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Improved method for the determination of Arsenic (III) in water by molecular absorption spectrophotometry

Akpénè Amenuvevega DOUGNA[1,2*], Kossitse Venyo AKPATAKU[1,2], Moctar L. BAWA[2], Tani Bamboctile TOUGOUDINE[2] and Gbandi DJANEYE-BOUNDJOU[2]

[1]*Laboratory of Organic Chemistry and Environmental Science, Faculty of Science and Technology, University of Kara, BP 404 Kara-Togo.*

[2]*Laboratory of Applied Hydrology and the Environment, Faculty of Science, University of Lomé, Lomé, Togo.*

[*]*Corresponding author; E-mail:* adougna@yahoo.fr; Tel: *+22890964323*

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ABSTRACT

Arsenic analysis is essential, as it is a carcinogenic compound according to the International Agency for Research on Cancer (IARC). Currently, there are techniques based on powerful equipment that allow quantification with very low detection limits as Inductively Coupled Plasma (ICP). However, groundwater pollution issues are not respectful of equipment's availability or local financial resources. Furthermore, standardised methods on the market are not sufficiently accessible to laboratories in developing countries that use them as a reference, and only methods available in reference books are commonly used. We have therefore modified the available colorimetric method, which has a quantification limit of 25 µg/L, to achieve a detection limit of 5 µg/L, which is lower than the WHO standard, in order to further identify groundwater samples that would present a high health risk due to consumption. Colorimetric method based on silver diethyldithiocarbamate has been used. The effects of various parameters such as arsenic initial concentration (up to 800 µg/L), volume of solution (35-120 mL) were evaluated on arsine quantification. The results reveal that for concentrations below 25 µg/L, a test volume of 100 mL is sufficient to measure arsenic concentrations in water up to 5 µg/L. However multiple extraction is indicated in the less concentrated samples (<5 µg/L) for reliable quantification. Experiments performed with the previously used zinc shots show that washing with appropriate mixture several times and drying allows arsenic quantification with 10% loss of zinc activity.

Keywords: Arsenic, Groundwater Pollution, Colorimetric Method, Trace Element, UV Visible Spectrophotometer.

INTRODUCTION

Arsenic is not a major problem only in water systems (Ehsan et al., 2020). It is a problem also in soils, sediments, vegetables, and fish, complicating exposure routes and health risks to the human body (Irunde et al., 2022). Arsenic contamination in potable groundwater resources could make it unfit for drinking purposes and may cause various health diseases, like kidney failure, heart problems, and hair loss (Ehsan et al., 2020). Arsenic is a ubiquitous, naturally occurring contaminant that is a common problem in many aquifers pumped for drinking water. Climate change could increase aquifers contamination, and over pumping could lead to the release of

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arsenic present in pore water within aquifers (Ullah et al., 2023). Anthropogenic sources of As contamination in groundwater sources are mining actions(Kassenga & Mato, 2009), industrial effluents, industrial discharge, land-filling of sewage sludge, and agricultural pesticides (Ahmad et al., 2021). Both sources of arsenic water contamination (N'guessan et al., 2017) represent a high motivation to find an affordable analytical method for monitoring groundwater, surface water, and drinking water production. This is of high concern in developing countries, particularly in sub-Saharan Africa, where arsenic contamination is problematic but sophisticated, and onerous lab testing facilities are limited or not readily available (Irunde et al., 2022; Reich et al., 2022).

Various analytical methods for determining arsenic have already been described in the literature, which are mainly based on spectroscopic, colorimetric, spectrophotometric, chromatographic and biological techniques (Rice et al., 2012; Bhat et al., 2022). The spectrophotometric method is relatively less expensive than other analysis techniques (Rodier et al., 2016). The colorimetric methods are easy to use and inexpensive regarding equipment and operator costs. They are useful for semi-quantitative determination of relatively high concentrations of arsenic in water (IARC Working Group, 2004) as the atomic absorption spectrometry method (Bodjona et al., 2024) with hydride generation or with graphite furnace (AFNOR, 1996). Other spectroscopic methods such as Inductively Coupled Plasma (ICP), X-ray fluorescence (XRF)(Pringle et al., 2022), and the chromatographic techniques have the advantage of offering high sensitivity for the determination of traces elements but require expensive and less accessible equipment (Rodier et al., 2016; Chattopadhyay et al., 2020).

It was in 1775 that C. W. Scheele discovered the volatile arsenic compound known as arsine (AsH_3) through his research on arsenic. On this basis, James Marsh continued his research into his qualitative test for arsenic, which led to a significant decrease in the criminal use of arsenic in society (Webster, 1947). He discovered that the action of zinc and dilute acid on arsenical material resulted in the production of arsine gas. This discovery later led to the quantitative determination of arsenic, known as *Gutzeit* test by quantifying AsH_3 (Budesinky, 1979; Reich et al., 2022) in hydrochloric solution.

The existing literature, has shown that there are two familiar methods based on molecular absorption spectrophotometry with quite different sample volumes, quantities of reagents and limits of quantification (AFNOR, 1993; Rodier et al., 2016). Colorimetric methods have also been developed and are desirable for portable arsenic monitoring because of the simplicity of the detection method. However, most commercial colorimetric kits are unreliable for arsenic concentration below 70 µg/L (Bhat et al., 2022), In the context of sustainable development, it is important to enable the determination of arsenic at a lower cost and to achieve a limit of quantification below 10 µg/L as arsenic concentration included in the WHO standards for drinking water (WHO, 2022). So, the current works, particularly intended for under-equipped laboratories, are designed to present a method with a limit of quantification below 10 µg/L by studying the influence of some parameters, such as the amount of reagents, sample volume, and procedure on arsine (AsH_3) dosage efficiency. The dissolved arsenic typically in the form of arsenite $\left(AsO_3^{3-}\right)$, arsenate $\left(AsO_4^{3-}\right)$ ions (Ouedraogo et al., 2020), is reduced into arsine (AsH_3) in an hydrochloric acid solution with zinc metal as the reducing agent.

MATERIALS ET METHODS

All chemicals were used without previous purification and were of analytical quality (ACS reagent) and trace metals basis. Zinc powder (≥ 99.995%), hydrochloric acid (HCl) (32 wt. % in H_2O, <0,01ppm of As), ACS reagent, sodium hydroxide (≥ 97.0%), pellets (< 0.002% As), copper(II) sulfate anhydrous powder (≥99.99%), potassium iodide (≥99.0%), Tin(II) chloride dihydrate (≥99.99%), pyridine (C_3H_5N) (≥99.0%),

lead(II) acetate trihydrate $Pb(CH_3COO)_2.3H_2O$ (≥99.999%), Arsenic trioxide As_2O_3 (≥99.995%), silver diethyldithiocarbamate $AgS_2CN(CH_2CH_3)_2$ (≥99.0%), were purchased from Sigma Aldrich. Sodium hydroxide (0,1N) and sulphuric acid (98%) were used to adjust the pH. Each experiment was carried out in triplicate and the mean value were reported. Thermo scientific spectrophotometer Genesys 10 UV-Vis was used for absorbance measurement.

Experimental procedure

The silver diethyldithiocarbamate method uses volatile arsine (AsH_3) gas to separate Arsenic from other possible interference with the sample matrix (IARC Working Group, 2004). First, lead acetate paper was prepared by immersing a filter paper in a solution of crystallized $Pb(CH_3COO)_2.3H_2O$ (100g/L). The wet paper filter was then dried gently in an oven (approx. 60°C) and kept airtight in a desiccator until used in arsine scrubber.

Zinc shot activation and regeneration.

The zinc granules were activated using the following method: 100 mL of distilled water, 10 drops of copper sulphate solution (100 mg/L) and 80 g of pure arsenic-free zinc granules were added to a beaker; after 10 min of reaction at a steady state, the mixture was stirred 2 or 3 times, then decanted, washed several times with distilled water and then dried. After the arsine dosage, zinc shots were regenerated before any use following the same activation process but with 15 drops of copper sulphate solution instead of 10, and then activated as new ones.

Arsine detection and analysis

Fisherbrand™ Arsine Generator was used (Figure 1), including Erlenmeyer flask, scrubber, and absorber. 35 mL of sample was introduced in Erlenmeyer flask. 5 mL of concentrated HCl, 2 mL of KI solution (150 g/L), and 8 drops of $SnCl_2$ (400 g/L) were added. After 15 min of reaction, the lead acetate paper was introduced in the arsine scrubber and 5mL of silver diethyldithiocarbamate solution (1g of $AgS_2CN(CH_2CH_3)_2$ dissolved in 0.2 L C_3H_5N) in the absorber. Then, 3g of pre-activated zinc was introduced into the generator. After waiting for at least 2 hours to ensure the release of arsenic hydride from the reducing mixture (containing zinc, $SnCl_2$, and KI), the absorbance of the bubbler solution following homogenization (Rodier et al., 2016) were measured. The spectrophotometric method is based on generating AsH_3 in HCl solutions with zinc.

Then the arsine absorber is disconnected, and the absorbance of the red-coloured complex is measured at 535 nm in a 1 cm flow-cell against a blank solution (Budesinky, 1979).

Calibration curves

An arsenic standard solution at 1 mg per litre was used to establish the calibration curves. A quantity of 0.132 g of anhydrous arsenic trioxide (As_2O_3) was dissolved in 5 mL of sodium hydroxide solution with a concentration of approximately 5 eq/L. This solution was neutralised to pH 7 using a dilute sulphuric acid solution. 1 mL of this stock solution was used to prepare the corresponding solution C = 1 mg/L. Successive dilutions were made with arsenic-free distilled water. The blank solution was treated following the same protocol as standards for each calibration curve.

Expression of arsenic concentration

The arsenic concentration in the sample was calculated by following equation:

$$[As]_{\mu g/L} = \frac{A_2 - A_1}{l} \times f \qquad (2)$$

Where

A_1 is absorbance of the blank solution

A_2 is absorbance of the sample solution

f is calibration factor ($mm.\mu g.L^{-1}$), determined from calibration curve

l is the length of optical cell

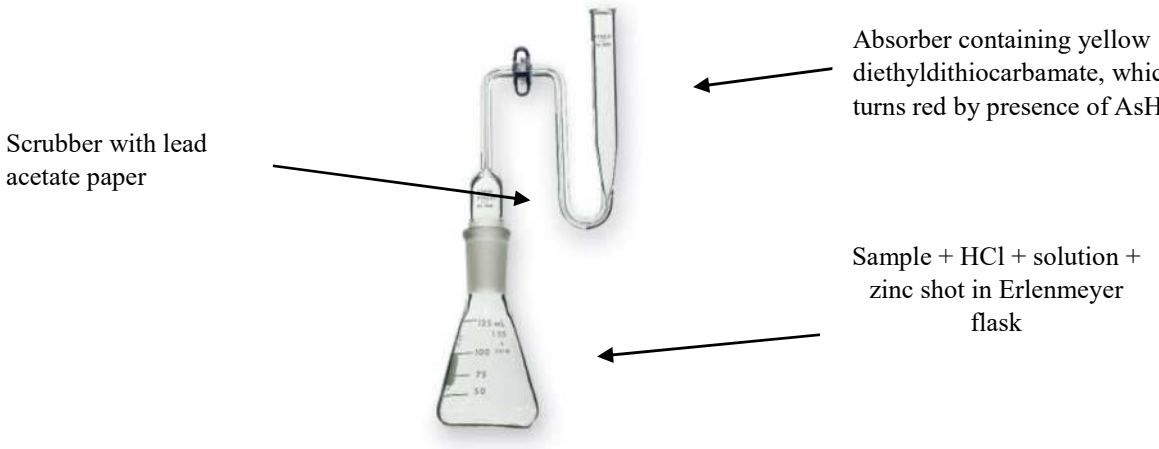

Figure 1: Arsine generator (from Fischer Scientific, modified).

RESULTS

Kinetics of arsenic formation and study of the stability of the coloured complex

Firstly, we studied the formation of the red complex attesting to the presence of arsenic in the sample. Samples were taken every 30 minutes from the bubbler and analysed using a spectrophotometer during 150 minutes. The results are shown in Figure 2. The stability of the complex was also studied by varying the initial concentration of arsenic present in the sample for 5 days (Figure 3). It can be observed that from 0 to 90 min, complex formation appears slow, but from 90 to 120 min it is rapid. After 120 min the curve stabilises showing the end of reaction.

Reuse of zinc shot.

With a perspective of sustainable chemistry and to optimize the use of the inputs to arsenic analysis, the shot used is rinsed and reused according to the protocol presented. The results are presented in Table 1. The absorbance obtained for the two types of shots show a significant difference. Globally, obtained absorbance with regenerated zinc shot shows a downward trend with the decrease of absorbance values compared to values obtained with new zinc shots.

Effect of concentration and volume of sample

With the aim of determining lower concentrations in water by spectrophotometric methods, we first studied the variation in sample volume and arsenic concentration on the formation of the coloured red complex. Four volume levels were studied: 35, 70, 105, and 120 mL for a concentration of 28.57 µg/L to assess the effect of volume on coloured complex formation and the results are represented on Table 2. The effect of arsenic concentration on red complex formation is also shown in Figure 5. The study was conducted up to 800 µg/L of arsenic. The results show that it is possible to analyse arsenic by colorimetric method up to 800 µg/L. However, with the increase of sample volume, the absorbance of red complex decrease with the same initial concentration.

Modified protocol for low concentration of arsenic

In light of the experiments carried out in the previous paragraph, we modified the protocol to measure low concentrations while considering the possibility of increasing the sample volume and detection limit (Figure 6 and **7**). For the modified protocol, a sample volume of 100 mL was used. The concentrations of the solutions used remained

unchanged from the initial protocol. Only the volumes used have changed: 10 mL of HCl, 4 mL of KI and 0.5 mL of $SnCl_2$ solutions. The rest of the protocol remains unchanged with a mass of 6 g of activated zinc. This modified protocol enabled to obtain calibration curves with acceptable R^2 (>0.98) and detection limit up to 5µg/L (Figure 7).

Trace Analysis by liquid evaporation and multiple extraction.

In order to be able to measure low concentrations of arsenic (< 5µg/L), different solvents (water, ethanol, hexane, petroleum ether and acetone) were used to perform a liquid-liquid extraction coloured complex solution. The experiments were not successful due to miscibility with pyridine. We tried then to concentrate by evaporation of the water.

500 mL of less concentrated arsenic solution (< 5µg/L) were prepared and then slowly evaporated to a volume of less than 100 mL. The solution was then completed up to 100 mL to reach the desired concentrations (2; 4 µg/L). For the control experiments, the same concentrations were prepared at a volume of 100 mL directly without evaporation. The results (Table **3**) obtained showed a significant difference between the expected and the obtained optical densities.

Then, to determine the low concentrations by carrying out a successive multiple extraction, a 100 mL volume in triplicate, each containing the same low concentrations of arsenic (< 5µg/L) was used. At the end of the third distillation, the global concentration corresponding at threefold extraction of diethyldithiocarbamate was read at spectrophotometer. The results are presented in Table 4 for three concentrations (0, 2 and 4) and show that the obtained values of absorbance are similar of expected ones.

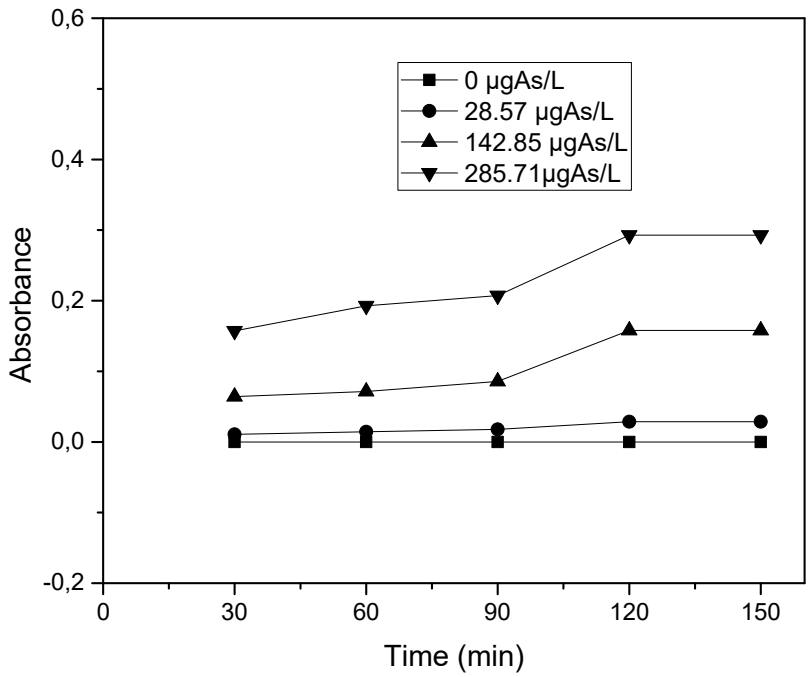

Figure 2: Kinetics of coloured complex formation.

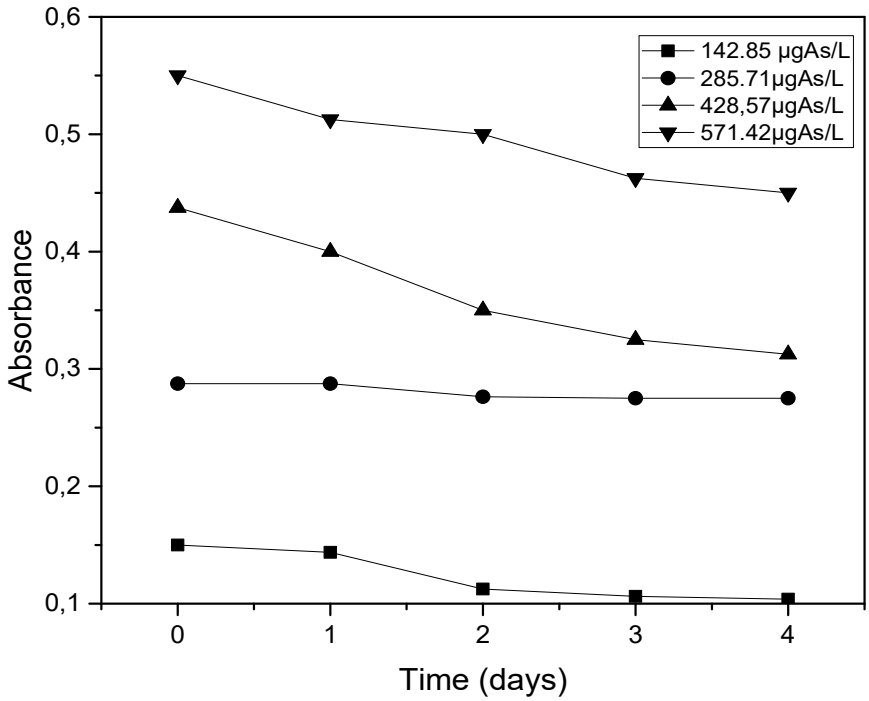

Figure 3: Stability of the coloured complex during 4 days.

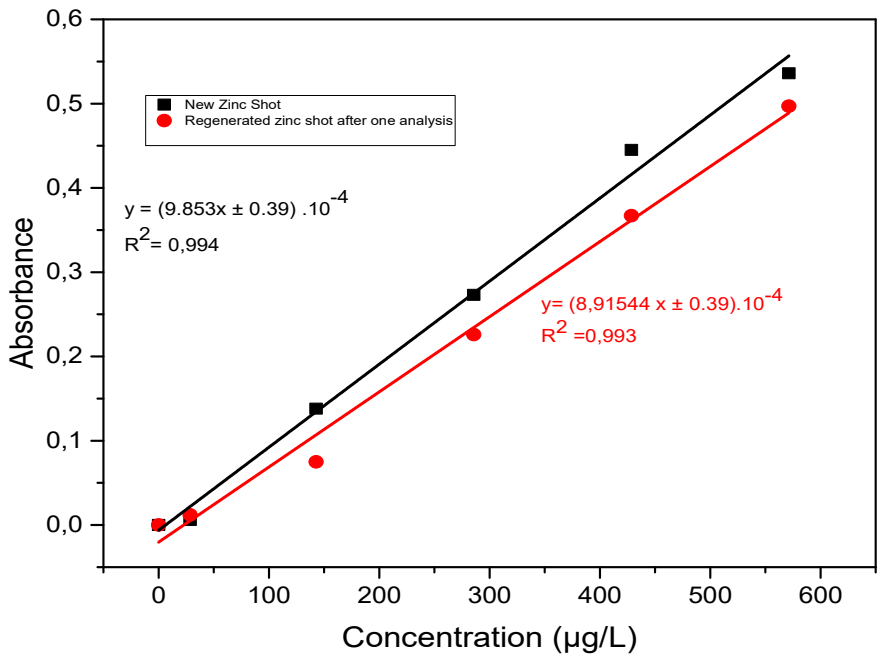

Figure 4: Comparative absorbance of red complex using new and regenerated zinc shot.

Table 1: Influence of zinc shot regeneration on arsenic quantification.

Concentration (µgAs/L)	DO	
	New Zinc Shot	Regenerated zinc shot after one analysis
0	0	0
28.57	0.006	0.012
142.85	0.138	0.075
285.71	0.273	0.226
428.57	0.445	0.367
571.42	0.536	0.497

Table 2: Effect of sample volume on red complex absorbance.

Volume (mL)	DO
35	**0.015**
70	**0.007**
105	**0.009**
120	**-0.003**

Table 3: Absorbances after concentration of the sample by liquid evaporation.

Concentration (µg/L)	DO	
	Control samples (100 mL)	Samples after evaporation (from 500 to 100 mL)
0	0.020	0.022
2	0.0036	0.068
4	0.0070	0.077

Table 4: Absorbance obtained by multiple extraction of different concentrations of As in a volume of 100 mL.

Global Concentration (µg/L)	ΔDO	
	Expected values	Obtained values for multiple extraction(*)
0×3	0.0022 (0 µg/L)	0.0024
2.0×3	0.032 (6 µg/L)	0.033
4.0×3	0.048 (12 µg/L)	0.058

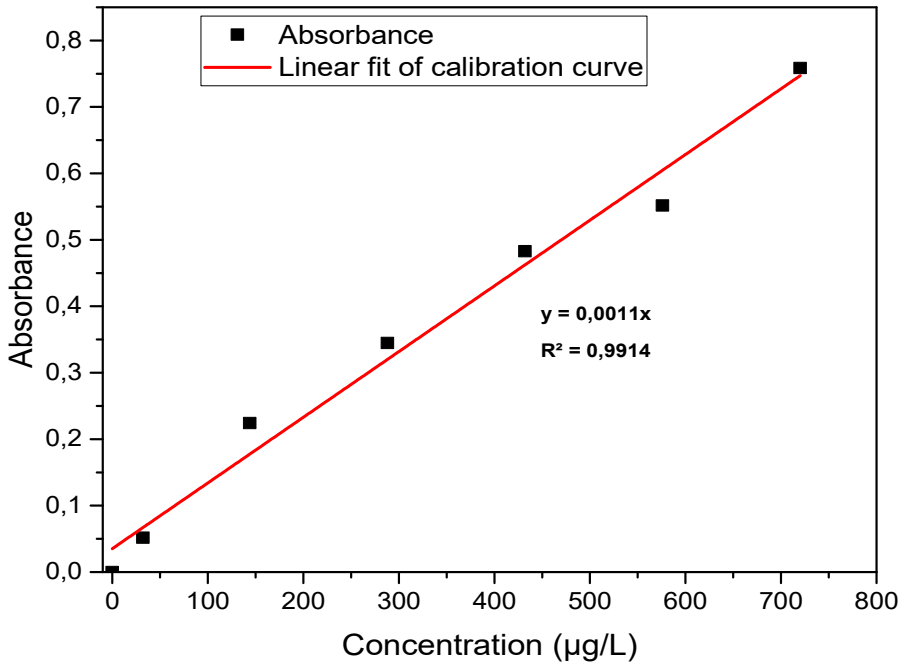

Figure 5: Calibration curve for arsenic determination between 0 and 800 µgAs/L.

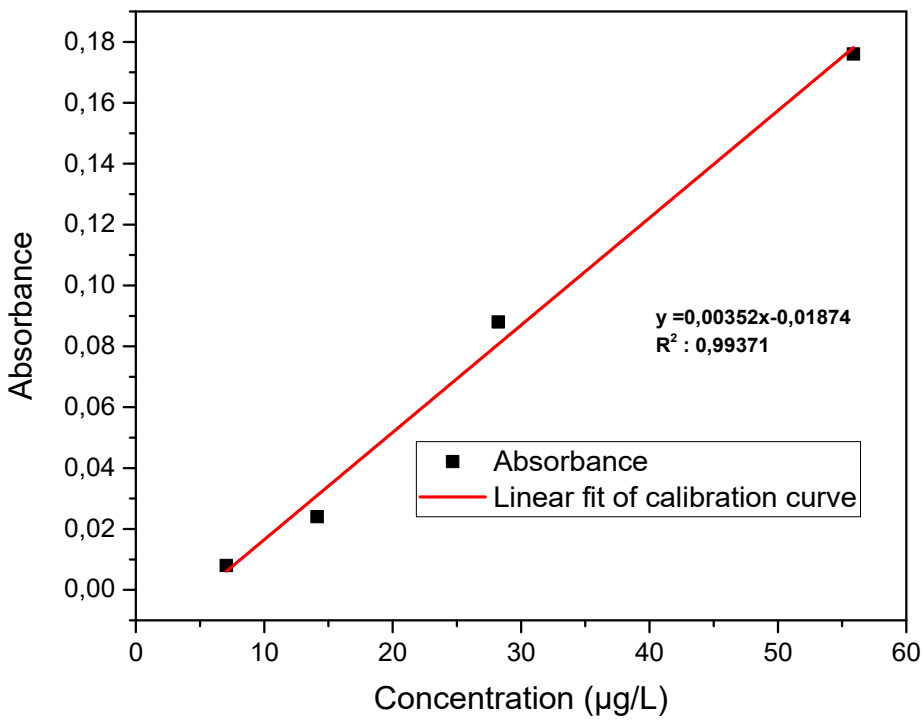

Figure 6: Calibration curve for arsenic determination between 7 and 60 µg/L.

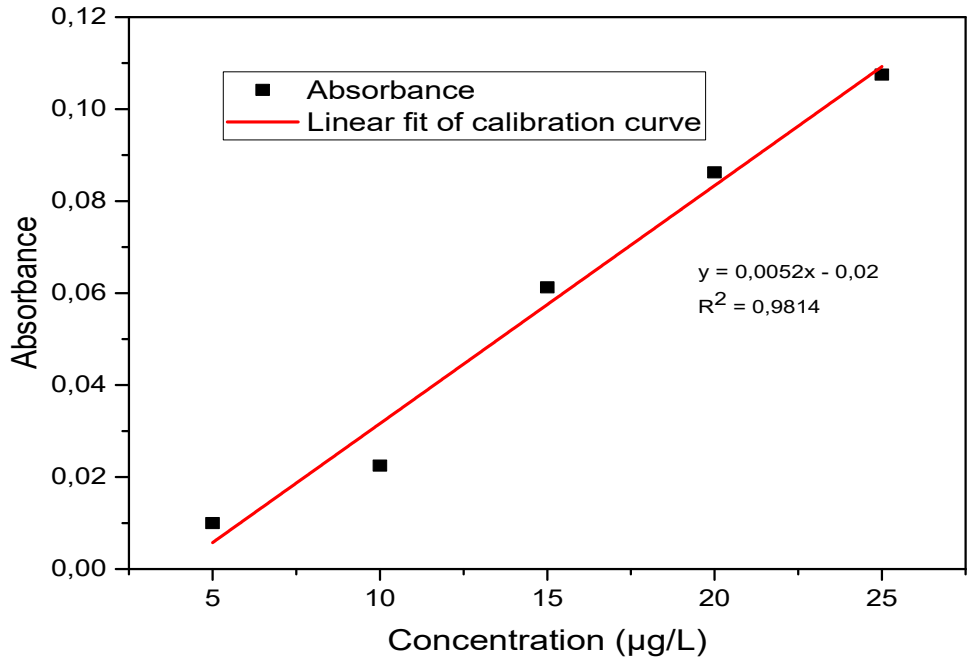

Figure 7: Calibration curve for arsenic determination under 25 µgAs/L.

DISCUSSION

According to Figure 2, the arsine reaction would be complete after two hours of reaction. The presence of arsenic is indicated by the colour of the yellow diethyldithiocarbamate, which turns red (Rodier et al., 2016) based on equation (1), presented only by Budesinky (1979).

$$AsH_3 + 6AgS_2CN(CH_2CH_3)_2 + 3C_3H_5N \rightarrow \underbrace{AsAg_3.3AgS_2CN(CH_2CH_3)_2}_{\text{Redcomplex}} + 3\left[SCN(CH_2CH_3)_2\right]S^- + 3C_3H_6N^+ \quad (1)$$

It appears that the higher is the initial concentration, the more the absorbance of colour complex tends to constant after 120 min. It could be argued that the rate of formation of the red complex is lower than the rate of formation of arsine. Indeed, the rate of arsine formation determines the concentration of red complex according to equation (1). Accumulation of AsH_3 would lead to an increase in its concentration and in the kinetics of red complex absorbance. The sudden increase in the rate of red complex formation after 90 min of the reaction could be explained by the increased probability of AsH_3 molecules meeting those of the complexing reagents. These kinetics are similar to other reactions whose kinetics depend on the initial concentration of the reactants (Dougna et al., 2015).

After hours of monitoring, the lowest concentration is almost zero due to degradation of $AsAg_3.3AgS_2CN(CH_2CH_3)_2$. The complex would be unstable, and the coloured complex responsible for the red coloration would be degraded according to equation (3) (Budesinky, 1979).

$$AsAg_3.3AgS_2CN(CH_2CH_3)_2 + 3C_3H_5N \rightarrow 6Ag + As\left[S_2CN(CH_2CH_3)_2\right]_3 + 3S_2CN(CH_2CH_3)_2^- + 3C_3H_6N^+ \quad (3)$$

These results are well in line with previous work, which suggests leaving the solution to stand for 2 hours to allow the arsine to be completely released (Rodier et al., 2016). There was a reduction of Ag^+ ions present in the red complex. This reaction would therefore be at the origin of the degradation of the red complex. Recent work has been carried out and confirm this reaction as authors tried to stabilise this complex using a silver-based polymer (Reich et al., 2022).

The reuse of zinc shots after appropriate washing (Figure 4) shows that the higher is the arsenic concentration, the greater is the difference between the two curves. This might suggest that the surface area available to the zinc has been reduced after an initial reaction, making the production of arsine gas slow or incomplete. Therefore, the aging of zinc shots after one analysis affects the release of the red complex since the ratio of two slopes (k_2/k_1: 8.915/9.853) is 0.90 showing that the reduction of zinc activity is linear with a correlation coefficient (R^2) greater than 0.99. Regenerated zinc shots can still reduce soluble arsenic (n.o: +III and IV) into arsine gas (n.o: -III). However, their reliability and efficiency for the analysis is an area to explore in future research in terms of regeneration procedures and optimal cycles.

Although the standard for arsenic is 10 µg/L (WHO, 2022), it is possible to measure arsenic up to a concentration of 800 µg/L (R^2 =0,9914). The results of Table 2 show that complex formation would be delayed as sample volume increase with a concentration of 28.57 µg/L (Rodier et al., 2016). These results could also be due to insufficient reagents for the formation of the coloured complex, which reflects the amount of arsenic formed. As the volume of the arsenic solution increases, the concentration of the reagents in the reaction medium decreases with the same quantity of diethyldithiocarbamate. One possibility would be to increase the analysis time to allow reaction between all the molecules. However, this would be detrimental to the stability of the complex that has already been formed due to sensitivity and Ag^+ reduction (Budesinky,1979; Rice et al., 2012). The protocol was therefore modified to quantify larger volumes (100 mL) for the eventual determination of very low arsenic concentrations.

Based on the value of sample absorbance without extraction, the experimental procedure could be oriented toward twice or threefold extraction based on LoQ of 5 µg/L. The standard method (AFNOR, 1993) recommends a sample volume of 350 mL. The modified method presented by other authors (Rodier et al., 2016) proposes a volume of 35 mL with a detection limit of around 25 µg/L. The modifications made in this study enabled us to quantify concentrations below 5 µg/L. Below this value, the multiple extraction of a volume of 100 mL enables low concentrations to be quantified (Table 4). A 100 mL sample is sufficient to measure arsenic concentrations in water up to 5 µg/L (Figure 6 and 7). Below this concentration, a multiple extraction would be required for quantitative analysis. This study shows that 35 mL as sample volume can therefore be used to measure arsenic concentration in water samples up to 10 µg/L, the WHO standard for arsenic concentration (WHO, 2022).

Conclusion

Arsenic, one of the carcinogenic water contaminants, is a source of concern due to its harmful impact on human health. Improving existing simple techniques is a key objective, enabling any small laboratory to carry out quantitative analyses and water to be classified in accordance with existing standards. The present study enabled to reduce the detection limit of an existing colorimetric method to 5 µg/L below the WHO standard. For expected concentrations above 5 µg/L, a sample volume of 100 mL is sufficient for quantitative determination of arsenic during two hours by changing the volume of involved solutions (HCl, KI and $SnCl_2$) and doubling the zinc's mass. The findings indicated also that multiple extraction can be used to analyse less concentrated samples (<5 µg/L). The aging of zinc shots revealed that they can be useful by performing several washing with an appropriate mixture. However, a loss of red

complex formation around 10% could occurred with previously used zinc shots.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

This work was carried out with all the authors. AAD analysed the data and drafted the manuscript. AAD, KVA, MLB, TBT and GD-B contributed to the interpretation of the data and the critical revision of the content of the article. MLB, initiator of the research activity, corrected the manuscript.

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