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Voltammetric Approach of Arsenic (Total) Determination in Blood using Sctrace Gold Electrode

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Abstract

Normal levels of Arsenic (As) in the blood of unexposed individuals arereported to be less than 1 µg/L. The quantitative determination of arsenic traces and its compounds is significant to assess its deleterious effects on human health. The use of conventional methods (AAS and ICP–MS) for trace metal detection extend several issues such as high cost of equipment, need of highly trained engineers and extensive sample preparations. An alternativevoltammetric method has been developed for arsenic determination in blood using scTrace Gold elcetrode. The scTrace Gold sensor holds all three electrodes together required for a voltammetric determination. The working electrode is a gold microwire whereas reference and auxiliary electrodes are screen printed electrodes. Human blood was processed by closed microwave digestion using nitric acid and hydrogen peroxide. Arsenic determination was carried out by standard addition method using primary solution being swept at a rate of 0.992 V/s and pulse amplitude of 0.020 V. Cleaning was done at – 1.0V for 60 seconds and potential was scanned from 0.4V to -1.0V on RDE/SSE at 2400 rpm speed. With this method, the total arsenic i.e., As(III) + As(V) in the sample can be determined. As(V) species being electrochemically inactive are reduced in-situ by nascent hydrogen to As (III). Along with the As(III) present in the sample, it is further reduced electrochemically to As(0) and deposited on the gold working electrode in the same step. During the subsequent stripping step the deposited As(0) is reoxidized to As(III) giving the analytical signal. Arsenic was deposited on the electrode at -0.250 V for 5 seconds. The deposited metal was swept by scanning the potential from -0.300V to 0.40 V using square wave mode. The stripping current was correlated with the concentration of the metal present in the sample. The detection limit of arsenic was found to be 0.9 µg/L and the calibration was linear up to a concentration of 20 µg/L.

Keywords: Arsenic, Blood, scTrace Gold Electrode, Voltammetry

1. Introduction

Phenolic compounds act as natural antioxidants and Environmental pollution is one of the most significant problems we are facing in the present era. Heavy metal pollution is one of the major causes of environmental pollution. Heavy metals are naturally occurring elements with high atomic weight and are released by natural and anthropogenic sources. Metals are systemic toxicants that are known to induce multiple organ damage, even at a low level of exposure[1]. In general, lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As) are some of the most toxic metals which targets essential organ systems of the body namely hepatobiliary, Gastro-Intestinal Tract (GIT), Cardiovascular System (CVS), renal, integumentary and Central Nervous System (CNS)[2]. According to the World Health Organization (WHO) these four elements rank

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among the priority metals that are of great public health concerns[3].

Arsenic (As), a naturally occurring metalloid, is ubiquitous in the environment and contaminates water as a result of geological and industrial pollution. It ranks first in the United States Environmental Protection Agency (USEPA) list of prioritized pollutants with a major influence on global health. Arsenic ranks 20th in abundance in the earth's crust, 14th in the seawater and 12th in the human body[4]. Since ancient times, arsenic has been labelled as "poison of kings" and "king of poisons"[5]. Arsenic exists in the environment as pentavalent (As^{5+}, arsenate) and trivalent (As^{3+}, arsenite) forms, however, trivalent (As^{3+}) arsenic compounds are generally more toxic than pentavalent (As^{5+}) compounds[5-6]. Arsenic is being exposed in the environment through the natural (air, water, soil) and anthropogenic sources (mining, smelting, etc.)[7]. Arsenic and its compounds are used commercially and industrially in the manufacture of transistors, lasers and semiconductors, processing of glass, pigments, textiles, paper, wood preservatives etc[8]. It is extensively available in the form of oxides or sulfides or as a salt of iron, sodium, copper, etc[9]. The International Agency for Research on Cancer has classified arsenic as a Group 1human carcinogen[10].

Arsenic is a protoplasmic poison due to its effect on sulphydryl group of cells interfering with cells enzymes, cell respiration and mitosis[11]. Its toxicity is exhibited by the inactivation ofvarious enzymes, especially those involved in cellular energy pathways, DNA synthesis and DNA repair[12].Acute and chronic exposure to inorganic arsenic causes deleterious effects on almost every organ system of the human body. Acute arsenic poisoning is associated with nausea, vomiting, abdominal pain and diarrhea[13]. Acute arsenic ingestion can damage mucous membranes of the GIT, causing irritation, vesicle formation and even sloughing[14]. Chronic arsenic toxicity results through the consumption of drinking water with high levels of arsenic over a longer period. Chronic ingestion of arsenite through contaminated water results in accumulation of arsenite and Monomethylarsonous Acid (MMA) (III) in vital organs and tissues leading to the incidence of atherosclerosis, hypertension, ischemic heart diseases, diabetes, hepatotoxicity, nephrotoxicity and cancer of the skin, bladder and lungs[15].

Permissible level of arsenic in ground water is reported to be 0.05 mg/L[16]. Normal human levels of arsenic in unexposed individuals is < 1µg /L in blood, <100 µg/L in urine, ≤ 1ppm in nails, ≤ 1ppm in hairs[17]. The Risk Assessment Information System database states that the acute lethal dose of inorganic arsenic to humans has been estimated to be about 0.6 mg/kg/day[18].

Some of the proposed mechanisms underlying arsenic-induced toxic effects are: Generation of oxidative stress, genotoxicity, cell proliferation, altered DNA repair and DNA methylation, oxidative stress, co-carcinogenesis and tumor promotion[19].

The biotransformation (detoxification process)of arsenic involves alternate reduction and oxidative methylation steps, with conversion of pentavalent to trivalent form by reduction,in presence of reduced glutathione. Reduction is then followed by oxidative methylation, in presence of S-adenosylmethionine (SAM), which acts as a methyl donor[20-21].On entering the blood stream, As (III) undergoes methylation in the hepatic cells by microorganisms to give rise to two major metabolites: Monomethylarsonic Acid (MMA) and Dimethylarsinic Acid (DMA). MMA (III) is not excreted and remains inside the cell as an intermediate product which is thought to be highly toxic compared to other arsenicals[21]. Trivalent form of inorganic arsenic (As^{3+}) is more mobile in natural sources (groundwater and soils) than the pentavalent form of arsenic (As^{5+}) and also constitutes the substrate for arsenic bio-transformationoccurring in the liver[22].

Several trace elements are retained in the body for prolonged periods and can be easily detected in order to assess exposure by analytical methods like Graphite Furnace atomic absorption spectrophotometry (GF-AAS), Hydride Generator Atomic Absorption Spectrophotometry (HG-AAS), Inductively Coupled Plasma-Mass Spectrophotometry (ICP-MS), etc. These methods became available several decades ago so that past and cumulated exposures could be well characterized through analysis of biological matrices, e.g. blood, urine, nails and hair[23]. Analyses of biological samples are frequently used to quantify and monitor the exposure of trace metals. Levels between 0.1 and 0.5 mg/kg of As in hair sample indicates chronic poisoning while 1.0 to 3.0 mg/kg of As indicates acute poisoning[12]. The toxicity of arsenic is manifestation of dose response effects and blood is reported to be an important indicator for the presence of arsenic in the human body[24]. Hence, it is necessary to keep a constant check on arsenic levels in biological samples.

Keeping in a view fact, an attempt has been made to develop new method for detection of total arsenic usingvoltammetrytechnique which isan inexpensive, convenient and less sophisticated analytical method for detecting trace heavy metals. Its application is even more rapid over other highprecision techniques such as AAS,ICP, ICP-MS due to minimal requirements for sample preparation and low acquisition cost.Voltammetry technique offers excellent sensitivity and extremely low limit of detection in comparison to other analytical procedures.

2. Materials and Methods

2.1 Materials

Beaker, volumetric flask, capillary tube, dropper, measuring cylinder (Borosil, India) were used as glasswares. Chemicals andreagents used in the present study included- sulphuric acid, potassium chloride, sulfamic acid, citric acid, nitric acid and were purchased from Merck (Germany). Standard solutions of arsenic were prepared from1000 parts per million (ppm) arsenic stock solution traceable to SRM from NIST (Merck, Germany). Ultrapure water from Rions India Lab Water System Pvt. Ltd. was used for dilution purpose.

2.2 Instrumentation

- 797 VA Computrace (Trace Metal Analyzer) (Figure 1) with scTRACE Electrode (Figure 2) from Metrohm (Switzerland) and
- Microwave Digestion System (Figure 3) ModelMDS-10, from Sineo(China) were used.



Figure 1. 797 VA Computrace (Trace Metal Analyzer).

(1)



(2)



Figure 2. (1) scTrace Gold Electrode (2) Complete assembly with scTrace Gold electrode and voltamteric vessel.



Figure 3. Microwave Digestion System (MDS-10).

2.3 Preparation of Cleaning Solution

In a 100 ml volumetric flask, 0.373g KCl was dissolved in approximately 80 ml ultrapure water and 2.78 ml of 96% H_2SO_4 was carefully added to it. Solution can become very hot. After cooling down to room temperature, the solution was made up to the mark using ultrapure water.

2.4 Preparation of Electrolyte

9.78g of Sulfamic Acid, 10.51g of Citric Acid and 3.35g KCl were dissolved in 100 ml ultrapure water. An ultrasonic bath was used to dissolve it.

2.5 Preparation of Standard Solution

1000 ppb, 100 ppb, 10ppb, and 1ppb As(V) standards were prepared from 1000ppm arsenic stock solution using ultrapure water.

2.6 Sample Preparation (Closed Digestion Method)

A blood sample was digested by using a microwave digestion system. The procedure followed for closed digestion of blood is depicted in flow chart (Figure 4) and the programming conditions followed for microwave digestion of blood sample is provided in Table 1.

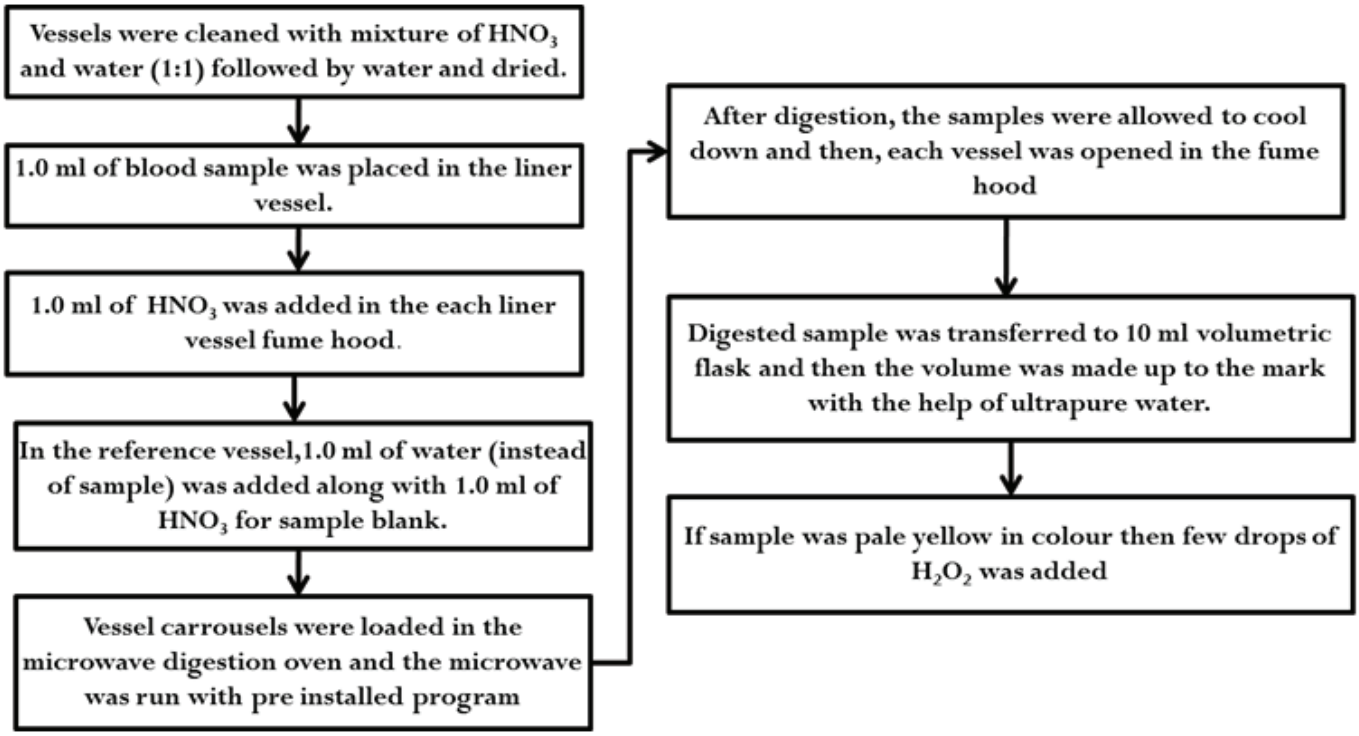


Figure 4. Flow chart showing the procedure followed for the digestion of human blood sample

Table 1. Programming conditions for microwave digester

Steps	Time (sec)	Starting temp (°C)	Ending temp (°C)
1	210	28	100
2	600	100	160
3	600	160	170

2.6 Activation of scTrace Gold Electrode

A new sensor needs to be activated. The activation has to be carried out prior to the first use. For activation of scTrace gold electrode, 10 ml of the cleaning solution was pipetted into the measuring vessel of TMA and 'Run' command was given under the voltammetric conditions given in Table 2. Curve obtained during the activation of scTrace gold electrode is presented in Figure5.

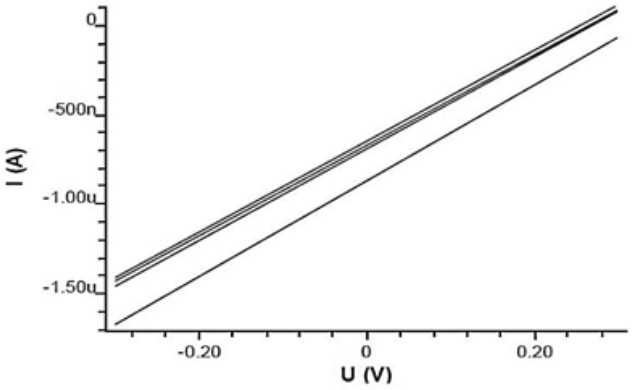


Figure 5. Curves obtained during activation of scTrace electrode.

Table 2. Voltammetric conditions for activation of scTrace gold electrode

Description	Parameters
General	
No. of Additions	0
No. of Replications	4
Voltammetric	
Electrode	RDE/SSE
Measuring Mode	DC-Sampled Direct Current
Stirring Speed	2400 min^{-1}
Hydrodynamic Measurement	No
Conditioning Cycles	
Start Potential	-1.5 V
End Potential	1 V
Pre-treatment	
No. of Cycles	10
Cleaning Potential	0.1 V
Cleaning Time	5 s
Deposition Potential	-0.3 V
Deposition Time	10 s
Sweep	
Equilibration Time	5 s
Start Potential	-0.3 V
End Potential	0.2 V
Potential Step	0.6 V
Potential Step Time	0.025 s
Sweep Rate	24 V/s

2.7 Determination of Total Arsenic

The sum of As (III) and As (V) in the sample was determined. The As (V) species is electrochemically inactive. During the determination, it was reduced in-situ by nascent hydrogen to As (III). Together with the As (III) present in the sample it was further reduced electrochemically to As (0) and deposited on the Gold working electrode in the same step. During the subsequent stripping step the deposited As (0) is re-oxidized to As (III) giving the analytical signal.

The electrode was washed well with ultrapure water. 10 ml ultrapure water and 1 ml of electrolytes were taken together in voltammetric vessel and voltammogram of blank was recorded under the voltammetric conditions provided in Table 3. After completion of blank voltammogram, 1 ml of the digested blood sample was added in the voltammetric vessel and voltammogram was recorded. After completion of the sample voltammogram, 1 ml of 100 ppb standard solution of Arsenic is added and voltammogram was recorded. Subsequently, 1 ml of 100 ppb standard solution was added in the same vessel and voltammogram was recorded for the second time.

Table 3. Voltammetric parameters of Sc trace Gold Electrode

Description	Parameters
General	
Measuring Mode	SqW-Square Wave
Electrode	SSE/RDE
Stirrer speed	2400 min^{-1}
No of addition	2
No of replication	2
Stirring Speed	2400 min^{-1}
Addition purge time	10 s
Initial purge time	0
Conditioning cycles	
Start potential	-0.2V
End potential	1 V
No. of Cycles	10

Pretreatment	
Cleaning potential	-1V
Cleaning time	60 s
Deposition potential	-0.25V
Deposition time	5 s
Sweep	
Equilibration time	5 s
Start potential	-0.3V
End potential	0.4 V
Voltage step	0.01V
Amplitude	0.02V
Frequency	100 Hz
Sweep rate (v)	1 V/s
Substance and calibration	
Calibration	Standard addition
Name	As (Total)
Peak Potential	0.0 V
Tolerance	0.05 V
Baseline	Linear

2.8 Storing and Cleaning of scTrace Gold Electrode

To maintain the scTrace gold the sensor can be electrochemically cleaned. The cleaning should be carried out before the sensor is stored and when it is put back into operation. But the cleaning can also be performed in between determinations to remove contaminations.

10 ml of the cleaning solution was pipetted into the measuring vessel and the procedure was carried out using the voltammetric parameters given in Table 4. If the cleaning is carried out properly, then the curve obtained is shown in Figure 6.

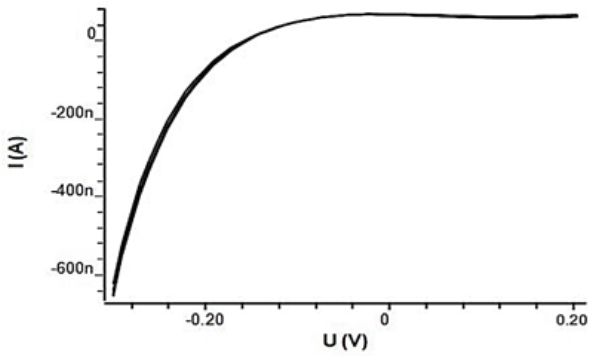


Figure 6. Curve obtained during cleaning of scTrace gold electrode.

When the electrode was not in use, then the electrode was kept cleaned and stored dry. After a series of determinations, it is recommended to clean the electrode using the cleaning procedure.Afterward, the electrode is rinsed thoroughly with ultrapure water. Care has to be taken that the gold microwire is not damaged when the sensor is removed or stored outside the measuring stand.

Table 4. Voltammetric parameters of Sc trace Gold Electrode

Description	**Parameters**
General	
No. of Additions	0
No. of Replications	4
Voltammetric	
Electrode	RDE/SSE
Measuring Mode	DC-Sampled Direct Current
Stirring Speed	2400 min^{-1}
Hydrodynamic Measurement	No
Conditioning Cycles	
Start Potential	-0.3 V
End Potential	1 V
No. of Cycles	10
Cleaning Potential	0.1 V
Cleaning Time	5 s
Deposition Potential	-0.3 V
Deposition Time	10 s
Sweep	
Equilibration time	5 s
Start potential	-0.3 V
End potential	0.2 V
Potential Step	0.01 V
Potential Step Time	0.025 s
Sweep Rate	0.4 V/s

3. Results and Discussion

Determination of other heavy metals like zinc, cadmium, lead, copper using deferential pulse anodic stripping voltammetry has been discussed in our previously published papers[25-26]. With respect to arsenic determination, we previously evaluated As (III) concentration in blood and urine samples using differential pulse cathodic stripping voltammetry[26]. The trivalent arsenic levels in blood and urine

samples were found to be 9µg/l and 841µg/l respectively after about twenty five days of homicidal poisoning through food and treatment as outpatient in hospital[26]. In the present study, we employed a simple, rapid and sensitive method for the determination of total arsenic content in blood sample using scTrace gold electrode. Briefly, it is a three-electrode system consisting of a working electrode, the reference electrode and auxiliary electrode. Voltammetry technique is based on the principle applying the potential and measuring current. Applying the potential between reference and working electrode and measuring current between the working and auxiliary electrode. The current is directly proportional to the concentration of metal. It is a two-step process in whichduring the first step, the metal is deposited onto the electrode and in the second step,metal is stripped out from the electrode. Thus, the stripped-out metalis directly proportional to the current and the current is directly proportional to the concentration of the metal.

The As (V) species is electrochemically inactive. During the determination, it is reduced in-situ by nascent hydrogen to As (III). Together with the As (III) present in the sample it is further reduced electrochemically to As (0) and deposited on the gold working electrode in the same step. During the subsequent stripping step, the deposited As (0) is re-oxidized to As(III) giving the analytical signal.

The electrode was washed well with ultrapure water two-three times. 10 ml ultrapure water, 1ml of electrolyte was taken in the voltammetric vessel and voltammogram of blank was recorded under the voltammetric conditions (Table 3). Briefly, 1 ml of digested sample was added into voltammetric vessel and voltammogram was recorded. Following the completion of the sample voltammogram, 1 ml of 100 ppb standard solution of arsenic was added to obtain a voltammogram and was repeated again. The voltammogram of standard and sample are presented in Figures 7-1 and 8-1. The extrapolation graph (Figures 7-2 and 8-2) gives the value of total As present. The software automatically calculated the final results by taking into consideration the dilution factor and sample size entered by the user. It was calculated by using the following formula:

$$\text{Final result} = \text{concentration} \times \frac{\text{Cell volume}}{\text{Sample amount}} \times \frac{\text{Multiplier}}{\text{Divisor}}$$

Wherein, divisor is sample amount taken for preparation and multiplier is dilution.

All the measurements were carried out by standard addition technique in which the first blank sample was taken into the voltammetric vessels and then the current was measured. The extrapolation curve was plotted between current vsconcentration. The extrapolation curve shows the amount of metal present in the sample solution. All the blank analysis was done with automatic blank subtraction,which is a feature of the instrument.

To maintain the life of scTrace gold sensor, it can be electrochemically cleaned. The cleaning should be carried out before storing the sensor and when it is put back into operation. However, cleaning can also be performed in between determinations to remove contaminations.

Due to its construction, the scTrace Gold is maintenance-free. It can be electrochemically cleaned as discussed. However, mechanical cleaning is neither necessary nor possible. The performance of the scTrace Gold will deteriorate with the number of determinations, signals will get smaller and curves are less reproducible, then it is the time to replace the sensor.

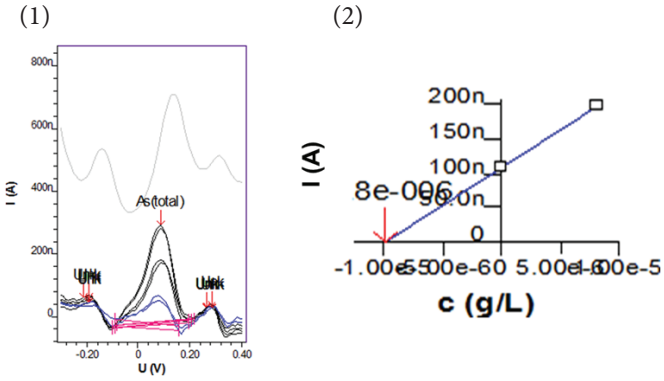


Figure 7. (1) Voltammogram of Arsenic standard
(2) Extrapolation graph of Arsenic standard.

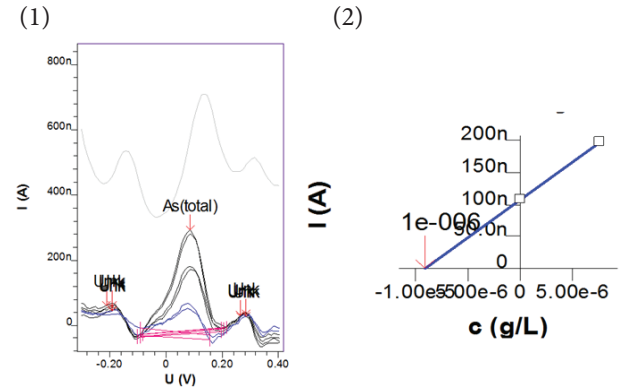


Figure 8. (1) Voltammogram of blood sample
(2) Extrapolation graph of blood sample.

3.1 Limit of Detection and Linear Working Range

The limit of detection was determined using the regression approach, where the limit of detection is calculated. The linear working range was read out from a calibration curve. The following table gives an overview of the limit of detection and linear working range depending on the time for the deposition.

Table 5. Limit of detection and linear working range

Deposition	Limit of Detection	Linear Working Range
30s	0.5µg/l	40µg/l
60s	0.9µg/l	20µg/l
90s	0.7µg/l	0.7µg/l

4. Conclusion

This article describes the determination of total arsenic in blood in trace level using voltammetric technique combined with ScTrace Gold Electrode. The technique developed is reproducible and can be used by the forensic scientist in any laboratory with very few chemicals, less sophistication and low maintenance cost

5. Acknowledgement

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6. Conflicts of Interest

The authors declare no conflicts of interest.

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