Chapter - 3

Method for the Analysis of Beryllium

OVERVIEW

Beryllium is a chemical element in the periodic table that has the symbol Be and atomic number 4. A bivalent element, beryllium is a steel grey, strong, light-weight yet brittle, toxic and potentially carcinogenic, alkaline earth metal, that is primarily used as a hardening agent in alloys (most notably beryllium copper).

History

The name beryllium comes from the Greek beryllos, beryl, from Prakrit veruliya, from Pali veuriya; possibly from or simply akin to a Dravidian source represented by Tamil veiruor, viar, “to whiten, become pale” [1,2]. At one time beryllium was referred to as glucinium (from Greek glykys, sweet), due to the sweet taste of its salts. This element was discovered by a French pharmacist and chemist Louis Nicolas Vauquelin in 1798 as the oxide in beryl and in emeralds. German chemist Friedrich Wöhler and French chemist A. A. Bussy independently isolated the metal in 1828 by reacting potassium and beryllium chloride [1].

Notable characteristics

Beryllium has one of the highest melting points (1287 °C) of the light metals. The modulus of elasticity of beryllium is approximately \( \frac{1}{3} \) greater than that of steel. It has excellent electrical and thermal conductivity, is non-magnetic and resists attack by concentrated nitric acid. It is highly permeable to X-rays and neutrons are liberated when it is hit by alpha particles, as from radium or polonium (about 30 neutrons/million alpha particles). At standard temperature and pressures beryllium resists oxidation when exposed to air [1,3].
Occurrence
The contents of beryllium in the earth’s crust [4], in soil [5] and in coal [6] are about 2–6, 1.2–2.1, and 2.5 µg g⁻¹, respectively. Beryllium is an essential constituent of about 100 out of about 4000 known minerals, the most important of which are bertrandite [Be₂Si₂O₇(OH)₂], beryl (Al₂Be₃Si₆O₁₈), chrysoberyl (Al₂BeO₄) and phenakite (Be₂SiO₄). Precious forms of beryl are aquamarine and emerald [1]. The most important commercial sources of beryllium and its compounds are beryl and bertrandite. Beryllium metal did not become readily available until 1957. Currently, most production of this metal is accomplished by reducing beryllium fluoride with magnesium metal [1].

\[
\text{BeF}_2 + \text{Mg} \rightarrow \text{MgF}_2 + \text{Be}
\]

Uses
Beryllium’s unique properties (it is a light metal with a very high melting point) make it very useful in industry. When it is used as an alloy, it increases thermal and electrical conductivity and strength; addition of just 2% beryllium to copper forms alloys that are six times stronger than copper alone [7]. The use of beryllium as an alloy, metal, and oxide in electrical components and aerospace and defence applications account for approximately 80% of its total use in the United States [8]. Pure beryllium metal or its alloys (with copper, aluminum, or other metals) are used in aircraft engine parts and disc brakes, X-ray transmission windows, space vehicle optics and instruments, aircraft and satellite structures, missile parts, nuclear reactor, neutron reflectors, nuclear weapons, fuel containers, precision instruments, rocket propellants, navigational systems, heat shields, mirrors, high-speed computers, audio components, electrical connectors and relays, springs, non-sparking tools, submarine cable housings and pivots, wheels, pinions, automotive electronics, molds for injection-molded plastics, telecommunications devices, home appliances, dental applications, golf clubs, bicycle frames, and many other applications [1,3,7, 9–13 ].

Toxicity
Beryllium and its salts are toxic substances and potentially carcinogenic [14]. The highest levels of human exposure to beryllium are through occupational exposure, which may occur via inhalation of beryllium dust or dermal contact with products containing beryllium. Chronic berylliosis is a pulmonary and systemic granulomatous disease caused by exposure to beryllium [12,13]. Acute beryllium disease in the form of chemical pneumonitis was first reported in Europe in 1933 and in the United States in 1943. Cases of chronic berylliosis were first described in 1946
among workers in plants manufacturing fluorescent lamps in Massachusetts. Chronic berylliosis resembles sarcoidosis in many respects, and the differential diagnosis is often difficult [1,3]. It may also cause eye and mucous membrane irritation, fatigue and weight loss [15]. Beryllium and beryllium compounds are known to be human carcinogens based on sufficient evidence of carcinogenicity in humans. Beryllium is classified by United States Environmental Protection Agency (USEPA) in Group B2 [15]. Beryllium and its compounds were first listed in the Second Annual Report on Carcinogens as reasonably anticipated to be human carcinogens based on carcinogenicity in experimental animals; however, the listing was revised to known to be human carcinogens in the Tenth Report on Carcinogens in 2002 [16]. The U.S. Department of Health and Human Services and the International Agency for Research on Cancer have determined that beryllium and its compounds are human carcinogens. EPA has estimated that lifetime exposure to 0.00004 mg beryllium/m³ can result in a one in a thousand chance of developing cancer [16]. EPA has found beryllium to potentially cause the following health effects when people are exposed to it at levels above the maximum contaminant level (MCL) for relatively short periods of time: inflammation of the lungs when inhaled; less toxic in drinking water. Beryllium has the potential to cause the following effects from a lifetime exposure at levels above the MCL: damage to bones and lungs; cancer [17].

Sources of beryllium in the environment
Beryllium is naturally emitted to the atmosphere by windblown dusts and volcanic particles. The major anthropogenic emission source to the environment is the combustion of coal and fuel oil, which releases particulates and fly ash that contain beryllium into the atmosphere. Other anthropogenic processes, such as ore processing, metal fabrication, beryllium oxide production and use, and municipal waste combustion, release only a fraction of the amounts emitted from coal and oil combustion. Deposition of atmospheric beryllium aerosols from both natural and anthropogenic sources is also a source of beryllium in surface waters. Some beryllium compounds are naturally present in soil, but the concentration of beryllium in localized soils can be increased because of the disposal of coal ash, municipal combusctor ash, industrial wastes that contain beryllium, and deposition of atmospheric aerosols [16].

Natural sources of beryllium and beryllium compounds in the atmosphere consist of windblown dust (5 metric tonnes/yr [11,000 lb/yr]) and volcanic particles (0.2 metric tonnes/yr [441 lb/yr]). Anthropogenic sources include industry (0.6 metric tonnes/yr [1,323 lb/yr]), metal
mining (0.2 metric tonnes/yr [441 lb/yr]), electric utilities (3.5 metric tonnes/yr [7,716 lb/yr]), and waste and solvent recovery (0.007 metric tonnes/yr [15 lb/yr]) [11].

Typical levels of beryllium that industries may release into the air are of the order of 0.01 µg/m³, averaged over a 30-day period, or 2 µg/m³ of workroom air for an 8-hour work shift. Compliance with the current U.S. Occupational Safety and Health Administration (OSHA) permissible exposure limit for beryllium of 2 µg/m³ has been determined to be inadequate to protect workers from developing beryllium sensitization. The American Conference of Governmental Industrial Hygienists (ACGIH), which is an independent organization of experts in the field of occupational health, has proposed a threshold limit value (TLV) of 0.05 µg/m³ in a 2006 Notice of Intended Change (NIC) [1].

Beryllium in water and soil

Beryllium enters waterways from the wearing away of rocks and soil. The sources of anthropogenic release of beryllium to surface waters include treated waste water effluents from beryllium or related industries and the runoff from beryllium-containing waste sites and from beryllium dust in the air from industrial activities settling over water. Most of the beryllium in water settles in the material on the bottom with sediment. A major portion of beryllium in soil does not dissolve in water and remains bound to soil, so it is not likely to move deeper into the ground and enter groundwater. Exposure to water-soluble beryllium compounds in the environment, in general, will pose a greater threat to human health than exposure to water-insoluble forms. [16]. In 1974, U.S. Congress passed the Safe Drinking Water Act. This law requires EPA to determine safe levels of chemicals in drinking water which do or may cause health problems. These non-enforceable levels, based solely on possible health risks and exposure, are called Maximum Contaminant Level Goals (MCLG) [17].

Beryllium is found in soil in amounts that vary over a wide range, but the typical concentration is 3 thousandths of a gram/kilogram (g/kg) of soil. Additional beryllium can be added by industrial activities. Soluble beryllium compounds can combine with other substances in the environment to form other beryllium compounds. Beryllium compounds may stay in the soil for thousands of years without moving downward into groundwater. In addition to the beryllium found naturally in minerals, beryllium metal and compounds that are left after humans mine and process the minerals can be released back into the environment as landfill waste [16].

As beryllium is a potential environmental pollutant, monitoring of beryllium level by trace analysis is demanded in atmospheric chemistry, geochemistry, industrial quality control and
occupational hygiene. Lobinski and Marczenko [18] have described the various analytical methods used to analyze beryllium in environmental media. Generally, atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma optical emission spectrometry (ICP-OES), PVC-based membrane sensors, particle induced gamma-ray emission and nuclear reaction analysis (PIGE-NRA), spectrofluorimetry are the common and recent techniques used in the determination of trace level Be. Many of these methods are highly expensive techniques. Spectrophotometry, however, continues to enjoy a wide popularity because of its rapidity, accuracy, precision and cost-effectiveness. The intent of this chapter is to provide a simple analytical technique, spectrophotometry for the determination of beryllium at trace levels.

**LITERATURE LOOK AT**

Most spectrophotometric methods for the determination of beryllium are based on ternary systems involving a triphenylmethane dye (e.g. Chrome Azurol S or Eriochrome Cyanine R) and a cationic surfactant [cetyltrimethylammonium bromide (CTA), cetylpyridinium bromide (CP) or Zephiramine)]. The methods are sensitive \( \varepsilon \approx 1 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1} \) whereas selectivity is considerably improved in the presence of EDTA. Marczenko [19] has reviewed several spectrophotometric methods for the determination of beryllium till 1986 with 88 references. Chrome Azurol S (Alberon) forms a coloured complex with beryllium, is the the popular and highly sensitive method for Be determination [20–23]. Alberon is so named because of its use as a reagent for aluminium and beryllium. The Chrome Azurol S (CAS) method is highly selective for beryllium in acetate buffer and in the presence of EDTA as masking agent. The absorbance of the complex depends on the pH, and on the concentration of CAS, EDTA, and the acetate buffer. The absorbance increases with increasing CAS concentration, and decreases with increasing EDTA and acetate concentrations. In the presence of hexamine buffer only a very small decrease in the sensitivity is observed. A pH of \( \sim 5 \) is the most suitable. Below this pH, the absorbance of CAS increases considerably, and above it, the absorbance of the beryllium complex is decreased more by EDTA.

The CAS method [19] involves the reaction of Be with CAS in presence of ascorbic acid and EDTA at pH \( \sim 5 \) (hexamine buffer). The absorption maximum of the binary complex is at \( \sim 570 \text{ nm} \) (against the reagent blank). The molar absorption coefficient is \( 1.5 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1} \) in presence of EDTA. In the absence of EDTA, the \( \varepsilon \) value increases to \( 2.4 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1} \). In the presence of cationic surfactant, such as CTA, the reaction of beryllium ions with CAS at the same
pH is several times more sensitive. Simultaneously, a large bathochromic shift of $\lambda_{\text{max}}$ of the reaction product (ternary complex) is observed. The molar absorption coefficient is $9.45 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ at 615 nm. At room temperature, the reaction is rather slow; measurements of the absorbance should be made after 1 h.

Besides CTA [24–26], other cationic surfactants have been proposed, namely: CP [27], Zephiramine [28–30], polyoxyethylene decylamine [31], Septonex [32], Sterolin ($\varepsilon = 1.06 \times 10^5$ L mol$^{-1}$ cm$^{-1}$) [33] and tetradecyldipyrudinium bromide (TPB) ($8.3 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ at 617 nm) [34]. Very high sensitivities in determining beryllium with CAS have been reached in the presence of non-ionic surfactants, such as OP-7 (polyhydroxyethylated alkylphenols) ($\varepsilon = 1.6 \times 10^5$ L mol$^{-1}$ cm$^{-1}$) [35], or Triton X-100 ($\varepsilon = 1.1 \times 10^5$ L mol$^{-1}$ cm$^{-1}$) [36].

Eriochrome Cyanine R (ECR) reacts with beryllium ions [37–40] similar to CAS. At pH 9.7, $\lambda_{\text{max}}$ of ECR is 435 nm and its water-soluble beryllium complex 525 nm. Molar absorption coefficient is $1.5 \times 10^4$ L mol$^{-1}$ cm$^{-1}$. EDTA, tartrate and cyanide are used as the main masking agents. In the presence of cationic surfactants, the sensitivity is increased several times, and significant bathochromic shifts are observed. In case of CTA, $\varepsilon = 8.65 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ at 590 nm, at pH ~7 (borate buffer) [41]. Similar sensitivities are reached by using Zephiramine [42], Septonex [32], and Hexadecyldipyrudinium chloride ($\varepsilon = 5.36 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ at 530 nm) [43].

Triphenylmethane chelating reagents, other than CAS and ECR, have also been used to determine beryllium, e.g. Aluminon [44], Xylenol orange [45], Methylthymol blue [46], Eriochrome Brilliant Violet B ($\varepsilon = 5.95 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ at 560 nm) [47], Sulphochrome ($\varepsilon = 1.4 \times 10^4$ L mol$^{-1}$ cm$^{-1}$) [48], and Chromal Blue G (CBG) ($\varepsilon = 3.1 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ at 610 nm) [49] (with CTA, $\varepsilon = 9.4 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ at 626 nm) [50].

Azo reagents have been used for the spectrophotometric determination of Be. The most important azo reagent is Beryllon II. It forms a blue complex with beryllium at pH 12–13 [51]; the molar absorption coefficient of the complex is $1.2 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ at 630 nm. Other important azo dyes proposed are: $p$-nitrophenylazo-orceinol [52], Arsenazo I [53], Thoron I ($1.36 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ at 523 nm) [54], Fast Sulphon Black F ($1.37 \times 10^4$ L mol$^{-1}$ cm$^{-1}$) [55], Eriochrome Black T (extraction with TOA in CHCl$_3$, $1.37 \times 10^4$ L mol$^{-1}$ cm$^{-1}$) [56], Calcichrome ($9.7 \times 10^3$ L mol$^{-1}$ cm$^{-1}$ at 625 nm) [57], the trisazosalicylic derivative of triphenylamine [58], 1-(2-thiazolylazo)-2-naphthol with Triton X – 100 ($2.25 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ at 555 nm) [59] and o-(2-hydroxy-5-methylphenylazo)benzoic acid [60].
Various organic reagents have found application in the spectrophotometric determination of beryllium, namely, quinalizarin [61], 2-phenoxyquinizarin-3,4-disulphonic acid [62], rufigallol [63], Naphthochrome Green G [64], 8-hydroxyquinaldine (3.5 × 10³ L mol⁻¹ cm⁻¹ at 380 nm) [65], and 2-hydroxyxalchone [66].

Investigation of ion-pair formation by beryllium with Alizarine Fluorine Blue and Adogen in slightly alkaline medium has been proposed by Salinas et al. [67]. The absorbance of the formed 1:2:2 Be-Alizarine Fluorine Blue-Adogen ion-association complex in the organic phase was measured at 480 nm. Beer's law was obeyed up to 0.36 µg mL⁻¹ of Be. The molar absorption coefficient was 5.35 × 10³ L mol⁻¹ cm⁻¹. Bhattacharjee et al. [68] have described a direct spectrophotometric determination of beryllium by complexion with 2-(p-sulfophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid (SPADNS) in the pH range 4.5-5.0. An apparent molar absorption coefficient of 1:1 Be-SPADNS complex at 585 nm was 1.0 × 10³ L mol⁻¹ cm⁻¹. These methods are less sensitive, poor selective and time consuming. Zhang et al. [69] have presented a method for the determination of beryllium with 4-(2-arsanophenylazo)-1,3-dihydroxynaphthalene. In buffer solution at pH 11.5, beryllium(II) reacts with the reagent to form an orange complex (1:2); the molar absorption coefficients are 4.0 × 10⁴ at 490 nm and 1.2 × 10⁴ L mol⁻¹ cm⁻¹ at 252 nm.

Shusen Luan et al. [70] described the spectrophotometric procedure for the determination of beryllium with Chlorophosphonazo III. In acetate buffer (pH 5.5), beryllium reacts with the reagent to form a 1:1 pink-coloured complex (ε = 3.0 × 10⁴ L mol⁻¹ cm⁻¹ at 550 nm). The calibration graph was linear for 1.0-6.5 µg Be/25 mL. Preeti Kaur et al. [71] have suggested spectrophotometric determination of beryllium with carminic acid. The absorbance of the resulting complex of Be-carminic acid complex at pH 4 was measured at 580 nm (2.25 × 10³ L mol⁻¹ cm⁻¹). Beer's law was obeyed from 0.4 to 1.6 µg mL⁻¹ of beryllium. The method is less sensitive and susceptible to associated metal ions.

The following organic reagents have also been recommended for spectrophotometric determination of beryllium: Chlorophosphonazo-mCF3 (ε = 2.73 × 10⁴ L mol⁻¹ cm⁻¹ at 603 nm) [72], N-phenyl-2-furohydroxamic acid (2.7 × 10⁴ L mol⁻¹ cm⁻¹ at 420 nm) [73], ECR-Benzalkoni Bromidium (9.1 × 10⁴ L mol⁻¹ cm⁻¹ at 586 nm) [74], CAS-Zephramine [75], 9-(2,4-dimethoxyphenyl)fluorine (DMPF) (9.85 × 10⁴ L mol⁻¹ cm⁻¹ at 542 nm) [76] and N-(4-methylphenyl)-3-oxobutanamide (8.65 × 10² L mol⁻¹ cm⁻¹) [77]. Some of these methods are less sensitive [72,73,77], time consuming [73,77] and poorer selective [72–77], and significantly interfere with associated ions in the beryllium determination.
An indirect spectrophotometric determination of beryllium by molybdenum blue method has been reported by Rachana Kaveeshwar et al. [78]. The method was based on the precipitation of beryllium as beryllium ammonium phosphate in acidic medium. This precipitate was dissolved in hot dilute HNO$_3$ and treated with ammonium molybdate to form yellow heteropoly molybdophosphoric acid which on reduction with malonyl dihydrazide (MDH) was converted to molybdenum blue. Beer’s law was obeyed at 780 nm in the range of 0.1-0.8 $\mu$g mL$^{-1}$ beryllium. The molar absorption coefficient of the method was $9.46 \times 10^3$ L mol$^{-1}$ cm$^{-1}$. The method has been successfully applied for determining beryllium in spiked water, biological samples, and standard beryl ore. The method is less selective and suffers from other reductants.

Molina-Diaz et al. [79] have developed a solid-phase spectrophotometric determination of beryllium in which Chrome Azurol S was used as a chromogenic reagent to form a blue complex which was easily and strongly sorbed on a dextran-type anion-exchange resin. The resin-phase absorbances at 594 and 800 nm were measured directly. The calibration graph was linear over the concentration range 0.010-0.085 $\mu$g mL$^{-1}$. A derivative spectrophotometric determination of beryllium with 5,8-dihydroxy-1,4-naphthoquinone in the presence of a non-ionic surfactant has been reported by Narinder Kumar Agnihotri et al. [80]. Absorption maxima and molar absorption coefficient of 1:2 (M:L) beryllium complex were 585 nm and $1.63 \times 10^4$ L mol$^{-1}$ cm$^{-1}$, respectively. Beer’s law was obeyed between 0.72 and 0.396 $\mu$g mL$^{-1}$ beryllium. Har Bhajan Singh et al. [81] described a procedure for the determination of trace amounts of beryllium using derivative spectrophotometry in non-ionic micellar medium. The method involves the formation of a 1:2 complex ($\varepsilon = 1.68 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ at 560 nm) with 1,4-dihydroxy-9,10-anthracenedione in an aqueous medium containing Triton X-100. Beer’s law was followed in the range 0.0036–0.36 $\mu$g mL$^{-1}$ of Be(II). Amin [82] has developed a procedure for the determination of trace amounts of beryllium based on solid-phase spectrophotometry. Beryllium reacts with aurantricarboxylic acid (ATCA) in the presence of ethylene-diaminetetraacetic acid to give a complex with a high molar absorption coefficient ($1.50 \times 10^7$ L mol$^{-1}$ cm$^{-1}$), which is fixed on a dextran-type anion-exchange gel (Sephadex DEAE A-25). The absorbance of the gel, at 575 and 750 nm, packed in a 1.0 mm cell, was measured directly. Calibration was linear over the range 0.03–1.0 $\mu$g L$^{-1}$ of Be.

Recently, Madrakian et al. [83] have studied a partial least-squares regression (PLS) for the spectrophotometric determination of beryllium in geochemical samples by Xylenol Orange as the chromogenic reagent in water media and in micellar media (CTA).
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A Thesis Submitted to the University of Mysore for the Award of Degree of Ph.D. in Chemistry

Very recently, a micelle-mediated extractive method for pre-concentration of ultra-trace quantities of beryllium as a prior step to its spectrophotometric determination has been developed by Abbas Afkhami et al. [84]. Chrome Azurol S (CAS) and cetyltrimethylammonium bromide (CTAB) were used as chelating agent and cationic surfactant, respectively. Linearity was obeyed in the range of 0.0009–0.018 μg mL⁻¹ of beryllium. Xin Zhuo et al. [85] have reported an extractive spectrophotometric determination of beryllium in dual-aqueous phase. In this method, the formed complex between beryllium and catechol violet was extracted into polyethylene glycol phase, which is prepared in acetate buffer of pH 3.5. The absorbance of the complex was measured at 591 nm (ε = 1.25 × 10⁵ L mol⁻¹ cm⁻¹).

Different experimental techniques for beryllium determination are reported in the literature which include fluorimetry [86,87], flow-injection system for potentiometry [88], ion chromatography [89,90], AAS [91,92], ICP-AES [93,94], ICP-OES [95], PVC-based membrane sensors [96,97], particle induced gamma-ray emission and nuclear reaction analysis (PIGE-NRA) [98] and laser-induced plasma spectrometer [99]. Some of these techniques are not affordable as a normal laboratory tool in most of the laboratories in Asian sub-continent.

In this chapter, the author has described a micelle-mediated sensitive spectrophotometric method for the determination of beryllium using haematoxylin.
Micellanized Spectrophotometric Method for the Determination of Beryllium Using Haematoxylin

3.1. INTRODUCTION

Haematoxylin or Natural Black 1 (C.I. 75290) is extracted from the wood of the logwood tree. It is chemically, 7,11b-dihydrobenz[b]indeno[1,2-d]pyran-3,4,6a,9,10 (6H)-pentol. Haematoxylin is one of the most commonly used stains in histology. Haematoxylin was synthesized by Dann et al. [100]. Haematoxylin trihydrate, white to yellowish crystals; redden on exposure to light. It is slightly soluble in water, but completely soluble in alcohol. It has the following structure:

![Structure of 7,11b-dihydrobenz[b]indeno[1,2-d]pyran-3,4,6a,9,10 (6H)-pentol (Haematoxylin)](image)

It has been used as a spectrophotometric reagent for the determination of boron [101], fluoride [102], tin [103], titanium [104], aluminium [105], gallium and indium [106], and ruthenium [107].

3.2. EXPERIMENTAL

3.2.1. Instrumentation

ANALYTIC JENA AG model SPECORD-50 and SYSTRONICS-166 digital spectrophotometers with 1.0 cm matched quartz cells were used for all absorbance measurements. The pH measurements were made with an ELICO model IL-610 digital pH meter.
3.2.2. Reagents

All chemicals used were of analytical reagent grade and doubly distilled water was used throughout.

**Standard beryllium(II) solution (1000 µg mL⁻¹):** This was prepared by dissolving 1.965 g of BeSO₄ · 4H₂O (Merck) in 0.2 M hydrochloric acid and standardized by phosphate method [108]. The working standard solution was freshly prepared every day by diluting with water.

**Haematoxylin (Sigma-Aldrich) solution (0.1%):** It was prepared by dissolving 250 mg of haematoxylin in 250 mL of ethanol.

**Cetyltrimethylammonium bromide (CTA) (Sigma-Aldrich) solution (0.1 M):** Prepared by dissolving 9.112 g of CTA in 250 mL of water by warming.

**Hexamine Buffer:** A 10% aqueous solution of hexamine (pH 9.0) was prepared and used. Hexamine buffer of pH 8.6 was prepared by adjusting the pH of 10% aqueous hexamine solution with dilute hydrochloric acid.

**Solutions of diverse ions:** Solutions of metal ions were prepared by dissolving the respective salts in water or in dilute acids. The solutions were standardized by conventional methods wherever necessary. Solutions of anions were prepared by dissolving the corresponding sodium or potassium salts in water.

3.2.3. Procedure for determination of beryllium as binary complex

In a 10 mL calibrated flask were placed standard solution containing not more than 2.5 µg of Be(II), 1.0 mL of haematoxylin (0.1%) and 3 mL of buffer (pH 9.0). The contents were diluted to the mark with water and the absorbance was measured after 1 h of mixing, at 555 nm against the reagent blank. The concentration of beryllium was established by reference to a calibration graph (Fig. 3.1) prepared with 0–2.5 µg of Be(II), following the above procedure.

3.2.4. Procedure for determination of beryllium as ternary complex

Various aliquots of standard solution containing 0.1–1.0 µg of Be(II) were transferred into a series of 10 mL calibrated flasks. Then, volumes of 1 mL each of the haematoxylin (0.1%) and CTA (0.05 M) were added followed by the addition of 3 mL of buffer (pH 8.6). The contents were diluted to the mark with water and the absorbance was measured after 1 h of mixing, at 592 nm against the reagent blank. A calibration graph was drawn (Fig. 3.1) and used to determine beryllium content in water samples.
3.2.5. Pretreatment and analysis of water samples

Tap and ground water samples were filtered through a filter-paper with a pore size of 0.45 μm (Millipore), preserved in nitric acid (1 mL of concentrated nitric acid per litre) in a polyethylene bottle that had been carefully cleaned with nitric acid. In order to eliminate the chlorine in tap water, the samples were treated for 5 min with 5 g of activated charcoal and filtered before being transferred into a polyethylene bottle. Beryllium in water samples was analyzed according to the general procedure described in Section 2.4, after separating it by cation-exchange column (in nitric acid-methanol medium) [109], to avoid the possible interference (matrix effect) of ions commonly associated with water samples.

3.3. RESULTS AND DISCUSSION

The developed method is based on the formation of a binary complex of beryllium with haematoxylin at pH 9.0. An attempt was made to increase the sensitivity of the method in the presence of micelle. The amplification of sensitivity was achieved by the addition of a cationic surfactant, CTA at pH 8.6. In comparison, the molar absorption coefficient of beryllium-haematoxylin in the presence of a micelle was nearly three times greater than in its absence.
3.3.1. Choice of surfactant
The effect of cetyltrimethylammonium bromide, zephiramine (cationic), and Triton X-100 (non-ionic) surfactants on the absorbance of beryllium-haematoxylin binary complex was studied at optimum experimental conditions ([Be] = 7.77 × 10⁻⁶ M). A maximum enhancement in the absorbance of the complex was occurred in presence of CTA (cationic). Therefore, CTA was selected and thus used as a micellar medium in the proposed procedure.

3.3.2. Absorption spectra
The absorption spectra of beryllium-haematoxylin binary complex at pH 9.0 (without CTA) and beryllium-haematoxylin in presence of CTA (ternary complex) at pH 8.6 ([Be] = 7.77 × 10⁻⁶ M; [haematoxylin] = 2.81 × 10⁻⁴ M; [CTA] = 5 × 10⁻³ M), and their corresponding reagent blanks were scanned in the wavelength range 350–700 nm. The above procedures (Sections 3.2.3 and 3.2.4) were followed for the formation of complexes. The absorption spectra are shown in Fig. 3.2. Beryllium-haematoxylin binary complex and its blank have maximum absorption at 555 nm (curve B) and at 478 nm (curve A), respectively. The addition of CTA to the haematoxylin at pH 8.6 is accompanied by hypsochromic shift (maximum absorption at 440 nm) with hypochromic effect (curve C). Moreover, bathochromic shift of the complex (maximum absorption at 592 nm) was observed with hyperchromic effect (curve D).

![Absorption spectra of haematoxylin complexes in aqueous and micellar media.](image)

Fig. 3.2. Absorption spectra of haematoxylin complexes in aqueous and micellar media. [Be] = 7.77 × 10⁻⁶ M; [haematoxylin] = 2.81 × 10⁻⁴ M; [CTA] = 5 × 10⁻³ M. 

A. Haematoxylin vs. buffer at pH 9.0; B. Be(II) + haematoxylin vs. haematoxylin at pH 9.0; C. Haematoxylin + CTA vs. CTA + buffer at pH 8.6; D. Be(II) + haematoxylin + CTA vs. haematoxylin + CTA at pH 8.6.
3.3.3. Optimization of experimental conditions

All variables were studied in order to fix the linear range for a quantitative determination of beryllium. The influence of following parameters with $7.77 \times 10^6$ M Be(II) in a final volume of 10 mL, are reported below.

3.3.3.1. Effect of pH

The effect of pH on the formation of beryllium-haematoxylin complex without and with micellar media was examined (Fig. 3.3), at the specified (Sections 3.2.3 and 3.2.4) wavelengths. It was found that the maximum absorbance of beryllium-haematoxylin binary complex was obtained when the solution was buffered in the pH range 8.8 – 9.2. Therefore, a buffer solution of pH 9.0 was used in the procedure. The absorbance of a ternary complex, beryllium-haematoxylin-CTA was found to be constant and maximum in the pH range 8.4 – 8.8. Hence, an optimum pH of 8.6 was maintained for the determination of beryllium in micellar medium.

![Absorbance vs pH](image)

**Fig. 3.3.** Effect of pH on the absorbance of (a) beryllium-haematoxylin and (b) beryllium-haematoxylin-CTA. Conditions as in Fig. 3.2.

3.3.3.2. Effect of the reagent concentration

The effect of haematoxylin concentration on the formation of beryllium-haematoxylin complex in non-micellar and micellar media was investigated (Fig. 3.4) at the optimum pH values. The maximum absorbance was observed in the range $2.11 \times 10^{-4}$ to $3.51 \times 10^{-4}$ M haematoxylin in both the media. Therefore, 1 mL of 0.1% haematoxylin was used in the proposed procedures to attain a final reagent concentration of $2.81 \times 10^{-4}$ M.
Fig. 3.4. Effect of haematoxylin concentration \((2.81 \times 10^{-3} \text{ M})\) on the absorbance of (a) beryllium-haematoxylin and (b) beryllium-haematoxylin-CTA. \([\text{Be}] = 7.77 \times 10^{-6} \text{ M}\).

3.3.3.3. Effect of CTA concentration

The effect of CTA concentration on the absorbance of beryllium-haematoxylin ternary complex was studied (Fig. 3.5) by keeping the fixed concentrations of beryllium \((7.77 \times 10^{-6} \text{ M})\) and

Fig. 3.5. Effect of CTA concentration \((0.05 \text{ M})\) on the absorbance of beryllium-haematoxylin. \([\text{Be}] = 7.77 \times 10^{-6} \text{ M}\).
haematoxylin (2.81 × 10⁻⁴ M) at the optimum pH of 8.6. The maximum sensitizing action of CTA was achieved in 5 × 10⁻³ M media of CTA in a final volume of 10 mL (1 mL of 0.05 M CTA in 10 mL reaction mixture). A lower concentration was accompanied by a decrease in the absorbance values.

3.3.3.4. Effects of temperature and time
The investigations were carried out at room temperature (27 ± 3°C). The absorbance of beryllium-haematoxylin binary and ternary complexes increased gradually for 1 h and remained constant for 1 h for binary complex and 2 h for ternary complex. Therefore, a standing time of 1 h was adopted in both procedures.

3.3.4. Stoichiometry of the complexes
The composition of beryllium-haematoxylin binary complex in the absence of micelle was determined by Job’s method of continuous variation (Fig. 3.6) and by the mole-ratio method (Fig. 3.7). From the graphs, the stoichiometry of the formed binary complex of beryllium-haematoxylin was 1:3 (metal to ligand). Attempt to determine the composition of the ternary complex was not made because more than 480-fold molar excess of CTA was required for complete complex formation.

![Fig. 3.6. Composition of beryllium-haematoxylin complex by the continuous variation method.](image-url)
3.3.5. Effect of interfering substances

The influence of various foreign substances on the determination of Be(II) was examined individually in micellar medium. The results are summarized in Table 3.1. The interference study indicated that the haematoxylin procedure is not selective for the determination of beryllium. Therefore, preliminary separation of beryllium is necessary in the real sample analysis.

3.3.6. Optical and Analytical characteristics

Calibration graph was linear \((r = 0.9999)\) over the concentration range 0.025 – 0.25 and 0.01 – 0.1 \(\mu g \ mL^{-1}\) of Be(II) for the beryllium-haematoxylin \([y = (2.7815)x + 0.0034];\) where ‘\(x\)’ is the concentration of Be(II) in \(\mu g \ mL^{-1}\)] and beryllium-haematoxylin-CTA \([y = (7.7939)x + 0.0017];\) where ‘\(x\)’ is the concentration of Be(II) in \(\mu g \ mL^{-1}\)], respectively. The value of molar absorption coefficient as determined by the least-squares method for ten results was \(2.54 \times 10^4 \ L \ mol^{-1} \ cm^{-1}\) for a binary beryllium-haematoxylin. The corresponding value in the presence of micelle was \(7.07 \times 10^4 \ L \ mol^{-1} \ cm^{-1}\). Sandell’s sensitivities were found to be 0.355 (a binary complex) and 0.127 (a ternary complex) ng cm\(^2\). The detection limit (DL = 3.3 \(\sigma /S\)) and quantitation limit (QL = 10 \(\sigma /S\)) [where ‘\(\sigma\)’ is the standard deviation of the blank \(n = 10\) and ‘\(S\)’ is the slope of the calibration curve] of beryllium determination were 74 ng mL\(^{-1}\) and 226 ng mL\(^{-1}\) in non-micellar, and 2.9 ng mL\(^{-1}\) and 8.9 ng mL\(^{-1}\) in micellar media, respectively.
Table 3.1. Effect of interfering substances

<table>
<thead>
<tr>
<th>Substance added (Substance/Be)</th>
<th>Molar ratio (Substance/Be)</th>
<th>Absorbance at 592 nm ([Be] = 0.05 μg mL⁻¹)</th>
<th>Interference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>0.392</td>
<td>-</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>10</td>
<td>0.159</td>
<td>-59.4</td>
</tr>
<tr>
<td>Mn(II)</td>
<td>10</td>
<td>0.181</td>
<td>-53.8</td>
</tr>
<tr>
<td>Co(II)</td>
<td>10</td>
<td>0.152</td>
<td>-61.2</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>100</td>
<td>0.380</td>
<td>-3.1</td>
</tr>
<tr>
<td>Pb(II)</td>
<td>10</td>
<td>0.221</td>
<td>-43.6</td>
</tr>
<tr>
<td>Ni(II)</td>
<td>10</td>
<td>0.434</td>
<td>+10.7</td>
</tr>
<tr>
<td>Cd(II)</td>
<td>10</td>
<td>0.418</td>
<td>+6.6</td>
</tr>
<tr>
<td>Ca(II)</td>
<td>100</td>
<td>0.401</td>
<td>+2.3</td>
</tr>
<tr>
<td>Ba(II)</td>
<td>100</td>
<td>0.396</td>
<td>+1.0</td>
</tr>
<tr>
<td>Mg(II)</td>
<td>100</td>
<td>0.254</td>
<td>-35.2</td>
</tr>
<tr>
<td>Al(III)</td>
<td>10</td>
<td>0.465</td>
<td>+18.6</td>
</tr>
<tr>
<td>La(III)</td>
<td>100</td>
<td>0.293</td>
<td>-25.3</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>100</td>
<td>0.325</td>
<td>-17.1</td>
</tr>
<tr>
<td>In(III)</td>
<td>10</td>
<td>0.451</td>
<td>+15.1</td>
</tr>
<tr>
<td>Ga(III)</td>
<td>10</td>
<td>0.458</td>
<td>+16.8</td>
</tr>
<tr>
<td>As(III)</td>
<td>10</td>
<td>0.357</td>
<td>-8.9</td>
</tr>
<tr>
<td>Zr(IV)</td>
<td>100</td>
<td>precipitated</td>
<td>-</td>
</tr>
<tr>
<td>V(V)</td>
<td>100</td>
<td>precipitated</td>
<td>-</td>
</tr>
<tr>
<td>EDTA</td>
<td>100</td>
<td>0.327</td>
<td>-16.6</td>
</tr>
<tr>
<td>Fluoride</td>
<td>100</td>
<td>0.325</td>
<td>-17.1</td>
</tr>
<tr>
<td>Phosphate</td>
<td>10</td>
<td>0.145</td>
<td>-63.0</td>
</tr>
<tr>
<td>Acetate</td>
<td>100</td>
<td>0.378</td>
<td>-3.6</td>
</tr>
<tr>
<td>Oxalate</td>
<td>10</td>
<td>0.197</td>
<td>-49.7</td>
</tr>
<tr>
<td>Tartrate</td>
<td>100</td>
<td>0.298</td>
<td>-24.0</td>
</tr>
<tr>
<td>Cyanide</td>
<td>10</td>
<td>0.294</td>
<td>-25.0</td>
</tr>
<tr>
<td>Thiocyanate</td>
<td>100</td>
<td>0.365</td>
<td>-6.9</td>
</tr>
</tbody>
</table>

3.3.6.1. Within-day and between-day precision studies

To ascertain the ruggedness of the method, five replicate determinations at different concentration levels of beryllium in presence of micelle were carried out. The within-day RSD values were ≤ 0.36%. The between-day RSD for different concentrations of beryllium obtained from the average of five determinations carried out over a period of 5 days were found to be ≤ 0.65%. The results indicate that the proposed method has an advantage of excellent reproducibility in both within- and between-days precision (Table 3.2).
Table 3.2. Within-day and between-day precision studies on the determination of beryllium in micellar medium

<table>
<thead>
<tr>
<th>Beryllium taken (µg)</th>
<th>Within-day</th>
<th>Between-day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beryllium found (µg)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>0.25</td>
<td>0.249 ± 0.0009</td>
<td>0.36</td>
</tr>
<tr>
<td>0.50</td>
<td>0.499 ± 0.0006</td>
<td>0.36</td>
</tr>
<tr>
<td>0.75</td>
<td>0.748 ± 0.0004</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*Average value of five determinations carried out over five days.

3.3.7. Applications

The proposed procedure utilizing haematoxylin in micellar medium has been applied successfully to the determination of beryllium in water samples as described in Section 3.2.4. The water samples tested negative for beryllium. Different amounts of beryllium(II) was spiked to the samples and analyzed for beryllium(II) by the proposed and Chrome Azurol S in presence of CTA (\(\lambda_{\text{max}} = 615 \text{ nm}\)) [19] methods. The results obtained are given in Table 3.3.

Table 3.3. Determination of beryllium in water samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Be(II) added (µg)</th>
<th>Proposed method</th>
<th>Reference method [19]</th>
<th>F-test</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Be(II) found* (µg)</td>
<td>RSD (%)</td>
<td>Recovery (%)</td>
<td>Be(II) found* (µg)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>Tap water</td>
<td>0.3</td>
<td>0.297 ± 0.0006</td>
<td>2.0</td>
<td>99.0</td>
<td>0.295 ± 0.0007</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.496 ± 0.0005</td>
<td>1.0</td>
<td>99.2</td>
<td>0.495 ± 0.0006</td>
</tr>
<tr>
<td>Ground water</td>
<td>0.4</td>
<td>0.392 ± 0.0007</td>
<td>1.8</td>
<td>98.0</td>
<td>0.394 ± 0.0008</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.593 ± 0.0006</td>
<td>1.0</td>
<td>98.8</td>
<td>0.592 ± 0.0006</td>
</tr>
</tbody>
</table>

\(^{a}\) Mean ± standard deviation (n = 5).

\(^{b}\) Tabulated F-value for (4, 4) degrees of freedom at \(P(0.95)\) is 6.39.

\(^{c}\) Tabulated t-value for 8 degrees of freedom at \(P(0.95)\) is 2.306.
3.3.8. Statistical evaluation of the results
The statistical analysis of the results of the proposed [haematoxylin in presence of CTA] and reference [Chrome Azurol S in presence of CTA] methods by F- and Student’s t-tests at 95% confidence level confirms the validity of the proposed method.

3.4. CONCLUSIONS
A simple, sensitive and reproducible spectrophotometric determination of beryllium was established by using haematoxylin in presence of a cationic surfactant, cetyltrimethylammonium bromide. The proposed method, owing to no need for solvent extraction, could be applied to assay of beryllium in different water samples. The proposed method has the advantage of excellent reproducibility both within-day and between-day precision (Table 3.2). The sensitivity in terms of molar absorption coefficient of the developed method is found to be higher or almost equal to many of the other spectrophotometric methods reported for beryllium (Table 3.4). The cost-effectiveness of both the technique and the reagent, and does not involve any stringent reaction conditions makes the method can be used as an alternative to the other for beryllium determination in real samples.
Table 3.4. Comparison of molar absorption coefficient ($\varepsilon$) of the present work with some other reported methods

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Ref.</th>
<th>$\lambda_{max}$ (nm)</th>
<th>$\varepsilon$ (L mol$^{-1}$ cm$^{-1}$)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematoxylin + Cetyltrimethylammonium bromide</td>
<td></td>
<td>592</td>
<td>$7.07 \times 10^4$</td>
<td>More sensitive.</td>
</tr>
<tr>
<td>Beryllon II</td>
<td>[40]</td>
<td>630</td>
<td>$1.20 \times 10^4$</td>
<td>Less sensitive.</td>
</tr>
<tr>
<td>Eriochrome Cyanine R + Hexadecylpyridinium chloride</td>
<td>[43]</td>
<td>530</td>
<td>$5.36 \times 10^4$</td>
<td>Sensitive.</td>
</tr>
<tr>
<td>Eriochrome Brilliant Violet B</td>
<td>[47]</td>
<td>560</td>
<td>$5.95 \times 10^4$</td>
<td>Sensitive.</td>
</tr>
<tr>
<td>Thoron I</td>
<td>[54]</td>
<td>523</td>
<td>$1.36 \times 10^4$</td>
<td>Less sensitive.</td>
</tr>
<tr>
<td>Calcichrome</td>
<td>[57]</td>
<td>625</td>
<td>$9.70 \times 10^4$</td>
<td>Very less sensitive.</td>
</tr>
<tr>
<td>1-(2-thiazolylazo)-2-naphthol + Triton X – 100</td>
<td>[59]</td>
<td>555</td>
<td>$2.25 \times 10^4$</td>
<td>Less sensitive.</td>
</tr>
<tr>
<td>8-hydroxyquinaldine</td>
<td>[65]</td>
<td>380</td>
<td>$3.50 \times 10^4$</td>
<td>Very less sensitive.</td>
</tr>
<tr>
<td>Alizarine Fluorine Blue and Adogen</td>
<td>[67]</td>
<td>480</td>
<td>$5.35 \times 10^4$</td>
<td>Very less sensitive.</td>
</tr>
<tr>
<td>2-(p-sulfophenylazo)-1,8-dihydroxy naphthalene-3,6-disulfonic acid</td>
<td>[68]</td>
<td>585</td>
<td>$1.00 \times 10^4$</td>
<td>Low sensitive.</td>
</tr>
<tr>
<td>Carminic acid</td>
<td>[71]</td>
<td>580</td>
<td>$2.25 \times 10^4$</td>
<td>Very less sensitive.</td>
</tr>
<tr>
<td>Chlorophosphonazo-mCF3</td>
<td>[72]</td>
<td>603</td>
<td>$2.73 \times 10^4$</td>
<td>Less sensitive.</td>
</tr>
<tr>
<td>N-phenyl-2-furohydroxamic acid</td>
<td>[73]</td>
<td>420</td>
<td>$2.70 \times 10^4$</td>
<td>Less sensitive.</td>
</tr>
<tr>
<td>N-(4-methylphenyl)-3-oxobutanamide</td>
<td>[77]</td>
<td></td>
<td>NF*</td>
<td>Least sensitive.</td>
</tr>
<tr>
<td>Molybdenum Blue method</td>
<td>[78]</td>
<td>780</td>
<td>$9.46 \times 10^4$</td>
<td>Very less sensitive.</td>
</tr>
<tr>
<td>1,4-dihydroxy-9,10-anthracenedione + Triton X-100</td>
<td>[81]</td>
<td>560</td>
<td>$1.68 \times 10^4$</td>
<td>Less sensitive.</td>
</tr>
</tbody>
</table>

*Not found in the abstract.
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