased, the prominent emission around 520 nm appeared while those of anthracene were not so significantly affected. For the ratiometric analysis, the ratio of fluorescence intensity at 400 and 520 nm was evaluated and plotted as a function of water content. The ratio varied in a large span from 200 to almost zero in response to the changes in water content, which signals effectively the water content in acetone with a detection limit of 0.11%.

The prominent fluorescence changes of the **An-Fl** system in response to the variations in water content seem to be due to the formation of the ring opened form of fluorescein in the presence of water.[15] In organic solvents, the equilibrium between lactone and ring opened form of the fluorescein is predominantly favored for lactone form.[16] However, as the water content increased, the interaction with water molecules resulted in the increase in the ring opened form. With this shift in equilibrium, the fluorescence as well as the UV-vis spectra changed significantly and a prominent fluorogenic and chromogenic signaling of water content could be realized. Although the detailed equilibrium involving the ring opened forms is not easy to follow, however, the ratiometric analysis resulted in a good signaling of the water content in acetone and acetonitrile. The structural charac-

teristics for the signaling behavior of fluorescein were confirmed by the closely related fluorescein derivative which can not establish the characteristic equilibrium between lactone and ring opened form of fluorescein moiety. The bis-pivaloyl ester **3**[17] which represented known closed lactone form of fluorescein does not produce any signaling behavior in response to the changes in water content in acetonitrile.



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In summary, a mixture of fluorescein and anthracene was found to be effective as an efficient probe system for the signaling of water content in organic solvents. The significant changes in fluorescein emission in response to the variations in water content were ratiometrically analyzed by employing relatively constant anthracene emission as an internal reference. The system works well in aprotic acetonitrile and acetone, especially in lower concentration range of less than 2% water.

Experimental Section

General. Anthracene and fluorescein were purchased from Aldrich Chemical Co. and used without any purification. ^{1}H and ^{13}C NMR spectra were measured on a Varian Gemini-2000 spectrometer. HRMS spectra were obtained with a Micromass Autospec Mass Spectrometer. UV-vis spectra were obtained with a Jasco V-550 spectrophotometer. Fluorescence measurements were performed using an Aminco-Bowman Series 2 Spectrometer. Acetonitrile used for the spectroscopic measurements was purchased from Aldrich Chemical Co. as 'anhydrous' grade having water content less than 0.001%. Acetone was spectroscopic grade and used after storing with molecular sieve.

Preparation of Bis-pivaloyl ester 3.[17] To a mixture of fluorescein (0.96 g, 2.88 mmol) and cesium carbonate (2.08 g, 6.38 mmol) in DMF was added pivalic anhydride (1.24 mL, 6.12 mmol) and stirred for 5 h at room temperature. The mixture was filtered and the volatiles were evaporated. The crude product was purified by the column chromatography (silica gel, CH_2Cl_2) to afford **3**. Yield: 89%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (d, J = 7.5 Hz, 2H), 7.65 (m, 4H), 7.17 (d, J = 7.5 Hz, 2H), 7.07 (d, J = 2.1 Hz, 2H), 6.84 (d, J = 8.4 Hz, 2H), 6.78 (dd, J = 8.7 Hz and 2.1 Hz, 2H), 1.36 (s, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.8, 169.5, 153.3, 152.8, 151.8, 135.5, 130.2, 129.0, 126.2, 125.4, 124.2, 117.9, 116.4, 110.4, 81.8, 39.2, 27.1. FAB-Mass (*m*-NBA) calcd for $C_{30}H_{29}O_7$ 501.2. Found 501.1.

UV-Vis and Fluorescence Spectra Measurements. Stock solutions of **1**, **2**, and **3** were prepared in anhydrous acetonitrile or acetone (1.0×10^{-3} M). Incremental amount of water was added to the stock solution of probe system in organic solvents by a microsyringe or micropipette. After this, the solution was diluted with organic solvents to make the required probe concentration as well as the water contents in between 0 and 50%.

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