CHAPTER 1

INTRODUCTION

Water is one of the major elements essential for sustenance of all forms of life and is available in abundance in nature covering approximately three fourths of the surface of the earth. The chemical nature of water is one of the most important criteria that determines its usefulness for a specific need and as such not all the waters are fit for drinking[1]. In nature, water contains cations and anions such as calcium, chloride, magnesium, fluoride, potassium, sodium, sulfate and nitrate. The presence of fluoride in water is widespread in several parts of Thailand. High fluoride contamination can cause detrimental to health such as dental or skeletal fluorosis.

1.1 Fluoride

1.1.1 The properties of fluoride[2-4]

Fluorine is a chemical element and is the lightest of the halogen group. Fluorine has the symbol F and its atomic number is 9. It is the seventeenth in order of abundance of elements in the earth's crust. The atomic weight of fluorine is 18.9984, together with the electron affinity of 333 kJ/mol. Fluorine itself is a greenish diatomic and poisonous gas. Fluorine has a strong and sharp odor. Fluorine, as fluoride, is an important anion, present in various environmental, clinical and food samples. Small amounts of fluoride are vital for the human organism, but it is toxic in larger amounts. Fluoride is widely used in making steel, chemicals, ceramics, lubricants, dyes, plastic and pesticides. Toothpaste and mouth rinses have fluoride added to prevent cavities. If drinking water supplies are low in fluoride, many communities add fluoride to help

prevent cavities. Some skin medicines and cancer treatment drugs also contain fluoride.

1.1.2 Fluoride in air[5]

Fluoride in the atmosphere may be in gaseous or particulate form, which is emitted from both natural and anthropogenic sources. Atmospheric fluorides can be transported over large distances as a result of wind or atmospheric turbulence or can be removed from the atmosphere via wet and dry deposition or hydrolysis. Fluoride released as gaseous and particulate matter is deposited in the general vicinity of an emission source, although some particulates may react with the other atmospheric constituents. The distribution and deposition of airborne fluoride are dependent upon emission strength, meteorological conditions, particulate size and chemical reactivity. In areas not in the direct vicinity of emission source, the mean concentrations of fluoride in ambient air are generally less than 0.1 μg/m^3. Levels may be slightly higher in urban than in rural locations; however, even in the vicinity of emission sources, the levels of airborne fluoride usually do not exceed 2-3 μg/m^3.

1.1.3 Fluoride in soil[5]

Fluoride is a component of most types of soil, with total fluoride concentrations ranging from 20 to 1000 μg/g in areas without natural phosphate or fluoride deposits and up to several thousand micrograms per gram in mineral soils with deposits of fluoride. Airborne gaseous and particulate fluorides tend to accumulate within the surface layer of soils but may be displaced throughout the root zone, even in calcareous soils. The clay and organic carbon content as well as the pH of soil are primarily responsible for the retention of fluoride in soils. Fluoride in soil is

primarily associated with the soil colloid or clay fraction. For all soils, it is the soluble fluoride content that is biologically important to plants and animals.

1.1.4 Fluoride in water[5,6]

Since some fluoride compounds in the earth's upper crust are soluble in water, fluoride is found in both surface water and ground water. In surface fresh water, however, fluoride concentrations are usually low 0.01 mg/l to 0.3 mg/l. In ground water, the natural concentration of fluoride depends on the geological, chemical and physical characteristics of the aquifer, the porosity and acidity of the soil and rocks, the temperature, the action of other chemical elements, and the depth of wells. Because of the large number of variables, the fluoride concentrations in groundwater can range from well under 1 mg/l to more than 35 mg/l. In Kenya and South Africa, the levels can exceed 25 mg/l. In India, concentrations up to 38.5 mg/l have been reported.

The transport and transformation of fluoride in water are influenced by pH, water hardness and the presence of ion-exchange materials such as clays. Fluoride is usually transported through the water cycle complexed with aluminium.

1.1.5 Fluoride in other places[5]

All foodstuffs contain at least trace amounts of fluoride. Elevated levels are present in fish. Tea leaves are particularly rich in fluoride; the amount of fluoride in brewed tea is dependent upon the concentration of soluble fluoride in the tea leaves, the level of fluoride in the water used in its preparation and the length of brewing period. The concentration of fluoride in food products is not significantly increased by the addition of superphosphate fertilizers, which contain significant concentrations of fluoride as impurities, to agricultural soil, due to the generally low transfer coefficient

from soil to plant material. However, a recent study suggests that, given the right soil conditions and application of sufficient fluoride as an impurity in phosphate fertilizers to soils, plant uptake of fluoride can be increased. The use of water containing relatively low levels of fluoride for crop irrigation generally does not increase fluoride concentrations in foodstuffs. However, this is dependent on plant species and fluoride concentrations in soil and water. The level of fluoride in foods is significantly affected by the fluoride content of the water used in preparation or processing, most notably in beverages and dry foodstuffs.

Dentifrice products for adults that are commercially available in many countries generally contain fluoride at concentrations ranging from 1000 to 1500 μg/g; some products designed for being used by children contain lower levels, ranging from 250 to 500 μg/g. Dental products such as toothpaste, mouthwash and fluoride supplements have been identified as significant sources of fluoride. Mouth rinses marketed for daily home use usually contain between 230 and 500 mg fluoride/l, whereas mouthwash products intended for weekly or biweekly use may contain 900-1000 mg fluoride/l.

1.1.6 Fluoride in natural water in Thailand[7]

In Thailand, fluoride contamination in water is mostly found in ground water. High concentration of fluoride in drinking water is hazardous to health. Those who consume water with high level of fluoride continuously for a long time can be at risk of fluorosis of their teeth and bones. In 1972, first quantitative survey of fluoride in drinking water was conducted in Chiang Mai Province in northern Thailand. Large-bone X-ray of several fluorosis cases were reported abnormal. In 1980, eleven cases of fluorosis were reported and all had a significant change in their bones. The X-rays

showed the accumulation of opacity along muscles and tendons. Fluoride in the range of 2-20 mg/l was found in urine. Of these, ten cases also had gallstones in their kidneys, three cases in their upper urinary tracts, and one case had to have a kidney dialysis. More investigation implied that all cases of fluorosis were related to drinking water which was contaminated by fluoride. A geochemical study of ground water in Chiang Mai found high fluoride concentration from 1.7-14 mg/l, similar to fluoride levels found in hot spring water from 17.3-20.5 mg/l. In this area, hot spring is the source of fluoride in ground water. It flows through rock fault and then contaminates ground water. In addition, fluoride concentration in ground water seems to increase after earthquake in the neighboring areas. The last National Dental Health Survey in 1994 found mottled enamel which is clinical sign of dental fluorosis in high proportion as 19-52% among schoolchildren in provinces of northern Thailand. In some areas within a province almost all children showed mottled enamel, suggesting widespread and seriousness of this problem in the region. Generally, ground water monitoring in Thailand find fluoride levels higher than the drinking water standard in the northern and western region. Sources of fluoride in these areas can be identified as follow:

a) Hot spring (as in Chiang Mai)

b) CaF_2 and apatite ores in decaying stones such as granite and pacmatile (as in Lumphun, Kanchanaburi and Surat Thani)

c) Industrial sources using hydrofluoric acid and phosphate fertilizer

1.1.7 Fluoride toxicity

Fluoride was first used to fight dental cavities in the 1940s, its effectiveness defended on two grounds[6]:

a) Fluoride inhibits enzymes that breed acid-producing oral bacteria whose acid eats away tooth enamel.

b) Fluoride ions bind with calcium ions, strengthening tooth enamel as it forms in children.

For the reasons above, it was found that small amounts of fluoride help prevent tooth cavities. According to World Health Organization (WHO) norms, the acceptable range of fluoride in drinking water is generally between 0.8-1.5 mg/l, in order to prevent dental and skeletal carries. The toxicity due to excessive consumption of fluoride more than 1.5 mg/l can lead to dental and/or skeletal fluorosis. The effect of fluoride is shown in the Table 1.1.

Table 1.1 Concentration of fluoride and biological effects[1]

Concentration of fluoride	Medium	Effect
0.002 mg/l	Air	Injury to vegetation
1 mg/l	Water	Dental caries reduction
2 mg/l	Water	Mottled enamel
8 mg/l	Water	10% osteosclerosis
50 mg/l	Food and water	Thyroid changes
100 mg/l	Food and water	Growth retardation
120 mg/l	Food and water	Kidney changes
2.5-5.0 g	Acute dose	Death

Generally, fluoride toxicity will manifest as any combination of;

1) Dental fluorosis[8,9]

Dental fluorosis is caused by a disruption in enamel formation which occurs during tooth development in early childhood. Enamel formation of permanent teeth, other than third molars (wisdom teeth), occurs from about the time of birth until approximately five years of age. After tooth enamel is completely formed, dental fluorosis cannot develop even if excessive fluoride is ingested. Older children and adults are not at risk for dental fluorosis. Dental fluorosis only becomes apparent when the teeth erupt. Because dental fluorosis occurs while teeth are forming under the gums, teeth that have erupted are not at risk for dental fluorosis. Classification criteria of dental fluorosis are as follows:

a) Normal: the enamel represents the usual translucent semivitriform type of structure. The surface is smooth, glossy and usually of a pale creamy white color.

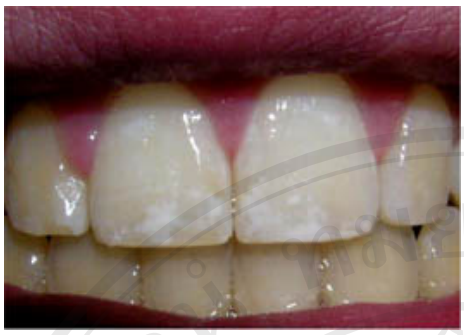
b) Questionable: the enamel discloses slight aberrations from the translucency of normal enamel, ranging from a few white flecks to occasional white spots. This classification is utilized in those instances where a definite diagnosis of the mildest form of fluorosis is not warranted and a classification of normal is not justified.

c) Very mild: small opaque, paper white areas scattered irregularly over the tooth but not involving as much as 25% of the tooth surface. Frequently included in this classification are teeth showing no more than about 1-2 mm of white opacity at the tip of the summit of the cusps of the bicuspids or second molars.

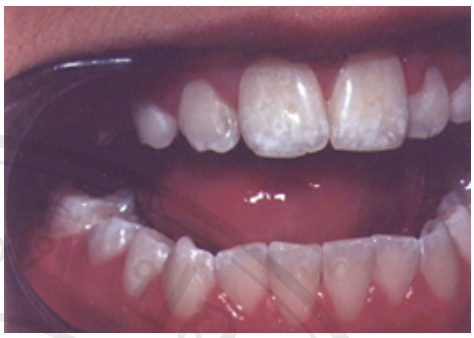
d) Mild: the white opaque areas in the enamel of the teeth are more extensive but do not involve as much as 50% of the tooth.

e) Moderate: all enamel surfaces of the teeth are affected and the surfaces subject to attrition show wear. Brown stain is frequently a disfiguring feature.

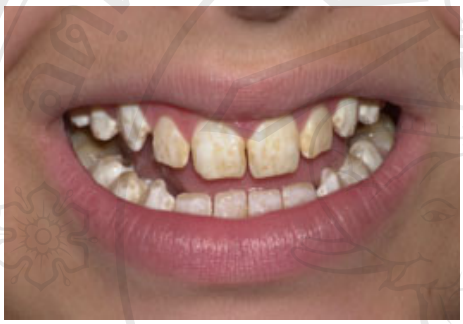
f) Severe: includes teeth formerly classified as moderately severe and severe. All enamel surfaces are affected and hypoplasia is so marked that the general form of the tooth may be affected. The major diagnostic sign of this classification is discrete or confluent pitting. Brown stains are widespread and teeth often present a corroded-like appearance.



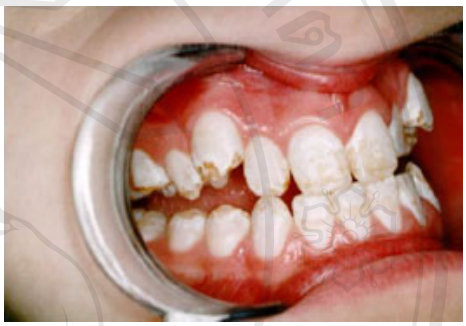
Very mild/mild Fluorosis



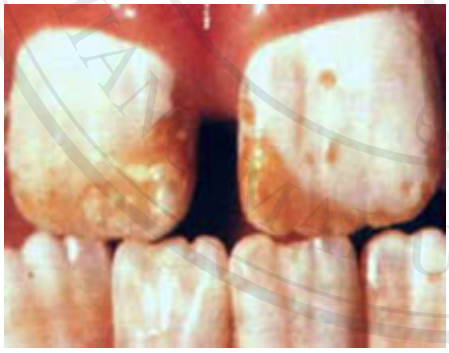
Mild Fluorosis



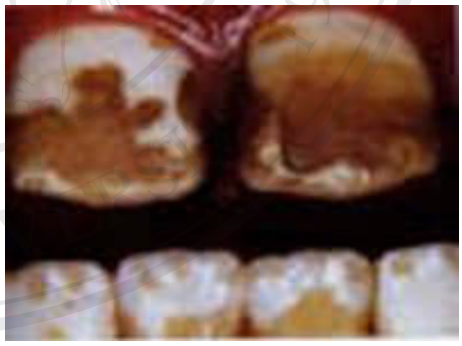
Moderate Fluorosis



Severe Fluorosis



Severe Fluorosis



Severe Fluorosis

Figure 1.1 Dental Fluorosis[9]

2) Skeletal Fluorosis[9]

A complicated illness caused by the accumulation of too much fluoride in the bones, has a number of stages. In the early clinical stage of skeletal fluorosis, symptoms include pains in the bones and joints; sensations of burning, pricking, and

tingling in the limbs; muscle weakness; chronic fatigue; and gastrointestinal disorders and reduced appetite. During this phase, changes in the pelvis and spinal column can be detected on x-rays. The bone has both a more prominent and more blurred structure. In the second clinical stage, pains in the bones become constant and some of the ligaments begin to calcify. Osteoporosis may occur in the long bones, and early symptoms of osteosclerosis (a condition in which the bones become more dense and have abnormal crystalline structure) are present. Bony spurs may also appear on the limb bones, especially around the knee, the elbow, and on the surface of tibia and ulna. In advanced skeletal fluorosis, called crippling skeletal fluorosis, the extremities become weak and moving the joints is difficult. The vertebrae partially fuse together, crippling the patient.

Figure 1.2 Skeletal fluorosis[10]

3) Non-Skeletal Manifestations[1]

This aspect of fluorosis is often overlooked because of the misconception prevailing that fluoride will only affect bone and teeth. Fluoride, when consumed in excess can cause several ailments besides skeletal and dental fluorosis viz.

a) Neurological manifestations: nervousness, depression, tingling sensation in fingers and toes, excessive thrust. Tendency to urinate frequently (polydypsia and polyurea are controlled by brain-appears to be adversely affected).

b) Muscular manifestations: muscle weakness, stiffness, pain in the muscle and loss of the muscle power.

c) Allergic manifestations: very painful skin rashes, which are perivascular inflammation present in women and children, pinkish, red or bluish red spots on the skin that fade and clear up in 7-10 days, they are round or oval shape.

d) Gastro intestinal problems: acute abdominal pain, diarrhea, constipation, blood in stools, bloated feeling (gas) tenderness in stomach, feeling of nausea and mouth sores.

e) Head-ache.

f) Loss of teeth (edentate) at an early age.

1.2 Methods for fluoride determination

1.2.1 Spectrophotometric methods[11-14]

Spectrophotometric determination of fluoride with organic reagents can be done using several methods such as Eriochrome cyanine R method, SPADNS method, Alizarin red S method and Alizarin fluorine blue method. These methods are based on the displacement reaction in which an organic ligand is replaced by fluoride in a

complex species, formed most often with Fe(III), Al(III), Th(IV) and Zr(IV). Because the spectrophotometric methods are subject to errors due to interfering ions, it may be necessary to distill the samples. When interfering ions are not present in excess of the tolerances of the method, the fluoride determination may be made directly without distillation.

a) Eriochrome cyanine R method

Zirconyl ion forms both monomolecular and bimolecular colored complexes with Eriochrome cyanine R in hydrochloric acid solution, depending on the acidity and the quantity of dye present. The structures of the complexes formed are believed to be as represented below:

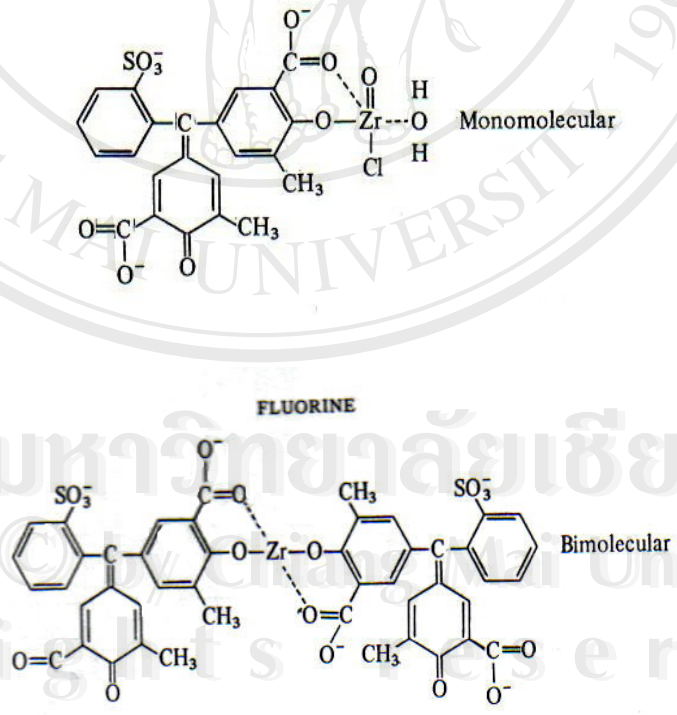


Figure 1.3 The structures of the complexes of zirconyl ions and Eriochrome cyanine R

When a limited quantity of fluoride is present in the reaction mixture, the zirconyl ion reacts preferentially with it, forming a complex of the composition ($ZrOF_2$). This reaction withdraws zirconyl ion from the colored complex and the results in a reduction of the total color of the system. This system involves complex equilibria in which acidity, zirconyl ions, Eriochrome cyanine R, fluoride, temperature and interfering ions all contribute to the amount of color formed. Therefore, no simple stoichiometric relationship exists between the fluoride, zirconium and dye.

b) SPADNS method

SPADNS method is based on the reaction between fluoride and a zirconium-dye lake. The dye lake itself is sodium 2-(parasulfophenylazo)-1,8-dihydroxy-3,6-naphthalene disulfonate, abbreviated as SPADNS. Fluoride reacts with the dye lake, dissociating a portion of it into a colorless complex anion (ZrF_6^{2-}) and the dye. As the amount of fluoride increases, the color produced becomes progressively lighter. This method provides a single, stable reagent, an instantaneous reaction, a high tolerance to sulfate as well as to other interferences and a satisfactory fluoride sensitivity for most purposes. It has been included as a standard method in the 13th edition (1971) of the standard methods. It can be used directly on many water samples without need for prior distillation. Its chemistry is very similar to the Eriochrome cyanine R method.

Figure 1.4 The structure of SPADNS

c) Alizarin red S method

Zirconyl chloride and Alizarin red S (sodium alizarin sulphonate) react, in acid solution, to form a brilliant reddish-violet lake. The complex gives a red-brown color if Alizarin red S is in excess and a violet color if the zirconium is in excess. This lake is destroyed by fluoride ions and the pale yellow color of the Alizarin reappears. Varying concentrations of fluoride produce a range of colors from red to yellow.

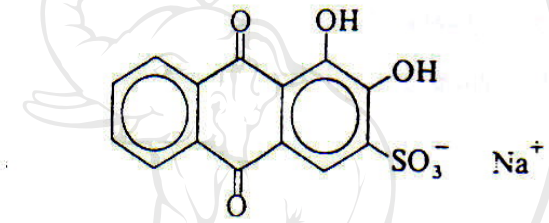


Figure 1.5 The structure of Alizarin red S

d) Alizarin fluorine blue method

A ternary complex containing equimolar amounts of a dye (Alizarin fluorine blue), lanthanum (III) and fluoride exhibits maximum absorbance at 625 nm. A comprehensive study of this method has shown that improved sensitivity and rate of color formation can be achieved by using a succinate buffer and a water-acetone solution.

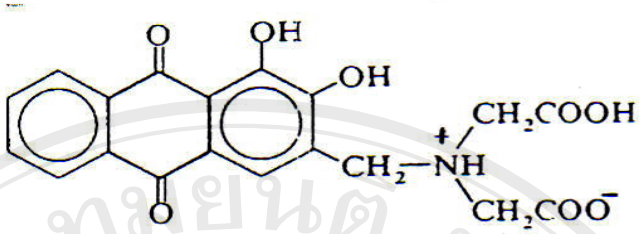


Figure 1.6 The structure of Alizarin fluorine blue

The determination of fluoride by spectrophotometric methods was studied by many workers: for examples, Dixon[15] has applied Eriochrome cyanine R method to determination of fluoride in the range 0-2.5 μg. It was found that this method was simple and rapid. Bellack and Schouboe[16] used SPADNS method for the determination of fluoride in the range of 0.00-1.40 mg/l in water samples. Sanchis[17] has adapted Alizarin red S method for fluoride determination in natural waters. It was found that this method is reliable, convenient and specially suited to routine determinations when a large number of samples are to be analyzed at any one time. Later on, Meyling and Meyling[18] modified zirconium-Alizarin red S method, accurate to within 0.05 mg/l of fluoride, for determining fluoride in water without prior distillation. Cardwell[19] adapted this method for flow-injection determination of fluoride in the range of 0.1-10 mg/l. For Alizarin fluorine blue method, the Analytical Methods Committee of the Society for Analytical Chemistry[20] has published a report based on a collaborative investigation of the Alizarin fluorine blue method for up to 40 μgF^- which increases both the sensitivity of the reaction and the stability of the complexes by using a 20% acetonitrile or acetone medium. Leon-gonzalez[21] used this method for determination of fluoride in the presence of sodium dodecyl sulfate at pH

4.6. It was found that this method provides a linear calibration graph for 0.08-1.2 mg/l fluoride. The relative standard deviation (n=10) was 0.2% at 0.60 mg/l fluoride and this method can be applied to sea and bottled mineral waters with satisfactory results. Rugrai[22] fabricated a fluoride test kit by using lanthanum-Alizarin fluorine blue method. It was found that this method has the effect of different factors such as type and concentration of metal, type and concentration of buffer, pH of buffer and the effect of acetone caused to the change of color. The result is found that the optimal composition of the reagent for fabricating the fluoride test kit is as follows: lanthanum mixed with Alizarin fluorine blue at equal concentration (0.002 M), 0.1 M of succinate buffer pH 4.6 and acetone.

1.2.2 Potentiometric method[23]

Potentiometric methods of analysis are based upon measurements of the potential of electrochemical cells in the absence of appreciable currents. Since the beginning of the twentieth century, potentiometric techniques have been used for the location of end points in titrimetric methods of analysis. Of more recent origin are methods in which ion concentrations are obtained directly from the potential of an ion-selective membrane electrode. Such electrodes are relatively free from interference and provide a rapid and convenient means for quantitative estimations of numerous important anions and cations.

The equipment required for potentiometric methods is simple and inexpensive and includes a reference electrode, an indicator electrode and a potential measuring device.

a) Fluoride-Ion Selective Electrode[12,24]

Fluoride electrode is a selective ion sensor. It is designed to be used with a standard calomel reference electrode and any modern pH meter having an expanded millivolt scale. The fluoride electrode consists of a single crystal of lanthanum fluoride as the membrane, bonded into a glass or an epoxy body. The cell may be represented by:

$$Ag/AgCl,\ Cl^-\ (0.3M),\ F^-(0.001M)\ /LaF_3/\ test$$

Only fluoride ions are mobile in the ionic conductor crystal. When the membrane comes in contact with a solution containing fluoride ions, a potential develops across the membrane. This potential is measured against an external (or internal) constant reference potential with a standard pH/mV meter or an ion meter and depends on the level of free fluoride ions in the solution. The Nernst equation describes the level of fluoride ions in the solution corresponding to the measured potential:

$$E = E_0 + S \log X$$

Where:

E = measured electrode potential

E_0 = reference potential (a constant)

S = electrode slope (~56 mV/decade)

X = level of fluoride in solution

The activity, X, represents the effective concentration of free fluoride ions in the solution. Total fluoride concentration, C_t, may include some bound as well as free fluoride ions. Since the electrode only responds to free ions, the concentration of the free ions, C_f, is found by:

$$C_f = C_t - C_b$$

where C_b represents the concentration of all bound or complexed fluoride ions. The activity is related to the free ion concentration by the activity coefficient, γ, by:

$$X = \gamma C_f$$

Activity coefficients vary, depending on total ionic strength, I, defined as:

$$I = \tfrac{1}{2} \Sigma C_x Z_x^2$$

where:

C_x = concentration of ion X

Z_x = charge of ion X

Σ = sum of all of the types of ions in the solution

In the case of high and constant ionic strength relative to the sensed ion concentration, the activity coefficient, γ, is constant and the activity, X, is directly proportional to the concentration.

b) Measurement Procedure

Direct measurement is a simple procedure for measuring a large number of samples. A single meter reading is all that is required for each sample. The ionic strength of samples and standards must be made the same by adjustment with TISAB for all fluoride solutions. The temperature of both sample solutions and standard solutions should be made the same.

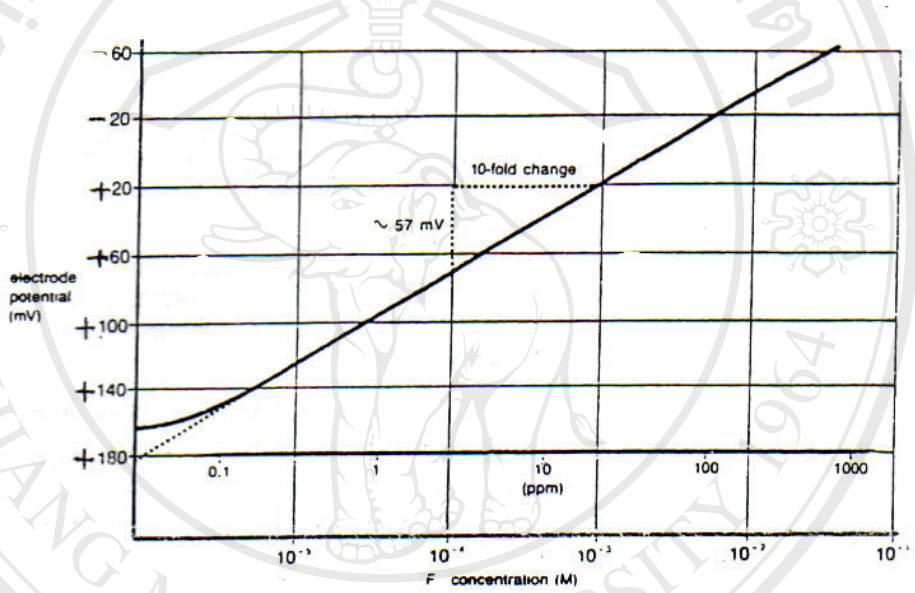


Figure 1.7 Typical fluoride electrode calibration curve

A fluoride electrode calibration curve is constructed on semi-logarithmic graph paper when using the pH/mV meter in the direct measurement procedure. The measured electrode potential in mV (linear axis) is plotted against the standard concentration (log axis). In the linear region of the curve, only three standards are necessary to determine a calibration curve. In the non-linear region, additional points must be measured. The direct measurement procedures given are for the linear portion

of the curve. The non- linear portion of the curve requires the use of low level procedures.

1.3 Ultraviolet and visible spectrometry[25-29]

The ultraviolet region of the spectrum is generally considered to range from 200 to 400 nm and the visible region from 400 to 800 nm. The corresponding energies for these regions are about 150 to 72 and 72 to 36 kcal mol^{-1}, respectively.

Molecular absorption spectroscopy is based on the measurement of the transmittance (T) or the absorbance (A) of solutions contained in transparent cells having a path length of b cm. Ordinarily, the concentration (c) of an absorbing analyte is linearly related to absorbance as represented by the equation:

$$A = -\log T = \log P_o/P = \varepsilon bc$$

All of the variables in this equation are defined in Table 1.2. This equation is a mathematical representation of Beer's law.

Table 1.2 Important terms and symbols for measurement of absorption

Term and symbol	Definition	Alternative name and symbol
Radiant power (P, P_0)	Energy of radiation (in ergs) impinging on a 1-cm^2 area of a detector per second	Radiation intensity I, I_0
Absorbance (A)	$\log P_0/P$	Optical density (D): extinction (E)
Transmittance (T)	P/P_0	Transmission (T)
Pathlength of radiation (b)	-	l,d
Absorptivity (a)	A/bc	Extinction coefficient (k)
Molar absorptivity (ε)	A/bc	Molar extinction coefficient

1.3.1 Instrument components

Instruments for measuring the absorption of ultraviolet and visible radiation consist of

1) Light source
2) Wavelength isolation device
3) Sample/reference compartment
4) Detector
5) Data output and data-processing device

A block diagram of the essential components of a typical ultraviolet and visible instrument is shown in Figure 1.8.

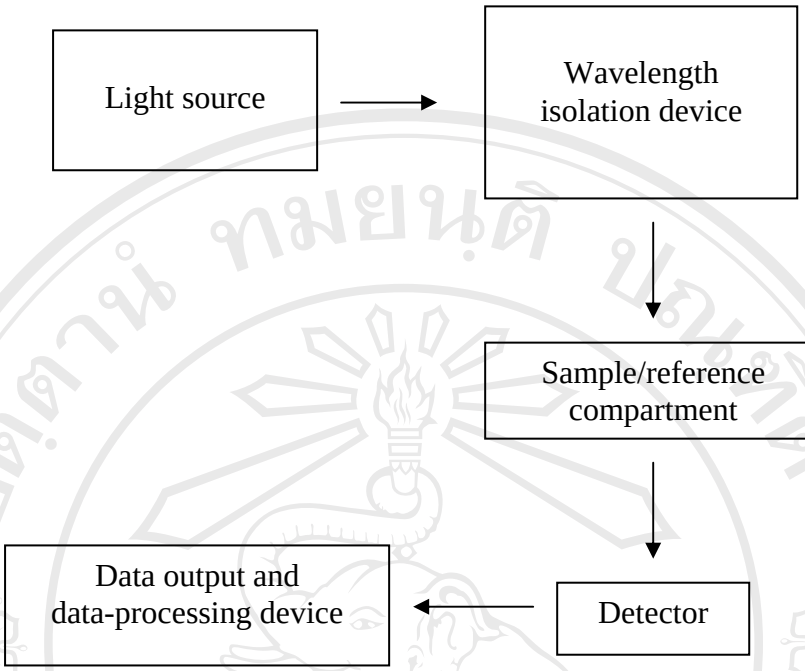


Figure 1.8 Block diagram of a typical instrument for making UV/Vis absorption measurements

The designs for photometers and spectrophotometers are shown in Figure 1.9 which can be in the form of single-beam, double-beam with beams separated in space and double-beam with beams separated in time.

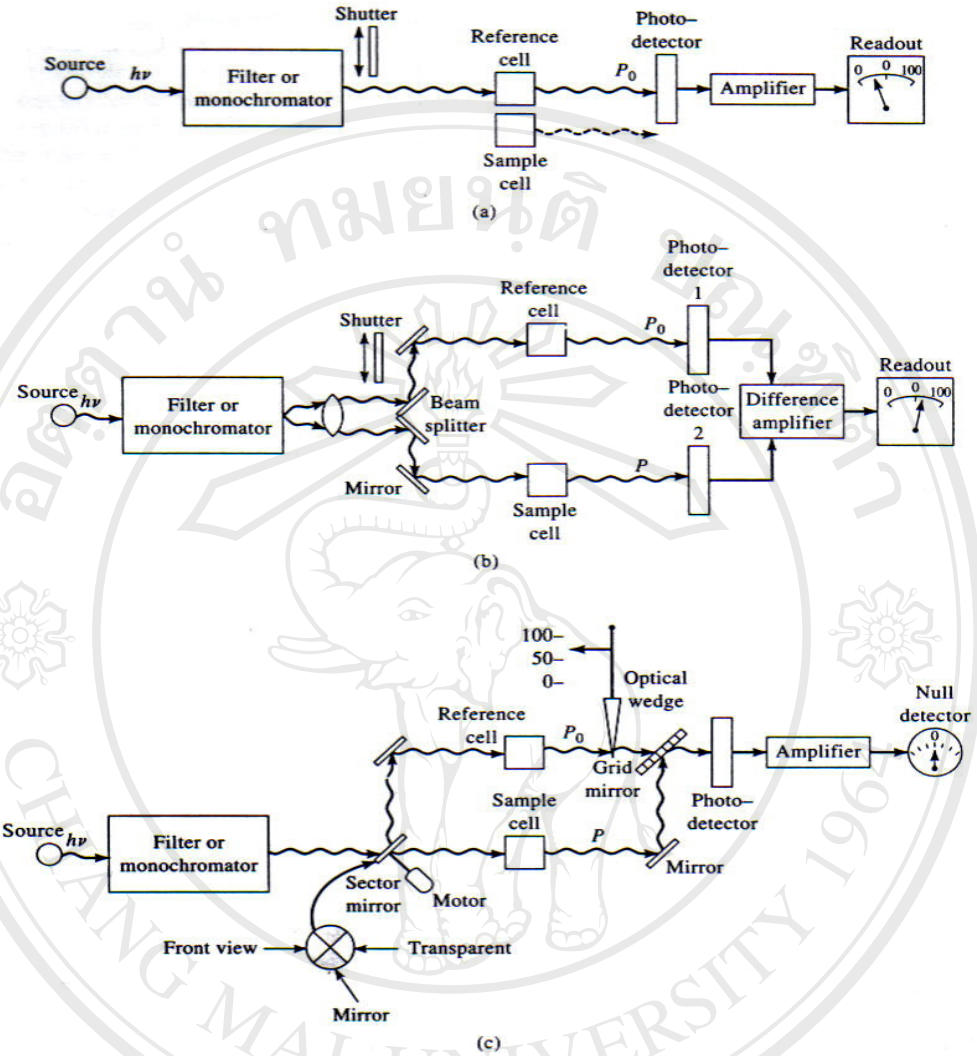


Figure 1.9 Instrument designs for photometers and spectrophotometers: (a) single-beam instrument, (b) double-beam instrument with beams separated in space, (c) double-beam instrument with beams separated in time

1.3.2 Quantitative analysis

UV/Vis spectroscopy is one of the most useful and widely used tools available to the chemist for qualitative and quantitative analysis, but only the quantitative aspect of the method will be mentioned in this work. In order to do the quantitative analysis, a calibration curve or a standard addition method is required.

a) Calibration curve

To use the calibration curve technique, several standards containing exactly known concentrations of the analyte are introduced into the instrument, and the instrumental response is recorded. Ordinarily, this response is corrected for the instrument output obtained with a blank. The resulting data are then plotted to give a graph of corrected instrument response versus analyte concentration.

b) Standard addition method

Standard addition method is particularly useful for analyzing complex samples in which the likelihood of matrix effects is substantial. A standard addition method can take several forms. One of the most common forms involves adding one or more increments of a standard solution to sample aliquots of the same size. This process is often called spiking the sample. Each solution is then diluted to a fixed volume before measurement.

1.4 The objective of the study

This research work is aimed to compare spectrophotometric methods for the determination of fluoride in water and apply the spectrophotometric methods for fabricating a simple fluoride test kit based on color comparison.