DIFFERENTIAL PULSE POLAROGRAPHIC DETERMINATION OF COPPER, CADMIUM, LEAD, NICKEL AND ZINC IN DRIED FISH TISSUE

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ABSTRACT

DIFFERENTIAL PULSE POLAROGRAPHIC DETERMINATION OF COPPER, CADMIUM, LEAD, NICKEL AND ZINC IN DRIED FISH TISSUE

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The objective of this project is to investigate the possibility of using the differential pulse polarographic technique in the analysis of fish tissue to determine quantitatively the trace metals, copper, lead, cadmium, zinc and nickel. The experimental procedure comprised of reducing the whole fish to a homogeneous mass, subjecting it to a dry ashing technique and taking up the resultant ash with acid. The fish tissue samples were analyzed by appropriate atomic absorption methodologies to establish the values for the levels of the analytes of importance. A reliable spiking technique was developed to permit elemental determination reasonably free from matrix perturbations. The level of each trace metal in the homogeneous fish samples were determined by dpp/spiking methodologies. The results obtained were compared with those from the atomic absorption approach. Where copper, zinc and nickel are concerned, the values from both approaches compared well. This was not the case with cadmium and lead, owing to the fact that the levels of the respective metals in the fish tissue are such that they approach the limits of detection by the dpp technique.

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INTRODUCTION

1.1 Purpose of Trace Element Determination in Fish

Published reports on chemical surveys of natural waters show aquatic environments are subject to trace heavy metal contamination [1-8]. Contaminant concentrations in natural waters vary in relation to substrate, the physical/chemical properties of the water and biological productivity. Generally, high concentrations of potentially toxic elements occur very close to sources of contamination. But tests conducted on other segments of the aquatic systems, quite removed from the source of contamination, reveal accumulation of trace elements. Hence plants and animals even at distances removed from sources of contamination can accumulate high levels of non-essential trace elements.

Information regarding concentration levels of trace metals in aquatic ecosystems is crucial for water quality monitoring and assigning toxicological standards. Several environmental scientists have worked in this field and have reported on trace metal contamination in various aquatic systems and their flora and fauna [9,10,11]. Determination of concentrations of metals in drinking water and fish and its relationship to water quality standards and/or incidence of mortality or disease have also been discussed by several authors [12,13,14]. The International Joint Commission, Great Lakes Research Advisory Board has published proceedings of two recent symposia on metal speciation, toxicity to aquatic organisms and water quality criteria for protecting aquatic life [15,16].

Plants may be used as biological indicators of trace element contamination in aquatic and terrestrial ecosystems. Rooted aquatic plants largely accumulate Cd, Ni and Hg [17]. Lead was found on the leaf surface of soybeans [18]. In marine environments algae are the principal accumulators of trace metals such as Cd, Cu, Pb and Zn [19]. The effects of these trace metals on aquatic flora have been studied by several authors. Sublethal amounts of lead retarded population growth in the case of the marine green algae by delaying cell division [20]. Various intracellular abnormalities also resulted from lead exposure. The presence of cupric ion was found to inhibit respiration and photosynthesis in some unicellular organisms [21]. Experiments on laboratory cultures of green algae show that 0.7 mg/ℓ Zn, 0.3 mg/ℓ Cu and 0.6 mg/ℓ Cd have adverse effects. These are a few examples of how trace metals influence aquatic vegetation.

General surveys of trace element concentrations in aquatic animal tissue have been conducted all over the world both in fresh water and marine environments [22,23,24,25,26]. Several authors have in recent years, determined growth rates and sensitivities of various life stages of fishes exposed to heavy metals so as to estimate maximum acceptable toxicant concentrations (MATC) [27,28,29]. A variety of approaches have been taken to examine sublethal or chronic effects of pollutants. These range from reproductive success to life span, including factors such as growth, adaptation to environment, feeding habits, respiration and effects on vital organs. Davies et al [27] found the rainbow trout to be particularly sensitive to lead when exposure began during development. The MATC for copper in a surface water of variable quality

based on survival, growth and reproduction of certain species of fish has been found to be 0.066 to 0.118 mg/ℓ Cu [28]. Several recent studies on toxicity of zinc on rainbow trout show that, juveniles are the most sensitive life stage [30]. A nickel concentration of 0.73 mg/ℓ caused a significant reduction of fecundity and hatchability of eggs [31]. There is evidence that cadmium can affect testicular steroidogenesis in the trout inhibiting biosynthesis of a ketotestosterone [32].

One of the general objectives of studies on trace elements in fish is to provide baseline values against which future levels of pollution may be measured. It permits exchange of information between industry, responsible for possible contamination of aquatic systems and the scientific community. Assessment of trace metal concentrations in drinking water are important for water quality monitoring and control. Baseline data and MATC values of toxicants help to determine biological effects of various hazardous metals on marine organisms. J. Valentine [33] assessed the relationships between concentrations of trace elements in water and mortality reates from arterio sclerotic heart disease and other causes. Food and population density were shown to be primary sources of many trace metals, than the water itself. Hence it is essential that various trace metal analyses be carried out for two principal causes.

(i) to determine maximum acceptable toxicant concentrations with respect to fish in any aquatic ecosystem,

(ii) to determine whether the fish are fit for human consumption.

1.2 Survey of Methods For Trace Metal Analysis in Fish

Various methods have been used to enable the determination of trace elements in fish tissue with reasonable accuracy. A new worker in the field of trace analysis today is confronted with a wide choice of methodology. The following are a few of the available methods.

1.2.1 Neutron activation analysis (NAA)

Since the earliest work of G. Hevesy and H. Levi [34], NAA has become one of the important methods for trace element analysis. It is an extremely sensitive method, being applied in medical and biological fields, air and water pollution control and in nutrition [35].

In NAA a standard containing an accurately known quantity of the element of interest is irradiated simultaneously with the sample. Provided identical conditions of irradiation are met for sample and standard, the ratio of the induced activity is used to obtain the unknown concentration of the element in the sample.

A large number of elements may be determined by NAA in the sub-microgram range. In many cases no preconcentration is necessary and there are no errors induced by reagents or contaminants. There are however, some problems associated with the method. Elements with long half-lives require very long activation periods during which highly radio-active samples may be produced from matrix elements. This requires several weeks of cooling to destroy the undesired product. Also, NAA is expensive to apply compared to other techniques.

1.2.2 X-ray fluorescence spectroscopy

This method is based on the principle that the intensity of the

characteristic x-radiation of a given wavelength is a function of the number of atoms of the corresponding element present in the portion of the sample that is emitting fluorescent radiation. The observed intensity of an analytical line of an element can be related to the weight of the element present in the sample. Attempts by several initial workers to obtain quantitative results by x-ray methods met with difficulties because of the direct exposure of the untreated sample to the excitation source [36,37]. Although this is a reasonably sensitive method, efficiencies of various excitations, absorption, scattering and diffraction phenomena are not known accurately enough to permit direct computation of concentration from intensity data. However, advances in the development of high intensity sealed x-ray tubes and other instrumentation have made the method adoptable for all elements heavier than atomic number 11.

1.2.3 Emission spectroscopy

The interactions of thermal and electrical radiation with an element, results in absorption by electronic orbital transitions and consequent excitation. The excited atoms return to the ground state emitting a pattern of electromagnetic radiation. This is the basis of emission spectroscopy. Emission spectroscopy, is an excellent tool for rapid survey analysis, and provides high sensitivity for most elements in the microgram range. The primary advantage of this method is that a large number of elements may be detected during a single exposure. Conventional dc or ac arc, and ion spark excitation, gas discharge sources, excitation in the vacuum ultraviolet region and laser microprobe excitation provide measurement of most elements in the periodic table.

1.2.4 Atomic absorption spectrophotomtry (AAS)

AAS is the study of the absorption of radiant energy by atoms. This absorption and its quantitative correlation with the concentration of the appropriate ions originally present in a sample solution forms the basis of analytical AAS. This method has been used more widely than any other method for trace analysis owing to its specificity, sensitivity, accuracy and the possibility of its application to most metallic elements [38,39]. Some difficulties may be encountered in atomizing refractory metals, however, some non-metals like sulphur may be determined by direct AA in the vacuum ultraviolet region [38]. Phosphorus may be determined by absorption of a meta stable line [39]. Still other like halides and specific ions like the cyanide ion may be determined by indirect procedures [40].

In general, flameless AAS techniques are more sensitive than flame techniques. Yet the technique of AAS as a whole is highly specific, extremely sensitive, relatively simple and not too expensive making it one of the best methods available for trace anlaysis.

1.2.5 Other methods

A multitude of methods other than those outlined above are available. Some like radioisotope dilution, stable isotope dilution, chromatography and spark source mass spectrometry are in fact sensitive enough even for ultra trace analysis. Yet they do not find wide application in the determination of trace metals in biological tissue due to the fact that they are time consuming. While working with biological samples time is an important factor since many samples deteriorate in less than 24

hours. Also these techniques require quite expensive instrumentation. Hence no single analytical technique can be labeled as 'the' most capable method. Though each method will meet some of the criteria for an ideal method, no technique has all the capabilities [41].

1.3 The Polarographic Method

1.3.1 General

Polarography is one of the older methods which is now rapidly advancing in the field of trace metal analysis.

Voltammetry is the study of the entire course of the applied potential vs current curve as this exists for an electode of very small area (a microelectrode) and a reference electrode of relatively large area. Polarography is that branch of voltammetry involving a dropping mercury electrode as the microelectrode. Recent advances in electronics have permitted the designing of extremely versatile multi-functional polarographic instrumentation. Polarographic techniques for trace analysis are now being applied in the field of pollution with particular reference to water analysis [42], pharmaceuticals [43] rocks and minerals [44,45] and pesticides [46].

Sensitivities or detection limits obtainable by polarographic techniques are often comparable to and, in certain instances, better than those obtained by atomic absorption techniques. For example, the detection limit for copper by flameless AA is about 10^{-8} M which is identical to the value of 10^{-8} M obtainable by differential pulse polargraphy. Anodic stripping voltammetry offers a detection limit of approximately 10^{-10} M. An important additional feature of polargraphy

is that this method is sensitive to the respective ionic species of the trace metals, and not to the total element content as in various other non-electro chemical methods such as AA, NAA, and Emission Spectroscopy. Polargraphy and voltammetry offer exceptional potentialities for the determination, specification and physiochemical characterization of many trace metals in aquatic systems since normal levels of most metal ions dissolved in sea water and animal tissue are very low [48].

1.3.2 Types of polarographic and voltammetric techniques applied to trace analysis

A considerable number of new voltammetric techniques have developed in the past two decades to supplement the conventional form of dc polargraphy which has been in existence for almost 50 years [49-52]. Rapid current dc polarography, Tast (current sampled) dc and derivative Tast dc polarography, normal pulse polarography, differential pulse polarography and ac polarography are valuable recent technqiues. Inverse or anodic stripping polarography has been cited as a very important method for very low concentration trace metal determination.

Polarographic techniques are most applicable in the field of marine and aquatic chemistry because sea water is a particularly favourable medium for the method. This is due to the fact that the presence of excess salt components in the water eliminates the need for an additional supporting electrolyte in many cases.

Several workers have applied the techniques of anodic stripping voltammetry (ASV) to the analysis of natural waters. Cu, Cd and Zn have been determined by Ariel and Eisner using the hanging mercury drop electrode [53]. Matson et al in 1965 developed a composite mercury

graphite electrode to determine Pb content of various natural waters [54]. Single electrode anodic stripping voltammetry has been used to determine Zn, Pb, Gd and Cu [55]. The sensitivity of conventional stripping techniques in trace analysis has been enhanced by differential anodic stripping voltammetry [56]. The technique has been extensively used in determination of Pb, Cd, Cu and Zn in biological materials [57,58]. Cd and Pb in canned beverages and foods have been determined by manual dc polarography [59]. Tapwater, natural and various waste waters have been analysed by differential pulse stripping voltammetry [60,61]. Because of its ability to determine the very low amounts of elements as may be present in the human body or in drugs, differential pulse polarography finds vast applications in the field of medicine.

1.4 Purpose of Experimental Work and Investigation

The purpose of this experimental work is to investigate the possibility of using differential pulse polarography to determine trace metals in fish tissue. This includes the methodology of preparing the tissue, supporting electrolyte and standard solutions prior to polarographic analysis.

The investigation will cover the determination of the trace elements Cu, Pb, Cd, Zn and Ni. These elements were selected as typical of the trace metals presently under investigation as to single and multiple toxicity to various fish species. Generally, the determination of trace metals in fish tissue is carried out by flame or flameless atomic absorption spectro photometry. The purpose of the application of differential

pulse polarography in this study is to devise a satisfactory technique, other than this methodology, for application to fish tissue. Such a satisfactory technique of analysis would provide an alternative where atomic absorption equipment is not available, and an auxillary and supporting technique where it is available.

CHAPTER 2

THEORY AND INSTRUMENTATION

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THEORY AND INSTRUMENTATION

2.1 Introduction

Basically the purpose of the experimental work, as outlined in the INTRODUCTION, is to explore the possibility of using the differential pulse polarographic technique in the analysis of fish tissue to determine quantitativety the trace metals copper, lead, cadmium, zinc and nickel. In order to carry out this objective the following general approach was taken.

(a) Fish had to be obtained with a known background of no exposure to experimentally induced high levels of the trace metal analytes of importance.

(b) The whole fish had to be reduced to a homogeneous mass for sampling purposes.

(c) A reliable dry ashing technique, with acid take-up of the resultant ash, had to be found and applied.

(d) The fish tissue sample had to be analyzed, by appropriately reliable atomic absorption methodologies, to establish values for the levels of the analytes of importance. These determinations were required to provide a comparison basis for the results obtained subsequently from the dpp technique.

(e) Supporting electrolyte (s) capable of permitting the polarographic determination of the analytes had to be reviewed and the most suitable chosen.

(f) The limits of detection for the dpp technique had to be explored for each analyte.

(g) A reliable spiking technique in the treatment of the fish tissue samples had to be developed in order to permit elemental determination reasonably free from possible matrix perturbations.

(h) Finally, the level of each trace metal in homogeneous whole fish samples had to be determined by dpp/spiking methodologies and the obtained results compared with those from the atomic absorption technique.

2.2 Voltammetric and Polarographic Principles

2.2.1 General

Much of the following derives from J.G. Dick [62], L. Meites [63] and J.B. Flato [64]. Voltammetry is the study of the diffusion current versus applied potential curve as this exists for a working electrode of very small area (microelectrode) and a reference electrode of relatively large area. When a mercury drop working electrode (dme) is used as the microelectrode, the technique is generally classed as polarography.

The area of the microelectrode used in these applications is relatively small, resulting in an extremely low current flow through the solution (in the microampere range). Because of the very low current value, the voltage drop through the solution is almost negligible and the applied potential will be virtually equal to the potential between the microelectrode and the reference electrode. With increasing applied potential between the microelectrode and the reference electrode, the

following situations are possible.

(i) When the applied potential is less negative than the potential value (E) for the galvanic cell, the galvanic cell half reactions:-

$$R - ne \rightleftharpoons O \qquad \text{microelectrode, negative}$$

$$Hg^{+} + 1e \rightleftharpoons Hg \qquad \text{SCE reference, positive}$$

will take place. O and R are the oxidized and reduced states for the ion species providing the negative electrode reaction, and Hg^{+} and Hg are the oxidized and reduced states for the positive electrode reaction. In this case current in the external circuit flows from the microelectrode to the reference electrode.

(ii) When the applied potential is exactly equal to the value of potential (E) for the galvanic cell, an almost zero current will flow. Potentiometric measurements are made under this condition in the normal current-voltage relationship. The value of the applied potential at this point, in conjunction with the known reference electrode potential, will yield the microelectrode potential. Through the Nernst relationship:-

$$E_{ind.} = E^{o}_{f',O/R} + \frac{0.059}{n} \log \frac{[O]}{[R]} \quad (25°C)$$

the value of $\frac{[O]}{[R]}$ may be obtained.

(iii) When applied potential is more negative than E, the cell reactions,

$$O + ne \rightleftharpoons R \qquad \text{microelectrode, cathode}$$

$Hg - 1e \rightleftharpoons Hg^{+}$ SCE reference, anode

will take place.

Whenever galvanic or electrolysis cell reactions occur the current flow through the solution will generally be porportional to the applied potential at the cathode, providing that the ion species O and R can be immediately brought to and removed from the electrode surface. When this is not the case, the current flow will largely depend on the transport situation for these ions. The ability to move the ion species O, to the microelectrode depends on three factors.

(a) the migration effect as related to the electrostatic attraction of the cathode for the species O.

(b) the convection effects related to the efficiency of solution mixing or stirring.

(c) the diffusion effect related to the rate at which O diffuses to the electrode surface on the basis of the difference in concentration of O in the bulk of the solution and at the electrode surface.

These effects yield respectively the migration current, convection current and diffusion current contributions to the total current. In polarographic work the rate of arrival of O at the electrode surface should depend basically on the diffusion current. Hence the convection effect and migration effect should be minimized. The convection effect is minimized by keeping the electrodes static, and by avoiding stirring the solution. The migration effect is minimized by providing in the solution, for cathodic microelectrode reduction processes, a high concentration of a positively charged ion. This ion should be attracted

to the cathode but, in the solution medium involved, should not be discharged or reduced. The concentration of such a substance, called the supporting electrolyte, must be relatively high compared to the concentration of O in the bulk of the solution.

Once the convection and migration current contributions have been minimized, O will reach the electrode surface almost exclusively on the basis of the diffusion and the current will then be dependent on this diffusion process. For the cathodic microelectrode reaction $O + ne \rightleftharpoons R$, we define [O] in the bulk of the solution as C_O and [O] at the microelectrode surface as C_O (x=o), where x is the distance into the solution from the microelectrode surface.

2.2.2 The plane-surface microelectrode

When the applied potential at a plane-surface cathode attains a slightly more negative value than the equilibrium potential for the original solution the reaction $O + ne \rightleftharpoons R$, will take place with the value of C_O (x=o) becoming less than C_O. The current 'i' through the solution will be controlled by the rate of diffurion of O to the electrode surface.

$$\text{Rate of diffusion of O} = D_O \frac{\Delta C_O}{\Delta x} \qquad (1)$$

where:-

Rate of diffusion of O = moles of O diffusing to the electrode surface per cm^2 per sec.

D_O = diffusion coefficient, cm^2/sec.

ΔC_O = $C_O - C_O$ (x=o), mol/cm^3.

Δx = distance from electrode surface to point x in solution where [O] equals C_O, Cm

The value of D_o depends upon the nature of the reaction $O + ne \rightleftharpoons R$. As the cathodic potential becomes more negative, the value of C_o $(x=o)$ decreases further, and with an applied potential value sufficiently negative to allow immediate reduction of O upon arrival at the electrode surface $C_o(x=o)$ becomes much less than C_o and approaches zero. Under these conditions, the value of $\Delta C_o = C_o - C_o(x=o)$ becomes entirely dependent on C_o. The value of Δx will increase as the electrolysis time increases since the front of the depleted [O] zone will move further into the solution with time. Consequently the current through the solution will be the diffusion current i_d. The number of moles of O arriving at the electrode surface per cm^2 per second is given by N in the expression

$$N = C_o \sqrt{\frac{D_o}{\pi t}} \qquad (2)$$

where:- C_o is in moles per cm^3, D_o is in cm^2 per sec, π has the conventional value and t is in seconds, representing the time from the start of the electrolysis. The current i_d is given by

$$i_d = nFAN \qquad (3)$$

$$= nFA\, C_o \sqrt{\frac{D_o}{\pi t}} \qquad (4)$$

where:- A = the surface area in square centimeters

F = the faraday at 96,490 C/equiv.

When C_o is expressed as millimoles/litre, i_d will be given in microamperes. Equation (4) is time dependent and it is not possible to guarantee that i_d measurements will be made at the same value of t for all systems. Hence a plane surface microelectrode is not immediately

adaptable to quantitative analytical purposes where i_d should be directly proportional to C_o.

2.2.3 The dropping mercury electrode (DME)

The time dependency of i_d may be significantly reduced when a dropping mercury electrode (dme) is used as a microelectrode. The dme consists essentially of a pure mercury reservoir connected to a glass capillary tube. The tube is fixed vertically in the solution and the head of mercury (the distance from the level in the reservoir to the capillary tip) is such as to yield a drop life of 2.5 to 6 secs. The drop size at the moment of dislodgement is generally about 0.5 mm in radius.

As opposed to the plane-surface electrode the dme is spherical with the surface area of the dme increasing during drop life and being given by:-

$$A = 4 \left(\frac{3}{4\pi d}\right)^{2/3} m^{2/3} t^{2/3} \qquad (5)$$

where:-

A = drop surface area cm^2 in time t

d = density of mercury g/cm^3

m = rate of flow of mercury into drop g/sec

t = time during drop life, sec.

In addition, in the case of the dme, the surface of the drop moves into the solution during drop life. This effect can be compensated for by multiplying the rate of diffusion by a factor of $\sqrt{\frac{7}{3}}$

Substituting the value for A in equation (4) and multiplying by $\sqrt{\frac{7}{3}}$,

$$i_d = nF\ 4\pi \left(\frac{3}{4\pi d}\right)^{2/3} m^{2/3}\ t^{2/3} \sqrt{\frac{7}{3}}\ \sqrt{\frac{D_o}{\pi t}}\ C_o \quad (25°C)$$

$$= 708\ n m^{2/3}\ t^{1/6}\ D_o^{1/2}\ C_o \qquad (6)$$

The above expression relating i_d and C_o is known as the Ilkovic equation [65] where i_d is in microamps, C_o in millimoles per litre, m is in milligrams per sec. The value 708 holds good at 25°C and includes the density of mercury as 13.53 g/cm^3.

For a single drop the diffusion current versus time curve is as shown in Fig. (1).

It can be seen that the diffusion current grows rapidly during the early stages of drop life, eventually reaching a plateau value. The plateau section of the current represents the i_d value based on C_o. Repetitive drops provide identical i_d values at drop dislodgement, and the current versus time curve is as shown in Fig. (2). The area under the i_d vs t curve to the value of $t = \tau$ yields the quantity of electricity consumed in the reduction of O to R during this time interval and quantity of electricity consumed, Q is

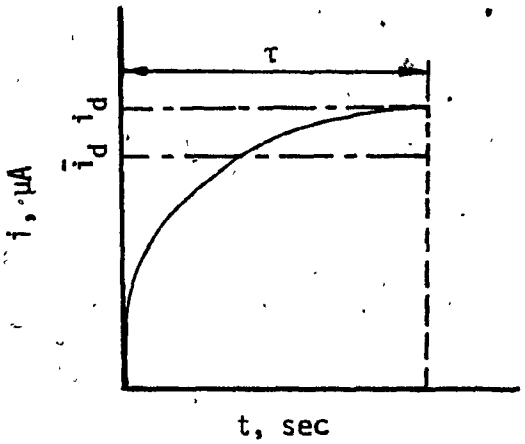
$$Q = \int_0^\tau i_d\ dt = \int_0^\tau 708\ n\ m^{2/3}\ t^{1/6}\ D_o^{1/2}\ C_o\ dt$$

$$= 607\ n\ m^{2/3}\ D_o^{1/2}\ C_o\ \tau^{7/6} \quad (25°C) \qquad (7)$$

If time average diffusion current over the entire drop life is defined as $\bar{i}_d$, then:-

$$Q = \bar{i}_d\ \tau$$

and, $$\bar{i}_d = 607\ n\ m^{2/3}\ D_o^{1/2}\ C_o\ \tau^{1/6} \quad (25°C) \qquad (8)$$



$$\bar{i}_d \tau = \int_0^\tau i \, dt$$

Figure 1: Single Mercury Drop:Current vs. Time

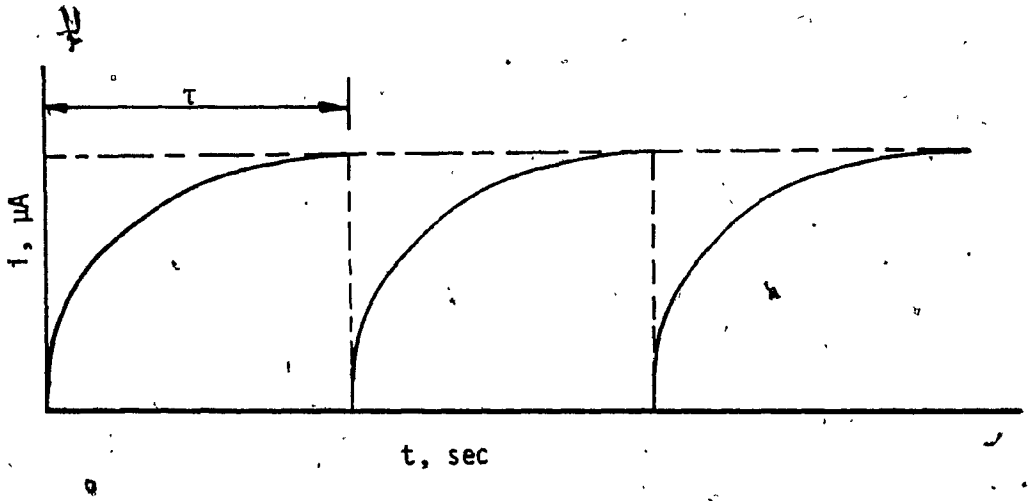


Figure 2: Repetitive Mercury Drops:
Current vs. Time

2.2.4 The polarographic wave

The above equation is based on the assumption that C_o (x=o) is negligible (practically = o) before drop dislodgement. In the event C_o (x=o) is not equal to zero the Ilkovic equation becomes,

$$\bar{i} = 607\, n\, m^{2/3}\, D_o^{1/2}\, \tau^{1/6} \left\{C_o - C_o\,(x=o)\right\} \quad (25°C) \qquad (9)$$

or $$\bar{i} = \bar{i}_d - 607\, n\, m^{2/3}\, D_o^{1/2}\, \tau^{1/6}\, C_o\,(x=o) \qquad (10)$$

Considering the reaction $O + ne \rightleftharpoons R$ if we define C_R as [R] at electrode surface,

$$C_o - C_o\,(x=o) = C_R\,(x=o)$$

Therefore,

$$\bar{i} = 607\, n\, m^{2/3}\, D_R^{1/2}\, \tau^{1/6}\, C_R\,(x=o) \qquad (11)$$

From equation (10) and (11)

$$\frac{C_o}{C_R}\,(x=o) = \frac{D_R^{1/2}}{D_o^{1/2}} \frac{\bar{i}_d - \bar{i}}{\bar{i}} \qquad (12)$$

Applying the Nernst relationship,

$$E_{O/R} = E^\circ_{O/R} + \frac{0.059}{n} \log \frac{D_R^{1/2}}{D_o^{1/2}} \frac{\bar{i}_d - \bar{i}}{\bar{i}}$$

$$= E_{1/2\,O/R} + \frac{0.059}{n} \log \frac{\bar{i}_d - \bar{i}}{\bar{i}} \quad (25°C) \qquad (13)$$

where $$E_{1/2\,O/R} = E^\circ_{O/R} + \frac{0.059}{n} \log \frac{D_R^{1/2}}{D_o^{1/2}}$$

A polarographic curve is obtained by plotting $\bar{i}$ vs $E_{O/R}$ and a typical plot is shown in Fig. (3).

2.2.5 Residual current

In the absence of any species O, the current through the solution with increasingly negative applied potentials should be zero, until hydrogen ion is discharged. But frequently this is not the case. A residual current i_r exists in the absence of the metal ion. This originates from two sources.

(i) the faradian current i_f involving the reduction of traces of impurities, including dissolved oxygen in the solution.

(ii) the condenser current i_c involving a double layer formed at the dme surface-solution interface, as a results of electrostatic interaction and adsorption effects. If the mercury drop is positive with respect to the solution anions are attracted and cations are repelled and vice versa. The interface is comparable to a capacitor of large capacity. The capacity of a capacitor with two parallel plates is proportional to the area of the plates. Since the area of the mercury drop increases during its growth the capacity of the double layer also increases continuously during drop life. The capacity c, is given by,

$$c = c' \, 4\pi m^{2/3} \left(\frac{3}{4\pi d}\right)^{2/3} t^{2/3} \qquad (14)$$

$$= p \, c' \, t^{2/3} \qquad (15)$$

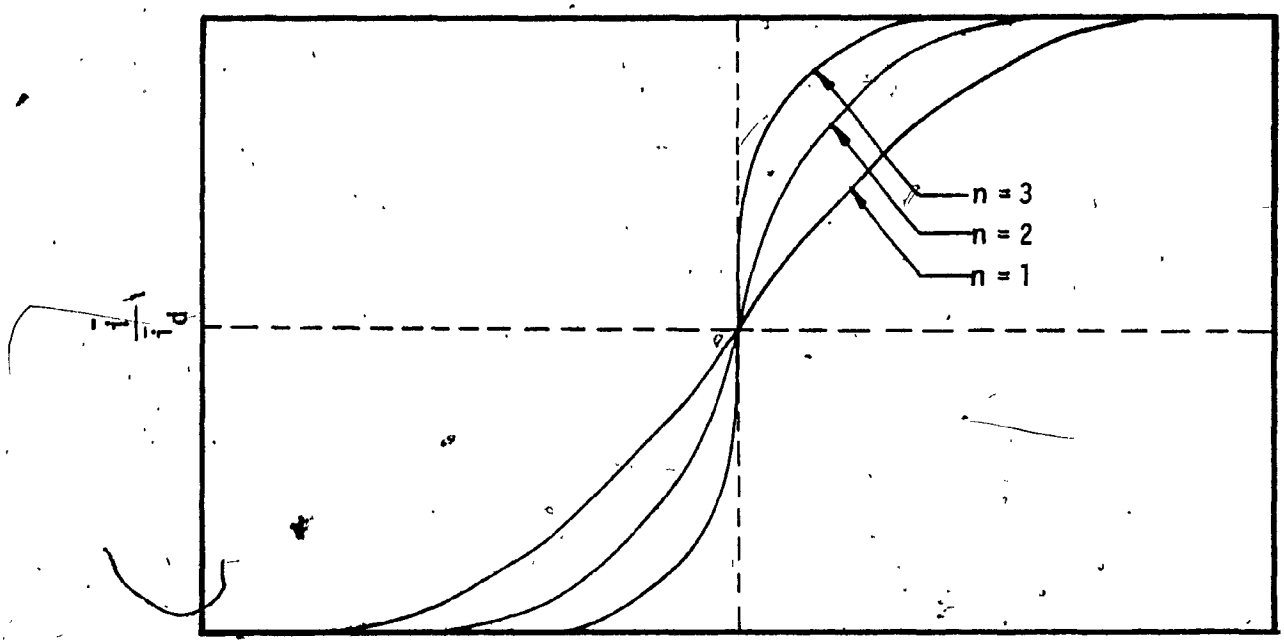


Figure 3: The Polarographic Curve

where:

c' = capacity for an electrode of area 1 cm^2

$p = 4\pi m^{2/3} \left(\frac{3}{4\pi d}\right)^{2/3}$

The capacity c must be kept charged at the difference of potential between electrode and solution and by definition of a capacity the following relationship is possible,

$$q = c\ (E - E_m) \qquad (16)$$

where:

q = quantity of electricity involved in the charge of the capacitor of capacity c

E_m = potential at which q = o

E = potential at which q is expressed.

Potentials E_m and E are measured with respect to some reference electrode (eg. SCE).

The electrode has a positive charge when $E > E_m$. Differentiation of equation (16) with respect to time results in the capacity current i_c.

$$i_c = \frac{dq}{dt} = \frac{dc}{dt}\ (E - E_m) + c\,\frac{dE}{dt} \qquad (17)$$

Since the potential of the dme is essentially constant during drop life, the term $\frac{dE}{dt}$ in equation (17) can be dropped. Combining the resulting equation with equation (15), the instantaneous capacity current is given by,

$$i_c = \frac{2pc'}{3t^{1/3}}\ (E - E_m) \qquad (18)$$

The average capacity current during drop life will be,

$$\bar{i}_{c\,\tau} = \int_0^{\tau} i_c \, dt$$

$$= \frac{pc' (E - E_m)}{\tau^{1/3}} \qquad (19)$$

In the above equation $\bar{i}_c$ is expressed in amperes, c' is in farads/cm^2, $(E-E_m)$ in volts and τ is in seconds. p may be calculated by expressing m in g/sec and d in g/cm^3.

Equation (19) shows that current varies linearly with the potential E and capacity c' C_o also varies with E and hence dependence of i_c on potential is quite complex. Capacity current is equal to zero when $E = E_m$.

Experimental currents measured with the supporting electrolyte alone are often larger than the value predicted in equation (19), because of the faradian current i_f. Thus the residual current is equal to the sum of the capacity current and the faradian current.

The order of magnitude of the average capacity current can be judged from the following example:

if c' = 20 μF cm

m = 10^{-3} g/sec

τ = 4 sec

$E-E_m$ = 1 volt,

i_c has a value of 0.054 μA.

The average diffusion current calculated from equation (8) for the same value of m and τ is,

$$\bar{i}_d = 2.40 x C_o$$

where, C_o is [O] in the bulk of the solution. If C_o is 10^{-5} moles/litre the average diffusion current

$$\bar{i}_d = 2.40 \times 10^{-5} A$$
$$= 0.024 \mu A$$

This value is much smaller than the value 0.054 μA, calculated for capacity current. The resulting wave is so distorted that precise determinations of i_d is virtually impossible. Hence conventional d.c. polarography cannot be easily applied to determine concentrations below 10^{-5} M [66].

2.2.6 Differential pulse polarography (DPP)

The differential pulse polarographic technique originally developed as an off shoot of square wave polarography, consists of superimposing a fixed height potential pulse at a regular interval on a slowly stepped potential as associated with dc polarography. The pulse is repeated for each drop and is synchronized with the period of maximum growth of the mercury drop. Figure (4) indicates the general situation.

The dpp instrumentation then samples the current flowing into the working electrode twice during each operating interval by use of electronic switching and crystal timing. The first current sample taken just before the application of the potential pulse would be equivalent to that obtained in the normal d.c. polarography. Immediately after the sample taking process, a sudden pulse of potential, usually between 5 and 100 mv is applied to the electrode. The application of this sudden change in potential produces a concurrent change in the current flow.

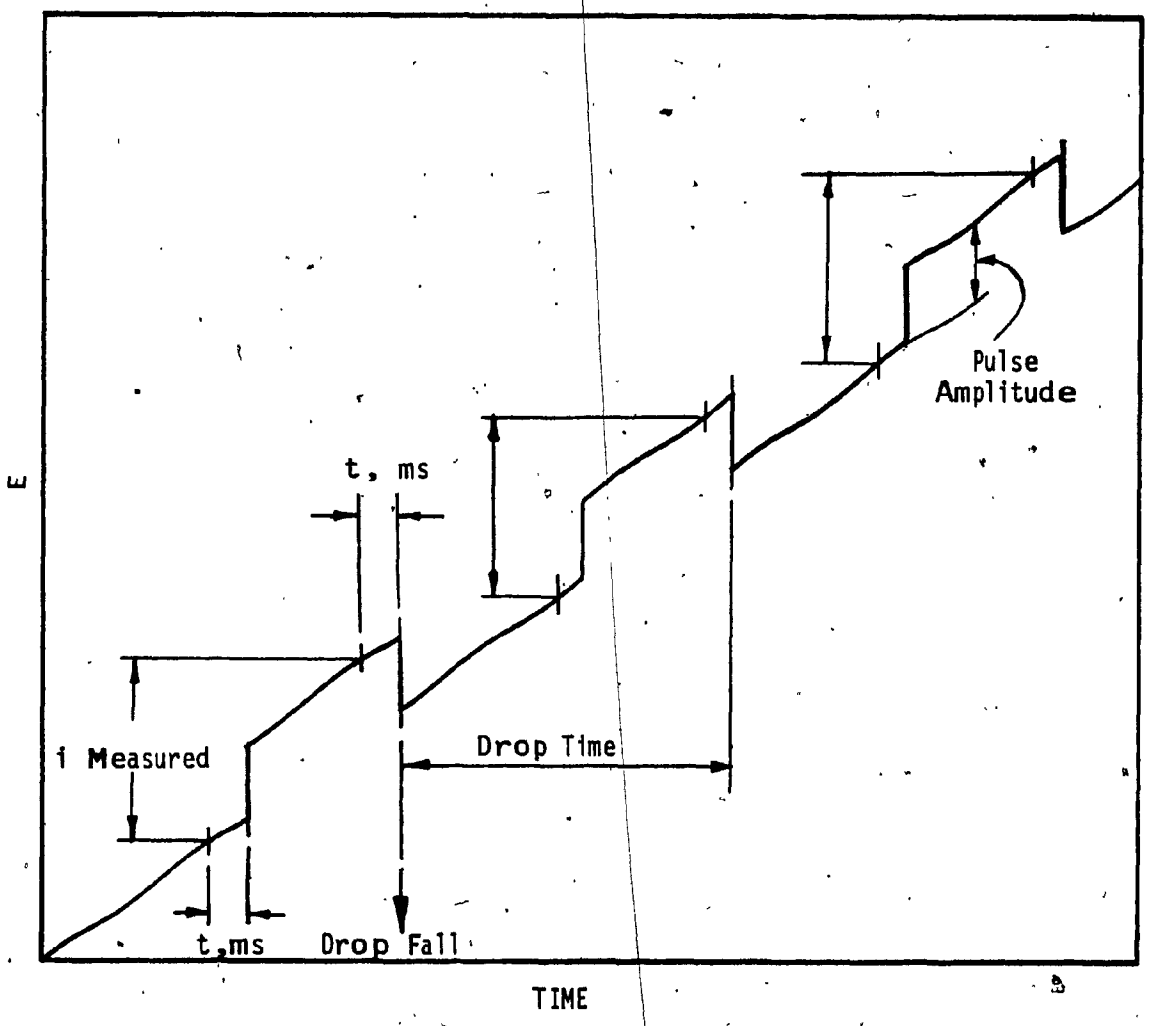


Figure 4: Potential Excitation Waveform used in Differential Pulse Polarography and Pulsed Stripping Voltammetry.

First, additional current must flow to change the double layer capacitance of the electrode to the new applied potential. Simultaneously an additional current may flow if the applied potential has changed to a potential where the equilibrium between O and R is shifted.

The pulse potential is maintained for a period of time long enough to allow the capacitance current to decay to a low value. During this time the faradaic current also decays somewhat but still does not reach the diffusion-controlled level. At the end of this period, a current sample is taken again. The difference between these two current samples (developed by applying the two signals stored in the memories to a differential amplifier) is then amplified and presented to the output of the system. This difference current-curve, which is proportional to the concentration of O, has the appearance of a peak rather than the usual polarographic step.

The dpp technique has many advantages over the conventional dc method. The most significant is that the influence of the capacitance current is minimized by the pulsing and sampling process, providing for peak relationships which improve resolution and for a considerable lowering of the detection limit for the concentration of O.

2.2.7 Pulsed and direct stripping voltammetry

Pulsed and direct anodic stripping voltammetry are techniques which are applicable exclusively in analysis of metals. They are based on the premise that significant increases in signal strength can be obtained by concentrating the metal of interest into a microelectrode before obtaining a reversed current relationship.

In the basic dc anodic stripping process, a suitable static

mercury microelectrode is initially maintained at a specific known potential, more negative than the reduction potential of the metal of interest and for a known length of time. The metal deposits on the electrode at a rate governed by its concentration and its rate of arrival at the electrode surface. It then forms an amalgam with the mercury. At the end of a known period of time, a specific amount of the metal has been deposited at the electrode surface. At the end of this process, a potential increasing in the positive direction is applied to the electrode, and the current obtained is measured as a function of potential so that a peak wave form is obtained, see Fig. (5).

Differential pulse anodic stripping voltammetry differs from dc anodic stripping voltammetry only in that the oxidation process is studied through the use of the same pulse-modulated ramps discussed under dpp. The deposition process is carried out in exactly the same way. Significantly lower detection limits can be obtained because of the concentration effect in the mercury microelectrode and the greater signal processing capabilities of the pulse modulation technique. Where dpp application is used, this allows shorter deposition times thus minimizing electrode instabilities.

2.3 Instrumentation

2.3.1 General

Throughout the experimental work specific atomic absorption spectrometric equipment and voltammetric/polarographic equipment was used. Following is a general description of the units involved, together with relevant operating parameters.

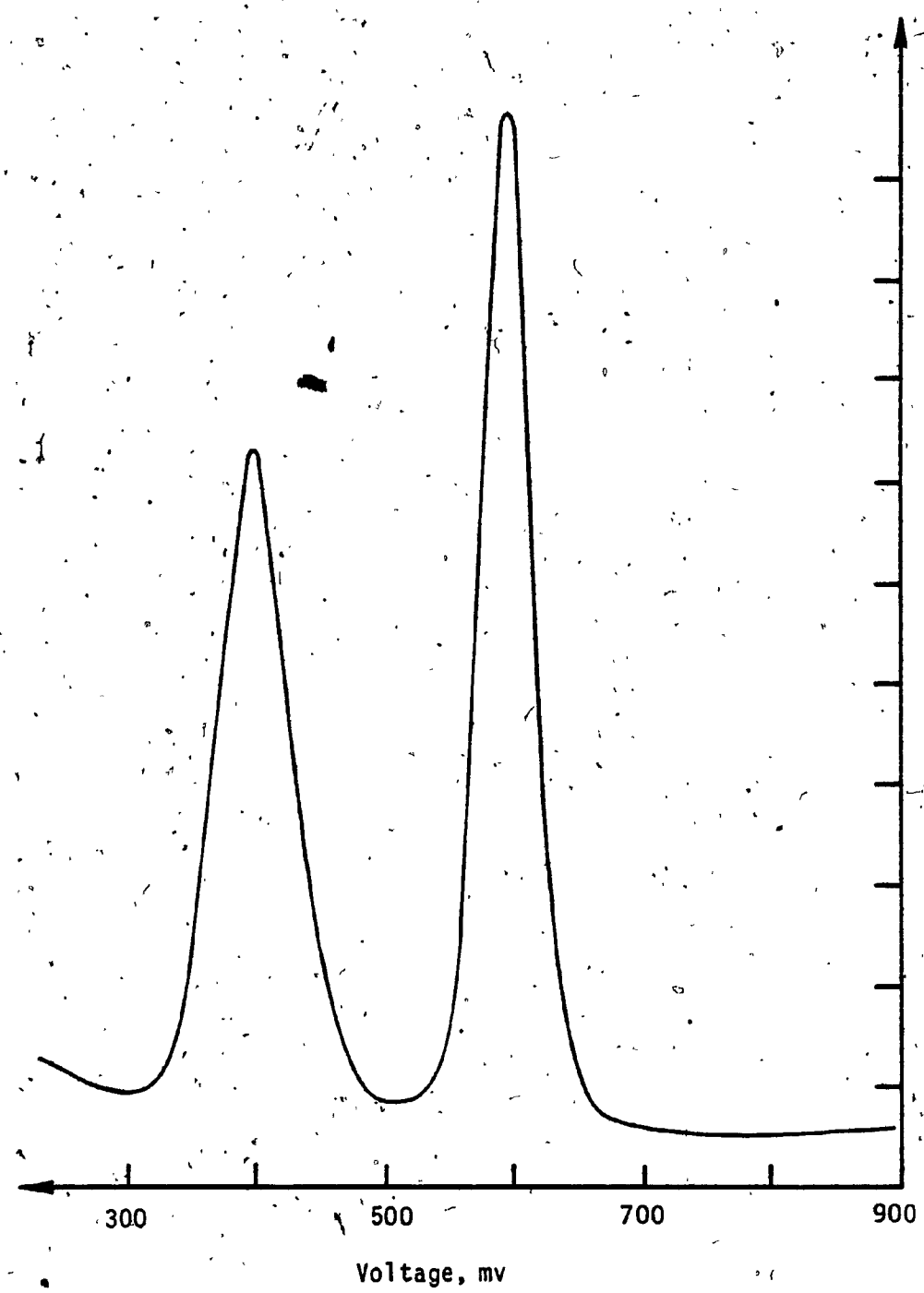


Figure 5: Anodic Stripping Voltametry,
Current vs Voltage.

2.3.2 Atomic absorption instrumentation

The following instrumentation was applied in flame and 'flameless' methodologies of trace metal detection and determination.

Atomic absorption spectrophotometer	Perkin-Elmer, Model 503
Background correction	(a) Normal double beam
	(b) D_2 beam
Recorder	Perkin-Elmer, Model 56
Carbon furnace	Perkin-Elmer, Model HGA 2100
High temperature lamp	Perkin-Elmer, Model 057-0450
Hollow cathode lamps and electrodeless discharge lamps	
Standard metal solutions	Fisher 1000 ppm standards.

2.3.3 Voltammetric/polargraphic instrumentation

Polarograph	Metrohm Herisau Polarecord Model E 506
Polarography stand	Metrohm Herisau, Model E505
Mode	DPP
Potential range (ΔU)	-3.0v, -2.5v, ..., -0.25v, +0.25v, +0.5v, ..., +2.0v
Voltage steps	The voltage range set on is divided along the standard polarogram length of 250 mm into 1000, 500, 250 or 125 equal voltage steps according to the setting of the "mm/t drop" switch.

Pulse amplitude	adjustable with 10-turn potentiometer, range ±100 mV.
Pulse duration	60 ms
Damping range	0, 1, 2, 3.
Drop times	0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 2.0, 3.0, 4.0, 5.0 and 6.0 sec.
Recorder	servo-compensating recorder
Paper	EA 998/3

CHAPTER 3

EXPERIMENTAL APPROACH AND RESULTS

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3.1 The Fish Tissue Specimen

A considerable quantity (500g) of whole fish in the form of rainbow trout *(Salmo gairdneri Rich.)* was obtained from the Department of Biological Sciences, Dr. P. Anderson's fish toxicology research group, Concordia University. These fish were supplied in two batches. One batch had never been exposed to experimental conditions involving elevated trace metal contents in their water and/or food. The second batch, unknown to the author at the time, had been exposed to high levels of nickel in the water and/or food. This second batch was obtained subsequent to the analysis of the first batch for copper, lead, cadmium and zinc, and was required to carry out determinations of nickel in fish tissue. It was appreciated, therefore, when detailed information was provided relative to the history of the second batch, that nickel contents higher than normal might be obtained for this portion of the investigation.

In the case of both batches, the whole fish were dried to constant weight at 65 ± 5°C. Each batch was subsequently macerated in 50 - 100 g weight lots, using a 2-inch test tube as a pestle and a polyethylene beaker as a mortar. The fish skin tends to form tissue balls under this form of maceration, and these were crushed immediately to avoid a time-hardening action which yields difficulties in the subsequent ashing procedure.

For each batch, the individual groups of macerated fish were

thoroughly mixed together, and each homogeneous batch was re-dried at 65 ± 5°C. Both batches were subsequently stored in a good desiccator.

3.2 The Dry Ashing Procedure Prior to Atomic Absorption and Polarographic Analysis

A methodology for the dry ashing of fish tissue was suggested by Dick et al [67], this technique being used to prepare fish tissue prior to the determination of arsenic by the atomic absorption hybride generation method. The same general technique was applied by De Luca [68] in the preparation of fish tissue for the determination of copper, lead, cadmium, zinc, nickel, vanadium, etc. For all of the metals listed, the temperature characteristics of the dry ashing procedure allowed ashing without significant metal loss. This technique was therefore applied in this study and involved:-

(a) Measuring out a 2.0000 ± 0.0002 g fish tissue sample from the homogeneous mass and transferring it to a 50 ml pyrex beaker.

(b) Providing an appropriate blank sample beaker.

(c) Placing the samples and blanks in a muffle furnace and subjecting them to a temperature cycle of:-

30 minutes at	100°C
30 minutes at	150°C
Increase of 50°C/30 minutes to	500°C
Overnight (ca 12 hours) at	500°C

This approach yielded heavy smoke at 250°-300°C.

(d) Removing the ashed samples and blanks, allowing them to cool to room temperature and adding 10 ml of concentrated nitric acid.

(e) Digesting the treated samples, without spattering, for 20-30 minutes, maintaining the 10 ml volume by periodic additions of

concentrated nitric acid.

(f) Evaporating the solution to dryness without any baking action.

To this point, the method of tissue preparation was identical regardless of whether the atomic absorption or polargraphic techniques were to be applied. Subsequent to this, the preparation procedures differ slightly. For the atomic absorption approach the following was carried out:-

(g) (1) Taking up the dried solution with 0.50 ± 0.02 ml of concentrated hydrochloric acid and swirling to dissolve as much residue as possible while warming at 60-70°C. Diluting the cooled solution with 24.50 ± 0.02 ml of 1 percent by volume hydrochloric acid.

For the polarographic approach, the take-up technique adopted was aimed at reducing the free HCl used, and consisted of:-

(g) (2) Taking up the dried residue with 0.200 ± 0.005 ml of concentrated hydrochloric acid and swirling to dissolve as much residue as possible while warming. Diluting with 29.80 ± 0.02 ml of the appropriate supporting electrolyte solution.

3.3 Atomic Absorption Determinations on Fish Tissue

3.3.1 Preparation of standard concentration solutions

Appendix A (A1-A2) indicates the stock solutions used and the preparative procedures for the standard concentration solutions required for the determination of each trace metal. The preparation of spiking solutions, differing from the standard solution ranges, is also shown in these sections.

3.3.2 Determination of trace metals in fish tissue

The following outlines the procedures adopted for the atomic absorption determination of the various trace metals.

Cadmium

The cadmium determinations were carried out by the "flameless" or graphite furnace methodology, this approach being dictated by the expected low levels of cadmium content.

Diluted stock solutions were used to prepare a set of six standard concentration solutions required to establish the concentration cadmium versus absorbance data. Using a blank for correction purposes, each standard solution was tested by injecting a 20 µℓ sample into the graphite furnace. The blank-corrected peak absorbance values were recorded. The operating parameters involved are shown in Appendix A (A-2). For each standard, multiple injections were carried out, and the peak absorbance values and associated average values are shown in Table A-1. The plot of peak absorbance versus cadmium concentration is shown in Figure A-1. It will be noted that, because of the extended concentration range, this plot showed a non-linear relationship to exist.

Six 2.0000 ± 0.0002 g dried fish tissue samples were weighted out, together with a blank sample, and these were subjected to the dry ashing and acid take-up procedures outlined in section 3.2. The final 25 ml solution volumes were sampled by 20 µℓ injections to the graphite furnace, and the resultant peak absorbance values, averages and standard deviations are indicated in Table A-2. Ignoring the possibility of a matrix-effect distrubance for the cadmium signal, the Table A-2 data can be used, with the Figure A-1 plot, to yield solution concentration

values for cadmium in each of the six samples and cadmium levels in dried tissue. These are shown in Tables A-3 and A-4. The overall average value and standard deviation results in a level of cadmium in the dried fish tissue of:-

$$0.048 \pm 0.006 \text{ ppm}$$

Following these tests, each of the six sample solutions was treated by a spiking technique. This involved taking 4.00 ml of the original solution and spiking it with 1.00 ml of a standard 0.200 ppm cadmium solution. 20 µℓ volumes of these spiked solutions were injected into the graphite furnace in a replicated test manner for each sample. The absorbance peaks, and associated average and standard deviation values are indicated in Table A-5.

Because of the non-linear nature of the Figure A-1 plot, the effect of the matrix on the cadmium signal, if such an effect exists, can not be corrected for by this particular application of the spiking method. Figure A-1 and the Table A-5 data were used so as to obtain, for each spiked solution, uncorrected cadmium concentration values. These are shown in Table A-6.

From these values, and using a specific calculation method, the ppm value for cadmium in dried fish tissue was determined. The calculation method, using sample S_1 as an example, involved:-

(a) spiked soln. conc. = 0.050^5 µg/ml = 0.252^5 µg/5ml

(b) spike value = 1.00 ± 0.01 ml of 0.200 ± 0.002 ppm = 0.200 ± 0.004 µg

(c) contribution of Cd from fish tissue = $0.252 - 0.200 \pm 0.004 = 0.052^5 \pm 0.004$ µg

(d) Cd in dried fish tissue = $$\frac{(0.052^5 \pm 0.004 \text{ µg}) \times (25.00 \pm 0.04 \text{ ml})}{(4.00 \pm 0.01 \text{ ml}) \times (2.0000 \pm 0.0002 \text{ g})}$$

$$= 0.16 \pm 0.01 \text{ ppm}$$

The value of ±0.01 represents a progressive maximum possible error.

Similar treatment applied to each of the 6 sample data yielded the values for dried fish tissue shown in Table A-7. The overall average and standard deviation for cadmium in dried fish tissue, as obtained by the spiking method, was:-

$$0.09 \pm 0.05 \text{ ppm}$$

The significant magnitude for the standard deviation has its origin in:-

(a) The fact that, outside the linear relationship zone, absorbance peak values tend to be erratic.

(b) The lack of linearity necessitated the use of the absorbance peak versus concentration plot, with further uncertainty in the concentration values obtained.

(c) The large spike value for cadmium relative to the low contribution of cadmium from the fish tissue resulted in variable by-difference at a low magnitude. Such variable and low by-difference values tend to yield relatively large variations in the final calculated data for cadmium in the fish tissue.

It is felt that the value of 0.09 ± 0.05 ppm, as obtained by the spiking technique, is likely to be significantly affected by points (a) to (c). On this basis it is worthwhile to note that the non-spiking procedure yielded a value of 0.048 ± 0.006 ppm for cadmium in the fish tissue. Using a similar fish background, but different batches, De Luca [68] obtained a value for this trace metal of 0.032 ± 0.002 ppm,

and this agrees reasonably well with the non-spiking value found for this study.

Copper

The copper determinations were carried out by the flame technique, this being based on the copper level being sufficiently high to permit its use.

Diluted stock solutions of copper were used to prepare a set of six standard concentration solutions required to establish the concentration copper versus absorbance data. Using a blank for correction purposes, each standard solution was aspirated and the blank-corrected absorbance values were recorded. The operating parameters are shown in Appendix A(A-3) and each solution was tested on a replicate basis using a 3-sec integration signal. Table A-8 shows the average blank-corrected absorbance values.

The Table A-8 data indicates a linear relation for absorbance versus concentration, with linear regression data:-

coefficient of correlation, $r = 0.0089$
intercept on absorbance axis, $b = 0.001$
slope, $m = 0.0606$

yielding a linear equation given by:-

$$\text{concentration Cu (ppm)} = \frac{\text{absorbance} - 0.001}{0.0606}$$

Six standard 2.0000 ± 0.0002 g dried fish samples were weighed, together with a blank, and were subjected to the dry ashing and acid take-up procedures indicated in section 3.2 The final 25 ml solution volumes were aspirated and the resultant replicate 3-second integrated absorbance values averaged. The data obtained is shown in Table A-9,

along with the solution concentration of copper as calculated from the linear equation. Use of the formula:-

$$\text{Cu in dried fish tissue (ppm)} = \frac{\text{orig.soln.conc.}(\mu g/ml) \times 25.00 \pm 0.04 \text{ ml}}{2.0000 \pm 0.0002 \text{ g}} \quad (20)$$

now yielded the values for copper in dried fish tissue shown in Table A-10, with an overall average and standard deviation of:-

Cu in dried fish tissue = 6.0 ± 0.5 ppm

The approach indicated above does not take into consideration the signal perturbation from the solution matrix, and a spiking technique was now applied to permit compensation for any such effect. This involved taking 4.00 ml of the original solution and spiking it with 1.00 ml of a 2.00 ppm copper standard solution. These spiked solutions were then aspirated and average absorbance values as indicated for the previous tests. The data obtained is shown in Table A-11. The formula shown below was now used to calculate the concentration of copper in the original solution before spiking. Solution S1 is used as the example.

(a) before spiking 0.031 absorbance = x µg/ml Cu

(b) after spiking 0.049 absorbance = $\frac{4 \times \mu g + 2 \mu g}{5 \text{ ml}}$ µg/ml Cu

$x = 0.51^2 \pm 0.01^7$ µg/ml

Subsequently the solution copper concentration for each sample was used in equation (20) to yield the ppm copper dried fish tissue. The data in these conncections is shown in Table A-12, and the overall average and standard deviation for copper in dried fish tissue using the spiking technique was found to be:-

Cu in dried fish tissue = 6.1 ± 0.4 ppm

It will be noted that the values for the copper content in dried tissue from both approaches agree quite well, the averages and standard deviations showing only random error differences when tested by the F(0.95) and t(0.95) tests.

$$F = \frac{(0.5)^2}{(0.4)^2} = 1.56 < F(0.95)_{n_1=6,n_2=6} = 5.05$$

$$t = \frac{6.1-6.0}{0.49}\sqrt{\frac{6x6}{6+6}} = 0.35 < t(0.95)_{n_1=6,n_2=6} = 2.23$$

It is felt, nevertheless, that the value obtained from the spiking technique is the more reliable one. The value obtained by De Luca [68] at 3.9 ± 0.3 ppm for copper in a similar fish type is appreciably lower, but represents work carried out on a different specimen batch.

Lead

The lead determinations were carried out using the graphite furnace methodology, this being required because of the lead level in fish tissue being relatively low.

Diluted stock solutions of lead were used to prepare a set of six standard solution concentrations required to obtain the concentration lead versus peak absorbance data. Using a blank for correction purposes, each standard solution was tested by injecting a 20 µℓ sample to the furnace. The blank corrected peak absorbance values were recorded. The operating parameters are shown in Appendix A (A-4). Each sample was tested with replicate injections, and the average peak absorbance values are shown in Table A-13. The plot of peak absorbance versus concentration is shown in Figure A-2. It will be noted that, because of the extended concentration range adopted to avoid aliquoting, this plot showed a

non-linear relationship.

Six 2.0000 ± 0.0002 g dried fish tissue samples were weighed out, together with a blank, and these were subjected to the dry ashing and take-up procedure outlined in section 3.2 The final 25 ml volume solutions were tested by injecting replicate 20 µl samples to the furnace, and the resultant peak absorbance values were averaged. The data obtained is shown in Table A-14. Ignoring the possibility of matrix-effects on the lead signal, the Table A-13 data was used, with the Figure A-2 plot, to yield solution lead concentrations and lead levels in the fish tissue. These data are shown in Tables A-15 and A-16. The overall average value and standard deviation results in a level for lead in the dried fish tissue by the direct method or non-spiking technique of:-

1.17 ± 0.06 ppm

Following these tests, each of the 6 sample solutions was treated by a spiking technique involving taking 4.00 ± 0.01 ml of original solution and spiking it with 1.00 ± 0.001 ml of a 0.500 ± 0.004 ppm standard lead soltuion. 20 µl samples from the spiked solutions were injected to the furnace as before, and the recorded data are shown in Table A-17.

Because of the non-linear nature of the Figure A-2 plot, the matrix effect on the lead signal could not be offset by the usual approach. However, Figure A-2 and Table A-17 data were used to yield, for each spiked solution, uncorrected lead concentration values. These are shown in Table A-18.

From these values, and using a specific calculation method, the ppm value for lead in dried fish tissue was determined. The calculation method, using sample S_1 data as an example, involved:-

(a) spiked soln. concen. = 0.183 µg/ml = 0.915 µg/5ml Pb

(b) spiked value = 1.00 ± 0.01 mℓ x 0.500 ± 0.004 ppm
= 0.50 ± 0.01 µg Pb

(c) concentration of Pb from fish tissue = $0.915 - 0.50 \pm 0.01$ µg = $0.41^5 \pm 0.01$ µg Pb

(d) Pb in dried fish tissue
$$= \frac{(0.41^5 \pm 0.01\ \mu g) \times (25.00 \pm 0.04\ ml)}{(4.00 \pm 0.01\ ml) \times (2.0000 \pm 0.0002\ g)}$$
$$= 1.30 \pm 0.03 \text{ ppm}$$

The value of ±0.03 ppm represents a progressive maximum possible error.

Similar treatment applied to each of the 6 sample data yielded lead values for dried fish tissue as indicated in Table A-19. The overall average and standard deviation for lead in dried tissue as determined by the spiking method was:-

$$1.24 \pm 0.04 \text{ ppm}$$

It will be noted that the values for lead in dried tissue from both the spiking and non-spiking techniques agree quite well. The F(0.95) test applied to the standard deviations show no evidence of other than random differences. The f(0.95) test applied to the average values shows a minor significance indicating a non-random source.

$$F = \frac{(0.06)^2}{(0.04)^2} = 2.25 < F(0.95)_{n_1=6, n_2=6} = 5.05$$

$$t = \frac{1.24-1.17}{0.05} \sqrt{\frac{6 \times 6}{6+6}} = 2.42 \text{ , } t(0.95)_{n_1=6, n_2=6} = 2.23$$

It can be assumed that the non-random difference in the average arises out of the greater effect of the matrix on the non-spiked solutions, although this is minimal. For this study, the spiked solutions value for lead in fish tissue will be used. De Luca [68] obtained a value for lead in similar fish, but a different batch, of 0.83 ± 0.08 ppm, and this agrees reasonably well with the value of 1.24 ± 0.04 ppm for this study.

Nickel

The nickel determinations were carried out by the flame method since, due to the origin of this fish tissue batch from a toxic nickel situation, high nickel levels were anticipated.

Diluted stock solutions of nickel were used to prepare six standard concentration solutions to obtain the concetration versus absorbance data. Using a blank for correction purposes, each reference solution was aspirated on a 3-sec integrated replicate basis. The operating parameters are shown in Appendix A (A-5) and Table A-20 indicates the average blank-corrected absorbances.

The Table A-20 data indicates a linear relationship with linear equation data of:-

coefficient of correlation, $r = 0.0095$
intercept on absorbance axis, $b = 0.000$
slope, $m = 0.0406$

yielding a linear equation given by:-

$$\text{concentration Ni (ppm)} = \frac{\text{absorbance}}{0.0406}$$

Six 2.0000 ± 0.0002 g dried fish tissue samples were weighed, together with a blank, and subjected to the dry ashing, etc., procedures outlined in section 3.2. Initial tests on these solutions of 25 ml

volume showed high nickel values. In order to maintain a concentration in the linear zone, 2.00 ± 0.001 ml of original solution was diluted to 10.00 ± 0.02 ml. These diluted solutions were aspirated and the replicate 3-sec integrated absorbance data recorded. Table A-21 shows the average absorbance values, the diluted soltuion concentration of nickel from the linear equation and the original solution concentration of nickel. Use of the formula:-

$$\text{Ni in dried fish tissue (ppm)} = \frac{\text{orig.soln.conc.}(\mu g/ml) \times 25.00 \pm 0.04\ m\ell}{2.0000 \pm 0.0002\ g} \quad (20)$$

now yielded the values for nickel in dried fish tissue by the non-spiking method. Table A-22 shows the data obtained, and yielded an overall average and standard deviation for this approach of:-

Ni in dried fish tissue = 44 ± 3 ppm

In order to compensate for the matrix effect (if any, since the dilution of the original solution reduces this effect), a spiking technique was applied. This involved taking 4.00 ± 0.01 ml of diluted solution and adding 1.00 ± 0.01 mℓ of a 3.00 ± 0.02 ppm nickel standard solution. These spiked solutions were aspirated as before, and the data obtained is shown in Table A-23. The formulae shown below were now used to calculate the concentration of nickel in the diluted solution and in dried fish tissue. Sampe S_1 is used as the example.

(a) diluted soln. before spike 0.026 absorbance = X µg/mℓ Ni

(b) diluted soln. after spike 0.044 absorbance = $\frac{4x+3}{5}$ µg/mℓ Ni

$$\therefore \quad X\ \mu g/m\ell \text{ (diluted soln.)} = 0.67^2 \pm 0.01^6$$

(c) $$\text{ppm Ni in dried fish tissue} = \frac{\text{dil.soln.conc.}(\mu g/m\ell) \times \text{aliq.factor} \times \text{orig.soln.vol.}}{\text{sample wt.}}$$

$$= \frac{(0.67 \pm 0.01) \times 5 \times (25.00 \pm 0.04\ ml)}{2.0000 \pm 0.000\ 2\ g}$$

$$= 42 \pm 1$$

Table A-24 shows the results of these calculations for all 6 samples, and the overall average and standard deviation for nickel in dried fish tissue by the spiking method was:-

Ni in dried fish tissue = 44 ± 2 ppm

It will be noted that the values for the nickel content from both approaches agree exactly. The standard deviation, tested by the F(0.95) test show no significant differences.

$$F = \frac{(3)}{(2)} = 2.25 < F(0.95)_{n_1=6, n_2=6} = 5.05$$

No comparison can be obtained relative to the De Luca [68] study, since the fish tissue for this study contained very high nickel levels. As a matter of interest, and for future reference, the De Luca study showed a value for nickel in dried tissue of 1.3 ± 0.2 ppm.

Zinc

The zinc determinations were made by the flame method because of the anticipated high zinc level in fish tissue.

Reference solutions for the determination of the concentration versus abosorbance relationship were prepared from diluted stock solutions and these, together with a blank, were aspirated on a 3-sec. integration replicate basis. The operating parameters are shown in Appendix A (A-6) and Table A-25 shows the obtained data. A linear relationship was indicated for concentration versus absorbance, with linear equation data of:-

coefficient of correlation, $r = 0.9987$
intercept on absorbance axis, $b = 0.003$
slope, $m = 0.1258$

yielding a linear equation given by:-

$$\text{concentration Zn (ppm)} = \frac{\text{absorbance} - 0.003}{0.1258}$$

Six 2.0000 ± 0.0002 g dried fish tissue samples were weighed out, with a blank, and treated by dry ashing, etc., as indicated in section 3.2. Initial tests on the final 25 ml volumes obtained showed a high zinc value. In order to obtain a solution concentration in the linear zone, 1.00 ± 0.01 ml of original solution was diluted to 20.00 ± 0.02 ml. These diluted solutions were aspirated as before, and Table A-26 shows the average absorbance values, the diluted solution zinc concentration and that for the original solution. Use of the formula:-

$$\text{Zn in dried fish tissue} = \frac{\text{orig.soln.conc.}(\mu g/ml) \times 25.00 \pm 0.04 \text{ m}\ell}{2.0000 \pm 0.0002 \text{ g}} \quad (20)$$

now yielded the values for zinc in dried fish tissue by the non-spiking method. Table A-27 shows this data, and provided an overall average and standard deviation for this approach of:-

Zn in dried fish tissue = 125 ± 4 ppm

In order to compensate for any matrix effect (expected to be minimal due to the high degree of dilution of the original solution), a spiking technique was applied. This involved taking 4.00 ± 0.01 ml of the 1:20 diluted solution and adding 1.00 ± 0.01 ml of a 2.00 ± 0.002 ppm zinc standard solution. These spiked solutions were aspirated as before, and the data obtained is shown in Table A-28. The formulae shown below were now used to calculate the concentration of zinc in the diluted solution and in the dried fish tissue. Sample S_1 is used as the example.

(a) diluted soln. before spike = 0.167 absorbance = X µg/ml Zn

(b) diluted soln. after spike = 0.106 absorbance = $\frac{4x+2}{5}$ µg/mℓ Zn

$\therefore$ X = 0.0511 ± 0.008 µg/mℓ (diluted soln.)

(c) ppm Zn in dried fish tissue = $\frac{\text{dil.solu.conc.}(\mu g/m\ell) \times \text{aliq.fact.} \times \text{orig.solu.vol.}}{\text{sample wt.}}$

$$= \frac{(0.511 \pm 0.008) \times 20 \times (25.00 \pm 0.04 \text{ ml})}{2.0000 \pm 0.0002 \text{ g}}$$

= 128 ± 2 ppm

Table A-29 shows the results of these calculations for all 6 samples, and the overall average and standard deviation for zinc in dried fish tissue by the spiking method was:-

Zn in dried fish tissue = 127 ± 2 ppm

It will be noted that the values for the zinc content from both approaches agree reasonably well. The standard deviations as tested

by the F test show no significant differences, as do the averages tested by the t test

$$F = \frac{(4)^2}{(2)^2} = 4 < F(0.95)_{\eta_1=6,\eta_2=6} = 5.05$$

$$t = \frac{1.28 - 1.25}{3.1}\sqrt{\frac{6\times6}{6+6}} = 1.64 < t(-0.95)_{\eta_1=6,\eta_2=6} = 2.23$$

The value obtained by De Luca [68] for zinc in similar fish tissue, but a different batch, was 121 ± 4 ppm, and this agrees relatively well with the data from this study.

The acceptable values for the various trace metals in dried fish tissue as determined by the atomic absorption methodology are shown in Table 1. These values will be used for comparative purposes with respect to the values to be determined by the differential pulse polarographic method.

3.4 Differential Pulse Polarographic Determinations on Fish Tissue

3.4.1 Study on expected polarographic concentrations of trace metals from dried fish tissue

It was anticipated that 2 g of dried fish tissue would be the sampling weight, and that these samples would eventually be contained in a total of 30 ml of solution. Based on the Table 1 analysis of fish tissue by the atomic absorption methodologies, these arrangements would yield final solution ppm values of:-

Cadmium	0.003
Copper	0.4
Lead	0.087
Nickel	2.8
Zinc	8.3

TABLE 1

TRACE METAL CONTENTS IN DRIED FISH TISSUE

BY ATOMIC ABSORPTION TECHNIQUES

Metal	ppm in dried fish tissue
Cadmium	0.048 ± 0.006
Copper	6.1 ± 0.4
Lead	1.24 ± 0.04
Nickel	44 ± 2
Zinc	127 ± 2

From this data it was decided that it would be probable that, during the preparation for dpp analysis, the 30 ml solution would have to be aliquoted for certain determinations.

From this data the following was tentatively concluded.

(1) The low level for the cadmium would likely render it improbable that this element would be successfully determined by dpp.

(2) The relatively low level for the lead content might render its accurate determination difficult.

(3) The high zinc level would most likely require aliquoting (1:10) of the final solution volume prepared for dpp.

(4) The accident of the second tissue batch having a high nickel content will allow easy determination of this element. The normal and expected level of about 1 ppm might have rendered the determination of nickel in the same category as that of lead.

3.4.2 Preparation of the supporting electrolytes

A study of various sources [63] indicated that, for the determination by dpp of copper, lead, cadmium and zinc, the single supporting electrolyte 0.2 M (3 pH) ammonium citrate would be the most suitable relative to the separation of the respective metal $E_{\frac{1}{2}}$ values. The same sources indicated that, for the determination of nickel, the most suitable electrolyte would be 2 M KCN. Appendix B (B-1) indicates the preparation of these supporting electorlytes.

Although the reagents used in these preparations contained minimal trace metal contents (less than 2 ppm), each supporting electrolyte was subjected to a dpp run to establish the level of peak value

for each trace metal. Since it was not anticipated because of signal to noise ratio increases, that a current sensitivity exceeding 1.5×10^{-10} A/mm of chart would be required, these blank runs were made at this sensitivity and at the parameters indicated in Appendix B (B-2). Table B-1 shows the peak values at the associated $E_{\frac{1}{2}}$ values in terms of chart mm. It will be noted that these showed minimal values. It will be appreciated that, when employing sensitivities less than 1.5×10^{-10} A/mm, blank values will be correspondingly less. For example, at 2.5×10^{-10} A/mm, each blank peak value in mm will be reduced by a factor of 1.67.

3.4.3 Preparation of standard concentration solutions for spiking and for sensitivity tests

The stock solutions used for auxillary standard solution preparation were identical to those indicated in Appendix A (A-1). The spiking and sensitivity test solutions were prepared in consideration of the expected trace metal levels in the solutions prepared for dpp, and these levels were outlined in section 3.4.1. Appendix B (B-3) indicates the preparation of these spiking and sensitivity tests standard solution concentrations.

3.4.4 Sensitivity values for analyte metals under dpp

Solutions of the two supporting electrolytes were prepared so as to contain amounts of the analyte metals generally similar to the anticipated solution amounts as derived from 2 g fish tissue samples. The preparation and concentrations of these solutions are shown in Appendix B (B-4).

The 18 ml 0.2 M ammonium citrate solution was tested in order of the decreasing $E_{\frac{1}{2}}$ values (i.e. Cu, Pb, Cd, Zn). The 25 ml nickel solution in 2 M KCN was tested for nickel only.

The 0.2 M ammonium citrate solution was tested as-is for copper and then tested again after a copper spike. The $E_{\frac{1}{2}}$ for the reaction $Cu^{2+} + 2e \rightleftharpoons Cu$ was determined. The solution was then tested as-is for lead and then after a lead spike. The $E_{\frac{1}{2}}$ for $Pb^{2+} + 2e \rightleftharpoons Pb$ was determined. This procedure was continued for cadmium and zinc, and for nickel in the 2 M KCN solution. For each element the sensitivity was determined in ppm/mm of chart at 1.5×10^{-10} A/mm sensitivity, this limit representing the mm peak added to the blank value. Table 2 shows the sensitivities and the $E_{\frac{1}{2}}$ values, the latter compared to literature values. All of the data associated with this portion of the study is given in Appendix B (B-5) and in Table B-2. Triplicate runs were carried out.

Figure 7 is a replicate of a typical dp polarogram, this being in the run to determine lead sensitivity.

3.4.5 Determination of Cu, Pb, Cd, Zn and Ni by dpp

Six samples of dried fish tissue were now prepared by weighing 2.0000 ± 0.0002 g and subjecting them to the dry ashing and take-up procedures outlined in section 3.2. Somewhat different dilution solutions were applied and three separate sets of samples were prepared. The purpose and dilutions for each set are noted below. Blank solutions were also prepared.

TABLE 2

THE SENSITIVITIES FOR Cu, Pb, Cd, Zn

AND Ni BY THE DPP METHOD

Metal	Sensitivity in mm of chart over blank at 1.5×10^{-10} A/mm	Exp. $E_{\frac{1}{2}}$ vs Ag/Agcl	*Exp. $E_{\frac{1}{2}}$ vs Ag/Agcl
Cu	0.007	-0.059	-0.071
Pb	0.009	-0.367	-0.39
Cd	0.004	-0.569	-0.571
Zn	0.005	-0.968	-1.00
Ni	0.005	-1.338	-1.32

*L. Meites, "Polarographic Techniques Interscience, N.Y., 2nd. ed., 1967, pp. 638-39

(a)	Set 1	determination of Cu, Pb, Cd	30.00 ± 0.04 ml final solution with 0.2 M ammonium citrate
(b)	Set 2	determination of Zn	20.00 ± 0.04 ml final solution with 0.2 M ammonium citrate
(c)	Set 3	determination of Ni	20.00 ± 0.04 ml final solution with 2 M KCN.

Copper

Each sample of set 1 was treated by taking 28.00 ± 0.04 ml of the original 30 mℓ solution, and the blank, and charging these to polarographic cells. The blank was tested, and the 28 ml solution was tested as-is and after spiking with a copper solution. The peak values at the $E_{\frac{1}{2}}$ for copper were blank-corrected. The parameters and the data recorded, with calculations, are shown in Appendix B (B-6-Copper). The calculation process, using sample 1 as the example, is shown below.

as-is 7.8 div - 0.25 div blank = 7.55 div = 15.1 mm = X µg/mℓ

spike $\frac{(28.00 \pm 0.04 \text{ mℓ}) \times X \text{ µg/ml} + (1.00 + 0.01 \times 10.00 \pm 0.05)}{29.00 \quad 0.04 \text{ ml}}$

$\frac{28x + 10}{29}$ µg/mℓ for 138 div - 0.25 div = 13.55 div = 27.1 mm

∴ X = 0.415 ± 0.004 µg/ml original solution

12.45 ± 0.12 µg/30 ml original solution

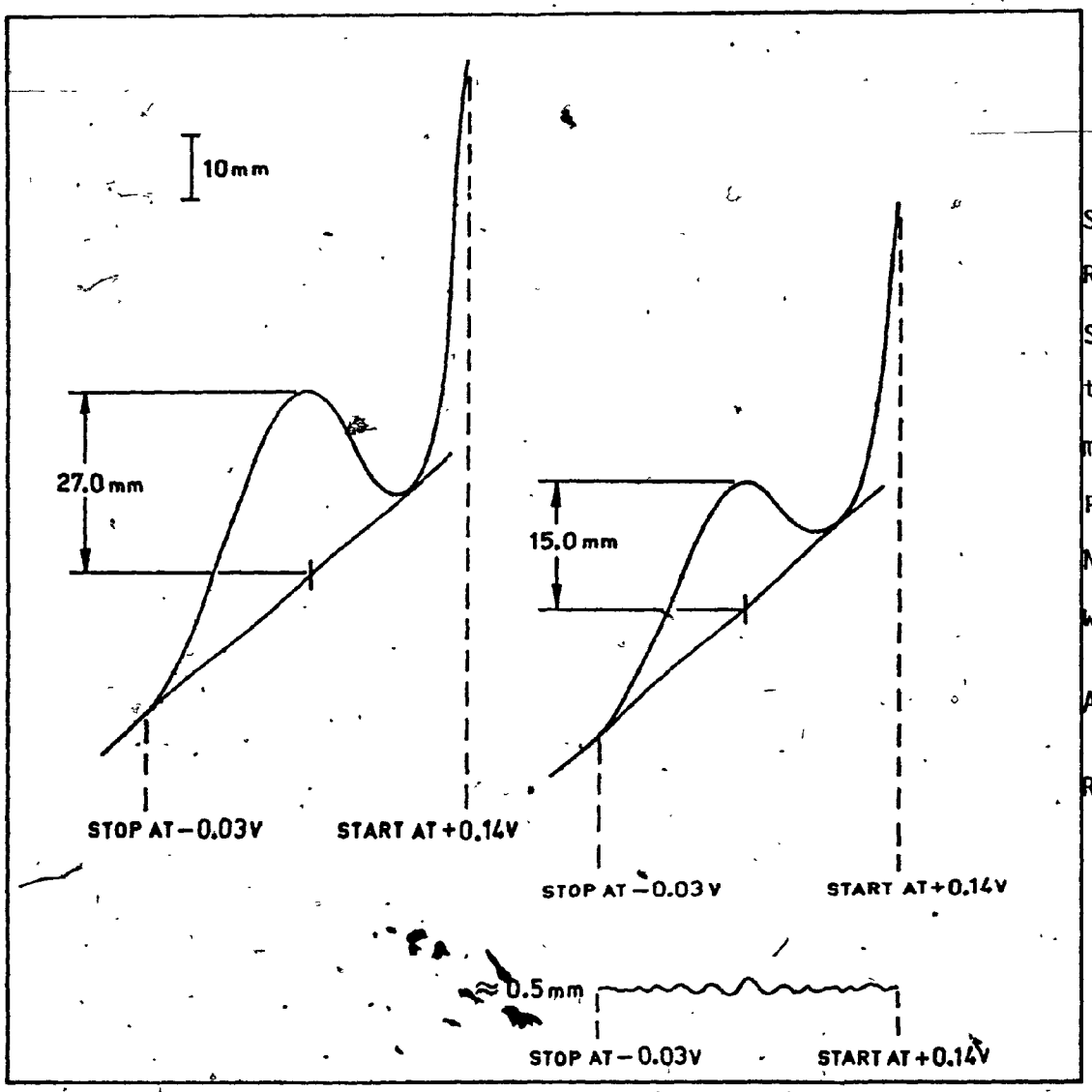
$$\frac{12.45 \pm 0.12 \text{ µg}}{2.0000 \pm 0.0002 \text{ g}} = \frac{6.22 \pm 0.06 \text{ µg}}{\text{g}}$$

∴ ppm Cu in dried fish tissue = 6.22 ± 0.06

Table 3 shows the individual data for the 6 samples, together with the overall average and standard deviation. Figure 6 shows a typical replicate polarogram for sample 4 of the series.

Lead

After the testing for copper was completed, the residual solutions



E vs Ag/Agcl (sat. Kcl)

Figure 6: Determination of Cu by dpp. Sample 4.

Sensitivity = 6 + 10^{-10} A/mm

Range = +0.2 to -2.00 V

Start/Stop = +0.14 to -0.3 V

t drop = 0.4 sec.

mm/t drop = 0.5

Pulse = -32 mV

N_2 degas = 15 mins.

Working electrode = DME

Auxillary electrode = Pt

Reference electrode = Ag/Agcl (sat.kcl)

TABLE 3

DETERMINATION OF COPPER IN FISH TISSUE BY DPP

Sample	Copper in fish tissue ± uncertainty (ppm)
1	6.22 ± 0.06
2	6.05 ± 0.06
3	5.92 ± 0.06
4	5.72 ± 0.06
5	5.87 ± 0.06
6	6.56 ± 0.06

Average and standard deviation = 6.0 ± 0.3 ppm

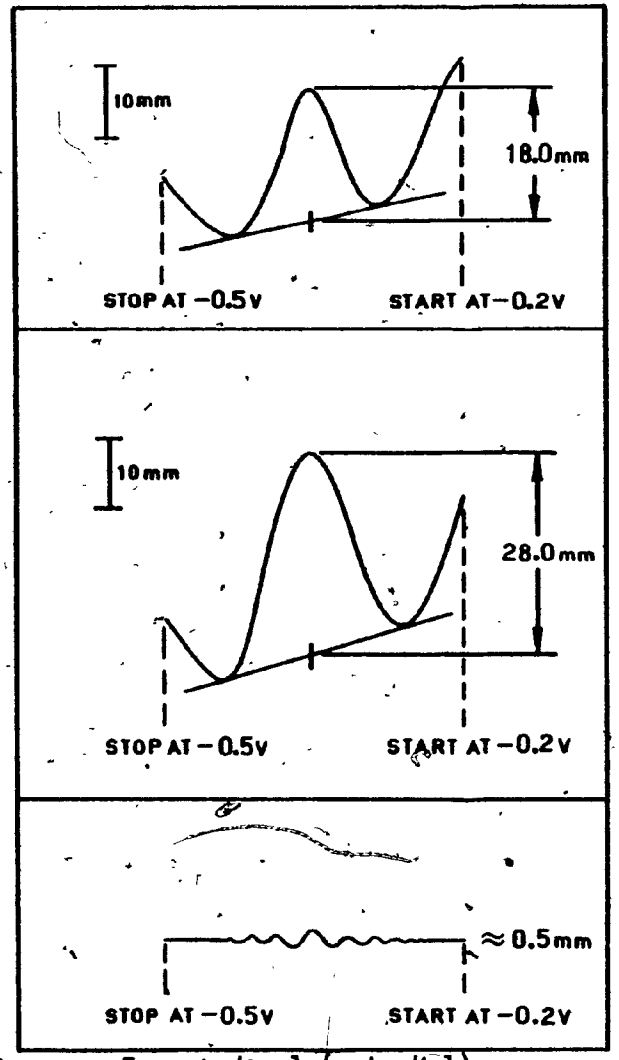
for each sample were tested as-is for lead and were retested after the addition of a lead spike. The peak values at the $E_{\frac{1}{2}}$ for lead were corrected for the blank. As shown for copper the lead was calculated from the as-is and spike data giving a value of lead free from the matrix effect. During the calculations due regard was paid to the volume dilution from the previous copper spike. The data and calculations are shown in Appendix B (B-6-lead). Table 4 shows the individual values and the overall average and standard deviation. (See Fig. 7)

Cadmium

After the testing for lead the determination of cadmium was carried out by testing the residues from the lead tests as-is and then spiked with cadmium. Appendix B (B-6-Cadmium) shows the operating parameters, the accumulated peak values at the $E_{\frac{1}{2}}$ for cadmium and the calculations for cadmium in fish tissue free from matrix effect. The calculations take into consideration the dilution effects arising out of the copper and lead determinations. Table 5 shows the individual values and the overall average. Figure 8 shows a replicate of the cadmium, cadmium spike and blank scans.

Zinc

The samples from set 2 were used in the determination of zinc. Because of the anticipated high zinc level, 2.00 ± 0.01 ml of the final 20 mℓ solution was diluted to 20.00 ± 0.04 ml. The blank was diluted accordingly. The diluted solution was tested as-is and spiked. The parameters and the data obtained are shown in Appendix B (B-6-Zinc). Table 6 shows the individual values and the overall average value for zinc in ppm in dried fish tissue.



Sensitivity = 1.5 + 10^{-10}A/mm

Range = +0.2 to -2.00 V

Start/Stop = -0.2 to -0.5 (approx.)V

t drop = 0.4 sec.

mm/t drop = 0.5

Pulse = -25 mV

N_2 degas = 15 mins.

Working electrode = DME

Auxillary electrode = Pt

Reference electrode = Ag/Agcl (sat.Kcl)

Figure 7 : Determination of Sensitivity of dpp to Lead.

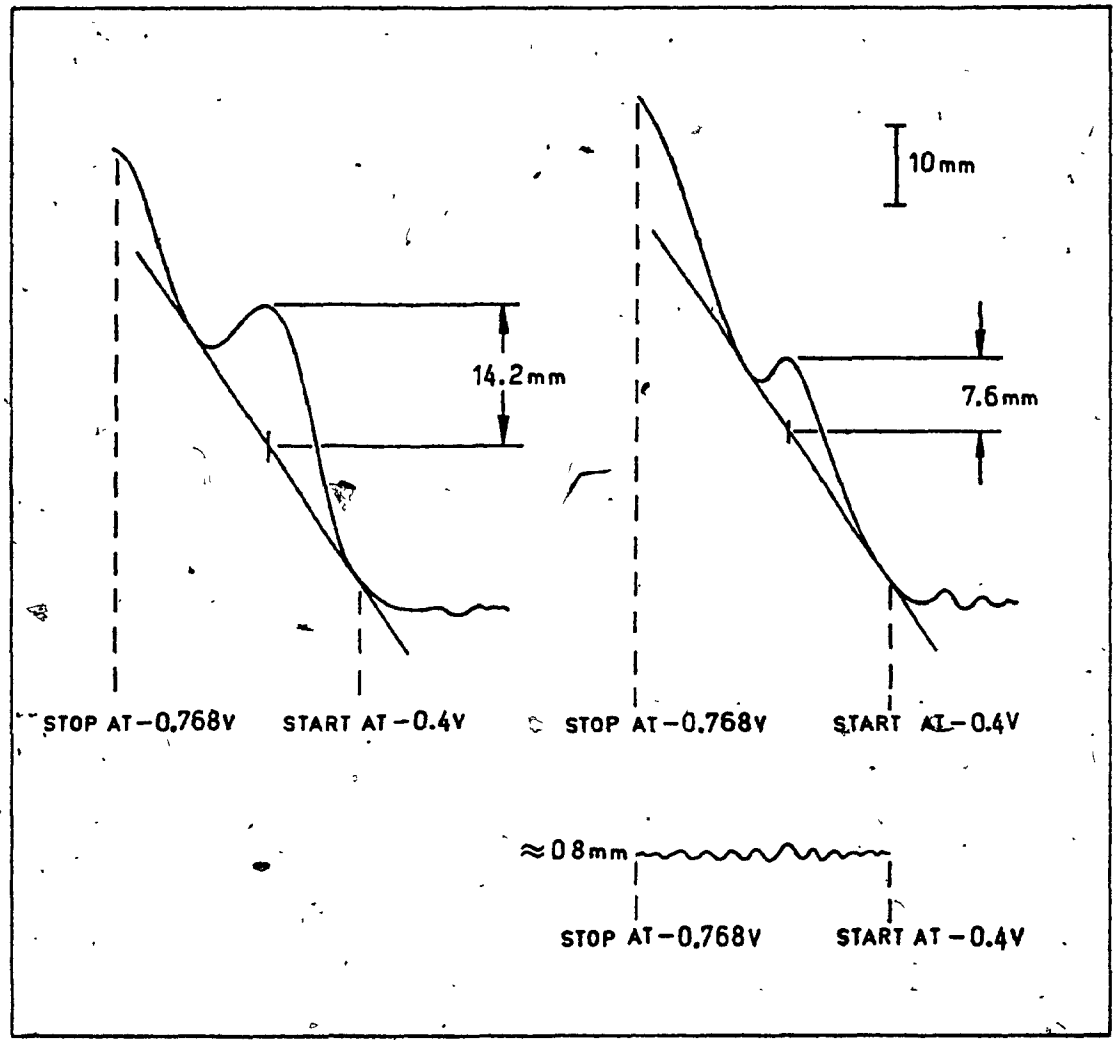


Figure 8: Determination of Cd by dpp. Sample 6.

Sensitivity	= 1.5×10^{-10} A/mm
Range	= +0.2 to -2.00 V
Start/Stop	= -0.4 to -0.776 V
t drop	= 0.4 sec.
mm/t drop	= 0.5
Pulse	= 32 mV
N degas	= 15 mins.
Working electrode	= DME
Auxillary electrode	= Pt
Reference electrode	= ag/agcl (sat.kcl)

TABLE 4

DETERMINATION OF LEAD IN

FISH TISSUE BY DPP

Sample	Lead in fish tissue ± uncertainty (ppm)
1	1.16 ± 0.08
2	1.20 ± 0.08
3	1.00 ± 0.08
4	0.97 ± 0.08
5	1.10 ± 0.08
6	1.18 ± 0.08

Average and standard deviation = 1.10 ± 0.09

TABLE 5

DETERMINATION OF CADMIUM

IN FISH TISSUE BY DPP

Sample	Cadmium in fish tissue ± uncertainty (ppm)
1	0.21 ± 0.02
2	0.17 ± 0.02
3	$0.08^{7} \pm 0.00^{6}$
4	0.29 ± 0.03
5	0.12 ± 0.01
6	0.14 ± 0.02

Overall average and standard deviation = 0.17 ± 0.07 ppm

TABLE 6

DETERMINATION OF ZINC IN FISH TISSUE BY DPP

Sample	Zinc in fish tissue ± uncertainty (ppm)
1	131 ± 1
2	126 ± 1
3	134 ± 1
4	131 ± 1
5	134 ± 1
6	128 ± 1

Overall average and standard deviation = 131 ± 3 ppm

Nickel

The samples from set 3 were used in the nickel determination, together with the associated blank. The solutions were tested both as-is and spiked. The operating parameters and the data collected are shown in Appendix B (B-6-Nickel). Table 7 shows the individual data together with the average and standard deviation for nickel in dried fish tissue.

3.5 Comparison of Atomic Absorption and dpp Data

Table 8 shows the atomic absorption and DPP values for the various metal analytes in dried fish tissue.

The individual averages and standard deviations were subjected to F and t tests to determine the quality of the differences. Table 9 indicates this situation. It will be noted that, where copper, lead, zinc and nickel are concerned any differences in the standard deviations have a random basis. Only for cadmium is the difference other than random. Where copper and nickel are concerned, the averages show only random differences. For zinc, the difference in the averages show some significance but only to a very minor degree. The averages for lead show a more significant difference and this may be attributed to the fact that the level of lead in the tested fish tissue solutions approached the limit of detection. Cadmium was obviously a problem, and the dpp determination was considered to be too close to the detection limits for reliable results to be secured. As for nickel, the difference in the averages, as shown in Table 9, is only random. Hence it may be said that the dpp technique may be used in the analysis of fish tissue and reliable results may be obtained in the cases of

TABLE 7

DETERMINATION OF NICKEL IN FISH TISSUE BY DPP

Sample	Nickel in fish tissue ± uncertainty (ppm)
1	46.3 ± 0.2
2	46.0 ± 0.2
3	42.6 ± 0.2
4	44.8 ± 0.2
5	45.4 ± 0.2
6	46.0 ± 0.2

Overall average and standard deviation = 45 ± 1 ppm

TABLE 8

COMPARISON OF ATOMIC ABSORPTION AND DPP VALUES

FOR Cu, Pb, Cd, Zn AND Ni IN DRIED FISH TISSUE

Metal	Level in dried fish tissue (ppm)	
	atomic absorption	dpp
Copper	6.1 ± 0.4	6.0 ± 0.3
Lead	1.24 ± 0.04	1.10 ± 0.09
Cadmium	0.048 ± 0.006	0.17 ± 0.07
Zinc	127 ± 2	131 ± 3
Nickel	44 ± 2	45 ± 1

TABLE 9

COMPARISON OF AVERAGES AND STANDARD DEVIATIONS FOR ATOMIC ABSORPTION AND DPP RESULTS ON TRACE METALS ANALYSES

For $n_1 = 6$, $n_2 = 6$

$F(0.95) = 5.05$
$t(0.95) = 2.23$

Metal	F(0.95) test	t(0.95)test
Copper	1.78	0.46
Lead	5.06	3.46
Cadmium	136	—
Zinc	2.25	2.47
Nickel	4.00	1.90

copper, nickel and zinc. In the case of lead and cadmium, the levels of the respective metals in the fish tissue are such that they approach the limits by detection by the dpp technique.

It must also be pointed out that the fish tissue used for nickel analysis was obtained from a particular batch that had been exposed to high levels of nickel in the water and/or food. [Section 3.1] With normal fish, the nickel content may not be as high as the values obtained during this analysis. De Luca [68] has obtained a value close to 1 ppm. If this is the case it is likely that nickel also might be poorly determined due to the fact that the value may be too close to the detection limits.

CHAPTER 4

CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH WORK

CHAPTER 4

CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH WORK

4.1 Conclusions

Tables 3-7 indicate the ppm values obtained for each of the metals Cu, Cd, Pb, Zn and Ni in the fish tissue by the differential pulse poloragraphic technique. Table 8 shows the values from both AA and dpp approaches. The quality of the differences were determined by subjecting the individual averages and standard deviations to the F and t tests.

For copper and nickel, the differences are none other than random. In the case of zinc, the difference in the averages shows significance to a very minor degree. For lead, the difference in the averages show a greater degree of significance and for cadmium, the difference is not at all random. On the basis of this it may be concluded that copper, nickel and zinc may be successfully determined in fish tissue, by the dpp technique. The differences encountered with lead and cadmium may be attributed to the fact that the levels of these metals are too close to the limits of detection by the dpp technique.

It must be stressed that the nickel values obtained during this analysis are usually high since that particular batch was exposed to high concentrations of nickel in the water and/or food. With normal fish the value is much lower (the De Luca study shows a value of 1.3 ± 0.2 ppm), in which case it is likely that nickel may also be poorly determined.

4.2 Suggestions for Future Research Work

In the conclusions drawn above, it has been shown that reliable results may not be obtained in the case of lead, cadmium and nickel by the dpp technique, owing to the fact that the levels of these metals in the fish tissue approach the limits of detection. Methods such as anodic stripping voltammetry (ASV) provide significant increases in signal strength by concentrating the metal of interest into a static mercury drop microelectrode before obtaining a reversed polarity stripping relationship. Based on the above reasoning it is recommended that future research work be carried out using ASV in the determination of Pb, Cd and Ni.

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APPENDIX A

A-1 Stock Solutions of Analyte Metals

The stock solutions involved the following as to metals, concentration and source. The uncertainty parameters have been assigned on the basis of normal laboratory preparative techniques.

Metal	Source	Correlation Cppml
Cadmium	Fisher Scientific So-C-118	1000.0±0.4
Copper	Fisher Scientific So-C-194	1000.0±0.4
Lead	Fisher Scientifc So-L-21	1000.0±0.4
Nickel	Fisher Scientific So-N-70	1000.0±0.4
Zinc	Fisher Scientific So-Z-13	1000.0±0.4

Copper

10.00 ± 0.02 ml of copper stock solution was diluted to 100.00 ± 0.88 ml to provide an auxilliary solution of 100.0 ± 0.3 ppm Cn. 10.00 ± 0.02 ml of the 100 ppm solution was then diluted to 100.00 ± 0.08 ml to provide a second auxilliary solution of 10.00 ± 0.06 ppm.

The 10 ppm solution was now used to prepare standard concentration solutions as follows:-

Volume of 10 ppm solution (ml)	Dilution volume (ml)	Concentration (ppm)
1.00 ± 0.01	100.00 ± 0.08	0.100 ± 0.002
2.00 ± 0.01	100.00 ± 0.08	0.200 ± 0.002
4.00 ± 0.01	100.00 ± 0.08	0.400 ± 0.003
6.00 ± 0.02	100.00 ± 0.08	0.600 ± 0.006
8.00 ± 0.02	100.00 ± 0.08	0.800 ± 0.006
10.00 ± 0.02	100.00 ± 0.08	1.000 ± 0.009

Cadmium

Dilution of the stock solution for cadmium was carried as for copper to yield an auxilliary solution of 10.00 ± 0.06 ppm. Since the cadmium determinations required the atomic absorption graphite furnace process, a further concentration reduction was obtained by taking 10.00 ± 0.02 ml of the 10 ppm solution and diluting it to 100.00 ± 0.08 ml. This yielded a solution of 1.000 ± 0.009 ppm.

The 1 ppm solution was now used to prepare standard concentration solutions as follows:-

Volume 1 ppm solution (ml)	Dilution volume (ml)	Concentration (ppm)
1.00 ± 0.01	100.00 ± 0.08	0.0100 ± 0.0002
2.00 ± 0.01	100.00 ± 0.08	0.0200 ± 0.0003
4.00 ± 0.01	100.00 ± 0.08	0.0400 ± 0.0005
6.00 ± 0.02	100.00 ± 0.08	0.0600 ± 0.0008
8.00 ± 0.02	100.00 ± 0.08	0.080 ± 0.001
10.00 ± 0.02	100.00 ± 0.08	0.100 ± 0.001

A spiking solution of 0.200 ± 0.002 ppm was prepared by taking 2.00 ± 0.001 ml of 10 ppm standard solution and diluting to 100.00 ± 0.08 ml.

Lead

Dilution of the stock solution for lead was carried as for copper down to an auxilliary solution of 10.00 ± 0.06 ppm.

The 10 ppm solution was now used to prepare the standard concentration solutions as follows:-

Volume 10 ppm solution (ml)	Dilution volume (ml)	Concentration (ppm)
0.50 ± 0.01	100.00 ± 0.08	0.050 ± 0.001
1.00 ± 0.01	100.00 ± 0.08	0.100 ± 0.002
2.00 ± 0.01	100.00 ± 0.08	0.200 ± 0.002
3.00 ± 0.01	100.00 ± 0.08	0.300 ± 0.003
4.00 ± 0.01	100.00 ± 0.08	0.400 ± 0.004
5.00 ± 0.01	100.00 ± 0.08	0.500 ± 0.004

Nickel

Dilution of the stock solution for nickel was carried out as for copper down to an auxilliary solution of 10.00 ± 0.06 ppm.

The 10 ppm solution was now used to prepare the standard concentration solutions as follows:-

Volumes 10 ppm solution (ml)	Dilution volume (ml)	Concentration (ppm)
1.00 ± 0.01	100.00 ± 0.08	0.100 ± 0.002
4.00 ± 0.01	100.00 ± 0.08	0.400 ± 0.003
8.00 ± 0.02	100.00 ± 0.08	0.800 ± 0.006
12.00 ± 0.04	100.00 ± 0.08	1.20 ± 0.01
16.00 ± 0.04	100.00 ± 0.08	1.60 ± 0.01
20.00 ± 0.04	100.00 ± 0.08	2.00 ± 0.02

A spiking solution of 3.00 ± 0.02 ppm was prepared by dilution 30.00 ± 0.04 ml of 10 ppm solution to 100.00 ± 0.08 ml.

Zinc

Dilution of the stock solution for zinc was carried out as for copper down to an auxilliary solution of 10.00 ± 0.06 ppm.

The 10 ppm solution was now used to prepare the standard concentration solutions as follows:-

Volume 10 ppm solution (ml)	Dilution volume (ml)	Concentration (ppm)
1.00 ± 0.01	100.00 ± 0.08	0.100 ± 0.002
2.00 ± 0.01	100.00 ± 0.08	0.200 ± 0.002
4.00 ± 0.01	100.00 ± 0.08	0.400 ± 0.003
6.00 ± 0.02	100.00 ± 0.08	0.600 ± 0.006
10.00 ± 0.02	100.00 ± 0.08	1.000 ± 0.009
12.00 ± 0.04	100.00 ± 0.08	1.20 ± 0.01

A spiking solution of 2.00 ± 0.02 ppm was prepared by diluting 20.00 ± 0.04 ml of 10 ppm solution to 100.00 ± 0.08 ml.

A-2 Determination of Cadmium by AA

The graphite furnace mode was applied for the determination of cadmium. The relevant operating parameters were:-

Cd hollow cathode lamp	228.8 nm
Slit	0.3 nm
Injection volume	20 µℓ
N_2 gas mode	normal
Drying temperature and time	120°C 20 sec
Charring terperature and time	250°C 30 sec
Atomizing temperature and time	2100°C 10 sec
Readout	Peak absorbance
Correction	D_2 beam

The standard solutions were injected into the graphite furnace and the peak absorbance values were obtained. Replicate injections were made and average peak absorbance values were calculated. The data obtained is shown in Table A-1 and the resultant plot is shown in Figure A-1.

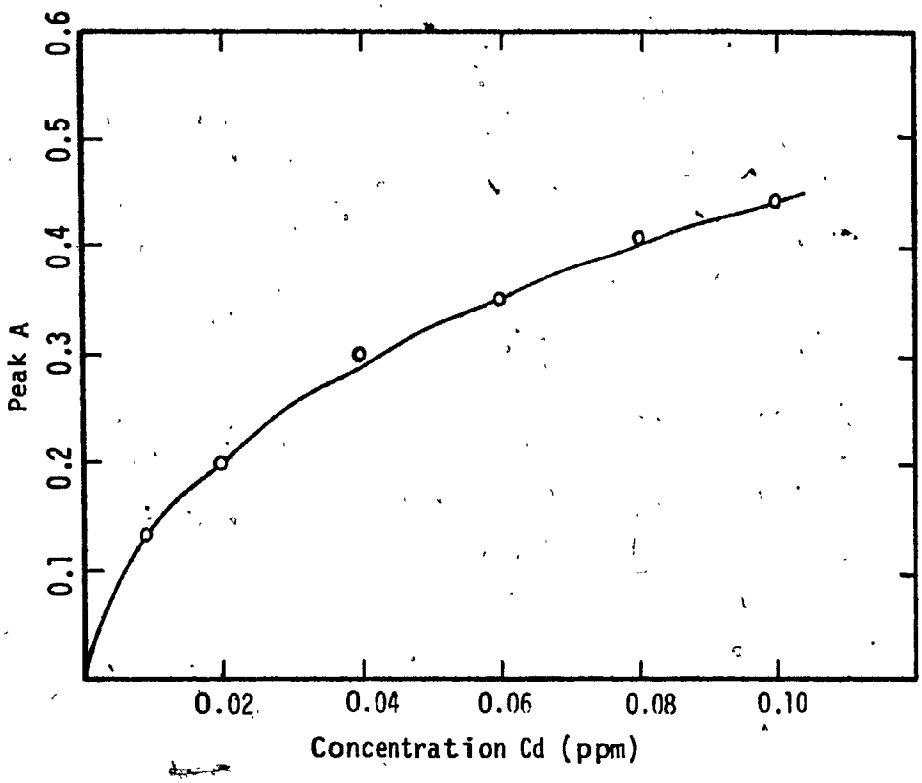

Figure A-1: Plot of Peak Absorbance versus Concentration for Standard Solutions of Cadmium.

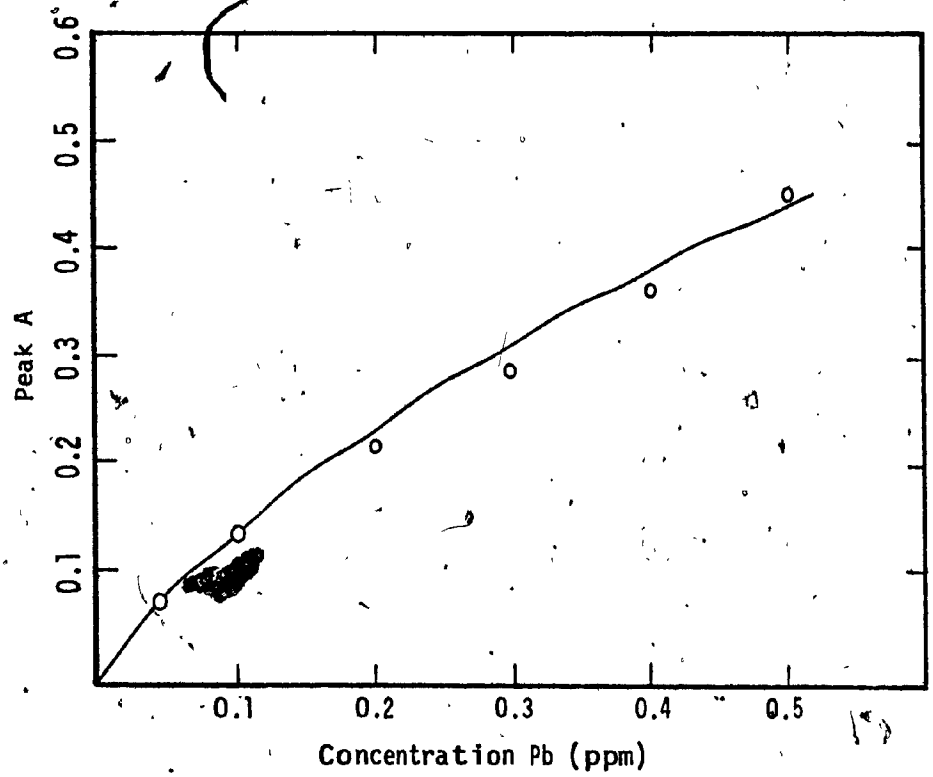

Figure A-2: Plot of Peak Absorbance versus Concentration for Standard Solutions of Lead.

TABLE A-1

Solution concentration ppm	Absorbance peaks (blank corrected)	Ave. peak absorbance
0.0100 ± 0.0002	0.154, 0.135, 0.134, 0.135	0.13^4
0.0200 ± 0.0003	0.200, 0.189, 0.190, 0.210, 0.204	0.199
0.0400 ± 0.0005	0.310, 0.302, 0.303	0.305
0.0600 ± 0.0008	0.366, 0.335, 0.349, 0.336	0.34^6
0.080 ± 0.001	0.408, 0.401, 0.408, 0.414	0.408
0.100 ± 0.001	0.449, 0.449, 0.453, 0.441	0.448

The testing of the fish tissue samples involved 20 µℓ injections from the 25.00 ± 0.04 ml solution representing 2.0000 ± 0.0002 g of fish tissue dry ashed and broken up as indicated in Section 3.2. These tests were carried on 6 samples of tissue and with replicate peak absorbance values. The average peak absorbances were calculated.

TABLE A-2

Sample	Absorbance peaks (blank corrected)	Ave. peak absorbance
1	0.055, 0.062, 0.055, 0.056	0.057 ± 0.003
2	0.054, 0.050, 0.050, 0.051	0.051 0.002
3	0.0.063, 0.055, 0.055, 0.048	0.055 0.006
4	0.046, 0.060, 0.047, 0.043	0.049 0.008
5	0.052, 0.054, 0.052, 0.054	0.053 0.001
6	0.063, 0.066, 0.062, 0.061	0.063 0.002

The average peak absorbance values and the plot of Figure A-1, yield solution concentration data, ignoring any matrix effects as follows:-

TABLE A-3

Sample	Ave. peak absorbance values	Solution concentration from the graph (ppm)
1	0.057 ± 0.003	0.0040 ± 0.0005
2	0.051 ± 0.002	0.0035 ± 0.0005
3	0.055 ± 0.006	0.0040 ± 0.0005
4	0.049 ± 0.008	0.0030 ± 0.0005
5	0.053 ± 0.001	0.0040 ± 0.0005
6	0.063 ± 0.002	0.0045 ± 0.0005

These solution concentration values can now be subjected to the calculation:-

$$\text{ppm Cd in fish tissue} = \frac{\text{solution conc. } (\mu g/ml) \times \text{solution volume } (ml)}{\text{sample weight } (g)}$$

$$= (\text{sample 1}) \quad \frac{0.0040 \pm 0.0005 \times 25.80 \pm 0.04}{2.0000 \pm 0.0002}$$

$$= 0.050 \pm 0.006 \text{ ppm}$$

Similar calculations for each sample yield individual values and an overall average and standard deviation of:-

TABLE A-4

Sample	ppm Cd in dried fish tissue
1	0.050 ± 0.006
2	0.043 ± 0.006
3	0.050 ± 0.006
4	0.038 ± 0.006
5	0.050 ± 0.006
6	0.056 ± 0.006

Overall average and standard deviation = 0.048 ± 0.006 ppm.

Each of the 6 samples taken were now treated to a spiking technique involving 4.00 ± 0.01 ml of original solution spiked with 1.00 ± 0.01 ml of 0.200 ± 0.002 ppm Cd standard solution. 20 µl volumes were injected for each spiked sample and the peak absorbances were read and averaged for multiple injections. The following data was obtained:-

TABLE A-5

Sample	Absorbance peaks (blank corrected)	Ave. peak absorbance
S_1	0.346, 0.340, 0.334, 0.330	0.338 ± 0.007
S_2	0.323, 0.331, 0.326, 0.334	0.328 ± 0.005
S_3	0.304, 0.319, 0.306, 0.309	0.310 ± 0.007
S_4	0.314, 0.328, 0.322, 0.320	0.321 ± 0.006
S_5	0.330, 0.333, 0.334, 0.325	0.330 ± 0.004
S_6	0.308, 0.312, 0.319, 0.319	0.314 ± 0.005

The concentration versus absorbance plot shown on Figure A-1 displays a non-linear relationship, so that matrix influences, if they exist, cannot be corrected for. Using Figure A-1 and Table A-5 values, the individual spiked solutions showed the following concentrations:-

TABLE A-6

Sample	Solu. concen. (ppm)
S_1	0.050^5
S_2	0.047^5
S_3	0.041^5
S_4	0.044^5
S_5	0.047^5
S_6	0.043^5

These solution concentrations for the 5 ml volume of spiked solution now yield, using sample S_1 as an example:-

Spiked soln. conc. = 0.050^5 µg/ml = 0.252^5 µg/5ml

Spike value = 1.00 ± 0.01 ml of 0.200 ± 0.002 ppm
= 0.200 ± 0.004 µg

Contribution of Cd from fish tissue = $0.252^5 - 0.200 \pm 0.004 = 0.052^5 \pm 0.004$ µg

Cd in dried fish tissue = $\frac{(0.052^5 \pm 0.004\ \mu g) \times (25.00 \pm 0.04\ ml)}{(4.00 \pm 0.01\ ml) \times (2.0000 \pm 0.0002\ g)}$

= 0.16 ± 0.01 ppm

Applying the same approach to the individual samples, the following data is obtained:-

TABLE A-7

Sample	ppm Cd in dried fish tissue
S_1	0.16 ± 0.01
S_2	0.12 ± 0.01
S_3	0.02 ± 0.01
S_4	0.07 ± 0.01
S_5	0.12 ± 0.01
S_6	0.05 ± 0.01

Overall average and standard deviation = 0.09 ± 0.05 ppm.

A-3 Determination of Copper by A.A.

The flame technique was applied in the determination of copper. The relevant parameters were:-

Cu hollow cathode lamp	324.8 nm
Slit	0.7 mm
Fuel	acetylene
Oxidant	air
Flame	lean
Read out	3-sec integration

The standard solutions were aspirated and the absorbance values were obtained. Replicate readings were obtained and averaged. Table A-8 shows the data obtained. The absorbance data showed a linear relationship and the linear equation was calculated.

TABLE A-8

Solution Concentration (ppm)	Absorbance blank corrected
0.100 ± 0.002	0.007 ± 0.000
0.200 ± 0.002	0.014 ± 0.001
0.400 ± 0.003	0.025 ± 0.000
0.600 ± 0.006	0.039 ± 0.000
0.800 ± 0.006	0.051 ± 0.001
1.000 ± 0.009	0.061 ± 0.001
Coefficient of correlation	r = 0.9989
Intercept on absorbance axis	b = 0.001
Slope	m = 0.0606

$$\text{Concentration (ppm)} = \frac{\text{absorbance} - b}{m} = \frac{A - 0.001}{0.0606}$$

The testing of fish tissue samples involved the aspiration of the 25.00 ± 0.04 ml solutions representing 2.0000 ± 0.0002 g of fish tissue dry ashed and taken-up as indicated in Section 3.2.

These tests were carried out on 6 samples of tissue and with replicate 3-sec integration absorbance readings. The average absorbance values and standard deviations are shown in Table A-9, together with the determination of the copper concentration for the solution as calculated from the linear equation.

TABLE A-9

Sample	Ave. blank-corrected absorbance ± s	Solution conc. Cu (ppm)
1	0.031 ± 0.001	$0.49^5 \pm 0.016$
2	0.029 ± 0.001	$0.46^2 \pm 0.015$
3	0.034 ± 0.000	$0.54^4 \pm 0.018$
4	0.026 ± 0.000	$0.41^2 \pm 0.014$
5	0.030 ± 0.000	$0.47^8 \pm 0.016$
6	0.029 ± 0.001	$0.46^2 \pm 0.015$

Using the formula:-

$$\text{ppm Cu in dried fish tissue} = \frac{\text{Soln. conc. } (\mu g/ml) \times 25.00 \pm 0.04 \text{ ml}}{2.0000 \pm 0.0002 \text{ g}}$$

The value of copper in dried fish tissue for each sample is shown in Table A-10.

TABLE A-10

Sample	ppm Cu in dried fish tissue
1	6.2 ± 0.2
2	5.8 ± 0.2
3	6.8 ± 0.2
4	5.2 ± 0.2
5	6.0 ± 0.2
6	5.8 ± 0.2

Overall average and standard deviation = 6.0 ± 0.5 ppm Cu.

Such determinations do not allow compensation for the effect of the solution matrix on the copper signal, if such exists, and a spiking technique was therefore carried to offset this effect. This involved taking 4.00 ± 0.01 ml of the original solution and spiking it with 1.00 ± 0.01 ml of a 2.00 ± 0.02 ppm standard Cu solution. These spiked solutions were now aspirated and replicate 3-sec integrated absorbance signals recorded. Table A-11 shows the data obtained:-

TABLE A-11

Sample	Ave. blank-corrected absorbance ± s
S_1	0.049 ± 0.001
S_2	0.048 ± 0.000
S_3	0.056 ± 0.001
S_4	0.046 ± 0.000
S_5	0.048 ± 0.000
S_6	0.047 ± 0.001

Using the calculation for samples S_1 as an example, the value of the original solution concentration of copper is now calculated from:

before spike 0.031 absorbance = X µg/ml

after spike 0.049 absorbance = $\frac{4X + 2}{5}$ µg/ml

∴ X µg/ml (original soln.) = $0.51^2 \pm 0.01^7$

Applying the formula:-

$$\text{ppm Cu in dried fish tissue} = \frac{\text{Soln. conc (µg/ml)} \times 25.00 \pm 0.04 \text{ ml}}{2.0000 \pm 0.0002 \text{ g}}$$

$$= 6.4 \pm 0.2$$

yields the value of Cu in dried fish tissue by the spiking methods, and this value should be free from any matrix effect. Table A-12 shows the final data obtained:-

TABLE A-12

Sample	Original solu. conc. Cu (µg/ml)	Cu in dried fish tissue (ppm)
S_1	$0.51^2 \pm 0.01^7$	6.4 ± 0.2
S_2	$0.46^7 \pm 0.01^5$	5.8 ± 0.2
S_3	$0.47^2 \pm 0.01^5$	5.9 ± 0.2
S_4	$0.41^2 \pm 0.01^3$	5.2 ± 0.2
S_5	$0.50^0 \pm 0.01^6$	6.2 ± 0.2
S_6	$0.48^7 \pm 0.01^6$	6.1 ± 0.2

Overall average and standard deviation = 6.1 ± 0.4 ppm.

A-4 The Determination of Lead by A.A.

The determination of lead was carried out by the graphite furnace technique. The relevant operating parameters were:-

Pb hollow cathode lamp	283.3 nm
Slit	0.7 mm
Injection volume	20 µl
N_2 gas mode	normal
Drying temperature and time	120°C 20 sec
Charring temperature and time	600°C 30 sec
Atomizing temperature and time	2200°C 10 sec
Readout	peak absorbance
Correction	D_2 beam

The standard solutions were injected and peak absorbance values were obtained. Replicate injections were made and the averages and standard deviations calculated. The data obtained

is shown in Table A-13 and the resultant plot of absorbance versus concentration is shown in Figure A-2.

TABLE A-13

Solution concentration (ppm)	Absorbance peaks (blank corrected)	Ave. peak absorbance
0.050 ± 0.001	0.073, 0.067, 0.068, 0.067	0.069 ± 0.003
0.100 ± 0.002	0.132, 0.136, 0.137, 0.140	0.136 ± 0.003
0.200 ± 0.002	0.219, 0.223, 0.211, 0.221	0.218 ± 0.005
0.300 ± 0.003	0.284, 0.283, 0.289, 0.279	0.284 ± 0.004
0.400 ± 0.004	0.373, 0.345, 0.393, 0.354	0.366 ± 0.021
0.500 ± 0.004	0.466, 0.459, 0.457, 0.473	0.464 ± 0.007

The testing of fish tissue samples involved 2.0000 ± 0.0002 g tissue dry ashed and taken-up as in Section 3.2. 20 µl volumes were injected to the furnace from the final 25.00 ± 0.04 ml volume. These tests were carried out on 6 samples of tissue and with replicate absorbance peak readings. The average peak absorbances were calculated.

TABLE A-14

Sample	Absorbance peaks (blank corrected)	Ave. peak absorbance
1	0.129, 0.117, 0.120, 0.123	0.122 ± 0.005
2	0.130, 0.125, 0.131, 0.128	0.128 ± 0.003
3	0.110, 0.111, 0.116, 0.120	0.114 ± 0.005
4	0.114, 0.119, 0.125, 0.134	0.123 ± 0.009
5	0.120, 0.130, 0.130, 0.124	0.126 ± 0.005
6	0.120, 0.119, 0.118, 0.119	0.119 ± 0.001

The average peak absorbance values and the plot of Figure A-2, yield solution concentration data, ignoring any matrix effect, of:-

TABLE A-15

Sample	Ave. peak absorbance values	Solution concentration from the graph (ppm)
1	0.122 ± 0.005	0.092^5 ± 0.003
2	0.128 ± 0.003	0.100^0 ± 0.003
3	0.114 ± 0.005	0.087^5 ± 0.003
4	0.123 ± 0.009	0.092^5 ± 0.003
5	0.126 ± 0.005	0.097^5 ± 0.003
6	0.119 ± 0.001	0.092^5 ± 0.003

These solution concentration values can now be subjected to the calculation:

$$\text{ppm Pb in dried tissue} = \frac{\text{Soln. conc. } (\mu g/ml) \times \text{soln. vol. (ml)}}{\text{Sample weight (g)}}$$

$$= (\text{Sample 1}) \frac{(0.092^5 \pm 0.003) \times (25.00 \pm 0.04 \text{ ml})}{2.0000 \pm 0.0002 \text{ g}}$$

$$= 1.16 \pm 0.03 \text{ ppm.}$$

Similar calculations for all samples yield the individual and overall data of:-

TABLE A-16

Sample	ppm Pb in dried fish tissue
1	1.16 ± 0.04
2	1.25 ± 0.04
3	1.09 ± 0.03
4	1.16 ± 0.04
5	1.22 ± 0.04
6	1.16 ± 0.04

Overall average and standard deviation = 1.17 ± 0.06 ppm.

Each of the six samples was now spiked using 4.00 ± 0.01 ml of original solution and 1.00 ± 0.01 ml of 0.500 ± 0.004 ppm standard lead solution. 20 µl volumes were injected, replicate peak absorbance value obtained and associated average values were calculated.

TABLE A-17

Sample	Absorbance peaks (blank corrected)	Ave. peak absorbance
S_1	0.190, 0.200, 0.201, 0.189	0.195 ± 0.006
S_2	0.209, 0.200, 0.196, 0.195	0.200 ± 0.006
S_3	0.194, 0.198, 0.199, 0.201	0.198 ± 0.003
S_4	0.220, 0.210, 0.209, 0.225	0.216 ± 0.008
S_5	0.196, 0.207, 0.203, 0.194	0.200 ± 0.006
S_6	0.210, 0.201, 0.202, 0.206	0.206 ± 0.004

The concentration versus absorbance plot shown in Figure A-2 does not indicate a quite linear relationship, so that solution matrix effects can not be corrected for by a simple calculation. Using Figure A-2 and Table A-17 values, the individual spiked solutions showed Pb concentrations of:-

TABLE A-18

Sample	Soln. conc. Pb (ppm)
S_1	0.183
S_2	0.177
S_3	0.181
S_4	0.179
S_5	0.176
S_6	0.181

These solution concentrations for the 5 ml spiked solution volume now yield, using sample S_1 as an example:-

Spiked soln. concen. = 0.183 µg/ml = 0.915 µg/5 ml

Spike value = 1.00 ± 0.01 ml + 0.500 ± 0.004 ppm Pb
= 0.50 ± 0.01 µg

Contribution of Pb from fish tissue = 0.915 - 0.50 ± 0.01 = 0.41^5 ± 0.01 µg

Pb in dried fish tissue = $\frac{(0.41^5 \pm 0.01\ \mu g) \times (25.00 \pm 0.04\ ml)}{(4.00 \pm 0.01\ ml) \times (2.0000 \pm 0.0002\ g)}$

= 1.30 ± 0.03 ppm.

Applying the same calculation mode to the Table A-18 data:-

TABLE A-19

Sample	ppm Pb in dried fish tissue
S_1	1.30 ± 0.03
S_2	1.20 ± 0.03
S_3	1.26 ± 0.03
S_4	1.23 ± 0.03
S_5	1.19 ± 0.03
S_6	1.26 ± 0.03

Overall average and standard deviation = 1.24 ± 0.04 ppm.

A-5 The Determination of Nickel by A.A.

The determination of nickel was carried out by the flame method. The relevant operating parameters were:-

Ni Hollow cathode lamp	232.0 nm
Slit	0.2 mm
Fuel	acetylene
Oxidant	air
Flame	lean
Readout	3-sec integration

The standard solutions were aspirated and the absorbance values obtained. Replicate readings were obtained and averaged Table A-20 shows the data obtained. The absorbance data showed a linear relationship and the linear equation was calculated.

TABLE A-20

Solution concentration (ppm)	Absorbance (blank corrected)
0.100 ± 0.002	0.004 ± 0.001
0.400 ± 0.003	0.016 ± 0.000
0.800 ± 0.006	0.032 ± 0.001
1.20 ± 0.01	0.050 ± 0.001
1.60 ± 0.01	0.066 ± 0.000
2.00 ± 0.02	0.080 ± 0.001

Coefficient of correlation $r = 0.995$

Intercept on absorbance axis $b = 0.000$

Slope $m = 0.0406$

$$\text{Concentration (ppm)} = \frac{\text{absorbance} - b}{m} = \frac{A - 0.000}{0.0406}$$

Fish samples were tested by preparing a final volume of 25.00 ± 0.04 ml of solution representing 2.0000 ± 0.0002 g of tissue dry ashed and taken-up as outlined in Section 3.2. Initial tests showed a high nickel value and, to maintain a test concentration within the linear range, 2.00 ± 0.01 ml of original solution was diluted to 10.00 ± 0.02 ml. Six samples were so prepared and aspirated. The average absorbance values were obtained from replicate 3-sec integrated values. Table A-21 shows the recorded data, together with the value of nickel in each diluted solution and in the original solution.

TABLE A-21

Sample	Ave. blank-corrected absorbance ±.5	Diluted soln. conc. Ni (ppm)	Original soln. conc. Ni (ppm)
1	0.026 ± 0.001	0.64 ± 0.02	3.2^0 ± 0.1
2	0.030 ± 0.000	0.74 ± 0.02	3.7^0 ± 0.1
3	0.028 ± 0.002	0.69 ± 0.02	3.4^5 ± 0.1
4	0.027 ± 0.001	0.66 ± 0.02	3.3^0 ± 0.1
5	0.028 ± 0.000	0.69 ± 0.02	3.4^5 ± 0.1
6	0.031 ± 0.000	0.76 ± 0.02	3.8^0 ± 0.1

Using the formula:-

$$\text{ppm Ni in dried fish tissue} = \frac{\text{orig. soln. conc. } (\mu g/ml) \times 25.00 \pm 0.04 \text{ ml}}{2.0000 \pm 0.0002 \text{ g}}$$

The value of nickel in dried tissue for each sample was calculated as:-

TABLE A-22

Sample	ppm Ni in dried fish tissue
1	40 ± 1
2	46 ± 1
3	43 ± 1
4	41 ± 1
5	43 ± 1
6	48 ± 1

Overall average and standard deviation = 44 ± 3.

The foregoing determination does not allow for the correction of any matrix effect if this exists, and a spiking technique was therefore provided to minimize this effect. 4.00 ± 0.01 ml of the 1:5 aliquoted solution was spiked with 1.00 ± 0.01 ml of 3.00 ± 0.02 ppm of standard nickel solution. These solutions were

aspirated and replicate 3-sec integrated absorbance signals recorded.

TABLE A-23

Sample	Ave. blank corrected absorbance ± 5
S_1	0.044 ± 0.001
S_2	0.048 ± 0.000
S_3	0.045 ± 0.000
S_4	0.046 ± 0.000
S_5	0.047 ± 0.001
S_6	0.050 ± 0.002

With sample S_1 as the example, the value of the Ni concentration in the spiked solution is calculated from:

diluted soln. before spike 0.026 absorbance = X µg/ml

diluted soln. after spike 0.044 absorbance = $\frac{4X + 3}{5}$ µg/ml

$\therefore$ X µg/ml (diluted solu.) = $0.67^2 \pm 0.01^6$

Applying the formula:-

$$\text{ppm Ni in dried fish tissue} = \frac{\text{dil. soln. conc. (µg/ml)} \times \text{aliq. fact.} \times \text{orig. sol. vol.}}{\text{sample weight}}$$

$$= \frac{(0.67^2 \pm 0.01^6) \times 5 \times (25.00 \pm 0.04\ \text{ml})}{2.0000 \pm 0.0002\ \text{g}}$$

$$= 42 \pm 1$$

This yields the value of Ni in dried fish tissue from the spiking technique, and this value should be free from any matrix effect.

Table A-24 shows the final data.

TABLE A-24

Sample	Diluted solu. concen. Ni (ppm)	Ni in dried fish tissue (ppm)
S_1	$0.67^2 \pm 0.01^6$	42 ± 1
S_2	$0.75^0 \pm 0.01^9$	47 ± 1
S_3	$0.74^3 \pm 0.01^8$	46 ± 1
S_4	$0.66^3 \pm 0.01^6$	41 ± 1
S_5	$0.68^3 \pm 0.01^7$	43 ± 1
S_6	$0.73^8 \pm 0.01^8$	46 ± 1

Overall average and standard deviation = 44 ± 2 ppm.

Note that, for the absorbance readings on the spiked solution, a difference of only ±0.001 units results in a difference of ±2 ppm Ni in the dried fish tissue.

A-6 Determination of Zinc by A.A.

The flame technique was used in the determination of zinc. The relevant parameters were:-

Zn hollow cathode lamp	213.9 nm
Slit	0.7 mm
Fuel	acetylene
Oxidant	air
Flame	lean
Readout	3-sec integration

The zinc standard solutions were aspirated and replicate 3-sec integrated absorbance values were obtained and averaged. Table A-25 shows the data, and a linear equation was calculated.

TABLE A-25

Solution Concentration (ppm)	Absorbance blank corrected
0.100 ± 0.002	0.013 ± 0.002
0.200 ± 0.002	0.028 ± 0.000
0.400 ± 0.003	0.059 ± 0.001
0.600 ± 0.006	0.079 ± 0.001
1.000 ± 0.009	0.128 ± 0.000
1.20 ± 0.01	0.154 ± 0.000

Coefficient of correlation $r = 0.9987$

Intercept on absorbance axis $b = 0.003$

Slope $m = 0.1258$

$$\text{Concentration (ppm)} = \frac{\text{absorbance} - b}{m} = \frac{A - 0.003}{0.1258}$$

Fish samples were tested by preparing a final volume of 25.00 ± 0.04 ml of solution representing 2.0000 ± 0.0002 g of tissue dry ashed and taken-up as indicated in Section 3.2. Initial tests showed a high zinc value and, to maintain a test concentration in the linear range, 1.00 ± 0.01 ml of original solution was diluted to 20.00 ± 0.02 ml. Six samples were prepared and aspirated. The replicate 3-sec absorbance values were used to obtain approriate averages. Table A-26 shows the recorded data, together with the value of zinc in each diluted solution and in the original solution.

TABLE A-26

Sample	Ave. blank-corrected absorbance ± 5	Diluted soln. conc. Zn (ppm)	Original soln. conc. Zn (ppm)
1	0.067 ± 0.001	0.508 ± 0.008	$10.1^{6} \pm 0.1^{6}$
2	0.069 ± 0.000	0.524 ± 0.008	$10.4^{8} \pm 0.1^{7}$
3	0.067 ± 0.002	0.508 ± 0.008	$10.1^{6} \pm 0.1^{6}$
4	0.066 ± 0.000	0.501 ± 0.008	$10.0^{2} \pm 0.1^{6}$
5	0.064 ± 0.001	0.484 ± 0.008	$9.6^{8} \pm 0.1^{6}$
6	0.064 ± 0.000	0.484 ± 0.008	$9.6^{8} \pm 0.1^{6}$

Using the formula:-

$$\text{ppm Zn in dried fish tissue} = \frac{\text{Soln. conc. } (\mu g/ml) \times 25.00 \pm 0.04 \text{ ml}}{2.0000 \pm 0.0002 \text{ g}}$$

The ppm Zn in dried fish tissue for each sample was calculated.

TABLE A-27

Sample	ppm Zn in dried fish tissue
1	127 ± 2
2	131 ± 2
3	127 ± 2
4	125 ± 2
5	121 ± 2
6	121 ± 2

Overall average and standard deviation = 125 ± 4 ppm.

The foregoing does not allow compensation for any matrix effect, although this should ne negligible relative to the high degree of solution dilution for the original solution. Nevertheless, a spiking method was applied involving 4.00 ± 0.01 ml of the 1:20 diluted solution and 1.00 ± 0.01 ml of a 2.00 ± 0.02 ppm standard

Zn solution. These spiked solutions were aspirated and the usual replicate data recorded.

TABLE A-28

Sample	Ave. blank-corrected absorbance ± S
S_1	0.106 ± 0.001
S_2	0.109 ± 0.002
S_3	0.107 ± 0.001
S_4	0.105 ± 0.000
S_5	0.102 ± 0.000
S_6	0.100 ± 0.000

With sample S_1 as the example, the value of Zn in the spiked solution was calculated.

Dilute soln. before spike 0.067 absorbance = X µg/ml

Dilute soln. after spiking 0.106 absorbance = $\frac{4X + 2}{5}$ µg/ml

$\therefore$ X = 0.511 ± 0.008 µg/ml (diluted solu.)

Applying the formula:-

$$\text{ppm Zn in dried fish tissue} = \frac{\text{dil. soln. conc. (µg/ml) x aliq. fact. x orig. soln. vol.}}{\text{sample weight}}$$

$$= \frac{(0.511 \pm 0.008) \times 20 \times (25.00 \pm 0.04)}{2.0000 \pm 0.0002}$$

$$= 128 \pm 2$$

yields the value for ppm Zn in dried fish from the spiking technique.

TABLE A-29

Sample	Diluted soln. conc. Zn (ppm)	Zn in dried fish tissue (ppm)
S_1	0.511 ± 0.008	128 ± 2
S_2	0.513 ± 0.008	128 ± 2
S_3	0.502 ± 0.008	125 ± 2
S_4	0.506 ± 0.008	126 ± 2
S_5	0.503 ± 0.008	126 ± 2
S_6	0.524 ± 0.008	131 ± 2

Overall average and standard deviation = 127 ± 2

Note that, for the absorbance readings on the spiked solutions, a difference of only ±0.001 units results in a difference of ±3 ppm Zn in dried fish tissue.

B-1 Preparation of Supporting Electrolyte

0.2M (3 pH) Ammonium Citrate

42.00 ± 0.01 g of Baker Analyzed Reagent citric acid was dissolved in 800 ml of glass-distilled water. A pH meter, combination glass/SCE electrode and a 3.00 ± 0.02 pH buffer solution were used for calibration purposes. After calibration, the glass/SCE combination electrode was immersed in the citric acid solution. Aqueous ammonia was now added until a pH of 3.0 ± 0.1 was obtained. The solution was then diluted to 1000 ml.

Preparation of Supporting Electrolyte 2M KCN

130.0 ± 0.1 g of Baker Analyzed Reagent potassium cyanide was dissolved in 500 ml of glass-distilled water and diluted to 1000 ml.

B-2 Scan of Supporting Electrolytes to Determine Peaks for Trace Metal Analytes

0.2M (3 pH) Ammonium Citrate

Scan Parameters

Mode	dpp
Sensitivity	1.5×10^{-10} A/mm
Scan Range	+0.2 to -2.00 V
Start/Stop	+0.14 to -1.176 V
t drop	0.4 sec
mm/t drop	0.5 mm
Pulse Voltage	-25 mV
N_2 Degas	15 mins
Working Electrode	D.M.E.
Auxilliary Electrode	Pt
Reference Electrode	Ag/AgCl (Sat. KCl)

APPENDIX B

2M KCN

Scan Parameters

Mode	dpp
Sensitivity	1.5×10^{-10} A/mm
Scan Range	+0.2 to -2.00 V
Start/Stop	-0.8 to -1.70 V
t Drop	0.4 sec
mm/t drop	0.5 mm
Pulse Voltage	-25 mV
N_2 Degas	15 mins
Working Electrode	D.M.E.
Auxilliary Electrode	Pt
Reference Electrode	Ag/AgCl (Sat. KCl)

The divisions (mm) of chart for the peak values at the respective $E_{1/2}$ values are shown in Table B-1.

TABLE B-1

Metal	Reaction	Chart div. $E_{1/2}$ peak or mm.
Cu	$Cu^{2+} + 2e \rightleftarrows Cu$	1.0 (2.0 mm)
Pb	$Pb^{2+} + 2e \rightleftarrows Pb$	2.0 (4.0 mm)
Cd	$Cd^{2+} + 2e \rightleftarrows Cd$	0.8 (1.6 mm)
Zn	$Zn^{2+} + 2e \rightleftarrows Zn$	1.5 (3.0 mm)
Ni	$Ni^{2+} + 2e \rightleftarrows Ni$	0.5 (1.0 mm)

B-3 Preparation of Standard Solutions for Sensitivity and Spiking Tests

Glass-distilled water was used throughout the dilution procedures.

Cadmium

10.00 ± 0.02 ml of cadmium stock solution was diluted to 100.00 ± 0.08 ml to provide an auxiliary solution of 100.0 ± 0.3 ppm Cd. 1.00 ± 0.01 ml of the 100 ppm solution was then diluted to 100.00 ± 0.08 ml to provide a solution of 1.00 ± 0.01 ppm Cd.

Copper

10.00 ± 0.02 ml of copper stock solution was diluted to 100.00 ± 0.08 ml to provide an auxiliary solution of 100.0 ± 0.3 ppm Cu. 10.00 ± 0.02 ml of the 100 ppm solution was diluted to 100.00 ± 0.08 ml to provide a 10.00 ± 0.05 ppm Cu colution.

Lead

10.00 ± 0.02 ml of lead stock solution was diluted to 100.00 ± 0.08 ml to provide an auxiliary solution of 100.0 ± 0.3 ppm Pb. 10.00 ± 0.02 ml of the 100 ppm solution was diluted to 100.00 ± 0.08 ml to provide a 10.00 ± 0.05 ppm Pb solution.

Nickel

10.00 ± 0.02 ml of nickel stock solution was diluted to 100.00 ± 0.08 ml to provide an auxiliary solution of 100.0 ± 0.3 ppm Ni. 20.00 ± 0.04 ml of the 100 ppm solution was diluted to 100.00 ± 0.08 ml to provide a solution of 20.0 ± 0.1 ppm Ni.

Zinc

10.00 ± 0.02 ml of stock zinc solution was diluted to 100.00 ± 0.08 ml to provide an auxiliary solution of 100.0 ± 0.3 ppm Zn. 10.00 ± 0.02 ml of the 100 ppm solution was diluted to

100.00 ± 0.08 ml to yield a solution of 10.00 ± 0.05 ppm Zn.

B-4 Preparation of Synthetic Solutions for Sensitivity Tests Related to dpp

Copper, Lead, Cadmium and Zinc:

The following additions were made to 10 ml of the supporting electrolyte 0.2M ammonium citrate. The solution was then diluted to 18.00 ± 0.04 ml. The zinc solution was treated separately to a final volume of 20.00 ± 0.04 ml.

Cu:- 0.900 ± 0.005 ml of 10.00 ± 0.05 ppm = 0.500 ± 0.006 ppm

Pb:- 0.300 ± 0.005 ml of 10.00 ± 0.05 ppm = 0.167 ± 0.004 ppm

Cd:- 0.400 ± 0.005 ml of 1.00 ± 0.01 ppm = 0.0222 ± 0.0004 ppm

Zn:- 2.50 ± 0.01 ml of 1.00 ± 0.01 ppm = 0.125^{0} ± 0.001^{7} ppm

Nickel:

The following addition was made to 10 ml of 2M KCN supporting electrolyte. The solution was then diluted to 25.00 ± 0.04 ml.

Ni:- 6.25 ± 0.01 ml of 20.0 ± 0.1 ppm = 5.00^{0} ± 0.04^{3} ppm

B-5 Sensitivity Tests for the Metal Analytes

The 0.2M ammonium citrate solution was tested progressively starting at the least negative $E_{1/2}$ value (i.e. Cu-Pb-Cd-Zn). The 2M KCN solution was tested for Ni only. The 0.2M solution was tested as is for copper and retested after a copper spike. This solution was then tested for lead and retested after a lead spike. The procedure was repeated for zinc. The 2M solution was tested as is for nickel and retested after a nickel spike. Each

system was run in triplicate.

Copper

Sensitivity	6×10^{-10} A/mm
Blank	0.25 div (0.5 mm)
Range	+0.2 to -2.00 V
Start/Stop	+0.112 to -0.256 V
t drop	0.4 sec
mm/t drop	0.5
Pulse Voltage	-25 mV
N_2 Degas	15 mins
Working Electrode	D.M.E.
Auxiliary Electrode	Pt
Reference Electrode	Ag/AgCl (Sat. KCl)

Start:- Cu = 0.9 ml/18 ml x 10 ppm = 0.500 ± 0.006 ppm

Peak:- (1) 9.50 - 0.25 = 9.25 div = 18.5 mm

(2) 9.75 - 0.25 = 9.50 div = 19.0 mm

(3) 9.25 - 0.25 = 9.00 div = 18.0 mm

Ave:- 9.25 ± 0.25 div = 18.5 ± 0.5 mm.

Spike:- 0.400 ± 0.005 ml of 10.00 ± 0.05 ppm (total volume 18.40 ± 0.04 ml)

Cu = 0.706^5 ± 0.09^8 ppm.

Peak:- (1) 13.50 - 0.25 = 13.25 div = 26.5 mm

(2) 13.50 - 0.25 = 13.25 div = 26.5 mm

(3) 13.75 - 0.25 = 13.50 div = 27.0 mm

Ave:- 13.33 ± 0.14 div = 26.7 ± 0.3 mm.

Sensitivity at 6×10^{-10} A/mm given by:

Start:- $\frac{0.500 \pm 0.006 \text{ ppm}}{18.5 \pm 0.5 \text{ mm}} = 0.0270 \pm 0.0009$ ppm/mm

Spike:- $\frac{0.706^{5} \pm 0.009^{8} \text{ ppm}}{26.7 \pm 0.3 \text{ mm}} = 0.0265 \pm 0.0006$ ppm/mm

Sensitivity at 1.5×10^{-10} A/mm = 0.0066 ± 0.0002 ppm/mm

$E_{1/2}$ (Cu) = -0.0614, -0.0600, -0.0558 = -0.059 ± 0.003 V vs Ag/AgCl (Sat. KCl)

Lead

Sensitivity	1.5×10^{-10} A/mm
Blank	2.0 div (4.0 mm)
Range	-0.2 to -2.00 V
Start/Stop	-0.2 to -0.576 V
t drop	0.4 sec
mm/t drop	0.5
Pulse Voltage	-25 mV
N_2 Degas	15 mins
Working Electrode	D.M.E.
Auxiliary Electrode	Pt
Reference Electrode	Ag/AgCl (Sat. KCl)

Start:- Pb = 0.3 ml/18.4 ml x 10 ppm x 0.163 ± 0.004 ppm

Peak:- (1) 11.0 - 2.0 = 9.0 div = 18.0 mm

(2) 11.0 - 2.0 = 9.0 div = 18.0 mm

(3) 10.0 - 2.0 = 8.0 div = 10.0 mm

Ave:- $8.6^{6} \pm 0.5^{7}$ div = $17.^{3} \pm 1.^{1}$ mm.

Spike:- 0.150 ± 0.005 ml of 10.00 ± 0.05 ppm (total volume 18.55 ± 0.04 ml)

Pb = $0.24^{2} \pm 0.01^{1}$ ppm

Peak:- (1) 16.0 - 2.0 = 14.0 div = 28.0 mm

(2) 16.5 - 2.0 = 14.5 div = 29.0 mm

(3) 15.5 - 2.0 = 13.5 div = 27.0 mm

Ave:- 14.0 ± 0.5 div = $28.^{0} \pm 1.^{0}$ mm.

Sensitivity at 1.5×10^{-10} A/mm given by:

Start:- $\frac{0.163 \pm 0.004 \text{ ppm}}{17.^{3} \pm 1.^{1} \text{ mm}} = 0.0094^{2} \pm 0.0005^{6}$ ppm/mm

Spike:- $\frac{0.24^{2} \pm 0.01^{1} \text{ ppm}}{28.^{0} \pm 1.^{0} \text{ mm}} = 0.0086^{4} \pm 0.0006^{5}$ ppm/mm

Sensivitity at 1.5×10^{-10} A/mm = 0.0090 ± 0.0005 ppm/mm

E 1/2 (Pb) = -0.372, -0.368, -0.360 = -0.367 ± 0.006 V
vs Ag/AgCl (Sat. KCl)

Cadmium

Sensitivity	1.5×10^{-10} A/mm
Blank	0.8 div (1.6 mm)
Range	+0.2 to -2.00 V
Start/Stop	-0.4 to -0.712 V
t drop	0.4 sec
mm/t drop	0.5
Pulse Voltage	-25 mV
N_2 Degas	15 mins
Working Electrode	D.M.E.
Auxiliary Electrode	Pt
Reference Electrode	Ag/AgCl (Sat. KCl)

Start:- Cd = 0.4 ml/18.55 + 1.00 = 0.0216 ± 0.0004 ppm

Peak:- (1) 3.5 - 0.8 = 2.7 div = 5.4 mm

(2) 3.2 - 0.8 = 2.4 div = 4.8 mm

(3) 3.8 - 0.8 = 3.0 div = 6.0 mm

Ave:- 2.7 ± 0.3 div = 5.4 ± 0.6 mm.

Spike: 0.200 ± 0.005 ml of 1.00 ± 0.01 ppm (total volume = 18.75 ± 0.04 ml)

Cd = 0.0320 ± 0.0006 ppm

Peak:- (1) 4.8 - 0.8 = 4.0 div = 8.0 mm.

(2) 4.5 - 0.8 = 3.7 div = 7.4 mm

(3) 5.0 - 0.8 = 4.2 div = 8.4 mm

Ave:- $3.9^{7} \pm 0.2^{5}$ div = $7.9^{4} \pm 0.5^{0}$ mm.

Sensitivity at 1.5×10^{-10} A/mm given by:

Start:- $\frac{0.0216 \pm 0.0004 \text{ ppm}}{5.4 \pm 0.6 \text{ mm}} = 0.0040 \pm 0.0004$ ppm/mm

Spike:- $\frac{0.0320 \pm 0.0006 \text{ ppm}}{7.9^{4} \pm 0.5^{0}} = 0.0040 \pm 0.0003$ ppm/mm

Sensitivity at 1.5×10^{-10} A/mm = 0.0040 ± 0.0004 ppm/mm

$E_{1/2}$ = -0.563, -0.572, -0.571, = -0.569 ± 0.0005 V vs Ag/AgCl (Sat.KCl)

Zinc

Sensitivity	1.5×10^{-10} A/mm
Blank	1.5 div (3.0 mm)
Range	+0.2 to -2.00 V
Start/Stop	-0.8 to -1.176 V
t drop	0.4 sec
mm/t drop	0.5
Pulse Voltage	-25 mV
N_2 Degas	15 mins
Working Electrode	D.M.E.
Auxiliary Electrode	Pt
Reference Electrode	Ag/AgCl (Sat. KCl)

Start:- $Zn = 2.5/20 \times 1.00 = 0.125^{0} \pm 0.001^{7}$ ppm

Peak:- (1) 14.0 - 1.5 = 12.5 div = 25.0 mm

(2) 14.5 - 1.5 = 13.0 div = 26.0 mm

(3) 13.8 - 1.5 = 12.3 div = 24.6 mm

Ave:- $12.6^{0} \pm 0.3^{6}$ div = 25.2 ± 0.7 mm

Spike:- 1.00 ± 0.01 ml of 1.00 ± 0.01 ppm (total volume 21.00 ± 0.04 ml)

$Zn = 0.166^{7} \pm 0.006^{6}$ ppm

Peak:- (1) 19.2 - 1.5 = 17.7 div = 35.4 mm

(2) 19.0 - 1.5 = 17.5 div = 35.0 mm

(3) 19.8 - 1.5 = 18.3 div = 36.6 mm

Ave:- $17.8^{3} \pm 0.4^{1}$ div = 35.7 ± 0.8 mm.

Sensitivity at 1.5×10^{-10} A/mm given by:

Start:- $\frac{0.125^{6} \pm 0.001^{7} \text{ ppm}}{25.2 \pm 0.7 \text{ mm}} = 0.0050 \pm 0.0002$ ppm/mm

Spike:- $\frac{0.166^{7} \pm 0.006^{6} \text{ ppm}}{35.7 \pm 0.8 \text{ mm}} = 0.0047 \pm 0.0002$ ppm/mm

Sensitivity at 1.5×10^{-10} A/mm = 0.0048 ± 0.0002 ppm/mm

$E_{1/2}$ = -0.965, -0.975, -0.965 = -0.968 ± 0.006 V vs Ag/AgCl (Sat. KCl)

Nickel

Sensitivity	6×10^{-9} A/mm
Blank	0.01 div (negligible)
Range	+0.2 to -2.00 V
Start/Stop	-0.8 to -1.70 V
t drop	0.4 sec
mm/t drop	0.5
Pulse Voltage	-25 mV

N_2 Degas	15 mins
Working Electrode	D.M.E.
Auxiliary Electrode	Pt
Reference Electrode	Ag/AgCl (Sat. KCl)

Start:- 6.25/25 x 20 = 5.00 ± 0.04 ppm

Peak:- (1) 12.5 div = 25.0 mm

(2) 12.2 div = 24.4 mm

(3) 12.8 div = 25.6 mm

Ave:- 12.5 ± 0.3 div = 25.0 ± 0.6 mm

Spike: 5.00 ± 0.01 ml of 20.0 ± 0.1 ppm (total volume = 30.00 ± 0.04 ml)

Ni = 7.50 ± 0.08 ppm

Peak:- (1) 18.8 div = 37.6 mm

(2) 18.8 div = 37.6 mm

(3) 19.0 div = 38.0 mm

Ave:- 18.9 ± 0.1 div = 37.8 ± 0.2 mm

Sensitivity at 6 x 10^{-9} A/mm given by:

Start:- $\frac{5.00 \pm 0.04 \text{ ppm}}{25.0 \pm 0.6 \text{ mm}}$ = 0.200 ± 0.007 ppm/mm

Spike:- $\frac{7.50 \pm 0.08 \text{ ppm}}{37.8 \pm 0.2 \text{ mm}}$ = 0.198 ± 0.002 ppm/mm

Sensitivity at 6 x 10^{-9} A/mm = 0.199 ± 0.007 ppm/mm

Sensitivity at 1.5 x 10^{-10} A/mm = 0.0050 ± 0.0002 ppm/mm

E 1/2 = -1.338, -1.339, -1.336 = -1.338 ± 0.002 V vs Ag/AgCl (Sat.KCl)

TABLE B-2

Metal	Sensitivity at 1.5×10^{-10} A/mm Based on 1 mm (ppm)	$E_{1/2}$ vs Ag/AgCl	$E_{1/2}$* (Actual)
Cu	0.007	-0.059	-0.071
Pb	0.009	-0.367	-0.39
Cd	0.004	-0.569	-0.571
Zn	0.005	-0.968	-1.00
Ni	0.005	-1.338	-1.32

B-6 Analysis of Fish Tissue by dpp for the Metal Analytes

Six samples of dried fish tissue were prepared by weighing 2.0000 ± 0.0002 g and subjecting them generally to the dry ashing and taken-up techniques referred to in Section 3.2. Three such sets were prepared, the purpose and final dilutions being as noted. Blank samples were carried along with each set.

Set 1	Cu, Pb, Cd,	30.00 ± 0.04 ml final dilution with 0.2M amm. cit.
Set 2	Zn	20.00 ± 0.04 ml final dilution with 0.2M amm. cit.
Set 3	Ni	20.00 ± 0.04 ml final dilution with 2M KCN

Copper

For each sample, 28 ml of blank and original solution were charged to polarographic cells and tested as-is and spiked at the following parameters (the blank was not spiked).

*As given by: L. Meites, "Polarographic Techniques", Interscience, N.Y., 2nd Edition, 1967.

Sensitivity	6×10^{-10} A/mm
Range	+0.2 to -2.00 V
Start/Stop	+0.14 to -0.308 V
t drop	0.4 sec
mm/t drop	0.5
Pulse Voltage	-32 mV
N_2 Degas	15 min
Working Electrode	D.M.E.
Auxiliary Electrode	Pt
Reference Electrode	Ag/AgCl (Sat. KCl)

The results obtained were:-

#1 as-is:- 7.8 - 0.25 blank = 7.55 div = 15.1 mm = X µg/ml

Spike:- $$\frac{28.00 \pm 0.04 \text{ ml} \times X \text{ µg/ml} + (1.00 \pm 0.01 \text{ ml} \times 10.00 \pm 0.05 \text{ ppm})}{29.00 \pm 0.04 \text{ ml}}$$

$$= \frac{28\,X + 10}{29} \text{ µg/ml for } 13.8 - 0.25 \text{ div} = 13.55 \text{ div} = 27.1 \text{ mm}$$

$\therefore$ X = 0.415 ± 0.004 µg/ml original solution

$\therefore$ $12.4^5 \pm 0.1^2$ µg/30 ml original solution

$$\frac{12.4^5 \pm 0.1^2 \text{ µg}}{2.0000 \pm 0.0002 \text{ g}} = \frac{6.22 \pm 0.06 \text{ µg}}{\text{g}}$$

$\therefore$ 6.22 ± 0.06 ppm Cu in dried fish tissue

#2 7.8 - 0.25 = 7.55 div = 15.1 mm = X µg/ml

$$\frac{28\,X + 10}{29} \text{ µg/ml for } 14.0 - 0.25 = 13.75 \text{ div} = 27.5 \text{ mm.}$$

X = 0.403 ± 0.004 µg/ml original solution

$\therefore$ $12.0^9 \pm 0.1^2$ µg/30 ml original solution

$\therefore$ 6.05 ± 0.06 ppm Cu in dried fish tissue.

#3 8.0 - 0.25 = 7.75 div = 15.5 mm = X µg/ml

$\frac{28\ X + 10}{29}$ µg/ml for 14.5 - 0.25 = 14.25 div = 28.5 mm.

∴ X = 0.395 ± 0.00^2+ µg/ml original solution

11.8^4 ± 0.1^2 µg/30 ml original solution

∴ 5.92 ± 0.06 ppm Cu in dried fish tissue.

#4 7.5 - 0.25 = 7.25 div = 14.5 mm = X µg/ml

$\frac{28\ X + 10}{29}$ µg/ml for 13.8 - 0.25 = 13.55 div = 27.1 mm.

∴ X = 0.381 ± 0.004 µg/ml original solution

= 11.4^3 ± 0.1^2 µg/30 ml original solution

∴ 5.72 ± 0.06 ppm Cu in dried fish tissue.

#5 7.8 - 0.25 = 7.55 div = 15.1 mm = X µg/ml

$\frac{28\ X + 10}{29}$ µg/ml for 14.2 - 0.25 = 13.95 div = 27.9 mm

∴ X = 0.391 ± 0.004 µg/ml original solution

= 11.7^3 ± 0.1^2 µg/30 ml original solution

∴ 5.87 ppm Cu in dried fish tissue.

#6 7.8 - 0.25 = 7.55 div = 15.1 mm = X µg/ml

$\frac{28\ X + 10}{29}$ µg/ml for 13.5 - 0.25 = 13.25 div = 26.5 mm

∴ X = 0.437 ± 0.004 µg/ml original solution

13.1^1 ± 0.1^2 µg/30 ml original solution

∴ 6.56 ± 0.06 ppm Cu in dried fish tissue.

Overall average and standard deviation = 6.0^5 ± 0.3^0.

Lead

For each sample, the residue from the blank and the spiked test for copper was tested as-is for the blank and residue solution and spiked with lead for the residue solution only.

Sensitivity	1.5×10^{-10} A/mm
Range	+0.2 to -2.00 V
Start/Stop	-0.2 to -0.464 V
t drop	0.4 sec
mm/t drop	0.5
Pulse Voltage	-32 mV
N_2 Degas	15 mins
Working Electrode	D.M.E.
Auxiliary Electrode	Pt
Reference Electrode	Ag/AgCl (Sat. KCl)

#1 as-is 10.00 - 2.0 = 8.0 div = 16.0 mm = 28/29 X µg/ml

Spike: $$\frac{(28.00 \pm 0.04 \text{ ml}) \times X \text{ µg/ml} + (0.300 \pm 0.005 \text{ ml} \times 10.00 \pm 0.05 \text{ ml})}{29.30 \pm 0.04 \text{ ml}}$$

$\frac{28X + 3}{29.3}$ µg/ml for 20.8 - 2.0 = 18.8 div = 37.6 mm

∴ X = 0.077 ± 0.005 µg/ml original solution

2.31 ± 0.15 µg/30 ml original solution

∴ 1.16 ± 0.08 ppm Pb in dried fish tissue.

#2 10.8 - 2.0 = 8.8 div = 17.6 mm = 28/29 X µg/ml

$\frac{28X + 3}{29.3}$ µg/ml for 22.4 - 2.0 = 20.4 div = 40.8 mm.

∴ X = 0.080 ± 0.005 µg/ml original solution

2.40 ± 0.15 µg/30 ml original solution

∴ 1.20 ± 0.08 ppm Pb in dried fish tissue.

#3 9.1 - 2.0 = 7.1 div = 14.2 mm = 28/29 X µg/ml

$\frac{28\ X + 3}{29.3}$ µg/ml for 20.3 - 2.0 = 18.3 div = 36.6 mm

∴ X = 0.067 ± 0.005 µg/ml original solution

2.00 ± 0.15 µg/30 ml original solution

∴ 1.00 ± 0.08 ppm Pb in dried fish tissue.

#4 8.9 - 2.0 = 6.9 div = 13.8 mm = 28/29 X µg/ml

$\frac{28\ X + 3}{29.3}$ µg/ml for 20.2 - 2.0 = 18.2 div = 36.4 mm

∴ X = 0.064 ± 0.005 µg/ml original solution

1.93 ± 0.15 µg/30 ml original solution

∴ 0.97 ± 0.08 ppm Pb in dried fish tissue.

#5 9.8 - 2.0 = 7.8 div = 15.6 mm = 28/29 X µg/ml

$\frac{28\ X + 3}{29.3}$ µg/ml for 21.0 - 2.0 = 19.0 div = 38.0 mm

∴ X = 0.073 ± 0.005 µg/ml original solution

2.20 ± 0.15 µg/30 ml original solution

∴ 1.10 ± 0.08 ppm Pb in dried fish tissue.

#6 10.3 - 2.0 = 8.3 div = 16.6 mm = 28/29 X µg/ml

$\frac{28\ X + 3}{29.3}$ µg/ml for 21.6 - 2.0 = 19.6 div = 39.2 mm

∴ X = 0.079 ± 0.005 µg/ml original solution

2.36 ± 0.15 µg/30 ml original solution

∴ 1.18 ± 0.08 ppm Pb in dried fish tissue.

Overall average and standard deviation = 1.10 ± 0.09 ppm.

Cadmium

For each sample tested, the residue from the blank and the spiked test for lead was tested as-is and spiked for cadmium at the following parameters:-

Sensitivity	1.5×10^{-10} A/mm
Range	+0.2 to -2.00 V
Start/Stop	-0.4 to -0.776 V
t drop	0.4 sec
mm/t drop	0.5
Pulse Voltage	-32 mV
N_2 Degas	15 mins
Working Electrode	D.M.E.
Auxiliary Electrode	Pt
Reference Electrode	Ag/AgCl (Sat. KCl)

#1 as-is 4.0 - 0.8 = 3.2 div = 6.4 mm = 28/29.3 X µg/ml

Spike: $$\frac{28.00 \pm 0.04 \text{ ml} \times X \text{ µg/ml} + (0.300 \pm 0.005 \text{ ml} \times 1.00 \pm 0.01 \text{ ppm})}{29.60 \pm 0.04 \text{ ml}}$$

$$\frac{28X + 0.3}{29.6} \text{ µg/ml for } 6.4 - 0.8 = 5.6 \text{ div} = 11.2 \text{ mm}$$

∴ X = 0.014 ± 0.001 µg/ml original solution

0.42 ± 0.03 µg/30 ml original solution

∴ 0.21 ± 0.02 ppm Cd in dried fish tissue.

#2 3.4 - 0.8 = 2.6 div = 5.2 mm = 28/29.3 X µg/ml

$$\frac{28X + 0.3}{29.6} \text{ µg/ml for } 5.8 - 0.8 = 5.0 \text{ div} = 10.0 \text{ mm}$$

∴ X = 0.011 ± 0.001 µg/ml original solution

0.34 ± 0.03 µg/30 ml original solution

∴ 0.17 ± 0.02 ppm Cd in dried fish tissue.

#3 3.0 - 0.8 = 2.2 div = 4.4 mm = 28/29.3 X µg/ml

$\frac{28\ X + 0.3}{29.6}$ µg/ml for 7.0 - 0.8 = 6.2 div = 12.4 mm

∴ X = 0.0058 ± 0.0004 µg/ml original solution

0.172 ± 0.012 µg/30 ml original solution

∴ 0.087 ± 0.006 ppm Cd in dried fish tissue.

#4 5.5 - 0.8 = 4.7 div = 9.4 mm = 28/29.3 X µg/ml

$\frac{28\ X + 0.3}{29.6}$ µg/ml for 8.0 - 0.8 = 7.2 div = 14.4 mm

∴ X = 0.019 ± 0.002 µg/ml original solution

0.58 ± 0.06 µg/30 ml original solution

∴ 0.29 ± 0.03 ppm Cd in dried fish tissue.

#5 3.2 - 0.8 = 2.4 div = 4.8 mm = 28/29.3 X µg/ml

$\frac{28\ X + 0.3}{29.6}$ µg/ml for 6.4 - 0.8 = 5.6 div = 11.2 mm

∴ X = 0.0078 ± 0.0006 µg/ml original solution

0.24 ± 0.02 µg/30 ml original solution

∴ 0.12 ± 0.01 ppm Cd in dried fish tissue.

#6 3.8 - 0.8 = 3.0 div = 6.0 mm = 28/29.3 X µg/ml

$\frac{28\ X + 0.3}{29.6}$ µg/ml for 7.1 - 0.8 div = 12.6 mm

∴ X = 0.0095 ± 0.0009 µg/ml original solution

0.28 ± 0.03 µg/30 ml original solution

∴ 0.14 ± 0.02 ppm Cd in dried fish tissue.

Overall average and standard deviation = 0.17 ± 0.07 ppm.

Zinc

For each sample, the 20.00 ± 0.04 ml solution originally made up was diluted to 1/10 by taking 2.00 ± 0.01 ml of original solution and diluting it to 20.00 ± 0.04 ml. The following parameters were used:-

Sensitivity	1.5×10^{-9} A/mm
Range	+0.2 to -2.00 V
Start/Stop	-0.8 to -1.176 V
t drop	0.4 sec
mm/t drop	0.5
Pulse Voltage	-32 mV
N_2 Degas	15 mins
Working Electrode	D.M.E.
Auxiliary Electrode	Pt
Reference Electrode	Ag/AgCl (Sat. KCl)

#1 as-is 16.6 - 0.2 = 16.4 div = 32.8 mm = X µg/ml

Spike: $$\frac{(20.00 \pm 0.04 \text{ ml}) \times X \text{ µg/ml} + (2.00 \pm 0.01 \text{ ml} \times 10.00 \pm 0.05 \text{ ppm})}{22.00 \pm 0.04 \text{ ml}}$$

$$\frac{20X + 20}{22} \text{ µg/ml}$$ for 26.5 - 0.2 = 26.3 div = 52.6 mm

∴ X = 1.31 ± 0.01 µg/ml dil. solution

26.2 ± 0.2 µg/20 ml dil. solution

26.2 ± 0.2 µg/2 ml original solution

26.2 ± 2 µg/20 ml original solution

∴ 131 ± 1 ppm Zn in dried fish tissue.

#2 16.1 - 0.2 = 15.9 div = 31.8 mm = X µg/ml

$\frac{20\ X + 20}{22}$ µg/ml for 26.1 - 0.2 = 25.9 div = 51.8 mm

∴ X = 1.26 ± 0.01 µg/ml dil. solution

25.2 ± 0.2 µg/20 ml dil. solution

25.2 ± 0.2 µg/2 ml original solution

252 ± 2 µg/20 ml original solution

∴ 126 ± 1 ppm Zn in dried fish tissue.

#3 17.1 - 0.2 = 16.9 div = 33.8 mm = X µg/ml

$\frac{20\ X + 20}{22}$ µg/ml for 27.0 - 0.2 = 26.8 div = 53.6 mm

∴ X = 1.34 ± 0.01 µg/ml dil. solution

13.4 ± 0.1 µg/ml original solution

268 ± 2 µg/20 ml original solution

∴ 134 ± 1 ppm Zn in dried fish tissue.

#4 18.1 - 0.2 = 17.9 div = 35.8 mm = X µg/ml

$\frac{20\ X + 20}{22}$ µg/ml for 28.9 - 0.2 = 28.7 div = 57.4 mm

∴ X = 1.31 ± 0.01 µg/ml dil. solution

13.1 ± 0.1 µg/ml original solution

∴ 131 ± 1 ppm Zn in dried fish tissue.

#5 17.1 - 0.2 = 16.9 div = 33.8 mm = X µg/ml

$\frac{20\ X + 20}{22}$ µg/ml for 27.0 - 0.2 = 26.8 div = 53.6 mm

∴ X = 1.34 ± 0.01 µg/ml dil. solution

13.4 ± 0.1 µg/ml original solution

∴ 134 ± 1 ppm Zn in dried fish tissue.

#6 16.4 - 0.2 = 16.2 div = 32.4 mm = X µg/ml

$\frac{20\ X + 20}{22}$ µg/ml for 26.4 - 0.2 = 26.2 div = 52.4 mm

∴ X = 1.28 ± 0.01 µg/ml dil. solution

12.8 ± 0.1 µg/ml original solution

∴ 128 ± 1 ppm Zn in dried fish tissue.

Overall average and standard deviation = 131 ± 3.0 ppm.

Nickel

For each sample, the 20.00 ± 0.04 ml original solution was tested as-is. The following parameters applied:-

Sensitivity	6×10^{-9} A/mm
Range	+0.2 to -2.00 V
Start/Stop	-1.00 to -1.528 V
t drop	0.4 sec
mm/t drop	0.5
Pulse Voltage	-32 mV
N_2 Degas	15 mins
Working Electrode	D.M.E.
Auxiliary Electrode	Pt
Reference Electrode	Ag/AgCl (Sat. KCl)

#1 as-is: 11.6 - 0 = 11.6 div = 23.2 mm = X µg/ml

Spike: $\frac{(20.00 \pm 0.04\ \text{ml}) \times X\ \mu g/ml + (5.00 \pm 0.01\ \text{ml} \times 20\ \text{ppm})}{25.00 \pm 0.04}$

$\frac{20\ X + 100}{25}$ µg/ml for 19.3 - 0 = 19.3 div = 38.6 mm

∴ X = 4.63 ± 0.03 µg/ml original solution

92.6 ± 0.6 µg/20 ml original solution

∴ 46.3 ± 0.3 ppm Ni in dried fish tissue.

#2 11.5 - 0 = 11.5 div = 23.0 mm = X µg/ml

$\frac{20\ X + 100}{25}$ µg/ml for 19.2 - 0 = 19.2 div = 38.4 mm

∴ X = 4.60 ± 0.02 µg/ml original solution

92.0 ± 0.4 µg/20 ml original solution

∴ 46.0 ± 0.2 ppm Ni in dried fish tissue.

#3 10.7 - 0 = 10.7 div = 21.4 mm = X µg/ml

$\frac{20\ X + 100}{25}$ µg/ml for 18.6 - 0 = 18.6 div = 37.2 mm

∴ X = 4.26 ± 0.02 µg/ml original solution

∴ 42.6 ± 0.2 ppm Ni in dried fish tissue.

#4 11.1 - 0 = 11.1 div = 22.2 mm = X µg/ml

$\frac{20\ X + 100}{25}$ µg/ml for 18.8 - 0 = 18.8 div = 37.6 mm

∴ X = 4.48 ± 0.02 µg/ml original solution

∴ 44.8 ± 0.2 ppm Ni in dried fish tissue.

#5 11.3 - 0 = 11.3 div = 22.6 mm = X µg/ml

$\frac{20\ X + 100}{25}$ µg/ml for 19.0 - 0 = 19.0 div = 38.0 mm

∴ X = 4.54 ± 0.02 µg/ml original solution

∴ 45.4 ± 0.2 ppm Ni in dried fish tissue.

#6 11.5 - 0 = 11.5 div = 23.0 mm = X µg/ml

$\frac{20\ X + 100}{25}$ µg/ml for 19.2 - 0 = 19.2 div = 38.4 mm

∴ X = 4.60 ± 0.02 µg/ml original solution

∴ 46.0 ± 0.2 ppm Ni in dried fish tissue.

All over average and standard deviation = 45 ± 1.