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Alizarin red S –Zinc(II) fluorescent ensemble for selective detection of Hydrogen Sulphide and assay with H_2S donor

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Alizarin red S based new fluorescent probe **ARS-Zn(II)** has been reported for the detection of H_2S in aqueous buffer solution. In presence of zinc ion, ARS displayed an increase in fluorescent intensity through formation of **ARS-Zn(II)** ensemble. Upon addition of H_2S to the ensemble cause the disassembly of metal and ARS, this restored the fluorescence intensity of ARS. This sensing ensemble exhibited H_2S-selectivity over other biothiols and biological relevant analytes. The calculated detecting limit of **ARS-Zn(II)** with H_2S was 92 nM. **ARS-Zn(II)** was also detect H_2S in serum under physiological condition. Moreover, as a practical application it could be used for the real time monitoring of H_2S released from H_2S-donor molecule benzoic (methyl carbonic) dithioperoxyanhydride and also applicable for live cell imaging. The reported ensemble **ARS-Zn(II)** has advantages like readily available, high selectivity with good sensitivity, fast response time, higher excitation (520 nm) and emission (625 nm) wavelength and real time fluorescence assay with H_2S donor.

Introduction

Hydrogen Sulfide (H_2S) is an unpleasant gas with a characteristic smell of rotten eggs. Endogenously produced H_2S has been recognized as one of the three gasotransmitters along with nitric oxide (NO) and carbon monoxide (CO).[1] The physiological levels of H_2S appear to be involved in various biological functions and essential for maintaining the human health along with other roles.[2] Unusual level of H_2S in human has been linked to diseases like Alzheimer's disease,[3] Down's syndrome[4] and hypertension.[5] The physiological and therapeutic importance of H_2S, leading to a quick progress in research activity involving H_2S.[6]

Number of fluorescent probes for H_2S has been developed in last decade.[7-12] However, selectivity over competing biothiols such as glutathione (GSH), cysteine (Cys) and homocysteine (Hcy), sensitivity, response time, multistep synthesis and biocompatibility are some of the serious limitations with the existing fluorescent probes.[13-16] These drawbacks call for a need of H_2S sensors with specific properties such as selective, sensitive, fast response and readily available; so that they can be widely used to tackle biological issues and pharmacological application. To deal with those demanding issues, we wish to report a displacement approach for the development of practically useful fluorescent probe for H_2S.

Dye displacement approach is a competitive binding supramolecular sensor system,[17-19] where an analyte displaces a dye from a receptor, as a results colour or fluorescent change was obtained, which can be related to the amount of analyte present.[20] In this regard, Alizarin red-S (ARS) has been successfully employed as an indicator in competitive binding assays for the detection of various analytes such as biological phosphates,[21, 22] saccharides,[23, 24] and anion.[25] ARS also has been known for the colorimetric detection of metal ions.[21, 27] Analyte mediated release of ARS from ARS-Copper complex ensemble has been reported for the selective detection of glutathione,[28] tiopronin,[29] proteins,[30] and L-cysteine.[31] Similar to copper, ARS is also known to form complex with zinc ion with high affinity.[32] However ARS-Zinc ion based fluorescent ensemble is not well documented for the detection of analytes using dye displacement method.

Herein, we wish to report a new probe **ARS-Zn(II)** (Scheme 1) for the selective detection of H_2S under biological condition. ARS shows ratiometric absorbance change and fluorescent enhancement upon coordination with zinc to form **ARS-Zn(II)** ensemble. In presence of H_2S the emission intensity of the ARS restored immediately as it separates from sensing ensemble by formation of ZnS. We have also used this ensemble to monitor the H_2S released from H_2S donor molecule in aqueous solution.

Experimental Section

Materials and reagent

Methoxycarbonylsulfenyl chloride, Thiobenzoic acid, Sodium sulfide were purchased from Sigma-Aldrich. Other chemical

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Electronic Supplementary Information (ESI) available: [Synthesis of H_2S Donor, Experimental methods and Some Uv-Vis and Fluorescence spectrum are available].

were purchased from Loba Chemie or Merck limited unless otherwise mentioned. Solvents used were analytically pure and used without further purification.

Instrumentation methods

^{1}H NMR was measured on a Bruker Avance 400 MHz. Mass spectrum was measured on Waters Q-Tof mass spectrometer. Cary eclipse fluorescence spectrophotometer was used to obtain the fluorescence spectra. UV absorption spectra were recorded on a Evolution 201 UV-Vis spectrophotometer using quartz cells of 1.0 cm path length. Live cell images were recorded by using Nikon Air Laser Scanning Confocal Microscope. Donor studies were performed on Synergy-HT Multimode Plate reader (BIOTEK). Buffer solutions were prepared by using deionized water and pH studies were done on HACH sension 2.

General procedures for spectroscopic measurements

Spectral study of ARS with Zinc: Absorbance and Fluorescence study was performed in 10 mM MeOH-PBS buffer (3/1 (v/v), pH = 7.4) at RT. For spectroscopic titrations 120 μl of ARS (0.5 mM) stock solution was diluted to 2000 μL by adding buffer. For metal complex formation, the titration was performed with different concentration of zinc perchlorate (0 – 30 μM) in buffer. Each spectrum was recorded in standard quartz cuvette cell of length 1 cm. The change in absorption was monitored at 530 nm. Emission spectrum was recorded upon excitation at 530 nm with excitation and emission slit width 10/10

Spectral study of ARS-Zn(II) vs H_2S: For the binding study with H_2S, 120μl of ARS (0.5 mM) was mixed with 40μl of zinc perchlorate (1.5 mM) in buffer and diluted to 2000 μL to form a **ARS-Zn(II)** complex. The concentration of [ARS] = 30 μM and [Zn(II)] = 30 μM was maintained for all titrations. UV-Vis and Fluorescence titration was done by adding different concentration of Na_2S (0 – 44 μM) to this solution. For all the experiments Na_2S has been used as a source for H_2S gas.

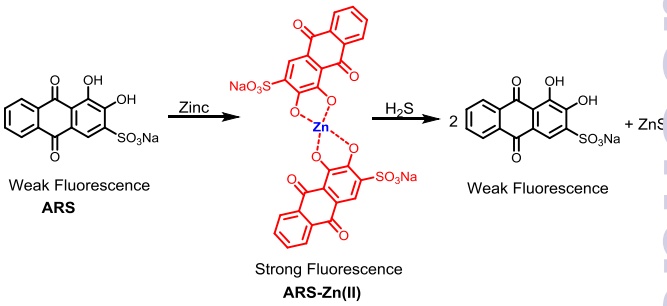
Fluorescence assay with H_2S Donor

For the fluorescence assay with H_2S donor molecules, 100 μl of **ARS-Zn(II)** (60 μM) was prepared in MeOH:PBS buffer (3/1,(v/v), 10 mM , pH = 7.4, 0.04% THF used as a co-solvent) and diluted by adding 134μl of buffer to this 6μl of glutathione (0.1 M, 100 equivalent) was added and fluorescence emission was monitored at 645 nm for 30 minutes at 35°C. The excitation wavelength was 530 nm and emission wavelength was 645/30 nm. The titration was done in 96 well- plate using Synergy-HT multimode micro plate reader.

Result and Discussion

Binding studies of ARS with Zn(II)

Alizarin and ARS are known for their complexation behaviour with different metal ions.[32-35] We have studied the complex formation ability of ARS with Zn(II) by using Ultraviolet-visible (UV-Vis) and fluorescent spectroscopy in aqueous buffer (MeOH/PBS buffer, 3/1, v/v) at pH 7.4. The UV-Vis spectra resulting upon addition of different concentration of zinc ar shown in Figure. 1.



Scheme 1: Proposed Sensing mechanism for the detection of H_2S using **ARS-Zn(II)** ensemble.

As shown in figure 1, upon addition of Zn(II) to the **ARS** induces a ratiometric change with increase in absorbance at 520 nm, along with decrease in wavelength at 425 nm and appearance of isosbestic point at 465 nm. Based on the UV–Vis spectral change, the binding mode was determined by a Job's plot using mixtures of ARS and zinc in buffer solution.

The relative **ARS–Zn(II)** complex concentration reached maximum when the molar fraction was 0.33, therefore the job plot was confirmed that a 2:1 (**ARS:Zn(II)**) complex formation (SI Fig 3), it was similar to the observation of ARS and Cu^{2+} coordination stoichiometry as reported earlier.[31] At neutral pH one of the phenolic OH groups of ARS has been deprotonated and it forms **ARS-Zn(II)** complex as shown in Scheme 1.

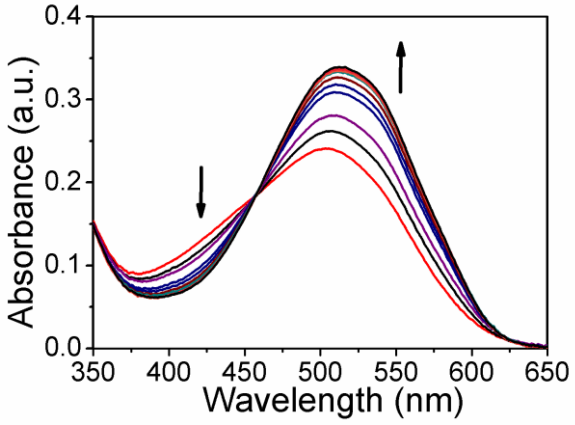


Fig 1: Absorption spectra of ARS (30 μM) in MeOH/ PBS buffer (3:1, v/v, pH = 7.4) in the presence of (0 – 30 μM) equivalent of Zn(II).

The ability of ARS to form complex with zinc ion was also established by using fluorescence measurement. ARS has been a natural dye with weakly fluorescent property in aqueous solution at neutral condition.[36]

As depicted in figure 2, with increasing the concentration of Zn(II) ion the fluorescence intensity in the orange region at 615 nm was increased gradually upon excitation at 530 nm, which was mainly due to a combination of chelation enhanced

fluorescence (CHEF) and internal charge transfer (ICT) mechanism.

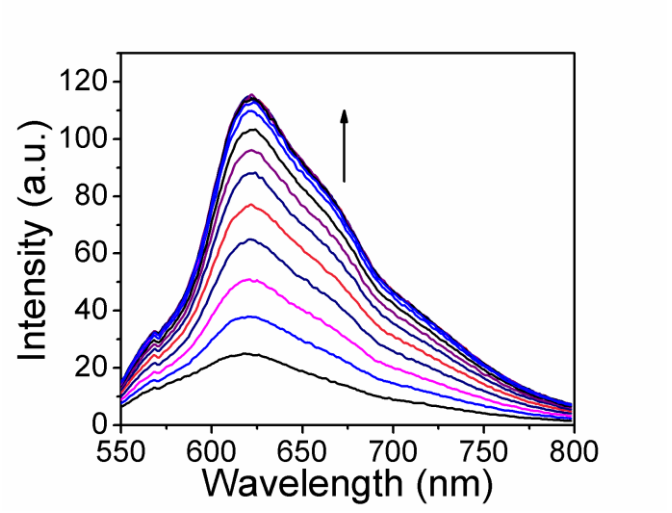


Fig 2: Fluorescence spectrum of ARS (30 μM) in MeOH/ PBS buffer (3:1, v/v, pH = 7.4) upon addition of 0 - 30 μM equivalent of Zn^{2+}.

Moreover, it was observed that Zn(II) ion induced almost 6 fold increases in fluorescence intensity of ARS. The fluorescent intensity of ARS reached maximum at 30 μM of zinc, which makes ARS as a more sensitive probe for zinc. But as described earlier, when the Zn(II) was added more than 1 equivalent the fluorescence intensity was dropped.[32] The association constant (K_a) was evaluated graphically by plotting $1/\Delta F$ against 1/[Zn(II)] as shown in SI Fig. 4. The data was linearly fitted according to the Benesi–Hilderbrand equation and apparent binding (K_a) value was obtained from the slope and intercept of the line. The K_a value was found to be 13826 M^{-1}. Zinc ion induced increase in fluorescence intensity and ratiometric change in the UV-Vis absorbance ensured the determination of H_2S with high sensitivity with **ARS- Zn(II)** sensing ensemble.

Effect of H_2S on the optical signal of ARS- Zn(II) ensemble

H_2S has been known for the formation of metal sulphides upon reaction with metal ions. Zinc ions are known to react with sulphide to form stable ZnS species,[37] which has low solubility products constant (K_{sp}) = 1.6 x 10^{-24}. So it was expected that, **ARS-Zn(II)** sensing ensemble would act as a potential sensor for the detection of H_2S through displacement of zinc thereby recovery of original ARS optical (fluorescent and absorbance) signal. Figure 3a illustrates the emission spectral changes of probe with H_2S at pH 7.4 in aqueous buffer solution. **ARS-Zn(II)** emitted strong fluorescence at 625 nm, upon addition of different concentration of H_2S to this solution, the fluorescent intensity was decreased. The decrease of fluorescence at 625 nm was achieved immediately (less than a minute) with a fast response time. Real time detection are important for the practical application, consequently the quick time response time of the **ARS-Zn(II)** sensing ensemble has the advantage as compared to DHAQ-Cu^{2+} ensemble reported earlier for H_2S detection, because it has response time about 4 minutes.[38]

In addition to that, the original fluorescent intensity of ARS almost 100% recovered, when the concentration of H_2S was about 42 μM. It confirmed that the H_2S disassemble the Zn(II) ion and ARS from the sensing ensemble. These results revealed that the applicability of the **ARS-Zn(II)** system for the detection of H_2S in aqueous medium. The fluorescence recovery could be used to plot calibration curve to afford quantitative measurement of H_2S in biological sample and H_2S releasing drugs.

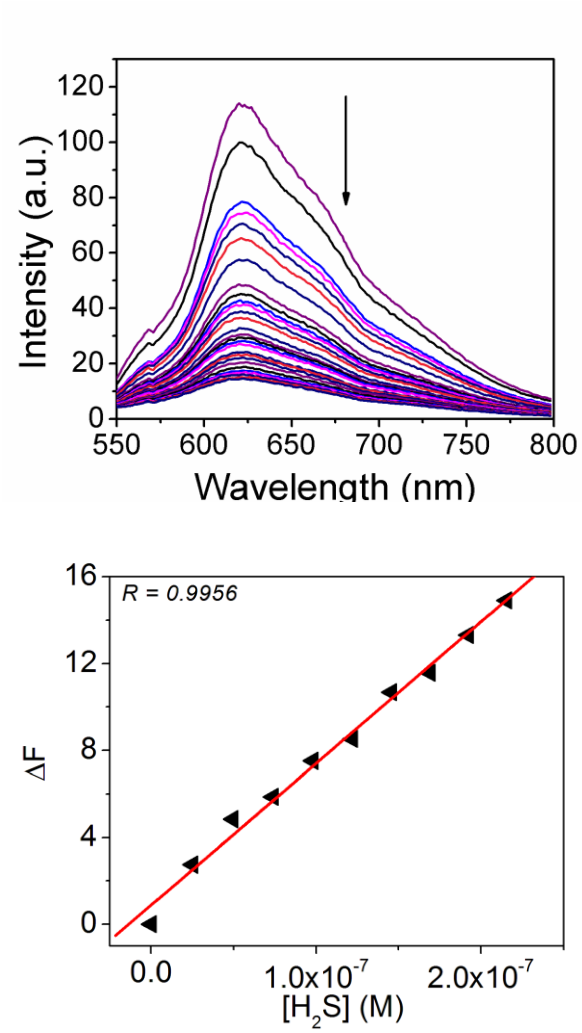


Fig 3: (A) Fluorescence spectrum of **ARS –Zn (II)** ensemble in MeOH/PBS buffer (3:1, v/v, pH = 7.4) upon addition of 0 - 42 μM equivalent of H_2S. (B) Detection limit plot from fluorescent titration of **ARS –Zn (II)** with H_2S.

Based on the change in emission intensity the detection limit of H_2S calculated for the sensing ensemble are 92 nM, which was better than other probes reported for H_2S, based on zinc displacement approach.[39-41] The detection limit was calculated by the equation 3σ/slope, where slope was obtained by plotting change in fluorescence intensity (ΔF) *vs* conc. of H_2S and σ = the standard deviation of the blank signal obtained without H_2S (Fig 3b).

Further, we have also monitored the UV-Vis absorbance change in presence of H_2S. As shown in figure 4, upon addition

of H_2S in to the **ARS-Zn(II)** the absorption band at 525 nm decreased gradually and restored to the original absorption state of ARS after adding 44 μM of H_2S. It indicates that the dye ARS and Zn(II) has been dissociated from the sensing ensemble in presence of H_2S.

Hence the results obtained from fluorescent and absorbance spectroscopy confirmed the convenience of using **ARS-Zn(II)** sensing ensemble for the detection of H_2S in aqueous buffer solution.

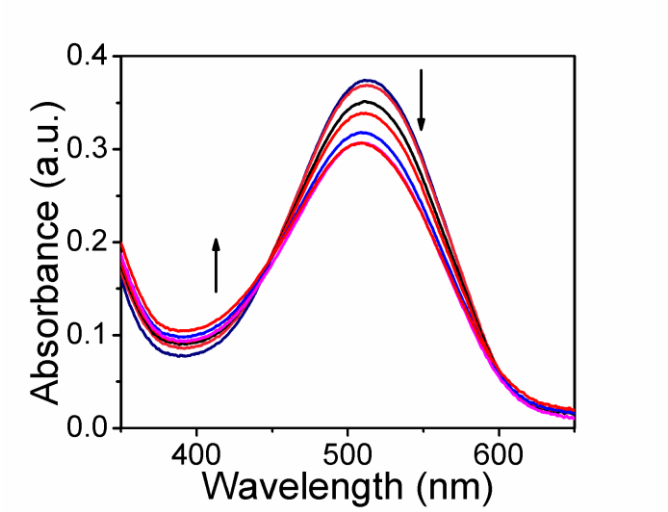


Fig 4: Absorption spectra of **ARS-Zn(II)** ensemble in MeOH/ PBS buffer (3:1, v/v, pH = 7.4) upon addition of 0–44 μM of H_2S.

Selectivity studies and effect of interference

The selectivity of the sensing ensemble **ARS-Zn(II)** for H_2S was examined by fluorescence spectroscopy under the same condition as discussed above. As shown in figure 5a, the probe checked with the range of physiological and biological important analytes such as reactive sulphur species (glutathione, cysteine, methionine, $S_2O_3^{2-}$) reactive nitrogen species ($NO^{\cdot}$, NO_3^- and N_3^-), biological phosphates (ATP, ADP, AMP, PPi, $H_2PO_4^-$ and HPO_4^{2-}) and other anions (F^-, Cl^-, Br^-, I^-, OH^-, $HCO_3^-$and CH_3COO^-). Among these biological phosphates (PPi, AMP and $H_2PO_4^-$), GSH and cysteine displayed little and other analytes exhibited insignificant change in the fluorescent spectra of **ARS-Zn(II)** as shown in Fig. 5a**.** At higher concentration of cysteine (1mM) and glutathione (1mM) notable change was obtained, but these change are less as compared to H_2S (30μM) (SI Fig 11).

These results confirms that the higher selectivity of H_2S among other physiological and biological important analytes. The decrease in fluorescence intensity with H_2S was due to the displacement of zinc and formation ZnS in aqueous solution. But non-bonding interaction of glutathione (GSH), cysteine and $H_2PO_4^-$ to the Zn(II) center could be responsible for the weak binding and small change in fluorescent intensity with these analytes.

Further, the selectivity of the probe with H_2S was also tested in presence of interfering species. As shown in Fig 5b. The competitive experiments revealed that minimal interference or no interference with detection in the coexistence of various species and H_2S. Accordingly, probe **ARS-Zn(II)** could be applicable for selective determination of H_2S even in presence of other biological species like ATP, ADP, AMP, PPi, GSH and cysteine. This result was also confirming the possible use of the probe even when H_2S co-exists with other biological relevant biothiols like glutathione, cysteine, methionine and phosphates.

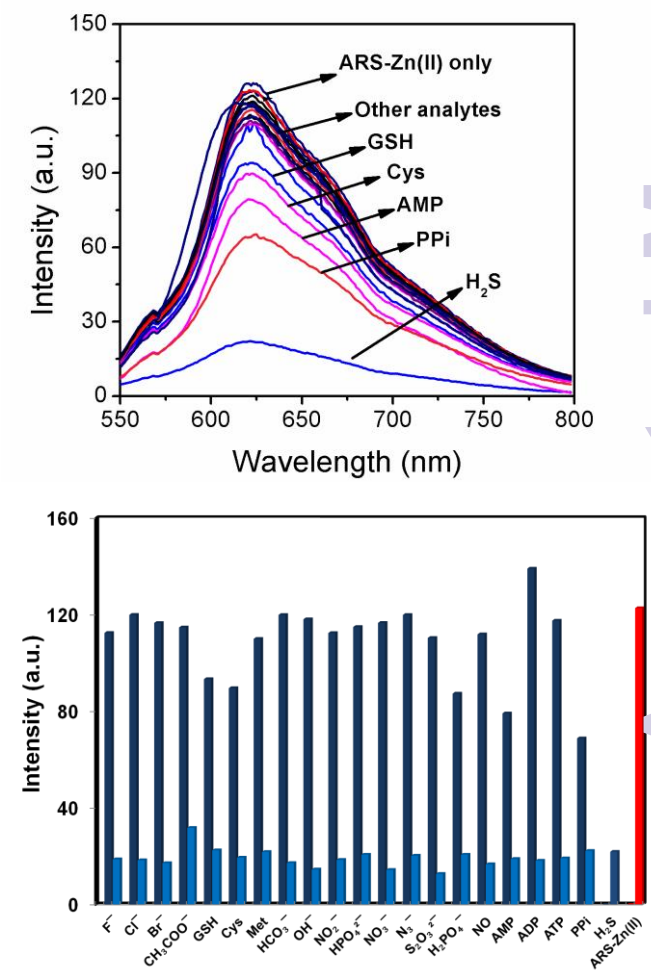


Fig 5: (a) Fluorescent spectra of **ARS-Zn(II)** ensemble in the presence of various analytes in Me-OH/ PBS buffer (3:1, v/v, pH = 7.4) at RT upon excitation at 530 nm. (b) Different analytes response of **ARS-Zn(II)** in buffer. [ARS] = 30 μM, [Zn(II)] = 30 μM [anion] = 300 μM, [H_2S] = 30μM. Red colour bar indicates only probe **ARS-Zn(II)**, dark blue colour indicates **ARS-Zn(II)** + anions and the Sky blue bar indicates **ARS-Zn(II)** + anions + H_2S.

Effect of pH

The maximum fluorescence of ARS has been greatly affected by pH of the solution.[42] The influence of pH on **ARS-Zn(II)** was studied using fluorescence measurement. The nature of the fluorescence profile shown in fig.6 could be a result of the stability of the **ARS–Zn(II)** sensing ensemble. At pH values less than 6, the competition between the OH-proton and the metal ion could occur. Therefore, the **ARS–Zn(II)** complex was less

stable, this prevented the H_2S detection, therefore, emission intensity does not change so much with H_2S at lower pH lesser than 6. Due to the high concentration of hydroxide ions, at higher pH (pH > 9.0) it is expected that Zn(II) ion may precipitate and consequently higher pH sensing ensemble not stable, therefore it may be not suitable for binding studies. These results described that the detection of H_2S using probe **ARS–Zn(II)** was pH dependent and maximum change in emission intensity with H_2S was obtained in the pH range of 6.0-8.0.

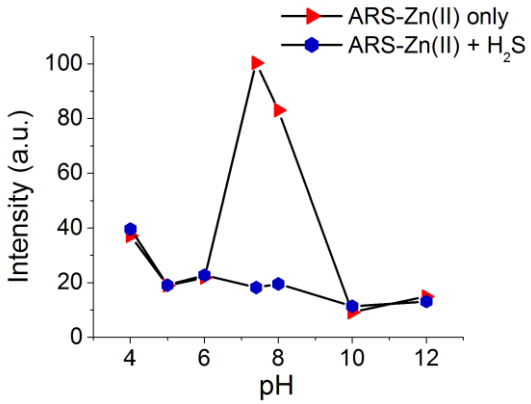


Fig 6: Effect of pH on the fluorescent intensity of **ARS-Zn(II)** ensemble and the ensemble with H_2S. [ARS] = 27 μM, **[Zn(II)]** =27 μM [H_2S] = 27 μM in Me-OH/ PBS buffer (3:1, v/v, pH = 7.4).

Recycling Sensing Performance

The reusability of any sensor is very important for practical application, which requires that the sensor device should be reversible. Therefore, the recycling sensing performance of the **ARS-Zn(II)** was studied through a disassembly and reassembly of ARS by H_2S and Zinc.

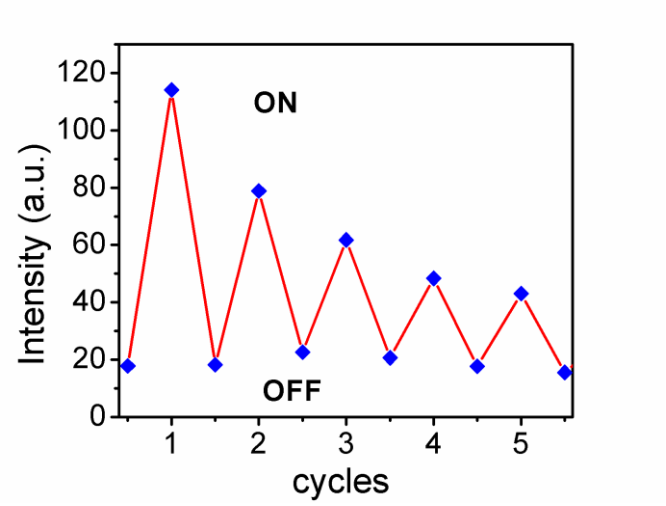


Fig 7: Fluorescent intensity of **ARS-Zn(II)** ensemble upon alternate addition of H_2S and Zn(II) [**ARS**]= 30 μM, [**Zn(II)**]=30μM, [**H_2S**]=42μM.

The reversibility study was carried out by monitoring the change in fluorescence of **ARS-Zn(II)** ensemble in a buffered solution. The fluorescent intensity was checked after displacing the Zn(II) by H_2S (42μM) and again, adding Zn(II) (30μM) to the ARS (30μM). As shown in Fig. 7 even after 5 cycles, the change in fluorescent or decrease in fluorescent for **ARS-Zn(II)** solution with H_2S were still relatively good. Thus, switching between the fluorescent **OFF-ON** states could be repeated more than five times without decomposition of the fluorescent indicator ARS dye. Therefore the results of high reversibility will be in favour of the real-life application.

Fluorescent study of ARS-Zn(II) with H_2S in serum

After confirming the sensitivity and selectivity of the probe in buffer solution, we have decided to explore the practical application of probe for the quantization of H_2S, in Human serum(HS) and Bovine Serum albumin (BSA).

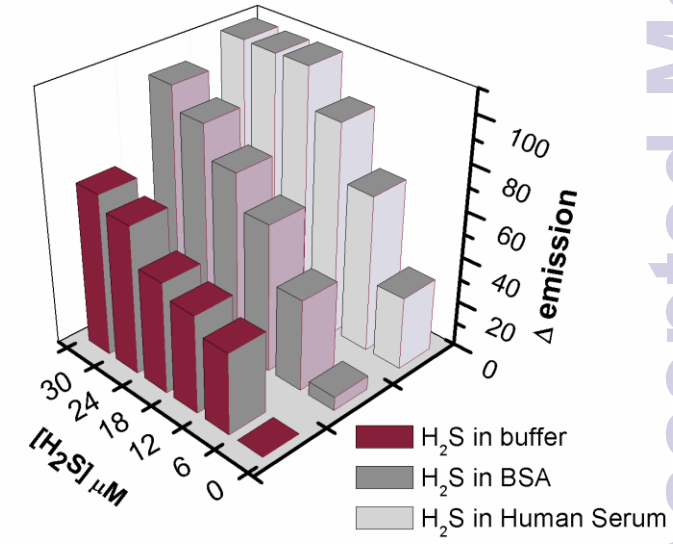


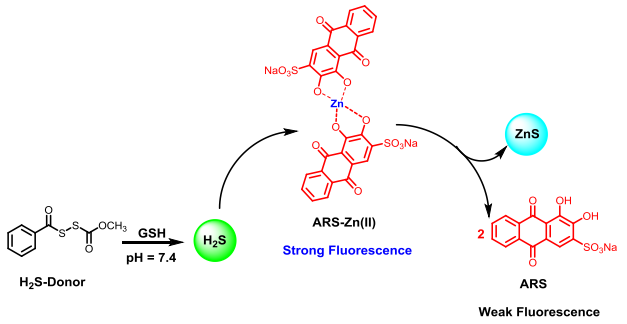
Fig 8: Fluorescent intensity of **ARS-Zn(II)** ensemble upon addition of H_2S spiked (6 μM, 12 μM, 18 μM, 24 μM, and 30 μM) buffer, Human serum and BSA.

As shown in Fig. 8 the background fluorescence of **ARS-Zn(II)** with blank HS and BSA not much changed. However upon addition of H_2S spiked (6 - 30 μM) HS or BSA, fluorescence intensity of the probe **ARS-Zn(II)** changed significantly. A linear calibration curve can be found between the change in fluorescent intensity at 620 nm and the concentration of H_2S. The change in fluorescence intensity at 620 nm increased in a linear fashion with a higher percentage H_2S in the serum solutions (SI fig. 12). This calibration curve could be useful for estimating the concentration of H_2S in biological fluid. These results confirmed that probe **ARS-Zn(II)** can be used to detect H_2S rapidly and quantitatively in biological samples.

Real time monitoring of H_2S from donor molecule

Recently development of novel H_2S donors has become a rapidly growing field, because synthetic H_2S donors are useful research tool as well as potential therapeutic agents.[6, 43] These donor molecules release H_2S *via* different mechanism with different rate. Therefore it is important to understand the rate of H_2S release from donor molecules. Various methods hav

been used to determine concentration of H_2S released by these donor molecules. Fluorescence assays were one of the promising methods, in this regard researchers always looking for better probe that uses fluorescent technique to measure and monitor the release of H_2S for donor. The present probe **ARS-Zn(II)** has high selectivity, sensitivity and quick response time for the detection of H_2S in biological condition. Thus, we have decided to make use of this new probe to monitor the H_2S released from donor molecule.



Scheme 2: Sensing mechanism for the detection of H_2S released from H_2S Donor molecule.

In this regard, we have prepared, H_2S donor benzoic (methyl carbonic) dithioperoxyanhydride as describe in the literature.[44]

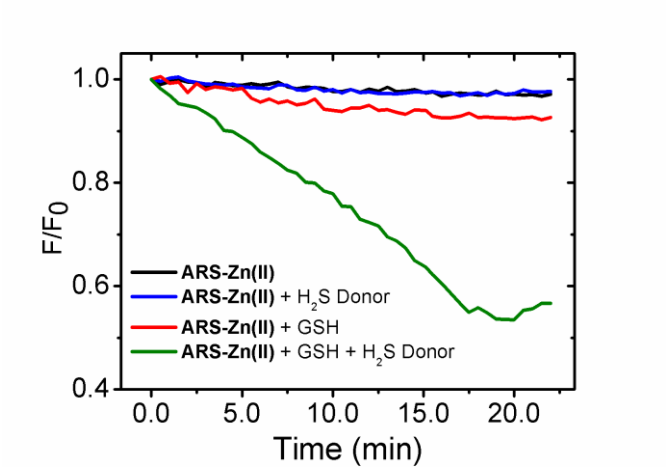


Fig 9: H_2S release monitored by the **ARS-Zn(II)** (2 μM) ensemble with H_2S donor (20 μM) and GSH (200 μM) at 35 °C.

Our aim was to examine the feasibility of using **ARS-Zn(II)** to monitor *in situ* production of H_2S from donor molecules. In a typical experimental setup, the sensing ensemble **ARS-Zn(II)** (1 mM, 100μL of the stock solution) in phosphate buffer (134 μL) was mixed with GSH (0.1 M, 6 μL of the stock solution). Then H_2S donor (1 mM, 60 μL of the stock solution in THF) was added just before starting the fluorescent measurement. Time dependent fluorescence measurement was performed in Synergy HT micro plate reader at 530/645nm as excitation/emission wavelength for 30 minutes period of time.

As shown in figure 9, **ARS-Zn(II)** could determine the H_2S release associated with decomposition of donor in presence of GSH by using multimode micro plate reader. The amount of H_2S released was expressed as relative fluorescence unit (F/F_0). The fluorescent assay showed that the spontaneous release of H_2S by donor molecules easily monitored by new probe **ARS-Zn(II)** ensemble based on fluorescent change.

We have also assessed that H_2S release assay in a temperature-dependent experiment by varying assay temperature from 30-50°C (SI Fig. 13), we found that the rate of H_2S release from donor was quite faster at higher temperature; it confirmed the stability of probe at higher temperature. Hence, the results demonstrated that the H_2S released from donor molecules can be monitored by probe **ARS-Zn(II)** in a real time.

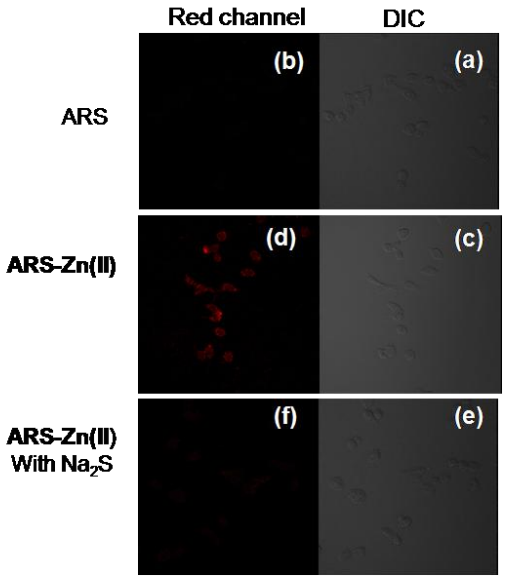


Fig 10: Fluorescence images of C6 cell lines. (a) DIC image of C6 cells treated with **A** (30 μM) only. (b) Fluorescence image of cells in red channel treated with **ARS** (30 μM) for 20 min at 37°C. (c) DIC image of cells treatment with **ARS** (30 μM) and then treated with 30 μM of Zn(II) ions. (d) Fluorescence images of cells in red channel upon treatment with 30 μM of **ARS** and then 30 μM Zn(II) ions for 20 min at 37°C. (e) DIC image of cells treatment with 30 μM of **ARS** and then treated with **30 μM** of Zn(II) ions followed by addition of Na_2S. (f) Fluorescence images of cells in red channel upon treatment with 30 μM of **ARS** and then treated with **30 μM** of Zn(II) ions followed by addition of 44 μM of Na_2S for 20 min at 37°C. Images were taken at λ_{ex} = 543 nm with 40X objective.

The spectroscopic properties of **ARS-Zn(II)** and its selectivity for H_2S appeared to be suitable for live cell image studies. Thus we have used cultured C6 cell lines to investigate the potential application of **ARS-Zn(II)** for the detection of H_2S in biological cells. As shown in Figure 10, C6 cells images were recorded after incubating with compound **ARS** for 30 min, and we did not observe any fluorescent signal. But strong fluorescence in the cells was induced after addition of Zinc and the fluorescence was disappeared upon treatment with H_2S. This result proves that probe **ARS-Zn(II)** can be used for the detection of H_2S in cultured cells.

Table 1: List of Zinc(II) based probes for the detection H_2S available in literature

Sensor Probe	LOD	Medium	Detection time	Reference
8-aminoquinoline based Zn-Complex	1.2 μM	CH_3CN-Tris-HCl	2 mins	40
7-mercapto-4-methylcoumarin based Zn- Complex	1 μM	Acetone-HEPES	ND	39
4-Methyl-2, 6-Diformyl Phenol based Zn- Complex	μM level	Ethanol-Tris-HCl	ND	41
ARS-Zn(II)	92 nM	Methanol-PBS	< 1 min	Present work

LOD = Limit of Detection, ND=Not Determined

Conclusions

In summary, we have reported Alizarin Red S based fluorescent sensing ensemble **ARS-Zn(II)** for the selective detection of H_2S in aqueous solution. The probe displayed good selectivity and remarkable sensitivity. Compared to other reported probes based on zinc complex for the detection of H_2S, we see that the present probe has number of advantage as listed below. (a) Probe **ARS-Zn(II)** are commercially available and very cheap, therefore this strategy eliminated the need of multi-step synthesis, purification and characterization by sophisticated analytical instrumentations, (b) longer excitation (λ_{exc} = 530 nm) and emission (λ_{em} = 625nm) wavelengths are more suitable for the biological purposes (c) fast response (<30 seconds) time with H_2S (d) High detection limit (92 nM) (e) Reversible ON-OFF-ON mode fluorescent change with H_2S and zinc makes **ARS-Zn(II)** as an recyclable probe for H_2S (f) Suitable for real time detection of H_2S released from H_2S Donor molecule. In addition, fluorescent response with H_2S is also applied in live cell imaging. We anticipate that sensing ensemble **ARS-Zn(II)** based fluorescent assays will be used in future as powerful tool to monitor the release of H_2S from biological and pharmacological samples.

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Notes and references

1. H. Kimura, *Mol. Neurobiol.*, 2002, **26**, 13-19.
2. R. Wang, *Physiol.Rev.*, 2012, **92**, 791-896.
3. L. E. Rojo, J. A. Fernández, A. A. Maccioni, J. M. Jimenez and R. B. Maccioni, *Arch. Med. Res.*, 2008, **39**, 1-16.
4. P. Kamoun, *Med. Hypotheses*, 2001, **57**, 389-392.
5. G. Yang, L. Wu, S. Bryan, N. Khaper, S. Mani and R. Wang, *Cardiovasc Res.*, 2010, **86**, 487-495.
6. J. L. Wallace and R. Wang, *Nat Rev Drug Discovery*, 2015, **14**, 329-345.
7. V. S. Lin and C. J. Chang, *Curr. Opin. Chem. Biol.*, 2012, **16**, 595-601.
8. M. Strianese, M. Staiano, G. Ruggiero, T. Labella, C. Pellecchia and S. D'Auria, *Methods Mol. Biol.,* 2012, **875**, 193-216.
9. C.-X. Duan and Y.-G. Liu, *Curr. Med. Chem.*, 2013, **20**, 2929-2937.
10. A. R. Lippert, *J. Inorg. Biochem.*, 2014, **133**, 136-142.
11. J. Li, C. Yin and F. Huo, *RSC Adv.*, 2015, **5**, 2191-2206.
12. N. Kumar, V. Bhalla and M. Kumar, *Coord. Chem. Rev.*, 2013, **257**, 2335-2347.
13. H. S. Jung, X. Chen, J. S. Kim and J. Yoon, *Chem. Soc. Rev.*, 2013, **42**, 6019-6031.
14. K. Wang, H. Peng and B. Wang, *J. Cell. Biochem.*, 2014, **115**, 1007-1022.
15. Z. Guo, G. Chen, G. Zeng, Z. Li, A. Chen, J. Wang and L. Jiang, *Analyst*, 2015, **140**, 1772-1786.
16. H. Peng, W. Chen, S. Burroughs and B. Wang, *Curr. Org. Chem.*, 2013, **17**, 641-653.
17. D. A. Jose, M. Elstner and A. Schiller, *Chem. - Eur. J.*, 2013, **19**, 14451-14457.
18. X. Lou, D. Ou, Q. Li and Z. Li, *Chem. Commun.* 2012, **48**, 846[illegible] 8477.
19. G. Ghale and W. M. Nau, *Acc. Chem. Res.*, 2014, **47**, 2150-2159.
20. B. T. Nguyen and E. V. Anslyn, *Coord. Chem. Rev.*, 2006, **250**, 3118-3127.
21. R. Villamil-Ramos and A. K. Yatsimirsky, *Chem. Commun*, 2011, **47**, 2694-2696.
22. A. Nonaka, S. Horie, T. D. James and Y. Kubo, *Org. Biomol. Chem.*, 2008, **6**, 3621-3625.
23. K. K. Ghosh, E. Yap, H. Kim, J.-S. Lee and Y.-T. Chang, *Chem. Commun.*, 2011, **47**, 4001-4003.
24. W. M. J. Ma, M. P. Pereira Morais, F. D'Hooge, J. M. H. van den Elsen, J. P. L. Cox, T. D. James and J. S. Fossey, *Chem Commun.*, 2009, 532-534.
25. Y. Kubo, T. Ishida, A. Kobayashi and T. D. James, *J. Mater. Chem.*, 2005, **15**, 2889-2895.
26. A. Safavi and M. Bagheri, *Anal. Chim. Acta*, 2005, **530**, 55-60.
27. A. Abbaspour and L. Baramakeh, *Sens. Actuators B: Chemical*, 2006, **114**, 950-956.
28. Z. Chen, Z. Wang, J. Chen and X. Chen, *Biosens. Bioelectron.*, 2012, **38**, 202-208.
29. Z. Chen, Z. Wang, J. Chen and W. Gao, *Talanta*, 2012, **9**[illegible] 774-779.

30. X. Yu and C. Mou, *Anal. Lett.*, 2011, **44**, 137-145.
31. F.-Y. Wu, W.-s. Liao, Y.-M. Wu and X.-F. Wan, *Spectrosc. Lett.*, 2008, **41**, 393-398.
32. L. Zhang, S. Dong and L. Zhu, *Chem. Commun.*, 2007, 1891-1893.
33. H. Kunkely and A. Vogler, *Inorg. Chem. Commun*, 2007, **10**, 355-357.
34. M. Doskocz, K. Kubas, A. Frąckowiak and R. Gancarz, *Polyhedron*, 2009, **28**, 2201-2205.
35. S. Sinha, P. Gaur, T. Mukherjee, S. Mukhopadhyay and S. Ghosh, *J. Photochem. Photobiol. B: Biology*, 2015, **148**, 181-187.
36. N. S. Allen and J. F. McKellar, *J. Photochem.*, 1976, **5**, 317-320.
37. P. Wu, J. Zhang, S. Wang, A. Zhu and X. Hou, *Chem. - Eur. J.*, 2014, **20**, 952-956.
38. Y. Wang, H. Sun, L. Hou, Z. Shang, Z. Dong and W. Jin, *Anal. Methods*, 2013, **5**, 5493-5500.
39. E. Galardon, A. Tomas, P. Roussel and I. Artaud, *Dalton Trans.*, 2009, 9126-9130.
40. Z. Dong, X. Le, P. Zhou, C. Dong and J. Ma, *New J. Chem.*, 2014, **38**, 1802-1808.
41. Z. Dong, X. Le, P. Zhou, C. Dong and J. Ma, *RSC Adv.*, 2014, **4**, 18270-18277.
42. G. Springsteen and B. Wang, *Chem. Commun.*, 2001, 1608-1609.
43. Z. J. Song, M. Y. Ng, Z.-W. Lee, W. Dai, T. Hagen, P. K. Moore, D. Huang, L.-W. Deng and C.-H. Tan, *MedChemComm*, 2014, **5**, 557-570.
44. T. Roger, F. Raynaud, F. Bouillaud, C. Ransy, S. Simonet, C. Crespo, M.-P. Bourguignon, N. Villeneuve, J.-P. Vilaine, I. Artaud and E. Galardon, *ChemBioChem*, 2013, **14**, 2268-2271.