Development of a portable and economical method of detecting trace metals from aqueous solution based on the coordination chemistry of 1,3,5-substituted hexahydrotriazines

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Development of a portable and economical method of detecting trace metals from aqueous solution based on the coordination chemistry of 1,3,5-substituted hexahydrotriazines

Rudy J. Wojtecki, Alexander Y. Yuen, Thomas G. Zimmerman, Gavin O. Jones, Hans W. Horn, Dylan Boday, James L. Hedrick, and Jeannette M. García*

The detection of trace amounts (<10ppb) of heavy metals from aqueous solution is described using 1,3,5-hexahyrdo-1,3,5-triazines (HTs) as a chemical indicator and a low cost fluorimeter-based detection system. This method takes advantage of the inherent property of HTs to coordinate strongly with metal ions in solution, a fundamental property that was studied using a combination of analytical tools (UV-Vis titrations, 1H-NMR titrations and computational modeling). Based on these fundamental studies that show significant changes in the HT UV signature when a metal ion is present HT compounds were used to prepare indicator strips that resulted in significant fluorescent changes when a metal was present. A portable and economical approach was adopted to test the concept of utilizing HTs to detect heavy metals using a fluorimeter system that consisted of a low-pressure mercury lamp, photo-detector, monolithic photodiode and amplifier, which produces a voltage proportional to the magnitude of the visible fluorescence emission. Readings of the prepared HT test strips were evaluated by exposure to two different heavy metals at the safe threshold concentration described by the U.S. Environmental Protection Agency (EPA) for Cr^{3+} and Ag^{2+} (100 ug/L and 6.25, respectively). This method of detection could observe the presence of either metal at these threshold concentrations.

Introduction

Contamination of water sources by heavy metals brought about by human influence from industrial discharge, urban runoff, and sewage effluents is a concern for both developing and develop countries.[1a] Although governments regulate limits of heavy metal concentrations for safe drinking water, it is also well-known that chronic exposure to heavy metals below these thresholds can cause adverse health effects. As people are becoming increasingly conscious and aware of these environmental concerns there is not only interest in more stringent regulation to ensure water safety but also demand for rapid, portable and economical methods to detect the presence of trace heavy metals from water. Herein, we will describe efforts to fundamentally understand the coordination chemistry of 1,3,5-hexahydro-1,3,5-triazines (HTs) and its utilization to detect trace amounts of heavy metals from aqueous solution.

The HT motif has attracted significant recent attention in its application for thermosetting materials.[1b] The unique reactivity of this functional group provides access to attractive material properties such as chemical recyclability and self-healing, properties that are generally elusive for highly-crosslinked thermosetting materials. While the polymeric materials based on this repeating unit have only recently been developed, many accounts of the respective small molecules – HTs– have been reported in the literature.[2] There are a wide spectrum of applications for HTs (realized and potential) such as national defense (hexahydro-1,3,5-trinitro-1,3,5-triazine – RDX[3]), insecticides (1,3,5-hexahydrotriazine-2-N-nitroimines[4]) polymerization catalysis (N-alkylated 1,3,5-hexahydrotriazines[5]), flame retardants[6] and antifungals[7a] to name a few.

While the synthesis of HTs has been extensively reported, significantly less attention has been focused on the co-ordination chemistry of this class of ligands. Structurally, HTs contain a six-membered ring containing three nitrogen atoms,
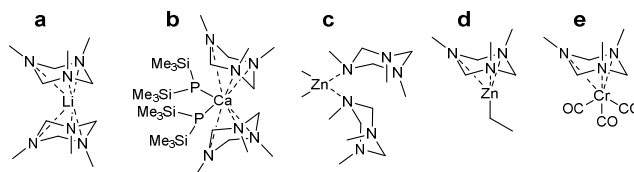

Figure 1. Selection of HT complexes with (a) Li^+, (b) Ca^{2+}, (c,d) Zn^{2+} and (e) Cr^{3+} ions previously reported.

is similar to many terdentate ligands (terpyridine,[8] 2,6-bisbenzimidazole pyridine,[9] amide pincer[10]) in its high local concentration of lone pairs of electrons and can be anticipated to provide a region for the strong association of cationic metals. Indeed, there have been detailed reports of the ability of HTs to associate with a number of mono- and multivalent metal ions such as lithium, calcium, zinc[11] and chromium (Fig. 1).[12]   However, reports of the formation of organometallic complexes using HTs have largely focused on descriptions of the complexes in the solid state and with a limited number of substituents.[13] The literature is scant with descriptions of such complexes in solution, though Zeigan and colleagues briefly described, in their method of preparing single crystals of d, the slow decomposition of the complex in a dichloromethane (DCM) solution.[4] In addition, using 2D-NOESY (nuclear Overhauser enhancement spectroscopy) they also showed the HT became chemically different by ligand coordination to zinc.

Interested in broadening the repertoire and fundamental understanding of the coordination chemistry of HTs in solution, here we present several fundamental studies of organometallic complexes formed between aromatic substituted HTs and a range of metal salts in solution using both nuclear magnetic resonance (^{1}H-NMR) and UV-Vis titrations.   In addition, these empirical studies were complemented by molecular modelling to provide a more nuanced understanding of not only the spatial arrangements of the complexes but the energies associated with their formation. These fundamental studies also provided the basis for a new application of these organometallic complexes for the detection of heavy metals in aqueous solution (we are unaware of any previous reported methods for metal detection using HTs). Taking advantage of the facile 1-step synthesis of the HT from commercial starting materials and a fluorometer-based method for the detection of metals using HTs, this approach may provide an additional low-cost method for the detection of heavy metals in solution.
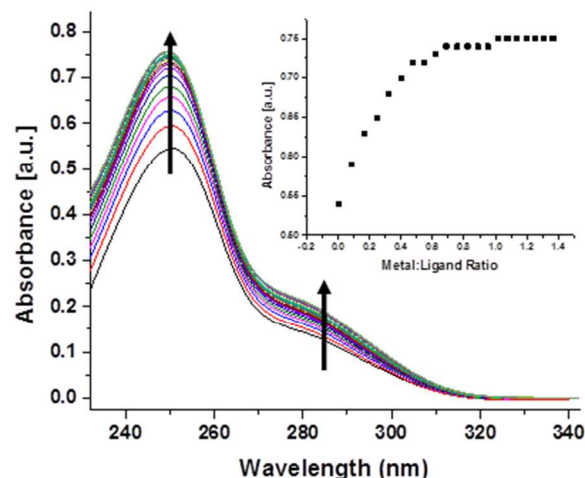
Design & Synthesis of HTs

A large spectrum of chemically diverse HTs can be produced through a facile 1-step synthesis that simply requires a primary amine and paraformaldehyde heated in an appropriate solvent (Scheme 1). With interest, in part, directed towards the use of this HT for UV-Vis characterization we focused synthetic efforts on two HTs containing aromatic chromophores (**2A-B**).
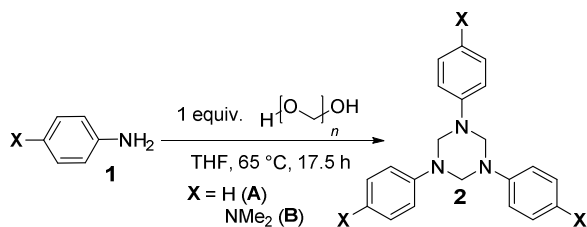
Scheme 1. Facile 1-step synthesis to access aromatic HTs.

Solution Studies of HT Complexes

The interaction between HTs and various metals of different atomic radii, oxidation state, and "hard" or "soft" properties were studied. These metal ions were selected to approximate metal binding behavior in applications in which heavy metals with varying oxidation states, hardness and atomic radii

Figure 2. UV-Vis titration of LiOTf with **2A** (25μM). Inset: plot of absorbance at λ=251 nm vs. molar ratio of LiOTf to ligand.

(Cd(II), Pb(II,IV), Hg(I,II), and As(III,V)) are present in contaminated water.[14] Metal binding studies were performed between metal

triflates and **2B** including LiOTf, Ca(OTf)$_2$, Eu(OTf)$_3$, Zn(OTf)$_2$, and AgOTf. ^{1}H NMR and UV-Vis studies of HTs to bound metal ions showed different reactivity with "soft" ions compared to "hard" ions (see SI for details). In order to rule out possible binding at the -NMe$_2$ group of **2B**, UV-Vis titrations of the unsubstituted **2A**.

UV-Vis Studies of HT Complexes

Addition of LiOTf to a 25μM solution of **2A** resulted in an increase in the ligand absorbance at 251 nm and 285 nm (Figure 2). Interestingly, no isosbestic points were observed during these titration experiments. The plot of the absorbance at 251 nm vs. the ratio of metal to ligand (M:L) revealed a significant change in absorbance at a M:L ratio of ca. 0.5, suggesting that two LiOTf ions bind to **2A**. A result consistent with previous literature reports.[15] When titrations were carried out with **2A** and a variety of other metal salts, the λ$_{max}$ shifted after the introduction of metal ions, even at M:L ratios as low as 0.12. A shift in the λ$_{max}$ may correspond with a chemical change of **2A** in the presence of each of these metal salts and this was explored by the use of ^{1}H-NMR titrations. NMR titrations reveal peak shifts characteristic of metal-binding consistent with UV-Vis titrations (**Figure S6**). Furthermore, based on these NMR titrations it was revealed that HT **2B** would undergo degradation from soft or borderline Lewis acid metal cations (Ag(I), Zn(II), and Eu(III) salts). The degradation products, a complex mixture involving aniline **1B** and other species, turned NMR samples of **2B** from colourless to strongly coloured samples (**Figure S30**).

In addition to the fundamental characterization of the association of HT to metal salts, in more concentrated forms visible color changes were observed.   For instance, the addition of Zn(OTf)$_2$ to a solution of HT **2B** lead to the formation of a yellow color. Thus, we were motivated to observe the absorbance spectra of these metal:HT solutions at longer wavelengths (**Figure 3**). The absorbance spectra at wavelengths above 340nm produced peaks that are distinct to the metal complexes.   For instance, at a wavelength of ca.
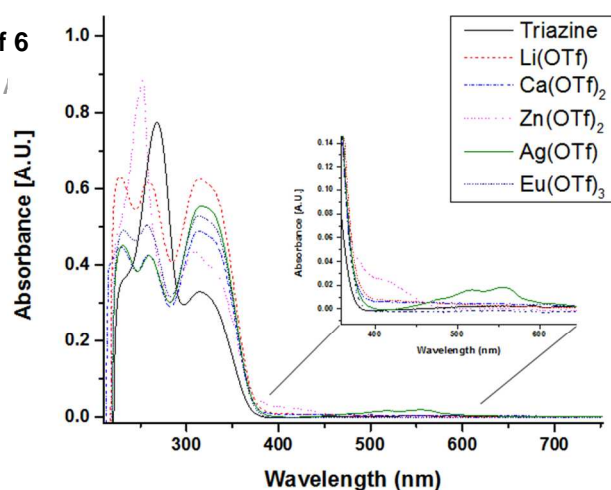
550 nm a broad peak was observed in the sample solution of **2B** + 1.0 equiv. of AgOTf consistent with a charge transfer type of metal-ligand interaction (**Figure 3**). The solution of 2B + 1.0 equiv. of Zn(OTf)$_2$ also produced distinct peaks at higher wavelengths a characteristic shoulder at ca. 400 nm was

Figure 3. UV-Vis absorbance spectra of HT **2B** and **2B** + **1** equiv. Li$^+$, Ca^{2+}, Zn^{2+}, Ag$^+$ and Eu^{3+} ions (all spectra have a conc. of **2B** at 25μM). Inset: expanded section of absorbance spectra.

observed. In the case of other metal containing solutions the presence of distinguishing peaks at higher wavelengths were difficult to observe and shoulders may not be as pronounced as
the case of Zn(OTf)$_2$. In addition, titrations with Zn(OTf)$_2$ produced a shift in λ_{max} as well as an increase in absorbance. This was the only metal in which an increase in absorbance was
noted. Observation of unique absorption spectra dramatically different from the parent ligand at various wavelengths may, however, provide a method of detecting and determining the presence of metal ions in solution and thus inspired such an investigation.

Computational Modelling of HT Complexes

To complement these studies and to determine trends in the complexation of metal ions to HT, we performed computational studies with the dispersion-corrected B3LYP-D3 density functional method (see SI for details) on the binding of group I and group II metal chlorides LiCl, NaCl, KCl and MgCl$_2$, as well as ZnCl$_2$ and AgCl to **2A** in MeCN solvent (**Table 1**).

Table 1. Binding free energies (ΔG_{bind}), in kcal/mol, for the complexation of ZnCl$_2$, AgCl and group I and II metal chlorides (MCl$_n$) with **2A**.

MCl$_n$	ΔG_{bind} (kcal/mol)
LiCl	−28.1
NaCl	−25.5
KCl	−21.4
MgCl$_2$	−50.7
CaCl$_2$	−50.7
ZnCl$_2$	−39.7
AgCl	−34.6

These studies reveal that all of the metal chlorides bind strongly to the HT with binding free energies ranging from −21 to −51 kcal/mol. Binding free energies decrease moving down the periodic table with, for example, LiCl being more strongly bound to the HT than NaCl which is in turn more strongly bound than KCl. Moreover, binding free energies increase moving across the periodic table with LiCl being less strongly bound to **2A** than MgCl$_2$. These results are qualitatively consistent with Pearson's HSAB theory with harder Lewis acidic metal chlorides being more strongly bound to the hard Lewis basic heterocyclic amine core of the HT.[17] Interestingly, the calculations reveal that ZnCl$_2$ and AgCl might be as strongly bound to the HT if not for the experimentally observed degradation.

HT Based Detector: Design & Performance

The motivation for studying the coordination chemistry of HTs is exploiting a metal ion induced change in their UV signature to develop a economical means of detecting heavy metals in aqueous solution. As our introduction suggests our interest in detection is primarily limited would primarily be utilized as a means of testing metal concentrations for safe drinking water. The scope of developing an economical method of metal detection is therefore limited to a narrow range of field measurments limiting the variation in pH (to at or near 7) and temperatures above freezing and below 50°C. This range of temperature and pH are well within the stability of the HT molecule which only shows degradation at low pH (<2) and temperatures above 250°C (based on thermal gravimetric analysis **Figure S5**).[1] The use of HTs are therefore, well suited for this targeted application.

Commercial methods for the detection of trace chemicals in aqueous solutions often utilize colorimeters and fluorometers.[18] In these methods of detection, generally, an aqueous sample to be tested is placed in a cuvette, followed by the addition of a chemical reagent whose fluorescence/colorimetric signature changes upon the presence of a trace chemical of interest. The resulting solution is then placed in an optical path for measurement. However, a lower cost alternative can instead use a detecting agent adhered to a substrate such as paper or silica based devices which increase the convenience and portability of field measurements for water quality and simplify the sample collection and measurement.[19]

Detector Characteristics

This more portable and economical approach was adopted to test the concept of utilizing HTs to detect trace amounts of heavy metals. We developed a detection system that implements a compact fluorometer. The fluorometer was built using a UV LED light source (UVTOP255TO39 Sensor Electronic Technology) with a center wavelength of 255 nm +/-10 nm providing 300 uW of optical power and a photodetector (OPT101 Texas Instruments) producing a voltage proportional to the magnitude of the visible fluorescence emission (**Figure 4**). The photodetector output voltage is digitized (ATmega328P microprocessor, Atmel) at 10 bits with a sample rate of 125 kHz. The conversion uses a 1.1 internal voltage reference resulting in a 1.074 mV
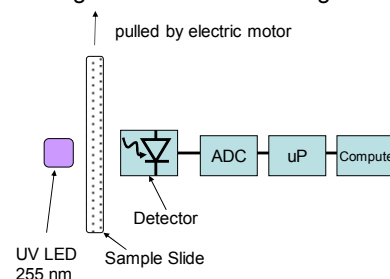
sensitivity.

Figure 4: Schematic representation of fluorometer based detection system.

The prepared test strip (Sample) is placed between the UV light source and a photo-detector, and 100 sequential readings are captured and averaged to reduce noise and achieve sub-bit resolution. The photodetector has negligible sensitivity at 254 nm (<1%) so no UV blocking filter is required and enables an in-line measurement, simplifying construction. The measured electrical noise is 0.22 mV peak-to-peak with a dark (light fully blocked) voltage of 5.5 mV. The signal-to-noise ratio for a typical measurement is on the order of 19 dB (17.6 mV signal for a silver metal concentration of 12.5 ug/L).

Sample readings are measured using an indicator strip consisting of silica impregnated with a fluorescent indicator adhered to strips of poly(ethylene terephthalate)(PET). The strip is mechanically pulled across the optical path of the LED and photodetector by a geared electric motor at a rate of X mm per second while the photodetector voltage is digitized and collected in a computer. The silica matrix provides a chemically inert hydrophilic surface for the HT **2B** indicator. Several methods were explored to coat the silica matrix with indicator including drop casting, dip coating and spray coating. Spray coating visually appears to provide the most uniform distribution of indictor and metal sample to the test strips, and was used to prepare all strips reported here.

During repeatability experiments it was observed that subsequent readings of a strip, performed by mechanically pulling the strip across the UV optical path, would produce a exposure phenomenon. The strip would exhibit a visible dark band tracing along the length of the strip, corresponding to the contact of the UV illumination on the strip. The exposed area would have a lower transmission, decreasing 4% on the first pass and 8.8% on the second pass, as illustrated in Figure S49.

Test strips were spray coated with HT **2B** from a dilute solution (conc.10 mM) from an organic solvent such as dichloromethane. Referring to Figure 5 b, the absence of flat plateaus in the UV light absorption of the sprayed segments reveals the variability of the coating method. Spin coating is often used in industry to deposit uniform thin films and should produce better uniformity that our spray coating method.[20] Other sources of signal variation are changes in silica coating thickness and tiny scratches that remove portions of the silica gel matrix. While these variations in the silica coating may be of little consequence for the strips intended use of thin layer chromatography, these imperfections produce dramatic (e.g. 20%) variations in detected signal and therefore must be controlled for commercial use. These developments however are outside the scope of this paper which seeks to establish a proof of concept method for the use of HTs for the detection of heavy metals.

Detector Performance

For a proof of concept detection system we explored the limits of detection based on safety thresholds for several heavy metals perscribed by the U.S. Environmental Protection Agency (EPA). To test the effectiveness of this detection system the indicator strips were exposed to two metals, AgOTf and $Cr(acac)_3$, at and above the safe threshold concentrations in water set-forth by the EPA (6.25 µg/L and 100 µg/L, respectively). The test strips were spray coated with these metal solutions, dried, then inserted into the fluorometer to obtain a reading. A plot of transmission intensity vs. strip position first encounters an area unexposed to UV light, establishing a zero transmission baseline, followed by deposited indicator mixed with metal solution, resulting in a transmission intensity responsive to silver metal concentration (**Figure 5A**). The plot indicates that AgOTf can be readily detected at concentrations as low as 6.25µg/L (0.024 µM), with detectable difference at the three concentrations tested. The response dependent on the metal being detected. Increasing concentrations of AgOTf lead to a decrease in transmission intensity. In the case of $Cr(acac)_3$ the inverse trend is observed. Increasing concentrations of $Cr(acac)_3$ lead to an increase in transmission intensity (**Figure 5B**). It is believed this behaviour results from a difference in the coordination chemistry of the two metals with **2B**. Further investigations will pursue the influence of coordination chemistry on detector response. In any case, it is clear that **2B** is sensitive enough to be used as a method of detecting trace amounts of metals in aqueous solutions.

With a proof of concept device sensitive enough to produce readings at EPA safe drinking water thresholds we sought to optimize the device performance by exploring the effect of light source. In a comparison study of signal quality using the low- pressure mercury lamp vs. a light emitting diode (LED) The mercury lamp tends to display a noisy baseline with significant fluctuations a result of the light source flickering at a

a.)

b.)

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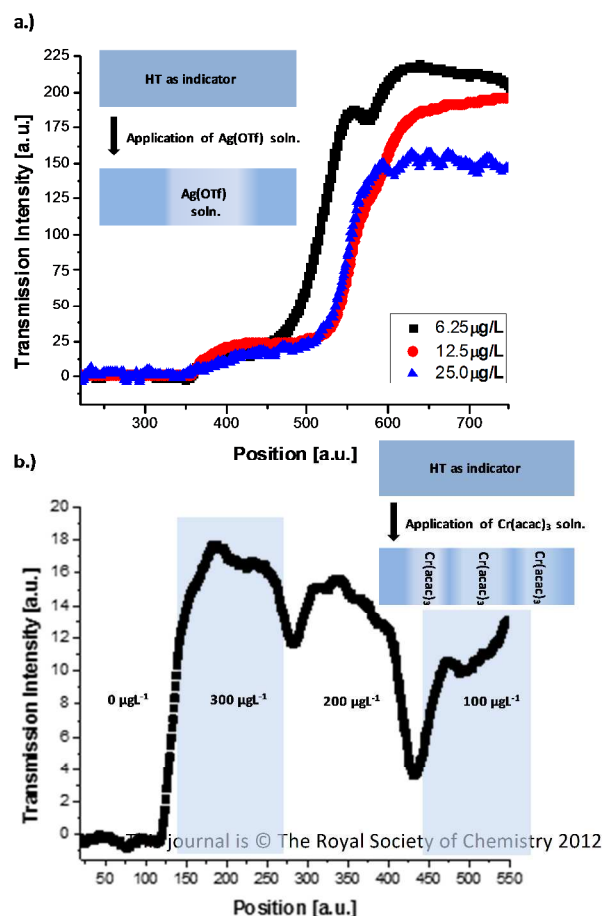
constant rate. However, with the use of a LED light source lead to a significant improvement in signal quality and this baseline noise

Figure 5. Transmission vs. position of test strips containing **2B** exposed to a trace amount of heavy metal, measured using fluorometer detection (**a**) Results from test strips exposed to varying concentrations of AgOTf. Inlet schematic illustrates area exposed to metal solution. (**b**) Test strip exposed to Cr(acac)$_3$ with inlet highlighting how test strip was exposed to different concentrations of metal solution.

is, in large part, eliminated (**Figure S42**). Using the LED light source blank indicator strips were tested and produce a largely stable baseline though over the length of the test strip (containing silica and indicator) show a small decrease in transmission from ca. 21 to 18% (**Figure S43**). When using the LED source and test strips exposed to a low concentration of AgOTf (6.25 ug/L) a signal was observed using this device, though the signal is only 1-2x the baseline signal (**Figure S44**).

Two of the most significant aspects of detection is, of course, reproducibility and detection limit. Therefore, we sought to explore the reproducibility of detecting a heavy metal at the lowest concentration possible for our device. Therefore we selected silver as the EPA's concentration for safe drinking water is 6.25 ug/L. Several test strips were prepared using the safe threshold silver concentration of 6.25 ug/L. The test strips were then dried and measured in a consistent manner with the previous results. At this low concentration we observe decreasing transmission intensity at the areas exposed to Ag^{2+}. While the intensity of this signal is not as dramatic as the use of the low-pressure mercury light source the results are reproducible and given the signal strength (only 1-2x the baseline) would suggest reliable readings would not be achievable at lower concentrations. Three concurrent runs of the same test strips lead to the same type of signals generated with no observable decrease in signal intensity (**Figure S44** & **S45**). Furthermore, doubling and tripling the concentration of Ag^{2+} lead to an increase in signal intensity (**Figure S46** & **S48**) this raw data also highlights the reproducibility of the signals generated from this detection method with only minor disparities in position, primarily due to slight differences in the areas spray coated with the Ag^{2+} sample.

Conclusion

In conclusion, the coordination chemistry of HTs have been explored by ^{1}H-NMR and UV-Vis titrations and found that in organic solvents HTs strongly coordinate with a variety of metal ions a conclusion supported by computational modelling. However, it appears that soft Lewis acid cations lead to the degradation of HTs in solution as evidenced by ^{1}H-NMR titrations while complexes formed with hard cations remained stable during observation. Furthermore, the unique UV absorbance traces motivated the exploration of these HTs as a method of detecting trace quantities of heavy metals using low cost components. It was demonstrated that quantities described as the threshold levels for safe water quality, as prescribed by the EPA, could be detected using a HT-based method. This method can be used to gather reproducible data with a limit of detection at or near the EPA threshold for silver ions (6.25 ug/L). Given the identified stability of HTs to mono and divalent cations (Li+ and Ca2+)

future work will explore the use of this detection method to selectively detect transition metals in a solution containing a mixture of ionic species.

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