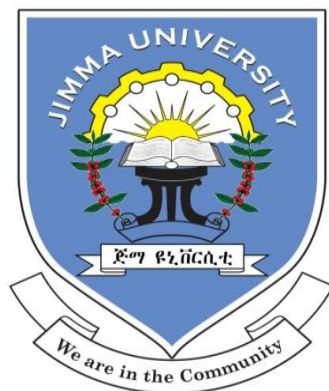JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF CHEMISTRY

SPECTROSCOPIC DETERMINATION OF FLUORIDE FROM GROUNDWATER USING

ERIOCHROME BLACK T (EBT) AS SPECTROMETRIC REAGENT:

A METHOD DEVELOPMENT

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JIMMA, ETHIOPIA

SPECTROSCOPIC DETERMINATIONS OF FLUORIDE FROM GROUNDWATER USING
ERIOCHROME BLACK T (EBT) AS SPECTROMETRIC REAGENT:
A METHODE DEVELOPMENT

A THESIS SUBMITTED TO COLLEGE OF NATURAL SCIENCES JIMMA UNIVERSITY
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IN CHEMISTRY (ANALYTICAL)

BY

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ACKNOWLEDGEMENTS

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List of Abbreviations

SPADNS   Sodium-2-p-sulfophenylazo-1, 8-dihydroxy naphthalene-3,6-disulfonate.

ISE   Ion selective electrode

UV/Vis   Ultra violet visible

ATPase   Adenosine triphosphatase

LOD   Limit of detection

LOQ   Limit of quantification

RSD   Relative standard deviation

EBT   Eriochrome Black T

SD   Standard deviation

GW   Groundwater

ABSTRACT

Fluoride health problem is a global issue as a result of groundwater use. Thus, trace level fluoride analysis is vital to take appropriate measures upon fluoride level in drinking water. Potentiometric method is an effective analytical technique for the determination of fluoride ion in various samples matrics. Spectroscopic methods are also found important to quantify fluoride levels from water, however, several factors hinder its easier determination. Among the bottlenecks, the use of expensive chemicals and tedious steps in preparing chemicals as well as toxicity of chemicals used (e.g., SPANDS method) are to mention few. In this study, spectrophotometric based method was developed for the determination of fluoride from groundwater using Eriochrome Black T (EBT) as spectroscopic reagent. Experimental parameters affecting determination of fluoride including ligand type, reaction time, pH and ligand to metal ratio were assayed and the optimum conditions were established. Calibration curve was constructed by preparing different standards of fluoride at seven concentration levels. The obtained calibration curve has good linearity with a coefficient of determination (R^2 = 0.9997). The limits of detection (LOD) and quantification (LOQ) were 0.19 and 0.64 mg/L, respectively. The precision studied in terms of intra-day and inter-day at two concentration levels showed %RSD values that were less than 7% in both cases. Applicability of the method was investigated by analyzing groundwater samples and demonstrated satisfactory recoveries which were 111.4 and 110.6 for 0.5 and 1.5 mg/L concentrations, respectively. The developed spectrophotometric method could be used as attractive alternative for the determination of fluoride from groundwater.

Keywords: Eriochrome Black T, spectrophotometry method, fluoride, groundwater

1 INTRODUCTION

Fluoride is a common element that is widely distributed in the earth's crust and exists in the form of fluoride in a number of minerals, such as fluorite (CaF_2), cryolite (Na_3AlF_6), monofluorophosphate and fluoroapatite ($Ca_5(PO_4)_3F$) [1]. It occurs in almost all waters from trace to high concentrations [2]. It forms mineral complexes with a number of cations like Al^{3+} and others. Though fluoride enters the body through water, food drugs, cosmetics, etc. Drinking water is major source of daily intake. It has been shown to cause significant effects in humans through drinking water [3]. Low concentrations of fluoride in ground water is an essential component for normal mineralization of bone, teeth and formation of dental enamel, but excessive exposure to fluoride in drinking water can give rise to a number of adverse effects [4–6]. Concentration in the range of 1.5–4.0 mg/L result in dental fluorosis and with prolonged exposure and higher fluoride concentration (4-10 mg/L) progresses to skeletal fluorosis. In dental fluorosis, there is dental mottling, which is characterized initially by opaque white patches on the teeth and in advanced stages leads to dental fluorosis (teeth display brown to black staining) followed by pitting of teeth surfaces. In skeletal fluorosis there is increase in bone density leading for thickness of long bones and calcification of ligaments [7–9]. The symptoms include pain in neck, backbone, in joints and there is stiffness in neck and rigidity in hip region [10-13]. One of the constraints in fluorosis mitigation work is the testing of fluoride in the water. World health organization (WHO) has set a limit value of 1.5 mg/L for fluoride in ground water [14]. So, there is narrow margin between the desired and harmful doses of fluoride in drinking water [15].

Fluoride concentration in various sample matrices is determined by various methods. The most important techniques are classified into five main categories: chromatography, electrochemical methods, Capillary zone electrophoresis, titration, and spectroscopy [16]. Each method has its own downsides, for instance, SPANDS method of spectroscopic determination posses lengthy solution preparations and also use toxic chemicals, ion chromatography is expensive, ion selective electrodes (ISE) is useful but require advanced cautions and buffer systems. Therefore, relatively simple, easier method of fluoride determination is found to be vital.

1.1 Statement of the problem

Fluoride occurs naturally in all types of water in different concentration levels. Surface waters and seawaters have low concentration of fluoride while groundwater may contain high levels of fluoride as it may be exposed to many inorganic fluoride-containing minerals. Major problems are being faced due to the presence of excess fluoride in groundwater in certain parts of Ethiopia. Low levels of fluoride can help to prevent dental cavities, however, higher levels of fluoride (above the desired limit) can lead to different health effect in children as well as adults [17]. Thus, several studies focused on the determination of fluoride including spectrophotometric, FISE and others. However, most of the methods posses several limitations for instance SPANDS method of fluoride analysis used toxic chemicals and require expensive chemicals. FISE also has bottlenecks because of its requirement of tedious buffer preparations [18]. Thus, to solve the aforementioned challenges or to bring better alternative, this study focused on the development of spectrophotometric method based on the use of organic dye (EBT) as spectrophotometric reagent for the determination of fluoride from groundwater.

1.2 Objectives

1.2.1 General objective

The general objective of this study was to develop a simple spectrophotometric method for the determination of fluoride from groundwater using EBT as spectrophotometric reagent.

1.2.2 Specific objectives

- ✓ To explore complexing reagents for the spectroscopic determination of fluoride.
- ✓ To optimize different parameters like working wavelength, reaction time, pH and ligand to metal ratio
- ✓ To validate the developed method in terms of linearity, limit of detection, limit of quantification, accuracy and precision before applying to real water sample.
- ✓ To apply the developed method for the analysis of the target analyte from the real groundwater sample.

1.3 Significance of the study

It is well known that the use of fluoride in small quantities [0.5-1.0 mg/L] taken from water is usually considered to have a beneficial effect, reducing dental carries, particularly among children. However, excessive intake results in pathological changes in teeth and bones, such as mottling of teeth or dental fluorosis followed by skeletal fluorosis. Therefore, development of analytical method for the determination of level of fluoride is crucial. In general, the findings of this study will have the following significances:

- ✓ It gives room for regular determination of fluoride in water sample including groundwater.
- ✓ The obtained results would be used as the background information about the levels of the target (fluoride) in groundwater to which the method was applied.
- ✓ The finding will also serve as resource materials for further studies.

2 LITERATURE REVIEW

2.1. Definition of fluoride

Fluorine is a univalent gaseous halogen, pale yellow-green in color and most chemically reactive electronegative of all other elements [19]. It does not usually occur in elemental form, and is found in ionic form in combination with various chemical compounds in minerals, e.g., cryolite, fluorapatite, and fluorspar, etc. The fluoride ion can exchange with hydrogen and the replacement of fluorine for hydrogen in natural compounds yields a very large number of compounds [20]. In aqueous solution it is commonly found as fluoride (F^-). It is the world's 13th most abundant element and constitutes 0.08% of the earth's crust and the lightest member of the halogen group [21]. Soil contains approximately 330 mg/L of fluorine [22, 23]. Some amount of fluorine is naturally present in the water, air, plants and animals. As a result, humans are exposed to fluorine through food, ground water and breathing air.

Fluorine in low level is essential for the maintenance and solidification of our bones and prevents dental decay. However, if it is consumed in excess, it may act in reverse way causing teeth decay, osteoporosis and harm to kidney, bone, nerve and muscle also. Fluorine being the most electronegative of all the elements possesses a strong tendency to acquire a negative charge and in solution forms F^- ions. Fluoride ions have the same charge and nearly the same radius as hydroxide ions and may replace each other in mineral structures [24]. Fluoride has been shown to cause significant effects in population through many ways like drinking water, air, dental products, food, beverages and salts [25]. A major cause of fluorosis is the inappropriate use of fluoride containing dental products such as toothpaste and mouth rinses [26].

2.2. Sources of fluoride

Unlike some of the other halogens, the majority of fluoride in the Earth's surface is derived from rock minerals, whereas other sources such as air, seawater and anthropogenic activities constitute a relatively small proportion. Traces of fluorides are present in many waters; higher concentrations are often associated with underground sources. In groundwater, for example concentrations vary with the type of rock the water flows through but do not usually exceed 10 mg/L [27]. In general fluoride is found in soil, water, food and air. The fluoride content in soil

normally ranges from 200 to 300 mg/L [28]. Virtually all foodstuffs contain at least trace amounts of fluoride [29]. Fluoride enters in human food-and-beverage chain in increasing amounts through the consumption of tea, wheat, spinach, cabbage, carrots and other foods items [30]. Principally fluoride is ingested into human body via water, food and beverages [31]. The amount of fluoride that a person intake in a day is usually less than 1.0 mg/L [28].

The major causes for the distribution, transportation and transformation of fluoride in the environment are: weathering and dissolution of minerals in water bodies, emissions from volcanoes, marine aerosols, coal combustion and process waters and waste from various industrial processes; including steel manufacturing, primary aluminum, copper and nickel production, phosphate ore processing, phosphate fertilizer production and use, glass brick and ceramic manufacturing, and glue and adhesive production [32].

2.2.1. Food and beverages

Drinking-water is typically the largest single contributor to daily fluoride intake [33]. However, fluoride intake from other sources is also considerable, especially from food [34]. Food is the main causative factor for fluorosis in areas with low fluoride concentration in the drinking water [35]. High fluoride concentration is often found in food items rich in minerals and trace elements. Teff flour, for instance, is considered a positive food item because of its high concentration of iron, zinc, and calcium [36]. The highest fluoride concentrations are found in the leaves and roots of plants [37].

Except for tea, most vegetables grown in low-fluoride areas have fluoride content less than 1mg/L [38]. Tea plants are found having high fluoride uptake and 97% of it gets accumulated in leaves [39]. The fluoride content of tea leaves is about 1000 times the soluble fluoride content of soil and 2 to 7 times the total fluoride content in soil [40].

With relatively high fish consumption in a mixed diet, the fluoride intake from fish alone would seldom exceed 0.2 mg/L F⁻ per day [41]. Milk typically contains low levels of fluoride, e.g. 0.02 mg/L in human breast milk and 0.02–0.05 mg/L in cow's milk [42]. Thus milk is usually responsible for only a small fraction of total fluoride exposure. Vegetables and fruits normally have low levels of fluoride (e.g. 0.1–0.4 mg/L and thus typically contribute little to exposure. However, higher levels of fluoride have been found in barley and rice which was about 2 mg/L.

The levels of fluoride in meat (0.2–1.0 mg/L) and fish (2–5 mg/L) are also relatively low [43]. The establishment of a water quality standard of 1 to 1.5 mg/L for fluoride consumption, through drinking water alone, is not enough to mitigate the adverse health effects of fluoride [44].

2.2.2. Air

Due to dust, industrial production of phosphate fertilizers, coal ash from the burning of coal and volcanic activity; fluorides are widely distributed in the atmosphere. However, air is typically responsible for only a small fraction of total fluoride exposure [45]. In non-industrial areas, the fluoride concentration in air is typically quite low (0.05–1.90 mg/L fluoride) [46].

2.2.3. Water

Fluoride in water derives mainly from dissolution of natural minerals in the rocks and soils with which water interacts. High fluoride concentrations are also often found in arid climatic conditions. Here, groundwater flow is slow and reaction times between water and rocks are therefore increased [47]. For a given individual, water consumption increases with temperature, humidity, exercise and state of health, and is modified by other factors including diet. Roughly, the closer to the Equator is the higher will be the water consumption [48].
As a result of this total daily fluoride exposure can vary markedly from one region to another. However, from several studies, a rough estimate of total daily fluoride exposure in a temperate climate would be approximately 0.6 mg/L per adult per day in an area in which no fluoride is added to the drinking-water and 2 mg/L per adult per day in a fluoridated area [49].

2.3. Health effect of fluoride

Fluoride has beneficial effects on teeth at low concentrations in drinking-water, but excessive exposure to fluoride in drinking-water, or in combination with exposure to fluoride from other sources, can give rise to a number of adverse effects. These range from mild dental fluorosis to crippling skeletal fluorosis as the level and period of exposure increases. Crippling skeletal fluorosis is a significant cause of morbidity in a number of regions of the world [50].

2.3.1. Dental fluorosis

Excessive, chronic ingestion of the fluoride ion during tooth formation can lead to tooth discoloration, which in most cases takes the form of slight, whitish spots that are barely visible and tend to fade over time. This change in the appearance of teeth is known as dental fluorosis which increases as fluoride concentration increases [51].

.Other etiological factors, such as exposure to amoxicillin at an early age, can have a similar effect on teeth appearance [52].

Fluoride-related fluorosis usually appears in children between birth and age 3, most commonly on the permanent central incisors [53], which are the most important teeth from an esthetic standpoint. With the exception of the third molars, enamel formation on permanent teeth starts around birth and is complete by about age 5. Later, dental enamel is completely mineralized and the risk of developing fluorosis disappears, even when fluoride is ingested in excessive amounts [54]. Dental fluorosis in its very mild, mild or moderate form is not considered to be a toxic effect. Mild or very mild fluorosis is often imperceptible to the untrained eye, but moderate fluorosis, caused by the ingestion of large quantities of fluoride during tooth formation, does affect tooth appearance which is dental decay [55]. Dental decay is the destruction of the outer coating of the tooth (enamel) through the action of bacteria in the dental plaque. If decay is untreated it spreads into the inner portion of the tooth causing a toothache and sometimes infection [56]. Calcium deficiency, co-exposure to certain minerals, malnutrition, respiratory, and various pathological conditions affecting urinary output and kidney function; may contribute to increases in the prevalence and severity of dental fluorosis and/or produce dental abnormalities that are indistinguishable from dental fluorosis.

Ingestion of very large quantities of fluoride during tooth formation can lead to severe fluorosis and enamel erosion, making teeth more vulnerable to decay. This undesirable effect is generally associated with water fluoride concentrations in excess of 4 mg/L [57].

However, is the possibility that those individuals exposed to fluoride levels above 2 mg/L and suffering from severe fluorosis might be at greater risk of developing caries due to the fluoride-induced pitting of the enamel which would allow food plaque to become entrapped in enamel defects and thereby induce decay. Evidence of an increase in decay rates in this segment of exposed populations would support the supposition that severe fluorosis is not merely an

undesirable cosmetic effect, but can also have adverse consequences with the potential to impact health [58].

When fluoride is present in oral fluids (i.e., saliva), fluorapatite, rather than hydroxyapatite, forms during the demineralization process. Fluoride ions (F^-) replace hydroxyl groups (OH^-) in the formation of the apatite crystal lattice as follows.

$$Ca_{10}(PO_4)_6(OH)_2 \rightarrow Ca_{10}(PO_4)_6F_2 \text{ [59].}$$

[60]
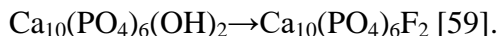
Figure 1 Picture showng dental fluorosis

2.3.2. Skeletal effects

Endemic skeletal fluorosis is well documented and is known to occur with a range of severity in several parts of the world, including India, China and northern, eastern, central and southern Africa. It is primarily associated with the consumption of drinking-water containing elevated levels of fluoride but exposure to additional sources of fluoride such as high fluoride coal is also potentially very important. This is compounded by a number of factors which include climate, related to water consumption, nutritional status and diet, including additional sources of fluoride and exposure to other substances that modify the absorption of fluoride into the body [61].

Fluoride is readily incorporated into the crystalline structure of bone, and will accumulate over time. Concerns about fluoride's effects on the musculoskeletal system are focused on a condition called skeletal fluorosis and also on increased risks of bone fracture. Models that estimate the accumulation of fluoride into bone (pharmacokinetic models) have been developed that are useful in understanding fluoride's effect on bone. Skeletal fluorosis is a bone and joint condition associated with prolonged exposure to high concentrations of fluoride.

Fluoride increases bone density and causes changes in the bone that lead to joint stiffness and pain [62].

In adults; stiffness of the back and neck muscles, unable to bend forward and to stand straight are some of the indicators of skeletal fluorosis. On the other hand, signs and symptoms of skeletal fluorosis in children include; pain in the lower limbs, knock knee, bow leg and anterior bowing of the lower limb bones [63].

Bones are largely composed of Calcium compounds, particularly carbonated hydroxyapatite $(Ca_{10} (PO_4)_6(OH)_2)$ or $Ca_5(PO_4)_3(OH)$ .When reacted with HF it forms an insoluble salt, CaF_2. This insoluble salt, CaF_2 is cleared by the body through excretion and as a consequence some of the calcium that would have been part of the bone matrix is washed away. This causes increased bone density with decreased bone strength [9].

Dimineralision Process: $Ca_5 (PO_4)_3(OH) + HF \rightarrow Ca_5 (PO_4)_3F + H_2O$

$$Ca_5 (PO_4)_3F \rightarrow 2Ca_3 (PO_4)_2 + CaF_2$$

Fluoride poisoning can be treated by the administration of soluble Calcium salt such as gluconate or chloride to produce insoluble CaF2 and to reverse the demineralization process.

Mineralisation Process: $3Ca2+ + 2PO_4^{\ 3-} \rightarrow Ca_3 (PO_4)_2$

$$3Ca_3 (PO_4)_2 + CaF_2 \rightarrow Ca_5 (PO_4)_3F$$

Figure 2 Picture showing skeletal fluorosis

2.3.3. Other problems

This aspect of fluorosis is often overlooked because of the notion prevailing that fluoride only affects bones and teeth [64]. Besides skeletal and dental fluorosis, excessive consumption of fluoride may lead to muscle fiber degeneration, low hemoglobin levels, deformities in red

blood cells, excessive thirst, headache, skin rashes, nervousness, neurological manifestations (it affects brain tissue similar to the pathological changes found in humans with Alzheimer's disease), depression, gastrointestinal problems, urinary tract malfunctioning, nausea, abdominal pain, tingling sensation in fingers and toes, reduced immunity, repeated abortions or still births, male sterility, etc. It is also responsible for alterations in the functional mechanisms of liver, kidney, digestive system, respiratory system, excretory system, central nervous system and reproductive system, destruction of about 60 enzymes. The effects of fluoride in drinking water on animals are analogous to those on human beings. The continuous use of water having high fluoride concentration also adversely affects the crop growth [65].

2.4 Different metal-dye complexes and their reaction with fluoride

2.4.1 Chrysin

Aluminum forms a complex with chrysin in the ratio of 1:3 that was studied by Job's method. The absorbance was measured at the wavelengths of the maximum difference (393 nm) in the electronic spectra between the ligand and the complex. The spectra of the reaction of various amounts of fluoride with the complex were compared [66]. In determination of fluoride by using aluminum to chrysin complex, colorless aluminum fluoride complex and free ligand were formed after the reaction of fluoride with the complex as follows.

$$Al\,[chrysin]_{3\,yellow}+ 6F^{-1}\rightarrow AlF_6^{-3}{}_{colorless}+3[chrysin]_{colorless}\ [67]$$
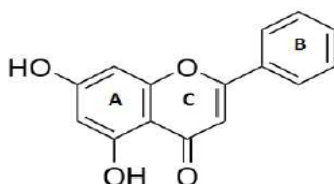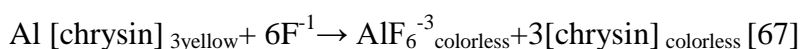
Figure 3 Structure of chrysin

The chemical structure of chrysin, with the presence of a double bond between C_2-C_3 in ring C, and the lacking of oxygenation at C_3 (Figure **3**.), is associated with numerous pharmacological properties. These include anti-microbial, anti-inflammatory, antispasmodic, anxiolytic, anthelmintic, anti-cancer, hypoglycemic, antiatherogenic, and anti-HIV [68].

Although chrysin possesses anti-cancer properties but poor bioavailability of this phenolic compound is an important obstacle for cancer treatment, which can be due to low absorption, quick metabolism and rapid systemic elimination. [69]

2.4.2 Reaction of fluoride with aluminum chrome azurol B complex

Fluoride reacts with the dark pink aluminum chrome azurol B complex to produce a colorless aluminum fluoride complex by replacement of the chrome azurol B by fluoride and liberation of the free ligand. This leads to a change in color from that of the complex, dark pink, to that of the free ligand, dark orange, according to the equation below. Aluminum reacts with fluoride to give compounds of the nature of (AlF_6^{-3}) or $(AlF_y(OH)_{6-y})^{-3}$ [70].

Al [chrome azurol B]$_{2\ pink}$ + 6F$^-$ $\rightarrow$ AlF$_6^{-3}$ $_{colorless}$ + [chrome azurol B]$_{2\ orange}$
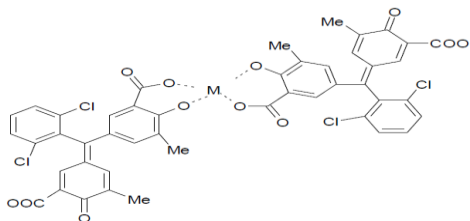
Figure 4 Structure of aluminum to chrome azurol B complex

The absorption spectra of the reaction of fluoride with the Al chrome azurol B complex showed that fluoride interacts to cause an increase in absorbance of the aluminum complex at 425 nm and a decrease in absorbance at 581 nm, due to the formation of the aluminum fluoride complex and release of the free ligand [71].

2.4.3 Reaction of Fluoride with the Resorcin Blue Complex

Aluminium forms a complex with Resorcin blue dye as the following structure.
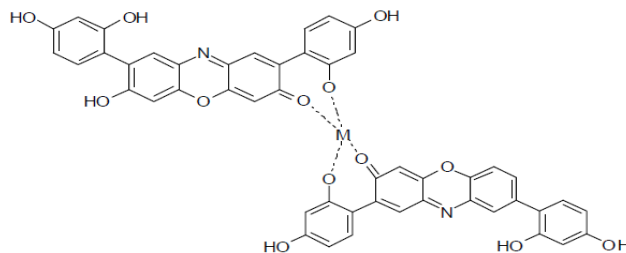
Figure 5 Possible structure for aluminum resorcin blue 1:2 complex

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Fluoride reacts with the brown aluminium resorcin blue 1:2 complex to produce a colorless aluminium fluoride complex by replacement of the resorcin blue by fluoride and liberating of the free ligand. This is resulting in a change in the color from that of the complex, brown to the color of the free ligand according to the equation below.

Al[resorcin blue]$_{2 \text{ brown}}$ + 6F^{-1} →AlF$_6$$^{-3}$$_{\text{colorless}}$ + [resorcin blue] $_{\text{blue}}$
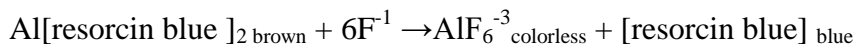
Fluoride interacts with complex under investigation to cause an increase in absorbance at 624 nm due to the releasing of the free ligand [71].

2.4.4 Reaction of fluoride with zirconium-SPADNS complex

Fluoride was analyzed spectrophotometrically using SPANDS (Sodium-2-(p-Sulfophenylazo)-1, 8-dihydroxynaphthalene-3, 6-disulfonate) as fluoride reagent. This method depends upon the bleaching action of fluoride on the intensity of color of the complexes formed between dyestuff and metal. A colored complex is formed between zirconium and SPANDS reagent. Fluoride ion reacts with dye- Lake dissociating a portion of it into a colorless complex anion [ZrF$_6$$^{2-}$] and dye. The rate of reaction between fluoride ion and Zirconium ion is influenced greatly by acidity of the reaction mixture and polyvalent metal ion present in the mixture [72].

Zr[SPADNS]$_{4\text{dark red}}$+6F^{-1} →ZrF$_6$$^{-2}$$_{\text{colorless}}$+4[SPADNS]

2.4.5 Reaction of aluminum quinalizarin complex with fluoride

Fluoride reacts with the pink aluminum quinalizarin 2:1 complex to produce a colorless aluminum fluoride complex by replacement of the quinalizarin by fluoride and liberating of the free orange quinalizarin ligand according to the equation below.

Al$_2$[quinalizarin]$_{\text{pink}}$+12F^{-1}→2AlF$_6$$^{-3}$$_{\text{colourless}}$ +[quinalizarin]$_{\text{orange}}$

Aluminum reacts with fluoride to give compounds of the nature of (AlF$_6$$^{-3}$) or (AlF$_y(OH)_{6-y}$)$^{-3}$. The absorption spectra of the reaction of fluoride with aluminum quinalizarin 2:1 complex showed that fluoride interacts to cause an increase in absorbance of the aluminum complex at 297 and 482 nm, and a decrease in absorbance at 264 and 553 nm [73].
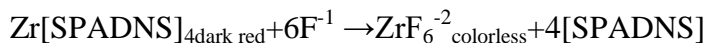
2.5 Eriochrome Black T (EBT)

Eriochrome Black T (EBT) is a solid brown–black powder with a faint metallic sheen. It is soluble in water and slightly soluble in ethanol [74]. Chemically it is sodium 1-(1-hydroxy-2-naphthylazo) -6-nitro-2-naphthol-4-sulphonate (NaH$_2$In) and also commonly called as Mordant Black 11. The sulphonic acid group on EBT is a strong acid and sodium salt of EBT is completely ionized in acid medium. Below pH 6, the aqueous solution of EBT exhibited a wine red colour, which is due to the presence of 1-(1-hydroxy-2-naphthylazo) - 6-nitro-2-naphthol-4-sulphonate anion (H$_2$In$^-$) [75].
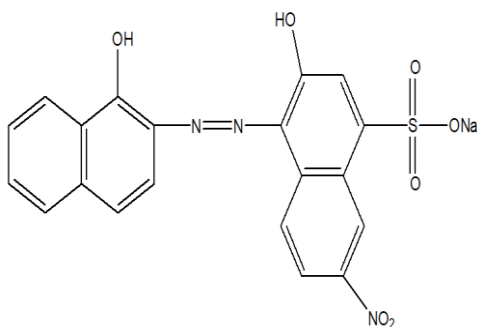
Figure 6. Chemical structure of EBT

It is a compound of anionic azo dyes that represents the class of carcinogenic dyes in nature. Eriochrome Black T is one of the essential azo dyes which are used in dyeing multi fibres, nylon, wool, and silk. It is also used as a complexometric titrant for estimation of various metal cations such as Ca^{2+}, Mg^{2+} and Zn^{2+} ions and to indicate the endpoint of a water hardness titration [76]. It has chelating properties. In analytic chemistry EBT is more often used in the inorganic analysis [77]. For the analysis of organic substances its application is limited. According to literature data it is known that EBT interacts with organic molecules, resulting in formation of ion associates, which are extracted by organic solvents [78, 79]. The visible-spectra of azo dyes (EBT) exhibit intense bands at 510–560 nm. The visible -spectra of azo dye to Al(III) complexes are very similar to those of free dyes while a 10–30 nm bathochromic shift of absorption maxima was observed. This effect can be caused by the displacement of the naphthalic hydrogens during chelation process. It is known that deprotonation of phenols is accompanied by a bathochromic shift of absorption band and increasing of absorbance. In addition, oxygen of ligand gives its lone pair to aluminium during chelation [80].

3. MATERIALS AND METHODS

3.1 Chemicals and reagents

Different analytical chemicals and reagents were used during the research work. These chemicals include sodium fluoride (99.0% NaF, Finkem, India) used to prepare standard solutions, Eriochrome lack T (Suvidhinath Laboratories, India) as ligand, Glacial acetic acid (CH_3COOH, 100%, Sigma-Aldrich Laborchemikalien, Germany) and sodium acetate (Sigma-Aldrich Laboratory, India) were used to prepare a buffer solution, concentrated hydrochloric acid (37%, Fisher Scientific UK Limited) and Ammonia solution (NH_4, 5%w/w, merk, Germany) to adjust the pH of the solution. Sodium chloride (Fisher Scientific chemicals, UK), nitric acid (69%, Research Lab Fine Chemical Industries, Mumbai, India) for the purpose of cleaning, Hydrated aluminium nitrate [$Al(NO_3)_3.9H_2O$, Nice chemical campany, India] was used for both the preparation of complex as the source of Al and for interference study as the source of NO_3^- ion. Sodium sulphate (Na_2SO_4, Blulux laboratory reagent, India) and hydrated aluminium chloride ($AlCl_3.6H_2O$) were also used as the source of SO_4^{-2} and Cl^- ion for interference study. Double distilled water was used in this study during the experimental studies.

3.2 Instruments and Equipments

Double beam UV-VIS spectrophotometer (Analytic Jena, Germany) was used to measure the absorbance of the sample, pH meter, (Bante instruments, pH902, USA) equipped with pH glass electrode was used to measure the pH of sample solutions. Electronic weight balance (Kern, ABJ-220NM, Germany) was used to weigh samples, quartz cuvette (1cm), Measuring cylinders (Duran, Germany), pipettes (Pyrex, USA), micropipettes micropipette (10-100 μL, 100- 1000 μL Dragonmed, Shangai, China) were used during measuring volumes of sample solutions and fluoride standard solutions, filter paper (whatman® no.542, 110mm, England), and other classical laboratory glassware's and apparatus were used during the study. Plastic funnels were also used for sample filtration.

3.3 Standard and working solution preparation

Sodium fluoride powder f was dried for 2hr in a desiccator before weighing. Fluoride stock solution was prepared by dissolving 2.21 g of dried sodium fluoride (NaF) in one liter distilled water [81]. This is to prepare a stock standard solution with 1000 mg/L fluoride concentration. The stock solutions were kept in refrigerator at 110°C until it was used. A range of standards of decreasing concentration are prepared by serial dilution from the stock standard solution. The stock solution was further diluted with distilled water as needed.

3.4 Preparation of Al to EBT complex

The complex solutions for the spectrophotometric measurements were prepared by mixing the proper aluminium to EBT ratio of 1.0×10^{-3} M of aluminium and 1.0×10^{-3} M of EBT ligand by weighing 0.1g of $Al(NO_3)_3.9H_2O$ and 0.12g EBT in 250 mL distilled water and then diluted to 5.0×10^{-5} M which was suitable for the spectrophotometric measurements.

3.5 Parameter optimization

The parameters like reaction condition of fluoride with a complex, maximum wavelength at which the complex absorbance was measured, initial concentration of fluoride, pH of the sample, the time to complete the reaction and the ligand itself was optimized.

3.5.1 Dye selection

The dyes including methylene orange, Brilliant green, Bromocresol green, methyl violet, malachite green and eriochrome black T were studied to select appropriate ligand (dye) for the fluoride determination. This is to find stable complex of aluminium to ligand for the determination of fluoride by spectrophometeric method.

3.5.2 Selection of scanning wavelength

As in the literature, the EBT exhibits an intense bands from 510-560 nm [80]. Based on this, the UV-VIs was scanned from 400–700 nm with the help of double beam spectrophotometer and the maximum absorbance of EBT with its complex of Al^{3+} was recorded.

3.5.3 Reaction of fluoride with the prepared complex

To optimize the reaction condition between the prepared complex and the fluoride different amounts of complexes were taken by making the concentration of fluoride constant and again by varying the concentration of fluoride with constant concentration of the complex.

3.5.4 Reaction time

The stability of the complex in water was examined by measuring the absorbance of the prepared solution at different time intervals (starting from initial to 15 min. to 48 hr).

3.5.5 pH study

The influence of pH on the formation of the complex was studied by carrying out the reaction in sodium acetate-acetic acid buffer solution of pH range 4.0–5.4.

3.5.6 Complexing ratio of metal to EBT complex

The complex solutions for the spectrophotometric measurements were prepared by mixing the proper aluminium to EBT ratio of 1.0×10^{-3} M of aluminium and 1.0×10^{-3} M of EBT ligand in distilled water, and then diluted to 5.0×10^{-5} M which was suitable for the spectrophotometric measurements. The molar combining ratio between aluminum and EBT was evaluated by Job's method of continuous variations. In this method, solutions of aluminum and EBT with identical molar concentrations (5.0×10^{-5} M) were mixed in varying volume ratios (1 + 9 to 9 +1 mL) in such a way that the total volume of each mixture was the same which is 10 mL [31]. The absorbance of the solutions was measured at wavelength of 544 nm and the mole fraction was calculated by using the following formula. For the reaction $M+nL \rightarrow ML_n$, the composition of the complex was given by the volume fraction ratio as follows:

$n = \dfrac{x}{1-x}$, Where n is the subscript of the ligand and

X: [1 - X], Where $X = \dfrac{V_m}{V_m + V_L}$ and V_M =Volume of metal, V_L =Volume of ligand

3.6 Sampling and determination of fluoride in a real groundwater samples

Groundwater sample was collected from Jimma University campus and transported to the laboratory, and the pH of the groundwater collected was measured to be 8.0. The other physicochemical characterstics were adopted from recent literature based on permission from authors. The water sample was filtered using filter paper no.1. Polythene bottle was used for sample collection. The sample was collected directly in the rinsed bottle without using any preservatives. After water sample was transported to the laboratory, fluoride analysis was performed using the developed method. The method under investigation was tested using a real groundwater sample by appropriate dilution. The water sample was diluted and then spike analysis has been performed. Fluoride was analyzed using Specord-200 plus double beam spectrophotometer. It was measured in the sample using the proposed spectrophotometric method.

3.7 Method validation

Analytical method validation plays a major role in the development of spectroscopic methods for the determination of different types of analytes. Validation is confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled. And method validation is the process of proving (through scientific studies) that an analytical method is acceptable for its intended use. It is usd to ensure confidence in the analytical data throughout product development. The validation was carried out in terms of linearity, the limit of detection, the limit of quantification, precision, accuracy and recovery studies [82–84].

3.7.1 Linearity (Calibration curve)

Linearity is the proportionality of measured value to the concentration. It was established using different calibration standards of fluoride in the range between 0.5–3.0 mg/L and the results were given below in results and discussion section.

3.7.2 LOD and LOQ

Limit of detection (LOD): -is the lowest concentration or mass of analyte in a sample that can be detected with a specified level of confidence but not necessarily quantified, under stated condition of the test. It was evaluated from LOD=$3.3\sigma/S$ Where, σ = the standard deviation of y-intercepts of regression lines and S = the slope of the calibration curve.

Limit of quantification (LOQ): -is the lowest amount of analyte in a sample which can be quantitatively determined with a suitable precision and accuracy.

LOD and LOQ were determined from the calibration curves and calculated based on the standard deviation of the response (y-intercepts of regression lines) and the slope using independent analytical curves by using the absorbance of the blank. LOQ=$10\sigma/S$ Where, σ = the standard deviation of y-intercepts of regression lines and S = the slope of the calibration curve.

3.7.3 Precision

The precision was determined by repeatability (intra-day), intermediate precision (inter-day) and reproducibility. Repeatability is a measure to express precision obtained with the same method, on the same test material, in the same laboratory, by the same operator, and using the same equipment within short intervals of time. Thus, in this research, repeatability was evaluated assaying three determinations at the same concentration, during the same day, under the same experimental conditions in morning session and afternoon session. Intermediate precision was determined by comparing the assays in three determinations at the same concentration within five different days. Precision (repeatability and intermediate precision) was expressed as relative standard deviation (RSD).

3.7.4 Interference study

The interference studies were evaluated by measuring the influence of anions such as chloride, nitrate, and sulphate on the determination of 1.5 mg/L fluoride. Therefore, the expected interfering anions were added in such concentrations for nitrate, sulphate and chloride in the range of 2–300, 25-500 and 40–700 mg/L, respectively [67, 70, 71].

3.7.5 Accuracy

Accuracy is an agreement between measured and real value. It is also defined as a measure of the difference between the expectation of the test result and the accepted reference value due to systematic method and laboratory error.

The accuracy of the proposed method was evaluated using recovery studies after standard addition of known amount of analyte of interest. Three different solutions of fluoride was prepared for each concentration levels (0.5 and 1.5 mg/L) and the accuracy was calculated on the basis of percentage recovery. The percentage recovery values were calculated by comparing amount obtained from the spiked samples with amount of the fluoride in which the concentration was actually added, as follows:

$$\%\text{Recovery} = \frac{C_{spiked} - C_{unspiked}}{C_{added}} \times 100$$, where C is the concentration of the sample

4. RESULTS AND DISCUSSION

4.1 Optimization of parameters

4.1.1 Dye selection

Several dyes have been tried as for the determination of fluoride including methylene orange, Brilliant green, Bromocresol green, methyl violet, malachite green and eriochrome black T. However, most of them fail to be sensitive and stable for analysis. As a result, the dyes fail to provide linear curve during the experiment. The ionic nature of the dyes could be also one possible factor to influence the complex formation. Accordingly, EBT is an anionic dye and can react with cation aluminium to form Al-EBT complex.Therefore, based on these informations and experimental trials, EBT was selected as spectrophotometric reagent for the determination of fluoride from groundwater.

4.1.2 Selection of wavelength

The visible spectra of EBT alone show a maximum absorbance at 535 nm. But when fluoride reacts with Al to EBT ($5.0x10^{-5}$ M) complex, it showed a maximum absorbance at 544 nm as shown in figure 7. This bathochromic shift observed because of the displacement of the naphthalic hydrogens during chelation process. It is known that deprotonation of phenols is accompanied by a bathochromic shift of absorption band and increasing of absorbance. Therefore, Electronic spectra of the complex shown a wavelength maximium at 544 nm, thus 544 nm was selected for further study.
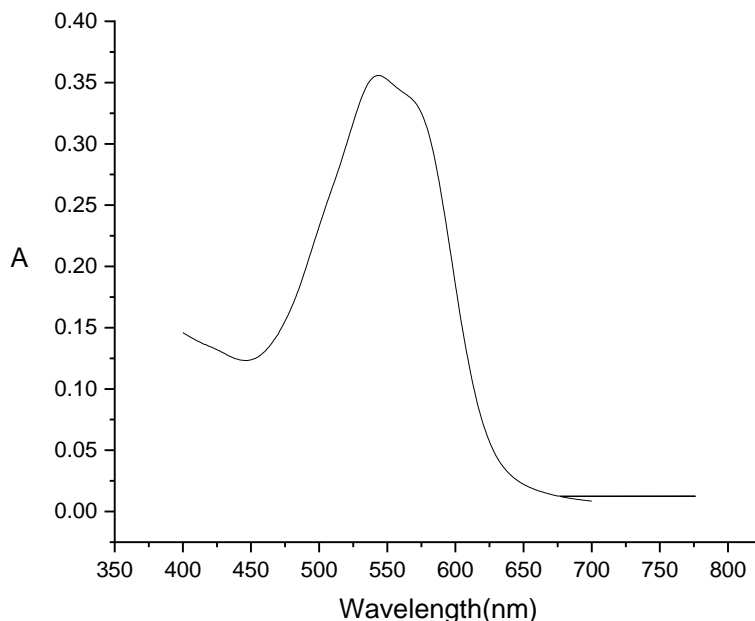
Figure 7. Electronic spectra of the complex in water at 5 x 10^{-5} M.

4.1.3 Reaction condition of fluoride with Al-EBT complex

The reaction between the metal to ligand complex of $(5.0\times10^{-5}\,\text{M})$ and fluoride calibration was attempted by varying the amount of fluoride as follows: [0.1, 0.3, 0.5, 1, 2, 3, 4 mg/L], [0.5, 1, 1.25, 1.5, 1.75, 2, 2.25 mg/L], [0.0, 1, 1.5, 2, 2.5, 3, 4] and [0.0, 0.5, 1, 1.5, 2, 2.5, 3 mg/L]. This gave the following squared correlation coefficient such as 0.9482, 0.9212, 0.9789 and 0.9986 respectively. The result showed that the standards of fluoride gives a good strong positive correlation in the range of 0.5-3.0 mg/L. And, again by making the concentration range of the fluoride (0.0, 0.5, 1, 1.5, 2, 2.5, 3 mg/L) and varying the concentration of the complex as $1.25\text{x}10^{-2}$ M, $2.0\text{x}10^{-4}$ M, $2.0\text{x}10^{-6}$ M and $5.0\text{x}10^{-5}$ M which gave squared correlation coefficient of 0.8791, 0.7756, 0.7910 and 0.999, respectively. Therefore, [0.0, 0.5, 1, 1.5, 2, 2.5, 3 mg/L] for fluoride and $5.0\text{x}10^{-5}$ M for complex were selected for further study. Fluoride reacts with the brown aluminium EBT complex to produce a colourless aluminium fluoride complex with replacement of EBT by fluoride and liberation of the free ligand. This leads to a change in colour from that of the complex, brown to that of the free ligand, light red

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(Figure **8**). Aluminium reacts with fluoride to give compounds of the nature (AlF_6^{-3}) or $(AlF_y(OH)_{6-y})^{-3}$ [80]. The absorption spectra of the reaction of fluoride with the Al-EBT complex shown that fluoride interacts to cause an increase in absorbance of the aluminium complex at 544 nm, due to the formation of the aluminium fluoride complex and release of the free ligand as shown in figure **8.**
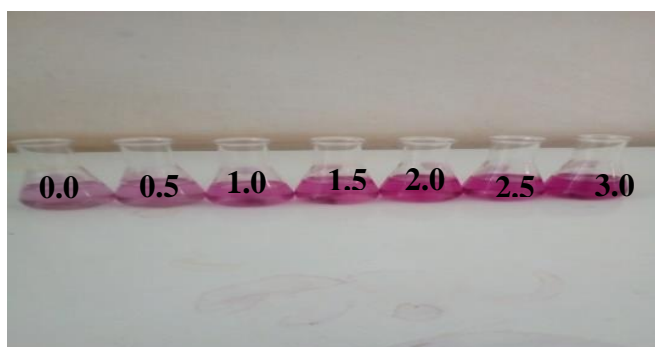
Figure 8. Shows the color increments of the solution after the addition of different amount of fluoride from 0.5–3.0 mg/L to 5.0×10^{-5}M metal-EBT 1:1 complex.

4.1.4 Optimization of time

The study of stability of the solution showed that absorbance of the solution increased up to 24hr and started to decrease after 24hrs. The increment of the absorbance was because intensity of the colour gradually increasesed as the concentration of fluoride increased from 0.5–3.0 mg/L up to 24hrs (figure 8), and started to decrease after 24hr due to the bleaching of the colour of the ligand. The bleaching of the colour was because the reaction between the complex and fluoride was completed at this time and the fluoride starts to replace EBT at all as follows:

$$Al[EBT] + 6F^- \rightarrow AlF_6^-{}_{colorless} + EBT$$

Table 1 Time effect on the determination of fluoride by EBT as a spectrophotometric reagent

Fluoride added in the complex	Absorbance reading at each time interval						
1.5 mg/L	At initial	2hr	6hr	12hr	24hr	36hr	48hr
	0.1230	0.2369	0.2729	0.3120	0.3684	0.2528	0.2017

4.1.5 Optimization of pH

High pH value may inhibit the reaction condition of fluoride with almunium-EBT complexes because as pH increases the concentration of OH^-, which has equal electrical charge and radius with fluoride increases and competes with it to form a complex with aluminium. That means the formation of $Al(OH)_3$ facilitated instead of the formation of aluminium fluoride. It is also known that low pH (below 4.5) is undesirable because fluoride ions combine with hydrogen ions (H^+) producing HF and HF_2 which can reduce the concentration of fluoride. At pH below 4.5, the UV-Vis may measure lower than the actual concentration of the fluoride ions. Based on this information, the pH of the sample was checked in between pH 4.0-5.4 and a maximum absorbance was found to be at pH 5.0, and it was selected for further study as the optimum pH.
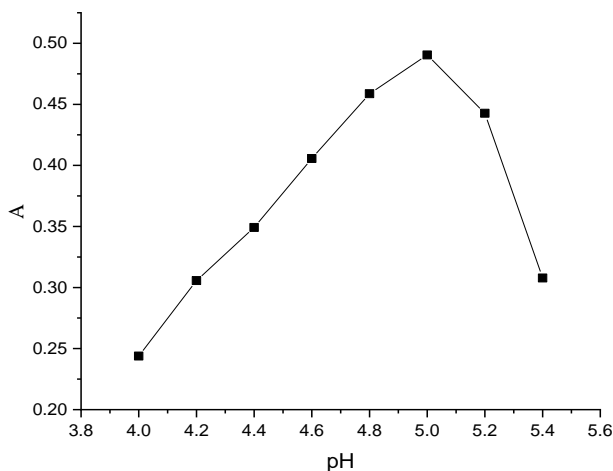
Figure 9. pH effect on determination of 1.5 mg/L fluoride using EBT as a spectrophotometric reagent

4.1.6 Molar ratio of metal to EBT complex

The results obtained from applying Job's method of continuous variation indicated the aluminium and EBT can form 1:1 complex because it gives the maximum absorbance at x=0.5 as showed in figure **10** below. The complex is possible because the lone electron pair of the oxygen atom in the Eriochrome black T is delocalized into the outer orbitals of the Al^{3+} ion, and a compound which is analogous to a donor-acceptor complex is formed. The proposed complex has a Π-bond, between the donor (oxygen atom) and the acceptor (Al^{3+} ion), which increased the binding energy of the central Al atom.

Aluminium Eriochrome Black T 1:1 complex is brown in water and changes this colour to light red after fluoride was added. This is because of the formation of aluminium fluoride complex and the release of free dye which responsible for the color.
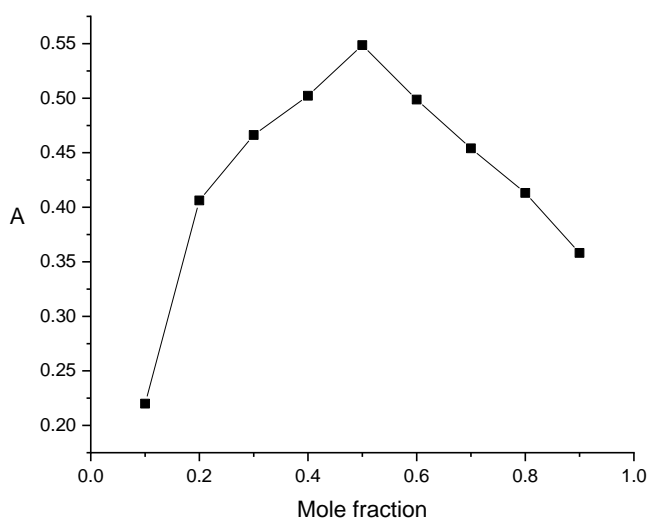
Figure 10 The graph of continuous variation method plotted absorbance versus mole fraction shows the ratio of complexes of 5.0×10^{-5} M aluminum to dye.

4.2 Method Validation

4.2.1 Calibration Curve

The spectrophotometric method was validated by using a series of concentration of fluoride transferred to cover the range of (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/L) by using the developed optimum conditions of pH 5.0. Each concentration level was read in triplicate the average value was taken. Then, standard calibration curve was plotted from the absorbance values obtained for the six standards of fluoride solutions (including the blank) by taking concentration (mg/L) on x-axis and absorbance values on y-axis. The linear regression equation was found to be y = 0.23373x + 0.2286 and R^2 = 0.9997, which is satisfactory for quantitative analysis of the analyte in a proposed groundwater sample as shown in Figure **11** below.

Equation	y = a + b*x
Intercept	0.22862 ± 0.00343
Slope	0.23373 ± 0.00176
R-Square (COD)	0.99977
Adj. R-Square	0.99972

Figure 11. Calibration curve for the determination of fluoride in the range from 0.5–3.0 mg/L in 5.0×10^{-5} M Al–EBT complex at 544 nm

4.2.2 Detection limit and Quantification limit

The limits of detection (LOD) and quantification (LOQ) of the method were calculated using 3 and 10 times the ratio of SD of the blank to slope the calibration curve, respectively. In this study, the values of LOD and LOQ were obtained 0.19 and 0.64, respectively. This result showed that the method can detect theoretically from 0.19 mg/L and quantify starting from 0.64 mg/L of fluoride in groundwater. Detail of the figures of the merits of the proposed method was presented in Table 2.

Table 2. Summary of sensitivity, detection limit, and quantification limit of the proposed methods

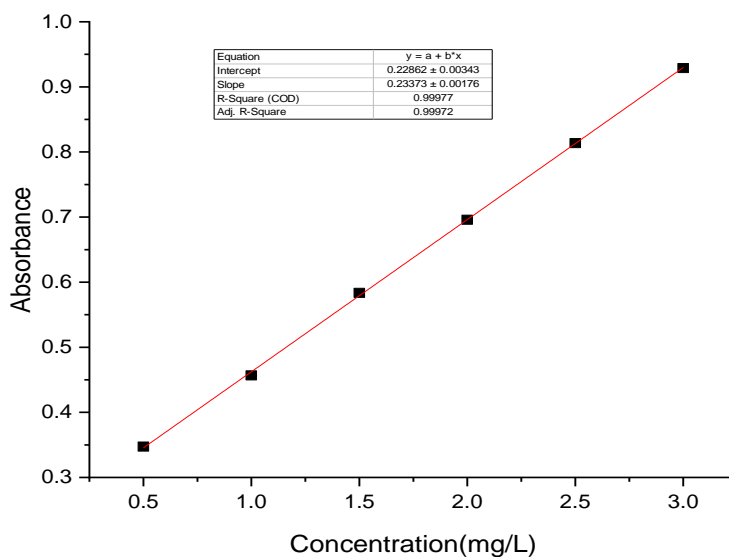|	
Wavelength (nm)	544
Sensitivity (mg/L)	0.2337
Detection limit (mg/L)	0.19
Quantification limit (mg/L)	0.64

4.2.3 Precision study

The precision of the proposed spectrophotometric method was investigated in terms of intra-day precision (repeatability) and inter-day precision (within-lab reproducibility). Intra-day precision was studied by preparing two different concentration levels (1.5 and 2.5 mg/L) fluoride and each concentration level was read in triplicate on the same day (in morning and afternoon session). Under the same experimental conditions, inter-day precision of the method was also evaluated at two concentration levels earlier used for intra-day precision studies, for five consecutive days with the same time interval. The relative standard deviation (RSD) of the Intra-day (repeatability) and inter-day (within-lab reproducibility) precisions of the proposed method were shown in Table **3**. The average %RSD of both intra and inter-day precisions of the two concentration levels were below 8%, which indicates the developed method was in the acceptable range of precisions for the analysis of the target analyte.

Table 3 Reproducibility, Intra-day and inter-day precision for determination of fluoride in 5.0 x 10^{-5} M aluminium EBT complex

Conc. of F^- in mg/L	Situation	
	Intra-day (%RSD)	Inter-day (%RSD)
1.5	7.30	6.94
2.5	8.18	6.75

4.2.4 Interference study

The interference studies were evaluated by measuring the influence of anions such as chloride, nitrate, and sulphate on the determination of 1.5 mg/L fluoride. Therefore, the expected interfering anions were added in such concentrations for nitrate, sulphate and chloride in the range of 2–300, 25-500 and 40–700 mg/L, respectively. From the studied ions SO_4^{2-} influenced the absorbance of the target analyte complex at high concentration within $\pm$ 5% that calculated based on the following formula:

$$Recovery\ (\%) = \frac{Absorbance\ of\ the\ interfering\ ion}{Absorbance\ of\ the\ analyte} x100$$ as summarized in Table 4.

Table 4. Effect of various amounts of interfering anions on 5.0 x 10^{-5} M aluminium EBT complex plus 1.5 mg/L fluoride at 544 nm

Absorbance of complex with 1.5 mg/L fluoride was 0.4235							
Cl^- in mg/L	40	70	100	300	500	600	700
Absorbance	0.4203	0.4244	0.4199	0.4200	0.4157	0.429	0.432
Recovery (%)	99.2	100.2	99.2	99.2	98.2	101.3	102
Absorbance of complex with 1.5 mg/L fluoride was 0.4375							
SO_4^{-2} in mg/L	25	50	75	100	200	300	500
Absorbance	0.437	0.434	0.432	0.4253	0.399	0.464	0.494
Recovery (%)	99.8	99.2	98.7	97.2	91.2	106.1	115.6
Absorbance of complex with 1.5 mg/L fluoride 0.3953							
NO_3^- in mg/L	2	5	25	50	100	200	300
Absorbance	0.3878	0.3914	0.3839	0.3904	0.3937	0.3822	0.3841
Recovery (%)	98.1	99	97.1	98.8	99.6	96.7	97.2

The data on interference showed that chloride and nitrate did not interfere with the determination of fluoride by this developed method. Whereas sulphate interfered with this method for determination of fluoride. This is due to the competition of SO_4^{2-} with fluoride to form a complex with the metal, thereby resulting in higher concentrations [85]. In the present work, when the amount of sulphate is higher than 200 mg/L sulphate shown to interfere with the determination of fluoride by changing the absorption at 544 nm.

4.2.5 Analysis of real sample and recovery studies

The developed spectrophotometric method was applied by performing relative recovery studies utilizing analysis of the groundwater sample. Relative recovery (%RR) studies were performed by spiking at two concentration levels earlier employed for precision studies. At each of the concentration levels, three samples were prepared and read in triplicate after 24hr. For each level, three blank samples (unspiked) were also prepared and analyzed by the same method. The %RR of the analyte was determined by comparing the concentration of the target analyte found from the spiked water sample with unspiked sample. The %RR of the target analyte for each level was shown in Table. The relative recovery (%RR) of the sample was calculated by:

%RR=$\frac{Cspiked-Cunspiked}{Cadded}$x100, where C is the amount of the sample

Table 5 %Recovery of groundwater sample in two different concentration level

Sample Type	Amount added	Amount unspiked	Amount spiking	%Recovery
GW-1	0.5 mg/L	2.500	3.0570	111.4
GW-2	1.5 mg/L	1.199	2.8590	110.6

The observed %RR of the target analyte were 111.4 and 110.6 , which briefly indicates the proposed method had acceptable relative recoveries for the determination of fluoride from groundwater samples. It was also observed that the contents of fluoride in the given water sample of 0.5 mg/L was above permissible limits of WHO recommended in drinking water which is 1.5 mg/L.

4.2.6 Comparison of methods in fluoride detection

The developed method for the determination of fluoride has been compared with related analytical techniques reported in the literatures employed to determine it from water sample. The comparison was made in terms of the calibration curve (linear), limit of detection (LOD), limit quantification (LOQ) and solvent type have been used and analytical method was shown below in Table **6**.

Table 6. Comparison of the LOD and LOQ of previously reported method development with present work.

Analytical methods	Solvent used	Linear Range (mg/L)	R^2	LOD (mg/L)	LOQ (mg/L)	Reference
Al to Quinalizarin	Ethanol	0.5-2.0	0.9977	0.1	0.3	[67]
Al to Resorcin blue	Methanol	0.2-1.0	0.9933	0.2	0.5	[72]
Al to Flavonoid chrysin	Ethanol	0.5-3.0	0.9914	0.1	0.3	[68]
Al to Malachite green	Ethanol	0.5-4.0	0.9878	0.1	0.3	[71]
Al to Chrome azurol B	Ethanol	0.5-4.0	0.9907	0.2	0.5	[71]
Zr to Resorcin blue	Ethanol	0.1-4.0	0.9930	0.07	0.2	[86]
Al to EBT	water	0.5-3.0	0.9997	0.19	0.64	Current work

As it can be seen from Table **6**, it was noted that the method developed in this study has provided better linearity, similar or better LOD, LOQ than the other methods. The technique was also uses conventional laboratory equipment as well as cheap solvents (water) that can be found in all research laboratories. Based on the experimental results, the spectrophotometric method developed can be considered one of the preferred alternatives for determining fluoride in groundwater sample.

5 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this work, the method was proposed for the determination fluoride by UV-Vis spectrophotometry. The accuracy of the method expressed by the percentage recoveries calculated from the spiking experiments of groundwater samples gave analytically acceptable results showing that the various matrices found in ground waters have insignificant effect on applicability of the developed method. Various optimization parameters influencing the determining efficiency of the methods have been carefully studied and optimal conditions were established. In conclusion, the obtained results demonstrated that the developed method was fully functional in the range of 0.5-3 mg/L of fluoride from ground water with 0.19 mg/L detection limit and 0.64 mg/L quantification limits. The relative percent recoveries ranging up to 111. This might help to control the amount of fluoride in the countries suffering from problem of fluoride, and to reduce different health effects.

5.2 Recommendation

Based on the finding of this study the analyst recommended that:

- ✓ The developed method should be further investigated for other samples/matrices in spectroscopic method determination of fluoride.
- ✓ The developed method could be used as alternative method for monitoring the level of fluoride in water and other similar matrices.
- ✓ The developed method could be used as new fluoride reagent for controlling the amount of fluoride in the countries suffers from fluoride health problem.

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