International Journal of Research in Engineering and Science (IJRES)
ISSN (Online): 2320-9364, ISSN (Print): 2320-9356
www.ijres.org Volume 11 Issue 8 ǁ August 2023 ǁ PP. 178-182

Reliable quantification of ultra-trace beryllium in environmental and biological samples by electrothermal atomic absorption spectrometry

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Abstract
Beryllium holds significant strategic importance owing to its distinctive physicochemical attributes. It frequently serves as a constituent in a multitude of alloys that find extensive applications within the aerospace and electronics sectors. However, it is crucial to note that beryllium ranks among the most toxic non-radioactive elements and is classified as a human carcinogen. Consequently, it commands special attention. From an analytical perspective, the primary goal is reliable quantifying beryllium at ultra-trace levels within diverse complex matrices. Among the numerous available detection methods, spectrometric approaches stand out as the most prevalent. This article focuses on the application of electrothermal atomic absorption spectrometry (ETAAS) in determining beryllium content in various environmental and biological samples. This can be done either directly or following the separation/pre-concentration using extraction techniques.

Keywords: *beryllium, electrothermal atomic absorption spectrometry (ETAAS), liquid-liquid extraction (LLE), solid-phase extraction (SPE), cloud-point extraction (CPE)*

Date of Submission: 13-08-2023 Date of acceptance: 28-08-2023

I. Introduction

Beryllium (Be) is a lightweight metal with a high melting point of 1287 °C. It is lighter than aluminium but approximately 40% stronger and about one-third more flexible than steel. These unique physicochemical properties lead to its utilization in preparing of numerous alloys. It enhances strength, increases electrical and thermal conductivity, improves resistance to corrosion and wear. As a result, Be and its alloys with other metals are often used in the production of various electrical devices, electronic instruments, telecommunications equipment, as well as structural components for airplanes, rockets, satellites, and nuclear reactors [1].

On the other hand, Be belongs to the group of toxic non-radioactive elements. Beryllium and its compounds can cause skin issues, such as various inflammations, ulcer formation, or granuloma formation. Berylliosis, a chronic disease of beryllium ("Chronic Beryllium Disease"; CBD), manifests as inflammation and granuloma formation in supportive lung tissues. Additional acute lung diseases (such as bronchitis, pneumonia, and alveolar tissue edema) [2] can occur upon inhalation of Be present in smoke, mist, or dust particles with sizes below 10 μm. Carcinogenic, teratogenic, and mutagenic effects of Be and its compounds have been confirmed in animal studies. The International Agency for Research on Cancer (IARC), based in Lyon, has classified beryllium as Group 1, which includes 119 items representing human carcinogens with well-demonstrated effects [3,4].

Even ultra-trace concentrations of Be in environmental samples (air, water, soil, and others) that have the potential to directly enter the food chain can pose serious risks. The effort to reliably quantify the ultra-trace concentrations of this analyte leads to the development of new procedures for both its separation and pre-concentration, as well as the quantification itself. One of the detection methods used for the reliable quantification of beryllium will be discussed in the following lines.

The fact that beryllium is an intriguing metal is indisputable. This is also supported by the number of publications. The number of publications dedicated to beryllium exceeds 21,000 (Web of Science database; August 2023). Of these, 5% are devoted to its determination, with spectrometric methods employed in 565 publications. Among them, spectrophotometry, flame atomic absorption spectrometry (FAAS), electrothermal atomic absorption spectrometry (ETAAS), inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS) belong to the most frequently used. Each of the mentioned methods has its pros and cons. This article will focus on the application of ETAAS in determining ultra-trace concentrations of Be in environmental and biological samples, either directly or after beryllium separation/pre-concentration using effective extraction procedures.

II. Electrothermal atomic absorption spectrometry

ETAAS belongs to the commonly utilized detection methods for quantifying Be in various matrices [5-12], although it is not entirely free from interferences [13-15]. Mitigation of some undesirable effects has been achieved by employing pyrolytically coated graphite tubes or metal atomizers. This has resulted in improved signal reproducibility and reduced memory effects in comparison to uncoated graphite tubes [7,16]. In a study where the stabilized temperature platform furnace (STPF) concept was employed, no undesirable effects were observed for the determination of Be in natural waters [17]. Another study utilizing pyrolytically coated graphite tubes and lanthanum as a modifier also reported no memory effect [16]. The achieved limit of detection in this study was 10 ng/L [16].

For reliable Be quantification in urine, lutetium as a semi-permanent modifier was employed, resulting in a limit of detection of 19 fg Be [11]. Most of the described ETAAS determinations report detection limits on the order of tens of ng/L [18]. However, even lower detection limits can be found in the published literature (Table 1). Nevertheless, in a case of analyzing highly complex matrices, achieving reliable quantification below 0.1 µg/L usually requires the use of an effective separation technique. This technique aids in concentrating the target analyte and can also eliminate unwanted matrix interferences caused by co-existing components.

Table 1. Some analytical characteristics achieved in the determination of beryllium by the ETAAS method.

Sample	Modifier	RSD (%)	ChM (pg)	LOD	Ref.
sediment	$Al(NO_3)_3$	2.8	0.73	80 ng/L	27
coal fly ash	none	2.7	1.32	NR	34
coal fly ash, water	$ZrOCl_2$	2.5	0.42	9 ng/L	1
natural water	Si	3.8	0.25	0.3 ng/L	35
natural water	$Mg(NO_3)_2$	8.0	3.7	70 ng/L	36
natural water, wastewater	Lu	4.8	0.45	25 ng/L	18
drinking water	none	2.3	NR	2.3 ng/L	37
human urine	none	5.6	1.19	85 fg	11
human urine	Lu	1.8	0.45	19 fg	11
human urine	$Mg(NO_3)_2$	3.3	0.48	58 fg	11
human urine	acac/NH_4OAc	3.0	1.9	0.37 µg/L	38
human urine	$(NH_4)P(Mo_3O_{10})_4$/AA	3.6	NR	20 ng/L	32
human blood serum	none	NR	0.22	2 ng/L	33
human blood	$Mg(NO_3)_2$	NR	0.20	7 ng/L	33
human hair	none	2.2	NR	2.0 ng/g	31
animal liver, muscle tissue	none	3.0	NR	0.18 ng/g	29
marine organism	none	3.0	NR	4.6 ng/g	30

RSD: relative standard deviation; ChM: characteristic mass; LOD: limit of detection; Ref.: reference; NR: not reported; acac: acetylacetone; AA: ascorbic acid

An advantage of metal atomizers is that they prevent carbide formation during electrothermal atomization [19,20]. The use of tungsten atomizers leads to improved signal reproducibility, faster heating, and reduced energy consumption [21,22]. Coating graphite atomizers with lanthanum or titanium carbide [23], zirconium [24,25], or thorium [6] has resulted in enhanced sensitivity of determination, increased signal reproducibility, lowered limit of detection [23], as well as extended lifetime of the used tubes and reduction of interferences. Employed modifiers included magnesium, aluminium, lutetium, and palladium [10-12,26]. A study employing a tungsten platform in a pyrolytically coated graphite tube with zirconium as a chemical modifier demonstrated that this approach provides a reliable means to increase sensitivity and reduce the limit of detection in Be determination [1].

The use of HF for the decomposition of solid samples results in a significant reduction of the absorption signal, which is attributed to the formation of BeF_2, which sublimes at 800 °C. Among various tested modifiers for Be determination in sediments after their decomposition in a mixture of HF and HNO_3, reliable results were achieved using $Al(NO_3)_3$. This finding was confirmed by analyzing 15 different certified reference materials [27]. The decomposition of rocks and sediments in a mixture of HF-HNO_3-HCl was employed for the determination of 12 different trace elements (including Be) [28]. An interesting finding in this case is that reliable Be quantification was achievable without the use of any modifier.

For the quantification of Be in biological samples (animal liver and muscle tissue, marine organisms, human hair) [29-31], sample decomposition in a mixture of HNO_3 and H_2O_2 using microwave irradiation was employed in all cases. Subsequently, solid-phase extraction was utilized, followed by dosing the obtained methanolic solution into an uncoated graphite tube. All measurements were done with no chemical modifier. The reliability of the obtained results was verified by analyzing certified reference materials (CRMs).

Sample decomposition in a mixture of HNO_3 and HCl using microwave irradiation was used for the quantification of Be (as well as As and Se) in the urine of workers in steel mills [32]. Reliable results for Be were achieved using a mixed modifier containing $(NH_4)P(Mo_3O_{10})_4$ and ascorbic acid. Higher concentrations of the

monitored elements were measured in samples from steel mill workers compared to a control group that did not work in steel mills. The conclusions of the study are intriguing and indicate that in samples where the concentration of As or Be was higher, the concentration of Se was almost always lower, and vice versa [32].
For the direct analysis of blood and serum samples, they were diluted using Nash reagent containing HNO_3, NH_4OH, Triton X-100, anti-foaming emulsion B, and EDTA [33]. While $Mg(NO_3)_2$ as a matrix modifier helped increase the sensitivity of Be determination in blood, there was no increase in sensitivity or improvement in other analytical characteristics in the case of serum. Reduced palladium (using citric acid) showed no positive effect on Be determination in blood or serum [33]. All measurements were conducted on pyrolytically coated graphite tubes, and peak areas were used for evaluation. The results showed statistically significant differences in Be content between blood and serum. Be concentrations in whole blood were approximately 30% higher compared to concentrations in serum [33].
Summarization of pyrolysis and atomization temperatures used in temperature programs for the determination of beryllium by the ETAAS method can be seen in Table 2.

Table 2. Pyrolysis temperatures and atomization temperatures used in temperature programs for the determination of beryllium by the ETAAS method.

Modifier	PT (°C)	AT (°C)	Note	Ref.
none	500	2800	LLE; back extraction of Be into HCl	34
none	600-900	2800	SPE; $Be(acac)_2$ eluted by methanol	37
none	600-1400	2900	SPE; $Be(acac)_2$ eluted by methanol	31
none	600-1000	2900	SPE; $Be(acac)_2$ eluted by methanol	30
none	800	2300	multielement ETAAS used	28
none	1000	2900	diluted blood serum samples	33
$Mg(NO_3)_2$	800	2800	diluted whole blood samples	33
$Al(NO_3)_3$	1500	2400	various modifiers tested	27
$Mg(NO_3)_2$	1300	2300	various modifiers tested	36
acac/NH_4OAc	600-1200	2800	diluted urine samples	38
$(NH_4)P(Mo_3O_{10})_4$/AA	1400	2500	decomposed urine samples	32
Lu	1500	2500	semi-permanent modifier used	11
Lu	1500	2500	FIA; precipitation of Be	18
$ZrOCl_2$	900/1000	2400	tungsten platform used	1

PT: pyrolysis temperature; AT: atomization temperature; Ref.: reference; acac: acetylacetone; AA: ascorbic acid; LLE: liquid-liquid exreaction; SPE: solid-phase extraction; FIA: flow injection analysis

III. Liquid-liquid extraction and electrothermal atomic absorption spectrometry

One of the extraction techniques applicable for the separation and pre-concentration of Be before its quantification through ETAAS is liquid-liquid extraction (LLE). Notable findings were reported in the context of isolating this analyte from coal fly ash discharged by thermal power plants, utilizing a β-diketone liquid chelating exchange (LCE) agent ($C_9H_{19}COCH_2COCH_3$). The resulting complex exhibited the composition [$Be(C_9H_{19}C(=O)CH=C(O)CH_3$] [34]. Investigation into experimental conditions such as pH, optimal selection of the extraction agent, extraction process kinetics, and the impact of various ions led to the deduction that achieving quantitative Be yields with LCE is feasible using cyclohexane at pH 9.5 and an extraction time of 2 minutes. Subsequently, the analyte was re-extracted into 2M HCl. The organic solvents tested included cyclohexane, isoamyl acetate, methyl isobutyl ketone, chloroform, carbon dioxide, and 1,2-dichloroethane. When assessing potential interferences, binary and multicomponent model solutions were prepared with a substantial excess of potential interferents. An intriguing finding in this scenario is that reliable results were obtained by measurements with no modifier when evaluating absorbance peak heights [34]. Background correction was accomplished using a deuterium lamp.

IV. Solid-phase extraction and electrothermal atomic absorption spectrometry

Another extraction technique that can be employed for the separation and pre-concentration of (ultra)trace Be is solid-phase extraction (SPE). The complexation of Be with acetylacetone (acac) in the presence of an acetate buffer solution (pH 6.0) was applied to analyze the liver and muscle tissue of poultry and livestock [29], as well as marine organisms (fish, shrimp, octopus, lobster, oyster, algae) [30], and human hair [31]. After sample decomposition in a mixture of HNO_3 and H_2O_2 using microwave irradiation and the formation of the complex ($Be(acac)_2$), this was captured in a cartridge prepared from a polypropylene tip containing C_{18} and subsequently eluted with methanol. The methanolic solution was introduced into an uncovered graphite furnace, and measurement evaluation was conducted from the absorbance peak heights. The reliability of the achieved results was confirmed through the analysis of CRMs of available biological matrices, or by the standard addition method (in case of unavailability of CRMs for marine organisms). Similarly, the $Be(acac)_2$ chelate captured on a Sep-Pak C_{18} cartridge and subsequently eluted with methanol was employed in separation and pre-concentration

of Be from natural waters. In this case as well, uncovered graphite furnaces were used for measurements, and the results were evaluated from the absorbance peak heights [37].

In another study, beryllium was adsorbed onto the surface of silica fibers from a slightly alkaline medium (pH 9.5), leading to its separation and pre-concentration from various types of natural water samples (lake water and seawater) [35]. The adsorbed forms were $(\text{-SiO})\text{Be}(\text{OH})_2^-$ or $(\text{-SiO})_2\text{Be}(\text{OH})_2^{2-}$. Three-layer filters containing silica fibers were used for separation, and 0.5M HCl was employed for desorption.

V. Cloud-point extraction and electrothermal atomic absorption spectrometry

Surfactant-assisted extraction, known as cloud point extraction (CPE) utilizes the properties of non-ionic and zwitterionic surfactants. These surfactants, when present in aqueous solutions, can form micelles and induce turbidity upon heating to a specific temperature referred to as the cloud point temperature (CPT). Beyond this threshold temperature, the initially homogeneous micellar solution undergoes phase separation, resulting in the formation of two distinct phases. The first is a surfactant-rich phase of small volume, primarily composed of the surfactant molecules. The second is a diluted aqueous phase in which the surfactant concentration is close to the critical micellar concentration (CMC) [39]. In the context of elemental analysis, this process usually involves the introduction of a chelating agent. The role of this agent is to generate hydrophobic chelates that can selectively reside within the hydrophobic cores of the micelles present in the surfactant-rich phase. Consequently, these hydrophobic chelates become conducive to extraction and pre-concentration, significantly enhancing the overall efficiency of the extraction process.

When considering beryllium, the utilization of a hydrophobic chelate formed with cupferron becomes relevant [40]. Consequently, optimizing the experimental conditions for a CPE procedure involving the non-ionic surfactant Triton X-114 can result in the reliable separation and pre-concentration of trace amounts of Be from natural water samples. Several pivotal experimental parameters require thorough optimization, including solution pH, chelating agent concentration, surfactant concentration, incubation temperature, and incubation time. Following optimization, it is possible to achieve a characteristic mass of 0.33 pg and a pre-concentration factor of 20 [40].

VI. Conclusion

The reliable quantification of (ultra)trace beryllium through the ETAAS method has been achieved by overcoming initial challenges associated with significant memory effects that arise when using an uncovered graphite furnace. The utilization of a pyrolytically coated graphite furnace, often in combination with a suitable chemical modifier, has proven instrumental in obtaining reliable results for the analysis of various environmental and biological samples. The application of extraction techniques has not only improved the analytical characteristics of ETAAS measurements, such as lowering the limit of detection for beryllium, but has also effectively removed matrix interferences caused by co-existing components. This dual effect has enabled the reliable quantification of ultratrace concentrations of beryllium with no issue.

Acknowledgement

The work was supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences under the contract VEGA 1/0135/22.

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