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1-Imino Nitroxide Pyrene, a Burst-type H^+ Fluorescent Switch

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Abstract: 1-Imino nitroxide pyrene **1** has been characterized as a H^+ fluorescent switch. The effects of various interfering species, solvents and the irradiation of ultraviolet light on the fluorescence intensity of **1** are also investigated. **1** fluoresces weakly in the 85% ethanol media containing no more than 4.5×10^{-5} mol/L of H^+ (HCl), but can produce a burst increase of about 4 times in the fluorescence intensity when the concentration of H^+ is increased to or over 4.9×10^{-5} mol/L. The extremely sharp response of **1** to H^+ occurs only within such a narrow concentration range. Moreover, the decrease of H^+ concentration through adding sodium hydroxide causes the fluorescence quenching, suggesting that **1** may serve as a fluorescent switch for monitoring the concentration change of H^+ around the point of 4.5×10^{-5} mol/L.

Keywords: Fluorescent switch, 1-imino nitroxide pyrene, H^+.

Nitronyl nitroxides and imino nitroxides are paramagnets and stable free radicals, which have been widely used as spin probes as well as for studies related to organic magnets[1-4]. Among these nitroxides, 1-imino nitroxide pyrene **1** and 1-nitronyl nitroxide pyrene are both representatives[4], and evidence accumulated indicating that the nitroxides attached to fluorophores can serve as strong intramolecular quenchers[2-3]. Based on this property, for example, Zhang *et al.* found that the fluorescence of pyrene can be tuned by virtue of chemical inputs[4], and Medvedeva *et al.* used 1-nitronyl nitroxide pyrene to explore superoxide dynamics and antioxidant status of biological systems[5]. On the other hand, since protonation/deprotonation of the nitrogen and oxygen atoms in the imidazole unit may change their electron-donating ability, thereby causing the change in fluorescence signal, such a behavior may be utilized for monitoring the H^+ alteration. In this communication we describe that **1** may serve as a H^+ fluorescent probe for monitoring the concentration change of H^+ around the point of 4.5×10^{-5} mol/L.

Experimental

1-Imino nitroxide pyrene (note that pyrene is carcinogenic) was prepared as reported previously[4]. All fluorescence measurements were made with a Hitachi F-2500 spectro-fluorimeter (Japan). Unless otherwise noted, the typical test solution was prepared by diluting 0.3 mL of ethanol solution of **1** (1×10^{-5} mol/L) to 2 mL with different solvents.

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Prior to measurement, the solution was irradiated with an ultraviolet light of 365 nm for 20 min.

Results and Discussion

The effect of different solvents on the fluorescence of **1** was first examined considering its low water solubility. As shown in **Table 1**, the probe **1** has a rather weak fluorescence in various solvents perhaps due to the expected intramolecular photoinduced electron transfer (PET)[6]. Moreover, the probe's fluorescence intensity is decreased with the increase of solvent's polarity, accompanying a small blue shift of 8 nm in the emission peak from 442 nm in dichloromethane to 434 nm in ethanol with a hardly altered excitation peak at 350 nm. It was noted that the fluorescence intensity from the solution of **1** is unstable and increases with time at room temperature, which may arise from the fact that **1** is sensitive to light irradiation. However, further experimental results showed that the fluorescence of the probe solution could reach a stable status after a 20 min irradiation with a 365 nm ultraviolet light, leading to about 2.7 fold fluorescence enhancement in all cases of the three solvents (**Table 1**). Furthermore, the lowest background fluorescence was observed from an ethanol solution of **1**. Thus, all subsequent experiments were done in 85% (v/v) ethanol media (the remaining 15% volume was for the introduction of aqueous samples) with 20 min ultraviolet light irradiation unless otherwise specified.

Figure 1B shows the typical fluorescence response of **1** to the change of H^+ concentration. It can be seen that the fluorescence is switched on once the H^+ concentration arises from 4.5×10^{-5} mol/L to 4.9×10^{-5} mol/L, and a burst increase of about 4 times in the fluorescence intensity occurs in such a narrow change of the H^+ concentration. Moreover, this fluorescence can be switched off (data not shown) by the decrease of H^+ concentration through adding sodium hydroxide solution, suggesting that **1** may serve as a fluorescent switch for monitoring the concentration change of H^+ around the point of 4.5×10^{-5} mol/L.

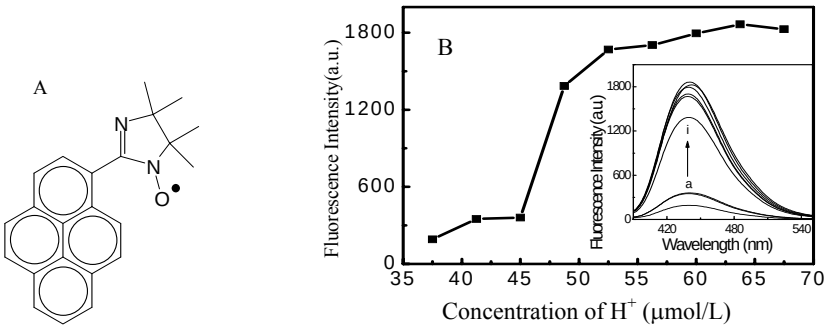
To test the selectivity of the probe, the effects of the various ions upon the fluorescence of **1** were then investigated. The results show that the selectivity of this probe for H^+ over other ions is good (**Table 2**), making it suitable for accurate reporting of H^+ concentration changes, and even possible for use in extreme pH ranges[8].

Table 1 Effect of solvents on the fluorescence of **1** (1.5 μmol/L)

Solvents	Dielectric constant [a]	Relative fluorescence intensity ($\lambda_{ex/em}$ = 350/442 nm)	
		Solution without 365 nm light irradiation	Solution with 365 nm light irradiation for 20 min
Dichloromethane	9.1	163	463
Acetone	20.7	43	114
Ethanol	25.3	16	41

[a] Data from Dean[7].

Figure 1



(A) Structure of **1**, and (B) the H^+-dependence of fluorescence intensity of **1** (1.5 μmol/L) in 85% ethanol media. The inset of (B) shows the variation of the corresponding fluorescence emission spectra [from (a) to (i)] with the H^+ concentration (as HCl) from 38 to 68 μmol/L at λ_{ex} = 350 nm.

Table 2 Effect of various species on the relative fluorescence intensity (***I***) of **1**[a]

M^{n+}	Blank	NaCl	K^+	Ca^{2+}	Mg^{2+}	Cu^{2+}	Fe^{3+}	Zn^{2+}	CO_3^{2-}	NO_3^-	PO_4^{3-}	SO_4^{2-}
I	1831	1703	1848	1873	1879	1738	1899	1867	1905	1859	1867	1907

[a] The fluorescence intensity of **1** (1.5 μmol/L) was measured in 85% ethanol media with 150 μmol/L of HCl and 6 μmol/L of various species (except that NaCl was 0.15 mol/L, K^+, Ca^{2+} and Mg^{2+} were 10 mmol/L) at $\lambda_{ex/em}$ = 350/442 nm.

The mechanism of the probe's fluorescence response to H^+ is complex due to the influence of solvent's polarity and the protonation/deprotonation of the nitrogen and oxygen atoms in the imidazole unit, but it may be interpreted largely according to PET principle, which has been widely used to design a variety of fluorescent ion sensing molecules[6,8]. It is known that the nitroxide group is a paramagnetic group, and electronegative nitrogen and oxygen atoms often serve as both H^+ receptors and electron donors in the PET process, which can quench the fluorescence of the proximity fluorophore by the intramolecular PET process[4,9]. This may explain why the probe exhibits weak fluorescence. On the other hand, further increasing of H^+ concentration to 4.9×10^{-5} mol/L may result in the complete protonation of the imino nitrogen atom, which prohibits the corresponding PET process and thereby switches on the fluorescence of **1** to a great extent.

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