CHAPTER 1

INTRODUCTION

1.1 Calcium, magnesium, iron, zinc and ethanol

1.1.1 Importance for the determination of calcium, magnesium, iron, zinc and ethanol

Calcium (1)

Calcium is an essential mineral for human body. Most of calcium is used in forming and maintaining bones and teeth. It also has important roles in blood and maintaining heartbeat rhythm. There are many sources of calcium such as dairy products (milk and milk product), non-dairy product (broccoli, sardines, oysters and kale) and supplements (calcium carbonate and calcium citrate).

Calcium is an important mineral for human body especially women. It reduces the risk of osteoporosis, the increased porosity or softening of bone, which usually occur in mature and postmenopausal women. However, too much of calcium consumption can cause the problems. The effects of calcium overdose are renal damage and the deposit of calcium in other areas of the body besides the bones. It can contribute to stone formation for people who are at risk of developing kidney stones.

Magnesium (2)

Magnesium is a mineral needed for formation of bone, protein, ATP and fatty acid, making new cells, activating B vitamins, relaxing muscles and clotting blood. The secretion and action of insulin also require magnesium. Magnesium also improves our natural immunity and protects the cells against a range of diseases.

The important roles of magnesium are maintaining normal muscle and nerve function, keeps heart rhythm steady, and bones strong. It is also involved in energy metabolism and protein synthesis. Green vegetables such as spinach provide magnesium because the center of the chlorophyll molecule contains magnesium. Nuts, seeds, and some whole grains are also good sources of magnesium (1).

Magnesium supplementation is usually suggested when a specific health problem or condition causes an excessive loss of magnesium or limited magnesium absorption. People who take some medicine (diuretic, antibiotic, and cancer treated medicine) have the increased loss of magnesium in urine. Besides, a gastrointestinal system disorders may cause a loss of magnesium or limited magnesium absorption (3).

Furthermore, $Mg(OH)_2$ is an active ingredients in antacids for neutralize the digestive acids. However, too much magnesium consumption will result in diarrhea. Magnesium toxicity is often associated with kidney failure that is when the kid5ney loses the ability to remove excess magnesium. This is why the determination of mgnesium in drug and magnesium supplements is important.

Iron (1)

Iron is essential for human health. It is required for the synthesis of the iron-porphyrin proteins hemoglobin, myoglobin, cytochromes, and cytochrome oxidase. It is carried in the blood bound to the plasma protein transferrin, and in the tissues it is stored in the form of ferritin, an iron-protein containing ferric hydroxide and ferric phosphate.

Iron is found in organ meats such as liver, kidney and heart, in egg yolks, dried pea and shellfish (4) In addition, fortification of food, e.g. flour, with iron is widespread. Supplemental iron is also taken by women during pregnancy and lactation.

Iron is not excreted in the urine but is lost from the body via the bile and feces and in menstrual blood. Because the rate of loss of iron from the body is doubled or tripled during menstruation, women require larger amounts of iron than men. Deficiency of iron leads to iron-deficiency anemia, in which the number of red blood cells is normal but the amount of hemoglobin in the cells is relatively low. It may be associated with a poor diet, or bleeding. Dietary supplements of iron can be administered to help alleviate this condition. The dosage of iron must be considered because high iron content in the body has been linked to cancer and heart disease (5). The assay of iron in pharmaceutical products is extremely important.

Zinc(4, 6)

Zinc is an essential trace element for human body. It is necessary for the formation of various metal-dependent enzymes, such as carboxypeptidase, collagenase, dipeptidase, alkaline phosphatase, carbonic anhydrase, phospholipase C, neutral protease. Zinc supports a healthy immune system, helps to maintain sense of

taste and smell, and is needed for DNA synthesis. Zinc also supports normal growth and development during pregnancy, childhood, and adolescence. Zinc is abundant in a wide variety of foods such as oysters, cereal, meat, beans and nuts.

The risk factors of zinc deficiency are inadequate intake and digestive diseases. Zinc is a part of several liver enzymes, one of which is important in the oxidation of alcohol to less toxic substances. A high level of alcohol in the body may cause the enzyme to break down, thus producing toxic conditions in the liver. Alcoholics having cirrhosis of the liver show a high level of zinc in the urine. Zinc toxicity has been seen in both acute and chronic forms. Intake of 150 to 450 mg of zinc per day have been associated with low copper status, altered iron function, reduced immune function and reduced levels of high density lipoproteins (the good cholesterol). Zinc salts-mainly $ZnSO_4.7H_2O$ is used as supplements to correct zinc deficiency. Level of zinc relates to change in levels of other minerals in the body, hence, the determination of zinc in supplements was considerable.

Ethanol (7, 8)

Ethanol (ethyl alcohol), CH_3CH_2OH, is a colorless, volatile and flammable liquid with a boiling point of 78.5 ^oC. It has a low melting point of -114.5 ^oC and density is 789 g/L. It is a very weak acid ($Ka = 10^{-16}$), and can lose a proton from the hydroxyl group to form the ethoxide ion $C_2H_5O^-$ (9).

Usefulness of ethanol

Ethanol is widely used as a solvent in laboratory and industry and as a raw material for the preparation of the other organic chemicals (10).

It is a powerful disinfectant in medicine and an extraction solvent or carrier both in pharmaceutical and food industry. A solution of 70-85% of ethanol is commonly used as a disinfectant; it kills organisms by denaturing their proteins and dissolving their lipids; it is effective against most bacteria and fungi, and many viruses, but is ineffective against bacterial spores.

It is a high-energy content in alcohol beverage. The amount of alcohol in an alcoholic beverage may be specified in percent alcohol by volume (ABV). Various beverages contain different percent alcohol such as beer (3-6%), wine (10-20%), whiskey (40%), etc. Determination of ethanol in beverages is important for the quality control that gives confidence in product quality. Moreover, the ethanol content has commercial value significance, which is regulate tax of each product.

1.1.2 Methods for the determination of calcium, magnesium, iron, zinc and ethanol

Calcium, magnesium, iron and zinc

Because of the assay of mineral content in pharmaceutical preparations that mentioned was critical. Many of analytical methods for the determination of calcium, magnesium, iron and zinc have been reported (Table 1.1). Among them used as standard methods for the determination of those cation is titrimetry (11) which measure the volume of a reagent reacting stoichiometrically with the analyte (12). However, this technique has a drawback such as tedious, time and reagents consuming.

Various complexing agents have been used to react with cations (calcium, magnesium, iron and zinc), and a complexation product can be detected by a visibility (eye) and an instrumental (colorimeter, spectrophotometer or fluorimeter) analysis. Although it was performed with flow injection analysis to decrease the reagent and time consuming (13-29) but the specific complexing agent are expensive.

From the literature, none of the complexing agent can be used for all of cations (calcium, magnesium, iron and zinc) in the same procedure.

The summary of analytical methods for the determination of calcium, magnesium, iron and zinc using complexing agent is shown in Table 1.1.

Table 1.1 Summary of analytical methods for the determination of calcium, magnesium, iron and zinc using complexing agent

Analyte(s)	Technique	Detail	Characteristic	Ref.
Ca	Complexo metric titration	-Sample: Milk -Titrant: EDTA -Indicator: Murexide		(13)
		-Sample: Slags -Titrant: EDTA -Indicator: 1-(2-Pyridylazo)-2-naphthol		(14)
		-Sample: Water -Titrant: EDTA -Indicator: 2-[(4-phenyl thioacetic acid)azo]-1,8-dihy droxynapthalene-3,6-disulpho nic acid -λ_{anal} = 495 nm	-Recovery: 100 %	(15)
	Spectro photometry	-Sample: Water -Complexing agent: 4-(2-pyridylazo) resorcinol (PAR) -Using diode-array detector	-LR: 0.10-4.0 μg mL^{-1} -LOD: 0.1 μg mL^{-1}	(16)
	FI-spectro photometry	-Sample: Animal Feeds -Complexing agent: Creosol phthalein complexone (CPC) in 2-amino-2-methylpropan -1-ol (AMP) -Using colorimeter as a detector	-LR: 0.5-5% -Sampling rate: 300 h^{-1}	(17)
		-Sample: Milk -Complexing agent: CPC in AMP -λ_{anal} = 580 nm	-LR: 250-1500 mg L^{-1} -Sampling rate: 180 h^{-1}	(18)
		-Sample: Water, Soil and Plant Materials -Complexing agent : Glyoxal bis(2-hydroxyanil) (GBHA) in ethanol -λ_{anal} = 555 nm	-LR: 0-10 ppm -RSD: <1% -Sampling rate: 180 h^{-1}	(19)

Table 1.1 Summary of analytical methods for the determination of calcium, magnesium, iron and zinc using complexing agent (continued)

Analyte(s)	Technique	Detail	Characteristic	Ref.
Ca	FI-spectro photometry	-Sample: Water -Complexing agent: Zn-EGTA, PAR -λ_{anal} = 505 nm	-LR: 0.8-7.2 mg L^{-1} -Sampling rate: 80 h^{-1}	(20)
		-Sample: Water -Complexing agent: Chlo rophosphonazo III (CPA-III) in HCl -λ_{anal} = 668 nm	-LR: 0.20-2.00 mg L^{-1} -LOD: 0.02 mg L^{-1} -Recovery: 96-100 % -Sampling rate: 200 h^{-1}	(21)
		-Sample: Brine -Complexing agent: 1-(2-hydroxy-4-diethyl amino-1-phenylazo)-2-hydroxynaphthalene-3,6-disulfonic acid in NH_3/NH_4^+ buffer pH 10.5 -λ_{anal} = 530 nm	-LR: 8-120 μg L^{-1} -RSD: 1.8 % (80 μg L^{-}) -LOD: 5 μg L^{-1}	(22)
		-Sample: Mineral Water -Complexing agent: Arsenazo III, Tris buffer pH 8.5 -Using diode-array detector	-LR: 0.2-1.5 μg mL^{-1} -Sampling rate: 50 h^{-1}	(23)
		-Sample: Milk -Complexing agents: CPC in AMP -λ_{anal} = 580 nm	-LR: 0-15 mg L^{-1} -RSD: < 1.1 % -Recovery: 97.0-103.8 % -Sampling rate: 50 h^{-1}	(24)
	FI-titration	-Sample: Tap Water -Titrant: EDTA -Indicator: Hydroxynapthol blue -λ_{anal} = 641nm	-LR: 0.3-0.6 mM -RSD: 0.2-0.5 % -Sampling rate: 85-109 h^{-1}	(25)
	SI-spectro photometry	-Sample: Water, Urine and Pharmaceuticals -Complexing agent: CPC -λ_{anal} = 580 nm	-LR: 0-20 ppm -LOD: 1.4 ppm -Sampling rate: 43 h^{-1}	(26)

Table 1.1 Summary of analytical methods for the determination of calcium, magnesium, iron and zinc using complexing agent (continued)

Analyte(s)	Technique	Detail	Characteristic	Ref.
Ca	Fluorimetry	-Sample: Serum -Complexing agents: Calcein blue -Masking reagent: triethanol amine and N-(2-hydroxy ethyl) iminodiacetic acid -λ_{exit} =405 nm -λ_{emiss}= 455 nm	-LR: 0-15.0 µM -RSD: 1.14 %	(27)
Mg	Complexo metric titration	-Sample: Slags -Titrant: EDTA -Indicator: 1-(2-Pyridylazo)-2-naphthol		(14)
		-Sample: Milk -Titrant: EDTA -Indicator: Palladiazo		(13)
		-Sample: Water -Titrant: EDTA -Indicator: 2-[(4-phenylthio acetic acid)azo]-1,8-dihydro xy napthalene-3,6-disulponic acid (PTAADNDA) -λ_{anal} = 495 nm	-Recovery: 96-101 %	(15)
	Spectro photometry	-Sample: Water -Complexing agent: PAR -Using diode-array detector	-LR: 0.15-2.5 µg mL^{-1} -LOD: 0.1 µg mL^{-1}	(16)
		-Sample: Water and Human Fluid -Complexing agent: Polyallyl amine 3-(4-formylphenylazo)-4, 5-dihy droxynapthalene-2,7-disulfonic acid (PA.FPNS) in Na_2HPO_4 NaOH buffer -λ_{anal} = 604 nm	-LR: 0-0.35 µg mL^{-1} -LOD: 15 ng mL^{-1} -Recovery: 93-104 %	(28)

Table 1.1 Summary of analytical methods for the determination of calcium, magnesium, iron and zinc using complexing agent (continued)

Analyte(s)	Technique	Detail	Characteristic	Ref.
Mg	FI-titration	-Sample: Tap Water -Titrant: EDTA -Indicator: Eriochrome black T -λ_{anal} = 641nm	-LR: 0.49-2.20 mM -RSD: 0.2-0.5 % -Sampling rate: 85-109 h^{-1}	(25)
	FI-spectro photometry	-Sample: Brine -Complexing agent: Ba(II) + EGTA + NH_3/NH_4^+ buffer pH 10.5 -λ_{anal} = 530 nm	-LR: 30 µg L^{-1} -RSD: 1.4 % (15 µg L^{-1}) -LOD: 0.6 µg L^{-1}	(22)
		-Sample: Water -Complexing agents: Chlorophosphonazo III (CPA-III) in triethanol amine/hydrochloric acid buffer pH 7 -λ_{anal} = 668 nm	-LR: 0.20-2.00 mg L^{-1} -LOD: 0.02 mg L^{-1} -Recovery: 90-104 % -Sampling rate: 200 h^{-1}	(21)
	SI-spectro photometry	-Sample: Pharmaceutical Preparations -Complexing agent: CPC -λ_{anal} = 570 nm	-LR: 0-20 mg L^{-1} -LOD: 0.24 mg L^{-1} -RSD: <2.0% -Sampling rate: 80 h^{-1}	(29)
	Polaro graphy	-Sample: Serum and Water -Measuring adsorptive wave of Mg-EBT at –0.7 V (vs. Ag/Hg electrode)	-LOD: $2x10^{-8}$ M	(30)
Fe(II)	Complexo metric titration	-Sample: Slags -Titrant: EDTA at pH 2 -Indicato: sulphosalicylic acid		(14)
	Spectro photometry	-Complexing agent: 1,10 phenanthroline -λ_{anal} = 515 nm	-LR: 1-12 µg mL^{-1} -RSD: 5 %	(31)

Table 1.1 Summary of analytical methods for the determination of calcium, magnesium, iron and zinc using complexing agent (continued)

Analyte(s)	Technique	Detail	Characteristic	Ref.
Fe(II)	Spectro photometry	-Sample: Glass -Complexing agent: (3-(2-pyridyl)-5,6-diphynyl-1,2,4-triazine-p-disul fonic acid (Ferrozine) -Using colorimeter as a detector	-RSD: 5 %	(32)
	FI-spectro photometry	-Sample: Water and Plant Material -Complexing agent: 1,10–phenanthroline -λ_{anal} = 512 nm	-LR: 0.1-30 μg mL^{-1} -RSD: <0.1 % -Sampling rate: 180 h^{-1}	(33)
		-Complexing agent: 1,10-phenanthroline -λ_{anal} =510 nm	-LR: 0.1-3.5 μg mL^{-1} -RSD: 2.% -Sampling rate: 60 h^{-1}	(34)
		-Sample: Multi-Vitamin Tablets -Complexing agent: Potassium thiocyanate -λ_{anal} = 480 nm	-RSD: 2.5-4.2 % -Sampling rate: 9-15 h^{-1}	(35)
		-Complexing agent: 2-(5-nitro-2-pyridylazo)-5-(N-propyl-N-Sulfopropyl amino)phenol (Nitro-PAPS) -λ_{anal} =582 nm	-LR: 0-100 ng mL^{-1} -LOD: 1.0 ng L^{-1} -Sampling rate: 15 h^{-1}	(36)
		-Sample: Pharmaceutical products -Complexing agent: 2,2-dipyridyl-2-pyridyl hydrazone (DPPH) in acidic medium -λ_{anal} =535 nm	-LR: 0-30 mg L^{-1} -Recovery: 99-102 %	(37)

Table 1.1 Summary of analytical methods for the determination of calcium, magnesium, iron and zinc using complexing agent (continued)

Analyte(s)	Technique	Detail	Characteristic	Ref.
Fe(II)	SI-spectro photometry	- Sample: Multi-Vitamin Preparations - Complexing agent: 1,10 phenanthroline -λ_{anal} = 512 nm	-LR: 0.25-5.0 mg L^{-1} -Sampling rate: 40 h^{-1}	(38)
Zn	Spectro photometry	-Sample: Pharmaceutical Formulations -Complexing agent: PAR -λ_{anal} = 493 nm	-LR: 0.18-2.00 μg mL^{-1}	(39)
	FI-spectro photometry	-Sample: Human hair, Pharmaceutical Cosmetic and Water Samples -Complexing agents: 1-(2-pyridylazo)-2-naphthol (PAN) immobilized on Dowex cation exchanger resin placed at flow cell -λanal = 555 nm	-LR: 0.2-4.0 μg mL^{-1} -LOD: 0.05 μg mL^{-1} -RSD: 2.1 %	(40)
		-Sample: Water, Plant Material -Complexing agent: Zincon -Using diode-array detector	-LR: 0.00-2.00 mg mL^{-1} -RSD: <0.02 % -Sampling rate: 45 h^{-1}	(41)
		-Sample: Beverage, Powdered Corn and Milk -Complexing agent: meso-tetra(4-trimethyl ammoniumphenyl) porphyrin (TAPP) -Using diode-array detector	-LR: 0.05-2.0 μg mL^{-1} -LOD: 5 ng mL^{-1} -Sampling rate: 30 h^{-1} -RSD: 2.8-7.1 % -Recovery: 89.6-103.2%	(42)

Ethanol

Standards methods, such as distillation and gas chromatography with FID (43) are laborious and time consuming. Many techniques have been reported including enzymatic methods using alcohol oxidase (AOD) (44-46) or alcohol dehydrogenase (47-52). Flow injection with enzymatic analysis was used for rapid determination by various detectors (amperometry, spectrophotometry and colorimetry). However, expensive and unstable enzymes utilized are the main drawbacks.

The direct measurement of ethanol by voltammetry has not been reported. While the differential-pulse polarographic detection after batchwise derivatization of ethanol has been studied (53).

Rapid on-line derivatization has been developed for chromatographic and flow injection technique (54). Flow injection voltammetry for electroactive species has widely been reported in the literature (55-58), but none of them involve conversion of ethanol to electroactive species.

The summary of analytical methods for the determination of ethanol is represented in Table 1.2.

Table 1.2 Summary of analytical methods for the determination of ethanol

Technique	Sample	Detail	Characteristic	Ref.
FI-enzymatic method	beer, wine	-Using immobilized alcohol oxidase (AOD) -Amperometric detection: monitoring oxidation current of H_2O_2 at 0.7 V	-LR: 0-15% v/v -Sampling rate: 30 h^{-1}	(44)
	bever-ages	-Using immobilized alcohol oxidase (AOD) -Amperometric detection monitoring oxidation current of H_2O_2 at 0.7 V	-LR: 0.0006-60% v/v -Sampling rate: 120-180 h^{-1}	(45)
	broth	-Enzymatic method using alcohol oxidase (AOD) at pH 7.5	-LR: 0.5-5.0% v/v	(46)
	wine	-Using NAD^+ in ADH at pH 9 and monitoring absorbance of NADH -Spectrophotometric detection :	-LR: 0.002-0.010% v/v -RSD: 0.7% -Sampling rate: 50-55 h^{-1}	(47)
	alcohol beverages	-Using enzyme-based sensor consist of ADH, electron mediator (hexacyanoferrate(III)) and gas-permeable membrane (silicone rubber or PTFE) -Sensor was set at 0.36 V.	-LR: 0-1.8 mM (silicone rubber) 0-0.08 mM(PTFE) -RSD: 1.0% (silicone rubber) and 0.5% (PTFE)	(48)
	water	-Using NAD^+ and ADH in phosphate buffer pH 8.0 -Fluorometric detection: NADH was monitoring (λ_{exite} = 360 nm, λ_{emiss} = 460 nm)	-LR: 0.05-0.5% v/v -RSD: 1.7%	(49)
Enzymatic method	beer, wine	-Using NAD+/ADH modified carbon pasted electrode -Amperometric detection monitoring oxidation current of NADH at 0.7 V vs. quasi reference platinum electrode	-LR: 0-10 mM	(50)

Table 1.2 Summary of analytical methods for the determination of ethanol (continued)

Technique	Sample	Detail	Characteristic	Ref.
Enzymatic method	serum	-Using NAD^+/ADH convert to NADH prior to complex with PMS-INT -Colorimetric detection : λ_{anal} = 505 nm	-LR: 11- 145 mg dL^{-1} -Recovery: 99% (11 to 145 mg dL^{-1})	(59)
SI-spectro photometr y	distilled liquors	-Reagents: potassium dichromate and sulfuric acid -Spectrophotometric detection : λ_{anal} = 600 nm	-LR: 1-6% v/v -RSD: <1% -Sampling rate: 19 h^{-1}	(60)
FI-enthal pimetry	fuel	-Measuring the heat of dilution	-LR: 87.66-96.07% v/v -RSD: 0.05% -Sampling rate: 110 h^{-1}	(61)
FI-NIR	liquor	-Extraction by chloroform prior to monitoring absorbance (at λ 2305-2636 nm)	-LR: 20-50% v/v -Injection rate: 240 h^{-1}	(62)
GC	human albumin	-Using a headspace sampler(equilibrated time 5 min, at 100 ^oC), a DB-wax capillary column (150 ^oC) and FID (250 ^oC)	-LR: 0.2-0.5 % v/v -LOD: 3.7 mg L^{-1}	(63)
	yogurt	-Using a headspace sampler, thick film capillary column coated with SE-54 and FID(200^oC)	-LR: 0.63-20 ppm -RSD: 5.29% (0.6 ppm) 0.74% (20 ppm)	(64)
	blood	-Using a headspace sampler (equilibrated time 22 min, at 60 ^oC), a Carbowax 1500 column (100 ^oC) and FID (200 ^oC)	-LR: 0.1-4 g L^{-1}	(65)

Table 1.2 Summary of analytical methods for the determination of ethanol (continued)

Technique	Sample	Detail	Characteristic	Ref.
GC	alcohol free beer	-Using a purge and trap injector, a WCOT CP-Sil 5 CB column(programmed temperature) and FID(220 ^{o}C)	-LR: 0-5% v/v	(66)
HPLC	body fluids	-Derivatization by using ADH, NAD and phenylhydrazine -Using an octadecylsilyl column	-LR: 0.008 – 5 g/L -Recovery: 98.2 % v/v	(52)
Differential-pulse polaro graphy	beer	-Regents: carbon disulfide, methyltrioctylammonium chloride -W.E.: DME -R.E.: SCE -A.E.: Pt -Supporting electrolyte: NH_4Cl + KH_2PO_4 + NaOH + eosin	-LR: 10-100 mg mL^{-1} -LOD: 0.02% v/v -Recovery: 94-104% v/v	(53)
Colori metry	aqueous solution	-Complexing reagent : diazotized p-aminobenzoic acid	-LR: 0.5-4.0 g mL^{-1} -RSD: 4.76%	(67)

1.1.2.1 Complexometry of calcium, magnesium, iron and zinc

Complexometry is the study of complex compound, including the metal ion and ligand. The concept of complex compounds or complexes originated in the work by Alfred Werner, who in 1913 was awarded the first Nobel Prize in inorganic chemistry. Currently, metal complexation is widely interest. It is studied not only by inorganic chemists, but also by physical, organic chemists, analytical chemists and environmentalists (68).

In analytical chemistry, the usefulness complex compounds may involve in:

1. Complexation titration

EDTA has widely been used for the determination of almost all metal ions directly and indirectly. EDTA forms complexes with various metal ions. Structure and formation constants of some metal-EDTA complexes are represented in Figure 1.1 and Table 1.3, respectively.

Table 1.3 Formation constants for metal-EDTA complexes (69)

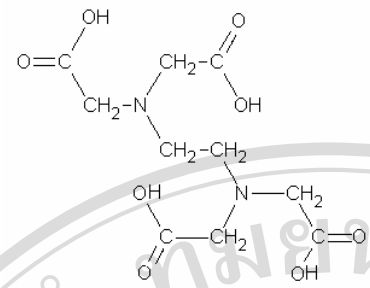
Cation	log K_f
Ca(II)	10.69
Mg(II)	8.79
Fe(II)	14.32
Zn(II)	16.5

Note: The stability constant is the equilibrium for the reaction: $M^{n+} + Y^{4-} \rightarrow MY^{n-4}$.

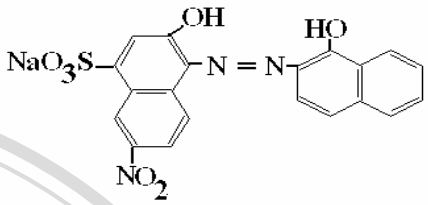
Values in table generally apply at 20 ^{o}C and ionic strength 0.1 M.

2. Metallochromic indicator

For complexometric titration, the metallochromic indicators for end point detection are important (70). Eriochrome Black T is widely employed for complexometric titration. The structure is shown in Figure 1.1 .



EDTA



EBT

Figure 1.1 Structure of EDTA and sodium 1- (1-hydroxy-2-naphthylazo)-6-nitro-2-naphthol-4-sulphonate (II) or Eriochrome Black T (EBT)

Some of mettalochromic indicators used for calcium, magnesium, iron and zinc titration are presented in Table 1.4.

Table 1.4 Some of metallochromic indicators for calcium, magnesium, iron and zinc (68)

Indicator	Color of M-In complexes	Applications	
		Direct titration	Back titration
Eriochrome black T	wine red	Calcium Magnesium Zinc	Iron
Calmagite	wine red	Calcium Magnesium Zinc	Iron
PAN	red	Zinc	Zinc
Murexide	red	Calcium	Iron
Pyrocatechol violet	blue	Magnesium Zinc	Iron
Hydroxynaphthol blue	red-violet	Calcium	-
Xylenol orange	red	Zinc	-

3. Auxiliary complexing agent

An ammonia/ammonium chloride buffer was used as an auxiliary complexing agent to prevent the undesired precipitation (70). At a too high pH, the EDTA complex is unstable because OH^- competes with EDTA for the metal ion and may precipitate the metal hydroxide or form inactive hydroxide complexes.

4. Color complex for instrumentation detection

The transition metal complexes frequently have distinctive color. The color of these complexes are due to light absorption at specific wavelengths in the visible region of the spectrum (71).

Some of ligands have been employed to complex with metal ions to produce a color complex such as calcium complexes (13-27, 33, 72, 73), magnesium complexes (22, 28), iron complexes (32-34, 36, 74, 75) and zinc complexes (39-41).

1.1.2.2 On-line derivatization of ethanol

Chemical derivarization offers an improved method for instrumentation analysis of ethanol such as in gas chromatography (76) and polarography (77). The desired product will be obtained by using derivatizing agent such as carbon disulfide. (77-79).

Normally, organic compounds which are reduced at the mercury dropping electrode must contain a highly polar or unsaturated bond (80). In the case of ethanol, an electroinactive species that can not be detected by voltammetry, the derivatization of ethanol to ethyl dithiocarbonate (xanthate), an electroactive species was performed. The reaction is shown in an equation below (77).

$$C_2H_5OH + CS_2 \xrightarrow{\text{NaOH \& TAHS}} C_2H_5OCS_2\ Na^+ + H_2O$$

(TAHS; tetrabutyl ammonium hydrogen sulfate)

From the equation, hydroxide ion is a base which is strong enough base to remove a proton from ethanol to give an ethoxide ion (C_2H_5-O^-), which reacts with carbon disulfide to form xanthate. A better transformation of this reaction when using TAHS as phase transfer catalyst has been described (77).

Osteryoung Square Wave Voltammetry (OSWV) has been reported for the determination of ethanol after a batchwise derivatization procedure (81) using the similar reaction that Chan and Lee (77) described.

On-line method is a technical management for various objectives. On-line monitoring for quantitative analysis has been reported by many workers (55, 82). The

objectives of on-line flow injection techniques are to enhance efficiency of separation (83), to preconcentrate (84), to react with reagent (85) and to convert the undetectable species to be detectable species (77, 79, 86-88).

1.1.3 Reasons for method development for determinations of calcium, magnesium , iron, zinc and ethanol

A novel cost-effective procedure for determination of calcium, magnesium, iron and zinc will be developed. The author expects that the proposed method can be reduced the analysis time, amount of chemical reagents and gave the suitable analytical characteristics for a control quality of some drug and mineral supplements products.

The development of on-line derivatization system for determination of ethanol that leads to reduced the derivatized reagent and convenience to analysis an electroinactive species.

1.2 Flow Injection Analysis

Flow Injection Analysis (FIA) is a widely used technique in analytical chemistry. In1975, Ruzicka and Hansen defined that a method based on injection of a liquid sample into a moving unsegmented continuous stream of a suitable liquid. The important characteristics of FIA are below;

1. Precision of sample injection
2. Reproducible time
3. Controlled dispersion of sample

Over the length of tubing, dispersion effect was occurred because the diffusion at the edges of sample zone from higher to lower concentration.

The dispersion coefficient of sample Ds is defined as the relationship between the concentration of the sample before (C_s^o) and after (C_s)dispersion step.

$$D_s = C_s^o/C_s$$

Figure 1.2 depicts components of FIA.

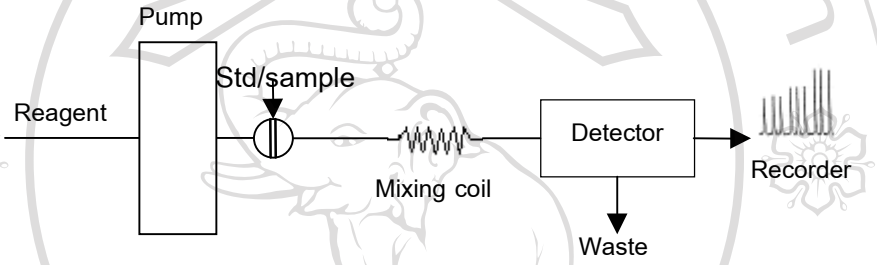


Figure 1.2 A simple flow injection manifold consisting of pump, injection valve, mixing coil, detector and recorder.

Several instrumental techniques can be combined with FIA for sample preparation, to increase the sampling rate and to performing of less-conventional batch procedures. Quantitative analysis of samples is achieved using a calibration graph, which could be a plot of peak height versus analyte concentration. In FIA, it is unnecessary for measuring at the condition of the complete reaction or at equilibrium. FIA is one of the most popular continuous-flow techniques due to its versatility and simplicity.

The success of an FIA application is dependent on the manifold design. In this work, FIA procedures are to be adapted for determination of calcium, magnesium, iron, zinc and ethanol.

Advantages of FIA (89)

FIA provides advantages over other conventional techniques because;

1. It is a versatile technique for many chemical reactions.
2. Some of chemical reaction are impossible to perform by batch technique such as the titration of ascorbic acid with potassium permanganate because the reagent is unstable, but can be done in FIA.
3. The physical and chemical conditions are not changed, steady state is not a necessary requirement. Therefore a short time analysis will be obtained.
4. Smaller sample and /or reagents required
5. Simplicity of construction and assembly
6. The capacity for a wide range of physical and chemical changes to be used for detection.

1.3 Detection system

1.3.1 Voltammetric detection of derivatized ethanol (89)

Voltammetric detection is based on the transport of an electroactive species towards the surface of a working electrode. It measures current at varying potential. A number of voltammetric experiments are used for routine analysis.

A chemical reaction of xanthate at mercury electrode has not been reported. However, carbon-sulfur bond is an electroactive functional group (80) that can reduced at electrode surface. From the literature, the determination of xanthate by polarographically has been described by Sun and Holzmann (90). They applied potential in the range of –0.1 to –0.7 V vs. SCE. Xanthate wave ($E_{1/2}$) was observed at

–0.26 V vs. SCE. After that Chan and Lee (53) have been developed the method for the determination of ethanol by differential-pulse polarography. A negative potential scan was initiated between 0.00 and –0.40 V vs. SCE at 2.5 mV s^{-1}. The peak potential at –0.175 V vs. SCE was reported.

Square wave voltammetry which is rapid, high sensitivity was selected for this work.

1.4 Research Aims

1. To develop flow injection procedures for the determination of some cations namely Ca(II), Mg(II), Fe(II) and Zn(II).

2. To develop flow injection - on-line derivatization voltammetry for ethanol determination.