Simple and Efficient Ratiometric Fluorescent Probes for Near-neutral pH in Aqueous Solutions

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Two ratiometric fluorescent pH probes of 2,6-diaminopyridine (DAPD) and 2-amino-5,7-dimethyl-1,8-naphthyridine (ADMND), though simple-structured, show good sensitivity to near-neutral pH range (6.0–8.0) in aqueous solutions. Further studies indicate that the 2-amino groups on pyridine or naphthyridine ring play an important role in the pH-dependent fluorescence spectral properties of these dyes.

Fluorescent pH probes in aqueous solutions have now attracted much attention since their potential applications in fluorescence pH microscopy, ratio imaging, as well as intracellular pH measurements.[1,2] However, protons still have not been studied as much as other ions (e.g. Ca^{2+}) by fluorescence microscopy,[3] a possible explanation for this is the absence of entirely satisfactory fluorescent indicators to measure pH in the relevant range (4.5–7.4).[4] Therefore, many works has been concerned about the exploration of new fluorescent pH indicators,[5] especially those applied to measure pH by means of a ratiometric method.[6–8] The greatest advantage of these ratiometric probes is their insensitivity to probe concentration or background fluorescence, thus the photobleaching or diffusion of the probes will not affect the measurements. Despite of their advantages, many ratiometric probes reported are rather complicated to synthesize.[3,6–8] Herein we report two simple ratiometric fluorescent probes of **1** and **3**, which are sensitive to near-neutral pH range (6.0–8.0) with acceptable quantum yields compared to reported probes (Figure 1).[3,8]

In fact, 2,6-diaminopyridine (**1**) is commonly used as an original material in chemical synthesis[9] and commercially available. Owing to its simplicity, the fluorescence properties of **1** are usually neglected by researchers. However, with careful experiment, we found that **1** could indeed serve as an efficient ratiometric fluorescent pH probe in aqueous solutions. In phosphate buffers of pH 4.0–10.0, **1** exhibited an absorption isosbestic point at 310 nm. When excited at this isosbestic point, it gave fluorescence in both the acidic and basic states with an isoemission point at 371 nm and exhibited shifted absorption and emission spectra (Figure 2), which was the most important requirement for a ratiometric probe. The bathochromic shift observed in both absorption and emission spectra upon decreasing the pH value was due to the increase in the electron-withdrawing power of the pyridine ring upon protonation of the pyridyl nitrogen atom.[8] The evolution of the fluorescence emission of an aqueous solution of **1** as a function of pH is shown in Figure 2. The acid–base reaction of **1** yields pK_a (**1**H$^+$) $= 7.0 \pm 0.1$ at 298 K.[10] With this neutral pK_a value, the ratio of fluorescence emission at 390 and 360 nm of **1** shows good sensitivity to pH 6.0–8.0 range. The plots in the response range (6.0–8.0) are approximately linear with abrupt slope, which is ideal for accurate measurements. Furthermore, the three curves in Figure 2 nearly overlap, with a deviation less than 5%, indicating that the ratio of fluorescence emissions (F_{390}/F_{360}) of **1** is insensitive to probe concentration even from 2.0×10^{-7} to 2.0×10^{-5} mol·L^{-1}.

The chemical modifications of **1** were also carried out to get a better understanding of the fluorescence spectral properties of this simple compound, thus **2**–**5** were synthesized. Direct acyla-

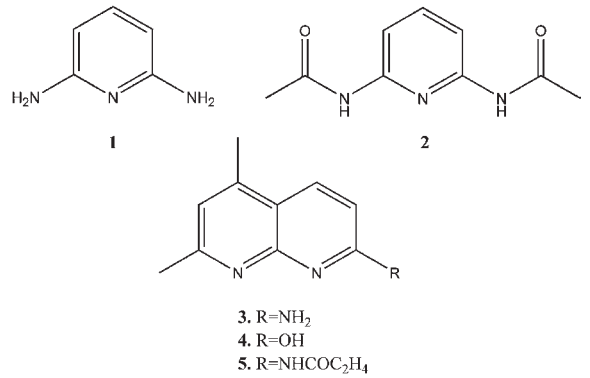

Figure 1. Molecular structures of **1**–**5**.

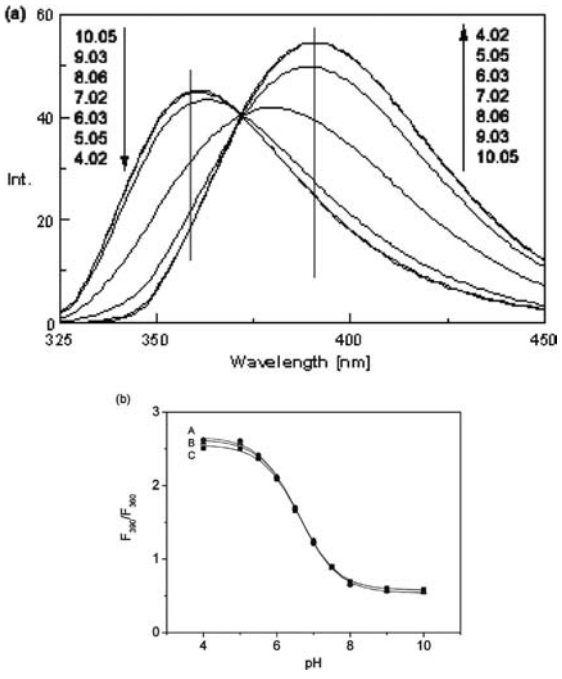

Figure 2. (a) Dependence of the emission spectra of a solution of 2.0×10^{-5} mol·L^{-1} **1** (in phosphate buffer, 298 K) on pH: from acidic to basic conditions: pH 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0. (b) Evolution of the ratio of the fluorescence emissions at 390 and 360 nm of **1**: (A) 2.0×10^{-5} mol·L^{-1} **1**; (B) 2.0×10^{-6} mol·L^{-1} **1**; (C) 2.0×10^{-7} mol·L^{-1} **1**.

Table 1. Spectral properties of **1** and **3–5** at 298 K.

Compound	$\lambda_{ab,max}{}^{a}$ /nm	$\lambda_{ab,max}{}^{b}$ /nm	$\varepsilon_{max}{}^{a}/10^3$ cm·L·mol^{-1}	$\varepsilon_{max}{}^{b}/10^3$ cm·L·mol^{-1}	$\lambda_{em,max}{}^{a}$ /nm	$\lambda_{em,max}{}^{b}$ /nm	$\Phi^{a,c}$	$\Phi^{b,c}$	$pK_a{}^{d}$
1	331	304	8.0	4.4	390	360	0.28	0.24	7.0 ± 0.1
3	347	335	9.2	7.0	401	380	0.65	0.26	7.2 ± 0.1
4	326	324	24	20	397	363	0.30	0.06	
5	346	334	13	10	378	388	0.13	0.02	

[a]Measurements were carried out in acidic phosphate buffers (pH = 4.0). [b]Measurements were carried out in basic phosphate buffers (pH = 4.0). [c]Fluorescence quantum yields were calculated at emission peaks using fluorescein as a standard (0.01 mol·L^{-1} NaOH, $\Phi = 0.95$).[11] [d]pK_a values were calculated following equation: $\log[(F_{max} - F)/(F - F_{min})] = pH - pK_a'$.

tion of the two amino groups (**2**)[9] would make the compound nearly nonfluorescent due to the strong electron-withdrawing power of the acyl groups. To avoid this vanishing of fluorescence, compound **3**[9] was introduced. The more delocalized naphthyridine ring in **3** made it more fluorescent (with a much higher quantum yield) than **1**. Figure 3 illustrates the evolution of the fluorescence emission of an aqueous solution of **3** as a function of pH. The fluorescence spectral properties of **3** were somewhat similar to those of **1**. However, when the amino group of **3** was modified to whether a hydroxyl group (**4**) or acrylamido group (**5**), the fluorescence of basic state of **4** and **5** became much weaker (Table 1) and no isoemission point could be observed, suggesting that **4** and **5** could not be used as ratiometric probes. Therefore, the 2-amino group on pyridine or naphthyridine ring plays an important role in the fluorescence spectral properties of these probes, making them applicable for ratiometric fluorescent pH measurements. An explanation for this phenomenon is that the lone electron pair of 2-amino group conjugates with the delocalized heteroaromatic ring and makes the probe more fluorescent in basic aqueous solutions.

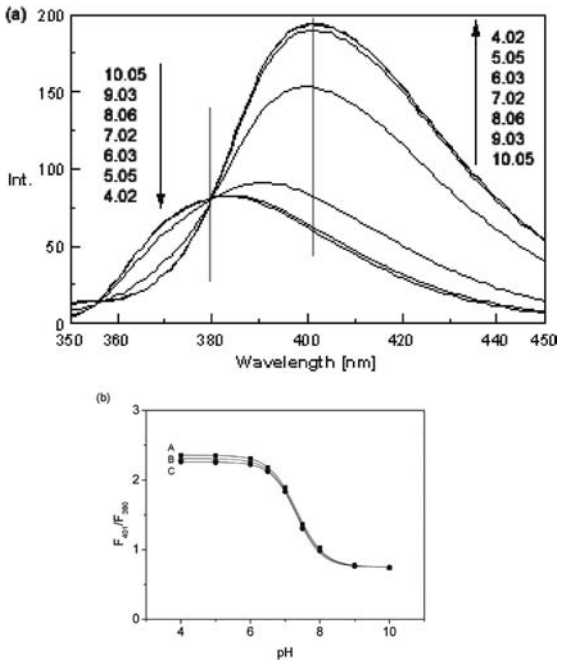

Figure 3. (a) Dependence of the emission spectra of a solution of 2.0×10^{-5} mol·L^{-1} **3** (in phosphate buffer, 298 K) on pH: from acidic to basic conditions: pH 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0. (b) Evolution of the ratio of the fluorescence emissions at 401 and 380 nm of **3** in aqueous solutions. (A) 2.0×10^{-5} mol·L^{-1} **3**; (B) 2.0×10^{-6} mol·L^{-1} **3**; (C) 2.0×10^{-7} mol·L^{-1} **3**.

In conclusion, two ratiometric fluorescent probes of **1** and **3** for near-neutral pH (6.0–8.0) in aqueous solutions have been reported in this paper. Their ratios of fluorescence emissions are found insensitive to probe concentration over a broad range. However, it has to be noted that, in accurate pH measurements of biological or clinical samples, the emission of some impurities at low wavelengths (360–380 nm) may give a considerable interference to these probes. For this reason, the exploration of new ratiometric fluorescent pH probes with longer emission wavelength based on **1** and **3** are now under our active investigation.

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