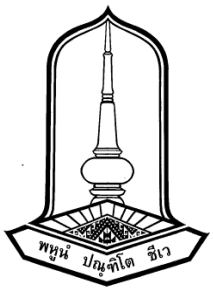

DEVELOPMENT OF FLOW INJECTION AND SEQUENTIAL INJECTION SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF COBALT USING NITROSO-R SALT AS REAGENT

NONGKRAN DUANGSIN

A thesis submitted in partial fulfillment of the requirement for
the Master of Science degree in Chemistry
at Mahasarakham University
March 2014

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The examining committee has unanimously approved this thesis, submitted by Mr. Hai Long Nguyen, as a partial fulfillment of the requirements for the Master of Fine Arts Program in Visual Arts at Mahasarakham University.

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ชื่อเรื่อง การพัฒนาวิธีโฟลอินเจคชันและซีเควนเชียลอินเจคชันสเปกโตรโฟโตเมตริกสำหรับการหาปริมาณโคบอลต์โดยใช้เกลือไนโตรโซอาร์เป็นรีเอเจนต์
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บทคัดย่อ

งานวิจัยนี้นำเสนอวิธีโฟลอินเจคชันอะนาลิซีส (เอฟไอเอ) และซีเควนเชียลอินเจคชันอะนาลิ - ซีส (เอสไอเอ) ที่ตรวจวัดด้วยเครื่องสเปกโทรโฟโตเมตรีสำหรั บหาปริมาณโคบอลต์ (II) ซึ่งอาศัยการเกิดปฏิกิริยาเชิงซ้อนระหว่างโคบอลต์ (II) และเกลือไนโตรโซอาร์ เกิดเป็นผลิตภัณฑ์สีส้มที่พีเอช 6.5 ซึ่งดูดกลืนแสงสูงสุดที่ 500 นาโนเมตร ได้ทำการหาสภาวะที่เหมาะสมสำหรับหาปริมาณโคบอลต์ทั้งสองวิธี สำหรับโฟลอินเจคชันอะนาลิซีส สเป กโทรเมตรี ให้ช่วงความเป็นเส้นตรงในช่วงความเข้มข้น 0.05 ถึง 2.0 มิลลิกรัมต่อลิตร มีสมการถดถอยเชิงเส้นเท่ากับ y = 0.0911x + 0.0076 และค่าสัมประสิทธิ์สหสัมพันธ์เท่ากับ 0.997 ซึ่งวิธีนี้มีขีดจำกัดต่ำสุดในการตรวจวัด (LOD) เท่ากับ 0.0013 มิลลิกรัมต่อลิตร ปริมาณต่ำสุดที่สามารถตรวจวัดได้ (LOQ) เท่ากับ 0.0042 มิลลิกรัมต่อลิตร ค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ของการวัดซ้ำ และการทำซ้ำจากการวัด 11 ครั้ง มีค่าน้อยกว่า 1.40เปอร์เซ็นและ 1.00เปอร์เซ็นตามลำดับ เปอร์เซ็นต์การได้กลับคืนอยู่ในช่วง 89.17 ถึง 109.23 เปอร์เซ็นต์ สำหรับตัวอย่างตัวอย่างวิตามินบีรวม เทคนิคที่พัฒนาขึ้นนี้สามารถประยุ กต์ใช้สำหรับการปริมาณโคบอลต์ในตั วอย่างวิตามินบีรวม ความเข้มข้นที่ตรวจพบอยู่ในช่วง 0.083 ถึง 0.379 มิลลิกรัมต่อลิตร

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คำสำคัญ: โฟลอินเจคชันอะนาลิซีส ซีเควนเชียลอินเจคชันอะนาลิซีส สเปกโทรโฟโตเมตริก

TITLE Development of flow injection and sequential injection spectrophotometric methods for determination of cobalt using nitroso-R salt as reagent

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DEGREE Master of Science degree in Chemistry

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ABSTRACT

A flow injection analysis (FIA) and sequential analysis (SIA) system with spectrophotometric detection were proposed for determination of Co(II). It was based on the reaction between Co(II) and nitroso-R salt forming an orange solution of Co(II)-nitroso-R salt in buffer pH 6.5 with the maximum absorption at 500 nm. The optimum parameters for determining Co(II) in both methods were investigated. Under the optimum conditions, FIA spectrophotometric method, the calibration graph was linear in the range of 0.05-2.0 mg L^{-1} of Co(II) with the regression equation:

$y = 0.0911x + 0.0076$ and a correlation coefficient (r^2) of 0.997. The detection limit (LOD) and the quatitation limit (LOQ) were 0.0013 and 0.0042 mg L^{-1}, respectively. The relative standard deviation (RSD) for repeatability and reproducibility were less than 1.40% and 1.00% (n=11), respectively. The accuracy of the method presented as a percentage recovery was found to be in the range 89.17 to 109.23 for vitamin B complex. The proposed FIA system has been satisfactorily applied to determine Co(II) in vitamin B complex with the amount of Co(II) in the range 0.083 to 0.379 mg.

For SIA spectrophotometric method the calibration graph was linear in the range of 0.05-7.0 mg L^{-1} of Co(II) with a regression eqution: $y = 0.0538x + 0.0158$ and a correlation coefficient of 0.998. The detection limit (LOD) and the quatitation limit (LOQ) were 0.0173 and 0.0575 mg L^{-1}, respectively. The relative standard deviation (RSD) for repeatability and reproducibility were less than 2.70% and 4.30% (n=11), respectively. The accuracy of the method presented as a percentage recovery was found to be in the range 88.10 to 101.40 for vitamin B complex. The proposed SIA system has

been satisfactorily applied to determine Co(II) in vitamin B complex with the amount of Co(II) in the range 0.091 to 0.404 mg.

Keyword: Flow injection analysis, Sequential injection analysis and Spectrophotometry

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LIST OF ABREVIATIONS

AR	analytical reagent
cm	Centimeter
FAAS	flame atomic absorption spectrometry
FIA	flow injection analysis
HPLC	high performance liquid chromatography
HC	Holding coil
i.d.	inner diameter
L	liter
LOD	limit of detection
LOQ	limit of quantitation
mg	Milligram
min	Minute
mL	milliliter
mm	millimeter
mg L^{-1}	milligram per liter
mLh^{-1}	mililiteter per hour
mol L^{-1}	mole per liter
nm	nanometer
RSD	relative standard deviation
SD	standard deviation
SIA	Sequential injection analysis
SV	Selection valve
µFA	micro-flow analysis
µg	microgram
µL	microliter
UV-Vis	ultraviolet visible spectrophotometry
v/v	volume by volume
w/v	weight by volume

CHAPTER 1

INTRODUCTION

Cobalt is known as transition elements that essential to human, animals and plants. It is the active center of coenzymes called cobalamin or vitamin B_{12}. In natural can be found in water, rocks, plant and animals. In addition, the vitamin B complx tablets also contain cobalt. The determination of cobalt in samples is usually made using analytical techniques of high sensitivity. Several techniques have been proposed and evaluated for the simultaneous quantification of cobalt such as spectrophotometry [1-6], atomic absorption spectrometry [7-10], chemiluminescence [11-12], high-performance liquid chromatography [13-15], inductively coupled plasma-optical emission spectrometry [16], and voltammetry [16-21] etc. However, many problems remain in analysis of cobalt, due to high reagent and sample consumption, high waste production and low sampling rate. Flow injection analysis is one of analytical technique that is simple, rapid, high sensitivity, consumption small amount of sample and reagent, and low waste production. Therefore flow injection analysis with spectrophotometric detection may solve these problems. In addition, the sequential injection (SI) technique is also another alternative that has advantages and performance similar to the flow injection (FI) technique. In this work, the determination of cobalt by flow injection analysis compared with sequential injection analysis and used nitroso-R salt as reagent will be performed.

1.1 Objectives of the Research

The Objectives of this research can be summarized as follows:

(1) To design and construct a flow injection and sequential injection with spectrophotometric detection system for determination of cobalt.

(2) To study the optimum conditions for the determination of cobalt

(3) To apply the proposed method for determination of cobalt in multivitamin tablets samples

1.2 Expected result obtained from the research

(1) A simple, rapid and low cost flow injection and sequential injection system were obtained.

(2) Reduction of chemical waste release was possible.

(3) Application of the proposed flow injection and sequential injection system for analysis cobalt were achieved.

1.2 Scope of the Research

(1) Literature review.

(2) Preliminary studies of Co(II)-nitroso-R-salt complex absorption spectrum measuring in the range of 200-800 nm using a spectrophotometer.

(3) Design a manifold for flow injection and sequential injection system.

(4) Investigation the optimum conditions for determination of cobalt by flow injection and sequential injection system such as pH, concentration of nitroso-R salt, flow rate, length of mixing coil, order of reaction and sample volume.

(5) Testing statistics of validation; precision, detection limit and accuracy.

(6) Application of the proposed flow injection and sequential injection system for determination of cobalt in real samples.

1.3 Definition of Terms

(1) Carrier: The solution of sample or reagent stream flow continuously, pushed by the pump, after, pass the injection valve, the reaction coil and transported toward a detector.

(2) Dispersion: Profile form of sample zone develops due to friction with walls, it's occurs inside narrow tubing whose form dependents on the geometry of manifold components sample or reagent volume and the flow velocity.

(3) FI gram: The signal was obtain from FIA system detection, corresponding between the times spent with the response such as absorbance, electrode potential, etc.

(4) Flow injection analysis (FIA): The analytical method base on the injection of a sample solution into a moving continuous carrier stream then, transported toward a detector that continuously records the signal.

(5) Holding coil: A small coil in SIA system, usually made of PTFE tubing as profile function of the chemical reaction of the solutions in completed before pass through detector.

(6) Laminar flow: A form of fluid flow in which a spread of sample zone is parabola owing to higher velocity at the center of tubing.

(7) Manifold: A diagram of continuously flowing solutions of FIA system. A simple manifold consist of pump, vale, mixing coil and detector which is designed according to the chemical reaction.

(8) Peak height: A maximum value of FIA signal, which is correlative to the concentration of the analyse.

(9) Reaction coil: A small coil, usually made of PTFE tubing as profile function of the chemical reaction of the solutions in completed before pass through detector.

(10) Residence time: The time between the sample injection and the signal of peak maximum, during the chemical reaction takes place.

(11) SI gram: The signal was obtain from SIA system detection, corresponding between the times spent with the response such as absorbance, electrode potential, etc.

(12) Sequential injection analysis (SIA): The analytical method base on the injection of solutions into system by selection valve and all solutions transported toward a detector that continuously records the signal.

(13) Turbulent flow: A form of fluid flow in which particle of the fluid move with irregular local velocities and pressures.

CHAPTER 2

LITERATURE REVIEW

2.1 Flow Injection Analysis (FIA) [16-18]

2.1.1 Introduction

Flow injection Analysis (FIA) was first introduced by Ruzicka and Hansen (1975). The principle is based on injection a suitable volume of a sample solution into a stream of carrier, which is continuously pushed through the narrow tube by the pump with a constant flow rate. The reagent stream is continuously pumped down another tube and mixed with the carrier stream containing the sample at the mixing reactor where the sample zone disperses and reacts with the components of the carrier stream. The injected sample zone is transported toward a detector that continuously records the absorbance, electrode potential, or other physical parameter as it continuously changes due to the passage of the sample material though the flow cell and further recorded. Usually change must be proportional to the analyze concentration if controlled experimental conditions as kept equal for both samples and standard.

2.1.2 Principle of FIA

A simple flow injection analyzer (Figure 2.1) consists of a pump, which is used to push the carrier or reagent stream through a narrow tube; injection valve which is used to inject a certain volume of sample solution into a carrier stream in a reproducible manner and a mixing coil where the sample zone disperses and reacts together with the reagent forming a species, then it was transported toward a detector and continuously recorded by computer. A typical recorder output as a peak is shown in Figure 2.2. The peak height (H), width (W), or area (A) are related to the concentration of the analyse. The time span between the sample injection (S) and the peak maximum is the residence time (T) during the chemical reaction takes place.

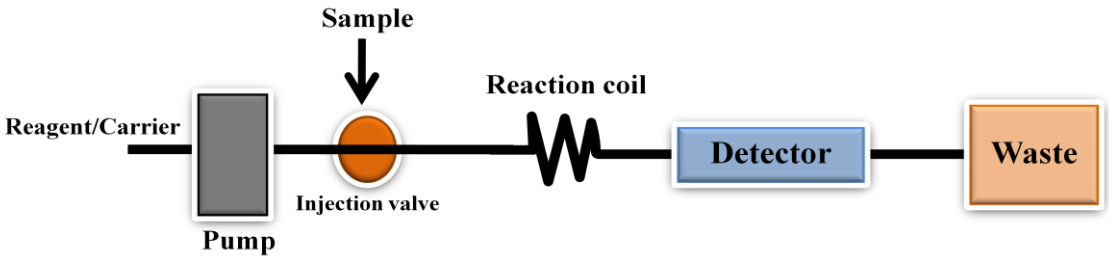


Figure 2.1 Flow injection analysis system [16].

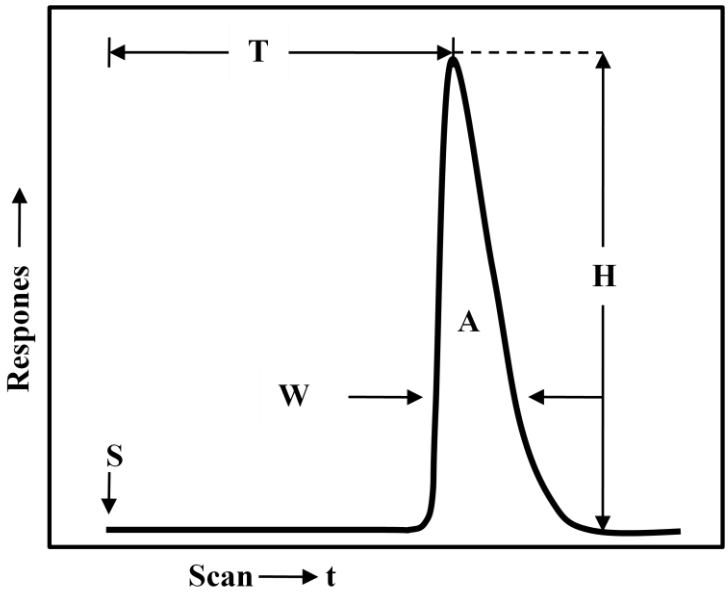


Figure 2.2 The FIA gram; S is injection position, H is the peak height, W is the peak width at a selected level, A is a peak area, T is the residence time [16].

2.1.3 Dispersion

The most common physical phenomenon in manipulation of sample zone in the FIA system is dispersion. The shape of the resulting zone is determined by two main processes: convective transport and diffusion transport. Convective transport occurs from mechanical flow driven by a propelling system. It consists of two processes: turbulent and laminar flows (Figure 2.3a). The turbulent flow occurs in transporting of liquid with air-segmentation. The laminar flow occurs for non-segmented liquids in narrow tubing. In FIA, laminar flow is predominant and causes the sample zone to

spread in a parabolic due to higher velocity at the center of tubing (about 2 times the average velocity)

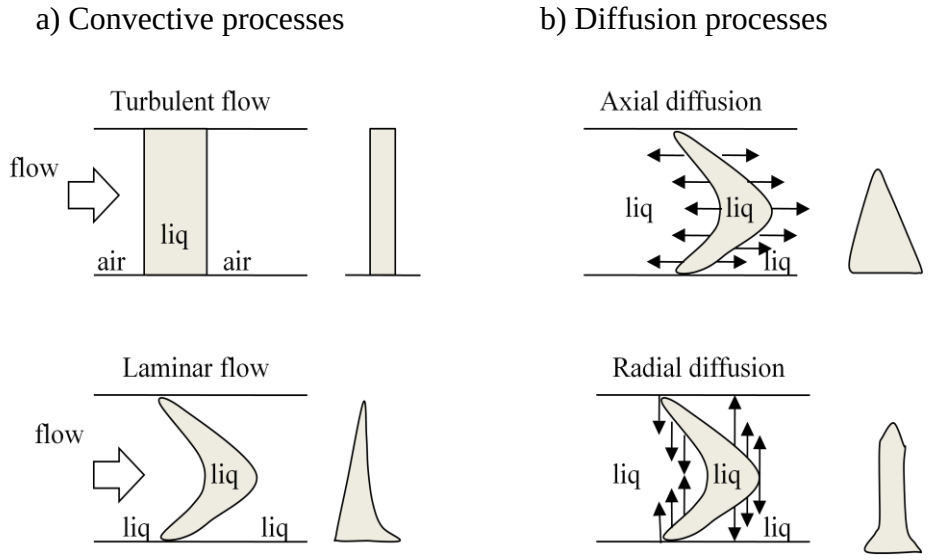


Figure 2.3 General types of transport in closed tubes and the recorded profiles at the detector.

Diffusion transport is caused by concentration gradients. There are two types of diffusion processes: axial and radial, as shown in Figure 2.3b. Axial diffusion is insignificant compared to convective flow, but the radial diffusion contributes more significantly to sample dispersion. This process, termed “secondary flow”, results in a washout effect accounting for the low mutual contamination of samples successively injected into the carrier stream and also serves to limit band spreading. At low flow rate it may even be the major mechanism for dispersion. In fact, flow injection analyses usually performed under conditions in which dispersion by both convective process and radical diffusion occurs as shown in Figure 2.4c.

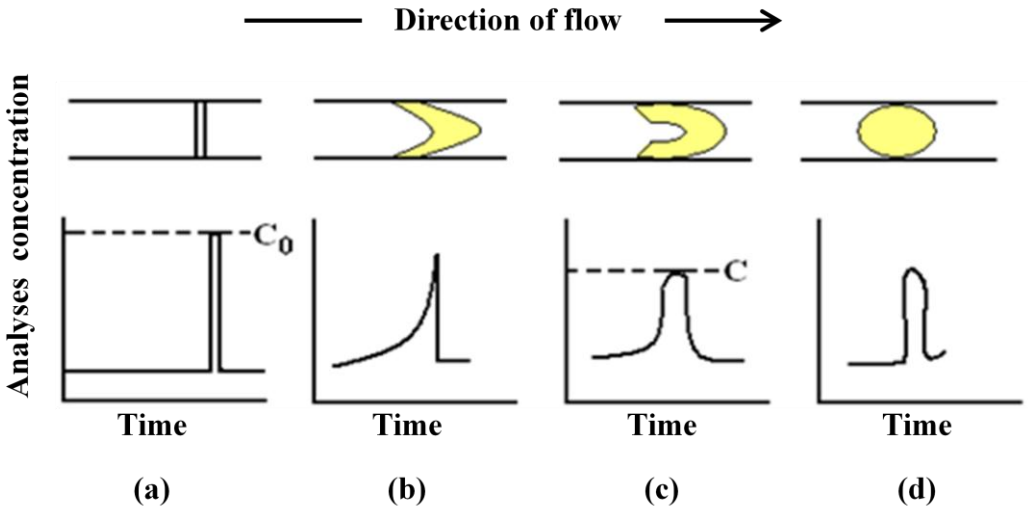


Figure 2.4 Effects of convection and diffusion on concentration profiles of analyses at the detector: (a) no dispersion; (b) dispersion by convective process; (c) dispersion by convective process and radical diffusion; (d) dispersion by diffusion [19].

A simple dispersion experiment is used to pursue dispersion by measure dispersion by means of the dispersion coefficient as shown in Figure 2.4. A sample solution is homogeneous and has the original concentration C^0 that would yield a square signal. The height of square signal would be proportional to the sample concentration (Figure 2.5, left). When the sample zone is injected, it forms a dispersed zone whose form depends on the geometry of the channel and flow velocity. Therefore, the response curve has the shape of a peak reflecting a continuum of concentrations (Figure 2.5, right), which composed of a certain concentration of individual elements of fluid.

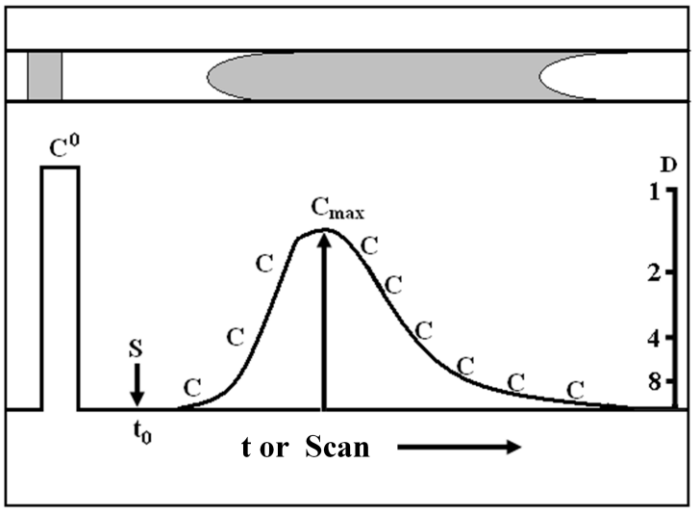


Figure 2.5 Dispersed sample zone in flow system; an original homogeneous samplezone (top left) disperses during its movement through a tubular reactor (top center), thus changing from an original square profile (bottom left) of original concentration C^0 to a continuous concentration gradient with maximum concentration C_{max} at the apex of the peak [18].

The dispersion coefficient (D) is defined as the ratio of the analyte concentration before and after the dispersion takes place:

$$D = C^0 / C_{max} \qquad (2.1)$$

Where C^0 is the original concentration of injected sample solution and C_{max} is the concentration of dispersed sample solution.

Dispersion may be considered in terms of the three general categories:

(1) Low dispersion systems ($D < 2$) are used whenever one intends to prevent the original concentration of the analyte in the injected fluid zone being diluted by the carrier.

(2) Medium dispersion systems ($2 < D < 10$) are also used in single channel FI systems, where reagents are used as carrier streams, to attain adequate mixing of sample and reagent.

(3) Large dispersion (D > 10) and medium systems are used to achieve sample dilutions, usually to bring the analyte concentration into an appropriate range for readout.

The FI experimental parameters which may influence the d3ispersion including sample volume, carrier flow rate, flow rate ratio between sample carrier and merging reagent and geometrical dimensions and configurations of manifold components. Varying the values of these parameters confers a significant degree of control over the dispersion characteristics and facilitates optimization of a flow injection system for many diverse applications.

2.1.4 FIA Instrumentation

The basic components of a simple FI manifold typically consist of a propulsion system, a sample introduction system, a reaction system and a detection system.

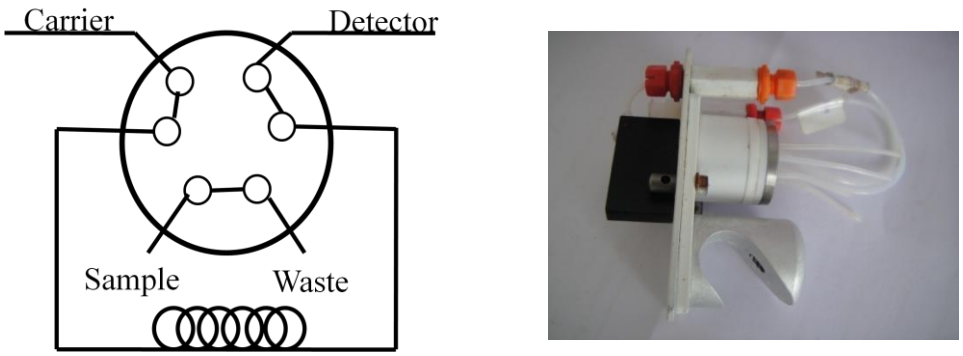
2.1.4.1 Propulsion system

The propulsion system is a propel device of the solution in the flow analysis systems. The several pump types are used in the FIA system for example peristaltic pump, syringe pump and pressurized bottle. A peristaltic pump is used most often in FIA system and other continuous flow analysis system, because it a highly versatile and compose several channels according to diameters of tube, which is might be obtain equal or different pumping rates. The peristaltic pump consists of a motor-driven wheel with external position rollers and a cam which is pushed against the rollers (Figure 2.6).

Figure 2.6 The peristaltic pump

2.1.4.2 Sample introduction system

The sample introduction system is the injector device to employ in FIA system, which is essential for the sample volume is injected quickly into the carrier stream. In addition, the injections must not disturb the flow of the carrier stream. The injection system employed in flow analysis is a needle syringe but it was a low of used in FIA system. Nowadays, the other injection systems have been utilized for example the rotary valve, proportional injector selection valve and multi-injection system (Figure 2.7).



(a) Injection valve (b) Injection system

Figure 2.7 (a) Injection valve and (b) Injection system

2.1.4.3 The transport and reaction system

The transport and reaction system in FIA manifold consist of a narrow tubing (PTFE tubing or Teflon) as used in connectors and reactors (Figure 2.8). The characteristic of connectors include either dual (linear or V-shaped) or triple (T- shaped and Y-shaped) ways, which is to join the tube to one tube another and to other parts of system.

(a) T-shaped and (b) Y-shaped

Figure 2.8 Connectors (a) T-shaped and (b) Y-shaped.

The reactor is placed of a merge solutions and the reaction product occurs was completed before through the detection system. Several types of the reactor such as straight, coil tube, Knitteed coil, Zig zag and Inert packing (Figure 2.9)

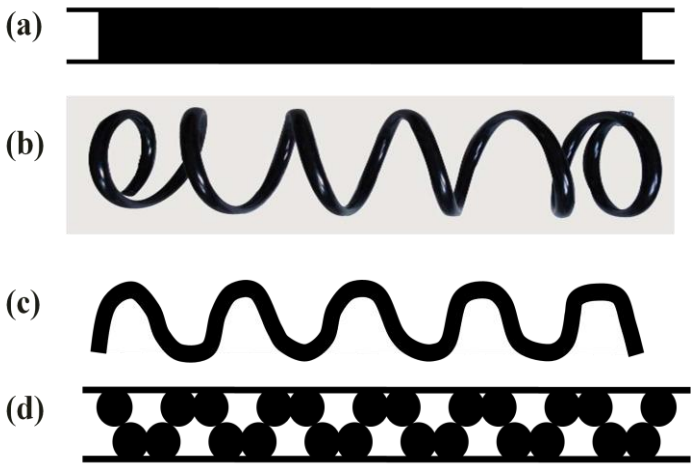


Figure 2.9 Types of the reactor (a) Straight, (b) Coil tube, (c) Zig zag and (d) Inert packing [20].

2.1.4.4 Detection system

The detection system is the sensing place of the FIA system, which is a monitoring of a provided reaction and property of product. A peak heights as a signal output was recorded. Several detector could be adapted suitable for flow through detection in FIA including the UV-VIS spectrophotometer, atomic absorption, inductively coupled plasma spectrometers, fluorimeter, radiometric and various electrochemical detectors. The information of analyze can be applies in qualitative and quantitative analysis.

2.2 Sequential Injection Analysis (SIA) [21-23]

Sequential injection analysis, introduced in 1990, is a simple and convenient concept of flow analysis. The basic components of the system are a peristaltic pump with only one carrier stream, a single selection valve, a single channel and a detector. The versatility of the technique is centred around the selection valve where each port of the valve allows a different operation to be performed. This technique is based on the sequential injection of a wash solution, sample zone and reagent zone(s) to create a stack of well-defined zones adjacent to each other in a holding coil. After the valve has been selected to the detector position, the flow in the carrier stream is reversed and the zones mutually disperse and penetrate each other to form a composite zone as they pass through a reaction coil to the detector.

The heart of the SI system is a multi-position valve. A well-defined volume of the sample is aspirated through a port of the valve in a suitable coil (Holding coil) that is positioned between the valve and the propulsion system. A fixed volume of the reagent is sequentially aspirated in the holding coil forming a zone that is in contact with the previously aspirated sample. Upon selection of the detector port, the stacked zones are propelled through a reaction coil and the product is formed on the overlapped regions of the sample and reagent zones. Comparing SIA and FIA for this simple sample manipulation, the following points can be made

1) SIA makes use of a simpler, more robust single channel manifold even with multi-component chemical systems. In FIA, additional flow channels are required for each reagent.

2) In SIA, the multi-channel peristaltic pumps commonly used in FIA are replaced by more accurate, robust syringe pumps.

3) With SIA, the sample and reagent consumptions are drastically reduced.

4) The single-channel operation of SIA enables the use of the same manifold to implement a wide range of assays.

5) In SIA, the selection valve provides a means for performing convenient automated calibration

6) In SIA, accurate handling of sample and reagent zones necessitates computer control, so automation becomes essential.

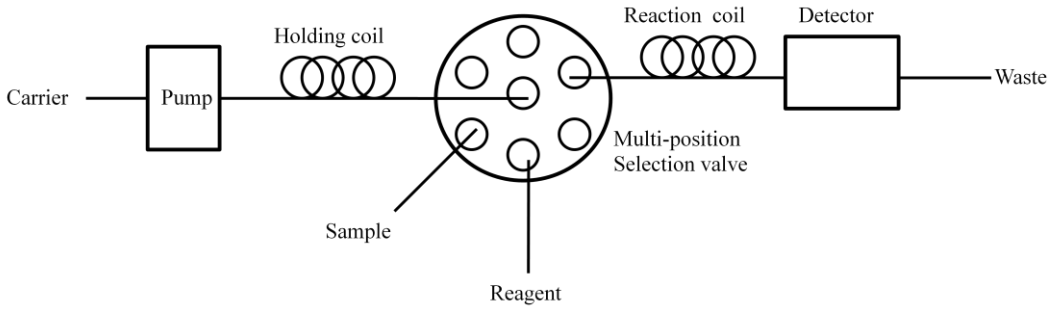


Figure 2.10 Basic SIA manifolds [22]

2.2.1 Programmable Flow of SIA [20].

Sequential injection uses programmable, bi-directional discontinuous flow, precisely choreographed by means of computer control. Sample and reagents are injected sequentially, by means of a multiposition valve, into a carrier stream using a single syringe pump placed upstream of the valve. Shown here are sample and reagent zones, at the interface where a detectable product is formed. Flow reversal (D, E) transports the reaction mixture into the detector (Figure 2.11). Each step can be

described as follows: A = The sample was loaded into the holding coil, B = The reagent was loaded into the holding coil, C = The stack zone was aspirated into the holding coil to improve mixing and dispersion, D = The product was produced and was propelled to the detector and E = The product was monitored by the detector and the signal was recorded.

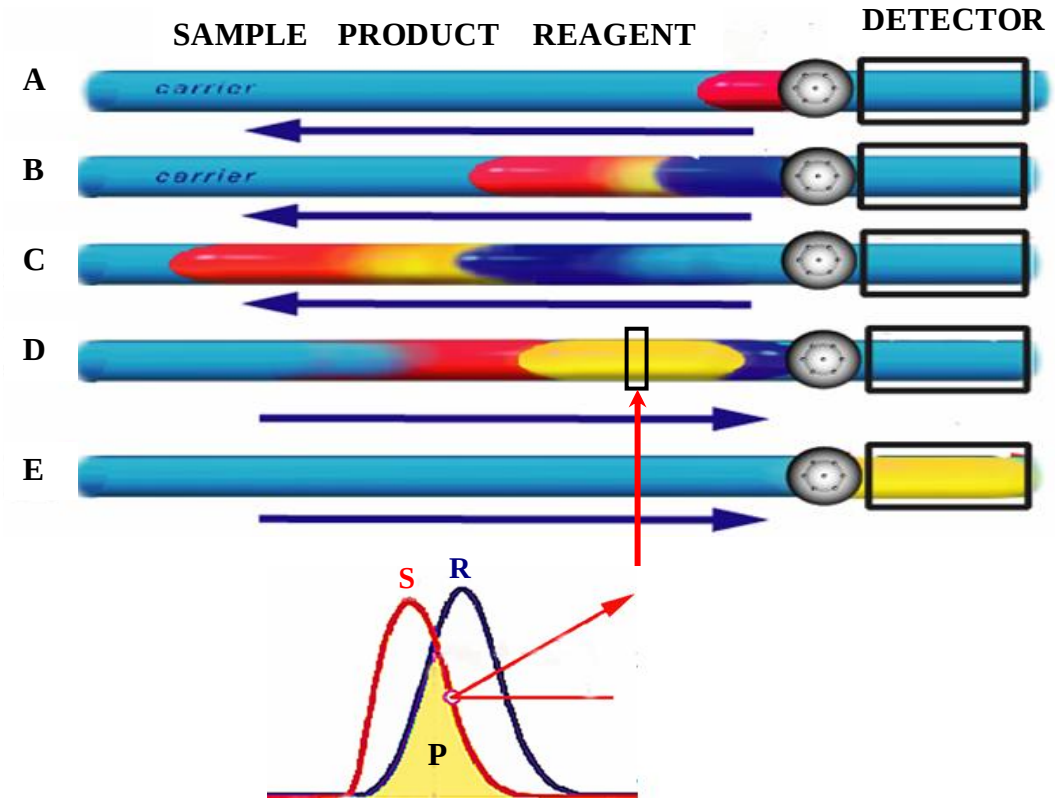


Figure 2.11 Structure of injected zones and concentration profiles as seen by the detector; R-reagent; S-sample; P- composite region where the analyte is transformed into a detector product [20].

2.2.2 Essential Compartments of SIA [23]

The SIA assembly includes the following essential parts:

(a) Pump

Syringe pumps have been most widely used to aspirate zones and propel the stack of zones through the detector. Some researcher have used peristaltic pump. The requirements for the pump are that it is precise, reproducible, bi-directional, and able to measure mall volumes. Computer control is imperative. However, it is relatively expensive requires priming before using and has a limited reservoir volumes.

(b) Selection Valve

The selection valve must allow random access of the ports. Small dead volume and zero cross contamination between ports are essential features of good selection valve. The common port is connected to the pump through the holding coil. Other ports are connected to reagent solutions, samples and the detector flow cell. The 10 port multi-position valve is by far the most widely used.

(d) Detector

The wide ranges of detectors that are employed for FIA are suitable for SIA. Almost detectors are inserted with suitable flow cell.

(e) Software

The important of SIA is the SIA program. This sequence of events results in the assembly of the stack of zones in holding coil and subsequent transport to the detector flow-cell. Microprocessor control is imperative. Several packages have been written to achieve this. Some software are used for SIA such as AnalySIA, Flow TEK^{TM}, Lab VIEW, and FIAlab.

2.2.3 Mixing and Zone overlap of SIA

Since the reaction product (Figure 2.12) is formed at the interface between the sample and reagent zones, it is essential to maximize zone overlap by increasing the amplitude of the forward flow. As the stacked zones are pushed into the holding coil (HC), axial dispersion is promoted, since the center of the stream travels at twice the mean flow velocity.

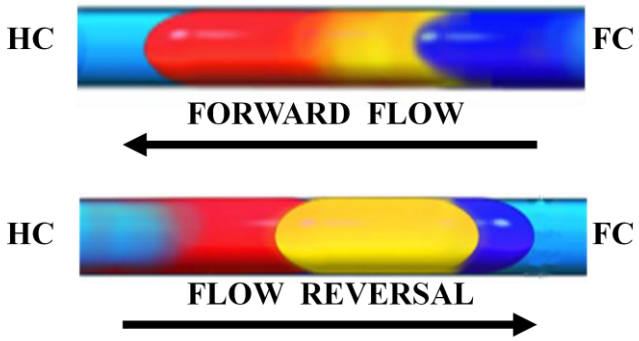


Figure 2.12 Forward and reversal flow of SIA system [20].

The resulting parabolic profile telescopes the trailing zone toward the leading edge of the sample zone, and the radial dispersion promotes mixing of adjacent parallel layers of sample and reagent. Upon flow reversal, the flow velocity profile is suddenly inversed. First, radial mixing is caused by local turbulence, and then axial dispersion and zone overlap are increased when the stacked zones travel downstream toward the flow cell (FC). Combined volumes of sample and reagents define the amplitude of flow reversal. When a spacer zone of carrier solution is injected, zone overlap and mixing are further promoted.

2.3 Cobalt [24]

Cobalt is a chemical element chemical element with symbol Co. A chemical element of cobalt having an atomic number 27 is one of the transition elements of the periodic table, the density of its at 25 ˚C is 8.90 g·cm^{-3}, melting point and boiling point

of 1768 K and 3200 K respectively. The electrical and thermal conductivity of 62.4 nΩ·m (20 ˚C) and 100 $W \cdot m^{-1} \cdot k^{-1}$ (300 K) respectively. This metal usually has an oxidation state +2 and +3.

2.3.1 Sources of cobalt

Cobalt is widely distributed in the environment and it is essential for good health in humans since it is a component of the vitamin B_{12}. The sources of cobalt in the environment are both natural and anthropogenic. Trace amounts of this metal occur in rocks, dust, soil, sediments, water, plants, animal tissues and fluids, and they are mobilized in volcanic eruptions, forest fires and biogenic emissions. Cobalt can be found in tea, coffee, fruits, vegetables, seafood and tobacco. The man-made sources are the by-products of burning of coal and oil, industrial processes, vehicular exhausts and sewage sludge.

2.3.2 Applications of cobalt

Cobalt is used to make alloys and mostly superalloys. Superalloys are used in situations where metals are placed under extreme stress, often at high temperatures. Addition cobalt used in making magnetic alloys. These alloys are used to make devices that must hold a magnetic field, such as electric motors and generators. Another application of cobalt is used in metallurgy, electroplating, nuclear technology, fertilizers, medicine (as vitamin B_{12}), as a drier for paint and colored pigments.

2.3.3 Toxicology of Cobalt

Cobalt has been used as a treatment for anemia. However, harmful health effects can occur when too much metal is taken into the body, such as pulmonary diseases, skin allergies and effects on the cardiovascular and hematological systems and thyroid gland. TheInternational Agency for Research on Cancer has determined that cobalt is possibly carcinogenic to humans based on animal data.

The general population is exposed to more cobalt from drinking water than from air and the exposure to cobalt from food is normally higher than intake from drinking water. The intake from food has been estimated to be 5.0–40.0 µg Co day^{-1}.

2.4 Disodium-1-nitroso-2-naphthol-3,6-disulphonate (Nitroso-R salt) [25-27]

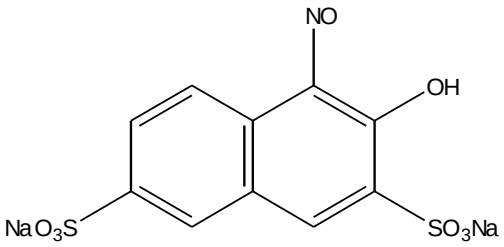


Figure 2.13 Chemical structure of disodium 1-nitroso-2-naphthol-3,6-disulphonate (Nitroso-R salt)

Disodium 1-nitroso-2-naphthol-3,6-disulphonate ($C_{10}H_5NNa_2O_8S_2$) (Figure 2.13) was introduced in 1921 by Klooster for detection of cobalt. It is a derivertive of 1-nitroso-2-naphthol. This reagent is specific for cobalt. The sulphonate groups in the molecule of nitroso-R-salt render this reagent and its cobalt complex soluble in water but insoluble in non-polar solvents. Hence, nitroso-R-salt is used to determination cobalt spectrophotometrically in aqueous medium. In acidic solution (pH ~4), cobalt (II) is oxidized to Co (III)**.**

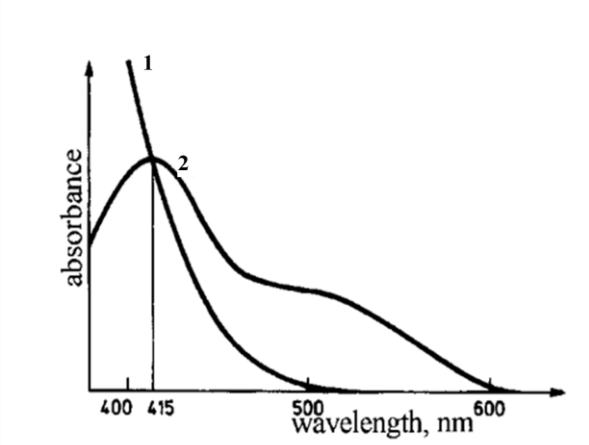


Figure 2.14 Absorption spectra of nitroso-R-salt in water (1) and Co (II)-nitroso-R salt complex (2) [25].

Figure 2.14 shows the absorption spectra of nitroso-R-salt in water and the absorption spectra of Co(II)-nitroso-R salt complex, The molar absorptivity of the red cobalt-nitroso-R salt complex at λ_{max} = 415 nm is 3.5×10^4 (a = 0.60). The absorbance of the Co (III) complex solution may be measured with higher sensitivity at 415-425 nm, or with lower sensitivity at 500-520 nm (a reagent blank, or water as reference), the reaction of cobalt with nitroso-R-salt is usually done in a hot weakly acidic medium buffered with sodium acetate. The solution is then made sufficiently acidic with hydrochloric or nitric acid to decompose the nitroso-R-salt complexs of other metals (e.g., Cu, Ni, Fe and Mn), which are less stable than cobalt (III) complex. Phosphate or fluoride masks iron (III), which has a yellow colour in hydrochloric acid medium.

2.5 The methods for determination of Cobalt

Several analytical techniques have been used for determination cobalt in various samples including spectrophotometric, atomic absorption spectrophotometry, a voltametry, Luminescence, High performance liquid chromatography, Flow injection analysis and Sequencetial injection analysis and a number of ways which is reported in the following.

2.5.1. UV-visible spectrophotometric method

In 1998, Jadhav *et al*. [28] presented a spectrophotometric method for determination of cobalt using benzeneacetaldehyde-4-hydroxy-α-oxo-aldoxime (*p*-hydroxy isonitrosoacetophenone or BAHA) as complexing agent. The reagent reacts with cobalt in the solutions buffered pH 9.0 to form a yellow color complex. The cobalt to BAHA ratio of 1:3 chelate is extracted in chloroform. The maximum absorption of cobalt-BAHA complex was found to be 390 nm, and stable for 3 h. The method applied for the determination of cobalt in synthetic mixtures, pharmaceutical samples for example injectables, liquid formulations and tablets, biological samples is cobalt SRM ray flour sample and alloys is high speed steel samples. In 2001, Jadhav *et al*. [29] developed a method for the determination of cobalt using UV-VIS spectrophotometer. This method is base on the reaction between cobalt (II) forms a yellow coloured complex with HIMH (isonitroso-5-methyl-2-hexanone), which can be extracted into

chloroform. The measurement of the absorbance maximum of the cobalt-HIMH complex was found at 400 nm. The method has been applied for the determination of cobalt in synthetic mixtures, pharmaceutical, biological and high speed steel samples. In 2001, Rezaei *et al*. [30] described a spectrophotometric procedure for the determination of the cobalt (II) and nickel (II) using pyrrolidinedithiocarbamic acid (PDC) to form a complex in the organic phas. The complexe was extracted with *p*-xylene. An artificial neural network (ANN) model was used to analyze the mixture spectra. The limit of detection for Co (II) and Ni (II) were 0.005 and 0.006 $\mu g \cdot mL^{-1}$, respectively. This procedure allows the simultaneous determination of the cited ions in alloy and synthetic samples. In 2004, Ghasemi *et al*. [31] described the spectrophotometric simultaneous for determination of cobalt, copper, and nickel in aqueous solutions with nitroso-R-salt as reagent. The absorption spectra recorded at the maximum wavelength (λ_{max}) of each complex were 503.5 nm for cobalt, 497.5 nm for copper and 490.0 nm for nickel. The linear calibration graph obtained for cobalt, copper and nickel were 0.4-2.6, 0.6-3.4, 0.5-5.5 $mg \cdot mL^{-1}$, respectively. The method was used for determination of cobalt, copper and nickel in synthetic and alloy samples. In 2004, Safavi *et al.* [32] reported a method for determination of nickel and cobalt in natural and waste water samples. Nickel and cobalt were preconcentration with cloud point extraction using 0.25% (w/v) Triton X-114 as surfactant after complex with 0.07 mM 2-amino-cyclopentene-1-dithiocarboxylic acid (ACDA), (pH 5) base on the maximum absorbance was measured 534 and 452 nm for Ni and Co, respectively by spectrophotometer. The linear of calibration graphs were in the range 20-500 and 20-200 $\mu g \cdot L^{-1}$ with detection limits of 10 and 7.5 $\mu g \cdot L^{-1}$ for Ni and Co, respectively. In 2007, Ahmed and Uddin [33] described a method for determination of cobalt using bis(salicyladehyde) orthophenylenediamine (BSOPD) as a reagent by double-beam UV-visible spectrophotometer detector. The experiment was performed in slightly acidic (0.0002-0.001 M H_2SO_4) 50% 1, 4-dioxanic medium to form a red-orange chelate with a molar ratio 1:1. The maximum absorbance of Co-BSOPD complex was obtained at 458 nm and remains constant for over 24 h. The linear calibration graph was obtained in the range of 0.1-15 $mg \cdot L^{-1}$ of Co (II) with a correlation coefficient value of 0.995. The application method was used for determination of cobalt in some environmental waters (potable and polluted), biological (blood and urine), soil samples and solution containing both cobalt (II) and cobalt (III).

The results of the proposed method were comparable with AAS and were found to be in good agreement. In 2009, Gharehbaghi *et al*.[34] described cold-induced aggregation microextraction (CIAME) method was determination of trace amounts of cobalt in several water samples using 1-(2-Pyridylazo)-2- naphthol (PAN) as the complexing agent. Analysis was carried out by a fiber optic-linear array detector spectrophotometer (FO-LADS) at 570 nm. In this method, very small amounts of 1- hexyl-3-methylimidazolium hexafluorophosphate [Hmim][PF_6] and 1-hexyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide [Hmim][Tf_2N] as hydrophobic ionic liquids (ILs) and extractant solvents were dissolved in the sample solution containing Triton X-114 (anti-sticking agent). After dissolving, the solution was cooled in the ice bath and a cloudy solution was formed of IL fine droplets due to the decrease of IL solubility. After centrifuging, the fine droplets of extractant phase were settled to the bottom of the conical-bottom centrifugen tube. Under the optimum conditions, the limit of detection (LOD) of the method was 0.14 ng mL^{-1} and the relative standard deviation (R.S.D.) for 30 ngmL^{-1} cobalt was 2.32%.

2.5.2. Atomic absorption spectrophotometric (AAS) method

In 2000, Zhang *et al.* [35] described a method for the determination of ultra-trace amounts of cobalt in seawater. Cobalt was pre-concentrated by co-precipitation with a combination of 8-quinolinol and nickel (II) as a carrier element and complexed with 1-nitroso-2-naphtol as an auxiliary complexing agent. The co-precipitates obtained were directly measured by graphite furnace atomic absorption spectrometry (GFAAS) using the solid-sampling technique. The co-precipitation conditions for ultra-trace amounts of cobalt with Ni/8-quinolinol/1-nitroso-2-naphtol complex were investigated in detail. They found that ultra-trace amounts of cobalt are co-precipitated quantitatively with Ni/8-quinolinol/1-nitroso-2-naphtol complex in the pH of 5.5 and 8.5. The concentration factor by the co-precipitation reached about 33,000-fold for 1000 ml of the aqueous sample. The proposed method has been applied to the determination of ultra-trace amounts of cobalt in seawater, and the detection limit for cobalt (3σ), is 1 ng·L^{-1} for 1000 mL portions of seawater samples. In 2001, Chen and Teo [36] studied the cloud point extraction for preconcentration of cobalt and nickel using octylphenoxypolyethoxyethanol (Triton X-114) 0.05% w/v as surfactant. The extraction

of cobalt and nickel complexed with 1-(2-thiazolylazo)-2-naphthol (TAN) is analyzed by flame atomic absorption spectrometry. The chemical variables affecting the separation phase and the viscosity affecting the detection process were optimized. Under the optimum conditions, preconcentration of 50 ml of sample in the presence of 0.05% Triton X-114 permitted the detection of down to 0.24 $\mu g \cdot L^{-1}$ of cobalt and 0.44 $\mu g \cdot L^{-1}$ of nickel. The proposed method has been applied to the determination of cobalt and nickel in water samples. In 2005, Ribeiroa *et al*. [37] developed two procedures for the determination of Co in biological samples by graphite furnace atomic absorption spectrometry (GF AAS): Direct solid sampling (SS) and dissolution with tetramethylammonium hydroxide (TMAH), using two different instruments: a conventional line-source (LS) graphite furnace atomic absorption spectrometer and a prototype high-resolution continuum source atomic absorption spectrometer, using the latter instrument for optimization of the furnace temperature program. For the direct introduction of the solid samples, certified reference materials (CRM) were ground to a particle size ≤50 μm. Alkaline treatment was carried out by placing about 250 mg of the sample in polypropylene flasks, adding 2 mL of 25% m/v tetramethylammonium hydroxide and de-ionized water. Due to its unique capacity of providing a 3-D spectral plot, a high-resolution continuum source (HR-CS) graphite furnace atomic absorption spectrometry was used as a tool to evaluate potential spectral interferences, including background absorption for both sample introduction procedures, revealing that a continuous background preceded the atomic signal for pyrolysis temperatures lower than 700 ºC. Molecular absorption bands with pronounced rotational fine structure appeared for atomization temperatures 1800 º C probably as a consequence of the formation of PO. After optimization had been carried out using high resolution continuum source atomic absorption spectrometry, the optimized conditions were adopted also for line-source atomic absorption spectrometry. Six biological certified reference materials were analyzed, with calibration against aqueous standards, resulting in agreement with the certified values (according to the t-test for a 95% confidence level) and in detection limits as low as 5 ng g^{-1}. In 2010, Lemos *et al.* [38] studied method for determination of cobalt and manganese in food seasonings using flame atomic absorption spectrometry (FAAS) after preconcentration with 2-hydroxyacetophenone-functionalized polyurethane foam. In this work, polyurethane

foam (PUF) functionalized with 2-hydroxyacetophenone through a covalent group (–N=C–) was applied as a preconcentration system for the determination of cobalt and manganese. PUF was reacted with 2-hydroxyacetophenone in a dioxane medium. The limits of detection obtained for the solid sample analysis were 0.4 $\mu g \cdot g^{-1}$ (Co) and 0.4 $\mu g \cdot g^{-1}$ (Mn). The method was applied to the determination of cobalt and manganese in several common food seasonings. In 2010, Berton and Wuilloud. [39] employed a liquid-liquid microextraction procedure based on an ionic liquid (IL-DLLME) using electrothermal atomic absorption spectrometry (ETAAS) for the determination of cobalt in environmental and biological samples. The reaction is base on cobalt was complexed with 1- nitroso-2-naphtol (1N2N) reagent at pH 4.0. The IL-DLLME procedure was then performed by using a few microliters of the room temperature ionic liquid (RTIL) 1-hexyl-3-methylimidazolium hexafluorophosphate $[C_6mim][PF_6]$ as extractant while methanol was the dispersant solvent. The resultant limit of detection (LOD) was 3.8 $\mu g \cdot L^{-1}$, the relative standard deviation (RSD) was 3.4% (at 1 μgL^{-1} of cobalt and n = 10), calculated from the peak height of absorbance signals.

2.5.3 Voltammetric method

In 1995, Giroussi *et al.* [40] developed the differential pulse adsorptive stripping voltammetry (DPASV) for determination of cobalt in vegetable animal foodstuffs. The method is based on the use of α-benzil dioxime (α-BD) as a chelating agent. The influence of pH on Co-α-BD complex is 0.1 M ammoni/ammonium chloride solution pH 9.50. The detection limit was 10 $mg \cdot mL^{-1}$. They found that, zinc interferes at a ratio of Zn/Co more than 10^6 with R.S.D less than 6%. In 2005, Korolczuk *et al.* [41] described a method for determination of Co (II) by adsorptive stripping voltammetry. In this system, Co (II)-nioxime reduction current was enhanced by the simultaneous presence of cetyltrimethylammonium bromide (CTAB) and piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES buffers; pH 7.4). The calibration plot for an accumulation time of 60 s is linear from 5×10^{-11} to 3×10^{-9} $mol \cdot L^{-1}$. The relative standard deviation is 3.8% for Co (II) determination at concentration 1×10^{-9} $mol \cdot L^{-1}$. The detection limit is 1.7×10^{-11} $mol \cdot L^{-1}$. The validation of the method is performed by the analyses of certified reference materials and comparing the result of Co(II) determination in river water sample by the adsorptive stripping voltammetry with those obtained by ETAAS. The advantage of this system is a low concentration of the

supporting electrolyte used and so a low blank current from reagents. The method can be applied to micro-trace cobalt determination in real samples without an additional preconcentration step.

2.5.4 Luminescence

In 1998, Mori I. *et al*. [42] presented a fluorophotometric method for determination of cobalt(II) and hydrogen peroxide using the reaction between fluorescein-hydrazide (fl-NH NH_2), and hydrogen peroxide, cobalt(II), respectively. The calibration graphs were liner in the range of 0-6.0 ng cobalt(II) and 0-1000 ng hydrogen peroxide per 10 ml at an emission wavelength (E_m) of 530 nm with an exicitation wavelength (E_x) of 508 nm, respectively. The calculated precisions were for 3.0 ng of cobalt(II) and 340 ng of hydrogen peroxide, the relative standard deviations (RSD) were 3.6 and 32.4% (n=8), respectively. The proposed method was applied to assay of cobalt or hydrogen peroxide in pharmaceuticals such as neuvita ace tab and alinamin tab.

2.5.5 High performance liquid chromatography (HPLC)

In 1997, Niwa *et al.* [43] studied a pre-column derivatizing reagent for the separation of metal ions in the reversed-phase high-performance liquid chromatography. The experimental base on the separation of 2-(4-Methyl-2-quinolylazo)-5-diethylaminophenol (QADP) used. The detection limits defined by S/N = 3 were 0.24 ng for Co, 0.22 for Ni and 0.53 for Cu. This method was applied to the determination of these metal ions in a standard steel sample. In 2008, Cheng *et al.* [44] developed an on-line configuration of microdialysis (MD), Au/TiO_2 nanoparticle preconcentration, and highperformance liquid chromatography-ultraviolet (HPLC-UV) detection method for the simultaneous measurement of cobalt (Co) and nickel (Ni) concentrations in water. The sample matrix was first cleaned with an MD system using a MD probe. A continuously flowing dialysate stream was introduced into tubing coated with Au/TiO_2 nanoparticles to adsorb metals, followed by elution by an acidic eluent. The enriched samples were derivatized on-line using 8-hydroxyquinoline. The separation of Co and Ni were achieved by using a LC-C_{18} column. The UV detection was performed at 319 nm. Detection limit (n = 3) was 0.05 and 0.18 $mg{\cdot}L^{-1}$ of Co and Ni respectively, linear range of Co was 1-20 $mg{\cdot}L^{-1}$ and Ni was 2-20 $mg{\cdot}L^{-1}$. The proposed method offers a

simple and reliable procedure to determine the levels of Co and Ni in environmental water samples.

2.5.6 Flow injection analysis

In 1995, Hernandez *et al*. [45] described a flow injection analysis for determination of Co (II), Cu (II) and Zn (II) using zincon (2-carboxy-2'-hydroxy-5' sulfoformazylbenzene) as reagent by spectrophotometric detection (Figure 2.15). The calibration curve was done by using a PSL method; the concentration ranges used to construct the calibration matrix were 0.397-1.590, 0.268-1.879 and 0.240-1.612 $mg \cdot L^{-1}$ for cobalt, copper and zinc, respectively. This method was applied to analysis of Cu (II) and Zn (II) in blood serum and Co (II), Cu (II) and Zn (II) in pharmaceutical formulations, the results obtained were compared with the standard AAS method.

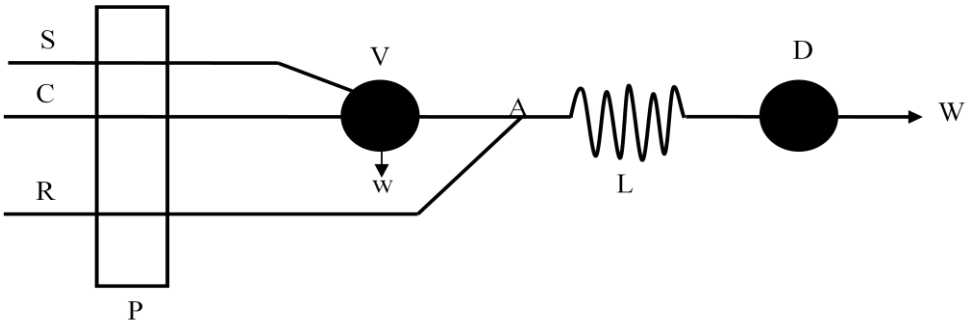


Figure 2.15 Schematic diagram of flow injection manifold. P = Peristaltic pump; S = sample 132 µL; C = carrier (0.5 $mol \cdot L^{-1}$ borate buffer); R = reagent (0.1 $mmol \cdot L^{-1}$ of Zincon); V = valve; A = mixing point; L = reactioncoil; W = waste; D = Detector [45].

In 1998, Nogueira *et al.* [46] described a flow injection system for determination of cobalt (II) (Figure 2.16). The procedure based on the catalytic effect of Co (II) on the oxidation of Tiron (1, 2-dihidroxybenzene-3,5-disulphonic acid disodium salt) by hydrogen peroxide, the wavelength was set at 416 nm. Linearity was observed up to 8.0 $mg \cdot L^{-1}$ Co, the detection limit was 0.2 $mg \cdot L^{-1}$ Co (3σ blank) and precise results (R.S.D. <1%) were detected. The system requires 0.12 mg Tiron per determination and handles 65 samples h^{-1}. The results suggest that HCl extraction and flow injection determination

of cobalt in faeces of bovines. The results have been successful compared with atomic absorption spectrometry.

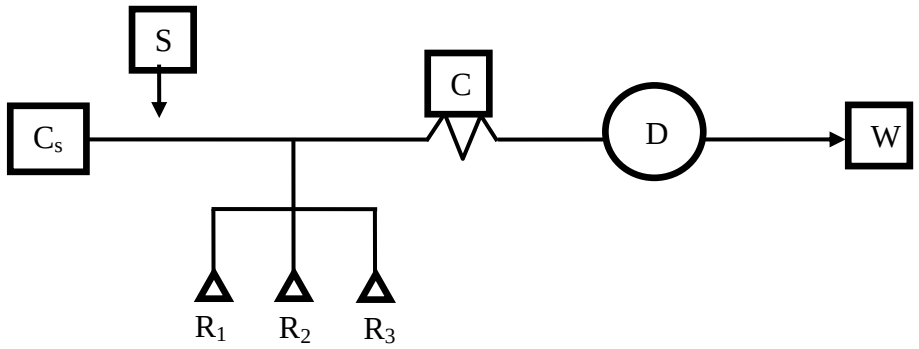


Figure 2.16 Flow injection system for cobalt (II) determination. C_S, carrier; R_1 masking solution; R_2, reagent; R_3,oxidant; S, sample/standard loop; C, reaction coil; D, detector and W, waste [46].

In 1999, Qiu-e *et al.* [47] presented the use of 5-(4-chlorophenylazo)-8-aminoquinoline (APAQCL) as a fluorescence reagent react with copper(II) and cobalt(II) in food samples and the synthetic mixture of Co(II) and Cu(II) by flow analysis couple with a fluorimetric detector . The proposed manifold as shown in Figure 2.17. The method based on the copper (II) reacts with APAQCL forming a fluorescence product at pH 4.5, 0.1 mol·L^{-1} Na_2B_4O solution and 0.1 mol·L^{-1} HCl buffer solution and the fluorescence product of cobalt (II) with APAQCL forms on pH 11.0, 0.1 mol·L^{-1} Na_2B_4O solution and 0.1 mol·L^{-1} NaOH buffer solution and in the presence of H_2O_2. The condition of two systems was 1.0% Tween-80 of a surfactant and 90 ˚C of reaction temperature. They found that the linear calibration graphs were obtained over the concentration range 0.010±1.20 mg·mL^{-1} of cobalt(II) and 0.050±5.00 mg·mL^{-1} of copper(II) with the detection limit of 0.003 (mg·mL^{-1}) of cobalt(II), A sample throughput was 52 h^{-1} and 0.010 mg·mL^{-1} of copper(II), respectively. The method was successfully applied to the determination of copper and cobalt in food samples.

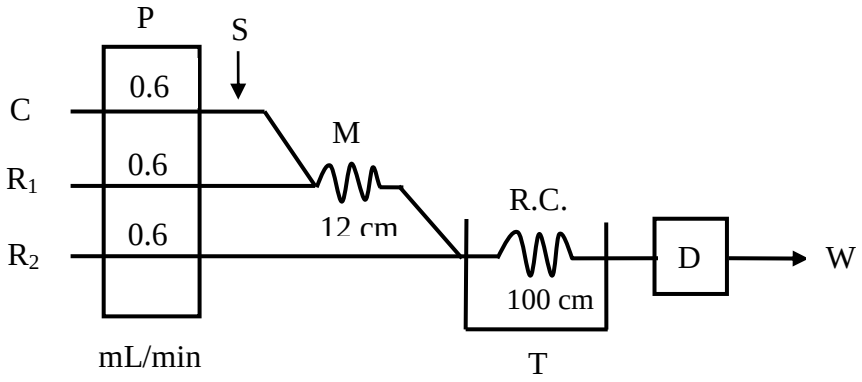


Figure 2.17 Manifold of the flow injection systems for the determination of copper(II) and cobalt(II). C, Carrier stream; R_1, APAQCL and Tween-80 mixing solution; R_2, buffer solution; M, mixing coil; R.C., reaction coil; P, peristaltic pump; T, thermostat; D, detector (spectrofluorimeter) the maximum wavelength of excitation and emission of copper and cobalt were measured at λ_{ex} /λ_{em} = 328 nm/368 nm and 330 nm/370 nm, respectively.; S, sample; W, waste. The optimum FIA conditions for the two systems were the same [47].

In 2001, Andac *et al*. [48] improved a flow injection analysis for the determination of cobalt (II) using 4-benzylpiperidinedithiocarbamate as a chromogenic reagent base on the measurement of the absorbance of cobalt (II)-4-benzylpiperidinedithiocarbamate (1:2) complex at 640 nm by spectrophotometric detection. In the procedure, a microcolumn (MC) containing cation-exchange resin was placed interactions between cobalt (II) and reagent, and for preconcentration of cobalt (II). The linear range of the proposed FI method was 0.6-100 $\mu g \cdot L^{-1}$, the limit of detection (signal/noise =3) was 0.1 $\mu g \cdot L^{-1}$ for a 10µL injection of cobalt (II) solution and a sample throughput of 60 h^{-1}. The relative standard deviation (R.S.D.) for 0.6-100 $\mu g \cdot L^{-1}$ cobalt (II) was <1.0% (n = 5). The precision and accuracy of the method were checked by analysis of certified reference metals, and the method was applied to the determination of cobalt (II) in river, sea and hot spring water samples. In 2001, Cassella *et al*. [49] designed and developed a flow injection system for determine cobalt using a PUF-TAC (2-(2-thiazolylazo)-pcresol) minicolumn to preconcentrate it from water

samples and using a flame atomic absorption spectrometer. The procedure, is based on on-line retention of Co(III) ions (generated in alkaline medium by Co(II) oxidation) in a minicolumn packed with a polyether type polyurethane foam loaded with TAC (2-(2-thiazolylazo)-p-cresol) and elution with 2 mol·L^{-1} HCl directly to the flame atomic absorption spectrometer nebulizer. For 2 min of preconcentration time (10.0 mL of sample volume) the system achieved a detection limit 3.2 mg·L^{-1}, a R.S.D. 5% at 20 mg·L^{-1} and an analytical throughput 24 h^{-1}. Whereas for 3 min of preconcentration time (15.0 ml of sample volume) a detection limit 2.4 mg·L^{-1}, a R.S.D. under 8% at 10 mg·L^{-1} and a sampling frequency 17 h^{-1}. Percentage of recovery were spiked with cobalt at concentrations between 25 and 100 mg·L^{-1} was found in the rang of 94.2% to 107 %. As can be seen, good recoveries were reached for analyzed samples. In 2003, Shabani *et.al.* [50] reported a flow injection analysis-atomic absorption spectrometric procedure for determination of trace quantities of cobalt with base upon on-line system and preconcentration of cobalt on a microcolumn of 2-nitroso-1-naphthol immobilized on surfactant coated alumina, a manifold as shown in Figure 2.18. The method was applied to the determination of cobalt in water samples, vitamin B_{12} and B-complex ampoules. The detection limit (3σ), 0.02 ng·mL^{-1}, precision (RSD), 2.8% for 20 ng·mL^{-1} and 1.7% for 70 ng·mL^{-1} of cobalt. The results compared well with those using a standard furnace atomic absorption spectrophotometric method (FAAS).

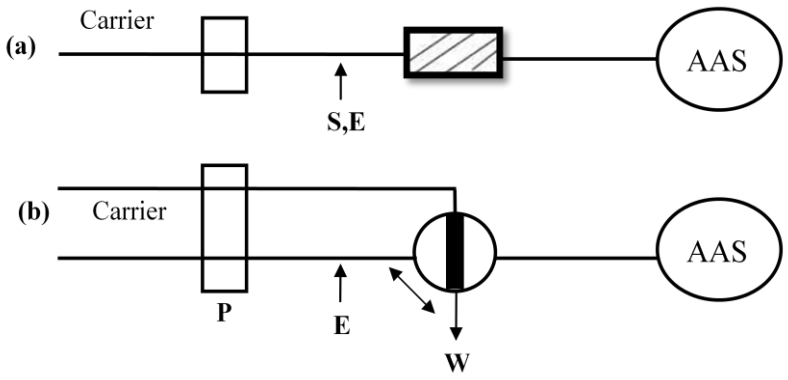


Figure 2.18 Schematic diagram of flow injection manifold. S, sample; E, eluent; W, waste; P, pump [50].

In 2006, Song *et al*. [51] developed a flow injection chemiluminescence system (Figure 2.19) for the determination of cobalt based on its significantly catalyzed effect on luminol-dissolved oxygen which was monitored using photomultiplier detector. The increment of CL signal was proportional to the concentration of cobalt, giving a linear range from 10 $fg \cdot mL^{-1}$ to 50 $pg \cdot mL^{-1}$ (r^2 = 0.9992) with the detection limit of 4 $fg \cdot mL^{-1}$. At a flow rate of 2.0 ml min^{-1} and a potential of -725 volt under identical condition. The method was applied to the determination of cobalt in egg yolk, fish tissue and human serum, agreed well with radioimmunoassay.

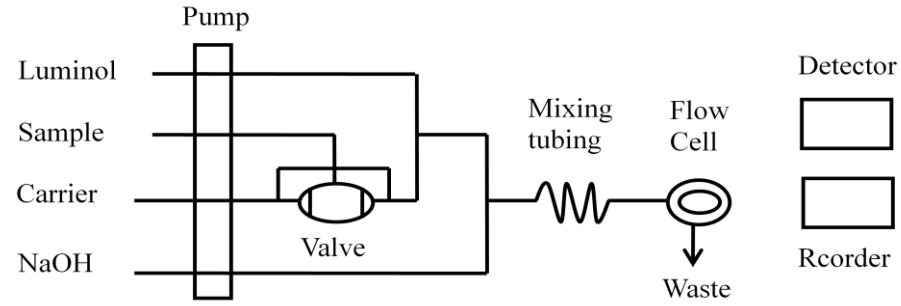


Figure 2.19 Schematic diagram of the flow injection system for determination of cobalt (II). luminol: $1.0x10^{-5}$ $mol \cdot L^{-1}$; sodium hydroxide: 0.03 $mol \cdot L^{-1}$ flow rate: 2.0 $mL \cdot min^{-1}$; high voltage: -725 V [51].

In 2006, Li *et al.* [52] improved a flow-injection chemiluminescence (CL) system for determination of Co^{2+} and Cu^{2+} using partial least squares (PLS) calibration as shown in Figure 2.20. This method is based on both Co^{2+} and Cu^{2+} catalysed the CL reaction of luminal-H_2O_2, and that their kinetic characteristics of Co^{2+} and Cu^{2+} are different in the luminol-H_2O_2 system. The CL intensity was measured and recorder at different reaction times of luminol-H_2O_2-Co^{2+}-Cu^{2+}, and the obtained data were processed by the chemometric approach of partial least squares. The experimental calibration graph of emission intensity versus Co^{2+} concentration was linear in the range 0.0002-0.4 $\mu g \cdot mL^{-1}$ and the detection limit was 0.08 ng·mL (3σ). The calibration graph of emission intensity versus Cu^{2+} concentration was linear in the range 0.02-20 $\mu g \cdot mL^{-1}$ and the detection limit was 6 ng mL^{-1}. The relative standard deviations (n = 11) were 4.4% for 0.02 $\mu g \cdot mL^{-1}$ Co^{2+} and 3.2% for 2 $\mu g \cdot mL^{-1}$ Cu^{2+}, respectively, and was successfully applied to the determination of both analyzes in real water sample.

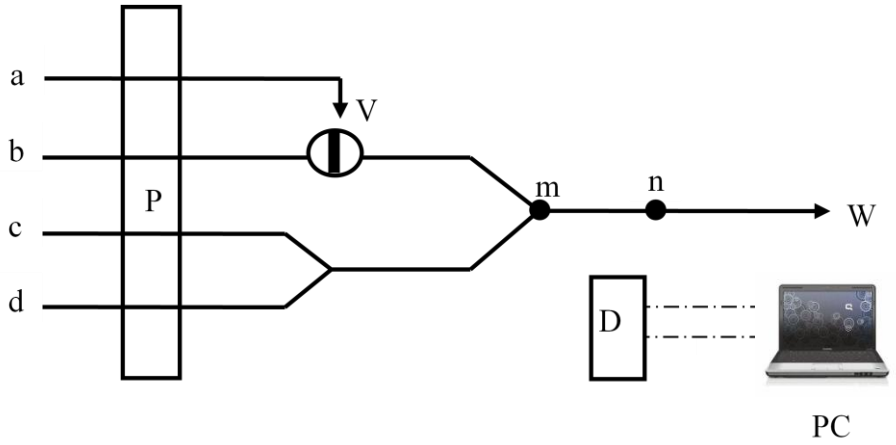


Figure 2.20 Schematic diagram of CL flow system: (a) sample; (b) H_2O; (c) H_2O_2; (d) luminol basic solution; (m) mixed-reagents point; (n) detected-signal point; P: peristaltic pump; V: injection-valve; W: waste; D: detector; PC: personal computer [52].

2.5.7 Sequential injection analysis (SIA)

In 1998, Taljaard and Staden [53] designed the sequential injection technique for kinetic determination of cobalt (II) and Ni (II) in water and soil sample as shown in Figure 2.21. The reactions are complexes of both metal ions with 4-(2-pyridylazo) resorcinol (PAR) at pH 8 was absorbance of measured at 510 nm. EDTA is added to masking agent for removing major interferences. This technique provided a sample throughput of 11 h^{-1} with a relative standard deviation better than 1.2%. The detection limits are 0.14 and 0.20 $mg{\cdot}L^{-1}$ for Ni (II) and Co (II) respectively. In 2001, Kubiak *et al*. [54] developed two Sequential injection system with adsorption stripping voltammetry at mercury drop electrode for determination of copper, lead, cadmium and cobalt. In the first, riboflavin determination by adsorption at mercury and the second used SIA system for determination of heavy metals by complexation with oxime and Dimethylglyoxime. The complex will be adsorbed on the mercury surface and then stripped off during a chathodic scan. The measuring system was applied to determination of riboflavin in vitamin pills. In the second case, metal ions were

determined. They have to be complexed be before deposition on the mercury surface. The detection limit < 0.1 mg mL^{-1}.

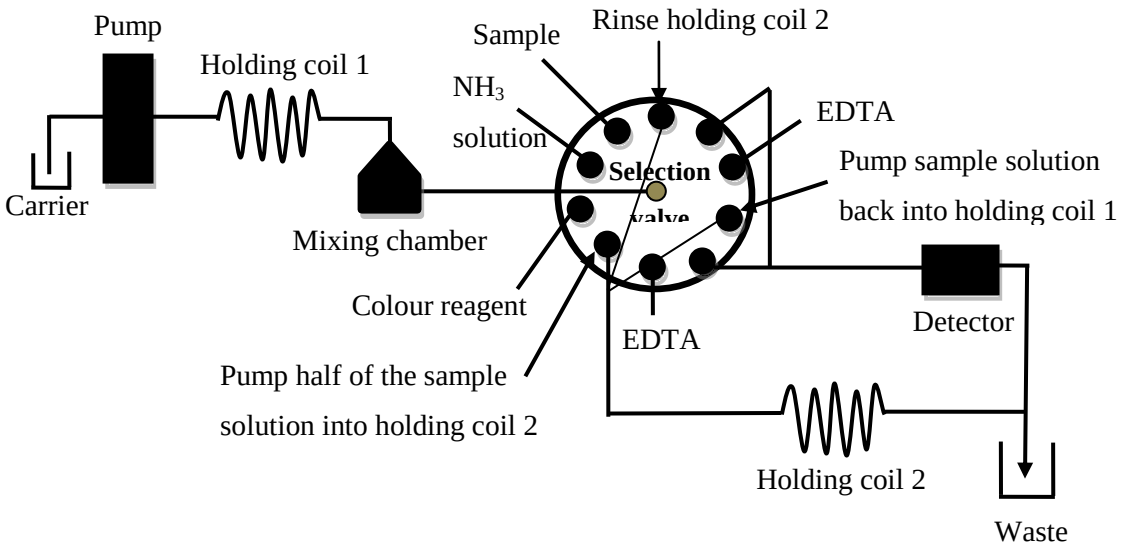


Figure 2.21 SIA system for the simultaneous determination of trace amounts of nickel (II) and cobalt (II) [53].

The literature review have been reported for the determination of cobalt(II) using nitroso-R salt as reagent is following description.

In 2002, Dzherayan *et al.* [54] studied the interaction of nitroso-R salt (NRS) and Co with presence of water-soluble polymers (WP), it able to change the kinetics base on the reaction of cobalt- nitroso-R-salt complex by flow injection analysis using spectrophotometric detection (Figure 2.22). The calibration graph was linear in the range 0.005-2 $\mu g \cdot mL^{-1}$. The effective molar absorptivity coefficient of the complex was equal to $(2.8 \pm 0.08)10^3$. A spectrophotometric determination method for cobalt with nitroso-R-salt in the presence of water-soluble polymers (before and after membrane preconcentration) and a colorimetric flow injection method were developed. For the flow injection-based spectrophotometric determination, the calibration graph was linear in the concentration range of 0-4.0 $\mu g \cdot mL^{-1}$ cobalt with a regression coefficient of 0.9992. The relative standard deviation (R.S.D.) for the determination of 1.0 $\mu g \cdot mL^{-1}$cobalt was 0.9% (ten replicate injections), and at all concentration

measured, the %R.S.D. of the data was below 5.0. The limit of detection was 0.2 $\mu g \cdot mL^{-1}$. The sample throughput rate was about 120 h^{-1}. The FI procedure has been developed to apply in river water samples after membrane preconcentration of cobalt.

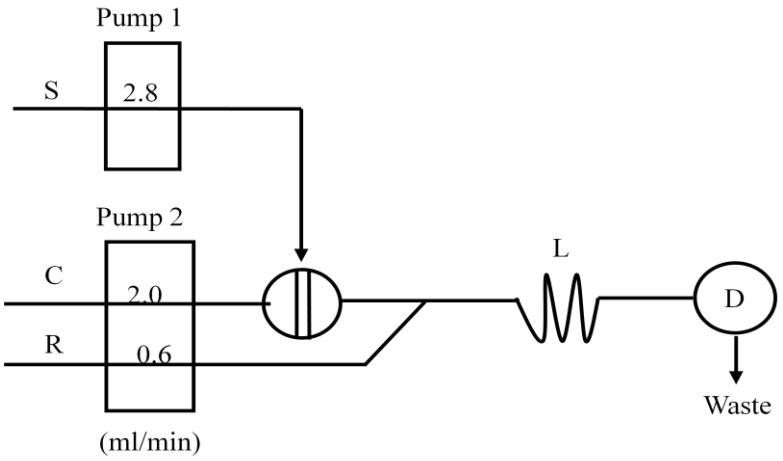


Figure 2.22 Schematic diagram of the flow injection manifold used for determining cobalt (II): C, carrier; R, reagent; S, sample; L, reaction coil; D, detector [54].

In 2003, Ahmed *et al*. [55] developed a convenient colorimetric assay method for determination of vitamin B_{12} content in pharmaceutical. This method is based on the decomposition of vitaminB_{12} by HNO_3 followed by subsequent formation of a stable colored complex (λ_{max} 435 nm) between the liberated cobalt ion and Nitroso-R salt. The proposed method was successfolly employed for determination of vitamin B_{12}. The percentage recovery value in proposed method was 99.7 %. The method suffered on interference by other components and formed colored complex was stable (for 12 h) at room temperature. Besides present method was simple, rapid and accurate over other methods and can be applied successfully for the analysis of vitamin B_{12} in pharmaceutical preparations. In 2004, Ghasemi *et al*. [56] applied to spectrophotometric simultaneous determination of cobalt, copper and nikel using nitroso- R salt in alloys by partial least squares. The experimental calibration set was composed with 36 sample solutions using a mixture design for three component mixtures. The absorption spectra were recorded from 470 to 600 nm. The method was used to determination of cobalt

copper and nickel in two sample alloys.

Table 2.1 The analytical methods for determination of cobalt using various reagents

Method	Reagent	Sample	LOD	Reference
UV-VIS spectrophotometry	Benzeneacetaldehyde-4-hydroxy-α-oxo-aldoxime (*p*-hydroxy isonitrosoacetophenone, (BAHA)	alloys	2.19 ng ml^{-1}	[28]
UV-VIS spectrophotometry	Isonitroso-5-methyl-2-hexanone (HIMH)	Synthetic mixtures, pharmaceutical, biological and steel	5.17 ng ml^{-1}	[29]
UV-VIS spectrophotometry	Pyrrolidinedithiocarbamic acid (PDC)	alloy and synthetic	0.005 µg ml^{-1}	[30]
UV-VIS spectrophotometry	disodium 1-nitroso-2-naphthol-3-6 disulphonate (nitroso-R salt)	Synthetic and alloy	-	[31]
UV-VIS spectrophotometry	2-amino-cyclopentene-1-dithiocarboxylic acid (ACDA)	Waste water	7.5 µg L^{-1}	[32]

Table 2.1 The analytical methods for determination of cobalt using various reagents (Continue)

Method	Reagent	Sample	LOD	Reference
UV-VIS spectrophotometry	Bis(salicyladehyde) orthophenylenediamine (BSOPD)	Environmental waters, biological, soil and solution containing both cobalt(II) and cobalt(III)	15 $ng \cdot mL^{-1}$	[33]
UV-VIS spectrophotometry	1-(2-Pyridylazo)-2-naphthol (PAN)	Water	0.14 $\mu g \cdot L^{-1}$	[34]
Atomic absorption spectrometry (AAS)	1-nitroso-2-naphtol	Sea water	1.0 ng L^{-1}	[35]
Atomic absorption spectrometry (AAS)	1-(2-thiazolylazo)-2-naphthol (TAN)	Water	0.24 $\mu g \cdot L^{-1}$	[36]
Atomic absorption spectrometry (AAS)	2-hydroxyacetophenone-functionalized polyurethane foam	Food seasonings	0.4 $\mu g \cdot L^{-1}$	[38]

Table 2.1 The analytical methods for determination of cobalt using various reagents (Continue)

Method	Reagent	Sample	LOD	Reference
Atomic absorption spectrometry (AAS)	1- nitroso-2-naphtol (1N2N)	Environmental and biological samples	3.8 $\mu g \cdot g^{-1}$	[39]
Voltammetry	α-benzil dioxime (α -BD)	Vegetable animal foodstuffs	10 $mg \cdot mL^{-1}$	[40]
Voltammetry	Nioxime	water	1.7×10^{-11} $mol \cdot L^{-1}$	[41]
Luminescence	Fluorescein-hydrazide (fl-$NHNH_2$)	Pharmaceuticals (Neuvitaace and Alinamin tab)	3.0 $ng \cdot mL^{-1}$	[42]
High performance liquid chromatography (HPLC)	2-(4-Methyl-2-quinolylazo)-5-diethylaminophenol (QADP)	steel	0.24 $ng \cdot mL^{-1}$	[43]
High performance liquid chromatography (HPLC)	Icrodialysis (MD), Au/TiO_2 nanoparticle	Environmental water	0.05 $mg \cdot L^{-1}$	[44]

Table 2.1 The analytical methods for determination of cobalt using various reagents (Continue)

Method	Reagent	Sample	LOD	Reference
Flow injection analysis (FIA)	2-carboxy-2'-hydroxy-5'sulfomazylbenzene zincon (Zincon)	Pharmaceutical formulations	-	[45]
Flow injection analysis (FIA)	1,2-dihidroxybenzene-3,5-disulphonic acid disodium salt (Tiron)	Faces of bovines	0.2 $\mu g \cdot L^{-1}$	[46]
Flow injection analysis (FIA)	5-(4-chlorophenylazo)-8-aminoquinoline (APAQCL)	Food	0.03 $mg \cdot L^{-1}$	[47]
Flow injection analysis (FIA)	4-benzylpiperidinedithiocarbamate	Water	0.1 $\mu g \cdot L^{-1}$	[48]
Flow injection analysis (FIA)	2-nitroso-1-naphthol	Water, vitamin B_{12} and vitamin B complex	0.02 $ng \cdot mL^{-1}$	[50]
Flow injection analysis (FIA)	Luminol-dissolved oxygen	Egg yolk, fish tissue and human serum	4 $fg \cdot mL^{-1}$	[51]

Table 2.1 The analytical methods for determination of cobalt using various reagents (Continue)

Method	Reagent	Sample	LOD	Reference
Sequential injection analysis (SIA)	4-(2-pyridylazo) resorcinol (PAR)	Water and soil	0.20 $mg \cdot L^{-1}$	[53]
Flow injection analysis (FIA)	disodium 1-nitroso-2-naphthol-3-6 disulphonate (nitroso-R salt)	Water-soluble polymer	0.2 $\mu g\ mL^{-1}$	[54]
Colorimetric assay	disodium 1-nitroso-2-naphthol-3-6 disulphonate (nitroso-R salt)	Vitamin B_{12}	-	[55]
Uv-vis Spectrophotometry	disodium 1-nitroso-2-naphthol-3-6 disulphonate (nitroso-R salt)	Alloys	-	[56]

A several methods are used determination of cobalt in real sample but many problems remain in analysis to high reagent and sample consumption, high waste production and low sampling rate. Therefore, flow injection and sequential injection analysis with spectrophotometric detection may solve these problems, both techniques are simple, rapid, consumption small amount of sample and reagent, low waste production and high sample throughput rate per hour. Thus, we are interesting compare flow injection with sequential injection method for determination cobalt using nitroso-R salt as reagent.

CHAPTER 3

MATERIALS AND METHODS

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
Acetic acid	$C_2H_4O_2$	Analytical Reagent	UNIVAR
Cobalt standard	Co(II)	Analytical Reagent	BDH
Di-sodium hydrogen Orthophosphate anhydrous	Na_2HPO_4	Analytical Reagent	UNIVAR
Hydrochloric acid	HCl	Analytical Reagent	Merck
Nitric acid	HNO_3	Analytical Reagent	Merck
Nitroso-R salt			
Potassium dihydrogen orthophosohate	KH_2PO_4	Analytical Reagent	CARLO ERBA
Sodium acetate	$C_2H_3NaO_2$	Analytical Reagent	UNIVAR
Sodium hydroxide	NaOH	Analytical Reagent	UNIVAR
Sulfuric acid	H_2SO_4	Analytical Reagent	UNIVAR

3.2 Instrument and Apparatus

3.2.1 UV-Vis spectrophotometer (Lambda 25, Perkin Elmer, USA)

3.2.2 Flow-through cell for spectrophotometer (Hellma, Germany)

3.2.3 Peristatic pump (Reglo Dig MS-4/8 V 1.12, Ismatec, Switzerland)

3.2.4 Ten port injection valve (VICI, Valco Instruments, USA)

3.2.5 Teflon tubing (Anachem, UK)

3.2.6 Tygon tubing (Cole-parmer, USA)

3.2.7 Atomic absorption spectrophotometer (AA-680, Shimadzu)

3.2.8 pH meter (Metrohm, Switzerland)

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

Parameters	Cobalt
Wavelength (nm)	240.7
HC lamp current (mA)	8
Slit width (nm)	0.2
Fuel gas flow rate (mL min^{-1})	2.0
Type of flame	Air/C_2H_2

3.3 Experimental

All chemicals used are of analytical reagent grade and all solutions were prepared with deionized water.

3.3.1 Preparation of standard solutions and reagents

3.3.1.1 Stock standard solution of Co(II) 100.00 mg L^{-1}

Stock standard solution of Co(II) was prepared by transferring 10.00 mL of 1000.00 mg L^{-1} Co(II) standard solution in 100.00 mL volumetric flask and adjusting volume to 100.00 mL with de-ionized water. Working standard solution of Co(II) was prepared by appropriate dilution of the stock standard Co(II) solution.

3.3.1.2 Stock solution of nitroso-R salt 1.0%w/v

The stock nitroso-R salt solution was prepared by dissolving 1.00 g of standard nitroso-R salt and adjusting volume to 100.0 mL using deionized water. Working nitroso-R salt solution was prepared by appropriate dilution and adjusting volume using buffer solution.

3.3.1.3 Buffer solution

Buffer solutions of pH 3.0-5.0 and pH 6.0-8.0 were prepared by mixing an appropriate ratio of 0.1 mol L^{-1} acetic acid with 0.1 mol L^{-1} sodium acetate and mixing an appropriate ratio of 0.07 mol L^{-1}potassium dihydrogen orthophosphate with 0.07 mol L^{-1} di-sodium hydrogen orthophosphate anhydrous, respectively. The required pH was performed by adjusting with 1.0 mol L^{-1} sodium hydroxide and 1.0 % v/v hydrochloric acid.

3.4 Methodology

3.4.1 The maximum absorption spectra

The absorption spectra of Co(II)-nitroso-R salt complexes was investigated by mixing of 0.01 % w/v of nitroso-R salt and 1.0 mg L^{-1} of Co(II), after that, it is transferred into the sample cell and scanned for the maximum absorption wavelength over the range from 200 to 800 nm using spectrophotometer. The maximum absorption wavelength was selected and used for further study.

3.4.2 Determination of Co(II) by FIA spectrophotometric method

The FIA manifold illustrated in Figure 3.1, a sample solution or Co(II) standard (S) was injected into a stream of nitroso-R salt as reagent solution (R) pumping at the constant flow rate. Subsequently, the mixtured solution were merged together at the reaction coil (L), where the complexation of Co(II)-nitroso-R salt was occurred. The color complex was pumped through the flow through cell placing on the spectrophotometer (D).

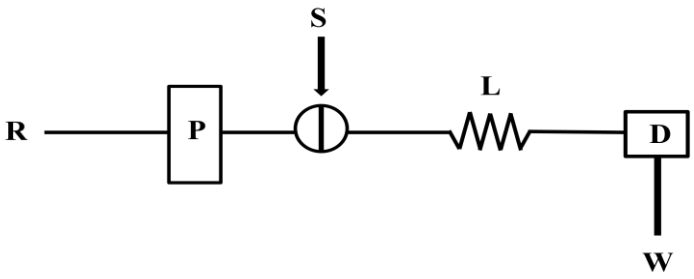


Figure 3.1 The FIA manifold for determination of Co(II); S, sample; L, reaction coil; P, pump; V, injection valve; D, detector; W, waste.

3.4.2.1 Optimization of the experimental conditions

The FIA condition was optimized by a univariate method. The value of one variable is changed while the other variables are fixed. In the experimental conditions, to select the optimum conditions for the highest absorbance with low standard deviation of Co(II)-nitroso-R salt complex. Five replicate measurements were performed for each studied parameter.

3.4.2.2 Effect of flow rate

The effect of flow rate on the absorption signal of Co(II)-nitroso-R salt complex was examined by variation of the flow rate over the range from 0.5 to 3.0 mL min^{-1} under the conditions; 1.0 mg L^{-1} of cobalt(II), 300 µL sample volume and 0.1 % w/v of nitroso-R salt. The flow rate which giving the highest absorbance and lowest standard deviation was selected for the further study.

3.4.2.3 Effect of the reaction coil length

The influence of reaction coil length on the absorption signal of Co(II)-nitroso-R salt complex in the FIA system was studied between 10.00 to 80.00 cm. under the conditions; 1.0 mg $L^{-1}$of Co(II), 300 µL sample volume, 0.1 % w/v of nitroso-R salt and 1.5 mL min^{-1} of flow rate. The reaction coil length providing the highest absorption signal was selected.

3.4.2.4 Effect of sample volume

The effect of sample volume on absorption signal of Co(II)-nitroso-R salt complex was examined by injecting sample volume in the range of 50 to 400 µL, under the conditions; 1.0 mg $L^{-1}$of Co(II), 0.1 %w/v of nitroso-R salt, 1.5 mL min^{-1} of flow rate and 40 cm of reaction coil length. The sample volume providing the highest absorption signal was chosen.

3.4.2.5 Effect of nitroso-R salt concentration

The effect of nitroso-R salt concentration on the absorbance of Co(II)-nitroso-R salt complex was investigated by varying the concentration of nitroso-R salt between 0.005 to 0.50 % w/v, under the conditions; 1.0 mg $L^{-1}$of Co(II), 300 µL sample volume, 1.5 mL min^{-1} of flow rate and 40 cm of reaction coil length. The concentration of nitroso-R salt providing the highest absorption signal was selected.

3.4.2.6 Effect of pH of nitroso-R salt reagent

The influence of pH on the absorption signal of Co(II)-nitroso-R salt complex was studied between pH 3.0 to 8.0 pH of the solutions providing the highest absorption signal was selected.

3.5.2 Determination of Co(II) by SIA spectrohpotometric method

The manifold of SIA spectrophotometric determination of Co(II) setup as shown in figure 3.2. It consists of a buffer solution reservoir (B) connection to the selection valve (S.V) position 1, a standard or sample solution of Co(II) (S) was placed at the selection valve position 2, a solution of nitroso-R salt (R) was placed at the selection valve position 3. A holding coil (H.C) is position between a peristaltic pump and selection valve. The absorbance of Co(II)-nitroso-R salt complex was measured at flow through cell (1.0 cm) placing in the spectrophotometer at 500 nm (D) and the signals was recorded using computer.

3.5.2.1 Procedure for determination of Co(II) by SIA

The SIA manifold design in this proposed method showed in Figure 3.2 which including carrier (C), pump (P), holding coil (HC) selection valve (SV), reagent (R), sample (S), buffer (B), detector (D), recorder (RD) and waste. The analytical procedure was operated by aspiration of the desired volumes of the solution containing of phosphate buffer solution pH 6.5 (B), standard Co(II) or sample (S) and nitroso-R salt reagent (R) into a holding coil to mix together forming a Co(II)-nitroso-R salt complex. Then the absorption of the complex was measured at 500 nm by transportation to a flow through cell placing in the UV-Vis spectrophotometer.

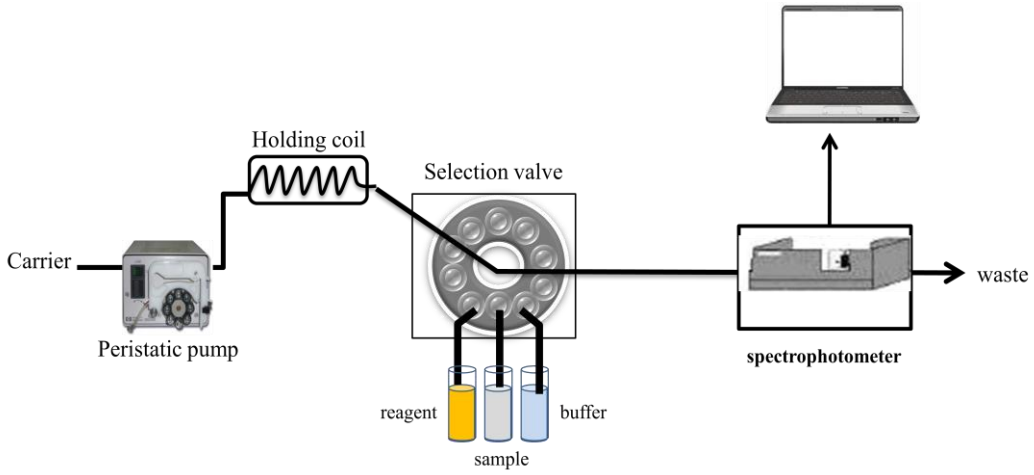


Figure 3.2 The SIA manifold system for determination of cobalt(II): C, carrier (Deionization water); P, pump (Reglo Dig MS-4/8 V 1.12, Ismatec, Switzerland); HC, holding coil (1.02 mm i.d., 200 cm long); SV, selection valve (VICI, Valco Instrument, USA); R, reagent (nitroso-R salt); S, sample (Co(II)); B, buffer (phosphate buffer solution pH 6.5); D, detector (UV-VIS spectrophotometer); RD, recorder (computer); W, waste.

3.5.2.2 Optimization of the experimental conditions

The SIA condition was optimized by a univariate method. The value of one variable are changed while the other variable are fixed. In the experimental conditions, to select the optimum conditions for the highest absorbance with low standard deviation of Co(II)-nitroso-R salt complex and five replicate measurements was performed for each studied parameter.

3.5.2.3 Effect of aspiration sequence of the solution

The effect of aspiration sequence of the solution; 1.0 mg L^{-1} of Co(II) (S), 0.1 % w/v of nitroso-R salt (R) and buffer solution pH 6.5 (B). Co(II)-nitroso-R salt complex was performed by changing the order of the solution in Table 3.3 using selection valve which was controlled by a personal computer. The aspiration order which giving the highest absorbance and the lowest standard deviation was selected for the further study.

Table 3.3 The study of aspiration sequence of the solution; 1.0 mg L^{-1} of Co(II) (S), 0.1 %w/v of nitroso-R salt (R) and buffer solution pH 6.5 (B).

Sequence order	Valve position
S-R-B	2-1-3
S-B-R	2-3-1
R-S-B	1-2-3
R-B-S	1-3-2
B-S-R	3-2-1
B-R-S	3-1-2

3.5.2.4 Effect of flow rate

The effect of flow rate on the absorption signal of Co(II)-nitroso-R salt complex was varied over the range from 0.50 to 4.0 mL min^{-1} under the conditions; 1.0 mg L^{-1} of Co(II) and 0.1 % w/v of nitroso-R salt. The flow rate giving the highest absorbance was selected for further study.

3.5.2.5 Effect of buffer aspiration volumes

The effect of buffer volume on the absorption signal of Co(II)-nitroso-R salt complex was examined by variation of buffer volume in the range of 10 µL to 90 µL, under the conditions; 1.0 mg L^{-1} of Co(II), 2.0 mL min^{-1} of flow rate and 0.1 %w/v of nitroso-R salt. The buffer volume of the solutions providing the highest absorption signal was selected for the further study.

3.5.2.6 Effect of nitroso-R salt aspiration volumes

The effect of nitroso-R salt volume on the absorption signal of Co(II)-nitroso-R salt complex was examined by injecting nitroso-R salt volume in the range of 10 µL to 60 µL, under the conditions; 1.0 mg L^{-1} of Co(II), 2.0 mL min^{-1} of flow rate and 0.1 % w/v of nitroso-R salt. The nitroso-R salt volume of the solutions providing the highest absorption signal was selected for the further study.

3.5.2.7 Effect of sample aspiration volumes

The effect of sample volume on the absorption signal of Co(II)-nitroso-R salt complex was examined by injecting nitroso-R salt volume in the range of 10 µL to 130 µL, under the conditions; 1.0 mg L^{-1} of Co(II), 2.0 mL min^{-1} of flow rate and 0.1 % w/v of nitroso-R salt. The sample volume of the solutions providing the highest absorption signal was selected for the further study.

3.5.2.8 Effect of nitroso-R salt concentration

The effect of concentration of nitroso-R salt on absorption signal of Co(II)-nitroso-R salt complex in the SIA system was studied in the range of 0.01 to 0.5 % w/v, under the conditions; 1.0 mg L^{-1} of Co(II), 2.0 mL min^{-1} of flow rate and 0.1 % w/v of nitroso-R salt. The concentration of nitroso-R salt providing the highest absorption signal was selected.

3.5.3 Analytical of figure merit

3.5.3.1 The linear calibration graph

Under the optimum conditions, the linearity range for determination of Co(II)-nitroso-R salt complex was performed by measurement the absorbance of the complex using the concentration of Co(II) in the range of 0.005 to 12.0 mg L^{-1} under the conditions; 0.1 % w/v of nitroso-R salt and phosphate buffer solution 6.5. The obtained absorbance was plotted as y-axis against the concentration of Co(II) as x-axis. After that, the linearity range had been examined and subsequently plot a calibration graph for determination of Co(II).

3.5.3.2 Precision

The precision of the proposed method was studied as the repeatability and reproducibility. The repeatability and reproducibility were accomplished by measurement 11 replicates and 11 duplicates of three standard Co(II) solutions covering different concentration levels: low, medium and high (0.05, 3.0 and 7.0 mg L^{-1}), respectively. The precision was expressed as the percentage relative standard deviation (%RSD) using equation 3.1 [57].

$$\%RSD = \frac{SD \times 100}{\bar{x}} \qquad (3.1)$$

Where % RSD = percentage relative standard deviation

SD = standard deviation

$\bar{x}$ = mean of data measurements

3.5.3.3 Limit of detection

The limit of detection (LOD) and limit of quantitation (LOQ) were accomplished by measurement 11 replicates of complex forming between 0.1 % w/v of nitroso-R salt and 1.0 mg L^{-1} of Co(II) in phosphate buffer solution pH 6.5. After that the absorbance 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 3.2 and 3.3, respectively [57].

$$LOD = 3\ SD \quad (3.2)$$

$$LOQ = 10\ SD \quad (3.3)$$

Where SD = standard deviation of measurement of a lowest calculation of iron(III) from calibration curve (n=11)

3.5.3.4 Accuracy

The accuracy of the proposed method was examined by addition of three concentrations; 0.05, 1.0 and 2.0 mg L^{-1} of Co(II) standard solutions into the sample solution under the optimum condition. The concentrations of Co(II) are calculated from the linear calibration graph. The accuracy was expressed as the percentage recovery which was calculated from the equation 3.4 [57].

$$\%Recovery = \frac{C_F - C_S}{C_A} \times 100 \quad (3.4)$$

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

3.5.3.5 Interferences

The effect of some possible interferences (Cu(II), Ni(II), Fe(II), Fe(III), Cr(II), Mn(II), Zn(II) and Al(II) which would be formed a complex compound with nitroso-R salt on the determination of Co(II) in samples was investigated by addition of the different concentration of the possible interference species into 1.0 mg L^{-1} standard Co(II) and the signals obtained were compared. Tolerance limits was determined for a maximum error of less than ±5%.

3.5.3.6 Sample preparation

The vitamin B complex was purchased from pharmaceutical store in Mahasarakham provice, Thailand

The samples were prepared using the standard method [58] 0.2000 g of powdered sample (10 tablets) were digested in 15 mL of HNO_3: HCl (2:1) on hot plate and heating until the lowest volumes as possible (about 1 mL). Then, cool down at room temperature, neutralization of pH 7.0 using 1.0 M NaOH and/or 1.0 % v/v HCl and adjusted to a certain volume using deionized water.

3.5.3.7 Comparison with standard method

The result obtained from the proposed SIA and FIA method for determination of Co(II) in vitamin B complex were compared with the standard FAAS method using One-way ANOVA, which was determining by statistical package for social sciences (SPSS, Version 16.0 for windows).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Flow injection spectrophotometric method for determination of Co(II) using nitroso-R salt as a complexing agent

4.1.1 Study of the maximum absorption spectra

The apsorption spectra of nitroso-R salt, Co(II) and Co(II)-nitroso-R salt complex were investigated over the range from 200-800 nm by UV-vis spectrophotometer using 0.1% w/v of nitroso-R salt and 1.0 mg L^{-1} of Co(II) solution. The absorbance of the complex comparison to the nitroso-R salt reagent and Co(II) was shown in Figure 4.1. It was found that, the characteristic of Co(II), nitroso-R salt and Co(II)-nitroso-R salt complex solution were colorless, yellow and orange, respectively. The maximum absorption of nitroso-R salt solution was 380 nm, and Co(II)-nitroso-R salt complex showed the maximum absorption signal at 400 nm. It can be explained that, the absorption band of nitroso-R salt solution found in the range of 350-450 nm was relatively high leading to interfere the absorbance of the complex. While the wavelength at 500 nm, the absorbance of Co(II)-nitroso-R salt complex was higher than nitroso-R salt solution which was not interfered the absorbance of complex. Therefore a wavelength of 500 nm was chosen as the optimum wavelength and used for the subsequent investigations.

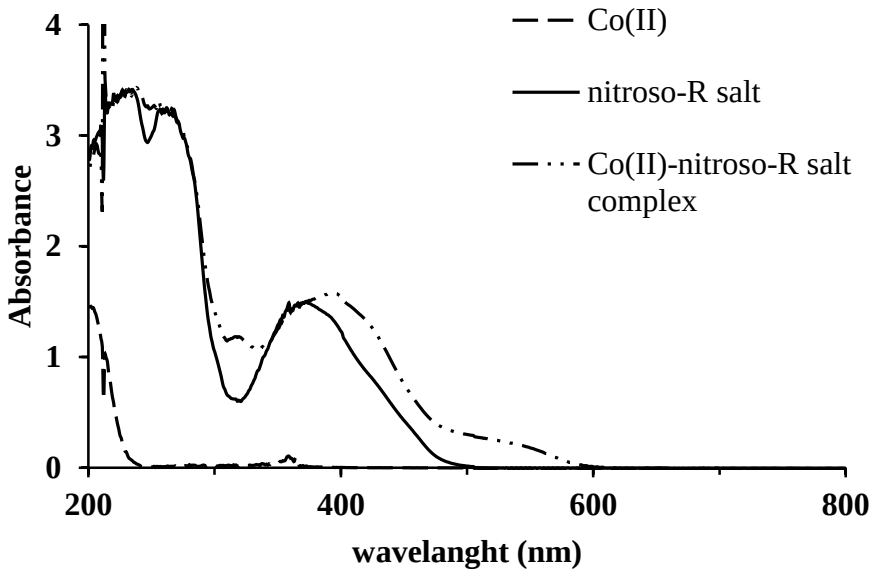


Figure 4.1 The absorption spectra of the studied solutions; 0.1% w/v of nitroso-R salt, 1.0 mg L^{-1} of Co(II) standard and Co(II)-nitroso-R salt complex.

4.1.2 Optimization of the flow injection system

As the previous studied in section 4.1.1, The FIA system (Figure 3.1) for determining Co(II) had been designed and obtimized by using 1.0 mg L^{-1} of Co(II) with 0.1 % w/v of nitroso-R salt and measured the absorbance at 500 nm. The studied parameters for finding the optimum value were included of flow rate, buffer solution, concentration of nitroso-R salt, reaction coil and sample injection volume using the univariation method.

4.1.2.1 Effect of flow rate

Flow rate of reagent and/or carrier were affected on the reaction time of Co(II)-nitroso-R salt complex due to dispersion and the reaction time. Higher flow rate caused low dispersion, incomplete mixing and insufficient time to form Co(II)-nitroso-R salt complex resulting to weak absorption signal and low precision was observed. Lower flow rate was sufficient time to form a complex and longer resident time of the complex caused large dispersion resulting to weak absorption signal and band boarding. The flow rate of nitroso-R salt was investigated in the range from 0.5-3.0 $mLmin^{-1}$

under the condition; 1.0 mg L^{-1} of Co(II), 0.1 %w/v of nitroso-R salt , 300 µL of sample volume. The results were shown in Table 4.1 and Figure 4.2. The absorbance was increased continuously while increasing flow rate to 2.0 mL min^{-1} above of which, the absorbance was decreased slightly. Therefore, the optimum flow rate was 2.0 mL min^{-1}.

Table 4.1 Effect of flow rate on the absorbance of nitroso-R salt complex. Under the conditions; 1.0 mg L^{-1} of Co(II), 0.1 %w/v of nitroso-R salt , 300 µL of sample volume.

Flow rate (mL min^{-1})	Absorbance					
	1	2	3	4	5	Mean ± SD
0.5	0.041	0.040	0.041	0.040	0.041	0.041 ± 0.0005
1.0	0.043	0.043	0.042	0.043	0.043	0.043 ± 0.0004
1.5	0.040	0.043	0.042	0.042	0.042	0.042 ± 0.0010
2.0	0.046	0.046	0.047	0.048	0.047	0.047 ± 0.0007
2.5	0.043	0.043	0.043	0.046	0.046	0.044 ± 0.0015
3.0	0.043	0.043	0.042	0.043	0.043	0.043 ± 0.0004

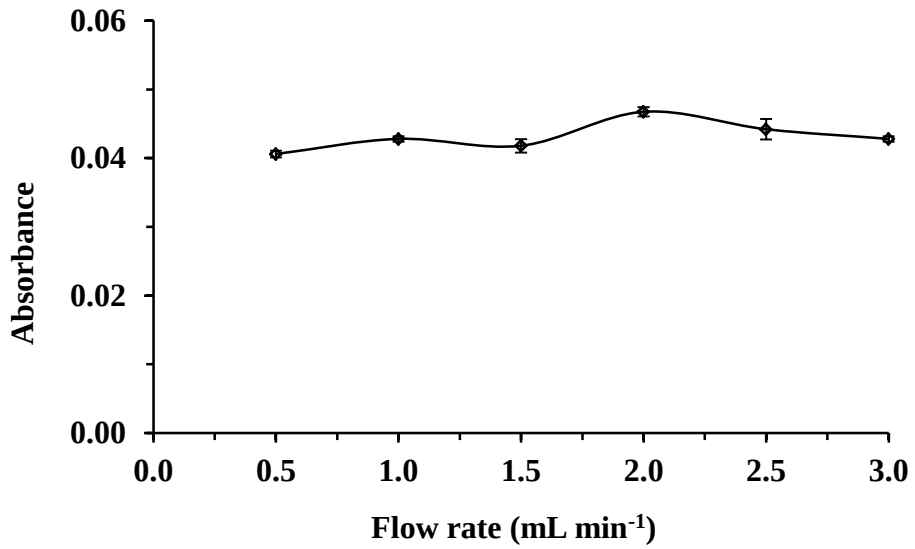


Figure 4.2 Effect of flow rate on the absorbance of nitroso-R salt complex. Under the conditions; 1.0 mg L^{-1} of Co(II), 0.1 %w/v of nitroso-R salt and 300 µL of sample volum

4.1.2.2 Effect of the pH

The pH of solution effected on the selectivity and also influenced on the stoichiometry of the complexes. At suitable pH of the solution, the complex was formed efficiently resulting to strong absorption signal was obtained. The effect of pH of buffer solution was studied over the range of 3.0-8.0. The results were shown in table 4.2 and Figure 4.3. It was found that, the absorbance of Co(II)-nitroso-R salt complex increased with increasing pH of the solution from 3.0-6.5 above of which the absorbance was decreased. Therefore, the phosphate buffer pH of 6.5 was selected as the optimum condition in provided the greatest sensitivity.

Table 4.2 Effect of various pH on the absorbance of Co(II)-nitroso-R salt complex under the conditions; 1.0 mg mL^{-1} of Co(II) and 0.1% w/v of nitroso-R salt.

pH	Absorbance					
	1	2	3	4	5	Mean ± SD
3.0	0.005	0.006	0.006	0.006	0.006	0.006 ± 0.0004
3.5	0.006	0.006	0.006	0.007	0.007	0.006 ± 0.0005
4.0	0.006	0.007	0.006	0.007	0.006	0.006 ± 0.0005
4.5	0.009	0.010	0.009	0.010	0.010	0.010 ± 0.0005
5.0	0.020	0.019	0.018	0.020	0.020	0.019 ± 0.0008
5.5	0.040	0.040	0.040	0.040	0.042	0.040 ± 0.0008
6.0	0.043	0.043	0.043	0.043	0.042	0.043 ± 0.0004
6.5	0.047	0.046	0.046	0.046	0.047	0.047 ± 0.0004
7.0	0.040	0.043	0.046	0.040	0.040	0.042 ± 0.0024
7.5	0.039	0.043	0.039	0.039	0.043	0.041 ± 0.0020
8.0	0.040	0.043	0.042	0.040	0.039	0.041 ± 0.0015

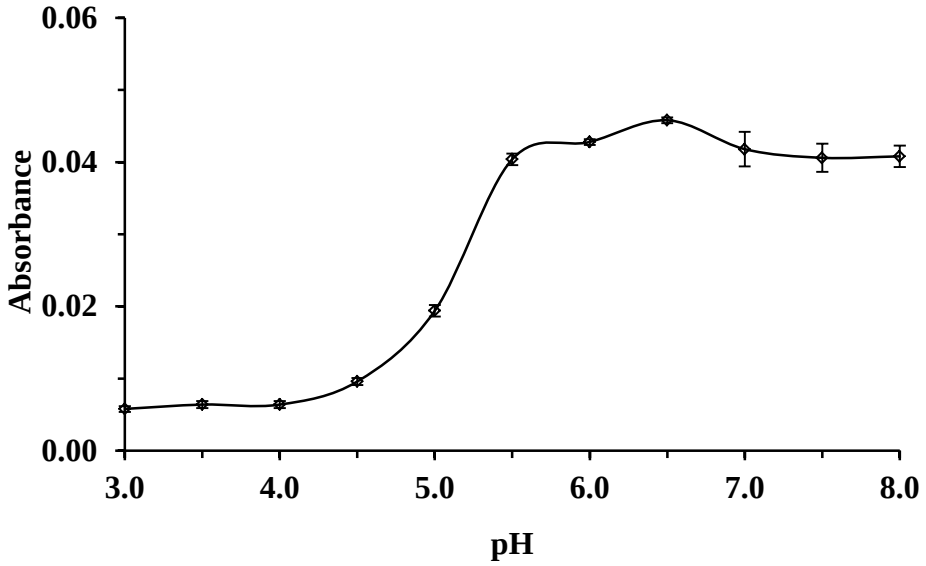


Figure 4.3 Effect of various pH on the absorbance of Co(II)-nitroso-R salt complex under the conditions; 1.0 mg mL^{-1} of Co(II) and 0.1% w/v of nitroso-R salt.

4.1.2.3 Effect of nitroso-R salt concentration

Effect of nitroso-R salt concentration on the determination of Co(II) was studied with the following FI system in Figure 3.2, the concentration of nitroso-R salt solutions were varied from 0.01-0.5% w/v. The results were shown in Table 4.3 and Figure 4.4. It was found that, the absorbance was increased as increasing concentration of nitroso-R salt in the range from 0.01-0.1 % w/v above of this concentration, the signal was decreased slightly owing to the amount of nitroso-R salt was greatest than required stoichiometry of the complex (Co(II):nitroso-R salt = 1:3) [54]. Consequently, a concentration 0.1 % w/v of nitroso-R salt was chosen as the optimum concentration in order to obtain the greatest precision.

Table 4.3 Effect of nitroso-R salt concentration on the absorbance of Co(II)-nitroso-R salt complex under the conditions; 1.0 mg L^{-1} of Co(II), 0.1% w/v of nitroso-R salt and flow rate of 2.0 $mLmin^{-1}$

Concentration of nitroso-R salt (%w/v)	Absorbance					
	1	2	3	4	5	Mean ± SD
0.01	0.025	0.026	0.027	0.027	0.026	0.026 ± 0.0007
0.05	0.050	0.050	0.052	0.052	0.052	0.051 ± 0.0010
0.10	0.057	0.058	0.058	0.057	0.057	0.057 ± 0.0005
0.20	0.057	0.055	0.055	0.054	0.055	0.055 ± 0.0010
0.30	0.055	0.057	0.057	0.055	0.055	0.056 ± 0.0010
0.40	0.053	0.053	0.053	0.053	0.056	0.054 ± 0.0012
0.50	0.051	0.054	0.054	0.053	0.053	0.053 ± 0.0011

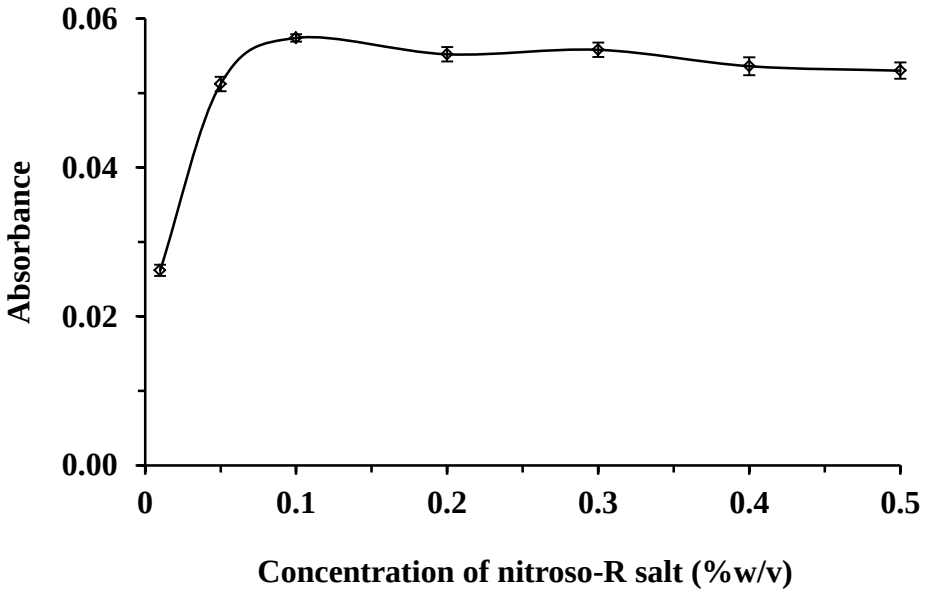


Figure 4.4 Effect of nitroso-R salt concentration on the absorbance of Co(II)-nitroso-R salt complex under the conditions, 1.0 mg mL^{-1} of Co(II) and phosphate buffer pH 6.5.

4.1.2.4 Effect of mixing coil length

The mixing coil length effects on absorbance of Co(II)-nitroso-R salt complex due to the complexation reaction of Co(II) and nitroso-R salt, resident time, back pressure and chemical consumption. A shorter mixing coil exhibited short resident time, low back pressure and less chemical consumption but nitroso-R salt could be not sufficient to complex with Co(II) than a longer mixing coil. The coil length was investigated in the range of 10 to 70 cm. The results are shown in Table 4.4 and Figure 4.5. It was found that, the absorbance of Co(II)-nitroso-R salt complex with reaction coil length of 40.0 cm was the greatest. Therefore, this coil length was selected as optimum to obtain the desire sensitivity, low back pressure.

Table 4.4 Effect of reaction coil length on the absorbance of Co(II)-nitroso-R salt complex**.** Under the conditions; 1.0 mg L^{-1} of Co(II), 0.1 %w/v of nitroso-R salt (pH 6.5), 2.0 mL min^{-1} of flow rate and 300 µL of sample volume.

Mixing coil length	Absorbance					
(cm)	1	2	3	4	5	Mean± SD
10	0.062	0.058	0.058	0.056	0.058	0.058 ± 0.0020
20	0.065	0.071	0.065	0.071	0.075	0.069 ± 0.0039
30	0.071	0.07	0.071	0.071	0.074	0.071 ± 0.0014
40	0.077	0.077	0.077	0.076	0.076	0.077± 0.0005
50	0.076	0.075	0.075	0.075	0.075	0.075 ± 0.0004
60	0.062	0.061	0.061	0.061	0.062	0.061 ± 0.0005
70	0.062	0.062	0.059	0.059	0.061	0.061 ± 0.0014

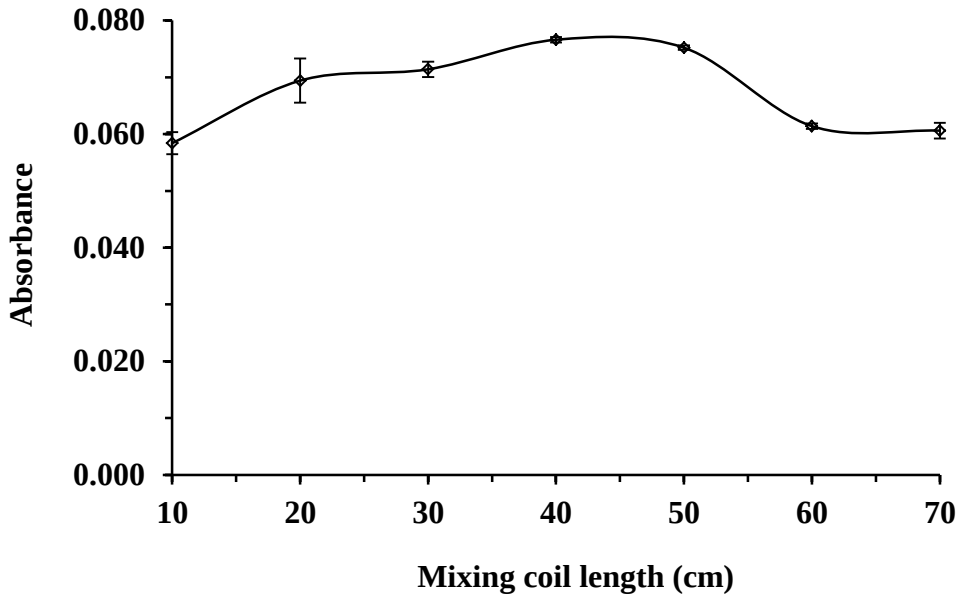


Figure 4.5 Effect of mixing coil length on the absorbance of Co(II)-nitroso-R salt complex**.** Under the conditions; 1.0 mg L^{-1} of Co(II), 0.1 %w/v of nitroso-R salt, 2.0 mL min^{-1} of flow rate and 300 µL of sample volume.

4.1.2.5 Effect of sample volume

The influence of sample volume on the absorbance of Co(II)-nitroso-R salt complex owing to dispersion and the profile of sample zone. Large sample volume caused peak broadening, double peak, and long resident time. In contrast at small sample volume, the reaction of complex is incomplete cause weak absorbance. Various sampling volume was examined by injecting sample volume in the range of 50-400 µL into the FIA system. The results were shown in Table 4.5 and Figure 4.6. As the results found that, initially the absorbance was increased while increasing the sample volume in the range from 50 to 300 µL above of which the absorbance was decreased and a broadening peak was obtained due to a large sample volume increasing the dispersion. Consequently, the sample volume of 300 µL was chosen as the compromise value and used throughout the experimental.

Table 4.5 Effect of sample volume on the absorbance of Co(II)-nitroso-R salt complex Under the conditions; 1.0 mg L^{-1} of Co (II), 0.1 %w/v of nitroso-R salt, 1.5 mL min^{-1} of flow rate and 40 cm of mixing coil length.

Sample volume (µL)	Absorbance					
	1	2	3	4	5	Mean± SD
50	0.037	0.036	0.035	0.036	0.035	0.036 ± 0.0007
100	0.042	0.041	0.041	0.041	0.039	0.041 ± 0.0010
150	0.050	0.049	0.048	0.048	0.048	0.049 ± 0.0008
200	0.065	0.065	0.065	0.065	0.066	0.007 ± 0.0004
250	0.082	0.088	0.082	0.088	0.083	0.085 ± 0.0028
300	0.089	0.089	0.087	0.089	0.089	0.089 ± 0.0008
350	0.088	0.083	0.088	0.083	0.082	0.085 ± 0.0026
400	0.083	0.079	0.089	0.085	0.085	0.084 ± 0.0032

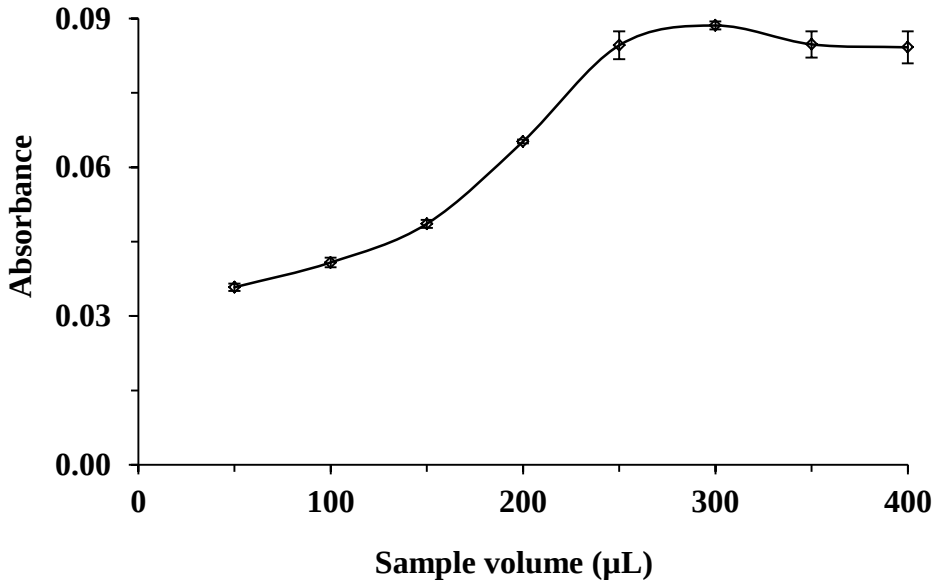


Figure 4.6 Effect of sample volume on the absorbance of Co(II)-nitroso-R salt complex. Under the conditions; 1.0 mg L^{-1} of Co(II), 0.1 %w/v of nitroso-R salt, 1.5 mL min^{-1} of flow rate and 40 cm of mixing coil length.

The FI system for determination of Co(II) using nitroso-R salt as reagent showed in Figure 3.1. The FIA parameters were studied and their optimum conditions are shown in Table 4.6.

Table 4.6 The studied parameters and their optimum value of the proposed FIA for determination of Co(II)

Parameters	**Studied range**	**Optimum value**
Wave length (nm)	200.0-800.0	500.0
Flow rate (mL min^{-1})	0.50-3.0	2.0
pH of buffer solution	3.0-8.0	6.50
Concentration of nitroso-R salt (%w/v)	0.005-0.50	0.10
Reaction coil length (cm)	10.0-80.0	40.0
Sample injection volume (µL)	50.0-400.0	300.0

4.1.3 Analytical characteristics of the method

The analytical characteristics of the proposed FIA such as the linearity for calibration graph, accuracy, precision and interferences were estimated under the optimum condition as showed in Table 4.5.

4.1.3.1 The linear range for calibration graph

The linearity range for a calibration graph was investigated by measurement the absorbance of Co(II)-nitroso-R salt complex using standard Co(II) concentration in the range of 0.01-5.0 mg L^{-1} under the optimum conditions as showed in table 4.6. Table 4.7 and Figure 4.8 showed the absorbance of Co(II)-nitroso-R salt complex at the studied concentration range, the relative plot of the absorbance versus the concentration of Co(II) range from 0.01-5.00 mg L^{-1}, and the FIA gram at the selected linearity range. Regarding to Figure 4.7 the linearity for determination of Co(II) was found in the range of 0.05-2.0 mg L^{-1}. The calibration graph was plotted and shown in Figure 4.9. The linear regression equation of $y = 0.0911x + 0.0076$ the correlation coefficient was 0.997.

Table 4.7 The absorbance of Co(II)-nitroso-R salt complex at Co(II) concentration in the range of 0.01-5.00 mg L^{-1} for calibration graph

Concentration of Co(II)(mg L^{-1})	Absorbance					
	1	**2**	**3**	**4**	**5**	**Mean ± SD**
0.01	0.004	0.003	0.003	0.003	0.003	0.003 ± 0.0004
0.05	0.007	0.007	0.007	0.007	0.006	0.007 ± 0.0004
0.10	0.015	0.014	0.015	0.014	0.014	0.014± 0.0005
0.50	0.056	0.057	0.056	0.056	0.057	0.057 ± 0.0005
1.00	0.097	0.097	0.096	0.097	0.096	0.097 ± 0.0005
1.50	0.135	0.136	0.135	0.136	0.135	0.135 ± 0.0005
2.00	0.160	0.161	0.159	0.148	0.148	0.155 ± 0.0059
2.50	0.180	0.180	0.181	0.179	0.180	0.180 ± 0.0006
3.00	0.187	0.188	0.187	0.185	0.184	0.186 ± 0.0015
3.50	0.182	0.189	0.190	0.190	0.191	0.188 ± 0.0033
4.00	0.192	0.192	0.190	0.191	0.191	0.191 ± 0.0007
4.50	0.204	0.210	0.205	0.198	0.199	0.203 ± 0.0044
5.00	0.194	0.196	0.192	0.192	0.195	0.184 ± 0.0016

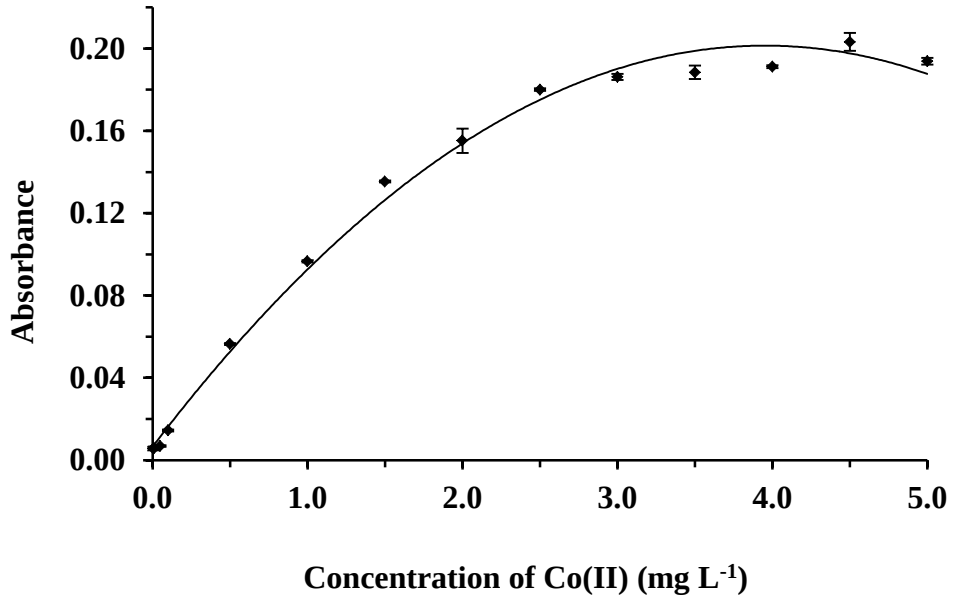


Figure 4.7 The absorbance of Co(II)-nitroso-R salt complex at Co(II) concentration in the range of 0.005-5.00 mg L^{-1} .

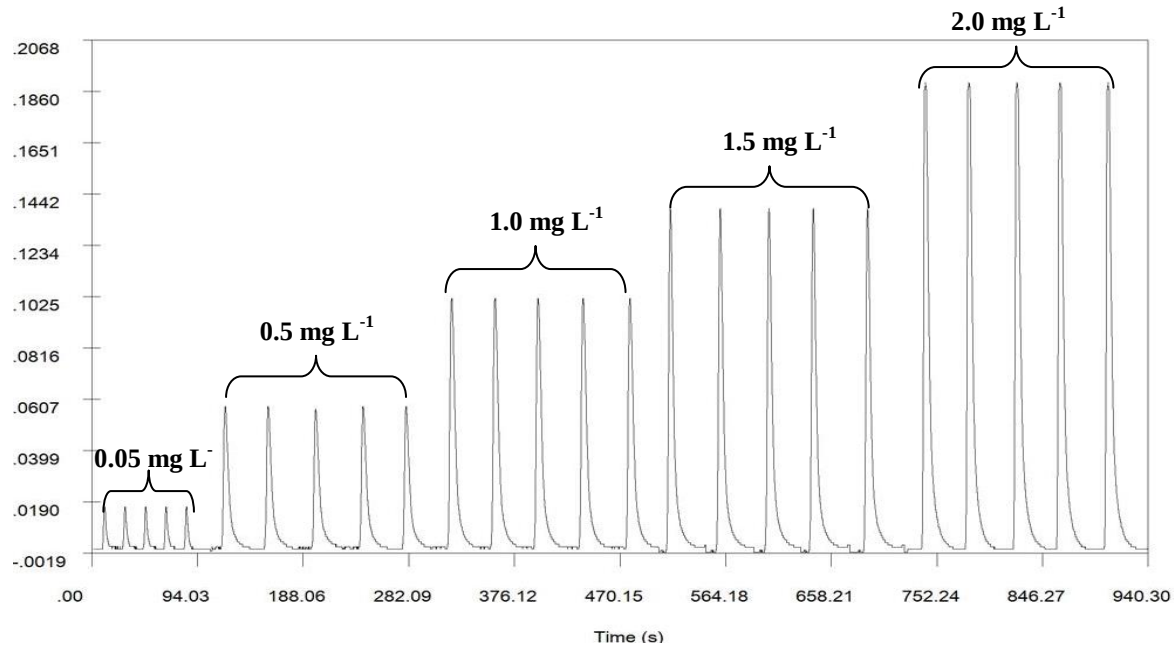


Figure 4.8 Analytical signal for determining Co(II) using the proposed FIA system under the optimum conditions

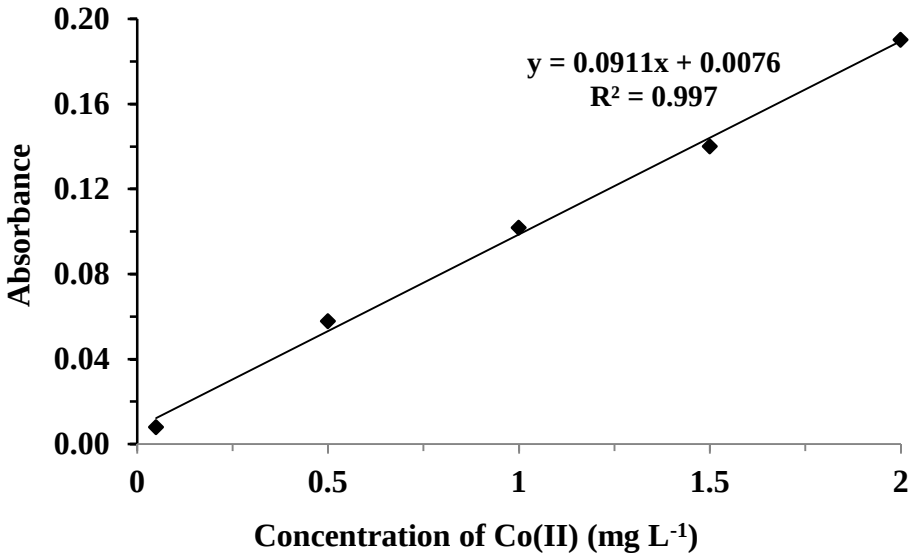


Figure 4.9 Calibration graph for determination of Co(II) at the concentration range from 0.05-2.0 mg L^{-1}.

4.1.3.2 Precision of the flow injection system

The precision of the proposed method (Table 4.8) was verified as repeatability and reproducibility by measurement the absorbance of 11 replicated covering different concentration of standard Co(II) (0.05, 1.0 and 2.0 mgL^{-1}), under the optimum conditions listed in Table 4.6. The relative standard deviation of repeatability and reproducibility were calculated and found to 0.41, 1.35 and 0.41 for repeatability and 0.55, 0.56 and 0.26 for reproducibility, respectively.

Table 4.8 The precision of the method based on repeatability and reproducibility.

Experimental number	Absorbance obtained from the Co(II) (mg L^{-1})					
	Repeatability			Reproducibility		
	0.05	**1.0**	**2.0**	**0.05**	**1.0**	**2.0**
1	0.007	0.101	0.192	0.008	0.102	0.190
2	0.007	0.101	0.191	0.008	0.102	0.191
3	0.007	0.101	0.190	0.008	0.101	0.190
4	0.007	0.102	0.192	0.008	0.102	0.191
5	0.007	0.101	0.191	0.008	0.102	0.191
6	0.007	0.103	0.192	0.008	0.103	0.190
7	0.007	0.100	0.190	0.008	0.102	0.190
8	0.007	0.105	0.191	0.008	0.101	0.191
9	0.007	0.101	0.191	0.008	0.102	0.190
10	0.007	0.103	0.190	0.008	0.101	0.190
11	0.007	0.103	0.190	0.008	0.102	0.191
Mean	0.007	0.102	0.191	0.008	0.102	0.190
SD	0.0001	0.0014	0.0008	0.0001	0.0006	0.0005
%RSD	0.41	1.35	0.41	0.55	0.56	0.26

4.1.3.3 Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of the proposed medthod were determined as the concentration of the analyte leading to three times and ten time of the standard deviation of blank, respectively. It was found that the LOD and LOQ of the proposed method were 0.0013 and 0.0042 mg L^{-1}, respectively.

4.1.4 Effect of interference

The effect of some possible interferences might be formed a complex with nitroso-R salt on the determination of 1.0 mg L^{-1} Co(II) in samples was investigated and

summarized in Table 4.9, the tolerance limits was determined for a maximum error of less than ±5%. It was seen that that, most of interferences ions did not interfere, except 10.0 mg L^{-1} of Ni(II), Fe(III) and Cu(II). However, Ni(II), Fe(III) and Cu(II) were not presented in medicine samples. Therefore, Ni(II), Fe(III) and Cu(II) can be considered to be no interference in case of Co(II) determination in vitamin B complex.

Table 4.9 Effect of interference ions on the determination of 1.0 mg L^{-1} Co(II). The tolerance limits was determined for a maximum error of less than ±5%

Interference ions	**Tolerance (mg L^{-1})**
Al(II), As(II), Cd(II), Ca(II), Pb(II), Mg(II)	200
Mn(II), Zn(II)	100
Ni(II), Cu(II), Fe(III)	10

4.1.5 Determination of Co(II) in vitamin B complex

The proposed FIA spectrophotometric method was applied to determination of Co(II) in vitamin B complex were purchased from the pharmaceutical store around Mahasarakham province. The contents of Co(II) in each samples were analyzed and calculated against the standard calibration curve. The results were given in Table 4.10. The amount of Co(II) in vitamin B complex were found in the range of 0.083-0.379 mg L^{-1}. The percentage recoveries for determination of Co(II) in medicine samples were in the range 89.17-109.23. It was also found that the amount of Co(II) contents in vitamin B complex were agreed well with the label and standard FAAS method.

Table 4.10 The amount of Co(II) in medicine samples determinationed by the proposed FIA spectrophotometric medthod comparison to the standard FAAS method.

Samples	**Co(II) added (mg L^{-1})**	**The amount of Co(II) found (mgL^{-1}) in FIA**	**Recovery (%)**	**The amount of Co(II) found (mgL^{-1}) in FAAS**	**Recovery (%)**
Neuvipex	-	0.379 ± 0.009	109.23	0.397 ± 0.027	95.60
	1.0	1.471 ± 0.005		1.353 ± 0.001	
Neurobion	-	0.251 ± 0.005	89.17	0.339 ± 0.028	96.95
	1.0	1.142 ± 0.005		1.308 ± 0.047	
Sambee	-	0.101 ± 0.005	101.27	0.126 ± 0.027	93.05
	1.0	1.114 ± 0.009		1.056 ± 0.027	
T-man	-	0.103 ± 0.003	98.57	0.087 ± 0.001	102.73
	1.0	1.088 ± 0.005		1.114 ± 0.027	
Vitamin B	-	0.097 ± 0.009	89.90	0.126 ±0.027	94.99
	1.0	0.982 ±0.001		1.075 ± 0.001	

4.2 Sequential injection spectrophotometric determination of Co(II) using nitroso-R salt as a complexing reagent

The sequential injection analysis (SIA) instrument for determining Co(II) according to manifold showed in Figure 3.2. Deionization water as a carrier stream was delivered by a high precision peristaltic pump (Reglo Dig MS-4/8 V1.12, Ismatec, Switzerland) with a computer control program. The sequence was started using a ten-port selection valve (VICI, Valco Instrument, USA) with a computer control program by aspiration of nitroso-R salt reagent solution into a holding coil (1.02 mm I.d., 200 cm long), followed by buffer solution and sample or standard solution, respectively. The peristaltic pump was use for delivering the complex through a flow-throughcell (1 cm. Hellma, Germany) placing in a spectrophotometer. A software name SENEE SIA version 1.0.0 and UVWINLAB (Lambda 25) were also used for controlling, recording and analyzing the data.

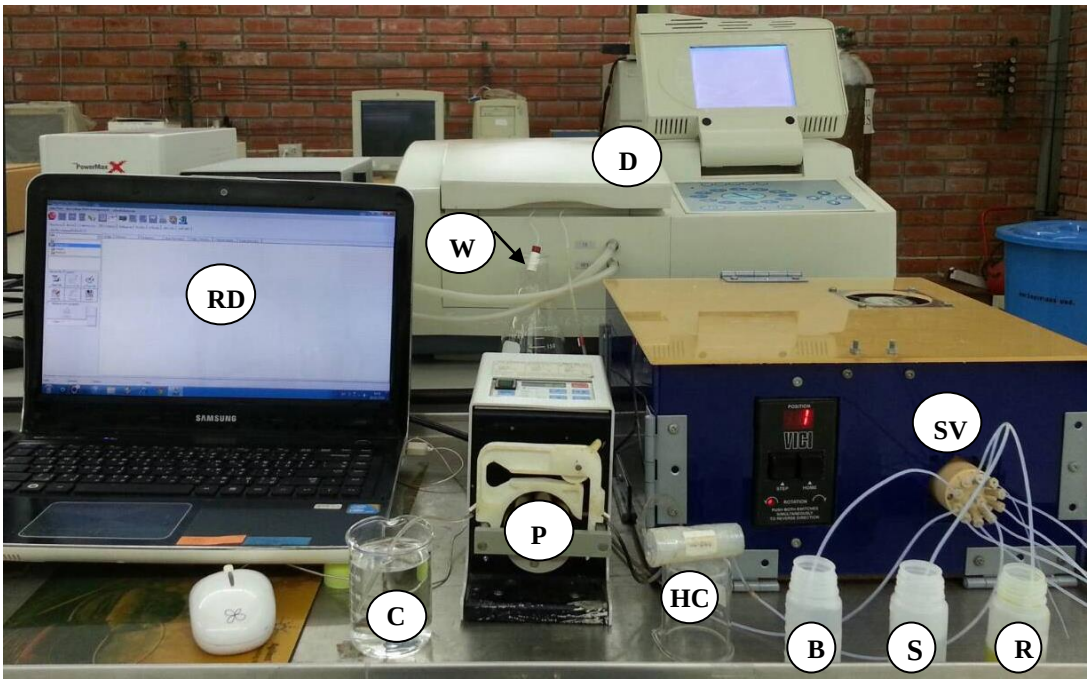


Figure 4.10 The SIA manifold system for determination of Co(II): C, carrier (DI water); P, peristaltic pump; HC, holding coil; SV, selection valve; R, reagent (nitroso-R salt); S, sample (cobalt(II)); B, buffer; D, detector; RD, recorder; W, waste.

4.2.1 Optimization of the sequential injection system

The conditions of determination of Co(II) were optimized by studying the influences of the various parameters, such as aspiration order, sample and reagent volumes, flow rate of carrier and reagent concentrations. The optimum value obtained by means of the univariate optimization procedure (changing one variable in turn and keeping the others at their optimum values). The optimal value for each parameter was selected from maximum response with a minimum standard deviation.

4.2.1.1 Aspiration sequence

The order of aspiration sequence were studied by drawing of reagent, buffer and standard/sample solutions to holding coil in various patterns as shown in Table 4.8. The probability of effective mixing of each zone could be penetrating to form the series that is giving the highest stable Co(II)-nitroso-R salt complex is considerable. As the results (Table 4.11 and Figure 4.11), the highest absorbance and precision of the studied series was found in series 1.(R-S-B)

Table 4.11 The absorbance of the Co(II)-nitroso-R salt complex at the different aspiration order, under the condition; 1.0 mg L^{-1} Co(II) standard solution, 0.1%w/v nitroso-R salt and phosphate buffer pH 6.5.

Series	Aspiration sequence	Absorbance SD (n=5)
1	R-S-B	0.045 ± 0.0005
2	R-B-S	0.006 ± 0.0004
3	B-R-S	0.008 ± 0.0004
4	B-S-R	0.011 ± 0.0004
5	S-R-B	0.039 ± 0.0005
6	S-B-R	0.005 ± 0.0004

Remark; S is 1.0 mg mL^{-1} Co(II) standard solution

R is 0.1 %w/v nitroso-R salt

B is 0.1 mol L^{-1} phosphate buffer

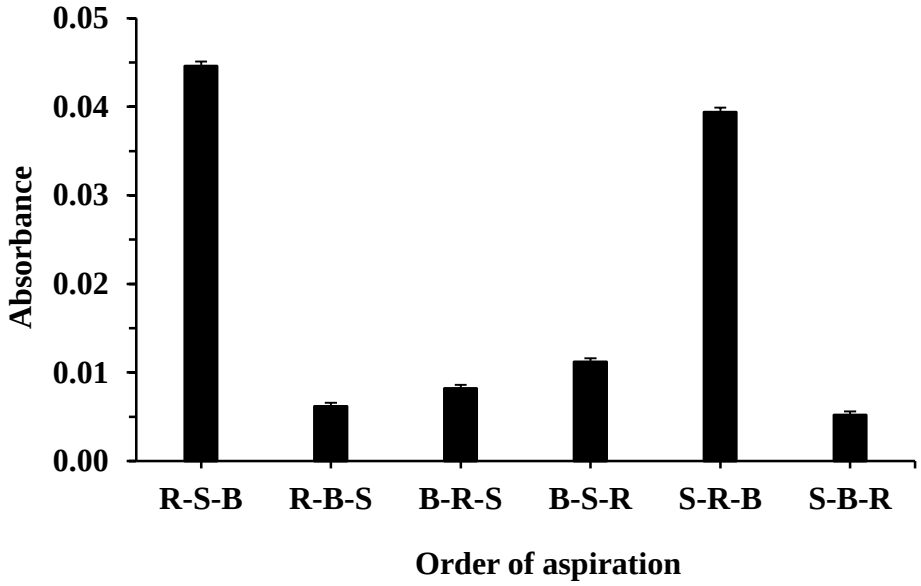


Figure 4.11 The absorbance of Co(II)-nitroso-R salt complex at the different aspiration sequence, under conditions; 1.0 mg L^{-1} Co(II) standard solution, 0.1%w/v nitroso-R salt and 1.0 mol L^{-1} phosphate buffer pH 6.5.

4.2.1.2 Effect of the flow rate

Flow rate was an important parameter affected on the SI system. Higher flow rate caused low dispersion, shorter residence time but higher back pressure. Lower flow rate can reduce back pressure but longer residence time. The greatest of flow rate on the absorption signal of Co(II)-nitroso-R sal was studied over the range of 0.5 to 4.0 mL min^{-1}, the result shown in Table 4.12 and Figure 4.12. It was seen that the absorbance was increased continuously while increasing flow rate to 2.5 mL min^{-1} above of which, the absorbance was decreased slightly. Therefore, the optimum flow rate was 2.5 mL min^{-1}.

Table 4.12 Effect of flow rate on the absorbance of Co(II)-nitroso-R salt complex. Under the conditions; 1.0 mg L^{-1} of Co(II), 0.10 % w/v of nitroso-R salt and phosphate buffer pH 6.5.

Flow rate	absorbance					
(ml/min)	1	2	3	4	5	Mean± SD
0.5	0.038	0.038	0.039	0.039	0.039	0.039 ± 0.0005
1.0	0.041	0.041	0.042	0.041	0.042	0.041 ± 0.0005
1.5	0.044	0.044	0.043	0.044	0.044	0.044 ± 0.0004
2.0	0.046	0.046	0.047	0.047	0.047	0.047 ± 0.0005
2.5	0.050	0.051	0.051	0.051	0.051	0.051 ± 0.0004
3.0	0.050	0.050	0.050	0.050	0.049	0.050 ± 0.0004
3.5	0.048	0.048	0.048	0.047	0.048	0.048 ± 0.0004
4.0	0.044	0.043	0.044	0.044	0.044	0.044 ± 0.0004

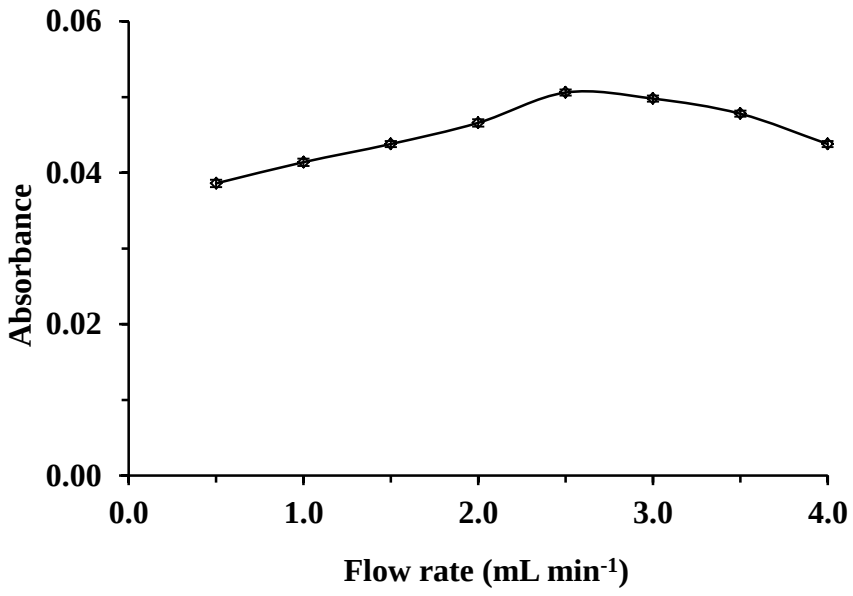


Figure 4.12 Effect of flow rate on the absorbance of Co(II)-nitroso-R salt complex. Under the conditions; 1.0 mg L^{-1} of Co(II), 0.1 % w/v of nitroso-R salt and phosphate buffer pH 6.5.

4.2.1.3 Effect of buffer volumes

The aim for optimization of this parameter is to minimize the consumption of reagent while maintaining the best sensitivity, accuracy and reproducibility of the procedure for the analyte of interest. The influence of buffer volume on the absorbance of Co(II)-nitroso-R salt complex was studied by controlling pump flow rate and changing the switching time of selection valve at phosphate buffer solution position over the range of 10 to 90 µL under the condition; flow rate 2.5 mL min^{-1}, 1.0 mg L^{-1} of Co(II) concentration, 0.1 %w/v of nitroso-R salt concentration and phosphate buffer pH 6.5. The result are shown in Table 4.13 and Figure 4.13. It was found that the absorbance was increased when increasing the aspiration volume of buffer at 60 µL, above which the absorbance was decreased. So a volume of 60 µL was chosen as the optimum.

Table 4.13 Effect of phosphate buffer volume on Co(II)-nitroso-R salt complex. Under the conditions; flow rate 2.5 mL min^{-1}, 1.0 mg L^{-1} of co(II) concentration, 0.1 %w/v of nitroso-R salt concentration and phosphate buffer pH 6.5.

Aspiration volumes of phosphate buffer (µL)	**Absorbance**					
	1	**2**	**3**	**4**	**5**	**Mean ± SD**
10	0.036	0.037	0.038	0.037	0.038	0.037 ± 0.0007
20	0.037	0.038	0.039	0.038	0.039	0.038 ± 0.0007
30	0.037	0.037	0.038	0.039	0.039	0.038 ± 0.0009
40	0.039	0.040	0.039	0.041	0.040	0.040 ± 0.0007
50	0.043	0.042	0.043	0.043	0.042	0.043± 0.0005
60	0.054	0.054	0.054	0.053	0.054	0.054± 0.0004
70	0.044	0.046	0.044	0.045	0.046	0.045± 0.0009
80	0.036	0.037	0.038	0.038	0.038	0.037 ± 0.0008
90	0.035	0.034	0.036	0.039	0.038	0.036 ± 0.0017

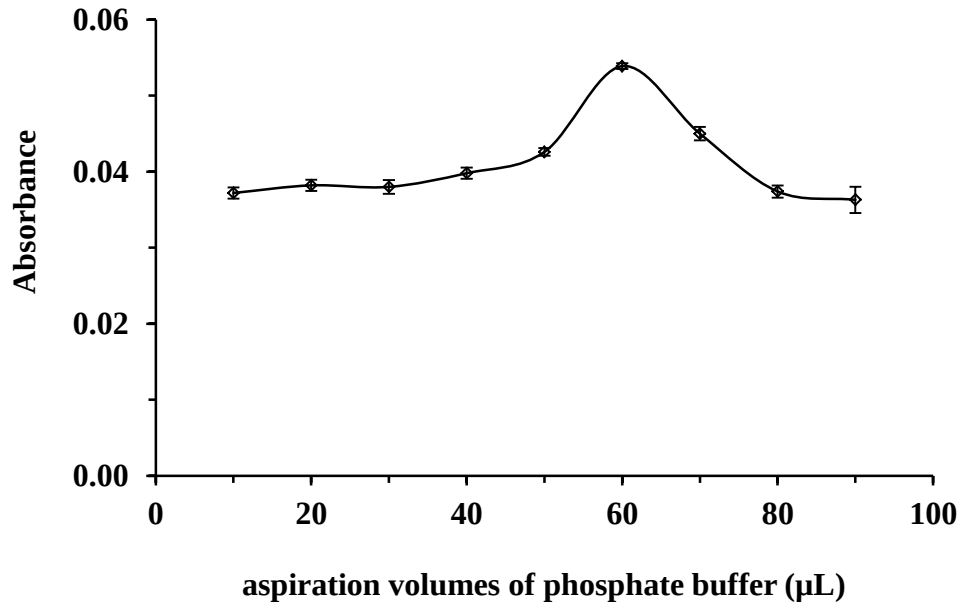


Figure 4.13 Effect of phosphate buffer volume on Co(II)-nitroso-R salt complex. Under the conditions; flow rate 2.5 mL min^{-1}, 1.0 mg L^{-1} of Co(II) concentration, 0.1 %w/v of nitroso-R salt concentration and phosphate buffer pH 6.5.

4.2.1.4 Effect of nitroso-R salt aspiration volumes

The influence of sample volume on the absorbance of Co(II)-nitroso-R salt complex was perform by controlling pump flow rate and changing the switching time of selection valve at nitroso-R salt solution position over the range of 10 to 60 µL under the condition; flow rate 2.5 mL min^{-1}, 1.0 mg L^{-1} of Co(II) concentration, 0.1 %w/v of nitroso-R salt concentration and phosphate buffer pH 6.5. The results were shown in Table 4.14 and Figure 4.14 It can be seen that the absorbances were increased with increasing reagent volume up to 20 µL, above this the absorbance was decreased. Thus, a 20 µL of nitroso-R salt solution was chosen as a optimum volume for subsequent measurements.

Table 4.14 Effect of nitroso-R salt volume on Co(II)-nitroso-R salt complex. Under the conditions; flow rate 2.5 mL min^{-1}, 1.0 mg L^{-1} of Co(II) concentration, 0.1 %w/v of nitroso-R salt concentration and phosphate buffer pH 6.5.

Aspiration volumes of nitroso-R salt (µL)	Absorbance					
	1	2	3	4	5	Mean ± SD
10	0.018	0.018	0.018	0.018	0.019	0.018 ± 0.0004
20	0.058	0.057	0.058	0.033	0.058	0.058 ± 0.0004
30	0.034	0.034	0.034	0.038	0.033	0.034 ± 0.0005
40	0.039	0.039	0.038	0.038	0.038	0.038 ± 0.0005
50	0.037	0.038	0.038	0.039	0.036	0.037 ± 0.0009
60	0.037	0.038	0.038	0.039	0.038	0.038 ± 0.0007

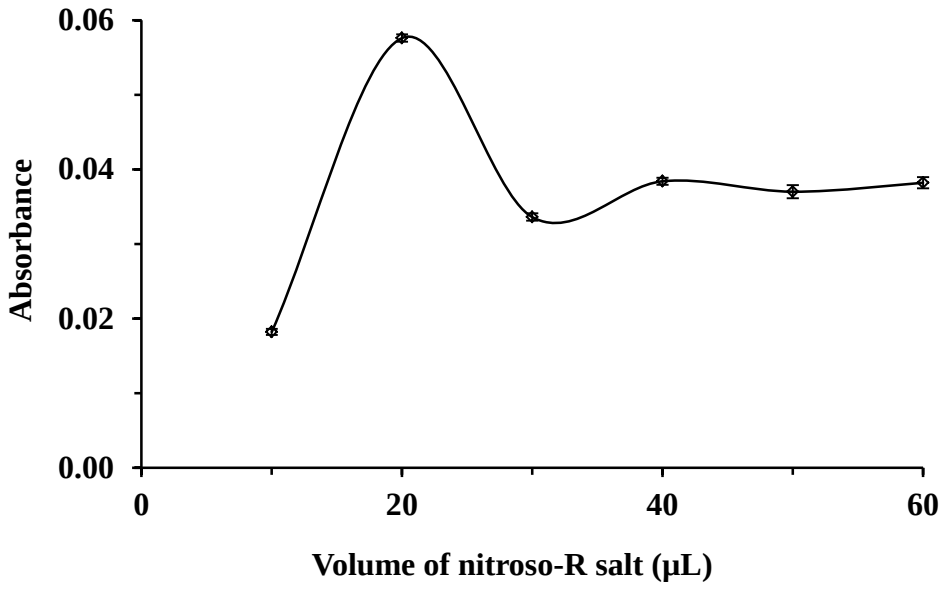


Figure 4.14 Effect of nitroso-R salt volume on Co(II)-nitroso-R salt complex. Under the conditions; flow rate 2.5 mL min^{-1}, 1.0 mg L^{-1} of Co(II) concentration, 0.1 %w/v of nitroso-R salt concentration and phosphate buffer pH 6.5.

4.2.1.5 Effect of sample aspiration volumes

The influence of sample volume on Co(II) determination was studied by controlling pump flow rate and changing the switching time of selection valve at sample position over the range of 10 to 100 µL. Table 4.15 and Figure 4.15. Shown initially, the absorbance increased rapidly with increasing sample volume up to 70 µL, above of which the absorbance keep constant with peak broadening owing to the effect of dispersion. Hence, the aspiration volume of 70 µL was considered to be optimum sample introduction volume, which was used throughout the experiments.

Table 4.15 Effect of sample/Co(II) standard volume on Co(II)-nitroso-R salt complex. Under the conditions; flow rate 2.5 mL min^{-1}, 1.0 mg L^{-1} of Co(II) concentration, 0.1 %w/v of nitroso-R salt concentration and phosphate buffer pH 6.5.

aspiration volumes of sample (µL)	**Absorbance**					
	1	**2**	**3**	**4**	**5**	**Mean ± SD**
10	0.023	0.023	0.023	0.022	0.022	0.023 ± 0.0005
20	0.027	0.027	0.027	0.026	0.026	0.027 ± 0.0005
30	0.030	0.031	0.030	0.031	0.030	0.030 ± 0.0005
40	0.033	0.035	0.035	0.036	0.036	0.035 ± 0.0011
50	0.037	0.040	0.039	0.040	0.040	0.039 ± 0.0012
60	0.049	0.049	0.048	0.048	0.048	0.048 ± 0.0005
70	0.064	0.065	0.065	0.066	0.064	0.065 ± 0.0005
80	0.063	0.066	0.066	0.065	0.064	0.065 ± 0.0012
90	0.065	0.065	0.064	0.065	0.065	0.065 ± 0.0004
100	0.062	0.065	0.064	0.065	0.065	0.064 ± 0.0012

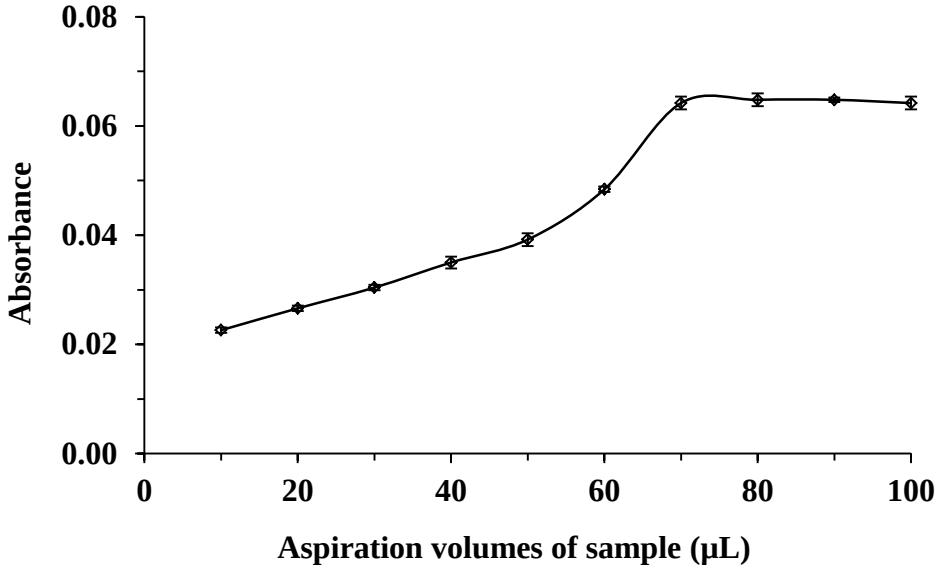


Figure 4.15 Effect of sample/Co(II) standard volume on Co(II)-nitroso-R salt complex. Under the conditions; flow rate 2.5 mL min^{-1}, 1.0 mg L^{-1} of Co(II) concentration, 0.1 %w/v of nitroso-R salt concentration and phosphate buffer pH 6.5.

4.2.1.6 Effect of nitroso-R salt concentration

Effect of nitroso-R salt concentration on the determination of Co(II) was studied examined in the range of 0.01-0.4%w/v. The results were shown in table 4.16 and Figure 4.16. It was found that, the absorbance was increased at the nitroso-R salt concentration range of 0.01-0.1%w/v. So, a concentration of 0.1 %w/v of nitroso-R salt was chosen as optimum.

Table 4.16 Effect of nitroso-R salt concentration on Co(II)-nitroso-R salt complex. Under the conditions; flow rate 2.5 mL min^{-1}, 1.0 mg L^{-1} of Co(II) concentration and phosphate buffer pH 6.5.

Nitroso-R salt concentration (%w/v)	Absorbance					
	1	2	3	4	5	Mean ± SD
0.01	0.055	0.055	0.055	0.056	0.056	0.055 ± 0.0004
0.05	0.061	0.062	0.062	0.062	0.062	0.062 ± 0.0004
0.1	0.074	0.073	0.074	0.074	0.074	0.074 ± 0.0004
0.2	0.070	0.071	0.07	0.071	0.071	0.071 ± 0.0004
0.3	0.064	0.065	0.065	0.065	0.064	0.065 ± 0.0004
0.4	0.060	0.062	0.062	0.062	0.062	0.062 ± 0.0008

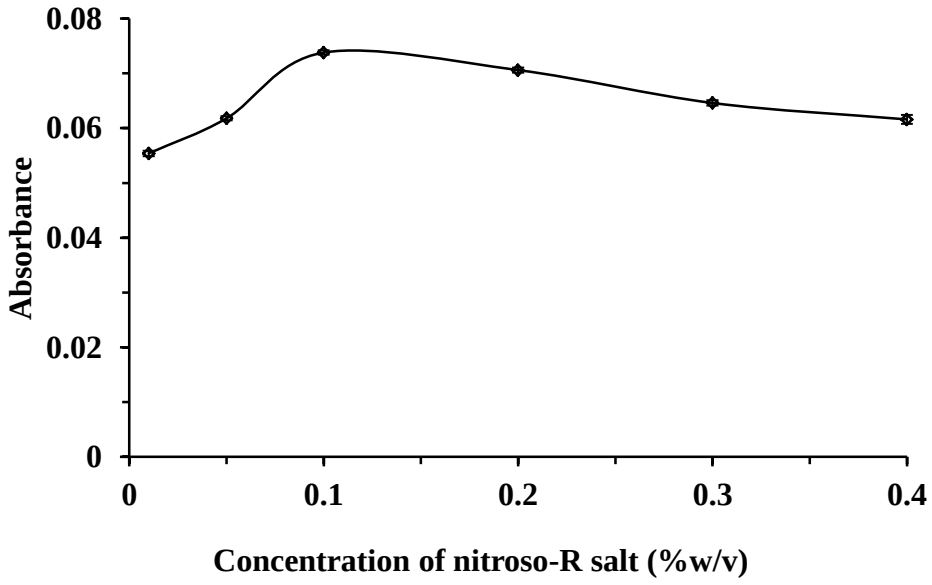


Figure 4.16 Effect of nitroso-R salt concentration on Co(II)-nitroso-R salt complex. Under the conditions; flow rate 2.5 mL min^{-1}, 1.0 mg L^{-1} of Co(II) concentration and phosphate buffer pH 6.5.

The studied parameters for SIA spectrophotometric determination of Co(II) using nitroso-R salt as reagent and their optimum value were summarized in Table 4.17.

Table 4.17 The studied parameters and their optimum condition of SIA spectrophotometric method for determination of Co(II).

Parameter	**Studied range**	**Optimum value**
Aspiration sequence		R-S-B
Flow rate (mL min^{-1})	0.50-4.0	2.50
Aspiration volume of phosphate buffer (µL)	10.0-90.0	60.0
Aspiration volume of nitroso-R salt (µL)	10.0-60.0	20.0
Aspiration volume of sample (µL)	10.0-100.0	70.0
Concentration of nitroso-R salt (%w/v)	0.01-0.40	0.10

4.2.2 Analytical characteristics of the method

The analytical characteristic of the proposed method such as the linear calibration curve, accuracy, precision and interferences were estimated under the optimum condition as showed in Table 4.17.

4.2.2.1 The linear range for calibration graph

The linear range of the proposed method was studied by aspiration of appropriate volume of Co(II) standard solution into SI system under the optimum condition. Linear calibration graphs were obtained for Co(II) standard over the concentration range of 0.005-12.0 mg L^{-1}(Table 4.18).

Table 4.18 The absorbance of Co(II)-nitroso-R salt complex for a linearity range measurement at Co(II) concentrations in the range 0.005-12 mg L^{-1}.

Concentration of Co(II) (mg L^{-1})	**Absorbance**					
	1	**2**	**3**	**4**	**5**	**Mean ± SD**
0.005	0.003	0.003	0.004	0.003	0.004	0.003 ± 0.0000
0.01	0.007	0.007	0.007	0.007	0.007	0.007 ± 0.0000
0.05	0.011	0.011	0.011	0.011	0.011	0.011 ± 0.0000
0.5	0.030	0.030	0.030	0.029	0.029	0.030 ± 0.0004
1.0	0.072	0.072	0.073	0.073	0.073	0.073 ± 0.0004
2.0	0.108	0.108	0.108	0.106	0.107	0.107 ± 0.0008
3.0	0.168	0.168	0.168	0.168	0.164	0.167 ± 0.0016
4.0	0.213	0.218	0.216	0.210	0.213	0.214 ± 0.0027
5.0	0.257	0.258	0.258	0.258	0.258	0.258 ± 0.0004
6.0	0.312	0.314	0.310	0.317	0.309	0.312 ± 0.0028
7.0	0.348	0.348	0.356	0.351	0.343	0.349 ± 0.0042
8.0	0.378	0.371	0.381	0.379	0.383	0.378 ± 0.0040
9.0	0.408	0.403	0.412	0.400	0.406	0.406 ± 0.0041
10.0	0.428	0.422	0.441	0.441	0.426	0.432 ± 0.0079
11.0	0.425	0.425	0.413	0.413	0.413	0.418 ± 0.0058
12.0	0.425	0.414	0.413	0.425	0.412	0.418 ± 0.0059

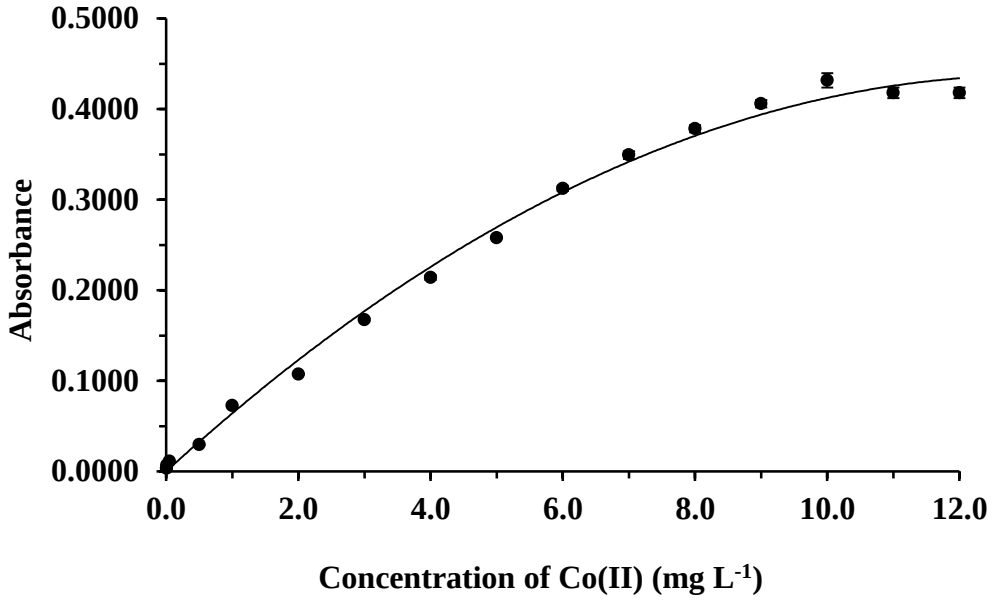


Figure 4.17 The absorbance of Co(II)-nitroso-R salt complex for a linearity range measurement at Co(II) concentrations in the range 0.005-12 mg L^{-1}.

Regarding to Figure 4.16 the linearity for determination of Co(II) was found in the range of 0.05-7.0 mg L^{-1}. The analytical signal was shown in Figure 4.18. The calibration graph was plotted and shown in Figure 4.19. The linear regression equation of Y = 0.0538x + 0.0158, Y is absorbance of Co(II)-nitroso-R salt complex and X is concentration of Co(II). The correlation coefficient for this line was 0.998.

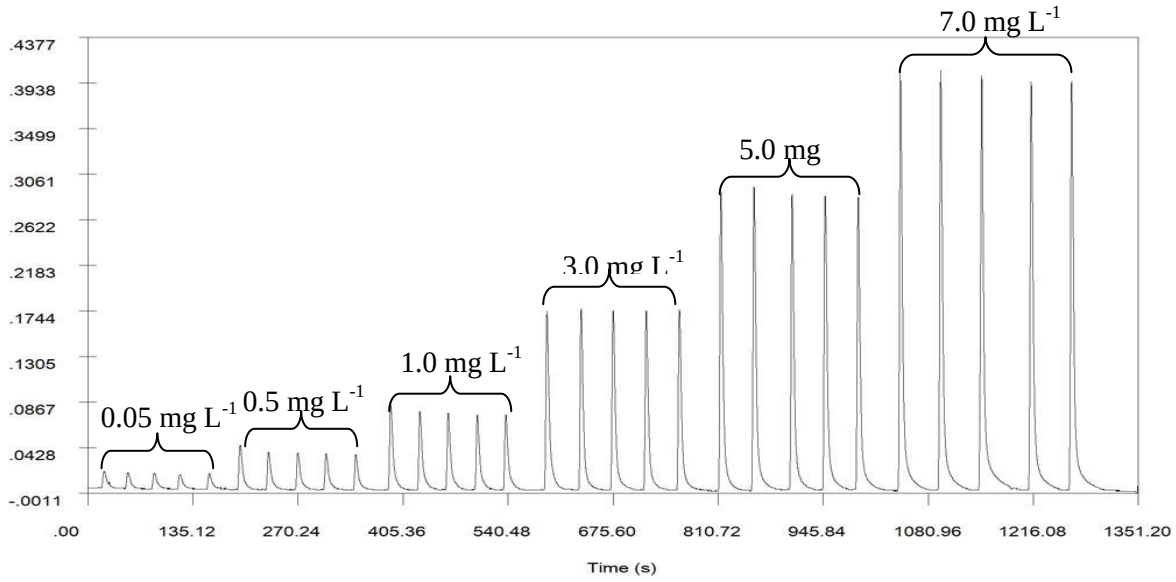


Figure 4.18 The analytical signal for determining Co(II) using the proposed SIA system under the optimum conditions.

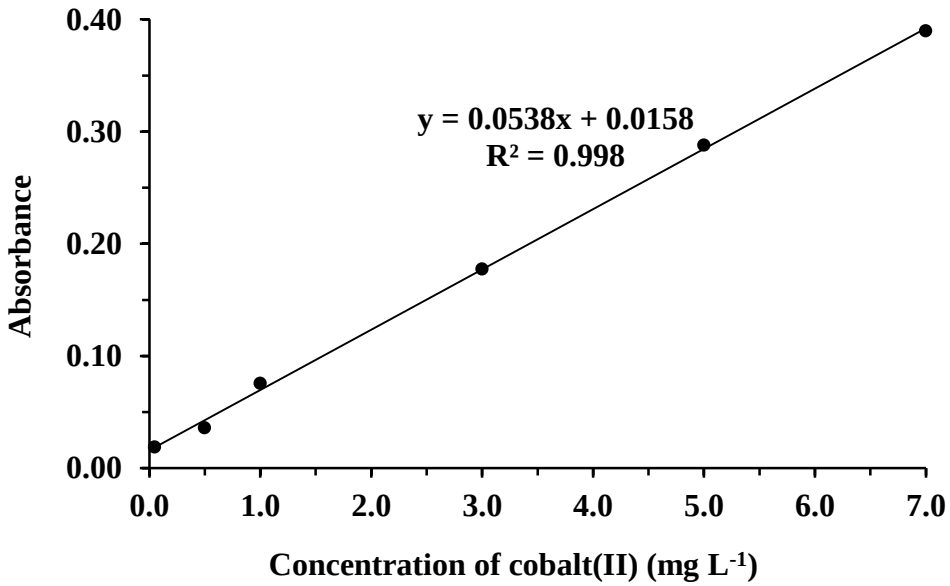


Figure 4.19 Calibration graph of the proposed SIA system for Co(II) determination.

4.2.2.2 Precision of the SIA spectrophotometric method

The precision of the proposed method (Table 4.17) was verified as repeatability and reproducibility by measurement the absorbance of 11 replicated covering different concentration of standard Co(II) (0.05, 3.0 and 7.0 mg L^{-1}), under the

optimum conditions listed in Table 4.19. The relative standard deviation of repeatability and reproducibility were calculated and found to be 2.64, 0.26 and 0.08 for repeatability and 4.23, 0.29 and 0.13 for reproducibility, respectively. Indicating that, the proposed SIA method is providing a precision.

Table 4.19 The precision of the method based on repeatability and reproducibility was performed, by 11 replicates of three standard.

Experimental number	Absorbance obtained from the cobalt(II) (mg L^{-1})					
	Repeatability			Reproducibility		
	0.05	3.0	7.0	0.05	3.0	7.0
1	0.011	0.172	0.367	0.011	0.171	0.365
2	0.010	0.171	0.367	0.011	0.171	0.366
3	0.011	0.172	0.367	0.011	0.171	0.366
4	0.011	0.172	0.367	0.011	0.172	0.365
5	0.011	0.171	0.367	0.011	0.172	0.365
6	0.011	0.171	0.367	0.012	0.171	0.365
7	0.011	0.172	0.367	0.012	0.171	0.365
8	0.011	0.172	0.367	0.012	0.172	0.365
9	0.011	0.172	0.366	0.012	0.171	0.365
10	0.011	0.172	0.367	0.011	0.172	0.366
11	0.011	0.172	0.367	0.011	0.172	0.366
Mean	0.011	0.172	0.367	0.0114	0.172	0.365
SD	0.0003	0.0004	0.0003	0.0005	0.0005	0.0005
%RSD	2.64	0.26	0.08	4.23	0.29	0.13

4.2.2.3 Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of the propose method were determined as the concentration of the analysis leading to three

times and ten time of the standard deviation of blank, respectively. It was found that the LOD and LOQ of the proposed method were 0.0248 mg L^{-1} and 0.0827 mg L^{-1}, respectively. Therefore the method is giving a high sensitivity

4.2.2.4 Effect of interferences

The effect of some possible interferences might be formed a complex with on the determination of 1.0 mg L^{-1} Co(II) in vitamin B complex was investigated and summarized in Table 4.20, the tolerance limits was determined for a maximum error of less than ±5% . It was seen that that, most of interferences ions did not interfere, except 10.0 mg L^{-1} of Cu(II) and Ni(II). However, Cu(II) and Ni(II) not present in medicine samples. Therefore, Cu(II) and Ni(II) can be considered to be no interfered.

Table 4.20 Effect of interference ions on the determination of 1.0 mg L^{-1} Co(II). The tolerance limits was determined for a maximum error of less than ±5%

Interference ions	**Tolerance (mg L^{-1})**
Ca(II), Mg(II)	300
Hg(II), Zn(II), Pb(II), As(II), Cd(II)	200
Fe(III),Fe(II)	20
Cu(II), Ni(II)	10

4.2.2.5 Determination of Co(II) in vitamin B complex

The proposed SIA spectrophotometric method was applied to determination of Co(II) in vitamin B complex which were purchased from a pharmaceutical Mahasarakham province. The contents of Co(II) in each samples were analyzed and calculated against standard calibration curve. The results were given in Table 4.19. The amount of Co(II) in vitamin B complex were found in the range of 0.091-0.404 mg L^{-1}. The percentage recoveries for determination of Co(II) in vitamin B complex were in the range 88.10-101.40. It also found that the amount of Co(II) content in vitamin B complex agreed well with the medicine label.

Table 4.21 The amount of cobalt(II) in medicine samples determinationed by the proposed SIA spectrophotometric medthod comparison to the standard FAAS method.

Samples	Co(II) added (mg L^{-1})	The amount of Co(II) found (mgL^{-1}) in SIA	Recovery (%)	The amount of Co(II) found (mgL^{-1}) in FAAS	Recovery (%)
Neuvipex	-	0.404 ± 0.017	94.23	0.397 ± 0.027	95.60
	1.0	1.346 ± 0.034		1.353 ± 0.001	
Neurobion	-	0.236 ± 0.001	88.10	0.339 ± 0.028	96.95
	1.0	1.117 ± 0.017		1.308 ± 0.047	
Sambee	-	0.115 ± 0.017	98.93	0.126 ± 0.027	93.05
	1.0	1.104 ± 0.030		1.056 ± 0.027	
T-man	-	0.103 ± 0.007	101.40	0.087 ± 0.001	102.73
	1.0	1.116 ± 0.017		1.114 ± 0.027	
Vitamin B	-	0.091 ± 0.001	90.50	0.126 ±0.027	94.99
	1.0	0.996 ±0.001		1.075 ± 0.001	

CHAPTER 5

CONCLUSIONS

5.1 Determination of Co(II) in medicine samples by flow injection spectrophotometric method

A flow injection spectrophotometric procedure for determination of Co(II) based on forming a complex with nitroso-R salt had been accomplished. A sample or standard solution was injection into a reagent stream of 0.1 % w/v nitroso-R salt at pH 6.5. The absorption of Co(II)-nitroso-R salt was measured at 500 nm. Several factors influencing the absorbance were optimized using a univariate method. The optimum conditions were summarized in Table 4.9. The linear calibration graph over the range of 0.05-2.0 mg L^{-1} of Co(II) was established with a regression equation: y = 0.0911x - 0.0076 and the correlation coefficient of 0.997. The limit of detection was 9.0×10^{-4}mg L^{-1}, and the limit of quantitation was 3.0×10^{-2}mg L^{-1} of Co(II). The reproducibility and repeatability calculated from 11 replicate injection of 0.05, 1.0 and 2.0 mg·L^{-1} Co(II) standard solution, reproducibility were less than 3.0%. The accuracy of method presented as the percentage recovery were in the range of 89.01 to 109.23%. The proposed FI system has been satisfactorily applied to determination of Co(II) in Vitamin B complex. The amount of Co(II) ion in medicine samples determined by such system were in the range of 0.083 to 0.379 mg L^{-1}.

5.2 Determination of Co(II) in medicine samples by sequential injection spectrophotometric method

The sequential injection analysis procedure for Co(II) determination base on complex with nitroso-R salt has been developed, the resulting of complex Co(II)-nitroso-R salt was measured at 500 nm. Optimum conditions for determining Co(II) were investigated by univariate method and the optimum conditions are summarized in Table 3.33.A linear calibration graph was over the ranges of 0.05-7.0 mg L^{-1} with a regression equation: y = 0.0538x + 0.0158 with the correlation coefficient of 0.998. The limit of detection was 0.0173mg L^{-1}, and the limit of quantitation was 0.0575 mg L^{-1} of

cobalt(II). The reproducibility and repeatability calculated from 11 replicate injection of 0.05, 3.0 and 7.0 mg·L^{-1} Co(II) standard solution, reproducibility were less than 3.0%. The accuracy of method presented as the percentage recovery were in the range of 88.10to 101.40%. The proposed FI system has been satisfactorily applied to determination of cobalt(II) in medicine samples. The amount of Co(II) ion in vitamin B complex determined by such system were in the range of 0.091 to 0.0404 mg L^{-1}.

5.3 The content of Co(II) found in medicine samples analyzed by the proposed FIA and SIA system comparison with the standard FAAS method

A comparison of the analytical characteristics between flow injection analysis and sequential injection analysis. The results obtained by flow injection method offers a better analytical performance in term of sensitivity. The FIA system is more sensitivity with a lower detection limit (0.0013 mg L^{-1}) and reproducibility (<1.0) than the SIA system which has a detection limit of 0.0173 mg L^{-1} and reproducibility less than 4.3. Nevertheless, the SIA system method is lower consumption of reagent than the FIA system and minimum waste production.

The amount Co(II) contents in vitamin B complex determined by two proposed methods (FIA and SIA) had been compared with those obtained by FAAS method using statistic ANOVA. The result are given in Table 5.1. It was found that the result obtained by the proposed FIA and SIA method were not significant different with those obtained by FAAS, owing to the calculated One-way ANOVA (Sig.□ 0.05, n=3) was less than the theoretical value at confident level of 95%.

Table 5.1 Comparative determination of Co(II) in vitamin B complex by FIA, SIA and standard FAAS method.

Sample	The amount of cobalt found (mgL^{-1})			
	FIA	**SIA**	**AAS**	**Label**
1.Neuvipex	0.38 ± 0.01	0.40 ± 0.02	0.40 ± 0.03	0.430
2.Neurobion	0.25 ± 0.01	0.34 ± 0.02	0.33 ± 0.03	0.350
3.Sambee	0.10 ± 0.01	0.12 ± 0.02	0.13 ± 0.03	0.110
4.T-man	0.10 ± 0.01	0.10 ± 0.02	0.09 ± 0.00	0.080
5. vitamin B	0.08 ± 0.01	0.09 ± 0.00	0.13 ± 0.03	0.130

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APPENDICES

APPENDIX A

Appemdix A1: Diagram of FIA

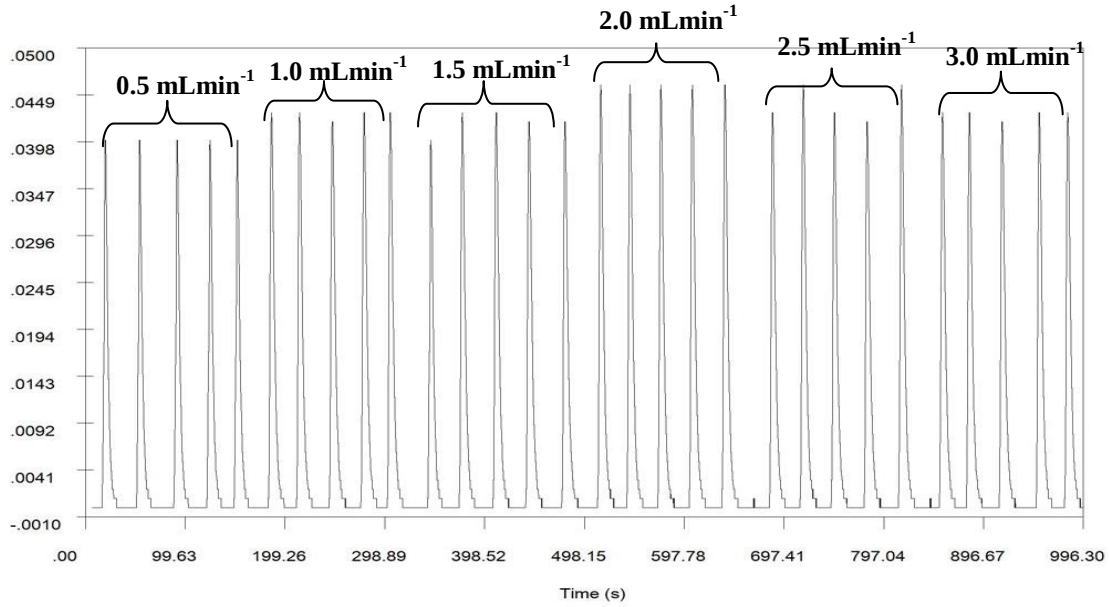


Figure A1 Analytical signal of flow rate over the range 0.5-3.0 mL min^{-1} of Co(II)-nitroso-R salt complex under conditions: 1.0 mg L^{-1} of Co(II) , 0.1% w/v of nitroso-R salt, 300 µL of sample volume and 40 cm of reaction coil length.

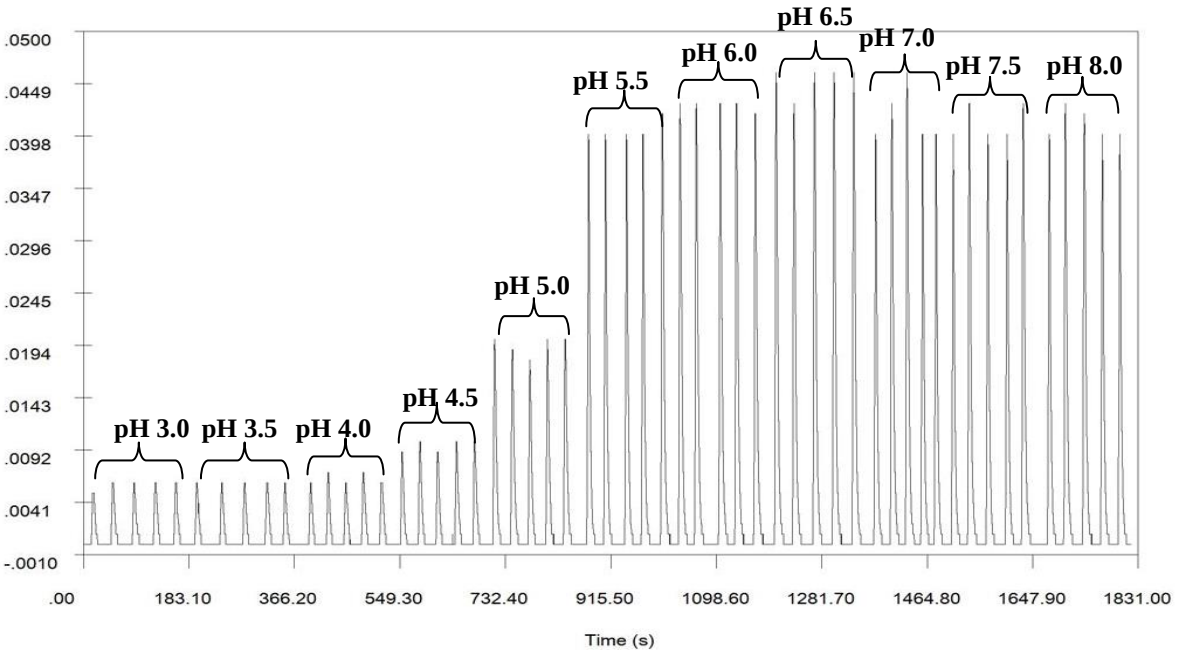


Figure A2 Analytical signal of pH in the range of 3.0-8.0 of Co(II)-nitroso-R salt complex under conditions: 1.0 mg L^{-1} of Co(II) , 0.1% w/v of nitroso-R salt, 300 µL of sample volume, 40 cm of reaction coil length and 2.0 mL min^{-1} of flow rate.

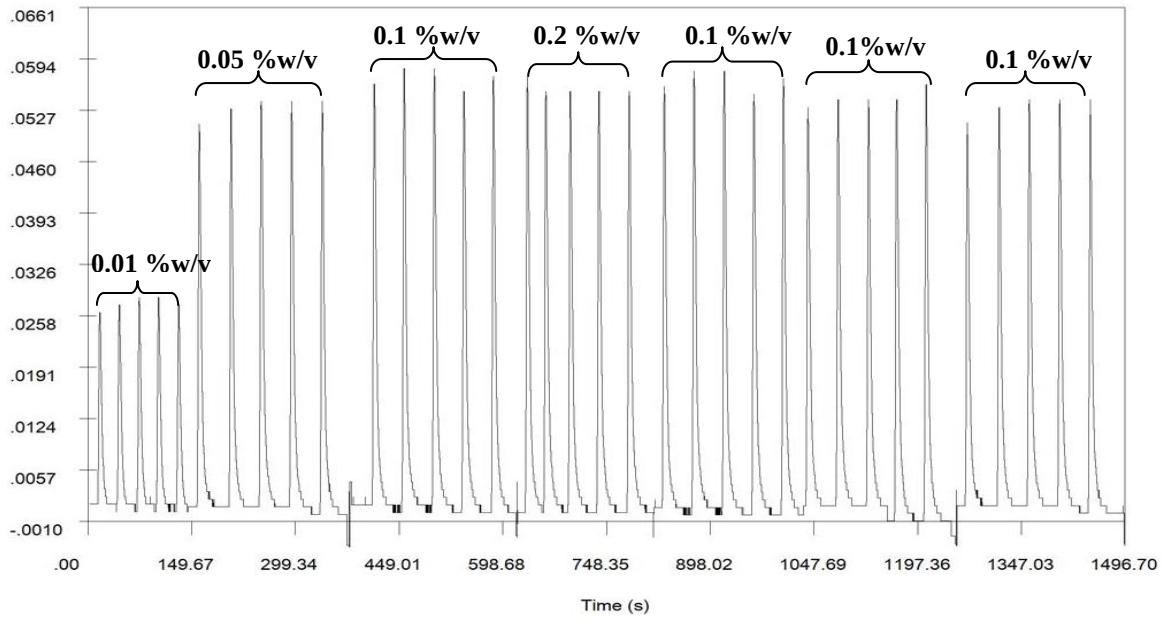


Figure A3 Analytical signal of nitroso-R salt concentration over the range 0.01-0.5 %w/v of Co(II)-nitroso-R salt complex under conditions: 1.0 mg L^{-1} of cobalt(II), 300 µL of sample volume and 40 cm of reaction coil length.

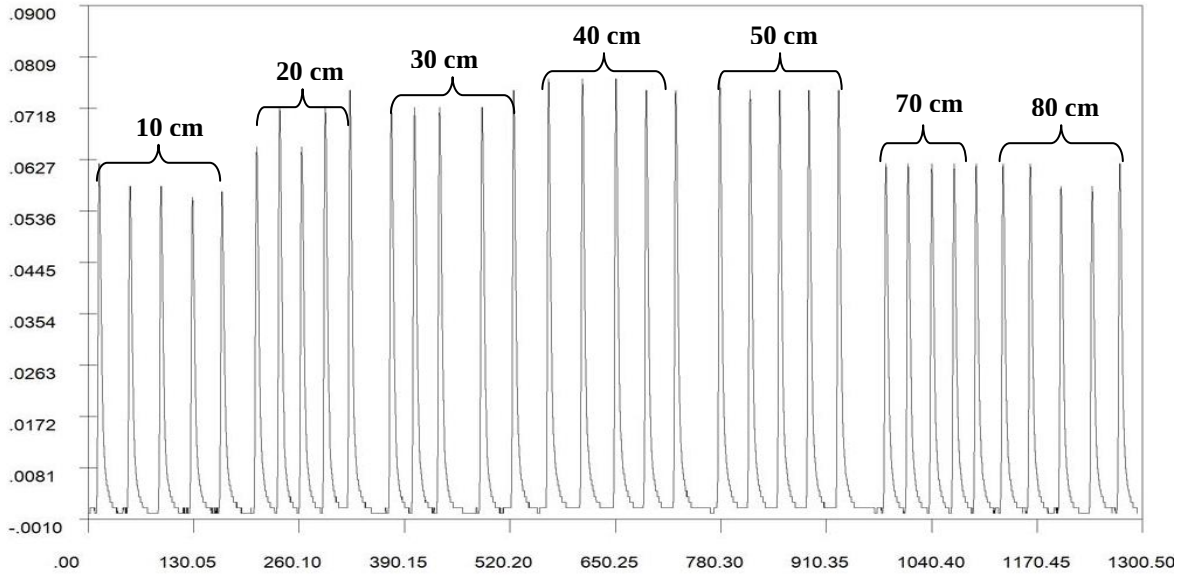


Figure A4 Analytical signal of mixing coil length over the range 10-70 cm of Co(II)-nitroso-R salt complex under conditions: 1.0 mg L^{-1} of Co(II) , 0.1% w/v of nitroso-R salt (pH 6.5), 300 µL of sample volume, 40 cm of reaction coil length and 2.0 mL min^{-1} of flow rate.

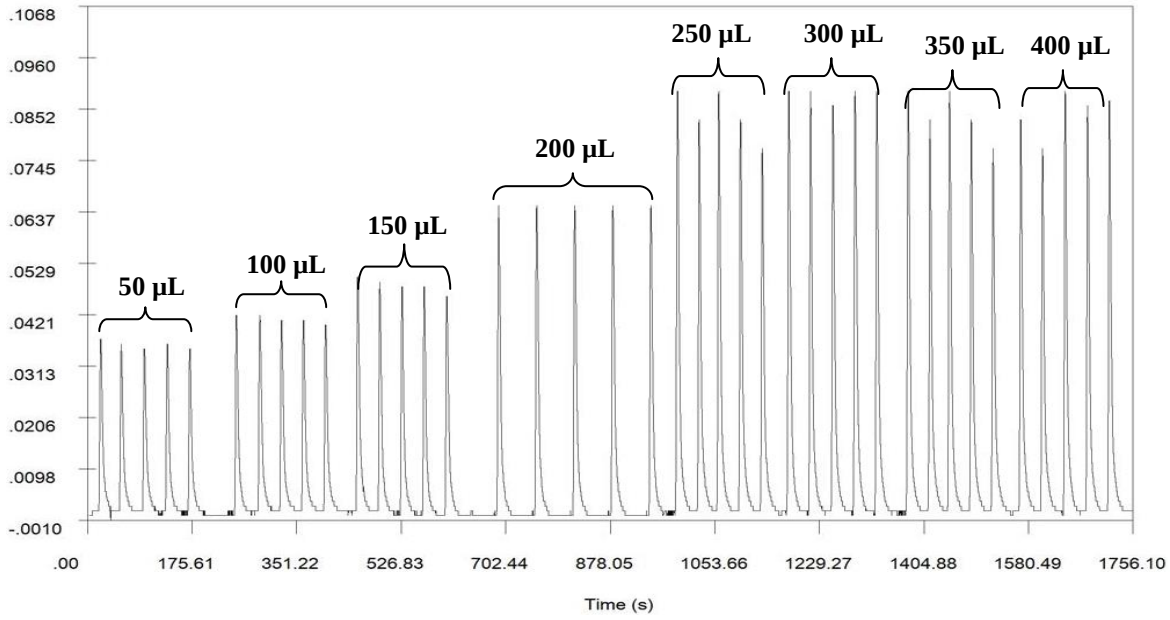


Figure A5 FIA gram of sample volume 50-400 µL of Co(II)-nitroso-R salt complex under under conditions: 1.0 mg L^{-1} of Co(II) , 0.1% w/v of nitroso-R salt (pH 6.5), 40 cm of reaction coil length and 2.0 mL min^{-1} of flow rate.

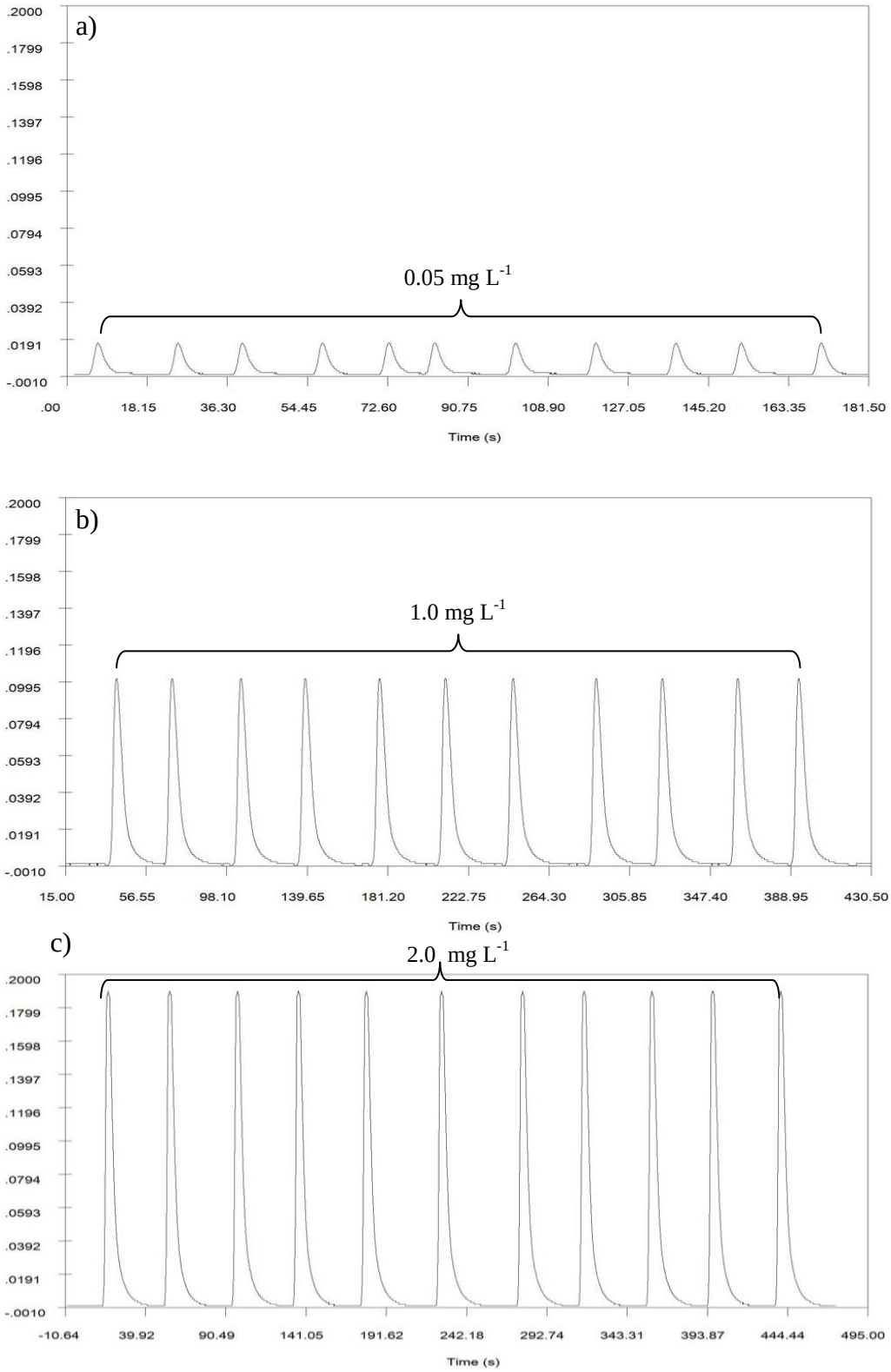


Figure A6 FIA gram of precision expressed as the relative standard deviation of repeatability; a) 0.05 mg L^{-1}, b) 1.0 mg L^{-1}, c) 2.0 mg L^{-1} of Co(II).

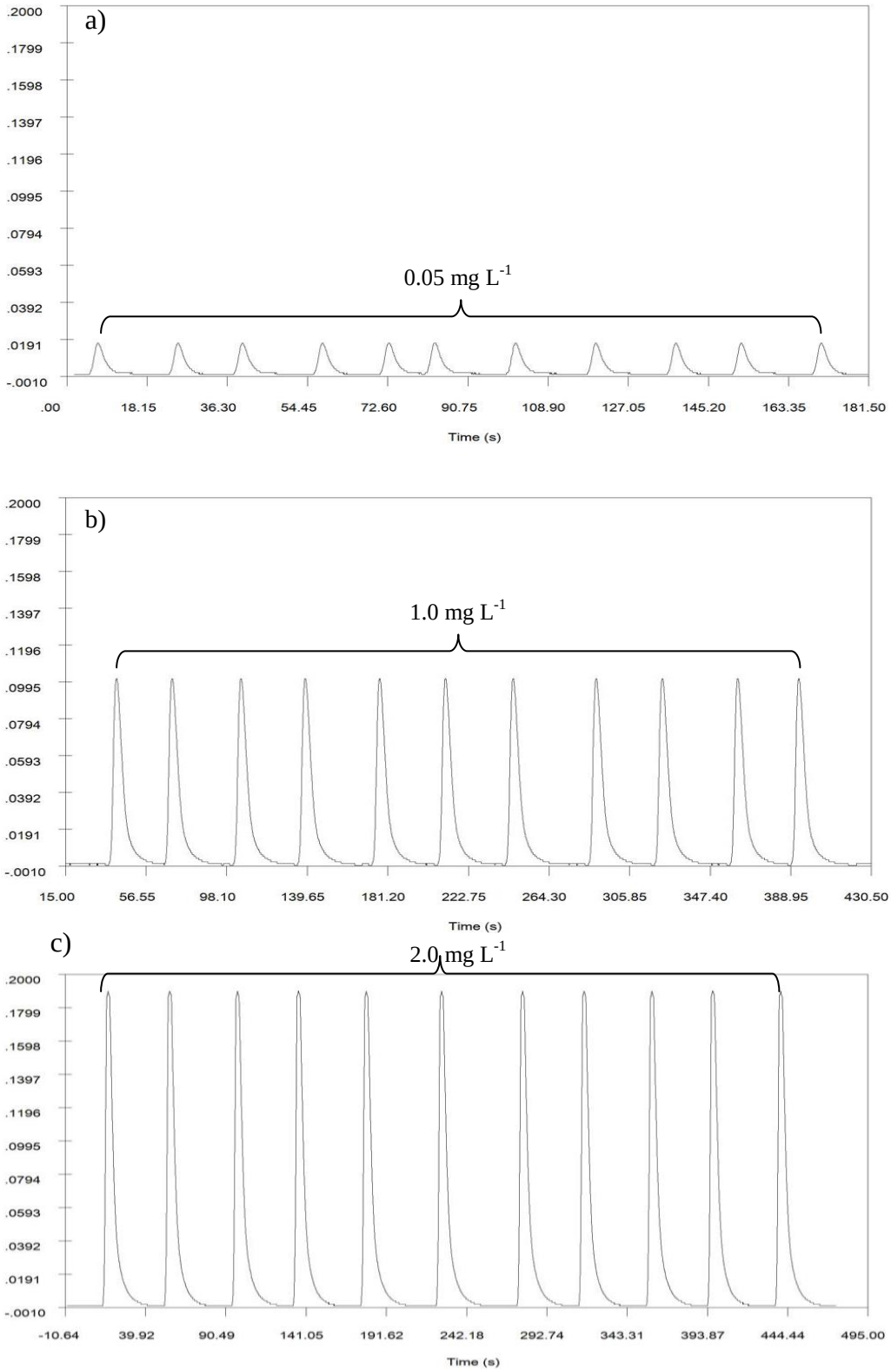


Figure A7 FIA gram of precision expressed as the relative standard deviation of reprocibility; a) 0.05 mg L^{-1}, b) 1.0 mg L^{-1}, c) 2.0 mg L^{-1} of Co(II)

Diagram of SIA

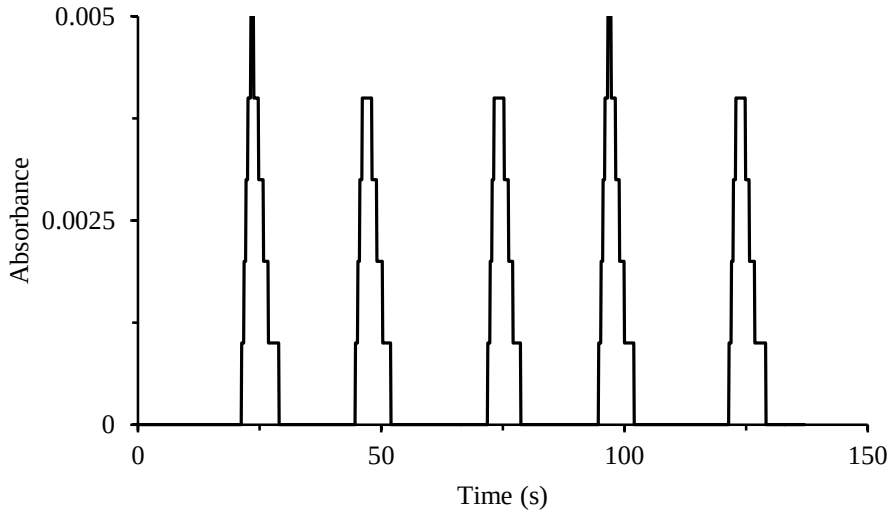


Figure A8 SIA gram of blank

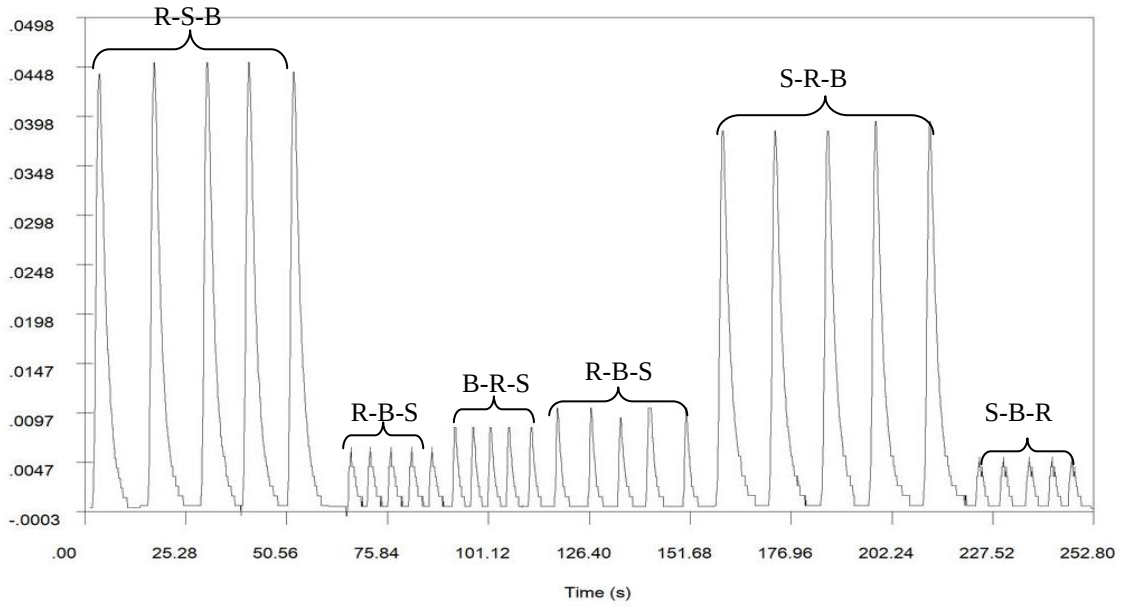


Figure A9 SIA gram of aspiration sequence on the absoebance of Co(II)-nitroso-R salt complex, under the experimental conditions; 1.0 mg L^{-1} of Co(II), 0.1 %w/v of nitroso-R salt.

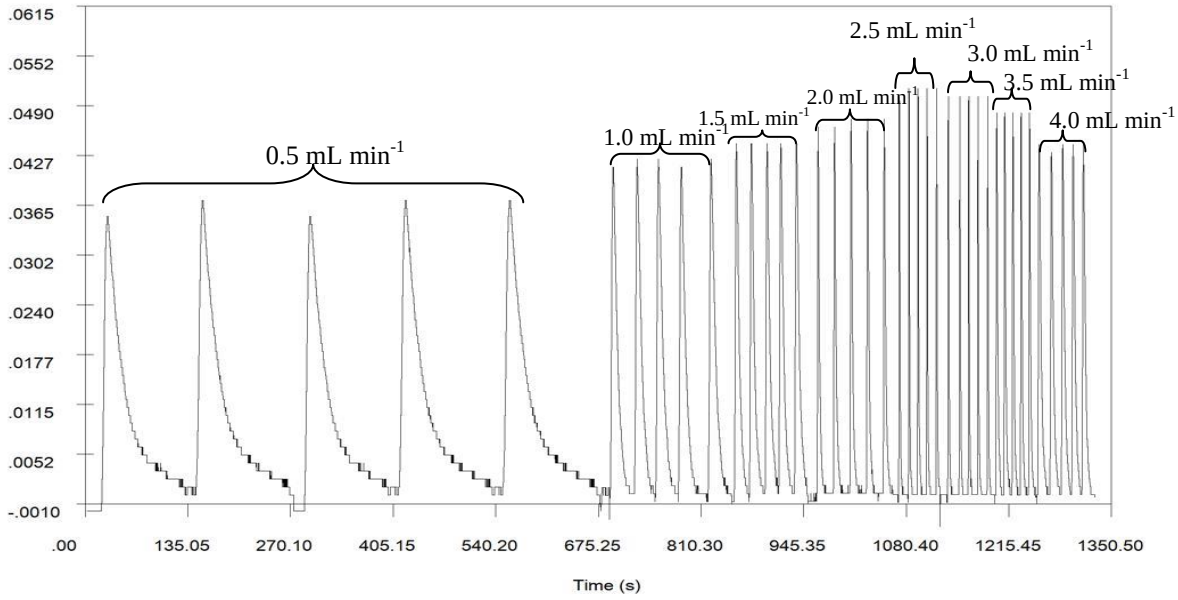


Figure A10 SIA gram of flow rate on the absorbance of Co(II)-nitroso-R salt complex, under the experimental conditions; 1.0 mg L^{-1} of Co(II), 0.1 %w/v of nitroso-R salt

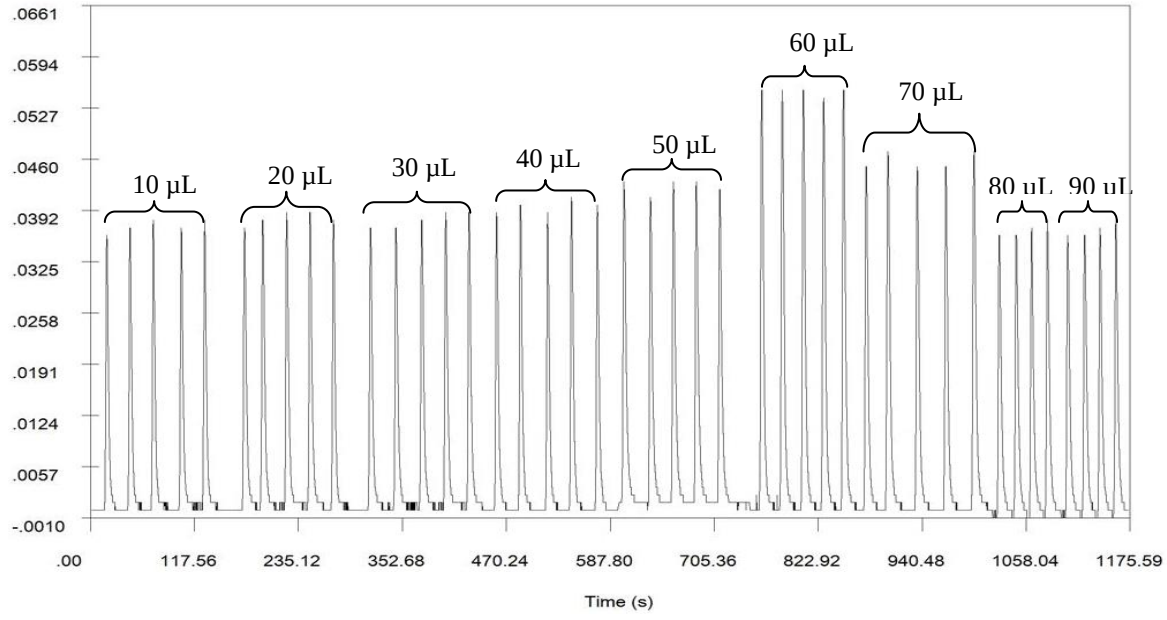


Figure A11 SIA gram of buffer aspiration volume on the absorbance of Co(II)-nitroso-R salt complex, under the experimental conditions; 1.0 mg L^{-1} of Co(II), 0.1 %w/v of nitroso-R salt and 2.5 $mLmin^{-1}$of flow rate.

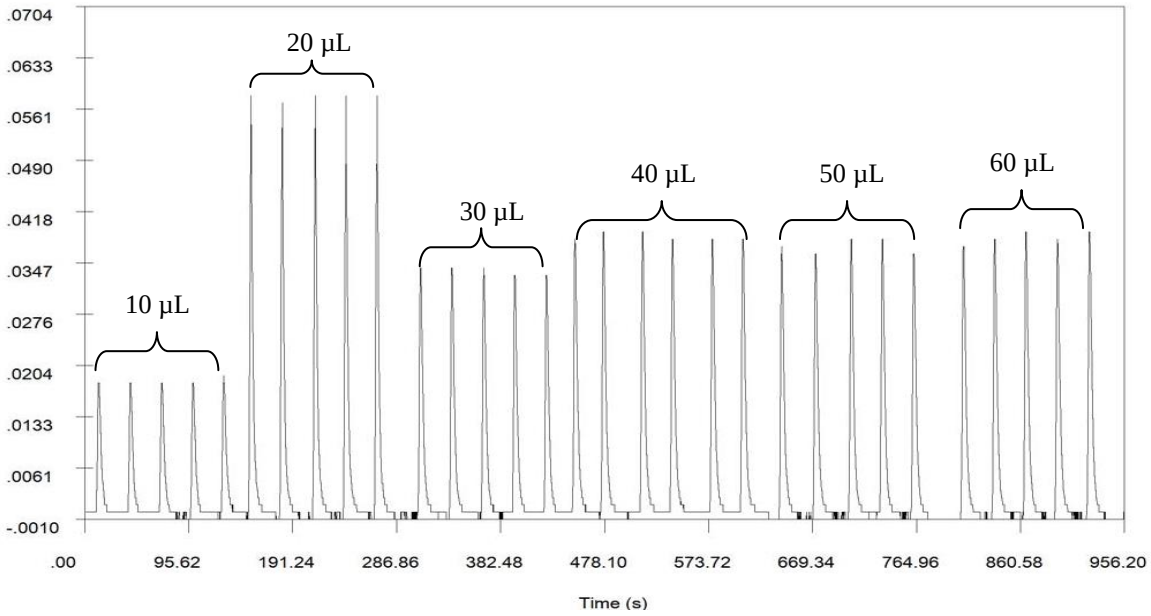


Figure A12 SIA gram of nitroso-R salt aspiration volume on the absoebance of Co(II)-nitroso-R salt complex, under the experimental conditions; 1.0 mg L^{-1} of Co(II), 0.1 %w/v of nitroso-R salt and 2.5 $mLmin^{-1}$of flow rate.

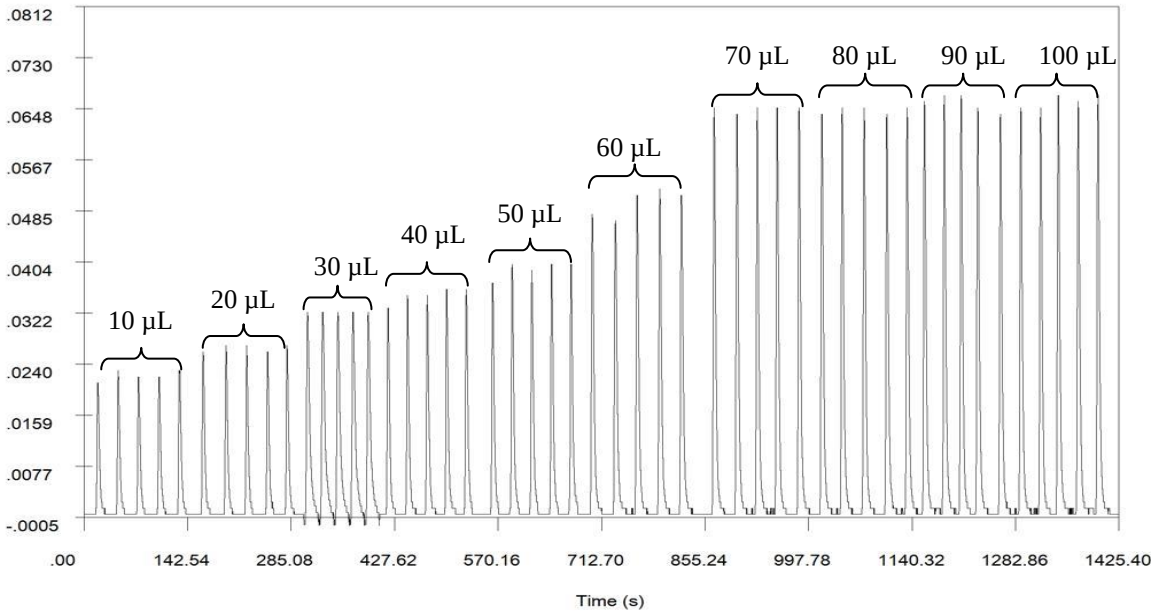


Figure A13 SIA gram of sample aspiration volume on the absoebance of cobalt(II)-nitroso-R salt complex, under the experimental conditions; 1.0 mg L^{-1} of cobalt(II), 0.1 %w/v of nitroso-R salt and 2.5 $mLmin^{-1}$of flow rate.

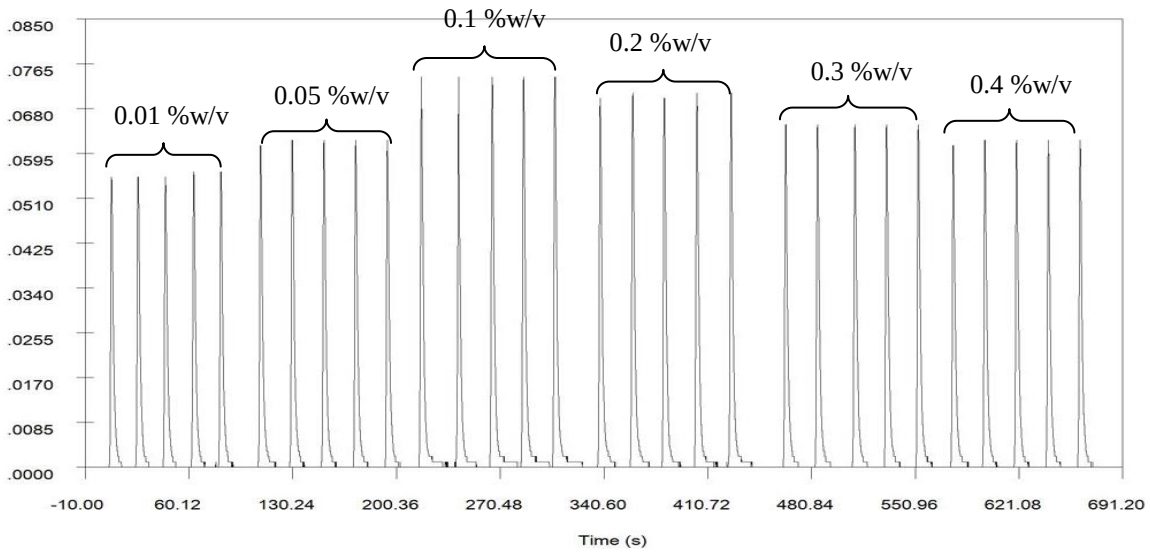


Figure A14 SIA gram of nitroso-R salt concentration on the absoebance of Co(II)-nitroso-R salt complex, under the experimental conditions; 1.0 mg L^{-1} of Co(II), 0.1 %w/v of nitroso-R salt and 2.5 $mLmin^{-1}$of flow rate.

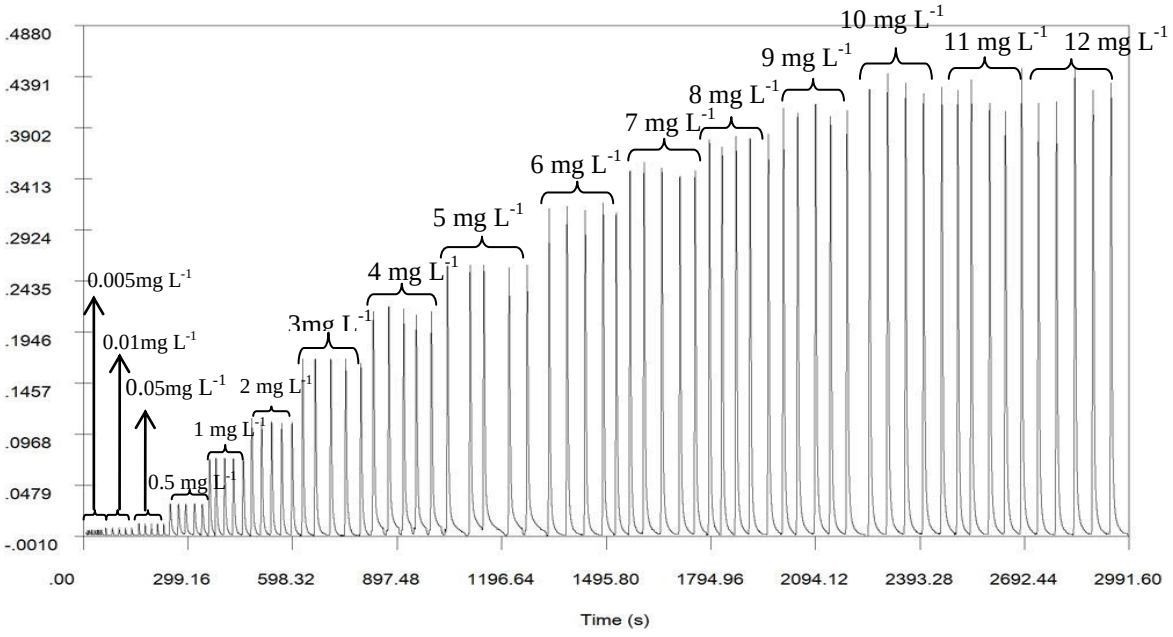


Figure A15 SIA gram of concentration of Co(II)-nitroso-R salt complex at Co(II) concentration 0.005-12.0 mg L^{-1}.

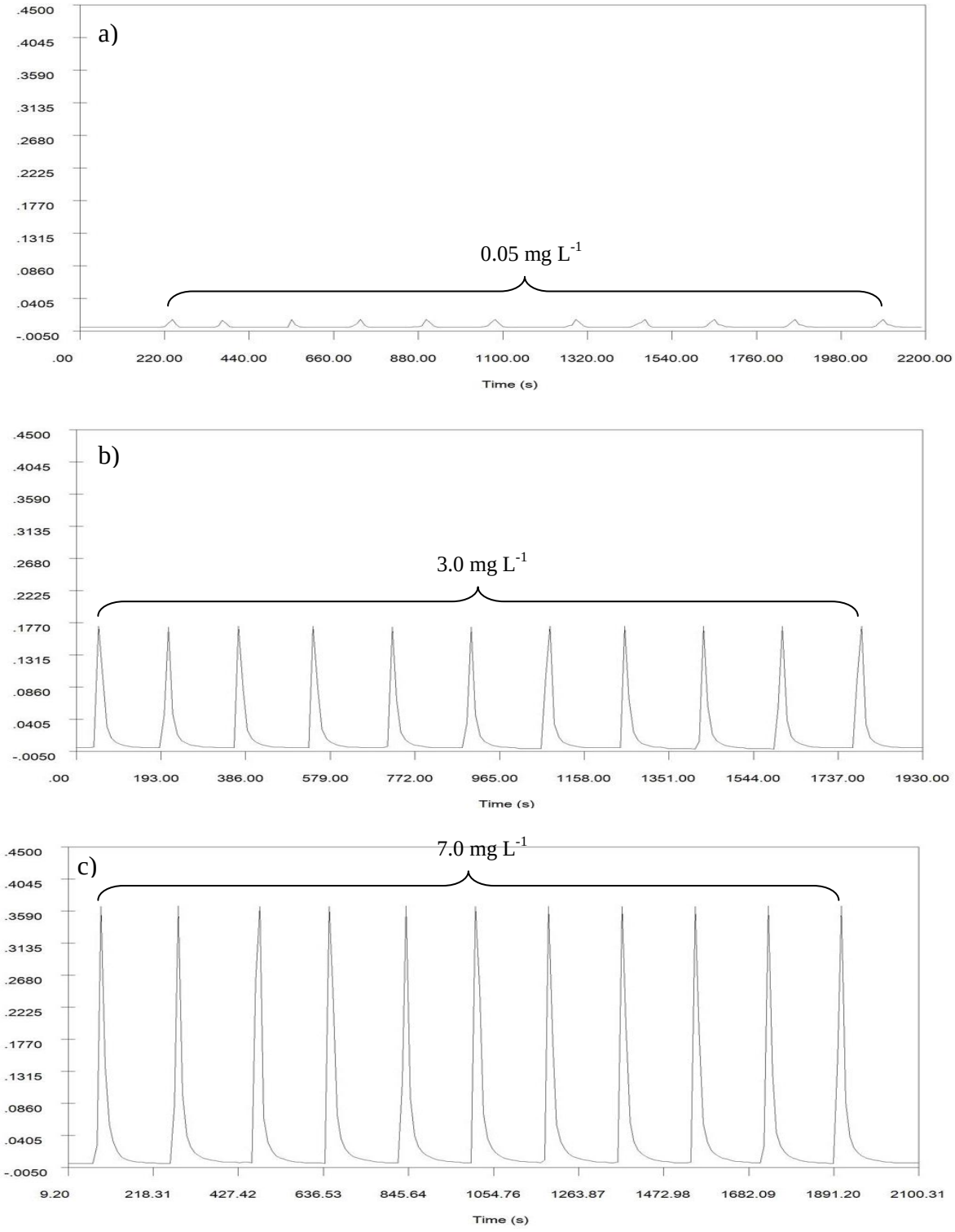


Figure A16 SIA gram of precision expressed as the relative standard deviation of repeatability; a) 0.05 mg L^{-1}, b) 1.0 mg L^{-1}, c) 7.0 mg L^{-1} of Co(II).

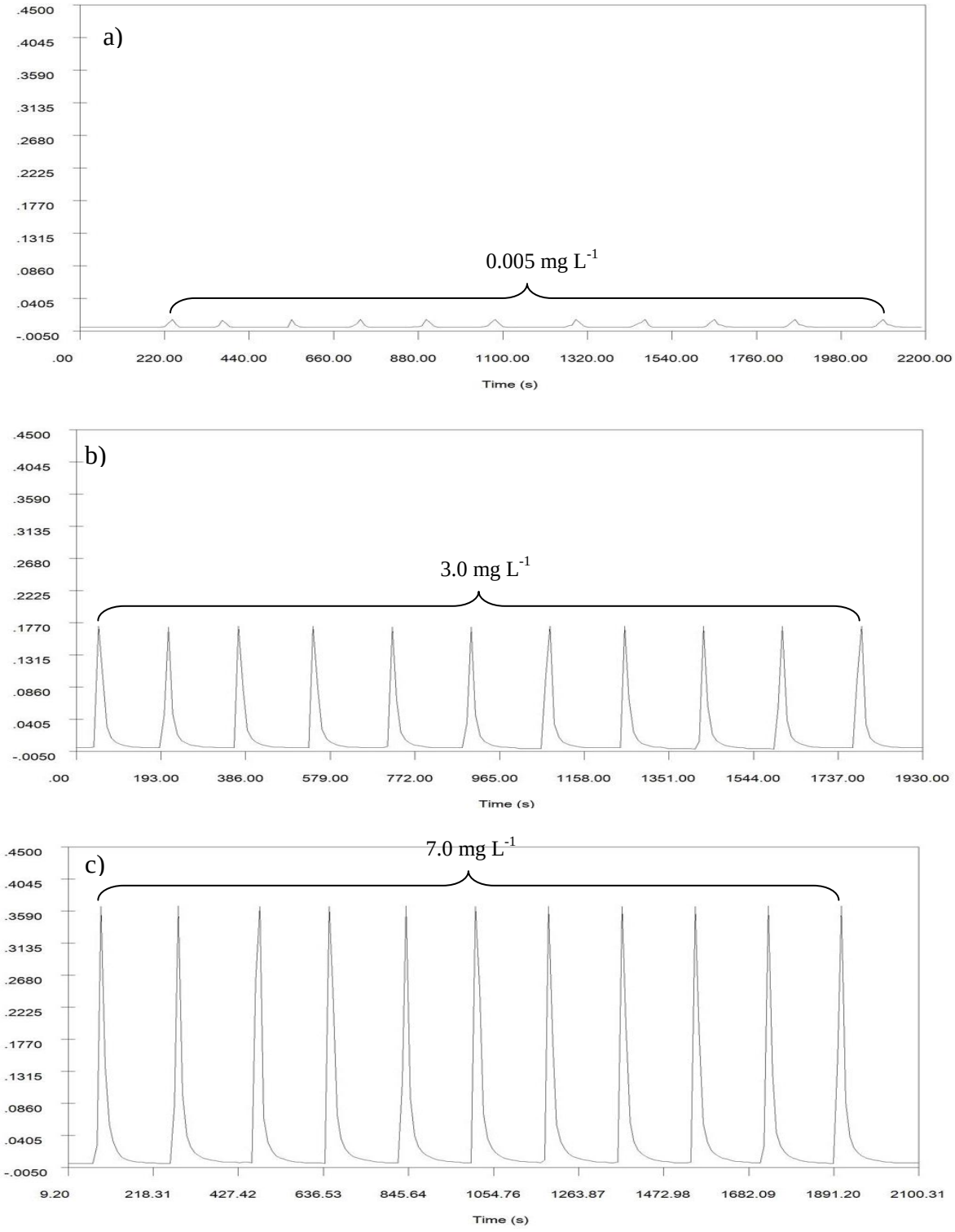


Figure A17 SIA gram of precision expressed as the relative standard deviation of reproducibility; a) 0.05 mg L^{-1}, b) 1.0 mg L^{-1}, c) 7.0 mg L^{-1} of Co(II).

APPENDIX B

APPENDIX B

B1. Precision

$$\%RSD = \frac{SD \times 100}{\overline{X}}$$

When %RSD = percentage relative standard deviation

SD = standard deviation

$\overline{X}$ = mean average

B1.1 Example

Experimental number	Absorbance obtained from the cobalt(II) (mg L^{-1})					
	Repeatability			Reproducibility		
	0.05	3.0	7.0	0.05	3.0	7.0
1	0.011	0.172	0.367	0.011	0.171	0.365
2	0.010	0.171	0.367	0.011	0.171	0.366
3	0.011	0.172	0.367	0.011	0.171	0.366
4	0.011	0.172	0.367	0.011	0.172	0.365
5	0.011	0.171	0.367	0.011	0.172	0.365
6	0.011	0.171	0.367	0.012	0.171	0.365
7	0.011	0.172	0.367	0.012	0.171	0.365
8	0.011	0.172	0.367	0.012	0.172	0.365
9	0.011	0.172	0.366	0.012	0.171	0.365
10	0.011	0.172	0.367	0.011	0.172	0.366
11	0.011	0.172	0.367	0.011	0.172	0.366
Mean	0.011	0.172	0.367	0.0114	0.172	0.365
SD	0.0003	0.0004	0.0003	0.0005	0.0005	0.0005
%RSD	2.64	0.26	0.08	4.23	0.29	0.13

$$\%RSD = \frac{SD \times 100}{\overline{X}}$$

$$\%RSD = \frac{0.0003 \times 100}{0.011}$$

$$= 2.64$$

B2. The detection limit and quantitation limit of FIA method

The detection limit and quantitation limit can be detected and calculated from equation as 3.2 and 3.3

$$LOD = 3SD$$

$$LOQ = 10SD$$

B2.1 Example

No	**Absorbance**	**Y = 0.0911x + 0.0076**
1	0.008	0.003
2	0.008	0.003
3	0.008	0.003
4	0.008	0.003
5	0.008	0.003
6	0.008	0.003
7	0.008	0.003
8	0.008	0.003
9	0.008	0.003
10	0.008	0.002
11	0.008	0.002
Mean		0.003
SD		0.0004

LOD;

$$LOD = 3SD$$

$$= 3\times 0.0004$$

$$= 0.0012 \text{ mg } L^{-1}$$

LOQ;

$$LOQ = 10SD$$

$$= 10 \times 0.0004$$

$$= 0.004 \text{ mg } L^{-1}$$

B3. Accuracy

The percentage error and percentage recoveries, which can be calculated from the equation as follows;

$$\%\text{Recovery} = \frac{|\text{Deteced valve} - \text{Found valve}|}{\text{Spiked Ascorbic Acid concentration}} \times 100$$

B3.1 Example

Co(II) add ($mg \cdot L^{-1}$)	**Absorbance**				**Found ($mg \cdot L^{-1}$)**	**Recovery (%)**
	1	**2**	**3**	**Average**		
0	0.013	0.014	0.014	0.014	0.404	100.0%
1.0	0.041	0.039	0.041	0.040	1.346	94.23%

$$\%\text{Recovery} = \frac{|\text{Deteced valve} - \text{Found valve}|}{\text{Spiked Ascorbic Acid concentration}} \times 100$$

$$\%\text{Recovery} = \frac{|1.346 - 0.404|}{1.0} \times 100$$

% Recovery = 94.23

B3. The student Oneway

Table B3.1 Descriptives

Neuvipex

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
AAS	3	.3973	.03349	.01933	.3141	.4805	.38	.44
FIA	3	.3790	.01100	.00635	.3517	.4063	.37	.39
SIA	3	.4040	.02078	.01200	.3524	.4556	.38	.42
Total	9	.3934	.02333	.00778	.3755	.4114	.37	.44

Table B3.2 Test of Homogeneity of Variances

Neuvipex

Levene Statistic	df1	df2	Sig.
3.599	2	6	.094

Table B3.3 ANOVA

Neuvipex

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	2	.001	.901	.455
Within Groups	.003	6	.001		
Total	.004	8			

B4. One coated tablet content of samples

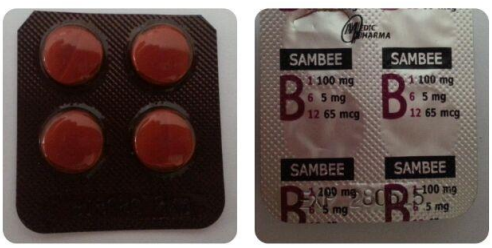
1. Neuvipex :

Thiamine propyl Disulfide (TPD)	25	mg
Vitamin B_2	2.5	mg
Vitamin B_6	25	mg
Vitamin B_{12}	250	µg

2. Neurobion :

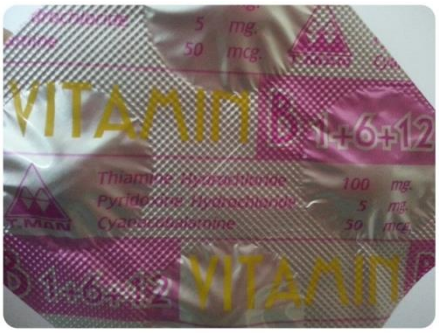
Vitamin B_1	100	mg
Vitamin B_6	200	mg
Vitamin B_{12}	200	µg

4. Sambee :



Vitamin B_1	100	mg
Vitamin B_6	5	mg
Vitamin B_{12}	65	µg

5. T-man



Vitamin B_1	100	mg
Vitamin B_6	5	mg
Vitamin B_{12}	50	µg

6. Vitamin B

Vitamin B_1	100	mg
Vitamin B_6	7.5	mg
Vitamin B_{12}	75	µg

BIOGRAPHY

BIOGRAPHY

Name	Miss Nongkran Duangsin
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Research output

Publication

1. Nongkran Duangsin, Uthai Sakee and Senee Kruanetr. **"Spectrometric method for the determination of cobalt(II) concentration using nitroso-R salt as reagent"** Proceeding of the *9thMahasarakham University Research Conference*. 12th-13thSeptember 2013,Mahasarakham, Thailand. P.728−738.

Conference

1. Nongkran Duangsin, Uthai Sakee and Senee Kruanetr. **"Spectrophotometric Method for Determination of Co(II) using nitroso-R salt as complexing agent"** *Pure and Applied Chemistry International Conference (PACCON 2014),* January 8-10, 2014, The convention hall Centara Hotel & convention Centre, Khon Kaen, Thailand. P. 112.

2. Nongkran Duangsin, Uthai Sakee and Senee Kruanetr. **"A simple spectrophotometric method for the determination Cyanocobalamin using nitroso-R salt as reagent"** *Pure and Applied Chemistry International Conference (PACCON 2012),* January 11-13, 2012, The empress convention center, Chiang Mai, Thailand. P. 069.

3. Nongkran Duangsin, Uthai Sakee and Senee Kruanetr.

"Spectrophotometric Method for Determination of Vitamin B_{12} Using Nitroso-R salt"

PERCH-CIC CONGRESS VII Theme: Towards a Sustainable Future Conference 2011, May 4-7, 2011, Jomtien Palm Beach Hotel & Resort Pattaya, Chonburi, Thailand. P. 68.