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Ratiometric Determination of Water Content in Acetonitrile by a Fluorescein Derivative Bearing Two Pyrene Subunits

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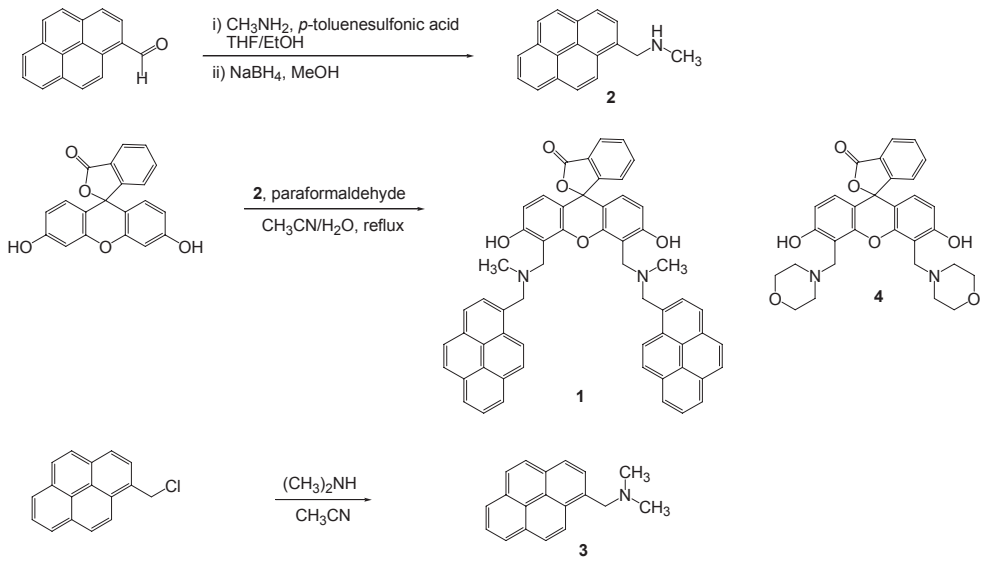
Measuring and controlling water content in organic solvents are factors of considerable importance in many chemical and industrial processes.[1,2] The Karl Fischer titration, based on a well-known water consuming reaction, has been the most widely used technique for the water content determination in many substances.[3] However, optical systems offer greater convenience and ease to use for routine work. Recently, a variety of optical signaling systems for metal ions and anions, such as chromogenic and fluorogenic sensors that convert chemical information or physical interactions into optically detectable signals have been developed as they are simple to manipulate and often allow naked-eye detection.[4] There have been many reports on optical sensing systems employing dye molecules,[5] including merocyanines,[6] flavones,[7] chalcone,[8] 3-hydroxychromone,[9] naphthalimide,[10] and indole derivatives[11] for the determination of water content in organic solvents.

Fluorescein has been used in wide-ranging applications, from biochemical research to molecular imaging, because of its high fluorescent quantum yield and excellent environment-sensitive responses.[12] Recently, the possibility of fluorescein for the determination of water in organic solvents has been investigated.[13] In that report, to fabricate a useful ratiometric sensing system, anthracene was introduced as an internal reference since its fluorescence is not significantly affected by changes in water content.

The pyrene subunit has been widely used as a reporting fluorophore for the construction of chemical sensors as their characteristic fluorescence features are strongly dependent on the environment.[14] Especially, the pyrene excimer, which is the result of a conformation between two nearby pyrene moieties, has been used extensively to elucidate structural characteristics of various chemical and biological systems.[15] Furthermore, the pyrene-fluorescein pair has been used as an efficient signaling system that employs the fluorescence resonance energy transfer (FRET) process.[16] In this study, we prepared a new fluorescein derivative, bearing two nearby pyrene moieties that could yield an excimer, as a ratiometric sensing system for probing the water content in acetonitrile. The pyrene-appended fluorescein exhibited well-defined ratiometric signaling behavior suitable for the determination of the water content in a representative water miscible organic solvent, acetonitrile.

Fluorescein-pyrene conjugate **1** was prepared by the Mannich reaction[17] of fluorescein with *N*-methylaminomethylpyrene **2** (paraformaldehyde, acetonitrile/H_2O = 7:3) in moderate yield (64%) (Scheme 1). The *N*-methylaminomethylpyrene **2** was prepared by condensation of 1-pyrenecarboxaldehyde with methylamine (*p*-toluenesulfonic acid, THF/EtOH), followed by reduction with sodium borohydride in methanol.[18] The mor-



Scheme 1. Synthesis of fluorescein derivatives (**1** and **4**) and aminomethyl pyrenes (**2** and **3**)

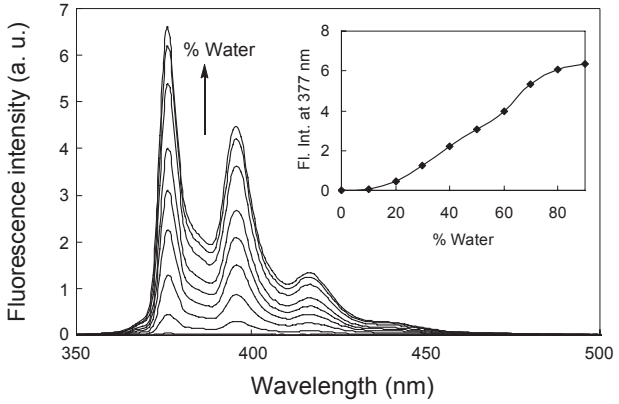


Figure 1. Fluorescence spectra of **3** in acetonitrile with varying water content. Inset shows the changes in fluorescence intensity at 377 nm as a function of water content. [**3**] = 5.0×10^{-6} M, λ_{ex} = 340 nm.

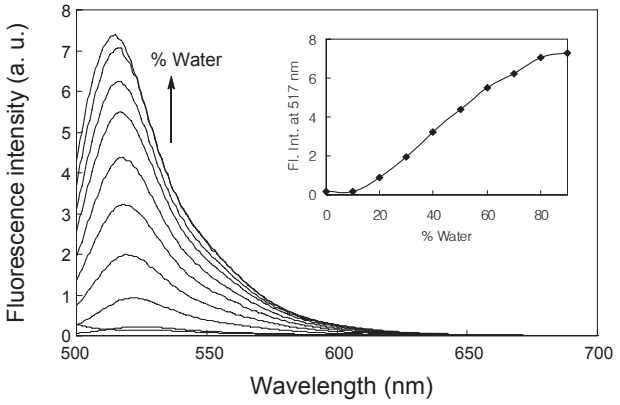


Figure 2. Fluorescence spectra of **4** in acetonitrile with varying water content. Inset shows the changes in the fluorescence intensity at 517 nm as a function of water content. [**4**] = 5.0×10^{-6} M, λ_{ex} = 494 nm.

pholine derivative of fluorescein **4**, as a model compound, was prepared similarly by the Mannich reaction of fluorescein with morpholine. Another model compound, *N*,*N*-dimethylaminomethylpyrene **3**,[19] was prepared by the reaction of 1-chloromethylpyrene with dimethylamine.

First, a preliminary study on the fluorescence responses of model compounds **3** and **4** toward changes in water content was carried out to assess the potential of compound **1** to report water content variations in acetonitrile. Significant changes in the fluorescence spectra, as well as fluorescence color of solution in response to the variation in water content, were observed in an aqueous acetonitrile solution. Based on this, the effects of the water content on the fluorescence spectra of **3** and **4**, as well as pyrene-fluorescein conjugate **1** in acetonitrile, were systematically investigated.

Dimethylaminomethylpyrene **3** exhibited very weak fluorescence in acetonitrile (Figure 1), due possibly to the presence of the nearby amino group that exhibits efficient PET from the nitrogen atom to the pyrene.[20] Upon treatment with incremental amounts of water, the fluorescence intensity of **3** at 377, 397, and 417 nm, typical of the pyrene fluorophore, progressively increased. The changes in fluorescence intensity at 377 nm were very large, with a 610-fold increase observed upon increasing the water content from 0 to 90%. In this case, the changes were quite linear in response to the variation of water content from 10 to 80% and could be used readily as a signaling tool for assessment of the water content in aqueous acetonitrile.

The smooth increases throughout the entire range of the water content changes in acetonitrile suggest that the fluorescence changes are probably due to general solvent effects induced by variation of the water content in the aqueous acetonitrile.[21] In addition to this general solvent effect, as the water content increased, the lone pair on the nitrogen atom can strongly interact with the water molecules; the effectiveness of the photoelectron transfer (PET) from the amino group to the pyrene thus becomes less pronounced.[22] This also resulted in a fluorescence increase in the pyrene moiety.

Next, the effects of the water content on the emission behavior of morpholinomethyl fluorescein **4** in acetonitrile were measured (Figure 2). In 100% acetonitrile, the fluorescence intensity centered at 517 nm was relatively weak, as in pyrene derivative **3**. With increases in the water content in acetonitrile, the fluorescence intensity was steadily increased, up to 80% water, with a minor blue shift ($\Delta\lambda_{max}$ = −10 nm). The changes in fluorescence intensity were due mainly to the shift in equilibrium towards the ring-opened structure of fluorescein,[23] as well as suppression of PET from the interaction of the nitrogen atom of the amine with water molecules in response to the increased water content. This emission behavior could also be readily used for signaling a water content of up to 80% in acetonitrile.

As has been discussed, signaling a water content of up to 80% in acetonitrile, using **3** or **4**, seems plausible by measuring the changes in fluorescence intensity of the pyrene or fluorescein moiety. However, the precise measurement of the changes in emission intensity is subject to error because the responses were measured at one specific wavelength. The conjugation of these two subunits might provide more useful ratiometric signaling behavior or a possibility of realizing the FRET process. As is well known, the ratiometry using two wavelength measurements is more adequate as it provides more precise measurements for normalizing variations in measuring conditions such as path length, photobleaching, and dye concentration.[24]

Compound **1**, having the two fluorophores of pyrene and fluorescein, exhibited moderate fluorescence emission characteristics of a pyrene monomer at 377, 397, and 418 nm, and a weak emission of fluorescein (Figure 3). As the water content increased, the emissions of pyrene between 377 - 418 nm, as well as fluorescein at 517 nm, were progressively enhanced. This might be due to the general solvent effect and suppression of the PET process by the increases in water content. However, expected FRET from pyrene to fluorescein seems to be inefficient as the water content increased, resulting in increased intensity in emissions of both pyrene and fluorescein. On the other hand, as can be seen from the Figure 3, another large (> 10-fold) fluorescence enhancement was observed at 460 nm, indicating that the intramolecular excimer between the two appended pyrene moieties formed as the water content increased. The fluorescent probe behavior was easily observed by the color change of the emissions from dark blue to bright sky blue, under illumination

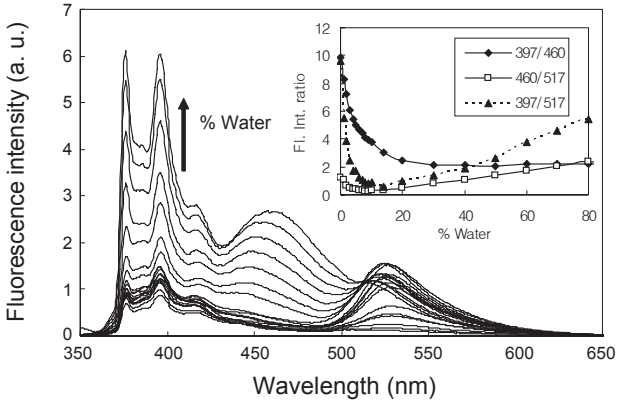


Figure 3. Fluorescence spectra of **1** in acetonitrile solution of varying water content. Inset shows the ratiometric behavior of I_{397}/I_{460}, I_{460}/I_{517}, and I_{397}/I_{517} as a function of water content. [**1**] = 5.0×10^{-6} M, λ_{ex} = 340 nm.

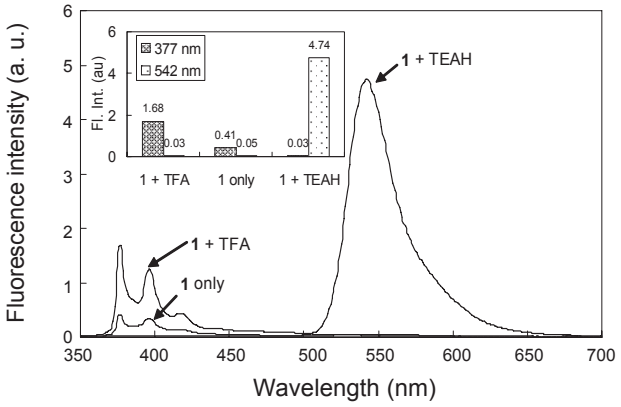


Figure 4. Effect of foreign acidic or basic materials on the fluorescence behavior of **1** in acetonitrile. [**1**] = 5.0×10^{-6} M, [TFA] = [TEAH] = 5.0×10^{-5} M, λ_{ex} = 340 nm.

with a UV lamp.

The changes in fluorescence of pyrene and fluorescein were analyzed by ratiometry using the ratio of two emissions from pyrene and fluorescein. First, variation in the ratio of the two pyrene fluorescence emissions (monomer/excimer, I_{397}/I_{460}) was plotted as a function of water content (inset of Figure 3). The ratio I_{397}/I_{460} varied smoothly from 9.9 to 2.2, as the water content in the aqueous acetonitrile increased. As can be seen from Figure 3, significant ratiometric changes were observed up to 20% water content. The ratiometry using the pyrene monomer and fluorescein emissions at 397 and 517 nm resulted in a more steeply decreasing ratio from 9.7 to 0.79, up to 10% water content; however, after this, the ratio slowly increased again as the water content increased. Another ratiometric analysis using a pair of emissions at 460 and 517 nm was not as pronounced, and varied only within a narrow range between 2.3 and 0.28.

These observations imply that pyrene-appended fluorescein **1** could be used as a simple ratiometric fluorescent probe for the determination of water content of up to 20% in acetonitrile, using the ratio of the emissions of the pyrene monomer and excimer. The detection limit of **1** for water in acetonitrile was found to be 0.45%. However, using the ratio I_{397}/I_{517} of the pyrene monomer and fluorescein provides signaling for less than 10% water, with a more sensitive detection limit of 0.24%.

Finally, the effect of foreign acidic or basic materials on the fluorescence behavior of **1** was tested (Figure 4). Compound **1** exhibited week emissions of pyrene around 377 - 418 nm in acetonitrile. Upon addition of 10 equiv of trifluoroacetic acid (TFA), these emissions of pyrene enhanced significantly, while the emission of fluorescein region was not changed. Enhanced fluorescence is due to the suppression of PET process from the amino group to pyrene by the protonation of the amino group of **1**. The absence of fluorescence in fluorescein region is due to the formation of non-fluorescent lactone form of fluorescein in acidic media. On the other hand, addition of 10 equiv of tetraethylammonium hydroxide (TEAH) resulted in a large decrease in pyrene emissions with significantly enhanced emission around 545 nm of fluorescein. That might be due to the formation of the strongly fluorescent ring-opened structure of lactone moiety of fluorescein as well as efficient FRET from pyrene to fluorescein.

In summary, a new fluorescein derivative appended with two pyrene moieties was synthesized and its fluorogenic signaling behavior for the determination of water content in acetonitrile was investigated. The compound showed a characteristic fluorescence change in response to the changes in water content in acetonitrile. The changes could be conveniently analyzed by ratiometry using both the monomer and/or excimer emission of pyrene, as well as the fluorescein emission. The ratiometric analysis of the fluorescence changes in the pyrene monomer and excimer emissions successfully provided a convenient means for the determination of a water content of up to 20% in acetonitrile.

Experimental Section

General. Fluorescein, 1-chloromethylpyrene, and 1-pyrenecarboxaldehyde were obtained from TCI and Aldrich Chemical Co. and used as received. Acetonitrile was purchased from Aldrich Chemical Co. as 'anhydrous grade' and was used without further purification. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra were obtained on a Varian VNS spectrometer. Mass spectral data were obtained with a Micromass Autospec mass spectrometer. Fluorescence spectra were recorded with a Amin-co-Bowman Series 2 Luminescence Spectrometer.

Preparation of 2. Compound **2** was prepared by the reaction of 1-pyrenecarboxaldehyde with methylamine followed by the reduction with $NaBH_4$ following the literature procedure.[18]

Preparation of 1. Compound **2** (250 mg, 1 mmol) was treated with paraformaldehyde (290 mg, 10 mmol) in 20 mL of CH_3CN and refluxed for 30 min, and then a solution of fluorescein (100 mg, 0.3 mmol) in 30 mL of H_2O/CH_3CN (1:1, v/v) was added to the mixture. The reaction mixture was heated under reflux for 1 day. The solvents were removed completely under reduced pressure and the residue was purified by the column chromatography (silica gel, CH_2Cl_2 : CH_3OH = 19 : 1, v/v) to afford compound **1** as an orange solid (162 mg, 64%). mp > 300 ^{o}C (decomp). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.18 (d, J = 9.3

Hz, 2H), 8.17 - 8.14 (m, 3H), 8.06 (d, J = 7.1 Hz, 2H), 8.03 (d, J = 9.0 Hz, 2H), 7.98 (d, J = 8.7 Hz, 4H), 7.91 (t, J = 7.6 Hz, 2H), 7.82 (s, 1H), 7.80 (d, J = 6.0 Hz, 2H), 7.78 (s, 1H), 7.73 (m, 2H), 7.20 (d, J = 7.6 Hz, 1H), 6.57 (d, J = 8.7 Hz, 2H), 6.52 (d, J = 8.7 Hz, 2H), 4.23 - 4.08 (m, 8H), 2.18 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.1, 159.6, 150.7, 135.9, 132.0, 131.0, 130.7, 130.5, 129.6, 129.0, 127.8, 127.7, 127.6, 127.5, 127.4, 127.0, 126.5, 125.6, 125.5, 125.4, 125.2, 124.7, 124.5, 124.4, 124.2, 124.1, 112.6, 110.7, 110.2, 59.0, 51.1, 42.2. HRMS (FAB+) m/z calcd for $C_{58}H_{43}N_2O_5$ $[M+H]^+$ 847.3172, found 847.3185.

Preparation of 3.[25] 1-Chloromethylpyrene (0.25 g, 1.0 mmol) was dispersed in 30 mL of dichloromethane and excess dimethylamine (10 mL) was added to the reaction mixture. The mixture was refluxed for 1 day and evaporated under reduced pressure. The residue was partitioned between dichloromethane and water. The organic layer was separated, washed with water, and evaporated under reduced pressure. Crude product was purified by column chromatography (silica gel, CH_2Cl_2 : CH_3OH = 9 : 1, v/v) to yield compound **3** (220 mg, 85%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.51 (d, J = 9.2 Hz, 2H), 8.21-8.16 (m, 2H), 8.14 (d, J = 2.2 Hz, 1H), 8.13 (s, 1H), 8.05 (s, 2H), 8.00 (t, J = 7.7 Hz, 1H), 7.96 (d, J = 7.7 Hz, 1H), 4.10 (s, 2H), 2.37 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 132.7, 131.3, 130.9, 130.8, 129.9, 128.2, 127.4, 127.1, 125.8, 125.0, 125.0, 124.9, 124.8, 124.3, 123.9, 62.5, 45.7. LRMS (DIP) m/z calcd for $C_{19}H_{17}N$ $[M]^+$ 259.14, found 259.17.

Preparation of 4. Morpholine (87 mg, 1 mmol) was treated with paraformaldehyde (290 mg, 10 mmol) in 20 mL of CH_3CN and refluxed for 30 min, and then a solution of fluorescein (100 mg, 0.3 mmol) in 30 mL of H_2O/CH_3CN (1 : 1, v/v) was added to the mixture. The reaction mixture was heated under reflux for 1 day. The solvents were removed completely under reduced pressure and the residue was purified by the column chromatography (silica gel, CH_2Cl_2 : CH_3OH = 19 : 1) to afford compound **4** as an orange solid (119 mg, 75%). mp > 300 °C (decomp). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.98 (d, J = 7.7 Hz, 1H), 7.67 (t, J = 7.5 Hz, 1H), 7.60 (t, J = 7.5 Hz, 1H), 7.19 (d, J = 7.7 Hz, 1H), 6.52 (m, 4H), 4.01 (m, 4H), 3.79 (br s, 8H), 2.68 (br s, 8H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.1, 159.9, 152.2, 149.8, 134.8, 129.7, 128.1, 127.5, 125.0, 124.0, 112.8, 110.2, 107.2, 84.2, 66.7, 54.7, 53.2. LRMS (DIP) m/z calcd for $C_{30}H_{30}N_2O_7$ $[M]^+$ 530.21, found 530.20.

Measurements of fluorescence spectra. The fluorescence spectra were measured at a constant concentration (5.0×10^{-6} M) of probe in acetonitrile containing varying amount of water. Mixed aqueous acetonitrile solutions were obtained by mixing appropriate amount of stock solution of probe (5.0×10^{-4} M in acetonitrile solution) with water and finally diluted with the acetonitrile to make the solution having desired water content and concentration of probe.

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