

# Analytical Methods

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4 **A fast and simple method to perform cyanide detection using ATP stabilized**  
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6 **gold nanoparticles combined with the Cu(DDTC)<sub>2</sub> complex**  
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9 *Alba González Castro, Elena Peña-Vázquez and Pilar Bermejo-Barrera\**  
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14 A method has been developed for the determination of cyanide using gold nanoparticles  
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16 stabilized with adenosine triphosphate (ATP), and in the presence of the Cu(DDTC)<sub>2</sub> complex.  
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18 Cyanide reacts with copper, and free diethyldithiocarbamate (DDTC) causes nanoparticle  
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20 aggregation. Reaction was performed in a buffered media (10 mM phosphate + 0.1 MNaCl) by  
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22 mixing stabilized gold nanoparticles ( $1.27 \times 10^{-9}$  M;  $19.2 \pm 4.0$  nm), the Cu(DDTC)<sub>2</sub> complex  
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24 and cyanide solutions. The influence of the measurement time after the addition of cyanide, the  
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26 pH of the reaction media and the concentration of the complex were evaluated. The selected  
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28 conditions were: time=1 min, [Cu(DDTC)<sub>2</sub>] = 0.1  $\mu$ M and pH =10.4. The analytical performance  
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30 of the assay was studied, with a LOD (0.059 mg L<sup>-1</sup>) which is under the level proposed by the  
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32 USEPA in drinking water (0.2 mg L<sup>-1</sup>).  
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## 1 Introduction

In recent years, the development of gold nanoparticles (AuNPs) based colorimetric and fluorescent assays has increased sharply. The applications include the analysis of cations (e.g. Hg(II), Cu(II), As(III), Ca(II), Al(III)), anions (nitrite, cyanide, fluoride, iodide,  $\text{PF}_6^-$ , oxoanions) and small organic molecules (such as cysteine, homocysteine, trinitrotoluene, melamine, cocaine, ATP, glucose, dopamine, etc).<sup>1</sup> The development of fast and simple methods for cyanide analysis<sup>2,3</sup> in environmental waters and wastewaters is appealing because of the high use of the compound in the industry and its high toxicity. Standard methods for the determination of cyanide include a distillation step to liberate hydrogen cyanide (HCN) and collect it in a NaOH solution. Afterwards, the alkaline distillate is analyzed using a titration with silver using p-dimethylaminobenzalrhodanine as indicator, a colorimetric method with chloramine-T in the presence of a pyridine-barbituric acid reagent, or a cyanide-selective electrode.<sup>4</sup> The detection level using the gravimetric titration with p-dimethylaminobenzalrhodanine is  $1 \text{ mg L}^{-1}$ . For concentrations lower than  $1 \text{ mg L}^{-1}$ , the colorimetric (approximate detection level:  $0.02 \text{ mg L}^{-1}$ ;  $0.003 \text{ mg L}^{-1}$  for the automated method<sup>5</sup>) or the potentiometric method (applicable concentration range:  $0.05\text{-}10 \text{ mg L}^{-1}$ ) should be used. Cyanide can also be determined in drinking waters by gas chromatography-mass spectrometry headspace analysis after the release of HCN under acidic conditions.<sup>6</sup> There are a broad range of detection techniques available, but new colorimetric and fluorometric methods seem suitable for fast and in-situ analyzers.<sup>2</sup>

Cyanide has been used in several studies to evaluate the stability of the surface of gold nanoparticles protected with different coatings.<sup>7-10</sup> The simplest detection methods are based on the reaction of cyanide with gold nanoparticles inducing their dissolution. The absorption peak of AuNPs disappears after the addition of potassium ferricyanide, and  $[(\text{Au}(\text{CN})_2)]^-$  is formed.<sup>11</sup>

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3 Thus, Pal and Bandyopadhyay<sup>12</sup> observed how the pink solution of AuNPs stabilized with Triton  
4 X-100 became colorless in the presence of cyanide. Polysorbate 40-stabilized gold  
5 nanoparticles<sup>13,14</sup> were used in colorimetric assays of hydrogen cyanide in cyanogenic glycoside-  
6 containing plants. When the plant tissue is damaged in a high salinity medium,  $\beta$ -glucosidase  
7 hydrolyzes the cyanogenic glycosides and the hydrogen cyanide formed dissolves the particles.  
8 Finally, Radhakumary and Sreenivasan<sup>15</sup> generated chitosan-AuNPs films suitable for  
9 dipchecking and on-site monitoring. The color change was observable by the naked eye above a  
10 concentration of 2 mg L<sup>-1</sup> of cyanide.  
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Fluorescence is an interesting and versatile possibility for the determination of the analyte using AuNPs. Shang and Dong<sup>16</sup> designed a fluorescent assay based on the inner filter effect (IFE) of metal nanoparticles that function as absorbers to modulate the emission of a fluorophore. This turn-on assay is based on the etching of AuNPs by cyanide in the presence of MDMO-PPV (poly[2-methoxy-5-(3,7-dimethyloctyloxy)-1,4-phenylenevinylene]). These authors<sup>17</sup> also developed a turn-on fluorescent assay based on the dissolution of AuNPs functionalized with the fluorophore Rhodamine B. Liu et al.<sup>18</sup> reported the use of an Au-nanocluster-based fluorescence sensor based on the cyanide etching induced fluorescence quenching of the Au nanoclusters. They analyzed several spiked water samples and achieved a good selectivity and a  $200 \times 10^{-9}$  M limit of detection. Lou et al.<sup>19</sup> reported a fluorescence method based on observing the dissolution of polymer coated AuNPs and analyzed several real water samples spiked with cyanide. Polyacetylene-functionalized AuNPs<sup>20</sup> also acted as a turn-on fluorescent detection system to quantify cyanide in presence of other anions, and in spiked water samples. Cyanide and iodide were analyzed selectively in high salinity solutions and edible salt samples using bovine serum albumin labeled with fluorescein isothiocyanate (FITC)-

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3 capped AuNPs.<sup>21</sup> In this case, when the analytes are deposited or etched the AuNPs, the probes  
4 are released and the fluorescence is restored. Finally, an assay based on IFE includes bifunctional  
5 Au-Fe<sub>3</sub>O<sub>4</sub> nanoparticles, with a “magnetic concentration-washing process” to reduce the  
6 interferences in the analysis.<sup>22</sup>  
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12 Surface enhanced Raman spectroscopy (SERS) has also been used to analyze cyanide  
13 using sensitive substrates containing gold nanoparticles.<sup>23,24</sup> Limits of detection at the 110 parts-  
14 per-trillion level<sup>25</sup> were achieved, valid for the analysis of environmental samples and with a  
15 good discrimination against other anions and cations. A new class of material has been  
16 developed for SERS consisting of AuNPs coated onto hollow buoyant silica microspheres<sup>26</sup>. The  
17 assays using this material are called “lab on a bubble” (LoB) and avoid the problem of particle  
18 aggregation in SERS.  
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29 Lou et al.<sup>27</sup> have reviewed the bibliography in the field of the fluorescent and  
30 colorimetric anion chemosensors using the displacement strategy (mainly from 2008 to 2011).  
31 Some methods for the detection of cyanide are based on the affinity of this anion for copper<sup>32,28-30</sup>  
32 to form [Cu(CN)<sub>x</sub>]<sup>n-</sup> species. Kim et al.<sup>31</sup> mixed ATP-stabilized AuNPs with Cu(II)-  
33 phenanthroline complexes. The phenanthroline became free when exposed to cyanide anions and  
34 caused the aggregation of the nanoparticles with a visible change in the color of the solutions.  
35 ATP stabilized particles are interesting for the detection of anions<sup>32</sup>, but methods have also been  
36 developed for the analysis of cations such as mercury<sup>33</sup> or for enzyme sensing assays.<sup>34</sup>  
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48 The objective of this study is the development of an assay for cyanide using gold  
49 nanoparticles stabilized with adenosine triphosphate (ATP), and in the presence of the  
50 Cu(DDTC)<sub>2</sub> complex. Some of the conditions for this assay were optimized and the analytical  
51 performance of the method (linearity, matrix effect in drinking water samples, sensitivity,  
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interferences) was evaluated.

## 2 Experimental

### 2.1 Chemicals

The following reagents were used in this study: sodium cyanide (Panreac Química SA, Barcelona, Spain), auric chloride ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ ) (Sigma-Aldrich, St. Louis, USA), adenosine triphosphate (ATP) (Sigma), sodium diethyl dithiocarbamate (DDTC) (Merck, Darmstadt, Germany) and copper chloride (Panreac). Sodium dihydrogen phosphate and sodium hydroxide (Merck) were used to prepare the buffer reaction media; silver nitrate (Probus, Barcelona, Spain), sodium chloride (Panreac) and p-dimethylaminobenzalrhodanine (Sigma) were used for the standardization of the stock solutions of cyanide (approximately  $1000 \text{ mg L}^{-1}$ ). This standardization was carried out on a regular basis using the 4500-CN<sup>-</sup> D Method of the AWWA-WEF-APHA4. Silver nitrate was used to produce the soluble cyanide complex,  $\text{Ag}(\text{CN})_2^-$ ; the excess of silver was detected by the indicator p-dimethylaminobenzalrhodanine.

All chemicals were of analytical grade and used without further purification. Ultrapure water (specific resistance of  $18 \text{ M}\Omega\text{cm}$ ) was obtained from a Milli-Q purification system (Millipore Corporation, Massachusetts, USA). All the glassware and material were cleaned using ultrapure water, soaked in nitric acid for 48 hours and rinsed again with ultrapure water at least three times. Afterwards, material was stored dry and covered.

### 2.2 Instrumentation

The UV-Vis spectrophotometer model U-2010 (Hitachi, Schaumburg, USA) was used for the measurements of absorbance and characterization of AuNPs. A PHILIPS CM-12 Transmission Electronic Microscope (Amstelplein, Germany) was used in this work for characterization. The

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3 microscope was equipped with a Mega View-II Docu camera to collect images at magnifications  
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5 of 730,000x, with a resolution of 0.2 nm.  
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### 8 **2.3 Synthesis of ATP stabilized AuNPs(sAuNPs)**

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10 Citric acid-stabilized nanoparticles were prepared by adding 10 mL of a 38.8 mM sodium citrate  
11 solution to 100 mL of boiling 1.0mM HAuCl<sub>4</sub>.3H<sub>2</sub>O with vigorous stirring.<sup>33</sup> The solution was  
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13 boiled and stirred for 15 minutes after the appearance of a deep red color, and afterwards it was  
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15 allowed to cool to room temperature with continuous stirring.  
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19 The sAuNPs were freshly prepared just before the performance of each set of  
20 experiments. In a similar procedure to that developed by Kim et al.<sup>31</sup>, a volume of 60 μL of a  
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22 1mM ATP solution was added to 4 mL of the synthesized AuNPs in a 10 mL volumetric flask.  
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24 After 5 minutes, the solution was diluted to the mark with 10 mM phosphate buffer (pH 7.0, 0.1  
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26 M NaCl) and subsequently used.  
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### 31 **2.4 Detection of cyanide using ATP stabilized AuNPs (sAuNPs)**

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33 The method is based on the use of sAuNPs in the presence of the Cu(DDTC)<sub>2</sub> complex. Cyanide  
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35 binds copper, and aggregates of sAuNPs are formed after the release of ATP.  
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38 Just after the preparation of the sAuNPs, the following amounts of sample (Milli-Q water or  
39 mineral water) and reagents were mixed to prepare the standards: 0.750 mL of sAuNPs + 15 μL  
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41 of 10 μM Cu(DDTC)<sub>2</sub> + X μL of 50mM buffer solution (pH 10.4) + 0.600mL of sample + Y  
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43 μL solution of 10-100 mg L<sup>-1</sup> cyanide standard (final volume = 1.5 mL).  
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### 3 Results and discussion

#### 3.1 Characterization of ATP stabilized AuNPs (sAuNPs)

The size of the AuNPs used during the optimization of the method was estimated using TEM  $19.2 \pm 4.0$  nm ( $n = 392$ ), and UV spectroscopy (17.9 nm).<sup>35</sup> The concentration of AuNPs in the solution ( $3.18 \times 10^{-9}$  M) was calculated using TEM data and the method proposed by Haiss et al.<sup>35</sup>, considering that the molar absorptivity ( $\epsilon$ ) of those nanoparticles is  $4.76 \times 10^8$  at 450 nm. Two batches of nanoparticles were used in the experiments with size in the 15-17.9 nm range (VIS spectroscopy data). The estimated concentration of the ATP stabilized nanoparticles was  $1.27 \times 10^{-9}$  M.

The aggregation of the sAuNPs in presence of cyanide was also observed by TEM (Fig. 1). The images show the sAuNPs ( $1.27 \times 10^{-9}$  M) + Cu(DDTC)<sub>2</sub> (0.1  $\mu$ M) at pH 10.4 before and after the addition of cyanide (final concentration 1 mg L<sup>-1</sup>).

#### 3.2 Optimization of operating conditions

Dithiocarbamate (DTC) reagents have been widely used for chelation and extraction of trace metals; the most common compound, NaDDTC (sodium diethyldithiocarbamate), was used for the determination of Cu and, less often, of Bi, Ni and other metals.<sup>36,37</sup> The solubility of sodium diethyldithiocarbamate in water is approximately 35.1 g L<sup>-1</sup>, and the compound is not stable in acidic solutions (decomposition into carbon disulfide and diethyl amine). However, the dithiocarbamate complexes are generally not soluble in water but can be dissolved in chloroform or methyl isobutyl ketone (MIBK). Thus, the solubility of the Cu(II) complex in water is  $3.2 \times 10^{-10}$  and the pK<sub>s</sub> is 30.2 (in 0.2 M NaCl at 25°C).<sup>36</sup> Some metal thiocarbamate complexes (M(DTC)<sub>n</sub>) form, in chloride solutions, complexes like M(DTC)<sub>n-m</sub>Cl<sub>m</sub>.<sup>37</sup> The overall (cumulative) formation constants,  $\beta_n$ , is the equilibrium constant for the reaction  $M + nL \leftrightarrow ML_n$ .



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3 The overall formation constant or stability constant for  $\text{Cu}(\text{DDTC})_2$  is:  $\log \beta_2 = 12.1$ , data that is  
4 an average from several measurements given in various studies.<sup>38</sup> In comparison, the constants  
5 for the  $\text{Cu}(\text{II})$ -cyanide complexes are higher:  $\log \beta_2 = 24$ ,  $\log \beta_3 = 26.6$  and  $\log \beta_4 = 30.3$ .<sup>39</sup>  
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7 Therefore, the DDTC from  $\text{Cu}(\text{DDTC})_2$  becomes free in the presence of cyanide ions, and the  
8 aggregation of the gold nanoparticles occurs. The formation constants for the complex  $\text{Cu}(\text{o-phenanthroline})$   
9 used by Kim et al.<sup>31</sup> in a previous study are:  $\log \beta_1 = 8.82$ ,  $\log \beta_2 = 15.39$  and  $\log$   
10  $\beta_3 = 20.41$  (calculated at 20°C and ionic strength: 0.1M)<sup>39</sup>, values that are also higher than those  
11 obtained for  $\text{Cu}(\text{DDTC})_2$ .  
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23 In this study, several variables influencing cyanide detection were investigated in the  
24 search for the optimum conditions to carry out the analysis: measurement time after the addition  
25 of cyanide, the concentration of the complex  $[\text{Cu}(\text{DDTC})_2]$ , and the pH of the reaction medium.  
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### 31 **3.2.1 Influence of measurement time after the addition of cyanide**

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34 The measurement time after the addition of cyanide to solutions containing the sAuNPs  
35 influences the recorded signal. The absorbances corresponding to a solution of a blank and a 1  
36 mg L<sup>-1</sup> standard solution of cyanide were measured at 1 min intervals. These solutions contained  
37 750  $\mu\text{L}$  of  $1.27 \times 10^{-9}$  M sAuNPs and 15  $\mu\text{L}$  of 10  $\mu\text{M}$   $\text{Cu}(\text{DDTC})_2$  (dilution to a final volume of  
38 1.5 mL with phosphate buffer). The signals for the blank, the standard and the differences  
39 between them are shown in Fig. 2A (measurements at 522 nm) and Fig. 2B (715 nm). In the  
40 following experiments, the calibration curves didn't show a good linearity at a measurement time  
41 of 6 min, which is the reason why measurements were finally performed 1 min after the mixture  
42 of the reagents. This was a shorter time than that used in the experiments by Kim et al.<sup>33</sup> (3 min).  
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60 This variation could be due to the difference between the stability of  $\text{Cu}(\text{DDTC})_2$  ( $\log \beta_2 =$

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3 12.1<sup>38</sup>) and the stability of the Cu(II)-phenanthroline complex ( $\log \beta_3 = 20.41^{39}$ ). Moreover, o-  
4 phenanthroline reacts slowly with some metals and also tends to dissociate slowly, especially in  
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11 Ni, V(IV) or Fe(II).<sup>36</sup> The measurement time after the addition of cyanide in the present study (1  
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13 min) is also shorter than in those methods based on etching AuNPs. In this case, the stabilization  
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15 times ranged from  $10^{12}$ - $20^{16,18}$  min to one hour<sup>21</sup>.  
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### 18 **3.2.2 Influence of [Cu(DDTC)<sub>2</sub>]**

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21 An experiment was performed to test the influence of the concentration of Cu(DDTC)<sub>2</sub> on the  
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23 analysis of cyanide. Solutions with increasing concentrations ( $10^{-4}$ ,  $10^{-3}$ , 0.01 and 0.1  $\mu$ M) of  
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25 [Cu(DDTC)<sub>2</sub>] were prepared and the absorbance was measured at 1 and 6 min after the mixing  
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27 of the reagents (750  $\mu$ L of  $1.27 \times 10^{-9}$  M sAuNPs + buffer + cyanide with a final concentration 1  
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29 mg L<sup>-1</sup> CN<sup>-</sup>). The biggest change in absorbance was observed for a 0.1  $\mu$ M solution of  
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31 [Cu(DDTC)<sub>2</sub>] (Fig. 3) The increase in the concentration of the complex [Cu(DDTC)<sub>2</sub>] is limited  
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33 by its low solubility.  
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### 39 **3.2.3 Influence of pH**

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42 Finally, the effect of the pH reaction medium was studied. Standards of 1 mg L<sup>-1</sup> of cyanide were  
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44 prepared at different pHs (7.0, 8.0, 9.0, 10.4) in a 10 mM phosphate/NaCl 0.1M buffer, with a  
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46 0.1 M concentration of [Cu(DDTC)<sub>2</sub>] and measuring at a time of 1 and 6 min. The measurements  
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48 at 715 nm were less affected by the pH than at 522 nm. Hydrogen cyanide is a weak acid with a  
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50 pKa of 9.2. Thus, HCN is the predominant form in neutral pH water samples. A careful  
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52 manipulation of cyanide-containing samples is required because of its toxicity; this is the reason  
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3 why a pH of 10.4 was selected for performing the measurements (Fig. 4) in the following  
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5 experiments.  
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### 8 9 **3.3 Analytical performance of the assay using sAuNPs**

#### 10 11 **3.3.1 Calibrations curves**

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13 Standards with concentrations of 0, 0.25, 0.5, 1.0, 2.5, 5.0, 7.5 mg L<sup>-1</sup> of cyanide were prepared  
14 and analyzed (Fig. 5). These standards included 750 μL of 1.27×10<sup>-9</sup>M, sAuNPs, and 0.1M  
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16 Cu(DDTC)<sub>2</sub> (diluting with buffer to a final volume of 1.5 mL). Similarly, standards were  
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18 prepared as reported in section 2.4, including 0.6 mL of a sample of mineral water.  
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22 A decrease in the signal was observed at 522 nm at low concentrations (equation of the  
23 calibration graph:  $A_{522} = -0.1849[\text{CN}^-] + 0.6304$ , with a regression coefficient  $r = 0.990$ ;  
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25 equation of the standard addition calibration graph:  $A_{522} = -0.1047[\text{CN}^-] + 0.9769$   $r = 0.995$ ). In  
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27 the case of the measurements at 715 nm, an increase in the signal was observed (calibration  
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29 graph:  $A_{715} = 0.1702 [\text{CN}^-] + 0.0374$ ,  $r = 0.997$ ); standard addition graph:  $A_{715} = 0.1573[\text{CN}^-] +$   
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31  $0.0867$ ,  $r = 0.996$ ). This increase in the signal was reflected in a gradual change in the color of  
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33 the standards (Fig. 6). The slopes of the calibration and the standard addition graphs were  
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35 statistically different (t-test, 95% confidence level); therefore, a matrix effect was present in the  
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37 analysis of cyanide in mineral water samples using sAuNPs. The variation of absorbance with  
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39 the added concentration of cyanide was observed, showing the response of the sAuNPs to free  
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41 cyanide. However, there was no natural water sample containing detectable cyanide in this study.  
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44 The absorption spectra of seven mineral water samples commercialized in Galicia (Spain) were  
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46 recorded and no traces of cyanide were found in them.  
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3 A control experiment was also performed: cyanide was added to a solution of sAuNPs  
4 under the same optimized conditions; a slightly change to a paler color was observed at 15 mg  
5 L<sup>-1</sup> of cyanide, probably due to the complexation with the gold nanoparticles.  
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### 10 11 **3.3.2 Limit of detection and quantification.** 12

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14 The limit of detection (LOD) and the limit of quantification (LOQ) are defined as 3SD/*m* and  
15 10SD/*m*, respectively. *SD* is the standard deviation of the blank (n=11), and *m* is the slope of the  
16 calibration graph. These criteria were used for the calculation of the limit of detection at 522 nm  
17 (LOD = 0.059 mg L<sup>-1</sup> and LOQ = 0.17 mg L<sup>-1</sup>). The response at low concentrations at 715 nm is a  
18 sigmoidal curve; therefore, a different criterion<sup>31</sup> was used for the calculation of the detection  
19 limit that was estimated after the extrapolation of the standard addition calibration graph to  
20 absorbance zero (LOD: 1.38 mg L<sup>-1</sup>). These values were calculated taking into account the  
21 dilution factor of the mineral water used in the standard additions (0.6 mL of water was diluted  
22 to a final volume of 1.5 mL).  
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37 The methods of analysis based on AuNPs and fluorescence present LODs ranging from  
38 0.002<sup>17</sup> to 0.026<sup>21</sup> mg L<sup>-1</sup>. The colorimetric assay based on the etching of polysorbate 40-  
39 stabilized AuNPs was capable of detecting 0.013 mg L<sup>-1</sup> after an incubation time of 30 min<sup>13</sup>.  
40 However, the LOD of the colorimetric method based on etching of Triton X-100 stabilized  
41 AuNPs<sup>12</sup> is 0.5 mg L<sup>-1</sup>. The limit of detection in the present study at 522 nm (0.059 mg L<sup>-1</sup>,  
42 taking into account sample dilution) is lower than those obtained using the gravimetric method  
43 (detection level 1 mg L<sup>-1</sup>)<sup>4</sup>, the indirect method using the complex of copper and zincon (0.13  
44 mg L<sup>-1</sup>), and the method developed by Kim et al.<sup>33</sup> with the phenanthroline-Cu complex and  
45 performing the measurements at 715 nm (0.36 mg L<sup>-1</sup>). The LOD is similar to the minimum  
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3 applicable concentration of the ion selective electrode (approximately  $0.05 \text{ mg L}^{-1}$ ).<sup>4</sup> The method  
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5 allows for the detection of the compound at the levels set for cyanide by the EPA in drinking  
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7 water ( $0.2 \text{ mg L}^{-1}$ )<sup>40</sup>, but it is still not enough to achieve the guideline level proposed by the  
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9 European Union ( $0.05 \text{ mg L}^{-1}$  in drinking water;  $0.07 \text{ mg L}^{-1}$  in mineral water).<sup>41,42</sup>

### 12 13 **3.3.3 Precision**

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15 Precision can be defined as the relative standard deviation (RSD) of a number of repeated  
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17 measurements ( $n=11$ ). The RSD was calculated for a standard containing  $1 \text{ mg L}^{-1}$  of cyanide. A  
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19 value of 2.3 % was obtained for the RSD(%) when the measurements were performed at 715 nm,  
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21 while a lower value of 0.3% was obtained at 522 nm . Those values are of the same order than  
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23 those observed in the literature, e.g. 3.7%<sup>16</sup>, 4.2%<sup>17</sup> or 1.4-3.4%<sup>20</sup>.

### 24 25 26 27 28 29 **3.3.4 Study of interferences**

30  
31 A study of the possible interferences in the analysis was carried out taking into account the  
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33 elements and anions that can be present in a sample of drinking water. The solution was spiked  
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35 with the interferent, and fluorescence was measured in the optimized conditions.  
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#### 38 39 40 **3.3.4.1 Anionic interferences**

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42 Standards were prepared containing sAuNPs and  $\text{Cu(DDTC)}_2$ , and measurements were  
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44 performed in the optimized conditions, adding to each standard a different interferent at a  
45  
46 concentration of  $10 \text{ mg L}^{-1}$ . Several anions were included in the study ( $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{HCO}_3^-$ ,  
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48  $\text{AcO}^-$  and  $\text{NO}_3^-$ ) to evaluate their effect on the absorbance of the sAuNPs. The anions  $\text{SO}_4^{2-}$  and  
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50  $\text{PO}_4^{3-}$  were the main interferents at that concentration, and a change in the color was observed.  
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56 Fig.7 A shows the color of the solutions, with a blue color in the solution of cyanide ( $10 \text{ mg L}^{-1}$ );  
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4 all the interferents are present at a concentration of 10 mg L<sup>-1</sup> except for SO<sub>4</sub><sup>2-</sup> and PO<sub>4</sub><sup>3-</sup>  
5  
6 (concentrations of 3 mg L<sup>-1</sup>). The interference of S<sup>2-</sup> was also evaluated, and a change in the  
7  
8 color was observed at 3 mg L<sup>-1</sup>.  
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11 In the bibliography, Kim et al.<sup>31</sup> and Lou et al.<sup>19,20</sup> reported a good selectivity for cyanide  
12  
13 above other anions but they didn't evaluate the effect of S<sup>2-</sup>. Shang et al.<sup>17</sup> had observed the  
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15 interference of S<sup>2-</sup>, I<sup>-</sup> and SCN<sup>-</sup> on the turn-on fluorescent detection of cyanide, and a good  
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17 selectivity for cyanide over other anions. However, the use of a fluorescent assay based on the  
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19 inner filter effect<sup>16</sup> and the method of Liu et al.<sup>18</sup> were very selective. Wei et al.<sup>21</sup> also observed  
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21 the restoration of the fluorescence of a gold nanoparticle-based fluorescent probe only in the  
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23 presence of S<sup>2-</sup>, I<sup>-</sup> and CN<sup>-</sup>. They proposed the use of hydrogen peroxide to develop a selective  
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25 sensor for I<sup>-</sup>, and the use of Pb<sup>2+</sup> to mask S<sup>2-</sup> and Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub> to mask I<sup>-</sup> in the determination of  
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27 cyanide.  
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### 3.3.4.2 Cationic interferences

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37 The cations also could affect the signals of absorbance in the assay. The absorbance was  
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39 measured after the addition of cyanide (10 mg L<sup>-1</sup>) or the interferent element at the maximum  
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41 concentration allowed by the EPA in drinking water (Ba: 2 mg L<sup>-1</sup>, Cr: 0.1 mg L<sup>-1</sup>, Pb: 0.15 mg  
42  
43 L<sup>-1</sup>, Cu: 1.3 mg L<sup>-1</sup>, Fe: 0.3 mg L<sup>-1</sup>, Mn: 0.05 mg L<sup>-1</sup>, Zn: 5 mg L<sup>-1</sup>, Ni: 0.02 mg L<sup>-1</sup>). In this case,  
44  
45 the color of the solutions were not affected by Ba, Cr, Pb, Cu, Fe, Mn, Zn and Ni at the levels  
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47 established by EPA legislation for drinking water (Fig. 7 B).  
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52 Metallic cations didn't affect the determination of cyanide using AuNPs stabilized with  
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54 Triton X-100<sup>12</sup>. Shang et al.<sup>16</sup> observed interferences due to metallic cations at concentrations  
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56 that are not usually present in real samples, and Liu et al.<sup>18</sup> proposed the addition of chelating  
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3 ligands such as 2,6-pyridinedicarboxylic acid, bovine serum albumine or glutathione to decrease  
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5 the interference of transition metals.  
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#### 10 **4. Conclusions**

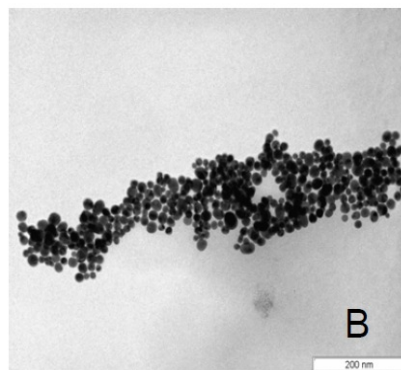
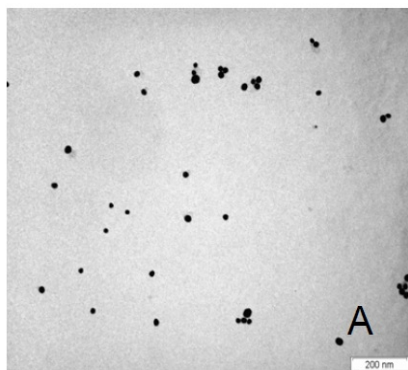
11  
12 The proposed method, using the  $\text{Cu}(\text{DDTC})_2$  complex to produce the aggregation of the sAuNPS  
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14 in the presence of cyanide present some advantages over other complexes used previously, such  
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16 as the Cu-phenanthroline complex. The new method is faster due to the bigger stability of the  
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18  $\text{Cu}(\text{DDTC})_2$  complex, and presents a better LOD, that it is enough to allow cyanide detection at  
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20 the levels proposed by the EPA in drinking waters.  
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#### 29 **Acknowledgments**

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32 This work has received the support of the Regional Government of Galicia, Spain (project  
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34 10PXIB2090032PR).  
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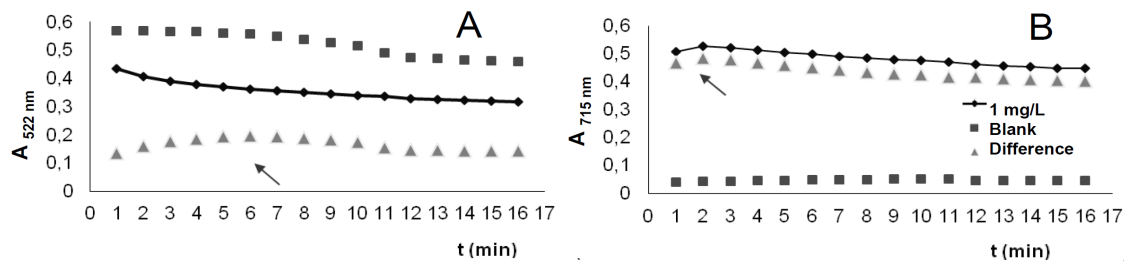
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3 **Fig.1** TEM images. A: Gold nanoparticles stabilized with ATP in presence of  $\text{Cu}(\text{DDTC})_2$

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6 B:Aggregation of the nanoparticles after the addition of cyanide

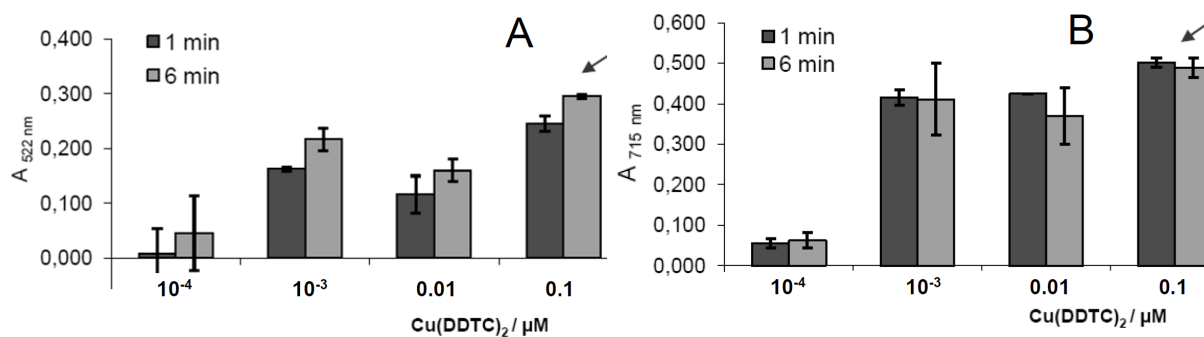




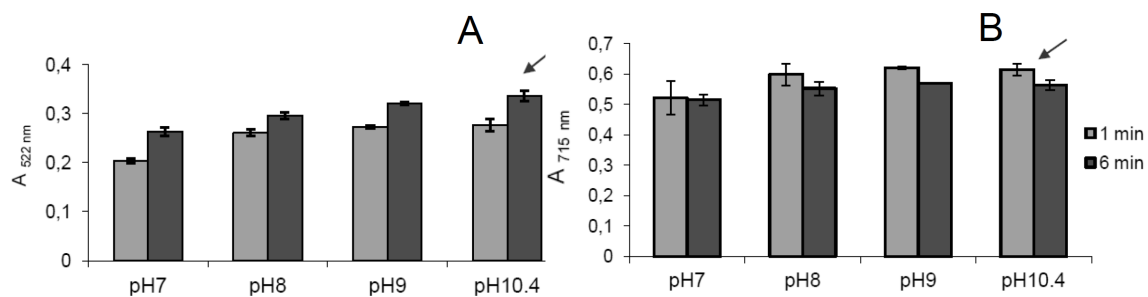
**Fig.2** Influence of measurement time after the addition of cyanide on the absorbance of ATP s-AuNPs. A: Measurements at 522 nm B: Measurements at 715 nm



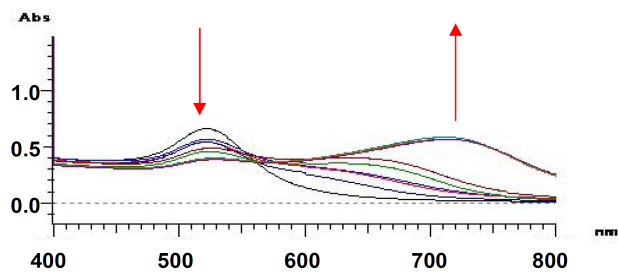
**Fig.3** Influence of  $\text{Cu}(\text{DDTC})_2$  concentration on the absorbance of ATP s-AuNPs. A: Measurements at 522 nm B: Measurements at 715 nm



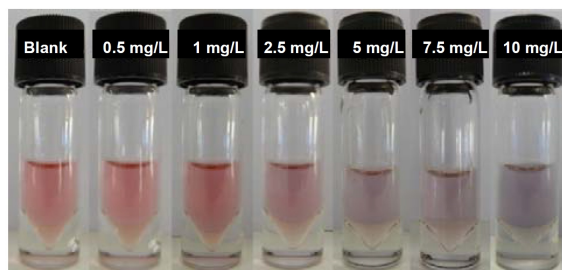
**Fig.4** Influence of pH on the absorbance of ATP s-AuNPs. A: Measurements at 522 nm B: Measurements at 715 nm



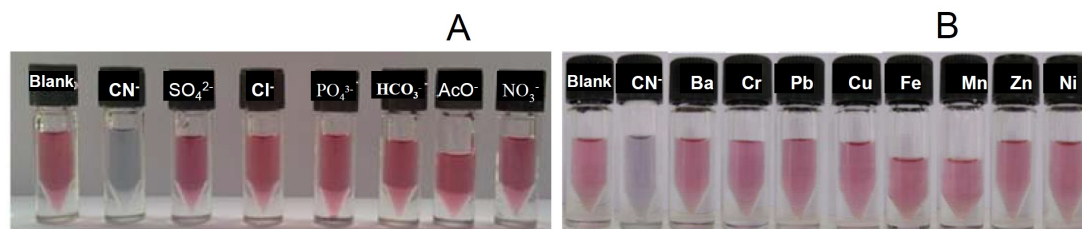
**Fig.5** Absorption spectra of stabilized AuNPs in the presence of 0.1M Cu(DDTC)<sub>2</sub> and increasing concentrations of cyanide (0, 0.25, 0.5, 1.0, 2.5, 5.0, 7.5 mg L<sup>-1</sup>)



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4 **Fig.6** Change of color to blue with increasing concentrations of cyanide  
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3 **Fig.7** Study of interferences using colorimetry. A: Anionic interferences ( $\text{SO}_4^{2-}$  and  $\text{PO}_4^{3-}$  at 3 mg  
4  $\text{L}^{-1}$  ;  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ ,  $\text{AcO}^-$  and  $\text{NO}_3^-$  at 10 mg  $\text{L}^{-1}$ ) B: Cationic interferences (cations at the maximum  
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6 concentrations allowed by the EPA in drinking water)  
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