Ligand-free gold nanoparticles as colorimetric probes for the non-destructive determination of total dithiocarbamate pesticides after solid phase extraction

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ABSTRACT

In this work, we describe a simple and sensitive non-destructive method for the determination of the total concentration of dithiocarbamate fungicides (DTCs) in real samples. The proposed method combines for the first time the benefits of an extraction method for sample clean-up and preconcentration with a sensitive colorimetric assay based on gold nanoparticle probes. In this two-step procedure, the target DTCs are isolated from the matrix and preconcentrated by solid phase extraction onto commercially available C_{18} sorbents. Following elution, the extract containing the target dithiocarbamates, free from most interferences and matrix components, is delivered into an aqueous dispersion of plain citrate-capped gold nanoparticles (AuNPs) which aggregate in response to DTCs coordination on AuNPs surface through multiple gold thiolate bonds. This aggregation is evidenced by changes in the spectral properties of the solution involving a decrease in the original absorbance of Au nanoparticles at 522 nm and the appearance of a new absorption band above 700 nm. An ensuing chromatic shift of the solution from wine-red to purple-blue is observed which is visual by naked eye at concentrations as low as $50\ \mu g\ L^{-1}$. Further improvement in the detection limits can be accomplished by scaling-down the method to micro-volume conditions alleviating the need to preconcentrate larger sample volumes. Overall, by combining sample clean-up and preconcentration with the strong affinity of DTC thiol group for the gold surface, the total concentration of dithiocarbamate pesticides was successfully determined in various water samples at the low and ultra-low $\mu g\ L^{-1}$ levels without resorting to destructive techniques, sophisticated instrumentation or post-synthetic modification of gold nanoparticles. Method application in real samples showed good analytical features in terms of recoveries (81.0–94.0%), precision (5.6–8.9%) and reproducibility ($\sim$9%) rendering the method as an attractive alternative to current methodologies for the determination of DTC fungicide residues in samples of environmental interest.

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1. Introduction

The determination of pesticides is a *leitmotif* environmental and food quality parameter that attracts a great amount of human labor and resources worldwide. Today, a large number of pesticides need to be monitored on a regular basis to ensure compliance with the legislation limits in every aspect of food production process, including the environment [1–3]. As the complexity of samples, the number of specimens and target compounds that need to be assessed increases, more sophisticated techniques are evolved to meet the continually increasing demands (e.g. LC–MS/MS, ORBITRAP/MS, GCxGC-TOF/MS, etc.) [4–6]. However, the associated costs and the required human and equipment resources concurrently increase. To address this issue, solutions have been sought to new analytical technologies amalgamating concepts such as miniaturization, nanoparticle techniques, multiplex detections, novel markers, etc. [7–9]. Such techniques afford fast, efficient and easy-to-operate procedures with reduced-cost and minimal resources in a wide variety of working conditions ranging from specialized laboratories to in-field surveys.

A unique category of such emerging analytical technologies are those based on nanometer-sized materials, especially those relying on the use of noble metal nanoparticles. Owing to their unique chemical and physical properties (optical, mechanical, size, etc.) these nanoparticles, have provided an unprecedented springboard for developing novel and efficient analytical applications for a vast gamut of analytes of clinical, biochemical, environmental and food interest [10–12]. Among others, the detection and determination of pesticide residues in environmental and food samples has just started to attract attention. The methods reported thus far either rely on direct signal readout from the interaction of pesticides with bare and chemically modified gold nano-assemblies

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(i.e. spherical or rods) [8,13–16] or on indirect detection based on the interference of pesticides on enzymatic or immune-affinity reactions which in turn effect the aggregation state or properties of gold nanostructured materials [17–19]. The former methods are simpler but they exhibit high detection limits and are generally prone to matrix interferences. On the other hand, the latter methods are more sensitive and free from most matrix interferences but they require long and cautious preparation procedures, costly reagents (i.e. biomolecules such as enzymes, antibodies, etc.) while they are further limited by the short life-time of biomolecules activity. Based on either of these principles, analytical methods for the determination of organochlorine, organophosphorus and carbamate pesticides have been described [13–19].

To date, only a limited number of reports have utilized nanomaterials as probes for the detection of dithiocarbamate fungicides (DTCs) employing fluorescence microscopy in home-made microfluidic devices [8] or the more selective and highly sensitive Surface Enhanced Raman Spectrometry (SERS) [14,15]. Nevertheless, there is still a lack of information regarding (a) the importance of DTC structure on the analytical response since only individual substances [8,14] or categories (i.e. dimethyldithiocarbamates) have been studied, (b) potential interferences from co-existing compounds such as other thiol containing pesticides (e.g. organophosphorous pesticides) and dithiocarbamate metabolites (i.e. ethylenethioure and propylenethiourea), and (c) the importance of non-specific interactions of the nanoparticle probes with abundant matrix components such as inorganic salts, metal ions and most importantly natural organic matter, which can modify the surface properties and aggregation of both bare and chemically modified AuNPs [20,21].

DTC pesticides are one of the most commonly used pesticides in agriculture. Monitoring data from around the world show that DTCs are the most frequently detected pesticides and they exhibit the highest frequency in exceeding the maximum residue limits (MRLs) [22–25]. In addition, current approaches for their determination in real samples are a difficult task which is feasible in two ways [26–28]. The first is a destructive approach that relies on the acidic hydrolysis of DTCs towards CS_2, which is then used as collective marker of the total concentration of DTCs in the samples. Although efficient, this approach is slow, necessitates high temperatures ($> 80\ ^\circ C$) and requires strict adherence to the experimental protocol to obtain accurate and reproducible results. In addition, the sample must not come in contact with any rubber or latex material that is a source of CS_2 contamination. The other approach involves initial extraction-preconcentration of intact DTCs followed by liquid chromatographic analysis coupled to molecular or mass selective detectors. This approach enables the determination of each individual DTC in the sample and it is faster since chromatographic analysis is accomplished in a short time. However, to account for matrix interferences, co-eluting species and instability problems of DTCs, different procedures are adopted depending on the experimental and analysis conditions that involve mobile phase modifiers, ion-pairs formation, derivatization reactions or alternative detectors (e.g. chemiluminescence, electrochemical, etc.) [26–28]. The use of MS detectors circumvents a few of these problems, especially with regards to selectivity, yet the analysis costs concurrently increase. On the other hand, the use of molecular imprinter polymers (MIPs) as a selective extraction sorbent that could provide the basis for alleviating many of these interferences, has not, to the best of our knowledge, been reported. Therefore, simple and cost-effective methods that enable the rapid assessment of total DTC concentration without destructive routes are not yet available.

With the above in mind, this work describes a simple non-destructive method for the determination of intact DTC pesticides. The method involves initial sample clean-up and preconcentration with solid phase extraction using commercially available C_{18} cartridges. The extract, free from most matrix components and interferences is delivered into a solution of citrate-capped AuNPs which rapidly aggregate in the presence of DTCs through the formation of gold-thiolate bonds. In that manner, the need to chemically modify AuNPs is alleviated, the determination of total DTCs is simplified and accelerated and detection limits can be pursed "on demand" depending on sample preconcentration. Owing to the strong interaction of gold with DTC thiol groups and the removal of most interferences during extraction, improved selectivity is also accomplished. To the best of our knowledge, this is the first study reporting on the fast, selective and sensitive determination of intact DTC pesticides by a non-destructive procedure combining an extraction method with an assay based on AuNP probes.

2. Experimental

2.1. Reagents

PESTANAL® analytical standards of dithiocarbamate (DTC) fungicides (Thiram, Ferbam, Ziram, Maneb, Mancozeb, Propineb and Zineb), dithiocarbamate compounds used for optimization (ammonium pyrrolidinedithiocarbamate and diethyldithiocarbamate) and $HAuCl_4 \cdot 3H_2O$, (min. 99.9%) for preparing gold nanoparticles were purchased from Sigma-Aldrich. Standard solutions of dithiocarbamates were prepared in acetone (Thiram, Propineb, Metiram, Ziram, Ferbam) or slightly alkaline doubly distilled water (Zineb, Maneb, Mancozeb, APDC and DETC) in dark glass containers and used immediately. Working solutions were prepared in methanol except for Zineb and Mancozeb which were used directly as aqueous standards and Ferbam which was prepared in acetonitrile. Chromabond syringe barrel cartridges (C18-ec, 500 mg, pore size 60 Å, particle size 45 μm, specific surface 500 $m^2\,g^{-1}$) were obtained from Macherey-Nagel (Duren, Germany). High purity solvents for pesticide residue analysis were purchased from Sigma-Aldrich and Fischer Scientific. All other reagents were of analytical grade and procured from major suppliers such as Alfa Aesar, Sigma-Aldrich and Merck.

2.2. Instrumentation

UV/vis spectra were recorded with matched quartz cells of 1 cm path length in a Jenway 6405 UV/vis spectrophotometer. Solid phase extraction was performed on a Supelco Preppy (Bellefonte, PA, USA) vacuum apparatus connected to a KNF vacuum pump.

2.3. Synthesis of gold nanoparticles

Gold NPs solutions were prepared by the standard citrate reduction method described by Huang [29], with slight modifications. Briefly, 100 mL of 0.25 mM $HAuCl_4 \cdot 3H_2O$ was brought to a boil under constant stirring. Then, 1.0 mL of 1% sodium citrate was added to the boiling solution to prepare a red colored colloidal solution. Heat supply was terminated after 5 min. The solution was equilibrated at room temperature prior to use and stored in a dark bottle at 4 °C for no more than 5 days.

The concentration and average size of the particles were determined from their UV–vis spectra using the extinction coefficients provided by Haiss et al. [30]. The estimated size and concentration were calculated after each synthetic cycle to ensure the reproducibility of the results. The prepared AuNPs showed a surface plasmon band at 522–525 nm indicating that they are well dispersed. The average size, calculated by the ratio of the absorbance of AuNPs at the