Ratiometric Probe for Rapid Naked Eye Detection of Toxic Hydrazine: Real Time Application in Strip Test, Spray Test and Soil Analysis

Natarajan Vijay1 · Sivan Velmathi1

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Abstract

Striking colorimetric probe (CynH) for abrupt detection of hydrazine under complete aqueous solution was achieved. The water soluble probe was designed with electron “push–pull” strategy by coupling of 4-hydroxy benzaldehyde and 2, 3, 3-trimethylindolinine. The positively charged N-propylated indolinine make the probe completely soluble in water. The probe yields eye catching selective detection of hydrazine over other competing analytes with high sensitivity. Obvious colour change was observed from colourless to appearance of bright pink colour with hydrazine. It reacts quickly with hydrazine within 2 min and makes the probe an effective candidate for practical application. The real time application was demonstrated using paper strip to detect hydrazine vapour. This probe is superior to earlier reported probes because of its effective sensing of hydrazine displayed with various applications including real-time strip based sensing, spray test and soil analysis. In all the examinations, the probe yields distinct response with rapid naked eye colour change which overcomes the drawbacks of previous reports.

Keywords Ratiometric probe · Hydrazine sensing · Colorimetric · Rapid response · Environmental analysis

Introduction

Hydrazine serves as one of the major reactive base in the field of pharmaceutical chemistry and agriculture industries because of its high alkalinity and reducing capability [1–3]. The repulsion between two nitrogen makes it more reactive in chemical synthesis and reactions. Its extensively high reducing nature promotes scavenging of oxygen from air though it applied to protect metal corrosion [4–7]. It can be absorbed by the human beings easily via oral, transdermal and inhalation because of its completely water soluble nature and can cause severe health effects [8, 9]. Some health effects due to hydrazine exposure can be highlighted such as fiery irritation to the eyes, inflammation and severe corrosion to the skin [10]. It can be lethal to the living systems due to its extremely high toxicity, expected to cause serious skin allergy, skin burn and respiratory injuries to the skin by direct contact with skin [11, 12]. Exposure to these kind of toxic chemicals for long period of time leads to anaemia, severe damage to the organs, abnormal changes in the metabolism and even worse can cause cancer [13]. As hydrazine is a potential carcinogen, for the sake of peoples health many government limits threshold limit value of toxic hydrazine in drinking water to be as low as 10 ppb and violation of this can cause mutagenesis and carcinogenic damage to liver, kidney lungs and central nervous system [14–17]. Hence, concern about detection of hydrazine in drinking water stimulated over these years as its large usage in industries and pharmaceuticals [18, 19]. Conventional detection mostly based on chromatographic, mass and electrochemical approaches handicapped in real time sensing of these toxic analytes due to their complicated detection procedure and tedious sample preparation [20–23]. Recently, colorimetric and fluorometric detection of toxic components in the environment has drastically increased due to their ease in operation and detection methods. Basically, colorimetric and fluorescent probes for hydrazine utilizes strong nucleophilicity of hydrazine to break or bind with the recognition unit to induce significant response [24–27]. In scheme 1 cleavage based and addition based recognition units for hydrazine was summarized [28–30]. In 2016 Tse et al. reported colorimetric probe for detection of hydrazine with ratiometric response. They have achieved 0.186 µM
hydrazine detection in 80% aqueous DMSO medium [31]. In 2020, Ruan et al. reported colorimetric and fluorometric probe with 1–8 µM detection limit in acetonitrile solvent medium. The probe yields effective naked eye response with hydrazine [32]. Recently in 2020 Fraga-Corral et al. reported a review that includes recent advancement in hydrazine detection and its application [33].

Sensor platform with organic probes handicapped in real time application due to their limitations such as constrained solvent medium, restricted pH range and prolonged reaction time. Herein, we have developed new colorimetric probe for effective sensing of hydrazine in complete aqueous medium. The probe yield rapid response towards hydrazine with distinct colour change from colourless to appearance of bright pink colour within few minutes of reaction time. The advantages of the probe was utilized effectively to monitor the hydrazine vapour in real time sensing using eco-friendly test strips and soil samples.

Experimental Section

Chemicals and Instruments

All the reagents and chemicals are obtained from commercial dealers and used to carry out all the experiments. Double distilled water was used for the complete studies. Bruker 500 MHz spectrophotometer for $^1$H and 125 MHz for $^{13}$C was employed to obtain the $^1$H and $^{13}$C NMR spectra by dissolving the samples in CDCl$_3$ or DMSO-d$_6$ solvent. Absorption spectra were collected through Shimadzu UV-2600.

Synthesis of Probe

Synthesis route was outlined in scheme 2. The detailed synthesis procedure for compound 1a and compound 2 given in supporting information. Compound 2 (604 mg, 2 mmol, 1 equiv.) and acetic anhydride (280 µL, 3 mmol, 1.5 equiv.) were dissolved in DCM and added with trimethylamine (420 µL, 3 mmol, 1.5 equiv.) and stirred at room temperature under nitrogen for 3 h. After completion of the reaction (followed by TLC) the residue was concentrated on vacuum and purified by silica column chromatography (Hexane: ethylacetate (9:1)) yield: 80% (0.6 g). $^1$H NMR (500 MHz, DMSO-d$_6$): 8.5 (d, $J = 15$ Hz, 1H), 8.3 (t, $J = 5$ Hz, 2H), 8.0 (d, $J = 5$ Hz, 1H), 7.9 (d, $J = 5$ Hz, 1H), 7.7 (d, $J = 20$ Hz, 1H) 7.6 (m, 2H), 7.3 (t, $J = 5$ Hz, 2H), 4.7 (t, $J = 7.5$, 2 H), 2.3 (s, 3H), 1.9 (q, $J = 7.5$, 3H), 1.8 (s, 6H) 1.0 (t, $J = 7.5$, 3H). $^{13}$C NMR (125 MHz, DMSO-d$_6$) δ (ppm): 182.6, 169.3, 154.7, 153.3, 144.4, 141.1, 132.6, 132.5, 130.0, 129.6, 123.6, 123.2, 116.0, 113.2, 52.9, 48.3, 26.1, 22.3, 21.4, 11.2. HR-EI Mass: Calculated for C$_{23}$H$_{26}$N$_2$O [M]: 348.21964; Found 348.15968 (ESI: S1–S6).

Photophysical Studies

To carryout photophysical studies standard stock solution of probe CyNH in DMSO was prepared with concentration of 0.0015 M and it is further diluted to lower concentration (µM) for sensing studies with double distilled water. All the analytes were dissolved in double distilled water to make 0.0015 M concentration for sensing applications.

Results and Discussion

Hydrazine sensing attracts much attention in recent days due to its extensively wide usage in chemical and pharmaceutical industries. In favour to detect the hydrazine contamination in water, we plan to design a small molecule organic probe to detect hydrazine with good selectivity and sensitivity under complete aqueous medium. In order to make water soluble organic system, we design a molecule with “electron push–pull” character. 2, 3, 3-Trimethylindolinine was N-alkylated and fused with 4-hydroxy benzaldehyde to make the water soluble probe (Scheme 2). Hydroxyl group was protected by acetic anhydride in order to tune the selective cleavage with hydrazine. Synthesised probe will react selectively with hydrazine to form an intermediate (Scheme 3). Finally acetohydrazide will cleave from the probe and colourful chromophore will form with bright pink colour.

It is obvious that, after reaction with probe, hydrazine becomes acetoxyhydrazide and loses its toxicity.
Naked Eye Sensing Studies

Herein, the synthesised probe was employed for naked eye sensing studies over amines and anions. The push–pull electron motion makes the probe completely soluble in water without any complication in sensing studies under aqueous medium. Selectivity experiment of probe (50 μM) was carried out with various analytes (500 μM) including amines and anions under aqueous medium. It yields selective response with hydrazine under aqueous medium with colour change from colourless to pink colour (Fig. 1). The probe yields distinct colour change with hydrazine within 2 min of reaction time. The colour change was due to the formation of bright chromophore after reaction with hydrazine.

Photophysical Studies

The absorbance spectrum of the probe was collected using Shimadzu UV-2600 spectrophotometer. The selectivity experiment of probe with various analytes in complete aqueous medium was carried out and respective absorption spectrum was recorded. Addition of 2 equiv. of hydrazine causes colour change from colourless to pink and forms new absorption peak at 525 nm. Other analytes does not evince any characteristic change in absorption spectrum of the probe as they do not interact with probe and doesn’t have any characteristic colour change even with addition of 10 equiv. The absorbance change for hydrazine detection was ratiometric with isosbestic point at 450 nm (Fig. 2). The addition of hydrazine evinced abrupt response in absorbance change with intense pink colour.

Selectivity and Sensitivity Experiment

The selectivity was further verified with competing interferents in order to achieve selective detection of hydrazine in the environment. The interference effect of other analytes such as aliphatic and aromatic amines and diamines, ammonia and reactive anions were verified. To the probe solution (50 μM in H₂O) various analytes (10 equiv.) were added and the absorbance at 525 nm was measured. To that solution 2 equiv. of hydrazine was added and the absorbance changes at 525 nm was measured again. It is obvious that addition of other analytes with probe does not have much effect and it does not yield significant absorbance change at 525 nm (Fig. 3 (Black bar)). Addition of hydrazine to the probe in the presence of other interferents causes distinct absorbance change at 525 nm and none of the analytes interfere with the sensing of probe towards hydrazine (Fig. 3 (Red bar)). The hydrazine undergoes nucleophilic addition at the trigger site and forms chromophore that is deep pink colour. The reaction was very fast and estimated to be completed within few minutes. In order to understand the reaction ability further in detail, we carried out gradual addition of hydrazine to the probe and respective absorbance was measured using spectrophotometer. It is inferred that the probe reacts extremely fast with hydrazine for each addition. The absorbance at 386 nm got decreased with increasing concentration of hydrazine and peak at 525 nm getting increased with increasing concentration of hydrazine and saturated with the addition of 1 equiv. of hydrazine (Fig. 4). It reveals that 1 equiv. of hydrazine is enough to complete the reaction with probe.

Further, absorbance at 386 nm and 525 nm was plotted together against increasing the concentration of hydrazine to monitor the ratiometric change of the probe with increasing concentration of hydrazine. Linear increase and decrease was observed for the probe at two different wavelength (Fig. 4 inset).

Kinetics Experiment

Abrupt response makes much attention on this probe to make real time working tool for practical sensing application of
hydrazine. The reaction time was monitored using UV–Vis spectrometer with increasing reaction time. Hydrazine (100 μM) was added to probe (50 μM) and absorbance was measured with interval of 1 min for 15 min. The absorbance at 386 nm decreased with increasing time; and absorbance at 525 nm was increased with respect to time and reaches maximum within few minutes (Fig. 5). The absorbance attains almost maximum

![Fig. 2 UV-Visible spectrum of probe CynH (50 μM) with analytes (100 μM hydrazine and 500 μM of other analytes) in water (3 mL). [Analyte details are provided in Fig. 1]](image)

![Fig. 3 Absorbance change at 525 nm probe CyNH (50 μM) added with hydrazine (2 equiv.) and other analytes (10 equiv.) in water (Black bar: Absorption of probe CyNH with analytes; Red bar: Absorption changes of probe CyNH with competing analytes (10equiv.) and hydrazine (2 equiv.))]
intensity within 10 min. The absorbance change at 386 nm and 525 nm was monitored by single plot in order to notice the ratiometric changes of probe with hydrazine over time. The increasing and decreasing of absorbance happens within 10 min and reaches maximum (Fig. 5 inset).

**Fig. 4** Incremental addition of hydrazine (0–100 µM) to probe CynH (50 µM) in water (3 mL)

**Fig. 5** Absorption changes of probe CynH (50 µM) with hydrazine (100 µM) upon increasing time in water (3 mL)
Real Time Application

The probe evinces eye catching colorimetric response towards hydrazine with immediate response. We want to utilize promising response of the probe towards hydrazine sensing to make a practical tool to detect hydrazine in real time. In prior to that, the limit of detection was calculated using linear plot method. The LOD was calculated as 97 nM (~3 ppb) (Fig. S6). The results of the probe was verified with reported probes and tabulated (Table S1) [34–40]. Compared to the previous reports our probe stands superior in selectivity, reaction time and detection limit under complete aqueous medium.

Strip Test

As it can detect nanomolar level hydrazine under complete aqueous medium, real time sensing of hydrazine with small tool was planned. In that motive, paper based test strip were prepared using filter paper and soaked into the aqueous solution of probe and dried at room temperature. The prepared paper strips were utilized like a litmus paper to detect hydrazine under aqueous medium and vapour phase. The strips were tested for the detection of hydrazine under aqueous medium. The strips were soaked into increasing concentration of hydrazine in aqueous solution and the colour change was monitored after 2 min. The strip yields distinct colour change with respect to increasing concentration of hydrazine (Fig. 6).

In second case, the hydrazine vapour was allowed to react with test strip and the changes were monitored. The test strips were pasted inside the glass vial cap containing test solution (1% hydrazine) and the colour change was monitored with increasing reaction time. We can see the appearance of pink colour due to reaction of probe with hydrazine vapour with increasing reaction time (Fig. 7). The hydrazine vapour reacts with the probe and makes colour change in the strip. The probe yields excellent response towards hydrazine under aqueous and vapour phase hydrazine detection.

Spray Test

The sensing applications were extended towards hydrazine in real time monitoring. Spray based sensing of hydrazine was demonstrated using probe CyNH (Fig. 8). In prior to that, hydrazine contaminated strips were prepared using whatman filter paper with drop of 0.1% hydrazine in double distilled water and the contamination spots were marked with pen. The test strip was sprayed with CyNH (50 μM in water) and immediate colour change was observed on the hydrazine contamination spot (Fig. 9). The colour change was quick and distinct with bright pink colour though we can monitor the changes with our naked eye without aid of any external instruments. The probe yields simple and effective response with hydrazine monitoring in real time with onsite information.
Soil Analysis

In order to observe the toxic chemical contamination in the environment we performed soil analysis for the detection of hydrazine in the soil. Initially, grounded field soil were pre-treated with different concentration of hydrazine (0.001, 0.005, 0.01, 0.05, 0.1, 0.5 and 1%) in double distilled water and dried. Under room temperature (Fig. 10a). Hydrazine pre-treated soils (~1 g) were added to the probe solution (50 μM in water) and the changes were monitored. Interestingly, hydrazine pre-treated soil yields immediate colour change with probe within 2 min with distinct colour change (Fig. 10b). The intensity of the colour change was increased with increasing concentration of hydrazine. The absorbance for the respective soil samples were measured. The absorbance at 525 nm was increased with increasing concentration of hydrazine and reaches maximum with addition of 0.05% of hydrazine and remains constant with higher concentration (Fig. 10c). We can monitor lower concentration upto 0.001% hydrazine in soil with distinct colour change. The results evinces effective detection of hydrazine in soil samples with rapid colour change.

Conclusion

In summary, water soluble probe (CynH) was designed and synthesised for detection of hydrazine under complete aqueous medium. Synthesised probe was successfully characterized by NMR and mass spectroscopic technique. The probe evinces eye catching sensing output towards hydrazine with the presence of other competing analytes under complete aqueous medium. The response with hydrazine was quick with distinct colour change. It yields sensitive response with hydrazine with LOD and LOQ 97 nM. Quick and sensitive response of the probe was utilized effectively to monitor the hydrazine in real-time with paper based test strip under
aqueous and vapour phase. The application was extended to monitor the hydrazine using spray method. For the environmental protection concern, the hydrazine contamination was monitored via soil test with probe solution. In summary, probe evinces better sensing application for hydrazine that can overcome the limitations of previous reports and can serve as good sensor for hydrazine in real time sensing application.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10895-021-02825-x.

**Authors’ Contributions** Sivan Velmathi contributed to the conception of the study and wrote the manuscript and Natarajan Vijay performed all the experiments and analysis and wrote the manuscript.

**Availability of Data and Material** Electronic supporting information file is available for synthesis procedure, NMR and MS analysis.

**Declaration**

**Consent to Participate** A statement regarding informed consent is not applicable.

**Consent for Publication** A statement regarding informed consent is not applicable.

**Conflicts of Interest** Not applicable.

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