CHAPTER I

INTRODUCTION

1.1 General knowledge about the analyses of interest [1, 2, 3, 4]

1.1.1 Sugar

Sugar is a sweetener that refers to sucrose or saccharose disaccharide. Sucrose is a white crystalline, odorless, sweet taste. It is the best known among the various sugars for flavoring. Manufacturing and preparing of foods may involve other types of sugar, such as fructose, generally obtained from corn or fruit. The molecular formula of is $C_{12}H_{22}O_{11}$. Consumption of sugar is annually about 20 kg per person. Sugar is necessary for daily life.

The chemical formation of sucrose is shown in **Figure 1.1**.

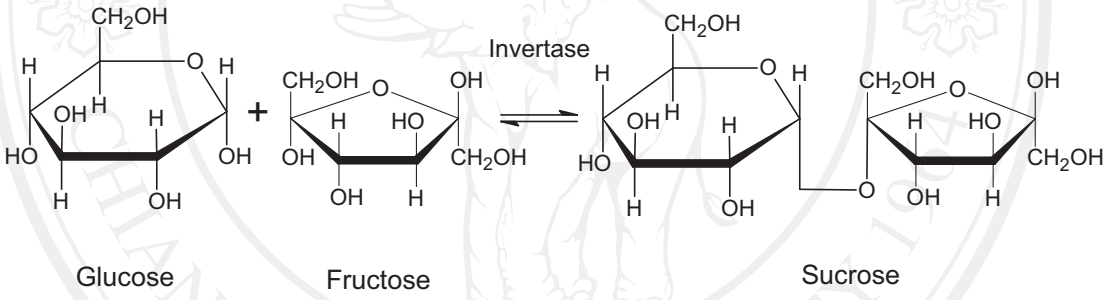

Figure 1.1 Chemical formation of sucrose

Sucrose is a disaccharide formed by acidic hydrolysis of glucose : fructose at 1:1 mole ratio. This formation involves enzymes called invertases that catalyze the hydrolysis of sucrose to a glucose-fructose mixture or sucrose through glycosides linkage. Sucrose is not a reducing sugar and does not exhibit mutarotation. Physical properties of sucrose are listed in **Table 1.1**.

Table 1.1 Physical properties of sucrose

IUPAC name	β-D-fructofuranosyl-(2→1)-α-D-glucopyranoside
Other names	sugar, saccharose, β-(2*S*,3*S*,4*S*,5*R*)-fructofuranosyl-α-(1*R*,2*R*,3*S*,4*S*,5*R*)-glucopyranoside
Molecular formula	$C_{12}H_{22}O_{11}$
Molar mass	342.30 g/mol
Appearance	white solid
Density	1.587 g/cm^3, solid
Melting point	186 °C decompose
Solubility in water	200 g/100 mL (25 °C)

1.1.2 Iron [5, 6]

Iron is a metallic chemical element with the symbol Fe and atomic number of 26. Iron is a group 8 and period 4 element and is therefore classified as a transition metal.

Usually there is difference between water soluble Fe (II) compounds and generally water insoluble Fe (III) compounds. The latter are only water soluble in strongly acidic solutions, but solubility increases when these are reduced to Fe (II) under certain conditions. Although Fe (II) is necessary trace used by almost all living organisms but may also involve bad effect to health. Because iron compounds such as $FeCl_2$ and $FeSO_4$ may cause toxic effects upon concentrations exceeding 200 mg, and are lethal for adults upon doses of 10-50 g. A number of iron chelates may be toxic, and the nerve toxin iron penta carbonyl is known for its strong toxic mechanism. Iron dust may cause lung disease.

Examples of physical properties of ammonium iron (II) sulfate ($[NH_4]_2[Fe][SO_4]_2{\cdot}6H_2O$) used in the experiment are listed in **Table 1.2**.

Table 1.2 Physical properties of ammonium iron (II) sulfate

IUPAC name	Iron (II) ammonium sulfate
Other names	Ferrous ammonium sulphate Ammonium iron sulphate Mohr's salt
Molecular formula	$FeH_{20}N_2O_{14}S_2$
Molar mass	392.14 g/mol [hexahydrate]
Appearance	Blue-green solid

1.1.3 Phosphorus/Phosphate [7, 8, 9, 10]

Phosphorus is the chemical element that has the symbol P and atomic number of 15. Biological productivity is mostly limited by the amount of phosphorus in water and soil. Phosphorus is present in natural waters as orthophosphate and undifferentiated organic phosphates. Phosphorus is found in rock as inorganic phosphates. As water runs over and through rocks, it carries off small amounts of minerals such as calcium, magnesium, and phosphates. Inorganic phosphates are plant nutrients and are taken in by plants with water and incorporated into organic phosphate compounds. Animals obtain their essential phosphorus from phosphates in water and plant material.

Natural waters contain phosphorus concentration of approximately 0.02 parts per million (ppm) which is a limiting factor for plant growth. On the other hand, large concentrations of this nutrient can accelerate plant growth and can be unbalanced ecologies system. This is the disadvantage of high quantity of phosphorus in environment. So the determination of phosphorus in nature is necessary.

The ionization products of orthophospheric acid in water are:

$$H_3PO_4 \rightleftharpoons H^+ + H_2PO^-_4$$

$$H_2PO_4 \rightleftharpoons H^+ + HPO^{2-}_4$$

$$HPO_4 \rightleftharpoons H^+ + PO^{3-}_4$$

The relationship of pH and activities of the various forms of orthophosphates (% mole fractions) is shown in **Figure 1.2**.

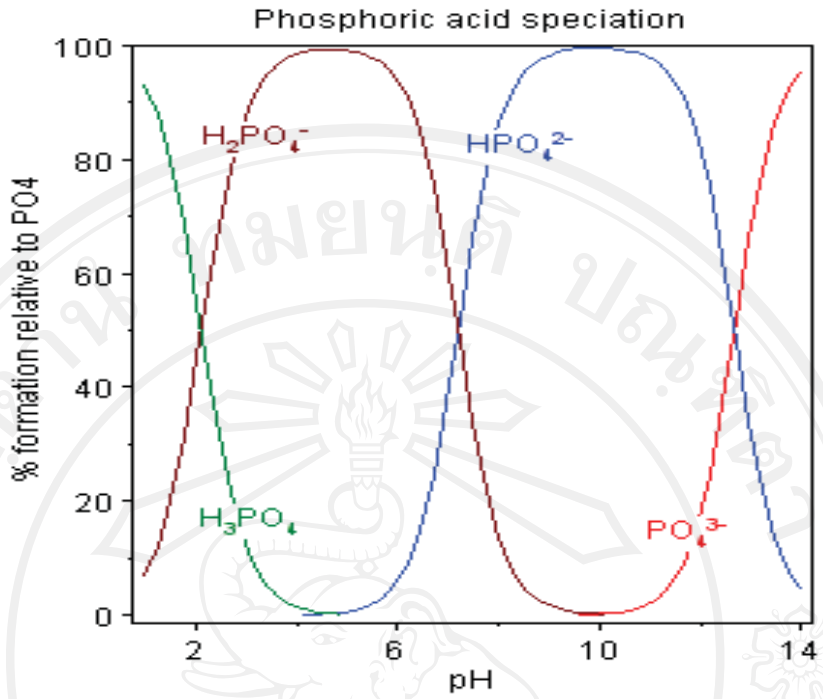

Figure 1.2 The relationship of pH and activities of the various forms of orthophosphates

Physical properties of phosphorus are listed in **Table 1.3**

Table 1.3 Physical properties of phosphorus

Name	Phosphorus
Appearance	colorless, waxy white, yellow, scarlet, red, violet, black
Density	(white) 1.823, (red) ≈ 2.2 – 2.34, (violet) 2.36, (black) 2.69 $g \cdot cm^{-3}$
Melting point	(white) 44.2 °C, (black) 610 °C Sublimation point (red) ≈ 416 – 590 °C, (violet) 620 °C
Boiling point	(white) 280.5 °C

Properties of potassium dihydrogen phosphate used in this study are listed in **Table 1.4**. It is a soluble salt which is a source of phosphorus and potassium and it is used as a fertilizer, a food additive, and a fungicide.

Table 1.4 Physical properties of potassium dihydrogen phosphate

IUPAC name	Potassium dihydrogen phosphate
Other names	Potassium phosphate monobasic Phosphoric acid, monopotassium salt
Molecular formula	KH_2PO_4
Molar mass	136.086 g/mol
Appearance	White powder
Density	2.338 g/cm^3
Melting point	252.6 °C
Boiling point	400 °C
Solubility in water	22 g/100 mL (25°C)
Solubility	insoluble in alcohol
Acidity	(pK_a) 4.4-4.7

1.2 The analytical methods

1.2.1 The analytical methods for sugar

Sugar is used extensively in foods and drinks industries. There were a variety of analytical methods available for the determination of sugar. Analytical methods applied to determine sugar in different samples are listed in **Table 1.5**.

Table 1.5 Analytical methods applied to determine sugar in different samples

Technique	Detection technique	Reagent	Linear or determination range	Detection limit	Sample	Analyze	Ref.
enzymatic reaction	spectrophotometer	Thio-NADP, G-6P-DH, G-1, 6-diP, PGM, 6PGDH, 6PGL, SP	serum and urine were 0–500 and 0–5000 µmol/l, respectively.	12-18 µmol/l	serum and urine	sucrose	11
enzymatic reaction	amperometric enzyme electrode	GOD, INV	-	-	-	sucrose	12
FIA	cyclic voltammetry	SP, PGM, G-6P-DH, $NADP^+$, NADPH, NADH, PIPES, G-6-P, G-1-P, G-1,6-diP, PEI, NAD^+	1-15 mmol/l	1 mmol/l	fruit juices	sucrose	13
FT mid-IR	spectrophotometer	-	-	-	sugar Cane Juices	sucrose	14
BIC	thermistor	INV, albumin	0.10-0.50 mol/l	-	sugar Cane Juices	sucrose	15
enzymatic reaction	amperometric enzyme electrode and spectrophotometer	GOx, INV, mutarotase, G-6P-DH, $[(C_{12})_2$ glu N^+ $Cl^-]$	Glucose (0.2 µmol/l -3mmol/l) Sucrose (10 µmol/l - 6mmol/l)	-	food, jams, fruit juices	glucose and sucrose	16
^{1}H NMR ERETIC	spectrometer	D_2O	0.1-200 mmol/l	-	sucrose esters	sucrose and sugar surfactants	17

Table 1.5 (continued)

Technique	Detection technique	Reagent	Linear or determination range	Detection limit	Sample	Analyze	Ref.
FIA	spectrofluorometer	SP, PGM, G-6P-DH,	0.1-200 µmol/l	0.2 µmol/l	soft drinks	sucrose	18
ATR-FTIR	spectrophotometer	sucrose, glucose, fructose, lactose, ethanol, glacial acetic acid, alanine, leucine, tryptophan, aspartic acid, threonine, cysteine and cellulose	-	0.15% (w/w)	beet root	sucrose	19
PLS-NIR	spectrophotometer	anhydrous D (+) - glucose, D (-) - fructose and sucrose	4.8-26.4 g/100 ml for total sugar, 0.6-18.6 g/100ml for glucose, 0.6-15.6g/100ml for fructose and 1.2-16.2 g/100 ml for sucrose.	0.2 g/l00 ml for total sugar and 0.2,0.4 and 0.5 g/100 ml for glucose, fructose and sucrose, respectively.	fruit juices	total sugar, glucose, fructose and sucrose	20
enzymatic reaction	conductometric biosensor	GOD, INV, BSA, GA, sucrose and KH_2P0_4–NaOH	-	-	juices	sucrose	23

Table 1.5 (continued)

Technique	Detection technique	Reagent	Linear or determination range	Detection limit	Sample	Analyze	Ref.
enzymatic reaction	spectrophotometer	INV, GOD, 4-aminoantipyrine, D-(+)-sucrose, D-(+)-glucose anhydrous, α,α,αTris-(hydroxymethyl)-methylamine, phenol-4-sulfonic acid sodium salt dihydrate, citric acid, sodium citrate, acetic acid, zinc acetate and potassium hexacyanoferrate (II)	100 to 1200 mg/l	20.3 mg/l	green and roasted coffee beans	sucrose	21
HPLC-MS	mass spectrometer	trigonelline, sucrose, caffeine, nicotinic acid, lead acetate,	-	11.9, 36.4, 18.5 and 5.0 ng/mL for caffeine, trigonelline, nicotinic acid and sucrose, respectively.	coffee (regular or decaffeinated green, ground roasted and instant)	caffeine, trigonelline, nicotinic acid and sucrose	22

Table 1.5 (continued)

Technique	Detection technique	Reagent	Linear or determination range	Detection limit	Sample	Analyze	Ref.
NIR	spectrophotometer	-	-	-	chocolate (dark, white, and milk)	sucrose	24
FIA	amperometric enzyme electrode (electropolymeriza-tion)	GOD, INV, mutarotase, 1,3-DAB and BSA	-	-	fruit juices	sucrose and glucose	25
Enzymatic reaction	ISFET	INV, GDH, BSA, NAD^+, phosphate buffer	-	-	biotechnological compounds	sucrose	26
Enzymatic reaction	potentiometric oxygen electrode	*Saccharomyces cerevisiae*, GOD, glucose, disodium phosphate–citric acid buffer	1×10^{-5} to 3×10^{-2} M	-	soft drinks	sucrose	27

Glucose-6-phosphate dehydrogenase; G6PDH, sucrose phosphorylase; SP, glucose-1,6-diphosphate tetracyclohexyammonium salt; G-1,6-diP, 6-phospho-gluconolactonase; 6PGL, phosphogluconate dehydrogenase; 6PGDH, phosphoglucomutase; PGM, thionicotinamide-adenine dinucleotide phosphate-oxidized form; thio-NADP, glucose oxidase; GOD, invertase; INV, β-nicotinamide adenine dinucleotide phosphate; $NADP^+$, β-nicotinamide adenine dinucleotide phosphate reduced form; NADPH, β-nicotinamide adenine dinucleotide disodium salt reduced form; NADH, piperazine-*N*,*N*%-bis [2-ethanesulfonic acid]; PIPES, D-glucose 6- phosphate; G-6-P, polyethylenimine; PEI, β-nicotinamide adenine dinucleotide free acid; NAD^+, glucose oxidase; GOx, N-(α-trimethylammonioacetyl)didodecyl-L-glutamate chloride); $[(C_{12})_2$ glu N^+ $Cl^-]$, deuterium oxide; D_2O, The partial least-squares; PLS, Near infrared spectroscopy; NIR, Attenuated total reflectance Fourier transform infrared spectroscopy; ATR-FTIR, High performance liquid chromatography–mass spectrometry; HPLC-MS, Bovine serum albumin; BSA, glutaraldehyde; GA, potassium-phosphate solution; KH_2P0_4–NaOH, Flow injection analysis; FIA, Batch injection analysis; BIC, Fourier transformed mid-infrared; FI mid-IR, 1,3-diaminobenzene; 1,3-DAB, Ion-sensitive field-effect transistor; ISFET, glucosedehydrogenase; GDH.

1.2.2 The analytical methods for Fe (II)

Iron is essential elements for living organisms including human being. Lack of this element in the daily diet may result in the development of serious diseases. However, excess uptake of iron through water pollution will affect in water supply pipes and drinking water quantity.

Determination of iron level in natural water is usually derived in the forms of Fe (II) and Fe (III). Several methods for determination of the Fe (II) are listed in **Table 1.6**.

Table 1.6 Analytical methods applied to determine the Fe (II) in different samples

Technique	Detection technique	Reagent	Linear or determination range	Detection limit	Sample	Analyze	Ref.
CZE	UV detection	$Fe(NH_4)_2(SO_4)_2.6H_2O$, $Fe(NO_3)_3.9H_2O$, H_3BO_3, $NH_2OH.HCl$, EDTA, CDTA, phen, HCl, H_2SO_4 and HNO_3	0.1-5 mgl^{-1} range for Fe (II) and in the 0.2-10 mgl^{-1} range for Fe (III)	0.06 mgl^{-1} for Fe (II) and 0.1 mgl^{-1} for Fe (III)	tap water and ground water samples	Fe (III) and Fe (II)	38
FIA	spectrophoto-meter	pure iron and zinc, HNO_3, 5-Br-PADAP, KH_2PO_4, NaOH, thiourea, ascorbic acid	iron is 0.1-1.8 µg ml^{-1} and for zinc is 0.2-5.0 µg ml^{-1}	-	the human hair	Fe (II) and zinc	39
differential pulse polarography	cyclic voltammetry	chloroform, perchloric acid, 8-hydroxyquinoline, tributylamine, TBACl, tri-BAP, tri-BA, Fe (III) oxinate, $Fe(NO_3)_2$, H_2SO_4	2-80 µM	1.5 µM	rock	Fe (III), Fe (II) and total iron	40
reversed-phase liquid chromatogra - phy	spectrophoto-meter and DAD	$Fe(NH_4)_2(SO_4)_2.6H_2O$, $FeNH_4(SO_4)_2.12H_2O$, H_2SO_4, 5-Br-PADAP, methanol, PAN, PAR, TAR, TAN, TAM, SDS, BDS, SDeS, ACN, ascorbic acid	20- 500 μgl^{-1} Fe(II) and Fe(III) ions	18 μgl^{-1}	water	Fe (III) and Fe (II)	41
FIA and catalytic reaction	spectrophoto-meter	HCl, H_2O_2, DPD, NH_4OAc buffer, acetic acid	up to 2 μgl^{-1}	0.08 μgl^{-1}	tap and natural water	Fe (III), Fe (II) and total iron	42

Table 1.6 (continued)

Technique	Detection technique	Reagent	Linear or determination range	Detection limit	Sample	Analyze	Ref.
FIA and photochemical reactions	spectrophotometer	H_2SO_4, HNO_3, ammonium thiocyanate, $Fe_2(SO_4)_3(NH_4)_2SO_4$.$24H_2O$, EDTA, $CuSO_4.5H_2O$, $PdCl_2$, PAN, xylenol orange	Fe (III) 20 μgml^{-1} Cu (II) 25 μgml^{-1} Pd (II) 25 μgml^{-1}	-	serum and ground water	Fe (III), Cu (II) and Pd (II)	43
flow-injection solid-phase	spectrophotometer	HCl, standard ferrous iron, Sodium acetate/acetic acid, hexamine/HCl, tris(hydroxymethyl)aminomethane/maleic acid, ascorbic acid, $NH_2OH.HCl$, TAN	50-1000 μgl^{-1}	15 μgl^{-1}	fresh water samples	Fe (II)	44
chemical and electrochemical analysis	screen-printed carbon sensor device	Iron(II) sulphate heptahydrate, iron(III) nitrate, phen, potassium hexacyanoferrate (III), potassium hexacyanoferrate(II), potassium chloride	0-5000 μgl^{-1}	10 μgl^{-1}	potable waters	Fe (II) and total iron	45

Table 1.6 (continued)

Technique	Detection technique	Reagent	Linear or determination range	Detection limit	Sample	Analyze	Ref.
AAS	spectrophoto-meter	H_2SO_4, HNO_3, H_2O_2, manganese and iron stock solutions	Mn 0.78 to 2.89 μgl^{-1} and Fe 0.88 to 9.22 μgl^{-1}	Manganese 27 μgl^{-1} Iron 40 μgl^{-1}	Wine	Iron and manganese	46
FIA and CPE	FAAS	Nitrate, triton X-114, sodium acetate, acetic acid, H_2SO_4, NR	Fe 2.5-200 $ngml^{-1}$ Cu 1.0-200 $ngml^{-1}$	Fe 0.7 $ngml^{-1}$ Cu 0.3 $ngml^{-1}$ (3s)	Spice samples	Iron and copper	47

Capillary zone electrophoresis; CZE, Ferrous ammonium sulfate hexahydrate; $Fe(NH_4)_2(SO_4)_2.6H_2O$, Ferrous (III) nitrate nonahydrate; $Fe(NO_3)_3.9H_2O$, Hydrogen borate; H_3BO_3, Hydroxylamine hydrochloride; $NH_2OH.HCl$, Ethylenediaminetetraacetic acid disodium salt dehydrate; EDTA, Trans-cyclohexane-1,2-diaminetetraacetic acid; CDTA, 1, l0-phenanthroline hydrochloride; phen, Hydrochloric acid; HCl, Sulfuric acid; H_2SO_4, Nitric acid; HNO_3, Flow injection analysis; FIA, 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol; 5-Br-PADAP, Potassium dihydrogen phosphate; KH_2PO_4, Sodium hydroxide; NaOH, Tetrabutylammonium chloride; TBACl, Tributylammonium perchlorate; tri-BAP, Ferrous (II) nitrate; $Fe(NO_3)_2$, Diode array detector detector; DAD, Iron alum; $FeNH_4(SO_4)_2.12H_2O$, 1-(2-pyridylazo)-naphthol; PAN, 4-(2′-pyridylazo)-resorcinol; PAR, 1-(2-thiazolylazo)-resorcinol; TAR, 1-(2-thiazolylazo)-naphthol; TAN, 2-(2-thiazolylazo)-5-diethylaminophenol; TAM, sodium dodecyl sulfate; SDS, sodium dodecylbenzenesulfonic acid salt; BDS, sodium 1-dodecanesulfonate; SDeS, Acetonitrile; ACN, Hydrogen peroxide; H_2O_2, *N*,*N*-dimethyl-*p*-phenylenediamine; DPD, Ammonium acetate buffer; NH_4OAc buffer, Atomic absorption spectrometry; AAS, Flame atomic absorption spectrometry; FAAS, Cloud point extraction; CPE, 3-amino-7-dimethylamino-2-methylphenazine or Neutral Red; NR, and octylphenoxypolyethoxyethanol; Triton X-114.

1.2.3 The analytical methods of phosphorus/phosphate

Phosphorus/phosphate is a growth limiting nutrient of aquatic plant. Therefore, it drains or fish pond water containing of phosphorus/phosphate was released into natural water, it may stimulate the rapid growth of plants. This is eutrophication problem in natural water. Determination of phosphorus/phosphate in drains or fish pond water is usually analyzed on total phosphate or total-P including phosphorus in the form of suspensions or those that combine with organic. Analytical methods applied to determine the phosphorus/phosphate in different samples are listed in **Table 1.7**.

Table 1.7 Analytical methods applied to determine the phosphorus/phosphate in different samples

Technique	Detection technique	Reagent	Linear or determination range	Detection limit	Sample	Analyze	Ref.
HPLC	spectrophoto-metric variable detector	potassium antimony tartrate, sulfuric acid, ammonium molybdate tetrahydrate, ascorbic acid	1 nM to 100 µM	1 nM	freshwater and saltwater	soluble reactive phosphate	28
enzyme reaction	amperometric biosensor	PyOD, nafion, PCS, citrate buffer, pyruvic acid, FAD, $MgCl_2$.6H2O, TPP	7.5 to 625 µM	3.6 µM	human salivary	phosphate	29
flow automated method with on-line photo-oxidation	colorimeter	peroxide, sulfuric acid, ammonium heptamolybdate, ascorbic acid, potassium antimony tartrate	tested up to 5 µM	0.02 µM	marine and fresh water	dissolved organic phospho-rus	30
Electro-chemical analysis	potentiometric titration	Na–Y zeolite, nujol, graphite powder, trisodium phosphate hexahydrate, potassium nitrate, potassium chloride, sodium arsenate, HDTMA-Br	1.58×10^{-5} to 1.00×10^{-2}M	1.28×10^{-5}M	a fertilizer	phosphate	31
FIA	luminol chemiluminescence detection	chelex 100, potassium antimony tartrate, sulfuric acid, ammonium molybdate tetrahydrate, luminol, borate buffer, sodium hydroxide	0.032–3.26 µg P l^{-1}	0.03 µg P l^{-1}	freshwaters	phosphate	32

Table 1.7 (continued)

Technique	Detection technique	Reagent	Linear or determination range	Detection limit	Sample	Analyze	Ref.
spectro-metry	spectrophoto-meter	ammonium molybdate, sodium sulphide, disodium hydrogen phosphate, sulfuric acid	0.3-12.24 ppm	-	sugarcane juices, water and detergent	phosphate	33
capillary Electropho-resis	UV detection	PMA, TEA, HMBr, ammonium heptamolybdate, L(+) ascorbic acid, potassium antimony(III) oxide-tartrate, potassium biphosphate, sulphuric acid, methanol, potassium hydroxide, sodium fluoride, sodium hydroxide, hydrogen peroxide, triton X-100, 1,2,4,5 benzenetetracarboxylic acid	-	0.45 µM	natural waters	phosphate	34
µFIA	spectrophoto-meter	potassium antimony tartrate, sulfuric acid, ammonium molybdate tetrahydrate, ascorbic acid, borate buffer	1-10 µg ml^{-1}	0.1 µgml^{-1}	water	phosphate	35
stopped-flow injection analysis	spectrophoto-meter	potassium antimony tartrate, sulfuric acid, ammonium molybdate tetrahydrate, ascorbic acid	0.5-3 µg P ml^{-1}	-	water	phosphate and silicate	36

Table 1.7 (continued)

Technique	Detection technique	Reagent	Linear or determination range	Detection limit	Sample	Analyze	Ref.
SIA	spectrophoto-meter	potassium antimony tartrate, sulfuric acid, ammonium molybdate tetrahydrate, ascorbic acid, nitric acid, oxalic acid	0.2 to 7 mg l^{-1} for P = PO_4^{3-} 5 to 50 mg l^{-1} for Si = SiO_3^{2-}	0.1 mg l^{-1} for P = PO_4^{3-} 1 mg l^{-1} for Si = SiO_3^{2-}	waters and sediments	phosphate and silicate	37

High performance liquid chromatography; HPLC, Piruvate oxidase; PyOD, Poly(carbamoyl) sulfonate; PCS, Flavin adenine dinucleotide; FAD, Thiamine pyrophosphate; TPP, Hexadecyltrimethyl ammonium bromide; HDTMA-Br, Flow injection analysis; FIA, Pyromellitic Acid; PMA, Triethanolamine; TEA, Hexane-1,6 bis(trimethyl)ammonium bromide; HMBr, Sequential injection analysis; SIA.

1.3 Simple lab-on-chip (LOC)

LOC is a down scaling analysis laboratory functions and processes to a miniaturized chip format. LOC devices can be fabricated from many types of material including various polymers, glass, or silicon, or combinations of these materials. The fabrication of LOC often uses photolithography or optical lithography process and hot embossing, injection molding and laser ablation. However, these processes require high technology and involve complicated process. Therefore, a simple LOC [50] was developed for quantitative analysis of sucrose syrup based on refractive index of solution, and for determination of phosphate and iron presented in natural water samples based on the formation of colored complexes.

A simple LOC offers advantages including fast analysis, simplicity of fabrication, low reagent and standard consumption, green analytical chemistry, portability of the instrument, and lower instrumentation cost compared to conventional method.

1.4 The aims of this research

The aim of this research work is to develop a simple lab-on-chip system for determination of sucrose syrup, iron and phosphate.