SUPPORTING INFORMATION

Generic assay of sulfur-containing compounds based on kinetics inhibition of gold nanoparticle photochemical growth

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Table S1. Experimental variables affecting the photochemical formation of AuNPs in the presence of sulfur containing compounds.

Parameter	**Range studied**	**Optimum value**
$AuCl_4^-$ (mM)	0.25-3.0	1.0
pH	2-12	3.5
Sensitizer (Citric acid-sodium citrate, mM)	1-40	5.0
Temperature (^oC)	4 - 70	≤ room temperature
Irradiation wavelength (nm)	254, 312, 365, Ambient light, room light	Any wavelength with inversely proportional relationship to irradiation time according to the order: $t_{254nm} < t_{312nm} < t_{365nm} < t_{ambient\ light} < t_{room\ light}$

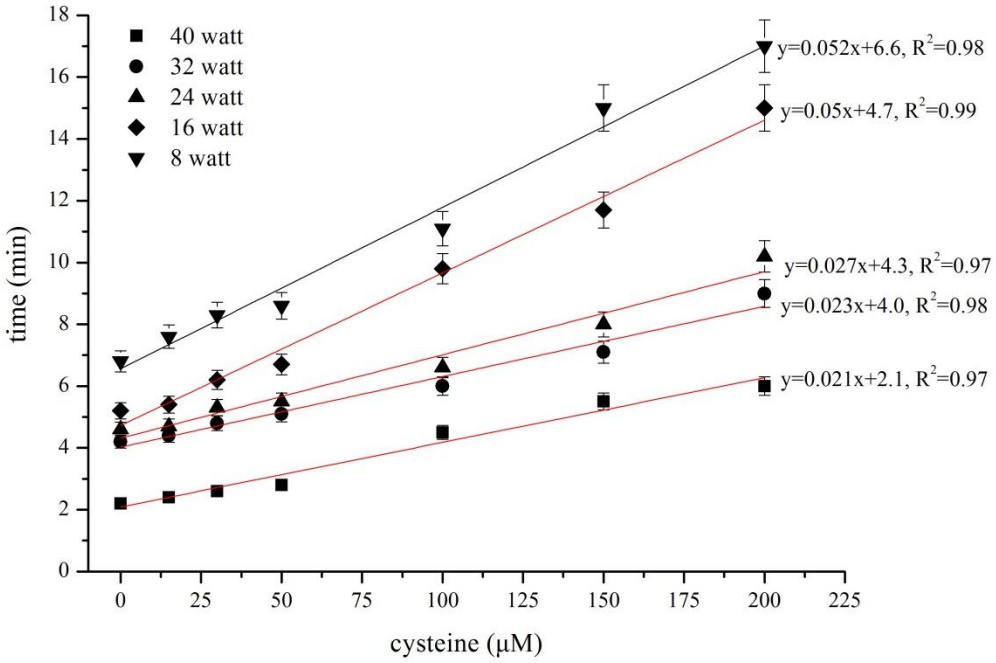
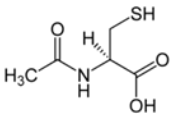
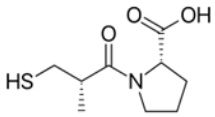
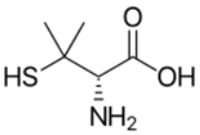
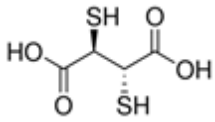
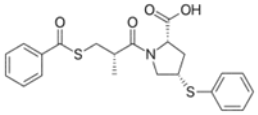
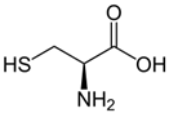
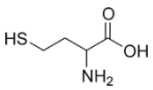
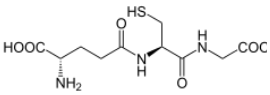
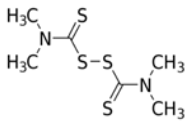
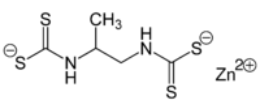
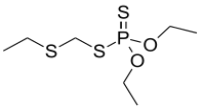
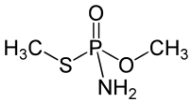


Figure S1. Dose-response curves with increasing cysteine concentrations at different UV light intensities (λ=254 nm).

Table S2. Analytical figures of merit for the determination of different sulfur-containing compounds based on kinetics inhibition of AuNPs photochemical formation as a metering unit.

Analyte	Calibration range	Calibration function	R^2	MDL	Structure
Inhibitors of Metallo-Lactamases					
acetylcysteine	15-150 μM	y=0.037x-0.7	0.96	15 μM	
DL-captopril	50-400 μM	y=0.024x-0.63	0.99	10 μM	
D- penicillamine	50-400 μM	y=0.06x-0.55	0.97	10 μM	
Meso-2,3-dimercaptosuccinic acid	50-400 μM	y=0.03x+1.9	0.99	10 μM	
Zofenopril	30-150 μM	y=0.05x+4.9	0.99	5 μM	
Biothiols					
Cysteine	15-200 μM	y=0.026x-0.45	0.99	15 μM	
Homocysteine	15-200 μM	y=0.02x-0.4	0.97	15 μM	
Glutathione	15-200 μM	y=0.057x-0.58	0.99	15 μM	
Dithiocarbamate pesticides					
Thiram	50-400 μg/L	y=- 8×10^{-3}x+1×10^{-3}	0.99	50 μg/L	
Propineb	50-300 μg/L	y=62×10^{-3}x+1.3×10^{-3}	0.98	50 μg/L	

Organophosphate pesticides					
Phorate	50-400 μg/L	y=2.1×10^{-3}x+0.42	0.97	15 μg/L	
Methamidophos	50-400 μg/L	y=1.8×10^{-3}x+0.34	0.98	12 μg/L	
Inorganic sulfur					
Sulfide	15-200 μM	y=0.013x+0.12	0.99	15 μM	S^{2-}

MDL: Method quantitation limit defined as the minimum level at which the analyte can be quantified by visual evaluation and in this method it is equivalent to the quantitation limit (LOQ).

Experimental protocols for the determination of sulfur-containing compounds in different samples

Determination of sulfur-containing compounds in drugs

The determination of sulfur-containing compounds in drugs was performed in a series of inhibitors of metallo-lactamases. We observed that the excipients present in commercial drugs (typically at concentrations higher than the active ingredient) influence the photoreduction of gold ions thus change the kinetics of the reactions in a different manner as compared to those observed in aqueous standard solutions of the compounds. Therefore, we applied the methods of standard-additions and matrix matched calibration. The method of standard additions was performed by spiking the unknown sample with standard solutions of the analyte at concentrations 5 and 10-fold higher than then excepted analyte concentration.[S1] For the method of matrix-matched calibration, a genuine drug with known concentration of the active ingredient was diluted sequentially in order to prepare a series of solutions with increasing concentrations of the active compound. Both methods produced equivalent results, therefore, any of these two methods can be selected depending on need or convenience.

The optimal experimental procedure for the determination of sulfur-containing compounds in drugs is as follows: An aliquot of 1.7 mL of the diluted sample was transferred into a 10 mL beaker. Then, 0.1 mL of citric acid-sodium citrate buffer pH 3.5 (0.1 M) and 0.2 mL of $AuCl_4^-$ solution (10 mM) were added and mixed manually. Photoreduction was performed under UV light irradiation at 254 nm (40W) and the formation of AuNPs was inspected by the unaided eye. The time delay between the formation of a red-purple coloration, in the blank and the sample solutions was used as the analytical signal. For the method of standard additions the time delay between the formation of a red-purple coloration, between

the sample and the spiked solutions was used to prepare the standard addition calibration curve.

Determination of biothiols in body fluids

The applicability of the assay for the determination of biothiols was evaluated in artificial urine fortified with 200.0 μM of cysteine. During this study we observed that the photo-reduction kinetics of gold ions is accelerated in artificial urine as compared to distilled water. As a result the sample changed color faster than the blank. In addition, colorimetric transitions occurred almost simultaneously ($\Delta t<0.1$ min) making it difficult to discriminate the presence of cysteine even at concentrations as high as 50.0 μM.

To overcome this problem we re-optimized the experimental conditions using artificial urine as sample matrix. We concluded that the optimum conditions for the analysis of biothiols in biological fluids are: a) 40 W of irradiation intensity under artificial (visible) light b) 1.0 mM of $AuCl_4^-$) and c) citrate concentration of 28.0 mM. Under these conditions (i.e. lower light intensity and higher concentration of sensitizer) the photoreduction kinetics in the absence and presence of cysteine could be clearly discriminated. It should be noted, however, that the color of the sample still changes faster than the blank in contrast to our observations in distilled water. Importantly, the sensitivity of the method was significantly improved. The dose-response curve (time vs concentration) in artificial urine was linear in the range of 1-20 μM (with a detection limit of 1 μM at $\Delta t=0.2$ min) as compared to distilled water under UV light, as previously discussed (linear range 15-200 μM with a detection limit of 15 μM at $\Delta t=0.2$ min).

We also observed that common biomolecules such as amino acids (i.e., glutamine, glutamic acid, asparagine, aspartic acid, glycine, valine, alanine), glucose, ascorbic acid, uric acid, urea, and creatinine, at physiologically relevant concentrations, do not interfere

with the detection of biothiols. Importantly, oxidized thiols species such as cystine were found to affect the photoreduction kinetics of gold ions in a manner analogous to cysteine due to cleavage of the disulfide bond (chemical cleavage upon interaction with gold and photochemical cleavage in the presence of citrate as electron donor).[S2] Therefore the method can be used to determine the total concentration of biothiols (both reduced and oxidized) as opposed to other methods which typically require reduction of the disulfide bonds prior to analysis. In contrast to these findings, however, amino acids with a basic chain (namely lysine, histidine and arginine) could inhibit the photoreduction kinetics even at low concentrations (i.e. 25 μM). This may possibly be attributed to electrostatic interactions between the positive charge of the amino acid chain and the negatively charged $AuCl_4^-$ species. Since the total concentration of basic amino acids in body fluids (lysine, histidine and arginine) is almost equal to that of total biothiols (mainly cysteine and homocysteine) we concluded that the method would infer false positive results. Therefore, basic chain amino acids must be removed prior to analysis. This could be made by using appropriate selective adsorbent materials such as strong cation exchange resins (for example Amberlite IR 100, Wolfatit C, Zeo-Karb 215, etc) or molecularly imprinted polymers.

Based on the above studies the following protocol is proposed for the determination of biothiols in body fluids: A protein-free aliquot of the sample, relieved from amino acids with a basic chain, was diluted as appropriate in distilled water. An aliquot of 1.25 mL of the diluted sample was placed in a beaker and 0.55 mL of 0.1 M citric acid-sodium citrate buffer pH 3.5 and 0.2 mL of $AuCl_4^-$ solution (10 mM), were added sequentially. A blank sample containing 120 mM of NaCl was prepared in a like manner. After mixing the beakers were irradiated simultaneously at 40W of artificial visible light and the formation of AuNPs was inspected by the unaided eye. The time delay between the formation of a

red-purple coloration, in the blank and the sample solutions was used as the analytical signal

Determination of sulfur-containing pesticides

For the determination of pesticides we examined the influence of organic solvents in the performance of the method considering that a sample pre-treatment step (such as solid phase extraction) would be required before analysis in order to extract and preconcentrate the target pesticides from environmental water samples. Therefore, an organic solvent extract would most probably be used for the analysis of pesticides rather than plain water. The dose-response curve of methamidophos (as a model pesticide) in the presence of 20% (v/v) methanol (Figure S2) shows that linearity is not affected, but the slope of the curve, and consequently the sensitivity of the method increases which is advantageous for the analysis of pesticides at trace levels. This finding may be attributed to the fact that polar organic solvents (such as acetone) accelerate the photoreduction kinetics of gold ions especially at the early stages of the irradiation.[S3]

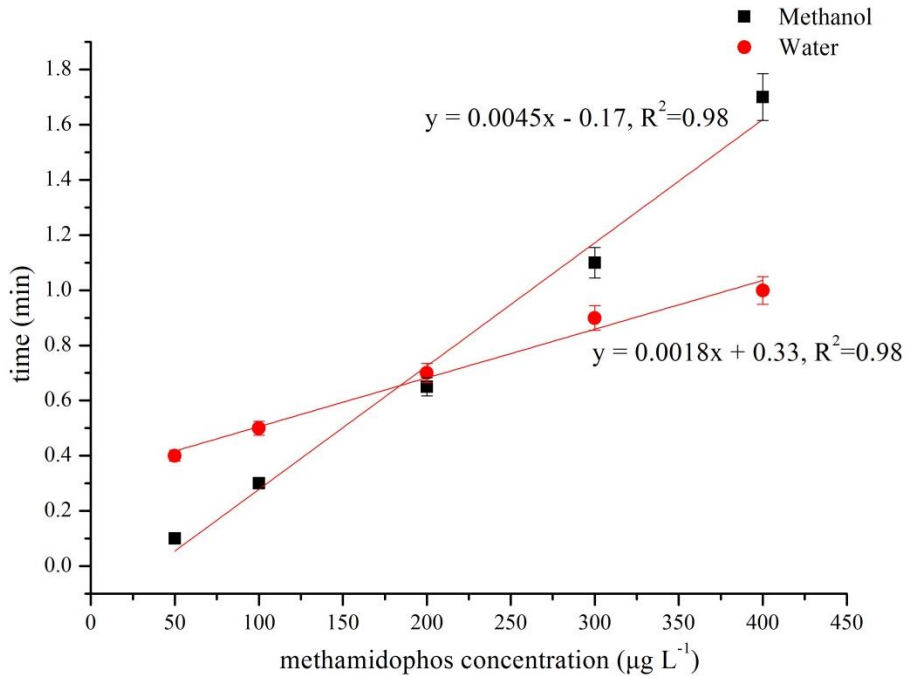


Figure S2. Calibration plots of methamidophos (time required for the photochemical formation of AuNPs vs. concentration) in methanol and water.

We also evaluated the response of the method after acidic and alkaline hydrolysis of the examined pesticides because their hydrolysis by-products are major metabolites in environmental samples. The results of Figure S3 show that the photoreduction kinetics of acidic and alkaline hydrolyzates are different as compared to those obtained from the parental compounds in distilled water but in all compounds the irradiation time increases with increasing pesticide concentration. This finding suggests that the method is responsive to both the parental compounds and their hydrolysis by-products; therefore, it can be used to obtain an estimate of the total concentration of sulfur containing pesticides in real samples.

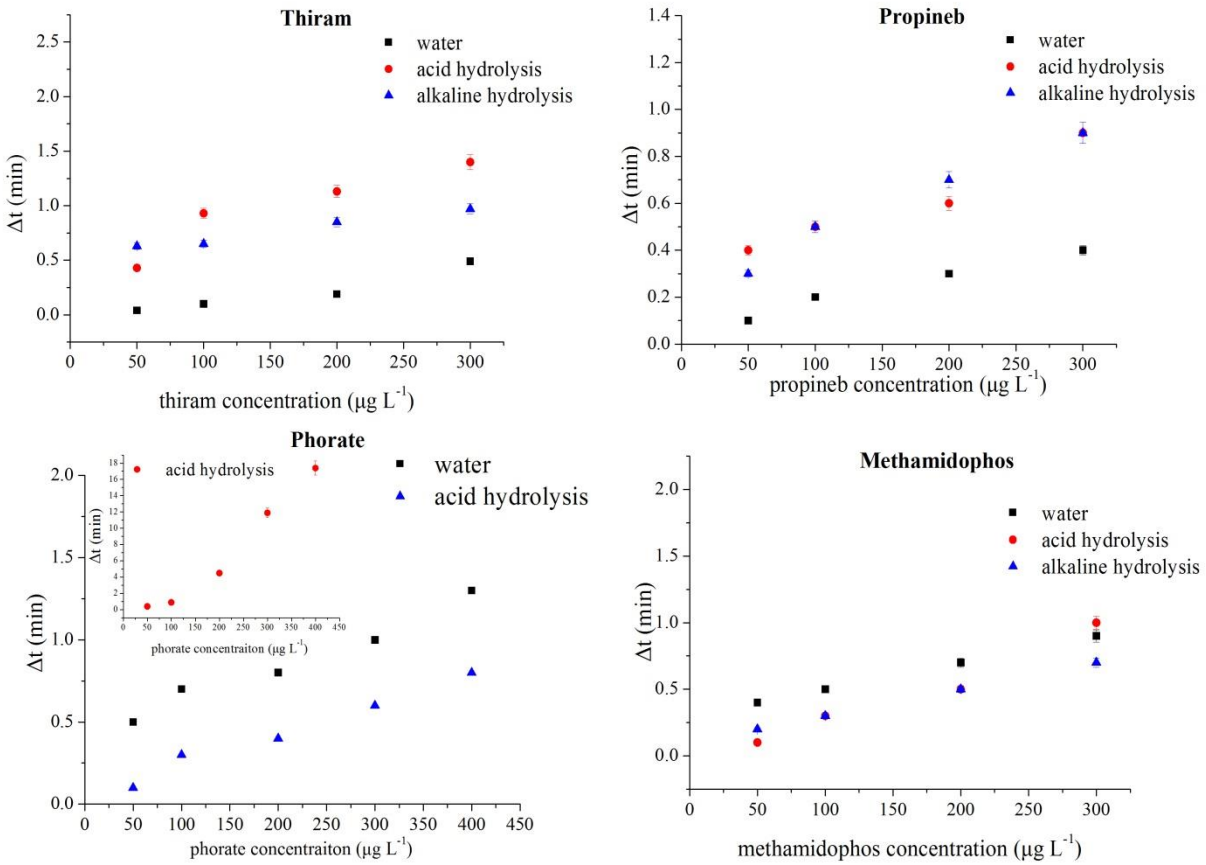


Figure S5. Dose-response curves obtained from the photoreduction of gold ions to AuNPs in the presence of dithiocarbamate and organophosphorus pesticides and their (acidic and alkaline) hydrolysis by-products.

On the basis of these observations, the determination of sulfur-containing pesticides was performed as follows: An aliquot of 0.8 mL of a methanolic extract solution containing the target pesticides or blank sample (methanol) was transferred into a 10 mL beaker and diluted with 0.9 mL of distilled water. Then, 0.1 mL of citric acid-sodium citrate buffer pH 3.5 (0.1 M) and 0.2 mL of $AuCl_4^-$ solution (10 mM) were added and mixed manually. Photoreduction was performed under UV light irradiation at 254 nm (40W) and the formation of AuNPs is inspected by the unaided eye. The time delay between the formation of a red-purple coloration, in the blank and the sample solutions was used as the analytical signal

Determination of sulfide

The influence of inorganic sulfur species in the photoreduction kinetics of Au ions to AuNPs was examined using standard solutions of Na_2S. Under the working conditions (pH=3.5) all sulfide should be present as hydrogen sulfide (H_2S). Unexpectedly, we found that sulfide accelerated the photoreduction kinetics in distilled water but slowed them down in real water samples. To investigate this phenomenon we spiked a series of bottled water samples with sulfide and studied the influence of water composition in the photoreduction kinetics. The results of this study, however, were not conclusive so we decided to adopt the method of standard additions as a strategy for the determination of sulfide in real water samples. Moreover, motivated by our previous findings in artificial body fluids, we irradiated the solutions with artificial (visible) light, instead of UV irradiation, in order to decrease photoreduction kinetics and facilitate the discrimination of color development between the blank and the sample solutions. Indeed, under visible light the time difference was clearly distinguishable offering an improvement to both precision and accuracy; specifically, the lowest detectable concentration decreased from 75 μM in distilled water and UV light irradiation (254 nm, 40 W, RSD=11.2%, n=5) to 15 μM in real water samples (under 40W of visible light irradiation, RSD=7%, n=5). This concentration (i.e. 15 μM sulfide) equals the maximum recommended sulfide concentration in drinking water by the World Health Organization and is much lower than the lowest odor nuisance concentration of sulfide in wastewater (i.e. 88 μM).

In summary, the experimental procedure for the determination of sulfide was as follows: aliquots of 1.7 mL of water sample were placed in a 10 mL beakers and fortified with 0.1 mL of citric acid-sodium citrate buffer pH 3.5 (0.1 M) and 0.2 mL of $AuCl_4^-$ solution (10 mM). Photoreduction was performed under visible light irradiation (40W) and the formation of AuNPs is inspected by the unaided eye. The time delay between the

formation of a red-purple coloration, between the sample solutions was used as the analytical signal to prepare a standard addition calibration curve.

References for Supporting Information

(S1) Ellison, S.L.R.; Thompson, M. Standard additions: myth and reality. *Analyst* **2008**, *133*, 992–997.

(S2) Lu, C.; Bucher, G.; Sander, W. Steady-state and time-resolved studies on photoinduced disulfide bond cleavage using aniline as an electron donor. *ChemPhysChem* **2004**, *5*, 399 – 402.

(S3) Dong, S.; Tang, C.; Zhou, H.; Zhao, H. Photochemical synthesis of gold nanoparticles by the sunlight radiation using a seeding approach. *Gold Bull*. **2004**, *37*, 187-195.