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Comparison of Three Separation Tecnniques for Arsenic (III) and Arsenic (V) in Sea Water

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COMPARISON OF THREE SEPARATION TECHNIQUES FOR ARSENIC(III) AND ARSENIC(V) IN SEA WATER

BY

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ABSTRACT

Separation and determination of arsenic species in sea water is an attractive area of current research primarily due to the effects the different oxidation states of the element have on its bioavailability and toxicity. Many separation procedures for the arsenic species in sea water prior to their determination by graphite furnace -, hydride generation - atomic absorption spectrophotometry or neutron activation technique have been reported.

Evaluation of three of these separation procedures based on (1) solvent extraction, (2) ion-exchange, and (3) thiol cotton is reported in this dissertation. The evaluation is based on the analytical parameters: Detection limits, Sensitivity, Reproducibility, Precision, Recovery, Accuracy, Cost and Time of analysis.

The separation procedure based on solvent extraction was found to be superior to the other two procedures for routine analysis of sea water samples.

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PREFACE

The large number of publications on the various techniques for separating and determining As(lll) and As(V) makes it important to evaluate these techniques before one makes a choice for his or her work.

In writing this dissertation on the comparison of arsenic speciation techniques, the standard form was adopted. The dissertation consists of four chapters and each chapter has subsections. There are two appendices and these are:

1. Equations and formulae utilized in the dissertation and blank analysis.
2. Characteristics of the sea water reference material (SRM) and the sea water from the Narragansett Bay.

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I

INTRODUCTION

1.1 Literature Review.

Interest in the separation and determination of the various species of arsenic in sea water stems from their toxicity and their biological transformations. It has been shown that different oxidation states of an element have different effects on its toxicity and availability[50,27,4,31].

Several analytical techniques have been developed to separate and determine arsenic(lll) and arsenic(V) in different types of sample matrix. These techniques include silver diethyldithiocarbamate method[61], Molybdenum blue method[47], Polarography[39], hydride generation technique[75], gas and liquid chromatographic methods[32,16], ion-exchange chromatography[54] and solvent extraction followed by hydride generation or graphite furnace atomic absorption spectrophotometry or neutron activation.[34]

The choice of techniques for the separation and determination of arsenic(lll) and arsenic(V) in sea water is rather limited due to the complex nature of the matrix and the low levels of arsenic species in this matrix ($\approx$0.06 - 2 ppb) see Table 1.[7] The techniques are reviewed below and summarized in Tables II, III and IV.

Whitehead et al.[71] and later on Lais et al.[61] have determined arsenic(lll) and arsenic(V) in aqueous medium.

TABLE 1. ARSENIC SPECIES IN SEA WATER[7] (ppb as As).

Source	As(III)	As(V)	* MAA	* DMAA
Sargasso	0.24	-	0.01	trace
LaJolla	0.019	1.75	0.017	0.12
(Calif.)	0.034	1.20	0.012	0.12
San Diego trough				
Surface	0.017	1.49	0.005	0.21
25mi	0.016	1.32	0.003	0.14
50mi	0.016	1.67	0.003	0.004
100mi	0.060	1.59	0.003	0.002
Tampa Bay (Fla.)	0.12	1.45	0.002	0.20
	0.62	1.29	0.08	0.29

* MAA - Methyl arsonic acid

* DMAA - Dimethyl arsinic acid.

TABLE II. DETERMINATION OF ARSENIC AND ARSENIC COMPOUNDS AT TRACE LEVELS BY COLORIMETRIC AND POLAROGRAPHIC METHODS: Detection Limits Given As ppb of As.[61,47,39]

Method	Total As	As(111)	As(V)	Alkylarsenic cpd.
Silver diethyl-dithiocarbamate (colorimetry)	20 10 100	20 10 100	20 10 100	$CH_3AsO_3H_2$ 20
Molybdenum blue (colorimetry)	1	1	1	
Differential Pulse Polarography	6	6	6	

Table III. DETERMINATION OF ARSENIC AND ARSENIC COMPOUNDS BY THE HYDRIDE GENERATION TECHNIQUE. (Detection Limits in ppb as As.) [75, 32]

Method	Total As	As(111)	As(V)	Alkylarsenic cpds	
Hydride generation/ DC discharge	0.02	0.02	0.02	$CH_3AsO_3H_2$	-
Hydride generation/	500ng	-	-	$CH_3AsO_3H_2$	1mg
				$(CH_3)_2$	1mg
Hydride generation/ GC-FID	0.0005	0.0005	0.0005	$CH_3AsO_3H_2$	0.0005
GC-ECD	0.0005	0.0005	0.0005	$(CH_3)_2AsOOH$	0.0005
GC-flame AA	0.0005	0.0005	0.0005	$(CH_3)_3AsO$	0.0005
Hydride generation/ heated quartz cell-AA preceded by separation by ion chromatography	-	10	10	$CH_3AsO_3H_2$	10
Hydride generation/ extraction with benzene or toluene/ collection in cold toluene/or liquid N_2 trap-GC-microwave emission spectrometry	-	-	0.25	$CH_3AsO_3H_2$	0.25
				$(CH_3)_2AsOOH$	0.25
Hydride generation flameless AA	1.0	-	-	-	

Table IV. DETERMINATION OF ARSENIC AND ARSENIC COMPOUNDS BY FLAMELESS ATOMIC ABSORPTION SPECTROMETRY AND BY CHROMATOGRAPHIC METHODS (Detection Limits In ppb as As.)[62,16]

Method	*Total Arsenic	Arsenite	Arsenate	*Alkylarsenic compounds
GFAA	0.5	-	-	-
Extration by diethyl-ammonium diethyldithio-carbamate/CCL_4 - GFAA	1.0	1.0	1.0	1.0
Extraction with dibutyl-dithiophosphate/Hexane-GFAA	-	0.006	0.006	-
HPLC - GFAA	-	100	100	100

* Total arsenic refers to arsenite + arsenate.

* Alkyl arsenic compounds as $CH_3AsO_3H_2$ or $(CH_3)_2ASO(OH)$.

Their method involve the reduction of arsenite and arsenate by zinc/HCl to arsine which is absorbed in pyridine solution of silver diethyldithiocarbamate. The resulting complex formed between arsenic and silver diethyldithiocarbamate was measured colorimetrically at a wavelength of 540nm. They report a detection limit of 10ppb. Although the instrumentation is simple, the high detection limit makes it difficult to apply the method to analysis of arsenic in sea water.

Spectrophotometric determination of arsenite, arsenate and phosphate in natural waters utilizing molybdenum blue method has been reported by Pilson et al.[47] The method is based on the selective reaction of arsenate with ammonium molybdate to form blue arsenomolybdate. The complex was extracted into an organic layer and measured at an absorbance of 865nm. Although the method has been successfully used to determine arsenite and arsenate in sea water the instability of the blue arsenomolybdate and the interferences from phosphates makes it less attractive for routine analysis of arsenic species in sea water.

Determination of arsenic(lll) at the parts-per-billion level by differential pulse polarography has been reported by Osteryoung et al.[55] A detection limit of 0.3ppb was reported in aqueous hydrochloric acid. The ionic content of sea water matrix makes it difficult to apply reliably polarographic method to the analysis of arsenic species.

The determination of arsenic(lll) and arsenic(V) by selective hydride generation and atomic absorption

spectrophotometry has been proposed by Aspell et al.[2] and later by Arbab-Zavar.[43] The method is not reliable due to the pH dependence on the selective hydride generation. Sea water matrix also poses serious interference in such a method.

It is only recently that gas and liquid chromatographic methods have been applied to the separation and determination of arsenic species. Stockton and Irgolic[70] separated As(lll), As(V), arsenobetaine, and arsenocholine by high-performance liquid chromatography (HPLC) using a reversed ion suppression technique. The detection limits they reported were rather high. Andreae[6] has determined methylarsine, dimethylarsine and trimethylarsine using gas chromatograph on a column packed with 16.5% silicone oil DC-550 on 80-100 mesh chromosorb WAW DMCS employing flame ionization or electron capture detector. Most arsenic compounds in sea water are not volatile enough for gas chromatographic separation and determination. Derivatisation of such compounds are required before gas chromatographic determination. Limited derivitizing compounds for arsenic species is a setback for gas chromatographic methods in arsenic speciation study.

The most frequently used methods for separating arsenic(lll) and arsenic(V) in sea water are based on ion-exchange and solvent extraction. A method based on thiol cotton has recently been reported by Yu et al.[82] The technique has good features for the separation of arsenic(lll) and arsenic(V) in sea water. These three methods which form the basis of my comparative study are reviewed extensively

below.

Arsenic Speciation by Solvent Extraction:

A solvent extraction technique for the separation and determination of As(lll) and As(V) using dithiocarbamates was probably first utilized by Shiro Gohda[34] to study the valency states of arsenic and antimony in sea water. In his method, As(lll) was separated from As(V) by extracting their diethyldithiocarbamates with chloroform and then back-extracting into dilute nitric acid. As(V) was collected in the aqueous phase by thionalide co-crystallization. The separated species were determined by neutron activation analysis. Although Gohda's method appears to be convenient, nitric acid back-extraction has several drawbacks associated with it as reported by J.J. Lo et al.[51] For example, the kinetics are generally slow and acid extraction is poor for certain metals.

Toshihiko Kamada[49] has also studied the extraction behavior of arsenic(lll) and arsenic(V) with ammonium pyrrolidinedithiocarbamate (APDC), sodium diethyldithiocarbamate and dithizone in organic (APDC-MIBK) solvents. He found the APDC - MIBK extraction system was the best and As(lll) was extracted in the pH range 4.0 - 5.6. Separation and determination of As(lll) and As(V) in natural waters based on the extraction of arsenic(lll) with ammonium-sec-butyl dithiophosphate, back-extraction into water, and measurement by graphite furnace atomic absorption spectrophotometry has also been reported by Chakraborti

et al.[19] A detection limit of 6 ng/L was reported.

The solution conditions and other parameters affecting the APDC - methyl isobutyl ketone extraction system for graphite furnace atomic absorption spectrophotometric determination of As(111) and As(V) have been studied by Subramanian et al.[72] They reported that arsenic(V) is not extracted over the entire range of pH studied. Using both direct and nickel matrix modifier, the results for total arsenic agreed with results by electrothermal atomization.

A comprehensive study for the differential determination of arsenic(111) and arsenic(V) by solvent extraction and electrothermal atomic absorption spectrophotometry has been recently reported by Puttemans and Massart.[62] With their method, arsenic(111) was extracted quantitatively from acidic media with APDC. As(111) was stripped off into aqueous medium by Cu(11) before analysis by graphite furnace atomic absorption spectrophotometry. Good extraction recoveries were reported. Recently, Amankwah et al.[5] have reported the separation and determination of arsenic(111) and arsenic(V) in sea water by solvent extraction and atomic absorption spectrophotometry by the hydride generation method. This method is reliable with a low detection limit of 0.031 ng/ml and a high sensitivity. To assess the applicability of this method to routine analysis of real samples, it has been chosen for comparative study against two other methods reported in literature.

Ion-Exchange Chromatography:

A considerable number of papers have been published in the literature concerning arsenic speciation using an ion-exchange chromatography. In order to identify and quantitate the metabolic products of arsenicals, Overby et al.[59] developed an ion-exchange method which was applicable to the detection and quantification of trace quantities of arsenic compounds. Column chromatography using a cationic ion-exchanger has been reported by Iverson et al.[47a] The method was used to study arsenic speciation in sediments. Due to the slowness of the digestion procedure and the elution system employed, Henry and Thorpe[39] have suggested an improved method for arsenic speciation involving an ion-exchange. In their method, both anion - and cation - exchange columns were used with detection of the arsenic species by differential pulse polarography. Later, Pacey and Ford[60a] reported a complete separation of all four species with the detection of the arsenic species by graphite furnace atomic absorption spectrophotometry. A relatively straightforward two-stage anion-exchange method for the speciation of arsenic and its application to environmental analysis has been reported by Aggett and Kadwani.[1] The method is, however, dependent on careful control of the pH. An automated ion-exchange chromatographic method for the separation and analysis of arsenite (AsO_3^{3-}), arsenate (AsO_4^{3-}), monomethyl arsonate (MMA), dimethyl arsenate (DMA), and P-aminophenyl arsonate (P-APA) has been reported

by Ricci et al.[64] Detection limits of less than 10 ng/nl were reported. A method which improves the detection limits obtained by Ricci et al.[64] has been reported by Haswell[38] This method is based on HPLC anion-exchange chromatography with post-column continuous hydride generation. The instrumentation of these reported methods is, however, complicated.

A simple method of separation of arsenic(lll) and arsenic(V) based on ion-exchange has been reported by W.H. Ficklin.[28] The method is an improvement on the work by Henry and Thorpe. The relatively low detection limit of the method and shorter analysis time makes it attractive to adopt for analysis of arsenic species in natural waters. The ion-exchange technique for speciation study reported here is a slight modification of the work by Ficklin.[28]

Thiol Cotton:

The use of cotton in ion-exchange experiments dates back to the 1950's.[36] Cotton is a cellulose, containing aliphatic alcohol groups which are easily oxidized to carboxyl groups.

Guthrie, in 1952, reported a method whereby both cation and anion-exchange groups may be introduced into cellulose in the form of cotton.[36] Later, Daul and co-workers[24] produced an ion-exchange cotton by the reaction of cotton with polyvinyl phosphate (prepared from urea phosphate and polyvinyl alcohol). Ion-exchange cottons made of half-esters of maleic, glutaric, and phthalic acids have also been

prepared.

Weakly basic anion-exchange groups have been introduced by treating the cotton with 2-aminoethylsulfuric acid ($NH_2CH_2OSO_3H$) in alkaline solution and then with the vapor of ethylene-imine.[60b] Similarly, strongly basic groups have been introduced by treating the cotton with 2-chloroethyl diethylamine ($ClCH_2CH_2N(C_2H_5)_2$) under alkaline conditions and then methylating with methyl iodide in absolute ethanol.[82]

The use of these ion-exchange cottons in separation science had been limited to organic compounds until the 1970's when they were first applied to the separation of heavy-metal ions. The form in which the ion-exchange was used was the thiol cotton. Yu et al.[81,83] have shown that the thiol group has very strong affinity for heavy-metal ions and that cotton impregnated with thioglycollic acid (thiol cotton) does absorb trace elements from water quantitatively. They also showed that there is a differential absorption of the elements depending upon their oxidation states on the thiol cotton.

Recently, Yu and Liu[82] have reported the determination of the various oxidation states of arsenic, antimony, selenium and tellurium by hydride generation after separation with thiol cotton. The method has excellent detection limits with good sensitivity. The method was, therefore, adopted for the comparative work reported in this dissertation.

1.2 Arsenic Speciation:

Speciation of an element as has been defined by Florence[30]

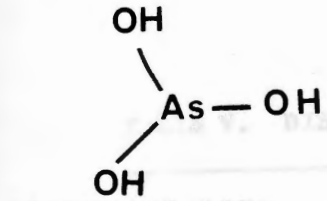
is the determination of the individual physico-chemical forms of that element which together make up its total concentration in a sample. This definition implies that in order to completely speciate an element in, for example, sea water sample, the various chemical forms in all sea water phases (liquid, colloidal and particulate) will have to be characterized.

The various chemical forms of arsenic which have been found to occur in sea water are arsenious acid [$As(OH)_3$], arsenic acid [$As\ O\ (OH)_3$], methyl arsonic acid [$CH_3\ As\ O\ (OH)_2$] and dimethyl arsenic acid [$(CH_3)_2\ As\ O\ (OH)$]. The structures of these acids are shown in Figure 1. All these forms have been found to occur in the liquid phase of sea water. These forms are either in the +3 state of arsenic as in arsenious acid or +5 state of arsenic as in arsenic acid, methyl arsonic acid and dimethyl arsenic acid.

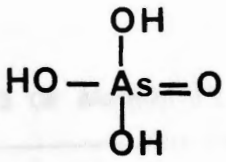
Equilibria.

Arsenious acid in aqueous solution exists in the monomeric and polymeric forms $HASO_2$, H_3ASO_3, $H_3[As\ (OH)_6]$, $HAs_2O_4^-$ and $H_2As_3O_6^-$. The pKa values for the dissociation of H_3AsO_3 are shown in Table V. The dissociations and their constants indicate that at pH range of 4-8, arsenious acid is essentially not dissociated.

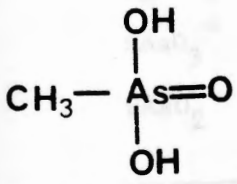
The equilibrium constants for the polymeric forms are not well studied. Attempt to study the equilibria of these polymeric forms has been made by Garrett et al.[33] and later by Voronova et al.[46] Table VI shows the equilibria and the



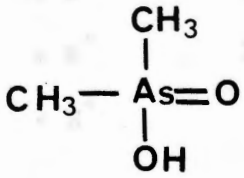
ARSENIOUS ACID



ARSENIC ACID



METHYL ARSENIC ACID



DI-METHYL ARSENIC ACID

Figure 1: Arsenic Species In Sea Water.

Table V. DISSOCIATION CONSTANTS OF ARSENIOUS ACID. [33]

Equilibria				pKa
H_3AsO_3	$\rightleftharpoons$	$H_2AsO_3^-$	$+ H^+$	8.9
$H_2AsO_3^-$	$\rightleftharpoons$	$HAsO_3^=$	$+ H^+$	12.1
$HAsO_3^=$	$\rightleftharpoons$	$AsO_3^{\equiv}$	$+ H^+$	13.3
$HAsO_2$	$\rightleftharpoons$	AsO_2^-	$+ H^+$	9.6

Table VI. EQUILIBRIUM CONSTANTS FOR ARSENIOUS ACIDS DETERMINED FROM SPECTROPHOTOMETRIC AND POTENTIOMETRIC MEASUREMENTS AT 22 + 0.5° C IN A MEDIUM OF NaCl SOLUTION.[46]

Ionic Strength		K 0	K 0.5	K 1.0
$2H_2AsO_3^-$ + H^+	$H5As_2O_6$	-	9.36×109	1.14×10^{10}
$2H_2AsO_3^-$ + $2H^+$	$H_6As_2O_6$	-	2.69×10^{18}	4.41×10^{15}
H_3AsO_3 + $H_2AsO_3^-$	$H_5As_2O_6^=$		4.49	6.92
$2H_3AsO_3$	$H_6As_2O_6$	0.12	0.62	1.64

K values of the various polymeric forms at different ionic strengths.

Arsenious acid being amphoteric can dissociate not only as an acid but also as a base as are depicted in the following equations,

$As(OH)_3 \rightleftharpoons AsO_3^{3-} + 3H+$ Acid. -1

$As(OH)_3 \rightleftharpoons As^{3+} + 3OH^-$ Base. -2

These two equations can be combined to establish the ratio of As^{3+} to AsO_3^{3-}. From equation 1 above,

$$K_1 = \frac{[AsO_3^{3-}]\ [H^+]}{[As(OH)_3]}$$

and from equation 2

$$K_1 = \frac{[As^{3+}]\ [OH^-]^3}{As(OH)_3}$$

Hence, $$\frac{[As^{3+}]}{[AsO_3^{3-}]} = \frac{K_2[H^+]^3}{K_1[OH^-]^3} \qquad \text{—— (3)}$$

But, $Kw = [H^+]\ [OH^-]$

and $$[OH^-] = \frac{Kw}{[H^+]}$$

Substituting for [OH-] is (3) we have

$$\frac{[As^{3+}]}{[AsO_3^{3-}]} = \frac{K_2[H^+]^6}{K1\ Kw}$$

therefore, $$\frac{K2}{K1Kw} = K = \frac{[As^{3+}]}{[AsO_3^{3-}]\ [H^+]^6} \qquad \text{—— (4)}$$

Equation 4 indicates that there is a possibility for As^{3+} ions to be present in a solution of carefully controlled acidic pH solutions. This is very important in solvent extraction experiment where arsenic should be in free As^{3+} ions for complexation with the ligand to take place.

Arsenious acid may also be formulated as $H_3As(OH)_6$ or $As(OH)_3(OH_2)_3$ as has been reported by Voronova et. al.[46] The dissociation equilibria of this form is depicted below,

$$As(OH)_3(OH_2)_3 \quad [As(OH)_4(OH_2)_2]^- + H^+$$

and

$$As(OH)_3(OH_2)_3 + H_2O \quad [As(OH)_2(OH_2)_4]^+ + OH^-$$

The dissociation constants for these equilibria can be evaluated using the arguments discussed above. These equilibria however do not suggest the formation of arsenious ion As^{3+} in 'slightly' acidic solution.

Arsenic acid, H_3AsO_4, unlike arsenious acid $As(OH)_3$ is almost exclusively acid in its behaviour. The dissociation equilibria of arsenic acid is shown below,

$$H_3AsO_4 + H_2O = H_2ASO_4^- + H_3O^+ \qquad pKa \quad 2.20$$

$$H_2AsO_4 + H_2O = HAsO_4^{2-} + H_3O^+ \qquad pKa \quad 6.97$$

$$HAsO_4^{2-} + H_2O = AsO_4^{3-} + H_3O^+ \qquad pKa \quad 11.53$$

These equilibria indicate that arsenic acid is essentially in some ionic form at analytical pH range of 4-8.

Organic Compounds of Arsenic.

The occurrence of two organic acids of arsenic in sea water has been reported by M.O. Andrea[9]. These acids are

methyl arsonic acid and dimethyl arsinic acid. These acids are formed by microbial transformation of the inorganic acids. Many reactions which produce organic arsenic compounds from inorganic arsenic compounds have been reported.[25] The conversion of inorganic to methylated arsenic compounds by microorganisms is well established. The mechanism as has been proposed by Challenger[20] is depicted below,

$$(O)\ As^{V}\ (OH)_3 \xrightarrow{2e} As^{III}(OH)_3 \xrightarrow{[CH_3^+]} CH_3\ As^{V}\ (O)\ (OH)_2 \xrightarrow{2e}$$

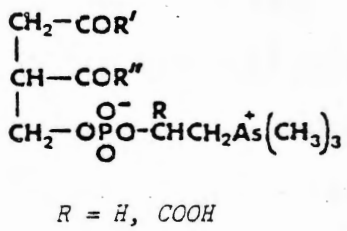
$$CH_3\ As^{III}\ (OH)_2 \xrightarrow{[CH_3^+]} As^{V}(O)(OH) \xrightarrow{2e} (CH_3)_2\ As^{III}\ (OH)$$

$$\xrightarrow{[CH_3^+]} (CH_3)_3 As^{V}\ (O) \xrightarrow{2e^-} (CH_3)_3\ As^{III}$$

Recently, trimethylarsoniumlactate and its derivatives and arsenobetaine have been found to be present in some sea water organisms.[14] Two other organo-arsenic species, O-phosphatidyltrimethylarsoniolactic acid and arsenic-containing sugar have also been isolated from the sea water organisms, Chaetoceros concavicornis and Ecklonia radiata, respectively.[26,15] The structures of these compounds are shown in Figure 2.

The arsenic cycle showing the transformations of arsenic compounds in sea water environment is shown in Figure 3.

Organo-arsenic compounds occur in very low concentration



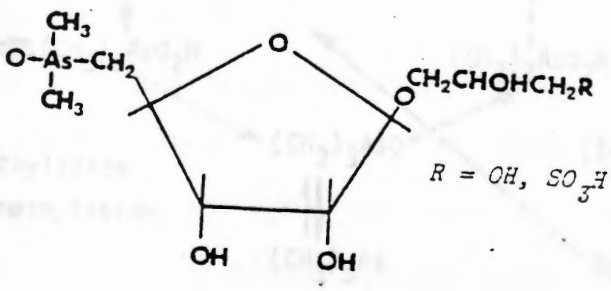


Figure 2: O-Phosphatidyltrimethylarsoniolactic Acid and Arsenic-Containing Sugar.

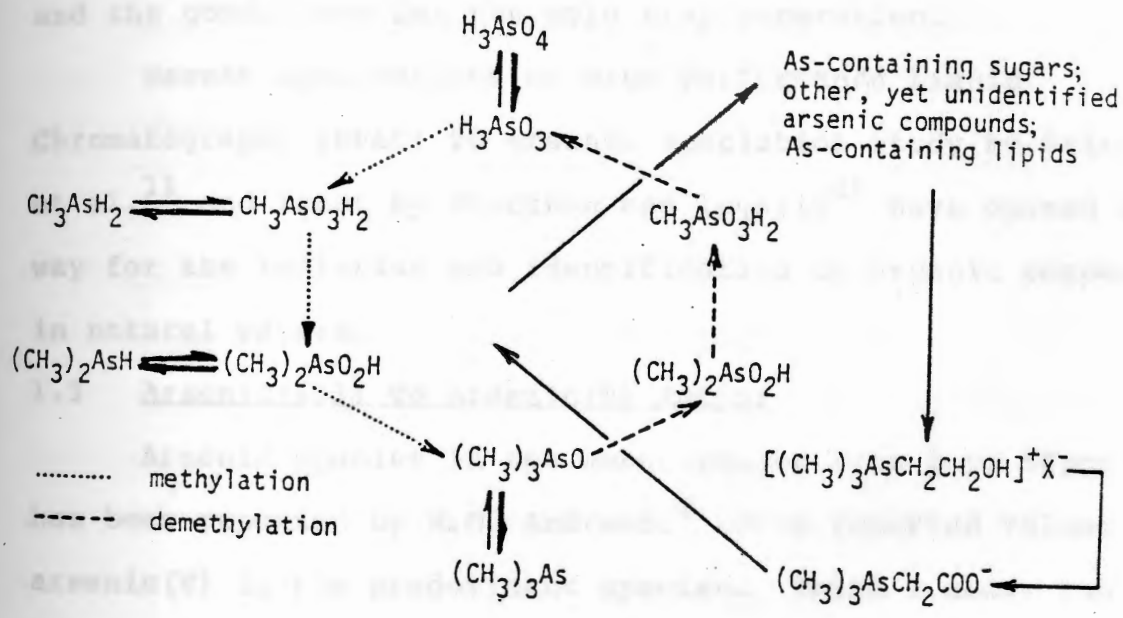


Figure 3: The Transformation Of Arsenic Compounds In The Environment.
Reproduced From; Irgolic K.J.; Speciation Of Arsenic Compounds in Water Supplies, June 1982.

levels (less than 0.5ppb) in sea water.[2,9] The analytical techniques for the determination of such low levels are not well established.[40]

The methods which are commonly used to speciate these organo-arsenic compounds is based on reduction with sodium borohydride followed by separation and detection of the arsines produced. Table VII shows the products obtained after reduction of the various arsenic compounds with borohydride and the conditions for the cold trap separation.

Recent applications of High Performance Liquid Chromatography (HPLC) to arsenic speciation study by Brinckman et al.[11] and later by Stockton and Irgolic[45] have opened a way for the isolation and identification of organic compounds in natural waters.

1.3 Arsenic(lll) To Arsenic(V) Ratio:

Arsenic species in sea water ranges from 1 to 50ppb as has been reported by M.O. Andreae.[8] From reported values arsenic(V) is the predominant species. Table I shows the various levels of arsenic(lll) and arsenic(V) in at different geographical locations.

Arsenic(lll) to arsenic(V) ratio is important in the study of arsenic transformations and its bioavailability in the marine environment. The ratio as reported by Andreae[8] and also by Braman and Foreback[10] ranges from 0.010 to 0.48. The low values for these ratios is attributable partly to oxidation of arsenic(lll) to arsenic(V)[47b] and methylation to methyl- and dimethyl- arsinic acids.[20]

Table VII. REACTIONS OF ANALYTICAL USE (with $NaBH_4$).

Compound	pKa_1	pH	Product	b.p.
$HAsO_2$	9.23	7	AsH_3	-55°C
H_3AsO_4	2.20	4.0	no reaction	
$CH_3AsO(OH)_2$	4.1	5.0	little reaction	
		1.5	CH_3AsH_2	2°C
$(CH_3)_2AsO(OH)$	6.2	1.5	$(CH_3)_2AsH$	36°

The ratio of arsenic(lll) to arsenic(V) also depends on the redox potential of the system, $AsO_4^{3-} + mH^+ + ne^- \rightleftharpoons H_2AsO_3^- + H_2O$

$$E = E^o - \frac{RT}{nf} \ln \frac{a(As^{3+})}{a(As^{5+})} - \frac{m}{n} \times 0.0591 \text{ pH}$$

where E^o = - 0.67 volts (As(V)/As(lll)) base.

m = # of moles of hydrogen ions

n = # of electrons.

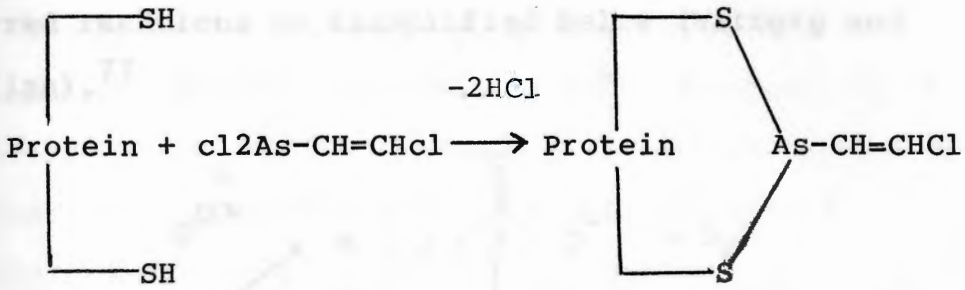
$a(As^{3+})$ and $a(As^{5+})$ are the activities of As^{3+} and As^{5+}.

The equation above indicates that As^{3+} to As^{5+} ratio is a pH dependent. If concentration constants are used instead of activity constants, then the ratio will vary as the ionic strength. Since salinity is a measure of ionic strength, changes in salinity will affect this ratio. The effect of salinity on As^{3+} to As^{5+} ratio in sea water has however not been reported.

1.4 Toxicity Of Arsenic Compounds.

Interest in the study of arsenic partly stems from its toxicity. The toxicity of arsenic compounds may be defined by one of the following modes of action as has been reported by McEwan and Stephenson;[53] a) mitotic inhibition, b) photosynthetic inhibition, c) respiratory inhibition and d) nucleic acid and protein synthesis interference. There are two major ways by which these modes of action come about. One involves As(lll) and the other involves As(V).

As(lll) exerts its toxic effects by means of enzyme sulfhydryl group interactions. From the chemical reactivity studies of As(lll) towards H_2S and R_2S compounds, Voegtlin[74] has concluded that the toxic action of As(lll) occurs as a result of its interaction with the SH groups of glutathione in cells, or possibly with the thiol groups of proteins (Johnson and Voegtlin).[48] For examples β-chlorovinyldichloroarsine (Lewisite) could react with proteins as depicted by the following equation



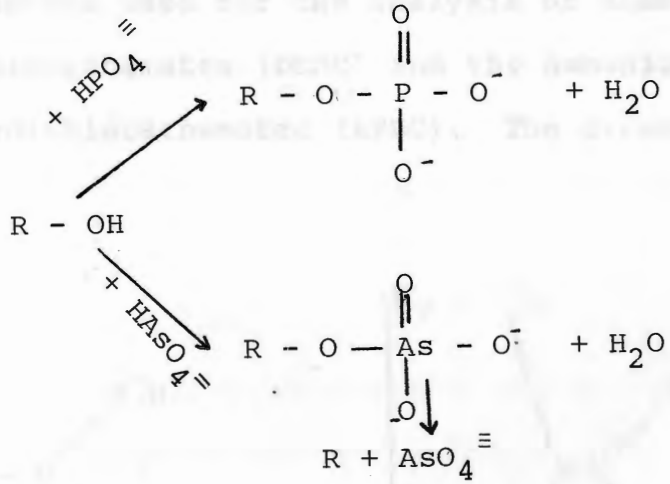
The way As(lll) inhibits enzyme activities is not the same for all enzyme systems. Extensive studies have been conducted on the inhibitory effects of As(lll), on a wide variety of isolated enzyme systems (Klebb, 1966).[79] Argopecten irradians are marine invertebrates. As(lll) toxic effects on these organisms have been studied by Nelson et al. (1956).[57] Their studies revealed that, Argopecten irradians showed a 96-hour LC_{50} for As(lll) at a concentration level of 3.49 $^{mg}/_{l}$ in sea water at 20°C and 25 $^{o}/_{oo}$. For As(lll) of concentration 40.6 $^{ms}/_{l}$, Curtis et al. (1979)[22] found a mean LC_{50} for the species, Palemonetes pugio.

As(V) on the other hand exerts its toxic effects in a different way since the arsenate ion does not react with the

free SH groups. The arsenate ion is isosteric and isoelectronic with Phosphate ion.

Arsenate ion Phosphate ion

Arsenate can therefore substitute for phosphate in enzyme catalyzed reactions as examplified below (Warburg and Christian).[77]



(A reaction scheme for arsenolysis of an arsenate ester in comparison with formation of a phosphate ester).

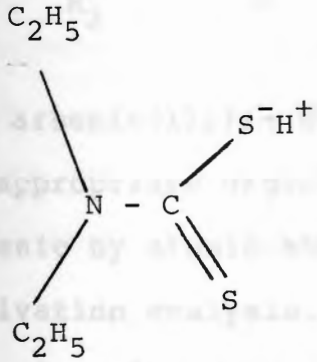
In the study by Sanders,[66] As(V) was found to inhibit the growth of the marine algae, Skeletonoma costatum in a culture at arsenic concentration level of 67nM. The addition of phosphate however removed this inhibition. Similar studies by Bottino et al.[18] showed that As(V) inhibited the growth

of Tetraselmis chui at the concentration range studied (10ppm).

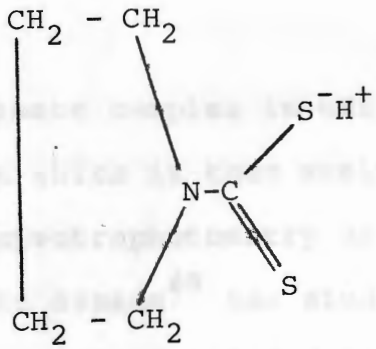
The two organic arsenic acids, methyl arsonic acid and dimethyl arsinic are however not toxic at the concentration levels at which As(III) and As(V) have been found to be toxic. These organic acids have LD_{50} of about 600-700 mg/Kg in mice.

1.5 Chemistry Of The Separation Of Arsenic(III) And Arsenic(V) By Dithiocarbamates, Ion-Exchange And Thiol Cotton.

Dithiocarbamates: By far the most important of the dithiocarbamates used for the analysis of arsenic are the diethyldithiocarbamates (DDDC) and the ammonium pyrrolidinedithiocarbamates (APDC). The structures are shown below:



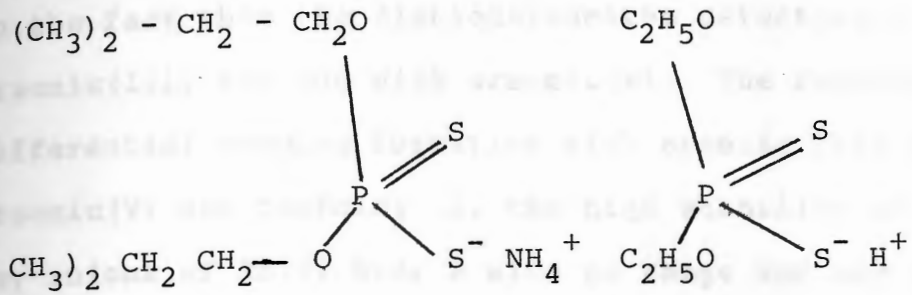
DDDC pKa = 4.04



APDC pKa = 2.95

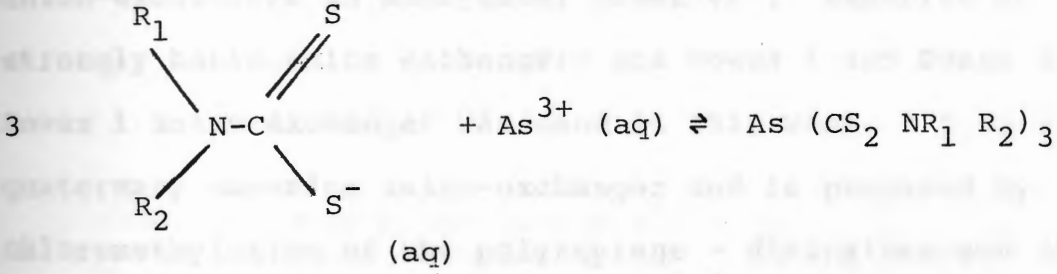
The use of dithiophosphates in the study of arsenic speciation has seldom been reported. The determination of arsenic(III) using ammonium-sec-butyl dithiophosphate[19] (ASBD) and diethyldithiophosphoric acid (HDEDTP)[54] has been

reported. The structures of these compounds are shown below:



ASBD HDEDTP

The dithiocarbamates form with arsenic(lll) analytically useful complexes of the general type as has been elucidated by et al.[21] The formation of these complexes which are pH-dependent can be represented by the equation.

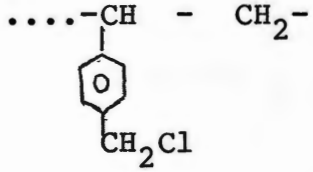


(aq)

The arsenic(lll) - dithiocarbamate complex is extracted into an appropriate organic solvent which is then analyzed for arsenic by atomic absorption spectrophotometry or by neutron activation analysis. Toshihiko Kamada[49] has studied various organic solvents for the extraction of these complexes. He reports the extraction efficiency of the solvents increases in this order: methyl isobutyl Ketone (MIBK) nitrobenzene (NB) carbon tetrachloride (CCL_4). The other solvents which have been reported to have good extraction efficiency are chloroform and Freon - TF.

The analytical application of dithiocarbamates is based on the fact that the dithiocarbamates selectively complex with arsenic(lll) but not with arsenic(V). The reasons for this differential complex formation with arsenic(lll) but not with arsenic(V) are twofold: 1. the high stability of the oxy-anions of As(V) over a wide pH range and the instability of dithiocarbamates in very acid solution and 2. the dithiocarbamates are generally soft bases and have the tendency to form stable complexes with soft acids. As(lll) is a soft acid whereas As(V) is a hard acid.

Ion-Exchange: The recent availability of resins containing quaternary ammonium ions has increased the utilization of anion-exchangers in analytical chemistry. Examples of strongly basic anion exchangers are Dowex 1 and Dowex 2. Dowex 1 anion-exchanger was used in this work. It is a quaternary ammonium anion-exchanger and is prepared by chloromethylation of the polystyrene - divinylbenzene (DVB) beads with chloromethyl ether.[52] The intermediate (1) then reacts with trimethylamine to produce the quartenary ammonium anion-exchanger shown in Figure 4.



(1)

The exchanger has the formula

$$= (CH_2CH)_1 + {}^{x}/_{100}\ (C_6H_4 - {}^{x}/_{100})\ CH_2N\ (CH_3)_3\ Cl$$

with equivalent weight of 216 + 0.5 x where x is mole percent

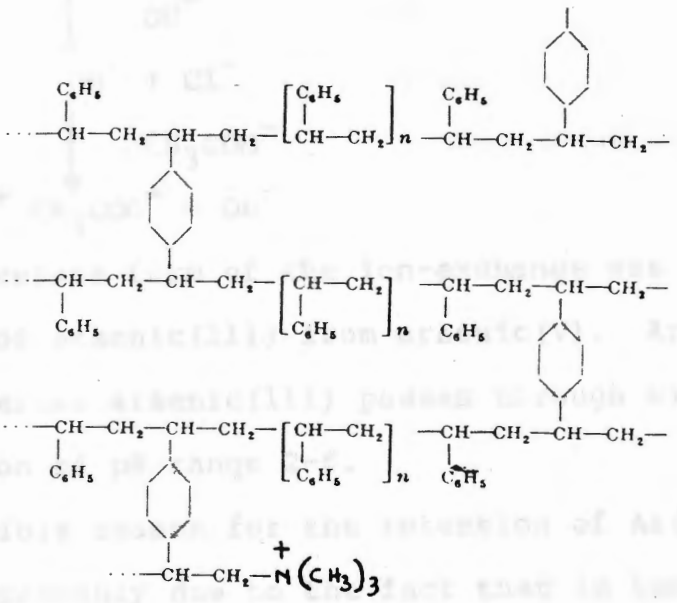


Figure 4: Chemical Structure Of A Styrene-divinylbenzene Resin. Reproduced From; Olof Samuelson, Ion Exchangers In Analytical Chemistry, 1953, John Wiley And Sons, Inc.

pure DVB. Some properties of Dowex 1 (analytical grade) are shown in Table VIII. Divinylbenzene is used as the crosslinking agent. The stoichiometry for the exchange process is represented below.

$$[R\ N(CH_3)_3]^+\ Cl^- + OH^-$$

$$\downarrow \quad OH^-$$

$$[R\ N(CH_3)]^+\ OH^- + Cl^-$$

$$\downarrow \quad CH_3COO^-$$

$$[R\ N(CH_3)_3]^+\ CH_3COO^- + OH^-$$

The acetate form of the ion-exchange was used for the separation of arsenic(111) from arsenic(V). Arsenic(V) is retained whereas arsenic(111) passes through without retention in a solution of pH range 2-6.

A possible reason for the retention of As(V) but not As(111) is probably due to the fact that in the pH range of 2-6 arsenic acid is in some ionic form whereas arsenious acid is not. This can be seen from the solution equilibria of the two acids shown below and which has been discussed earlier on in this thesis.

Arsenous acid

$$HAsO_2 + H_2O \rightleftharpoons H_3O^+ + AsO_2^- \qquad pKa\ 9.23$$

Arsenic acid

$$H_3AsO_4 + H_2O \rightleftharpoons H_2AsO_4^- + H_3O^+ \qquad pKa\ 2.20$$

$$H_2AsO_4 + H_2O \rightleftharpoons HAsO_{43-}^{2-} + H_3O^+ \qquad pKa\ 6.97$$

$$HAsO_4^{2-} + H_2O \rightleftharpoons AsO_4^{3-} + H_3O^+ \qquad pKa\ 11.53$$

Thiol Cotton: Cellulose is the chief component of cotton. The formula of cellulose is $(C_6H_{10}O_5)_n$ and the

Table VIII

Properties of Dowex 1 AG

Type of polymeric matrix	**Styrene-divinylbenzene**
Functional group	$- CH_2 N^+(CH_3)_3$
Form	Cl^-
Cross-linking (X)	8
Granulation (mesh)	20 - 15
Capacity (meq/g)	3.2
Stability	150° (Cl^-; 50° (OH^-)
Range of Use of pH	0 - 14

structure is shown in Figure 5. Reactions of cellulose are well known and include nitration, acetate formation and xanthate formation. The reactions occur at sites where there are free -OH groups.

The formation of thiol cotton is similar to the formation of cellulose acetate. Thioglycolic acid in the presence of acetic anhydride, acetic acid and a little sulfuric acid is impregnated into the cellulose structure of the cotton. The chemical nature of the thiol cotton is not well understood. However, it seems reasonable to suggest that both cellulose acetate and cellulose thioglycolate are formed and probably responsible for the adsorption of arsenic(lll) ions.

1.6 Hydride Generation Technique For The Determination Of Arsenic

Since the introduction of hydride generation technique for the analysis of arsenic by Holak[41], several papers have been published on this subject (12,29,73,69,67,56). The method is presently well-established and it is frequently used for routine analysis of hydride - forming elements at trace levels.

The basic principle for analysis of arsenic by the hydride generation technique is that arsenic(lll) is selectively reduced to arsine by sodium borohydride solution in an acidic medium. The reaction can be represented by the following general equations although the mechanism for the

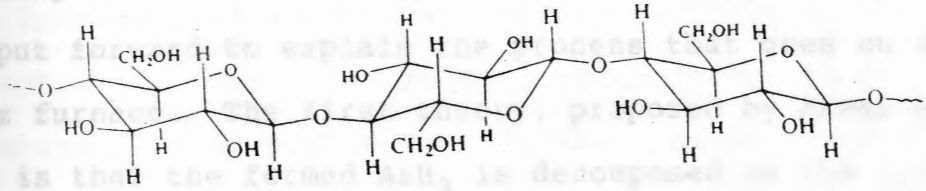


Figure 5. STRUCTURE OF CELLULOSE

reduction is not well understood.

$$BH_4^- (aq) + 3H_3O^+ \longrightarrow B(OH)_3 + 10H^-$$

$$As^{3+} + 3H^- \longrightarrow AsH_3$$

The gaseous arsine produced is swept into a pre-heated quartz furnace at a temperature of approximately 900°C by a carrier gas, usually argon or helium. Sometimes nitrogen or hydrogen gas is used.

In the furnace, AsH_3 is decomposed into arsenic and hydrogen atoms. Atomization followed by absorption of arsenic resonance radiation makes it possible to measure the amount of arsenic present in the sample. Two mechanistic theories have been put forward to explain the process that goes on in the quartz furnace. The first theory, proposed by Akman et al.[3], is that the formed AsH_3 is decomposed on the quartz surface before the atomization temperature is reached and the metallic arsenic is vaporized as As_4, which is then decomposed to As_2 dimers and atomized by gas-phase dissociation. This is shown schematically below

$$AsH_3(g) \longrightarrow As\ (s) \longrightarrow As_4(g) \longrightarrow As_2(g) \longrightarrow As(g)$$

The other theory has been proposed by Welz et al.[80] From their experimental results, they concluded the atomization of volatile hydride-forming elements in a heated quartz cell must be due to collisions with free H radicals according to the

following scheme;

$$AsH_3 + H \longrightarrow AsH_2 + H_2$$

$$AsH_2 + H \longrightarrow AsH + H_2$$

$$AsH + H \longrightarrow As + H_2$$

Any arsenic dimer formed is further atomized by collision with H radicals according the the mechanism:

$$As_2 + H \longrightarrow AsH + As$$

$$AsH + H \longrightarrow As + H_2$$

The possibility for the formation of the dimer is according to the equilibria:

$$As_4 \rightleftharpoons 2\ As_2 \rightleftharpoons 4\ As$$

Under mild acidic conditions, arsenic(V) is not reduced by borohydride solution. This is probably due to the fact that arsenic(V) is exclusively in the arsenate form. The arsenate ion might, however, by very slightly ionized into As^{5+} ions in very strong acidic conditions and a reduction to arsine by borohydride solution might then take place according to the following equilibria:

$$H_3AsO_4 + 5\ H^+ \rightleftharpoons As^{5+} + H_2O$$

$$As^{5+} + 2H^- \rightarrow As^3 + 2H^+$$

$$As^{3+} + 3H^- \longrightarrow AsH_3$$

$[H^-]$ - from borohydride solution

2 METHODOLOGY

2.1 Experimental.

Apparatus.

Analysis of Arsenic Species:

A Perkin-Elmer Model 403 Atomic Absorption Spectrophotometer equipped with an electrothermal quartz cell furnace, a chart-recorder and an Arsenic Electrodeless Discharge line (EDL) source powered by an 8-watt power supply source was used for all analysis. The Perkin-Elmer MHS-10 Mercury/Hydride system was connected to the quartz cell via two traps containing calcium chloride to remove water vapor. The carrier gas used was argon and the flow rate was regulated by a flowmeter. Figure 6 shows the assembly of the system.

The electrothermal quartz cell was 1.7 cm in diameter by 18.8 cm long with open ends. Graphite sleeves were used for heat dissipation. Nichrome wire (diameter = 0.0253 ins., resistance = 1 ohm/ft) was wound around the tube to a sufficient length (about 4 ft) to reach a temperature of approximately 900^{o}C by resistance heating. To insulate the tube and to maintain a uniform temperature distribution around the tube, asbestos was wrapped around it followed by glass cloth. Power to the resistance wire was supplied by a variable transformer.

Solvent Extraction. For the extraction, 500ml separatory funnels were used. Corning pH Meter Model 7 was used for all

pH measurements. Ashing was done on a Corning PC-100 Hot Plate. Mechanical shaking was performed on a Lab-line model 3575.

REAGENTS.

All solutions were prepared from analytical grade chemicals. Distilled demineralized water was used for all solutions.

Standard Arsenic(III) Solution (1 mg/ml). This was prepared by dissolving 1.322 g of As_2O_3 primary standard in 1000ml basic solution.

Standard Arsenic(V) Solution (1 mg/ml). This was prepared by dissolving 4.165 g of $Na_2HAsO_4 \cdot 7H_2O$ in 1000ml solution.

Sodium Borohydride Solution (5%). This was prepared by dissolving 5g of sodium borohydride powder in 100ml demineralized water followed by the addition of 1 pellet of potassium hydroxide. The solution was prepared as needed. The mineral acids, concentrated nitric acid and concentrated hydrochloric acid, were used as purchased.

Synthetic sea water was prepared by dissolving 254.0g of NaCl, 105.0g of $MgCl \cdot 6H_2O$, 39.1g of Na_2SO_4, 11.0g of $CaCl_2 \cdot 2H_2O$, 7.2g of KCl, 2.03g of $SrCl_2$, 0.27g of H_3BO_3 and 19.2g of $NaHCO_3$ in 10 liters of demineralized water.

Ammonium Acetate Buffer pH 4.5. Equal volumes of 4N sodium acetate and 4 N glacial acetic acid were mixed. The pH was adjusted to 4.5 with ammonium hydroxide or acetic acid.

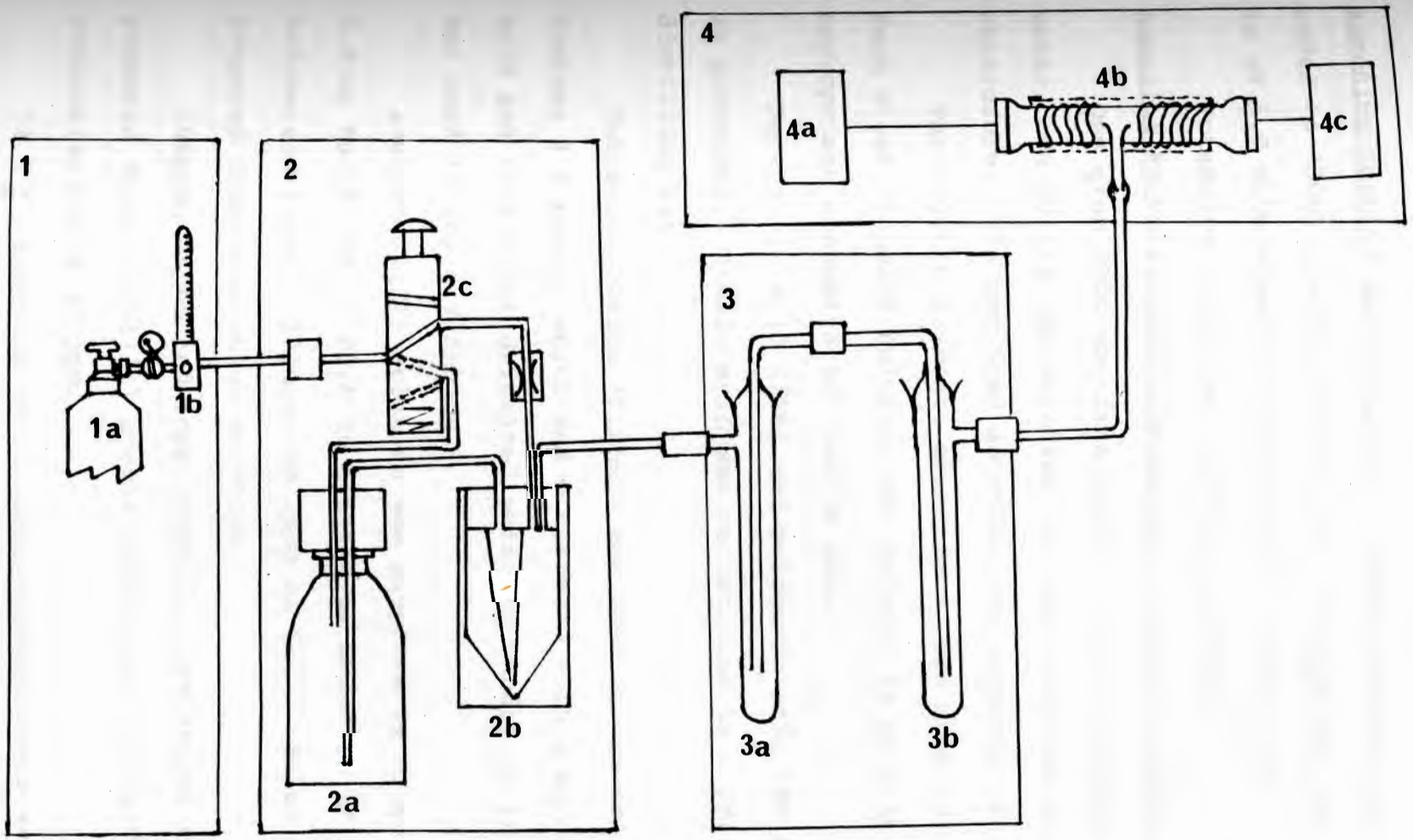


FIGURE 6 Perkin-Elmer Atomic Absorption Spectrophotometric-MHS-10 Hydride Generation Assembly

1a, Argon Gas Cylinder; 1b, Flowmeter; 2, Perkin-Elmer MHS-10, 2a, sodium borohydride container; 2b, reaction vessel; 2c, plunger; 3a&b, contains calcium chloride pellets to remove water vapor; 4, Perkin-Elmer Atomic Absorption Spectrophotometer 403; 4a, arsenic electrodeless discharge lamp; 4b, electrothermal quartz cell furnace; 4c, monochromators and detector.

Ammonium Acetate Buffer pH 6.0. 470ml ammonium hydroxide were added to 430ml glacial acetic acid. The pH was then adjusted to pH 6.0 with ammonium hydroxide or acetic acid.

Potassium iodide was used as purchased.

Ammonium Pyrrolidinedithiocarbamate (APDC) Solution (1%).

One gram APDC was dissolved in 100ml demineralized water. To purify the solution, it was extracted with chloroform. The purified solution was prepared as needed.

The organic solvent, chloroform, was used as purchased. Each stock standard solution was diluted to give an appropriate concentration before use.

Perchloric acid (70%) and sulphuric acid 18M were used as purchased. Nitric acid was redistilled in a pyrex distilling kit.

Potassium iodide (Fisher) was used in the solid form. Whatman # 4 paper, which was pretreated with 1 molar nitric acid and sufficient deionized water to render it acid-free, was used in the dry ashing.

1000ppm Cu(11) solution was prepared by dissolving 2.65og Cu(11) Cl_2 . $2H_2O$ in a litre of solution in a volumetric flask . 200ppm and 2ppm of Cu(11) solution were prepared from this stock solution.

100ppm, 10ppm and 1ppm nickel(11) solutions were prepared from nickel(11) atomic absorption standard in concentration of 997ppm.

1% ($\frac{W}{V}$) ammonium pyrolidinedithiocarbamate was prepared by dissolving 0.500g APDC in 50ml of deionized

water. This was filtered through an 0.45 m filter membrane followed by extraction with chloroform.

Pyrrolidine was used as purchased from Fisher.

Atomic absorption standards, lead (996ppm), iron (1013ppm), cobalt (988ppm), cadmium (1000ppm) were obtained from Alpha Analytical Laboratories.

Two molar HCl was prepared from the concentration solution.

2.2 Procedure.

Solvent Extraction:

All glassware was cleaned by washing several times in 4 molar nitric acid and then rinsing in demineralized water until it was neutral to litmus paper.

Synthetic sea water or natural water was spiked with an appropriate concentration of arsenic(lll) and arsenic(V) solution. This procedure was used in the various studies.

Natural sea water was collected from Narragansett Bay. It was filtered through a 0.45μm filter membrane to remove any particulate matter. Analysis of the filter membrane showed no retention of arsenic. The sea water was stored at a pH of 2 in a polyethylene bottle. The pH of 2 was attained by using hydrochloric acid. It was assumed that the As(lll) / As(V) ratio did not change at that pH value. Total arsenic was determined by reducing arsenic(V) to arsenic(lll) in one batch of sea water using potassium iodide. The sea water was acidified to acidic pH using hydrochloric acid before potassium iodide was added. To each liter sea water sample 2g

potassium iodide was added. The added potassium iodide did not interfere with the extraction procedure. This is probably due to the chemistry of the reduction reaction which can be represented by the reaction equation shown below:

$$2H^+ + 3I^- + AsO_4^{3-} = AsO_3^{3-} + I_3^- + H_2O$$

(I^- and I_3^- are not complexed with APDC.)

Extraction:

A 300ml sea water (synthetic or natural) sample was placed in a 500ml separatory funnel. Using the ammonium acetate buffers, the pH was brought to within the range 4.0 - 4.5. Two milliliters of purified 1% APDC were added to the solution followed by 25ml of chloroform. The solution was shaken for 20 minutes on a horizontal mechanical shaker. After the separation of the two layers, the chloroform layer was drained into a 150ml Erlenmeyer flask. The aqueous layer was discarded. Wet ashing was performed on the chloroform layer according to the following method: 10ml of concentrated nitric acid and 10ml of 70% perchloric acid were added to the chloroform layer. To prevent bumping and spattering, glass beads were added and the flask was fitted with a short-stem funnel. The set-up is shown in Figure 7. This was boiled to white dense fumes of perchloric acid on a hot plate. Ten ml of demineralized water was then added to the sample in the flask and again boiled to white dense fumes of perchloric acid. The resulting solution was then cooled to approximately 40^oC. About 0.1gm of potassium iodide was added to reduce arsenic(V) to arsenic(lll). The solution was made up to 50ml

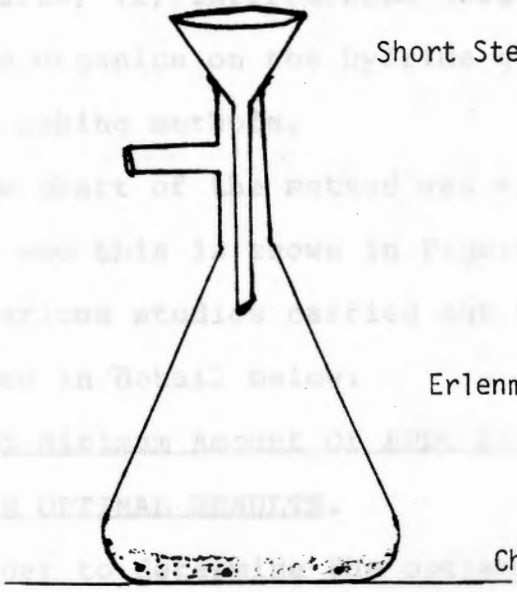


Figure 7: Wet Ashing Set-up.

in a volumetric flask with 1 molar hydrochloric acid. Atomic absorption spectrophotometric analysis of arsenic(lll) via the hydride generation technique was performed on 10ml aliquots of the preconcentrated sample. Arsenic(lll) levels in the aliquots were determined by the calibration curve method.

The method was used to study the pH effect on the complexation of As(lll) with APDC. Other parameters investigated are (1) the amount of APDC needed to obtain optimal results, (2) interference studies of some trace elements and organics on the hydride generation of arsenic, and (3) wet ashing methods.

A flow chart of the method was made to facilitate its routine use and this is shown in Figure 8.

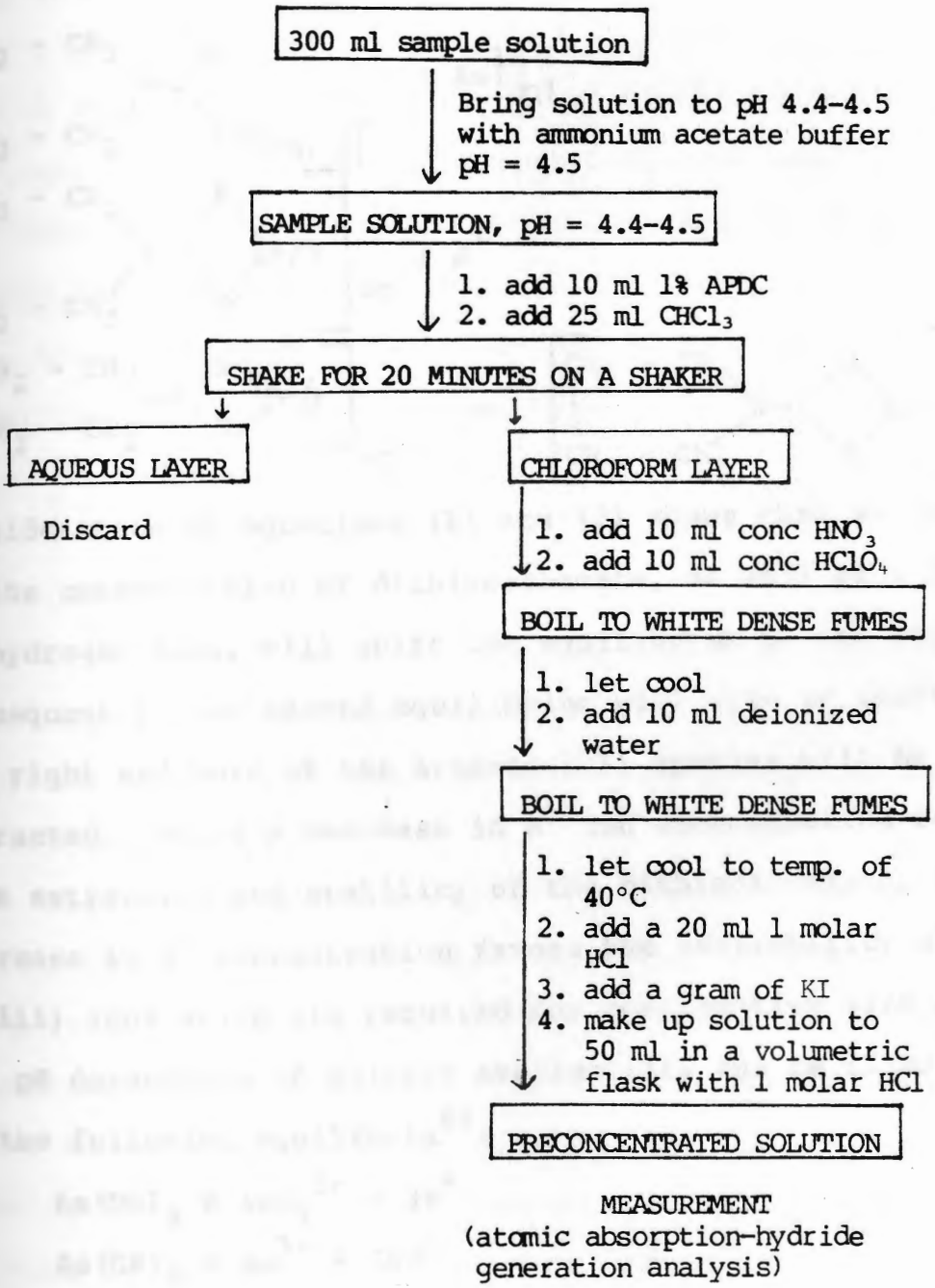
The various studies carried out to establish the method are discussed in detail below:

2.3 pH And Minimum Amount Of APDC Required To OPTAIN OPTIMAL RESULTS.

In order to determine the optimal pH for the complexation reaction between the APDC and arsenic(lll), and the minimal amount of APDC for optimal results separate experiments were carried out using standard solutions. The experimental method followed is the same as that used for the speciation study.

For the pH study As(lll) concentration was fixed at 1ppb. The experiment was repeated at different pH values (range of 3.5 - 6.0) in the APDC preconcentration step.

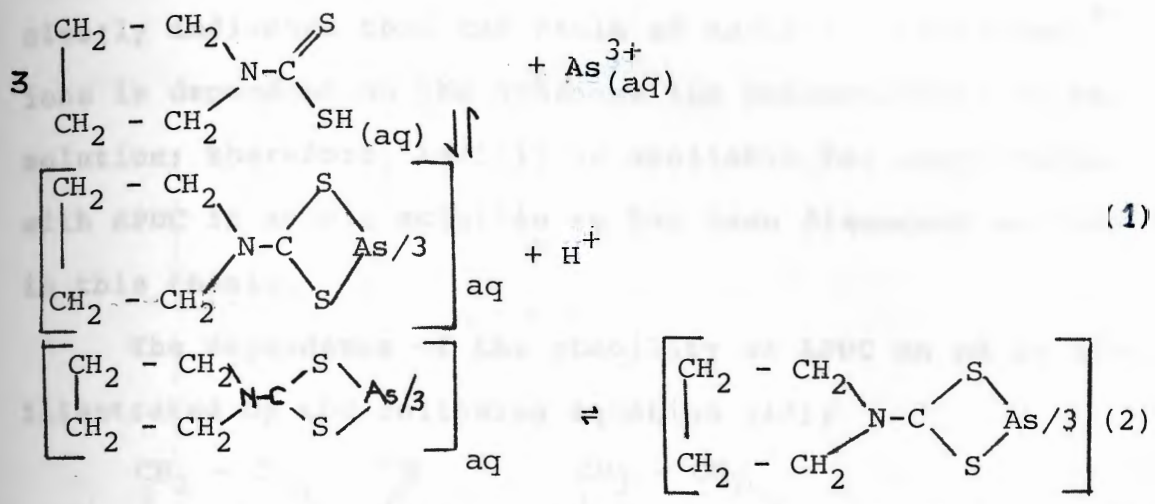
Figure 8: Flow Chart Diagram For The Solvent Extraction Procedure.



Discussion.

pH Effect:

The reaction between arsenic(lll) and ammonium pyrrolidine dithiocarbamate is pH dependent. The reaction equation is depicted below:



(1)

(2)

Consideration of equations (1) and (2) shows that an increase in the concentration of dithiocarbamate, as well as a decrease in hydrogen ions, will shift the equilibrium to the right. Consequently, the second equilibrium will also be shifted to the right and more of the arsenic(lll) species will be extracted. While a decrease in H^+ ion concentration favors more extraction and stability of the dithiocarbamate, an increase in H^+ concentration favors the availability of As(lll) ions which are required for complexation with APDC. The pH dependence of As(lll) availability can be illustrated by the following equilibria[63]:

$$As(OH)_3 \rightleftharpoons AsO_3^{3-} + 3H^+$$

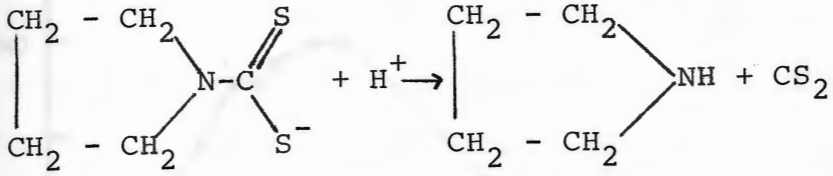
$$As(OH)_3 \rightleftharpoons As^{3+} + 3OH^-$$

and according to mass action law

$$\frac{[As^{3+}]}{[AsO_3^{3-}]\ [H^+]^6} = \text{Constant}$$

The product $[H^+]\ [OH^-]$ is constant and this expression clearly indicates that the ratio of As(lll) ions to AsO_3^{3-} ions is dependent on the hydrogen ion concentration of the solution; therefore, As(lll) is available for complexation with APDC in acidic solution as has been discussed earlier on in this thesis.

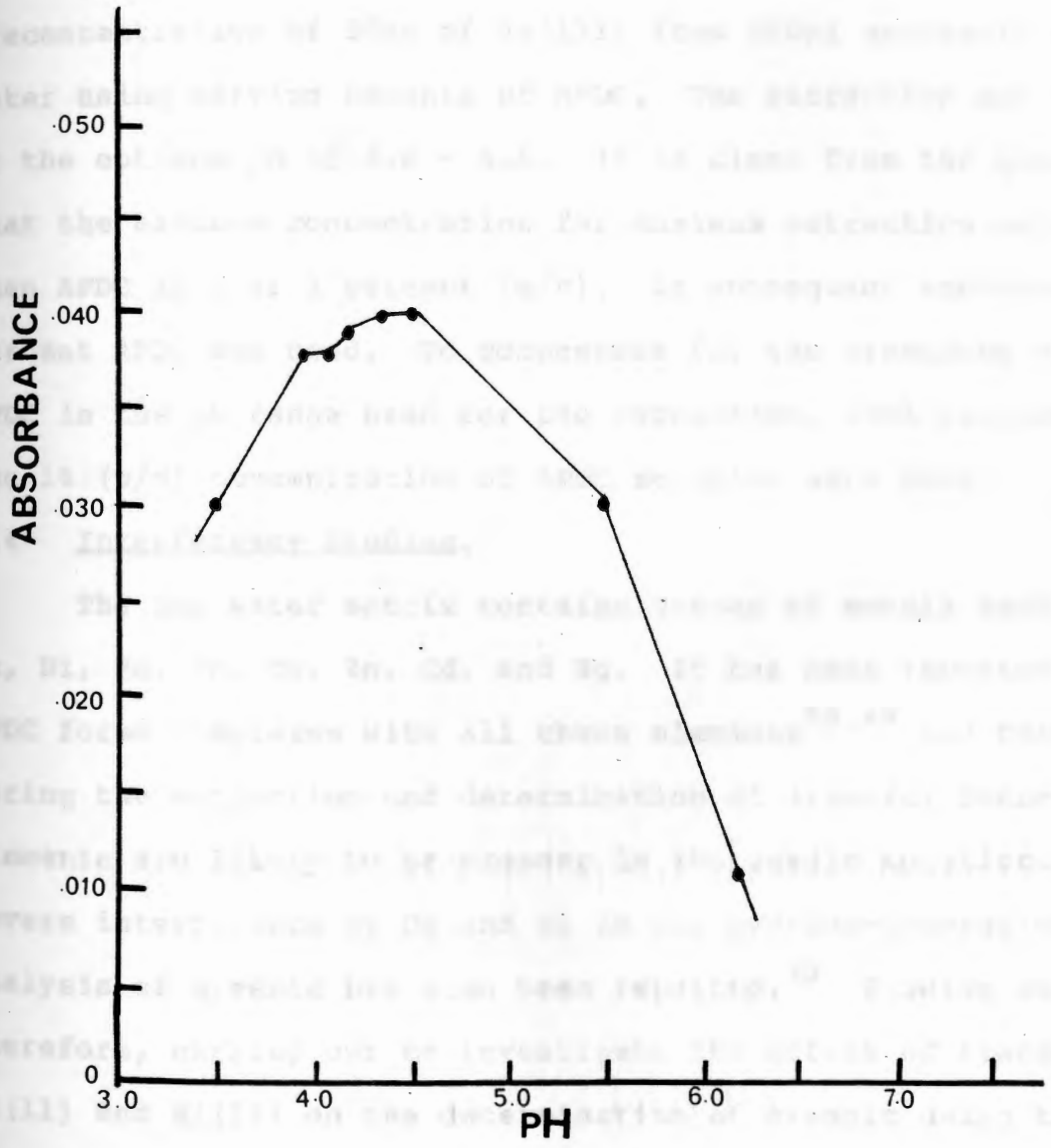
The dependence of the stability of APDC on pH is also illustrated by the following equation (14):



It is clear from these equations that any analytical method to be used for the study of arsenic involving complexation with APDC requires a careful control of the pH of the medium.

Figure 9 shows the study of the effect of pH on the complexation and extraction for the method developed. The study indicates that the optimal pH for complexation and extraction is within the 4.0 - 4.5 range. The decrease in the amount of arsenic(lll) extracted at pH greater than 5 is probably due to non-availability of arsenic(lll) ions. The decrease in efficiency at low pH (< 4.0) is probably due to the breakdown of the APDC to pyrrolidine and carbon disulfide.

Figure 9: Absorbance Versus pH of Sample Solution (in APDC preconcentration step.)



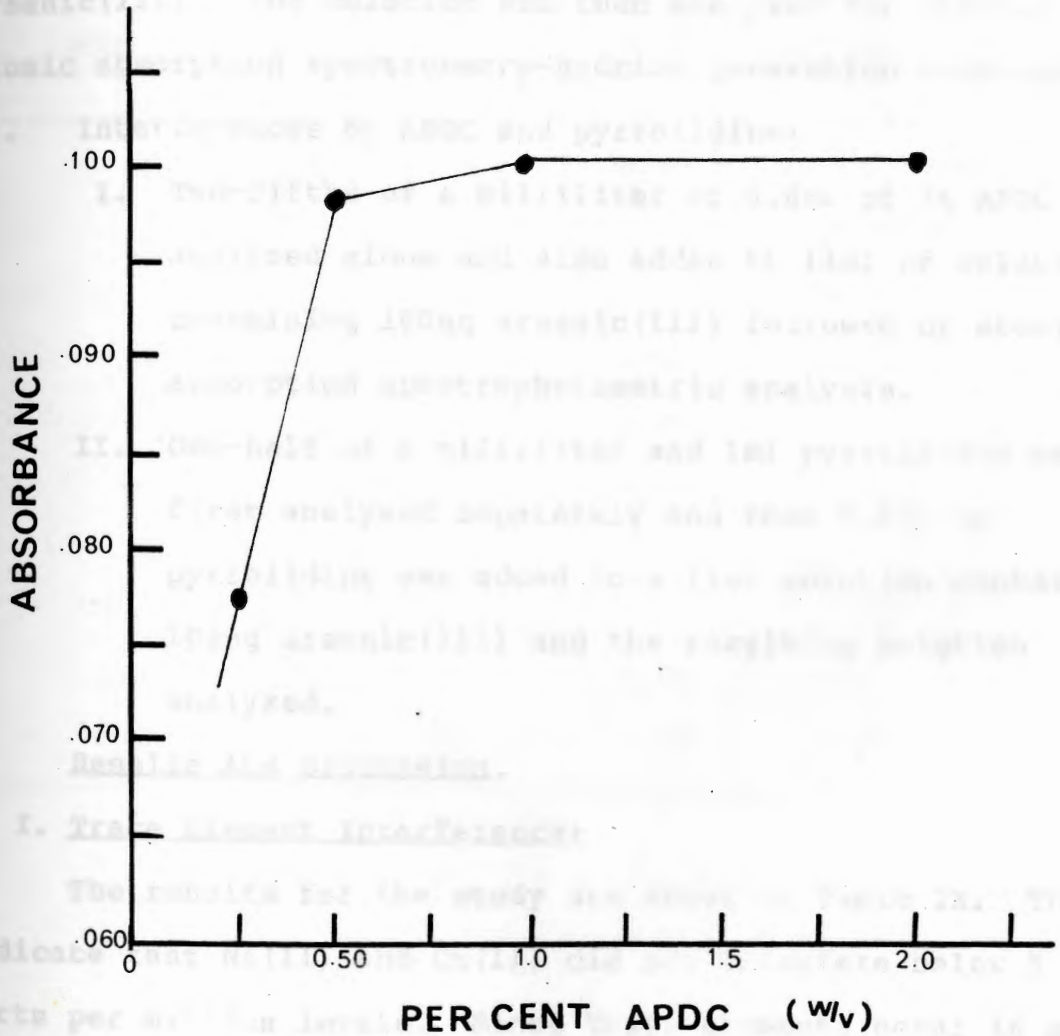
Concentration Dependence Of APDC:

From equation (1) an increase in APDC concentration should result in a shift of the equilibrium to the right. Consequently, more of the APDC-arsenic(lll) complex will be extracted. The optimum concentration of APDC necessary to bring about maximum extraction of the complex was studied. Figure 10 shows a graph of atomic absorption signal versus percent APDC. The graph was obtained from the preconcentration of 50ng of As(lll) from 300ml synthetic sea water using varying amounts of APDC. The extraction was done at the optimum pH of 4.0 - 4.5. It is clear from the graph that the minimum concentration for maximum extraction occurs when APDC is 1 or 2 percent (w/v). In subsequent analyses 1 percent APDC was used. To compensate for the breakdown of APDC in the pH range used for the extraction, 10ml portions of the 1% (w/v) concentration of APDC solution were used.

2.4 Interference Studies.

The sea water matrix contains traces of metals such as Cu, Ni, Fe, Pb, Co, Zn, Cd, and Hg. It has been reported that APDC forms complexes with all these elements[58,49] and that during the extraction and determination of arsenic, these elements are likely to be present in the sample solution. Severe interference by Cu and Ni in the hydride-generation analysis of arsenic has also been reported.[23] Studies were, therefore, carried out to investigate the effect of traces of Cu(ll) and Ni(ll) on the determination of arsenic using the hydride-generation technique. Studies were also conducted to

Figure 10: Absorbance Versus Percent of APDC $(\frac{W}{V})$.



investigate the interference effects of APDC and pyrrolidine on the hydride generation of arsenic.

Procedure.

a). Interferences of trace elements:

One ml of the appropriate concentration of the trace element was added to 11ml solution containing 100ng of arsenic(111). The solution was then analyzed for arsenic by atomic absorption spectrometry-hydride generation technique.

b). Interferences by APDC and pyrrolidine:

I. Two-fifths of a milliliter or 0.6ml of 1% APDC were analyzed alone and also added to 11ml of solution containing 100ng arsenic(111) followed by atomic absorption spectrophotometric analysis.

II. One-half of a milliliter and 1ml pyrrolidine were first analyzed separately and then 0.5ml of pyrrolidine was added to a 11ml solution containing 100ng arsenic(111) and the resulting solution analyzed.

Results And Discussion.

I. Trace Element Interference:

The results for the study are shown in Table IX. They indicate that Ni(11) and Cu(11) did not interfere below 5 parts per million levels. Since these elements occur in sea water below 5ppm (1) one should not worry about their interferences when using a hydride generation technique to analyze for arsenic in sea water matrix. There were no

Table IX. INTERFERENCE STUDIES OF CU(11) AND NI(11) ON As(111) ANALYSIS.

Added ion Concentration, mg/ml	As(111) absorbance	
	Cu(111)	Ni(11)
0	0.880	0.877
1	0.875	0.870
2	0.875	0.867
3	0.874	0.865
4	0.874	0.867
5	0.594	0.560
10	-	0.440
100	-	0.165
200	0.484	-
1000	0.440	0.011

interferences from Fe(lll), Pb(ll), Co(ll), Zn(ll), Cd(ll) and Hg(ll) at 100 parts per million levels.

A few reports have suggested a possible interference mechanism by these elements. Smith et al.[69a] have suggested that these elements form a precipitate with borohydride solution. Elemental nickel or copper is probably formed and this precipitate prevents the evolution of arsine from the reaction mixture.

Another reason for the interference has been offered by Saleh and Al-Daher.[23] They explain that the elemental copper or nickel form a surface for chemisorption of arsine. Another reason they have offered is that arsine gas is decomposed on the copper metal surface according to the reaction equation depicted below and thus arsine is not made available for atomization in the quartz tube.

$$nCu + AsH_3 \quad Cu_nAs + H_2$$

Brown et al.[17] have attributed this interference of copper to the reaction of NO_3^- ion, if present in solution, with the reduced copper to produce the species NO_2^-, NO_2, NO which interfere with the analysis since these species might absorb the same resonance energy as arsenic.

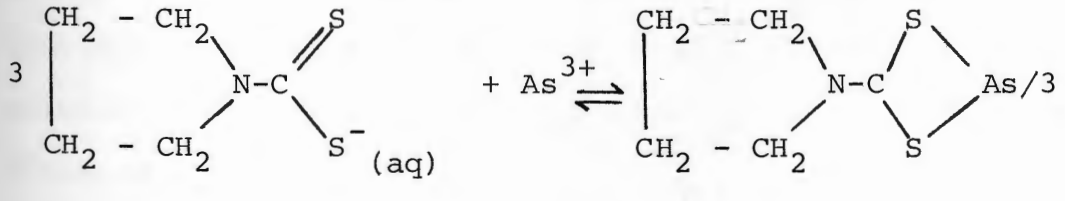
Although the explanations cited above somewhat clarify the chemical nature of these interferences, further studies are needed to completely establish the nature of these interferences and the mechanism of how they interfere.

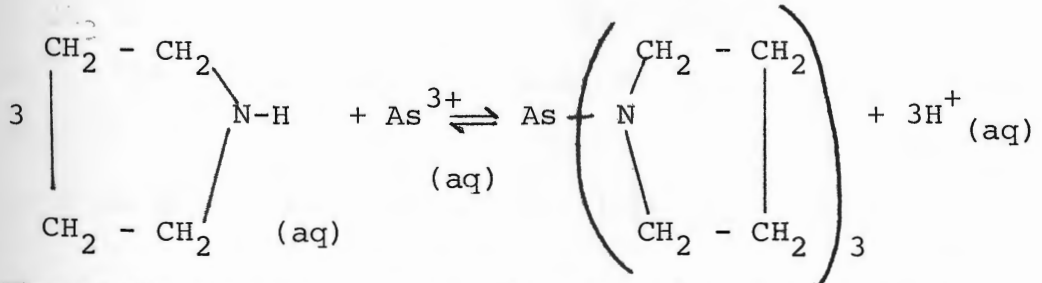
Interferences of APDC And Pyrrolidine:

Studies were also conducted to investigate the interference effects of APDC and pyrrolidine on the hydride generation of arsenic.

Table X shows some quantitative work done of the effect of pyrrolidine and APDC on the analysis of 100ng of arsenic(lll). The trend is that both the absorption peaks due to the 100ng arsenic(lll) alone and APDC or pyrrolidine alone decrease in height when APDC or pyrrolidine is added to the 100ng As(lll) before analysis.

A possible explanation for this observation is that sodium borohydride reacts with APDC or pyrrolidine to produce some gaseous products which absorb the same resonance energy as arsenic. The decrease in the peak heights of arsenic(lll) in the presence of either APDC or pyrrolidine is due to the fact that APDC or pyrrolidine react with the arsenic(lll) ions and, therefore, remove them from the solution matrix according to the following equations:





The peaks observed after addition of the various amounts of

Table X. ABSORBANCE VALUES OBTAINED FROM VARYING THE AMOUNTS OF APDC AND PYRROLIDINE ADDED TO 100ng OF As(111).

A. Pyrrolidine

Amount of pyrrolidine added (ml)	Absorbance
0	0.523
0.4	0.473
0.5	0.330
1.0	0.152

B. APDC

Amount of APDC (1%) added	Absorbance
0	0.501
0.4	0.420
0.6	0.280

C. APDC aline in 0.6M HCl

	Absorbance
0.6	0.501
0.4	0.245

APDC or pyrrolidine to the arsenic(lll) solution before analysis as shown in Table $\bar{X}$ are due to the unreacted APDC or pyrrolidine. This means that all of the 100ng arsenic(III) ions were used by the APDC or pyrrolidine according to the equations above.

During the preliminary studies, appropriate concentrations of arsenic(III) were extracted with APDC into a chloroform layer. The arsenic(III) was stripped off from the APDC-As(III) complex into an aqueous layer using concentrated nitric acid. The aqueous layer was analyzed for arsenic directly without wet ashing by the hydride-generation technique. Figure 11 is a calibration curve obtained by analyzing the various concentrations of arsenic by the extraction method described above. Figure 12 is a standard addition curve obtained from the same method of extraction and analysis. It appears from the graphs that a decrease in the absorption peak height of the extract is proportional to an increase in concentrations of arsenic(III) ions in the sample solution which are unexpected trends for calibration and standard curves.

This unusual trend can be accounted for by equation 3.[68] The source of pyrrolidine is from the reaction between APDC or APDC - As(III) complex and concentrated nitric acid used to strip off arsenic(III) from its complex with APDC.

Figure 11: Calibration Curve For Extraction System Without Wet Ashing.

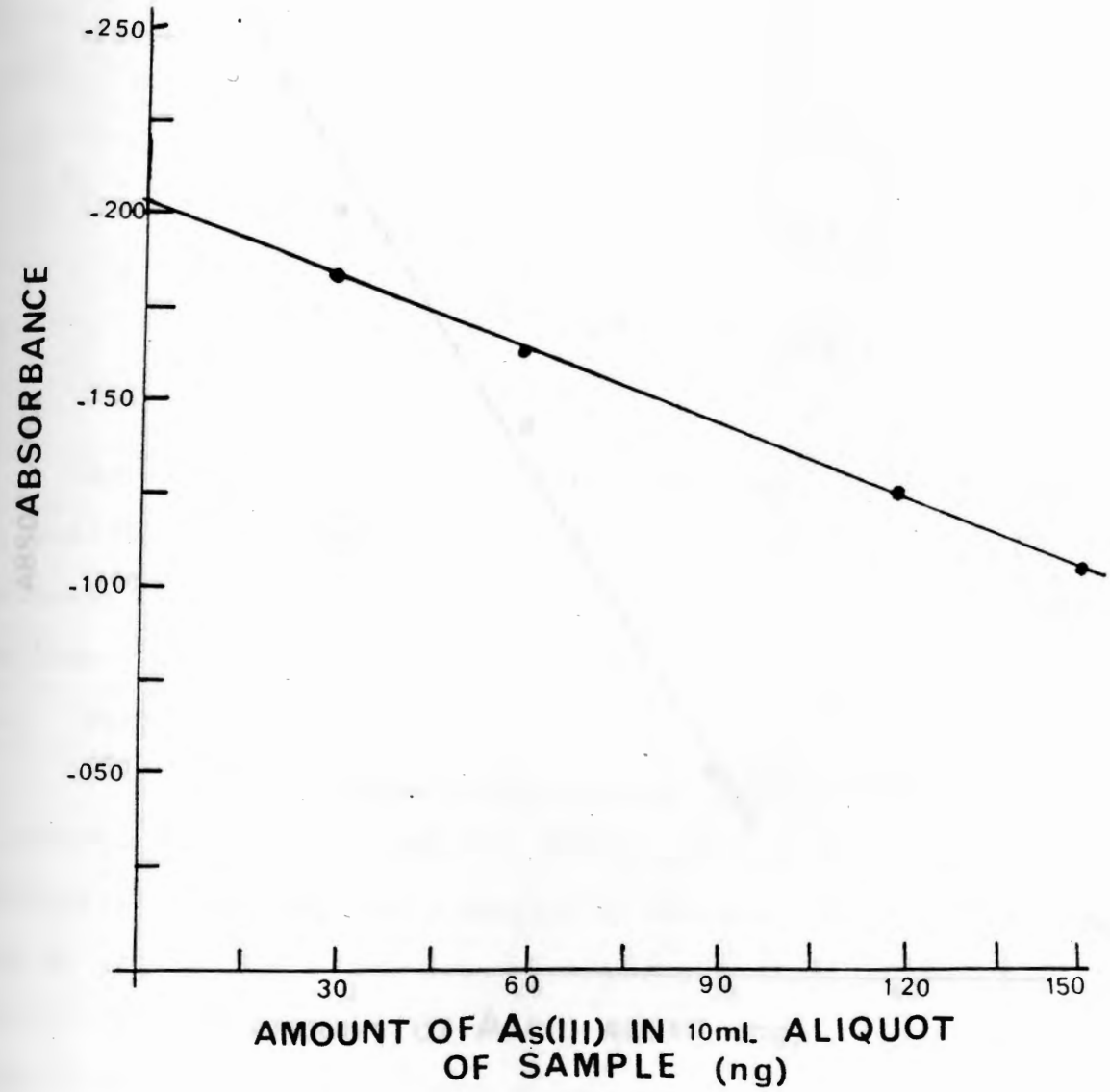
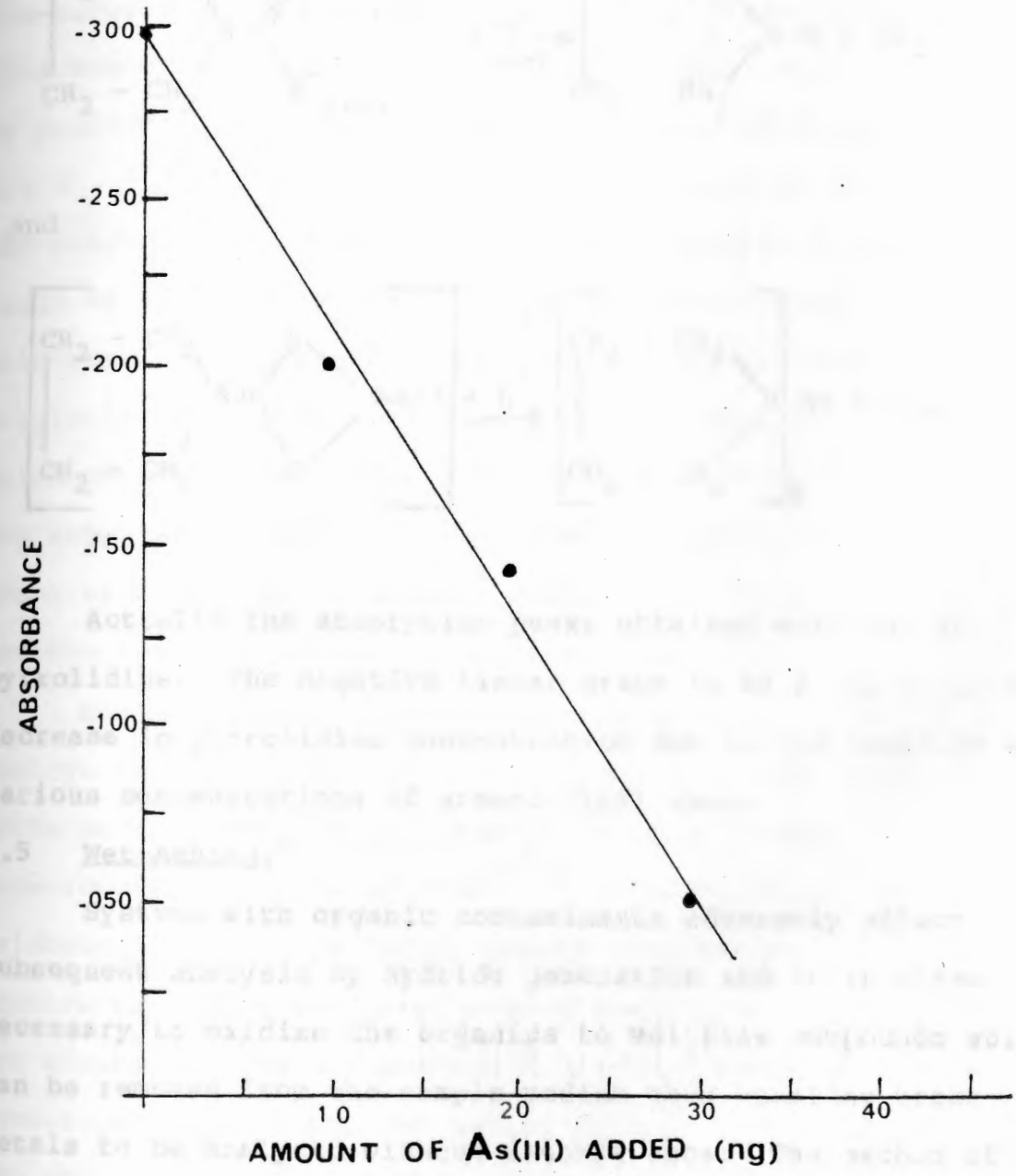
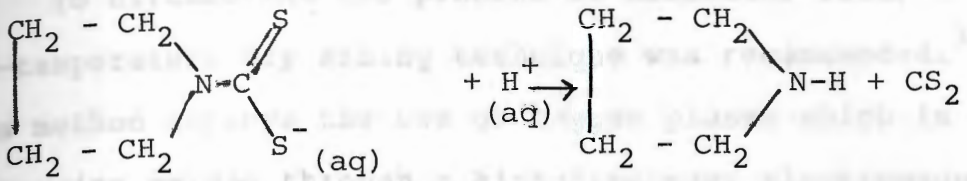


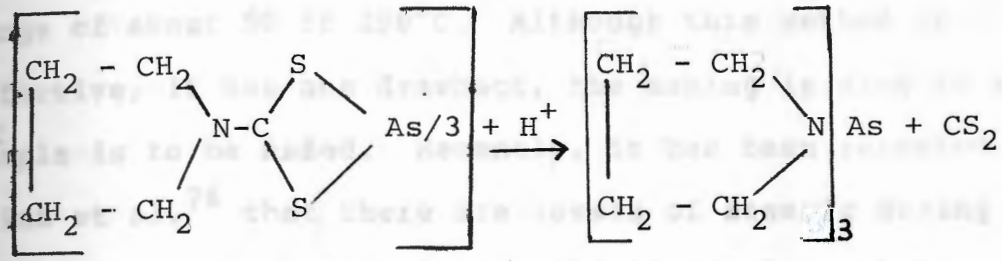
Figure 12: Standard Addition Curve For the Extraction System Without Wet Ashing.



The reaction equations are depicted below:



and



Actually the absorption peaks obtained were due to pyrrolidine. The negative linear graph is as a result of a decrease in pyrrolidine concentration due to its reaction with various concentrations of arsenic(III) ions.

2.5 Wet-Ashing.

Systems with organic contaminants adversely affect subsequent analysis by hydride generation and it is often necessary to oxidize the organics to volatile compounds which can be removed from the sample medium thus enabling trace metals to be analyzed without interference. The method of ashing has been used to bring about this oxidation. There are several methods for ashing which have been reported in literature. Each report is based on one of the following classifications: (a) dry or wet depending on the application of a gaseous or liquid reagent, (b) higher or lower

temperature and, (c) high or normal pressure.

To circumuvent the problem of elemental loss, a low-temperature dry ashing technique was recommended.[30] This method employs the use of oxygen plasma which is formed by passing oxygen through a high-frequency electromagnetic field. In this process, oxygen is activated and reacts with the sample. This causes a slow burning within a temperature range of about 50 to 250°C. Although this method is effective, it has one drawback, the ashing is slow if a large sample is to be ashed. Recently, it has been reported by Walsh et al.[76] that there are losses of arsenic during the low temperature ashing of atmospheric samples. Low temperature ashing of samples containing arsenic, therefore, becomes less attractive for sample preparation.

Another method which has been used is wet ashing; it employs the use of concentrated strong oxidizing minerals acids such as HNO_3, H_2SO_4, $HClO_4$, (HF), hydrogen peroxide and mixtures of them[78,49] to bring about the oxidation of organics during sample preparation. This method appears to be the preferred once since trace element losses are minimal during the ashing.[65] In this study an investigation into the wet ashing methods, HNO_3/H_2SO_4, $HNO_3/HClO_4$, $HNO_3/H_2SO_4/HClO_4$ and dry ashing with oxygen plasma has been carried out.

Procedure.

Dry Ashing: 0.3ml of 100ng/ml As(lll) with lml each of pyrrolidine and APDC added as organic matter solution were

collected on a pretreated Whatman #4 filter paper. After drying it in air the paper was dry ashed in a Low Temperature Asher. At the end of the ashing, the ash residue was dissolved in 2 molar hydrochloric acid and the solution was made up to 25ml with hydrochloric acid in a volumetric flask. Ten ml aliquots of this solution were analyzed for arsenic by atomic absorption-hydride generation technique.

Wet Ashing: Thirty ml of 1ppb arsenic(111) solution with 1ml each of pyrrolidine and APDC as organic matter were pipetted into a 250ml erlenmeyer flask. A small short-stem funnel was placed in top. Ten ml of concentrated nitric acid and 10ml of perchloric acid (70%) were added. This was evaporated to dense white fumes of perchloric acid. After cooling, 10ml of deionized water was added and the sample was again evaporated to white dense fumes of perchloric acid. This solution was transferred quantitatively into a 25ml volumetric flask. Ten ml aliquots of this solution were used for analysis.

Results And Discussion.

Table XI gives the percent recovery for the various systems used in the ashing. It is seen from the table that there is a serious loss of arsenic during dry ashing. This is in agreement with values reported by Walsh et al.[76]

This table also indicate that destruction of organic matter before the determination of arsenic can be achieved by wet ashing. These results also agree with reported values[49]

Both perchloric acid and nitric acid are strong oxygen

Table XI. DATA FOR THE STUDY OF DIFFERENT SYSTEMS FOR WET ASHING OF 30ng OF As(111).

	System	Per cent Recovery
1.	Dry ashing with oxygen plasma	50 + 10
2.	$HNO_3/HClO_4$	99 + 0.5
3.	$HNO_3/HCLO_4$	100 + 1
4.	$HNO_3/H2SO_4/HClO_4$	100 + 0.5

donors and it is the donated oxygen atom which destroy the organic matter.

Because of the oxidizing property of these acids, arsenic(lll) is oxidized to arsenic(V) during the process of ashing. The following equation illustrates the oxidation of arsenic(lll) to arsenic(V) by nitric acid.

$$As_2O_3 + 4HNO_3 + H_2O \longrightarrow 2H_3AsO_4 + 4NO_2 \uparrow$$

However, arsenic(V) is not reduced to arsine by borohydride solution. There is therefore the need to reduce arsenic(V) to arsenic(lll) before analysis by the hydride generation technique. The best system for the reduction was found to be potassium iodide among other reducing systems tried and this method was used in this experiment.

2.6 Separation By Ion-Exchange.

Experimental:

The ion-exchange columns were made from 50ml burettes with a diameter of 10mm. The burettes were cut to 30cm long. A separatory funnel was used as a reservoir. see Figure 13.

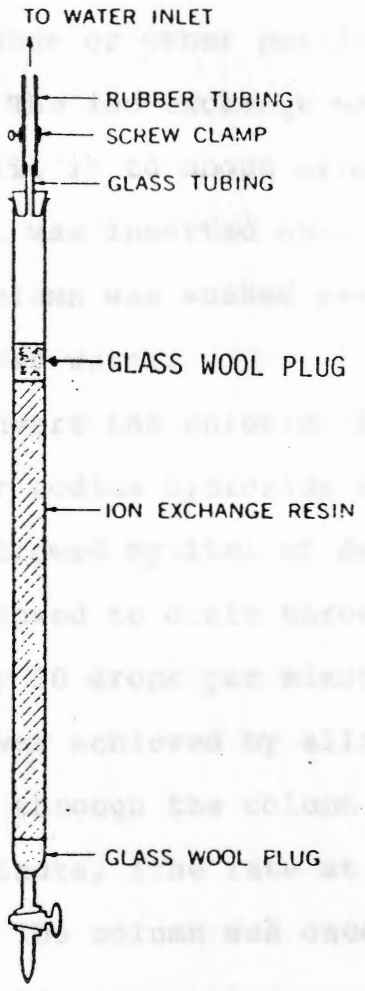
Reagents:

Ion-exchange slurry was prepared by mixing approximately 10g of the ion-exchange Dowex 1 x 8 (20-50 mesh) with a minimum amount of water.

Working solutions, 1 molar acetic acid, 1 molar hydrochloric acid and 1 molar sodium hydroxide were prepared from the concentrated solutions.

Standard solutions of arsenic(lll) and arsenic(V) were prepared from their stock solutions.

Figure 13: Buret Ion-Exchange Resin Column.



Glass wool was treated with 4 molar nitric acid before use.

Procedure:

A small amount of the pretreated glass wool was inserted to the bottom of the burette column. This was necessary to prevent the ion-exchange or other particles from passing through the column. The ion-exchange was then slurry packed into the column to fill it to about 10cm in length. Pretreated glass wool was inserted onto the surface of the ion-exchange. The column was washed several times with deionized demineralized water.

In order to convert the chloride form to the acetate form, 15ml of 1 molar sodium hydroxide was passed through the column. This was followed by 15ml of demineralized water. Each addition was allowed to drain through the column at the rate of approximately 30 drops per minute. The acetate form of the ion-exchange was achieved by allowing 15ml of 1 molar acetic acid to drain through the column at a flow rate of about 20 drops per minute, (the rate at which the effluent leaves the column). The column was once again washed with 15ml demineralized water.

The separation of arsenic(lll) from arsenic(V) on the column was based on the fact that arsenic(V) was retained on the acetate form of the ion-exchange, whereas arsenic(lll) was not retained. The pH of the sample solution was maintained in the range 2-6. Appropriate volume of the sample solution (either standard or natural sample) were passed through the

column through a reservoir as shown in Figure 7. The effluent was collected at a rate of about 20 drops per minute. The column was washed with 15ml of demineralized water and the washings were added to the sample effluent. Ten milliliters aliquots of this were used for analysis for arsenic(lll) by the hydride generation technique.

The arsenic(V) retained was eluted with 1 molar hydrochloric acid. The effluent was collected at the same flow rate as that for arsenic(lll). Thirty milliliters effluent were collected for analysis by the hydride generation atomic absorption spectrophotometric method. The column was washed with 30ml deionized demineralized water and was ready for the separation of another sample.

2.7 Separation By Thiol Cotton.

Experimental.

The thiol cotton columns were of the same dimensions as that of the ion-exchange. A separatory funnel was used as a reservoir.

Reagents:

High quality cotton was used for the separation. Acetic anhydride, glacial acetic acid, sulphuric acid, methyl thioglycollate and dithioglycollic acid were used as purchased.

Procedure:

Preparation of Thiol Cotton.

A solution mixture of methyl thioglycolate (30ml), acetic anhydride (25ml), glacial acetic acid (15ml),

concentrated sulphuric acid (0.2ml) and water (5ml) was prepared in a brown bottle. The solution was mixed thoroughly by shaking. Approximately 10g of absorbent cotton were added. The bottle was swirled around a few times. The bottle was closed and left in an oven at a temperature range of 40^{o} - 50^{o}C for 4 days. At the end of 4 days, the cotton was removed from the bottle and washed several times with demineralized water and the cotton was collected on a Buchner funnel using a suction pump. The washings were continued until they were neutral to litmus paper. The cotton was dried in the oven at 40^{o} - 50^{o}C. When it was established that it was properly dried, the thiol cotton was kept in a brown bottle.

Separation Procedure.

The burette column used was as described previously. A small plug of glass wool was inserted in the restriction above the stopcock. Approximately 1g of the thiol cotton was then introduced into the column. A small plug of glass wool was inserted to cover the surface of the thiol cotton.

The sample solution in 1 molar HCl was passed through the column. The effluent was collected at a flow rate of 20 drops per minute. During the passage through the column, arsenic(lll) was absorbed onto the thiol cotton. The effluent, which contains arsenic(V), was treated as follows to reduce the arsenic(V) to arsenic(lll): At the acidity of 1M HCl 10ml of 20% KI, 10ml of 20% thiourea were added. This was heated for 3 - 5 min in a boiling water bath. The reduced

solution was passed through the column at a rate of 20 drops per minute to extract the arsenic(lll).

Arsenic(lll) was desorbed from the thiol cotton by eluting slowly with 3ml of hot concentrated hydrochloric acid. The eluate was collected in a 10ml graduated cylinder. One milliliter of 20% potassium iodide solution was added and the solution made up to the 10ml mark. Arsenic in the 10ml solution was determined by the hydride generation/atomic absorption spectrophotometric method.

2.8 Analysis By The Hydride Generation Technique.

1. Install the proper light source for the element to be determined and set the correct lamp current.
2. Switch ON the Spectrophotometer and allow time for warmup (electrodeless discharge lamps require 10 to 30 minutes to achieve operational stability).
3. Select the correct values for wavelength, slit, etc. for the element to be determined. For As the wavelength is 193.7nm.
4. Tilt the quartz cell out of the light beam.
5. Perform lamp alignment and wavelength adjust Procedures.
6. Perform fine alignment of the quartz cell assembly.
7. Set operating mode on the Spectrophotometer suitable for operation with the MHS-10. The MHS-10 generates time-dependent, peak-shaped signals. The use of a chart recorder is generally essential.
8. Zero the display on the Spectrophotometer.

9. Switch on electrical power to the Recorder and select the required range and chart drive. Set the recorder pen to zero and 100%.
10. Prepare the reductant solution as given in the Experimental section. Fill the reductant reservoir bottle up to the shoulder and screw it into the fitting on the Analyzer Assembly.
11. Prepare standard and sample solutions as necessary.
12. Turn on the inert gas supply to the Analyzer Assembly and increase the pressure to 250kPa (2.5 bar; 2.5 kg/cm^2; 36 psig).
13. Dispense an aliquot of a standard solution into the reaction flask (maximum final sample volume 50ml).
14. Wait for a minimum of 15 seconds to allow the system to be purged free of air when the quartz cell has reached its optimal temperature for atomization.. For As the baseline falls as air is purged from the system; wait until the baseline is stable before actuating the plunger.
15. Start the Recorder chart drive.
16. Push down and hold the plunger to dispense reductant into the sample solution.
17. Observe the tracing on the recorder chart. When peak maximum has been achieved, release the plunger to stop the flow of reductant.
18. Wait for completion of the reaction (recorder pen

returns to the baseline), then remove the reaction flask from the flange and empty it. Stop the recorder chart drive.

19. Rinse the reaction flask with diluted acid to remove possible $NaBH_4$ residues.
20. Prepare the next standard solution or sample and dispense it into the reaction flask.
21. Perform the determination by following the above steps.
22. Analyze all further samples in turn.

Optimization Of The Three Separation Methods.

In order to do the experiments under optimum conditions, optimization experiments were carried out using synthetic sea water spiked with appropriate amount of arsenic.

The ion-exchange and the thiol cotton systems were optimized for the effluent flow rates. Optimization of the solvent extraction method was based on the factors, pH at the APDC preconcentration step, amount of APDC used in the complexation reaction with As(lll). Ashing conditions were determined in separate experiments as has been discussed previously.

Determination of AAS conditions for optimum results was done by direct hydride introduction into the pre-heated quartz tube. The per cent ($\frac{W}{V}$) of $NaBH_4$ solution required for optimum results was also determined.

3

RESULTS

3.1 Accuracy

In order to assess the reliability of the methods, accuracy experiments were carried out using standard reference materials. The sea water standard reference materials (SRM) were obtained from the Marine Analytical Chemistry Standards Program in Canada. The background of the collection of the sea water and the nature of the sea water matrix is presented in Appendix 2.

Recovery experiments were also conducted to assess the accuracy of the methods. The results are shown in Tables XII - XV. Synthetic sea water were spiked with known amounts of arsenic(111) and arsenic(V). The recovered amounts were expressed as percentages.

3.2 Sensitivity And Detection Limits:

Sensitivities were calculated from the slopes of the calibration curves of each method. In practice, the values obtained correspond to the concentration of arsenic that will give an absorbance value of 0.0044.

*Detection limits were calculated from the formula D.L. = $\bar{X}$ + 3 σ where $\bar{X}$ is the average value of ten blanks determination, σ is the standard deviation and 3 is a probability factor at the 99% confidence level.

Results for the sensitivity and detection limits studies are given in Tables XVI - XVIII.

Table XII. RESULTS FOR ACCURACY STUDIES ON STANDARD REFERENCE MATERIALS (in ppb).

	SOLVENT EXTRACTION	ION EXCHANGE	THIOL COTTON	SRM
As(111)	0.50 + 0.05	0.34 + 0.05	0.39 + 0.03	-
As(V)	1.05 + 0.02	1.14 + 0.06	1.06 + 0.05	-
Total As	1.55 + 0.03	1.48 + 0.04	1.45 + 0.06	1.65 + 0.19

Table XIII. RECOVERY OF ARSENIC(111) AND ARSENIC(v) IN 300ML SAMPLE VOLUME.

(Solvent Extraction)

Sample No.	As(111) added (ng)	As(V) added (ng)	Percent As(111) recovered	Percent As(V) recovered
1.	30	-	103.3	-
2.	300	30	100.6	96.7
3.	30	300	100.0	98.7
4.	40	40	95.0	95.0
5.	10	10	105.5	100.0

Table XIV. RECOVERY OF ARSENIC(111) AND ARSENIC(V) (ng in 300ml sample volume.)

Sample No.	(Ion-Exchange) As(111) added (ng)	As(V) added (ng)	Percent As(111) recovered	Percent As(V) recovered
1.	30	-	90	-
2.	300	30	90	110
3.	30	300	88	90
4.	40	40	95	90
5.	10	10	95	95

Table XV. RECOVERY OF ARSENIC(111) AND ARSENIC(V) (ng in 300ml volume sample.)

Sample No.	As(111) added (ng)	As(V) added (ng)	(Thiol Cotton) Percent As(111) recovered	Percent As(V) recovered
1.	30	-	90	-
2.	300	30	90	90
3.	30	300	95	95
4.	40	40	95	100
5.	10	10	95	95

Table XVI. MEANS AND STANDARD DEVIATIONS FOR SENSITIVITY AND DETECTION LIMITS STUDIES.

	(SOLVENT EXTRACTION)
SENSITIVITY ($^{ng}/_{ml}$)	0.14 + 0.006
DETECTION LIMITS ($^{ng}/_{ml}$)	0.006 + 0.007

Table XVII. MEANS AND STANDARD DEVIATIONS FOR SENSITIVITY AND DETECTION LIMITS STUDIES.

	(Ion-Exchange)
SENSITIVITY ($ng/_{ml}$)	1.0 + 0.05
DETECTION LIMITS (ng/ml)	0.060 + 0.001

Table XVIII. MEANS AND STANDARD DEVIATIONS FOR SENSITIVITY AND DETECTION LIMITS STUDIES.

	(Thiol Cotton)
SENSITIVITY ($^{ng}/_{ml}$)	0.50 + 0.07
DETECTION LIMITS ($^{ng}/_{ml}$)	0.022 + 0.005

3.3 Reproducibility And Precision.

Tables XIX - XXIV show the results for reproducibility and precision studies. The reproducibility data were obtained from within - run analysis. Precision data were obtained from between - run analysis on 5 consecutive days. Reproducibility studies were conducted on standard arsenic solutions. For precision studies, the sea water samples were spiked with known amounts of arsenic(lll) and arsenic(V). Standard deviation and per cent relative standard deviation were calculated from 5 determinations.

3.4 Cost And Time Of Analysis.

The cost of analysis was calculated by the cost-benefit analysis method similar to the one reported by Horne.[180] The time reported here is the sampling time which defines the number of analysis per unit time.

Tables XXV - XXVII show the cost and the time of analysis calculated.

Table XIX. PRECISION DATA FOR ARSENIC(111) AND ARSENIC(V) IN NATURAL SEA WATER FOR A 300ml SAMPLE.

(Solvent Extraction)

Sample no.	As(111) added (ng)	As(V) added (ng)	As(111) found (ng)	As(V) found (ng)
1.	150	150	148	146
2.	150	150	146	145
3.	150	150	148	148
4.	150	150	148	147
		MEAN (X)	147.5	146.5
		STANDARD DEVIATION	0.89	1.2
		PER CENT RSD (Sr)	0.6	0.8

Table XX. PRECISION DATA FOR ARSENIC(111) AND ARSENIC(V) IN NATURAL SEA WATER FOR A 30ml SAMPLE.

Sample no.	As(111) added (ng)	(Ion-Exchange) As(V) added (ng)	As(111) found (ng)	As(V) found (ng)
1.	15	15	13.9	17.5
2.	15	15	11.9	14.0
3.	15	15	13.5	15.0
4.	15	15	13.0	15.0
5.	15	15	14.2	12.0
		MEAN (X)	13.3	14.8
		STANDARD DEVIATION	1.8	2.0
		PER CENT RELATIVE STANDARD DEVIATION	13.5	13.5

Table XXI. PRECISION DATA FOR ARSENIC(111) AND ARSENIC(V) IN NATURAL SEA WATER FOR A 30ml SAMPLE.

(Thiol Cotton)

Sample no.	As(111) added (ng)	As(V) added (ng)	As(111) found (ng)	As(V) found (ng)
1.	15	15	13.5	14.3
2.	15	15	12.8	12.3
3.	15	15	17.5	17.5
4.	15	15	18.0	18.5
5.	15	15	16.0	18.0
		MEAN (X)	15.6	16.1
		STANDARD DEVIATION	2.3	2.7
		PER CENT RELATIVE STANDARD DEVIATION	14.7	16.8

Table XXII. DATA FOR REPRODUCIBILITY STUDIES (ng in 300ml sample volume.)

Analysis	(Solvent Extraction) As(111) added (ng)	Peak Height (Absorbance)
1.	80	0.495
2.	80	0.495
3.	80	0.495
4.	80	0.480
5.	80	0.500
	MEAN (X)	0.493
	STANDARD DEVIATION	0.0076
RELATIVE STANDARD DEVIATION		1.5

Table XXIII. DATA FOR REPRODUCIBILITY STUDIES (ng in 125ml sample volume.)

Analysis	(Ion-Exchange) As(111) added (ng)	Peak Height (Absorbance)
1.	33	0.099
2.	33	0.110
3.	33	0.099
4.	33	0.099
5.	33	0.120
	MEAN (X)	0.1054
	STANDARD DEVIATION	0.0095
PER CENT RELATIVE DEVIATION		9

Table XXIV. DATA FOR REPRODUCIBILITY STUDIES (ng in 30ml sample volume.)

Analysis	(Thiol Cotton) As(111) added (ng)	Peak Height (Absorbance)
1.	8.3	0.077
2.	8.3	0.070
3.	8.3	0.077
4.	8.3	0.099
5.	8.3	0.101
	MEAN (X)	0.0869
STANDARD DEVISATION		0.014
PER CENT RELATIVE DEVIATION		16.7

Table XXV

TIME OF ANALYSIS AND COST

(Solvent Extraction)	
TIME OF ANALYSIS (mins)	45
COST PER SAMPLE ANALYSIS ($)	2.26

Table XXVI

TIME OF ANALYSIS AND COST

	(Ion-Exchange)
TIME OF ANALYSIS (mins)	20
COST PER SAMPLE ANALYSIS ($)	1.86

Table XXVII

TIME OF ANALYSIS AND COST

(Thiol Cotton)

TIME OF ANALYSIS (mins)	20
COST PER SAMPLE ANALYSIS ($)	1.86

4

DISCUSSION

4.1 Accuracy.

Accuracy of analytical methods is usually determined by two methods, (a) Reference method and (b) Recovery experiments. Both methods were applied to establish the accuracy of the three separation methods used in this study.

(a) Reference Method.

The results for the accuracy study is shown in Table XII. Per cent error from the true value determined by anodic stripping voltammetry and hydride generation methods is tabulated below.

Method	Per cent error
Solvent extraction	6.0
Ion-Exchange	10.3
Thiol cotton	12.1

The per cent errors are deviations expressed as per centage of the true value. All the three separation methods have tolerable errors. The error due to thiol cotton is comparatively higher than the other two. The accuracy of measurement depends primarily upon the analyte type, the sample maxtrix, and the chemical steps involved in the sample preparation. The causes of the error may be explained in terms of the steps involved in the sample preparation since the analyte type, the sample matrix are the same in all the three cases considered. In fact accuracy refers to the

systematic errors encountered during the sample preparation and analysis. The relatively large errors from Ion-exchange and thiol cotton may probably be correlated to the adsorption efficiency of As(lll) to the thiol cotton and As(V) to the ion-exchange resin.

(b) Recovery Experiments.

Recovery experiments are another way to ascertain the accuracy of an analytical method. A known amounts of As(lll) and As(V) were added to synthetic sea water. Separation with subsequent analysis for these species as described in the experimental sections were carried out. The per cent recovery of these species are shown in Table XXVIII. At the concentration levels studied, 0.05 - 1.5ppb, all the separation methods showed good recovery. The inaccuracies may be due to losses during the sampling preparation step. The analysis of the blank (see appendix 1) showed very little contribution to the inaccuracies noted in these recovery experiments.

4.2 Sensitivity And Detection Limits.

Detection limits were determined using the formula D.L. = X + 3σ (ng/ml) where X is the average value of ten blank determinations, σ is the standard deviation of the blank determinations, and the value 3 is the probability factor at the 99.86% confidence level. Table XXX shows the comparative data for the detection limits studies. The separation method involving solvent extraction had a detection limit lower by a factor of 10 than the other two methods. This makes the

Table XXVIII. COMPARATIVE DATA FOR RECOVERY OF As(111) AND As(V).

As(111) added (ng)			As(V) added (ng)			Percent As(111) recovered			Percent As(V) recovered		
*S.E.	*I.E.	*T.C.	S.E.	I.E.	T.C.	S.E.	I.E.	T.C.	S.E.	I.E.	T.C.
30	30	30	-	-	-	103.3	90	90	-	-	-
300	300	300	30	30	30	100.6	90	90	96.7	110	90
30	30	30	300	300	300	100.0	88	95	98.7	90	95
40	40	40	40	40	40	95.0	95	95	95.0	90	100
10	10	10	10	10	10	105.5	95	95	100.0	95	95

* S.E. - SOLVENT EXTRACTION

* I.E. - ION-EXCHANGE

* T.C. - THIOL COTTON

solvent extraction method more attractive for studies of samples with low levels of arsenic species. The high values for ion-exchange and thiol cotton methods are probably due to contamination during the conditioning of the ion-exchange and the preparation of the thiol cotton, producing relatively high values for their blanks.

Sensitivity values were obtained from the slopes of the calibration curves. This corresponds to the mass of the element that will produce a peak of 1% absorption or 0.0044 absorbance units. The results obtained from the sensitivity studies are also shown in Table XXXI. The sensitivity values obtained are high enough for the methods to be adopted for the study of low-level arsenic species in natural waters.

4.3 Precision And Reproducibility.

Precision:

Precision of an analytical method may be studied in two ways; (a) Day-to-day reproducibility studies and (b) within-run reproducibility studies. In this dissertation, the former definition is referred to as Precision and the latter as Reproducibility. Both studies were conducted to evaluate the analytical techniques under consideration.

Precision:

The results for precision studies is given in Table XIX - XXI and the means and standard deviations for the three methods are tabulated in Table XXIX. The standard deviations and the relative standard deviations were calculated from the formula given in Appendix 1.

Table XXIX. COMPARATIVE DATA FOR PRECISION STUDIES.

	SOLVENT EXTRACTION		ION-EXCHANGE		THIOL COTTON	
	As(111)	As(V)	As(111)	As(V)	As(111)	As(V)
STANDARD DEVIATION	0.82	2.8	1.8	2.0	2.3	2.7
PERCENT RELATIVE	5.4	2.2	13.5	13.5	14.7	16.8

Table XXXI. COMPARATIVE DATA FOR SENSITIVITY AND DETECTION LIMITS.

	SOLVENT EXTRACTION	ION-EXCHANGE	THIOL COTTON
SENSITIVITY (ng/ml)	0.14 + 0.006	1.0 + 0.05	0.50 + 0.07
DETECTION LIMITS (ng/ml)	0.006 + 0.0007	0.06 + 0.005	0.02 + 0.005

The precision of measurements for a given sample depends primarily upon the sampling method, the source stability, and the instrumental system. All these should be about the same for the three methods studied. In order to compare the two methods against solvent extraction based on the calculated standard deviations, F-test was used. The formula is:

$$F = \frac{S_2^2}{S_1^2}$$ where

S_2 = standard deviation of the Ion-exchange method or the thiol cotton method.

S_1 = standard deviation of the solvent extraction method.

	F-Value	
	As(lll)	As(V)
Solvent extraction/Ion-exchange	4.82	0.51
Solvent extraction/Thiol cotton	7.87	0.93

The tabulated F-Value is 6.26

Solvent extraction/Ion-exchange methods have comparable standard deviation for both As(lll) and As(V) since the calculated values are less than the tabulated value.

Solvent extraction/Thiol cotton methods have comparable standard deviations for As(V) but not for As(lll).

The t-test was used to estimate whether there is statistical difference in the results by the two methods. The pooled standard deviation Sp was calculated using

the formula:

$$S_p = \sqrt{\frac{\Sigma(X_{11} - \bar{X}_1)^2 + \Sigma(X_{12} - \bar{X}_2)^2}{N_1 + N_2 - 2}}$$

and the paired t-test was calculated using the formula

$$\pm t = \frac{\bar{X}_1 - \bar{X}_2}{S_p} \sqrt{\frac{N_1 N_2}{N_1 + N_2}}$$

The calculated results are shown below:

	t-calculated	
	As(III)	As(V)
I. Solvent extraction/Ion-exchange	0.06	0.008
II. Solvent extraction/Thiol cotton	-0.02	0.03

The tabulated t for seven degrees of freedom ($N_1 + N_2 - 2$) at the 95% confidence level is 2.365 so there is no statistical difference in the results for the paired methods I and II. The results mean that the ion-exchange and the thiol cotton methods perform equally as well as the solvent extraction method with respect to precision.

Reproducibility:

Reproducibility studies were conducted on synthetic sea water samples which have been spiked with approximately 0.27ppb As(III) concentration to study within-run precision. The means and standard deviations from the data are shown in Table XXX. The results expressed as per cent relative standard deviation were computed from data obtained from analysis of 5 samples.

Table XXX. COMPARATIVE DATA FOR REPRODUCIBILITY STUDIES AT 0.27ppb ARSENIC(111) CONCENTRATION.

SEPARATION TECHNIQUE	PER CENT RELATIVE STANDARD DEVIATION
SOLVENT EXTRACTION	1.5
ION-EXCHANGE	9.0
THIOL COTTON	16.7

an equation given by:

$$K_t = \frac{a}{b} + b \qquad \text{—— 1}$$

where a and b are constants, Kt = cost per analysis for a series of n analyses per unit time.

More explicit equation for fixed costs per day is given by:

$$K_f = \cdot L\frac{(1 + \frac{S}{100} \cdot T_1)}{T_2 \cdot T_3} + \frac{G}{T_4} + E_f + R_f \qquad \text{—— 2}$$

where

L = catalogue price of apparatus;

S = service cost per year as a percentage of L;

T_1 = T_2 minus guarantee period in years;

T_2 = expected number of years that the apparatus can be used;

T_3 = number of work days per year;

G = cost of glassware;

T_4 = expected number of days that glassware can be used;

Ef = fixed costs of materials per series of analyses;

Rf = fixed costs of reagents per series of analyses;

More simplified equation is given as:

$$Kv = (Ep + Rp) \cdot n + P.tn$$

where:

Ep = cost of material per sample;

Rp = cost of reagents per sample;

P = cost of labor per minute;

tn = time required for n series of analyses.

Summary of cost per series of analysis based on the work by Horne[42] is given below.

The calculations of the indicated amounts are given in Appendix I.

Item	100 Samples Solvent Extraction	100 Samples Ion-Exchange	100 Samples Thiol Cotton
Instrument, maintenance and servicing over 10 years	$134.62	$134.62	$134.62
Labor/time of analysis/day	72.16	32.08	32.08
Materials: e.g. Glassware, reagents, other cost	19.23	19.23	19.23
Total Daily Cost	226.01	185.93	185.93
Cost Per Sample	2.26	1.86	1.86

With 100 samples to be analyzed the difference in cost is significant for the methods studied. The cost per sample is expected to decrease with larger number of samples. Although the estimated cost per analysis is based on instrumental price, labor, reagents, etc., there are other factors one must consider in selecting an analytical method based on cost. These include accuracy and precision of the

method. An estimated cost for a method with a low accuracy and poor precision should be less than the method with high accuracy and good precision. Although these factors do not appear in the equation for calculating cost, they exercise much influence in cost-benefit analysis. Cost as a function of the number of samples taken to establish a fixed precision has been treated by Marcuse.[52b]

Time:

Equations 2 and 3 above indicate that cost of analysis is a time dependent parameter. Time factor is made of two characteristics;

(a) the dead time, td, of an analysis vs the time that elapses between the sampling and the reporting of the results which is the analysis time.

(b) the sampling time, ta, which defines the number of analyses per unit time which can be carried out by an analyst. It is also equal to the time between two successive samplings.

The time reported in this thesis is the analysis time td. The cost of analysis was based on the analysis time. The comparative data on the cost and the analysis time of the three separation methods is given in Table XXXII.

Although the time of analysis is an important parameter in estimating the cost of analysis, it is seldom reported. Kateman et al.[49b] has made a rough estimation of the times required for the various manipulations in routine analysis and

Table XXXII. COMPARATIVE DATA FOR TIME OF ANALYSIS AND COST.

TIME OF ANALYSIS	SOLVENT EXTRACTION	ION-EXCHANGE	THIOL COTTON
COST PER SAMPLE ANALYSIS	2.26	1.86	1.86

is shown below:

Classification of Analyses

Manipulation	Class	Standard Time (minutes)
Addition, transferring, mixing, weighing, crushing, dissolving filtration, heating, cooling, titration	1	2 1/2
	2	5
Extration, measurement with AA,FES,UV-VIS,AES,XF	3	15

Classes 1, 2 and 3 all apply in the three separation methods evaluated. The number of manipulations multiplied by the standard time gives the total time in a given class. The sum of the total time in each class gives the time of analysis.

The number of manipulations in solvent extraction is higher than the other two and therefore accounts for its longer time. There are other factors which may influence the time of analysis. These include, waiting time when queues develop, standard of training of the analyst and the reliability of the AA instrument.

Conclusions:

The solvent extraction method of separation was found to be superior to the other methods with respect to detection limits, accuracy, precision and reproducibility. The method was, however, inferior with respect to cost and time of analysis. The cost difference would, however, not overshadow the better analytical variabilities.

Although all the three separation methods possess analytical features for the separation of As(lll) and As(V), solvent extraction method is recommended for routine use in the separation and determination of As(lll) and As(V). The choice of a method, of course, depends upon other factors such as the availability of instrumentation, the nature of the matrix and the concentration levels required for the determination.

One other important factor one must consider in selecting an analytical technique is safety. Is the method safe enough to be used for routine analysis? The risk factor with these methods considered is the use of dangerous reagents. The use of perchloric acid in the wet ashing in the solvent extraction procedure makes it less attractive. The pungent smell of thioglycolic acid in thiol cotton method is nuisance although not toxic. The risks associated with the use of AA and sodium borohydride reagent can be eliminated by good laboratory practice. The risks associated with the three separation methods are not cost-effective and a good analyst can reduce the risk to an acceptable level.

It is suggested that future comparative work should include the newer separation methods, ion chromatography and High performance liquid chromatography if their instrumentation is available.

Appendix 1. Equations And Formulae Utilized In The Dissertation And Blank Analysis.

EQUATIONS AND FORMULAE UTILIZED IN THE DISSERTATION:

A. Equations and formulae utilized in the statistical analysis of the data are those recommended by the Analytical Chemistry Division Commission on Analytical Nomenclature[24] and are listed below:

(1) Mean, $\bar{X} = 1/n \ \Sigma n$

(2) Deviation, $d = |X-\bar{X}|$

(3) Standard Deviation or Error, $S \ (= \frac{1}{n-1} \ \Sigma d^2) \ 1/2$

(4) Relative Standard Deviation, $Sr = S\sqrt{X}$

(5) Percent Relative Standard Deviation, % RSD = Sr x 100

B. The Detection Limit was calculated based on the formula, D.L. = $\bar{X}$ + K according to Zief and Mitchell[84] where D.L. is the Detection Limit, $\bar{X}$ is the average value of the blank, is the standard deviation of the blank, and the factor K has been assigned the value of 3.

C. The analytical sensitivity as has been defined by Price[61b] is the concentration which will absorb 1% of the incident resonance radiation of that element.

D. The standard working curves (calibration) were based on the theoretical linear relationship between the concentration of the absorbing species in the light path and the absorbance as is expressed by Beer-Lambert's Law; Absorbance = a b c

where a = molar absorptivity

b = path length

c = concentration in molarity

Absorbance (a) is defined by:

$$A = \log \frac{1}{T} = \log I_o/I$$

I_o and I are the intensity of the resonance radiation entering and leaving the cell.

Calibration Curves are shown in Figures 14, 15, 16.

Figure 14: Calibration Curve. (Solvent Extraction).

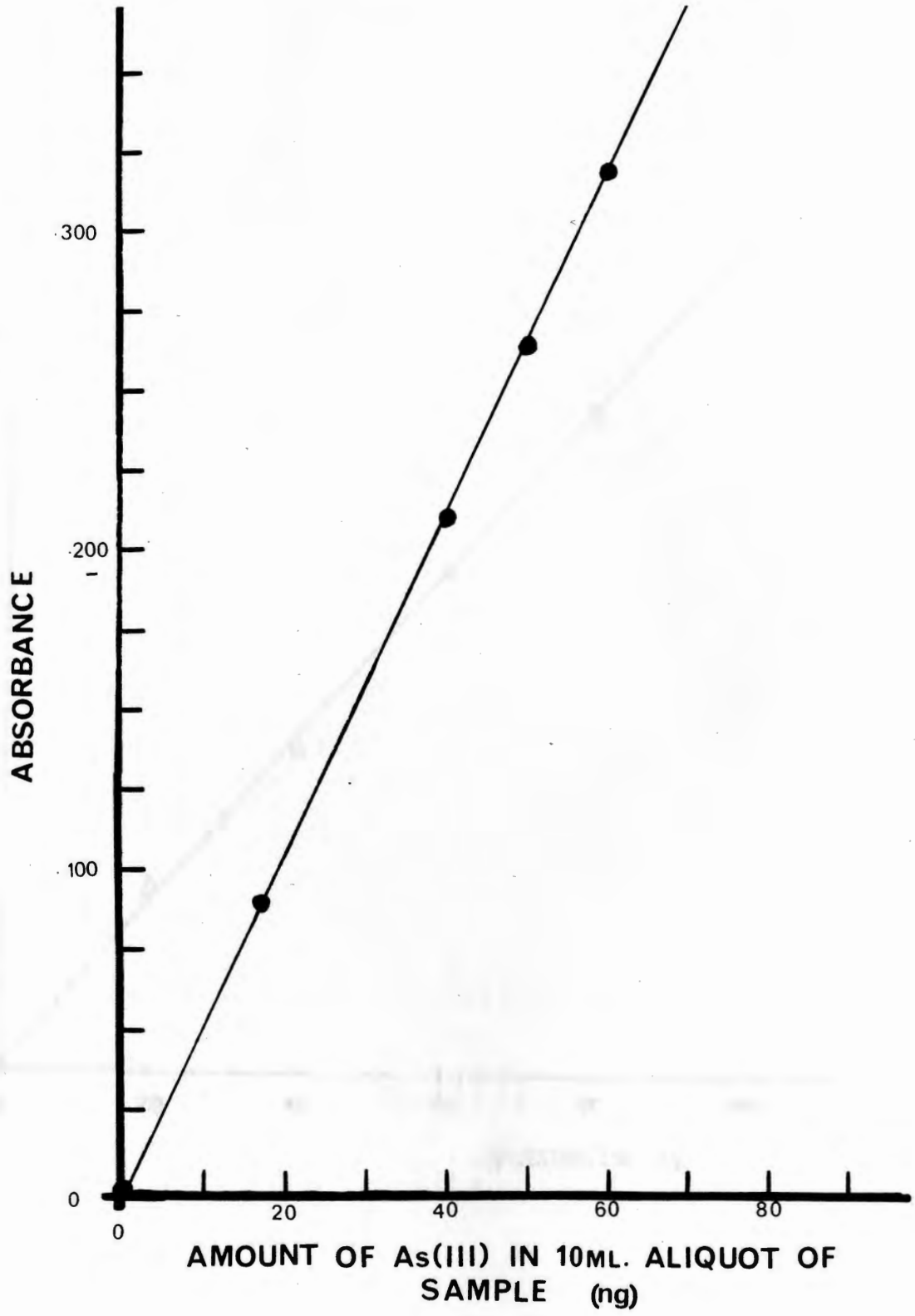


Figure 15: Calibration Curve. (Ion-Exchange).

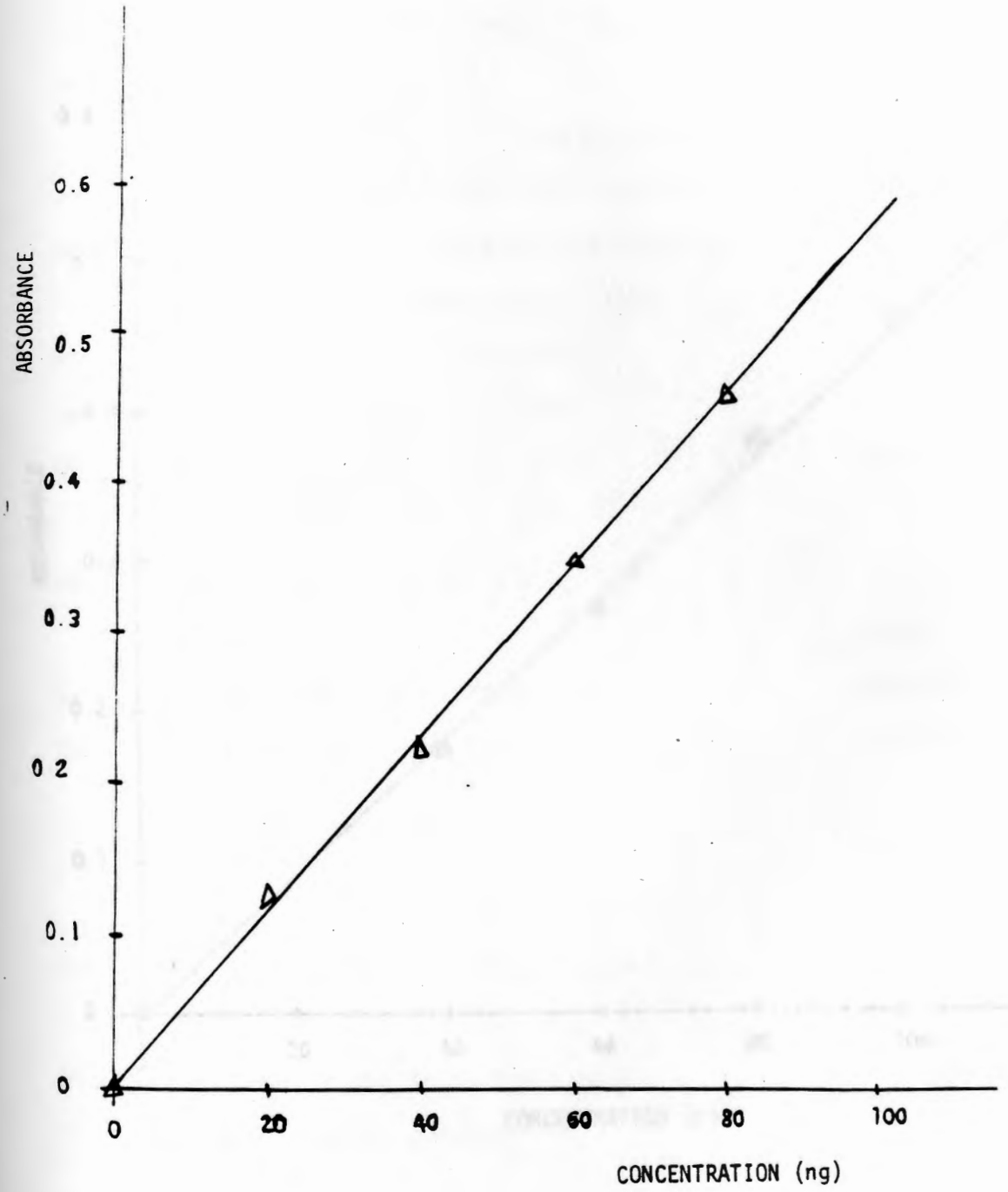
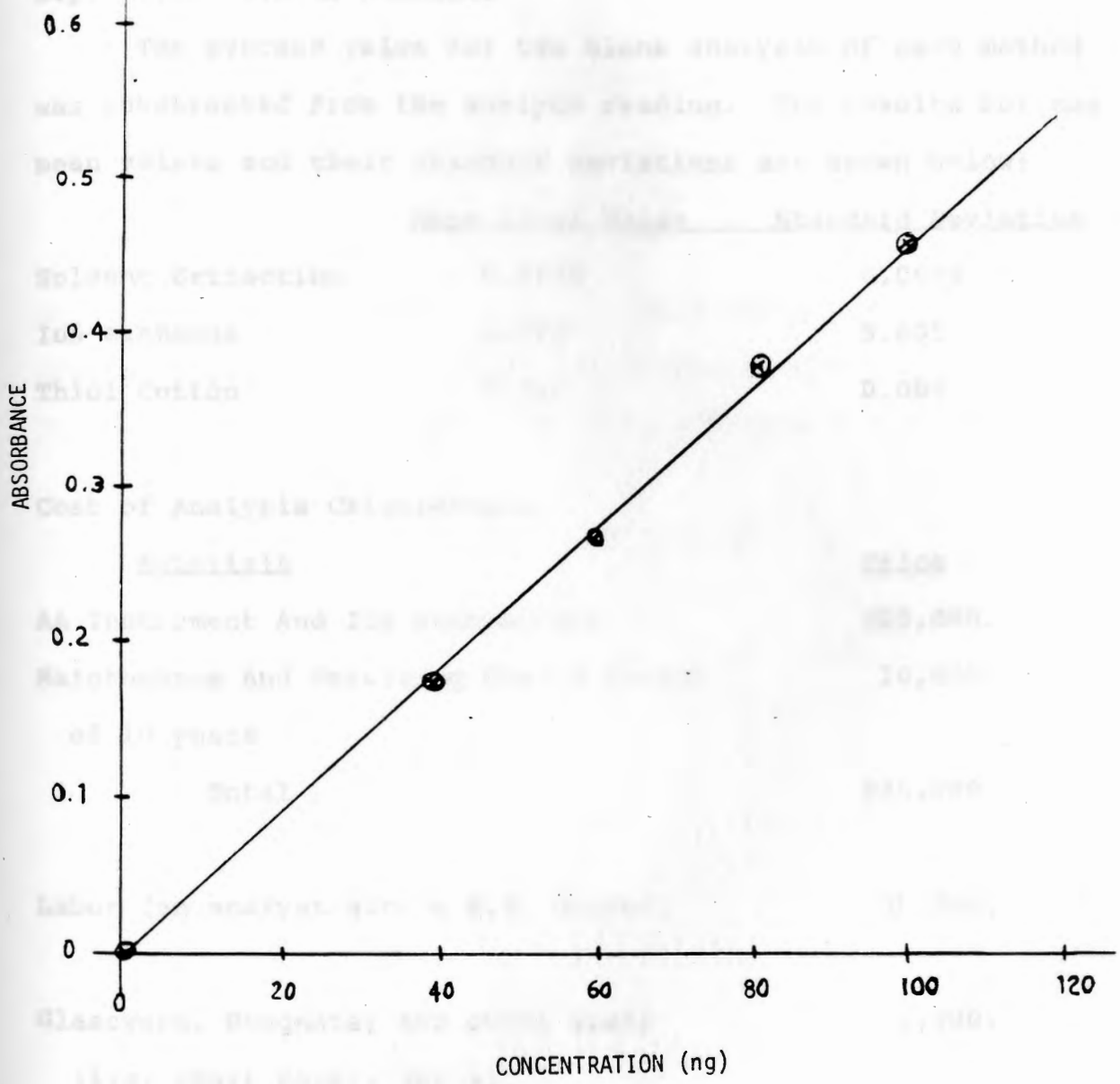


Figure 16: Calibration Curve. (Thiol Cotton).



BLANK ANALYSIS.

To correct for non-specificity, blank analysis is required. To compensate for the reading given by substances other than the analyte, which are present in the sample, synthetic sea water was used as the blank in all the three separation methods studied.

The average value for ten blank analysis of each method was substracted from the analyte reading. The results for the mean values and their standard deviations are shown below:

	Mean blank Value	Standard Deviation
Solvent Extraction	0.0045	0.0005
Ion-exchange	0.045	0.005
Thiol Cotton	0.016	0.004

Cost of Analysis Calculations.

Materials	Price
AA Instrument And Its Accessories	$25,000.
Maintenance And Servicing Over a Period of 10 years	10,000.
Total	$35,000.
Labor (an analyst with a M.S. degree)	25,000.
Glassware, Reagents, and other costs (i.e. chart paper, xerox)	5,000.
Total # of working days/year	260

	Materials	Price
1.	Daily cost for Instrument and Maintenance and Servicing = $134.62	$134.62
2.	Daily labor cost (based on time of analysis)	
	= $96.15/8 hrs	
	= 12.02/hr.	

Solvent extraction; $9.02/time of analysis x 8 = 72.16/Day

Ion-exchange; 4.01/time of analysis x 8 = 32.08/Day

Thiol Cotton 4.01/time of analysis x 8 = 32.08/Day

3. Daily use of Glassware, Reagents, etc.

Appendix 2. Characteristics Of The Sea Water Reference Material (SRM) And The Sea Water From The Narragansett Bay.

SEA WATER REFERENCE MATERIAL (SRM) FOR TRACE METALS:

The sea water reference material for the accuracy studies had total arsenic concentration (As(lll) + As(V) of 1.65 ± 0.19. The uncertainties represent 95% tolerance limits for an individual subsample. That is, 95% of samples from any bottle would be expected to have concentrations within the specified range of 95% of the time. Arsenic concentration quotated was determined by Anodic stripping Voltammetry and Hydride generation atomic absorption spectrometry.

The sea water was collected at the 1300 metre level, southeast of Bermuda, in the vicinity of ocean station's' (32^{o} 10'N, 64^{o} 30'W). It is representative of a North Atlantic open ocean water. The salinity is $35.07^{o}/_{00}$.

Collection was with 12 litre GO FLO samplers (General Oceanics). These PVC samplers were internally coated with teflon. They were modified at the Bedford Institute of Oceanography, Dartmouth, N.S. in order to reduce trace metal contamination. The samplers were acid leached and rinsed with ultrapure water prior to deployment.

The sea water was acidified to pH 1.6 with high purity nitric acid solution immediately upon collection. It was transferred to 50-litre acid leached polypropylene carboys conditioned with ultrapure water acidified to pH 1.6. Previous storage experiments indicated that the integrity of

a sea water sample with respect to total trace metal contents could be maintained for at least two years using this procedure.

The sea water was homogenized in an 800-litre polyethylene tank in a clean room at the Division of Chemistry in Ottawa, and immediately bottled in 2-litre polyethylene bottles. The tank and bottles had been previously acid leached and pH conditioned.

Randomly selected bottles were chosen for the analytical determinations. Results from different bottles showed no significant differences, nor was there any correlation between values obtained and bottle sequence. Thus, it is assumed that the trace metal concentrations of all bottles are essentially the same.

SEA WATER FROM NARRAGANSETT BAY:

The natural sea water sample used in this experiment was Narragansett Bay water. The level of arsenic concentration in this water as has been determined by Johnson et al. is 1.65ppb by neutron activation method.

The water sample was collected from the Bay behind the Narragansett Marine Laboratory. The salinity as determined by Mohr titrimetric method is $18^{o}/_{oo}$.

Collection was done using 1-litre acid leached polypropylene bottle. The water was immediately acidified to pH of 2 using redistilled hydrochloric acid. The sea water was filtered through 0.45μm filter membrane to remove any

particulate matter before any determination. When not in use the sea water was stored in a refrigerator at a temperature of about 4°C.

Critical values of F for a one-tailed test ($P = 0.05$)

ν_2 \ ν_1	1	2	3	4	5	6	7	8	9	10	12	15	20
1	161.4	199.5	215.7	224.6	230.2	234.0	236.8	238.9	240.5	241.9	243.9	245.9	248.0
2	18.51	19.00	19.16	19.25	19.30	19.33	19.35	19.37	19.38	19.40	19.41	19.43	19.45
3	10.13	9.552	9.277	9.117	9.013	8.941	8.887	8.845	8.812	8.786	8.745	8.703	8.660
4	7.709	6.944	6.591	6.388	6.256	6.163	6.094	6.041	5.999	5.964	5.912	5.858	5.803
5	6.608	5.786	5.409	5.192	5.050	4.950	4.876	4.818	4.772	4.735	4.678	4.619	4.558
6	5.987	5.143	4.757	4.534	4.387	4.284	4.207	4.147	4.099	4.060	4.000	3.938	3.874
7	5.591	4.737	4.347	4.120	3.972	3.866	3.787	3.726	3.677	3.637	3.575	3.511	3.445
8	5.318	4.459	4.066	3.838	3.687	3.581	3.500	3.438	3.388	3.347	3.284	3.218	3.150
9	5.117	4.256	3.863	3.633	3.482	3.374	3.293	3.230	3.179	3.137	3.073	3.006	2.936
10	4.965	4.103	3.708	3.478	3.326	3.217	3.135	3.072	3.020	2.978	2.913	2.845	2.774
11	4.844	3.982	3.587	3.357	3.204	3.095	3.012	2.948	2.896	2.854	2.788	2.719	2.646
12	4.747	3.885	3.490	3.259	3.106	2.996	2.913	2.849	2.796	2.753	2.687	2.617	2.544
13	4.667	3.806	3.411	3.179	3.025	2.915	2.832	2.767	2.714	2.671	2.604	2.533	2.459
14	4.600	3.739	3.344	3.112	2.958	2.848	2.764	2.699	2.646	2.602	2.534	2.463	2.388
15	4.543	3.682	3.287	3.056	2.901	2.790	2.707	2.641	2.588	2.544	2.475	2.403	2.328
16	4.494	3.634	3.239	3.007	2.852	2.741	2.657	2.591	2.538	2.494	2.425	2.352	2.276
17	4.451	3.592	3.197	2.965	2.810	2.699	3.614	2.548	2.494	2.450	2.381	2.308	2.230
18	4.414	3.555	3.160	2.928	2.773	2.661	2.577	2.510	2.456	2.412	2.342	2.269	2.191
19	4.381	3.522	3.127	2.895	2.740	2.628	2.544	2.477	2.423	2.378	2.308	2.234	2.155
20	4.351	3.493	3.098	2.866	2.711	2.599	2.514	2.447	2.393	2.348	2.278	2.203	2.124

ν_1 = number of degrees of freedom of the numerator and ν_2 = number of degrees of freedom of the denominator.

The *t*-distribution

Value of *t* for a confidence interval of	95%	98%	99%
Critical value of $\|t\|$ for *P* values of	0.05	0.02	0.01
Number of degrees of freedom			
1	12.71	31.82	63.66
2	4.30	6.96	9.92
3	3.18	4.54	5.84
4	2.78	3.75	4.60
5	2.57	3.36	4.03
6	2.45	3.14	3.71
7	2.36	3.00	3.50
8	2.31	2.90	3.36
9	2.26	2.82	3.25
10	2.23	2.76	3.17
12	2.18	2.68	3.05
14	2.14	2.62	2.98
16	2.12	2.58	2.92
18	2.10	2.55	2.88
20	2.09	2.53	2.85
30	2.04	2.46	2.75
50	2.01	2.40	2.68
∞	1.96	2.33	2.58

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