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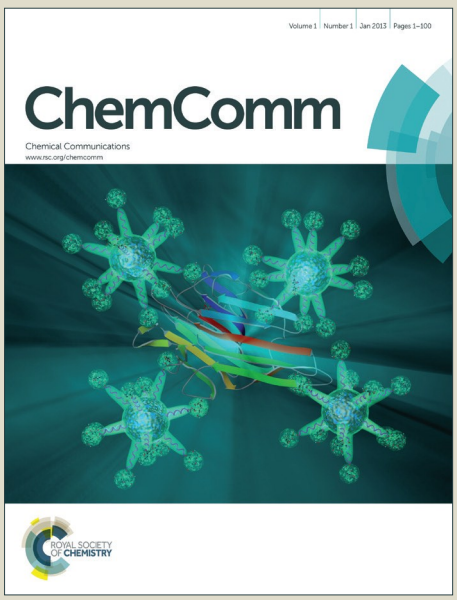

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ARTICLE TYPE

Comment on 'Fluorescence sensing of arsenate at nanomolar level in a greener way: naphthalene based probe for living cell imaging'

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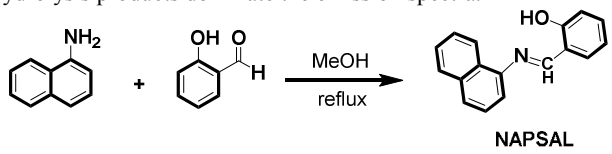
The naphthalene based probe (NAPSAL) described in the entitled communication is not stable in water, and therefore NAPSAL is unsuitable as an aqueous arsenate sensor.

Arsenic (As) is a highly poisonous element that exists in the environment in different chemical forms.[1] New field portable methods of detection are crucial for characterizing the environmental effects of arsenic. A. Sahana et al. published a communication entitled "Fluorescence sensing of arsenate at nanomolar level in a greener way: naphthalene based probe for living cell imaging" (*Chem. Commun*., 2013, **49**, 7231).[2a] This work, and subsequent work, [2b,c,d] describes a Schiff base compound (**NAPSAL**) capable of functioning as a hydrogen bond based fluorescent 'turn-on' sensor for arsenate ion at nanomolar concentrations in aqueous environments. In addition, the probe has been used to image arsenate in living cells.[2a] It's known that Schiff bases (imines) are good ligands for metal ions and that fluorescent moieties incorporating them are appealing sensors for optically detecting metal ions.[3] Thus, the reported study was noteworthy because this is the first work describing a Schiff base used as an anion sensor. The proposed sensing mechanism (nanoaggregate formation by hydrogen bonding between NAPSAL and arsenate) might open a new approach for sensing arsenic oxyanions. Unfortunately, it is problematic to perform arsenate ion sensing with this compound; ^{1}H NMR titration and fluorescence titration experiments instead show that spectral changes attributed to arsenate sensing are those for **NAPSAL** undergoing hydrolysis in water. The reported absorption and fluorescence spectra characteristics attributed to arsenate sensing can be produced by varying the pH of solution containing the hydrolysis products of **NAPSAL**.

NAPSAL (Scheme 1) was prepared following the method described in the supporting information of the literature.[2a] The NMR (^{13}C and ^{1}H NMR spectra) and HR-MS spectra, as well as C,H,N analyses confirmed the **NAPSAL** compound as reported (see ESI). However, fluorescence titration experiments (λ_{ex} = 377 nm), show that **NAPSAL** doesn't respond significantly to arsenate (two commercially available salts Na_2HAsO_4 and KH_2AsO_4 were tested). In addition, arsenite, which may be formed from arsenate under reducing conditions, also failed to cause a change in fluorescence. As shown in Fig.1, the addition of arsenate in buffered solution causes little change in the fluorescence of NAPSAL and the emission profile is similar as NAPSAL that has undergo hydrolysis in aqueous environment (fig. S9 in ESI). Within 10 to 20 minutes, fluorescence of the hydrolysis products dominate the emission spectra.



Scheme. 1 Synthesis of NAPSAL

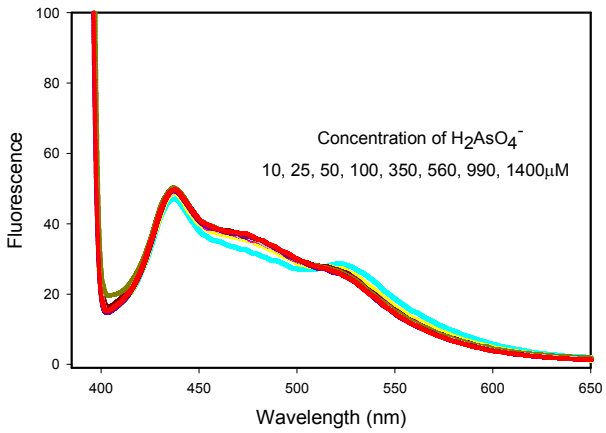


Fig. 1 Fluorescence titration spectra of NAPSAL (10 μM) in HEPES buffer (0.1 M, ethanol/water = 1/9, v/v, pH 7.4) on gradual addition of $H_2AsO_4^-$(10-1400μM). Spectra were measured 2 hour after sample preparation when background spectral changes without added arsenate stabilized.

T. K. Chondhekar, et al. have reported that a similar Schiff base (N-salicylidene-m-methyl aniline) is prone to hydrolysis in acidic and neutral pH with relatively short half-lives.[4] This raised the possibility that **NAPSAL** decomposed in the aqueous solution used for sensing. To test this speculation, ^{1}H NMR spectra were measured after addition of D_2O to a **NAPSAL** solution (D_2O:CD_3OD=1:5); several new peaks appear as seen in Fig. 2b. This confirms that **NAPSAL** decomposed partially after the addition of some (20%) D_2O. Fig. 2c and Fig. 2d show the ^{1}H NMR spectra of NAPSAL solution after addition of authentic salicylaldehyde and 1-naphthylamine, respectively. The addition of the expected hydrolysis products causes an increase in intensity of the new decomposition peaks. This proves that NAPSAL initially undergoes hydrolysis to starting materials.

Importantly, the addition of salicylaldehyde increases the intensity of the proton at 10.02ppm, which was previously attributed to an OH group associated with arsenate binding.[2]

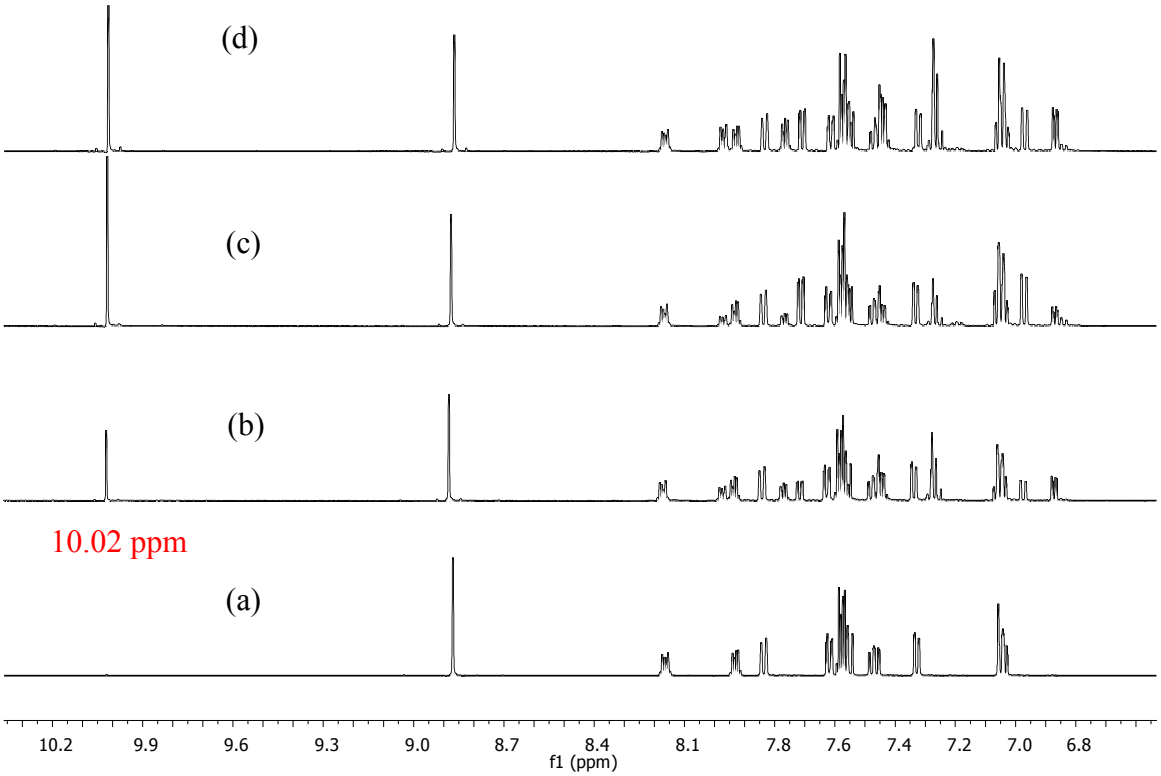


Fig. 2 ^{1}H NMR spectra of 20 mM NAPSAL in D_2O and CD_3OD solvent mixtures (1:5), which is near the maximum amount of water that can be used without precipitation at 20 mM concentration. (a) spectra measured immediately after sample preparation; (b) spectra measured 12 hours after sample preparation; (c) addition of approximately 1equivalent of commercial salicylaldehyde to the NAPSAL solution; (d) addition of approximately 1 equivalent of commercial 1-naphthylamine to the NAPSAL solution.

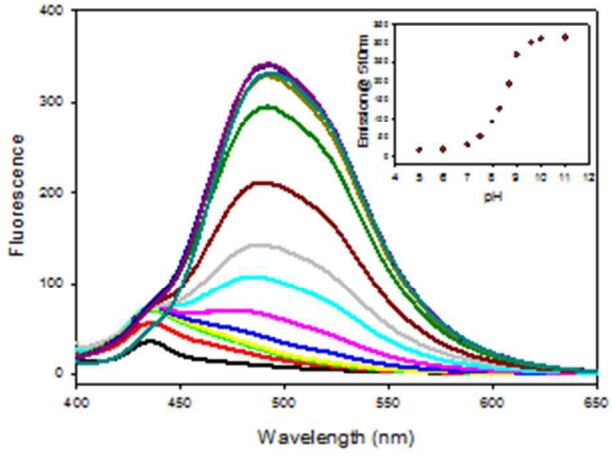


Fig.3 Change of emission spectra of NAPSAL (10 μM) in aqueous solution (ethanol:H_2O = 1:9) on changing the pH value (5-11), inset shows the emission intensity at 510 nm.

After the confirmation of the instability of NAPSAL in the presence of water by ^{1}HNMR, one possibility was that variations in pH were a cause for the reported change of fluorescence properties of NAPSAL aqueous solution, because the decomposition products (salicylaldehyde and 1-naphthylamine) are both pH sensitive compounds, and deprotonated salicylaldehyde is highly fluorescent. To test this speculation, pH dependence experiments were performed. As shown in Fig. 3, NAPSAL aqueous solutions (ethanol:H_2O = 1:9) exhibit a weak green-yellow emission (λ_{ex} = 377 nm), whose intensity drastically increases as the pH value increases from 5 to 11. The obtained emission spectrum appears the nearly the same as the emission profile reported in the literature and attributed to arsenate sensing (Fig.2 of reference 2). Instead of using NAPSAL, Fig. 3 can be reproduced simply with a 1:1 mixture of salicylaldehyde and 1-naphthylamine. The evidence suggests that the NAPSAL hydrolysis products are responsible for observed changes in the fluorescence spectrum. Since NAPSAL can undergo hydrolysis readily, and produces pH dependent highly fluorescent products, it is unlikely to be a useful sensor in aqueous solution.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Synthesis and characterization details of NAPSAL. See DOI: 10.1039/b000000x/

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