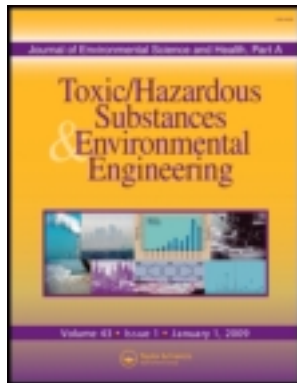


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### Digital analysis technique for uncertainty reduction in colorimetric arsenic detection method

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# Digital analysis technique for uncertainty reduction in colorimetric arsenic detection method

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This article proposes an alternative to increase the reliability and reproducibility of a colorimetric method to measure arsenic (As) concentrations. The method of analysis developed incorporates a digital analysis technique to eliminate the operator dependence of results, and As concentrations are quantitatively determined from digital levels computed from photographs of the colorimetric reaction that emerges during the test. This technique allows the sensitivity of the detection to be increased at low concentration ranges, which is of fundamental importance for the detection of As given the current acceptable limit for drinking water. The results obtained show a very good correlation between As concentrations determined by means of analytical laboratory techniques and the method proposed in this research.

**Keywords:** Arsenic, test kit, drinking water, digital level, detection.

## Introduction

Arsenic is a naturally occurring element found in many aquifers around the world, in concentrations that may affect human health if groundwater is used for drinking. The Environmental Protection Agency (EPA) and the World Health Organization (WHO) determined that the allowable As concentration for drinking water is 0.01 mg/L. Shallow and deep groundwater in an extended area in the center and east of Argentina contain arsenic in concentrations higher than those recommended by these current international regulations.<sup>[1–4]</sup> Similar situations are found in Vietnam, Bangladesh, Nepal, India and West Bengal, but in these cases the potential numbers of the affected populations are of fundamental importance.<sup>[5–6]</sup> Millions in these countries use groundwater for drinking, even when, in many cases, the As concentration is unacceptable, producing health risks such as cancer and skin alterations.<sup>[7–10]</sup>

There are many techniques for the detection of arsenic in water, from field test kits to analytical laboratory methods, including: colorimetry, gravimetry, x-ray fluorescence, atomic absorption spectrophotometry, among others. Each of these techniques has advantages and disadvantages in their precision, cost and sensitivity of measurement. As test kits follow the colorimetric methodology, are either

qualitative or semi-quantitative, and are mostly used as an in situ measurement technique.<sup>[11]</sup>

Results obtained with field test kits are based on visual observations and the comparison of colors, which restricts their accuracy and repeatability, given that sensitivity varies from operator to operator.<sup>[12]</sup> This explains why different authors have obtained poor correlations between results obtained by means of test kits and those obtained in analytical laboratories,<sup>[13]</sup> while others successfully obtain reliable As concentrations using test kits.<sup>[14–16]</sup> The advantage of test kits is that they are capable of providing fast and low-cost results, can be easily used, and need only small amounts of water, thus avoiding problems related to sample preservation and transportation from the field to the laboratory.

Most colorimetric measures of arsenic concentrations are based on Gutzeit's well-known method. Several authors have worked on improving this method to avoid interference, lower the detection limit and diminish the reaction time.<sup>[17]</sup> In general, test kits provide reliable As measurements for field applications and the accuracy of results increases with the reaction time of the test.<sup>[15]</sup> Test kits nowadays enable arsenic concentrations to be detected almost from ~1 ppb, and under favorable conditions they can also measure arsenic speciation.<sup>[11,18]</sup> Indeed, colorimetric methods can provide results nearly as accurate and reliable as those from analytical laboratories when the reaction is automatically evaluated by means of a color detector.<sup>[12]</sup>

The purpose of this work is to develop an analysis method to determine reliable arsenic concentrations either

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in the field or the laboratory by using low cost colorimetric methods. Arsenic measurements were performed with commercial test kits, but with an alternative analysis of the color that emerges in the paper strips during the tests in order to increase the reliability and reproducibility of the measurements. The proposed method consists in digitalizing images of the paper strips after the As measurements and performing a digital analysis of these pictures.

## Materials and methods

### Solutions

Synthetic and natural waters were tested to verify the accuracy of the proposed method in detecting As either in the laboratory or in the field. Synthetic samples were used for developing, calibrating and testing the As detection method proposed in this research, while natural waters were used to verify that reliable concentrations can also be obtained in the presence of other ions in the test solution.

Synthetic solutions containing different arsenite ( $[\text{AsO}_3]^{-3}$ ) concentrations were prepared from the solution of arsenic trioxide ( $\text{As}_2\text{O}_3$ ) in an alkaline medium obtained with sodium hydroxide (NaOH) and deionized water. Arsenic concentrations ranged from 0 to 3 mg/L, thus covering the concentrations recorded in most aquifers in the Center of Argentina. Natural waters were sampled from eleven monitoring wells in the Provinces of Cordoba, La Pampa and Chaco in the central and northeastern Argentina. The natural water samples were extracted in sterile containers at the wellhead or water supply system. All the samples were preserved at 5°C during transport and storage until the time of analysis.

### Colorimetric arsenic detection method

Macherey–Nagel Quantofix Arsen 10<sup>®</sup> and Arsen 50<sup>®</sup> arsenic test kits were used. These test kits discriminate the following concentrations in different colors: 0, 0.01, 0.025, 0.05, 0.1 and 0.5 mg/L for Arsen10<sup>®</sup> and 0, 0.05, 0.1,

0.5, 1, 1.7 and 3 mg/L for Arsen 50<sup>®</sup>. The presence of selected ions produces interference in the arsenic determinations only for very high concentrations (e.g., more than 1000 mg/L of  $\text{Ca}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ , etc. – data provided by the manufacturer).

The standard procedure followed for the determination of As concentrations with these test kits was as follows. A volume of 20 mL or 5 mL of liquid was introduced into the reaction vessel for the Arsen 10<sup>®</sup> and Arsen 50<sup>®</sup>, respectively. Then, a standard spoonful with  $0.53 \pm 0.02$  g of powdered zinc provided by the manufacturer was added to the liquid and the solution was lightly swirled during 2 s. After that, a standard spoonful with  $0.58 \pm 0.02$  g of powdered hydrochloric acid, also provided by the manufacturer, was added to the liquid. The test strip was placed with its mercury (II) bromide field in the reaction vessel, taking care to keep the test field always dry (above the water level). From this moment, arsine vapors liberated from the reaction between the As, zinc and hydrochloric acid reacted with the paper strip, for 30 and 20 min for the Arsen 10<sup>®</sup> and Arsen 50<sup>®</sup>, respectively. The test strip was removed from the reaction vessel and dipped into distilled water for 2 s.

The color that emerges in the test field after reaction is related to the As concentrations. The manufacturer of the test kits proposes a comparison between the test field color and a qualitative reference color scale. However, an improved analysis was developed, given that the comparison of colors is rather subjective and operator-dependent.

The test strip was placed in a base and 10 photographs were taken within the first minute after soaking it in distilled water. The distance between the camera and the paper strip, lighting conditions and camera setting were kept constant for all tests (Fig. 1). The picture of each test field had 9 cm<sup>2</sup> and 125,316 pixels.

Color, or red (R), green (G) and blue (B) pictures, were transformed into 8-bit gray scale images as follows:

$$\text{GL} = 0.299 \text{ R} + 0.587 \text{ G} + 0.114 \text{ B} \quad (1)$$

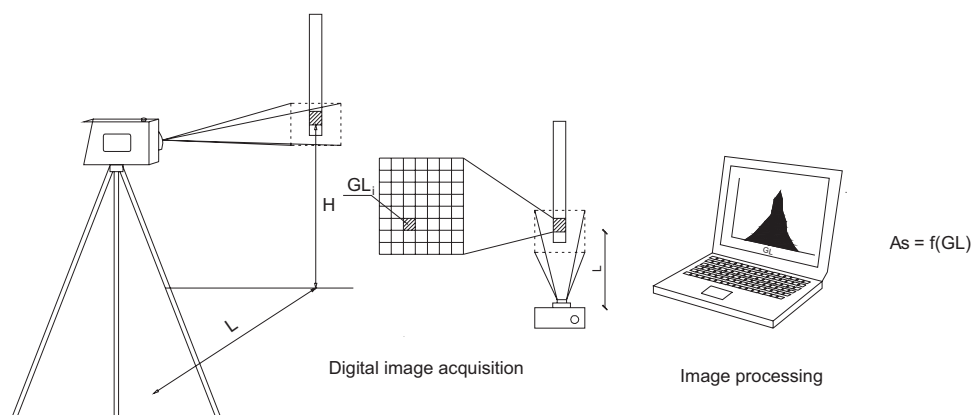


Fig. 1. Setup for the image acquisition and analysis of the test field color to determine As concentrations.

where GL is the gray level or digital level, and for 8-bit images, runs from 0 (black) to 255 (white).

Histograms of GL were obtained for each picture with the aid of the open source software ImageJ developed by Rasband.<sup>[19]</sup> Digital levels obtained from each picture were statistically analyzed to improve the reliability of the proposed method. This procedure enabled the GL obtained to be associated with the As concentration of each test solution, comparing concentrations of selected samples determined from the image analysis of colorimetric reactions (IACR) developed in this work with concentrations obtained by using Gaseous Hydride/Atomic Absorption Spectrometry (GHAA), following the procedure suggested by the USEPA.<sup>[20]</sup> Limit of detection (LOD) and limit of quantification (LOQ) using this technique were 0.02 ppb and 0.06 ppb, respectively.

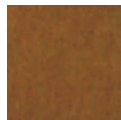

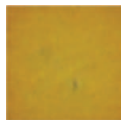
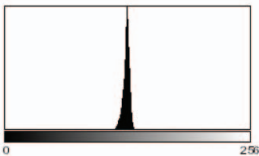

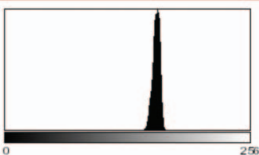

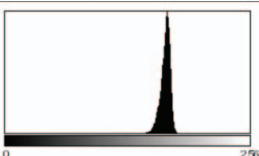
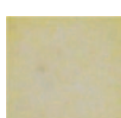
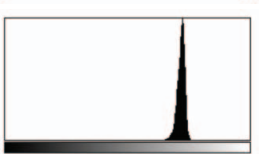
## Results and discussion

### Colorimetric analysis of reaction

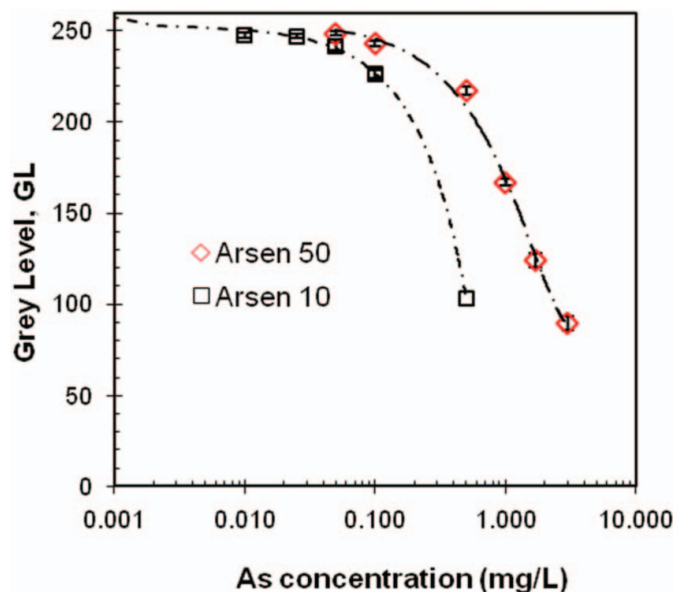
Different GL histograms of the acquired images were obtained for each paper strip after reaction with different As

concentrations (Fig. 2). Results showed that the higher the As content, the lower the mean GL, yet the coefficient of variation (COV) of gray levels remained almost constant. Figure 2 shows images obtained after testing solutions with known As concentrations, as well as the statistical properties of each picture. These measurements were performed at the As concentrations used as reference values for the Arsen 10<sup>®</sup> test kit. Similar analysis was performed for the Arsen 50<sup>®</sup>. The x-axis of the histograms represents GL values and the y-axis the number of pixels. The corresponding histograms of gray levels clearly showed similar Gauss distributions, displaced toward the right, with a higher digital level as the As concentration decreased.

All pictures and histograms were processed identically in order to avoid scatter in the data related to the test conditions. Histograms were corrected by stretching, which involves assigning GL = 0 to a known black color target and GL = 255 to a known white color target, which were included in all pictures. Average GLs were computed from the corrected histograms for each picture and were then related to the As concentration in the test solution. This was very useful in standardizing the GL of the pictures and avoiding environmental errors.

As [mg/L]	Image	Histogram of gray levels	Gray levels				
			Min	Max	Mean	SD	COV
0.5			92	109	103.0	4.43	4.3%
0.1			221	232	226.6	4.12	1.8%
0.05			238	245	241.7	2.16	0.9%
0.025			242	251	247.1	2.94	1.2%
0.01			242	252	247.4	3.83	1.5%

**Fig. 2.** Digital images, histograms, and statistical properties of gray level distributions at different As concentrations (color figure available online).



**Fig. 3.** Relationship between As concentration and gray levels obtained from digital image analysis of sensitive paper strips (color figure available online).

#### Calibration curve and analysis of uncertainty

A total of 13 tests were performed in solutions with known As concentrations equal to the reference values used in each test kit, and 10 pictures were taken and analyzed for each test. This allowed calibration curves to be obtained that related mean As concentrations to GL when using the Arsen 10<sup>®</sup> (Eq. 2) and Arsen 50<sup>®</sup> (Eq. 3) test kits (Fig. 3).

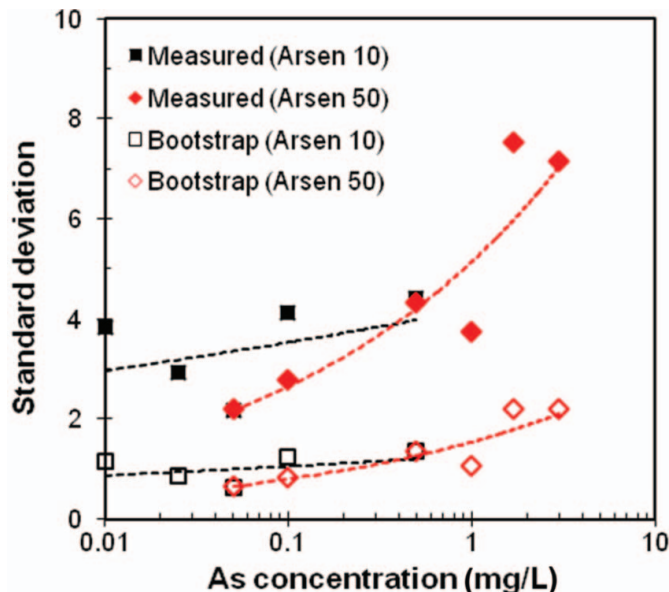
$$|\text{As}| = -0.0033 \text{ GL} + 0.8421 \quad (2)$$

$$|\text{As}| = 9\text{E}^{-05} \text{ GL}^2 - 0.0481 \text{ GL} + 6.4734 \quad (3)$$

The coefficients of determination  $R^2$  for the Equations (2) and (3) were 0.998 and 0.985 respectively. Arsenic cannot be determined outside the concentration range shown in Figure 3. Error bars shown in Figure 3 represent a 95% confidence interval.

Equations 2 and 3 enabled As concentrations to be determined between the discrete values established by the commercial test kits. Thus the accuracy of measurements was increased given that there was no further need of operator judgment. Light, test strip positions and camera settings were changed to verify the repeatability of the method. Formulas similar to Equations 2 and 3 were obtained and even with the fitting constants changed, the coefficients of determinations were as high as those reported in this research.

An uncertainty analysis was performed with the bootstrap technique.<sup>[21]</sup> This allowed errors and probabilistic distributions of As concentrations to be estimated by randomly creating a series of “ $n$ ” independent As values from an original data set with “ $n$ ” As measurements. The prob-

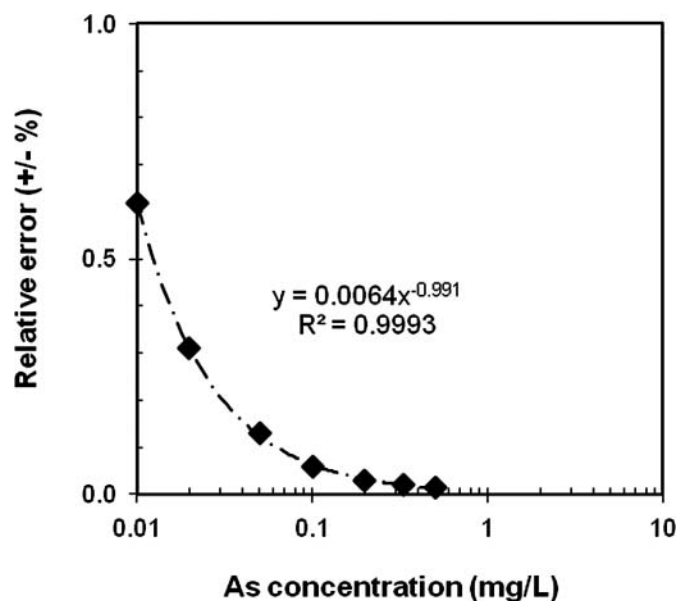


**Fig. 4.** Influence of As concentration on the standard deviations of GL with and without bootstrap analysis (color figure available online).

abilistic distribution and statistical parameters were then computed for each new set of data. The uncertainty analysis was performed using the code developed by Garcia et al.<sup>[22]</sup> A total of 1,000 new values were generated for each concentration analyzed, which helped to increase the accuracy of the method, and reduced the time needed for the development of reliable correlations.

The accuracy of measurements was directly related to the standard deviation of GL, which varied from 2.2 to 4.4 when As concentrations measured with the Arsen 10<sup>®</sup> were between 0 mg/L and 0.5 mg/L. In the case of measurements performed with the Arsen 50<sup>®</sup>, the standard deviation of GL fell to between 2.2 and 7.5 when concentrations ranged from 0 mg/L to 3 mg/L (Fig. 4). However, the COV remained almost constant and lower than 4.3% even when the standard deviation of GL slightly increased with the As concentration.

The standard deviation and COV of GL reduced as the number of experiments increased. Significant decreases in the standard deviations were observed when simultaneously analyzing physical experiments (tests) and numerical data generated by means of the bootstrap analysis (Fig. 4). The maximum standard deviations of GL fell to 1.3 and 2.2 for the Arsen 10<sup>®</sup> and Arsen 50<sup>®</sup>, respectively. However, the expected or mean values suffered negligible changes within the test range of As concentrations as a consequence of the bootstrap analysis. The reliable detection limit was close to 6.5  $\mu\text{g/L}$  and its accuracy varied with the arsenic level. Expected relative errors decreased significantly as the concentration increased (Fig. 5). The

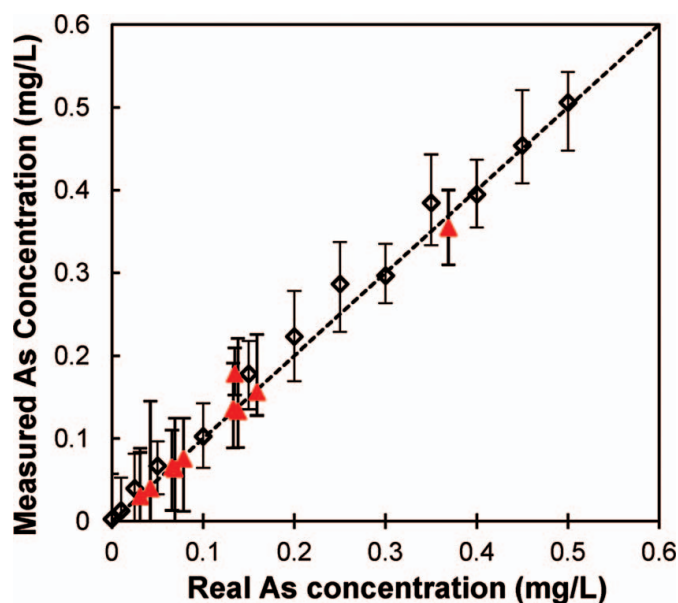


**Fig. 5.** Influence of As concentration on the relative error for a 95% confidence interval.

relative error for 10  $\mu\text{g/L}$  is about  $\pm 6.2 \mu\text{g/L}$  with a 95% confidence.

Table 1 presents the 11 samples of natural waters, their place of extraction and As concentrations detected by means of GHAA and with the IACR method developed in this work. Natural water samples were collected in situ and then analyzed following the same experimental procedure used for prepared samples and with GHAA. The results show that there is quite a good correlation between the mean concentrations determined from the two methods, although the LOD of the technique proposed here is significantly higher than that of GHAA.

Figure 6 presents the correlation between the As concentrations determined by using the method developed in this work and the real concentrations, either known for the syn-



**Fig. 6.** Comparison of arsenic concentrations determined from the analysis proposed in this work and laboratory analyses by Gaseous Hydride/Atomic Absorption Spectrometry (GHAA); line represents a 1:1 ratio (color figure available online).

thetic waters or determined by means of GHAA in the case of natural waters. The 45-degree line shows a perfect correlation between real concentrations and that determined by the method developed in this work. The results provided a coefficient of correlation higher than 0.98 for the concentration range tested (Fig. 6). Therefore, the IACR method proposed in this work clearly helped in improving the accuracy of measurements, significantly reducing the importance of potential error from environmental conditions or operator judgment, and providing more reliable quantitative As concentrations.

**Table 1.** Arsenic concentrations [mg/L] in natural water samples determined from the image analysis of colorimetric reactions (IACR) proposed in this work and laboratory analyses by means of Gaseous Hydride/Atomic Absorption (GHAA).

Sample location	IACR		GHAA	
	Mean values	Standard deviation	Mean values	Standard deviation
Monte Buey, Cordoba	0.178	0.022	0.135	0.04
Colonia Las Pichanas, Cordoba (sampling well No. 1)	0.133	0.038	0.138	0.20
Colonia Las Pichanas, Cordoba (sampling well No. 2)	0.354	0.033	0.369	0.30
Colonia Las Pichanas, Cordoba (sampling well No. 3)	0.136	0.032	0.133	0.10
Bell Ville, Cordoba (sampling well)	0.156	0.028	0.159	0.40
Bell Ville, Cordoba (tap water)	0.031	0.042	0.031	0.01
Realico, La Pampa (tap water)	0.040	0.054	0.042	0.40
Realico, La Pampa (sampling well No. 1)	0.030	0.035	0.031	0.06
Realico, La Pampa (sampling well No. 2)	0.065	0.034	0.066	0.10
Charata, Chaco (sampling well No. 1)	0.063	0.047	0.069	0.04
Charata, Chaco (sampling well No. 2)	0.076	0.041	0.079	0.04

## Conclusions

The findings of this research suggest that the Arsen 10<sup>®</sup> and Arsen 50<sup>®</sup> test kits can be used to detect the presence of arsenic in groundwater and drinking water for rapid, easy and low cost measurements of arsenic concentrations.

Detection of arsenic by using test kits is based on a colorimetric method and visual observations. However, the accuracy and repeatability of their results are often low, given that sensitivity varies from operator to operator. To avoid this problem, a new operator-independent analysis is proposed in this research, which consists in digitalizing the reaction seen during the test and performing a digital image analysis.

Results indicate that the accuracy increases significantly when the digital image analysis is performed. The Arsen 10<sup>®</sup> and Arsen 50<sup>®</sup> were capable of detecting arsenic concentrations as low as 0.065 mg/L, and, for 10 µg/L, the guideline value for arsenic set by the World Health Organization, the relative error is ± 6.2 µg/L with a 95% confidence. Additionally, the evaluation of expected relative errors at different arsenic concentration shows that the accuracy of determinations increases with the arsenic level.

The arsenic concentrations determined by using the method developed in this work perfectly correlate with those determined by Gaseous Hydride/Atomic Absorption Spectrometry. The proposed technique thus improves the accuracy of colorimetric methods, provides reliable and repeatable arsenic measurements, and significantly reduces potential errors associated with environmental conditions and operator judgment.

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