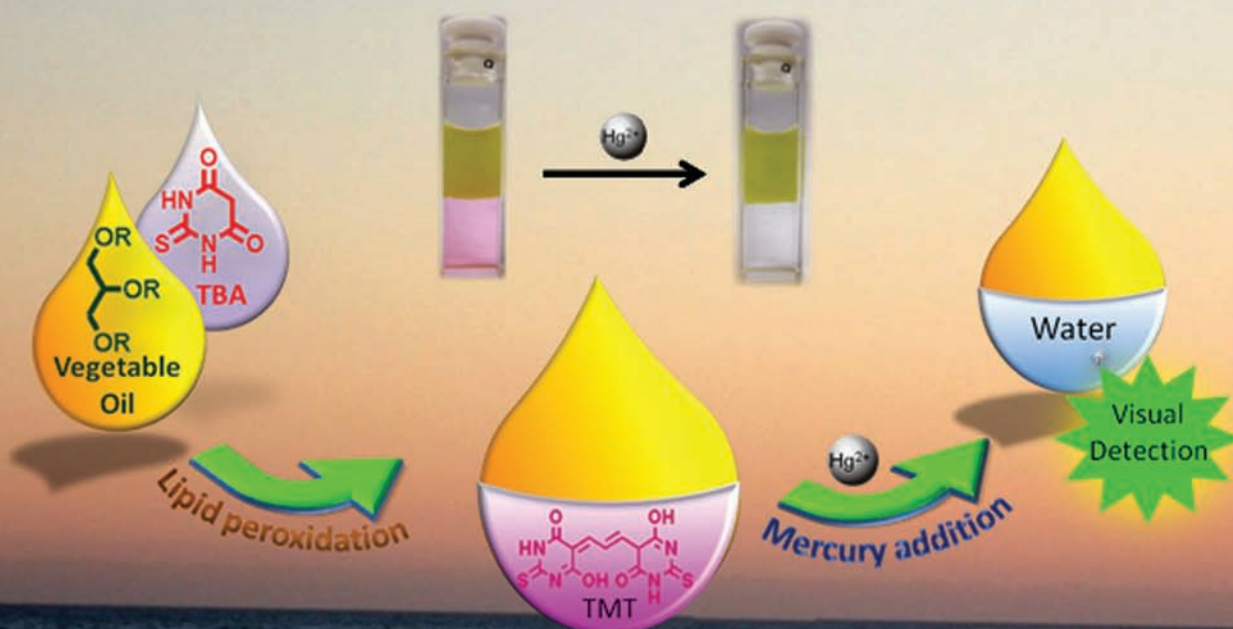


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conditions

A vegetable oil derived chemodosimeter for the selective detection of Hg^{2+} in aqueous media: a potential green laboratory method†

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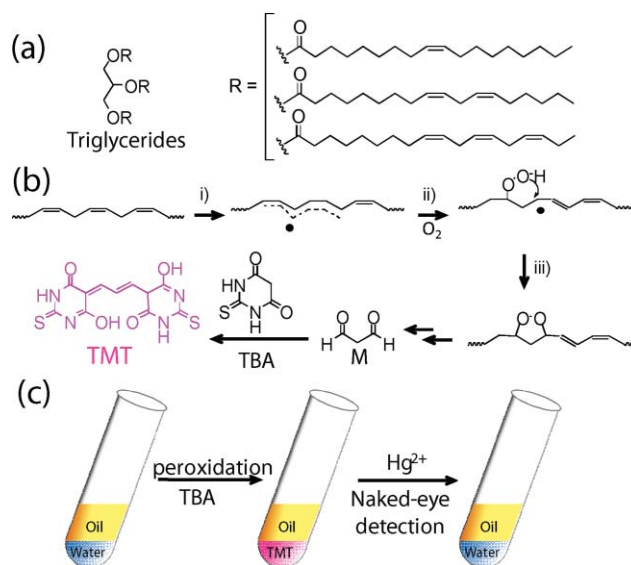
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An *in situ*-generated byproduct from the naturally occurring autoxidation of vegetable oil has been utilized to develop a thione-containing chemodosimeter for the selective detection of aqueous mercuric ions (Hg^{2+}) at nanomolar concentrations through a one-pot synthesis/detection technique.

Nature's vast repertoire of functional materials, generated from simple starting materials, is no better exemplified than in the oxidative drying of oils to form excellent natural coatings.¹ The efficient utilization of such a mechanism and materials in the modern lab would be an ideal path to a sustainable and green approach for future technologies.² The lipid peroxidation of vegetable oil is a natural phenomenon,^{3a,b} and is credited for enabling the development of functional materials.^{3c,d} Thus, prudent utilization of natural/renewable resources to develop functional materials is a new prolific approach.^{2b} Here, we have used an intermediate that is formed during the autoxidation of vegetable oils to develop an efficient detecting agent for toxic mercury.

Divalent mercury (Hg^{2+}) is the chief form of mercury pollutant that bioaccumulates in aquatic organisms at an alarming rate. Hg^{2+} acts as a chronic pollutant, even at sub-micromolar concentrations, and is best exemplified by the disastrous 1956 Minamata incident.⁴ Therefore, the foremost demand is to develop highly selective Hg^{2+} detecting agents that can detect mercury in environmental conditions, such as in an aqueous media. Even though numerous electronic-,^{5a,b} ionophore-^{5c-e} and chemodosimeter-⁶ based Hg^{2+} sensors are known, most of them involve multi-step synthesis, extensive sample preparation and sophisticated equipment for analyses, cumulatively incurring high operation expenditure.

Hg^{2+} , the most thiophilic heavy metal ion, is known to stoichiometrically react with sulfur-containing species (thiones in particular, e.g. 2-thiobarbituric acid (TBA)), thereby proportionately affecting their electronic properties, which in turn can be exploited for Hg^{2+} ion detection.⁶ Interestingly, as part of a peroxidation confirmation test, TBA reacts with *in situ*-generated malonaldehyde (M) in a 2 : 1 stoichiometry to form a 'TBA dimer-like' adduct; i.e. two TBA molecules are linked by a malonaldehyde moiety, commonly referred to as TMT [TBA-M-TBA] (Scheme 1).^{7a,b} Thus, we hypothesise that Hg^{2+}



Scheme 1 (a) The chemical structure of vegetable oil. (b) The general mechanism of lipid peroxidation: (i) initiation by abstraction of a hydrogen radical from the unsaturated fatty acid, (ii) reaction with O_2 to produce a lipid peroxy radical and (iii) formation of a cyclic peroxide; subsequently, cascade of reactions generate malonaldehyde (M) *in situ*, which can react with TBA to form the pink adduct (TMT). (c) A schematic presentation of an immiscible mixture of vegetable oil and water; peroxidation and the addition of TBA resulted in the formation of a pink coloured adduct, which disappeared upon the addition of Hg^{2+} ions, leading to its visual detection.

may react with thione-rich TMT, affecting the inherent spectral properties of TMT, to enable us to detect Hg^{2+} ions. In addition, unlike TBA, TMT, being colored, can act as a colourimetric 'naked-eye' detector, a prerequisite for rapid on-site detection. Taking advantage of this phenomenon, in this report, a judicial attempt has been made to utilize vegetable oil and its inherent autoxidation tendency to develop a novel thione-containing selective probe for Hg^{2+} that holds the potential to be used as a green and simplistic laboratory test for detecting mercury in water.

Esters of polyunsaturated fatty acid (PUFAs) with glycerol, known as triglycerides, are primary components in vegetable oils. Upon exposure to atmosphere triggers the free-radical mediated naturally occurring autoxidation process, in which the polyunsaturated hydrocarbon chains undergo peroxidation to subsequently produce malonaldehyde *in situ* (Scheme 1), a major secondary degradation product along with other carbonyl compounds.⁸ In the presence of TBA, malonaldehyde readily undergoes condensation reaction in a 2 : 1 molar ratio to produce

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a thione containing cyanine dye, TMT (Scheme 1). We envision that thione-rich TMT would interact with Hg^{2+} akin to TBA to detect Hg^{2+} ions.

Rapidly we have evaluated this hypothesis to use vegetable oil as an efficient probe for Hg^{2+} metal ions. Commercially available PUFAs containing vegetable oils (such as soyabean oil, corn oil, grape seed oil *etc.*) and aqueous solution of TBA (100 μM), were taken in a 20 mL vial (1 : 1 v/v ratio, 4 mL of each phase, Fig. 1a) and heated in a boiling water bath for 30 min. The reaction progress was monitored by the formation of characteristic absorption maxima for TMT at 532 nm (Fig. 1b ii). The reaction was completed in 30 min. Subsequently, the aqueous layer was used for the absorption and fluorescence measurements. Interestingly, upon addition of Hg^{2+} (10 μM) to the aqueous solution, pink colour disappeared and absorption maxima at 532 nm was significantly reduced (Fig. 1b iii) which is attributed to specific interaction of Hg^{2+} with TMT. However, though TMT is a predominant component, presence of trace amount of unreacted TBA cannot be ruled out. Thus, to unambiguously demonstrate that indeed *in situ*-generated TMT has the ability to act as a probe for Hg^{2+} ions, TMT has been chemically synthesized, and its ability to sense Hg^{2+} in its pure form has been extensively investigated.

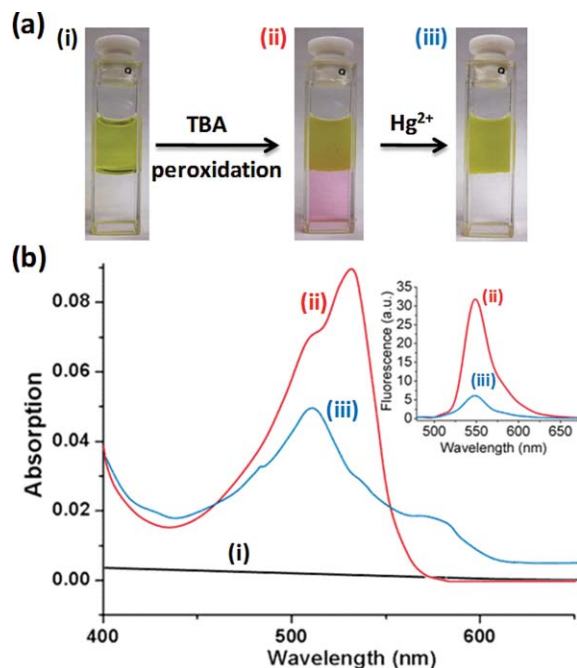


Fig. 1 a) Images of lipid peroxidation mediated *in situ* synthesis of TMT from vegetable oil; immiscible mixture of vegetable oil and 100 μM of aq. TBA solution. (b) Absorption spectra of aqueous layer i) before ii) after addition of TBA, iii) after addition of 10 μM of aq. Hg^{2+} . Inset shows fluorescence spectra of same samples.

TMT was synthesized using a single-step method⁹ (see the ESI for synthesis and characterization, Scheme S1†) and was subjected to detailed Hg^{2+} selective detection studies. TMT, a pink-coloured adduct (absorption and emission maxima at 532 and 549 nm, respectively), displayed an adequately high molar extinction coefficient ($1.3 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$), which was vital

for naked-eye colourimetric detection. Additionally TMT being water soluble, all the solutions and the titrimetric experiments were formulated in millipore water.

The sensitivity of TMT towards aqueous mercury was determined by ratiometrically titrating the TMT solution against Hg^{2+} ions. The changes in the absorption and fluorescence spectra of TMT upon increasing the concentration of Hg^{2+} is shown in Fig. 2. As the concentration of Hg^{2+} was increased, the absorption band at 532 nm decreased, along with the concomitant development of a new band at $\sim 415 \text{ nm}$ through an isosbestic point at 464 nm. The first noticeable change in the absorption is seen at 25 nM of Hg^{2+} , indicating the nanomolar sensitivity of the detection event (Fig. S1, ESI†). A further increase in the concentration of Hg^{2+} to 1.2 μM resulted in a near-complete disappearance of the absorbance at 532 nm, leading to a visual colour change from pink to colourless. Correspondingly, in the fluorescence spectrum, with an increase in concentration of Hg^{2+} , we observed a gradual decrease in the emission band at 549 nm (inset of Fig. 2). In fact, the fluorescence intensity was appreciably lowered at Hg^{2+} concentrations as low as 10 nM or $\sim 2 \text{ ppb}$, which is the maximum contamination level set by the Environmental Protection Agency in drinking water.¹⁰ The study of the relative changes in fluorescence intensity of TMT with increasing Hg^{2+} concentration indicated the reaction of TMT with Hg^{2+} as being of a 1 : 2 stoichiometry, with the reaction being virtually spontaneous, which in turn confirms the interaction of the thione groups with Hg^{2+} ions (Fig. S2, ESI†).

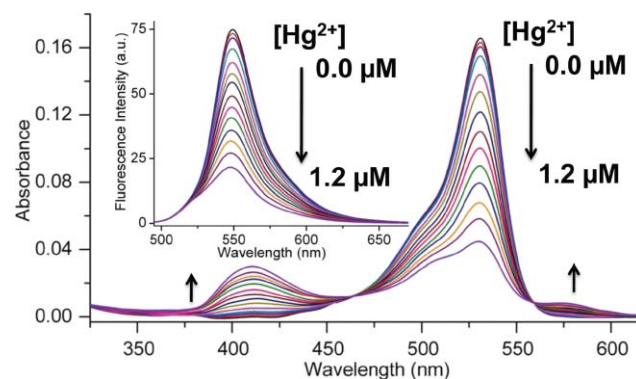


Fig. 2 Changes in the absorption spectrum of TMT (1.2 μM) in water upon adding aq. Hg^{2+} (0–1.2 μM). The inset shows the corresponding change in the emission spectrum of TMT upon adding Hg^{2+} (excitation wavelength 464 nm).

To determine the selectivity of TMT toward metal ions, the interaction of TMT with representative ions of alkali, alkaline earth and transition metal families, such as Na^+ , K^+ , Li^+ , Ag^+ , Mg^{2+} , Hg^{2+} , Cu^{2+} , Cd^{2+} , Ca^{2+} , Pb^{2+} , Zn^{2+} and Fe^{3+} , were studied. As shown in Fig. 3, under identical conditions, except for Ag^+ , all of the other metal ions (including the thiophilic metals Cu^{2+} , Pb^{2+} and Cd^{2+}) gave negligible changes in the absorption and fluorescence properties of TMT, even after adding an excess of metal ion (Fig. S3, ESI†).

The absorption and fluorescence changes of TMT upon increasing the concentration of Ag^+ was found to be similar

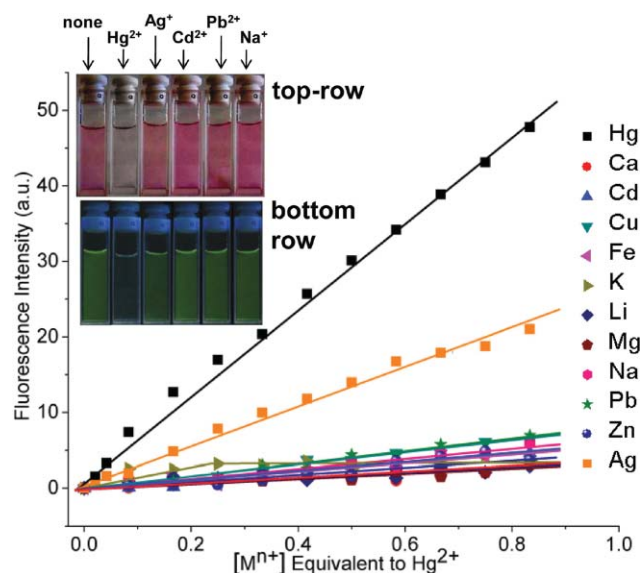


Fig. 3 The fluorescence response of TMT (1.2 μM) towards various metal ions. The inset shows the visual change in the absorption (top row) and fluorescence (bottom row) of TMT upon adding various metal ions.

to that of Hg^{2+} , except that the extent of the response was significantly low compared to that for Hg^{2+} (Fig. 3). The sensitivity of TMT towards Hg^{2+} is three-fold greater than towards Ag^+ , making it possible to differentiate between the detection events of the two metal ions. In addition, the selectivity of TMT towards Hg^{2+} can be observed visually. The bright pink colour (inset of Fig. 3, top row) and the fluorescence (inset of Fig. 3, bottom row) of TMT remained unchanged upon adding other metal ions, whereas with Hg^{2+} , a colour change from pink to colourless and complete quenching of the fluorescent intensity was observed.

To understand the practical usage in different conditions, the efficiency of TMT towards Hg^{2+} was tested in the presence of different metal ions, as well as in different pH solutions. In the presence of high concentrations of other competing metal ions (1 equiv. each of Na^+ , K^+ , Li^+ , Ag^+ , Mg^{2+} , Cu^{2+} , Cd^{2+} , Ca^{2+} , Pb^{2+} , Zn^{2+} and Fe^{3+}), TMT exhibited selectivity towards Hg^{2+} (Fig. S4, ESI†). Interestingly, TMT was found to be effective for the colourimetric determination of Hg^{2+} over the entire pH range (pH 2–12, Fig. S5, ESI†).

In the literature, it is well documented that Hg^{2+} ions promote the irreversible desulfurization of thione-containing compounds.¹¹ Similarly, TMT also has two thione groups; thus, the desulfurization process upon treating with Hg^{2+} could be similar to that of the reported examples. The occurrence of the reaction between TMT and Hg^{2+} was confirmed by adding a concentrated solution of the strong chelating agent EDTA.^{9f} The addition of EDTA (1000 equiv.) to a TMT– Hg^{2+} (1:2) solution did not alter the characteristic 415 nm band, indicating that an irreversible reaction between TMT and Hg^{2+} had taken place (Fig. S6, ESI†). In addition, the minimum detection limit for the absorption and fluorescence measurements were found to be in the nanomolar region, 25 and 10 nM, respectively. Furthermore, the detection limits for the

visual colour (for naked-eye detection) and fluorescence change were found to be in the sub-micromolar [Hg^{2+}] range, 0.7 and 0.5 μM , respectively (data not shown). Taken together, TMT could serve as a rapid, efficient and on-site detector for practical applications.

In summary, we have developed a thione-containing selective chemodosimeter for Hg^{2+} from vegetable oil. The Hg^{2+} -mediated desulfurization of TMT induced alterations in the chromogenic and fluorogenic properties of the dye, which were used for the ratiometric determination of Hg^{2+} . Nanomolar (ppm) levels of aqueous Hg^{2+} could be rapidly and selectively detected using the TMT dye, even in the presence of competitor metal ions. TMT possesses other advantages, such as: (i) colourimetric 'naked-eye' detection of Hg^{2+} at sub-micromolar concentrations, (ii) efficient at a wide range of pHs, and more notably, (iii) detection of nanomolar levels of Hg^{2+} ions. In a nutshell, the lipid peroxidation tendency of vegetable oil was utilized to develop a dye *in situ* that could be used *per se* for Hg^{2+} detection. This *in situ* synthesis/detection method for Hg^{2+} could be used as a promising green laboratory method for the detection of mercury.

Acknowledgements

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References

- 1 K. F. Lin, in *Bailey's Industrial Oil and Fat Products: Industrial and Nonedible Products from Oils and Fats*, ed. S. Fereidoon, John Wiley & Sons, Hoboken, 6th edn, 2005, vol. 6, ch. 9, pp. 307–351.
- 2 (a) *Green Chemistry: Theory and Practice*, ed. P. T. Anastas and J. C. Warner, Oxford University Press Inc., New York, 1998, pp. 160; (b) P. Vemula and G. John, *Acc. Chem. Res.*, 2008, **41**, 769–782.
- 3 (a) G. Gündüz, A. H. Khalid, I. A. Mecidoglu and L. Aras, *Prog. Org. Coat.*, 2004, **49**, 259–269; (b) H. Yin and N. A. Porter, *Antioxid. Redox Signaling*, 2005, **7**, 170–184; (c) A. Kumar, P. K. Vemula, P. M. Ajayan and G. John, *Nat. Mater.*, 2008, **7**, 236–241; (d) H. Deka and N. Karak, *Prog. Org. Coat.*, 2009, **66**, 192–198.
- 4 (a) S. Ekino, M. Susa, T. Nimomaya, K. Imamura and T. Kitamura, *J. Neurol. Sci.*, 2007, **262**, 131–144; (b) B. Weiss, *Toxicol. Sci.*, 2007, **97**, 223–225.
- 5 (a) T. Krawczynski vel Krawczyk, M. Moszczynska and M. Trojanowicz, *Biosens. Bioelectron.*, 2000, **15**, 681–691; (b) Q. Zhao, T. Cao, F. Li, X. Li, H. Jing, T. Yi and C. Huang, *Organometallics*, 2007, **26**, 2077–2081; (c) J. Wang and X. Qian, *Chem. Commun.*, 2006, 109–111; (d) L. Praveen, V. B. Ganga, R. Thirumalai, T. Sreeja, M. L. P. Reddy and R. L. Varma, *Inorg. Chem.*, 2007, **46**, 6277–6282; (e) M. Tian and H. Ihmels, *Chem. Commun.*, 2009, 3175–3177.
- 6 (a) B. Liu and H. Tian, *Chem. Commun.*, 2005, 3156–3158; (b) J. Ros-Lis, M. D. Marcos, R. Martinez-Manez and J. Soto, *Angew. Chem., Int. Ed.*, 2005, **44**, 4405–4407; (c) Y. K. Yang, K. J. Yook and J. Tae, *J. Am. Chem. Soc.*, 2005, **127**, 16760–16761; (d) S.-K. Ko, Y.-K. Yang, J. Tae and I. Shin, *J. Am. Chem. Soc.*, 2006, **128**, 14150–14155; (e) M. H. Lee, B.-K. Cho, J. Yoon and J. S. Kim, *Org. Lett.*, 2007, **9**, 4515–4518; (f) M. H. Lee, S. W. Lee, S. H. Kim, C. Kang and J. S. Kim, *Org. Lett.*, 2009, **11**, 2101–2104.
- 7 (a) R. Kahl and A. G. Hilderbrandt, *Food Chem. Toxicol.*, 1986, **24**, 1007–1014; (b) K. Kikugawa, *Recent Res. Dev. Lipids Res.*, 1997, **1**, 73–96.
- 8 (a) N. A. Porter, S. E. Caldwell and K. A. Mills, *Lipids*, 1995, **30**, 277–290; (b) C. Schneider, *Mol. Nutr. Food Res.*, 2009, **53**, 315–321.

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- 9 R. B. Pegg, F. Shahidi and C. R. Jablonski, *J. Agric. Food Chem.*, 1992, **40**, 1826–1832.
- 10 *Mercury Update: Impact of Fish Advisories*, EPA Fact Sheet EPA-823-F-01-011, EPA, Office of Water, Washington, DC, 2001.
- 11 (a) M.-Y. Chae and A. W. Czarnik, *J. Am. Chem. Soc.*, 1992, **114**, 9704–9705; (b) G. Hennrich, H. Sonnenschein and U. Resch-Genger, *J. Am. Chem. Soc.*, 1999, **121**, 5073–5074; (c) G. Zhang, D. Zhang, S. Yin, X. Yang, Z. Shuai and D. Zhu, *Chem. Commun.*, 2005, 2161–2163; (d) K. C. Song, J. S. Kim, S. M. Park, K.-C. Chung, S. Ahn and S.-K. Chang, *Org. Lett.*, 2006, **8**, 3413–3416; (e) G. Hennrich, W. Walther, U. Resch-Genger and H. Sonnenschein, *Inorg. Chem.*, 2001, **40**, 641–644.