DEVELOPMENT OF FLOW INJECTION-HYDRIDE GENERATION-PHASE SEPARATION INTERFACES FOR THE DETERMINATION OF ARSENIC IN NATURAL WATERS AND RICE USING ATOMIC ABSORPTION SPECTROMETRY.

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

I wish to dedicate this work to my dearest mum Mrs Salome Njeri Mbugua for her love and encouragement in every step in my life.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
Abs	Absorbance
Al-B	Alumina B (plus) cartridge
Al-N	Alumina N (plus) cartridge
AsB	Arsenobetaine
AsC	Arsenocholine
AsS	Arsenosugars
Atm	Atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BDL	Below limit of Detection
BGC- D_2	Background corrector-Deuterium
BOC (K)	British Oxygen Company (Kenya)
CN	Cyano propyl (plus) cartridge
Conc.	Concentration
CV-AAS	Cold Vapour Atomic Absorption Spectrometry
D_2	Deuterium
df	Degree of freedom
DMAA	Dimethyl arsinic acid
EPA	Environmental Protection Agency
FAAS	Flame Atomic Absorption Spectrometry
F_{calc}	F calculated
FIA	Flow Injection Analysis
FI-HG-FAAS	Flow Injection Hydride Generation Flame Atomic Absorption Spectrometry

FI-HG-FTIR	Flow Injection Hydride Generation Fourier Transform Infra Red
F_{tab}	F tabulated
GC	Gas Chromatography
HG-AFS	Hydride Generation Atomic Fluorescence Spectrometry
HG-FAAS	Hydride Generation –Flame Atomic Absorption Spectrometry
HG-GFAAS	Hydride Generation –Graphite Furnace Atomic Absorption Spectrometry
HG	Hydride Generation
HG-ICP-AES	Hydride Generation – Inductively Coupled Plasma Atomic Emission Spectrometry
HG-ICP-MS	Hydride Generation Inductively Coupled Plasma Mass Spectrometry
HMSO	Her Majesty's Stationery Office
Ho	Null Hypothesis
HPLC - HG-AAS	High Performance Liquid Chromatography Hydride Generation Atomic Absorption Spectrometry
HPLC - HG-AFS	High Performance Liquid Chromatography Hydride Generation Atomic Fluorescence Spectrometry
HPLC-HG-ICP-MS	High Performance Liquid Chromatography- Hydride Generation – Inductively Coupled Plasma Mass Spectrometry
HPLC	High Performance Liquid Chromatography
i.d	internal diameter
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
JKUAT	Jomo Kenyatta University of Agricultue and Technology

LC-ICP-MS	Liquid Chromatography Inductively Coupled Plasma Mass Spectrometry
LOV	Lab-on-Valve
m/v	mass per unit volume
MMAA	Monomethyl arsonic acid
NAA	Neutron Activation Analysis
ODS	Octadecylsilane
OEHHA	Office of Environmental Health Hazard Assessment
Pa	Pasca
PHG	Public Health Goals
ppb	parts per billion
PQL	Practical Quantifiable Level
RSC	Royal Society of Chemistry
S	Sensitivity
SIS	Sequential Injection Analysis
S/N	Signal/noise ratio
$\mathbf{S_{blank}}$	Standard deviation of blank
SPE	Solid Phase Extraction
TARDA	Tana and Athi River Development Authority
$\mathbf{t_{calc}}$	t-calculated
TMAO	Trimethyl arsenoxide
$\mathbf{t_{tab}}$	t-tabulated
USEPA	United States Environmental Protection Agency
v/v	volume per volume
WHO	World Heath Organization
XRFS	X-ray Fluorescence Spectrometry

ABSTRACT

High levels of arsenic (As) in drinking water supplies and some foods have been reported to cause health problems in some parts of the world. This has resulted in review of the regulatory maximum permissible levels by WHO and other national bodies to be reviewed downwards. There is therefore a need to develop new methods for analysis of arsenic in water and foods that will be accurate and reproducible for routine analysis of large number of samples. Flow injection analysis (FIA) coupled with Atomic Absorption Spectrometry (AAS) was investigated for determination of arsenic in water and rice samples after hydride generation. The optimized basic FIA manifold gave a detection limit (LOD) of 0.74 μgL^{-1} (<1.0ppb) above the 3σ of the blank.

Further increased mass flux of the gaseous hydride reaching the atomizer was improved by use of a modified gas-liquid phase separator and better detection limit was achieved.

Retention capacity of various cartridges for As was investigated and a flow injection manifold for pre-concentration of As was employed with on-line solid-phase extraction cartridges. Alumina N (plus) and alumina B(plus) cartridges showed retention of arsenic with Alumina B showing slightly higher retention capacity for As than Alumina-N (Al-B = 74% and Al-N =

72%). Breakthrough volumes for Al -B was 160mls for 10ppb As solution at a flow rate of 6.7ml min^{-1}.

The new method was applied for the analysis of water and rice samples. Water samples analyzed showed no significant arsenic contamination or less than 10μgL^{-1}, below the WHO guidelines value.

Bottled water samples showed no As contamination and locally produced Kenyan rice showed no detectable arsenic as it was below the instrumental detectable limits. However, rice from Asian countries was found to contain As, Vietnam rice had 3.7 μg Kg^{-1}, Pakistan rice 7.9 μg Kg^{-1} and Singapore rice 5.6 μg Kg^{-1}. The concentration of As obtained in rice were lower than the maximum contaminant levels (10μg Kg^{-1}) set by WHO and other world bodies.

Close monitoring of imported rice is therefore crucial. It is recommended that preconcentration of As be done *in situ* using alumina cartridges as it was found to have the highest retention capability of As and this method will be suitable for routine environmental monitoring.

CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Arsenic is a trace element that is currently of major environmental concern internationally as pollutant but occurs in very low concentrations. Exposure of the general population to arsenic occurs through arsenic present in drinking water and some foods grown in such waters. Over the recent years, the growing global population and lack of safe drinking water have led to the exploitation of groundwater resources in many parts of the world. Consequently, according to Sharpe (2003), people have inadvertently tapped into another problem; that of arsenic rich waters from deep-water aquifers. Furthermore, in many countries, borehole water is used for bottling and is sold as mineral water. Some countries also rely on imported foods such as rice sourced from such countries that are known to have arsenic problem. For this reason proper monitoring of the so-called mineral waters and imported foods has become very crucial all over the world.

The high levels of arsenic in drinking water supplies have been reported to cause health problems in many parts of the world particularly in the Asian continent and United States of America. This has resulted in review of the regulatory maximum permissible levels by WHO and other national bodies downwards. The highly toxic nature of arsenic has increased the importance of the reliable determination of this element not only in environmental samples and foods but also in biological samples (Doull *et al.*, 1980). Its ultra trace determination especially in natural waters therefore requires continued improvement and development of more superior analytical methods in terms of sensitivity, detection limits, accuracy and reproducibility for routine analysis of large number of samples.

This work focused on sensitivity and selectivity enhancement by developing a suitable flow injection-hydride generation method that utilized solid-phase extraction as a sample preconcentration system.

The commercial gas-liquid separator in current use for the determination of As has been known to offer high sensitivity measurements but this often gets compromised due to slower response time because of high dead volumes in the cell system.

A novel gas-liquid phase separator was fabricated which was expected to offer reduced dead volumes of the hydride vapour – liquid mixture by reducing the hold up volume capacity. Its geometry would ensure increased mass flux of the hydride vapour reaching the AAS for atomization, and consequently would offer better signal.

Using the novel gas-liquid separator, quartz atomization cell and AAS, analysis of As in natural waters, bottled mineral waters and some local and imported rice was carried out.

Optimization for the flow injection system, hydride generation and separation, atomization, and the overall system was carried out, and samples analyzed to obtain comparative analytical data.

1.2 STATEMENT OF THE PROBLEM

Determination of As, which occurs in nature in trace amounts, has in the recent past posed an analytical challenge. Its analysis cannot be performed by dissolution followed by direct nebulization into the atomic absorption spectrometer because of the complex matrix effects and the complex chemical forms in which As exists, all of which compromise the detection and sensitivity of such a procedure. Further more its concentration in a variety of samples is far below the detection limit of a flame atomic absorption spectrometer. The sensitivity of the instrument for As is also low.

Two approaches have been used to increase the sensitivity of FAAS for the determination of As. These include the separation of the analyte from the sample matrix and increasing the mass flux of analyte to the atomizer. Though existing methods combine the two approaches in the FI-HG-FAAS system, new instrumental strategies could be devised that further increase the mass flux of As to the AAS instrument, thereby improving on the sensitivity of the analytical procedure. Careful design, optimization and operation of a hydride generation system would help in achieving even lower detection limits for the determination of As.

Flow injection methods interphased with hydride generation and atomic absorption spectrometry have been known to offer limited sensitivity (Ralph, 1995). Proposals have been advanced that apply preconcentration methods such as liquid-liquid, solid phase extraction and co-precipitation to improve on selectivity and sensitivity. According to Jason (2004), solid phase extraction (SPE) method is by far the largest subsection of the off-line preconcentration section widely studied, and little if not none have been documented for on-line preconcentration of As. Presently FIA methods for arsenic determination have not involved preconcentration on solid phase cartridges. Studies were therefore done using commercial Se-Pak (Millipore Waters)

cartridges; Alumina B, Alumina N, Octadecyl silane(C-18) and CN cartridges to determine their retention ability for As. The cartridge that showed high retention capacity was interfaced online with the FI-HG-FAAS system.

Three commercially available gas liquid separators have been used to free the gaseous hydride from the reaction liquid mixture; the continuous generation type, the batch generation and the flow injection type (Ralph, 1995). However, the detection limits and sensitivity have been found to suffer because not all the generated gaseous hydride is separated out from the reaction mixture and subsequently purged by the inert gas for atomization into the absorption cell. Improvement of detection limits may therefore be achieved by using a modified gas-liquid phase separator, which may lower the dead volumes that characterize the commercial one. The modified gas-liquid phase separator may also offer a cheap and more reproducible method with high separation efficiency in the determination of As using FI-HG-FAAS method in a Kenyan situation.

1.3 OBJECTIVES OF THE STUDY

1.3.1 BROAD OBJECTIVES

The overall objective of the research work was to develop a more sensitive and selective analytical method for the determination of As. A FI-HG-FAAS method incorporating online/offline preconcentration on solid phase cartridges and a modified gas-liquid phase separator was applied in the determination of As in natural waters, bottled mineral waters and some selected local and imported rice in Kenya.

1.3.2 SPECIFIC OBJECTIVES

The specific objectives of the project were:

(i) To study the retention characteristics of various solid phase cartridges for As element: (Alumina B, Alumina N, C-18 and CN Waters Sep-pak cartridges.)

(ii) To develop and optimize a flow injection manifold for preconcentration and analysis of As employing on-line solid-phase extraction cartridges with HG-FAAS.

(iii) To test a modified gas-liquid phase separator for improved optimized detection.

(iv) To optimize the overall conditions for the determination of As by the FI-HG-FAAS method using on-line preconcentration system and the modified gas-liquid phase separator.

(v) To determine the As level in natural waters and some selected rice samples in Kenya, (local and imported rice).

1.4 SIGNIFICANCE AND JUSTIFICATION OF THE STUDY

Increased global concern over As contamination of waters and some foods has necessitated the review of the maximum contaminant levels in a downward trend. WHO, whose guidelines for As is $10\mu gL^{-1}$, has compiled reported cases of arsenic in drinking water in countries such as India, Argentina, China, Bangladesh, Chile, Ghana, Hungary, Mexico, Thailand and the United States among others. Consequently, a number of countries are coming up with drafts of legislation for public health goals (PHG), which propose even lower levels of As in drinking water than the WHO guidelines. For example in Canada the new draft proposes $4\mu gL^{-1}$ of As levels in drinking water (OEHHA, 2003), while Environmental Protection Agency of USA (USEPA, 1996), proposes $5\mu g\ L^{-1}$. Following the 2000 presidential election in USA, the Bush Administration reopened the topic to consider the levels of $3\mu g\ L^{-1}$, $5\mu g\ L^{-1}$, $10\mu g\ L^{-1}$ and $20\mu g\ L^{-1}$ and re-examine the risk and the cost issues. The final rule, setting the standard at $10\mu g\ L^{-1}$, came into effect on February 22, 2002 and required public water systems to meet the new standard by January 2006 (Sharpe, 2004). England has already started implementing its new regulation requiring the maximum limits for As to be $10\mu g\ L^{-1}$ (HMSO, 2004).

If such legislations are accepted, all drinking water providers and regulators in those countries or states will have those new levels as their long-term objective levels of As in drinking water. Technologies for routinely monitoring arsenic in drinking waters and foods as well as removing it from water supplies are therefore required to comply with such more stringent regulations.

However many existing analytical techniques do not have such capabilities or will have difficulties of determining As in drinking water accurately and reliably. For those that seem to be more sensitive such as ICP-AES and ICP-MS, they are too

expensive for the developing countries like Kenya. This makes it necessary to develop new analytical methods to cope with the changing global regulatory standards.

Though there has not been much documentation on the As situation in Kenya, the global concern on the arsenic crisis warrants some tests of possible contamination of borehole waters from some potential areas in Kenya, particularly the coast region where a lot of mineral mining and agricultural prospects is taking place. Environmental impact assessment in these areas will entail looking at such aspects and the results of this study will give an insight into the issue of As situation in some parts of Kenya.

While there is currently no international standard for arsenic levels in food, WHO advocates a maximum arsenic level in water of 10µg L^{-1}, which is also the standard currently used by EPA. But many developing countries including Kenya still use a standard of 50µg L^{-1}.

These findings cause considerable concern and suggest that ingestion of rice is a major source of arsenic exposure in Bangladesh and elsewhere in regions with subsistence rice diets. A number of countries in the region have similar problems with tubewell contamination. Two areas, Vietnam and West Bengal in India are particularly likely candidates for finding arsenic in rice (American Chemical Society, 2002).

Kenya being one of the importers of rice from some of these Asian countries needs to re-evaluate and assess the arsenic levels of rice imports from these countries in order to safeguard the health of its citizens.

The trend for lower regulatory limits have continued and will continue, requiring therefore that the analytical services laboratory must refine techniques or upgrade

instrumentation to achieve the analyte sensitivities required to meet the lower water-quality compliance criteria.

A modified gas-liquid phase separator coupled with online preconcentration in FI-HG-FAAS system employed in this study was evaluated and applied for the determination of As in natural waters and some selected foods in Kenya, specifically local and imported rice. This is because imported and local rice must be critically evaluated in terms of safety and quality, considering that much of the rice imported to Kenya comes from the Asian countries which have been documented to have high levels of arsenic in their waters and soils due to geographical factors.

Since As element is of significant concern from an environmental standpoint, the improvement of the currently used FI-HG-FAAS in terms of sensitivity and selectivity would readily offer a faster and more accurate analytical method for the determination of trace amounts of As in natural waters and some selected foods. It is expected that the method would have a major advantage over the slower graphite furnace technique and the more expensive methods such as ICP-AES and ICP-MS, particularly for the developing countries in which resources for water sampling and analysis are often inadequate (DeMenna *et al.*, 1994; Howard and Salou, 1997).

CHAPTER TWO: LITERATURE REVIEW

2.1 INTRODUCTION

The name Arsenic is derived from the Greek word *arsenikon,* which means yellow orpiment. Arsenic exhibit metallic as well as non-metallic characteristics and corresponding chemical properties, hence it is metalloid. Arsenic is one of the oldest human poisons known to mankind. It has six specific characteristics (Azcue and Nriagu, 1994):

- It is a virulent poison on acute ingestion.
- It is extremely toxic on long term exposure to very low concentrations.
- It is not visible in water and food.
- It has no taste.
- It has no smell.
- It is difficult to analyse, even when occurring in concentration twice as high as WHO guidelines.

2.2 OCCURRENCE OF ARSENIC

Arsenic is an ubiquitous element in the earth's crust. It is widespread in the earth's crust, occurring at high concentrations as its trioxide (arsenolite) or sulphides (realgar and orpiment). It also occurs in association with iron (arsenopyrite), cobalt (cobaltite), nickel (nickel glance), lead, zinc, copper or gold-bearing minerals (Ralph, 1995).

As is transported in the environment mainly by water, although other natural and anthropogenic sources of exposure to arsenic including burning of arsenic-rich coal, mining and smelter activities are of increasing international concern.

As is a naturally occurring el``ement in rocks, soils and the waters that contact them. Other major sources of arsenic include: agricultural run-off and industrial effluents

from metallurgical sites and manufacture of glassware/ceramics, dyes, herbicides, pesticides, petroleum refining, wood and hide preservatives, fertilizers, and phosphate detergents.

2.3 ENVIRONMENTAL CHEMISTRY OF ARSENIC

Chemical forms of arsenic in natural water consist of 70% of pentavalent inorganic arsenic (arsenate), 28% of trivalent inorganic arsenic (arsenite) and 2% of organic compounds. In the aquatic environment, transformation of arsenic from inorganic to organic forms can be mediated by freshwater algae, bacteria, fungi, plants, animals and man. In the case of organic form, species such as arsenosugars, arsenobetaine (AsB), arsenocholine (AsC), monomethylarsonic acid (MMAA), dimethylarsinic acid (DMAA) and trimethyl arsenoxide (TMAO) are well known for arsenic (Stoeppler, 1992). Arsenic in its various chemical forms and oxidation states is released into the aquatic environment by various processes and industrial discharges, as stated earlier. On release to aquatic environment, the arsenic species enter into methylation / demethylation cycle, while some are bound to the sediments or taken up by biota where, they could undergo metabolic conversion to other organo-arsenicals. Arsenic generally exists in the inorganic form in water samples. Under different redox conditions arsenic is stable in the +5, +3, 0, and -3 oxidation states. The pentavalent (+5) arsenic or arsenate species include AsO_4^{3-}, and $H_2AsO_4^-$. The trivalent (+3) arsenic or arsenite species include $As(OH)^{4-}$, $AsO_2(OH)^{2-}$, and AsO_3^{3-}. The pentavalent arsenic species are predominant and stable in the oxygen-rich aerobic environment, whereas the trivalent arsenic species are predominant in the moderately reducing anaerobic environment such as groundwater (Ghosh and Yuan, 1987, Alloway *et al*., 1997). As^0 and As^{-3} are rare in aquatic environments.

Methylated or organic arsenic occurs at concentration less than 1 ppb, and is not of major significance in drinking water treatment (Edwards, 1994).

2.4 PROPERTIES OF ARSENIC

Arsenic is a chemical element in the nitrogen family, existing in both yellow and grey crystalline forms. Although some forms of the Arsenic are metal-like, it is best classified as metalloid and non metal. Some of the significant properties of Arsenic are listed in Table 2.1, (Ralph, 1995; Azcue and Nriagu, 1994).

Table 2.1 Physical Properties of Arsenic

Parameter	**Value**
Atomic Number	33
Atomic Weight	74.92158
Melting point	814 ^{0} C at 36 atm
Boiling point	613 ^{0} C (sublimes)
Oxidation number	-3, 0, +3, +5
Electronic configuration	$1s^2$, $2s^2$,$2p^6$,$3s^2$,$3p^6$,$3d^{10}$,$4s^2$,$4p^3$

2.5 TOXICITY AND HEALTH EFFECTS OF ARSENIC

In nature As is present in both inorganic and organic forms. As appears to be an essential trace element, yet it also shows evidence of toxicity at levels which are regarded as normal for many trace elements. Recognized as a toxic element for centuries, arsenic today poses a major human health concern because it can contribute to long-term morbidity and carcinogenecity, (particularly lung and skin cancer) and mortality (Senapati and Alam, 2001, Steve and Hill, 2004). Although arsenic has been long known to be carcinogenic to humans, the full extent of arsenic-health related problems has still to be fully identified and studied. In order to understand the health effects of arsenic, it is imperative to develop chemical, toxicological and analytical approaches that provide accurate, precise and fast identification of its molecular and metabolic forms. According to the US Environmental Protection Agency (USEPA), when man continues to consume water over maximum contaminant level for arsenic (50ppb), the carcinogenic risk is 2.5×10^{-3}. This value is about 250-fold higher than the general permissible carcinogenic risk of 10^{-5}-10^{-6} used by USEPA (WHO, 1981; USEPA, 1996).

Pigmentation and keratosis of the skin are the two most common health effects arising from chronic arsenic exposure. In addition, arsenic has been associated with the occurrence of internal malignancies including liver cancer (e.g., hepatocellular carcinoma and angiosarcoma), bladder, kidney and lung cancers (Centeno, 2002; Sharpe, 2004). Increased risks of lung and bladder cancer and of arsenic-associated skin lesions have been observed at drinking water arsenic concentrations of less than 50ppb. Immediate symptoms of an acute poisoning typically include vomiting, oesophageal and abdominal pain, and diarrhoea (Sharpe, 2003).

The toxicity of arsenic depends on its chemical forms too. Inorganic arsenic is more toxic than organic arsenic, and arsenite is more toxic than arsenate (Smith, 1992).

2.6 GLOBAL SITUATION OF ARSENIC CONTAMINATION

The extent of the arsenic contamination in drinking water globally has not been adequately documented. Reliable data on exposure and health effects are rarely available, but there are many countries in the world where arsenic in drinking water has been detected at concentration greater than the WHO Guideline Value of 10ppb or the prevailing national standards. These include Argentina, Australia, Bangladesh, Chile, China, Hungary, Nepal, India, Mexico, Peru, Thailand, and the United States of America. Countries where adverse health effects have been documented include Bangladesh, China, India (West Bengal), and the United States of America (Sharpe, 2003).

Bangladesh, for instance, being the largest alluvial delta of the world and the country being crisscrossed by hundreds of rivers has experienced catastrophic impacts over arsenic poisoning to its citizens over the years. Guidelines for maximum acceptable concentrations for Arsenic pollutant in water for human consumption has been set as 10μg L^{-1} (10ppb) by World Health Organization (WHO) while Canada had set theirs as 25μg L^{-1}, United Kingdom and USA 50μg L^{-1} before the review (WHO, 1981). Because of the increased international concern over As contamination, countries are reviewing their legislation over tolerance limits of As in drinking water towards lower levels than the already existing ones.

2.7ANALYTICAL METHODS FOR ARSENIC DETERMINATION

Both classical and instrumental methods of analysis have been used in the determination of arsenic. Determination of As in environmental and biological samples has undergone tremendous development with respect to the trace analysis procedures and instrumentation. Since nanogram level detection limits are needed to avoid large sample sizes and enormous preconcentration steps, several analytical methods offering this advantage, have been developed during the last decade for the determination of amount and speciation of As.

2.7.1 CLASSICAL METHODS OF THE DETERMINATION OF ARSENIC

During the development of classical chemical analytical techniques, it was discovered that certain elements at one end of the periodic table had the property of forming a stable, gaseous hydride when reacted with a strong reducing agent, which could then be thermally reduced to the free metal. This is the foundation of the classic Marsh test for arsenic according to DeMenna *et al.*, (1998). Protocols for the hydride generation procedure were originally developed using highly reactive metals in the presence of strong acid solution, for instance, zinc in 25% hydrochloric acid or magnesium in 10% sulfuric acid. The gaseous discharge from the reaction flask was purged with inert argon gas and burned either in an air/acetylene flame or, preferably, in an argon/hydrogen flame of an atomic absorption system. This served two purposes namely (i) to thermally decompose the hydride compound into the element and (ii) minimize the atmospheric oxygen background absorption, which can obscure hollow-cathode lamp discharges below the 200-nm limit of most system configurations and reduce the signal/noise ratio. As chemical technology advanced

and more reagents became available to the analytical laboratory, the strong (and safe) reducing agents sodium borohydride and cyanoborohydride became the reagents of choice in improving both the reproducibility and the overall sensitivity of hydride generation. Other more efficient compounds such as lithium aluminum hydride have been found to be too dangerous for routine use in an analytical laboratory. Some researchers also noted that the presence of certain materials either enhanced or inhibited the formation of the desired hydride species; these can now be promoted or eliminated, respectively, using specific reagents in the reaction process. The classical methods include the gravimetric as well as titrimetic methods, both of which are no longer in common use. However they are still employed for the measurement of high concentrations of As (Wolfgang, 1995).

2.7.2 INSTRUMENTAL METHODS OF THE DETERMINATION OF ARSENIC

Various types of instrumental methods of analysis have been adapted for the determination and speciation of arsenic species. Hydride generation techniques coupled with atomic absorption, atomic emission, and mass spectrometry have found wide application in the determination of trace amounts of several elements, including arsenic and selenium. Such methods include Hydride Generation-Flame Atomic Absorption Spectrometry (HG-FAAS), Hydride Generation-Graphite Furnace Atomic Absorption Spectrometry (HG-GFAAS), Hydride Generation- Atomic Emission Spectrometry (HG-AES), Hydride Generation Atomic Fluorescence Spectrometry (HG-AFS) and relatively new X-ray fluorescence Spectrometry (XRFS), Neutron Activation Analysis (NAA), Hydride Generation Inductively Coupled Plasma Atomic Emission Spectrometry (HG-ICP-AES) and Hydride Generation Inductively Coupled

Plasma Mass Spectrometry (HG-ICP-MS). The latter two methods exhibit high degree of sensitivity. However their high cost in terms of instrumentation, installation and maintenance, besides the unique chemical and spectral interferences involved have inhibited their popularity (Wolfgang, 1995).

Speciation of inorganic arsenic in environmental samples is usually accomplished by chromatographic separation, chelating-extraction or elution of As^{+3} and then reduction of As^{+5}. For speciation purposes, separation has been effected by first coupling with Gas Chromatography (GC) or High Performance Liquid Chromatography (HPLC), as in HPLC-HG-AFS (Bohari, 2001). ICP-AES and ICP-MS are increasingly common techniques for the analysis of arsenic as both methods generally provide lower detection limits than absorbance detection methods (Facchetti and Pitea, 1995). However, their instrumentation is expensive and this limits their use.

Hyphenated methods of separation and detection such as HPLC-HG-ICP-MS, HPLC-HG-AAS, LC-ICP-MS and FI-HG-FTIR for individual and simultaneous determination of As, Sb and Sn have also been developed and reported by Andreae, (1997).

Atomic spectrometry with hydride generation interface and electronspray/nanospray mass spectrometry have also been shown to be most useful for arsenic speciation research. However Serife and Chris, (2001), in their study involving 25 commmercial and water utility laboratories, determined that the practical quantifiable level (PQL) for arsenic in drinking water was $4\mu gL^{-1}$. This is however insufficient to comply with the more stringent environmental regulations for arsenic.

The detection limits for As in particular have been reported for the various analytical methods (ATSDR, 2003). These are summarized in Table 2.2.

Table 2.2: Detection limits of As from various analytical methods

Method	Detection limit (µg L^{-1})
Gutzeit Spot test	0.5
Silver diethyldithiocarbamate (Ag-DEDTC) color	1
HG-AAS	0.1
Molybdenum, Mo-blue color	0.1
HG-ICP-AES	0.1
GFAAS	0.5
Proton Induced X-ray Emission, PIXIE	0.2
ICP-MS	0.03
Neutron Activation Analysis, NAA	Sub ng quantities

The possibility of using methods based on AAS for total metal determination and speciation studies makes this area of research available to groups who do not have access to more expensive and sophisticated instrumentation such as ICP-MS.

Perhaps, of all the above instrumental methods mentioned, the most commonly used is the flow injection-hydride generation-flame atomic spectrometry, (FI-HG-FAAS) for which this study would be the focus in terms of further development.

2.8 FI-HG-FAAS METHOD FOR ARSENIC DETERMINATION

2.8.1 FLOW INJECTION ANALYSIS

Flow Injection Analysis (FIA) is an automated, continuous flow approach to perform chemical analysis, based on injecting a small, well-defined volume of sample into a continuously flowing unsegmented carrier stream - to which appropriate auxiliary reagent streams can be added, whereby a concentration gradient of the sample is created. All of the, virtually innumerable, concentrations represented in the gradient can potentially be exploited for the readout. Thus, in contrast to conventional air segmented continuous flow procedures (and all batch methods), FIA does not rely on complete mixing of sample and reagent(s), (physical homogenisation). Combined with the inherent exact timing of all events it is neither necessary to wait until all chemical reactions have proceeded to equilibrium (chemical homogenisation). These feats, which allow transient signals to be used as the readout, do not only permit the procedures to be accomplished within a very short time (typically in less than 30 seconds), but have opened new and novel avenues to perform an array of chemical analytical assays. These are either very difficult and in many cases directly impossible to implement by traditional means.

The simplest flow analyzer consists of a pump, which is used to propel the carrier stream through a narrow tube, an injection valve, a microreactor in which the sample zone disperses and reacts with the components of the carrier stream, forming a species that is sensed by a flow-through detector and recorded. In the case of the FIA technique the physical equilibrium (flow homogenization) is never reached at the moment of detection.

Moreover, it is not necessary for the chemical equilibrium to be obtained at the moment of detection, which accounts for its high sample throughput.

FIA, which was originally invented and developed by Ruzicka and Hansen in 1975, is nowadays used all over the world, as reflected in the fact that more than 15,000 FIA-papers have appeared in the scientific literature (Welz and Schubert, 1991; Ralph, 1995).

In recent years, FIA has been supplemented by SIA (Sequential injection Analysis) and LOV (Lab-on-Valve), methods that have furnished additional features to the original FIA-concept. Thus, in SIA a multiposition selection valve is used for aspiration of sample as well as reagent(s), allowing not only the consumption of these solutions to be minimized and hence resulting in extremely low generation of waste materials (green chemistry), but also novel procedures to be made. In this context should especially be mentioned the determination of low and ultra-low concentrations of metal ions in complex matrices, and the extraction/determination of potentially polluting metals in solid materials, such as soils, sludges, and foods, in order to ascertain the bioavailability of these metals in the materials.

2.8.2 HYDRIDE VAPOUR GENERATION

According to Jason, (2004), and Dedina and Tsalev, (1995), chemical vapour generation, combined with AAS detection in the forms of cold vapour generation for the determination of Hg and Cd (CV-AAS), and FI-HG-FAAS for elements forming gaseous covalent hydrides (As, Bi, Ge, Sb, Se, Sn, Te and even In, Pb and Tl), remain the most commonly and widely used powerful analytical procedure for the determination of these elements in biological and environmental samples.

Hydride generation is a chemical derivatization process that produces volatile hydrides upon chemical treatment of a sample with a reducing agent, typically sodium borohydride (Ure *et al*., 1992). The generation of the volatile hydride and its

introduction into the atomization cells requires the separation of this gaseous hydride from the carrier liquid. This has offered several significant advantages over conventional solution-phase pneumatic nebulization of samples (Wolfgang, 1995) some of which include:

(i) elimination for the need for the nebulizer or spray chamber assembly,

(ii) better detection limits (at the μgL^{-1} level or lower),

(iii) automation of the methods,

(iv) enhancement of transport efficiency, approaching 100%,

(v) presentation of a homogeneous vapour to the atomizer and,

(vi) possibility of speciation studies and coupling with different techniques.

However, as the sample preparation and derivatization method most often employed, it also suffers from interferences, which must be evaluated and minimized.

Presently, reduction of analyte by tetrahydroborate in acidic medium is employed almost universally for hydride generation (HG). The inherent disadvantage of the borohydride (BH_4^-)-acid reduction is the risk of contamination of the reducing agent, which often controls the limit of detection (LOD) of the determination.

Howard and Salou, (1997), has reported derivatization of the arsenic compounds using $NaBH_4$ as the derivatisation reagent and the corresponding proposed reactions in the production of arsine.

Flow injection system used for hydride generation has been shown to offer a number of distinct improvements over the batch system; these include high absolute sensitivity, reduced sample and reagent consumption, reduced interface effects, ease of incorporation of elaborate sample pretreatment, high sample frequency and ease of automation (Ralph, 1995). As an efficient sample introduction method, hydride

generation enhances sensitivity normally by 10- to 100-fold over the more commonly used liquid sample nebulization procedure.

The analyte element can also be separated from almost all other accompanying materials in the sample through the hydride generation process. When the gaseous hydrides/liquid mixture is introduced directly to the AAS-detector, a combination of interferences such as sample matrix, spectral and chemical interferences are encountered, consequently compromising the overall sensitivity and selectivity, and detection limits deteriorate. The detection limits for As are rarely better than $1mgL^{-1}$ but often worse (Stoeppler, 1992). The usual practice is therefore to have the hydrides produced in a generator flushed out of the generator using inert gas such as argon and thus the hydride is separated from the sample matrix.

Separation modules therefore become necessary in the analytical process in order to increase the selectivity and sensitivity of the overall determination by removing undesirable interferents and preconcentrating the analyte(s), respectively.

By appropriately coupling the separation technique and a flow injection manifold, separation/preconcentration processes can be performed automatically and analytical data can be improved.

2.9 PRECONCENTRATION OF ARSENIC

Researchers all over the world are developing preconcentration procedures at an increasing rate. The motivation for this effort appears to be largely the need to extend the capability of procedures in which the quantitative measurements are made by FAAS, but as the procedures also separate analytes from matrix components, this may also be considered a motive for the method development (Tyson *et al.*, 2004).

Although analytical instruments have been significantly improved over the years, preconcentration methods for the determination of trace arsenic in natural waters are usually required during the pretreatment of samples in order to improve on the detection limits of certain trace elements such as As and Se whose concentrations are too low. For this to occur, the available preconcentration methods must be optimized. Various preconcentration methods have been reported which include: Solvent extraction, coprecipitation with lanthanum or hafnium, thorium or zirconium, lead-pyrrolidinedithiocarbamate, solid phase extraction, or the extraction of arsenic (III) with a marcroporous resin impregnated with bis(2- ethylhexyl) ammonium-bis(2-ethylhexyl)-dithiocarbamate and thionalide loaded on silica gel (Stoeppler, 1992). Preconcentration of the hydrides has also been done at cryogenic temperatures from which detection limits for As and Se have been significantly lowered to the picogram levels. Solid phase extraction (SPE) once again dominates as the method of choice, but there seems to be no emerging consensus as to what the best material might be for any given element. Use of the solid-phase extraction method by employing Al-La loaded chelating resin as the adsorbent material for As was reported in a study carried out by Trung, (2001). This chelating resin was found to be one of the most suitable solid phase extraction material for preconcentration of As. It provided not only a cheaper and simple preconcentration method but also enhanced sensitivity for As determination by HG-FAAS. Serife and Chris, (2001), have been reported to have developed a sensitive and inexpensive method that can be used for routine speciation of arsenic using SPE cartridges and quantified using FI-HG-AFS. Li-Li and Zhang, (2003), have also described a method for in situ preconcentration and determination of trace arsenic in botanical samples by HG-GFAAS with Pd-Zr as chemical modifier. A recent improvement of the hydride generation for the speciation of arsenic in

natural freshwaters by HPLC-HG-AFS has also been reported by Bohari, (2001), and Serife and Chris, (2001). By far the most frequently used bonded phases are the reversed phase packings, mainly the octadecylsilane (ODS or C-18) bonded silica, which have been considered nearly universal adsorbents, and to a lesser extent, the octyl (C-8) bonded silica (Ravindranath, 1989). There has not been much documentation on the common Sep-Pak (Millipore Waters) cartridges such as Alumina A, Alumina N,

C-18, C-8, and CN as packing materials, in terms of whether they preconcentrate As and then interfacing of the cartridges on-line with FI-HG-FAAS.

CHAPTER THREE: MATERIALS AND METHODS

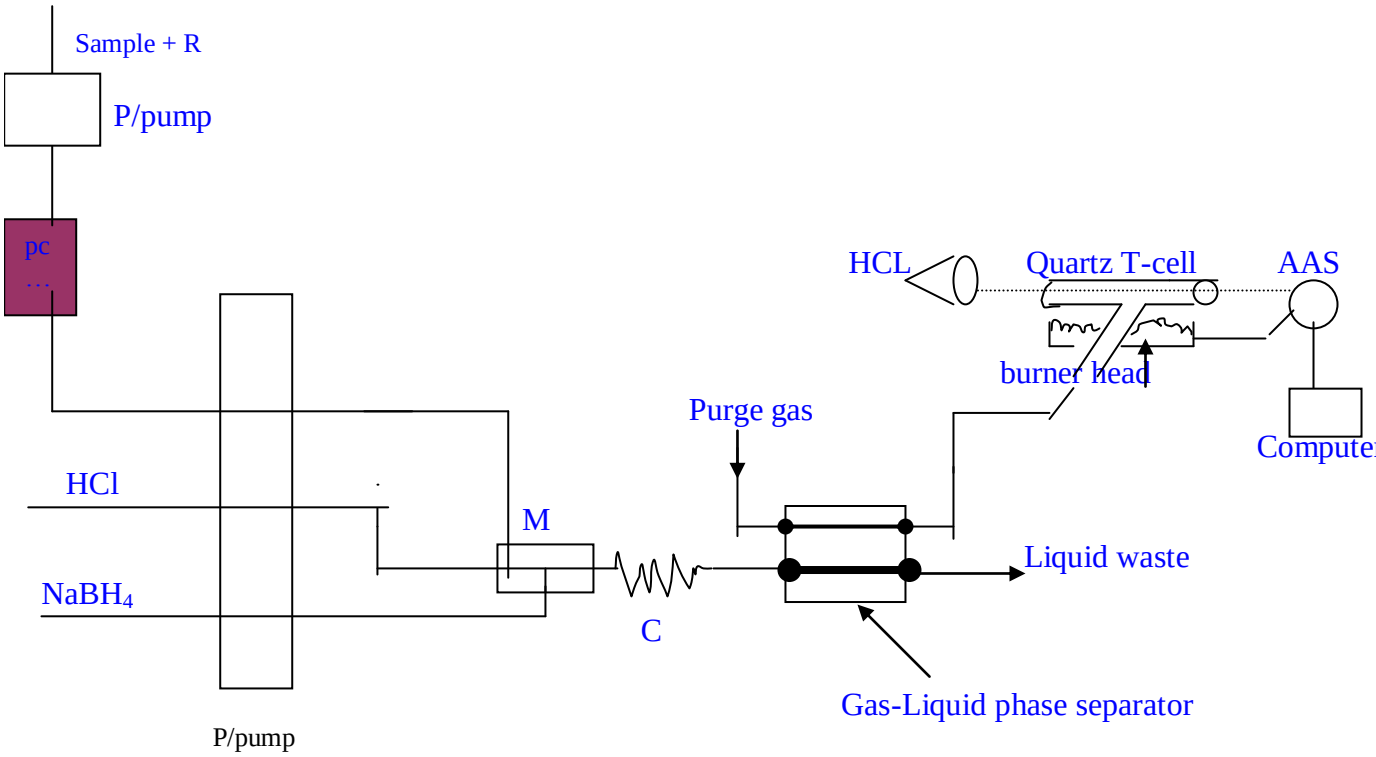
3.1 APPARATUS AND CALIBRATION OF EQUIPMENT

A Shimadzu hydride generator HVG-1 and Shimadzu AA6200 AAS equipped with a deuterium ($D_{2)}$ lamp background corrector, connected to a computer installed with software for metal analysis (WIZAARD) for system control and data analysis were used throughout in FAAS. The AAS was fitted with a hollow cathode lamp for arsenic, operated at 193.14 nm resonance line at lamp current of 15mA.

Flow injection (F1) system consisted of a peristaltic pump (from Shimadzu) with three channels, each of which was to propel the sample, HCl and $NaBH_4$.

Compressor pump for air which was a small size recipro-air compressor of maximum pressure of 0.49mPa and flow rate at 20-22 L min^{-1} was used (from Nishiba Electric Co. Ltd, Japan). Air-acetylene flame was used with air flow rate of 8.0 L min^{-1} at 0.49mPa pressure, while that of acetylene was 2.0 L min^{-1}. Argon was used as purging gas at 70ml min^{-1} and 0.32mPa pressure.

Flow injection (F1) system consisted of a three channel peristaltic pump (Shimadzu) in which the flow rates of 5M HCl and 0.4% $NaBH_4$ (in 0.4%NaOH m/v) reagents were both set at 2.2ml min^{-1} while calibration standards (concentration range = 1-13ppb) and sample flow rates were maintained at 6 ml min^{-1}. A 5% HCl (v/v) solution was used as the blank and to both the blank and the calibration standards, potassium iodide (20%m/v) in 10%ascorbic acid (m/v) were added as pre-reductants. Upon aspiration of the blank and the standards under the above described conditions, a plot of absorbance against concentration was obtained. Intrapolation of the calibration curve was used in the subsequent experiment for the determination of the retention capability of arsenic by various cartridges.



Key
R – Prereduction reagents, for As; $KI_{(aq)}$ /Ascorbic acid mixture in $HCl_{(aq)}$
P/Pump –peristaltic pump
PC – preconcentration minicolumn
M - manifold
C – reaction coil
HCL – Hollow cathode lamp
AAS – atomic absorption spectrometer

Figure 3.1 Diagram of FIA system connected to gas-liquid phase separator

A modified gas-liquid phase separator was constructed at Kenyatta University, Chemistry Department, and the diagram of the FIA system connected to the phase separator as given by Figure 3.1 above.

3.2 REAGENTS AND MATERIALS

All reagents were at least of analytical reagent grade.

Sodium hydroxide, ascorbic acid, potassium iodide and 37% hydrochloric acid were from Prolabo, nitric acid (Rasayan) and sodium borohydride, 95-97% sulphuric acid (Riedel-de Häen).

Standard solutions were prepared by serial dilution of 1000 ppm As stock solution (Riedel-de Häen) in 5% HCl to the manufacturer's recommended working range of 5 to 20ppb. Deionised water was used throughout this work, while the prepared reagents and samples were stored in a refrigerator at 4^{o}C in polyethylene bottles until use.

Acetylene and argon gases (analytical grades from British Oxygen Company, BOC Kenya) were used as the fuel and purge gases in HG-FAAS respectively. Air was from the small recipro-air compressor at a flow rate of 22 L min^{-1} and pressure of 0.49 mPa.

Alumina B, Alumina N, C-18 and CN cartridges (Sep-Pak Waters) were used to study the retention characteristics of solid phase for As.

3.3 EXPERIMENTAL

3.3.1 OPTIMIZATION OF INSTRUMENTAL PARAMETERS

Univariate approach was used for optimizing the following selected parameters while keeping the rest of the instrumental parameters constant as per the manufacturer's recommendations.

(i) Sample and reagents flow rates

(ii) Concentrations of $NaBH_4$ and HCl

Preliminary optimization of the flow rates was carried out using 5M HCl and 0.4% (m/v) $NaBH_4$ as described for As determination by the instrument manufacturer

(Shimadzu, 1992). The reagents flow rate was varied between 1-2.5 $mlmin^{-1}$ using the knob on the peristaltic pump while keeping the 10ppb As sample flow rate at 6 $mlmin^{-1}$. The reagents flow rate, which showed the highest absorbance, was taken as the optimized reagents flow rate and was used to optimize the sample flow rate by varying the sample flow rate adjustment knob on the peristaltic pump. The highest absorbance obtained was recorded and these flow rates combination were used in the subsequent experiments for optimization of the reagents concentration.

Reduction of arsenic to arsenic hydride with sodium borohydride via the acid media has been reported to be considerably efficient. Hydrochloric acid remains the acid of choice although sulphuric and nitric acids have also been used in hydride generation media. Optimum acidity ranges depend on the element and also often tailored to suit specific matrices (Ralph, 1995). In the optimization experiment for the reagents concentration, a range of 1-9M HCl and 0.2-0.9% $NaBH_4$ (m/v) were used. 0.2 % m/v $NaBH_4$ was prepared in 0.5% NaOH (m/v) in 500ml volumetric flask using deionized water. 3M, 5M, 7M and 9M HCl solutions were prepared in different 250ml volumetric flasks by dilution of an appropriate volume of the 37% HCl (analytical grade) using deionized water.

Calibration standards of 3ppb, 5ppb, 7ppb, 9ppb, 11ppb and 13ppb of As were prepared and preliminary calibration curves were obtained when 0.2% $NaBH_4$ (m/v) was aspirated along each of the four sets of HCl concentrations (3M, 5M, 7M and 9M HCl) through the FI-HG-FAAS. A 10ppb As standard solution was aspirated as the sample and its absorbance recorded.

The procedure was repeated with 0.4%, 0.6% and 0.9% $NaBH_4$ (m/v) reagents against all the four sets of 3M, 5M, 7M and 9M HCl solutions, and for each set, 10ppb As standard was aspirated as the sample and its absorbance recorded.

The optimization was based on signal to noise ratio (S/N), the noise taken as the variation of the blank, (Standard deviation of the blank, σ_{Blank}), lowest detection limit recorded coupled with high sensitivity. S/N was calculated as mean absorbance of sample /3σ_{Blank}.

Analytical blank was the 5% (v/v) HCl treated with the pre-reductants, 20% KI (m/v) in 10% Ascorbic acid (m/v), just as the standards and sample.

The 10ppb As-standard sample and the blank absorbance measurements were read from the univariate experimental design tabulated in Table 3.1.

Table 3.1 Experimental design for instrumental optimization

Experiment Serial Number	$NaBH_4$ concentration %, (m/v)	HCl concentration (M)
1	0.2	3
2	0.2	5
3	0.2	7
4	0.2	9
5	0.4	3
6	0.4	5
7	0.4	7
8	0.4	9
9	0.6	3
10	0.6	5
11	0.6	7
12	0.6	9
13	0.9	3
14	0.9	5
15	0.9	7
16	0.9	9

3.3.2 SELECTION OF SOLID-PHASE PRECONCENTRATION CARTRIDGES FOR ARSENIC

Four Sep-Pak (Waters) cartridges; Alumina B plus, Alumina N plus, C_{18} and CN were assessed for As retention capability. Each of the cartridge was first conditioned by passing 20ml of distilled water using a 25ml glass (Pyrex) syringe at about 6.5 ml min^{-1}, followed by 20ml of 5% HCl and then followed again by 20ml distilled water. The conditioning of each cartridge was followed by loading 20ml of 10ppb As standard solution prepared in 5% HCl acid (v/v). The eluate was collected in a 25ml plastic conical flask, 2ml of 20% KI in 10% (m/v) ascorbic acid were added in each eluted solution and aspirated to FI-HG-FAAS for As determination.

3.3.3 DETERMINATION OF THE BREAKTHROUGH VOLUME

The cartridge identified to give the highest retention capability of As was conditioned as given earlier. It was then connected to a 300ml burette containing 10ppb As standard solution (treated with proportionate KI and ascorbic acid) as the sample on one end and the other end was connected to a peristaltic pump (chromatograph ATTA: AC2110), which then was connected to F1-FAAS. The flow rate through the cartridge was maintained at 6.65ml min^{-1}. The online preconcentration and subsequent determination of the breakthrough volume of the cartridge was carried out using the apparatus set up as shown in Figure 3.1.

The absorbance of the 10ppb As standard solution passing through the cartridge was read after every 2 minutes. The volume at which maximum retention capability occurred was recorded by measuring absorbance after every 2 minutes while maintaining the flow rate at 6.65ml min^{-1}. The maximum cumulative volume that

passed through the system, up to the time the AAS recorded traces of As was noted. This volume was used to determine the breakthrough volume based on the mean weight of the adsorbent. The alternate way was to use cumulative time, in which case breakthrough volume was calculated as the cumulative volume per gram of the adsorbent as follows.

$$\text{Breakthrough volume (ml/g)} = \frac{\text{cumulative time (min) x flow rate (ml/min)}}{\text{adsorbent weight (g)}}$$

Replicate analysis was performed.

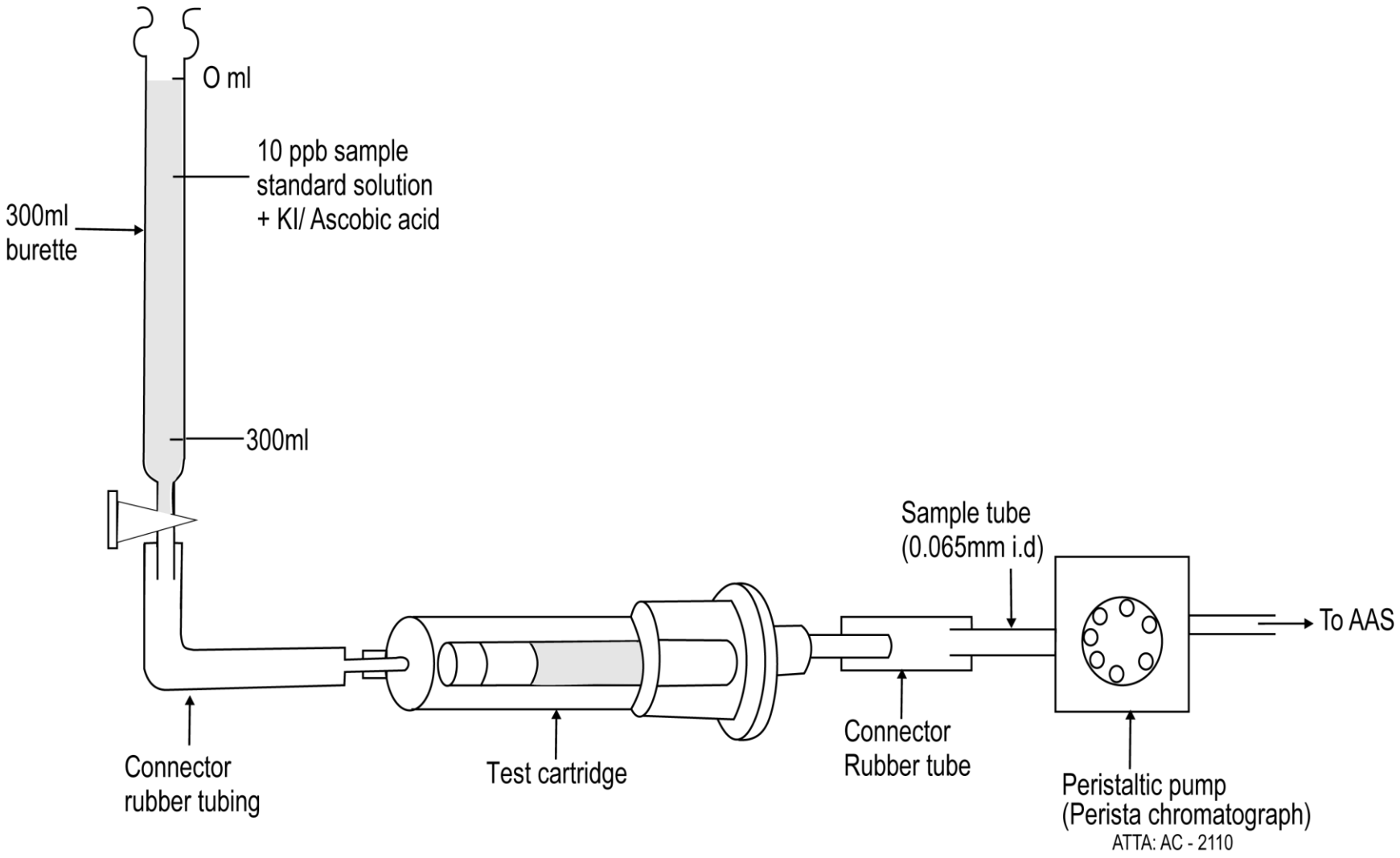


Figure 3.2 Apparatus Set Up for Online Determination of Breakthrough Volume of Sep-Pak (Waters) Cartridges

3.3.4 DETERMINATION OF THE OPTIMUM CONCENTRATION OF HCl AS ELUTION MEDIUM FOR ARSENIC

0.05%, 0.5%, 5% , 10%, 15% and 20% (v/v) of HCl solutions were prepared using distilled water. Using a 20ml capacity syringe, 20ml of each HCl solution prepared above were passed through the cartridges, whose break through volume had been previously determined. The eluates were collected in separate plastic conical flasks. 2ml of 20% KI (m/v) in 10% (m/v) ascorbic acid were added in each sample and the solutions aspirated through the FI- HG-FAAS for As determination. In order to eliminate variations in the signal intensities due to different acid concentration media, standard solutions were also prepared in the corresponding eluents. The absorbance readings were read twice as the optimized FI-HG-FAAS parameters were maintained.

3.3.5 CONSTRUCTION OF A MODIFIED GAS-LIQUID PHASE SEPARATOR

The commercial gas-liquid phase separator commonly used is shown in Figure 3.2(a). It consists of two compartments of glass cells linked together. The separation takes place in the compartment having gas-liquid mixture inlet from reaction coil and an inlet for the purging gas, argon. The hydride vapour separated is purged to the heated quartz cell of the AAS through the outlet at the top of the commercial separator. The second compartment facilitates continuous drain of the liquid mixture wastes through the outlet at the bottom of the cell.

The design of the modified gas-liquid phase separator shown in Figure 3.2(b) was proposed, which may lower the dead volumes that characterize the commercial one. The

modified gas-liquid phase separator may also offer a cheap and more reproducible method with high separation efficiency in the determination of As using FI-HG-FAAS method in a Kenyan situation. It was fabricated at Kenyatta University's Chemistry Laboratory, which offered glass blowing services. The inlet glass tubing for the hydride vapour – liquid mixture and that of argon gas used for purging the generated hydride vapour measured 3.14 mm (i.d) each. The outlet tube for the hydride vapour measured 3.8 mm (i.d), and was connected to Teflon tubing that connected the gas-liquid phase separator to the quartz T-cell for atomization in the AAS. The outlet to the drain measured 9.6mm (i.d), which was connected using plastic tubing to a waste plastic reservoir.

Schematic diagrams for the commercial gas-liquid separator and the fabricated one are given in Figure 3.2 (a) and (b) respectively.

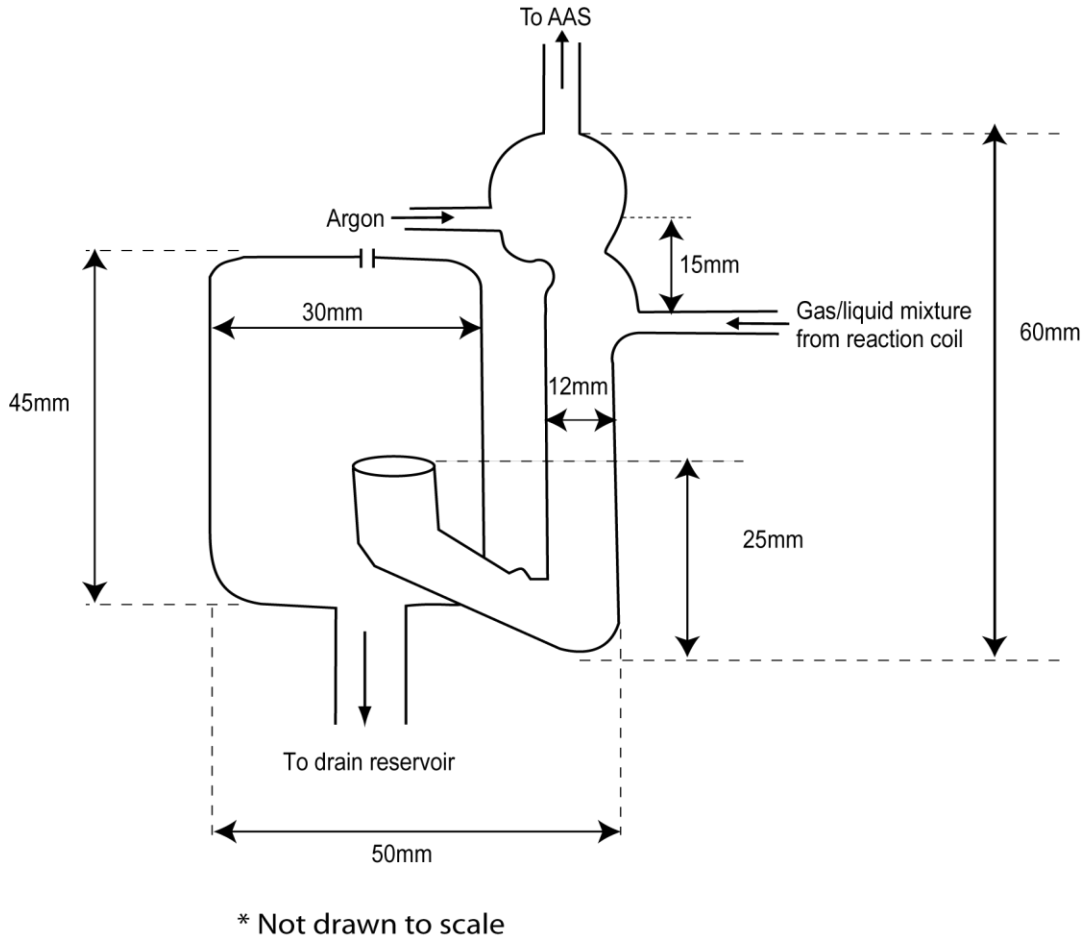


Figure 3.3(*a*) Commercial gas-liquid phase separator

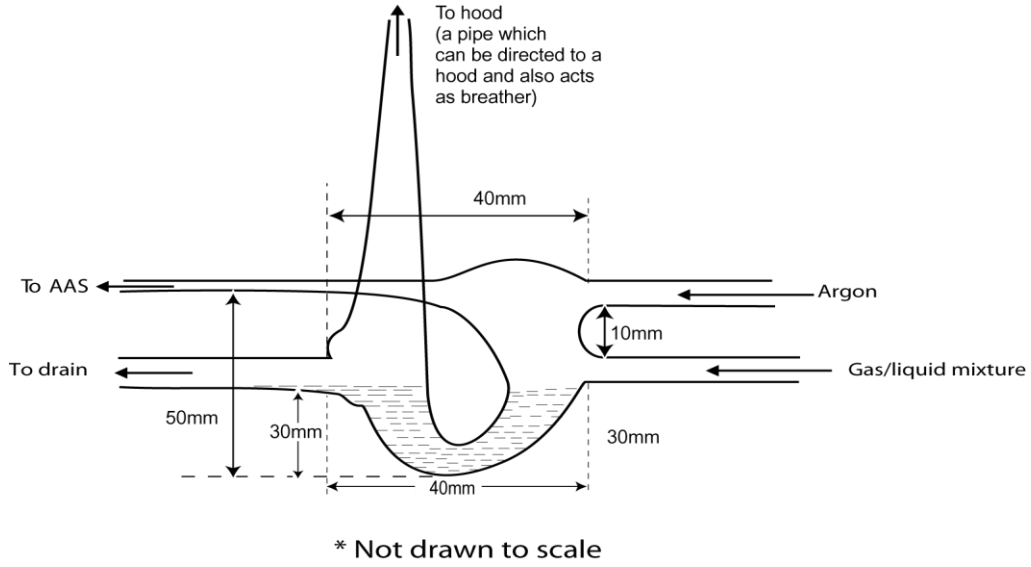


Figure 3.3 (*b*) Modified gas-liquid phase separator (Fabricated).

3.3.6 EVALUATION OF THE ANALYTICAL PERFORMANCE OF THE FABRICATED GAS-LIQUID PHASE SEPARATOR

The analytical capability of the optimized FI-FAAS was assessed for Arsenic by systematic evaluation of linear working range, sensitivity of the instrument, mean absorbance of 10ppb As control standard, standard deviation, relative standard deviation (% precision), and detection limit at 3 standard deviation above the blank.

Quantitation was by calibration curve method. The above parameters were compared against those obtained for the currently used commercial gas-liquid phase separator and an *F*-test was done to check whether the mean values obtained by the two methods were significantly different at 95% confidence level.

3.3.7 APPLICATION OF THE NEW METHOD

The developed method was applied to determine the As content in natural waters from shallow boreholes from Ganze area in the coastal region of Kenya, where Tana and Athi River Development Authority (TARDA) are planning to start a massive rice and sugar irrigation project. It was also applied in determination of As in commercial bottled water in the Kenyan market and some selected rice samples in the Kenyan market, both local and imported rice from some Asian countries which have been documented to suffer prevalent arsenic crisis.

3.3.7.1 ANALYSIS OF WATER SAMPLES

Thirty three (33) borehole water samples (each 500ml) from various selected areas of Ganze constituency were randomly collected as described by the EPA sampling method, As-Analysis method 1632 (USEPA, 1996). The sample names based on their sources are given in Table 3.2(a).

The samples were preserved through acidification to pH 2 with Hydrochloric acid (3ml of 6M HCl per 1 L sample) and stored in polyethylene bottles at $4^{o}C$ for a minimum of 48 hours before analysis to allow the As absorbed on the container walls to completely dissolve in the acidified samples.

9 samples of bottled mineral waters from different bottling companies in Kenya were also randomly collected from supermarkets in Thika area to investigate if they contained As. These are tabulated with respect to their brand names, the bottling company and water source as given in Table 3.2(b).

Table 3.2(a) Borehole water samples from Ganze

Sample number	Sample name
6	Mikinduni
2	Laini 'A'
9	Laini 'C'
26	Boji New
19	Bondeni Church
16	Tune 'A'
33	Kijijio
8	Maweni
22	Prison
12	Madogo Mosque
20	Madogo Water. Project
4	Hadampia
27	Vukoni village
11	Charidende
24	Makerere Mnguuweni
10	Mororo mosque
15	Emmaus

Sample number	Sample name
1	Bohoni
29	Lafuma Bondeni
18	Madogo ‘A’
13	Mororo Bondeni Well
28	Ghamano
5	Laini ‘B’
32	Ovo
21	Bububu
7	Mara mtu
23	Fangudho
3	Galamani
31	Handampia‘B’ Islamic

Table 3.2 (b) Bottled mineral water samples and their sources

Brand name	Bottling Company	Source
1. Keringet	Crown Foods Ltd	Keringet, Molo
2. Aquamist	Aquamist Ltd	Artesian Well, Rift valley

3. Joy	Crown Foods Ltd	Thika
4. BrownHill	Koba Water Ltd	Spring Natural Water Kiambaa
5. Sweetwaters	MicFood Executives Ltd	Nairobi
6.Ole Mara	Ole Mara Foods	Kikuyu and Narok.
7. Summer House	L.M Enterprises	Nairobi
8. Highlands	Highlands Mineral Water Co. Ltd	Nyeri
9. Dasani	Beverage Services Kenya Ltd (Coca-Cola Company)	Nairobi

20ml of each borehole water and bottled water sample was pipetted into a 50ml volumetric flask and 2ml KI/ascorbic acid mixture added before aspirating through the HG-AAS that incorporated the fabricated gas-liquid phase separator for As determination. Replicate analysis was performed for each sample.

3.3.7.2 ANALYSIS OF RICE SAMPLES

Locally grown rice**,** Mwea Pishori (from Mwea irrigation scheme, Kenya) was randomly collected from the supermarkets in Thika. From the same supermarkets, imported rice from three Asian countries; Pakistan, Singapore and Vietnam were also randomly collected. The rice samples were separately ground into powder form, dried in oven at 80^0C for 5 hours and then stored in a desiccator.

0.5g of each sample was acid digested (USEPA method; triacids mixture of HNO_3 : H_2SO_4: $HClO_4$, 6:3:1), in 5mls of the acid mixture at between 70^0C – 120^0C for 2 hours until approximately 1ml remained or SO_3 fumes were observed.

The contents were allowed to cool and 10ml of 5% HCl (v/v), were added and the mixture boiled for a few minutes. The mixture was allowed to cool, filtered through Whatman #42 into a 50ml volumetric flask and filled to the mark with 5% HCl. Blank digestion was also carried out in the same way by subjecting 5ml of the acid mixture through the same treatment as the rice samples. The digested rice samples and the blank were aspirated into HG-AAS for As determination after treating 20ml of each sample with 2ml of KI/Ascorbic acid mixture.

CHAPTER FOUR: RESULTS AND DISCUSSION

The results of the experiments performed as described in chapter three, on selection and evaluation of solid-phase extraction cartridges are presented first. Sample preparation including clean-up and preconcentration is an essential component of method development for trace analysis. This is followed by results on optimization of instrumental parameters and evaluation of fabricated gas-liquid phase separator.

Analytical performances of the commercial and fabricated gas-liquid separators are compared. The modified method was applied for the analysis of borehole and bottled water and also some imported and local rice after triacid digestion. The data was processed using WIZAARD software for metal analysis installed in computer connected to the Shimadzu HVG-AAS instrument and evaluated using Excel algorithm, all based on external standards calibration method.

4.1 CALIBRATION OF EQUIPMENT

Upon aspiration of the blank and the standards under the conditions described in section 3.1, absorbance data obtained gave a calibration curve in Figure 4.1. Intrapolation of the calibration curve was used in the subsequent experiment for the determination of the retention capability of arsenic by various cartridges.

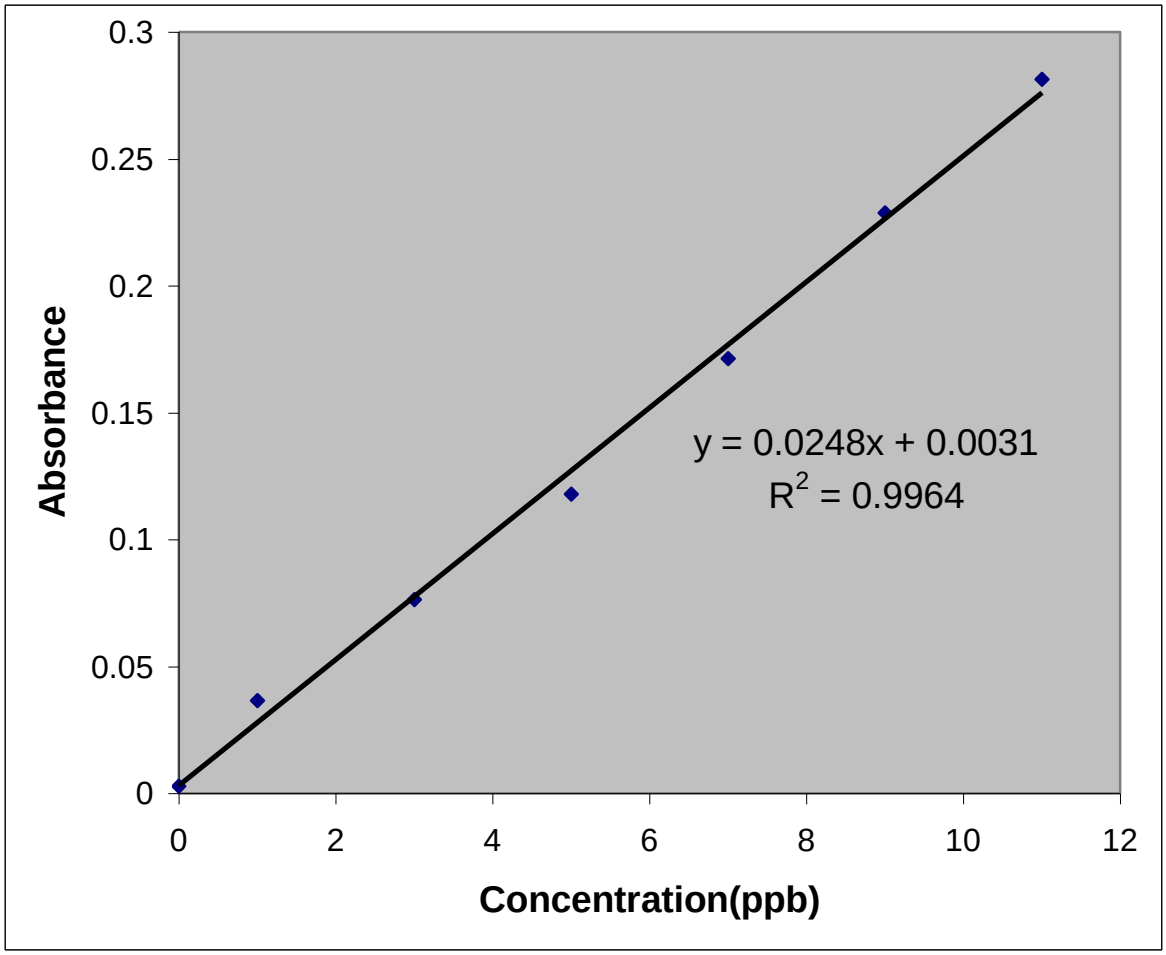


Figure 4.1 Calibration curve used for determination of As-retention capability of cartridges.

4.2 DETERMINATION OF RETENTION CAPABILITY OF ARSENIC BY VARIOUS CARTRIDGES

Four Sep-Pak (Millipore Waters) cartridges; Alumina B plus, Alumina N plus, C_{18} and CN were tested for As retention capability as described in Section 3.3.2.

Each of the cartridges was first conditioned by passing 20ml of deionized water using a 25ml glass (Pyrex) syringe at 6.5 ml min^{-1}, followed by 20ml of 5% HCl and then followed again by 20ml deionized water. This flow rate was chosen and maintained based on the fact that flow rate is a very critical factor in sample loading. It has been reported that excessive flow rate is one of the most common causes of poor recovery, yet it is also one of the most common uncontrolled variables in solid phase extraction (Millipore Waters, 1992).

The clean-up and conditioning of each cartridge was followed by loading 20ml of 10ppb As. Arsenic standard solution was prepared in 5% HCl acid (v/v) for each of the cartridges having been chosen as the suitable solvent on the basis that it enhances the arsine generation since it has been used as one of the reagents for hydride generation. The absorbance for each eluate from test cartridges was recorded and the recovery of the 10ppb As solution with the corresponding per cent of arsenic retained were calculated and tabulated (Table 4.1).

Table 4.1 Results for As-retention capability of cartridges: Alumina B (plus), Alumina N (plus), C18 (plus) and CN (plus)

Cartridge Type	Mean Absorbance $\overline{X} \pm \sigma$, (n=3)	Recovery of 10ppb standard (%)	Amount of As Retained (%)
CN	**0.2222± 0.0071**	**88.3±0.3**	**11.7±2.5**
C18	**0.2279±0.0046**	**90.6±0.2**	**9.4±1.9**
Al-N	**0.0719±0.0059**	**27.7±0.4**	**72.3±3.8**
Al-B	**0.0689±0.0053**	**26.5±0.2**	**73.5±1.9**

From the data given in Table 4.1**,** alumina B (plus) cartridges showed the highest ability to retain arsenic, followed very closely by alumina N (plus). CN (plus) and C18 (plus) cartridges showed inferior ability for retention of arsenic with C18 showing the lowest retention ability. The recovery of arsenic in the four cartridges shows an inverse relationship with respect to their retention capabilities so that the cartridge showing highest recovery value (C18) show the least retention capability of arsenic. This shows that CN and C18 cartridges may not be suitable for pre-concentrating arsenic. However, alumina B (plus) and alumina N (plus) cartridges may be used for pre-concentrating arsenic as they showed relatively higher retention capability and lowest recovery values than C18 and CN cartridges.

The retention capability of alumina B was not significantly different from that of alumina N, (from t-test$_{(\Upsilon=2)}$ and *F*-test$_{(\Upsilon=4)}$ for $n_{Al\text{-}A}$= 3, $n_{Al\text{-}N}$ = 3), at 95% confidence level (Appendix 4). However alumina B (plus) showing slightly higher retention ability for

arsenic than alumina N (plus), was chosen for further work and its breakthrough volume determined online. The retention capacities of the four cartridges were also calculated (see Appendix 1), based on the mean weight of packing material (Millipore Waters, 1992), and tabulated in Table 4.2.

Table 4.2 Calculated retention capacities of cartridges

Cartridge	**Mean weight of packing material (mg/cartridge)**	**Amount of As Retained (%)**	**Retention capacity (µg /100g adsorbent)**
Alumina B (Plus)	1710	73.5±1.9	8.5
Alumina N (Plus)	1710	72.3±3.8	8.4
CN (Plus)	360	11.7±2.5	6.5
C18 (Plus)	360	9.4±1.9	5.3

The calculated retention capacity of alumina B (plus) was highest compared with alumina-N (plus), CN (plus) and C18 (plus) cartridges. The retention capacity data for the cartridges under test was directly proportional to the amount of arsenic retained, with the amount of arsenic retained increasing with increase in the retention capacity of the cartridges. By alumina B (plus) cartridge showing the highest ability to retain arsenic and also the highest retention capacity, it means that the active sites of the alumina B (plus)

adsorbent not only had the highest ability to bind with the arsenic analyte but also did so most strongly.

4.3 DETERMINATION OF BREAKTHROUGH VOLUME OF THE ALUMINA B (PLUS) CARTRIDGES

10ppb As standard solution was continually passed through the Alumina B (plus) cartridge at a flow rate of 6.7ml min^{-1} using a peristaltic pump until arsenic was detected in the AAS signal. The time taken for the signal to be observed was used to calculate the amount of As retained. The results of the replicate determination of the average breakthrough volume was also calculated based on the mean weight of packing material (Millipore Waters, 1992), and tabulated in Table 4.3.

Table 4.3 Average breakthrough volume of the Al-B (plus) cartridges as determined online.

Cartridge Al – B Plus	Sample Flow Rate (ml min^{-1})	Cumulative Time Taken for Arsenic to be detected (Minutes)	Average Breakthrough Volume (ml of 10ppb sample/g adsorbent)
Al – B	6.7	23.9	93.7

The average breakthrough volume for Alumina B (plus) cartridge was 93.7ml of 10ppb sample per gram of adsorbent, and the total volume of the 10ppb As solution that was loaded through the cartridge to achieve maximum retention capacity was calculated to be 160.13ml (sample flow rate x cumulative time taken for As to be detected). This indicated that beyond this volume no more As would be retained. This is because when the amount of arsenic sample loaded onto the packing material exceeds the packing material's capacity, the excess arsenic elutes. Considering the percentage retention of As by Al – B was 74% as given earlier in Table 4.1, the amount of As retained at this breakthrough volume was calculated to give 59 µg L^{-1}. From calculations, it therefore means close to 60 µg L^{-1} of arsenic was retained in the cartridge at 93.7 ml/g adsorbent breakthrough volume when the sample flow rate was maintained at 6.7ml min^{-1}. Thus, a preconcentration factor of about 6 was achieved for the Alumina B (plus), which was close to the theoretical preconcentration factor of 8 expected (Millipore Waters, 1992).This may be attributed to possible fluctuations in the sample flow rates which in some instances could have been higher and thus the time allowed for the As to bind strongly with the adsorbent species could have been shorter.

4.4 ELUTION OF ARSENIC FROM CARTRIDGES

In general, after sufficient amounts of the component of interest is retained in the cartridge, it is eluted with a suitable solvent, capable of unbinding the analyte from the adsorbent's active sites. The choice of eluting medium for any analyte depends on physical and chemical characteristics of the packing material, cartridge configuration and elution protocol, (Millipore Waters, 1992). The flow rates when eluting are just as

important as during the bed conditioning and sample loading in order to achieve good recoveries. Different acid media such as nitric, perchloric, hydrochloric and acetic acids have been compared and reported by Li-Li and Zhang, (2003) and Ralph (1995) in which hydrochloric acid was found easier to control than the other acid media in arsine generation. Hydrochloric acid was therefore chosen as the elution medium on the basis that it is an important component in the arsenic hydride generation and whose concentration affects the amount and rate of arsine generated. Concentration range of 0.05%-20% HCl (v/v) was chosen for this experiment and 20ml of each HCl solution was passed through the loaded cartridge using the 20ml syringe at a flow rate of 6.5ml min^{-1}. The eluate was aspirated to the AAS for arsenic determination.

The absorbance for different concentrations of HCl was recorded and the respective recoveries calculated. Concentration of HCl was given in per cent (v/v) for convenience purposes, otherwise percent concentrations were converted to molar concentrations by calculation and the results are given in Table 4.4 while Figure 4.2 gives a plot of the absorbance of eluted sample against the acid concentration.

Table 4.4 Effect of HCl concentration as eluting media

Alumina B (plus) cartridges	Acid concentration % (v/v), (M)	Average absorbance of eluted sample	Recoveries (%)
Al-B_1	0.05 (0.0163)	0.0104	13.6
Al-B_2	0.5 (0.1630)	0.0156	21.8
Al-B_3	5.0 (1.6300)	0.0216	32.7
Al-B_4	10.0 (3.2500)	0.0495	78.9
Al-B_5	15.0 (4.8800)	0.0427	66.7
Al-B_6	20.0 (6.5000)	0.0419	65.3

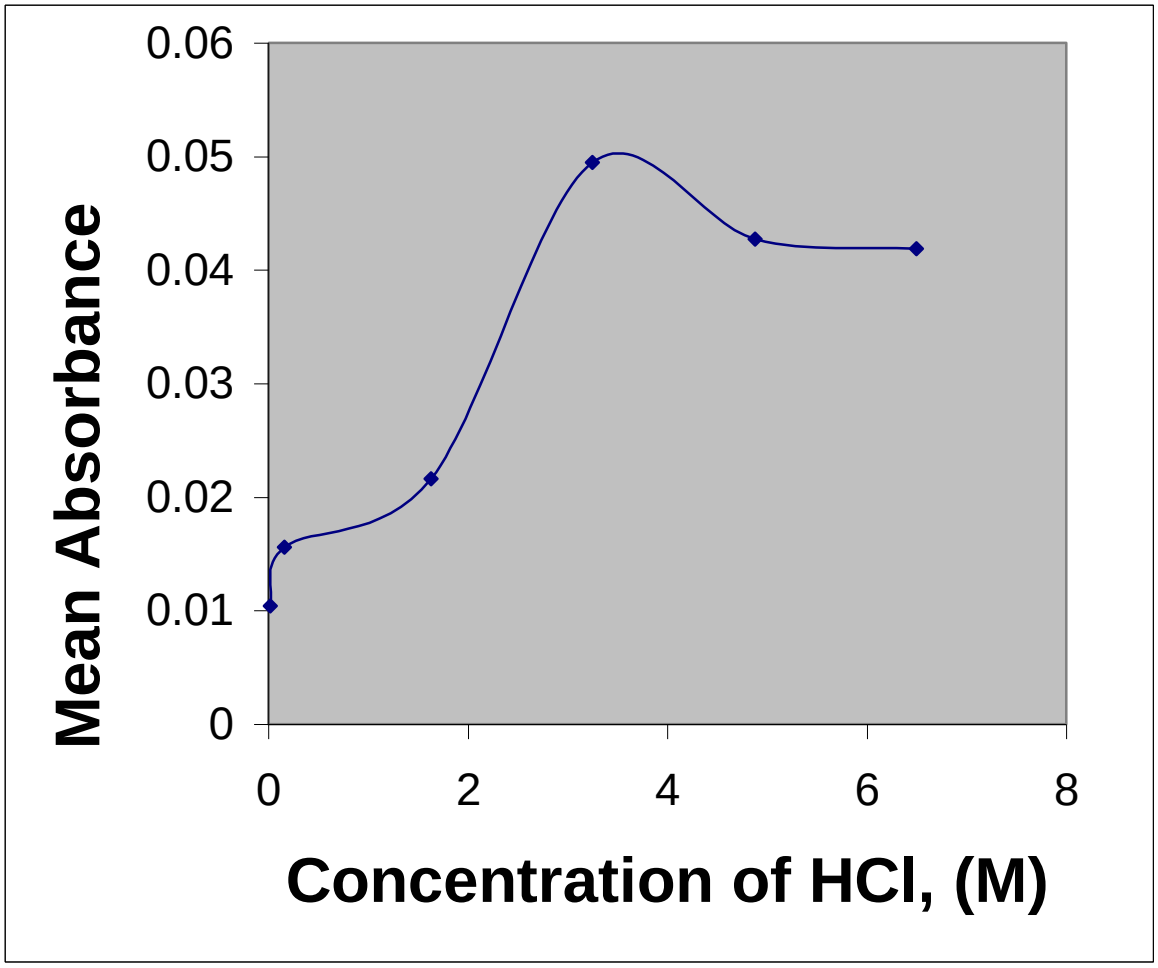


Figure 4.2 Effect of HCl concentration as elution medium for 10ppb As from alumina B (plus) cartridge.

From the data in Table 4.4 and Figure 4.2, there was a general increase of the signal (absorbance) as the concentration of the HCl eluting media was increased in the range of 0.05% - 10.0%. Concentrations above 10% gave slightly lower signal absorbance. This could perhaps be attributed to the possibility of impairment of selectivity by the eluting ions and hence lowers the sensitivity. In addition to this, 15% - 20% HCl, coupled with the manifold reagent of 7M HCl required in the generation of the hydride vapour would

make the reactant (HCl) to have elevated concentrations, leading to higher reaction rate for arsenic hydride generation and hydrogen gases. Consequently, this may cause turbulence and hence unnecessary noise in the AAS instrument, as the background absorption was observed to increase at high concentrations of HCl acid.

With relatively lower concentration of HCl, the elution of As retained in the alumina B (plus) column was poor, but showed improvement as the acid concentration was increased within the range of 0.05 – 10% v/v. It was recognized that 78.9% of arsenic retained on the alumina B (plus) cartridge can be eluted using 20mls of 10% (v/v), (3.25M) HCl. Therefore 10% HCl (v/v) solution was used in the subsequent experiments as eluting medium.

4.5 OPTIMIZATION OF THE INSTRUMENTAL PARAMETERS

Optimization of the instrumental working conditions was based on the best compromise obtained between signal to noise ratio (S/N), lowest detection limit, percentage recovery coupled with high sensitivity and precision (Douglas *et al.*, 2004). In addition to this, linearity could be considered along the above parameters as described by Alison *et al.*, (1998). However, in practice the working concentration range is usually limited by the sensitivity of the detector as per the manufacturer's recommendations.

4.5.1 REAGENTS AND SAMPLE FLOW RATES IN FIA SYSTEM

The efficiency of continuous flow hydride generation relies on several factors, such as reactants flow rates and concentrations, or gas flow rates. The reagents flow rates and concentrations were optimized independently for the FI system.

Preliminary optimization of the flow rates was carried out using 5M HCl and 0.4% (m/v) $NaBH_4$ as described for As determination by the instrument manufacturer (Shimadzu, 1992). The reagents flow rate was varied between 0.5 - 2.5 ml min^{-1} by variation of the peristaltic pump speed while keeping the 10ppb As sample flow rate at 6 ml min^{-1}. The reagents flow rate, which resulted in the highest absorbance, was taken as the optimized reagents flow rate and was used to optimize the sample flow rate by varying the sample flow rate adjustment knob on the peristaltic pump. The highest absorbance obtained was also recorded. Graphs of absorbance against the reagents flow rate and sample flow rates are plotted in Figure 4.3(a) and Figure 4.3 (b) respectively.

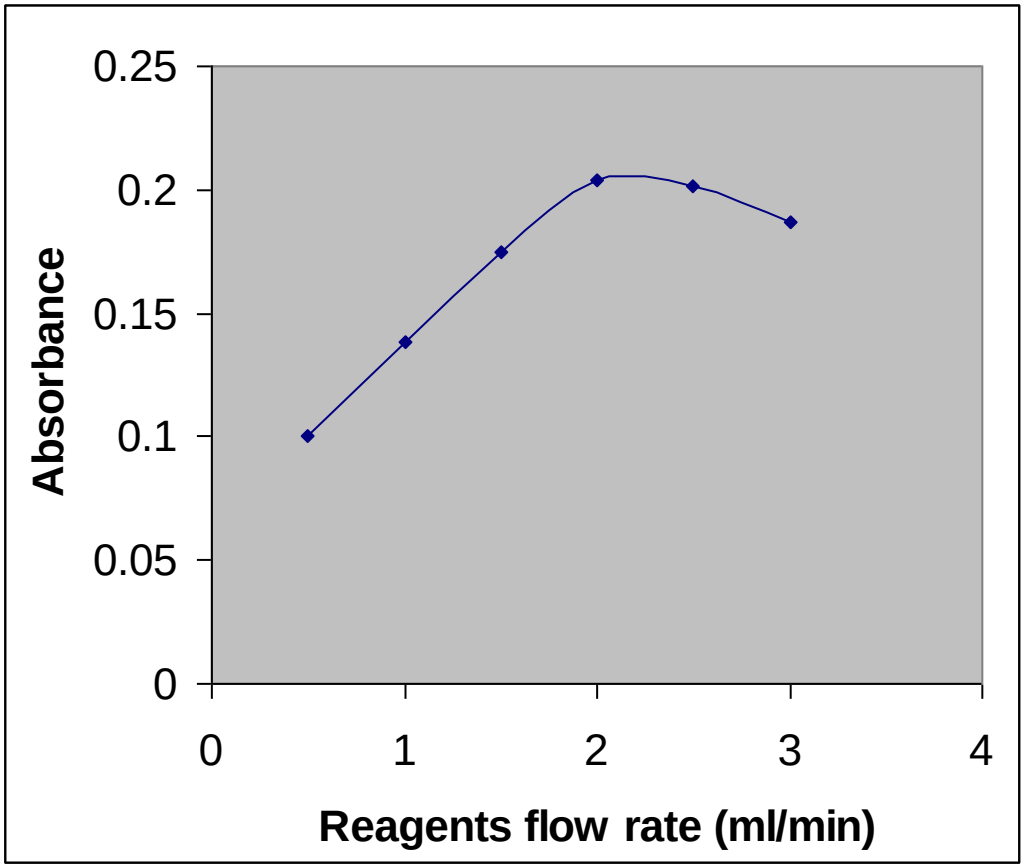


Figure 4.3 (a) Graph of absorbance against reagents flow rate.

From Figure 4.3(a), increased reagents flow rate results in increased absorbance signal up to about 2.2ml min^{-1} at a constant sample flow rate of 6ml min^{-1}. Further increase in the reagents flow rate above 2.2ml min^{-1} led to a decrease in the absorbance signal. This flow rate was used in the optimization of the sample flow rate as given in Figure 4.3(b).

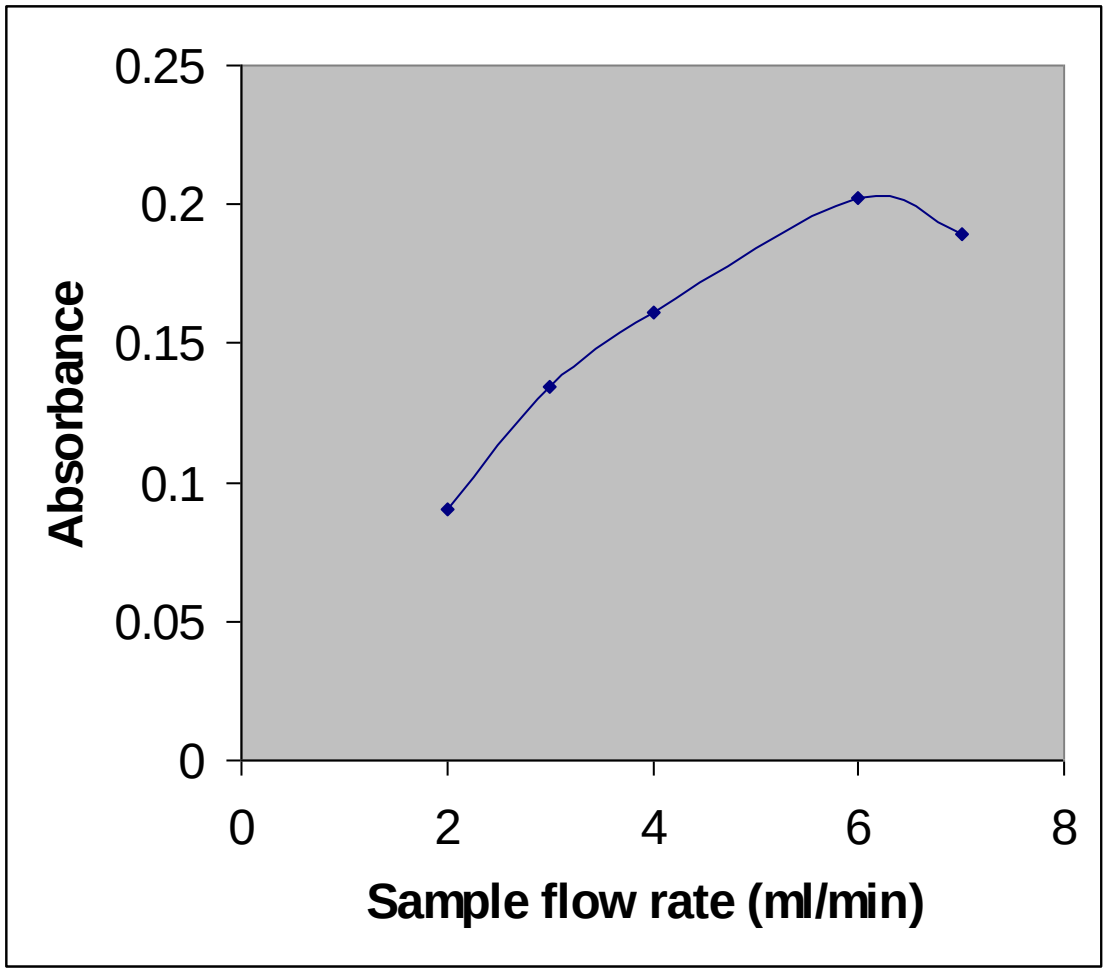


Figure 4.3 (b) Graph of absorbance against sample flow rate.

Optimized flow rates for both HCl acid and $NaBH_4$ were achieved at 2.2ml min^{-1} and sample flow rate was achieved at 6ml min^{-1}.

These flow rates combination were used in the subsequent experiments for optimization of the reagents concentration.

4.5.2 CONCENTRATION OF HYDRIDE GENERATION REAGENTS

The concentration of hydrochloric acid was varied between 3M and 9M and sodium borohydride in the range of 0.2% to 0.9% (m/v) to give absorbance readings of the 10ppb arsenic standard solution given in Table 4.5

Table 4.5 Effect of HCl and $NaBH_4$ concentrations on absorbance signal.

	Mean absorbance, (n = 3)			
[$NaBH_4$] (%) / [HCl] (M)	**0.2**	**0.4**	**0.6**	**0.9**
3	0.2262 ± 0.0071	0.2434 ± 0.0063	0.2247 ± 0.0077	0.1073 ± 0.0146
5	0.1573 ± 0.0075	0.2024 ± 0.0065	0.1577 ± 0.0032	0.1344 ± 0.0028
7	0.1124 ± 0.0032	**0.3101** ± 0.0043	0.1792 ± 0.0017	0.1493 ± 0.0018
9	0.1769 ± 0.0027	0.3029 ± 0.0131	0.2268 ± 0.0024	0.2873 ± 0.0069

From the data given in Table 4.5, the highest signal was recorded when a combination of 7M HCl acid was used with 0.4% $NaBH_4$ stabilized in 0.5% NaOH (m/v). However, it is inadequate to conclusively apply these parameters without subjecting the results for each set of reagents combination (HCl and $NaBH_4$ solutions) to calculations and comparison

of analytical figures of merit. These were based on the calibration equations obtained for each trial experiment and recovery levels of 10ppb arsenic standard solution, as 5% HCl (v/v) was used as the blank.

The analytical parameters of merit considered for this work were sensitivity, precision, signal/noise ratio, detection limits and percentage recovery which were calculated for each of the trial experiments in Table 4.5. The sensitivity was taken as the slope of the calibration curve and precision was expressed as the relative standard deviation of the 10ppb As solution (n=3). Both the signal/noise ratio and detection limit were calculated based on absorbance/$3\sigma_{blank}$ and $3\sigma_{Blank}$/sensitivity respectively. The trial experiment, which gives comparatively better compromise among the calculated parameters, gives the optimal concentration of HCl and $NaBH_4$ solutions for efficient hydride generation. The data for the analytical figures of merit is presented in Table 4.6.

Table 4.6 Analytical figures of merit for optimization of HCl and $NaBH_4$ concentrations under set instrumental conditions.

Experiment Number	Concentration of $NaBH_4$ % (m/v)	Concentration of HCl (M)	Calibration Equation y = Absorbance x = Concentration	Correlation Co-efficient r	Sensitivity (Abs/ugL^{-1})	RSD (Precision) (%) (n=3)	S/N Ratio Abs/3σ_{blank}	Detection Limit (3σ_{Blank}/S) µg L^{-1}	Recovery (%)
1	0.2	3	y = 0.012x + 0.0902	0.9988	0.0120	1.8	10.2	1.85	98
2	0.2	5	y = 0.0125x + 00261	0.9990	0.0125	4.8	3.9	3.22	105
3	0.2	7	y = 0.097x + 0.0184	0.9993	0.0970	2.8	5.4	2.13	97
4	0.2	9	y = 0.0185x + 0.0004	0.9971	0.0185	1.5	1.6	5.50	95
5	0.4	3	y = 0.0115x + 0.1204	0.9979	0.0115	2.6	1.3	16.36	107
6	0.4	5	y = 0.0097x + 0.1048	0.9980	0.0097	3.2	1.9	10.73	104
7	0.4	7	y = 0.0206x + 0.1002	0.9989	0.0206	0.8	20.7	0.74	101

Experiment Number	Concentration of $NaBH_4$ % (m/v)	Concentration of HCl (M)	Calibration Equation y = Absorbance x = Concentration	Correlation Co-efficient r	Sensitivity (Abs/ugL^{-1})	RSD (Precision) % (n=3)	S/N Ratio Abs/3σ_{blank}	Detection Limit (3σ_{Blank}/S) µgL^{-1}	Recovery (%)
8	0.4	9	y = 0.0289x + 0.0217	0.9978	0.0289	4.3	12.8	0.82	97
9	0.6	3	y = 0.0127 x + 0.0814	0.9974	0.0127	3.4	2.7	6.65	108
10	0.6	5	y = 0.0122 x + 0.0405	0.9966	0.0122	2.0	5.2	2.48	96
11	0.6	7	y = 0.0167x + 0.0062	0.9982	0.0167	0.9	3.4	3.20	104
12	0.6	9	y = 0.0203x + 0.0294	0.9984	0.0203	1.1	8.4	1.30	97
13	0.9	3	y = 0.0075x + 0.0317	0.9998	0.0075	13.6	3.6	4.00	101
14	0.9	5	y = 0.0117x + 0.0291	0.9967	0.0117	2.1	5.0	2.31	90
15	0.9	7	y = 0.012x + 0.0318	0.9984	0.0120	1.2	9.2	1.35	98
16	0.9	9	y = 0.0119x + 0.1716	0.9992	0.0119	2.4	3.0	8.07	97

From the data given in Table 4.6, the highest signal/noise ratio was 20.7, achieved by the 0.4% $NaBH_4$ – 7M HCl trial experiment. This acid – borohydride combination also gave the lowest detection limit of 0.74 μg L^{-1} coupled up with highest precision of 0.8%. It also gave comparatively the best percentage recovery of 101, and a sensitivity of 0.0206 Abs/μg L^{-1}.

Whereas highest sensitivity was 0.0289Abs/μg L^{-1} for a 0.4% $NaBH_4$ with 9M HCl combination, the respective signal/noise ratio and percentage recoveries were lower than those of the 0.4% $NaBH_4$ – 7M HCl trial experiment and it also recorded higher detection limit (4.3%). The calibration curves for these two sets of experiments that showed very close and comparable parameters are given in Figure 4.4.

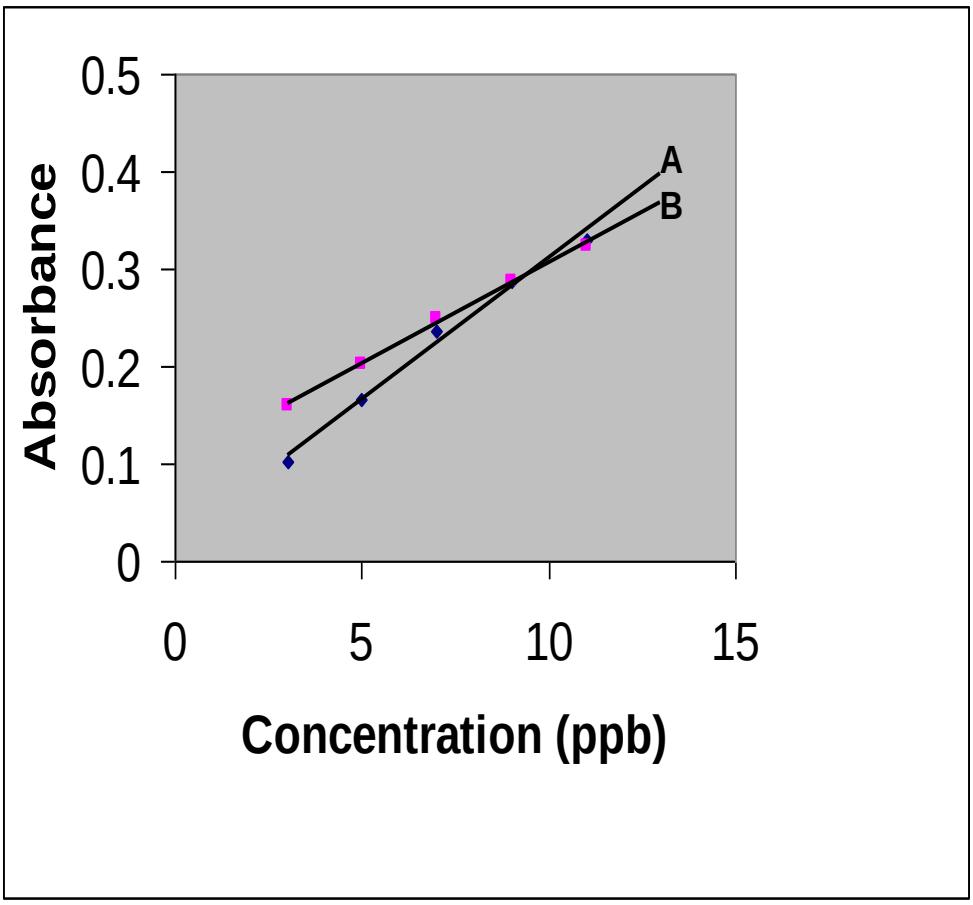


Figure 4.4 Calibration curves for optimization of HCl and $NaBH_4$.

- Legend A: 0.4% $NaBH_4$ with 9M HCl, (Abs = 0.0289conc + 0.0217, r = 0.9978).
- Legend B: 0.4% $NaBH_4$ with 7M HCl, (Abs = 0.0206conc + 0.1002, r = 0.9989).

From the results obtained, increased concentrations of both the sodium borohydride and hydrochloric acid solutions favour the generation of arsine vapour. At very low acid concentration, the rate of reaction for hydride generation is very slow and consequently no much arsine is generated in the reaction coil, which explains the high detection limits for all experimental sets involving 3M HCl solution. Except for the experimental sets that

used very low $NaBH_4$ concentrations of 0.2% (m/v), the general trend from Table 4.6 shows a general decrease of detection limits (improvement) for each set where increased concentration of acid and sodium borohydride, safe for experimental sets where 9M HCl (experiment no. 8) or very high concentrations of sodium borohydride (0.9%) were used, (experiment no.13 and 16). However at very high acid and borohydide concentrations, poor precision and detection limits were achieved coupled with lower signal/noise ratio. This can be attributed to the high reaction rate for arsine generation which causes pressure fluctuations (higher pressures) due to high amounts of arsine vapours, hydrogen gas and water vapour produced and which may cause turbulence in the FI-AAS system. This will bring about instrumental noise.

In this regard, 0.4% (m/v) $NaBH_4$ in 0.5% (m/v) NaOH, combined with 7M HCl were chosen for optimum hydride vapour generation because they gave the best compromise between signal/noise ratio and detection limits coupled with precision, sensitivities and recoveries. Instrumental operating parameters are summarized in Table 4.7 and the optimum concentrations were used in the subsequent experiments for hydride generation.

Table 4.7 Summary of instrumental operating parameters for arsenic analysis using F1-HG-FAAS

Parameter	**Description**
System type	Flame heated quartz tube
Element analyzed	Arsenic
Wavelength (nm)	193.7
Slit width (nm)	0.7
Slit height	Normal
Measurement mode	Continuous flow method
Sampling mode	Manual
Flame type	Air – acetylene
Instrument mode	Absorbance
Lamp current (mA)	12mA – 15mA
Acetylene flow rate	2.0 L min^{-1}
Air flow rate	8.0 L min^{-1}
Read time (s)	120
Carrier gas	Argon
Carrier gas flow rate	70 ml min^{-1}: 0.32m Pa supply pressure
Carrier solution	HCl
Carrier solution concentration	7M HCl
Carrier solution flow rate	2.2 ml min^{-1}

Reagents solution details	**Description**
HCl: (i) Acid concentration	7 M
(ii) Acid flow rate	2.2 (ml min^{-1})
$NaBH_4$: (i) Concentration	0.4% in 0.5% NaOH (m/v)
(ii) Flow rate	2.2 (ml min^{-1})
Sample flow rate	6.0 (ml min^{-1})
Lamp mode	BGC – D_2

4.6 GAS - LIQUID PHASE SEPARATORS

After online production of arsenic hydride, it is necessary to transport it to the heated quartz cell. The reaction mixture composed of arsenic hydride, hydrogen, water vapour and reagent residues are fed to the gas-liquid phase separator by argon carrier gas. In the separator, the gas phase is separated from the liquid and stripped or purged to the heated quartz cell where the arsenic hydride is pyrolyzed and atomized for the atomic absorption analysis.

The modified gas-liquid phase separator was fabricated as per the proposed specifications described previously in Section 3.3.5. The inner diameters for the inlet and outlet for the two gas-liquid separators were relatively the same in order to achieve compatibility with the tubings available for the peristaltic pump, but where need be, connectors were used in-between. However, the main feature in the fabricated gas-liquid separator was that the arsenic hydride generated was purged almost at the same level with the purging gas itself, thereby reducing the time taken for the arsine to reach the quartz cell for pyrolysis and atomization. This would further eliminate any pressure fluctuations and minimize high dead volumes that result to poor reproducibility, typical of the commercial gas-liquid separator. High dead volumes cause loss of the arsenic analyte by adsorption on the glass of the gas separator cell (Laborda *et al.*, 1996), consequently increasing detection limits and lowering efficiency of separation of the gaseous hydride from the gas-liquid mixture. The geometry of the fabricated gas separator offered a remedy to these problems typical of the currently used commercial gas-liquid separator (Figure 3.2(a) and (b)).

A given volume level of the gas-liquid mixture in the modified gas-liquid phase separator was maintained to avoid fluctuations of the signal.

Another feature in the new design was an extended outlet tube which was connected to a plastic tubing that acted as a breather as well as directing any waste gases to the hood for health safety, a feature not present in the currently used commercial gas-liquid phase separator.

The analytical performance of the fabricated cell was compared with the currently used commercial gas-liquid phase separator. The calibration graphs of the two gas-liquid phase separators are given in Figure 4.5 and their comparative statistical summary given in Table 4.8.

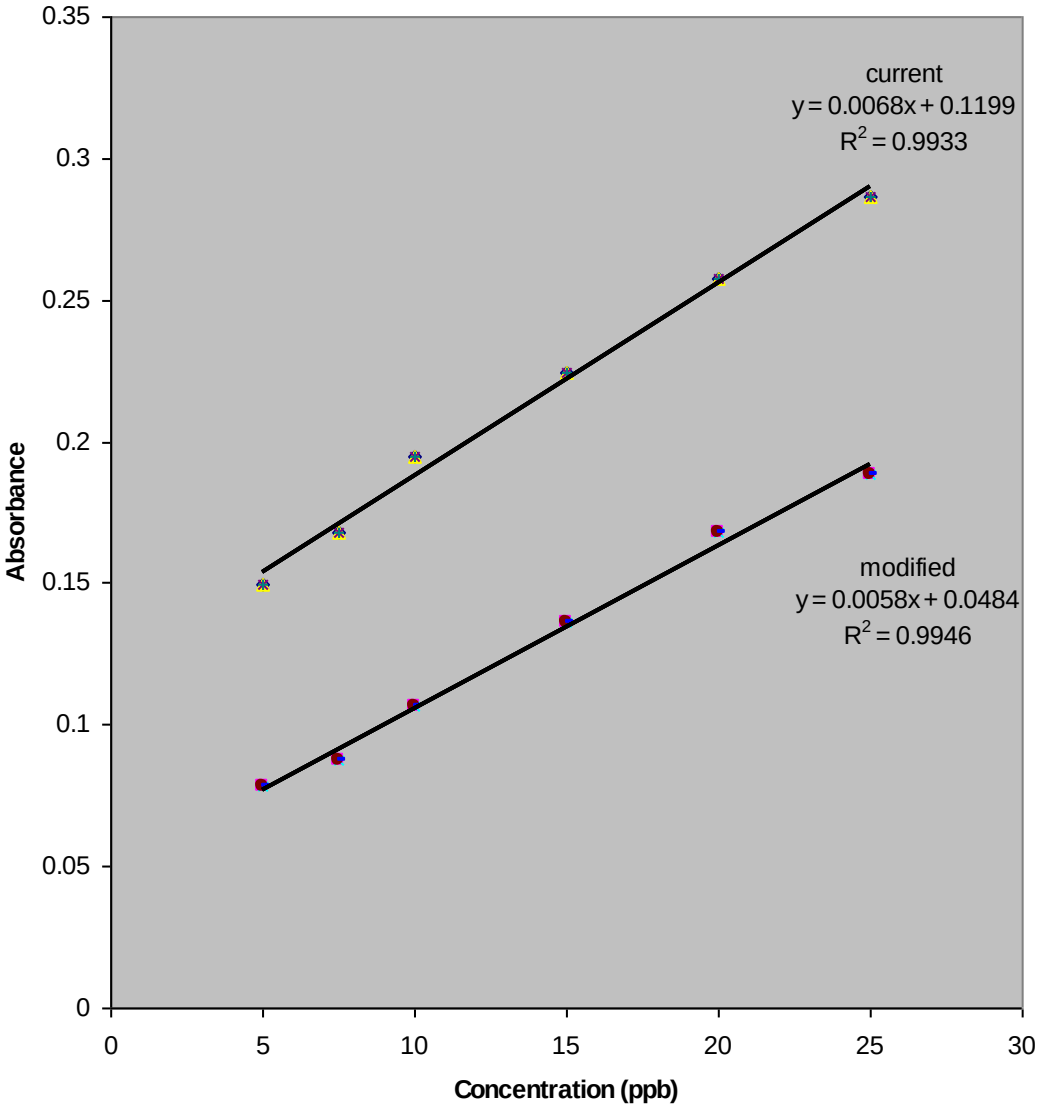


Figure 4.5 Calibration graphs for the two gas-liquid phase separators.

Table 4.8 Statistical summary for the two gas-liquid phase separators under similar instrumental conditions.

Parameters	**Current**	**Modified**
Mean absorbance of 10ppb sample, $\overline{X}_{abs}$	0.1910 ± 0.0063 (n=5)	0.1029 ± 0.0016 (n=8)
Standard deviation, σ_x	0.0063	0.0016
Recovery of 10ppb standard (**ppb**) **(%)**	105.3	94.7
Sensitivity, (**S**) (Abs/μg L^{-1})	0.0068	0.00575
Regression equations Correlation coefficient, **(r)**	Abs = 0.0068 conc. + 0.1199 0.9966	Abs = 0.0575conc. + 0.0484 0.9973
Mean of blank $\overline{X}_B$	0.0360	0.0183
Standard deviation of blank, σ_B	0.0617	0.0071

Relative standard deviation, RSD; (% Precision)	3.3%	1.55%
Signal/Noise ratio ($\overline{X}_{abs}/3\sigma_B$)	1.03	4.83
Detection limit, DL ($3\sigma_B$/ **S**), μg L^{-1}	27.2	3.67

From the results in Figure 4.5 and Table 4.8, the fabricated gas-liquid separator showed comparatively almost the same sensitivity. However, the fabricated gas-liquid phase separator exhibited slightly lower sensitivity of 0.00575 Abs/μg L^{-1}, compared to that of the currently used commercial gas separator (0.0068 Abs/μg L^{-1}). A possible explanation is that an ideal interfacing of the fabricated cell to FIA and AAS could not have been practically achieved.

The blank standard deviation was 0.0071 for the modified method, which translated to better detection limits, about 7 times better than the current method. The precision was also better for the modified method (1.55%), giving a 2-fold improvement.

4.7 STATISTICAL EVALUATION OF THE METHODS.

The mean and standard deviation of the commercial and fabricated gas-liquid separators as tabulated in Table 4.8 were used to evaluate the two methods. It was found using t-test that the mean results of As concentration obtained by the modified method and the current method were significantly different at the 95% confidence level, ($p<0.05$ level of significance), (refer to appendix 2, 3 and 4). However the variance of the two methods were not significantly different at the 95% confidence level (refer Appendix 5). The fabricated cell offered better precision (RSD 1.55%) than the currently used commercial cell (RSD 3.3%), indicating that the fabricated cell offers superior reproducibility of results than the commercial gas separator, about twice better than the latter.

4.8 ARSENIC IN WATER AND RICE SAMPLES USING THE FABRICATED GAS – LIQUID PHASE SEPARATOR

Water samples were analyzed for arsenic without digestion while rice was digested with triacids, HNO_3: H_2SO_4: $HClO_4$ (6:3:1) as per USEPA method described in Section 3.3.7.2.

The optimized instrumental conditions used for the fabricated gas liquid phase separator gave a calibration curve in Figure 4.6 which was used to analyze the water samples randomly collected from 33 boreholes in the Ganze areas (coast province of Kenya). Nine bottled mineral water samples were also analyzed together with samples of rice grown in Kenya (Mwea pishori) and imported rice from Asian countries, Pakistan, Vietnam and Singapore. A 5ppb As-solution was used as a control standard for every 4 to 5 set of

measurements of samples within a 95% confidence interval of 4.86 ± 0.18 ppb ($\overline{X}$ = 4.86, σ = 0.17, n=6, $t_{(5,\ 95\%)}$ = 2.57). Any data outside this confidence interval was rejected and correction had to be done. The results on arsenic in water from Ganze area, bottled waters and rice are given in Tables 4.9 (a), (b) and (c) respectively.

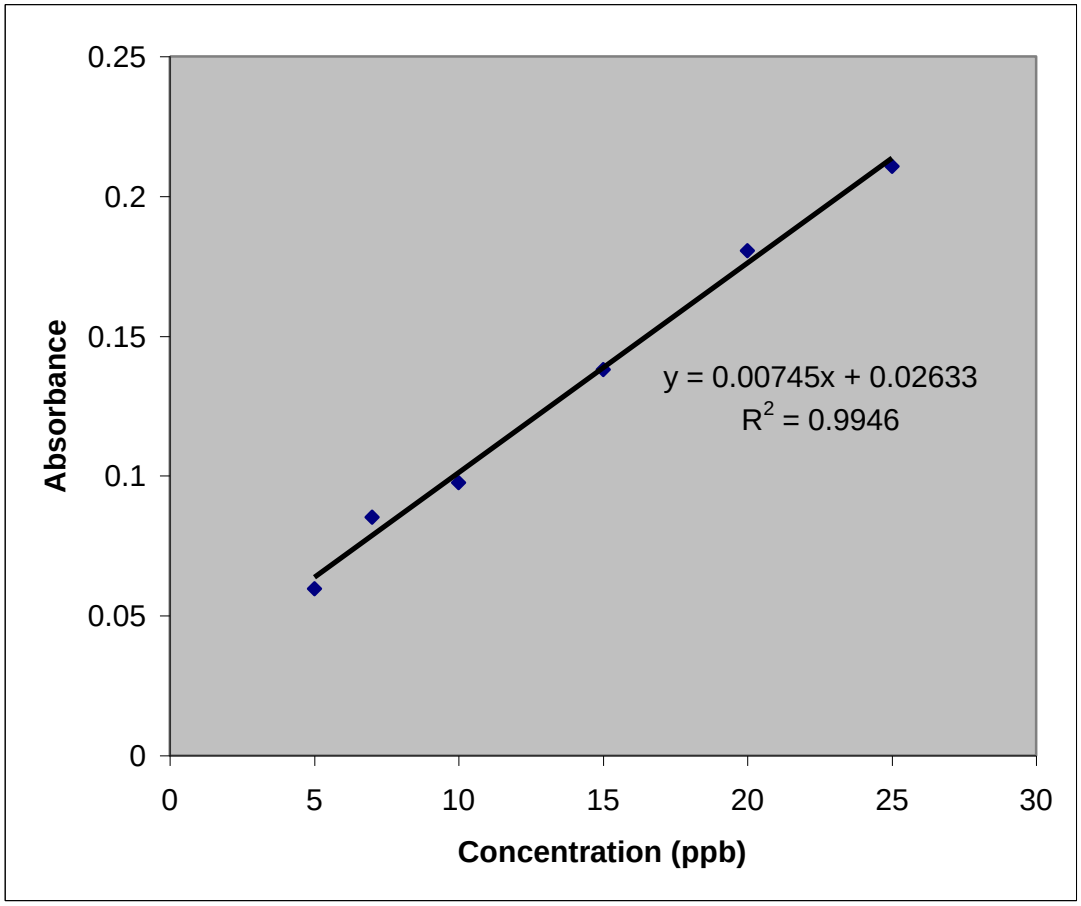


Figure 4.6 Calibration curve used for determination of As in water and rice using modified gas-liquid phase separator.

Table 4.9(a) Results for analysis of Ganze borehole water

Sample number	Sample name	Mean absorbance	Mean concentration (ppb)
6	Mikinduni	-0.1746	BLD*
2	Laini 'A'	-0.1724	BLD
9	Laini 'C'	-0.1556	BLD
26	Boji New	-0.177	BLD
19	Bondeni Church	-0.1693	BLD
Blank	Blank	0.0179	BLD
5ppb Standard	Control Standard	0.0621	4.80
16	Tune 'A'	0.0021	BLD
33	Kijijio	0.0665	5.39
8	Maweni	0.0194	BLD
22	Prison	0.02	BLD
5ppb Standard	Control Standard	0.0615	4.72
12	Madogo Mosque	0.0183	BLD

[BLD* = Below Limit of Detection, $\leq$ 3.7ppb]

Sample number	Sample name	Mean absorbance	Mean concentration (ppb)
20	Madogo Water. Project	-0.0071	BLD*
4	Hadampia	-0.0156	BLD
27	Vukoni village	-0.0013	BLD
11	Charidende	0.014	BLD
Blank	Blank	0.016	BLD
5ppb Standard	Control Standard	0.0643	5.10
24	Makerere Mnguuweni	0.0072	BLD
10	Mororo mosque	-0.0057	BLD
15	Emmaus	-0.0019	BLD
1	Bohoni	-0.0049	BLD
29	Lafuma Bondeni	-0.0033	BLD
18	Madogo 'A'	0.0683	5.64
Blank	Blank	0.0206	BLD
5ppb Standard	control standard	0.0631	4.94

[BLD* = Below Limit of Detection, ≤ 3.7ppb]

Sample number	Sample name	Mean absorbance	Mean concentration (ppb)
13	Mororo Bondeni Well	0.01215	BLD*
28	Ghamano	0.00795	BLD
5	Laini 'B'	0.00112	BLD
32	Ovo	0.0125	BLD
Blank	Blank	0.01875	BLD
5ppb Standard	Control Standard	0.0609	4.64
21	Bububu	0.0324	BLD
7	Mara mtu	0.0189	BLD
23	Fangudho	0.0272	BLD
3	Galamani	0.0102	BLD
31	Handampia'B' Islamic	0.03	BLD
Blank	Blank	0.0187	BLD
5ppb Standard	Control standard	0.0631	4.94

[BLD* = Below Limit of Detection, ≤ 3.7ppb]

Sample number	Sample name	Mean absorbance	Mean concentration (ppb)
Blank	Blank	0.0167	BLD
17	Boji Old	0.00315	BLD
25	Kelokelo	0.0111	BLD
14	Makere Maji safi	0.0108	BLD
30	Maweni	0.0198	BLD

[BLD* = Below Limit of Detection ≤ 3.7ppb]

Table 4.9 (b) Results for analysis of commercial bottled water

Sample number	Sample name	Mean absorbance	Mean concentration (ppb)
01	Keringet	-0.008	BLD
02	Aquamist	-0.0174	BLD
03	Joy	-0.0132	BLD
04	BrownHill	-0.0148	BLD
05	Sweetwaters	-0.0179	BLD
06	Ole Mara	-0.0204	BLD
07	Summer House	-0.0174	BLD
08	Highlands	-0.0244	BLD
09	Dasani	-0.0152	BLD

[BLD* = Below Limit of Detection $\leq$ 3.7ppb]

Table 4.9 (c) Results for analysis of rice samples

Sample number	Sample name	Mean absorbance	Mean concentration using dilution factor of 1.1 (ppb)
Digested Blank	Blank	0.01783	BLD
MP1	Mwea Pishori (Kenya)	0.02695	BLD
V1	Vietnam	0.05125	3.7
P1	Pakistan	0.0797	7.9
S1	Singapore	0.0642	5.6

[BLD* = Below Limit of Detection ≤ 3.7ppb]

From the data in Table 4.9(a), only two water samples (6.1%) of the borehole water samples from Ganze contained arsenic, with Madogo 'A' showing the highest levels of 5.64ppb, followed closely by Kijijio, 5.39ppb. The presence of As in some waters in Ganze, which is in the Kenyan Coast province, may be attributed to the kind of rocks and soils underneath. However, some anthropogenic factors may not be ruled out, especially considering that some of these areas have people carrying out irrigation using some waters from Tana river and of which some industrial effluents may be finding their way to these places. The rest of the borehole waters (93.9%) did not show any detectable arsenic, (BLD). The levels of arsenic in commercial bottled waters were all below the

limit of detection, (BLD) as shown in table 4.9(b). In the case of rice samples, local rice (Mwea Pishori) showed no detectable arsenic as it was below the limit of detection, indicating that rice from Kenya has As levels that are too low to cause any health concern. However, the imported rice from the Asian countries showed to contain arsenic, with Pakistan recording the highest (7.9ppb), followed by Singapore (5.6ppb) and Vietnam (3.7ppb). The results confirm the great concern over the arsenic crisis in the Asian countries, although the countries whose rice is imported in Kenya seemed to have moderately lower levels than the regulatory maximum permissible levels by WHO and Kenya Bureau of Standards, (10ppb). Hence they are relatively safe for human consumption, although the effects of bioaccumulation cannot be ruled out.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

Optimization of the basic FIA manifold with AAS using univariate approach showed that increased concentration of HCI improves the analytical performance of the FI-HG-AAS. In this regard, 0.4% (m/v) $NaBH_4$ in 0.5% (m/v) NaOH was used with combination of 7M HCl for optimum hydride vapour generation.

Retention characteristics of various solid phase cartridges for arsenic showed that alumina B (plus) and alumina N (Plus) cartridges retained arsenic and are therefore suitable for preconcentration of arsenic in natural waters.

Alumina B (plus) showed slightly higher retention capacity of As than alumina N (Plus), with close to 60 μg $L^{-1}$of As retained in the cartridge at 160ml breakthrough volume when the flow rate was maintained at 6.7 ml min^{-1}. In this regard, alumina B plus cartridge is preferred to preconcentrate As from natural waters. Preconcentration factor of 6 was achieved for Alumina-B (plus).

Elution of retained As was achieved by using HCl solution of a 10% (v/v) concentration. It showed that high HCl concentration of about 3.25M favoured elution of As compared to low concentration. However, the concentration should be moderately high to minimize instrumental noise.

Preconcentration factor could be increased by a factor higher than obtained using a more refined flow system approach that uses smaller eluting volumes, say about 2ml – 5ml. However this could not be achieved under the current FI-HG-AAS system as the reaction

time was relatively high, (2min), which therefore required between 15ml and 20ml of the sample. Significant decrease in the desorption volume would explain the increase in the preconcentration factor.

The enrichment factors calculated for this method are in the range of 6 to 8 based on the initial concentration. It is suggested that this method should be used for the preconcentration of trace arsenic in environmental waters and foods.

The current commercial gas-liquid phase separator may be modified to achieve improved analytical performance of the FI-HG-AAS. The modified gas liquid phase separator showed remarkable improved optimized detection and it also offered a new feature that would minimize the As-exposure to the analyst in the laboratory.

In the application of the modified method, the analysis of water samples from some parts of Kenya showed very low levels of arsenic and many of them showed no traces or undetectable traces of arsenic. This shows that waters from such areas are therefore safe from arsenic contamination.

Mineral waters bottled from boreholes or natural springs in Kenya contain no detectable arsenic and this is an assurance to consumers of such waters that it is safe for drinking with respect to arsenic contamination.

From the analysis of rice, locally grown rice from Mwea in Kenya (Mwea Pishori) contain insignificantly low levels of arsenic.

However, the imported rice from Asian countries (Vietnam, Singapore and Pakistan), not surprising, contains arsenic. Vietnam had 3.7μg L^{-1}, Singapore 5.6μg L^{-1} and Pakistan 7.9μg L^{-1}, indicating Pakistan rice has the highest levels of arsenic.

This shows that locally produced rice in Kenya is the safest while the imported rice may not be safe as it does have significantly higher levels of arsenic. However, the values are below the regulatory maximum permissible levels by WHO (10 μg L^{-1}) and other National bodies.

5.2 RECOMMENDATIONS AND FUTURE WORK

Owing to constant review of As standards by various bodies, there is need for new instrumental designs to cope with the trends and challenges. These have proved to be costly especially in the developing countries. It is recommended that the existing instruments can be modified in certain aspects of their instrumentation, to improve the detectability of arsenic without necessarily having to buy new and highly expensive instruments.

Proper and close monitoring of the imported rice in Kenya need to be carried out and the people are encouraged to eat their homegrown rice particularly Mwea Pishori as it contains insignificantly low levels of arsenic.

Profiling of the arsenic occurrence in Kenya should be carried out. For those water samples with As-content above 5 μg L^{-1}, some anthropogenic factors may have resulted to such relatively higher values than expected. This calls for further studies to assess the arsenic profile in the areas affected.

Speciation and selective retention of arsenic species on the alumina cartridges may also form the basis for preconcentration of the four arsenic species; As (III), As (V), MMA and DMA. The use of tandem cartridges for the selective retention and elution of arsenic species may particularly be useful for speciation of arsenic in water. Separation of arsenic

species may be carried out in the field and will be suitable for routine environmental monitoring.

The flow rates and sampling speed as well as improving the sample throughput are possible areas of interest that may improve the overall performance of the system. Incorporating an auto sampler may bring about considerable improvement to this aspect.

Similar studies may also be carried out for the element selenium (Se), and other elements that form hydride vapours and analyzed using the developed method of FI-HG-AAS. In addition, other methods that would enhance gas-liquid phase separation may be studied, like the use of a membrane gas-liquid phase separator.

The developed analytical system and methodology for arsenic determination can be tested for commercial use in Kenyan laboratories.

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APPENDICES

Appendix 1: Calculation of retention capacity of the cartridges

(a) ALUMINA B (PLUS) CARTRIDGE

Before loading, 20mls contained 10ppb As.

This contained 2×10^{-7}g As.

After loading with 20mls of 10ppb, 2.7ppb was eluted, as from Table 4.1.

Thus 20mls contained 2.7ppb As = 5.4×10^{-8}g = 0.54×10^{-7}g

Amount of As retained =2×10^{-7}g - 0.54×10^{-7}g = 1.46×10^{-7}g As.

Mean weight of packing material (mg/cartridge) for alumina B (plus) and alumina N (plus) cartridges = 1710mg /cartridge), while that of CN (plus) and C18 (plus) was 360mg /cartridge each, as provided by the manufacturer, (Millipore Waters, 1992).

Thus,

1.46×10^{-7}g As was retained by 1.71g of Adsorbent

$\frac{1.46 \times 10^{-7} \times 100}{1.71}$ ← 100g of Adsorbent

$= 0.85 \times 10^{-5}$g

$= 8.5 \times 10^{-6}$ g = 8.5µg

= 8.5µg /100g adsorbent

(b) ALUMINA N (PLUS) CARTRIDGE

Mean weight of packing material = 1710mg /cartridge

Before loading, 20mls contained 10ppb As

This contained 2×10^{-7}g As

After loading with 20mls of 10ppb As, 2.8ppb was eluted.

Since 20mls contained 2.8ppb = 0.56×10^{-7}g

Amount of As retained = 2×10^{-7}g - 0.56×10^{-7}g = 1.44×10^{-7}g As

Thus retention capacity for Al - N (plus)

Thus,

1.44×10^{-7}g As was retained by 1.71g of Adsorbent

$\frac{1.44 \times 10^{-7} \times 100}{1.71}$ ← 100g of Adsorbent

= 8.42µg/100g absorbent.

(c) C18 (PLUS) CARTRIDGE

Mean weight of packing material = 360 mg/cartridge

Before loading, 20mls contained 10ppb As

This contained $2x10^{-7}$g As

After loading with 20mls of 10ppb As, 9.06 ppb was eluted.

9.06 ppb contained $18.12 \times 10^{-8} = 1.81 \times 10^{-7}$g As was eluted.

Amount of As retained = 2×10^{-7}g $- 1.81 \times 10^{-7}$g = 0.19×10^{-7}g As

Thus,

0.19×10^{-7}g As was retained by 0.36 g of Adsorbent

$\frac{0.19 \times 10^{-7} \times 100}{0.36}$ ← 100 g of Adsorbent

= 5.3 µg/100g absorbent

(d) CN (PLUS) CARTRIDGE

Mean weight of packing material = 360 mg/cartridge

Before loading, 20mls contained 10ppb As

This contained $2x10^{-7}$g As

After loading with 20mls of 10ppb As, 8.83 ppb was eluted.

8.83 ppb contained $17.66 \times 10^{-8} = 1.766 \times 10^{-7}$g As was eluted.

Amount of As retained = 2×10^{-7}g $- 1.766 \times 10^{-7}$g = 0.234×10^{-7}g As

Thus,

0.234×10^{-7}g As was retained by 0.36 g of Adsorbent

$\frac{0.234 \times 10^{-7} \times 100}{0.36}$ ← 100 g of Adsorbent

= 6.5 µg/100g absorbent

Appendix 2: The Student's t-Distribution Table (Groeneveld, 1988).

Value of t for confidence interval of :	90%	95%	98%	99%
Critical value of |t| for *P* value of :	0.1	0.05	0.02	0.01
Number of degrees of freedom:				
1	6.31	12.17	31.82	63.66
2	2.92	4.3	6.96	9.92
3	2.35	3.18	4.54	5.84
4	2.13	2.78	3.75	4.6
5	2.02	2.57	3.36	4.03
6	1.94	2.45	3.14	3.71
7	1.89	2.36	3	3.5
8	1.86	2.31	2.9	3.36
9	1.83	2.26	2.82	3.25
10	1.81	2.23	2.76	3.17
12	1.78	2.18	2.68	3.05
14	1.76	2.14	2.62	2.98

The critical values of value of |t| are appropriate for a *two*-tailed test.

Appendix 3: Table showing the critical values of *F* for two tailed test (*P*= 0.05)

(Groeneveld, 1988).

v_1 / v_2	1	2	3	4	5	6
1	667.8	799.3	364.2	899.6	912.8	937.1
2	38.51	39.00	39.17	39.86	39.3	39.33
3	17.44	16.04	15.44	15.1	14.68	14.73
4	12.22	10.45	9.978	9.688	9.364	9.197
5	10.01	8.434	7.776	7.388	7.146	6.978
6	8.812	7.28	6.599	6.227	5.933	5.82
7	8.072	6.642	5.89	5.523	5.285	5.119
8	7.571	6.089	5.61	5.053	4.817	4.552
9	7.208	5.713	5.078	4.718	4.484	4.32
10	6.937	5.546	4.826	4.468	6.236	4.072

Appendix 4: Calculations for test of significance for Alumina B (plus) and Alumina N (plus) cartridges based on amount of As retained by each.

Parameter	Alumina B (plus) (n=3)	Alumina N (plus) (n=3)
Mean, $\overline{X}$, (ppb)	7.35	7.23
σ	0.09	0.11
σ^2	0.0081	0.0121

Setting the null hypothesis, Ho: There is no significant difference between the variance of Al-B and Al-N at 95% confidence level.

$F\text{calc} = \sigma^2_N / \sigma^2_B = 0.0121 / 0.0081 = 1.49$

$F\text{tab}_{(2,2,\ 95\%)} = 39.00$

Since $F\text{tab}_{(2,2,\ 95\%)} > F\text{calc}$, Ho is retained and hence $\sigma_N \approx \sigma_B$.

Therefore pooling the two variances, we have:

$$S^2_{pooled} = \frac{(n_B-1)\,\sigma^2_B + (n_N-1)\sigma^2_N}{(n_B-1) + (n_N-1)}$$

$$S^2_{pooled} = \frac{(2x0.0081 + 2x\,0.0121)}{(2+2)} = 0.0101$$

$S_{pooled} = 0.1005$

$$t_{calc} = \frac{|\overline{X}_N - \overline{X}_B|}{S_{pooled}\sqrt{(1/n_B + 1/n_N)}}$$

$$t_{calc} = \frac{0.12}{0.1005\sqrt{(⅓+⅓)}} = 1.462$$

$t_{tab(4,\,95\%)} = 2.78$

Since $t_{calc} < t_{tab(4,\,95\%)}$, Ho is accepted.

Therefore, there is no significant difference between retention capability of alumina B (plus) and alumina N (plus) for arsenic at 95% confidence level.

Appendix 5: Calculated mean, standard deviation for currently used commercial gas-liquid separator and the fabricated separator.

Parameter	**Current Gas separator, A**	**Modified gas separator, B**
Mean of 10ppb standard sample, $\overline{X}$, (ppb)	10.53 ± 0.73	9.47 ± 0.303
σ^2	0.53	0.09
Number of samples, n	5	7

Appendix 6: Calculations on statistical evaluation of the methods.

Setting null hypothesis, Ho: There was no significant difference between variance of current method A and modified method B at 95% confidence level.

By letting the standard deviation of the 10ppb sample for current method A be S_A and for modified method B be S_B.

Then,

$F_{calc} = S^2_A / S^2_B = 0.53/0.09 = 5.889$

$F_{tab, (4,6, 95\%)} = 6.227$, (from Appendix 3)

Since $F_{calc} = 5.889 < F_{tab, (4,6, 95\%)} = 6.227$, then $S_A \sim S_B$, hence Null Hypothesis, $H_{o,}$ was retained. Thus, the two variances obtained for the two methods were not significantly different at 95% confidence level.

t-test

Setting the Null Hypothesis, Ho: there was no significant difference between mean values obtained by the current method A and the modified method B at 95% confidence level.

Since $S_A \sim S_B$, the two variances are pooled,

$$S^2_{pooled} = \frac{(n_A-1)\,\sigma^2_A + (n_B-1)\sigma^2_B}{(n_A-1) + (n_B-1)}$$

$$S^2_{pooled} = \frac{4 \times 0.53 + 6 \times 0.09}{(4 + 6)} = 0.266$$

$S_{pooled} = 0.516$

$$t_{calc} = \frac{|X_A - X_B|}{S_{pooled}\sqrt{(1/n_A + 1/n_B)}}$$

$$t_{calc} = \frac{|10.53 - 9.47|}{0.516\sqrt{(1/5 + 1/7)}} = 3.51$$

$t_{tab\ (10,\ 95\%)} = 2.23$ (refer Appendix 2)

Since $t_{calc} = 3.51 > t_{tab(10,\ 95\%)} = 2.23$, H_o was rejected.

Therefore, there was significant difference between mean values obtained by the current method A (currently used commercial gas-liquid separator) and the modified method B (fabricated gas-liquid separator) at 95% confidence level.