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A Simple, Stable, and Highly Sensitive Spectrophotometric Method for the Determination of Arsenic(III) from Different Biological Media in the Presence of Nanosilica-Cysteine Composite

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Abstract: This paper describes a selective and fairly stable colorimetric approach to determine trace amounts of arsenic conjugated with nanosilica-cysteine composite in various aqueous and biological samples in milligram per liter (mg/L) using Leucocrystal Violet (LCV) as a chromogenic reagent. Attenuated total reflectance-Fourier-transform infrared (ATR-FTIR) spectroscopy analysis was applied to characterize the composite. Novelty of this method is dealing with the presence of nanosilica which is reflected in the difficulty of obtaining a clear solution. The maximum absorbance is measured and Beer's law shows linearity over the concentration range of (0.75 to 5.00 mg/L) of As(III) at 590 nm. The molar absorptivity, Sandell's sensitivity, and detection limit of the method were found to be 6.00×10^5 L/mol.cm, 8.55×10^{-2} μg/cm^2, and 0.043 mg/L, respectively. The optimum reaction conditions and other analytical parameters were evaluated. Arsenic was successfully detected in a variety of aqueous and biological samples using the proposed method.

Keywords: Arsenic determination, nanosilica, spectrophotometry, Leucocrystal Violet, cysteine.

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1. INTRODUCTION

Arsenic is a chemical element that has the symbol As, atomic number 33, and standard atomic weight of 74.922 g/mol. Arsenic is a metalloid, its compounds are found all over the natural world, and have both metallic and nonmetallic properties. The two most prevalent oxidation states are trivalent (arsenite) and pentavalent (arsenate) (1-3). Arsenic is primarily absorbed by humans through drinking water, which is regarded as the most important source globally. According to the NRC's Washington 2001 report, drinking water containing inorganic arsenic species has detrimental effects on one's health when exposed for an extended period of time, including damage to the gastrointestinal tract, skin and internal cancers, cardiac damage, and vascular disorders. According to WHO recommendations (4), the maximum permissible level of arsenic in drinking water is 10 μg/L. According to Kohlmeyer and his co-workers (5), certain regions in India, Bangladesh, China, and Mongolia, groundwater arsenic levels exceed 1000 ng/mL. Arsenic's toxicity, availability, and environmental mobility are completely unrelated to its chemical form. Arsenic can be found in many environmental matrices, including natural water and soil, in various oxidation states, and organic and inorganic forms. An accurate assessment of the environmental and biological effects of arsenic necessitates precise knowledge of the system's arsenic compounds, which has increased the demand for analytical methods for their determination at trace levels (6). In literature, various approaches to the analysis of arsenic have been described, including spectrophotometry (7), cathodic and anodic stripping voltammetry (8,9), hydride generation atomic absorption spectrometry (10), ion chromatography and coupled plasma mass spectrometry (11), neutron activation analysis (12), potentiometry (13), hydride generation atomic fluorescence spectroscopy (14), and electrothermal atomic absorption spectrometry (15). The spectrophotometric methods are the most

popular among these because of their low cost performance, but in general, the other methods are expensive and require trained staff. Spectrophotometry is a simple and sensitive method for the determination of arsenic. An extensive range of reagents is appropriate for the spectrophotometric determination of arsenic, according to the literature review, such as ammonium-hexamethylenedithiocarbamate (16), 2,4-dihydroxy benzophenone-2-amino thiophenol (17), azure B (6), ammonium pyrrolidine dithiocarbamate (18), Toluidine blue or Safranine O (19), Leuco malachite green (20), Rhodamine B (21), and Leucocrystal Violet (22). The majority of these methods are constrained by the following: interference from a lot of ions, low sensitivity, and the need for heating or extraction into organic solvents (23). As for these spectrophotometric methods, The LCV method is easily carried out without the use of solvent extraction (22,24,25). The process relies on the release of iodine from the reaction of As(III) with potassium iodate in an acidic medium. Iodine that has been released oxidizes the dye, which results in colour development.

Modified silica nanoparticles find wide use as supports of catalytic systems, fillers in the production of nanocomposites, optical electronic devices, electrochemical sensors, photonics (26), and also in drug delivery (27). Silica particles with a hydrophilic surface layer are biologically inert, which determines their promising use in medicine and biotechnology (27). The structures of functional silica nanoparticles can be very diverse (26). However, nanotechnology in the biomedical and environmental domains has vastly increased the chances of integrating various inorganic metals (i.e. arsenic) due to the help of adsorption and binding forces (27). But the problem is dealing with this irritated nano-system while the determination of the focused ion/s. The presences of nano particles as suspended particles interfere with the spectrophotometric determination. For this reason, a brand-new method must be developed to address the deficiencies in arsenic determination in the presence of nano silica currently in use. The goal of the current work is to address the shortcomings that currently exist and offer a spectrophotometric determination of arsenic at ppm level (mg/L) that is simple, sensitive, and inexpensive.

2. EXPERIMENTAL SECTION

2.1. Instruments

Utilizing RADWAG® AS 220.R2, Electronic Balance, weight was measured. The pH of solutions was measured using BANTE pH–meter (PHS-25CW). Centrifugation was achieved using (DJB Lab Care-AIC PK 130) at 4000 RPM speed. Samples were shaken using GFL-85 thermostatic shaker. DHP-9052 heating incubator was used to heat the samples. GRIFFIN (1-150) vacuum oven was used to dry samples at 25.0ºC, and 630 mm Hg. The attenuated total reflectance-Fourier transform infrared spectrum was recorded on a Bruker Vertex 70-FT-IR spectrometer at room temperature coupled with a vertex Pt-ATR-FTIR accessory. A method was used to calculate the As(III) concentration utilizing Vis-spectrophotometer from METASH model V-5100, and a 1.0 cm quartz cell. Sharp (SJ-K145-SL3) Refrigerator, China used to cool (5.0ºC) and freeze investigated samples (-3.0ºC).

2.2. Reagents and solutions

Silicon dioxide (SiO_2) nano powder, 10-20 nm particle size (BET), 99.5% trace metals basis, L-Cysteine ($C_3H_7NO_2S$) (≥ 98%) from non-animal source and Leucocrystal Violet (4,4′,4″-Methylidynetris(N,N-dimethylaniline) from Sigma Aldrich, Ninhydrin ($C_9H_6O_4$) from Bio Basic Inc. Potassium iodate (KIO_3) and hydrochloric acid (HCl) (37%) from VWR Chemicals, cadmium(II) chloride ($CdCl_2$) and sodium nitrate ($NaNO_3$) (99%) from Riedel-de Haën, arsenic trioxide (As_2O_3) (99.5%) from BDH Chemicals Ltd Poole England. Ortho-phosphoric acid (H_3PO_4) (85%) from Labchem Laboratory Chemicals. Nitric acid (HNO_3) (69.5%) from Scharlau and glacial acetic acid (CH_3COOH) from Tedia. Sodium hydroxide pellets (NaOH) from Merck and absolute ethanol (C_2H_5OH) (99.9%) from BBC Chemicals for Lab. Normal saline solution (0.9% w/v NaCl) and dextrose 5% solution from DEMO Pharmaceutical Industry, Greece. Ringer lactate solution from Pharmaceutical Solutions Industry, in Saudi Arabia. Qualitative filter paper (Whatman 2) from Whatman International Ltd., England. Quantitative filter paper (grade 94) and Glass microfiber (grade 161) from Ahlstrom, USA, hydrophobic PTFE and hydrophilic Nylon (0.22 µm syringe filters) were obtained from Hawach Scientific, China.

2.3. Spectrophotometric procedure for As(III)

2.3.1. Preparation of Leucocrystal Violet indicator solution

Leucocrystal violet solution was prepared by adding 250 ± 0.1 mg of Leucocrystal Violet i.e. 4,4',4"-methylidynetris-(N,N'-dimethylaniline) into 200.0 ± 0.1 mL of distilled water with 3.0 ± 0.1 mL of 85% phosphoric acid in a 1 L volumetric flask and shaken gently until the dye dissolved. The content of the flask was diluted to 1 L with distilled water. This indicator was stable for several months and was used as a spectrophotometric reagent in the determination of As(III) concentration.

2.3.2. Preparation of standard curve of As(III)

The stock solution of arsenic (1000 ± 1 mg/L) was prepared by dissolving 500 ± 0.1 mg of arsenic trioxide in 20 ± 0.1 mL of 2 g ± 0.1 mg NaOH, which was neutralized by adding dilute HCl to make acid. The solution was then made up to the mark in a 500 mL volumetric flask by adding distilled water. From stock solution, a working solution of 100 ± 1 mg/L has been prepared. These two solutions were used to build up an analytical calibration curve with different concentrations (0.75, 1.25, 1.50, 2.50, 3.00, 4.00, and 5.00 mg/L).

2.3.3. Optimizing experimental conditions

The experimental conditions were optimized by studying the influence of the following parameters (reagents concentration, temperature and duration of heating, and pH) with 6.5 mL of 5.00 mg/L As(III) solution.

2.3.4. Amount of potassium iodate

Through various volumes (0.10 - 1.00 mL) and different concentrations (0.5 – 2.0 %), the impact of potassium iodate concentration on the reaction system was investigated. This amount was added to As(III) aliquot in acidic media in which As(III) reacts with potassium iodate to release iodine quantitatively.

2.3.5. Amount of acid
Four acids (sulfuric, phosphoric, hydrochloric, and acetic acid) were tested for their effect on the liberation of iodine in the procedure. Different volumes (0.10 - 0.50 mL) of acid concentration ranges from 0.1 – 0.5 M were evaluated to see how well iodine was released from iodate by the reaction with As(III).

2.3.6. Volume of LCV
A volume range (0.10 – 1.00 mL) of 0.025 % LCV was investigated to achieve the optimum conditions in order to obtain the desired violet colour, which is produced when LCV is selectively oxidized by the freed iodine when As(III) reacts with potassium iodate.

2.3.7. Volume of NaOH
LCV is oxidized to crystal violet (CV) in a mild acidic medium (i.e. pH~4.5). A range of (1 - 10 drops) of 2.0 M NaOH solution was investigated to find the right number of drops that required reaching pH~4.5.

2.3.8. Effect of temperature and duration of heating
For complete colour development, the reagent system requires heating. A heating incubator was used to investigate the effect of temperature and the duration of heating on an aliquot of 7.5 ± 0.1 mL of reaction mixture containing 5.00 mg/L As(III) to achieve the required colour. A temperature range of (25, 30, 35, 40, 45, 50) ± 0.5ºC for 10, 15, 20, 25, and 30 min duration time were instigated.

2.3.9. Effect of pH
Before making a spectrophotometric determination, the pH of the medium affects the formation and stability of the CV indicator. The effect of pH was studied by varying the pH from 2.00 ± 0.01 to 9.00 ± 0.01 using drops of 0.1 M HCl or 0.1 M NaOH for an aliquot of 7.5 ± 0.1 mL of reaction mixture containing 5.00 mg/L As(III).

2.4. Modification of nanosilica with cysteine
A 36 g ± 0.1 mg (0.6 mole) of the nanosilica was dissolved in 600.0 ± 0.1 mL of distilled water then adjust the pH to 5.60 ± 0.01. A 36 g ± 0.1 mg (0.3 mole) of cysteine was added to nanosilica solution and shaken using a magnetic stirrer for 48 hrs. Then the mixture was filtered by centrifugation for 30 min at 4000 rpm and in a vacuum oven, the solid was dried at 25 ± 0.5ºC for 5 days (yield 90%). The product is labeled as (SiO_2-Cys).

2.5. Loading modified form of nanosilica with arsenic trioxide
A 50 ± 0.1 mg of (SiO_2-Cys) was dissolved in 25.0 ± 0.1 mL of 50 ± 1 mg/L As(III) at pH 6.00 ± 0.01 and 25.0 ± 0.5ºC, shook for 96 hour, then centrifuged and dried in vacuum oven at 25.0 ± 0.5ºC for another 5 days. The product (SiO_2-Cys/ATO) is called modified nanosilica with ATO.

2.6 Characterization using Attenuated total reflectance-Fourier Transform Infrared (ATR-FTIR) Spectroscopy Analysis
SiO_2-Cys and SiO_2-Cys with As(III) (SiO_2-Cys/ATO) spectra of ATR-FTIR were recorded using a Vertex 70-FT-IR spectrometer (Bruker, Germany) at room temperature coupled with a vertex Pt-ATR-FTIR accessory.

2.7. Obtaining a method for the determination of As(III) in presence of nanosilica-cysteine composite from aqueous media
A 10 ± 0.1 mg of prepared (SiO_2-Cys/ATO) was added to vessels containing 50.0 ± 0.1 mL of distilled water and shook for 48 hr at 250 rpm. A blank sample was prepared in the same manner using (SiO_2-Cys). In order to deal with colloidal nanoparticles solution, different filtration techniques are investigated before applying determination procedure; including centrifugation, gravity filtration using qualitative and quantitative filter papers, and vacuum filtration (suction) using previous types of filter papers in addition to microfiber filter paper. Micro-filters (0.22 µm hydrophilic and hydrophobic) were also tried. Moreover, cooling at 5.0 ± 0.5ºC for 24 hr, freezing at (-3.0 ± 0.5ºC) for 12 hr, and settling for 24, 48 or 72 hr have been investigated. Each technique/ method was followed by 60 min centrifugation at 4000 rpm to insure separation. After that an aliquot of 6.5 ± 0.1 mL was taken out for subsequent steps of the determination process, and readings were compared with a blank water sample.

2.8. Determination of As(III) in presence of nanosilica-cysteine composite from different media
Adding 10 ± 0.1 mg of (SiO_2-Cys/ATO) to vessels containing 50.0 ± 0.1 mL media solution (normal saline, dextrose, ringer lactate, water (all at pH=7.40 ± 0.01), and 0.1 M HCl were shook at 250 rpm for 48 hr and 37.5 ± 0.5ºC. After that the samples were settled for 48 hr and an aliquot was taken out for the determination procedure of As(III) ions. The concentration of As(III) in each sample was determined by comparison with a calibration curve based on the absorption maximum at 590 nm according to the proposed procedure.

3. RESULTS AND DISCUSSION

3.1 Characterization using ATR-FTIR Spectroscopy Analysis
The ATR-FTIR analysis was performed in order to establish the changes in the functional groups of SiO_2-Cys to insure loading of As(III). The spectrum of SiO_2-Cys (Figure 1b) shows distinctive peaks at three main wavenumbers: 1077, 800 and 453 cm^{-1} which corresponds to the asymmetric, symmetric modes of Si–O–Si, bending O-Si-O, respectively and a characteristic peak at 962 cm^{-1} for the silanol group stretching vibration (28). The red shift in asymmetric Si–O–Si band from original 1060 cm^{-1} on nanosilica to 1077 cm^{-1} on SiO_2-Cys indicated the interaction of amino acid with surface silanols of nanosilica (29).

Another peaks: 1583 cm^{-1} (COO^- asymmetric stretching), 1486 cm^{-1} (N-H bending) and 1406 cm^{-1} (COO^- symmetric stretching) were also observed. The existence of COO^- and N-H peaks showed that cysteine is present as a zwitterion molecule (30).

ATR-FTIR spectra of the bare As_2O_3 (Figure 1c) shows the prominent peak of As–O stretching vibration at 802 cm^{-1} and another peak at 474 cm^{-1} which is related to As–O bending (31).

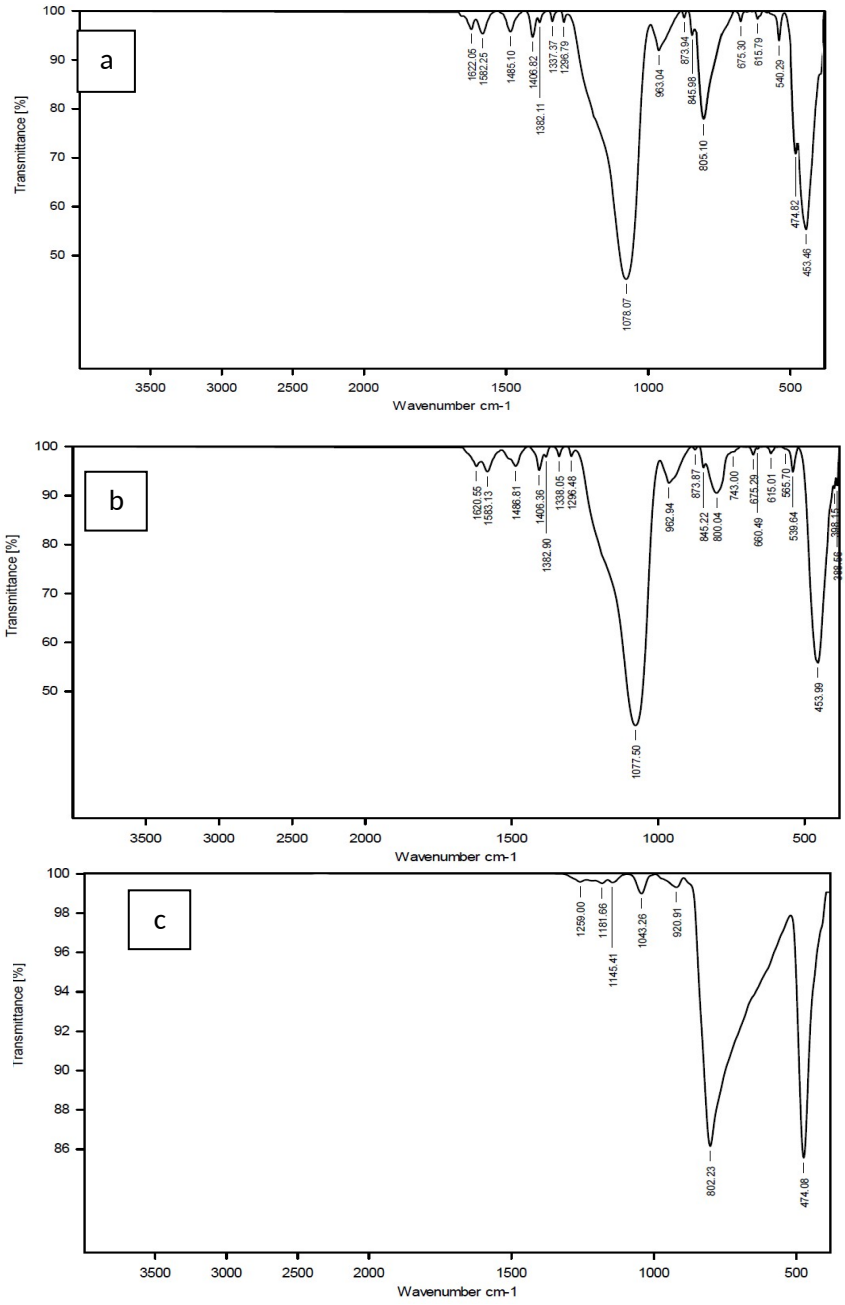


Figure 1: ATR-FTIR spectra for a) SiO_2-Cys/ATO, b) SiO_2-Cys, and c) ATO.

3.2. Reaction mechanism

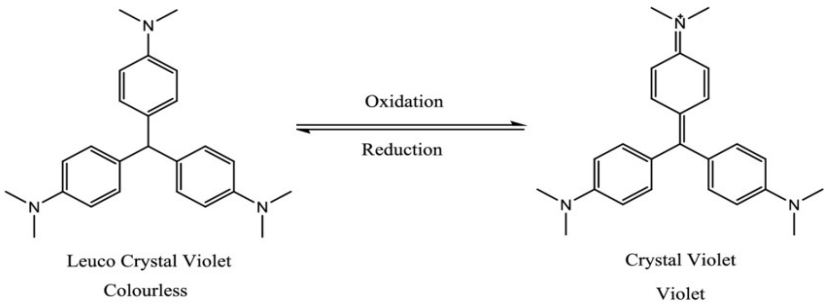
In acidic medium of low pH (pH~1), As(III) reacts with potassium iodate to release iodine quantitatively. Crystal violet (CV) dye is produced (Scheme 1) when LCV is selectively oxidized by the freed iodine to give a violet colour in the presence of sodium hydroxide (pH~4.5)(22). The reaction steps are as follows:

Step 1

$$5H_3AsO_3 + 2KIO_3 + 2HCl \rightarrow 5H_3AsO_4 + I_2 + H_2O$$

Step 2

$$I_2 + 2LCV \rightarrow 2I^- + 2CV^+$$



Scheme 1: LCV oxidation to CV

From Figure 1, the highest absorption for CV dye was at 590 nm while the blank had a negligible absorbance at this wavelength.

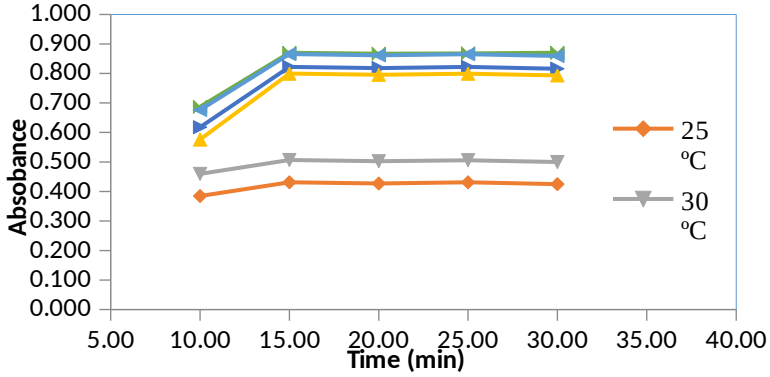


Figure 2: Absorption spectra of a) CV dye [3 mg/L As] versus reagent blank, b) reagent blank versus distilled water.

The experimental setup was perfected by looking at the impact of the following parameters with 5.00 mg/L As(III) in an approximately final volume of 7.50 ± 0.1 mL in order to make the colour system as sensitive as possible.

3.3. Amount of reagents

The reaction system's response to the concentration of potassium iodate was studied with (0.10 - 1.00 mL) of different concentrations (0.5 – 2.0 %). According to Table 1, it was found that 0.50 mL of 1 % KIO_3 or 1.00 mL of 0.5 % KIO_3 solution was sufficient for quantitative liberation of iodine.

It was noticed that hydrochloric acid is more suitable and efficient than other acids (sulfuric, phosphoric, and acetic acid) liberation of iodine from iodate by the reaction with As(III). A concentration of 0.5 M hydrochloric acid through different volumes (0.25 - 0.50 mL) in an overall volume of 7.5 mL of reaction mixture was effective for the liberation of iodine from iodate by the reaction with As(III); precisely 0.25 ± 0.01 mL of 0.5 M hydrochloric acid (Table 2). So adding this amount of HCl to the reaction mixture prior to the subsequent steps is the most appropriate for the reaction process.

A 0.025 % LCV was considered to be the most appropriate concentration to use in the determination of As(III) as obtained by Agrawal and his team (22). It was noticed that the values of absorbance were constant in the volume range 0.25 – 0.50 mL of 0.025 % LCV under the optimum conditions (Table 3). Lower concentrations of the indicator caused a drop in absorbance, and higher concentrations caused turbidity to form. As a result, an optimum volume of 0.25 ± 0.01 mL of 0.025 % LCV was used in the method to obtain the desired dye colour. Nevertheless, in order to oxidize LCV to CV, a mild acidic medium (i.e. pH~4.5) is achieved using 1-2 drops of 2 M NaOH solution to the final reaction mixture and was adequate to reach the desired results.

Table 1: Effect of potassium iodate amount on the reaction system.

KIO_3 Concentration (w/v)	KIO_3 Volume (mL)	Absorbance of 5.00 mg/L As(III)		
0.5 %	0.10 ± 0.01	N/A	N/A	N/A
	0.25 ± 0.01	0.167	0.164	0.168
	0.50 ± 0.01	0.332	0.328	0.329
	0.75 ± 0.01	0.504	0.511	0.508
	1.00 ± 0.01	0.669	0.660	0.665
1.0 %	0.10 ± 0.01	0.133	0.133	0.130
	0.25 ± 0.01	0.339	0.337	0.331
	0.50 ± 0.01	0.666	0.669	0.670
	0.75 ± 0.01	0.660	0.668	0.663
	1.00 ± 0.01	0.658	0.665	0.665
1.5 %	0.10 ± 0.01	0.208	0.199	0.200
	0.25 ± 0.01	0.490	0.498	0.500
	0.50 ± 0.01	0.567	0.560	0.556
	0.75 ± 0.01	0.656	0.666	0.658
	1.00 ± 0.01	0.664	0.660	0.657
2.0 %	0.10 ± 0.01	0.256	0.261	0.265
	0.25 ± 0.01	0.643	0.637	0.638
	0.50 ± 0.01	0.649	0.656	0.651
	0.75 ± 0.01	0.661	0.653	0.655
	1.00 ± 0.01	0.651	0.661	0.658

Table 2: Effect of acid amount on the reaction system.

Acid	Acid Concentration (M)	Acid pH	Acid Volume (mL)	Absorbance of 5.00 mg/L As(III)		
Sulfuric acid	0.1	1.00	0.10 ± 0.01	0.220	0.214	0.218
			0.25 ± 0.01	0.305	0.299	0.303
			0.50 ± 0.01	0.320	0.314	0.318
	0.3	0.52	0.10 ± 0.01	0.433	0.426	0.431
			0.25 ± 0.01	0.541	0.534	0.539
			0.50 ± 0.01	0.558	0.552	0.556
	0.5	0.30	0.10 ± 0.01	0.663	0.656	0.661
			0.25 ± 0.01	0.785	0.779	0.784
			0.50 ± 0.01	0.799	0.792	0.797

Phosphoric acid	0.1	1.55	0.10 ± 0.01	N/A	N/A	N/A
			0.25 ± 0.01	N/A	N/A	N/A
			0.50 ± 0.01	0.118	0.111	0.116
	0.3	1.28	0.10 ± 0.01	0.226	0.219	0.224
			0.25 ± 0.01	0.256	0.249	0.254
			0.50 ± 0.01	0.308	0.301	0.306
	0.5	1.16	0.10 ± 0.01	0.378	0.372	0.376
			0.25 ± 0.01	0.416	0.408	0.413
			0.50 ± 0.01	0.518	0.511	0.516
Hydrochloric acid	0.1	1.00	0.10 ± 0.01	0.238	0.231	0.236
			0.25 ± 0.01	0.317	0.308	0.313
			0.50 ± 0.01	0.328	0.321	0.327
	0.3	0.52	0.10 ± 0.01	0.456	0.449	0.454
			0.25 ± 0.01	0.567	0.559	0.564
			0.50 ± 0.01	0.578	0.571	0.576
	0.5	0.30	0.10 ± 0.01	0.678	0.671	0.676
			0.25 ± 0.01	0.815	0.809	0.813
			0.50 ± 0.01	0.818	0.810	0.816
Acetic acid	0.1	2.88	0.10 ± 0.01	N/A	N/A	N/A
			0.25 ± 0.01	N/A	N/A	N/A
			0.50 ± 0.01	N/A	N/A	N/A
	0.3	2.64	0.10 ± 0.01	N/A	N/A	N/A
			0.25 ± 0.01	0.111	0.105	0.109
			0.50 ± 0.01	0.118	0.111	0.116
	0.5	2.53	0.10 ± 0.01	N/A	N/A	N/A
			0.25 ± 0.01	0.113	0.109	0.116
			0.50 ± 0.01	0.132	0.125	0.130

Table 3: Effect of LCV amount on the reaction system.

LCV Concentration	LCV Volume (mL)	Absorbance of 5.00 mg/L As(III)		
0.025 %	0.10 ± 0.01	0.635	0.629	0.633
	0.25 ± 0.01	0.866	0.867	0.863
	0.50 ± 0.01	0.863	0.869	0.857
	0.75 ± 0.01	> 1.3	> 1.3	> 1.3
	1.00 ± 0.01	> 1.3	> 1.3	> 1.3

3.4. Effect of temperature and duration of heating

Under optimum conditions, the reagents system required heating at 45.0 ± 0.5ºC in a thermostat oven for complete colour development (Figure 2), since absorbance was markedly affected below this temperature. However, duration time of 15 min is efficient to achieve the required colour. The development of the colour was unaffected by an increase in temperature (> 45ºC) or by duration of heating (> 15 min).

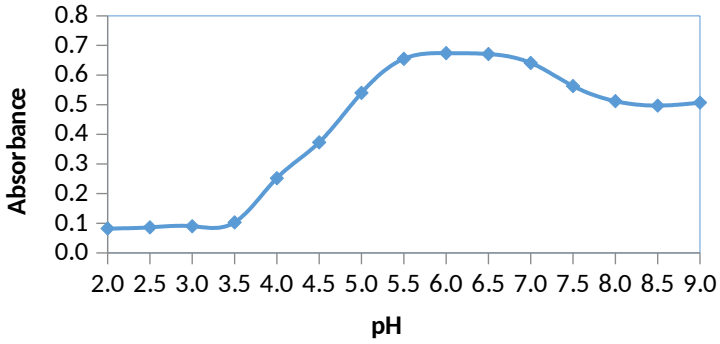


Figure 3: Effect of temperature and duration of heating on the reaction system through average absorbance of three determinations.

3.5. Effect of pH

The formed CV dye was pale violet in colour below pH 5.00 (after addition of 2.0 M NaOH). The colour of CV dye (Figure 3) developed to the full violet colour with decreasing acidity to 5.50 – 6.50 with an optimum pH of 6.00 ± 0.01. An increase of pH above 6.50 severely affected the stability and sensitivity of the dye. Colour development did not take place below pH 3.50. It was also found that a 15 min time period is needed for a complete colour development after adjusting the pH prior to the colorimetric measurement. The formed dye was stable for several weeks.

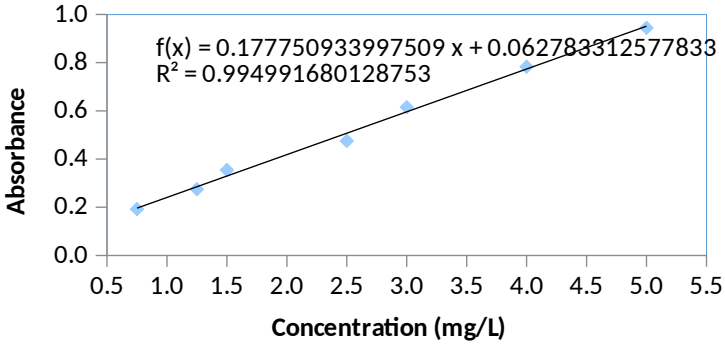


Figure 4: Effect of pH on the absorbance on CV dye [5 mg/L As(III)]

3.6. Optimum procedure for the determination of As(III) in aqueous solutions

Under the proposed reaction conditions, 0.50 ± 0.01 mL of 1 % potassium iodate and 0.25 ± 0.01 mL of 0.5 M HCl were added to a 6.50 ± 0.01 mL aliquot of As(III) solution and the mixture was shaken gently at 150 rpm for 5 min, followed by addition of 0.25± 0.01 mL of LCV and 2 drops of 2.0 M NaOH solution. The solution was kept in an incubator at 45.0 ± 0.5ºC for 15 min. The pH was adjusted to 6.00 ± 0.01 and stand for 15 min before the colorimetric measurement. The absorbance was measured at 590 nm against a reagent blank that was prepared in the same manner as mentioned above.

3.7. Analytical data

A linear correlation was found between absorbance and concentration of As(III). The calibration graph (Figure 4) shows linear relationship with a coefficient of determination (R2= 0.995) in the concentration range (0.75 – 5.0 mg/L) of As(III). This calibration graph was used to obtain As(III) concentration in solutions.

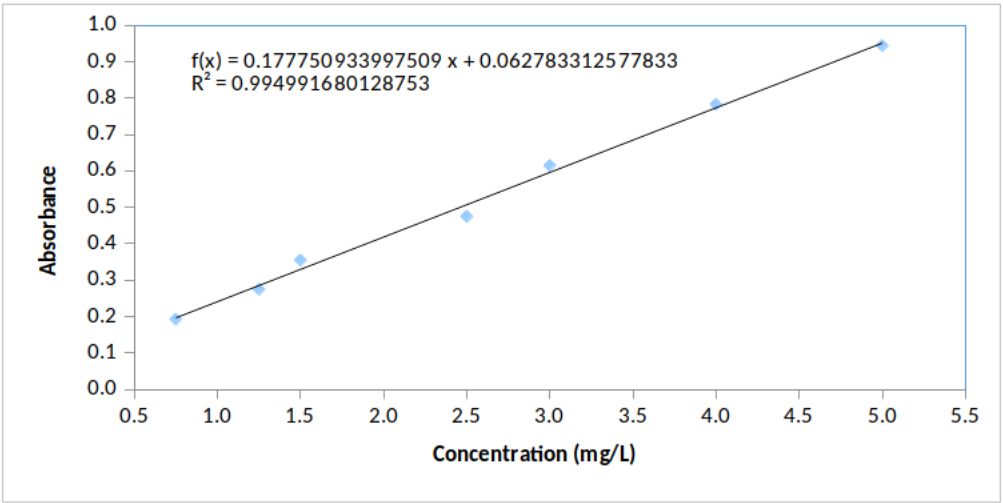


Figure 5: Calibration graph for As(III) determination

Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses (32). Herein, the absorbance values of six blank water samples were: 0.156, 0.150, 0.153, 0.153, 0.155, and 0.157 in which the standard deviation (σ = 0.0025).

Limit of detection (LOD) and limit of quantification were calculated according to ICH guidelines as LOD = 3.3 × σ/S and LOQ = 10 × σ/S, where σ is standard deviation of response and S is slope of calibration curve (32). LOD and LOQ values were found to be 0.047 ± 0.002 and 0.143 ± 0.006 mg/L, respectively.

The molar absorption coefficient (ε) could be calculated from Beer–Lambert law (A = εbc), where A is absorbance, c is concentration of the absorbing species in mol/L and b is path length in cm. So for ε, the equation becomes ε = A/bc. However, in a graph of A versus c, the slope will be εb. Since the path length in our experiment is 1 cm, and converting the slope from 0.117 mg/L to 5.9× 10^{-7} mol/L, the molar absorption coefficient is 1.69× 10^{6} L/mol.cm.
Sensitivity of proposed method is determined by calculating Sandell's sensitivity (33), which can be defined as the concentration of the analyte in ppm (mg/L) which will give an absorbance of 0.001 in a cell of 1 cm path length. Sandell's sensitivity is expressed as: (0.001 * 1cm)/slope, and was found to be 0.0855 μg/cm^2.

The accuracy of the method was established by analyzing As(III) at three concentration levels covering the specified range and the precision was ascertained by calculating the relative standard deviation of ten replicate determinations on the same solutions at three concentration levels are presented in Table 4. The relative error and relative standard deviation indicates the high accuracy and precision for this method.

Table 4: Evaluation of accuracy and precision

Amount added (mg/L)	Amount found (mg/L)*	RE (%)	SD (mg/L)	RSD (%)
1.25 ± 0.02	1.22 ± 0.04	0.020	0.015 ± 0.01	1.26 %
	1.23 ± 0.03	0.016		
	1.20 ± 0.05	0.008		
3.00 ± 0.04	2.94 ± 0.11	0.020	0.025 ± 0.01	0.86 %
	2.89 ± 0.07	0.017		
	2.91 ± 0.13	0.010		
5.00 ± 0.07	4.90 ± 0.22	0.018	0.020 ± 0.01	0.41 %
	4.94 ± 0.16	0.012		
	4.92 ± 0.19	0.014		

*Mean value of five determinations
RE - Relative Error; SD - Standard Deviation; RSD - Relative Standard Deviation

3.8. Obtaining a method for the determination of As(III) in presence of nanosilica-cysteine composite from aqueous media

Much attention is given in the recent time to functionalized derivatives of silica nanoparticles. The structures of functional silica nanoparticles can be very diverse (26). However, the solutions of silica nanoparticles form stable colloids, these suspensions considered to be a disadvantage while dealing with colorimetric detection. Colorimetric methods for the determination of arsenic in environmental and biological samples using LCV were conducted, but none has proven effective when dealing with colloidal solutions in presence of silica nanoparticles (22-24), or in environmental and biological samples (7,17,20-21,34).

Therefore, development of a method in order to deal with the nanoparticles solution is applied by using different filtration techniques and methods. Table 5 illustrates the absorbance achieved for blank and spiked samples. According to results shown in Table 5, using centrifugation as a single filtration technique at different times did not resolve the interference caused by the presence of nanosilica which is clear from the SiO_2-Cys absorbance results. So for the gravity and suction filtration techniques, neither SiO_2-Cys nor SiO_2-Cys/ATO absorbance results seems real, even with applying different types of filter papers. However, solutions obtained from previous techniques still having colloid properties. Using a 0.22 µm hydrophilic (Nylon) and hydrophobic polytetrafluoroethylene (PTFE) filters shows an improvement in results but still not reaching the desired ones. Having the samples cooled in the refrigerator at 5.0 ± 0.5ºC for 24 hr prior to the determination process shows a similar behaviour to the filters technique. Freezing the samples at -3.0 ± 0.5ºC and thawing after 12 hr lead up to settling the particles in the solution including the As(III) ions which result in a failure in the determination process of SiO_2-Cys/ATO. From Table 5, it's noticed that leaving the sample (suspension) to settle for 48 hr prior to the determination process has the best absorbance relative to blank water sample, in which it could be explained that this period of time is necessary for the settling of the colloid nanoparticles and achieve a much better clear solution. Increasing settling time did not improve the results. Regarding to other filtration techniques, it was found that a turbid solution is always achieved which gives false measurement values. As a result, we developed a suitable procedure commensurate with the constraints that accompany the detection process in order to achieve actual results for the determination of arsenic in such systems.

3.9. Determination of As(III) in presence of nanosilica-cysteine composite in different media

When dealing with drug delivery and stability, various biological solutions are options that could be used. The most popular ones were chosen as shown in Table 6. After the solutions have been shaken as in the proposed procedure, they were left to settle for 48 hr prior to the determination process. It can be noticed that the highest stability is in Ringer lactate and the lowest is in 0.1 M HCl.

Table 6: Determination of 5.00 mg/L As(III) in presence of nanosilica-cysteine composite from different media at 25ºC

Media	pH	Absorbance of SiO_2-Cys*	Absorbance of SiO_2-Cys/ATO*	Amount of As(III) detected (mg/L)
Normal saline	7.40 ± 0.01	0.131	0.361	0.949
Dextrose	7.40 ± 0.01	0.102	0.236	0.407
Ringer lactate	7.40 ± 0.01	0.102	0.219	0.311
Water	7.40 ± 0.01	0.125		0.475
0.1 M HCl	1.00 ± 0.01	0.122	0.376	1.085

*Mean value of three determinations

**Table 5 is on the next page.*

Table 5: Absorbance of blank and nanosilica-cysteine composite solutions using different filtration techniques and methods.

Filtration technique/ Method		Centrifugation Time (min)	Absorbance of blank water	Absorbance of SiO_2-Cys*	Absorbance of SiO_2-Cys/ATO*
Centrifuge		30	0.064	> 1.5	> 1.5
		60		1.301	> 1.5
		120		1.292	> 1.5
Gravity filtration	Qualitative filter paper	60	0.064	> 1.5	1.497
	Quantitative filter paper			1.292	1.355
Vacuum filtration (suction)	Qualitative filter paper	60	0.064	1.355	> 1.5
	Quantitative filter paper			1.286	> 1.5
	Glass microfiber			1.065	> 1.5
0.22 µm filters	Hydrophilic Nylon	60	0.064	0.581	1.136
	Hydrophobic PTFE			0.579	1.089
Settling	24 h	60	0.064	0.242	0.799
	48 h			0.153	0.301
	72 h			0.150	0.299
Cooling (5.0ºC)	24 h	60	0.064	0.568	1.286
Freezing (-3.0ºC)	12 h	60	0.064	0.260	N/A

*Mean value of three determinations

4. CONCLUSION

The use of Leucocrystal Violet as a reagent for the spectrophotometric measurement of arsenic is described in this paper. It provides sensitivity, selectivity, simplicity, and cost-effectiveness. Since there is no extraction steps involved into an organic solvent, it is considered a green method. The method's usefulness is demonstrated by the method's satisfactory applicability to the determination of arsenic in environmental and biological samples in presence of nanosilica-cysteine composite. Spectrophotometry is still a common and necessary method for determining arsenic at trace levels despite the availability of many sophisticated alternatives, particularly in laboratories in developing nations with limited budgets due to factors like instrument's low cost, simplicity, minimal maintenance requirements, and no consumables required. However determination of arsenic in urine and serum samples in presence of nanosilica-cysteine composite has not been investigated yet, in which could be a scope of future work.

5. CONFLICT OF INTEREST

There is no conflict of interest to report.

6. REFERENCES

1. Tatken RL, Lewis RJ. Registry of Toxic Effect of Chemical Substance. 1981–82 ed. US Dept of Health and Human Services, Cincinnati; 1983.

2. Cullen WR, Reimer KJ. Arsenic Speciation in the Environment. Chem. Rev. 1989;89:713-764. Available from: <URL>.

3. Ghazy SE. Removal of Cadmium, Lead, Mercury, Tin, Antimony, and Arsenic from Drinking and Seawaters by Colloid Precipitate Flotation. Sep. Sci. Technol. 1995;30:933. Available from: <URL>.

4. AWWA. Water quality and treatment. American water works association, Washington, DC; 1992.

5. Kohlmeyer U, Jantzen E, Kuballa J, Jakubik S. Benefits of high resolution IC-ICP-MS for the routine analysis of inorganic and organic arsenic species in food products of marine and terrestrial origin. Anal Bioanal Chem. 2003;377:6–13. Available from: <URL>.

6. Cherian T, Narayana B. A New Spectrophotometric Method for the Determination of Arsenic in Environmental

and Biological Samples. Anal Lett. 2005; 38: 2207–2216. Available from: <URL>.

7. Dasgupta PK, Huiliang HL, Zhang GF, Cobb GP. Photometric measurement of trace As(Ⅲ) and As(V) in drinking water. Talanta. 2002;58:153-164. Available from: <URL>.

8. Ferrerira MA, Barros AA. Determination of As(Ⅲ) and Arsenic(V) in natural waters by cathodic stripping voltammetry at a hanging mercury drop electrode. Anal Chim Acta. 2002;459:151-159. Available from: <URL>.

9. Kopanicia M, Novotny L, Determination of traces of arsenic(Ⅲ) by anoidic stripping voltammetry in solutions, natural waters and biological materials. Anal Chim Acta. 1998;368:211-218. Available from: <URL>.

10. Bundelaska JM, Stafilov T, Appadjian S. Direct analysis of natural waters for arsenic species by hydride generation atomic absorption spectrometry. Int J Environ Anal Chem. 2005;85:199-207. Available from: <URL>.

11. Heitkemper DT, Vela NP, Stewart KR, Westphal CS. Determination of total and speciated arsenic in rice by ion chromatography and inductively coupled plasma mass spectrometry. J Anal At Spectrom. 2001;16:299-306. Available from: <URL>.

12. Boadu M, Osae EK, Golow AA, Serfor-Armah Y, Nyarko BJ. Determination of arsenic in some water bodies, untreated ore and tailing samples at Konnongo in Ashanti region of Ghana and its surrounding town and villages by instrumental neutron activation analysis. J Radioanal Nucl Chem. 2001;249:581-585. Available from: <URL>.

13. Gupta VK, Agarwal S. PVC based 5,10,15,20-tetrakis (4-methoxyphenyl) porphyrinatocobalt(Ⅱ) membrane potentiometric sensor for arsenite. Talanta. 2005;65:730-734. Available from: <URL>.

14. Gomez MM, Kovecs M, Palacios MA, Pizarro I, Camara C. Effect of the mineralization method on arsenic determination in marine organisms by hydride generation atomic fluorescence spectroscopy. Microchim Acta. 2005;150:9-14. Available from: <URL>.

15. Abdolmohammad-Zahen H, Jouyban A, Amini R. Ultratrace determination of arsenic in water samples by electrothermal atomic absorption spectrometry after pre-concentration with Mg-Al-Fe ternary layered double hydroxide nano-sorbent. Talanta. 2013;116:604-610. Available from: <URL>.

16. Karayunlu S, Ay U. Spectrophotometric determination of total inorganic arsenic with hexamethylene ammonium-hexamethylenedithiocarbamate in nonionic triton X-100 micellar media. J Anal Chem. 2010;65:244-248. Available from: <URL>.

17. Deepa K, Lingappa Y. A simple spectrophotometric method for the determination of arsenic in industrial and environmental samples using 2,4-dihydroxy benzophenone-2-amino thiophenol. Spectrochim Acta, Part A. 2014;124:102-107. Available from: <URL>.

18. Yuji S, Kato T, Nukatsuka I, Ohzeki K. Spectrophotometric determination of arsenic(Ⅲ) based on solid phase extraction of the arsenic-APDC complex and the conversion to the copper complex. Bunseki Kagaku. 2003;52:1153-1158. Available from: <URL>.

19. Pasha C, Narayana B. Determination of arsenic in environmental and biological samples using toluidine blue or safranine O by simple spectrophotometric method. Bull Environ Contam Toxico. 2008;81:47-51. Available from: <URL>.

20. Revanasiddappa HD, Dayananda BP, Kumar TN. A sensitive spectrophotometric method for the determination of arsenic in environmental samples. Environ Chem Lett. 2007;5:151-155. Available from: <URL>.

21. Pillai A, Sunita G, Gupta VK. A new system for the spectrophotometric determination of arsenic in environmental and biological samples. Anal Chim Acta. 2000;408:111-115. Available from: <URL>.

22. Agrawal O, Sunita G, Gupta K. A Sensitive Colorimetric Method for the Determination of Arsenic in Environmental and Biological Samples. J. Chin. Chem. Soc. 1999;46:4. Available from: <URL>.

23. Stancheva K., Pasha C. Spectrophotometric Determination of Trace Amounts of As(III) and As(V) Using Safranine O and Fuchsine as New Reagents. Oxid. Commun. 2016;39(2):1538–1546. Available from: <URL>.

24. Kamaya M, Ito Y, Otomura Y, Ginatullina E. Comparative Study of Leuco Dyes as Reagents for Spectrophotometric Determination of Arsenic (Ⅲ). Asian J. Appl. Sci. 2015;3(1).

25. Moore R, Holt K, Zhao H, Salas F, Hasan A, Lucero D. Sorption of Arsenic from Drinking Water to Mg(OH)2 Sorrel's Cements, and Zirconium Doped Materials. Environmental Decisions and Program Development Sandia National Laboratories, United States; 2002.

26. Gorbatsevich OB, Kholodkov DN, Kurkin TS, Malakhova YN, Strel DR, Buzin AI, et al. Synthesis and properties of water soluble silica nanoparticles. Russ. Chem. Bull. 2017;66(3):409-417. Available from: <URL>.

27. Bajpai S, Tiwary S, Sonker M, Joshi A, Gupta V, Kumar Y, et al. Recent Advances in Nanoparticle-Based Cancer Treatment: A Review. ACS Appl. Nano Mater. 2021;4:6441−6470. Available from: <URL>.

28. Fan T, Liu Y, Feng B, Zeng G, Yang C, Zhou M, Zhou H, Wang X. Biosorption of cadmium(II), zinc(II) and lead(II) by Penicillium simplicissimum: Isotherms, kinetics and thermodynamics. J. Hazard. Mater. 2008;160(2):655–661. Available from: <URL>.

29. Du M, Zheng Y. Modification of silica nanoparticles and their application in UDMA dental polymeric composites. Polym. Compos. 2007;28:198–207. Available from: <URL>.

30. Khataee A, Movafeghi A, Nazari F, Vafaei F, Dadpour M, Y. Hanifehpour Y, Joo S. The toxic effects of L-Cysteine-capped cadmium sulfide nanoparticles on the aquatic plant Spirodela polyrrhiza. J. Nanopart. Res. 2014;16:2774-2784. Available from: <URL>.

31. Jadhav V, Sachar S, Chandra S, Bahadur D, Bhatt P. Synthesis and Characterization of Arsenic Trioxide Nanoparticles and Their In Vitro Cytotoxicity Studies on Mouse Fibroblast and Prostate Cancer Cell Lines. J. Nanosci. Nanotechnol. 2016;16:7599–7605. Available from: <URL>.

32. Miller JN, Miller JC. Statistics and Chemometrics for Analytical Chemistry, Prentice Hall, UK, 2005.

33. Shetty D, Narayana B, Samshuddin S. Sensitive methods for the spectrophotometric determinations of some anti-malarial drugs. J. Chem. Pharm. Res. 2012;4(3):1647-1653.

34. Echioda S, Adepeju O, Salisu S, Abdulrasheed A, Chindo I, Kolo A. UV-Vis Spectrophotometric Determination of Selected Heavy Metals (Pb, Cr, Cd and As) in Environmental, Water and Biological Samples with Synthesized Glutaraldehyde Phenyl Hydrazone as the Chromogenic Reagent. EJ-CHEM. 2021;2(3). Available from: <URL>.