DETERMINATION OF COBALT (II) ION IN BEVERAGES BY VOLTAMMETRY

PIYAPORN MANGSANGKAM

A thesis submitted in partial fulfillment of the requirement for
the Master of Science degree in Chemistry
at Mahasarakham University
February 2014
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The examining committee has unanimously approved this thesis, submitted by Miss. Piyaporn Mangsangkam, as a partial fulfillment of the requirements for the Master of Science degree in Chemistry at Mahasarakham University.

Examining Committee

P. Chantiratikul
.... Chairman
(Asst. Prof. Piyanete Chantiratikul, Ph.D.) (Faculty Graduate Committee)

S. Kr.
.... Committee
(Senee Kruanetr, Ph.D.) (Advisor)

Uthai Sak
.... Committee
(Asst. Prof. Uthai Sakee, Ph.D.) (Co-advisor)

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.... Committee
(Watsaka Siriangkhawut, Ph.D.) (Faculty Graduate Committee)

W. Ruengsitagoon
.... Committee
(Assoc. Prof. Wirat Ruengsitagoon, Ph.D.) (External expert)

Mahasarakham University has granted approval to accept this thesis as partial fulfillment of the requirements for the Master of Science degree in Chemistry.

T.
....
(Assoc. Prof. Thiensak Mekkapan-opas, M.Sc.)
Acting Dean of the Faculty of Science

S. Saikrasun
....
(Assoc. Prof. Sunan Saikrasun, Ph.D.)
Acting Dean of the Faculty of Graduate Studies
..........February 28, 2014

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Piyaporn Mangsangkam

ชื่อเรื่อง การวิเคราะห์หาปริมาณโคบอลต์ (II) ในเครื่องดื่มด้วยเทคนิคโวลแทมเมตรี
ผู้วิจัย นางสาวปิยพร มังสังคำ
ปริญญา วิทยาศาสตรมหาบัณฑิต **สาขาวิชา** เคมี
กรรมการควบคุม อาจารย์ ดร.เสนีย์ เครือเนตร
ผู้ช่วยศาสตราจารย์ ดร.อุทัย สาขี
มหาวิทยาลัย มหาวิทยาลัยมหาสารคาม **ปีที่พิมพ์** 2557

บทคัดย่อ

งานวิจัยนี้ได้ศึกษาวิธีการหาปริมาณโคบอลต์ (II) ด้วยเทคนิคโวลแทมเมตรี โดยการวัดค่ากระแสการแพร่ของสารประกอบเชิงซ้อนระหว่างโคบอลต์กับไดเมทิลไกลออกซีมที่ขั้วไฟฟ้าใช้งาน ซึ่งทำการให้ศักย์ไฟฟ้าแบบดิฟเฟอเรนเชียลพัลส์เข้าไปในขั้วไฟฟ้าใช้งานในช่วง -800 ถึง -1300 มิลลิโวลต์ ด้วยอัตราการสแกน 15 มิลลิโวลต์ต่อวินาที พบว่า ศักย์ไฟฟ้าครึ่งคลื่นของสารประกอบเชิงซ้อนโคบอลต์-ไดเมทิลไกลออกซีม เท่ากับ -1022 มิลลิโวลต์ ได้ช่วงความเป็นเส้นตรงในช่วง 1.0-100 ไมโครกรัมต่อลิตร และค่าสัมประสิทธิ์สหสัมพันธ์ (r^2) เท่ากับ 0.999 มีขีดจำกัดต่ำสุดในการตรวจวัด (3SD) เท่ากับ 1.14 ไมโครกรัมต่อลิตรและปริมาณต่ำสุดที่สามารถตรวจวัดได้ (10SD) เท่ากับ 4.80 ไมโครกรัมต่อลิตร ความถูกต้องและความแม่นยำที่ทำการทดลอง 11 ครั้ง สำหรับความเข้มข้นของโคบอลต์3 ความเข้มข้น (1.0, 50.0 และ 100.0 ไมโครกรัมต่อลิตร) ของการทำซ้ำภายในวันเดียวกัน เท่ากับ 3.24 เปอร์เซ็นต์, 0.13 เปอร์เซ็นต์และ 0.06 เปอร์เซ็นต์และ การทำซ้ำระหว่างวัน เท่ากับ 5.07 เปอร์เซ็นต์, 0.19 เปอร์เซ็นต์ และ 0.09 เปอร์เซ็นต์ตามลำดับ ณ สภาวะที่เหมาะสมของเทคนิคโวลแทมเมตรี สามารถประยุกต์ใช้วิเคราะห์หาปริมาณโคบอลต์ในตัวอย่างเครื่องดื่มได้ค่าร้อยละการย้อนกลับคืนอยู่ในช่วง 84.44 ถึง 99.54 เปอร์เซ็นต์ นำไปเปรียบเทียบวิธีทางสถิติกับวิธีมาตรฐานเฟรมอะตอมมิกแอบซอร์พชันสเปกโทรเมตรีด้วยวิธีที-เทสท์ พบว่าไม่มีความแตกต่างกันที่ความเชื่อมั่น 95 เปอร์เซ็นต์

คำสำคัญ: โวลแทมเมทรี, โคบอลต์, ไดเมทิลไกลออกซีม

TITLE Determination of cobalt (II) ion in beverages by voltammetry
CANDIDATE Miss. Piyaporn Mangsangkam
DEGREE Master of Science degree in Chemistry
ADVISORS Dr. Senee Kruanetr, Ph.D.
Assistant Professor Dr. Uthai Sakee, Ph.D.
UNIVERSITY Mahasarakham University **YEAR** 2014

ABSTRACT

In this work, the method for determination of Co(II) ion using voltammetry was studied by measurement of the diffusion current of Co(II)-dimethylglyoxime at hanging mercury drop electrode (HMDE) as working electrode. A differential pulse mode was applied the potential in the range of -800 to -1300 mV at the scan rate 15 mV/s for electrolysis of Co(II)-dimethylglyoxime complex in voltammetric cell. It was found that the highest diffusion current of Co(II)-dimethylglyoxime complex the half wave potential at -1022 mV. The linear range for the determination of Co(II) in beverages sample was over the range of 1.0-100 µg/L of cobalt with a correlation coefficient (r^2) of 0.999. The limit of detection (3SD) and limit of quantification (10SD) were 1.14 and 4.80 µg/L, respectively. The repeatability and reproducibility as a percentage of the relative standard deviation calculated from 11 replicate measurements of three concentrations (1.0, 50.0 and 100.0 µg/L Co(II) standard solution) were 3.24 %, 0.13% and 0.06%, for repeatability and 5.07%, 0.19% and 0.09% for reproducibility. The proposed method was successfully applied to determine the Co(II) in beverage samples with the percentage recoveries in the range of 84.44 - 99.54%. The results obtained by proposed methods are not significantly different from those obtained by the flame atomic absorption spectrophotometric method at a confidence interval of 95% verified by Student*t-test*.

Keyword: Voltammetry, Cobalt, Dimethylglyoxime

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List of Abbreviations

AAS	atomic absorption spectrophotometry
AR	analytical reagent
cm	centimeter
°C	degree celcius
FAAS	flam atomic absorption spectrophotometry
FIA	flow injection analysis
HPLC	high performance liquid chromatography
hrs	hours
i.d.	inner diameter
in.	inch
L	liter
LOD	limit of detection
LOQ	limit of quantitation
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mg/L	milligram per liter
mol/L	mole per liter
nm	nanometer
RSD	relative standard deviation
SD	standard deviation
µL	microliter
µg	microgram
UV-VIS	ultraviolet visible spectrophotometry
w/v	weight by volume
v/v	volume by volume

CHAPTER 1

INTRODUCTION

Cobalt is a biologically essential trace element. Serving function as an essential component of vitamin B_{12} and participates in a number of enzymatic reactions. The absence of cobalt can cause serious physiological problems such as anemia and neuropathy. Since the concentration of cobalt is extremely low in various natural samples. In natural it can be found in water, soil, rocks, plants and animals [1-2]. Nowadays, some beverage such as energy drink generally contains vitamin B_{12} for improvement of the nutrition. Determination of Co(II) in beverage is of great interest because cobalt is important for living species as complexed vitamin B_{12}. Many techniques such as spectrophotometry [3-6], atomic absorption spectrometry [7], chemiluminescence [8] high performance liquid chromatography [9-10] and inductively coupled plasma-mass spectrometry (ICP-MS) [11] have been applied for determination of Co(II) are good sensitivity but there are high reagent and sample consumption and high waste production. Voltammetry is one of the analytical technique that is simple, high sensitivity, consumption small amount of sample and reagent and low detection limit. Therefore voltammetry is a good choice for determination of Co(II) in beverage samples. In these study was determination of Co(II) in beverages based on measurement the diffusion current of the Co(II)-dimethylglyoxime complex by voltammetry.

1.1 Purposes of the research

1.1.1 To study the voltammetric method for determination of Co(II) in beverage samples

1.1.2 To optimize the electrochemical parameters for determination of Co(II) using voltammetry

1.1.3 To apply the proposed method for determination of Co(II) in beverage samples

1.2 Hypothesis of the research

The different type of supporting electrolyte, concentration of dimethylglyoxime, applying potential and scan rate may be effected on the diffusion current of Co(II)-dimethylglyoxime complex.

1.3 Expected results obtained the research

1.3.1 The optimum conditions for determination of Co(II) using voltammetry was obtained.

1.3.2 Application of the proposed method for determination of Co(II) in beverage samples was achieved.

1.4 Scope of the research

The scope of this work has been classified to five main parts as follows;

1.4.1 The literature and the analytical method for determining Co(II) was reviewed.

1.4.2 The diffusion current of Co(II)–dimethylglyoxime complex was scan in the applying potential range of -800 to -1300 mV.

1.4.3 Optimum parameters for determination of Co(II) in beverage samples by voltammetry such as concentration of dimethylglyoxime, type of supporting electrolytes, applying potential and scan rate for differential mode was studied.

1.4.4 The validation of the proposed method such as precision, detection limit and accuracy was performed.

1.4.5 The proposed method was applied for determination of Co(II) in beverage samples comparison with standard method.

1.5 Definition of terms

Voltammetry: An analytical technique based on the measurement of the current flowing through an electrode dipped in a solution containing electro-active compounds, while a potential scanning is imposed upon it.

Diffusion Current (i_d): The limiting current when the rate of electrolysis is controlled by the rate of diffusion of species to the electrode.

Half-wave potential: An important feature can be derived from the voltamogram. It is the potential corresponding to one half the limiting current i.e. $i_d/2$ and is a characteristic for each element and thus used for qualitative analysis.

Polarization: A change in the equilibrium potential of an electrochemical reaction

CHAPTER 2

LITERTURE REVIEW

2.1 Cobalt

Cobalt, is a chemical element with symbol Co, atomic number 27 with molecular weight of 59 g/mol, that is essential for the healthy functioning of many plants and animals and it usually found in food as vitamin B_{12}. Cobalt is the active center of coenzymes called cobalamin or vitamin B_{12}. Cobalt is stored in red blood cells with smaller amounts in kidney, liver, pancreas and spleen. Cobalt helps with the repair of the myelin sheath, increases the effectiveness of glucose transport from the blood into body cell and DNA synthesis and nervous system synthesis in brain [12].

2.1.1 Sources of cobalt

Cobalt can be found in water, soil, rocks, plants and animals. In food cobalt is a component of the vitamin B_{12}. In food, cobalt is a component of vitamin B_{12} [13]. Source of cobalt contents in various foodstuffs are shown in Table 2.1

Table 2.1 Source of cobalt contents in various foodstuffs [13]

Food Group	**Concentration (mg/kg)**	**Food Group**	**Concentration (mg/kg)**
Bread	0.006	Meats	0.008
Breakfast Cereals	0.008	Poultry and game	0.002
Rice	0.010	Offal	0.033
Other Cereals	0.001	Fish	0.007
Milk	0.001	Crustaceans and molluscs	0.046
Cheese	0.018	Vegetable (excluding potatoes)	0.006
Eggs and derivatives	0.005	Pulses	0.008
Butter	0.018	Fruits	0.009
Oils	0.018	Dried fruits and nuts	0.041

2.1.2 Applications of cobalt

Cobalt is used as a component of several alloys, including carboloy and stellite that are used to make very hard cutting tools. Cobalt is also used in some stainless steels. Alnico, an alloy of aluminum, nickel, cobalt and other metals are used to make high-strength permanent magnets. Cobalt is also used in electroplating to give a hard surface that is resistant to oxidation and as a blue colorant in pottery enamels and glass. High-energy gamma rays emitted during the radioactive decay of cobalt-60 can be used to detect flaws in metal components and in brachytherapy to treat various types of cancer [14].

2.1.3 Toxicology of cobalt

Cobalt dust is a mild irritant to the eyes and the skin. Symptoms of ingestion include hypotension, pericardial effusion, vomiting and convulsions. Inhalation of cobalt dust and fumes has caused shortness of breath, dermatitis with hyperemia and vesiculation. Additionally, cardiac effects, congestion of the liver, kidneys, conjunctiva and immunological effects have been observed [15].

2.2 Dimethylglyoxime (DMG)

Dimethylglyoxime (2,3-Butanedione Dioxime) is a chemical compoundwith the formula of$C_4H_8N_2O_2$ and molecular weight of 116.12 g/mol, it’s intensely white colour or off white powder and solid. Dimethylglyoximeis the dioxime derivative of the diketone and it’s coordination complexes are of theoretical interest as models for enzymes and as catalysts. Many related ligands can be prepared from other diketones[16-18]. The chemical structure of dimethylglyoxime is shown in Figure 2.1

Figure 2.1 Structure of dimethylglyoxime [18]

2.3 Voltammetry

Voltammetry was developed from the discovery of polarography in 1922 by the Czech chemist Jaroslav Heyrovsky. It comprises a group of an electroanalytical method in which information about the analyte is derived from the measurement of current as a function of applied potential obtained under conditions that encourage polarization of an indicator, or working electrode. Generally, the working electrodes in voltammetry are microelectrodes having surface areas of a few square millimeters. The current measured is usually the current due to a specific oxidation or reduction process involving the analyte species at the surface of one of the electrodes. A three-electrode system includes a working electrode, at which the oxidation or reduction process of interest occurs, a reference electrode, such as the SCE (Saturated Calomel Reference Electrode) or silver-silver chloride electrode and an auxiliary or counter electrode, which carries the bulk of the current (instead of reference electrode). The three electrodes are connected to the power source, which is a specially designed circuit for precise control of the potential applied to the working electrode and often called a potentiostat or polarograph (Figure 2.2) [19].

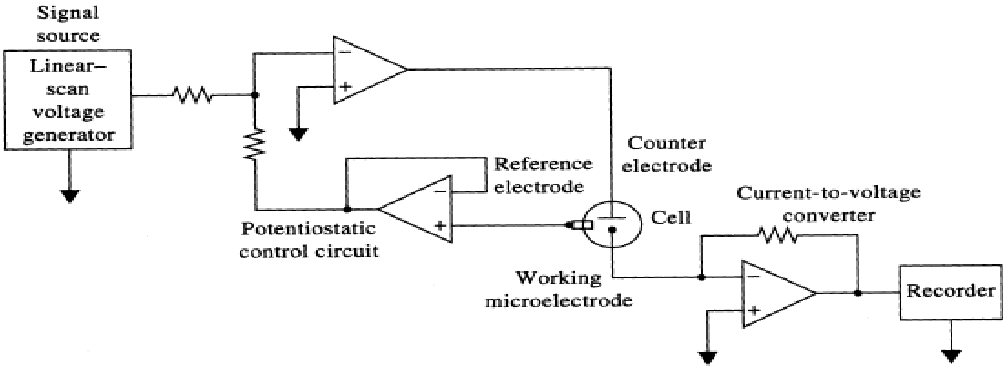


Figure 2.2 Block diagram of the major components of an electroanalytical system for performing voltammetric analysis [20]

In addition to diffusion, mass transport can also occur by migration or convection. Migration is the movement of a charged ion in the presence of an electric field. In voltammetry, the use of a supporting electrolyte at concentrations 100 times of the species being determined eliminates the effect of migration. Convection is the movement of the electroactive species by thermal currents, density gradients present in the solution, or by stirring the solution or rotating of the electrode. Convection must be eliminated or controlled accurately to provide controlled transport of the analyte to the electrode.

In voltammetry, a variable potential excitation signal is impressed upon an electrochemical cell containing a microelectrode. This excitation signal elicits a characteristic current response upon which the method is based. The waveforms of the common excitation signals used in voltammetry are linear scan, normal pulse, differential pulse, square wave and triangular or cyclic voltammetry.

Linear scan voltammetry

Linear scan voltammetry (Figure 2.3) is the simplest technique, in which the voltage applied to the cell increases linearly as a function of time. The current in the cell is then recorded as a function of time, and thus as a function of the applied voltage.

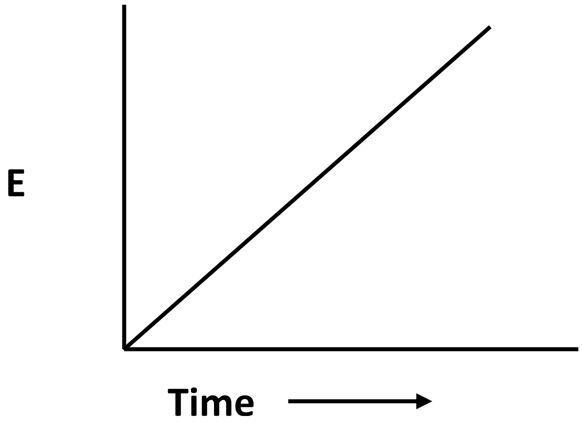


Figure 2.3 Diagram of the potential waveform used in linear scan voltammetry [20]

Normal pulse voltammetry

Normal pulse voltammetry consists of a series of pulses of increasing amplitude applied to successive drops at a preselected time near the end of each drop lifetime. Diagram of the potential waveform used in normal pulse voltammetry as shown in Figure 2.4.

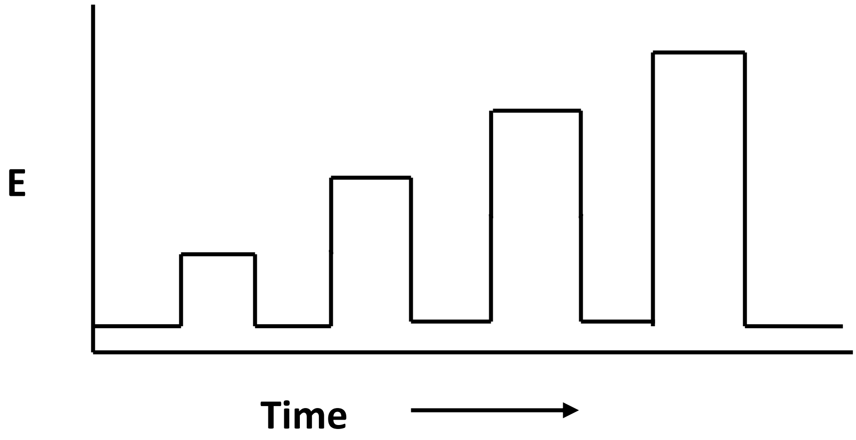


Figure 2.4 Diagram of the potential waveform used in normal pulse voltammetry [20]

Differential pulse voltammetry (DPV)

Differential pulse voltammetry if a series of periodical constant pulse of potential is superimposed to a linear scanning, a consistent enhancement of the signal is achieved. 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 performed. Diagram of the potential waveform used in differential pulse voltammetry shown in Figure 2.5.

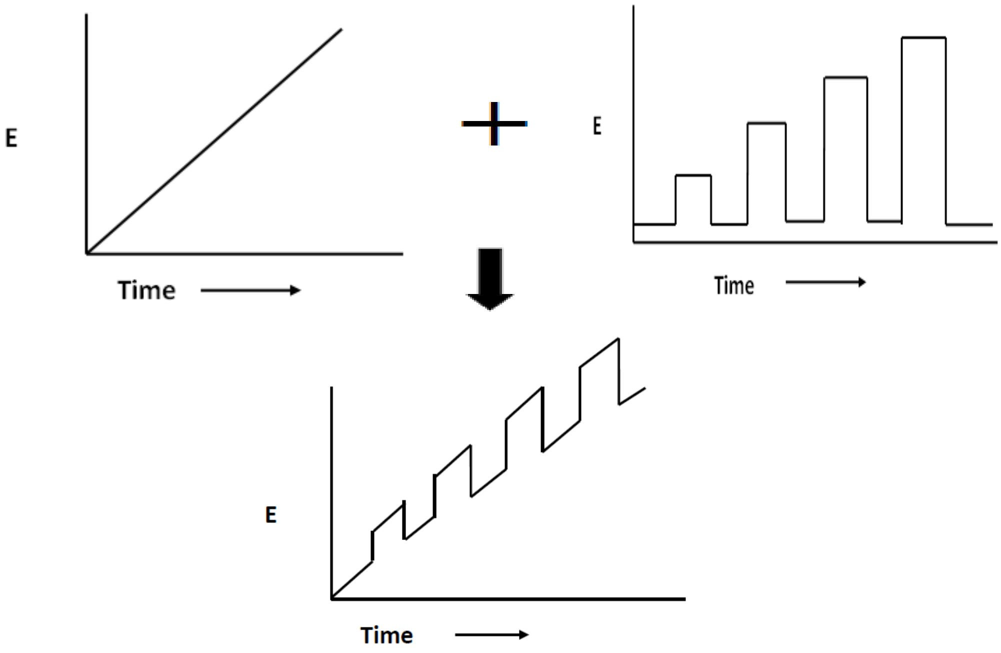


Figure 2.5 Diagram of the potential waveform used in differential pulse voltammetry [20]

Square wave voltammetry (SWV)

Square wave voltammetry this technique represents a further development of the preceding one. A rapid step scanning of potential is applied to the electrode and, moreover on each step is superimposed a high frequency square wave. The current is sampled two times at the end of the two half waves. If the amplitude of the wave is very little and the redox system is reversible, during the first half wave the electro active compound can be reduced (or oxidized), while the second half wave, at the contrary, it can be oxidized (or reduced). The two current are then summed up and so, the sensitivity is increased. Diagram of the potential waveform used in square wave voltammetry shown in Figure 2.6.

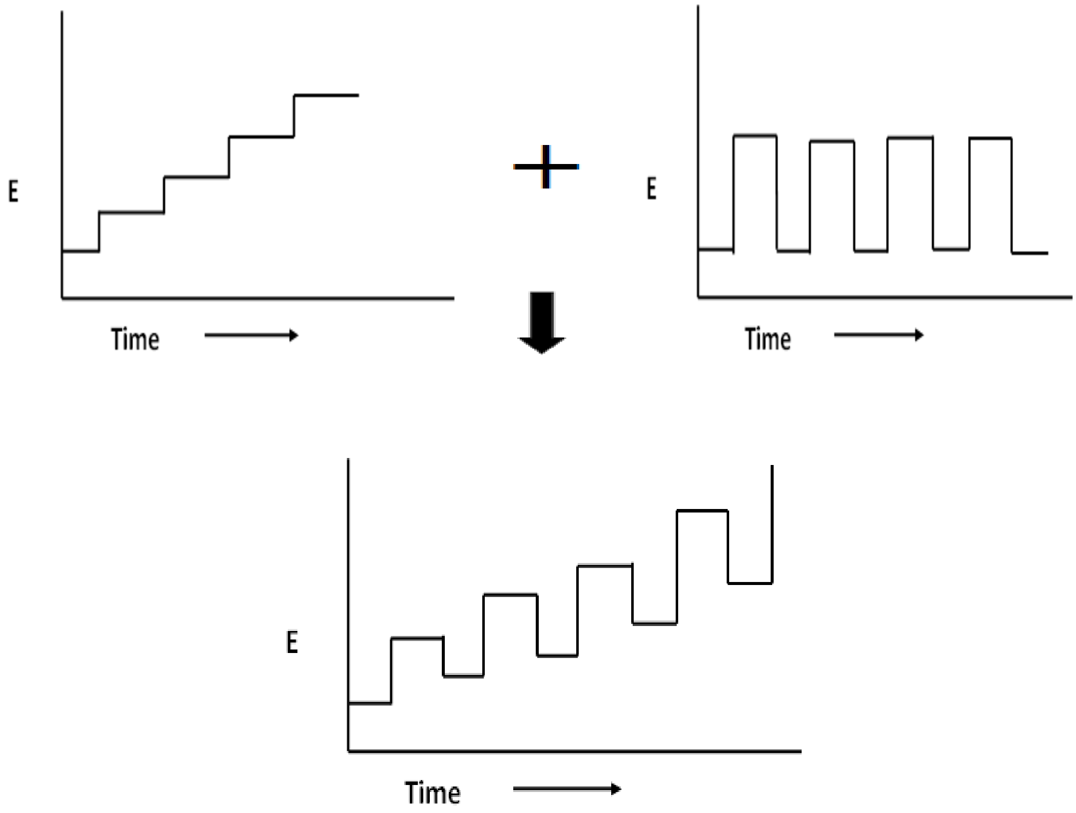


Figure 2.6 Diagram of the potential waveform used in square wave voltammetry [20]

Cyclic voltammetry (CV)

Cyclic voltammetry (Figure 2.7) has become an important and widely used for the study of redox processes, for understanding reaction intermediates, and for obtaining stability of reaction products. This technique is based on varying the applied potential at a working electrode in both forward and reverse directions (at some scan rate) while monitoring the current.

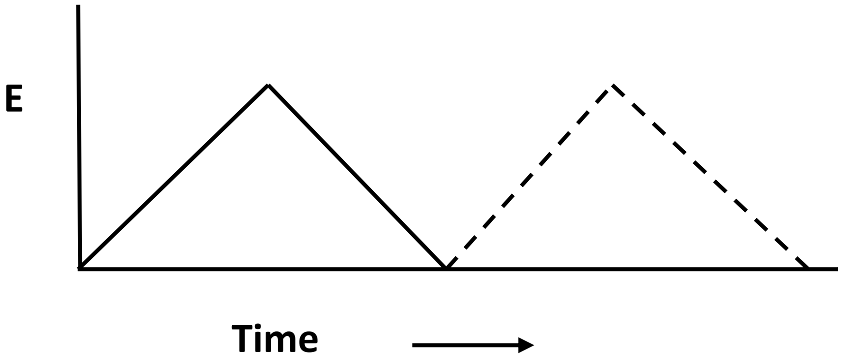


Figure 2.7 Diagram of the potential waveform used in cyclic voltammetry [20]

The basic components of a modern electroanalytical system for voltammetry (Figure 2.2) are a potentiostat, computer and the electrochemical cell. In some cases the potentiostat and computer are bundled into one package, whereas in other systems the computer and the A/D and D/A converters and microcontroller are separate, and the potentiostat can operate independently

The potentiostat

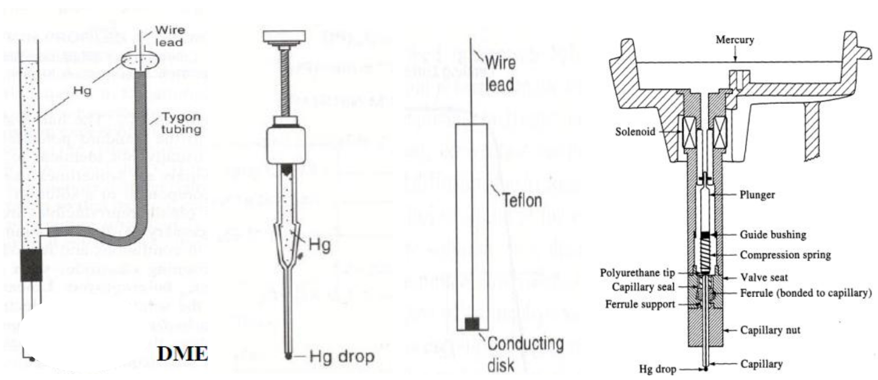
The task of applying a known potential and monitoring the current falls to the potentiostat. The most widely used potentiostats today are assembled from discrete integrated-circuit operational amplifiers and other digital modules. In many cases, especially in the larger instruments, the potentiostat package also includes electrometer circuits, A/D and D/A converters, and dedicated microprocessors with memory.

The electrodes and cell

A typical electrochemical cell consists of the sample dissolved in a solvent, an ionic electrolyte, and three (or sometimes two) electrodes such as working electrode, reference electrode and auxiliary electrode

Working electrode

The working electrode of an electrode system is the electrode, on the surface of which, the analyte is oxidized or reduced. The active potential of the working electrode is measured against a standard reference electrode. Mercury is useful because it displays a wide negative potential range (because it is difficult to reduce hydrogen ion or water at the mercury surface), its surface is readily regenerated by producing a new drop or film, and many metal ions can be reversibly reduced into it. The most commonly used working electrodes is mercury electrode (DME, HMDE, SMDE) showed in Figure 2.8. Other commonly used electrode material is disk electrode such as graphite, gold and glassy carbon.



DME **HMDE** **Disk electrode** **SMDE**

Figure 2.8 Working electrode use in voltammetry; DME, Dropping mercury electrode; HMDE, Hanging mercury drop electrode and SMDE, Static mercury drop electrode [20]

Reference electrode

The reference electrode should provide a reversible half-reaction with Nernstian behavior, be constant over time, and be easy to assemble and maintain. The most commonly used reference electrodes for aqueous solutions are the calomel electrode and the silver/silver chloride electrode (Ag/AgCl). These electrodes are commercially available in a variety of sizes and shapes.Reference electrodes should be constructed using half-cell components that are stable over time and with changing temperature, present at well-defined values of activity. The reference half-cell with which most of us are familiar is the standard hydrogen electrode (SHE), composed of an inert solid like platinum on which hydrogen gas is adsorbed, immersed in a solution containing hydrogen ions at unit activity.

Auxiliary electrode

Auxiliary electrode or counter electrode is the complementary electrode to the working electrode across which the potential is applied to achieve the oxidation or reduction. The most commonly used material for the auxiliary electrode is platinum, due to its inertness and the speed with which most electrode reactions occur at its surface. Other, less expensive materials may also be used as auxiliary electrodes.

The advantage of voltammetric analysis over the other analytical technique is a combines the processes of sampling, reagent addition, mixing, and measurement in one system. It is also a simple technique, rapid, economical reliable, more cost effective of analytical instrumentation, samples or reagents are low consumed with little waste release and practical technique with wide expands applications in quantitative analysis [21].

2.4 Composition and preparation of beverages sample

Composition of beverages sample such as B-ink consist of vitamin A, vitamin B_{12}, vitamin C, biotin, sugar and protein, energy drinks consist of vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_9, sugar, caffeine and protein and fruit juice consist of vitamin A, vitamin B_{12}, vitamin B_6, vitamin C, sugar and protein. The brief reviews for preparation of beverages sample are following description.

In 1996, Mena *et al.* [22] The samples were prepared by two different procedures such as wet digestion and microwave digestion. The samples of beer, cider and cava were degased in an ultrasonic bath prior to analysis. A 5 mL portion of sample was treated with 1 mL of 65% HNO_3 and a few micrograms of V_2O_5 in Pyrex tubes placed in a digestion block and heated at 120 °C for 90 min. The solutions were left to cool to room temperature, transferred to a calibrated flask and diluted to a final volume of 10 mL with deionized water. Acid mineralization in a microwave digestion bomb. A 2.0 mL portion of sample was treated with 2.5 mL of 65% HNO_3 and a few micrograms of V_2O_5 in the internal capsule of the microwave acid digestion bomb; the bomb was sealed and placed in the microwave oven at the maximum setting for 90 s. The bomb was cooled to -18 °C over a 45 min period, before the capsule containing the sample was removed and the mixture was brought to a final volume of 25 mL with deionized water

In 1997, Nkono and Asubbiojo [23] Determination of heavy metals (Co, Cu, Zn, Cd, Pb, Cr, Se and Ni) in bottled waters and soft drinks. Drinking water plays a major role in the intake of a nutritional and toxic trace elements in human. Elements such as Co, Cr, Fe, Mn, Mo, Ni, Se, Sn and V. Preparation of soft drink. The bottled (drinking) waters were acidified with $HNO_{3.}$ A 100 mL aliquot of the mineral water was digested with HNO_3 in a beaker at 120 °C until a clear solution was obtained. All the samples were then stored at 4 °C in a refrigerator prior to analysis. They found that the contents of cobalt in drinking water comparison with World Health Organisation (WHO) is lower than limits of WHO.

In 1999, Onianwa et al. [24] Heavy metals composition of foods for example, iron, zinc, copper, chromium cobalt and manganese are essential, while lead, cadmium, nickel and mercury are toxic at certain levels. In 1976 reports that, in Germany, beverages contribute 43, 36 and 31% of the total lead, cadmium and mercury, respectively, in the diet. Similar data are available from other countries. Beverages sample such as tea, coffee, fruit juice, soft drink and wines were preparation by dry-ashing. Solid samples were dry-ashed in furnace at 550 °C and the ash dissolved in 1 M nitric acid. Liquid samples were heated to dryness with a little concentrated nitric acid in an evaporating dish on a regulated hot-plate. The caked caramelous mass formed in most cases was then ashed in the same dish inside a furnace and the ash also dissolved with 1 M nitric acid. The contents of cobalt in beverages comparison with World Health Organisation (WHO) is lower than limits of WHO.

In 2006, Su´arez-Luque*et al*. [25] determination of heavy metals in beverages sample such as red wine, white wine, peach, apple juice, grape, pineapple juice, orange juice and orange soft drink. Preparation of sample followed by 1 mL of sample was dissolved in 100 mL of Milli-Q water, filtered through 0.4 µm membrane and injected directly without any other sample treatment. K^+, Ca^{2+}, Na^+ and Mg^{2+} were found in all samples and traces of Ni^{2+} were found in orange juice.

In 2006, Maduabuchi*et al*. [26] preparation of beverages and fruit drinks. Five milliliters of each sample was used. The samples were digested by adding 15 mL of nitric acid and making it up to 50 mL with deionised water. This was heated until the solutions were fully digested and reduced to 10 mL. The solutions were allowed to cool and then filtered.

2.5 The methods for determination of Co(II)

Several literature reviews for the determination of cobalt have been reported. For example UV-visible spectrophotometric method [27-37], atomic absorption spectrophotometric method [38-42], flow injection analysis method [43-50], capillary electrophoresis [51], luminescence [52-53], high performance liquid chromatography [54-56], inductively coupled plasma [57-58] and voltammetric method [59-74].

In this research, voltammetric method proposed for determination of Co(II) in beverage samples are simple, high sensitivity, consumption small amount of sample and reagent and low detection limit. The brief reviews are following description.

Determination of Co(II) by voltammetry

In 1989, Zhang *et al*. [59] determination of cobalt in sea water by the differential pulse cathodic stripping voltammetry comparison with cathodic stripping square-wave voltammetry. The method is based on measurement of the diffusion current of cobalt-dimethylglyoxime complex at a hanging mercury drop electrode. It was found that the adsorption potential of cobalt complex is obtained at -0.7 V. The sea water samples were collected from the Mediterranean Sea. Samples were filtered immediately through Nuclepore filters (0.4 µm pore diameter) under a pressure of nitrogen. The samples were placed in polyethylene bottles and acidified to a pH of about 2 by 150 mL of 10 M HCl to each 100 mL of sample, then stored at low temperature (4 °C) until required for analysis and the interfering effect of other metal ions in sea water such as Fe^{3+}, Mn^{2+}, Cr^{3+}, Cu^{2+}, Pb^{2+}, Cd^{2+} and Zn^{2+} was investigated by adding the appropriate amounts of the metal ion solutions to obtain concentrations of 8, 16 and 24 mg/L in a solution containing 0.06 mg/L of cobalt. Iron (III), Manganese (II) and Chromium (II) did not interfere at any concentration.

In 1995, Giroussiet *al*. [60] studied the differential pulse adsorptive voltammetry for determination of cobalt in vegetable animal foodstuffs using α-benzildioxime (α-BD) as a chelating agent. They found that the diffusion current for cobalt complex was appeared at the potential of 0.25 V. Vegetable animal foodstuffs were prepared by digestion. A weighed amount of sample (0.5 g of dried cereals or hay and 0.2 g of dried alfalfa meal or pasture grass), as well as certain volumes of working standard solutions were transferred into separate by Kjeldahl flasks. To each flask 4.5 mL of the digestion acid mixture were added. The flasks were heated on a digestion shelf and the temperature gradually increased until the appearance of white sulfur trioxide fumes; the heating was continued for at least 15 min, in order to reduce the volume of the sulfuric acid digest to 0.5 mL. After the flasks were removed from the digestion shelf and cooled down to room temperature, 5 mL of water were added to each flask and the whole content was quantitatively transferred into l0 mL volumetric flasks and diluted with water up to the mark. In case of precipitation, the digest was centrifuged and the supernatant was used for the analysis. The interfering effect of other metal ions in vegetable animal foodstuffs such as zinc interferes at a ratio Zn/Co > 10^6.

In 1996, Zhang *et al*. [61] described the construction and behaviour of a mixed binder carbon paste electrode (MBCPE) containing dimethylglyoxime (DMG) system for the cathodic stripping voltammetric determination cobalt (II) in rice, tea and human hair samples. The derivative voltammograms were recorded from -0.80 to - 1.40 V at a scan rate of 250 mV/s. The response of the cobalt (II) was appeared at the potential of -1.00 V. The samples prepared by digestion. A 0.50 - l .00 g of human hair was washed with acetone, chloroform and shampoo and then, thoroughly with deionized water. The samples were digested in a solution containing 6M $HC1O_4$ and 3 M HNO_3 at 200°C until the sample was colourless and heated to dryness. The residue was dissolved with 2.0 mL 0.10 M HCl solution and then 3.50 mL of buffer solution (pH 4.80) was added to the solution, rice samples were dried at 60°C for 8 h and later, powdered in a mortar and tea samples were washed with deionized water in order to remove silt and thereafter dried at 60 °C for 12 h for powdering it 2.0-4.0 g of the rice or tea samples were then, weighed and digested in a solution containing 6 M $HClO_4$ and 3 M HNO_3, followed by those steps mentioned in the section on analysis of hair samples. The interferences of other metal ions in rice, tea and human hair samples such as Ca^{2+}, Mg^{2+},

Ba^{2+}, Al^{3+}, Cu^{2+}, Cd^{2+}, Fe^{3+}, Mn^{2+}, Pb^{2+} and Cr^{3+} was investigated by adding the appropriate amounts of the metal ion solution to l0 mL of a solution containing 5.0x 10^{-7} Co (II). Interference was taken as the level causing an error in excess of 10%. The amounts that did not interfere were 2.5 mg each of Ca^{2+}, Mg^{2+}, and Ba^{2+}, 1.0 mg of $A1^{3+}$ and Cr^{2+}, 0.5 mg of Mn^{2+}, O.l0 mg each of Cu^{2+}, Zn^{3+} and Cd^{2+}, 0.050 mg each of Fe^{3+} and Pb^{2+}.

In 1996, Gao*et al*. [62] described a method for determination of cobalt (II) by catalytic adsorptive differential pulse voltammetry. The method is based on accumulation of a cobalt complex, $[CoSCNNO]^{+}$ on a hanging mercury drop electrode, followed by measurement of the catalytic current of the adsorbed complex. Applying the potential in the range of -0.2 to -0.7 V with a scan rate of 10 mV/s and a pulse amplitude of 50 mV/s. It was found that the reduction of cobalt (II) at the potential of -1.1 V. The interfering effect of other metal ions was tested on 50 nM cobalt. The results showed that the addition of 2.0 mM Ca^{2+} or Cu^{2+}, 0.5 mM Mn^{2+}, 0.15 mM $A1^{3+}$, 0.2 mM Cr^{3+}, 0.1 mM Pb^{2+}, 0.02 mM Cd^{2+}, 0.01 mM Au^{3+} and 5.0 µM Fe^{3+} effect on the determination of cobalt but no effect was observed at concentrations lower than 50 µM.

In 2000, Ensafi*et al*. [63] determination of Co(II) in steel sample using the adsorptive differential pulse voltammetry. The method is based on the accumulation of Co(II) complex with 2-minocyclopentene-1-dithiocarboxylic acid on a hanging mercury drop electrode, followed by measurement of the current of the adsorbed complex. The differential pulse voltammogram was applying the potential from –0.8 to –1.5 V with a scan rate of 10 mV/s. Accumulation potential of cobalt (II) at -0.60 V. The interfering effect of existing ions is tested on100 ng/mL cobalt such as Ba^{2+}, Cu^{2+}, Mg^{2+}, Cr^{3+}, Ca^{2+}, Hg, Mn^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, Fe^{3+} and Zn^{2+}. It concluded that many ions do not affect the determination of cobalt (II) when present in a 1000-fold to 50-fold excess.

In 2000, Savafi and Shams [64] described the method for determination of cobalt in mineral water by catalytic adsorptive cathodic stripping voltammetry. The method is based on adsorptive accumulation of the cobalt-MTB (methyl thymol blue) complex onto a hanging mercury drop electrode, followed by reduction of the adsorbed species by voltammetric scan using differential pulse modulation scan from 0.7 to 1.3 V with a scan rate of 20 mV/s. The accumulation potential of Co(II) at -0.50 V. Possible interference by other metals with the catalytic adsorptive stripping voltammetry of cobalt was investigated by the addition of the interfering ion to a solution containing 0.10 ng/mL of cobalt using the optimized conditions. Ions that tested at the 1.0 µg/mL and found not to interfere in determination of cobalt were alkaline and alkaline earth metals, Mn^{2+}, Zr^{4+}, Pb^{2+}, Cu^{2+}, Se^{4+}, Cd^{2+}, Al^{3+}, Cr^{3+}, V^{4+}, Ag^{+}, Fe^{2+}, Fe^{3+}, Cl^{-}, Br^{-}, F^{-} and Hg^{2+} did not interfere at the 300 ng/mL while Zn^{2+} and Ni^{2+} did not interfere at 150 ng/mL.

In 2000, Sancho *et al*. [65] described the method for the determination of cobalt in refined beet sugar by adsorptive cathodic stripping voltammetry using differential pulse mode at the hanging mercury drop electrode (HMDE) as their dimethylglyoxime complexes. Accumulation potential of Co(II) at -0.60 V. Beet sugar sample prepared by wet digestion, concentrated nitric acid (5 mL) and 10 mL of 40% m/v hydrogen peroxide were added to 0.5 g of extrapuresaccharose dissolved in 5 mL of deionized water. This solution was placed in a 100 mL vessel and introduced 20 min in the microwave oven for digestion. The power of the magnetron was linearly increased from 250 to 600 W during the process. After digestion of organic matter, the resulting solution was diluted to 25 mL with deionized water. A control reagent blank was conducted simultaneously. The performance of the procedure was compared with electrothermal atomic absorption spectrometry (ETAAS).

In 2000, Lu *et al*. [66] modified carbon paste electrode. tri (3,5-dimethylpyrazoyl)-1,3,5-triazine was chosen as modifier to determine Co(II) in water by the differential pulse anodic stripping voltammetry. Applying the potential range of 0.3 to 0.2 V. They found that the oxidation peak of Co(II) was observed at 0.03 V. The effected of other metal ions could interfere with cobalt (II) determination with an accumulation time of 1 min, no interference was found for additions of $2.0x10^{-5}$mol/L each of Na^{+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Ca^{2+}, Ba^{2+}, Mg^{2+}, Sn^{2+}. However Fe^{3+} interfere significantly by decreasing the cobalt (II) signal and this corresponds with the value obtained from the comparison with standard method of atomic absorption spectrometry (AAS).

In 2001, Ellwood *et al*. [67] developed the method for determination of extent of cobalt complex with organic ligands (Co–nioxime complex) in seawater by cathodic stripping voltammetry. Static mercury drop electrode was used working electrode, reference electrode was used silver/silver chloride, a glassy carbon was used the counter electrode. The potential of the cobalt (II) was appeared at −0.8 V.

In 2003, Kajic*et al.* [68] determination of cobalt in serum by adsorptive stripping voltammetry. The method is based on the complexation of Co(II) with dimethyglyoxime on a hanging mercury drop electrode (HMDE). Applying the potential of the working electrode in the range of -0.1 to -0.9 mV with a scan rate of 6.7 mV/s. It was found that the response of the cobalt complex was appeared at the potential of -0.8 V. The serum sample prepared by wet digestion, 0.5 mL of serum was digested with 0.2 mL of 98% H_2SO_4 and 0.5 mL of 65% HNO_3. The mixture of sample and acids was heated at about 150 °C over a burner. After a few minutes nitrous oxides evaporated and the solution carbonated. The remaining carbonaceous matter was oxidized by adding 4 aliquots of 0.15 mL H_2O_2. The residual peroxide was finally expelled by more vigorous heating to the point of thermal digestion of sunphuric acid at about 250 °C. After cooling, the digested sample was neutralized with NH_3 and transferred into an acid washed vessel by rinsing the Kjeldahl. The pH value was further adjusted to 9.0 using the addition of NH_3 and the value obtained from the comparison with standard method of ETAAS and ICP-MS.

In 2004, Morfobos*et al.* [69] developed the method for determination of cobalt (II) in river water and Iron ore after complexation with dimethylglyoxime (DMG) by square wave adsorptive stripping voltammetry on a rotating-disc bismuth-film electrode. Applying potential in the range of -0.7 to -1.3 V. They found that the potential of the cobalt (II) was appeared at −1.3V. The preparation of samples was done by dissolved samples (2.00 g) in 10 mL of 6 mol/L HNO_3 under mild heating and this solution was diluted to 100 mL and the metal ions can also interfere with the measurement of Co(II) such as Pb(II), Hg(II), Cu(II), Fe(II), Al(III), Cd(II), Ti(IV), Ca (II) and Mn(II) added at a 1000-fold mass concentration excess over Co(II) did not. It is well known that excess Zn (II) is likely to interfere with the determination of Co(II).

In 2005, Korolczuk *et al.* [70] described a catalytic system for determination of Co(II) in river water sample by adsorptive stripping voltammetry. The method is based on the enhancement of the current observed in the cobalt-nioxime-cetyltrimethylammoniumbromide-piperazine-*N*,*N*_-bis(2-ethanesulfonic acid) complex, while the potential was scanned from −0.7 to −1.2V at a scan rate of 50 mV/s. The response of Co(II) was appeared at the potential of -1.2 V. The applicability of the proposed method was validated by the analysis of the reference materials and by a comparison with the results obtained using ET-AAS method for samples containing higher levels of Co(II).

In 2005, Korolczuk et al. [71] develop plated lead film electrode. The method is based on a diffusion current of Co(II) with dimethylglyoxime for determination of cobalt in tea leaves and rain water by adsorptive stripping voltammetry. A three electrode cell containing a glassy carbon working electrode, a Platinum wire as a counter electrode and silver/silver chloride as a reference electrode was used. While the potential was scanned from −0.675 to −1.2V at a scan rate of 50 mV.The response of Co(II) was appeared at the potential of -1.2 V. The determination Co(II) is not influenced by 10^3-fold excess of Fe(III), Zn(II), Mn(II) and Cu(II).

In 2005, Hus´akov´ *et al*. [72] determination of cobalt in human urine by adsorptive stripping voltammetry comparison with atomic absorption spectrometry. The methods are based on adsorptive accumulation of a cobalt–nioxime (1,2-cyclohexanedione dioxime) complex on a hanging mercury drop electrode, followed by a stripping voltammetric measurement of the catalytic reduction current of the adsorbed complex. the potential was scanned from −1000 to −1200 mV in the differential pulse mode using a pulse amplitude of 50 mV. They found that the adsorptive accumulation of the Co–nioxime complex on a HMDE at −800 mV.

In 2006, Gedaminskiene*et al*. [73] to studied the method for determination of cobalt by adsorptive stripping voltammetry using dimethylglyoxime as a chelating agent at the hanging mercury drop electrode (HMDE) comparison with mercury film (MFE) electrodes. The potential of the Co(II) complex was obtained at -0.8 V.

In 2011, Mohadesi*et al*. [74] developed the method for determination of Co(II) in water samples by stripping voltammetry on the unmodified carbon paste electrode. The method is based on a diffusion current of Co(II)-Nitroso-S complex was adsorbed on the carbon paste electrode. Adsorbed complex was detected by cathodic differential pulse voltammetric scan from +0.1 to -0.6 V at 25 mV/s and the potential of the Co(II) complex was obtained at -0.3 V. These waters sample were exchanged with distilled water used for the preparation of a acetate buffer (pH 5.0) and the general procedure was used on these resultant solutions. The results obtained by the proposed method were compared with FAAS method

CHAPTER 3

METHODOLOGY

3.1 Chemicals and reagents

All chemicals and reagents used in this work are listed in Table 3.1

Table 3.1 List of chemicals used in this work

Chemicals	**Formula**	**Grade**	**From Company**
Ammonia	NH_3	AR	CARLO ERBA
Ammonium chloride	NH_4Cl	AR	CARLO ERBA
Aluminum nitrate	$Al(NO_3)_3\cdot 9H_2O$	AR	UNIVAR
Calcium chloride	$CaCl_2\cdot 2H_2O$	Extra pure	Merck
Chromium nitrate	$Cr(NO_3)_3$	pure	Merck
Cobalt standard solution 1000 mg/L	Co(II)	AR	BDH
Dimethylglyoxime	$C_4H_8N_2O_2$	-	-
Iron(III) nitrate	$Fe(NO_3)_3$	AR	CARLO ERBA
Potassium chloride	KCl	AR	CARLO ERBA
Potassium hydroxide	KOH	AR	UNIVAR
Lead(II) chloride	$PbCl_2$	AR	CARLO ERBA
Manganese sulphate	$MnSO_4$	Extra pure	Merck
Nickel nitrate	$Ni(NO_3)_2$	AR grade	CARLO ERBA
Nitric acid	HNO_3	AR	Merck
Sodium acetate	CH_3COONa	AR	UNIVAR
Sodium hydroxide	NaOH	AR	UNIVAR
Zinc sulfate	$ZnSO_4\cdot 7H_2O$	AR	CARLO ERBA

3.2 Instrument and apparatus

3.2.1 Voltammeter (Ω Metrohm; 693 VA Processor, 694 VA Stand and Automatic voltage stabilizer Series 101) including working electrode (HMDE), reference electrode (Ag/AgCl) and auxiliary electrode (Platinum wire)

3.2.3 pH meter (Metrohm; 713 pH Meter, Switzerland)

3.2.3 Hot plate

3.2.4 Flame atomic absorption spectrometry (FAAS)

3.3 Experimental

3.3.1 Principle for determination of Co(II) by voltammetry

In this work, the determination of Co(II) based on measurement the diffusion current of the complex forming between Co(II) and dimethylglyoxime at hanging mercury drop electrode (HMDE). The chemical reactions are shown in equation 3.1-3.3 [75].

$$Co^{2+} + DMG \rightleftharpoons Co(DMG)_2 \qquad (3.1)$$

$$Co(DMG)_2 + NH_4^+ \rightleftharpoons Co(DMG)_2H^+ + NH_3 \qquad (3.2)$$

$$Co(DMG)_2H^+ + e^- \rightleftharpoons Co(DMG)_2H \qquad (3.3)$$

As the equation, the reaction between Co(II) and dimethylglyoxime (DMG) to form a Co(II)-dimethylglyoxime complex. Then it was protonated to form a Cobalt-dimethylglyoximecation by ammonium buffer solution, Finally, the Co(II)-dimethylglyoximecation was reduced at HMDE and subsequently measurement the diffusion current of Co(II)-dimethylglyoxime complex which is proportional to the concentration of Co(II).

3.3.2 The method for determination of Co(II) by voltammetry

In this studies, the method for determination of cobalt using voltammetry was done by measurement the diffusion current of Co(II)-dimethylglyoxime complex at hanging mercury drop electrode (HMDE) as working electrode, a silver-silver chloride (Ag/AgCl) used as reference electrode and a platinum wire as auxiliary electrode. A differential pulse mode was applied at for electrolysis of Co(II)-dimethylglyoxime complex in voltammetric cell.

3.3.3 Preparation of standard solutions and reagents

3.3.3.1 Stock standard solution of Co(II) 2 mg/L

Stock standard solution of Co(II) was prepared by transferring 0.2 mL of 1000 mg/L Co(II) standard solution in 100 mL volumetric flask and adjusting volume to 100 mL with ammonia ammonium chloride buffer pH 9. Working standard solution of Co(II) was prepared by appropriate dilution of stock standard Co(II) solution.

3.3.3.2 Dimethylglyoxime stock solution 400 mg/L

The stock dimethylglyoxime solution was prepared by dissolving 0.025 g dimethylglyoxime in ammonia ammonium chloride buffer pH 9 and adjusting volume to 50 mL. Working solution of dimethylglyoximewas prepared by appropriate dilution of stock dimethylglyoxime solution with buffer solution.

3.3.3.3 Buffer solution

Buffer solutions pH 9 was prepared by mixing an appropriate ratio of 0.1 mol/L ammonium chloride with 0.1 mol/L ammonia and mixing an appropriate ratio of 83.33 mL ammonia with 167.67 mL ammonium chloride, respectively. The required pH was performed by adjusting with 1.0 mol/L sodium hydroxide and 1.0 % v/v hydrochloric acid.

3.3.4 Preliminary study of the diffusion current

The diffusion current of Co(II)-dimethylglyoxime complexes was investigated by mixing of 100 mg/L of dimethylglyoxime, 50 µg/L of Co(II) and 1 mL of ammonium chloride (supporting electrolyte) make up the volume to 10 mL using ammonia ammonium chloride buffer pH 9 after that, transfer into the votammetric cell and scan for the diffusion current over the applying potential range from -800 to -1300 mV.

3.3.5 Optimization of the experimental conditions

The experimental conditions such as type of supporting electrolyte, measurement time, pulse amplitude, pulse time, step time and dimethylglyoxime concentration was optimized by a univariate method. Five replicate measurements was performed for each studied parameter. The parameter that providing the highest diffusion current with low standard deviation was selected.

3.3.5.1 Optimization of supporting electrolyte

Study the supporting electrolyte solutions such as potassium chloride (KCl), ammonium chloride (NH_4Cl), potassium hydroxide (KOH) and sodium acetate (CH_3COONa) using 50 µg/L Co(II), 100 mg/L dimethylglyoxime and supporting electrolyte. A supporting electrolyte providing the highest diffusion current signal and lowest standard deviation was selected. After that was studies the concentration of supporting electrolyte.

3.3.5.2 Optimization of measurement time (t.meas)

The effect of measurement time on the diffusion current of Co(II)-dimethylglyoxime complexes was investigated in the range of 5-30 ms using 50 µg/L Co(II), 100 mg/L dimethylglyoxime and ammonium chloride (NH_4Cl) supporting electrolyte. The equilibration time which provides the highest diffusion current signal and lowest standard deviation was selected.

3.3.5.3 Optimization of step time (t.step)

The effect of voltage step time on the diffusion current of Co(II)-dimethylglyoxime complexes was investigated in the range of 0.1-1 s using 50 µg/L Co(II), 100 mg/L dimethylglyoxime and ammonium chloride (NH_4Cl) supporting electrolyte. The voltage step time which provides the highest diffusion current signal and lowest standard deviation was selected.

3.3.5.4 Optimization of pulse time (t.pulse)

The effect of time pulse on the diffusion current of Co(II)-dimethylglyoxime complexes was investigated in the range of 20-50 ms using 50 µg/L Co(II), 100 mg/L dimethylglyoxime and ammonium chloride (NH_4Cl) supporting electrolyte. The equilibration time which provides the highest diffusion current signal and lowest standard deviation was selected.

3.3.5.5 Optimization of pulse amplitude (U.ampl)

The effect of pulse amplitude on the diffusion current of Co(II)-dimethylglyoxime complexes was investigated in the range of -1 to -80 mV using 50 µg/L Co(II), 100 mg/L dimethylglyoxime and ammonium chloride (NH_4Cl) supporting electrolyte. The pulse amplitude which provides the highest diffusion current signal and lowest standard deviation was selected.

3.3.5.6 Optimization of dimethylglyoxime concentration

The effect of dimethylglyoxime concentration on the diffusion current of Co(II) - dimethylglyoxime complexes was investigated in the range of 1-250 mg/L using 50 µg/L Co(II), dimethylglyoxime and ammonium chloride (NH_4Cl) supporting electrolyte. The concentration of dimethylglyoxime which provide the highest diffusion current signal and lowest standard deviation was selected.

3.3.6 Analytical figures of merit

3.3.6.1 The linear calibration graph

The linear calibration graph for determining Co(II) was performed by measurement the diffusion current of Co(II)–dimethylglyoxime complex by voltammetry was investigated the concentration of Co(II) in the range of 0.5-1000.0 µg/L, 100 mg/L dimethylglyoxime under the optimum conditions: 15 ms of measurement time (t.meas), 0.6 s of step time (t.step), 20 ms of pulse time (t.pulse), -40 mV of pulse amplitude (U.ampl), 0.8 M ammonium chloride supporting electrolyte and measurement 5 replicate.

3.3.6.2 Precision

The precision of the proposed method was studied as the repeatability and reproducibility. The repeatability was accomplished by measurement 11 replicates. The reproducibility was accomplished by measurement 11 duplicate of three Co(II) standard solutions covering different concentration levels: low, medium and high (1.0, 50.0 and 100.0 µg/L), respectively and calculated as the relative standard deviation from equation 3.4. [76]

$$\%RSD = \frac{SD \times 100}{\bar{x}} \tag{3.4}$$

When

%RSD = percentage relative standard deviation

SD = standard deviation

$\bar{x}$ = mean average

3.3.6.3 Accuracy

The accuracy of the proposed method was examined by addition certain concentrations of 10, 30 and 50 μg/L of Co(II) standard solutions, respectively into sample under the optimum experimental condition. The concentrations of Co(II) was calculated from the linear calibration graph. Then, the percentage recoveries was calculated from the equation 3.5 [76].

$$\%\text{Recovery} = \frac{C_F - C_S}{C_A} \times 100 \qquad (3.5)$$

Where

C_F = concentration of Co(II) found in added sample

C_S = concentration of Co(II) in original sample

C_A = concentration of Co(II) standard added

3.3.6.4 Limit of detection

The limit of detection (LOD) and limit of quantitation (LOQ) are analyzed as minimum concentration, which can be detected and calculated from the equation 3.6 and 3.7 [76], respectively.

$$\text{LOD;} \quad 3\ \text{SD} \qquad (3.6)$$

$$\text{LOQ;} \quad 10\ \text{SD} \qquad (3.7)$$

Where

SD = standard deviation of measurement of a lowest calculation of Co(II) from diffusion current (n=11)

3.3.6.5 Effect of interferences

The effect of some possible interfering ions such as some metals ion (Zn^{2+}, Ni^{2+}, Ca^{2+}, Cd^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+} and Mg^{2+}) on the determination of Co(II) in beverage samples was investigated by varying the concentration of interfering ions (μg/L) to 50 μg/L Co(II). The tolerance value is defined as the foreign species concentration causing error smaller than ± 5% for determining the Co(II).

3.4 Sample preparation

3.4.1 Sample collection

Beverage samples (B1-B10) were collected supermarket in Mahasarakham University, Thailand. All samples were filtered through a No.1 whatman filter paper

3.4.2 Sample preparation

Beverage samples was prepared by dry ashing method. Liquid samples of 40 mL was heated to evaporation to dryness in hot plate. Dried samples or caramelous samples consist of cobalt, sugar and organic matrix in the crucible, a placing the crucible into the furnace. Initial temperature was set at lower than 100°C and increase temperature at a maximum to 550°C for 6 hour, cooled down to 50 °C in furnace. The ash of samples was dissolved using concentration nitric acid 2 mL, then adjusting pH to 7 using NaOH and made up total volume to 25 mL using ammonia ammonium chloride buffer pH 9 in volumetric flask [24]. The result obtained from the proposed voltammetric method was compared with the standard AAS method using Student's t-test, which was determining by statistical package for social sciences (SPSS, Version 16.0 for windows).

3.4.3 The efficiency of sample digestion

Beverage samples (B1-B10) collected from supermarket in Mahasarakham University was used to evaluate the efficiency of sample digestion method. It was performed by addition 10 μg/L standard Co(II) solutions into the sample prior to the digestion method in section 3.4.2 and investigated by voltammetric method. The efficiency was expressed as the percentage recovery (%Recovery).

3.4.4 Determination of Co(II) using standard FAAS method

The linear calibration graph for determining Co(II) using the standard FAAS method was investigated using standard Co(II) concentration of 1.0, 3.0, 5.0, 8.0 and 10.0 mg/L under the operating parameters for FAAS were shown in Table 3.2. Beverage samples were prepared using the method as section 3.4.2 and measured using the flame atomic absorption spectrometer (AA-680, Shimadzu, Japan). The Co(II) content in each samples were calculated by addition certain concentrations of 1, 3 and 5 mg/L of Co(II) standard solutions.

Table 3.2 The operating parameters of flame atomic absorption spectrometer (FAAS) for determination of Co(II)

Parameters	**Value**
Wavelength (nm)	240.7
HC lamp current (mA)	8
Slit width (nm)	0.2
Fuel gas flow rate (mL/min)	2.0
Type of flame	Air/C_2H_2

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Preliminary study

The diffusion current of Co(II)-dimethylglyoxime complexes was investigated by mixing 100 mg/L of dimethylglyoxime, 50 µg/L of Co(II) and 1 mL of 0.8 M ammonium chloride (supporting electrolyte) adjusting to 10 mL using ammonia ammonium chloride buffer pH 9 after that, transfer into the votammetric cell. As the equation 3.1 - 3.3, the reaction between cobalt (II) and dimethylglyoxime (DMG) to form a Co(II)-dimethylglyoxime complex. Then, it was protonated to form a Co(II)-dimethylglyoxime cation by ammonium buffer solution, Finally, the Co(II)-dimethylglyoximecation was reduced at HMDE when applying the potential range from -800 to -1300 mVwith a scan rate of 15 mV/s together with measurement of the diffusion current which is proportional to the concentration of Co(II). It was found that the response of the Co(II)-dimethylglyoxime complexes was appeared at the potential of -1022 mV and the response of dimethylglyoxime was appeared at the potential of -927.89 nA (Figure 4.1).

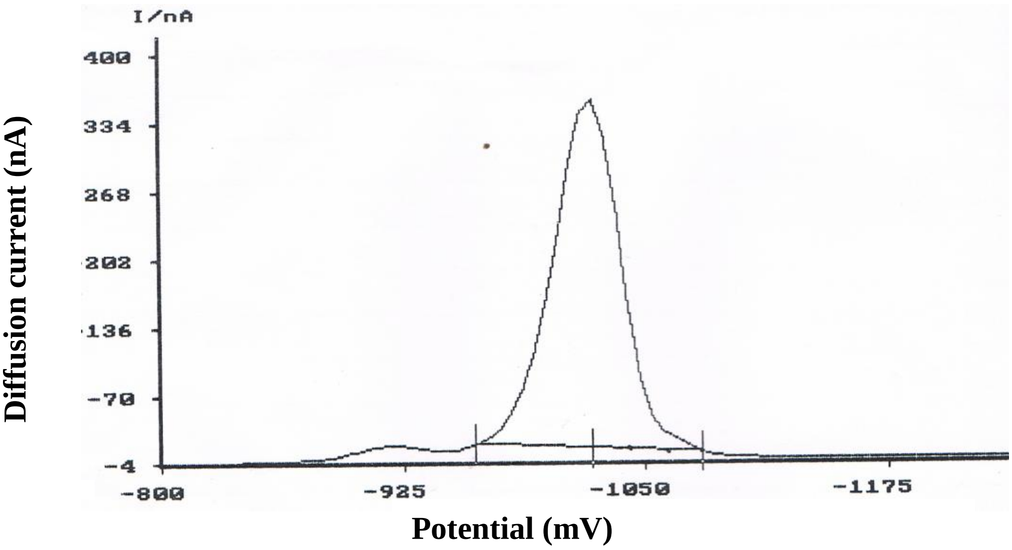


Figure 4.1 Voltammogram of Co(II) at the applying the potential in the range of -800 to -1300 mV with a scan rate of 15 mV/s in a 0.8 M NH_4Cl solution

4.2 Optimization of the experimental conditions

4.2.1 Optimization of supporting electrolyte

Study the supporting electrolyte solutions such as potassium chloride (KCl), ammonium chloride (NH_4Cl), potassium hydroxide (KOH) and sodium acetate (CH_3COONa) using 50 µg/L Co(II), 100 mg/L dimethylglyoxime and supporting electrolyte. The greatest of supporting electrolyte on the diffusion current signal of Co(II)-dimethylglyoxime complexes was studied over the range of 1-3 M. The results were shown in Table 4.1 and Figure 4.2. As the results ammonium chloride providing the highest diffusion current with low standard deviation. Therefore it was selected as the suitable electrolyte.

The suitable concentration of ammonium chloride over the range of 1-3 M were investigate using 50 µg/L of Co(II). The results were shown in Table 4.2 and Figure 4.3. It was seen that when increase the concentration of ammonium chloride up to 0.8 M providing the highest diffusion current but the concentration of ammonia ammonium chloride over 0.8 M the diffusion current was decrease less to poor electrolysis and lower diffusion current. Therefore ammonium chloride concentration of 0.8 M was selected.

Table 4.1 The effect of supporting electrolyte on the diffusion current of Co(II) – dimethylglyoxime complexes

Concentration of various electrolyte (M)	**Diffusion current (I.peak) (nA) (n=5)**				
	1.0	**1.5**	**2.0**	**2.5**	**3.0**
KCl	44.28±1.29	44.47±1.20	42.34±0.56	41.22±0.76	42.82±0.33
NH_4Cl	49.16±1.22	46.55±1.59	45.05±1.29	45.98±1.46	45.63±1.08
CH_3COONa	47.79±1.31	45.46±0.76	42.52±1.18	43.62±1.20	39.72±1.13

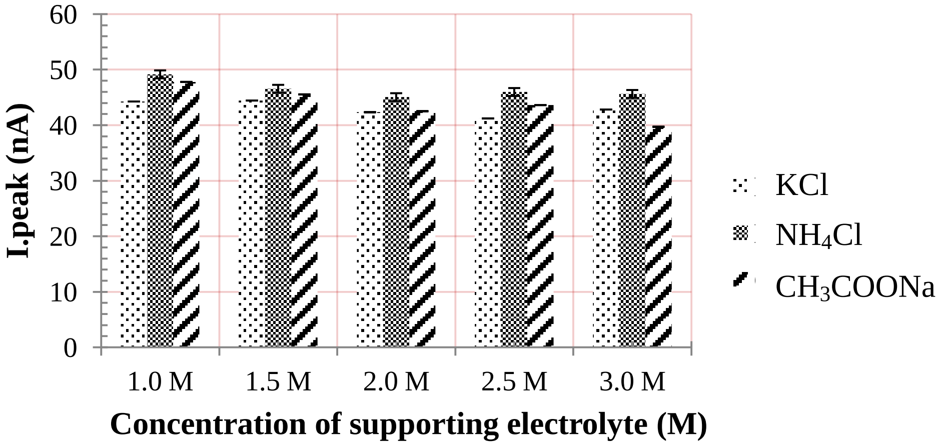


Figure 4.2 The effect supporting electrolyte on the diffusion current signal of Co(II)-dimethylglyoximecomplex, conditions; t.meas = 20 ms, t.step = 0.1 s, t.pulse =40 ms and U.ampl = 10 mV.

Table 4.2 The effect of the concentration of ammonium chloride on the diffusion currentof Co(II)-dimethylglyoxime complexes

Conc. of NH_4Cl (M)	**Diffusion current (I.peak) (nA)**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0.2	70.23	69.42	69.19	71.56	70.02	70.08	0.93
0.4	69.21	68.92	69.42	69.50	68.76	69.16	0.32
0.6	67.95	68.53	68.71	67.49	67.35	68.01	0.61
0.8	79.97	79.89	79.66	80.52	79.82	79.97	0.29
1.0	65.12	65.43	66.19	65.71	65.92	65.67	0.37
1.2	61.94	62.19	63.27	62.78	62.54	62.54	0.46
1.4	60.82	59.54	61.21	60.45	60.61	60.52	0.55
1.6	63.93	63.25	62.29	63.09	63.78	63.27	0.58
1.8	58.41	59.24	58.09	58.73	59.26	58.75	0.46
2.0	56.32	56.21	57.04	56.91	56.65	56.63	0.32
2.2	56.22	55.14	53.64	55.51	55.27	55.16	0.94
2.4	53.29	53.45	52.72	52.61	52.98	53.01	0.36
2.6	56.08	55.01	56.59	57.25	55.14	56.01	0.95
2.8	56.94	56.38	55.44	55.42	56.19	56.07	0.65
3.0	62.04	61.78	62.79	63.78	62.94	62.67	0.79

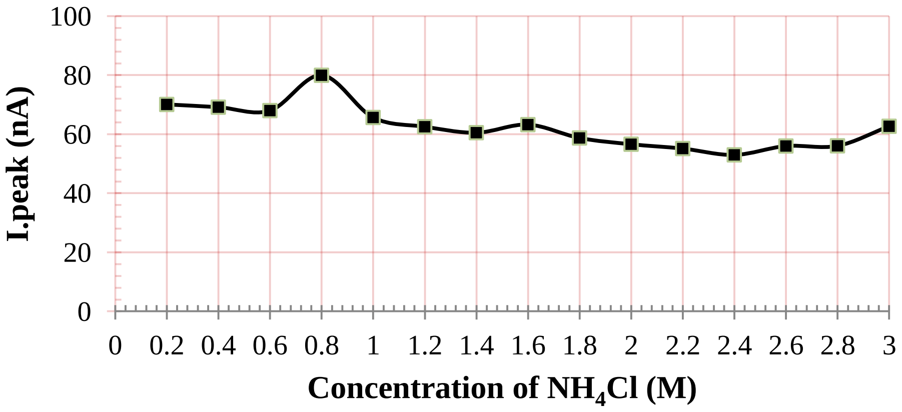


Figure 4.3 The effect of the concentration of ammonium chloride supporting electrolyte on the diffusion current signal of Co(II)-dimethylglyoxime complex

4.2.2 Effect of measurement time (t.meas)

The effect of measurement time on the diffusion current of Co(II)-dimethylglyoxime complexes for short time analysis was investigated in the range of 5-30 ms using 50 µg/L Co(II), 100 mg/L dimethylglyoxime and 0.8 M ammonium chloride (NH_4Cl) supporting electrolyte. The results were shown in Table 4.3 and Figure 4.4, it was found that the diffusion current was increased with increasing measurement time up to 15 ms and then decreased. The measurement time of 15 ms considered to be highest diffusion current with low standard deviation was selected.

Table 4.3 The effect of measurement time on the diffusion current of Co(II)-dimethylglyoxime complexes

time measurement (ms)	**Diffusion current (I.peak) (nA)**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
1	307.47	305.62	308.48	306.68	305.45	306.74	1.27
5	317.03	316.00	319.49	316.37	317.58	317.29	1.37
10	350.04	349.40	351.69	350.94	351.73	350.76	1.03
15	389.34	386.40	386.13	388.67	386.24	387.36	1.53
20	318.69	317.08	317.77	316.09	317.54	317.43	0.95
25	333.49	332.06	335.47	332.17	334.47	333.53	1.47
30	326.42	325.75	326.40	327.80	326.78	326.63	0.75

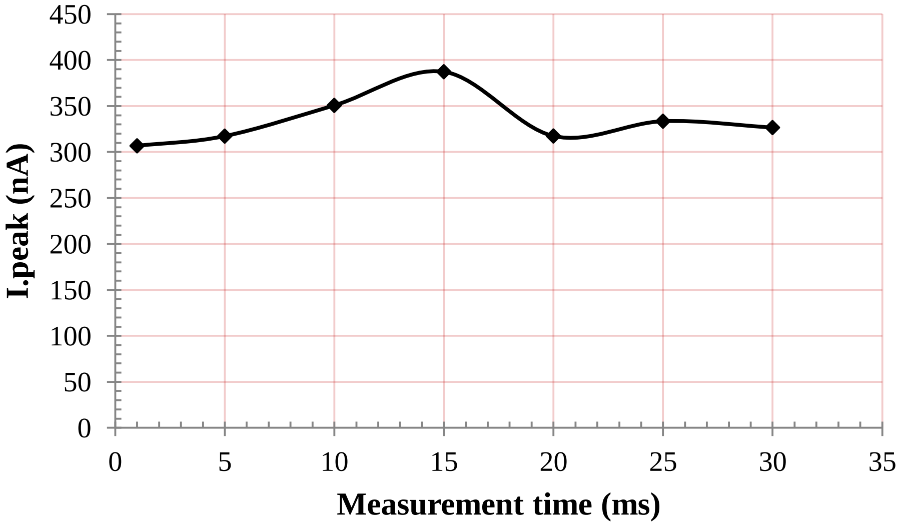


Figure 4.4 The effect of measurement time on the diffusion current using 50 µg/L Co(II), 100 mg/L dimethylglyoxime and supporting electrolyte

4.2.3 Effect of step time (t.step)

The effect of step time on the diffusion current of Co(II)-dimethylglyoxime complexes for sensitivity of analysis was investigated in the range of 0.1-1 seconds. The result were shown in Table 4.4 and Figure 4.5. It was found that when increasing the step time the diffusion current was increased. As an incentive for Co(II)-dimethylglyoxime complexes ($Co(DMG)_2H^+$) movement was polarization at the working electrode is more active. Resulting in higher diffusion current. But when increased step time more than 0.6 seconds, the diffusion current was stable slightly and higher standard deviation. Step time of 0.6 seconds was selected as the optimum condition.

Table 4.4 The effect of step time on the diffusion current of Co(II)-dimethylglyoxime Complexes

Step time (s)	**Diffusion current (I.peak) (nA)**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0.1	155.52	158.50	159.18	153.86	151.00	155.61	3.37
0.2	264.61	263.91	262.56	264.85	261.03	263.39	1.59
0.3	318.72	317.27	317.76	315.96	312.43	316.43	2.44
0.4	403.62	401.41	401.03	403.31	402.91	402.46	1.16
0.5	450.68	453.48	455.97	453.09	456.71	453.99	2.42
0.6	491.01	493.22	491.70	491.86	492.39	492.04	0.83
0.7	548.23	547.08	547.26	544.72	540.86	545.63	2.96
0.8	565.47	562.70	565.53	562.43	568.06	564.84	2.33
0.9	578.06	572.43	575.53	576.11	575.47	575.52	2.02
1.0	596.35	590.12	589.83	590.04	589.78	591.22	2.87

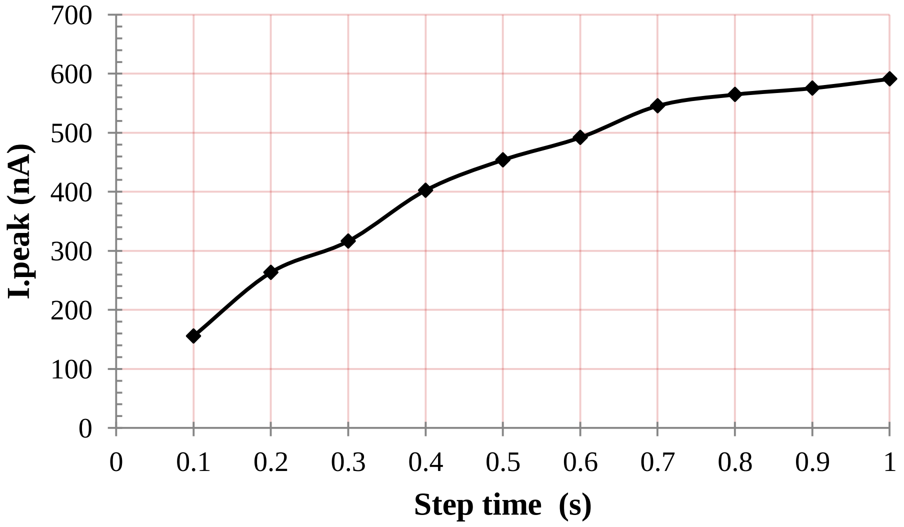


Figure 4.5 The effect of step time on the diffusion current of Co(II)-dimethylglyoxime complexes

4.2.4 Effect of pulse time (t.pulse)

The effect of pulse time on the diffusion current of cobalt (II)-dimethylglyoxime complexes for incentive of Co(II)-dimethylglyoxime complexes (Co(DMG)2H$^+$) movement to polarization at the working electrode is more active of analysis was investigated in the range of 20-50 ms. The results were given in Table 4.5 and Figure 4.6, when increasing the pulse time, the diffusion current was decreased. The diffusion current of Co(II)-dimethylglyoxime complex with pulse time of 20 ms was greatest. Therefore, The equilibration time of 20 ms which provides the highest diffusion current signal and lowest standard deviation was selected.

Table 4.5 The effect of pulse time on the diffusion current of Co(II)-dimethylglyoxime complexes

Time pulse (ms)	**Diffusion current (I.peak) (nA)**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
20	931.13	936.29	934.56	932.17	931.65	933.16	2.19
25	845.03	845.19	844.29	845.94	846.06	845.30	0.72
30	745.13	746.39	744.58	745.72	743.38	745.04	1.15
35	558.17	559.32	553.30	559.73	558.30	557.76	2.58
40	486.58	482.45	488.82	487.06	485.35	486.05	2.37
45	425.63	423.08	429.63	423.74	427.91	425.99	2.76
50	385.00	383.14	384.52	388.17	384.66	385.09	1.86

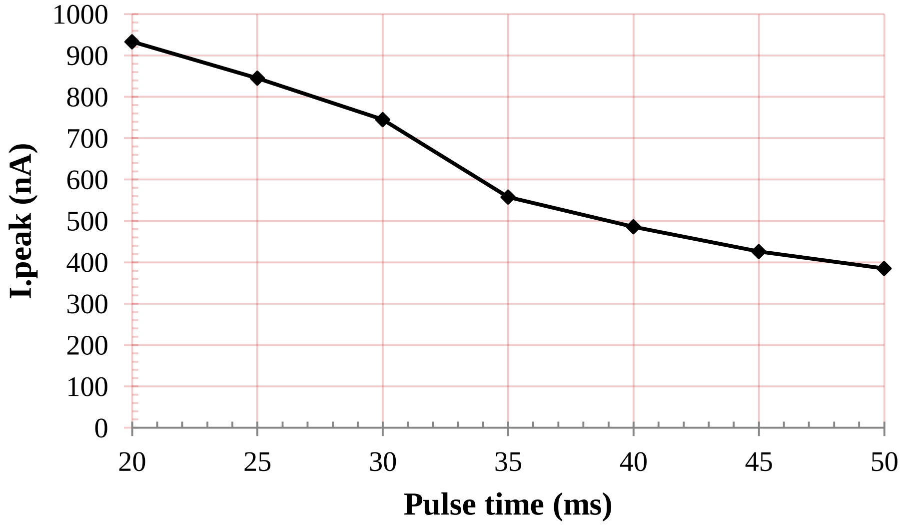


Figure 4.6 The effect of pulse time on the diffusion current of Co(II)-dimethylglyoxime complexes

4.2.5 Effect of pulse amplitude (U.ampl)

The effect of pulse amplitude on the diffusion current of the Co(II)-dimethylglyoxime complex for sensitivity of analysis was studied in the range of -1 to -80 mV. The results were shown in Table 4.6 and Figure 4.7, It was seen that the diffusion current was increased continuously while increasing pulse amplitude to – 45 mV above of which, the diffusion current was slightly stable. Therefore, the optimum pulse amplitude was – 45 mV.

Table 4.6 The effect of pulse amplitude on the diffusion current of Co(II)-dimethylglyoxime complexes

Pulse amplitude	**Diffusion current (I.peak) (nA)**						**SD**
(-mV)	**1**	**2**	**3**	**4**	**5**	**Avg.**	
1	3.19	1.96	2.27	2.79	3.15	2.67	0.54
5	16.48	17.66	18.19	16.07	16.72	17.02	0.88
10	48.14	45.10	47.24	45.00	48.50	46.79	1.66
15	80.04	81.21	80.82	80.40	81.01	80.69	0.47
20	131.00	131.31	131.37	133.10	134.50	132.26	1.50
25	202.27	205.85	201.94	202.60	207.25	203.98	2.40
30	286.53	287.13	284.86	289.72	283.32	286.31	2.42
35	369.45	368.81	366.25	367.69	363.53	367.15	2.36
40	508.43	509.90	510.10	510.56	512.61	510.32	1.51
45	661.80	661.13	660.72	659.61	659.47	660.55	0.99
50	792.93	792.71	798.48	781.02	795.78	792.18	6.67
55	935.55	930.78	929.57	930.15	929.48	931.48	2.54
60	949.15	947.11	940.03	941.94	940.23	943.23	4.18
65	953.53	950.78	957.84	955.06	954.17	954.28	2.55
70	952.08	953.83	955.91	951.46	952.88	953.23	1.74
75	963.78	968.54	960.05	961.81	960.46	962.93	3.46
80	968.38	961.40	962.74	965.99	961.46	963.99	3.08

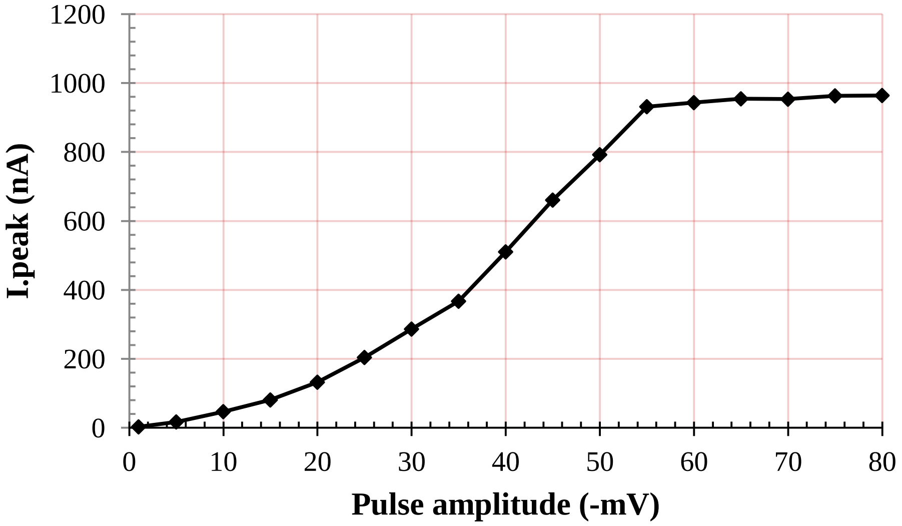


Figure 4.7 The effect of pulse amplitude on diffusion current of Co(II)-dimethylglyoxime complex

4.2.6 Effect of dimethylglyoxime concentration

The effect of dimethylglyoxime concentration on the diffusion current of Co(II)- dimethylglyoxime complexes was studied in the range of 1-250 mg/L using 50 µg/L Co(II). The results were shown in Table 4.7 and Figure 4.8. It was found that, when increasing dimethylglyoxime concentration, the forming complexes with cobalt was increased, but when increasing dimethylglyoxime concentration over 100 mg/L the diffusion current was increased slightly and higher standard deviation. Consequently, a concentration of 100 mg/L of dimethylglyoxime was chosen as a compromise between sensitivity and reagent cost.

Table 4.7 The effect of dimethylglyoxime concentration on the diffusion current of Co(II)-dimethylglyoxime complexes

Concentration of dimethylglyoxime (mg/L)	**Diffusion current (I.peak) (nA)**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
1	22.36	21.40	21.42	21.33	21.06	21.51	0.49
5	77.18	78.80	78.02	77.09	78.75	77.97	0.82
10	136.94	134.67	133.22	135.59	136.70	135.42	1.53
50	295.96	295.70	298.70	294.38	295.17	295.98	1.64
100	430.21	431.33	430.59	429.55	429.58	430.25	0.75
150	519.44	518.51	515.66	517.15	516.78	517.51	1.48
200	521.41	521.57	523.13	524.78	520.98	522.37	1.57
250	523.20	522.08	520.78	520.96	521.54	521.71	0.98

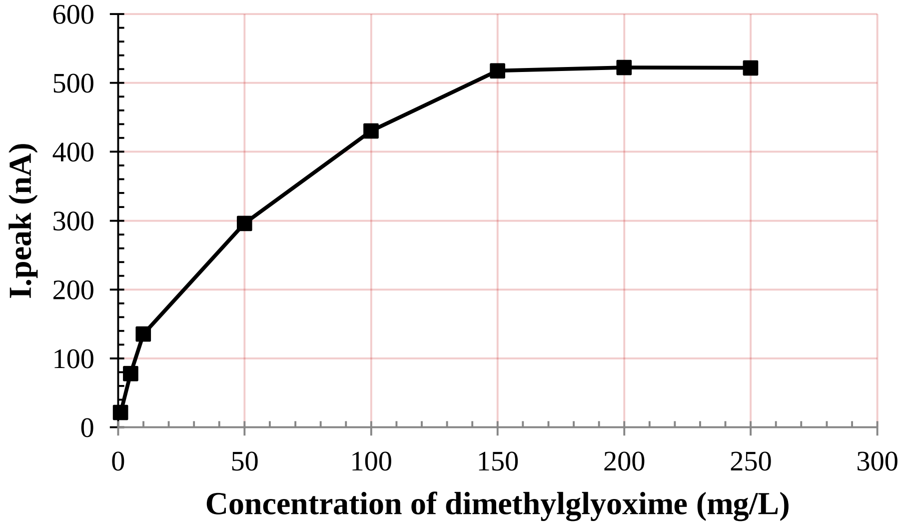


Figure 4.8 The effect of dimethylglyoxime concentration on the diffusion current of Co(II)-dimethylglyoxime complexes

4.2.7 Optimum experimental conditions

The studied parameters and their optimum valuewere shown in Table 4.8

Table 4.8 Optimum conditions for Co(II) determination using the proposed voltammetric method

Parameter	**Studied range**	**Optimum value**
Supporting electrolyte (NH_4Cl) (M)	0.2–3.0	0.8
Measurement time (t.meas) (ms)	5.0–30.0	15
Pulse amplitude (U.ampl) (mV)	(-1)–(-80)	-45
Pulse time (t.pulse) (ms)	20–50	20
Step time (t.step) (s)	0.1–1.0	0.6
Concentration of dimethylglyoxime (mg/L)	1–250	100

4.3 Analytical figures of merit

4.3.1 The linear calibration graph

The linearity range for a calibration graph was investigated by measurement the diffusion current of Co(II)–dimethylglyoxime complex using standard Co(II) concentration over the range of 0.5-1000.0 µg/L using the proposed voltammetric method under the optimum conditions. Table 4.9 and Figure 4.10 showed the diffusion current of Co(II)–dimethylglyoxime complex obtain from the proposed voltammetric method.

Table 4.9 The diffusion current Co(II)–dimethylglyoxime complex at Co(II) concentration in the range of 0.5-1000.0 μg/L for calibration graph

Concentration of Co(II) (μg/L)	**Diffusion current (nA)**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0.5	3.17	2.25	2.47	2.90	2.38	2.51	0.25
1.0	23.23	24.86	22.78	23.56	22.92	23.47	0.83
5.0	76.70	75.66	77.86	76.21	75.62	76.41	0.92
10.0	118.53	115.22	116.79	116.69	117.21	116.89	1.17
50.0	517.64	515.61	516.64	515.39	517.95	516.65	1.16
100.0	1040.00	1040.00	1043.00	1042.00	1045.00	1042.00	2.12
200.0	1720.00	1725.00	1725.00	1726.00	1720.00	1723.20	2.95
300.0	2240.00	2242.00	2246.00	2245.00	2240.00	2242.00	2.79
400.0	2770.00	2775.00	2771.00	2770.00	2773.00	2771.80	2.17
500.0	2820.00	2823.00	2824.00	2820.00	2821.00	2821.60	1.82
600.0	3150.00	3152.00	3156.00	3150.00	3153.00	3152.20	2.48
700.0	3320.00	3321.00	3320.00	3325.00	3326.00	3322.40	2.88
800.0	3580.00	3581.00	3585.00	3584.00	3580.00	3582.00	2.35
900.0	3500.00	3503.00	3505.00	3506.00	3505.00	3502.60	2.51
1000.0	3410.00	3414.00	3415.00	3410.00	3412.00	3412.20	2.28

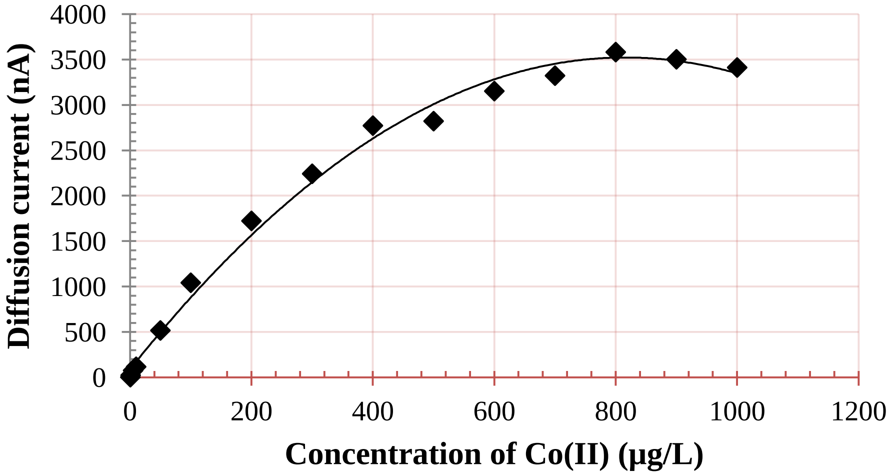


Figure 4.9 The diffusion current Co(II)–dimethylglyoxime complex at Co(II) concentration in the range of 0.5-1000.0 µg/L using the proposed voltammetric method under the optimum conditions

Regarding to Figure 4.9 the linearity for determination of Co(II) was found in the range of 1.0-100.0 µg/L. The calibration graph was then reploted and shown in Figure 4.10. The linear regression equation was Y = 10.21X + 16.01, where Y is diffusion current of Co(II)–dimethylglyoxime complex and X is the concentration of cobalt (II). The correlation coefficient for this line was 0.999.

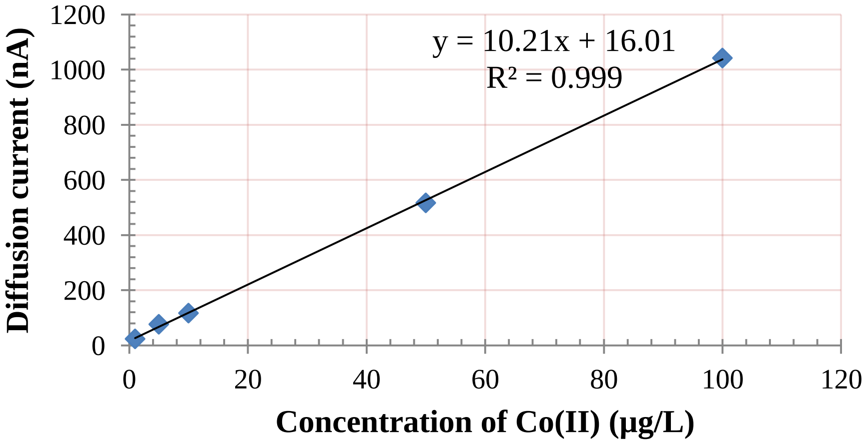


Figure 4.10 Calibration graph of the proposed voltammetric method for Co(II) determination

4.3.2 Limit of detection

The limit of detection (LOD) and limit of quantitation (LOQ) was accomplished by measurement 11 replicates of complex forming between 100 mg/L dimethylglyoxime and a lowest concentration of Co(II) (1.0 µg/L) in ammonia ammonium chloride buffer solution pH 9. After that the diffusion current values were then used to calculate the standard deviation (SD) of the linear calibration graph. Finally the limit of detection (LOD) and limit of quantitation (LOQ) of the proposed methods was calculated using equations 3.6 and 3.7, respectively. See an example LOD calculation in appendix B. It was found that the limit of detection (3SD) and limit of quantitation (10SD) were 1.14 and 4.80 µg/L, respectively.

4.3.3 Precision

The precision of the proposed voltammetric method based on repeatability and reproducibility were performed on 11 replicates and 11 duplicates of three Co(II) standard solutions covering different concentration levels: low, medium and high (1.0, 50.0 and 100.0 µg/L), respectively. The results were shown in Table 4.10. It was found that, the relative standard deviation (R.S.D.) of each concentration was 3.24, 0.13 and 0.06% for repeatability, respectively and 5.07, 0.19 and 0.09% for reproducibility, respectively. Indicating that, the proposed voltammetric method was providing a high precision.

Table 4.10 The precision study using three standard Co(II) solutions covering different concentration levels: low, medium and high (1.0, 50.0 and 100.0 µg/L)

Experimental number	**Diffusion current of Co(II) standard (µg/L)**					
	Repeatability			**Reproducibility**		
	1.0	**50.0**	**100.0**	**1.0**	**50.0**	**100.0**
1	24.10	514.54	1042.00	23.71	514.30	1042.32
2	23.35	514.02	1043.00	22.24	514.02	1044.29
3	23.72	514.13	1042.00	24.86	515.37	1043.83
4	24.89	513.98	1041.00	25.84	516.26	1042.21
5	25.85	513.21	1043.00	23.20	515.41	1043.26
6	24.46	513.71	1042.00	23.78	514.28	1044.56
7	23.91	512.23	1043.00	24.25	516.79	1045.15
8	23.33	514.04	1042.00	25.60	516.62	1042.73
9	24.90	512.97	1043.00	22.04	514.68	1043.17
10	24.72	514.24	1042.00	23.43	515.81	1044.63
11	25.12	513.55	1042.00	23.78	515.28	1042.91
$\bar{X}$	24.39	513.19	1042.27	23.89	515.35	1043.55
SD	0.79	0.67	0.65	1.21	0.96	0.99
%RSD	3.24	0.13	0.06	5.07	0.19	0.09

4.4 Effects of interferences

The effect of some possible interfering ions such as some metals ion (Zn^{2+}, Ni^{2+}, Ca^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+} and Mg^{2+}) on the determination of Co(II) in beverage samples was investigated by varying the concentration of interfering ions (µg/L) to 50.0 µg/L Co(II). The tolerance value is defined as the foreign species concentration causing error smaller than ± 5% for determining the Co(II) . The results are shown in this table 4.11. It can be seen that Zn^{2+}, Ni^{2+} and Ca^{2+}were tolerated in large amounts 50-fold and 10-fold of Fe^{3+}, Mn^{2+}, Cu^{2+} and Mg^{2+}did not interfere.

Table 4.11 Effect of interference ions on the determination of 50.0 μg/L Co(II). The tolerance limits was determined for a maximum error of less than ±5%

Interference ions	**Tolerance concentration ratio**
Zn(II), Ni(II), Ca(II)	50
Fe(III), Mn(II), Cu(II), Mg(II)	10

4.5 The efficiency of sample digestion

The efficiency of sample digestion was performed by addition 10 μg/L standard Co(II) solutions into the beverage samples (B1-B10) prior to the digestion method in section 3.4.2. The results were given in Table 4.12 showed the content of Co(II) in beverage samples analyzed by voltammetric method and the percentage recovery was perfumed by addition 10 μg/L Co(II) standard solutions into samples, it was over the range of 84.44-99.54%. It also found that the amount of Co(II) content in beverage samples agreed well with the Co(II) content in label.

Table 4.12 Recovery of Co(II) in beverage samples analyzed by the voltammetric method

Sample	**Co(II) added (μg/L)**	**Co(II) content found (μg/L)**	**Recovery (%)**
B-ing fine (B1)	-	0.047±0.62	89.05
	10.0	8.952±0.64	
Carabao (B2)	-	0.056±0.39	84.44
	10.0	8.500±0.63	
Lipovitan-D (B3)	-	0.086±0.39	99.54
	10.0	10.040±0.78	
Looktung (B4)	-	0.056±0.39	86.94
	10.0	8.750±0.78	
M 150 (B5)	-	0.112±0.80	88.78
	10.0	8.990±0.50	
Orange shake (B6)	-	0.082±0.43	98.81
	10.0	9.963±0.73	
Red bull (B7)	-	0. 087±0.63	95.83
	10.0	9.670±0.47	
Vitamix C120 (B8)	-	0.036±0.34	92.39
	10.0	9.275±0.68	
Vitamix V500 (B9)	-	0.035±0.34	98.34
	10.0	9.868±0.68	
5 day colours (B10)	-	0.074±0.62	94.14
	10.0	9.488±0.74	

4.6 Application to sample

The proposed voltammetry was applied to the determination Co(II) in beverages sample. The beverages samples were collected from super market, Mahasarakham province, Thailand (B1 to B10). The results are given in Table 4.13 showed the compared with those obtained by frame atomic absorption spectrophotometry. The content of Co(II) ion in beverages sample analyzed by the proposed voltammetry were in the range of 0.86-2.80 and 0.85-2.82 µg/L, respectively. As the results found that the proposed device agreed well with those obtained by FAAS, because the calculated Student's *t*-test was less than the theoretical value at confident level of 95% (see appendix B for more detail).

Table 4.13 The content of Co(II) found in beverage samples analyzed by the proposed voltammetric method comparison with the standard FAAS method

Sample	**The amount of Co(II) found (µg/L)**		**Co(II) content in label (µg)**
	Voltammetry	**FAAS**	
B-ing fine (B1)	1.18±0.62	1.20±0.53	1.24
Carabao (B2)	1.41±0.92	1.43±0.65	1.50
Lipovitan-D (B3)	2.16±0.39	2.15±0.46	2.20
Looktung (B4)	1.41±0.39	1.40±0.55	1.50
M150 (B5)	2.80±0.80	2.82±0.63	2.90
Orange shake (B6)	2.04±0.43	2.07±0.60	2.20
Red bull (B7)	2.18±0.40	2.19±0.71	2.20
Vitamix C120 (B8)	0.89±0.34	0.90±0.57	0.91
Vitamix V500 (B9)	0.86±0.63	0.85±0.48	0.97
5 day colours (B10)	1.85±0.53	1.86±0.62	1.92

CHAPTER 5

CONCLUSIONS

5.1 The proposed voltammetric method for determining Co(II) in beverage samples

The method for the determination of Co(II) based on measurement the diffusion current of the complex forming between Co(II) and dimethylglyoxime at hanging mercury drop electrode (HMDE) as working electrode, a silver-silver chloride (Ag/AgCl) used as reference electrode and a platinum wire as auxiliary electrode. A differential pulse mode was applied at for electrolysis of Co(II)-dimethylglyoxime complex in voltammetric cell. The chemical reactions are shown in equation 3.1-3.3. As the equation, the reaction between Co(II) and dimethylglyoxime (DMG) to form a Co(II)-dimethylglyoxime complex. Then it was protonated to form a Co(II)-dimethylglyoximecation by ammonium buffer solution, Finally, the Co(II)-dimethylglyoximecation was reduced at HMDE and subsequently measurement the diffusion current of Co(II)-dimethylglyoxime complex which is proportional to the concentration of Co(II). It was found that the highest diffusion current of Co(II)-dimethylglyoxime complex scanning the potential at -1022 mV. Under the optimum conditions, the linear range for the determination of Co(II) in beverages sample was over the range of 1.0-100.0 µg/L of Co(II) with a correlation coefficient (r^2) of 0.999. The limit of detection (3SD) and limit of quantification (10SD) were 1.14 and 4.80 µg/L, respectively. The repeatability and reproducibility calculated from 11 replicate measurement of three concentration (1.0, 50.0 and 100.0 µg/L Co(II) standard solution) were found to be 3.24, 0.13 and 0.06 for repeatability, 5.07, 0.19 and 0.09 for reproducibility, respectively. The proposed voltammetry was successfully applied to the determination of Co(II) in beverages samples with the percentage recovery was over the range of 84.44-99.54%.

5.2 Suggestion for further work

The method for determination of Co(II) in beverage sample can be applied for determination of vitamin B_{12} in beverages sample too because Co(II) serves its paramount function as an essential component of vitamin B_{12} and is important for living species as complexed vitamin $B_{12.}$ The amount of Co(II) found is relative to the amount of vitaminB_{12} in sample.

REFERENCES

REFERENCES

[1] Ensafi AA H. Mansour HR, Zarei K and Gholivand MB. Determination of ultra-trace amounts of cobalt by adsorptive differential pulse voltammetry using ACDA (2-amiocyclopentene-1-dithiocarboxxylic acid). Science.I.R.Iran. 2000; 11: 93-97.

[2] Sancho D, Deban L, Campos I, Pardo R and Vega M. Determination of nickel and cobalt in refined beet sugar by adsorptive cathodic stripping voltammetry without sample pretreatment. Food Chemistry 2000; 71: 139-145.

[3] Garcia Rodriguez AM, Garcia de Torres A, Cano Pavon JM and Bosch Ojeda C. Simultaneous determination of iron, cobalt, nickel and copper by UV-visible spectrophotometry with multivariate calibration. Talanta 1998; 47: 463-470.

[4] Jadhav SB, Utekar SS, Kulkarni AJ, Varadarajan A and Malve SP. Benzeneacetaldehyde-4-hydroxy-α-oxo-aldoxime as a new analytical reagent for the spectrophotometric determination of cobalt. Talanta 1998; 46: 1425–1432.

[5] Shemirani F and Shokoufi N. Laser induced thermal lens spectrometry for cobalt determination after cloud point extraction. Analytica Chimica Acta 2006; 577: 238–243.

[6] Oleszczuk N, Castro JT, Silva MM, Welz B, Maria das Gracas AK and Maria Goreti RV. Method development for the determination of manganese, cobalt and copper in green coffee comparing direct solid sampling electrothermal atomic absorption spectrometry and inductively coupled plasma optical emission spectrometry. Talanta 2007; 73: 862-869.

[7] Moreira FR, Maia CB and Avila AK. Titanium as a chemical modifier for the determination of cobalt in marine sediments. Spectrochimica Acta 2002; 57: 2141–2149.

[8] Zhang X, Zhou Q, Lv Y, Wu L and Hou X. Ultrasensitive determination of cobalt in single hair by capillary electrophoresis using chemiluminescence detector. Microchemical 2010; 95: 80–84.

[9] Amin AS. Study on the solid phase extraction and spectrophotometric determination of cobalt with 5-(2-benzothiazolylazo)-8-hydroxyquinolene. Arabian Journal of Chemistry 2011; In Press, Corrected Proof.

[10] Baliza PX, Gomes Teixeira LS and Lemos VA. A procedure for determination of cobalt in water samples after dispersive liquid–liquid microextraction. Microchemical 2009; 93: 220–224.

[11] Hutton EA, van Elteren JT, Ogorevc B and Smyth MR. Validation of bismuth film electrode for determination of cobalt and cadmium in soil extracts using ICP–MS. Talanta 2004; 63: 849–855.

[12] Leggett RW. The biokinetics of inorganic cobalt in the human body. The Science of the total environment. 2008; 259-269.

[13] Biego GH. Cobalt in food. [Online] 2006 [cited July 2013]; Avalable from: http:// www. atsdr.cdc. gov/tfacts33.html.

[14] Argonne National Laboratory. Human Health Fact Sheet. n.p: n.p.; 2005.

[15] Masten S. Toxicological Summary for Cobalt Dust. North Carolina: Research Triangle Park; 2002.

[16] Flinn Scientific, Inc. Material safety data sheet. n.p: n.p.; 2002.

[17] Semon WL and Damerell VR. Organic syntheses. [Online] 2010 [cited July 2013]; http:// www.orgsyn. org/orgsyn/chemname.asp?nameID=35095.

[18] Hihara G, Satoh M, Uchida T, Ohtsuki F and Miyamae H. Mechaochemical syntheses of complexs through solid-solid reactions of divalent transition metal salts (Ni(II) or Cu(II) with dimethylglyoxime. Solid State Ionics 2004; 172: 221-223.

[19] Skoog DA, Holler FJ and Neiman TA. Principle of Instrumental Analysis. 5th ed. Philadelphia: Saunders College Publishing; 1998.

[20] Skoog DA, Holler FJ and Crouch S.R. Principle of Instrumental Analysis. 6th ed. Philadelphia: Saunders College Publishing; 1998.

[21] Borland LM. Improving the accuracy of chemical measurements in the brain: new insights into dopaminergic mechanisms by fast-scan cyclic voltammetry and microdialsis. [Ph.D. Thesis]. USA: University of Pittsburgh; 2005.

[22] Mena C, Cabrer C, Lorenzo ML and Lopez MC. Cadmium levels in wine, beer and other alcoholic beverages: possible sources of contamination. The Science of the Total Environment 1996; 181: 201-208.

[23] Nkono NA and Asubiojo OI. Trace elements in bottled and soft drinks in Nigeria-a preliminary study. The Science of the Total Environment 1997; 208: 161-163.

[24] Onianwa PC, Adetola IG, Iwegbue CMA, Ojo MF and Tella OO. Trace heavy metals composition of some Nigerian beverages and food drinks. Food Chemistry 1999; 66: 275-279.

[25] Su´arez-Luque S, Mato I and Huidobro JF, Simal-Lozano. Rapid capillary zone electrophoresis method for the determination of metal cations in beverages. Talanta 2006; 68: 1143–1147.

[26] Maduabuchi JMU, Nzegwu CN, Adigba EO, Aloke RU, Ezomike CN, Okocha CE, Obi E and Orisakwe OE. Lead and cadmium exposures from canned and non-canned beverages in Nigeria: A public health concern. The Science of the Total Environment 2006; 366: 621–626.

[27] Carvalho MS, Fraga ICS, Neto CM and Silva Filho EQ. Selective determination of cobalt using polyurethane foam and 2-(2-benzothiazolylazo)-2-p-cresol as a spectrophotometric reagent. Talanta 1996; 43: 1675-1680.

[28] Jadhav SB, Utekar SS, Kulkarni AJ, Varadarajan A and Malve SP. Benzeneacetaldehyde-4-hydroxy-α-oxo-aldoxime as a new analytical reagent for the spectrophotometric determination of cobalt. Talanta 1998; 46: 1425–1432.

[29] Pancras JP, Puri BK, Taher MA, Dehzoei AM and Sheibani A. Preconcentration of trace cobalt with the ion pair of 2-(5-bromo-2-pyridylazo)-5 diethylaminophenol and tetraphenylborate onto microcrystalline naphthalene or column method and its determination by derivative spectrophotometry. Talanta 1998; 46: 1107-1113.

[30] Ozturk BD, Filik, Tutem E and Apak R. Simultaneous derivative spectrophotometric determination of cobalt(II) and nickel(II) by dithizone without extraction. Talanta 2000; 53: 263-269.

[31] Jadhav SB, Tandel SP and Malve SP. Extraction and spectrophotometric determination of cobalt(II) with isonitroso-5-methyl-2 hexanone. Talanta 2001; 55: 1059-1064.

[32] Rezaei B, Ensafi AA and Shandizi F. Simultaneous determination of cobalt and nickel by spectrophotometric method and artificial neural network. Microchemical 2001; 70: 35-40.

[33] Ghasemi J, Ahmadi Sh and Torkestani K. Simultaneous determination of copper, nickel, cobalt and zinc using zincon as a metallochromic indicator with partial least squares. Analytica Chimica Acta 2003; 487: 181–188.

[34] Ghasemi J, Shahabadi N and Seraji HR. Spectrophotometric simultaneous determination of cobalt, copper and nickel using nitroso-R-salt in alloys by partial least squares. Analytica Chimica Acta 2004; 510: 121-126.

[35] Zarei K, Atabati M and Malekshabani Z. Simultaneous spectrophotometric determination of iron, nickel and cobalt in micellar media by using direct orthogonal signal correction-partial least squares method. Analytica Chimica Acta 2006; 556: 247–254.

[36] Ahmed MJ and Uddin. MN. A simple spectrophotometric method for the determination of cobalt in industrial, environmental, biological and soil samples using bis(salicylaldehyde)orthophenylenediamine. Chemosphere 2007; 67: 2020-2027.

[37] Niazi A and Yazdanipour A. Simultaneous spectrophotometric determination of cobalt, copper and nickel using 1-(2-thiazolylazo)-2-naphthol by chemometrics methods. Chinese Chemical Letters 2008; 19: 860–864.

[38] Hiraide M, Chen ZS and Kawaguchi H. Coprecipitation with disulfide for the determination of traces of cobalt in water by electrothermal atomic absorption spectrometry. Talanta 1996; 43: 1131–1136.

[39] Zhang Q, Minami H, Inoue S and Atsuya I. Determination of ultra-trace amounts of cobalt in seawater by graphite furnace atomic absorption spectrometry after pre-concentration with Ni/8-quinolinol/1-nitroso-2-naphthol complex. Analytica Chimica Acta 2000; 407: 147-153.

[40] Chen J and Teo KC. Determination of cobalt and nickel in water samples by flame atomic absorption spectrometry after cloud point extraction. Analytica Chimica Acta 2001; 434: 325-330.

[41] Berton P and Wuilloud RG. Highly selective ionic liquid-based microextraction method for sensitive trace cobalt determination in environmental and biological samples. Analytica Chimica Acta 2010; 662: 155-162.

[42] Lemos VA, Santos LN and Bezerra MA. Determination of cobalt and manganese in food seasonings by flame atomic absorption spectrometry after preconcentration with 2-hydroxyacetophenone-functionalized polyurethane foam. Food Composition and Analysis 2010; 23: 277–281.

[43] Ma R and Adams F. Flow injection sorbent extraction with dialkyldithiophosphates as chelating agent for nickel, cobalt and manganese determination by atomic absorption spectrometry. Analytica Chimica Acta 1995; 317: 215-222.

[44] Taljaard RE and Staden JFv. Simultaneous determination of cobalt(II) and Ni(II) in water and soil samples with sequential injection analysis. Analytica Chimica Acta 1998; 366: 177-186.

[45] Nogueira ARA, Souza GB, Batista LAR, Arruda MAZ and Miyazawa M. Catalytic determination of cobalt by flow injection spectrophotometry: application in animal faeces analysis using hydrochloric acid as extracting solution. Animal Feed Science and Technology 1998; 74: 79-84.

[46] Fujimoto T, Teshima N, Kurihara M, Nakano S and Kawashima T. Catalytic determination of cobalt at sub-nanogram levels using the oxidative coupling of 3-methyl-2-benzothiazolinone hydrazone with N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline by manual and flow-injection methods. Talanta 1999; 49: 1091–1098.

[47] Haji Shabani AM, Dadfarnia S and Dehghan K. On-line preconcentration and determination of cobalt by chelating microcolumns and flow injection atomic spectrometry. Talanta 2003; 59: 719-725.

[48] Staden JFv and Taljaard RE. Determination of Lead(II), Copper(II), Zinc(II), Cobalt(II), Cadmium(II), Iron(III), Mercury(II) using sequential injection extractions. Talanta 2004; 64: 1203–1212.

[49] Song Z, Yue Q and Wang C. Flow injection chemiluminescence determination of femtogram-level cobalt in egg yolk, fish tissue and human serum. Food Chemistry 2006; 94: 457–463.

[50] Li B, Wang D, Lv J and Zhang Z. Flow-injection chemiluminescence simultaneous determination of cobalt(II) and copper(II) using partial least squares calibration. Talanta 2006; 69: 160-165.

[51] Zhang X, Zhou Q, Lv Y, Wu L and Hou X. Ultrasensitive determination of cobalt in single hair by capillary electrophoresis using chemiluminescence detector. Microchemical 2010; 95: 80-84.

[52] Minggang L, Xiaohu L and Fang Y. A new chemiluminescence system for determination of cobalt. Talanta 1990; 37: 393-395.

[53] Mori I, Takasaki K, Fujita Y and Matsuo T. Selective and sensitive fluorometric determinations of cobalt(II) and hydrogen peroxide with fluorescein-hydrazide. Talanta 1998; 47: 631-637.

[54] Khuhawar MY and Lanjwani SN. High-performance liquid chromatographic separation and determination of cobalt(II), cobalt(Ill) and iron(II) using bis(salicylaldehyde)tetramethylethylenediimine. Chromatography A 1995; 695: 132-135.

[55] Ghaedi M, Ahmadi F and Soylak M. Preconcentration and separation of nickel, copper and cobalt using solid phase extraction and their determination in some real samples. Hazardous Materials 2007; 147: 226–231.

[56] Cheng GW, Lee CF, Keng Chang Hsu, Wu HL and Huang YL. On-line microdialysis–nano-Au/TiO2–high-performance liquid chromatography system for the simultaneous determination of cobalt and nickel in water. Chromatography A 2008; 1201: 202-207.

[57] Khorrami AR, Hashempur T, Mahmoudi A and Karimi AR. Determination of ultra trace amounts of cobalt and nickel in water samples by inductively coupled plasma-optical emission spectrometry after preconcentration on modified C_{18}-silica extraction disks. Microchemical 2006; 84: 75-79.

[58] Beiraghi A, Babaee S and Roshdi M. Simultaneous preconcentration of cadmium, cobalt and nickel in water samples by cationic micellar precipitation and their determination by inductively coupled plasma-optical emission spectrometry. Microchemical 2011.

[59] Zhang H, Wollast R, Vire JC and Patriarche GJ. Simultaneous Determination of Cobalt and Nickel in Sea Water by Adsorptive Cathodic Stripping Square-wave Voltammetry. Analyst 1989; 114: 1597-1602.

[60] Giroussi S, Voulgaropoulos A, Ayiannidis AK, Golimowski J and Janicki M. Determination of cobalt in vegetable animal foodstuffs by differential pulse adsorptive voltammetry using α-benzil dioxime. The Science of the Total Environment 1995; 176: 135-139.

[61] Zhang ZQ, Liu H, Zhang H and Li YF. Simultaneous cathodic stripping voltammetric determination of mercury, cobalt, nickel and palladium by mixed binder carbon paste electrode containing dimethylglyoxime. Analytica Chimica Acta 1996; 333: 119-124.

[62] Gao Z, Siow KS and Yeo L. Determination of cobalt by catalytic-adsorptive differential pulse voltammetry. Analytica Chimica Acta 1996; 320: 229-234.

[63] Ensafi AA, Mansour HR, Zarei K and Gholivand MB. Determination of ultra-trace amounts of cobalt by adsorptive using ACDA (2-aminocyclopentene-1-dithiocarboxylic acid). Science I.R. Iran 2000; 11: 93-97.

[64] Safavi A and Shams E. Highly sensitive and selective measurements of cobalt by catalytic adsorptive cathodic stripping voltammetry. Talanta 2000; 51: 1117–1123.

[65] Sancho D, Deban L, Campos I, Pardo R and Vega M. Determination of nickel and cobalt in refined beet sugar by adsorptive cathodic stripping voltammetry without sample pretreatment. Food Chemistry 2000; 71: 139-145.

[66] Lu X, Wang Z, Geng Z, Kang J and Gao J. 2,4,6-tri(3,5-Dimethylpyrazoyl)-1,3,5-triazine modified carbon paste electrode for trace Cobalt(II) determination by differential pulse anodic stripping voltammetry. Talanta 2000; 52: 411–416.

[67] Ellwood MJ, Constant MG and Berg VD. Determination of organic complexation of cobalt in seawater by cathodic stripping voltammetry. Marine Chemistry 2001; 75: 33–47.

[68] Kajic P, Milosev I, Pihlar B and Pisot V. Determination of trace cobalt concentrations in human serum by adsorptive stripping voltammetry. Trace element in medicine and biology. 2003; 3: 153-158.

[69] Morfobos M, Economou A and Voulgaropoulos A. Simultaneous determination of nickel(II) and cobalt(II) by square wave adsorptive stripping voltammetry on a rotating-disc bismuth-film electrode. Analytica Chimica Acta 2004; 519: 57–64.

[70] Korolczuk M, Tyszczuk K and Grabarczyk M. Determination of traces of cobalt in the presence of nioxime and cetyltrimethylammonium bromide by adsorptive stripping voltammetry. Talanta 2005; 65: 1003–1007.

[71] Korolczuk M, Tyszczuk K and Grabarczyk M. Adsorptive stripping voltammetry of nickel and cobalt at in situ plated lead film electrode. Electrochemistry Communications 2005; 7: 1185–1189.

[72] Hus´akov´a L, Bobrowsk A, Sr´amkov´a J, Kr´olicka A and Vytˇras K. Catalytic adsorptive stripping voltammetry versus electrothermal atomic absorption spectrometry in the determination of trace cobalt and chromium in human urine. Talanta 2005; 66: 999–1004.

[73] Gedaminskiene V and German N, Armalis S. Adsorptive stripping voltammetry of cobalt at a hanking mercury drop and mercury flim electrode : a comparative study. Chemija (Vilnius) 2006; 24: 94-98.

[74] Mohadesi A, Teimoori E, Taher MA and Beitollah H. Adsorptive Stripping Voltammetric Determination of Cobalt (II) on the Carbon Paste Electrode. Electrochemical science 2011; 6: 301-308.

[75] Cordon F, Ramı´rez SA and Gordillo GJ. Adsorption and electrochemical reduction of Co(II)-dimethylglyoxime on mercury. Electroanalytical Chemistry 2002; 534: 131-141.

[76] Hibbert B. Quality assurance for the analytical chemistry laboratory. New York. Oxford university Press, Inc. 2007, pp. 241-254.

APPENDICES

APPENDIX A

DATA

Appendix A

A1. Analytical diffusion current of the voltammetry

Voltammogram of supporting electrolyte

Voltammogram of Co(II)-dimethylglyoxime complex at the supporting electrolyte solutions such as potassium chloride (KCl), ammonium chloride (NH_4Cl), potassium hydroxide (KOH) and sodium acetate (CH_3COONa) using Co(II) 50.0 µg/L, 100 mg/L dimethylglyoxime and supporting electrolyte. The greatest of supporting electrolyte on the diffusion current signal of Co(II)-dimethylglyoxime complexes was studied over the range 1-3 M.

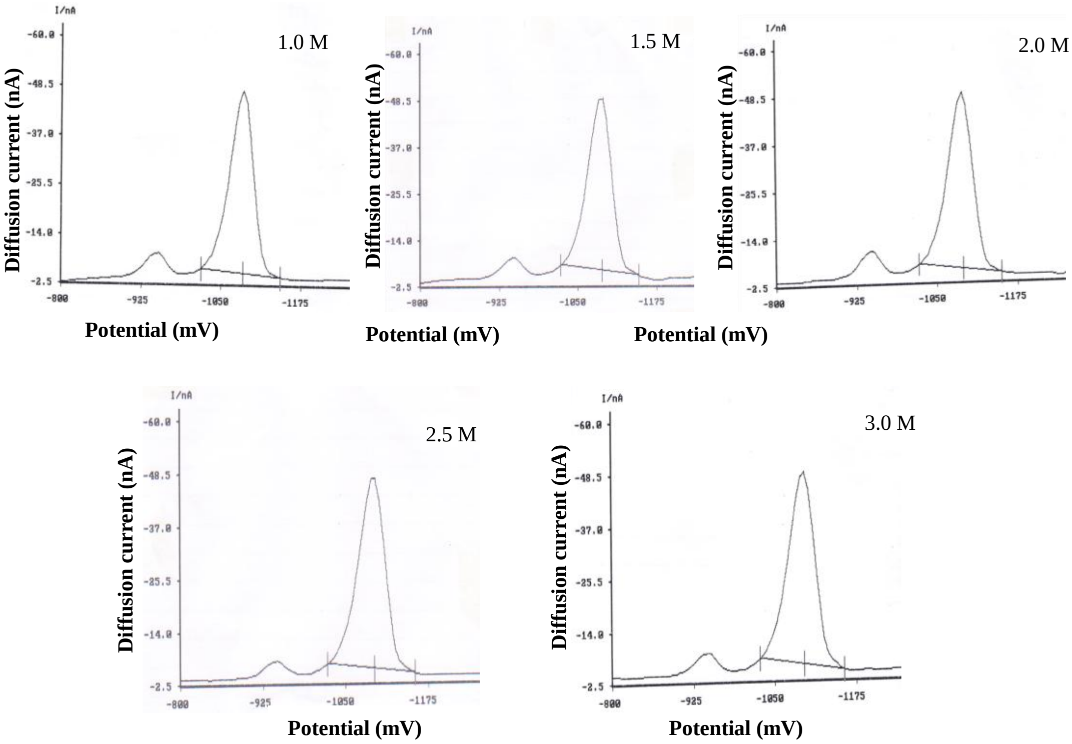


Figure A1 The voltammogram of Co(II)-dimethylglyoxime complex at the supporting electrolyte solutions (KCl) in the range of 1.0 to 3.0 M

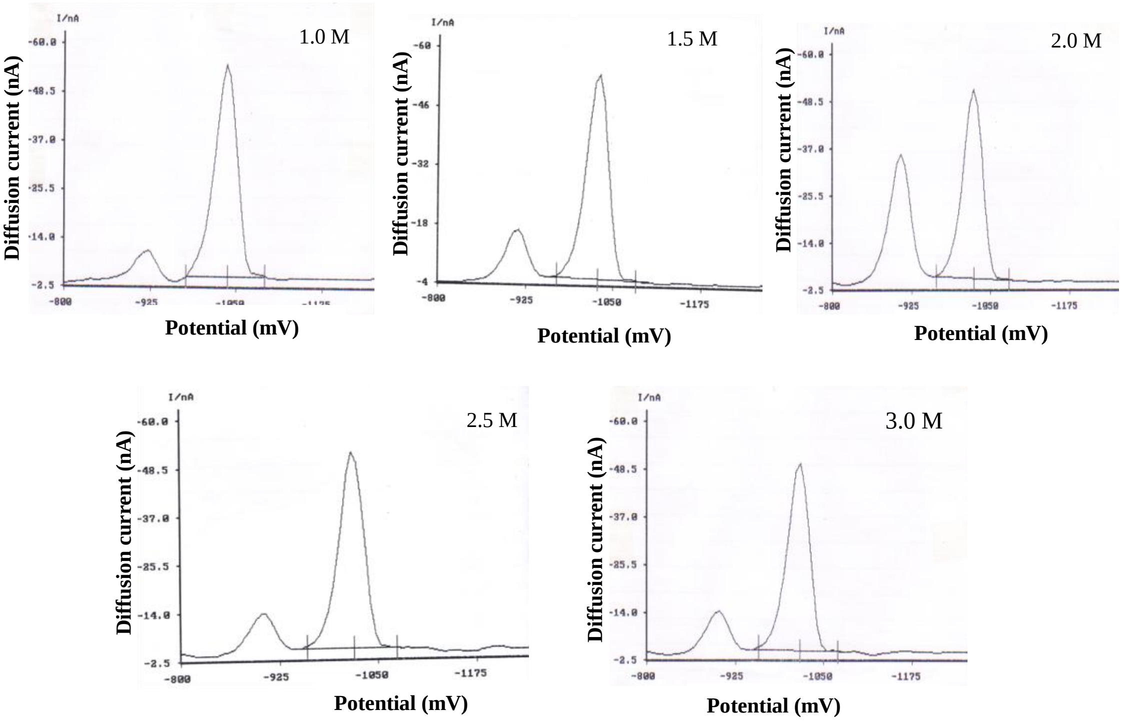


Figure A2 The voltammogram of Co(II)-dimethylglyoxime complex at the supporting electrolyte solutions (NH_4Cl) in the range of 1.0 to 3.0 M

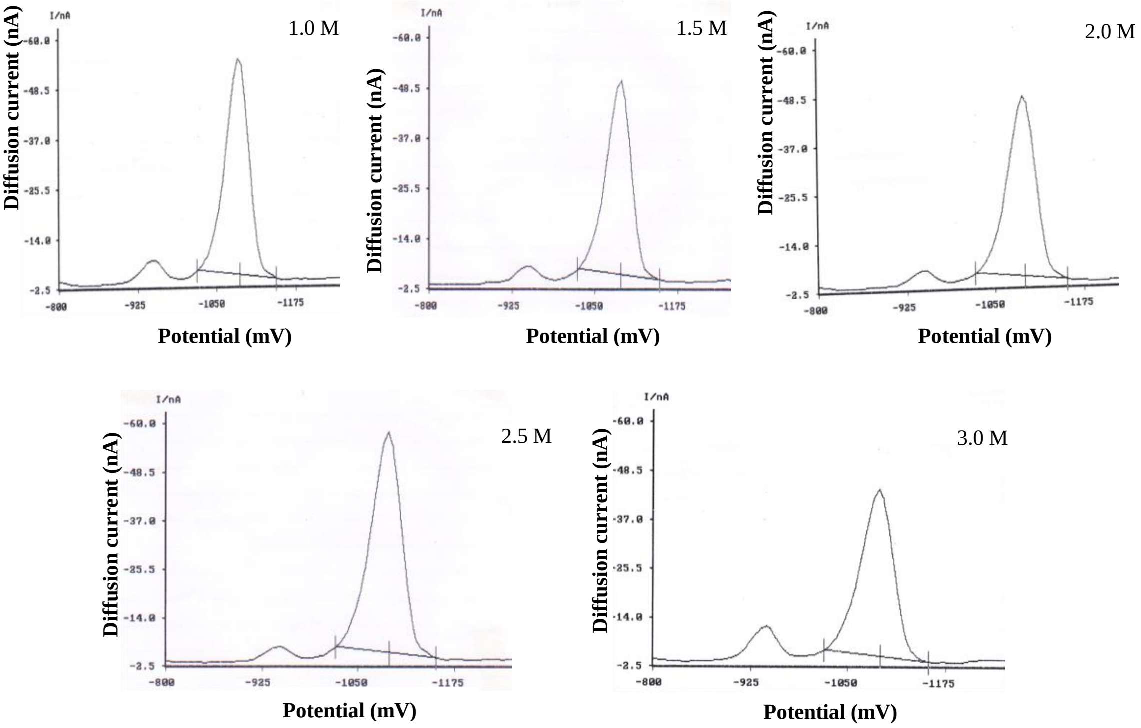


Figure A3 The voltammogram of Co(II)-dimethylglyoxime complex at the supporting electrolyte solutions (CH_3COONa) in the range of 1.0 to 3.0 M

Voltammogram of measurement time (t.meas)

Voltammogram of Co(II)-dimethylglyoxime complex at measurement time in the range of 1 to 30 ms using 50.0 µg/L Co(II), 100 mg/L dimethylglyoxime and 0.8 M ammonium chloride (NH_4Cl) supporting electrolyte.

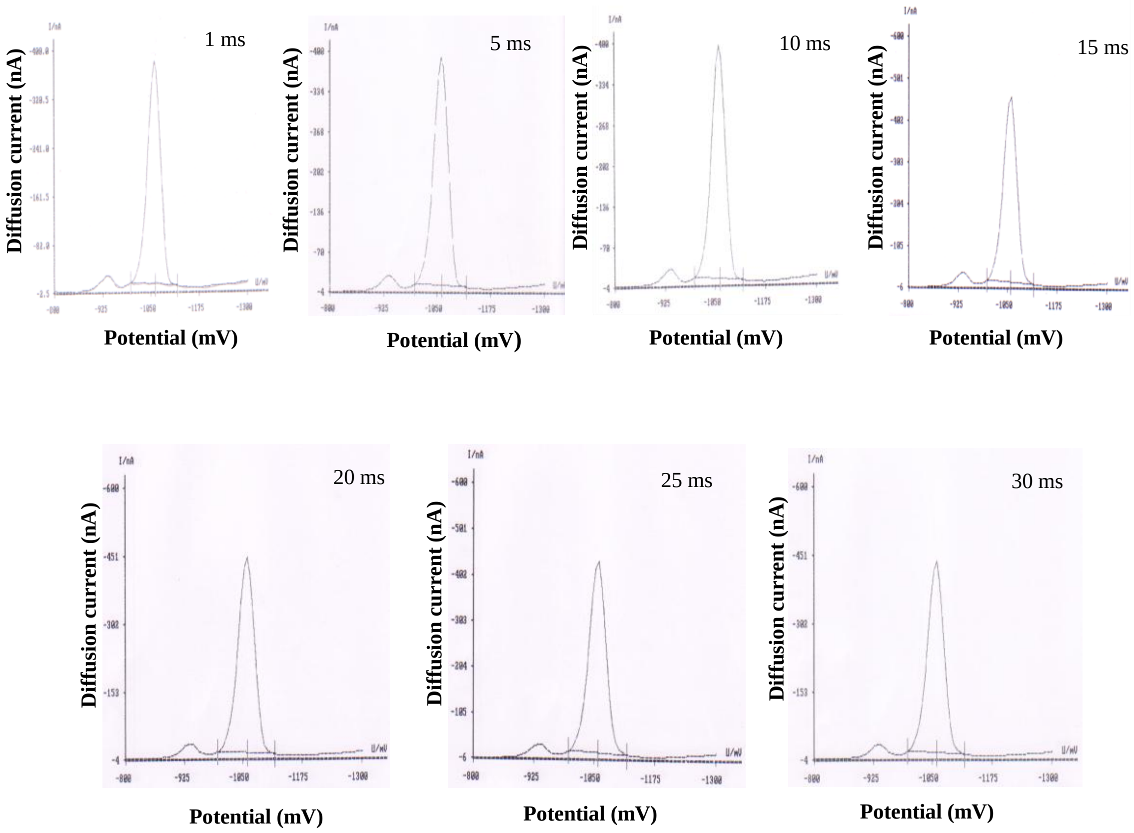


Figure A4 The voltammogram of Co(II)-dimethylglyoxime complex at measurement time in the range of 1 to 30 ms

Voltammogram of of step time (t.step)

The voltammogram of Co(II)-dimethylglyoxime complex at step time in the range of 0.1 to 1.0 second using 50.0 µg/L Co(II), 100 mg/L dimethylglyoxime and 0.8 M ammonium chloride (NH_4Cl) supporting electrolyte.

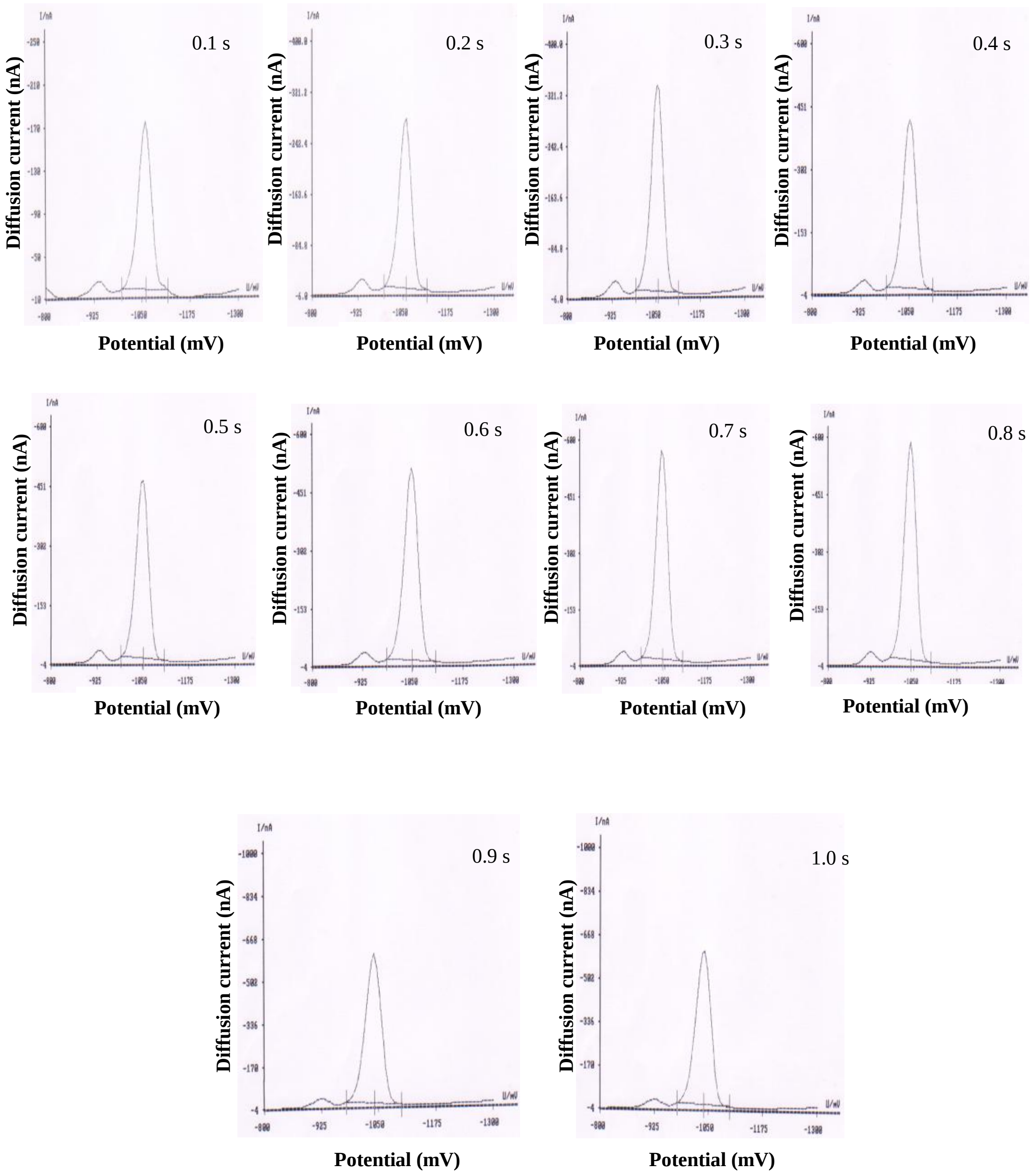


Figure A5 The voltammogram of Co(II)-dimethylglyoxime complex at step time in the range of 0.1 to 1.0 second

Voltammogram of pulse time (t.pulse)

The voltammogram of Co(II)-dimethylglyoxime complex at pulse time in the range of 20 to 50 ms using 50.0 µg/L Co(II), 100 mg/L dimethylglyoxime and 0.8 M ammonium chloride (NH_4Cl) supporting electrolyte.

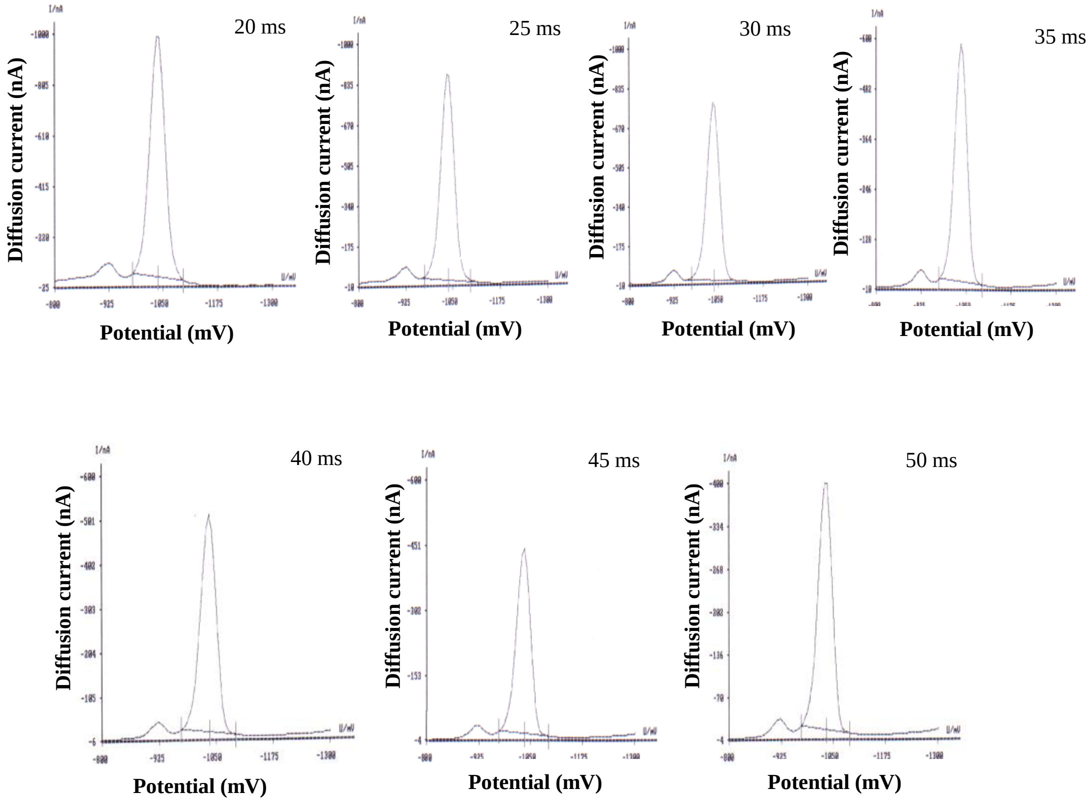


Figure A6 The voltammogram of Co(II)-dimethylglyoxime complex at pulse time in the range of 20 to 50 ms

Voltammogram of pulse amplitude (U.ampl)

The voltammogram of Co(II)-dimethylglyoxime complex at pulse amplitude in the range of -1 to -80 mV using 50.0 µg/L Co(II), 100 mg/L dimethylglyoxime and 0.8 M ammonium chloride (NH_4Cl) supporting electrolyte.

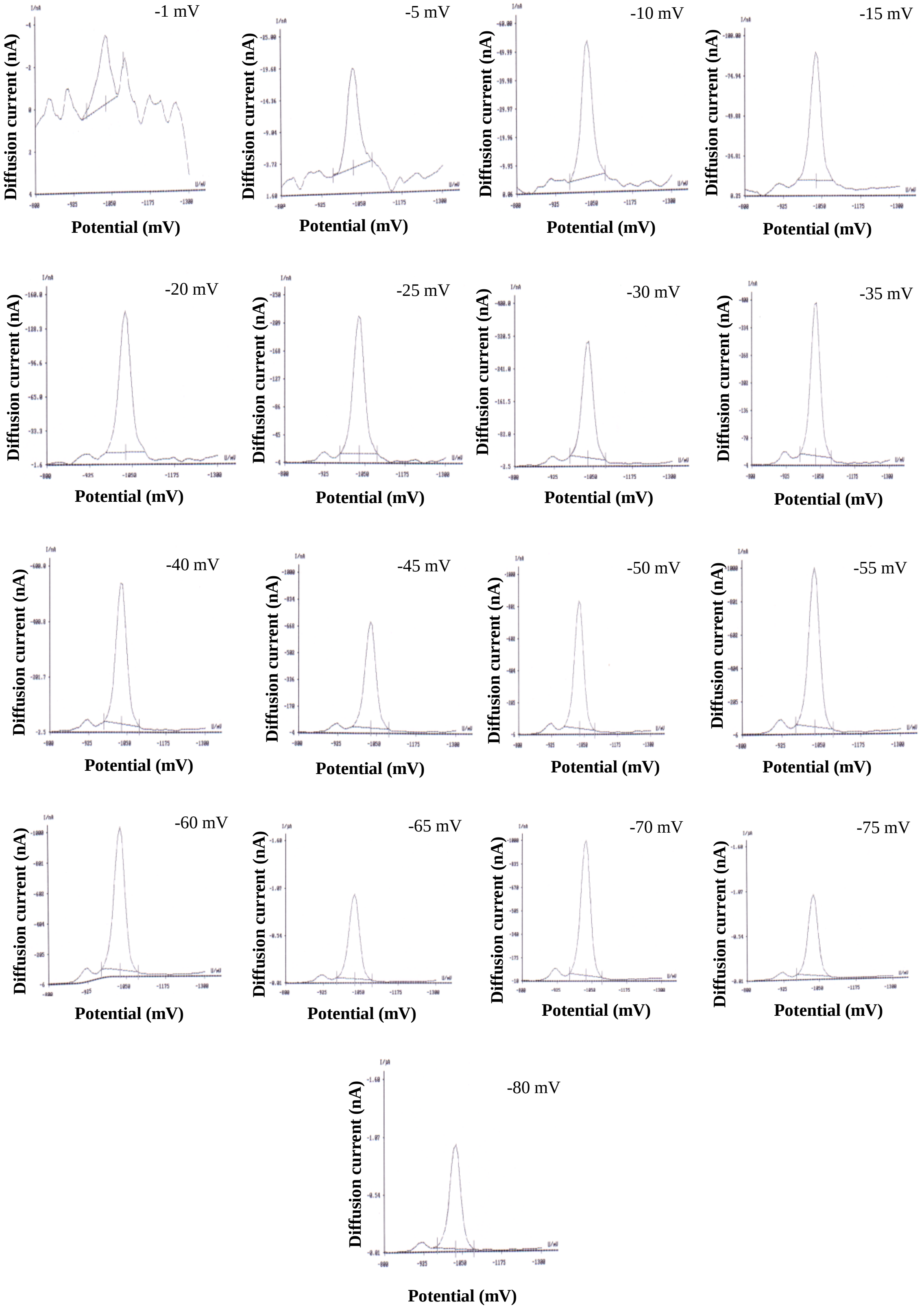


Figure A7 The voltammogram of Co(II)-dimethylglyoxime complex at pulse amplitude in the range of -1 to -80 mV

Voltammogram of dimethylglyoxime concentration

The voltammogram of Co(II)-dimethylglyoxime complex at the concentration of dimethylglyoxime in the range of 1 to 250 mg/L using 50.0 µg/L Co(II) and 0.8 M ammonium chloride (NH_4Cl) supporting electrolyte under the optimum conditions

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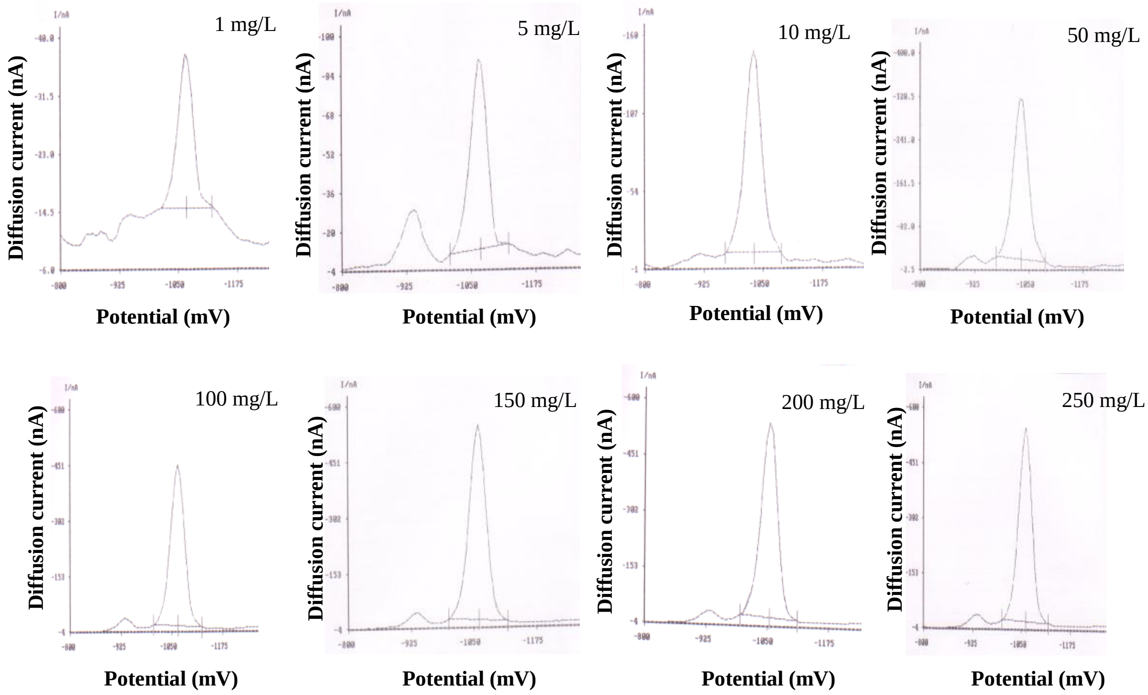

Figure A8 The voltammogram of Co(II)-dimethylglyoxime complex at the concentration of dimethylglyoxime in the range of 1 to 250 mg/L

Voltammogram of linear calibration graph

The voltammogram of Co(II)-dimethylglyoxime complex at the linear calibration graph in the range of 1.0 to 100.00 µg/L using 100 mg/L dimethylglyoxime, 0.8 M ammonium chloride (NH_4Cl) supporting electrolyte under the optimum conditions. The voltammogram were shown in Figure A9.

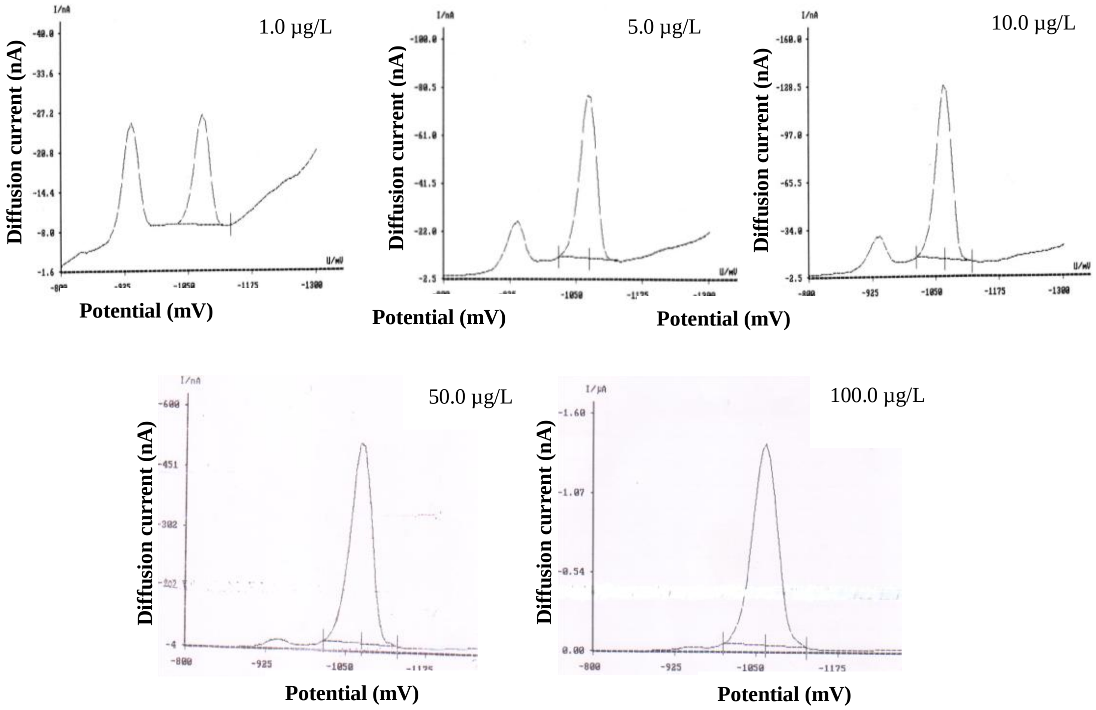

Figure A9 The voltammogram of Co(II)-dimethylglyoxime complex at the linear calibration graph in the range of 1.0 to 100.00 µg/L using 100 mg/L dimethylglyoxime, 0.8 M ammonium chloride (NH_4Cl) supporting electrolyte under the optimum conditions

A2. Application to sample

Determining Co(II) using the proposed voltammetric method

The proposed voltammetric method was applied for determining Co(II) in beverages sample under the optimum condition. Figure A10 to A19 showed the voltammogram of Co(II)-dimethylglyoxime complex in beverages sample and Table A1 to A10 showed the diffusion current of Co(II)-dimethylglyoxime complex in beverages sample.

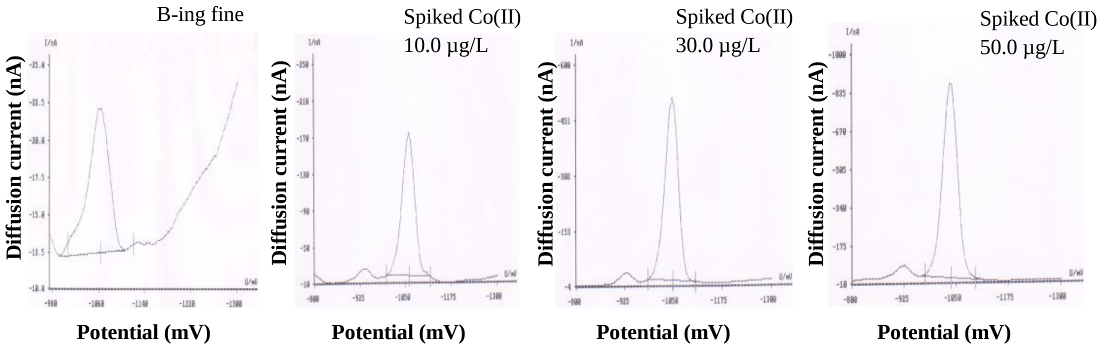

Figure A10 The voltammogram of Co(II)-dimethylglyoxime complex in beverages sample in beverage samples (B-ing fine)

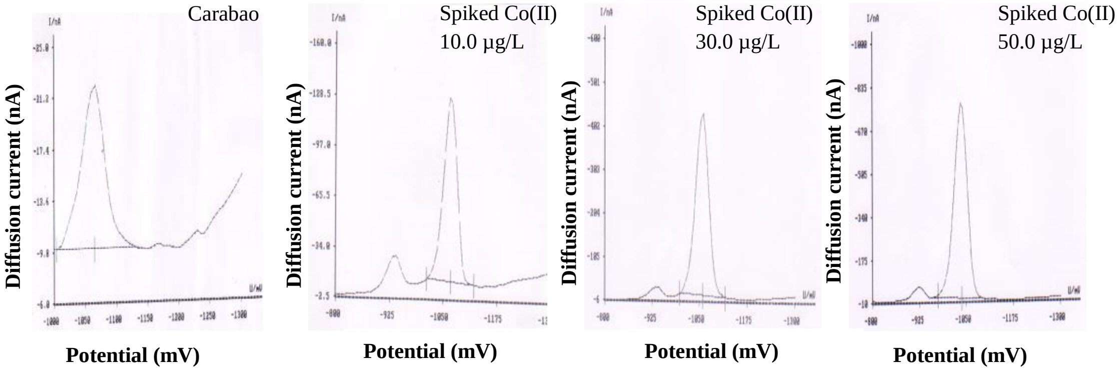

Figure A11 The voltammogram of Co(II)-dimethylglyoxime complex in beverages sample in beverage samples (Carabao)

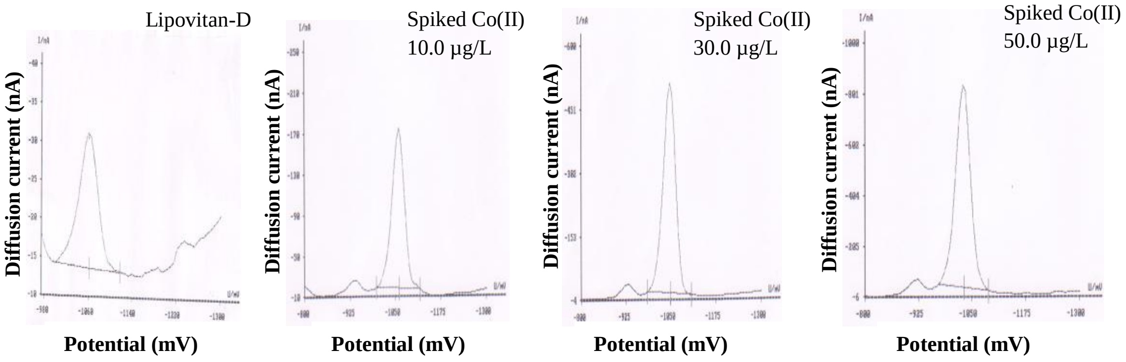

Figure A12 The voltammogram of Co(II)-dimethylglyoxime complex in beverage samples (Lipovitan-D)

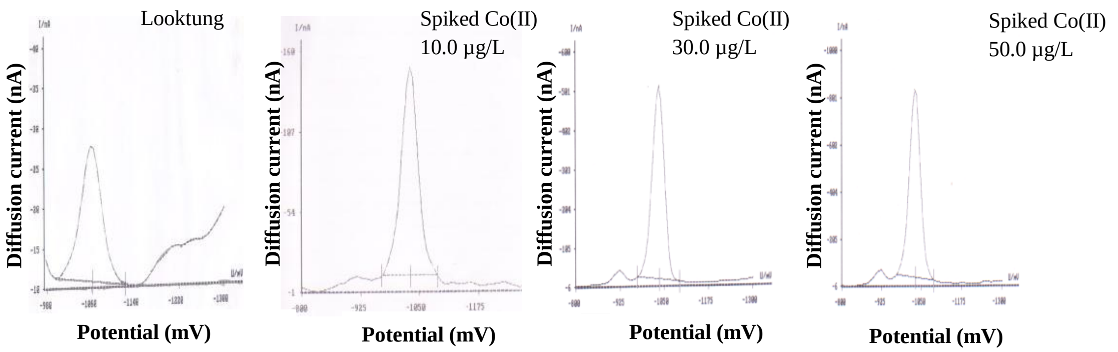

Figure A13 The voltammogram of Co(II)-dimethylglyoxime complex in beverage samples (Looktung)

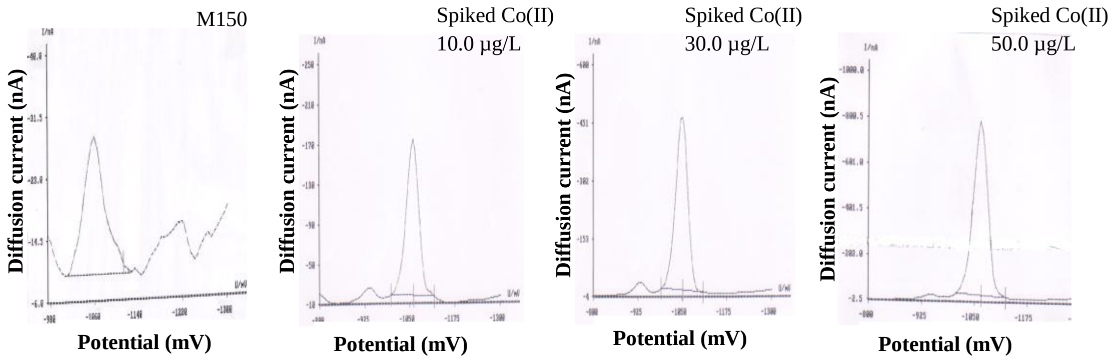

Figure A14 The voltammogram of Co(II)-dimethylglyoxime complex in beverage samples (M150)

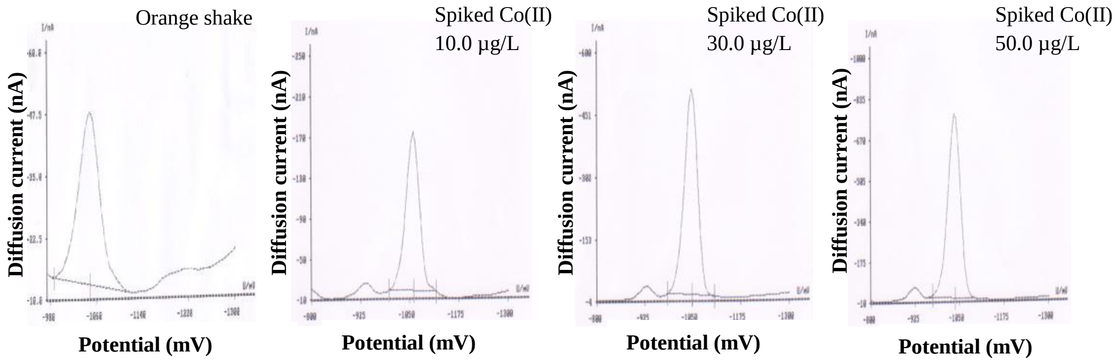

Figure A15 The voltammogram of Co(II)-dimethylglyoxime complex in beverage samples (Orange shake)

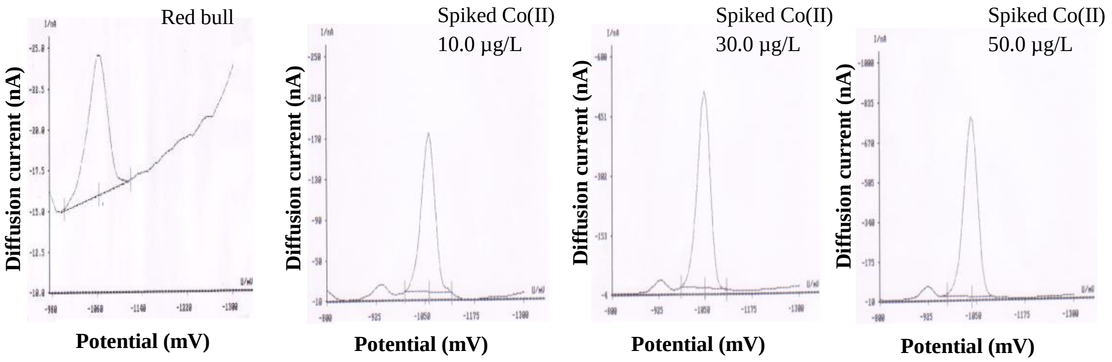

Figure A16 The voltammogram of Co(II)-dimethylglyoxime complex in beverage samples (Red bull)

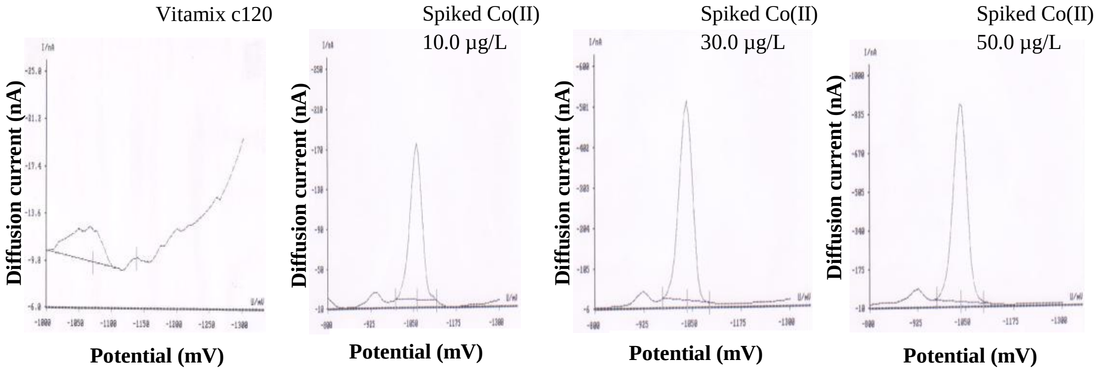

Figure A17 The voltammogram of Co(II)-dimethylglyoxime complex in beverage samples (Vitamix c120)

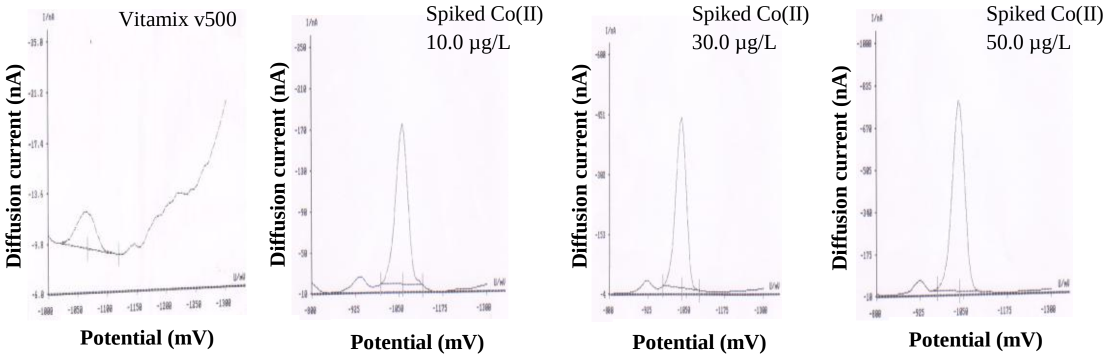

Figure A18 The voltammogram of Co(II)-dimethylglyoxime complex in beverage samples (Vitamix v500)

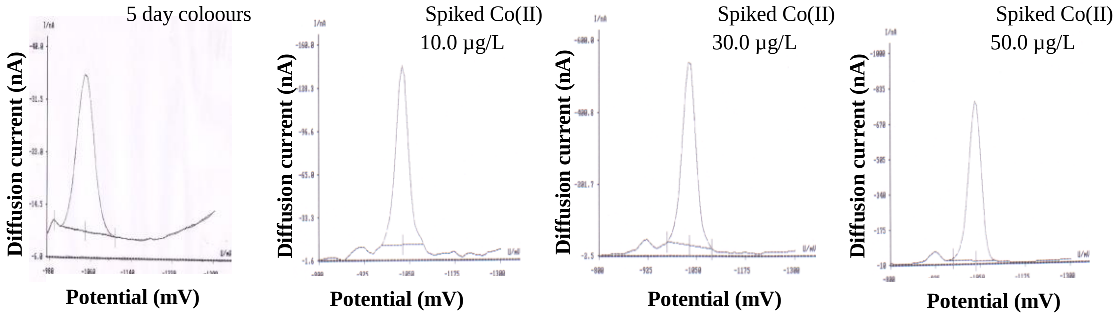

Figure A19 The voltammogram of Co(II)-dimethylglyoxime complex in beverage samples (5 day colours)

Table A1 The diffusion current of Co(II)-dimethylglyoxime complex in beverages sample (B-ing fine) using the proposed voltammetric method

Spiked Co(II) concentration (µg/L)	**Diffusion current (I.peak) (nA)**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0	22.74	24.65	23.78	24.67	22.84	23.74	0.94
10.0	150.97	151.62	151.68	150.66	152.94	151.57	0.89
30.0	462.91	462.57	460.98	461.62	461.58	461.93	0.79
50.0	764.41	763.48	764.28	764.54	762.48	763.84	0.86

Table A2 The diffusion current of Co(II)-dimethylglyoxime complex in beverages sample (Carabao) using the proposed voltammetric method

Spiked Co(II) concentration (µg/L)	**Diffusion current (I.peak) (nA)**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0	25.72	25.21	26.35	26.78	25.12	25.84	0.71
10.0	128.79	129.12	130.45	128.34	129.45	129.23	0.79
30.0	486.08	486.21	487.74	487.07	486.35	486.69	0.70
50.0	717.41	718.85	718.45	719.71	717.52	718.39	0.96

Table A3 The diffusion current of Co(II)-dimethylglyoxime complex in beverages sample (Lipovitan-D) using the proposed voltammetric method

Spiked Co(II) concentration (μg/L)	**Diffusion current (I.peak) (nA)**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0	27.62	28.41	27.54	26.30	27.12	27.35	0.77
10.0	148.54	149.21	149.75	148.64	149.39	149.11	0.51
30.0	490.01	488.11	488.13	489.33	490.91	489.29	0.99
50.0	726.89	727.78	728.45	728.69	727.98	727.96	0.69

Table A4 The diffusion current of Co(II) in beverages sample (Looktung) using the proposed voltammetric method

Spiked Co(II) concentration (μg/L)	**Diffusion current (I.peak) (nA)**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0	23.53	24.82	22..54	23.85	23.63	23.85	0.59
10.0	134.27	133.75	134.35	134.45	135.76	134.56	0.75
30.0	481.57	283.63	282.74	482.68	482.45	482.61	0.74
50.0	722.56	723.78	723.36	722.41	721.85	722.79	0.77

Table A5 The diffusion current of Co(II)-dimethylglyoxime complex in beverages sample (M150) using the proposed voltammetric method

Spiked Co(II) concentration (μg/L)	**Diffusion current (I.peak) (nA)**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0	39.41	38.29	40.02	38.04	39.32	39.11	0.83
10.0	160.21	159.61	158.28	159.17	158.04	159.06	0.91
30.0	445.12	446.81	447.01	446.22	445.45	446.12	0.83
50.0	736.51	736.12	735.35	735.28	736.31	735.91	0.56

Table A6 The diffusion current of Co(II)-dimethylglyoxime complex in beverages sample (Orange shake) using the proposed voltammetric method

Spiked Co(II) concentration (µg/L)	**Diffusion current (I.peak) (nA)**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0	31.65	32.54	33.62	32.61	31.94	32.47	0.76
10.0	159.68	159.63	158.56	159.57	159.94	159.48	0.53
30.0	485.08	485.22	485.13	484.28	484.21	484.78	0.49
50.0	718.82	717.61	717.69	717.43	718.23	717.96	0.57

Table A7 The diffusion current of Co(II)-dimethylglyoxime complex in beverages sample (Red bull) using the proposed voltammetric method

Spiked Co(II) concentration (µg/L)	**Diffusion current (I.peak) (nA)**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0	29.35	28.71	30.16	29.14	29.52	29.38	0.53
10.0	150.32	149.87	151.34	149.63	150.21	150.27	0.66
30.0	484.28	483.12	483.56	484.78	485.62	484.27	0.99
50.0	731.94	730.12	732.35	731.87	731.46	731.55	0.86

Table A8 The diffusion current of Co(II)-dimethylglyoxime complex in beverages sample (vitamix c120) using the proposed voltammetric method

Spiked Co(II) concentration (µg/L)	**Diffusion current (I.peak) (nA)**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0	7.56	6.27	6.07	7.75	6.27	6.78	0.80
10.0	148.21	149.04	147.32	148.43	148.37	148.27	0.62
30.0	490.55	490.27	491.54	491.31	490.96	490.93	0.52
50.0	738.13	738.43	740.32	740.28	740.02	739.44	0.98

Table A9 The diffusion current of Co(II)-dimethylglyoxime complex in beverages sample (Vitamix V500) using the proposed voltammetric method

Spiked Co(II) concentration (μg/L)	**Diffusion current (I.peak) (nA)**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0	5.82	4.70	5.27	5.39	6.23	5.48	0.58
10.0	132.73	134.27	133.57	133.92	134.89	133.88	0.80
30.0	478.32	479.48	479.75	480.37	480.57	479.29	0.96
50.0	691.28	692.18	693.64	692.73	692.03	692.30	0.86

Table A10 The diffusion current of Co(II)-dimethylglyoxime complex in beverages sample (5 day colours) using the proposed voltammetric method

Spiked Co(II) concentration (μg/L)	**Diffusion current (I.peak) (nA)**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0	29.63	29.75	28.74	29.73	29.56	29.48	0.42
10.0	151.78	150.25	152.53	152.26	151.59	151.68	0.88
30.0	487.47	487.16	488.37	486.19	486.48	487.14	0.86
50.0	756.32	756.35	757.43	757.35	757.47	756.98	0.59

Determining Co(II) using the standard FAAS method.

The linear calibration graph for determining Co(II) using the standard FAAS method was investigated using standard Co(II) concentration of 1.0, 5.0, 5.0, 8.0 and 10.0 mg/L under the operating parameters for FAAS were shown in Table 3.2. The results were shown in Table A11. Figure A20 showed the obtained linear regression equation of Y = 0.0231x - 0.0047 with the correlation coefficient of 0.9975.

Table A11 The absorbance of Co(II)-dimethylglyoxime complex for calibration graph using the standard FAAS method

Concentration of Co(II) (mg/L)	**Absorbance**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
1.0	0.015	0.015	0.016	0.016	0.015	0.015	0.0005
3.0	0.065	0.066	0.065	0.067	0.065	0.066	0.0009
5.0	0.112	0.114	0.113	0.112	0.112	0.113	0.0009
8.0	0.185	0.187	0.186	0.185	0.185	0.186	0.0008
10.0	0.221	0.221	0.220	0.222	0.221	0.221	0.0007

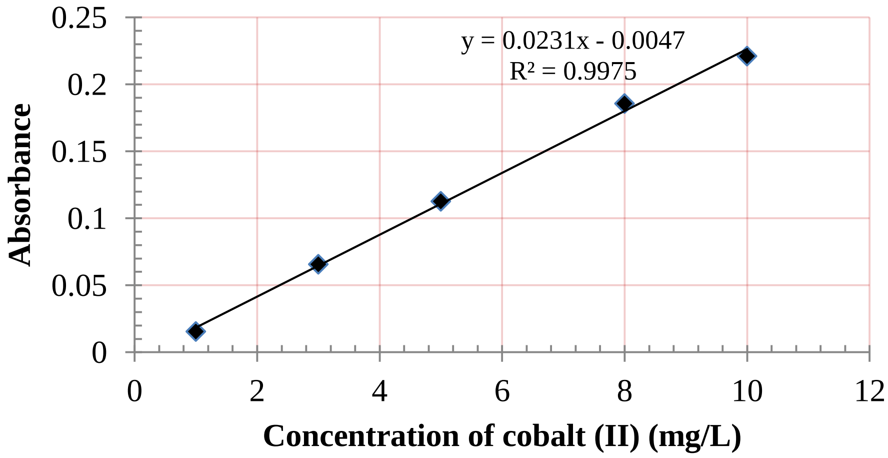

Figure A20 Calibration graph of the standard FAAS method for Co(II) determination

The standard FAAS method was applied for determining Co(II) in beverages sample. Table A12 to A21 showed the obtained absorbance of Co(II) determination in beverages sample

Table A12 The absorbance of Co(II) determination in beverages sample (B-ing fine) using the standard FAAS method

Spiked Co(II) concentration (mg/L)	**Absorbance**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0	0.017	0.017	0.016	0.015	0.016	0.016	0.0008
1	0.037	0.039	0.038	0.037	0.036	0.037	0.0011
3	0.076	0.075	0.074	0.076	0.074	0.075	0.0010
5	0.112	0.113	0.113	0.112	0.112	0.112	0.0005

Table A13 The absorbance of Co(II) determination in beverages sample (Carabao) using the standard FAAS method

Spiked Co(II) concentration (mg/L)	**Absorbance**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0	0.016	0.017	0.018	0.016	0.016	0.017	0.0008
1	0.030	0.029	0.030	0.028	0.029	0.029	0.0009
3	0.069	0.070	0.071	0.070	0.069	0.069	0.0012
5	0.100	0.099	0.098	0.099	0.100	0.100	0.0008

Table A14 The absorbance of Co(II) determination in beverages sample (Lipovitan-D) using the standard FAAS method

Spiked Co(II) concentration (mg/L)	**Absorbance**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0	0.018	0.016	0.015	0.015	0.016	0.016	0.0009
1	0.038	0.037	0.036	0.037	0.036	0.037	0.0008
3	0.074	0.076	0.075	0.076	0.074	0.075	0.0010
5	0.112	0.110	0.113	0.112	0.110	0.111	0.0013

Table A15 The absorbance of Co(II) determination in beverages sample (Looktung) using the standard FAAS method

Spiked Co(II) concentration (mg/L)	**Absorbance**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0	0.015	0.015	0.016	0.017	0.015	0.016	0.0011
1	0.029	0.030	0.031	0.029	0.029	0.030	0.0014
3	0.071	0.070	0.071	0.072	0.073	0.071	0.0009
5	0.103	0.104	0.105	0.104	0.103	0.104	0.0013

Table A16 The absorbance of Co(II) determination in beverages sample (M 150) using the standard FAAS method

Spiked Co(II) concentration (mg/L)	**Absorbance**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0	0.018	0.017	0.018	0.017	0.018	0.018	0.0005
1	0.038	0.036	0.037	0.038	0.036	0.037	0.0010
3	0.077	0.075	0.076	0.077	0.075	0.076	0.0010
5	0.112	0.114	0.111	0.1112	0.114	0.112	0.0014

Table A17 The absorbance of Co(II) determination in beverages sample (Orange shake) using the standard FAAS method

Spiked Co(II) concentration (mg/L)	**Absorbance**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0	0.017	0.018	0.019	0.020	0.020	0.019	0.0013
1	0.039	0.040	0.038	0.038	0.040	0.039	0.0008
3	0.068	0.069	0.070	0.070	0.068	0.069	0.0011
5	0.113	0.112	0.114	0.113	0.112	0.113	0.0011

Table A18 The absorbance of Co(II) determination in beverages sample (Red bull) using the standard FAAS method

Spiked Co(II)	**Absorbance**						**SD**
concentration (mg/L)	**1**	**2**	**3**	**4**	**5**	**Avg.**	
0	0.020	0.019	0.021	0.020	0.020	0.020	0.0007
1	0.041	0.040	0.041	0.042	0.041	0.041	0.0008
3	0.071	0.073	0.072	0.071	0.071	0.072	0.0009
5	0.119	0.117	0.119	0.118	0.118	0.118	0.0012

Table A19 The absorbance of Co(II) determination in beverages sample (vitamix c120) using the standard FAAS method

Spiked Co(II)	**Absorbance**						**SD**
concentration (mg/L)	**1**	**2**	**3**	**4**	**5**	**Avg.**	
0	0.014	0.015	0.015	0.014	0.016	0.015	0.0009
1	0.029	0.029	0.028	0.029	0.028	0.029	0.0008
3	0.057	0.060	0.058	0.059	0.057	0.058	0.0008
5	0.088	0.087	0.086	0.086	0.088	0.087	0.0010

Table A20 The absorbance of Co(II) determination in beverages sample (Vitamix V500) using the standard FAAS method

Spiked Co(II)	**Absorbance**						**SD**
concentration (mg/L)	**1**	**2**	**3**	**4**	**5**	**Avg.**	
0	0.015	0.016	0.015	0.014	0.016	0.015	0.0008
1	0.030	0.029	0.028	0.030	0.028	0.029	0.0010
3	0.059	0.060	0.057	0.059	0.057	0.058	0.0013
5	0.089	0.088	0.086	0.086	0.088	0.087	0.0013

Table A21 The absorbance of Co(II) determination in beverages sample (5 day colours) using the standard FAAS method

Spiked Co(II) concentration (mg/L)	**Absorbance**						**SD**
	1	**2**	**3**	**4**	**5**	**Avg.**	
0	0.018	0.017	0.016	0.017	0.016	0.017	0.0008
1	0.030	0.030	0.031	0.033	0.029	0.031	0.0015
3	0.069	0.068	0.069	0.070	0.070	0.089	0.0008
5	0.099	0.098	0.097	0.100	0.100	0.099	0.0013

APPENDIX B

CALCULATION

APPENDIX B

B1. Calculation of Co(II) content in sample label

Example **(**Looktung sample of 150 mL contains vitamin B_{12} content of 5.0 µg/L)

Vitamin B_{12} of 1355.37 g contains Co(II) content of 58.933 g

Vitamin B_{12} in the label of 5.0 x10 $^{-6}$ g contains Co(II) content of

$$\frac{58.933 \text{ g} \times 5.0 \times 10^{-6} \text{ g}}{1355.37}$$

$$= 0.22 \times 10^{-6} \text{ g}$$

sample solution of 150 mL contains Co(II) content of 0.22×10^{-6} g

sample solution of 40 mL contains Co(II) content of $\frac{0.22 \times 10^{-6} \text{ g} \times 40 \text{ mL}}{150 \text{ mL}}$

$$= 0.0587 \times 10^{-6} \text{ g}$$

sample solution of 40 mL contains Co(II) content of 0.0587×10^{-6} g

sample solution of 1000 mL contains Co(II) content of $\frac{0.0587 \times 10^{-6} \text{ g} \times 1000 \text{ mL}}{40 \text{ mL}}$

$$= 1.50 \text{ µg/L}$$

The amount of Co(II) content in the other samples were calculated as above and the results were shown in table 4.13

B2. Calculation of Co(II) content in beverage sample (Looktung) analyzed by votammetric method

The proposed method was examined by addition certain concentrations of 10.0, 30.0 and 50.0 µg/L of Co(II) standard solutions, respectively into sample under the optimum experimental condition. The Co(II) content in sample was calculated from the linear calibration graph amount of 1.13 µg.

Example

sample solution of 1000 mL contains Co(II) content of 1.13×10^{-6} g

sample solution of 10 mL contains Co(II) content of $\dfrac{1.13 \times 10^{-6}\text{ g} \times 10\text{ mL}}{1000\text{ mL}}$

$= 0.0113 \times 10^{-6}$ g

sample solution of 5 mL contains Co(II) content of 0.0113×10^{-6} g

sample solution of 25 mL contains Co(II) content of $\dfrac{0.0113 \times 10^{-6}\text{ g} \times 25\text{ mL}}{5\text{ mL}}$

$= 0.0565 \times 10^{-6}$ g

sample solution of 40 mL contains Co(II) content of 0.0565×10^{-6} g

sample solution of 150 mL contains Co(II) content of $\dfrac{0.0565 \times 10^{-6}\text{ g} \times 150\text{ mL}}{40\text{ mL}}$

$= 0.211 \times 10^{-6}$ g

sample solution of 150 mL contains Co(II) content of 0.211×10^{-6} g

sample solution of 1000 mL contains Co(II) content of $\dfrac{0.211 \times 10^{-6}\text{ g} \times 1000\text{ mL}}{150\text{ mL}}$

$= 1.41$ µg/L

The amount of Co(II) content in the other samples were calculated as above and the results were shown in table 4.13

B.3 Calculation of Co(II) content in beverage sample (Looktung) analyzed by FAAS method

The FAAS method was examined by addition certain concentrations of 1.0, 3.0 and 5.0 mg/L of Co(II) standard solutions, respectively into sample. The Co(II) content in sample was calculated from the linear calibration graph amount of 40.056 mg.

Example

sample solution of 1000 mL contains Co(II) content of 40.056 mg

sample solution of 25 mL contains Co(II) content of $\frac{40.056\text{ mg} \times 25\text{ ml}}{1000\text{ ml}}$

$= 1.0014$ mg

So, the Co(II) content in Looktung sample was 1.0014 – 1.0 = 0.0014 mg (1.4 µg)

The amount of Co(II) content in the other samples were calculated as above and the results were shown in table 4.13

B4. The percentage relative standard deviation

The percentage relative standard deviation (%RSD) is referred to the precession of the measurements or the instrumental. It can be calculated using the equation 3.4.

$$\%RSD = \frac{SD \times 100}{\overline{X}} \quad (3.4)$$

When

%RSD = percentage relative standard deviation

SD = standard deviation

$\overline{X}$ = mean of data measurements

Example

Table B.1 The percentage relative standard deviation (%RSD)

Experimental number	Diffusion current of Co(II) standard (µg/L)					
	Repeatability			Reproducibility		
	1.0	**50.0**	**100.0**	**1.0**	**50.0**	**100.0**
1	24.10	514.54	1042.00	23.71	514.30	1042.32
2	23.35	514.02	1043.00	22.24	514.02	1044.29
3	23.72	514.13	1042.00	24.86	515.37	1043.83
4	24.89	513.98	1041.00	25.84	516.26	1042.21
5	25.85	513.21	1043.00	23.20	515.41	1043.26
6	24.46	513.71	1042.00	23.78	514.28	1044.56
7	23.91	512.23	1043.00	24.25	516.79	1045.15
8	23.33	514.04	1042.00	25.60	516.62	1042.73
9	24.90	512.97	1043.00	22.04	514.68	1043.17
10	24.72	514.24	1042.00	23.43	515.81	1044.63
11	25.12	513.55	1042.00	23.78	515.28	1042.91
$\bar{X}$	24.396	513.193	1042.273	23.885	515.347	1043.551
SD	0.7909	0.6659	0.6467	1.2118	0.9592	0.9996
%RSD	3.24	0.13	0.06	5.07	0.19	0.09

$$\%RSD = \frac{SD \times 100}{\bar{x}}$$

$$\%RSD = \frac{0.7909 \times 100}{24.396}$$

$$= 3.24$$

B5. The percentage recovery

The accuracy of the proposed method was examined by addition certain concentrations of 10.0, 30.0 and 50.0 µg/L of Co(II) standard solutions, respectively into sample under the optimum experimental condition. The concentrations of cobalt was calculated from the linear calibration graph. Then, the percentage recoveries was calculated from the equation 3.5.

$$\%\text{Recovery} = \frac{C_F - C_S}{C_A} \times 100 \qquad (3.5)$$

Where

C_F = concentration of cobalt (II) found in added sample

C_S = concentration of cobalt (II) in original sample

C_A = concentration of cobalt (II) standard added

Example

Looktung

sample solution of 1000 mL	contains Co(II) content of	$1.13\text{ x }10^{-6}$ g
sample solution of 10 mL	contains Co(II) content of	$\frac{1.13\text{ x }10^{-6}\text{ g} \times 10\text{ mL}}{1000\text{ mL}}$
		= $0.0113\text{ x }10^{-6}$ g
sample solution of 5 mL	contains Co(II) content of	$0.0113\text{ x }10^{-6}$ g
sample solution of 25 mL	contains Co(II) content of	$\frac{0.0113\text{ x }10^{-6}\text{ g} \times 25\text{ mL}}{5\text{ mL}}$
		= 0.06 µg

Looktung + add Co(II) 10 µg/L

sample solution of 1000 mL	contains Co(II) content of	$174.91\text{ x }10^{-6}$ g
sample solution of 10 mL	contains Co(II) content of	$\frac{174.91\text{ x }10^{-6}\text{ g} \times 10\text{ mL}}{1000\text{ mL}}$
		= $1.7491\text{ x }10^{-6}$ g
sample solution of 5 mL	contains Co(II) content of	$1.7491\text{ x }10^{-6}$ g
sample solution of 25 mL	contains Co(II) content of	$\frac{1.7491\text{ x }10^{-6}\text{ g} \times 25\text{ mL}}{5\text{ mL}}$
		= 8.75 µg

$$\%\text{Recovery} = \frac{C_F - C_S}{C_A} \times 100$$

$$\%\text{Recovery} = \frac{8.75 - 0.06}{10} \times 100$$

$$\%\text{ Recovery} = 86.99$$

The amount of Co(II) content in the other samples were calculated as above and the results were shown in table 4.12

B6. Limit of detection

The limit of detection (LOD) and limit of quantitation (LOQ) can be calculated from the linear regression line of the calibration graph using equation 3.6 and 3.7, respectively.

$$\text{LOD} = 3\text{SD} \qquad (3.6)$$

$$\text{LOQ} = 10\text{SD} \qquad (3.7)$$

Example

Table B.3 Limit of detection data solution

No	Diffusion current	Y = 10.21x + 16.01
1	24.69	0.8501
2	23.53	0.7365
3	24.78	0.8589
4	25.41	0.9207
5	23.16	0.7003
6	23.38	0.7218
7	24.95	0.8756
8	24.73	0.8541
9	25.27	0.9069
10	24.75	0.8560
11	23.53	0.7365
$\overline{X}$		0.8198
SD		0.4804

LOD;

LOD = 3SD

= 3×0.4804

LOD = 1.14 µg/L

LOQ;

LOQ = 10SD

= 10×0.4804

LOQ = 4.80 µg/L

B7. Student’s *t*-test

The obtained content of Co(II) in beverages samples analyzed by the proposed voltammetric method and the standard FAAS method was compared using Student’s *t*-test

Example

The statistical data of the obtained content of Co(II) in sample (Looktung) was shown in Table B4 and Table B5.

Table B4 Group Statistics

Group Statistics

Method		N	Mean	Std. Deviation	Std. Error Mean
Cobalt	Voltammetry	5	1.4104	.38901	.17397
	AAS	5	1.4500	.65179	.29149

Table B5 Independent Samples Test

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Cobalt	Equal variances assumed	6.799	.031	.112	8	.914	.03800	.33946	-.74479	.82079
	Equal variances not assumed			.112	6.529	.914	.03800	.33946	-.77659	.85259

Considering to the Levene's Test for Equality of Variances colume in Table B5 found that Sig. = 0.031 was less than 0.05, it was indicatedthat the equal variances not assumed. Therefore, Sig. (2-tailed) = 0.914 was more than 0.05 ($p > 0.05$), it was indicated that the obtained content of Co(II) in sample (Looktung) analyzed by the proposed voltammetric method and the standard FAAS method were not significantly difference at confident level of 95%.

BIOGRAPHY

BIOGRAPHY

Name		Miss Piyaporn Mangsangkam
Date of birth		March 14, 1986
Place of birth		Kalasin, Thailand
Institutions attended		
	2008	Bachelor of Science degree in Chemistry, Mahasarakham University
	2014	Master of Science degree in Chemistry, MahasarakhamUniversity
Position and Work Place		-
Contact address		150 Moo 6 Tumbol Ummao, Aumphur Yangtalad, Kalasin 45160, Thailand

Research grants & awards

Center of Excellence for Innovation in Chemistry (PERCH-CIC)

Poster presentation

[1] Mangsangkam, Piyaporn, Sakee, Uthai and Kruanetr Senee. Determation of vitamin B_{12} by voltammetry. PERCH-CIC CONGRESS VII Theme: Towards a Sustainable Future Conference 2011, May 4-7, 2011, Jomtien Palm Beach Hotel & Resort Pattaya, Chonburi, Thailand. S1-P97

[2] Mangsangkam, Piyaporn, Sakee, Uthai and Kruanetr Senee. Determination of cyanocobalamin by voltammetry. Pure and Applied Chemistry International Conference (PACCON 2012), January 11-13, 2012, The empress convention center, Chiang Mai, Thailand. P. 929

[3] Mangsangkam, Piyaporn, Sakee, Uthai and Kruanetr Senee. Study the voltammetric method for determination of cobalt. The 38th Congress on Science and Technology of Thailand (STT 38), October 17-19, 2012, Empress convention center, Chiang Mai, Thailand. P. 91