FLOW INJECTION ANALYSIS (FIA)
DETERMINATION OF LEAD BY HYDRIDE GENERATION
IN BANDED CORAL SKELETONS

by

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ABSTRACT

A flow injection hydride generation direct current plasma atomic emission spectrometric (FI-HG-DCP-AES) method was developed for the determination of lead at ng.ml⁻¹ level. Potassium ferricyanide (K₃Fe(CN)₆) was used along with sodium tetrahydroborate(III) (NaBH₄) to produce plumbane (PbH₄) in an acid medium. The design of a gas-liquid separator (hydride generator) was tested and the parameters of the flow injection system were optimized to achieve a good detection limit and sample throughput.

The technique developed gave a detection limit of 0.7 ng.ml⁻¹(3σb). The precision at 20 ng.ml⁻¹ level was 1.6 % RSD with 11 measurements (n=11). Volume of sample loop was 500 µl. A sample throughput of 120 h⁻¹ was achieved. The transition elements, Fe(II), Fe(III), Cd(II), Co(II), Mn(II), Ni(II) and Zn(II) do not interfere in this method but 1 mg.l⁻¹ Cu(II) will suppress 50 % of the signal from a sample containing 20 ng.ml⁻¹ Pb.

This method was successfully applied to determine lead in a calcium carbonate (CaCO₃) matrix of banded coral skeletons from Si-Chang Island in Thailand.
ACKNOWLEDGEMENTS

"Kob-Khun" means thank you in Thai. I would like to say "Kob-Khun" to all friends, staffs and faculty members in Chemistry department and the Technical Services department of Brock University for all your helps and supports during the past 2 years. For the samples, coral skeletons, I would thank Dr. J. Cameron Lewis and his student, Mr. Chon Poosuwan, for diving to collect all those samples from Si-Chang Island in Thailand. I am grateful to Canadian International Development Agency (CIDA) for the scholarship. To my advisor,

Prof. Ian D. Brindle

"I would not be at this far without you...Kob-Khun"
TABLE OF CONTENTS

ABSTRACT..................................................................................................................I
ACKNOWLEDGEMENTS..............................................................................................II
LIST OF FIGURES........................................................................................................V
LIST OF TABLES............................................................................................................VI
INTRODUCTION............................................................................................................1
1. WHAT IS FIA (FLOW INJECTION ANALYSIS) ?....................................................1
   1.1 Principle of FIA.................................................................................................3
   1.2 Applications of FIA..........................................................................................7
   1.3 Advantages of FIA...........................................................................................8
   1.4 FIA as a sub-division of the injection technique...............................................8
2. WHAT IS HYDRIDE GENERATION ?......................................................................9
   2.1 Plumbane..........................................................................................................11
   2.2 An oxidant is needed for plumbane generation...............................................12
   2.3 Proposed mechanism of plumbane generation...............................................13
   2.4 Transition metal interferences in plumbane generation....................................16
3. WHY DETERMINE LEAD IN CORAL SKELETONS ?...........................................17
EXPERIMENTAL..........................................................................................................20
Apparatus.....................................................................................................................20
Reagents and Preparation.............................................................................................22
Analytical Procedure....................................................................................................24
   1. DCP-AES Operating Condition..........................................................................24
   2. FLOW Injection Manifold..................................................................................25
3. Data Collection and Steps of Determination……………………………………..26
4. Sample Preparation………………………………………………………………….28
5. Transition Metal Interference Study………………………………………………..28

RESULT AND DISCUSSION…………………………………………………………….29
Optimization of Operating Parameters………………………………………………..29
  1. Gas-Liquid Separator…………………………………………………………………29
  2. Transportation Line of Plumbane……………………………………………………30
  3. Sample Loop…………………………………………………………………………31
  4. Concentration of NaBH4 and K3Fe(CN)6……………………………………………32
  5. Argon carrier gas flow rate…………………………………………………………..33
  6. Reagents and Carrier Flow Rate……………………………………………………34
  7. pH……………………………………………………………………………………34
  8. Signal Enhancement by Easily Ionized Element (K)………………………………35

Determination of Lead in the Blank Solution…………………………………………37
Calibration Curve………………………………………………………………………..38

Determination of Trace Amount of Lead in Banded Coral Skeletons…………………40
  1. Study of Matrix Interference…………………………………………………………41
  2. The Amount of Lead in Coral Skeletons from 5 Stations…………………………42
  3. The Mass Spectrum of Residue from Dissolved Corals…………………………44

Study of Transition Metal Interference…………………………………………………45

FUTURE WORK……………………………………………………………………….46
CONCLUSION…………………………………………………………………………47

REFERENCES………………………………………………………………………….48
LIST OF FIGURES

Figure 1 : A flow injection system ................................................................. 2

Figure 2 : Comparison of mixing and dispersion conditions in
batch method(a) and FIA (b).............................................................................3

Figure 3 : Transport types in a narrow-bore tubing in FIS..........................4

Figure 4 : Cross section of a sample zone dispersion in a carrier
stream demonstrating the dilution effect......................................................5

Figure 5 : A relation of signal and traveling sample zone in FIA...............6

Figure 6 : Reduction potential versus pH diagram for hydride-forming elements.....10

Figure 7 : CaCO₃ lattice with different ways foreign cations can be
incorporated into the lattice...........................................................................17

Figure 8 : A cross section of Porites lutea (Order: Scleractinia): coral
skeleton samples used in this study.............................................................18

Figure 9 : The system of FIAlab™ 2000 from Alitea USA Medina Co.........20

Figure 10 : FI manifold for the determination of lead by FI-HG-DCP-AES......25
Figure 11: Computer Display of data collection

Figure 12: Rear view of the injection valve (from Alitea USA Medina Co.)

Figure 13: Gas-liquid separator designs

Figure 14: Calibration curves of lead determination using different length of transportation line L2 (see Figure 10)

Figure 15: The optimum amount of NaBH₄ and K₃Fe(CN)₆

Figure 16: Effect of argon carrier gas flow rate to a peak area signal

Figure 17: Signal enhancement by KCl aspiration into spray chamber

Figure 18: Calibration curve of lead determination

Figure 19: A map of 5 stations around Si Chang Island in Thailand, where samples were collected

Figure 20: Standard addition curve of Pb determination in coral Station 4

Figure 21: The mass spectrum of yellow organic material residue

Figure 22: Cu(II) interference in 20 ng.ml⁻¹ lead determination
# LIST OF TABLES

Table 1: Physical and thermodynamic properties of plumbane..........................11

Table 2: Oxidants with their conditions in plumbane generation........................12

Table 3: Selected reduction potentials relevant to this study..............................13

Table 4: DCP-AES Operating Conditions..........................................................24

Table 5: Steps of Determination...........................................................................27

Table 6: Conditions for plumbane generation in FIS............................................36

Table 7: Tests of lead determination in the blank solution....................................37

Table 8: The amount of lead (ng.g⁻¹) in banded coral skeletons
from 5 stations (n=3)..............................................................................................42

Table 9: Determination of lead in coral station 4 band 1 in
three sampling areas..............................................................................................43

Table 10: Interference of transition ions on Pb solution (20 ng.ml⁻¹ concentration)....45
INTRODUCTION

Health problems from exposure to lead\(^1\) make it significant to study and necessary to develop methods of lead determination at trace levels. Current lead determinations at ng.ml\(^{-1}\) levels are performed using a preconcentration technique prior to analysis by atomic absorption (AA) spectrometry.\(^2\),\(^3\),\(^4\),\(^5\) Without a preconcentration technique, the determination can be achieved by the graphite furnace AA method\(^6\), which is sometimes difficult because of the problem of matrix interference. Recently, a kinetic method for the determination of lead has been reported as a catalyst from the reduction of resazurin by sodium sulfide using stopped-flow FIA. The method was reported to have a 1 ng.ml\(^{-1}\) detection limit.\(^7\) In this kinetic method, a prior extraction must be performed before the determination to remove interferences from transition metals. The problem of matrix interferences and time-consuming are unavoidable for all these methods. The hydride generation technique is one solution for these problems, due to the fast generation of plumbane (PbH\(_4\)) and its removal from a solution to the gas phase. According to the growth in demand for trace element monitoring in the environment, an automatic system, FIA, is being applied to many methods of determination in laboratories.\(^9\) The trace concentrations of lead in calcium carbonate (CaCO\(_3\)) in a matrix such as coral skeletons were determined to investigate whether the technique overcomes the problem of matrix interference without requiring laboratories pre-extraction steps. In this study, the direct current plasma atomic emission spectrometer (DCP-AES) was used as a detector. Atomic absorption spectrometry (AAS) could also be used as a detector for this method.

1. What is FIA?

Flow injection analysis (FIA) was first described in 1975 by Ruzicka and Hansen.\(^8\) FIA is based on an injection of a sample plug into a carrier stream of a reagent, which flows through a microreactor into a detector or sensor of choice. As the reacted plug passes through the detector, a transient signal is recorded and displayed in the form of a peak, usually within 20 seconds. Over the past 20 years, FIA has proved itself to be extremely versatile: it has been developed for use in many areas of chemical research and technology. Consequently, there have been many publications on this topic and FIA instrumentation is commercially available from a number of manufacturers world-wide. Some representative products have been reported in a recent product review.\(^9\) Some companies also advertise on the internet. They also provide the researcher with information about the principles and applications of FIA.\(^10\),\(^11\)
The general steps of determination in flow injection systems (FIS) are clearly demonstrated in the two-channel FIA system illustrated in Figure 1.12

**Figure 1**: A FI system consists of a two-channel pump, an injection valve, a coiled reactor and a detector (D). Step 1: the reagent (navy blue) is added continuously to the carrier stream (light blue). Step 2: the injected sample zone (red) is merged with carrier stream to form a reacted sample zone (yellow) pass through the reaction coil. Step 3: The reacted sample zone is detected by the detector. The resulting reaction product forms a concentration gradient corresponding to the concentration of the analyte throughout the entire sample zone length. (from reference 12)
1.1 Principles of FIA.

There are three basic principles of FIA:\[^{13}\] 
- Sample injection
- Reproducible and controllable dispersion
- Exact timing

In order to make FIA possible, these three things have to occur. Sample injection can be performed by an injection valve with a sample loop. Two types of injection valve, the rotary valve and the commutator (sliding) valve, are primarily used in flow injection systems (FIS).\[^{14}\] They are commercially available from different companies.\[^{15}\] Reproducible and controllable dispersion, which is different from any batch method (as shown in Figure 2) is required in FIA.\[^{16}\]

![Figure 2: Comparison of mixing and dispersion condition in batch method (a) and FIA (b) (from reference 16 p. 7)](image-url)
The reproducible dispersion occurs in a continuously flowing stream in narrow-bore tubing, which has an inner diameter between 0.3-0.8 mm. Laminar flow and two types of diffusion transport occur in the narrow-bore tubing in FIS as shown in Figure 3.

![Laminar Flow and Diffusional Transport](image)

**Figure 3:** Transport types in a narrow-bore tubing in FIS. (from reference 17 p.59)

This reproducible and controllable dispersion is called dynamic dispersion and it never reaches equilibrium or steady-state before the sample zone reaches the detector. Thus, the third principle, exact timing, is needed in order to achieve high precision results. Dynamic dispersion promotes reaction between analyte and reagent (as shown in Figure 1), which enhances sensitivity. At the same time, dispersion also causes dilution of the analyte, as can be seen clearly in Figure 4. The figure shows cross sections of the sample zone traveling along a single-channel FIA.
Cross Sections

Figure 4: A cross section of a sample zone dispersion in a carrier stream demonstrated a dilution effect. (from reference 18)
However, the dilution problem can be solved by increasing the sample loop size to generate an undiluted center of dispersion zone. The sample loop size is usually 50-500 µl. At the same total flow rate of manifold, the bigger the sample loop, the longer the analysis time, which reduces sample throughput. In some cases, with concentrated samples, where dilution is desirable, the problem turns out to be an advantage. Dispersion is reproducible at any given instant of time, if the factors which affect dispersion are constant. These factors include flow rate, tubing inner diameter, type of reactor (e.g. coil, knotted, static mixer, straight, serpentine), length of tubing, and sample loop size.\textsuperscript{13} Dispersion is evaluated by the dispersion coefficient $D$, proposed by Ruzicka and Hansen\textsuperscript{13}, and expressed in the equation

$$D = \frac{C^o}{C} \quad \ldots \ldots \ldots \ldots 1$$

where $C^o$ is the original concentration of a sample solution, contained within the valve cavity prior to injection. If it could be scanned by a detector, it would yield a square signal the height of which would be proportional to the sample concentration (Figure 5 bottom left). When the sample zone is injected, it follows the movement of the carrier stream, forming a dispersed zone. Therefore, the response curve has the shape of a peak reflecting a continuum of concentration (Figure 5 bottom right), forming a different concentration gradient, $C$.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{dispersion_diagram.png}
\caption{A relation of signal and traveling sample zone in FIA. (from reference 13 p.24)}
\end{figure}
If the analytical readout is based on maximum-peak-height measurement, the maximum concentration of recorded curve $C_{\text{max}}$ has to be considered; thus, equation 1 is expressed as

$$D = \frac{C_0}{C_{\text{max}}} \quad \ldots \ldots \ldots . \quad 2$$

$D$ is therefore dimensionless and its value is greater than one. The $D$ value reflects the dilution factor of the sample in the carrier stream. For example, if $D=2$ this means the sample solution has been diluted 1:1 with the carrier stream.

1.2 Applications of FIA.

The first development in flow analysis from "beaker" chemistry was Air-Segmented Flow Analysis System (SFA). SFA is based on the segmentation of the flow by air bubbles, which is hardly more than an old-fashioned "beaker traveling on a conveyor belt"\(^{19}\), but present day FIA is very versatile, as can be seen from scope of its application described here.

1.2.1 Serial assay and random access FIA

This process represents the great advantage of FIA in many fields such as in pollution, in the clinical field, and in agricultural, pharmaceutical, industrial and food applications. It is a process where several components can be determined in the same sample or in a series of samples without any sample pretreatment. One of its most obvious application is in the clinical field where it is used to determine glucose, urea, ammonia, pH, electrolytes and other clinically important species in whole blood instead of plasma or serum as in the traditional method.\(^ {13}\)

1.2.2 Reversed flow FIA (rFIA)

This is based on the injection of reagent(s) into a sample carrier stream and is particularly useful when expensive reagents are necessary.\(^ {13}\) To reduce pollution, rFIA also minimizes the quantity of reagent(s) discharged to waste. It is suitable for applications in which the sample is in abundant supply, as in the case of the environmental field of water analysis.\(^ {20}\)
1.2.3 Enhancement of detector performance.

FIA enhances detector performance or instrument response by means of increased sensitivity, ability to distinguish between different species, and conversion of non-detectable species into detectable ones. The enhancement is performed by these several techniques.

- Solvent extraction,21,22,23,24
- Ion-exchange column,25,26
- On-line reagent production for reagents, which are impractical to handle under normal analytical conditions.13
- Indirect determination techniques, which have the same idea as back-titration. An example of this process is the indirect determination of sulfate by lead selective electrode.27

1.3 Advantages of FIA.

The advantages of flow injection system (FIS) over an continuous system can be summarized as follows:

- Economy in reagent consumption, due to a small amount of sample being used in FIA.
- High rate of sampling, since FIS is also a powerful technique for separations procedures, which are often involved in a sample pretreatment stage.16
- High accuracy and high precision, due to the principle of FIA.
- In some cases, such as potentiometric methods, FIA can be used to improve detection limit of methods compared to the detection limits achieved in batch systems.28
- Computer controllable system, the software packages can be written in BASIC29 or FORTH30 computer language.

1.4 FIA as a sub-division of the injection technique.

The injection technique from its name describes a process of the injection of a sample zone into a carrier stream of fluid, with subsequent continuous detection19 which is consistent with four types of chemical analysis in the present: FIA, liquid chromatography, capillary electrophoresis and field-flow fractionation. Thus, Ruzicka and Christian19 have proposed that novel approaches to injection techniques will be identified and will become an integral part of chemical sensors and analytical instrument in the future.
2. **What is hydride generation?**

Hydride generation is an effective technique of sample introduction to the atomizer compared with the conventional nebulizer technique. In the process of hydride generation, covalent volatile hydrides are produced from redox reaction of analyte with sodium tetrahydroborate and are transported to the atomizer. Almost 100% of the analyte can easily be detected by separating it in a gaseous form. Hydride generation was first introduced by Holak\(^1\) in 1969 to determine arsenic by atomic absorption spectrometer. Since then hydride generation combined with atomic absorption spectrometric detection has become one of the most powerful analytical tools in trace element analysis. From an analysis of reduction potentials, shown in Figure 6\(^2\), Tsalev, et al. showed that the elements As, Se, Bi, Sn, Ge, Sb, Te and Pb should be formed under various conditions of pH and electrode potential. Indium has recently been reported as a hydride-forming element. The method is still under investigation. Two papers\(^3\),\(^4\) have described the method of indium hydride generation, but practical applications to real samples have not been reported so far. Yan et al.\(^4\) and Ebdon et al.\(^5\) reported a determination of thallium by using a continuous-flow vapor generation system, the volatile species produced was thought to be TIH. Cadmium was also reported by Sanz-Medel et al.\(^6\) and Guo and Guo\(^7\) to form a cold vapor by reduction with tetrahydroborate under appropriate condition. The advantages of hydride generation atomic absorption spectrometry (HG-AAS) over other AAS techniques such as the flame and graphite furnace methods are:

- increased transport efficiency, due to the changing of analytes into gaseous form, almost 100% transport of analyte can be achieved.
- higher selectivity, only hydride-forming elements can be separated from the matrix as a way of reducing spectral interferences.

As a result, detection limits at the ng.ml\(^{-1}\) level or lower were achieved for the elements cited above.
Figure 6: Reduction potential versus pH diagram for hydride-forming elements
(from reference 32, p.945)

Considering the advantages described above it is not surprising that HG-AAS has become popular for the determination of hydride-forming elements at low concentrations. Several reviews have described the application of HG-AAS or other atomic spectrometric methods.\textsuperscript{38,39,40,41} However, the conditions, interferences and mechanisms of this technique for each element remain unclarified and require further investigation. For lead determination, hydride generation has not yet become a standard technique.
2.1 Plumbane.

Plumbane is a hydride of lead with the chemical formula PbH₄. Some physical and thermodynamic properties of plumbane have been reported, as shown in Table 1.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiling point</td>
<td>-13 °C</td>
</tr>
<tr>
<td>Melting point</td>
<td>-135 °C</td>
</tr>
<tr>
<td>Heat of formation</td>
<td>59.7 kcal.mol⁻¹</td>
</tr>
<tr>
<td>M-H bond energy</td>
<td>49.0 kcal.mol⁻¹</td>
</tr>
</tbody>
</table>

From Table 1, it is clear that plumbane is unstable at room temperature and difficult to generate. In 1974 Thompson and Thomerson⁴⁴ were the first to report the generation of plumbane for analytical purposes. Plumbane was generated by the direct reaction of an acidified sample (0.2 M HCl) and sodium tetrahydroborate (NaBH₄) solution (1% m/V). Atomization was carried out in a silica tube mounted in an air-acetylene flame. The efficiency of conversion of lead ion to plumbane was less than 5% and gave a detection limit of 0.1 μg.ml⁻¹. This sensitivity obtained for lead was low compared to other hydride-forming elements. Later in 1976, Fleming and Ide⁴⁵ and Vjian and Wood⁴⁶ discovered the sensitivity enhancement by the use of the oxidant K₂Cr₂O₇ and H₂O₂, respectively, together with NaBH₄. These two papers were the starting point of plumbane generation as an analytical technique in atomic absorption spectrometry. Several oxidants and a number of organic reagents were introduced to enhance the generation of plumbane.
2.2 An oxidant is needed for plumbane generation.

Since 1976, from the discovery of sensitivity enhancement by an oxidant in plumbane generation, several oxidants have been studied. The successful oxidants, with acceptable sensitivity, are summarized in Table 2, with their conditions for plumbane generation.

### Table 2: Oxidants with their conditions in plumbane generation.

<table>
<thead>
<tr>
<th>Oxidant</th>
<th>Conditions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>(NH₄)₂S₂O₈</td>
<td>- in HNO₃, H₂SO₄ or HClO₄ acid media, pH~1-2</td>
<td>32,47,48,49</td>
</tr>
<tr>
<td>E° = 2.01 V</td>
<td>- need pre-oxidation time between 20-40 sec.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- in tartaric acid or citrate and acetate buffer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH 3-7 with lower sensitivity.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- the highest HG efficiency was 97.5%</td>
<td></td>
</tr>
<tr>
<td>H₂O₂</td>
<td>- in HNO₃, HCl, H₂SO₄ or HClO₄ acid media, pH~1-2</td>
<td>48,50,51,52</td>
</tr>
<tr>
<td>E° = 1.78 V</td>
<td>Need pre-oxidation time between 20-40 sec.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>unless a catalyst Ni(II), V(V) or Fe(II) is present.</td>
<td></td>
</tr>
<tr>
<td>K₂Cr₂O₇</td>
<td>- in HNO₃ or HCl acid media, pH~1-2</td>
<td>47,53,54</td>
</tr>
<tr>
<td>E° = 1.33 V</td>
<td>gave very poor sensitivity.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- in lactic, malic or tartaric acid gave good sensitivity.</td>
<td></td>
</tr>
<tr>
<td>K₃Fe(CN)₆</td>
<td>- in H₂SO₄ or HCl acid media, pH~0-2 with</td>
<td>42,55</td>
</tr>
<tr>
<td>E° = 0.36 V</td>
<td>good sensitivity.</td>
<td></td>
</tr>
</tbody>
</table>

It should be noted that a new reaction medium of Nitroso-R-salt (2-nitroso-1-naphthol-3,6-disulfonic acid, disodium salt) in an acid system was reported with a characteristic concentration of 0.8 ng.ml⁻¹ of Pb determination by hydride generation.⁵⁶ The mechanism suggested involved the oxidation of Pb(II) to Pb(IV) while chelated with Nitroso-R-salt (NRS), analogous to the oxidation/chelation of Co(II) to a Co(III)(NRS)₃. Chen et al.⁵⁷ used a similar procedure to study the effect of some organic reagents as a chelating agent in plumbane generation. A characteristic concentration of 1.3 ng.ml⁻¹ of Pb from a chelating agent PAN-S (1-(2-pyridylazo)-2-naphthol-6-sulfonic acid) was reported. The proposed mechanism is the direct generation of plumbane from chelated Pb(II) instead of metastable Pb(IV).
2.3 Proposed mechanism of plumbane generation.

The mechanism of plumbane generation is still unclarified. There are two proposed mechanisms suggested in literature.

I. An oxidant is added to oxidize Pb(II) to Pb(IV) and Pb(IV) is then reduced by NaBH₄ to generate plumbane (PbH₄).

II. An oxidant is added as a depolarizer to solve a problem of overpotential on lead caused by hydrogen gas. Pb(II) is reduced by NaBH₄ to generate plumbane (PbH₄).

$E^0$ for Pb²⁺/Pb⁴⁺ is -1.65 (see Table 3). Only relatively powerful oxidizing agents would be able to carry out the reaction.

**Table 3**: Selected reduction potentials relevant to this study.

<table>
<thead>
<tr>
<th>Electrode reaction</th>
<th>$E^0$ (volts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb + 2H⁺ + 2e⁻ = PbH₂</td>
<td>-1.507</td>
</tr>
<tr>
<td>Pb²⁺ + 2e⁻ = Pb</td>
<td>-0.126</td>
</tr>
<tr>
<td>Fe(CN)₆³⁻ + e⁻ = Fe(CN)₆⁴⁻</td>
<td>0.360</td>
</tr>
<tr>
<td>Fe³⁺ + 2e⁻ = Fe²⁺</td>
<td>0.771</td>
</tr>
<tr>
<td>Cr₂O₇²⁻ + 14H⁺ + 6e⁻ = 2Cr³⁺ + 7H₂O</td>
<td>1.330</td>
</tr>
<tr>
<td>Pb⁴⁺ + 2e⁻ = Pb²⁺</td>
<td>1.650</td>
</tr>
<tr>
<td>H₂O₂ + 2H⁺ + 2e⁻ = 2H₂O</td>
<td>1.770</td>
</tr>
<tr>
<td>S₂O₈²⁻ + 2e⁻ = 2SO₄²⁻</td>
<td>2.010</td>
</tr>
</tbody>
</table>

The oxidant used in this study is K₃Fe(CN)₆. It also cannot oxidize Pb(II) to Pb(IV) due to its low reduction potential (see Table 3). The mechanism II was adopted to explain the generation of plumbane. Potassium ferricyanide (K₃Fe(CN)₆) was added to be a depolarizer. The overpotential, which inhibits the dissociation of sodium tetrahydroborate will not occur. The further reduction of Pb(II) to plumbane is permitted.
Plumbane might be generated from Pb(II) as shown in Equation 3.

\[ \text{Pb}^{2+} + 2\text{OH}^- + \text{H}_2\text{O} \rightarrow \text{Pb(OH)}_2 + \text{H}_2\text{O} \]

There are no relevant thermodynamic data available on PbH$_4$. Hence it would not be possible to calculate the reduction potential for this species to demonstrate that plumbane is more easily to generate from Pb(IV) than Pb(II). However, there is a reduction potential of PbH$_2$ instead of PbH$_4$ available in an analytical handbook.\textsuperscript{59,61} The trend of reduction should be the same, thus the following calculation was performed.

The $E^0_{\text{Pb}^{2+}/\text{PbH}_2}$ and $E^0_{\text{Pb}^{4+}/\text{PbH}_2}$ are calculated from Meites method.\textsuperscript{59}

Given

\[
\begin{align*}
C + n_{C,B} e^- & = B \\
B + n_{B,A} e^- & = A
\end{align*}
\]

Then

\[
C + (n_{C,B} + n_{B,A})e^- = A \\
E^0 = \frac{(n_{B,A})E^0_{B,A} + (n_{C,B})E^0_{C,B}}{n_{B,A} + n_{C,B}}
\]

i) $E^0_{\text{Pb}^{2+}/\text{PbH}_2}$

\[
\begin{align*}
\text{Pb}^{2+} + 2e^- & = \text{Pb} & E^0 & = -0.126 \text{ V} \\
\text{Pb} + 2\text{H}^+ + 2e^- & = \text{PbH}_2 & E^0 & = -1.507 \text{ V} \\
\text{Pb}^{2+} + 2\text{H}^+ + 4e^- & = \text{PbH}_2 & E^0 & = 2(-1.507) + 2(-0.126) \frac{2 + 2}{2} = -0.8165 \text{ V}
\end{align*}
\]

ii) $E^0_{\text{Pb}^{4+}/\text{PbH}_2}$

\[
\begin{align*}
\text{Pb}^{4+} + 2e^- & = \text{Pb}^{2+} & E^0 & = 1.65 \text{ V} \\
\text{Pb}^{2+} + 2e^- & = \text{Pb} & E^0 & = -0.126 \text{ V} \\
\text{Pb}^{4+} + 4e^- & = \text{Pb} & E^0 & = 2(-0.126) + 2(1.65) \frac{2 + 2}{2} = 0.762 \text{ V}
\end{align*}
\]
\[
Pb^{4+} + 4e^- = Pb \quad E^0 = 0.762 \quad V
\]
\[
Pb + 2H^+ + 2e^- = PbH_2 \quad E^0 = -1.507 \quad V
\]
\[
Pb^{4+} + 2H^+ + 6e^- = PbH_2 \quad E^0 = \frac{2(-1.507) + 4(0.762)}{2+4} = 0.006 \quad V
\]

The calculation does show that PbH_2 is more easily reduced from Pb(IV) than Pb(II). However, the redox reaction in plumbane generation is very powerful. Both species might be reduced very easily. Further investigations are needed in order to explain the mechanism of plumbane generation.
2.4 Transition metal interferences in plumbane generation.

The most serious reported interferences from transition metals in plumbane generation are from Cu, Ni and Fe. The magnitude of these interferences depends on the method of hydride generation (batch, continuous, flow injection analysis), on the atomization system and on the reagents added. H$_2$O$_2$-HNO$_3$ appears to be the best compromise system in terms of sensitivity, reagent blank and interference effect. Flow injection hydride generation has been reported to improve the interference problem from transition metals in the determination of Bi in hydride generation. The reason for this might be the reduction of sample-reagent contact time. The proposed mechanism of transition element interferences is a prereduction of transition metal to a lower oxidation states or to a free metal. The free metal atom forms a precipitate, which can either coprecipitate the analyte or adsorb the volatile hydride and catalytically decompose it.

The effect of transition metal interferences on lead hydride generation is contradictory, both enhancement and depression of the analytical signal have been reported. Furthermore, Fe(III) and Ni(II) have been reported to be catalysts in the H$_2$O$_2$-HNO$_3$ system of plumbane generation. Several techniques have been adopted to eliminate or reduce these problem of interferences. On-line removal by chelating resin or cation-exchange material is one of those techniques. Jin and Taga used a separation technique to extract lead with dithizone into chloroform and back-extract into acid. Coprecipitating lead with MnO$_2$ in acidic solution to eliminate copper interference was used by Vijan and Sadana. To avoid tedious work, the use of masking reagents such as citric acid-potassium cyanide mixture, thiourea, 1,10-phenanthroline, sodium cyanide with oxalic acid and sulfosalicylic acid with sodium cyanide dissolved in NaBH$_4$ have been reported.
3. Why determine lead in coral skeletons?

Sea water contains cations and anions of many major, minor and trace elements in specific concentration. They are incorporated, by varying degrees, in the calcium carbonate shells or skeletons.\textsuperscript{70} The ways that minor and trace elements are incorporated into CaCO\textsubscript{3} minerals were described by McIntire\textsuperscript{71} and Zemann.\textsuperscript{72}

i) by direct substitution for Ca in the CaCO\textsubscript{3} lattice (diadochic).

ii) by emplacement interstitially between the lattice planes

iii) by occupation of vacant lattice positions due to structure defects.

iii) by adsorption due to remnant ionic charges.

iiii) by presence in non-carbonate components, such as fluid inclusions and silicate impurities.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{CaCO3_lattice.png}
\caption{CaCO\textsubscript{3} lattice with different ways foreign cations can be incorporated into the lattice (from reference 71, p.238).}
\end{figure}
Lead is one of the toxic trace elements found in sea water. Industrial discharge and transportation are sources of lead contamination into the environment. A determination of these trace element ratio in shell or skeleton to sea water was used by biologists to identify biological controls such as growth rate, nutrient uptake and fluid pumping. In environmental science, these data were used to determine environmental conditions. Corals, which lay down growth rings like trees, can be used as a record of past environmental conditions. *Porites* corals from the Great Barrier Reef in Australia showed yellow-green fluorescent bands under UV light. The yellow-green fluorescent bands came from fulvic acid in soil. The bands match rainfall and soil runoff records from the Australian mainland. Thus, old *Porites* corals from around the world may provide a record of rainfall and soil runoff from centuries past.
Figure 8: A cross section of *Porites lutea* (Order: Scleractinia): coral skeleton samples used in this study.
EXPERIMENTAL

Apparatus

• A Beckman SpectraSpan V Direct Current Plasma Atomic Emission Spectrometer (DCP-AES) equipped with a modified sample tube head for plumbane introduction was described elsewhere. A data collection program was written by the Technical Services department of Brock University to collect transient signals produced by the flow injection system.

• FIAlab™ 2000 (Alitea USA Medina, Washington USA.): a manually operated flow injection analysis system consisting of an 8-roller, 4-channel variable speed peristaltic pump and a specially designed Upchurch kit with a 6-channel injection valve (as shown in Figure 9), fittings, pump tubing and connecting tubes. This 0.51 mm. i.d. Teflon connecting tubing (Upchurch Cat. no. 1548) was used throughout the manifold. Only two channels of peristaltic pump were used to pump K₃Fe(CN)₆ and NaBH₄ with 1.02 mm. i.d. Tygon peristaltic pump tubing (Upchurch Cat. no. 1553).

Figure 9: The system of FIAlab™ 2000 from Alitea USA Medina Co.
• 4-channel peristaltic pump (Gilson Minipuls 312) was used to pump the sample into a sample loop, carrier into the carrier line and to pump waste solution out of the gas-liquid separator. SMA Flow Rated pump tubes (Technicon Instrument Corporation, Terrytown, NY 10591), Part: 116-0549P17, Collar color: purple/orange was used for these three channels.

• A gas-liquid separator which has a volume of 13 cm$^3$ was made in-house and is shown in Figure 13.

• Rotameter for controlling argon flow to hydride generator.

• All glassware was primarily soaked overnight in 50% nitric acid and left in 10% nitric acid when they were not in use.
Reagents and preparation

All reagents used were analytical grade except as stated.

1. Reagents.

- Argon, Welding grade argon (Union carbide Mississauga, Ontario, CA)
- Distilled water from Chemical store, Chemistry Dept., Brock university.
- Hydrochloric acid: HCl lot no.241219 (Caledon Laboratories LTD. Georgetown, ON. CA. L7G 4R9)
  Preparation: HCl 0.05 M was diluted from 0.1 M HCl, which was prepared by measuring 8.3 ml of concentrated HCl in 10.0 ml graduated cylinder and diluting with distilled water to a litre in a volumetric flask.
- Lead nitrate: Pb(NO3)2 lot no.725291 (Fisher Scientific Co., Chemical Manufacturing Division Fair Lawn, New Jersey 07410)
  Preparation: 1.6 g. of lead nitrate was weighed to the nearest 0.1 mg and dissolved in 500 ml distilled water and 20 ml concentrated HNO3 respectively and diluted to 1 litre by distilled water to make a stock standard solution of 1000 mg/l Pb(II). Solutions of Pb(II) 10 mg/l and 1 mg/l were prepared by dilution. These two solutions were used to prepared a 100 ml of each standard from a series of 2, 5, 10, 20 ng/ml Pb(II).
- Nitric acid: HNO3 lot no.209839 (BDH Chemicals Toronto, Ontario, CA)
- Potassium chloride: KCl lot no. 20095 (Caledon Laboratories LTD. Georgetown, ON. CA. L7G 4R9)
- Potassium ferricyanide: K3Fe(CN)6 lot no.732676 (Fisher Scientific Co., Chemical Manufacturing Division Fair Lawn, New Jersey 07410).
  Preparation: K3Fe(CN)6 1.5% m/V was prepared by dissolving of 7.5 g K3Fe(CN)6 in 500 ml distilled water and was kept in a brown Nalgene plastic bottle in a refrigerator for use.
- Sodium hydroxide: NaOH, Laboratory Reagent (BDH Chemicals Toronto, Ontario, CA)
• Sodium tetrahydroborate: NaBH₄ lot no. 10H3440 and 83H3437 (Fisher Scientific Co., Chemical Manufacturing Division Fair Lawn, New Jersey 07410)  

Preparation: NaBH₄ 1.5% m/V in 0.15% m/V NaOH was prepared freshly and filtered before use.

2. Reagents for transition metal interference study.

All reagents for interference study were dissolved in distilled water to make a stock standard solution of 1000 mg.l⁻¹ of interference ions. A solution of 10 mg.l⁻¹ of each ion was prepared by dilution to be used for spiking. Ferric chloride was digested in 5 ml. concentrated nitric acid for 30 min. to make a stock standard solution.

• Ammonium ferrous sulfate hexahydrate: (NH₄)₂SO₄·Fe(SO₄)·6H₂O Laboratory Reagent (BDH Chemicals Toronto, Ontario, CA )

• Cadmium chloride: CdCl₂·2.5H₂O (BDH Chemicals Toronto, Ontario, CA )

• Cobalt nitrate hexahydrate: Co(NO₃)₂·6H₂O (Aldrich Chemical Co., Inc. Milwaukee WI 53233 USA )

• Copper sulfate pentahydrate: CuSO₄·5H₂O (BDH Chemicals Toronto, Ontario, CA )

• Ferric chloride: FeCl₃ lot no. 06224CN (Aldrich Chemical Co., Inc. Milwaukee WI 53233 USA).

• Manganese chloride tetrahydrate: MnCl₂·4H₂O (BDH Chemicals Toronto, Ontario, CA ).

• Nickel nitrate hexahydrate: Ni(NO₃)₂·6H₂O (Fisher Scientific Co., Chemical Manufacturing Division Fair Lawn, New Jersey 07410).

• Zinc chloride: ZnCl₂ lot no. 11630MN (Aldrich Chemical Co., Inc. Milwaukee WI 53233 USA ).
Analytical procedure

1. DCP-AES Operating Conditions.

The studies were performed by using the operating conditions for DCP-AES as given in Table 4.

**Table 4: DCP-AES Operating Conditions.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>368.3  nm.</td>
</tr>
<tr>
<td>Plasma position</td>
<td>-1</td>
</tr>
<tr>
<td>Entrance slit</td>
<td>100 x 300 μm.</td>
</tr>
<tr>
<td>Exit slit</td>
<td>100 x 300 μm.</td>
</tr>
<tr>
<td>Ar pressure to sleeves</td>
<td>50 psi</td>
</tr>
<tr>
<td>Ar pressure to nebulizer</td>
<td>25 psi</td>
</tr>
<tr>
<td>Voltage supply to PMT</td>
<td>1500 volts</td>
</tr>
</tbody>
</table>
2. Flow Injection Manifold.

The Flow injection (FI) manifold adapted from previous work as shown in Figure 10 was used for the determination.

![Diagram](image)

**Figure 10**: FI manifold for the determination of lead by FI-HG-DCP-AES. L= Sample loop, V= Injection valve, P1, P2= Peristaltic pump, GL= Glass gas-liquid separator, Ar= Argon gas flow 1.3 L/min., L1=1.51 mm. id. Upchurch Tygon pump tube no.1552 connected with Teflon tube the same inner diameter all together is 10 cm. long, L2 = 100 cm, 0.50 cm. id. Tygon tubing, C= carrier 0.1 M HCl, R1= 1.5% m/V K$_3$Fe(CN)$_6$, R2=NaBH$_4$ 1.5% m/V in 0.2 % m/V NaOH, S=Sample in 0.1 M HCl. W= waste
Figure 1: The simplified circuit diagram of the device with all components labeled. 

[Diagram of the circuit with labels and connections]
3. Data Collection and Steps of Determination.

As mentioned before in the Apparatus section, a new software program was written by the Technical Services department of Brock University to collect transient signals produced by the flow injection system. Figure 11 is a plot of 4000 points from a total of 4096 points (The number of points was limited by the use of the Excel program). A baseline was calculated by taking an average signal of all data points in the first 9 sec. This baseline value was subtracted from each point in the next 18 sec. to generate a signal value. All the signal values over the 18 sec. were added up to give the total peak area. Total time of 30 sec. per sample was used in this determination, which allows a sample throughput of 120 h⁻¹.

![Figure 11: Computer Display of data collection which takes 30 sec./sample/screen. During the first 9 sec., baseline data were collected. In the next 18 sec., the analytical signal was collected.](image-url)
Table 5: Steps of Determination.

<table>
<thead>
<tr>
<th>Step</th>
<th>Injection valve</th>
<th>Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>load position</td>
<td>Sample is aspirated from ch.4, passed to ch3. (as shown in Figure 12) into sample loop and out at ch.6 and through ch.5 passed through the pump into the waste container. At the same time carrier is aspirated from ch1. passed through ch.2 to mix with K₃Fe(CN)₆ and NaBH₄ to generate H₂ gas into the gas-liquid separator. Ar carrier gas flushes all the gaseous products to the plasma to generate the baseline data in the first 9 sec.</td>
</tr>
<tr>
<td>2</td>
<td>injection position</td>
<td>When a signal hits the first cursor at 9 sec., the valve will be switched into the injection position. The sample plug will be flushed into the carrier line to mix with the two reagents to generate plumbane and will pass to the plasma to generate the analyte signal.</td>
</tr>
<tr>
<td>3</td>
<td>load position for the next sample</td>
<td>When the signal hits the second cursor at 27 sec., the valve will be switched back to the load position to load the next sample, while baseline data are collected for subtracting from the signal of the next sample.</td>
</tr>
</tbody>
</table>

Figure 12: Rear view of the injection valve (from Alitea USA Medina Co.).

Banded coral skeletons were bleached overnight in 10% household bleach (to remove algae that stayed symbiosis with corals) two times and were left under running tap water for an hour. After that, they were washed with distilled water and dried overnight in a fume hood. Coral skeletons were scraped out from each band by a sharpened silica-base tool. 0.1 g. was removed and weighed to the nearest 0.1 mg and was placed in a 20 ml Erlenmeyer flask. Distilled water 5 ml. was added to the flask, following by 0.30 ml. concentrated HCl acid, measuring by graduated pipette. After the coral was dissolved, some yellow organic material was left in the flask. The solution was transferred into a 25 ml. volumetric flask with distilled water and made to volume. The small amount of yellow organic material settled at the bottom of the flask. The material was collected and submitted for mass spectral analysis.

5. Transition metal interference study.

A solution of 10 mg.l⁻¹ of each transition ion was diluted from a 1000 mg.l⁻¹ stock standard solution and spiked into a 20 ng.ml⁻¹ Pb(II) solution, which was 0.1 M in HCl, to make a 1 mg.l⁻¹ and 10 mg.l⁻¹ of each ion in a 20 ng.ml⁻¹ Pb(II) solution. Copper solutions were prepared at 0.1, 0.2, 0.3, 0.4, 1 and 2 mg.l⁻¹ in 20 ng.ml⁻¹ Pb(II). All these solution were analyzed and their signals compared with the signal from a solution containing only 20 ng.ml⁻¹ Pb(II) to find the percent recovery.
RESULTS AND DISCUSSION

Optimization of Operating Parameters.

Determination of lead by hydride generation in a continuous flow system has been reported to achieve a detection limit of 1.1 ng.ml\(^{-1}\).\(^{55}\) In FIS, as stated in the introduction, only a small amount of sample (50-500 \(\mu\)L) is used to give a transient signal in determinations. In order to get the same detection limit as in a continuous system, the following parameters were optimized in addition.


**Figure 13**: Gas-liquid separator designs. The second one was successfully used in this study.

![Gas-liquid separator designs](image)

The first gas-liquid separator was adapted from previous work\(^ {75}\) by constructing in two layers with a frit in the inner layer. This frit caused a problem due to the appearance of a white salt that was being generated in the process of hydride generation. The frit was clogged by the salt, and the argon carrier gas could not flow up to the plasma, resulting in no signal being produced. This white salt was suspected to be produced by a reduction of Prussian blue \((Fe_{2+}[Fe^{II}(CN)_6]_{3\text{.xH}_2O})\), which was observed in the waste solution. \(Fe(III)\) in \(K_3Fe(CN)_6\) might be reduced by \(NaBH_4\) to \(Fe(II)\) and produces Prussian Blue, which is further reduced to a white salt, which is called Everitt's salt \((K_2[Fe^{II}Fe^{II}(CN)_6])\).\(^ {76}\)

The second gas-liquid separator was modified from the first one by eliminating the frit and the inner layer. The direction of argon flow was also changed to flow from left to right to flush all plumbane up to plasma instead of from bottom to top. The size is half the size of the first one, resulting in a smaller dead volume.
UNITED STATES OF AMERICA

ExxonMobil Corporation v. Shell

In this case, the United States District Court for the Northern District of California ruled that ExxonMobil Corporation was not liable for the costs of cleaning up a large oil spill in the Gulf of Mexico that occurred in 2010. The court determined that ExxonMobil was not the proximate cause of the spill, as Shell Oil Company had been negligent in its operations. The court also found that ExxonMobil had not breached any contractual obligations to Shell. The case was significant because it involved one of the largest oil spills in history and had significant environmental and economic implications.

ExxonMobil Corporation v. Shell Oil Company

Docket No. 09-CV-00055

United States District Court

Northern District of California

Plaintiff: ExxonMobil Corporation

Defendant: Shell Oil Company

In a ruling on February 24, 2012, the court held that ExxonMobil was not liable for the costs of cleaning up the oil spill. The court found that ExxonMobil was not on notice of the potential for a spill at the time it entered into the contract with Shell Oil Company to facilitate the sale of Shell's interest in the Gulf of Mexico oil field. The court also found that ExxonMobil did not breach any contractual obligations to Shell and that Shell had been in breach of its own duty to conduct a full environmental assessment of the area.

The court noted that ExxonMobil had taken steps to ensure the safety of its operations and that it was not responsible for the spill. The court also emphasized that it was not the role of the court to determine whether or not the spill was preventable.

The court concluded that ExxonMobil was not liable for the costs of cleaning up the spill and that Shell Oil Company should be responsible for those costs.
2. Transportation line of plumbane.

Plumbane is generated right after the point at which the NaBH₄ line was merged with the carrier line. Thus, there are two transportation lines, as shown in Figure 10; one is after merging point of NaBH₄ to gas-liquid separator (L1) and the other one is from the gas-liquid separator to the plasma (L2). Due to the instability of plumbane, these two lines were recommended to be kept as short as possible and to use a bigger inner diameter of tubing than usual in FIS to leave enough space for plumbane gas to be generated. The optimum length of L1 is 10 cm. For tube lengths shorter than 10 cm., less signal was produced. For L2, due to a difficulty of working in a small space close to plasma, 10 cm. and 100 cm. of length were compared. The result is shown in standardization curves in Figure 14, which shows that a tube of 100 cm. gave about 10% higher slope than one of 10 cm. length.

**Figure 14**: Calibration curves of different length of transportation line L2. (see Figure 10)

![Calibration curves of different length of transportation line L2](image)

<table>
<thead>
<tr>
<th>Length</th>
<th>Series 1</th>
<th>Series 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>cm.</td>
<td>slope</td>
<td>slope</td>
</tr>
<tr>
<td>100</td>
<td>5.5± 0.1 x 10⁴</td>
<td>4.9± 0.1 x 10⁴</td>
</tr>
</tbody>
</table>
This result might be explained by the observation that waste solution from the gas-liquid separator can be flushed up to extinguish the plasma or drops of water can build up in the transportation line. This build up can cause noisy plasma because of different size of aerosol droplets which obstruct the flow of plumbane to the plasma. In 100 cm. length of L2, which about 90 cm. further away from the plasma than in 10 cm, drops of water were dried in the line before reaching the plasma. In other words, 10 cm length of L2 limited argon flow rate resulting in less plumbane being flushed to the plasma. Therefore, 100 cm was used in this study.

3. Sample loop.

Sampling time and signal are two factors that influence the choice of sample loop size, as stated in the introduction. The bigger the sample loop, the higher the signal up to some value which would be the same as a continuous flow value. The bigger the sample loop, the longer the analysis time, which reduces sample throughput. In this study 500 µL was a suitable compromise size of sample loop between analysis time and sensitivity.
4. Concentration of NaBH₄ and K₃Fe(CN)₆

In a continuous system, large amounts of both reagents, 2.5 % m/v NaBH₄ and 5 % m/v K₃Fe(CN)₆, are used in lead determination by hydride generation. One of the advantages of FIA is that it needs smaller amounts of reagents for the same determination of lead by hydride generation as shown in Figure 15.

**Figure 15**: The optimum amount of NaBH₄ and K₃Fe(CN)₆

<table>
<thead>
<tr>
<th>Series</th>
<th>Concentration (m/v) NaBH₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series 1</td>
<td>0.5</td>
</tr>
<tr>
<td>Series 2</td>
<td>1.0</td>
</tr>
<tr>
<td>Series 3</td>
<td>1.5</td>
</tr>
<tr>
<td>Series 4</td>
<td>2.0</td>
</tr>
</tbody>
</table>

The signal does not change much with concentrations of K₃Fe(CN)₆ varying from 0.5 to 2.0 % m/v. In contrast, for NaBH₄, the signal obviously increases from 0.5 to 1.5 % m/v of NaBH₄. The signal stays almost the same, however, for 1.5 and 2.0 % m/v of NaBH₄. As a result, 1.5 % m/v for both reagents was chosen.
5. Argon carrier gas flow rate.

The flow rate of argon carrier gas affects the peak area signal. The higher the flow rate, the bigger the peak area was found, up to a point at which the peak area stays the same or gets smaller. The constant peak area might be explained by a constant amount of transported analyte, resulting in an upper limit of peak area could be achieved as the argon flow rate increases. In addition, the argon flow rate is high, it causes change in the plasma position. The plasma image moves away from the entrance slit, resulting in less emission radiation of analyte being presented to the monochromator which shows as a smaller peak area. This relation between peak area and argon flow rate is shown in Figure 16.

**Figure 16**: Effect of argon carrier gas flow rate to a peak area signal.
6. Reagents and carrier flow ratio.

An attempt to vary concentration of reagents, instead of varying the flow rate, was used at the beginning of this study and failed to improve the sensitivity of method. The reason for this can be explained by the dilution factor. Since the sample slug in a carrier stream is diluted when it merges with the reagent line, the carrier flow rate should be kept higher than the reagent flow rate to achieve as small a dilution factor as possible. The 1:1:1 flow ratio of HCl : NaBH₄ : K₃Fe(CN)₆, which was used at the beginning gave a dilution factor of 3. This means that only 33 % of original (injected) concentration of lead was actually delivered to the reagents. A better flow ratio was found to be 10: 2.8: 2.8, which gives a dilution factor of 1.56. The peak area for the same concentration of lead standard solution was 3 times the peak area of 1:1:1 ratio because 64 % of injected lead concentration was delivered, resulting in a higher sensitivity of the method.

7. pH

Sodium hydroxide, a stabilizer for NaBH₄ and the HCl carrier, affects the pH of the system. Because the reaction takes place very rapidly in a very small (inner diameter 1.5 mm.) tubing, an attempt to measure pH of plumbane solution at the end of transportation line L1 was made by using pH paper. The result was that a pH 8 was found. However, by a calculation in a system of 0.1 M HCl, 0.2 % NaOH and a dilution factor of 1.56, pH of the reaction should be equal to - log( 0.1 / 1.56 ) = 1.19, which is in the range that has been reported in a literature. But this calculation neglected the concentration of NaOH, which is important. A mixture of 0.1 %m/v NaOH and 0.05 % m/v HCl was found to give the same signal as 0.2 % m/V NaOH and 0.1 % m/v HCl at the same dilution factor. But 0.1 % m/v NaOH cannot stabilize 1.5 % m/V NaBH₄ quite well. This lack of stabilization can be seen from the appearance of hydrogen gas bubbles, which were produced in the line, making a variation of signal. The pH of sample solution must be adjusted to be the same pH as the carrier to control the ultimate pH of the reaction. In addition, the generated hydrogen gas from different pH might change the plasma position, which affects the signal.
8. Signal enhancement by easily ionized element (K)

The advantage of a replacement of distilled water by a solution of easily ionized elements nebulized into the spray chamber in continuous hydride generation has been investigated in previous work. In lead determination by hydride generation in K$_2$Cr$_2$O$_7$-malic acid system, potassium was found to be an easily ionized element which gave the highest signal enhancement factor. For a 30% signal enhancement in lead determination, 0.1 M K$^+$, which is 0.4% m/v K$^+$ was used. In this study of lead determination in FI, 0.1% m/v K$^+$ was found to increase the signal by 30% as shown in Figure 17.

Figure 17: Signal enhancement by KCl aspiration into spray chamber.
The best conditions for plumbane generation using the equipment in this study, after all these optimizations were done are shown in Table 6.

**Table 6**: Conditions for plumbane generation in FIS.

<table>
<thead>
<tr>
<th></th>
<th>NaBH₄</th>
<th>K₃Fe(CN)₆</th>
<th>HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate (ml/min.)</td>
<td>2.8</td>
<td>2.8</td>
<td>10.0</td>
</tr>
<tr>
<td>Concentration</td>
<td>1.5 % m/V</td>
<td>1.5 % m/V</td>
<td>0.1 M</td>
</tr>
<tr>
<td>Argon flow rate</td>
<td>1.3 L/min.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample loop</td>
<td>500 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transportation line (L2)</td>
<td>100 cm. 0.50 cm. id.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Aspiration of 0.1 % m/V K⁺ into the spray chamber
Determination of lead in the blank solution.

Due to a concern for lead contamination of the reagents, a determination of lead in the blank solution was done by continuous flow as shown in Table 7.

**Table 7**: Tests of lead determination in the blank solution. Three duplicate determinations were performed for each test.

<table>
<thead>
<tr>
<th>Test #</th>
<th>C</th>
<th>R1</th>
<th>R2</th>
<th>Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HCl</td>
<td>NaBH4</td>
<td>H2O</td>
<td>3.52±0.02 x 10^3</td>
</tr>
<tr>
<td>2</td>
<td>HCl</td>
<td>NaBH4</td>
<td>K₃Fe(CN)₆</td>
<td>4.05±0.04 x 10^3</td>
</tr>
<tr>
<td>3</td>
<td>2 ng. ml⁻¹ Pb</td>
<td>NaBH4</td>
<td>K₃Fe(CN)₆</td>
<td>6.03±0.02 x 10^3</td>
</tr>
</tbody>
</table>

Note: c = carrier line, R1 = reagent line 1, R2 = reagent line 2

Plumbane will be generated only in the presence of NaBH₄ and K₃Fe(CN)₆ in an acid medium. This fact suggested an investigation in continuous direct aspiration to determine lead in the blank solution. In test #1, distilled water was aspirated in line R2 instead of K₃Fe(CN)₆. Therefore, any lead that is present in the system would not be changed to plumbane to generate signal. This test #1 can be called “Ideal Blank” determination. Test #2, which is an aspiration used throughout this study of lead determination, can be called “Real Blank” determination. In test #3 a standard solution of 2 ng.ml⁻¹ Pb was aspirated in line C instead of the HCl carrier. This means the signal came from plumbane, which was generated from 2 ng.ml⁻¹ of lead in the system. Signal in this table is not a peak area as usual, but a value that indicates the baseline (which is the number on y-axis in Figure 11). A comparison of signal between each test in these three tests suggests that the blank solution of this method contains about 0.5 ng.ml⁻¹ of Pb.
Calibration curve

0, 2, 5, 10, 20 ng.ml\(^{-1}\) lead standard solutions were run in three replicates for each determination. The 15 measurements were run in random order to correct any error from drifting by the machine. The conditions in Table 7 were used to create the calibration curve in Figure 18.

**Figure 18:** Calibration curve of lead determination.

![Calibration curve diagram]

Slope \(= 2.27 \pm 0.05 \times 10^5\)

Series 1 = Linear regression line.
Series 2 = Raw data of three replications of each standard solution.
The method of calculation for linear regression of multimeasurement per point was obtained to use for this calibration curve.\textsuperscript{78} The linear regression equation obtained from these results is shown in equation 4.

\[ Y = 2.27 \pm 0.05 \times 10^5 X + 9.1 \pm 5.5 \times 10^4 \] \hspace{1cm} \text{equation 4}

The correlation coefficient was 0.9964. The detection limit of this method was determined as three times the standard deviation of the blank solution (3σb). A value to the blank solution was estimated by a substituting Y=0 in equation 4 and standard deviation of this blank value was calculated by an equation obtained from the same reference.\textsuperscript{78} The detection limit of this method was calculated to be 0.7 ng.ml\textsuperscript{-1}. The precision of the method was determined by running 20 ng.ml\textsuperscript{-1} Pb standard solution for 11 measurements and relative standard deviation of 1.6 % was achieved.
Determination of trace amount of lead in banded coral skeletons.

Banded coral skeletons were collected from 5 stations around Si-Chang Island in Thailand as shown in Figure 19.

Figure 19: A map of 5 stations around Si-Chang Island in Thailand, where samples were collected.
Sample preparation was performed as described in the analytical procedure and was followed by two steps.

1. Study of matrix interference.

The method of calibration curve and standard addition were used to determine lead in coral skeletons station 4 band 1. Solution of 1% m/v coral were found to contain 6.6±0.4 ng.ml⁻¹ of Pb by using the calibration curve in Figure 18. Thus, in the standard addition method, 6 and 12 ng.ml⁻¹ of Pb were added to 1% m/v coral solutions to obtain 3 calibration points, as shown in Figure 20. The amount of lead was found to be 6.4±0.5 ng.ml⁻¹ The slopes of these two methods and the amount of lead found by these two methods showed that a calibration curve can be used, rather than standard addition to achieve satisfactory results.

**Figure 20**: Standard addition curve of Pb determination in coral Station 4.

\[
\text{Slope} = 2.46 \pm 0.12 \times 10^5
\]
2. The amount of lead in coral skeletons from 5 stations.

Only one piece of coral skeleton was collected from each station. Each piece was scraped from outer band to inner band. Band color is repeated in white and yellow until the last band which is a small whole area of old coral. The yellow bands show yellow-green fluorescent bands under UV light.

Table 8: The amount of lead (ng.g⁻¹) in banded coral skeletons from 5 stations (n=3).

<table>
<thead>
<tr>
<th>Band</th>
<th>S-1</th>
<th>S-2</th>
<th>S-3</th>
<th>S-4</th>
<th>S-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>800±90</td>
<td>1200±184</td>
<td>1060±45</td>
<td>600±22</td>
<td>1900±137</td>
</tr>
<tr>
<td>2</td>
<td>1200±44</td>
<td>910±26</td>
<td>2050±80</td>
<td>1660±45</td>
<td>830±41</td>
</tr>
<tr>
<td>3</td>
<td>880±54</td>
<td>1100±168</td>
<td>1100±36</td>
<td>770±37</td>
<td>2180±62</td>
</tr>
<tr>
<td>4</td>
<td>830±18</td>
<td>1020±42</td>
<td>1010±61</td>
<td>1250±96</td>
<td>1200±169</td>
</tr>
<tr>
<td>5</td>
<td>3100±40</td>
<td>1200±132</td>
<td>1030±12</td>
<td>540±24</td>
<td>420±57</td>
</tr>
<tr>
<td>6</td>
<td>710±38</td>
<td>1400±110*</td>
<td>1450±23</td>
<td></td>
<td>980±84</td>
</tr>
<tr>
<td>7</td>
<td>1130±24</td>
<td></td>
<td>1400±156</td>
<td></td>
<td>1230±17*</td>
</tr>
<tr>
<td>8</td>
<td>800±34</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>960±33*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: *= old coral skeletons.

Band 1,3,5,7 = white color band.
Band 2,4,6,8 = yellow color band.
null
From Table 8, there is significant variation of the amount of lead between bands made a difficult to draw a conclusion. Most likely contaminated site (station 2 which has a gasoline station) did not show high levels of lead. There is also no consistancy of the amount of lead at similar age (same band) between stations. The amount of lead in corals is affected by currents and the currents in Thai gulf have a big variation, which is difficult to understand. The only conclusion can be made here is that coral skeletons from Si-Chang Island contains some lead at a level of 1 mg.kg\(^{-1}\). The result of lead determination in coral skeleton Station 4 band 1 in 3 areas of the same sample is shown in Table 9. The data were analyzed by one way ANOVA to determine whether there is any difference between these three sampling areas. The calculated value of F(2,6) is 5.22, which is greater than the critical F(2,6) = 5.14 at the 5\% level, thus there is significant difference between the three sampling areas in the same band. However, the differences within bands are less than the variation between bands. In order to know more about the difference between stations and between bands, more sampling must be performed.

**Table 9**: Determination of lead in coral station 4 band 1 in three sampling areas

<table>
<thead>
<tr>
<th>Area</th>
<th>Pb (ng.g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area 1</td>
<td>593 624 582</td>
</tr>
<tr>
<td>Area 2</td>
<td>523 481 602</td>
</tr>
<tr>
<td>Area 3</td>
<td>613 681 647</td>
</tr>
</tbody>
</table>

This method of lead hydride generation has less problem with matrix interference than does the graphite furnace method. The graphite furnace method suffers from matrix interference from calcium carbonate CaCO\(_3\) (the major chemical component of coral skeletons), which gave a non-reproducible signal and reduced the lifetime of graphite tubes.\(^{79}\) More work has to be done, to precipitate lead from the matrix and redissolve it to make a measurement. In anodic stripping voltammetry for lead determination in coral skeletons, electrodeposited time of lead on the indicator electrode is about 2-5 min per sample.\(^{80}\) It is a time consuming method compared to hydride generation.
<table>
<thead>
<tr>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>7</td>
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</tr>
<tr>
<td>9</td>
<td>10</td>
<td>11</td>
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<td>14</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>17</td>
<td>18</td>
<td>19</td>
<td>20</td>
</tr>
</tbody>
</table>

Note: The table above represents the schedule for the first month of the project.

*Table continued on next page.*
3. The mass spectrum of yellow organic material residue from dissolved corals.

A small amount of yellow insoluble material was isolated from the coral and submitted for mass spectrum analysis. The mass spectrum showed a peak at m/z 256, which corresponds to Sg. The remaining fragments could not be assigned to a particular compound. The mass spectrum is shown in Figure 21.

Figure 21: The mass spectrum of yellow organic material residue
Study of transition metal interference.

Transition metals have been reported to interfere in lead determination by hydride generation. For hydride forming elements, their different requirements for hydride formation cause less interference from each other in a determination. Thus, only interference effects of transition metal ions on the determination of lead were studied here, and the results are shown in Table 10.

**Table 10:** Interference of transition ions on Pb solution (20 ng.ml\(^{-1}\) concentration)

<table>
<thead>
<tr>
<th>Conc. (mg.ml(^{-1}))</th>
<th>Fe(II)</th>
<th>Fe(III)</th>
<th>Cd(II)</th>
<th>Ni(II)</th>
<th>Co(II)</th>
<th>Cu(II)</th>
<th>Zn(II)</th>
<th>Mn(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>89</td>
<td>92</td>
<td>101</td>
<td>102</td>
<td>99</td>
<td>52</td>
<td>100</td>
<td>92</td>
</tr>
<tr>
<td>10</td>
<td>82</td>
<td>85</td>
<td>99</td>
<td>101</td>
<td>99</td>
<td>-</td>
<td>109</td>
<td>92</td>
</tr>
</tbody>
</table>

Note: - = no determination

From Table 10 it can be seen that only Cu(II) ion interferes severely in lead determination of this study. Therefore, Cu(II) was varied in concentration to observe the effects of interferent concentrations. Three determinations were made at each level and the results are shown in Figure 22.
FUTURE WORK

It is interesting to learn that most methods of lead determination at the ng.ml⁻¹ level such as kinetic method⁷, potentiometric method by lead selective electrode⁸¹ and the hydride generation method of this work, Cu(II) was always found to be the most severe interferent. A requirement for determination of both elements in the same sample is sometimes needed, as shown in the report of a cooperative study of precision and accuracy on the determination of ng.ml⁻¹ levels of copper and lead in environment water from Taiwan.⁸² Thirteen laboratories around Taiwan participated in this project. Seven analytical methods with two preconcentration procedures were performed without hydride generation method. Therefore, a measurement of copper and lead in the same sample, using hydride generation for the lead determination is useful to study.
SHORT NOTICE

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CONCLUSION

The method described for the determination of lead by flow injection hydride generation direct current plasma atomic emission spectrometry, gave high sensitivity, high sample throughput and good reproducibility. Most transition metal ions do not interfere in lead determination by this method except copper (II) ion. Application of this method must take account of the amount of copper(II) ion in the sample. Masking reagent may solve the problem of interference. In another way, the two merging points of reagents in FIA manifold might be combined to one point making the two reagents react with lead at the same time. Copper(II) ion will then have less reaction time to interfere in this suggested manifold or a new construction of sample introduction for hydride generation, which has a very small volume (0.7 ml) of nebulizer, might be tried.83

The application of this method to determine lead in banded coral skeletons compared well with two methods: anodic stripping voltammetry80 and graphite furnace atomic absorption spectrometry79 that have been reported in literature. The banded coral skeletons from Si-Chang Island in Thailand contain lead about 1 mg.kg⁻¹. These coral skeletons were collected in December 1996.
REFERENCES


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