

CHAPTER II



EXPERIMENTS

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Informations concerning chemical speciation are relied on the quantity of anions and cations of interest. In this case the dissolved cations in question are Cd^{2+} , Pb^{2+} , Cu^{2+} , and the anions present in abundance i.e. SO_4^{2-} , Cl^- , HCO_3^- , CO_3^{2-} and OH^- . Moreover, since in the study of speciation, ionic strength is used instead of an individual ion concentration, the concentration of other cations other than mentioned above but also present i.e. Na^+ , K^+ , Ca^{2+} and Mg^{2+} had to be known. In this work several analytical techniques were employed for the determination of each of the ions of interest. They were the well known methods and were used without complications with the exception of the differential pulse anodic stripping voltammetry which was employed in the analyses of cadmium, lead and copper ions in the sample. The operation is adequately simple but the interpretation of the results was found to be rather subjective. One of the reasons of introducing this technique in this work was that it has a reputation of being one of the sensitive instrumental techniques but has never been introduced in Thailand for the purpose of trace element analyses in sea water while neither of the widely known techniques such as atomic absorption spectrophotometry and neutron activation analysis was provided to yield satisfactory results. The principle of DPASV was described in the following section.

2.1 Principle of Differential Pulse Anodic Stripping Voltammetry (DPASV)

Voltammetry is concerned with the study of voltage-current-time relationships during electrolysis carried out in a cell where one electrode is of relatively large surface area, and the other (the working electrode) has a very small surface area and is often referred to as a micro-electrode. The micro-electrode is usually constructed of some inert, conducting material such as gold, platinum or carbon.

In anodic stripping voltammetry, the material to be analyzed is first pre-concentrated into a stationary mercury electrode by the application of a controlled potential more negative than the reduction potentials of all various species determined. This process causes the species to be reduced at the electrodes, from the ionic state in which they are present in the solution to the metallic form, and these metals atoms amalgamate with the mercury. At the end of a suitable length of time, determined preliminary by the concentration of material in the solution, a wave form consisting of a positive-going linear ramp with amplitude pulse of 50mv superimposed with the process is applied to this mercury electrode. As the potential is made more positive, the various metals present in the amalgam are oxidized, each beginning the oxidation process at its own redox potential and redissolved in the solution as ions. Current is generated due to this reaction, and the amount of current depends on the concentration of each of the material being oxidized at any given point, hence current measurement was used as the means for the quantitative determination of the ion concerned.

The basic stripping technique which employed a simple d.c. scan (for the stripping process) has been known for many years. It has long been acknowledged that this technique is very sensitive, but it is equally well known that the technique suffers from a number of severe practical limitations, arising from the problems encountered when very long deposition times are employed to increase sensitivity. One cannot amplify the signal by an excessively large amount since one also amplifies all the contributing noise factors, so that excessive deposition times must be used for adequate sensitivity. However, by employing the pulse modulation and by obtaining two current samples, one just before the pulse and a second during the last portion of the pulse, which will be referred to as current sampling later, the normal dc. stripping wave form can be differentiated. From the sampling technique a number of the noise sources can be minimized or effectively eliminated, and the resultant extracted signal can then be amplified to a much greater degree.

An additional advantage of the method is that as the potential is pulse through that at which stripping occurs, the analyte is oxidized from the electrode during the pulse but does not have time to diffuse away from the proximity of the electrode before the pulse ends. As the pulse is removed, the potential returns to a level at which reduction occurs. Consequently, a significant portion of the analyte stripped during the pulse is replated during the rest period between pulses as long as that period is cathodic of the reduction potential. As a result, the same analyte may make repetitive contributions to the current measured while it can contribute any once in a linear scan operation. Differential

pulse stripping offers sensitivity enhancements of one to several orders of magnitude and appears to offer the highest signal-to-noise of any stripping technique (37,38). The differential pulse stripping wave form is shown in Fig 2.1.

It is desirable to perform quantitative measurement by a method of standard addition to compensate for matrix difference. The method is used when the peak height is proportional to the concentration of the substance being determined. The concentration of the unknown solution can be achieved by calculations (39). If V is the volume of the unknown solution in cm^3 , C_x its concentration, i_1 its peak height at the peak potential; and if i_2 is the peak height resulted after the adding $v \text{ cm}^3$ of a standard solution whose concentration is C_s , one has

$$i_1 = k C_x$$

and

$$i_2 = \frac{k (V C_x + v C_s)}{V + v}$$

therefore

$$C_x = \frac{i_1 V C_s}{i_2 v + (i_2 - i_1) V}$$

2.2 Collection and Preparation of Samples

The samples were collected on October, 12, and December, 18, 1979 at four stations from the Tha Chin River along the estuary in Samut Sakhorn Province (Fig 2.2). Station 1 represented sample at the river mouth, station 2 in front of Wat Kamphra, station 3 south of Khlong Maha Chai, and station 4 north of Phra Puthalertlar Bridge. Surface and subsurface water, at one metre below, were collected and filtered, as

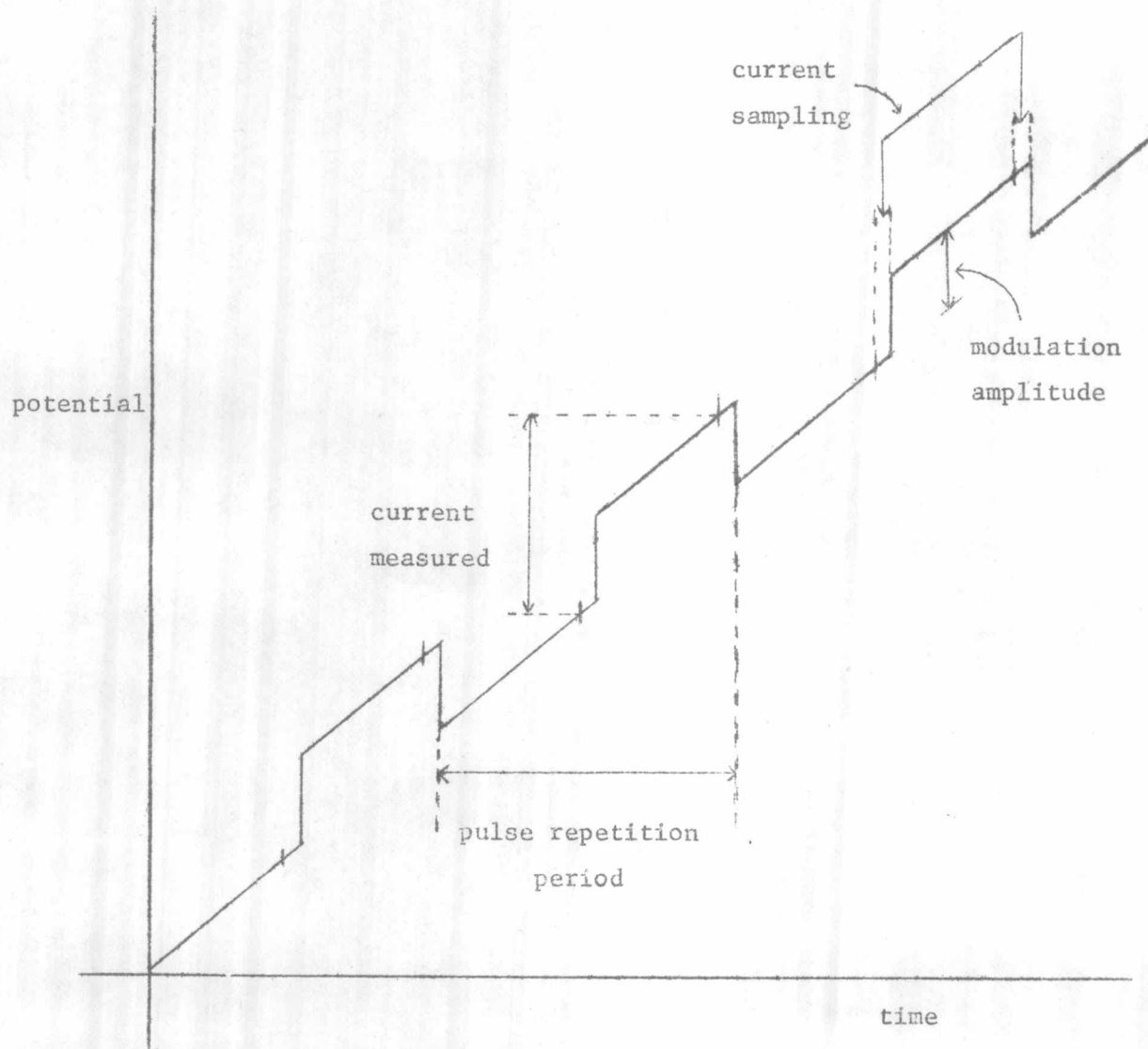
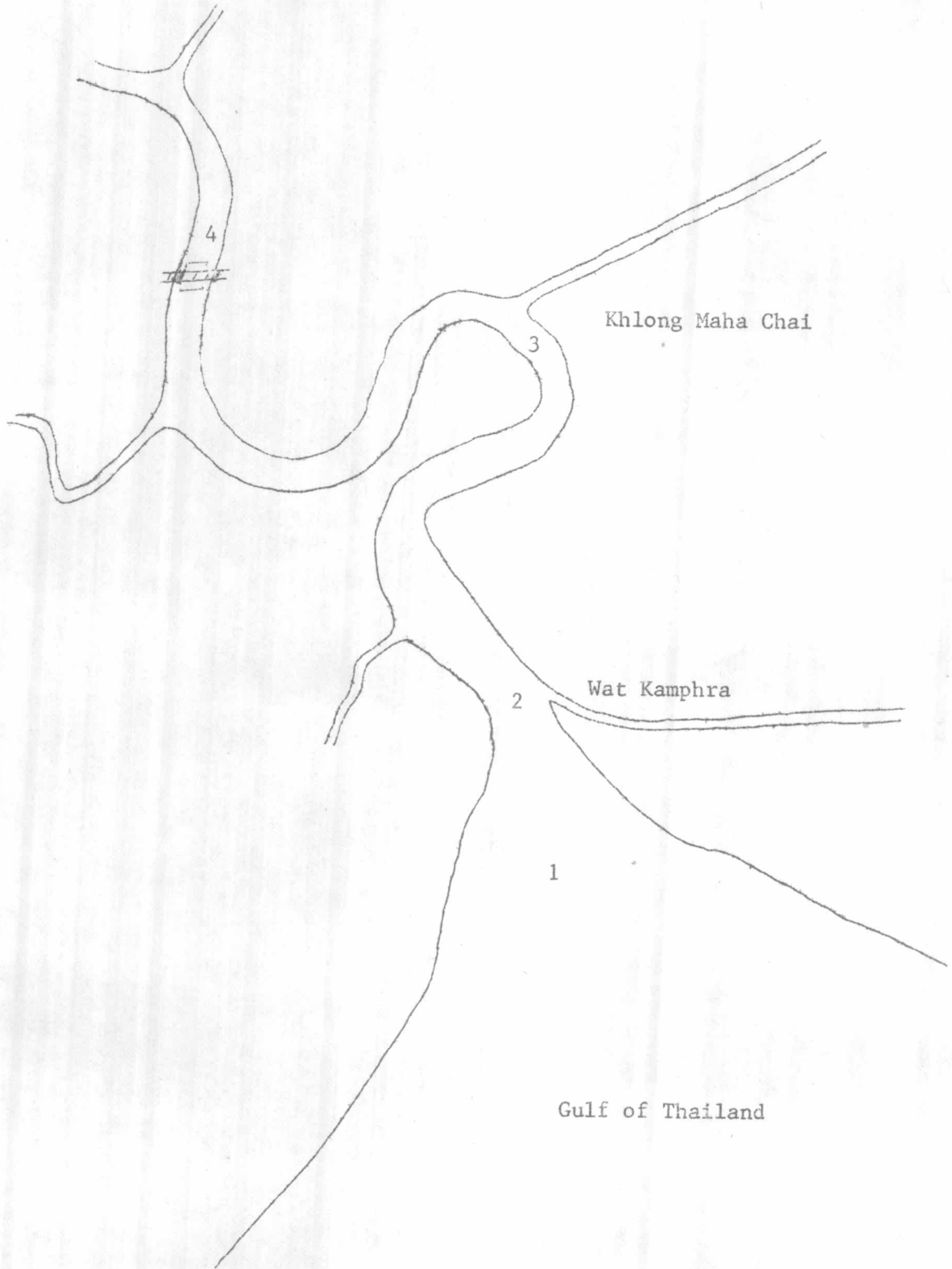


Figure 2.1 The differential pulse stripping wave form.



scale 1:50,000

Figure 2.2 Sampling stations in the Tha Chin River estuary.

soon as received, through 0.45- μ m Millipore Filter to separate particulate matter. The filtrates were stored in polyethylene bottles which had been cleaned with nitric(V) acid rinsed with double deionized water and finally with the samples (40).

2.3 Experimental

In this study, double deionized water and A.R. grade of reagents were used throughout except otherwise mentioned.

2.3.1 Anions Analyses

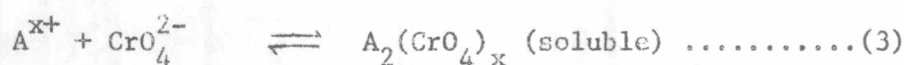
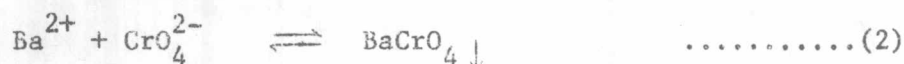
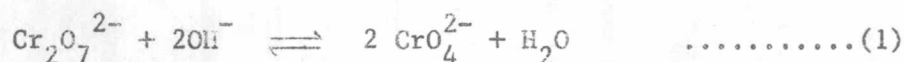
The method of spectrophotometry was employed in the determination of SO_4^{2-} while potentiometric method was chosen for the determination of Cl^- , CO_3^{2-} and HCO_3^- . For the determination of OH^- , unfortunately however that there was no existing suitable analytical method, the determination was therefore carried out by calculation using known values of pH and K_w of the samples.

2.3.1.1 Determination of Sulphate Ion by Spectrophotometric Method

Usually sulphate solution is colourless hence cannot be detected spectrophotometrically and an indirect method is needed whereby colourless solution is changed by chemical reactions to a coloured one (41). Using a series of chemical processes Iwasaki et al. (42) have developed a "recipe" which was suitable for the determination of a small amount of sulphate ions in water sample and was adopted in this work. The principle is described below.

SO_4^{2-} in the sample are allowed to replace $\text{Cr}_2\text{O}_7^{2-}$ in BaCr_2O_7 solution which is added in excess at and acid pH. At this stage all of SO_4^{2-} are converted to BaSO_4 and is precipitated. As a result, the supernatant liquid contains all of the added $\text{Cr}_2\text{O}_7^{2-}$ with the counter ions being the rest of the added Ba^{2+} as well as the cations that used to be counter ions of the sulphate ions. The latter will be represented by " A^{x+} ". The quantity of " A^{x+} " can be used to determine the amount of sulphate ion.

Next, the pH of the above mixture is raised and the following reactions take place,



Now, the rest of Ba^{2+} are in the form of the precipitate of BaCrO_4 leave behind the soluble chromate formed by the reaction (3) in the solution which becomes yellow in colour and is suitable for the spectrophotometric measurement. This work measured the intensity of the colour of the resulting solution at 370 m μ wavelength (42,43) using a Varian Techtron Spectrophotometer model 635 equipped with a Varian Techtron Recorder model 7040 A.

a). Preparation of Reagents

-Suspended barium chromate solution

2.5 G of barium chromate ((VI) were dissolved in 200 cm³ of acetic acid-hydrochloric acid mixture. The mixture was prepared by

taking 2.0 cm^3 of conc. HCl and 2.9 cm^3 of conc. CH_3COOH and adjusting to 200 cm^3 with water.

-Ammonia solution containing calcium ion

1.85 G of $\text{CaCl}_2 \cdot 2 \text{ H}_2\text{O}$ were dissolved in 500 cm^3 of 25 percent ammonia solution. The resulting solution was stored in a polyethylene bottle.

-Standard sulphate solution

A stock solution containing $1.00 \text{ mg SO}_4^{2-} \text{ cm}^{-3}$ was prepared by dissolving 0.1315 g of dried K_2SO_4 in double deionized water and dilute to the make of a 100.0 cm^3 volumetric flask.

b). Procedure

Exactly 0.50 cm^3 of sample was transferred to a 50.0 cm^3 volumetric flask followed by the addition of 3.0 cm^3 of well shaken suspended barium chromate(VI) solution. The content was mixed thoroughly and settle for 2 minutes after which 1.00 cm^3 of the clear supernatant of ammonia solution containing calcium ions was gently added using a dropping pipette. 10.0 cm^3 of ethanol were added and the mixture was diluted to the marked line and shaken for 1 minute, allowed to settle for 10 minutes. The solution was filtered through whatman No 42 filter paper and the absorbance of the filtrate was measured at $370 \text{ m}\mu$ with reagent blank as reference. The content of the sulphate ion was obtained from the calibration curve which constructed from the results of an identical experiment as above using standard sulphate ion solutions containing 0.30, 0.60, 0.90, 1.20, 1.50 and 1.80 mg of sulphate.

2.3.1.2 Determination of Chloride ion by Potentiometric Titration.

Chloride ion was determined by precipitation titration with silver nitrate. Ethanol was added to reduce the solubility of the precipitate and thus increase the potential change at the equivalence point (44). Because the precipitate occurred could also adsorb other ions, the adsorption was decreased upon the addition of KNO_3 . It was found that by acidifying the solution with nitric acid, the complexation of SO_4^{2-} and CO_3^{2-} with Ag^+ ion became insignificant and the chloride determination was more reliable (45).

The potential measurements were obtained with a pH-meter manufactured by Radiometer, Copenhagen type PHM 28. Silver and saturated calomel electrodes were used as an indicator electrode and a reference electrode respectively.

a). Preparation of Reagents

-Standard silver nitrate solution

25.4835 G of reagent grade silver nitrate were dissolved and diluted in a 500.0 cm^3 volumetric flask. The solution was standardized with sodium chloride solution (46).

-Acidic potassium nitrate

5.0555 G of potassium nitrate were dissolved in water, 3.12 cm^3 of nitric acid were added and diluted to 1000.0 cm^3 in a volumetric flask.

b). Procedure

10.0 cm³ of sample were diluted with 50.0 cm³ of acidic potassium nitrate solution and then with 50.0 cm³ ethanol. The solution was titrated with standard silver nitrate (44).

The equivalence point of the titration was obtained by Gran's plot (47) (Appendix I and Fig 3.2).

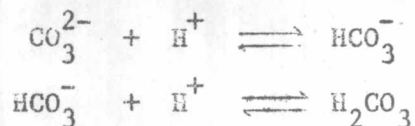
2.3.1.3 Determination of Carbonate and Hydrogencarbonate Ions by Potentiometric Method.

Sea water contains several protolytic species e.g. borate, carbonate etc. in various stages of protonation. The concentration of protolytic species are characterized by the total alkalinity (At) and pH. The total alkalinity is defined as

$$\text{At} = [\text{B(OH)}_4^-] + 2 [\text{CO}_3^{2-}] + [\text{HCO}_3^-] + [\text{OH}^-] - [\text{H}^+]$$

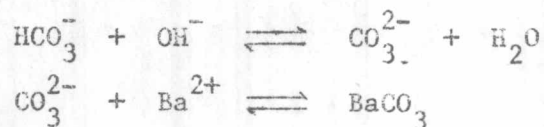
and the carbonate alkalinity is $2 [\text{CO}_3^{2-}] + [\text{HCO}_3^-]$ with slightly carbonic acid and carbon dioxide (26, 48).

If sea water is titrated with acid, two equivalence points will be obtained and the difference of them gives the carbonate and hydrogencarbonate content.



The amount of hydrogencarbonate ion can be determined by adding sodium hydroxide to convert hydrogencarbonate ion to carbonate ion and precipitate the carbonate as barium carbonate, the amount of sodium

hydroxide used indicates the amount of hydrogencarbonate in the solution (46).



The pH measurements between the titrations were performed with a pH-meter (Radiometer Copenhagen Type PHM 28), coupled with a glass electrode and saturated calomel electrode pair.

a). Preparation of Reagents

About 0.05 mol.dm⁻³ of standard hydrochloric acid and carbonate-free sodium hydroxide solution were prepared according to Vogel (46). Standard hydrochloric acid was standardized with anhydrous sodium carbonate whereas carbonate-free sodium hydroxide underwent the standardized process with standard hydrochloric acid.

b). Procedure

100.0 cm³ of sample were titrated with standard hydrochloric acid to give the amount of carbonate and hydrogencarbonate ions. To another aliquot of sample, 5.0 cm³ of carbonate-free sodium hydroxide was added and carbonate as barium carbonate precipitated. The excess of carbonate-free sodium hydroxide was immediately determined by the titration with standard hydrochloric acid.

The equivalence points were obtained by Gran's Plot (47) (Appendix II and Fig 3.3).

2.3.1.4 Calculation for Activity of Hydroxide Ion.

As has been mentioned previously that it was not practical to determine the concentration of OH^- experimentally, instead the activity of OH^- was calculated from pH and the ionic product of water, K_w , by the following relationships.

$$a_{\text{H}^+} = 10^{-\text{pH}}$$

and
$$a_{\text{H}^+} + a_{\text{OH}^-} = 10^{K_w}$$

therefore
$$a_{\text{OH}^-} = 10^{K_w} - 10^{-\text{pH}}$$

Generally, the K_w value of the dilute solution was assumed to be 14.0 but for sea water it is linearly dependence on salinity (49) where five salinities of 20, 25, 30, 35 and 40% was studied and shown in Fig 2.3 . In this study, values of K_w of samples which had salinity less than 20% were determined by extrapolating the graph.

2.3.2 Cations Analyses

Here, sodium and potassium ions were determined by the flame photometric method while calcium, and magnesium ions were determined by the atomic absorption spectrophotometric method. These methods were specific and sensitive for the purpose.

Trace quantities of calcium, lead and copper ions were simultaneously determined by the differential pulse anodic stripping voltammetry, the principle of which was described in section 2.1. This method was suitable for the work on the accounts that no interference from major ions such as alkaline earth had to be considered (50) and the

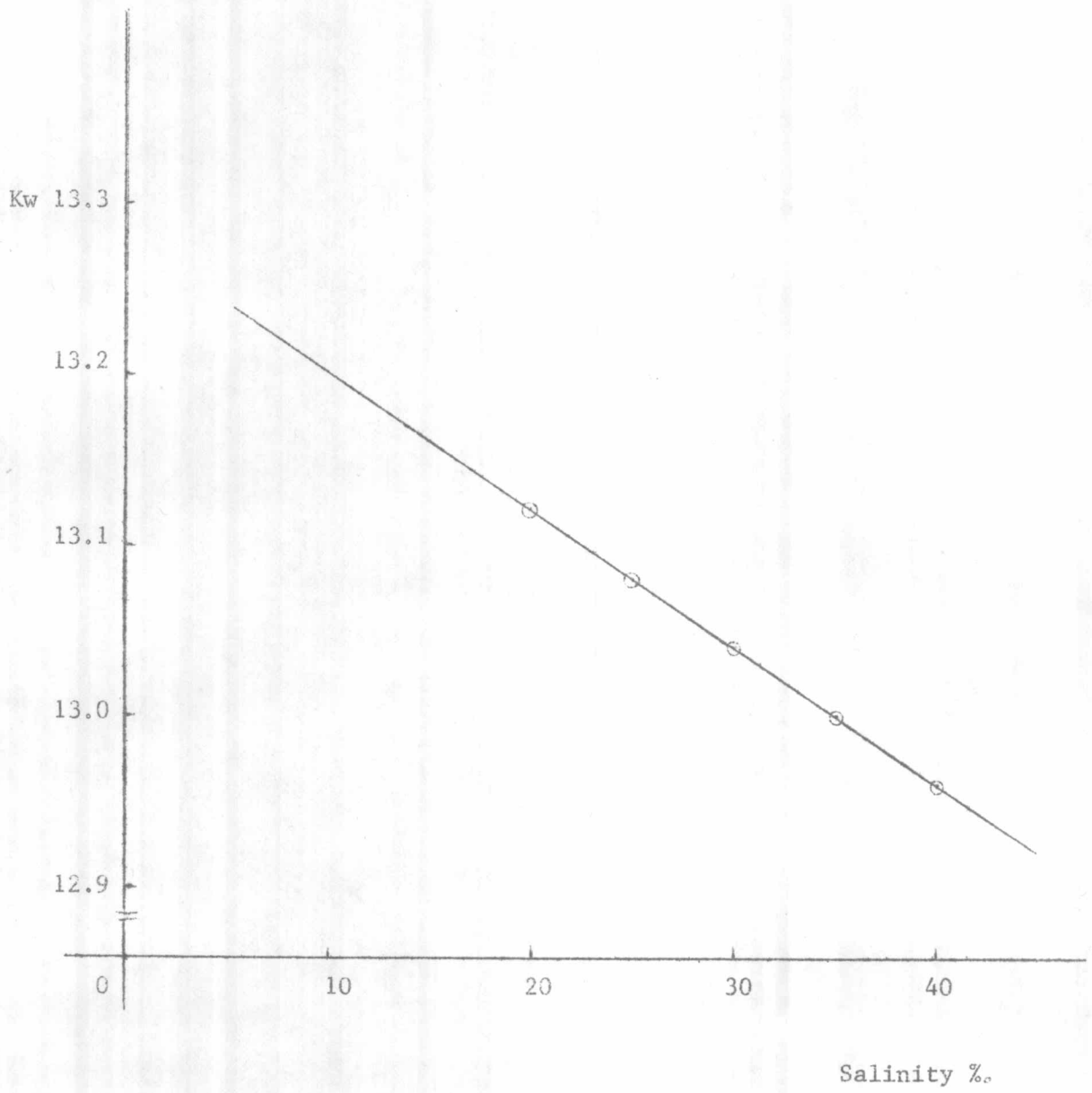


Figure 2.3 Linear dependence of K_w on salinity

technique was highly sensitive, unlike other analytical methods, the reason being that the preconcentration step was unnecessary therefore minimizing a quantity loss. Moreover, the addition of supporting electrolyte was no need since in each sample sodium chloride was already present in a considerable amount (38).

2.3.2.1 Determination of Sodium and Potassium ion by Flame Photometric Method.

Flame photometric method has been applied to the analyses of a wide variety of materials. The most important applications are for the determination of alkali metals. For the determination of sodium in natural water by the flame photometric method, the co-existing ions especially potassium, calcium and magnesium interfere with the determination. Likewise, the determination of potassium by this method was found to be distributed by the presence of sodium, calcium and magnesium. Some correction procedure is needed to minimized the errors. This is accomplished with a so-called radiation buffer, which is added to the sample as well as to the standards employed for the calibration. Radiation buffers are solutions that have been saturated with all elements likely to be present in the sample other than the one being determined. The amounts of the various elements added in this way are ordinarily large relative to the quantities originally present in the sample; thus, a uniform level of concentration is established in both standards and samples (51, 52).

In sodium and potassium determination, the relative intensity were obtained with a EEL (Evans Electro Selenium Ltd.) model 100 Flame Photometer, equipped with a EEL 230 Air Compressor.

a). Preparation of Reagents.

-Standard sodium ion solution

A stock solution containing $1.00 \text{ mg Na} \cdot \text{cm}^{-3}$ was prepared by dissolving 0.2542 g of NaCl in water and adjusting to 100.0 cm^3 in volumetric flask. The $100 \text{ } \mu\text{g Na} \cdot \text{cm}^{-3}$ solution was prepared by diluting the stock solution.

-Standard potassium ion solution

A stock solution containing $1.00 \text{ mg K} \cdot \text{cm}^{-3}$ was prepared by dissolving 0.1907 g of KCl and diluting with water to the mark of 100.0 cm^3 volumetric flask.

-Radiation Buffer

Radiation buffer for determination of sodium ion was obtained by saturating water with CaCl_2 , KCl , and MgCl_2 while radiation buffer for determination of potassium ion NaCl , CaCl_2 and MgCl_2 were used instead.

b). Procedure

-Determination of Sodium Ion

The calibration graph was prepared by introducing 1.50 cm^3 of sodium radiation buffer to a series of 25.0 cm^3 volumetric flasks. A series of the $100 \text{ } \mu\text{g} \cdot \text{cm}^{-3}$ standard sodium solution of volume 0.50 , 1.00 , 1.50 , 2.00 and 2.50 cm^3 were added at a time to the radiation buffer and diluted to the mark. The emission intensity was measured against sodium filter by using the highest concentration of standard sodium ion solution

to set a maximum intensity, then the lower concentrations were measured as a relative intensity.

The amount of sodium ion in a sample was obtained by carrying out the identical experiment as described above and by using the calibration graph to convert the relative intensity to concentration.

-Determination of Potassium Ion

The calibration graph was prepared by introducing 1.50 cm³ of potassium radiation buffer to a series of 25.0 cm³ volumetric flasks containing 0.25, 0.50, 0.75, 1.00 and 1.25 cm³ of 1.00 mg K⁻³.cm standard potassium ion solutions and diluted to the mark. The emission intensity was measured by the same procedure as sodium but the potassium filter were used instead.

The amount of potassium ion in the sample was also determined from the calibration graph.

2.3.2.2 Determination of Calcium and Magnesium ion by Atomic Absorption Spectrophotometric Method (AAS).

Atomic absorption spectrophotometry is usually used for the determination of alkali earth metals i.e., calcium, magnesium. The greatest advantage is due to its specificity of determination so that the mixtures of two or more of these elements which are a problem in emission flame photometry, may easily be analyzed. From the point of view of atomic absorption, magnesium differs somewhat from the rest, its determination is one of the most sensitive; much higher than the sensitivity of the others. Magnesium has its main resonance line in the ultraviolet region,

the others in the visible region so that modulated light sources must be used with acetylene-air flames. For determination of calcium, the sensitivity of $0.1 \mu\text{g} \cdot \text{cm}^{-3}$ is lower than that of emission flame photometry but its main advantage is higher in specificity (53,54).

a). Reagents

-Calcium nitrate standard solution for AAS, (BDH) with following specification

$$1 \text{ cm}^3 = 1.00 \text{ mg} = 24.9 \text{ mmol} \cdot \text{dm}^{-3}$$

-Magnesium nitrate standard solution for AAS, (BDH) with the following specification

$$1 \text{ cm}^3 = 1.00 \text{ mg} = 41.1 \text{ mmol} \cdot \text{dm}^{-3}$$

b). Procedure

-Determination of Calcium Ion

A calibration graph for calcium ion determination was prepared by diluting a series of 0.50, 1.00, 1.50, 2.00, 2.50, 3.00 and 3.50 cm^3 of $100 \mu\text{g} \cdot \text{cm}^{-3}$ of standard calcium ion solution in 25.0 cm^3 volumetric flask. The content of calcium ion in samples was obtained from the calibration graph.

-Determination of Magnesium Ion

A calibration graph was prepared by diluting a series of $10 \mu\text{g} \cdot \text{cm}^{-3}$ standard magnesium ion solution of 0.20, 0.40, 0.60, 0.80, 1.00 and 1.20 cm^3 in 25.0 cm^3 volumetric flasks. The amount of magnesium ion

in samples was also obtained from the calibration graph.

In calcium and magnesium determination, the absorbances were obtained with Atomic Absorption Spectrophotometer, Varian Techtron, model AA 5 with calcium and magnesium hollow cathode lamp. Other operating conditions were shown below.

Table 2.1 Operating conditions for AAS analyses of calcium and magnesium ions.

Ion	Wavelength (mm)	slit width (μ)	lamp current (mA)	flame
Ca ²⁺	422.7	100	5	air-acetylene
Mg ²⁺	285.2	100	3	air-acetylene

2.3.2.3 Determination of Cadmium, Lead and Copper Ions by Differential Pulse Anodic Stripping Voltammetry (DPASV).

In this study, the DPASV was used to determine cadmium, lead and copper both at natural pH and at $0.16 \text{ mol. dm}^{-3} \text{ HNO}_3$. At natural pH free metal ions and some electroactive labile metal complexes are detected. For the determination of total concentration of metals ions, the sample's pH has to be very low, Batley and Florence (55) found that the optimum condition being that should be acidified to $0.16 \text{ mol. dm}^{-3} \text{ HNO}_3$.

Voltammograms were obtained with a PAR (Princeton Applied Research Inc.) model 174A Polarographic Analyzer, equipped with a PAR model 315 Automate Electro-Analysis Controller and a Hewlett-Packard 7040A x-y Recorder.

The cell employed in all DPASV is a conventional H-type with two compartments (Fig 2.4), one compartment served as the saturated calomel electrode and the other served as the container for the test solution. The two compartments are separated by a cross member filled with 4% agar-saturated potassium chloride gel, which is held in position by a sintered glass disc E. The three electrodes used were a glassy carbon electrode (GCE) as a working electrode, saturated calomel electrode (SCE) as a reference electrode and a platinum wire as an auxiliary electrode.

a). Preparation of Electrodes and solutions

-Preparation of a Reference Electrode.

Salt bridge was prepared first by adding 0.4g of agar-agar to ca. 10 cm^3 of saturated potassium chloride solution and the mixture was warmed on a hot plate with stirring. The clean and dry cell was clamped so that the cross member was vertical, the agar gel was transferred into the cross member by means of a dropper. The cell was allowed to stand undisturbed until the gel had set. No air bubble should be present in the KCl-agar salt bridge. When the H cell is not in used, the test solution compartment should be kept filled with the double deionized water to prevent the agar plug from drying out.

To set up the SCE electrode, a saturated solution of KCl and Hg_2Cl_2 was first prepared. Pure mercury was placed in the bottom of the dry electrode compartment of the H cell for a depth of 0.5 cm. The mercury was then covered with a layer of calomel paste. The latter was prepared by rubbing pure Hg_2Cl_2 , mercury and saturated KCl solution

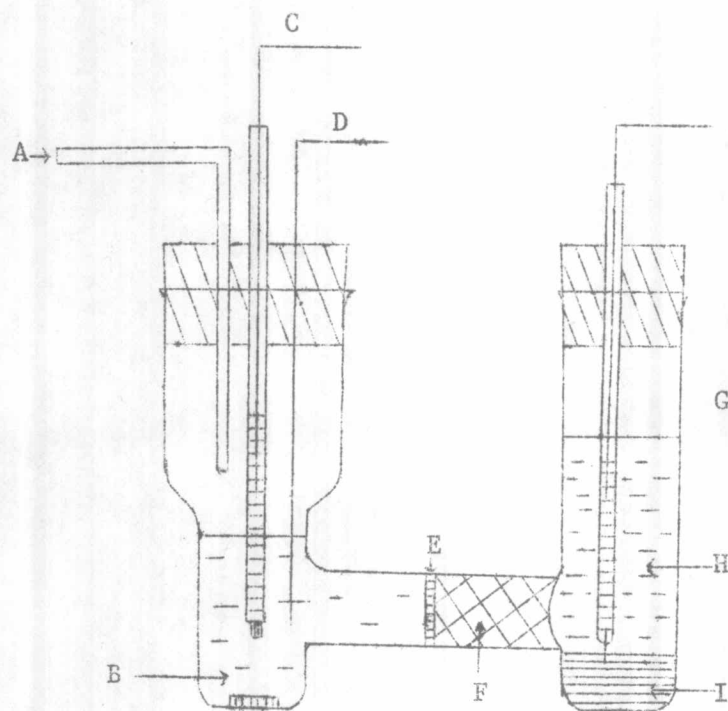


Figure 2.4 The H cell used in DPASV analysis. The labelling letters are A.-purified N_2 , B- a sample solution C- the GCE working electrode, D- platinum wire auxiliary electrode, E- sintered glass disc, F- agar plug, G- the SCE reference electrode, H- an aqua saturated with KCl and Hg_2Cl_2 , and I- Hg coated with calomel paste and solid KCl.

in a clean beaker with a clean dry glass rod. The electrode compartment was filled with a saturated solution of KCl which was also saturated with Hg_2Cl_2 , and the rubber bung carrying the glass tube associated with electrical connection was then inserted. (45)

-Preparation of a Working Electrode

A piece of 10 mm. glassy carbon rod (Vitreous Carbon rod 3mm.dia., from Ringsdorff, Bonn. Germany) was glued in a 4-mm (ID) soft glass tube with Aradite epoxy (Ciba-Geigy Limited, Basle, Switzerland). A fresh glassy carbon surface was prepared by polishing with a piece of silicon carbide paper then washed with deionized water and gently rubbed with a piece of filter paper to get rid of dust and moisture. (56)

-Standard Metal Ion Solution

Stock solutions containing $1.00 \text{ mg} \cdot \text{cm}^{-3}$ of each metal were prepared by dissolving the appropriate amount of the nitrate salt of the corresponding metal with double deionized water.

Standard solutions containing $0.10\text{-}1.00 \text{ } \mu\text{g} \cdot \text{cm}^{-3}$ were prepared by successive dilution from the stock solutions.

-Preparation of Sample Solutions.

The test solution for determination of cadmium, lead and copper ions at natural pH could be directly pipetted from the sample. But for the determination of total metals ions 25.0 cm^3 of a sample were pipetted into a beaker and 0.25 cm^3 of concentrated nitric acid was added. The solution was boiled 5 minutes and cooled to room temperature. This solution was made to 25.0 cm^3 in a volumetric flask with deionized water.

b). Procedure

To obtain a voltammogram of the test solution, the instrument was set as followed:

potential scan rate	5 mV/s
scan direction	"+"
potential range	3.0 V
initial potential	-0.9 V
modulation amplitude	50 mV
operation mode	diff. pulse.
display direction	"_"
pulse repetition time	0.5 s
current range	as needed
deposition time	3-15 min
condition time	3 min
nitrogen purge time	10 min
equilibrium time	30 s

Before introducing any test solution into the cell, the test solution compartment was washed with ca. 3 mol.dm⁻³ HNO₃, rinsed with double deionized water for several times and finally with the test solution. 10.0 cm³ of test solution and 0.05 cm³ of 0.008 mol.dm³ of mercuric nitrate solution were added to the cell (57). The purified nitrogen gas bubbled through the solution for 10 minutes, then was blanketed over the solution. The electrode was conditioned for 3 minutes and deposit at -0.9 V for an appropriate period of time. Allow 30 seconds waiting period at the end of each deposition time, then start to record the anodic

stripping voltammogram while the potential was scanned to 0 V with a scan rate of 0.5 mV.S^{-1} . For the addition method, standard solution of cadmium, lead and copper ion were subsequently added by means of a pipette to the cell. Again, the purified nitrogen gas was bubbled through the solution for 1 minute and blanketed over the solution and the voltammogram was recorded as before.