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Voltammetric Determination of Iron in Powder Milk

Dissertation submitted in partial fulfillment for the Degree of Bachelor of Science in Forensic Science

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ABSTRACT

In this study, voltammetric method which is one of the electro analytical techniques is described. The research focused on adsorptive stripping voltammetry (AdSV) in the presence of catechol for confirmation of electro analytical properties of iron. The analysis was carried out after acid digestion procedure then measurement was made using phosphate buffer 7.0 as the supporting electrolyte. The electrodes used in this analysis were hanging mercury dropping electrode (HMDE) which act as the working electrode, a platinum electrode as auxiliary electrode and Ag/AgKCl reference electrode respectively. Optimum voltammetric parameter for the analysis of the sample such as initial potential, final potential, deposition potential, deposition time and scan rate were determined prior to sample analysis using iron standard solution at 1 ppm. The result showed that the optimum voltammetric parameters for the determination of iron in powder milk were, initial potential, (E_i) of 0 V, final potential (E_f) of -0.7 V, deposition potential E_{acc} of -0.6 V, deposition time of 0 s and scan rate of 0.030 V/s were obtained. The pH 7.0 was found out to be the optimum voltammetric condition for the determination of iron in samples. 3 different samples of powder milk were analyzed. The result showed that, the sample A and B contained 0.5776 and 0.3075 ppm of trace iron respectively and no iron was detected in sample C

INTRODUCTION

Iron is an essential element in health aspect. Most of the human body's iron is contained in red blood cell's' hemoglobin, and iron-deficiency is the most common cause of anemia. However, the control of this potentially toxic substance is important, because it can affect many aspects of human health and disease. The most important group of iron-binding proteins is the heme molecules, which contain iron at their centers. Humans use variants of heme to carry out redox reactions and electron transport processes. These reactions and processes are required for oxidative phosphorylation. That process is the principal source of energy for human cells, and without it, the cells would die (Andrews, 2000).

Humans also use iron in the hemoglobin of red blood cells, in order to transport oxygen from the lungs to the tissues and to export carbon dioxide back to the lungs. Besides, iron is an essential component of myoglobin to store oxygen in muscle cells. Humans have no physiologic regulatory mechanism for excreting iron. So human bodies tightly regulate iron absorption and recycling. Most humans prevent iron overload solely by regulating iron absorption. Those who can't regulate absorption well enough get disorders of iron overload and those who have deficiency in iron absorption will lead to iron deficiency such as anemia. Most of the iron in the body is hoarded and recycled by the reticuloendothelial system which breaks down aged red blood cells. However, people lose a small but steady amount by sweating and by shedding cells of the skin and the mucosal lining of the gastrointestinal tract. The total amount of loss for healthy people in the developed world amounts to an estimated average of 1 mg a day for men and 1.5 – 2

mg a day for women with regular menstrual periods (Andrews, 2000). This steady loss means that people must continue to absorb iron. They do so via a tightly regulated process that under normal circumstances protects against iron overload.

Milk is one of the important sources of iron. The breast milk from mother is usually enough for the infant, but nowadays many of the mothers didn't have time to breastfeeding and alternatively they prefer to use powder milk. The minerals content in powder milk is controlled in order to ovoid any excess amount or deficiency of the minerals that can lead to disease. Some of the technique used for determination of mineral content in powder milk such as Inductive couple plasma-atomic emission spectroscopy (ICP-AES), Inductive couple plasma mass spectroscopy (ICP-MS), and Graphite furnace atomic absorption spectroscopy (GF-AAS) by the powder milk manufacturer to control their product quality. However, they are too expensive. Voltammetric becomes the technique of choice because it require relatively inexpensive instrumentation and are capable of determining element accurately at trace to ultra trace levels and have demonstrated ability for multi-element determination.

Stripping Analysis

The term electrochemical stripping analysis is applied to a family of procedures involving a preconcentration of the determinant onto the working electrode, prior to its direct or indirect determination by means of an electro analytical technique. Such a combination of an effective accumulation step with an advanced measurement procedure results in a very low detection limit, and makes stripping analysis one of the most important techniques in trace analysis (Fogg, 1999). The original stripping analysis method involved the cathodic electro deposition of amalgam forming metals onto a hanging mercury drop electrode, followed by the anodic voltammetric determination of the accumulated metal during a positive-going potential scan. Numerous advances, however, have led to the development of alternative preconcentration schemes and advanced measurement procedures that further enhance the scope and power of stripping analysis. As a consequence, numerous variants of stripping analysis exist currently, differing in their method of accumulation and measurement. A recent stripping analysis was adsorptive stripping voltammetry.

Even though anodic stripping voltammetry (ASV), cathodic stripping voltammetry (CSV), and adsorptive stripping voltammetry (AdSV) each have their own unique features, all have two steps in common. First, the analyte species in the sample solution is concentrated onto or into a working electrode. It was the crucial preconcentration step that results in the exceptional sensitivity that can be achieved. During the second step, the preconcentrated analyte is measured or stripped from the electrode by the application of a potential scan. (Brainina et al, 1993). Any number of potential waveforms can be used for the voltammetric techniques stripping step. The

most common is differential pulse due to the discrimination. Important conditions that should be held constant include the electrode surface, rate of stirring, and deposition time. Every effort should be made to minimize contamination.

Adsorptive stripping voltammetry (AdSV) is quite similar to anodic and cathodic stripping methods. The primary difference is that the preconcentration step of the analyte is accomplished by adsorption on the electrode surface or by specific reactions at chemically modified electrodes rather than accumulation by electrolysis (Bard et al, 1980). Many organic species such as heme, chlorpromazine, codeine, and cocaine have been determined at micromolar and nanomolar concentration levels using adsorptive stripping voltammetry (AdSV). Inorganic species such as Molybdenum and ferum had also been determined. In this research, The AdSV technique was applied with the presence of catechol as the adsorptive agent.

LITERITURE REVIEW

Historically, the branch of electrochemistry we now call voltammetry developed from the discovery of polarography in 1922 by the Czech chemist Jaroslav Heyrovsky, for which he received the 1959 Nobel Prize in chemistry. The early voltammetric methods experienced a number of difficulties, making them less than ideal for routine analytical use. Numerous advances during the 1980s and 1990s, however, have led to the development of alternative preconcentration schemes and advanced measurement procedures that further enhance the scope and power of stripping analysis (Esteban, 1994). As a consequence, numerous variants of stripping analysis exist currently, differing in their method of accumulation and measurement.

In 1989, an analytical procedure for the determination of iron(III) and total iron in wines based on adsorptive stripping voltammetry is described by Wang and Mannino. Iron (III) was determined by using Solochrome Violet Red as chelating agent while catechol was used for the determination of the total iron content. Each chelate was adsorbed on the hanging mercury electrode and the reduction current of the accumulated chelate was measured (Wang et al, 1989).

In 1991, as opposed to many spectroscopic techniques, trace metal determination by adsorptive stripping voltammetry (AdSV) is performed directly in brine. Summary routine analysis of brine for trace metals is important for safe and economical production in the alkali chloride electrolysis. With minimal sample preparation chromium

(VI), iron (III), nickel (II), cobalt (II), titanium (IV), manganese (II), molybdenum (VI) and vanadium (V) can be determined within minutes. The influence of parameters such as pH-value, supporting electrolyte solution, concentration of complexing reagents and possible interferents had been investigated for optimal experimental conditions (Romanus et al, 1991).

Gao and Siow (1995) described about a highly sensitive and selective voltammetric procedure is for the determination of trace amounts of iron. The procedure is based on the adsorptive collection of an iron-thiocyanate-nitric oxide complex on a hanging mercury drop electrode. The adsorbed complex catalyzes the reduction of nitrite in solution, which gives a detection limit of 40 ppt iron with 30 s accumulation. The stripping current increases linearly with iron concentration up to 80 ppb. Most of the common ions, except cobalt, do not interfere with the determination of iron. The procedure was applied to determine iron in biological samples, natural waters and analytical-grade chemicals.

The electrochemical behavior of iron makes its polarographic determination very difficult. Therefore, for the analysis of trace amounts of iron most of the reported electroanalytical procedures are based on adsorptive stripping voltammetry. For example, in the presence of catechol, I-nitro-2-naphthol, solochrome violet KS, or 2-(5-bromo-2-pyridylazo) 5-diethyl-aminophenol, trace amounts of iron can be determined after adsorptive accumulation of the iron complexes with these ligands (Gao et al, 1995).

The toxic effect caused by iron in kidney was performed on experimental studies with mice following administration of a metallic solution of this species to simulate the iron corrosion products of a metallic implant. To quantify the total levels of iron present in this organ, an electrochemical method was chosen based on the application of square wave voltammetry using adsorptive collection of iron-catechol on a mercury film microelectrode (MFM). The optimal working conditions to produce a very stable and reproducible iron peak in the digested kidney samples were found to be pH = 7.2 provided by 8.0 mol/L PIPES buffer, catechol concentration of 3.0 x 10^{-4} mol/L, deposition potential -1.80 V and deposition time 20 s. These results were compared with those obtained by atomic absorption spectrometry (AAS) indicating a good performance of the electrochemical method used. The analytical results show an increase of iron concentration with treatment time, which indicates that this metal ion is partially accumulated in the kidney. This accumulation induces with time some morphological alterations as evidenced by the histological analysis (Trace, 1998).

A procedure for iron determination in quartz and silica glass materials based on adsorptive stripping voltammetry (AdSV) in the presence of catechol is described by M. Gawry and Golimowski in 1999. Hanging mercury drop electrode as a working electrode was used. The optimized conditions include pH 7.1, accumulation potential -0.1 V, accumulation time 60 s, 10 mV s^{-1} scan rate, and 25 mV pulse amplitude. In case of 60 s accumulation time the obtained detection limit was 2. 2 g/L Fe. The possibility of simultaneous determination of iron and copper in the same solution is shown. Atomic

absorption spectroscopy (AAS) technique was applied as a reference method to AdSV measurements (Gawry and Golimowski, 1999).

Determination of Cd, Co, Cr, Cu, Fe, Ni and Pb in milk, cheese and chocolate Combined analytical procedures consisting of wet digestion step followed by instrumental determination - differential pulse cathodic stripping voltammetry (DPCSV) or electro thermal 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 are described and compared. Wet digestion using a mixture of HNO_3-$HClO_4$-H_2O_2 is proposed for complete matrix decomposition prior to trace analyte determination by DPCSV or ETAAS. A mixture of HNO_3-H_2O_2 is used for slurry preparation. Optimal instrumental parameters for trace analyte measurements are presented (Karadjova et al, 2000).

Voltammetric technique require relatively inexpensive instrumentation and capable of determining element accurately at trace to ultra trace levels and have demonstrated ability for multi-element determination (Inam et al, 2000). It becomes the technique of choice, since most of the sensitive and selective method recently available such as Inductive Couple Plasma-Atomic Emission Spectroscopy (ICP-AES), Inductive Couple Plasma Mass Spectroscopy (ICP-MS), and Graphite furnace atomic absorption spectroscopy (GF-AAS) were too expensive to be used in routine analysis (Sham et al, 2004).

OBJECTIVES

The objectives of this study are:

1. To determine iron qualitatively and quantitatively in various brands of powder milk.
2. To study optimum voltametric parameters and optimum voltammetric condition for detection of iron in milk using Differential Pulse Adsorptive stripping Stripping Voltammetry technique.
3. To develop a method for detection of iron in powder milk

MATERIALS AND METHODS

1. Chemicals and Reagent

The chemicals reagent used in the preparation of solution are HNO_3 and HCl for sample digestion, Phosphate tablet pH 7.0, for phosphate buffer, and Catechol for complex agent with iron.

2. Glassware

Voltammetric cell, volumetric flask 100 ml, 50 ml and 25 ml, beaker 50 ml, serological pipette 10 ml, pipette filler, analytical pipette 100 µl, pipette tip, bulb pipette and amber bottle

3. Sample collection

3 sample of powder milks, A (Lactogen 1), B (Dumex) and C (Omega Plus) were used in this research. These samples were purchased from the supermarket around Kota Bharu, Kelantan. After suitable wet digestion, the samples were analyzed using Metrohm 757 VA Computerace Voltammetric Analyzers combined with Multimode Electrode (MME) system. The amount of trace iron in samples A and B were stated on the packaging label and none for sample C.

Sample A

Sample B

Sample C

Figure 1; Powdered milk samples that have been used

4. Instrumentation

A

B

C

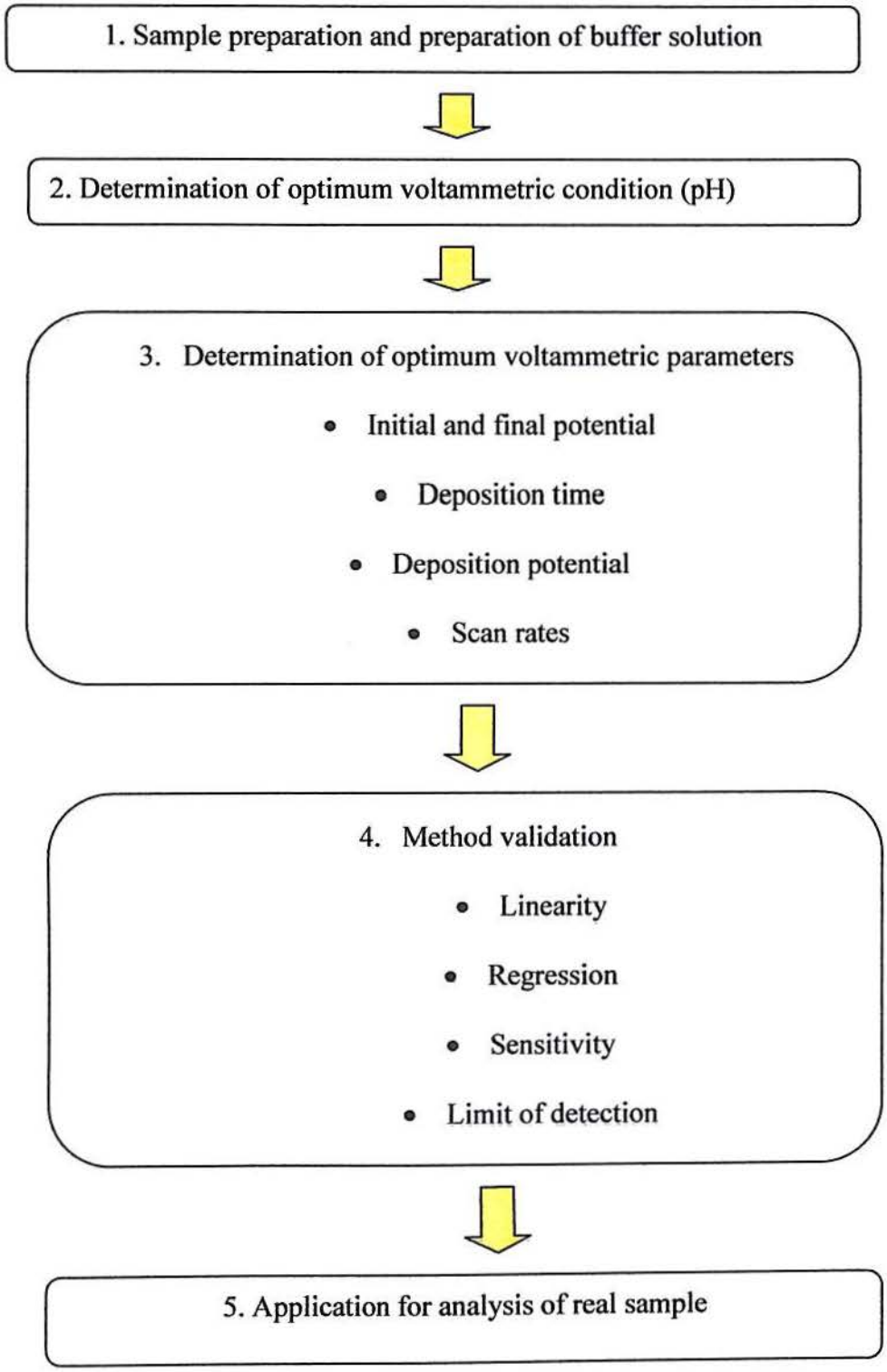
Figure 2; 757 VA Computerace Voltammetry

A; Voltammetric cell of 757VA Computerace Voltammetry, B; Computer Integrated system, C; Multimode electrode

A Metrohm 757 VA Computerace Voltammetry with three electrode system was used to determine the presence of iron in the powder milk samples. The electrode system consists of working electrodes, a platinum electrode, as the auxillary electrode and an $Ag/AgCl/KCl_{sat}$ reference electrode. Hanging mercury drop electrode (HMDE) was used as the type of working electrode. The voltammetric parameters such as initial potential E_i, final potential E_f, deposition potential, E_{acc}, deposition time t_{acc}, scan rate v, and experimental condition such as pH of supporting electrolyte were optimized prior to the sample.

5. Experimental

Systematic working procedure for determination of iron in powder milk



1 Sample preparation and preparation of buffer solution

a. Sample preparation

Standard EPA- Wet digestion for solid sample

1. 5 gram from each powder milk was weighted by using evaporating dish and labeled as A (Lactogen 1), B (Dumex) and C (Omega Plus)
2. 20 ml of HNO_3 was added to the sample, and placed on the hot plate
3. Then, the sample was evaporate until residue left
4. HNO_3 (10 ml each addition) was added continuously until colorless
5. After that, 10 ml of HCl was added (The release of brown fumes)
6. Evaporation process was continued until a clear solution is obtained.
7. The sample was evaporated again until residues left
8. Then, the sample was dissolved in deionized distilled water
9. Finally, the sample was transferred to 25 ml volumetric flask and ready for voltammetric analysis

b. Preparation of buffer solution

Ready to use solution-phosphate tablet buffer was dissolved in 100 ml distilled water by using magnetic stirrer

c. Catechol solution (1 mol/L)

2.75 g catechol was dissolved in 25 ml deoxygenated ultra pure water. The solution was stored in the dark and allowed to stand 1 hour before use. The stability of the solution depends on the purity of the substance and range from one to several days.

d. Preparation of standard iron solution from stock solution (10 mg/L)

10 ppm of standard solution was used for optimization.1 ml Fe is taken from stock solutions (1000 ppm) and filled up to 100 ml with ultra pure water.

2 Determination of optimum voltammetric condition (pH)

The optimum pH for supporting electrolyte for sample analysis was determined. A series of pH were tested.

3. Determination of optimum voltammetric parameters for sample analysis

All analysis was done at selected pH. Triplicate measurements were performed for each selected parameter which were initial potential, final potential, deposition time, deposition potential and scan rate.

4. Method validation

A series of different volumes; 200, 400, 600, 800, 1000, and 1200 µL of iron standard (10 ppm) were analyzed against the selected optimum voltammetric parameters and optimum condition (pH). Based on the results, a calibration curve was drawn. The linearity, regression, sensitivity, standard deviation and limit of detection of analysis were calculated.

5. Application for analysis of real samples

9 ml of phosphate buffer was used as supporting electrolytes.100 µL of catechol was added to the voltammetric cell. Then, 1ml of each sample was added and analyzed at optimum voltammetric condition and parameters. In this study, 3 brand of powder milks labeled as A (Lactogen 1), B (Dumex), and C (Omega Plus) were analyzed. Triplicate measurements were completed for each sample analysis.

RESULT

The findings of this research are presented in form of table and graph. All finding are as follows

A. Optimum voltammetric condition (pH)

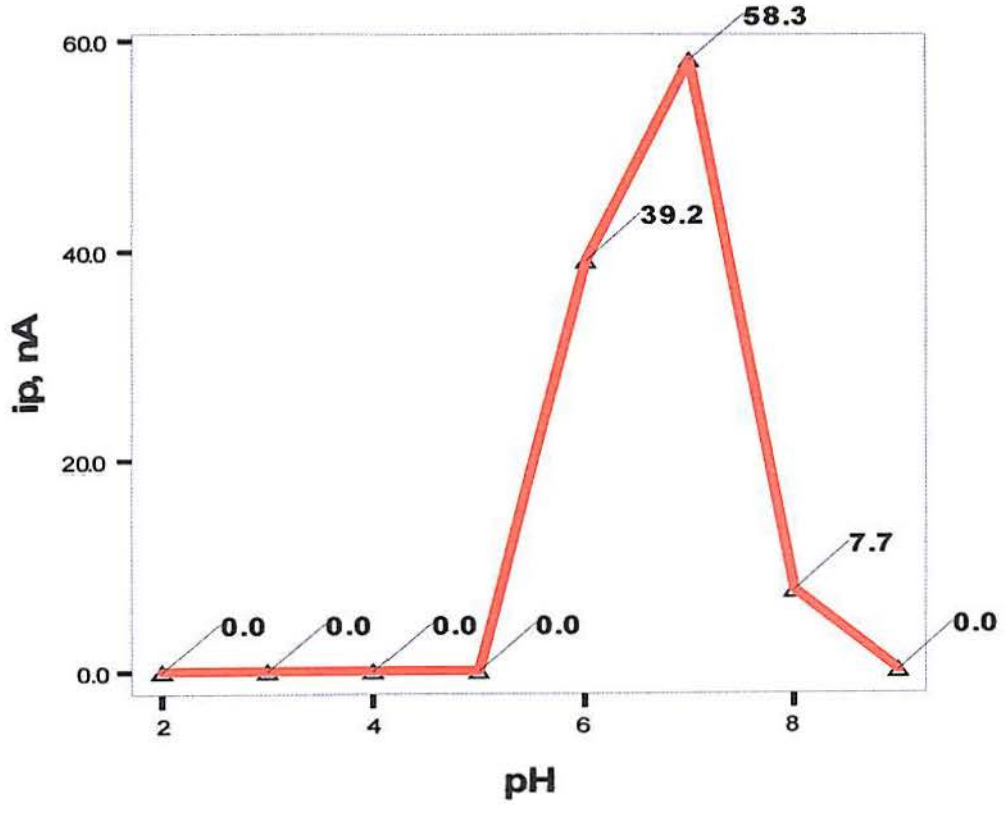


Figure 3; Graph of peak height, ip (nA) for iron at 1ppm versus pH of supporting electrolyte

Optimum pH condition for determination of trace iron in powder milks was pH 7