

UNIVERSITI SAINS MALAYSIA



**Voltammetric Determination of Nickel in Chocolate by
Differential Pulse Cathodic Stripping Analysis**

Dissertation submitted in partial fulfilment for the Degree of Bachelor of
Science (Health) in Forensic Science

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CERTIFICATE

This is to certify that the dissertation entitled

**Determination of Nickel in Chocolate by Differential Pulse
Cathodic Stripping Voltammetry**

is the bonafide record of research work done by

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ABBREVIATIONS

AAS	- Atomic Absorption Spectroscopy
ASV	- Anodic Stripping Voltammetry
BRB	- Britton-Robinson Buffer
CSV	- Cathodic Stripping Voltammetry
DMG	- dimethylglyoxime
DPAdSV	- Differential Pulse Adsorptive Stripping Voltammetry
DPASV	- Differential Pulse Anodic Stripping Voltammetry
DPCSV	- Differential Pulse Cathodic Stripping Voltammetry
ETAAS	- Electrothermal Atomic Absorption Spectrometry
FI-AAS	- Flame Ionization Atomic Absorption Spectroscopy
ICP-AES	- Inductively Coupled Plasma Atomic Emission Spectroscopy
ppb	- parts per billion
ppm	- parts per million
WHO	- World Health Organization

ABSTRACT

The determination of nickel in the chocolate was performed by Differential Pulse Cathodic Stripping Analysis(DPCSV). Chocolate was a complex sample with a high content of organic compounds such as sugar, protein, fat, lecithin and many more and its analysis generally involves digestion procedures that might include the risk of losses and/or contamination. Wet digestion by using concentrated nitric acid and 1:1 ratio of distilled water-hydrochloric acid on the sample was selected on this study because of its availability and low cost . Sample was then processed to the sample analysis stage. Prior to the sample analysis by DPCSV, the optimum pH for the supporting electrolyte, Britton-Robinson Buffer (BRB), 0.04 M and optimum parameters for the analysis was determined. Optimum pH for the supporting electrolyte, BRB was pH 4. Initial potential, E_i of -0.68 V, final potential, E_f of -1.15 V, scan rate of 0.050 V/s, deposition potential of -1.4 V, equilibration time of 5 s and deposition time, t_{acc} of 20 s were the optimized parameters used in this study. The validation of methodology was performed by plotting a calibration curve peak height, i_p versus different concentrations of nickel (5 ppm) as the stock solution. A linear graph with the regression equation of $\hat{Y} = 0.820 + 23.322X$, correlation coefficient of 0.966, standard deviation of 1.129, sensitivity of 23.322 nA/ppm and limit of detection of 0.145 ppm was obtained. 3 different brands of chocolate were analyzed. The results in all samples show absence of nickel.

INTRODUCTION

Nickel is one among the heavy metals that play an important role in our body system. It is usually present in minute amount in our system and the excessive intake will cause harmful effect to our body. According to the dietary reference intake of nickel, the tolerable upper intake level, UL of nickel is 0.2 mg/day for children 1 to 3 years old, 0.3 mg/day for children 4 to 8 years old and 1 mg/day for adult, pregnant women and lactating women. UL is the maximum level of daily nutrient intake that is likely to pose no risk of adverse effect. UL represent total nickel intake from food, water and supplement. (Trumbo. P, 2001)

The main routes of nickel intake for humans are inhalation, ingestion and absorption through the skin. Here, the route of nickel intake is by ingestion through the food. In most food products, the nickel content is less than 0.5 mg/kg fresh weight. Cacao products and nuts may, however, contain as much as 10 and 3 mg/kg, respectively. Total diet studies indicate a total average oral intake of 200–300 µg/day (WHO, 2001).

Nickel can produce serious effects on human health. There are two types of effects; toxicological effects and carcinogenic effects on human body. For toxicological effects, severe lung damage has been recorded following acute inhalation exposure to nickel carbonyl. Reversible renal effects (in workers), allergic dermatitis (most prevalent in women), and mucosal irritation and asthma (in workers) have been reported following exposure to inorganic nickel compounds. Renal effects and dermatitis presumably relate nickel uptake by both inhalation and ingestion, in addition to cutaneous contact which

result in dermatitis. As far as carcinogenic effects, studies linking to nickel uptake from the environment and cancer incidence in the general population are not available yet. But there is agreement that nickel refinery workers exposed by inhalation to various nickel compounds in the past are at a significantly higher risk for cancer of the lungs and the nasal cavity than the non-occupationally exposed population. Laryngeal cancer, kidney cancer, and cancer of the prostate or bone have also been found in nickel workers, but the epidemiological evidence does not indicate a relationship to nickel exposure or to any other occupational origin (WHO, 2001).

Nickel can usually be detected at higher concentration in the chocolate, especially in chocolate with cocoa-based product. Nickel is the main known contaminant resulting from the manufacturing process of chocolate. Chocolate hardening is done by hydrogenation of unsaturated fats using Raney nickel (50% aluminium and 50% nickel) as catalyst. Other methods by which nickel can contaminate toffees are raw materials containing nickel, their processing and canning for transportation and storage in nickel containers.

Many methods can be used to determine nickel such as Atomic absorption spectrometry (AAS) method and Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) method. Due to its electroactive property, nickel can be determined by voltammetric technique.

Stripping voltammetry is a sensitive electro analytical technique for the determination of trace amounts of metals in solution. The technique consists of three

steps. First, metal ions are deposited onto an electrode which is held at a suitable potential. The solution is stirred during this step to maximize the amount of metal deposited. Second, stirring is stopped so that the solution remains undisturbed. Third, the metal deposits are stripped from the electrode by scanning the potential. The observed current during the stripping step can be related to the amount of the metal in the solution.

The stripping step may consist of a positive or a negative potential scan, creating either an anodic or cathodic current respectively. Hence, Anodic Stripping Voltammetry (ASV) and Cathodic Stripping Voltammetry (CSV) are the two specific stripping techniques.

Cathodic stripping voltammetry (CSV) is the most suitable method for field analyses because of its relatively simple and portable instrumentation, minimum sample preparation, rapid analysis time (<10 min per sample), small sample volume, and ease of quantification (Y. He et. al., 2002). It also can detect nickel up to 50 ppt (**Application Bulletin of Metrohm no 231/2e**).

Differential pulse is a series of periodical constant pulse of potential that is superimposed to a linear scanning produces a consistent enhancement of the signal. Moreover, if the difference between the current just before and at the end of the pulse is measured, a reading less influenced by the capacitive current can be obtained. In this way this differential reading of the current generates a peak shaped voltammogram. The

technique is very sensitive and detection limits range near 10 – 100 µg/l (Pierpaolo Protti, 2001).

The concentration of nickel in a sample using DPCSV can be detected by three ways. First, by using the chelating agent such as DMG (or more known as DPAdSV). Second, by the use of some organic compounds such as mercaptopurine-riboside (MPR), which could catalyzed the reduction of nickel ion and thirdly, by direct reduction of $[\text{Ni}(\text{H}_2\text{O})_6]^{2+}$.

LITERATURE REVIEW

Many studies have been conducted in order to determine the concentration of nickel in various types of samples. All studies used many types of methodology, such as AAS, ICP-MS and electro analytical technique.

Reinhard Kissner in his study stated that nickel can be determined as bis-(dimethylglyoximato)-nickel(II) in industrial waste water samples and in urine by fast cathodic linear sweep voltammetry with a rate of 10 V s^{-1} after adsorption onto a static mercury electrode (Reinhard Kissner, 1988). The peak width at half-height was 60 mV. For determinations as low as to 1 ppb of Ni(II), only 40 s accumulation time were required without accelerating measures like stirring or potential application. Resolution and sensitivity were thus similar to differential pulse voltammetry but the duration of a measuring cycle was only 45 s (40 s accumulation, 80 ms for sweep and 5 s for readout). The direct determination of Ni(II) in urine failed, whereas it was possible to determine nickel concentration in the waste water. Concurrent adsorption was the main interference.

Another study of nickel determination was reported by using of adsorptive stripping voltammetry, in which the determination of nickel, aluminium, selenium and arsenic were performed by the addition of 2,5-dimercapto-1,3,4-thiadiazole (DMTD) that has been used as a chelating agent (Li Chiang e.t. al, 1989). By a suitable choice of deposition potential, deposition time, reagent reaction time and other operating

conditions, the determination of the four elements could be achieved simultaneously. According to this study, the use of benzyltrimethyl ammonium methoxide as a digesting solvent, it was possible to apply the procedure to the direct determination of the four elements in biological samples.

Constant current potentiometry was used for nickel and cobalt determination in water (Eduardo Pinilla Gil and Peter Ostapczuk, 1993). All parameters like deposition potential and time, buffer and DMG concentration, and current density which have an influence on the determination sensitivity were optimized [deposition potential: -0.5 V vs. Ag/AgCl (saturated KCl); buffer concentration 0.01 mol/l, pH 9.3; DMG concentration 0.05 mmol/l and deposition time 10 s, current: -15 μ A]. No significant differences of the determination sensitivity between cathodic stripping voltammetry and constant current potentiometry were found.

In Poland, a research was conducted with necessary conditions for simultaneous nickel and cobalt determination in environmental samples, such as oak wood and soil, based on cathodic adsorptive stripping voltammetry (Jadwiga Opydo, 2001). Ni(II) and Co(II), complexed with dimethylglyoxime, were determined using a hanging mercury drop electrode. Optimum conditions were found to be as follows: accumulation time 90 s, accumulation potential -0.80 V vs. SCE, supporting electrolyte 0.2 mol dm^{-3} ammonia-ammonium chloride buffer (pH = 9.4) + 0.05 mol dm^{-3} NaNO₂ and dimethylglyoxime 2×10^{-4} mol dm^{-3} . A linear current-concentration relationship was observed up to 7.5×10^{-7} mol dm^{-3} for Ni(II) and 7.0×10^{-7} mol dm^{-3} for Co(II). Excess amounts of zinc(II)

interfering with cobalt peaks were masked by complexation with EDTA. Wood and soils were mineralized by applying a microwave digestion system, using mixtures hydrogen peroxide and nitric acid or nitric acid and hydrogen fluoride, respectively. The developed procedure was used to determine pollution of oak wood and soil with nickel and cobalt in different regions of Poland.

The determination of nickel content, along with lead and cadmium contents were studied in 69 different brands of chocolates and candies available in local markets of suburban areas of Mumbai, India by using Atomic Absorption Spectroscopy (Sudhir Dahiya e.t. al, 2003). The majority of these chocolates and candies were made mainly from cocoa, milk solids, dry fruits, fruit flavours and sugar. Out of 69 brands of chocolates and candies analysed, 23 were cocoa-based, 22 milk-based and another 24 were of fruit flavour and sugar-based. According to this study, concentration of nickel ranged from 0.041 to 8.29 $\mu\text{g/g}$ with an average of 1.63 $\mu\text{g/g}$. Cocoa-based chocolates are found to have higher concentration of heavy metals compared to milk-based chocolates, fruit flavour- or sugar-based candies. The higher concentration of these elements in the cocoa-based chocolates was mainly due to their high concentrations in the raw materials such as cocoa beans, cocoa solids, and cocoa butter.

The study of combined analytical procedures consisting of wet digestion step followed by instrumental determination such as differential pulse cathodic stripping voltammetry (DPCSV) or electrothermal atomic absorption spectrometry (ETAAS) - as well as a direct analysis method - slurry sampling ETAAS - for the determination of Cd, Co, Cr, Cu, Fe, Ni and Pb in milk, cheese and chocolate were described and compared

(I.Karadjova e.t. al., 2004). Wet digestion using a mixture of nitric acid-perchloric acid-hydrogen peroxide was proposed for complete matrix decomposition prior to trace analyte determination by DPCSV or ETAAS. In this study, 1-phenyl-propan-1-pentylsulfonylhydrazon-2-oxime was selected as complexing agent for simultaneous determination of Cd, Co, Cu, Ni and Pb. Optimal instrumental parameters for trace analyte measurements were presented. The reliability of the procedures has been verified by analyzing standard reference materials.

Ni, Co, and Zn are among toxic metals of significance for environmental surveillance. Therefore, determination of these elements in wastewater is very important. This study reports the use of an adsorptive cathodic stripping voltammetric technique for the simultaneous determination of Ni(II), Co(II), and Zn(II) with dimethylglyoxime (DMG) as a chelating agent is described by Rezaei **(B. Rezaei and E. Rezaei, 2005)**. Voltammograms of Ni(II), Co(II), and Zn(II) initially contained three peaks corresponding to these metals. However, the peaks overlapped. Therefore, the effect of organic solvents was studied, and the results showed that the use of a suitable ratio of ethanol-water (1:5) solvent and a particular pH, provided peaks that were distinctly separated. The metals can be quantified at concentrations above 0.03, 0.02, and 0.1 $\mu\text{g/mL}$ for Ni, Co and Zn respectively. The relative standard deviation, RSD (%) at concentration levels of 0.10 $\mu\text{g/mL}$ for Ni(II), 0.10 $\mu\text{g/mL}$ for Co(II), and 0.30 $\mu\text{g/mL}$ for Zn(II) is 2.3, 2.0, and 3.3%, respectively. The influence of pH, DMG concentration, scan rate, accumulations time, and potential was investigated. The method was satisfactorily used for determination of the metals under study in water and wastewater.

OBJECTIVES

The objectives of this study are:

- i. To determine nickel qualitatively in various brands of chocolate.
- ii. To determine nickel quantitatively in various brands of chocolate.
- iii. To develop a method of direct determination of nickel concentration in sample.
- iv. To develop optimum voltammetric parameters for detection of nickel in chocolate by using Differential Pulse Cathodic Stripping Voltammetry technique.
- v. To develop an optimum voltammetric condition parameter for the detection of nickel in chocolate samples by using Differential Pulse Cathodic Stripping Voltammetry technique.
- vi. To develop a method for detection of nickel in chocolate.

MATERIALS AND METHODS

1) Samples collection:

3 Brands of chocolate have been purchased from supermarket which were:

1. Chippy chip(labelled as A)
2. Romako (labelled as B)
3. Kokotube (labelled as C)



Figure 1: Three brands of chocolate, from left; Kokotube, Romako and Chippy chip

2) Chemicals and reagents:

Boric acid (Merck), glacial acetic acid (Merck), orthophosphoric acid (Merck), 32% acid hydrochloric (Merck), Nickel stock solution (Merck) and nitric acid (67%)

3) Glassware:

Voltammetric cell, 1000 ml volumetric flask, 100 ml volumetric flask, 25 ml volumetric flask, 50 ml beaker, serological pipette 10 ml, glass funnel, evaporating dish, 100 ml conical flask, pipette fillet, analytical pipette 100 μ l, pipette tip, bulb pipette and amber bottle.

4) Sample preparation:

- 4.1) The sample was mixed thoroughly to achieve homogeneity. All equipment used for homogenization was cleaned and rinsed by 10% acid nitric to minimize the potential of cross-contamination. For each digestion procedure, the sample was weighed to 5 g (dry weight) and transferred to an evaporating dish.
- 4.2) 20 mL of concentrated HNO_3 was added and the solution is mixed. The sample was heated on the hot plate to $95^\circ\text{C} \pm 5^\circ\text{C}$ and evaporated until residue was left.
(If brown fumes generated, it indicates the oxidation of the sample by HNO_3)
- 4.3) 10 mL of concentrated HNO_3 was added until the solution becomes colorless.
- 4.4) 10 ml 1:1 HCl was added into the solution and evaporated until clear.
- 4.5) 10 mL of concentrated HNO_3 was continued added until the solution becomes colorless.
- 4.6) When the colorless solution was obtained, it was evaporated and left a residue.
- 4.7) After cooling, particulates in the digest were removed by filtration using Whatman No. 41 filter paper.
- 4.8) The solution was transferred into a volumetric flask and diluted to 50 mL with double distilled water. The sample was ready for analysis.

5) Instrumentation:

Determination of nickel in chocolate was determined by using Metrohm 757 VA Computrace Voltammetric Analyser combined with Multimode Electrode (MME) System. The electrode system consists of:

- i. Working electrode: Hanging Mercury Drop Electrode(HMDE)
- ii. Reference electrode : Ag/AgCl/KCl_{sat}
- iii. Auxilliary electrode : platinum

The voltammetric parameter for sample analysis was optimized first. These voltammetric parameters include:

- i. Initial potential, E_i
- ii. Final potential, E_f
- iii. Deposition potential, E_{acc}
- iv. Deposition time, t_{acc}
- v. Scan rate, v

Before starting the analysis, the supporting electrolyte was purged with nitrogen gas for 1200 seconds to remove dissolved oxygen in the solution. A cyberscan 1000 pH had been utilized to check the pH value of the supporting electrolyte in the voltammetric cell prior to any analysis.



Figure 2: Metrohm 757 VA Computrace Voltammetric Analyser

5) Methodology:

The method used in this study is shown in chart 1.

EXPERIMENTAL

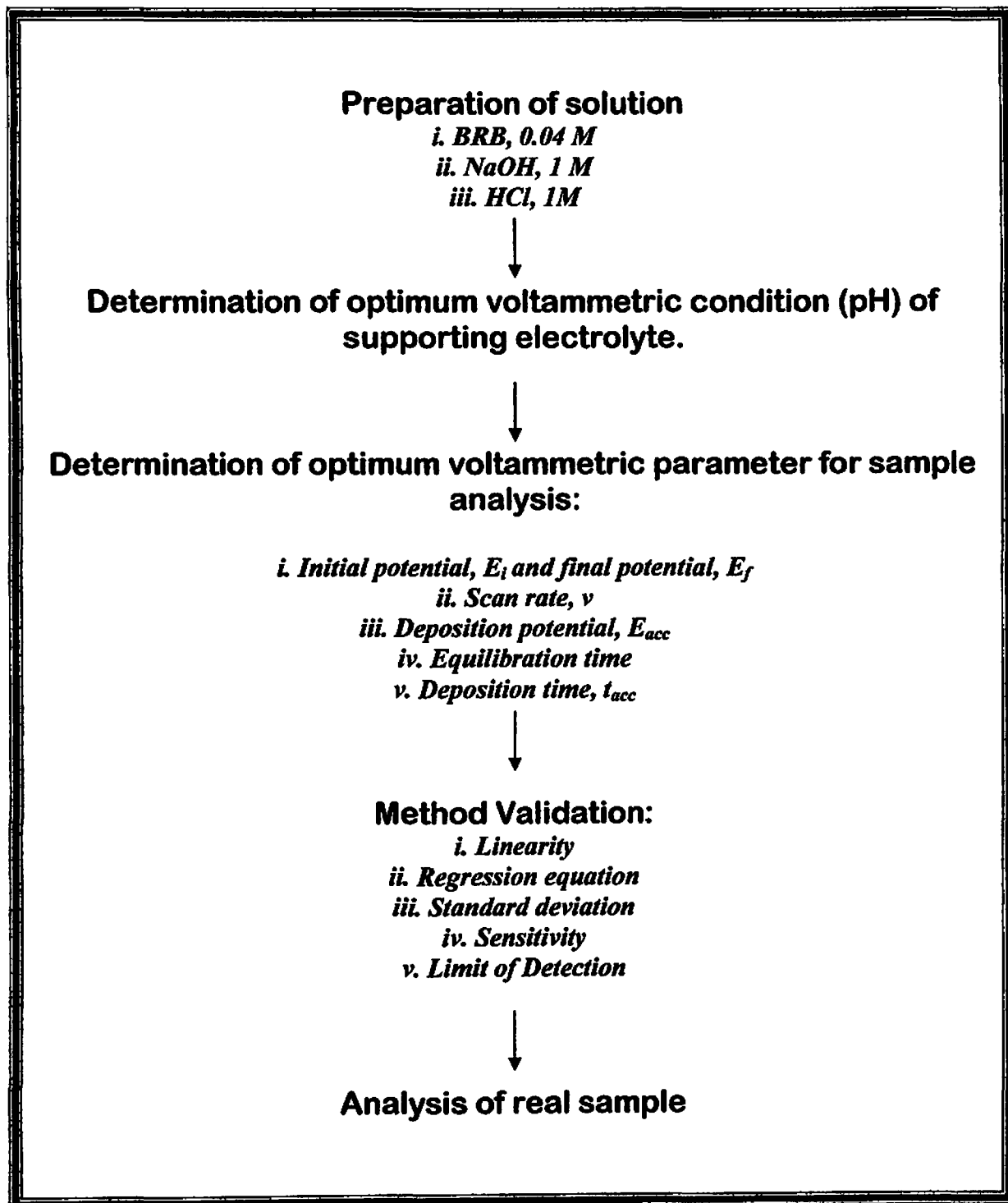


Chart 1: Flow chart of methodology

1) Preparation of solution:

i. Britton-Robinson Buffer (BRB), 0.04 M

2.47 g of boric acid (Fluka) was dissolved in 1000 mL volumetric flask containing distilled water, 2.30 mL glacial acetic acid (Merck) and 2.70 mL orthophosphoric acid (Merck). The solution then diluted to the 1 liter mark of volumetric flask.

ii. 100 mL HCl, 1M

9.8 mL of 32% HCl was added to 100 mL volumetric flask. Distilled water then was filled up to 100 mL mark and this solution was transferred into an amber bottle.

iii. 100 mL NaOH, 1M

4 g of NaOH powder was dissolved into 100 mL of distilled water. The solution was transferred into an amber bottle.

iv. Supporting electrolyte

- a. 9 mL of BRB, 0.04 M for optimization procedure using lead standard solution**
- b. 9 mL of BRB, 0.04 M for sample analysis.**

2) Determination of optimum voltammetric condition (pH) of supporting electrolyte.

9 mL of BRB, 0.04 M was mixed with 1 ml of 50 ppm of nickel standard solution. pH of supporting electrolyte was adjusted by adding HCl or NaOH into the solution. Then, the supporting electrolyte was analyzed by voltammetry. Peak height, i_p of nickel in the electrolyte with adjustable pH was recorded. A series of pH was tested. Result was presented by a graph of peak height, i_p versus pH (figure 3).

3) Determination of optimum voltammetric parameter for sample analysis:

9 mL of BRB, 0.04 M was mixed with 1 ml of 50 ppm nickel standard solution for optimization of voltammetric parameter. All analysis was performed at the pH which had been selected during the determination of optimum voltammetric condition (pH) of supporting electrolyte previously. The parameters which optimized include:

i. Initial potential, E_i and final potential, E_f

The initial potential, E_i and final potential, E_f for sample analysis were determined. The optimized initial potential, E_i was the potential that exclude hydrogen potential and the optimized final potential, E_f was the potential before the mercury potential.