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Highly water-soluble, OFF–ON, dual fluorescent probes for sodium and potassium ions

Premchendar Nandhikonda, Michael P. Begaye, Michael D. Heagy *

New Mexico Institute of Mining and Technology, Department of Chemistry, Socorro, NM 87801, United States

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ABSTRACT

Fluorescent probes 1 and 2 were conveniently synthesized from 4-sulfo-1, 8-naphthalic anhydride, potassium salt, and 4′-aminobenzo-15-crown-5 and also from 4′-aminobenzo-18-crown-6. These sensors exhibit dual emission and ratiometric absorption, are water soluble, and show good selectivity for sodium and potassium ions.

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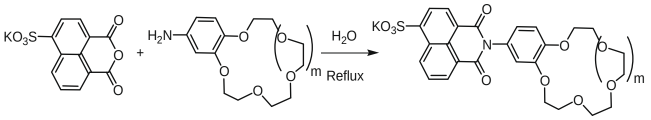
The development of wavelength ratiometric probes for Na^+ and K^+ has important implications in clinical research such as hyponatremia and hypertensive screening.[1] In blood, the concentrations of K^+ and Na^+ are near 4.5 and 120 mM, whereas in resting cells the concentrations are effectively reversed at 140 and 4 mM, respectively.[2a] Presently, wavelength ratiometric probes are available but problems regarding solubility, adequate spectral changes, and selectivity persist.[2] Therefore, designs for new probes of sodium and potassium which function in water with high selectivity remain highly desirable for biological studies. Because of their ability to selectively bind to alkali and alkaline earth metal cations, crown ether derivatives have attracted considerable attention.[3] Crown ethers have been widely used as the receptor component in conjunction with the fluorophore which makes them attractive targets as fluorescent probes. To date, sodium and potassium chemosensors based on such fluorescent platforms as benzofuranisophthalate[1a], 4-(dimethylamino)benzonitrile,[4] coumarin groups,[5] phenolichydroxyl,[6] anthracenylmethyl,[7] spirobenzopyran,[8] and 4-amino-1,8-napthalimide[9] have all been explored.

A relatively unexplored class of fluorescent platforms for ion sensing are 1,8-naphthalimides (NIs).[10] Recently, there has been an upsurge in the number of reports on photophysics and applications of NI-based dyes.[11] In the case of *N*-arylnaphthalimide, the dicarboximide functionality features a molecular orbital arrangement that augments the effects of donor/acceptor groups on both the NI ring and *N*-aryl component. *N*-Phenylnaphthalimides have been found to exhibit dual fluorescence when appropriately substituted at both the *N*-phenyl aromatic ring and naphthalene system.[12] These compounds display dual luminescence with two clearly resolved emission bands in the visible region from the locally excited (LE) state and a strongly red shifted long wavelength emitted by an internal charge-transfer (ICT) state. Dual fluorescence (DF) compounds feature the advantage of ratiometric detection as they reduce or eliminate distortions caused by photobleaching, indicator concentrations, and illumination stability.[13] In this report, we introduce two NI-based ion sensors that provide ratiometric absorption spectra as well as DF emission for internally calibrated ion analysis of sodium and potassium. Therefore, the crown ether probes **1** and **2** were synthesized based on DF systems previously reported by our group.[12]

Compounds **1** and **2** were conveniently synthesized from 4-sulfo-1,8-naphthalic anhydride, potassium salt, and 4′-aminobenzo-15-crown-5 and 4′-aminobenzo-18-crown-6, respectively, which is shown in Scheme 1. No chromatography was required and isolated yields for both **1** and **2** were nearly quantitative. Figure 1 shows ratiometric absorption spectra of **1** with increasing concentrations of Na^+ in buffered ($[CH_3(CH_2)_3]_4NCl$) water. The absorption spectrum of **1,** which originally peaked at 345 nm, was blue shifted to 293 nm with increasing concentration of Na^+. This spectrum was accompanied by the appearance of one isosbestic point at 311 nm. When probe **1** was assayed with K^+ little change or no changes were observed (spectra not shown).

Figure 2a shows ratiometric absorption spectra of **2** with increasing concentration of K^+ in buffered (tetrabutyl ammonium chloride) water. The absorption spectrum of **2,** which originally peaked at 345 nm, was also blue shifted to 300 nm with increasing concentration of K^+.

In the presence of K^+**,** the absorption spectrum was accompanied by the appearance of two isosbestic points at 357 and



1: m = 1 ; 98%
2: m = 2 ; 98%

Scheme 1. Synthesis.

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* Corresponding author. Tel.: +1 575 835 5417; fax: +1 575 835 5364.
E-mail address: mheagy@nmt.edu (M.D. Heagy).

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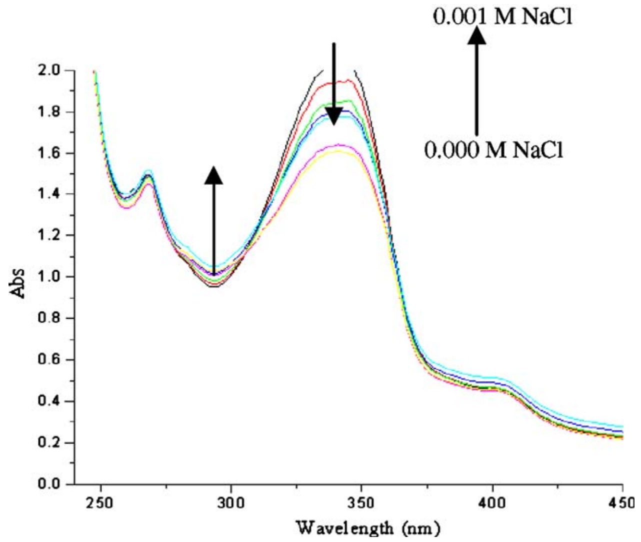

Figure 1. Absorption spectra for 1 (1×10^{-5} M) with NaCl (1×10^{-3} M) aqueous solution.

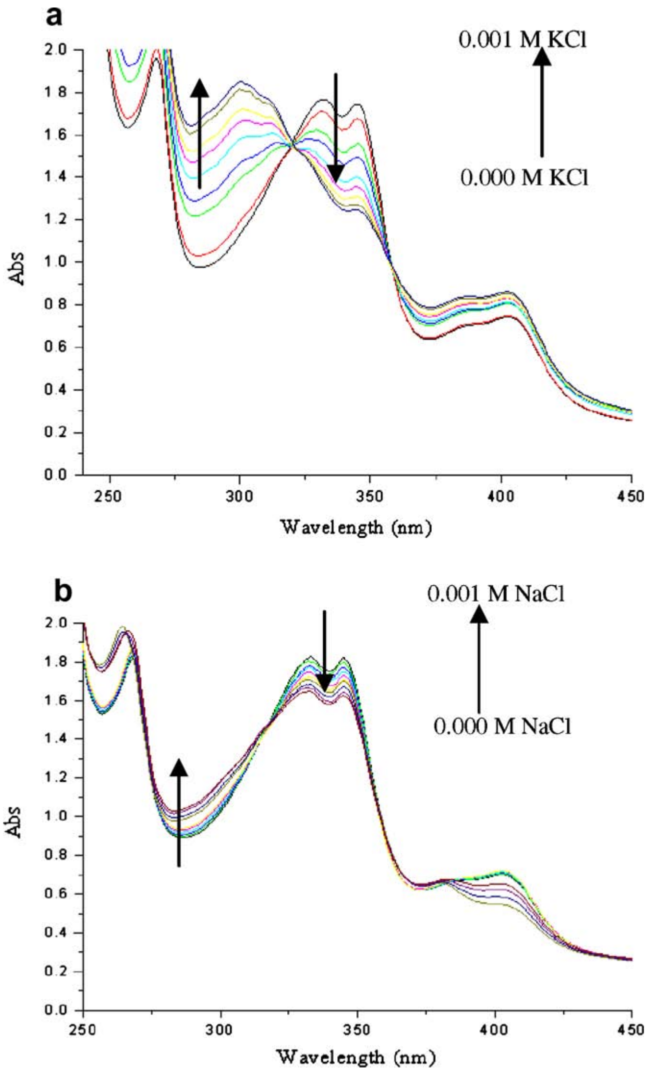

Figure 2. (a) Absorption spectra for 2 (1×10^{-5} M) with KCl (1×10^{-3} M) aqueous solution. (b) Absorption spectra for 2 (1×10^{-5} M) with NaCl (1×10^{-3} M) aqueous solution.

320 nm. In the case of Na^+, the absorption spectrum was also blue shifted to 310 nm. This titration resulted in relatively little change in optically density, and in the appearance of one isosbestic point at 320 nm as shown in Figure 2b. Therefore, **1** selectively binds Na^+ over K^+ whereas **2** displays higher sensitivity to K^+ relative to Na^+.

In Figure 3, sensor **1** shows a substantial increase in its dual emission with Na^+ in aqueous media. Fluorescence intensity of **1** was enhanced from 0 to 0.5 mM of NaCl in an aqueous solution containing tetrabutylammonium chloride (pH 7.2). The fluorescence intensity of both emission peaks (382 and 424 nm) increased concomitantly up to 0.5 mM and then leveled off at higher concentration. However, the addition of K^+ did not change fluorescence intensity of sensor **1**. Therefore, it shows that **1** selectively binds Na^+ even in the presence of K^+ because of potassium rejection from the smaller size of the 15-crown-5 cavity. Job plots confirm the 1:1 binding stoichiometry. Based on the fluorescence assay, the apparent K_D for Na^+ is 1.12 mM.

Fluorescence intensity of **2** was enhanced from 0 to 6 mM of KCl in aqueous medium containing tetrabutylammonium chloride (pH 7.2) which is shown in Figure 4. In the case of compound **2**, fluorescence intensity was not enhanced proportionally with sodium. The fluorescence intensity of both emission bands (382 and 441 nm) also showed an OFF–ON signaling in the presence of analyte. As the range of K^+ ion in human blood is between 3.5 and 5.3 mM, this response falls exactly within the dynamic range of our probe. Job plots confirm the 1:1 binding stoichiometry. Based on the fluorescence assay, the apparent K_D for K^+ is 0.4 mM. Table 1 shows the dissociation constants for **1** and **2** obtained from absorption and fluorescence spectra.

The crown ether moieties of compounds **1** and **2** were synthesized based on DF systems previously reported by our group.[13] Fluorescence intensities of 15-crown-5 (**1**) and 18-crown-6 (**2**) were substantially enhanced upon addition of Na^+ and K^+ cations. This OFF–ON signal is attributed to the electron-accepting properties of NI. Thus, alkali metal cations disrupt the PET quenching process that occurs between oxygen lone pairs of crown ether and NI fluorophore. Most of the earliest and best-known sodium (SBFI) and potassium (PBFI) probes contain azacrown groups that exhibit lower affinities relative to the oxycrown systems. While the azacrown systems provide an ideal optical range for sodium ion, they are unsuitable for extracellular potassium sensing. Mainly because PBFI exhibits insufficient binding strength and interference from sodium, as well as shows small spectral shifts for potassium.

In conclusion, whereas numerous Na^+ and K^+ sensors have been developed for water solubility, ratiometric probes, and ideal binding constants, they are sizable systems compared to probes **1** and

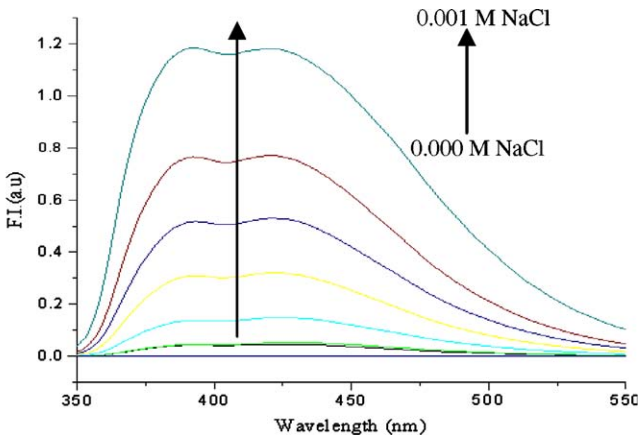

Figure 3. Fluorescence response for 1 (1×10^{-5} M) with NaCl (1×10^{-3} M) aqueous buffered system.

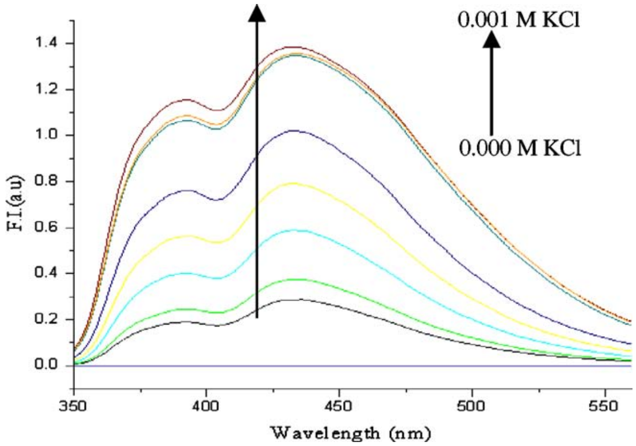

Figure 4. Fluorescence response for 2 (1×10^{-5} M) with KCl (1×10^{-3} M) aqueous buffered system.

Table 1
Spectroscopic data of **1** and **2** compounds

Sensors	$K_{D(absorb)}$	$K_{D(fluoresce)}$	λ_{ex}	λ_1	λ_2
1	1.5 ± 0.002 mM	1.12 ± 0.002 mM	345 nm	374 nm	435 nm
2	0.6 ± 0.002 mM	0.4 ± 0.002 mM	345 nm	380 nm	440 nm

2. Such large molecules can be an issue in their ability to enter cells. Furthermore, our one-step synthesis holds considerable synthetic advantage compared to the elaborate multistep approaches required by previously reported probes. Finally, these DF probes represent a rare example of the 'OR–OR switching in a reversible excited state' mechanism. Such signaling devices obviate the need of adding reference dyes, FRET pairs, or other dye ensemble systems necessary for internal calibration.[12]

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Supplementary data

Synthetic procedures, spectral data, NMR, IR, and elemental analysis as well as Job plot diagram. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.02.197.

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