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ARTICLE TYPE

pK_a Modulation in Rhodamine Based Probes for Colorimetric Detection of Picric Acid

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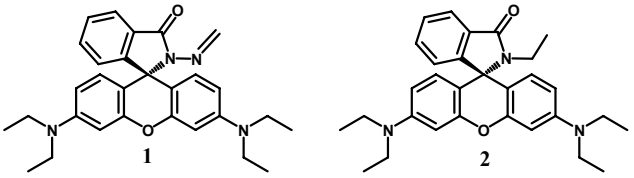
Tuning of pKa in acid sensitive rhodamine spirolactam derivatives as a function of solvent medium resulted in selective detection of picric acid from its lower nitro phenolic analogues and few other carboxylic acids.

The contrast colour change due to structural transformation of organic molecules in colligation with pH is promising mode for selective detection of various acidic/alkaline analytes. Rhodamine spirolactam derivatives (RSL), a class of xanthene dyes, being such acid responsive probes are suitable for such prototype approach. These derivatives are also known for their usage in detection of various metal ions[1] relying on a structure-function correlation. The analyte triggers a structural transformation in RSLs from their colourless spirolactam conformation to a conjugated pink coloured ring-opened amide form to result in a signal modulation which can be visually perceived as well as instrumentally monitored. Although RSLs have shown to recognize the acidic environment of different systems in various studies,[2] selective detection of an organic acid from a solution containing mixture of its possible degraded acidic analogues, to the best of our knowledge, is not explicitly known. The acid mediated ring opening of spirolactam form of an RSL depends upon strength of acidic entity measured in pKa. It is apparent that pKa value of an acid varies if non-aqueous solvent is employed as a co-solvent along with water, because of its varied dissociation ability in different solvent medium. Concurrently, most of RSL derivatives have poor solubility in water, thus, the sensing ability of acids with RSL would vary with solvent medium. Strong acids such as mineral acids could open the spirolactam ring elegantly irrespective of the solvent medium due to their much lower pKa values as compared to RSLs. The selectivity among them may possibly not be induced as pKa of RSLs are not in the match scale with those of mineral acids. Therefore, considering wide spectrum of pKa values of many organic acids and strength relative to RSLs, their selective detection is possible, in principle, based on their different dissociation ability in different solvent medium. Herein we report selective detection of such an organic acid, picric acid (**PA**), from a mixture of nitro phenols and carboxylic acids through pKa compatibility approach.

Picric acid (**PA**) is an explosive material of nitro aromatics family that also have been utilized for various applications[3], however, causes mild to severe damage on human health depending upon the intake. Thus, their detection in traces is highly desirable in environmental perspective. Although various efficient materials and methods are known for their quantifiable detection, colorimetric/fluorometric probes have perceived much attention recently due to their high sensitivity and real time monitoring. Such probes are categorized into two groups based on their interaction with **PA** through (a) the electron deficient phenyl ring[4] and (b) the acidic hydroxyl group.[5] The former type of probes, however, lack in good selectivity if **PA** is part of a mixed analyte containing other nitro aromatics, because the donor-acceptor complex formation is feasible too for all nitro aromatics. The latter type show high selectivity for **PA** among nitro aromatic explosives as those interfering entities generally do not have the acidic hydroxyl group. However, their selectivity gets severely affected if other sensitive acidic components present in the mixed analyte. For example, degradation of **PA** lead to possible acidic products such as dinitrophenol, nitrophenol, phenol, etc,[6] which may affect the selective detection of **PA**. Thus, probes that selectively identify **PA** from both nitro aromatics and other acidic components are certainly advantageous. In a different methodological approach, we have reasoned to choose RSL derivatives, as they are inactive towards nitro aromatic compounds and anticipated to detect **PA** selectively from other acidic analytes based on varied dissociation ability of the acids in different solvent medium.

Scheme 1



1 **2**

Two simple RSLs, **1**, the Schiff base and **2**, the alkyl derivative, were synthesized by two and one steps reaction respectively for the investigation. **1** was obtained by synthesizing rhodamine B hydrazide followed by the reaction with formaldehyde and **2** was

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obtained by refluxing rhodamine B hydrochloride with excess ethylamine in ethanol. The molecular ion peaks at 469.3 and 470.4 $(m/z)^+$ in the positive ion ESI-MS spectra inferred to formation of **1** ($C_{29}H_{32}N_4O_2$) and **2** ($C_{30}H_{35}N_3O_2$) respectively. The unique chemical shift in their ^{13}C NMR spectrum, at 64.27 ppm for **1** and 64.88 ppm for **2** in $CDCl_3$ corresponding[7] to a quaternary carbon(C_9) atom, has confirmed their existence in spirocyclic conformation.

If the pKa of acids is variable in different solvent medium, the pKa of RSL, for ring opening process mediated by that acid, should also be variable in such conditions. Thus, to investigate the variation in pKa values of **1** in water/non-aqueous solvent mixture of varied compositions, a series of pH titrations were carried out in water/acetone mixture by varying the amount of water from 15-60% in the 10μM solution of **1**. The absorbance at 558 nm is measured against pH range from 2-10 for each mixture, and the pKa values obtained[8] from each experiment is plotted against the percentage of water as shown in Fig.1a.

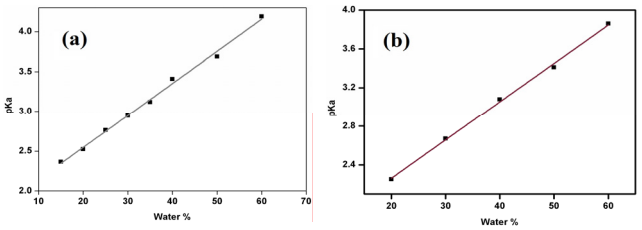
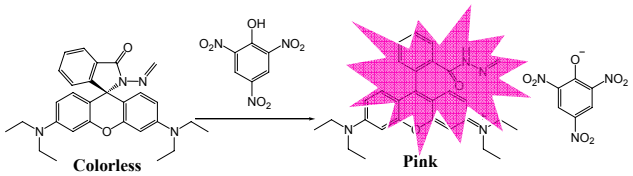


Fig. 1. Plot of water percentage versus the pKa values of (a) **1** and (b) **2**.

A linear increase in pKa values of **1** with respect to increase in the percentage of water is observed within the range of 15-60% of water in water/acetone mixture. The extrapolation of straight line towards zero and 100% of water gave pKa of lowest of 1.89 and highest of 6.19 respectively. Thus, it is presumed to be convenient to tune pKa of RSL derivatives by simply varying the solvent medium. pKa variation with respect to substituents of different bulkiness in spirocyclic nitrogen is known.[9] Further, it is apparent that the observed increase in pKa values of **1** due to increase in percentage of water in the water/acetone binary mixture medium, has a reverse effect with respect to the trend would be observed for the pKa values of acids in such conditions. Thus, it is not straight forward to correlate the pKa values of acids with RSL for ring opening process. However, a qualitative idea could be drawn from the pKa window of RSL for selective detection of an acid by choosing an appropriate solvent system for a particular acidic analyte. The reverse trend of pKa values of RSLs and the acidic analyte in a particular range facilitates a match in their respective pKa in that suitable medium which in turn, induce opening of the spiro-ring of RSLs. Within a pKa match scale for a RSL-organic acid couple therefore, a strong acid with higher dissociation abilities could be discriminated from other acids comparatively of moderate strengths in a solvent medium where the percentage of non-aqueous solvent is exclusive or near exclusive in aqueous medium. This is because such condition does not favour the dissociation of relatively weaker acids. However, increase in polarity of solvent medium may also facilitates those organic acids with moderate dissociation constant to open spirolactam ring of RSL along with the acids of comparatively higher strength in that pKa range. In other words, any acid whose pKa is lower than that of RSLs should, in principle, be compatible for its proton-mediated spirolactam ring opening to exhibit spectroscopic signalling responses as well as structural transformation induced colour change. The analyte being **PA** here, a strong acid with a pKa value of 0.38 in aqueous medium (ESI, Table ST1), has been anticipated to be selectively detected from other acidic analytes of moderate strength by using a non-aqueous solvent medium. Thus, all experiments were carried out in acetone, a non-aqueous medium, which is already known to be used as a processing solvent for the detection of **PA** due to high solubility of **PA**.[10] In addition to **1**, pH titration of **2** at varied percentage of water in water/acetone solvent medium have also exhibited similar trend of increase in the pKa (Fig. 1b). The extrapolated pKa range for **2** was estimated to be from 1.41 to 5.66. Notably, generation of pink colour after addition of the acid during pH measurement was observed to be instantaneous for **1**, however followed a slow kinetics for **2**.

Considering acetone as a solvent medium based on pH titration experiments explained above, evaluation of signalling responses of **1** with **PA** was assessed by differentiating UV-Vis absorption spectra of **1** and its mixture with **PA** in acetone. The colourless solution of **1** confirmed its existence in near exclusive spirolactam conformation, which remained stable for several days. However, addition of **PA** to **1** exhibited an instantaneous pink colouration in resultant solution, attributed to ring opening of its spirolactam form through a proton transfer from the Lewis acid (**PA**) as depicted in Scheme 2.

Scheme 2



As anticipated, the absorption spectra of **1**(25μM) in 450-650 nm range showed no appreciable absorption (Fig. 2a). Addition of **PA** to **1** immediately exhibited a strong peak with maximum of 558 nm ($\varepsilon_{\mathbf{1+PA}}$ =26760 $dm^3mol^{-1}cm^{-1}$) indicating the opening of its spirolactam ring. However, addition of **PA** to the colourless solution of alkyl derivative **2**(25μM) in acetone did not result in such instantaneous colour change, instead, the pink colour generated slowly and saturated after 12h (Fig. 2b) along with absorption enhancement at 558nm ($\varepsilon_{\mathbf{2+PA}}$ =16788 $dm^3mol^{-1}cm^{-1}$).

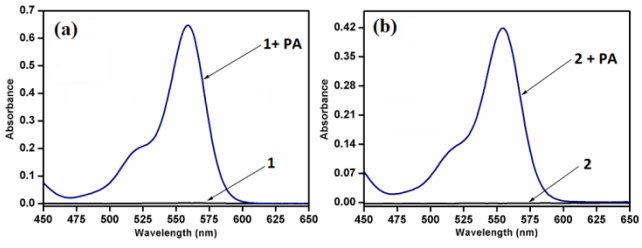


Fig. 2. Absorption spectra of (a) **1** and **1**+**PA** (b) **2** and **2**+ **PA** in acetone.

Therefore, the absorbance measurements for **2** with **PA** were carried out here onwards after 12h of addition. Such slow

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generation of pink colour supports the slow reaction rate(ESI, Fig. S3) in **2** for ring opening process assessed during pH titration experiments. The reactions of **1** and **2** with **PA** follow first order kinetics where the rate is much faster for **1** with $k > 0.4643\ s^{-1}$ in comparison to that of $2.77\times10^{-5}\ s^{-1}$ for **2**. The rate constants obtained therefore supported **1** to be an appropriate probe for rapid detection of **PA**. The NMR and ESI-MS spectral analyses of the probes with **PA** have further confirmed the formation of **1**+**PA** and **2**+**PA** complexes and support their spirocyclic ring-opening as observed through their absorption at 558nm upon equimolar **PA** addition. The quaternary carbon(C_9) peaks corresponding to spirocyclic conformation, which appeared at 66.04 and 64.73 in acetone-d_6 for **1** and **2** in their ^{13}C NMR spectrum respectively, were not observed when **PA** was added. This indicated their structural transformation to ring-opened conformation in their picrate complexes, which is too supported by their ESI-MS spectral pattern. The stoichiometric ratio of the complex formed was evaluated from the absorption spectral pattern using continuous variation method (Job's plot), which indicated to the formation of 1:1 molecular complex of **1** and **2** with **PA** (ESI, Fig. S4).

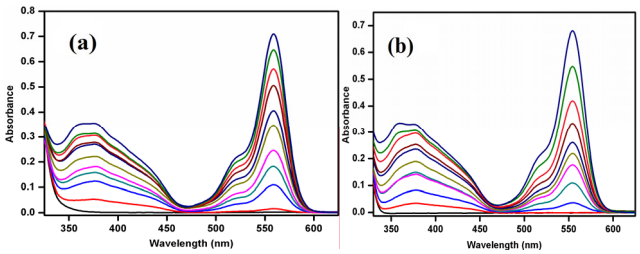

Fig. 3. Absorption spectral pattern of (a) **1**(30μM) and (b) **2** (40μM) upon addition of **PA** (0-60μM) in acetone medium.

The UV-Vis absorption titration of **1** and **2** with **PA** showed a steady increase in the peak intensity with respect to increased concentration of **PA** (Fig. 3). The equilibrium constants thus obtained for **1** and **2** were estimated to be $3.458\times10^{4}\ M^{-1}$ and $1.731\times10^{4}\ M^{-1}$ respectively which inferred to the formation of moderately stable complexes (ESI, Fig. S5). Further, **1**(10μM) and **2**(10μM) could detect as low as 4μM concentration of picric acid in acetone where the change in colour could be observable through naked eye (ESI, Fig. S6).

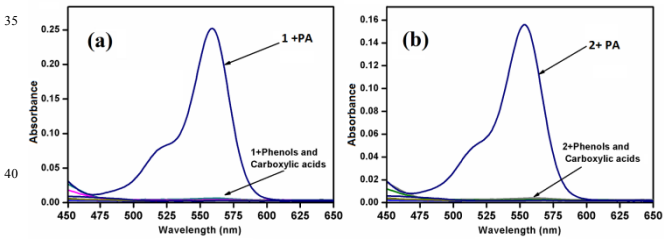

Fig. 4. Absorption spectra of the mixtures of (a) **1**(10μM) and (b) **2**(10μM) alone and in presence of various analytes such as **PA**, other nitrophenols and few carboxylic acids. [analytes] = 20-30 μM, acetone.

In order to evaluate the colorimetric signalling responses of **1** and **2** with different acidic analytes including the nitrophenolic derivatives, the UV-Vis absorption spectral measurements were carried out using a 10 μM solution of **1** or **2** in acetone added with a solution(20-30 μM) of phenol, 4-nitrophenol, different isomers of dinitrophenol, and carboxylic acids such as oxalic, malonic, succinic, glutaric, adipic, benzoic, 4-nitrobenzoic, 4-hydroxybenzoic, 4-aminobenzoic, acetic, trimesic acids in acetone. No appreciable absorption transition in 450- 650 nm window was observed for either **1** or **2** with any of these acids utilized here, indicating the inability of the acidic analytes to open the spirolactam ring (Fig. 4). It is interesting to note that the degraded acidic products of **PA** such as phenol, 4-nitophenol and dinitrophenols with higher pKa values than **PA** itself, minimum of 3.97 for 2,6-dinitrophenol in aqueous medium, have also failed to render spectroscopic signals and colour change mediated by the ring-opening process. Therefore, these probes suitable enough to detect **PA** selectivity among its possible degraded nitro phenols and some other carboxylic acids.

In the process of selective detection of **PA** with **1** and **2**, influence of various acidic analytes were assessed by measuring absorbance at 558 nm for individual mixtures containing the acid, **1** or **2** and **PA**. Addition of **PA** (20 μM) to the solution containing either **1** or **2** and any of these acids exhibited a strong absorption peak (A_{558}) to a comparable extent of that observed for these probes in presence of **PA** only. On contrary, none or negligible changes in absorption intensities of **1**+**PA** and **2**+**PA**(ESI, Fig. S7) were observed upon addition of those interfering analytes, which ascertained that the detection of **PA** is not affected in presence of these acidic products investigated here. In addition, the formation of the complexes of **1** and **2** with **PA** was found to be reversible as the pink colour disappeared along with lowering of A_{558} absorption almost to an extent to that of analyte free probe upon subsequent addition of triethylamine. Further addition of excess of **PA** facilitated the absorption transition at 558nm and regenerated the pink colour again, thus, establishes the probes' reusability.

In general, RSLs are utilized as a dual mode probe for analyte detection. However, fluorescence experiments exhibiting spectral maxima at 580nm pertaining to **PA** mediated ring opening of **1** and **2** did not show notable higher sensitivity; only 2μM solution of **PA** could be detected with **1** and **2** which is two-fold less than that in colorimetric mode. Considering operational module vis-à-vis sensitivity of detection and with the advantage of naked eye detection, the colorimetric mode for detection of **PA** is thus preferred here.

In summary, the RSL derivatives **1** and **2** are identified as a reversible probes for selective detection of **PA** among its degraded acidic analogues. Although both **1** and **2** could detect the lower limit of 4 μM of **PA** through naked eye/colorimetric mode, **1** was observed to be promising for real time monitoring in comparison to **2** as the former derivative showed a faster response time. A qualitative idea from the pKa window of **1** and **2** obtained due to different solvent medium helped to discriminate **PA** from various acidic analytes including nitro phenols and some carboxylic acids utilizing acetone as a non-aqueous solvent medium that did not favour for other acidic analytes to open the spirolactam ring. It may be argued that other stronger acids such as sulfonic derivates with much lower pKa values than that of these RSLs should facilitate the ring-opening process to render

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colorimetric signals; however, **1** and **2** could be demonstrated here to selectively detect **PA** among its other nitrophenolic analogues considering its utility and mechanism of its acidic degradation and the pKa compatibility factor. Based on pKa compatibility of RSLs, modulation of their stereo-electronic environment and subsequent tuning as a function of solvent medium, probes for selective detection of other organic acids are envisaged to be developed and we are currently working along this direction.

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Notes and references

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†Electronic Supplementary Information (ESI) available: Experimental details for synthesis of **1** and **2**, their corresponding characterization, pH titrations, equilibrium constant determination, other photo-physical, ESI-MS and NMR experiments. See DOI: 10.1039/b000000x/

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