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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 μg 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 μg 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 (~9%) rendering the method as an attractive alternative to current methodologies for the determination of DTC fungicide residues in samples of environmental interest.

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

surface plasmon resonance peak to the absorbance at 450 nm, was 25 ± 4 nm. For verification, the average size was calculated using the initial concentration of gold, according to the following equation:

$$d = \left(\frac{(A_{spr}(5.89 \times 10^{-6})}{C_{Au} e^{C_1}} \right)^{1/C_2}$$

where A_{spr}=absorbance at the surface plasma resonance peak, C_{Au}=concentration of gold and C_1, C_2=empirical parameters ($C_1 = -4.75$, $C_2 = 0.314$) experimentally derived from standard solutions [30]. The calculated size was 24 ± 3 nm, which is close to the initial calculation. Finally, the particle concentration of AuNPs, calculated by dividing the absorbance at 450 nm with the molar decadic extinction coefficient at $\lambda = 450$ nm [30] was 0.44 ± 0.08 nM.

2.4. Real samples

River, lake and tap water samples were filtered through 0.2 μm membrane filters (Schleicher and Schuell, Dassel, Germany). A portion of the samples was retained for direct analysis. The remaining sample was fortified with known amounts of DTCs and used for recovery studies.

2.5. Procedure

2.5.1. Solid phase extraction

For the extraction of DTCs, the C_{18} cartridges were conditioned by sequential application of acetone, ethyl acetate, methanol and finally double distilled water. The aqueous sample was percolated through the cartridge with a flow rate of 2.5–3.0 mL min^{-1} and double distilled water (2 mL) was rapidly passed to desalt the samples. The sorbent was sucked dry and the target analytes were eluted under vacuum with 6 mL of a mixture of ethyl acetate/dichloromethane (80:20) in graduated tubes followed by application of 1 mL of acetone. The eluant solvent mixture was then evaporated under a gentle stream of nitrogen and the residue was re-constituted with 100 μL of HPLC-grade methanol.

2.5.2. AuNPs assay

Typically, 50 μL of DTC standard solution or extract and 10 μL of 1.0 M HCl were added into the AuNPs aqueous solution and vortexed for a few seconds to ensure complete mixing. The mixture was incubated in an ice-bath at approximately 5 °C for 10 min with mild interim mixing. The chromatic change of the AuNP solution was monitored by UV/vis spectrophotometry against blank. Determination of the total DTCs concentration was performed against Thiram calibration curve.

2.5.3. Micro-volume AuNPs assay

Scaling-down achieved using a commercially available quartz micro-cell of 1 cm path length with a total capacity of 0.7 mL. The lowest volume that could accurately be measured with this cell was 200 μL. To this end, 160 μL of 0.44 nM AuNPs aqueous solution was fortified with 50 μL of methanolic extract (or Thiram standard solutions) and 10 μL of HCl 0.1 M. The procedure was then followed as in the AuNP assay.

3. Results and discussion

3.1. Optimization of the spectrophotometric response

The instability of DTC pesticides has been reported to be a main source of error during their analysis with various techniques [27]. To avoid similar problems, the optimization study was performed with two compounds that belong to the general category of dithiocarbamates but they exhibit good stability in aqueous and polar solvents [31]. Ammonium pyrrolidinedithiocarbamate (APDC) and diethyldithiocarbamate (DETC) were therefore used for the optimization study which was conducted univariately, by varying one variable at a time, at a DTC working concentration level of 100 μg L^{-1}. Unless otherwise stated, the reported concentrations of DTCs refer to the final AuNP solution, which was prepared by adding 50 μL of a DTC standard solution to 1950 μL of AuNPs aqueous suspension.

Starting with method development and optimization, we investigated the most appropriate AuNPs size and concentration. Various sizes were prepared following two common synthetic routes that produce citrate-capped AuNPs of 9–90 nm [29] and CTAB coated AuNPs between 8 and 32 nm [32]. Indisputably, citrate coated AuNPs of 25 nm in size produced the best results (Fig. S1-a) while CTAB coated NPs exhibited trivial aggregation (not shown) most probably due to interference from free CTAB [15]. At the optimum AuNPs, the signal exhibited a monotonous increase up to the maximum AuNPs concentration of 0.43 nM (Fig. S1-b) which was therefore selected as optimum.

The influence of pH on the interaction of DTCs with AuNPs is a crucial parameter in the proposed method because; (a) it can cause protonation of DTCs at acidic conditions or decomposition of monoalkyl DTCs at pH > 7 [33–35], (b) accelerate the decomposition and hydrolysis rates of most DTCs [34] and (c) partially

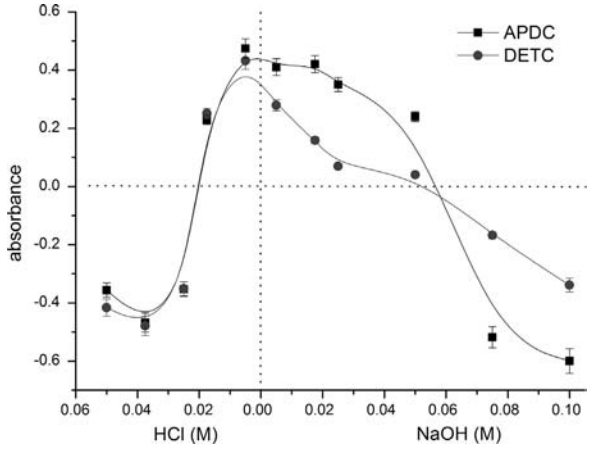

Fig. 1. Absorbance signal of AuNPs in the presence of 100 μg L^{-1} of dithiocarbamates under different pH conditions.

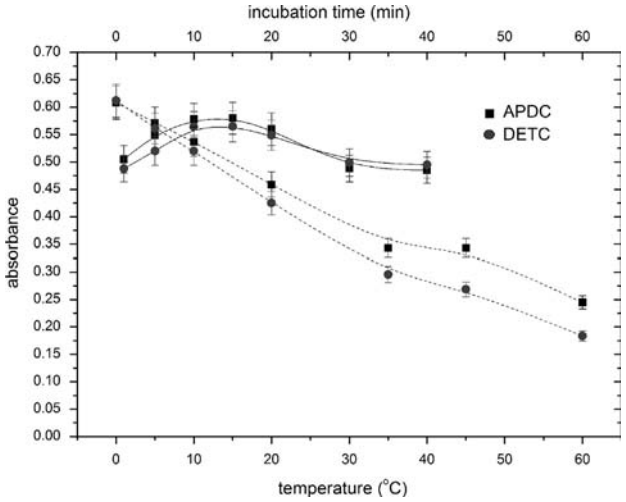

Fig. 2. Effect of incubation time and temperature on the absorbance signal of AuNPs in the presence of 100 μg L^{-1} of dithiocarbamates. Solid lines correspond to incubation time and dash lines to temperature axis.

neutralize the carboxyl moieties of the citrate coating of AuNPs [33]. To find a compromise between these factors, net absorbances (sample minus blank) of APDC and DETC were recorded in the presence of dilute HCl and NaOH. The graphic profile illustrated in Fig. 1 shows that the optimum signals for both DTCs were attained at slightly acidic conditions (i.e. 5×10^{-3} M HCl) that was employed throughout the remaining work.

The importance of incubation time and temperature on the analytical signal response of DTCs was monitored over a time span of 5–40 min and for a temperature range of 0-60 °C. As shown in Fig. 2 the signal reaches its maximum value between 10 and 15 min slightly attenuating up to 30 min possibly due to instability of the DTCs. Accordingly, increasing temperature induced a monotonous decline in the absorbance signal, in concurrence with the reported thermal liability of DTCs [36,37]. Therefore, the samples were incubated at cold conditions (~5 °C) for 10 min prior to measurement.

3.2. Response to dithiocarbamate pesticides

The response of DTC pesticides under the optimized experimental conditions defined above, were assessed for each pesticide individually by adding appropriate volumes of DTC standard solutions to the AuNP suspension. Compounds representing all three sub-classes of DTCs were examined including: dimethyldithiocarbamates (Ferbam, Thiram, Ziram), ethylenebis(dithiocarbamates) (Mancozeb, Maneb, Metiram, Zineb) and propylenebis (dithiocarbamates) (Propineb).

The spectrum profile of all DTCs at a concentration level of 10 mg L^{-1} was also recorded to ensure that there is no overlap with the recorded signal in the developed AuNP assay. Most DTCs exhibited a small peak at approximately 540 nm (< 0.1 a.u.) and another larger peak below 300 nm, with the only exception of Ferbam which exhibited an additional absorption band at 335 nm. On the other hand, in the presence of AuNPs, all DTCs exhibited absorption maxima above 700 nm suggesting the lack of spectrum overlap with the pure compounds even at elevated concentrations. The absorption spectra profile of the DTC-AuNPs mixtures with increasing concentrations are depicted in Fig. S2 (Supplementary material) and the corresponding data are gathered in Table 1. As we can observe, the signal intensity is greatly affected by the structure of DTCs. The absorbance signal decreases according to the order: dimethyldithiocarbamates > ethylenebis (dithiocarbamates) > propylenebis (dithiocarbamates) while DTC mimics such as APDC and DETC also trigger intense aggregation of AuNPs similar to that of dimethyldithiocarbamates. Accordingly, the colorimetric response of each DTC, evidenced by a visual red-to-blue transition of the AuNP suspension, follows a similar pattern. A typical visual effect with increasing concentrations is presented in Fig. 3 on the example of Thiram.

In line with these observations, the ratio of absorbance at maximum wavelength to the absorbance at the surface plasmon resonance (SPR) of the AuNPs (A_{max}/A_{spr}) exhibits a rectilinear response to DTCs concentration. The calibration plots, embedding the corresponding calibration data, are presented in Fig. S3 (Supplementary material). From both data sets it is also revealed that the only DTC that did not trigger the aggregation of AuNPs was the polymeric Zineb (net absorbance signal 1×10^{-3} at 250 μg L^{-1}), possibly due to fast hydrolysis rate in water [38].

Superficially, this structure-related response can be attributed to the similarity and differences in the structure of the three DTC categories. All compounds contain several sulfur bonds which undergo cleavage upon interaction with gold nanoparticles [39] leading to gold thiolate bonds [14] which assemble onto the surface in a bidentate manner [40]. Similarly, other DTCs such as dimethyldithiocarbamates and propylenebis (dithiocarbamates) undergo such interactions but their polymeric structure and their complexes with metal ions, exerts a different effect which generally follow a structure-depended pattern. It is characteristic that polymeric DTCs exhibited the lowest absorbance signals, especially those containing Zn^{2+} (Mancozeb and Zineb). Any differences in the spectra or sensitivity of DTCs belonging to the same categories are assumed to be due to adsorption of some analyte molecules in a monodentate configuration with the same or different nanoparticles, steric hindrance and ion association effects (mainly for polymeric DTCs such as Maneb and Mancozeb) or multi-dendate interactions with the same or different nanoparticles. The latter may explain the significant higher signal of Metiram as compared to other polymeric DTCs, due to the presence of a polyethylenebis(thiuram disulfide) moiety along with the ethylenebis(dithiocarbamate) ion. On the other hand, Mancozeb, which contained both Mn^{2+} and Zn^{2+}

Fig. 3. Colorimetric response of AuNPs to increasing Thiram concentrations. From left to right: 25 μg L^{-1}; 50 μg L^{-1}; 75 μg L^{-1}; 100 μg L^{-1}; 125 μg L^{-1}; 175 μg L^{-1}; and 250 μg L^{-1} Thiram.

Table 1
Analytical figures of merit for the quantitative detection of dithiocarbamate pesticides.[a]

Dithiocarbamate	Regression equation ($y=ax+b$)	Regression coefficient (R^2)	Linear range (μg L^{-1})[a]	Limit of detection (μg L^{-1})[b]	Lower level of visual detection (μg L^{-1})[c]
Thiram	$y=2.629x+0.036$	0.9976	25–175	10.8	≥75
Ferbam	$y=3.480x+0.005$	0.9995	25–175	0.72	≥50
Ziram	$y=2.885x-0.023$	0.9947	25–175	10.6	≥75
Maneb	$y=0.801x-0.030$	0.9985	50–250	46.8	≥100
Metiram	$y=2.341x-0.038$	0.9984	25–250	19.4	≥75
Mancozeb	$y=0.048\ln(x)+0.140$	0.9905	75–250	63.3	≥250
Propineb	$y=0.383x-0.020$	0.9968	75–750	71.8	≥200
APDC	$y=4.576x-0.009$	0.9971	25–150	3.6	≥50
DETC	$y=3.243x-0.018$	0.9963	25–150	7.8	≥50
Zineb	n.a.[d]	n.a.	n.a.	n.a.	n.a.

[a] Concentration levels correspond to the final concentration in the 2 mL AuNP suspension and were prepared by adding 50 μL of DTC standard solution (4 mg L^{-1}) into 1950 μL of AuNP aqueous suspension containing 5×10^{-3} M HCl.
[b] Limit of detection (LOD) calculated as three times the signal-to-noise ratio (3 S/N).
[c] Concentration level above which the color of the sample solution can be discriminated from the blank.
[d] n.a. = Not applicable.

showed a very weak absorbance, which is probably related to its complex with Zn^{2+} ions, as previously discussed.

3.3. Selection of the extraction method

In view of the lack of a selective extraction protocol for DTC pesticides, a multi-residue extraction methodology was only available option. However, multicomponent methods for simultaneous extraction of several groups of substances are inevitably a compromise, as the extraction conditions and cannot be optimal for all organic compounds. Therefore, the extraction protocol adopted in this work was based on a multi-residue method previously optimized through multivariate experimental design for 28 pesticides of different categories [41], with some modifications. The extraction procedure involves initial conditioning of the C_{18} sorbent material with sequential application of acetone, ethyl acetate, methanol and water while pesticides elution is accomplished with a mixture of ethyl acetate:dichloromethane (4:1). This extraction protocol was favorable for the indented application because most DTCs (except Ferbam and Mancozeb) exhibit some solubility in ethyl acetate (Thiram, Metiram, Maneb, Ziram) or dichloromethane (Propineb) therefore this elution solvent is appropriate for most of the target compounds. To aid the elution of Ferbam, which has some solubility in solvents with high dielectric constant, 1 mL of acetone was also applied.

3.4. Selectivity and interferences

For the interference study, a uniform criterion was adopted for all potential interferences. According to this criterion, a compound was considered to interfere with the analysis of DTCs, when the error in the analysis of 25 μg L^{-1} of Thiram exceeded ± 7%. Due to the SPE step involved in the proposed method, inorganic salts and alkaline earth-metal ions that would otherwise interfere with analysis by promoting the aggregation of AuNPs [42] are removed (desalting out) (Table 2) posing as not threat to the detection of DTCs. However, co-extraction of other organic compounds, mainly pesticide residues, should be taken into consideration. To this end, the potential interference of common pesticides on the determination of DTCs with the developed assay was investigated in more detail (Table 2). Organochlorine, carbamate, triazine and organophosphorous pesticides were examined both separately and in mixtures with DTCs (represented by Thiram) in order to assess their interaction with the AuNPs and their potential synergism or competition to the DTC-induced aggregation of AuNPs. Organochlorine (aldrin, dieldrin), triazine (atrazine) and carbamates (carbaryl, carbofuran) had no effect either alone or in mixtures with DTCs, apparently due to the lack of active moieties that could bind to the gold surface (Table 2). Organophosphorous pesticides on the other hand, due to the presence of thiol groups were found to cause the aggregation of AuNPs. The most potent effect was exerted by Fenthion followed by Methidathion and Chloropyrifos. Other common organophosphorous pesticides such as, Parathion and Fenthion derivatives such as Fenthion sulfone and Fenthion sulfoxide did not had any effect up to the maximum concentration examined (250 μg L^{-1}). Mixtures of Thiram with organophosphorous pesticides showed that Chloropyrifos and Methidathion could be tolerated at concentrations up to three and four times that of Thiram, respectively, while only Fention could enhance the absorbance even at equimolar concentrations. These results can be explained taking into consideration the structure of the examined pesticides. Most organophosphorous pesticides have one active (i.e. thiol) moiety therefore they require higher concentrations to affect the aggregation state of AuNPs. On the other hand, Fenthion which has 2 thiol groups, exerts a stronger effect, similar to that of DTCs.

Table 2
Minimum tolerance of diverse organic and inorganic compounds to the determination of DTCs.

Interference[a]	Tolerance
Organochlorine pesticides	
Aldrin	> 10[b]
Dieldtin	> 10[b]
Triazines	
Atrazine	> 10[b]
Carbamates	
Carbaryl	> 10[b]
Carbofuran	> 10[b]
Organophosphorus pesticides	
Fention	≤ 1[c]
Fention sulfone	> 10[b]
Fention sulfoxide	> 10[b]
Parathion	> 10
Methidathion	≤ 4
Chloropyriphos	≤ 3
Dithiocarbamate metabolites	
ETU	> 10[b]
PTU	> 10[b]
Other	
Acetamiprid	> 10[b]
Matrix components	
Natural organic matter (as humic acid)	> 80[b]
Alkaline earth metals (Ca^{2+}, Mg^{2+})	> 5000[b]
Metal ions (Fe^{3+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+})	> 5000[b]
Inorganic anions (PO_4^{3-}, Cl^-, NO_3^-, SO_4^{2-})	> 5000[b]

[a] A compound was considered to interfere when the error in the analysis of 25 μg L^{-1} of Thiram exceeded ± 7%.
[b] Maximum level examined.
[c] No longer registered for use in EU, USA, Canada and New Zealand.

Despite these interactions the presence of organophosphorous pesticides, poses as no significant threat to the detection of DTCs. That is because: (1) DTCs are the most frequently detected pesticides and they exhibit the highest frequency in exceeding the maximum residue limits (MRLs) [22–25]. Therefore, their concentration is usually higher than most pesticides (2) Fenthion, which exerts the strongest interference, is no longer registered for use on food producing plants in most countries (European Union, USA, Canada and New Zealand).

Beyond the parental compounds, ethylene thiourea (ETU) and propylene thiourea (PTU) which are the main metabolites of ethylenebisdithiocarbamates and prophylenebisdithiocarbamates, respectively, were also assessed. Direct spiking of ETU or PTU in the AuNP suspension was found to induce intense aggregation, suggesting that their presence will lead to an overestimation of the actual concentration of intact DTCs. However, when aqueous standard solutions of either ETU or PTU were extracted through the SPE cartridge, no aggregation was observed. This may be attributed to the poor retainment of ETU and PTU on hydrobobic sorbent materials such as C18 [43] and to significant losses during the evaporation step due to oxidative degradation [44].

Another pesticide, belonging to the category of insecticides that could interfere with the determination of DTCs, is Acetamiprid because it contains a cyano group that shows strong affinity for the gold surface [16] and it is favorably extracted by the C_{18} material [41]. However, direct addition of Acetamiprid to Thiram-AuNP mixtures did not have any significant effect possibly because it contains a single active group (i.e. cyano) therefore its interaction with AuNPs is favored at higher concentrations (in analogy to most organophosphorous pesticides as discussed previously). Another reason is that

the interaction of Acetamiprid with AuNPs is favorably accomplished and under different experimental and reaction conditions, than those adopted in this work [16].

The last interference examined was natural organic matter because it can enhance the solubility of organic pollutants, block the active sites of the adsorbent [45] and most importantly associate with AuNPs changing their properties and aggregation behavior [20,21]. To evaluate its influence in the determination of DTCs, the overall procedure was applied to 10 mL of aqueous standard solutions containing 25 μg L^{-1} Thiram and 2 mg L^{-1} of humic acid. Evidently, the presence of humic acid did not affect the determination of Thiram suggesting that it is removed during extraction either due to its low affinity for hydrophobic surfaces at neutral conditions [46], or because it cannot be eluted from the C_{18} material with ethyl acetate which is a poor solvent for HA [47]. For samples burdened in HA (e.g. eutrophicated waters) the addition of Na_2SO_3 has been reported to efficiently mitigate the interference of HA during the SPE [48]. The addition of Na_2SO_3 can therefore safely be employed because the sample is desalted out during SPE.

3.5. Calibration and analytical features of the AuNP and AuNP micro-volume assays

Calibration was performed against increasing concentrations of Thiram. This fungicide was selected for several reasons: (a) it is one of the most-used DTC fungicides [26], (b) it is already used by several authorities to express the total concentration of DTCs [49] c) it is one of the few DTCs (along with Ziram and Propineb) where specific MRLs which have been established by EU (European Community, Commission Directive 2007/57/EC). Calibration of the AuNP assay was performed within the linear range established in Table 1 above, by extracting 20 mL of aqueous standard solutions containing 5–50 μg L^{-1} of Thiram. After extraction and evaporation of the elution solvent, the extract was re-constituted to 100 μL with methanol, affording a preconcentration of 250 times. A 50 μL aliquot was then added to a 1950 μL AuNP suspension and the signal was recorded against blank. The detection limit, defined as three times the signal-to-noise ratio, was as low as 1.2 μg L^{-1} which is satisfactory for environmental surveillance applications based on the established criteria for dithiocarbamates (as Thiram) set by Australia and Japan (3 and 6 μg L^{-1}, respectively) [49]. However, to meet the MRLs of total pesticide residues in water set by EU (0.5 μg L^{-1}) [50], larger sample volumes need to be extracted.

The precision of the method was then evaluated by estimating the method repeatability and reproducibility. Method repeatability, estimated as the relative standard deviation (RSD) of seven sequential measurements of 25 μg L^{-1} Thiram, was 5.97%. Method reproducibility was calculated at the same Thiram levels for five different days yielding a mean RSD value of 8.96% which was deemed as satisfactory. The robustness of the method was evaluated by synthesizing fresh AuNP solutions daily for a period of 5 days. Analysis of variance (ANOVA) showed that no significant changes in the measured signal ($p < 0.05$) suggesting that the method is robust for variation in the AuNP characteristics within the experimental error ($\pm$ 5%).

To accomplish detection limits at the sub-μg L^{-1} levels with minimal sample volume requirements the method was scaled down to micro-volume conditions. Under these conditions, 50 μL of the methanolic extract was delivered into a smaller volume of AuNPs solution ($\sim$160 μL) thus affording lower dilution of the methanolic extract. In that manner, SPE of 10 mL of aqueous standard solutions offers a preconcentration of 100 times but the concentration of AuNPs in solution is concurrently diluted to 0.32 nM. As a result AuNPs were saturated at lower DTC concentrations, yielding a rectilinear absorbance signal response over the range of 1.0–10.0 μg L^{-1} offering detection limits as low as 0.25 μg L^{-1}. In that manner, the micro-volume assay offers further convenience and increased sample throughout by extracting smaller sample volumes. The precision and reproducibility of the micro-volume assay, at a concentration level of 2.0 μg L^{-1} and for five replicates ($n=5$) was 6.65% and 9.58%, respectively.

By and large, the analytical features of the assays, both in terms of sensitivity and reproducibility, are comparable to previous chromatographic methods for DTC analysis based on destructive and non-destructive techniques [23–26], including recently published methods employing nanoscale gold materials [8,14,15].

3.6. Analysis of real samples

A series of water samples with different quality characteristics and matrix components (tap, river and lake) were used for the determination of DTC residues. All samples were fortified with two concentrations of Thiram and extracted through the C_{18} cartridges. In addition, a 10 mL aliquot of each sample was spiked with 1.0 μg L^{-1}. Following extraction onto the C_{18} cartridges, analysis was performed with the micro-volume assay. Extraction of lake water samples was carried out in the presence of 5 mM Na_2SO_3 to reduce the potential influence of humic acids.

The recoveries gathered in Table 3 show that the method affords recoveries higher than 70% which is the minimum acceptable recovery for each fortification level [51] and in the range of 81.0–94.0%, which lies within the usual recovery levels of DTCs from water samples [26,27,52,53]. The RSD from triplicate analysis of real samples lied between 5.6 and 8.9% which was deemed as satisfactory. Altogether, these results suggest that the method is tolerant of matrices representative of environmental water samples and can be applied to the determination of DTC residues in

Table 3
Recovery of dithiocarbamate pesticides from spiked water samples.

Sample	AuNPs assay[a]				Micro-volume assay[a]			
	Spiked (μg L^{-1})	Found (μg L^{-1})	Recovery (%)	RSD (%)[b]	Spiked (μg L^{-1})	Found (μg L^{-1})	Recovery (%)	RSD (%)[b]
Tap water	5.0	4.6	92.0	5.6	1.0	0.93	93.0	6.2
	10.0	9.4	94.0	5.6				
River water	5.0	4.2	84.0	7.1	1.0	0.81	81.0	7.3
	10.0	8.9	89.0	6.4				
Lake water	5.0	4.2	84.0	8.2	1.0	0.82	82.0	8.9
	10.0	8.2	82.0	7.6				

[a] All concentrations refer to the initial sample (25 mL in the AuNP assay and 10 mL in the micro-volume AuNP assay).
[b] Relative standard deviation from triplicate analysis.

water samples as well as for the determination of pesticide residues in the surface of crops.

4. Conclusions

A simple non-destructive method combining solid phase extraction and AuNPs as detection probes was developed for the determination of the total concentration of dithiocarbamate pesticides. Solid phase extraction relieved the sample from matrix components and most interferences while affording analyte preconcentration. The utilization of ligand-free AuNP nanoprobes for signal read-out offered amplified sensitivity and satisfactory selectivity due to the high affinity of DTCs for the gold surface, attributed to the presence of several thiol groups in their molecules. In this manner, the determination of dithiocarbamates was accomplished at the low $\mu g\,L^{-1}$ levels with small or minimal sample volume requirements, without resorting to destructive techniques, costly instrumentation, derivatization reactions or post-synthetic modification of gold nanoparticles surface. Method application was successfully demonstrated in real samples of environmental interest at concentrations relevant to the maximum residue limits. More selective sorbent materials, such as molecular imprinted polymers that could further enhance the application range of the proposed assay in more complex matrices and in-field applications using portable photometers, are currently under consideration.

Novelty statement

The determination of pesticides with gold nanoparticle (AuNP)-based assays has just attracted to receive attention. Surprisingly, methods for the determination of dithiocarbamate fungicides (DTCs) are still at a very early stage, despite the fact dithiocarbamates are the most popular pesticide category worldwide. This work describes a methodology combining the benefits of solid phase extraction (SPE), with AuNPs as colorimetric probes. The sensitivity of the method is greatly enhanced by combing sample preconcentration with the high sensitivity offered by AuNPs. In parallel, sample clean-up by SPE along with the strong coordination of DTCs through their thiol groups on the AuNPs surface affords improved selectivity. By combining these aspects into one method: (1) the determination of DTC pesticides in real samples with good analytical features in terms of selectivity, sensitivity, recoveries and reproducibility is accomplished; (2) simple detectors, common instrumentation and commercially available reagents are used; and (3) simple citrate capped gold nanoparticles are employed without post-synthetic modifications. Least but not last, by scaling down the method to micro-volume conditions, the detection of dithiocarbamates at the sub-ppb levels with minimal reagents consumption and sample volume requirements is demonstrated.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.10.063.

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