DETERMINATION OF BORON IN WATER SAMPLES BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

NAİL ENGİN ŞİMŞEK

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
CHEMISTRY

SEPTEMBER 2012

Approval of the thesis:

DETERMINATION OF BORON IN WATER SAMPLES BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

submitted by **NAİL ENGİN ŞİMŞEK** in partial fulfillment of the requirements for the degree of **Master of Science in Chemistry Department, Middle East Technical University** by,

Prof. Dr. Canan Özgen
Dean, Graduate School of **Natural and Applied Sciences** ____________________

Prof. Dr. İlker Özkan
Head of Department, **Chemistry** ____________________

Prof. Dr. O. Yavuz Ataman
Supervisor, **Chemistry Dept., METU** ____________________

Examining Committee Members:

Prof. Dr. R. Sezer Aygün
Chemistry Department, METU ____________________

Prof. Dr. O. Yavuz Ataman
Chemistry Department, METU ____________________

Prof. Dr. G. İnci Gökmen
Chemistry Department, METU ____________________

Prof. Dr. Orhan Acar
Atatürk Vocational High School, Gazi University ____________________

Assoc. Prof. Dr. Gülay Ertaş
Chemistry Department, METU ____________________

Date: 10.09.2012

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name: NAİL ENGİN ŞİMŞEK

Signature

ABSTRACT

DETERMINATION OF BORON IN WATER SAMPLES BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

Şimşek, Nail Engin
M. Sc., Department of Chemistry
Supervisor: Prof. Dr. O.Yavuz Ataman

September 2012, 91 pages

Boron (B) is a rare element on Earth crust with a natural abundance of 0.001%. However, boron content of water and soils may be significantly high in the regions with rich boron reserves. In addition, extensive use of agrochemicals in soils as well as various natural processes increases the boron concentration in water. Despite B is an essential element for all living creatures, it may pose risks at high level exposures. World Health Organization (WHO) has recommended a daily intake of 1 to 13 mg B for adults.

Turkey has almost 70% of world boron reserves principally in four regions: Kütahya, Emet; Balıkesir, Bigadiç; Eskişehir, Kırka and Bursa, Kemalpaşa. The boron content of water in these regions may go up to significant levels. Therefore, it is important to determine B in drinking water from these regions.

Electrothermal atomic absorption spectrometry (ETAAS) is a relatively sensitive technique for determination of boron. However, the technique suffers from formation of molecular boron compounds. Therefore, use of chemical modifiers and pyrolytically coated graphite tubes modified with refractory carbide forming elements (Ta, W, Zr, Pd, Ru, Os) were utilized to develop a reliable and sensitive method. Based on optimization studies, Tantalum (Ta) coated tube and co-injection of 5.0 µL 0.01 mol/L $Ca(NO_3)_2$, 5.0 µL 0.05 mol/L citric acid together with 15.0 µL sample solution prepared in 1000 mg/L $Mg(NO_3)_2$ have been chosen as optimum conditions. Optimum temperatures for pyrolysis and atomization temperatures were determined as 1100 and 2700 °C, respectively. Under these conditions, a detection limit of 0.088 mg/L and a characteristic mass of 186 pg for 15.0 µL sample volume were obtained. The accuracy of the method was checked by EnviroMAT-Waste Water EU-L-1 CRM and NIST 1573a Tomato Leaves SRM analyses.

Drinking water samples were collected from Balıkesir, Bigadiç and Kütahya, Emet and analyzed by the developed method. Samples were also analyzed by more sensitive techniques; ICP-OES and ICP-MS for a comparison study. The results are compatible with each other.

Keywords: Boron determination, ETAAS, chemical modifiers, water samples

ÖZ

ELEKTROTERMAL ATOMİK ABSORPSİYON SPEKTROMETRİ YÖNTEMİ İLE SU NUMUNELERİNDE BOR TAYİNİ

Şimşek, Nail Engin
Yüksek Lisans, Kimya Bölümü
Tez Yöneticisi: Prof. Dr. O. Yavuz Ataman

Eylül 2012, 91 sayfa

Bor (B) %0.001 doğal bolluk oranı ile yer kabuğunda az bulunan bir elementtir. Bununla birlikte, zengin bor rezervlerinin bulunduğu bölgelerde toprak ve su içerisinde bulunan bor içeriği önemli ölçüde yüksek olabilir. Buna ek olarak tarım kimyasallarının aşırı kullanımı ve çeşitli doğal süreçler su içerisindeki bor derişimini arttırmaktadır. B, tüm canlılar için gerekli bir element olmasına rağmen yüksek seviyede maruziyetler risk oluşturabilir. Dünya Sağlık Örgütü (WHO) yetişkinler için günde 1 ila 13 mg arasında bor alımını tavsiye etmektedir.

Türkiye dünya bor rezervlerinin yaklaşık olarak %70 ine sahiptir. Bu rezervler temel olarak dört bölgede bulunmaktadır: Kütahya, Emet; Balıkesir, Bigadiç; Eskişehir, Kırka and Bursa, Kemalpaşa. Bu bölgelerin sularında bor miktarı erişimi önemli seviyelere ulaşmaktadır. Bu nedenle, bu bölgelerde içme sularında bor tayini önemlidir.

Elektrotermal atomik absorpsiyon spektrometre (ETAAS) bor tayini için kullanılan nispeten duyarlı bir tekniktir. Fakat bu teknikte moleküler bor türlerinin oluşumundan ötürü duyarlılık düşmektedir. Bu çalışmada güvenilir ve duyarlı bir yöntem geliştirmek için kimyasal değiştiricilerden ve refrakter karbür oluşturan elementlerle (Ta, W, Zr, Pd, Ru, Os) işleme sokulmuş pirolitik kaplı grafit tüp kullanımından faydalanılmıştır. Optimizasyon çalışmaları sonucu Tantalum (Ta) ile modifiye edilmiş tüp kullanımı ve 5.0 µL 0.01 mol/L $Ca(NO_3)_2$ ve 5.0 µL 0.05 mol/L sitrik asitin 1000 mg/L $Mg(NO_3)_2$ ortamında hazırlanmış 15.0 µL örnek çözeltisi ile birlikte enjeksiyonu optimum koşullar olarak belirlenmiştir. Optimum külleme ve atomlaştırma sıcaklıkları sırasıyla 1100 and 2700 °C dir. Bu koşullar altında gözlenebilirlik sınırı 0.088 mg/L ve 15.0 µL örnek hacmi için karakteristik kütle 186 pg olarak bulunmuştur. Yöntemin doğruluğu EnviroMAT-Waste Water EU-L-1 CRM ve NIST 1573a Tomato Leaves SRM analizleri ile denenmiştir.

İçme suyu örnekleri Bigadiç ve Emet’ten toplanmış ve geliştirilen yöntem ile analiz edilmiştir. Ayrıca karşılaştırma yapabilmek için analizler daha duyarlı teknikler olan ICP-OES ve ICP-MS ile de yapılmıştır. Sonuçlar birbiriyle uyumludur.

Anahtar Kelimeler: Bor tayini, ETAAS, kimyasal değiştiriciler, su numuneleri

To My Beloved Family...

ACKNOWLEDGEMENTS

I would like to express my sincere gratitudes to my supervisor Prof. Dr. O. Yavuz Ataman for his guidance, support and significant contributions to my work and me personally.

I would like to express my gratitudes to Prof. Dr. Lemi Türker for his guidance and support since the very first days at the department.

I would like to thank Assoc. Prof. Dr. Gülay Ertaş for her different angle of view, suggestions, support as well as friendly way of communication.

I am grateful to Emrah Yıldırım for his helps and contributions to my work and more importantly for sincere friendship and enjoyable memories that we share.

I would like to thank my dear lab mates Selin Bora, Pınar Akay, Feriye Şenol, Erhan Özdemir, Njaw Njie and Mohamed Attwa Ibraheim for their help and friendship.

Very special thanks to my “oldies but goldies” friends; Deniz, Döndü, Hakan, Gözde, İpek, Seda, Şeniz, Utku for this never ending and precious friendship.

Another special thanks to two of my brothers; Bora Gerçeker and F. Ümit Akçaoğlu for sharing this life either at happy or tough times with me.

Finally, words cannot express the thanks that I owe to my big family. It is an irreplaceable relief to feel your love and support always by my side.

TABLE of CONTENTS

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
AES	Atomic Emission Spectrometry
CRM	Certified Reference Material
ET-AAS	Electrothermal Atomic Absorption Spectrometry
FAAS	Flame Atomic Absorption Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
NAA	Neutron Activation Analysis
PE	Polyethylene
PG	Pyrolytic Graphite

PTFE	Polytetrafluoroethylene
SRM	Standard Reference Material
RSD	Relative Standard Deviation
SIMS	Secondary Ion Mass Spectrometry
THGA	Transversely Heated Graphite Atomizer
TIMS	Thermal Ionization Mass Spectrometry
TPG	Total Pyrolytic Graphite

CHAPTER 1

INTRODUCTION

1.1. Boron

1.1.1. Properties

Boron is a Group 3A element with atomic number of 5 and is represented by the symbol 'B'. It is a metalloid, which carries characteristics of both metals and nonmetals. There are two stable isotopes of boron in nature; ^{10}B and ^{11}B, with percentages of 19.9% and 80.1% respectively, giving an average atomic weight of 10.81 g/mol **(Farhat et al., 2012).**

Boron is never found as a free element on earth **(Argust, 1998)** but particularly bound to oxygen as borates or less often as boric acid (H_3BO_3) and hardly bound to fluorine as in the BF_4^- ion **(Farhat et al., 2012).**

Boron was first isolated in 1808 by Joseph-Louis Gay-Lussac and Louis-Jacques Thenard and independently by Sir Humphry Davy by heating boron oxide (B_2O_3) with potassium **(Encyclopedia Britannica, 2012).** It was recognized as an element in 1824 by Jons Jakob Berzilius. The name boron is derived from the Arabic word *Buraq* which is a word for the mineral borax **(Wikipedia, 2012).**

Elemental boron exists either in amorphous or crystalline forms. Amorphous boron is a brown powder. It is unreactive to oxygen, acids, water and alkalis yet it reacts with HF forming volatile BF_3, which has a boiling point of -100.3 ^{0}C. Crystalline boron has a dark grey color. It behaves as a semiconductor at room temperature **(Nemodruk, Karalova, 1964).**

The most important oxidation state of boron is +3. The lower oxidation state +1 has no significance in boron industry as well as in the chemistry of naturally occurring boron compounds **(Power, Woods, 1997).**

Boron is an interesting element and it draws attention as much as carbon with a variety of chemical properties **(Greenwood, Earnshaw, 1984).** This interest originates from its being an electron-deficient element, holding a p-orbital unfilled in the valence shell. This feature is responsible for formation of three-centre, two-electron bonds in boron compounds. This type of bonding can be observed mostly in boron hydride derivatives, which have played an active role in the development of molecular orbital theory and organic synthesis methodology. Two Nobel awards, in 1976 to W. N. Lipscomb and in 1979 to H.C. Brown (shared with G Wittig), have proved the importance of B compounds to bonding theory and organic chemistry **(Power, Woods, 1997).**

Two natural isotopes of boron are NMR active. They both have spins of greater than 1/2 and are quadrupolar. ^{11}B and ^{10}B have spins of 3/2 and 3, respectively. ^{11}B is easy to detect by NMR owing to its lower quadrupole moment and relative sensitivity, which is 0.17 (H = 1) **(Emsley, 1991).**

1.1.2. Boron Minerals and Distribution

Boron has a natural abundance of 0.001% on Earth crust. The rarity of the element is attributed to its being one of the light elements, which are left out in the normal chain of thermonuclear reactions in stars **(Reeves, 1974).** Fragmentation reactions in which lighter nuclei are generated from heavy ones are the most probable mechanism for its production **(Power, Woods, 1997)**. Correspondingly, *Crosswell* proposed that boron may be generated by explosion of massive stars **(Crosswell, 1992).**

In spite of its low natural abundance, boron is a common element in rocks, soil, and water **(Camacho-Cristobal, Rexach, Gonz´alez-Fontes, 2008).** Boron concentrations are in the range of 5 - 10 mg/kg in rocks **(Shorrocks, 1997)**, 3 - 30 μg/L in rivers **(Power, Woods, 1997)** and around 4 - 5 mg/L in ocean **(Lemarchand et al., 2000).** The concentration in soils may vary from 2 to 100 mg/kg though most of the soils have less than 10 mg/kg boron **(Camacho-Cristobal, Rexach, Gonz´alez-Fontes, 2008).**

Rich and economically worth boron deposits are particularly found in dry regions with hydrothermal sources or former volcanic eruptions. Primarily, Turkey, USA and Russia are the countries having important boron reserves **(Matterson, 1980).** Turkey has approximately 70% of the boron reserves of the world. The reserves are mainly located in four regions, which are Kütahya–Emet, Balıkesir–Bigadiç, Eskişehir–Kırka and Bursa–Kemalpaşa **(Özpeker, 2002).**

There are more than 150 boron minerals on Earth, yet only a number of them are commercially important. Of these minerals, *Tincal*, *Colemanite* and *Ulexite* are the ones with more economic value and Turkish reserves are mainly composed of these minerals.

They are given names based on the mineral content of calcium, sodium and magnesium. Ones containing sodium are Tincal, ones containing calcium are Colemanite and ones containing both sodium and calcium are Ulexite. **(Yiğitbaşıoğlu, 2004).**

Together with Tincal, Colemanite and Ulexite, other minerals of economic importance are listed in Table 1 with their names and structural formulae **(Kartal, Gürtekin, 2002).**

Table 1. 1 Important boron minerals and their chemical formulae

NAME	FORMULA
Tincal	$Na_2B_4O_7.10H_2O$
Kernite	$Na_2B_4O_7.4H_2O$
Ulexite	$NaCaB_5O_9.8H_2O$
Probertite	$NaCaB_5O_9.5H_2O$
Colemanite	$Ca_2B_6O_{11}.5H_2O$
Pandermite	$Ca_4B_{10}O_{19}.7H_2O$
Ascharite	$Mg_2B_2O_5.H_2O$
Datolite	$Ca_2B_2Si_2O_9.H_2O$
Sassolite (natural boric acid)	$B(OH)_3$

The first boron mining plant in Turkey was started in 1865 with mining of a kind of calcium borate, *Pandermite*. In the same years, some borate ores of ulexite and colemanite were discovered in California and Nevada **(Woods, 1994).**

Turkey is a leading exporter of colemanite to boric acid producers in Europe. In 1960, sodium borate ores were found in Kırka, Eskişehir and since then other deposits have been found and expanded in Anatolia **(Woods, 1994).** Owing to rich boron reserves, Turkey is the biggest producer of borate products, including natural minerals as well as products such as refined borax decahydrate, borax pentahydrate, anhydrous borax, and boric acid **(Sprague, 1992).** Table 2 includes these products with boron oxide contents in percentages.

Table 1. 2 Refined borate products and mineral concentrates of importance

Product Name	**Formula**	**B_2O_3 (%)**
Borax pentahydrate	$Na_2B_4O_7.5H_2O$	47.8
Borax decahydrate (tincal)	$Na_2B_4O_7.10H_2O$	36.5
Colemanite	$Ca_2B_6O_{11}.5H_2O$	50.8
Ulexite	$NaCaB_5O_9.8H_2O$	43.0
Boric acid	H_3BO_3	56.3
Anhydrous borax	$Na_2B_4O_7$ (glass)	69.2

1.1.3. Applications and Field of Use

Since the end of World War II, there has been a growing interest in applications of B. Certain boron compounds have attracted more attention for military applications. Some of these include using boron hydrides as plane and rocket fuel or using metal or metalloid derivatives of boron for applications in protecting armor or electronics **(Power, Woods, 1997).**

However, these practices could not find a wide range of applications in boron industry, where the production is highly dominated by boron-oxygen compounds, such as boric acid and inorganic borate salts (**Thompson, 1980).**

One of the main industrial use of boron compounds is the production of borosilicate glass (e.g. Pyrex), which has a low coefficient of thermal expansion and is very well resistant to thermal shocks. Another field of use is in detergents and bleaches for which sodium perborate ($NaBO_3$) serves as a source of active oxygen. Boron compounds are also of major importance in agriculture for making various agricultural chemicals, pest controls, fertilizers and soil supplements **(Power, Woods, 1997).**

Unusually high cross section of ^{10}B for thermal neutron capture with 3.837×10^{-28} m^2 (3837 barns) enable its use in nuclear shielding **(Hawthorne, 1993).** Additionally, boron carbide shields are used as control rods in nuclear reactors owing to boron serving as a neutron absorber. The nuclear reaction is given as follows:

$$^{10}B + ^{1}n \rightarrow ^{7}Li + \alpha \text{ particles (2.31 MeV)} + \text{gamma ray (478 KeV)}$$

Moreover, by taking advantage of neutron absorbing property, boric acid (H_3BO_3) is dissolved in cooling water of reactors to control the extent of reaction.

Besides, ^{10}B is used in neutron capture therapy for malignant tumours owing to its high thermal cross section **(Hawthorne, 1993).**

Another field of use is in semiconductor industry as a dopant. Boron is mixed with pure semiconductors like silicon, germanium and silicon carbide in small amounts to alter their conductive properties for use in transistors and diodes **(May, Spanos, 2006).**

1.1.4. Importance of Boron for Plants

The essential role of boron for plants has been recognized for a long time **(Warington, 1923).** Among other micro-nutrients, plants are commonly deficient in boron which affects their vital functions and regeneration capacity as well. In the first place, boron deficiency inhibits growing tissues and leads to anomalies on carbohydrate metabolism, nucleic acid synthesis, and pollen tube growth **(Blevins, Lukaszewski, 1998; Goldbach, Wimmer, 2007).** Moreover, generation of cell wall with different physical properties is resulted from boron deficiency **(Fleischer et al., 1999).** Plants lacking boron are brittle, while plants with high boron levels may have too flexible bodies **(Loomis, Durst, 1992).**

Boron is also required for maintenance of the structure and functions of plasma membranes **(Çakmak, Römheld, 1997).** Boron deficiency alters plasma membrane permeability which inhibits uptake of a number of nutrients **(Çakmak, Kurz, Marschner, 1995).** Therefore, it also affects the growth of plants indirectly by changing mineral metabolism.

Despite the indispensable role of boron for plants, it poses a serious risk in high amounts. As the soil is not generally rich in boron, this is particularly due to excess fertilization or irrigation with water in high levels of boron. Additionally, arid areas with low rainfall are source of boron toxicity **(Nable, Banuelos, Paull, 1997).** B toxicity causes a reduced growth of shoots and roots, changes chlorophyll content of leaves and so affects photosynthesis rates negatively **(Nable et. al. 1997; Reid 2007).** Accordingly, anomalies in metabolism and even death of plants are resulted from high B levels **(Nable, Cartwright, Lance, 1990).**

1.1.5. Importance of Boron for Humans and Animals

Boron is determined to be an essential element for humans by the Expert Committee on Trace Elements in Human Nutrition of the World Health Organization **(WHO, 1996).** It is almost all absorbed by body and present in body fluids and tissues mostly in the form of boric acid, $B(OH)_3$. Boron intake is generally through drinking water, vegetables with green leaves, legumes and fruits **(Howe, 1998).** Meat, fish and dairy products contain low levels of boron. For humans and animals, the effect of boron has not been explained at all as was done for plants **(Kabay, Güler, Bryjak, 2010).** Experiments on animals show that lack of boron intake may disrupt bone health and also deteriorate brain functions and immune system **(Devirian, Volpe, 2003; Nielsen, 2009).** On the other hand, being exposed to high doses of boron causes toxicological effects on skeletal and cardiovascular systems of animals, particularly mice, rats and rabbits **(Heindel et al., 1992; Price et al., 1996).** Additionally, upon high doses in animals, it was observed that male reproductive capabilities are affected negatively and at even higher doses inhibition of sperm production and atrophy are resulted **(Fail et al., 1998).** However, there has been no such indication for humans.

Newnham mentioned that boron is crucial for the metabolism and use of calcium in bones and deficiency in boron causes bone losses **(Newnham, 1994).**

According to *Nielsen*, boron has a crucial role in the function or composition of several body systems, including the brain, skeleton and immune system as well as in treatment of arthritis **(Nielsen, 1994).** Recent evidence proves that boron and borates may have anticarcinogenic properties. The study by *Korkmaz et al.,* concluded that high levels of boron exposure decrease the risk of cervical cancer-related pathological findings **(Korkmaz et al., 2006).**

In this study, occurrence of adverse cytological findings in cervical smears of women, whose boron exposure varies from little to much, was investigated. Women live in boron-rich sites had no cytopathological symptoms of cervical cancer, while 15 women from the boron-poor sites were of cytopathological findings. Moreover, the study proposes that high level of boron intake through drinking water lowers the probability of cervical cancer development.

Additionally, effect of boron on human reproduction system was examined. *Şaylı et al.* interviewed 927 subjects (230 female, 697 male) randomly selected from Balıkesir, Kütahya and Eskişehir, where rich boron deposits are found. People were exposed to boron through diet or working at mines, or both. In conclusion, there has been no negative sign of boron exposure on fertility and reproduction **(Şaylı, Tüccar, Elhan, 1998).** In another study on the employees of boron industry in China, it was realized that that occupational exposure to boron does not affect the sperm count of employees **(Ping et al., 2006).**

Despite the roles of boron in body have not been detailed at all, a range of 1 to 13 mg/day for adults is recommended based on both human and animal research data (**WHO, 1996).** Additionally, there are some limits for boron content in drinking water. A guideline value of 0.5 mg/L is determined by WHO **(WHO, 2009).** Guideline value stands for the upper limit of a chemical, which does not cause health concerns upon life-time consumption. Moreover, in 2005, EU has added a new standard for boron content in drinking water which is 1 mg/L **(Weinthal et. al, 2005).** Similarly, Turkish Standard numbered TS 266 concerning water for human consumption dictates a limit of 1 mg/L boron in drinking water **(TSI, 2005).**

1.2. Techniques for Boron Determination

1.2.1. Spectrophotometric Methods

Spectrophotometry, which involves the use of specific reagents for the color development of boron solutions, is one of the methods for boron determination. As the color of the solution is developed, absorbance is measured at a wavelength specific to the coloring reagent **(Farhat et al., 2012).** Some of the mostly used reagents include curcumin, absorbance of the resulting solution is measured at 550 nm **(Mair, Day, 1972),** carmine at 605 nm **(Ammar, Abdelhedi, Flox, Arias, Brillas, 2006)** and azomethine-H at 410 nm **(Spencer, Erdmann, 1979).** Among them, azomethine-H is the most preferred one due to it provides a relatively fast, simple and sensitive method **(Sah, Brown, 1997).**

Another approach is spectrofluorimetry in which fluorescence of samples are measured subsequent to the addition of reagents that produce fluorescent boron compounds **(Farhat et al., 2012).** Alizarin Red S (**Campana, Barrero, Ceba, 1992)**, chromotropic acid **(Lapid, Farhi, Koresh, 1976)** and dibenzoylmethane **(Aznarez, Bonilla, Vidal, 1983)** are the reagents that form fluorescent compounds with boron. These methods are pH and temperature dependent and they suffer from interferences.

The spectrophotometric methods, in general, suffer from interferences. pH and temperature ranges are also critical. These reasons as well as relatively low sensitivity limit the use of spectrophotometric methods for boron determination **(Sah, Brown, 1997).**

1.2.2. Non-spectrophotometric Methods

Potentiometry is one of these methods in which boron in the sample is converted into tetrafluoroborate ion (BF_4^-) which is then measured via a BF_4^- selective electrode **(Carlson, Paul, 1968).** This method, however, is severely affected by the sample matrix which may shift the potential. The potentiometric method cannot find a wide range of applications but for analysis in some fields such as borophosphosilicate glass, rocks and ores, it is somehow preferred **(Sah, Brown, 1997).**

Moreover, boron analysis is performed through neutron activation analysis (NAA) methods. It is preferred by nuclear industry, where boric acid is used in the primary coolant of pressurized water reactors to control the nuclear reaction **(Nordmann, 2004).** Boron concentration is determined through bombardment of boron samples with neutrons which produce α-particles and γ-particles, of which one or both are monitored to correlate with ^{10}B stable isotope abundance **(Farhat, 2012).**

$$^{10}B + \text{neutron} \rightarrow {}^{7}Li + \alpha \text{ particles (2.31 MeV)} + \text{gamma ray (478 KeV)}$$

NAA is advantageous as it is a nondestructive method with minimum sample preparation yet it is not offered for liquid samples, which may pose threat of radioactive leakage. Besides, it requires access to nuclear reactors for production of neutrons which is not easily accessible **(Sah, Brown, 1997).**

1.2.3. Atomic Spectrometry

For boron determination a wide range of atomic spectrometry techniques are employed. These include Flame Atomic Spectrometry, either Flame Atomic Absorption Spectrometry (FAAS) or Flame Atomic Emission Spectrometry (FAES), Electrothermal Atomic Absorption Spectrometry (ETAAS), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Thermal Ionization Mass Spectrometry (TIMS) and Secondary Ion Mass Spectrometry (SIMS).

FAAS and FAES are conventional atomic spectrometry techniques. Absorption of generated atoms or emissions of excited species are measured to determine the analyte concentration. These methods are not proper for boron determination with respect to poor sensitivity, memory effects and interferences **(Papaspyrou et al., 1994)** and often require separation and pre-concentration steps.

The ETAAS presents a more sensitive method for boron determination. However, it is still one of the most challenging analytical practices **(Welz, Sperling, 1999)** due to formation of stable boron oxides, carbides and nitrides as well as memory effects, which result a persistent plateau in signals **(Luguera M., Madrid Y., Camara C, 1991).** To make a reliable and sensitive analysis**,** use of chemical modifiers, pyrolytic graphite tubes or platforms coated with refractory carbide forming elements, tantalum, tungsten, zirconium, etc., and an optimum temperature program are indispensable.

Inductively Coupled Plasma techniques are more commonly used for boron analysis. ICP is a type of plasma source, which provides high temperatures (8000 to 10 000K) for optical emission (ICP-OES) and mass spectrometry (ICP-MS) techniques **(Sah, Brown, 1997).**

ICP-OES detects electromagnetic radiation emitted from excited atoms and ions produced by the plasma source. Wavelengths of interest for boron analysis are 249.678 nm, 249.773 nm **(Kasemann et al., 2001)** and 182.52 nm **(Duffy, Thomas, 1996).** The detection limit and precision of B determination by ICP–OES are better than all other spectrophotometry, FAAS/FAES, ETAAS and neutron activation methods mentioned above.

ICP coupled with a mass spectrometer (ICP-MS) has brought a further improvement in sensitivity for boron determination. In this technique, ions based on their mass-to charge ratio (m/z) are measured rather than emissions of the excited atoms and ions **(Farhat et al., 2012).** The ability of ICP–MS to measure the boron isotope ratios makes it especially suitable for biological samples and body fluids, where boron concentrations are in µg/L levels **(Brown, Hu, 1996).**

Thermal Ionisation Mass Spectrometry (TIMS) is another plasma technique for boron determination. This method is especially used for isotopic determination of BO_2^+ or BO_2^- salts of Na, Cs or Rb **(Nakamura et al., 1992).** It presents high accuracy and precision **(Eisenhut et al., 1996)**, yet it demands sample preparation steps **(Bassett, 1990)** and long analysis times, **(Johnson, 1989)** which make it less preferred for routine B determination.

The last plasma technique for boron determination is the Secondary Ion Mass Spectrometry (SIMS). Unlike other plasma techniques, SIMS does not require sample preparation and analyte purification. Therefore, it is proper to determine B and its isotope ratios in solid samples **(Chu et al., 1994).** In this technique, energetic primary ion beam of Ga^+, Cs^+ or O^- causes sputtering of the surface atoms from the small area of the sample. Then, secondary ions generated from the sample at mass 10 or 11 are detected by mass analyzer **(Sah, Brown, 1997).**

Drawbacks of this method are high cost, difficulty in determining absolute B concentration due to different matrices that may provide different ion yield, poorer resolution than TIMS and difficulty in the analysis of volatile phases of B **(Aggrawal, Palmer, 1995).**

Among all the techniques discussed above, plasma based techniques; ICP-OES and ICP-MS offer the most sensitive and reliable results within a wide range of applications, which require low detection limits. However, especially the latter still remains very expensive in terms of instrumental and operational costs. In addition, the method is not totally free from matrix interferences and baseline elevation is observed due to retainment of boron on glass parts of instrument including nebulizer, spray chamber and torch. Hence, many researchers tend to prefer other techniques in terms of economical and practical reasons.

ETAAS is a promising alternative for determination of boron. It still allows working in low detection limits. Despite the technique suffers from pre-atomization losses and memory effects, treatment of graphite tubes and use of chemical modification improve the sensitivity.

1.3. Atomic Absorption Spectrometry (AAS)

The beginning of optical spectroscopy is grounded in 1672, when Sir Isaac Newton described his observations that sunlight is split into various colors when it is passed through a prism. Scientists kept on doing experiments to realize the nature of the light in following eras. In 1802, black lines in the sun’s spectrum were first noticed by Wollaston. Thereby, the close relation between light and matter was brought to attention. Later in 1860, Kirchhoff and Bunsen demonstrated the principle of atomic absorption upon their experiments that matter absorbs light at the same wavelength that it emits light. However, practical significance of this technique was not understood for a long time **(Welz, Sperling, 1999).** Later in 1955, Walsh published the paper "*The application of atomic absorption spectra to chemical analysis*" and introduced a new instrumental analysis, which is Atomic Absorption Spectrometry (AAS) **(Walsh, 1955).**

Walsh and his colleagues developed the former hollow cathode lamp (HCL), which was first used by Paschen in 1916, and used it as radiation source **(Welz, Sperling, 1999).** Reliable sealed hollow cathode lamps made this type of source applicable and one of the first AAS designs of Walsh and colleagues shown in Figure 1.1 was manufactured in Australia in early 1960s **(L'vov, 2005).**

Working principle of AAS is that ground state gas-phase atoms absorb radiation at a specific wavelength and make transitions to excited state. The amount of absorption determines the elemental concentration. An AAS instrument consists of a radiation source, an atomization unit, a wavelength selection unit, a detector and data processing system. AAS instruments can be classified as Flame AAS and Electrothermal AAS based on atomization units.

Figure 1. 1 An early design of AAS by Walsh et al. (L'vov, 2005)

1.3.1. Flame Atomic Absorption Spectrometry (FAAS)

Flame atomization is the oldest AAS technique. In this technique, solution of a sample is converted into aerosol in a nebulizer and transported into the flame. Flame serves as an atomizer and its main function is to generate ground state atoms of the element of interest. The success of the analysis is directly proportional to atomization degree of the element, which is in turn dependent on efficiency of the atomizer **(Lajunen, Perämäki, 2004).**

Two types of flames are commonly used in AAS which are air-acetylene flame and the nitrous oxide-acetylene flame. Air-acetylene flame is mostly preferred as it offers a suitable environment and a sufficient temperature for about 30 elements.

The nitrous oxide-acetylene flame provides a hotter environment (3150 K), compared to air-acetylene flame (2500 K) and therefore it is favored for analysis of refractory elements such as aluminium, titanium, zirconium **(Dean, 1997).**

Although flame atomization is a simple and inexpensive technique for a significant number of elements, it has some limitations that affect the extent of applications. First, the sample introduction system, e.g. nebulizer/expansion chamber does not work efficiently. At most 10% of the solution can be converted into aerosol and so it requires large volumes of solutions. Second, the residence time is limited. Thirdly, viscous samples such as blood, serum, oils etc require dilution with a solvent. In order to avoid such problems, nonflame methods involving electrical heating have been developed **(Dean, 1997).**

1.3.2. Electrothermal Atomic Absorption Spectrometry (ETAAS)

Electrothermal or graphite furnace atomic absorption spectrometer was developed by the Russian chemist and physicist Boris L'vov in 1959. Although the technique was introduced by L'vov three years after the establishment of flame atomization methods, it took ten years longer for it to be recognized and production of first commercial instrument at the end of 1960s **(Welz, Sperling, 1999).**

In this technique, a graphite tube serves as an atomizer into which a sample solution, usually 10-50 µL, is introduced through a dosing hole on the tube. Then the tube is heated electrically by passing a high current in three steps; drying, ashing (pyrolysis) and atomization. As the analyte is atomized within a short time (generally 1-2 s), a peak-shaped signal is obtained. Peak height or more reliably peak area (integrated absorbance) is used to determine the analyte concentration in measurement solution **(Welz, Sperling, 1999).**

In comparison to FAAS, ETAAS is a superior technique as it allows to;

i. Use small volumes or masses of samples
ii. Analyze solid samples with minimal sample preparation
iii. Work in low detection limits as there are no flame gases to dilute and remove the analyte atoms as well as highly efficient sample transportation is achieved.

1.4. History of ETAAS

After rediscovery of AAS by Walsh in 1955, L'vov followed and supported his ideas. However, unlike Walsh he started to use graphite cuvettes as atomizer in his experiments **(Welz, Sperling, 1999).** Actually, graphite cuvette was not first used by L'vov. Furnace history starts in nineteenth century **(Marshall, Ottaway, Ottaway, Littlejohn, 1986).** However, L'vov was the first person to use an electrically heated furnace as atomizer. He did not only develop this technique but also determined the theoretical principles and published them in 1966 in Russian and in 1970 in English **(Welz, Sperling, 1999).**

L'vov with his furnace design, which is shown in Figure 1.2, aimed to volatilize the analyte in a short time and thereby get rid of any losses and interferences. He placed samples on a graphite electrode and then introduced vertically into a pre-heated graphite tube. **(Lajunen, Perämäki, 2004).** The graphite tube was 5 to 10 cm in length and 3 mm in diameter. To accelerate the whole process, the electrode was first heated by a direct current arc and then by an electric current flowing through the electrode and furnace **(Welz, Sperling, 1999).** To prevent oxidation of graphite, the tube and electrode were held within a water-cooled glass casing which is full of argon **(Lajunen, Perämäki, 2004).**

Even during the 1960's L'vov and co-workers were able to determine more than 40 elements with absolute limits of detection between 0.01 pg and 10 pg using his graphite cuvette. At the same time he was able to show that matrix influences were much lower than in the flame technique. Graphite furnace AAS was used for analysis of water, high purity reagents, metals, alloys, semiconductors, rock samples and radioactive substances **(Welz, Sperling, 1999).**

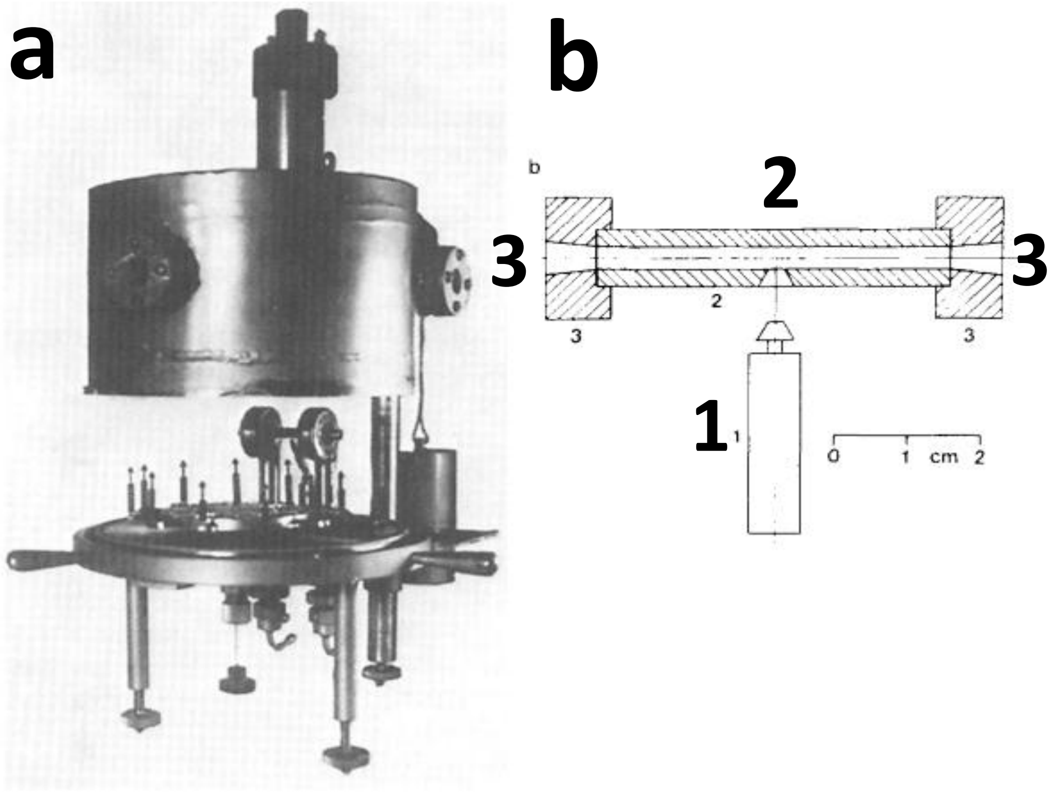
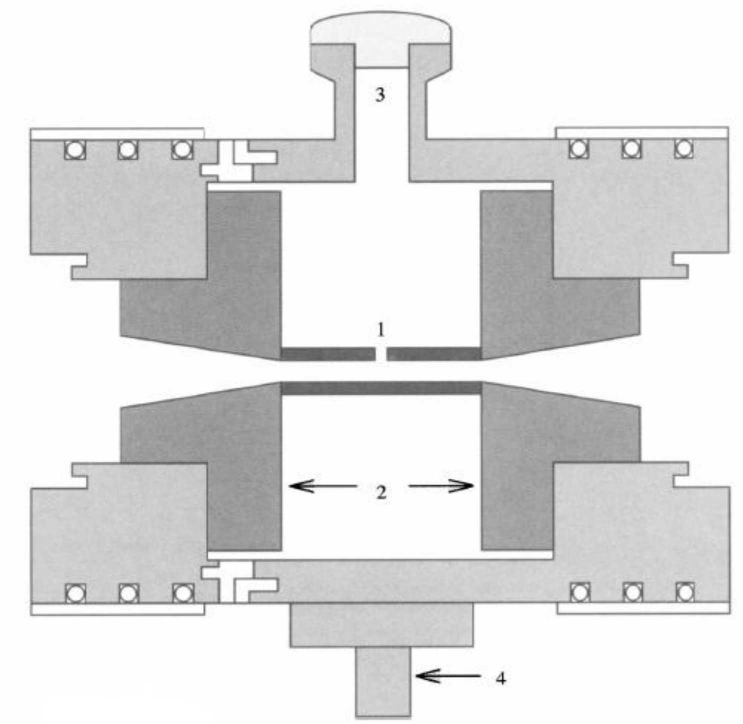


Figure 1. 2 Graphite cuvette by L'vov. **a** – argon chamber with atomizer; **b** – schematic cross-section. 1- graphite electrode; 2- graphite tube; 3- contacts (Welz, Sperling, 1999).

Other than L'vov and his group, very few researchers showed interest in the graphite furnace as an atomizer. *Nikolaev* and *Aleskovskii* **(Nikolaev, Aleskovskii, 1963)** also experienced the advantages of graphite cuvette and developed methods for metallurgical needs. *Woodriff et al.* **(Woodriff, Ramelow, 1968)** developed an isothermally heated furnace differing from that of L'vov in length. In this design, sample was placed in a capsule. However, this method did not provide a further improvement. Later, *West et al*. **(West, Williams, 1969)** submitted a simplified graphite atomizer in which a carbon rod served as an atomizer. The rod was placed between two electrodes and whole system was inside a glass chamber of full of argon. The rod was heated electrically then measurement took 5-10 seconds and next sample could be introduced after a few minutes. Despite the primary promising results, difficulties with sample introduction as well as arising interferences mainly attributed to the huge temperature difference between carbon rod and environment kept the technique away from progress.

The most significant step in the development of ETAAS was taken by Massmann, who reported his first results in 1965 **(Massmann, Ehrlich G. (Editor), 1966)**. He simplified the furnace relatively. In the Massmann's furnace, there was no electrode for sample introduction; sample was directly placed on the tube wall and atomized from the wall. The atomization could be acquired by rapid heating rather than using a pre-heated tube. The graphite tube, which was 5 cm long and 6.5 mm wide, was heated by passing a high current (500 A) at low voltage (10 V) along the tube. Unlike the L'vov furnace, Massmann furnace was not kept in a closed argon chamber therefore a continuous argon flow through the tube was maintained to prevent oxidation of graphite. The tube and graphite end-cones were housed inside a stainless steel water-cooled jacket. Schematic cross-section is given in Figure 1.3. The operating cycle of this model was based upon three steps: (i) The drying step to remove the solvent; (ii) The pyrolysis or ashing step to remove any matrix components; (iii) The atomization step to produce free atoms of the element to be determined **(Welz, Sperling, 1999).**



1. Graphite tube
2. Steel holders
3. Sample inlet port
4. Mounting holder

Figure 1. 3 The Massmann furnace (Lajunen, Perämäki, 2004)

With his system Massmann could not achieve as sensitive methods as much of L'vov. The reasons were attributed to (i) continuous gas flow through the tube which decreases the residence time of atoms; (ii) slower heating rate than that of using an arc; (iii) observation of background attenuation.

L’vov clarified these items in his book **(L’vov, 1970).** Despite these weaknesses (which at that time were not known by majority), all commercial electrothermal atomizers built in 1970s and 1980s were based on Massmann’s principle. The first commercial graphite furnace, the HGA-70, introduced by Perkin-Elmer in 1970 is shown in Figure 1.4 **(Welz, Sperling, 1999).** As the instrument was more practical than L’vov design, it gained general acceptance. In addition, applications confirmed the higher sensitivity of the technique in two to three powers of magnitude compared to flame AAS **(Manning, Fernandez, 1970).** Although ETAAS was recognized as a sensitive technique, there was almost none paper with no concern of matrix and spectral interferences. These were mostly attributed to non-isothermal conditions of atomizers. To eliminate this problem, two of the most prominent solutions that L’vov proposed are platform atomization **(L'vov, Pelieva, Sharnopolsky, 1977)** and probe atomization **(L'vov, Pelieva, 1978)** other than tube (wall) atomization. Later, transversely heated graphite atomizers (THGA) have been developed to provide a homogeneous temperature distribution in the graphite tube. This considerably reduces matrix influences and makes analysis almost free of memory effects **(Bozsai, Melegh, 1995).**

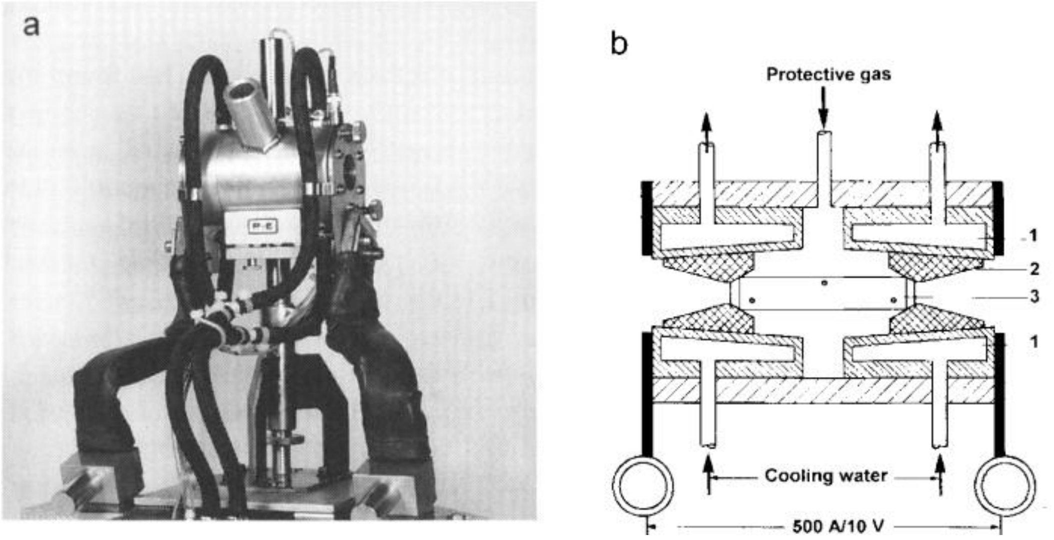


Figure 1. 4 The Perkin – Elmer HGA-70, the first commercial graphite furnace (HGA = Heated Graphite Atomizer). **a** – atomizer; **b** – schematic cross-section; 1 – cooling chambers; 2 – graphite contacts; 3 – graphite tube (Welz, Sperling, 1999)

1.5. Modern ETAAS Systems

Some modern ETAAS systems employ transversely heated graphite furnaces rather than previously used longitudinally heated furnaces. Transversely heated furnaces provide a uniform temperature profile over the entire tube and by this way interferences and memory effects due to condensation of atoms on cooler ends are avoided **(Lajunen, Perämäki, 2004).** Figure 1.5 shows the comparison of both furnace types.

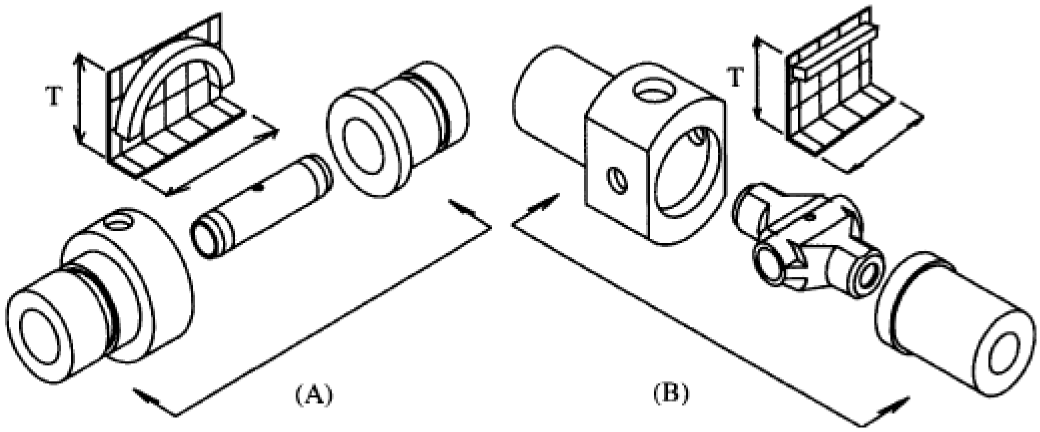


Figure 1. 5 Comparison of structures of the longitudinally heated (A) and transversely heated (B) graphite furnaces (Lajunen, Perämäki, 2004)

A graphite tube with an inner diameter of 3 to 6 mm and 20 to 40 mm in length is used. Sample injection is performed through the hole in the upper center of the tube. The temperature of the tube can be varied from ambient to 3000 ^{0}C. Temperature control is maintained in two ways. One is based on the current applied and the other one is by the optical temperature sensor (pyrometer); in most modern instruments, these both controls are utilized. The tube is housed inside stainless steel water-cooled electrodes that are electrically connected to the power supply. The tube is heated by a high current flowing between these electrodes and the tube. By the help of water-cooling system, the tube is cooled down. A purge gas, usually argon, is flown through the internal and external walls of the tube to exclude oxygen and prevent combustion of the tube **(Butcher, Sneddon, 1998).** A complete graphite furnace system is shown in Figure 1.6.

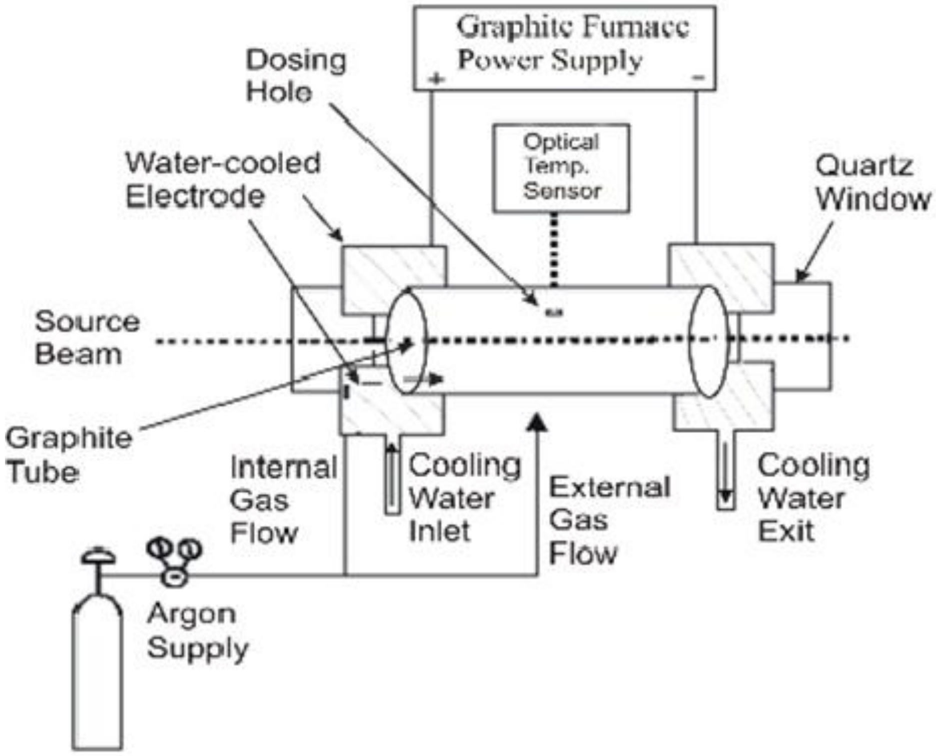


Figure 1. 6 Graphite furnace system (Butcher, Sneddon, 1998)

1.5.1. Sample Introduction

Modern ETAAS systems employ auto samplers for sample introduction. Auto samplers present some advantages over manual pipetting. First one is precision of sample injection on the same location each time. Second one is prevention of any contamination arising from pipette tips and lastly generation of a productive system **(Boulo, Soraghan, Sadler, Littlejohn, Creeke, 1997).** Auto sampler consists of a pump system, a mechanical arm, a tray on which solutions are placed in cups and a container for cleaning agent.

Plastic tubing, which is attached to the arm, is inserted into the solutions and draws a user-specified volume (generally 5 to 50 µL) and delivers it into the graphite tube. Then the tubing is rinsed with cleaning agent (5% HNO_3 solution or simply deionized water) **(Butcher, Sneddon, 1998).**

A dentist's mirror or charge-coupled device (CCD) camera is used to observe the sample introduction process. The CCD camera is very useful for setting the alignment of the tubing as well as ensuring all of the liquid is deposited correctly in the tube.

1.5.2. Furnace Temperature Programme

Analytical methods by ETAAS require a well-planned temperature programme. It is started following the sample introduction and generally composed of four steps: (i) Drying; (ii) Pyrolysis (ashing); (iii) Atomization; (iv) Cleaning. An optimum programme should allow the removal of concomitants in drying and pyrolysis stages. This includes both vaporization of solvents and decomposition of organic matter. In addition, vaporization of analyte and atomization of all by the end of the temperature programme is of importance **(Butcher, Sneddon, 1998).** Each heating step is explained below.

1. Drying. During this step the solvent part is evaporated from the sample. It must be performed gently in order to prevent spattering of the sample. For wall atomization, a temperature below 100 ^{0}C is preferred for starting and in a few seconds it is increased slowly in the range of 110 to 150 ^{0}C. On the other hand, for platform and probe atomization higher drying temperatures (around 200 - 250 ^{0}C) and longer hold times are required.

2. Pyrolysis. Following the drying step, the temperature of the furnace is increased generally in the range of 400-1000 ^{0}C for a period of time called ashing (or pyrolysis) stage. It is aimed to separate the analyte from matrix components and reduce interference effects. Organic part of the sample is ashed and high boiling point components are removed. However, use of a high pyrolysis temperature or keeping the time longer leads to loss of analyte before atomization. This is particularly critical for volatile elements such as Pb, Cd, As, Se.

3. Atomization. As the name implies, the analyte is atomized in this stage. Temperature of the graphite tube is increased with a maximum power heating (>1000 ^{0}C/s) as high as 2000 - 3000 ^{0}C. A high heating rate is required in order to prevent volatilization of analyte prior to atomization temperature.

4. Cleaning. Cleaning is the last step of a furnace cycle and is applied to remove any deposits on the tube surface. The temperature is increased to just above atomization temperature for few seconds. A cleaning temperature of 2500-3000 ^{0}C is preferred commonly. As the cleaning step is completed, the tube is allowed to cool down by the help of water cooling system and thereby a new furnace cycle can be started **(Butcher, Sneddon, 1998; Lajunen, Perämäki, 2004).**

1.6. Other Atomization Types

1.6.1. Platform Atomization

The environment of a Massmann furnace was not stable during atomization. The sample was placed on a cold tube wall which was then rapidly heated through atomization. After vaporization, temperature difference between middle and ends of the tube were considerable which led to interferences **(Lajunen, Perämäki, 2004).**

L'vov et al. **(L'vov, Pelieva, Sharnopolskii, 1977)** proposed that sample should not be injected onto tube wall, but onto a 'platform' positioned in the tube. A platform is a thin rectangular piece of graphite which is shown in Figure 1.7. As the tube and platform are made of same piece of graphite, it is generally called as 'integrated platform'. Platform is set in a minimal contact with the tube only at ends and thereby there is no direct heat transfer through the platform. When the tube and the gas phase reach the atomization temperature and are largely in equilibrium, the platform is heated by the radiation and the hot gas. As a result of retarded heating of the platform (Figure 1.8), the analyte is atomized into an environment stable in temperature which causes less chemical and spectral interferences **(Lajunen, Perämäki, 2004).** Platform atomization is particularly preferred for volatile elements such as lead, cadmium, arsenic, selenium.

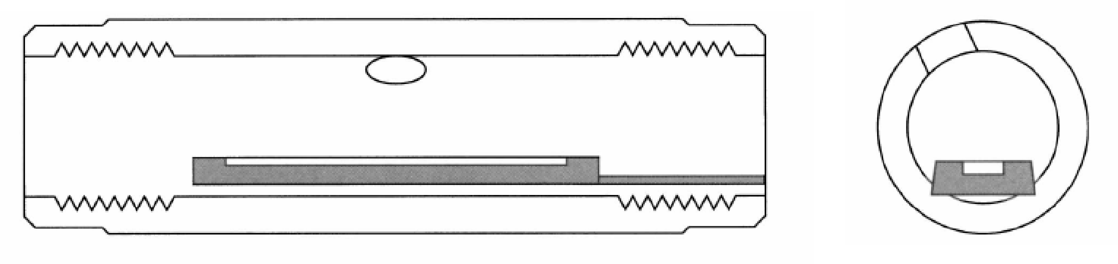


Figure 1. 7 Graphite tube with L'vov platform (Lajunen, Perämäki, 2004)

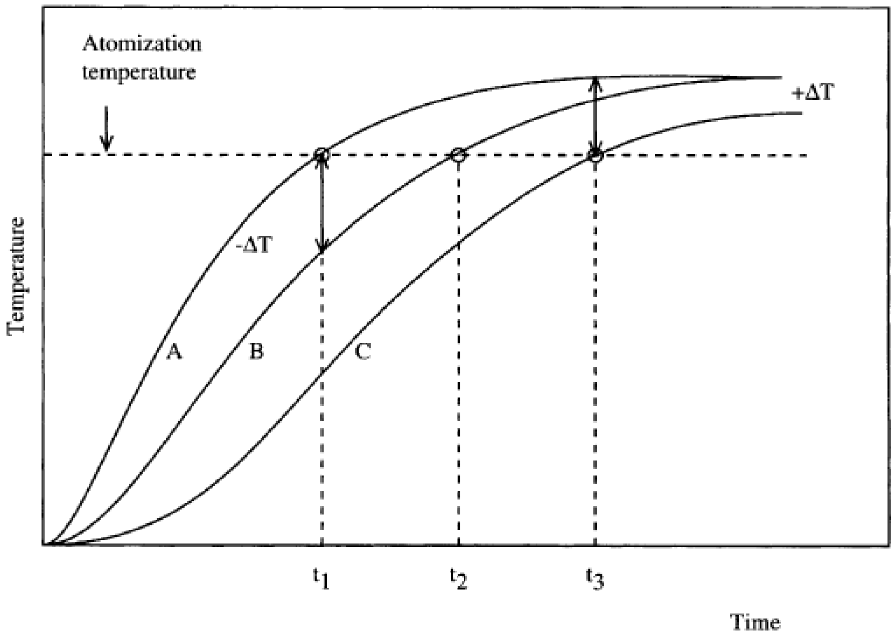


Figure 1. 8 Temperatures of graphite tube wall (A), gas (B), and L'vov platform (C) versus time (Lajunen, Perämäki, 2004)

1.6.2. Probe Atomization

Probe atomization is an alternative approach to achieve high vapour temperatures in the graphite furnace and isothermal atomization of the sample in order to minimize chemical interference effects. The technique was first suggested by *L'vov et al.* **(L'vov, Pelieva, 1978).** In this technique, sample is introduced onto a graphite probe and inserted into the tube then drying and ashing steps are completed within the tube. Later, the probe is withdrawn and the tube is heated to just below atomization temperature. When the temperature of the tube has been stabilized, probe is automatically re-introduced into the furnace and vaporization of analyte into hot gas environment is maintained. Final step is cleaning **(Lajunen, Perämäki, 2004).**

Since the probe is not in contact with the graphite tube, it is heated by radiation during drying, pyrolysis and atomization stages therefore higher furnace temperatures compared to wall atomization are required for each stage **(Lajunen, Perämäki, 2004)**.

Probe atomization has not been as much sensitive as platform atomization. Some drawbacks have been effective such as weak chemical and mechanical strength of probe and also probe head must be of very thin section to obtain a low thermal mass. In addition, spreading of sample droplet is the case for most of the times. Therefore, only a limited volume of sample can be introduced. These reasons led this technique to lose its reputation and it is no longer preferred **(Lajunen, Perämäki, 2004)**.

1.7. Atomizer Materials and Design

An ideal material for tube atomizers must be chemically inert, have good thermal and electrical conductivity. Additionally, having low porosity, a low expansion coefficient and a high melting point are desired properties. From the possible materials available, graphite nearly satisfies all these requirements **(Lajunen, Perämäki, 2004).**

Another option is atomizers made of metals such as tantalum, tungsten, molybdenum. *Sychra et al*. **(Sychra, Kolihová, Vyskočilová, Hlaváč, 1979)** constructed a tungsten atomizer and performed determinations of a number of elements. Optimum conditions and analytical figures of merit were found similar to graphite tube atomizers, however, advantages as low cost, long lifetime and better analytical performance, particularly for elements forming refractory carbides were apparent. These metal furnaces could achieve very fast heating rates that improved the peak height sensitivity considerably. On the other hand, signals are extremely narrow, leading to difficulties in their evaluation **(Shan, Radziuk, Welz, Sychra, 1992).** Moreover, as the surfaces are not totally inert to samples, memory effects are commonly observed.

Tubes made from normal graphite are named ‘electrographite tubes (EG)’. This material can be easily worked and has favorable thermal and electrical properties. However, at higher temperatures EG becomes increasingly porous, which leads to diffusion of atom vapour through the tube wall. Additionally, some elements (boron, aluminium, titanium, etc) form stable carbides on graphite surface. These problems may be overcome to a large extent by coating the tubes with pyrolytic graphite (PG) or making the tubes entirely pyrolytic graphite (total pyrolytic graphite (TPG)) **(Welz, Sperling, 1999; Lajunen, Perämäki, 2004).**

Pyrolytic graphite (PG) layer is formed on an electrographite tube in an inert atmosphere by pyrolyzing hydrocarbons, e.g. methane at about 2000 ^{0}C. This process usually forms a pyrolytic graphite layer of 30 to 50 µm in thickness. Pyrolytic graphite tube presents some advantageous properties such as low gas permeability and good resistance to chemical attacks. Carbide formation is also reduced on the tube and therefore carbide forming elements can be determined with an improvement in analytical sensitivity. However, pyrolytic graphite tubes also have a limited lifetime. The coating is getting thinner as the material sublimes in time. Therefore, reduction in sensitivity is observed after a number of analyses and it should be replaced with a new tube **(Lajunen, Perämäki, 2004).**

Total pyrolytic graphite (TPG) tubes have the properties of low gas permeability and high chemical resistance as pyrolytic graphite tubes do. Moreover, the mass of the tube can be reduced using this material which means a lower thermal mass.

This in turn increases heating rate. A study by *De Loos-Vollebregt et al*. **(Loos-Vollebregt de, Galan de, 1986)** concluded that higher heating rate provides higher sensitivity in peak height. However, the long term tests showed that surface of TPG can be attacked and there can be dramatic changes in the magnitude and shape of the signals. It may not be possible to have reproducible signals at any time during the lifetime of the tubes. Since the expectations were not met, TPG's have not come into general use **(Welz, Schlemmer, Ortner, Wilhelm, 1989).**

1.8. Chemical Modification

Chemical modification or matrix modification was first introduced by Ediger in 1973 **(Ediger, 1975).** The function of chemical modifiers was to remove the sample matrix at the pyrolysis stage, while keeping the analyte in the atomizer. This was attained through injection of modifier(s) into a graphite atomizer simultaneously with a test sample.

Later, *Kuzovlev et al.* **(Kuzovlev, Kuznetsov, Sverdlina, 1973)** came up with the idea of coating the graphite tubes with high melting point carbides to improve the sensitivity for boron determination by ETAAS. Since then, interest in this field has continued increasingly. International Union for Pure and Applied Chemistry (IUPAC) defines chemical modification as use of reagents in order to influence processes taking place in the atomizer in the desired way **(Ure, Butler, L'vov, Rubeska, Sturgeon, 1992).**

Boron determination by ETAAS cannnot be imagined without use of proper chemical modification. Modifiers are utilized to increase sensitivity by both retaining the analyte to higher temperatures and also reducing or eliminating interferences in electrothermal AAS methods. They may be added into analyte solutions directly or co-injected into tube with analyte during analyses. Another way of use is as tube coating materials.

Throughout the years, there has been a great number of modifiers proposed by many scientists. In order to control the modifier inflation, *Schlemmer* and *Welz* offered a mixture of magnesium nitrate and palladium nitrate as universal modifier **(Schlemmer, Welz, 1986).** It was later proven by *Welz et al*. **(Welz, Schlemmer, Mudavaki, 1992)** that universal modifier could be used for more than 20 elements successfully. Today, low detection limit analyses for both volatile and involatile elements such as arsenic, selenium, lead, boron, chrome, titanium, etc. cannot be imagined without use of modifiers. Most commonly used modifiers belong to the groups of (i) metal nitrate salts; (ii) high melting carbides; (iii) organic compounds (organic acids, EDTA, etc); (iv) nitric acid and corresponding ammonium salts, (v) ammonium phosphates; (vi) transition metals with higher oxidation states (salts of W(VI), Mo(VI), Zr(IV), etc.); (vii) compounds of platinum-group metals **(Volynskii, 2003).**

1.9. Memory Effect

One of the major challenges of boron determination by ETAAS is the formation of stable boron carbides on the tube surface which causes a permanent memory effect **(Barnett, Ebdon, Evans, Ollivier, 1991).** Boron atoms are slowly released from refractory boron carbide **(Goltz, Chakrabarty, Sturgeon, Hughes, Gregory, 1995)**. Therefore, the analyte signal cannot reach the baseline even after an atomization time of more than 5 seconds. As a result, signal tailing is observed. In addition, *Wiltshire et al*. **(Wiltshire, Bolland, Littlejohn, 1994)** proposed that the tailing is due to the atomization profile of boron atoms, which are once formed go through a series of condensation-vaporization steps.

To minimize memory effect on analyses, a blank must be run between successive sample injections. However, even this practice cannot prevent the memory effect at all and the tail tends to increase and flatten. Besides, repetitive firings reduce the tube lifetime considerably.

In order to solve these problems, solutions were examined which are injected into the tube during a cleaning cycle and assumptively form volatile boron species such as boron trifluoride (BF_3) **(Luguera, Madrid, Camara, 1991).** NaF **(Barnett, Ebdon, Evans, Ollivier, 1991)** and NH_4F HF **(Burguera, Burguera, Rondon, Carrero, 2001)** that were experienced as effective in reducing memory effects relatively, though not completely.

1.10. Aim of the Study

Up to date, our research group has worked on boron determination by ICP-OES and ICP-MS. Not only sensitive methods were developed but also a number of studies on boron and health relationship were done **(Bora, 2010; Korkmaz, Uzgören, Bakırdere, Aydın, Ataman, 2006; Korkmaz, Yenigün, Bakırdere, Ataman, Keskin, Müezzinoğlu, Lekili, 2011**)

This study was aimed at performing boron studies with an alternative technique, ETAAS. To develop a reliable and sensitive method by ETAAS would vary our capability. For this study, water samples collected from Balıkesir, Bigadiç and Kütahya, Emet were analyzed by the relevant method.

CHAPTER 2

EXPERIMENTAL

2.1. Chemicals and Reagents

All reagents used throughout this study were of analytical grade or high purity. 1000 mg/L boron stock solution was prepared by dissolving appropriate amount of H_3BO_3 (Merck, Germany) in 1.0 mol/L HNO_3. Working solutions were prepared in 1% (v/v) HNO_3 by successive dilutions. Dilutions were made using 18 MΩ·cm deionized water obtained from a Millipore (Molsheim, France) Milli-Q water purification system which was fed using the water produced by Millipore Elix 5 electro deionization system. Working standard solutions were acidified with proanalysis grade HNO_3 (Merck, Germany). Boron concentrations of the calibration standards were 0.50, 1.0, 2.0 and 5.0 mg/L. High purity Ar (99.999%) was used throughout the study.

All of the solutions were prepared by using 100-1000 µL and 500-5000 µL range Eppendorf micropipettes. Working solutions were stored in polyethylene and plastic containers in order to prevent boron contamination from glassware. All the containers and labware used throughout the study were immersed in acid tanks with 10% (v/v) HNO_3 at least 24 hours and rinsed with deionized water before use.

1000 mg/L W standard solution was prepared by dissolving an appropriate amount of $Na_2WO_4.2H_2O$ (Merck) in deionized water. Since acidification caused precipitation of tungstic acid, it was stored in an alkaline medium. 1000 mg/L standard solutions of Ta (High Purity Standards, USA) in 2% HNO_3, Zr (Ultra Scientific, USA) in 2% HNO_3, Rh and Pt (Inorganic Ventures, USA) in 5% HCl were used. 1000 mg/L Os solution was prepared by dissolving appropriate amount of Ammonium Hexachloroosmate(IV) in 5% HCl.

1000 mg/L $Mg(NO_3)_2$ standard solution was prepared by dissolving a proper amount of $Mg(NO_3)_2.6H2O$ in 1% (v/v) HNO_3. In addition, 0.01 mol/L metal nitrate solutions were prepared by dissolving appropriate amounts of $Mg(NO_3)_2.6H2O$, $Ca(NO_3)_2.4H2O$, $Ni(NO_3)_2.6H2O$ (Riedel-de Haen AG, Germany) and $Pd(NO_3)_2$ (Merck, Germany) in deionized water.

0.05 mol/L organic acid solutions were prepared by dissolving certain amounts of L(+) Ascorbic acid (Carlo Erba, Italy), Citric acid (AnalaR, UK), Oxalic acid (AnalaR, UK) and Tartaric acid (Baker & Adamson, USA) in deionized water.

10.0 g/L NaF solution, used as cleaning agent, was prepared by dissolving a proper amount of NaF (Merck, Germany) in deionized water.

2.2. Samples

Water samples were collected from Balıkesir, Bigadiç and Kütahya, Emet where rich boron reserves are found. These are either tap water or from local fountains. Sampling was made into 50 mL polypropylene centrifuge tubes. The tubes were filled and washed with regarding samples at first hand. This washing procedure was repeated three times for each tube and then the sampling was performed.

2.3. Instruments

2.3.1. Atomic Absorption Spectrometer

Varian Spectra AA 140 (Victoria, Australia) atomic absorption spectrometer equipped with Varian GTA 120 graphite tube atomizer was used. The instrument employs a deuterium background correction system. Instrument control and data process are performed with SpectrAA software (version 5.1). Sample introduction was carried out by Varian PSD 120 programmable sample dispenser, which is a detachable part of the instrument.

Pyrolytically coated graphite tubes (Varian) were used as atomizer. Boron hollow cathode lamp was obtained from Perkin-Elmer with part number of N 202-5304.

Table 2. 1 Operating parameters for ETAAS

Parameter	Value
Wavelength, nm	249.8
Lamp Current	10 mA
Slit Width, nm	0.5
Atomization Type	Wall
Measurement Mode	Peak Height

2.3.2. Inductively Coupled Plasma Optical Emission Spectrometer

Water samples were analyzed by ICP-OES for the comparison of the results with ETAAS analysis. For this study, Leeman Labs Inc. DRE (Direct Reading Echelle) with an axial view configuration Inductively Coupled Plasma Optical Emission Spectrometer (Leeman Labs, Hudson, NH) was used. Determination of boron was done by selecting the 249.773 nm emission line at which the highest signal to noise ratio was obtained. Indium was used as an internal standard. 230.606 nm emission line gave the best signal for In determination. Boron/indium signal intensity ratios were used for results. The operating conditions for ICP-OES were as follows:

Table 2. 2 Operating parameters for ICP-OES

Parameters	Optimum Value
Power, kW	1.2
Frequency, MHz	40.68
Coolant Gas Flow Rate, L/min	19
Auxiliary Gas Flow Rate, L/min	0.5
Nebulizer Pressure, psi	35
Sample Flow Rate, mL/min	0.5

2.3.3. Inductively Coupled Plasma Mass Spectrometer

Water sample from Emet was analyzed by ICP-MS for comparison of the result with ETAAS analysis. For this study, Thermo X Series Inductively Coupled Plasma Mass Spectrometer (Thermo Scientific, USA) was used. Flow injection system with a loop volume of 500 µL was used for sample introduction. Sampler and skimmer cones of the instrument are made of Nickel.

Boron determination was done by evaluating both ^{11}B and ^{10}B signals. Internal standard, Be, has one isotope at mass number 9 with 100% abundance. $^{11}B/^{9}Be$ signal intensity ratios were used for results. The operating conditions for ICP-MS were as follows:

Table 2. 3 Operation parameters for ICP-MS

Parameters	Optimum Value
Extraction Lens Voltage, V	-208
Lens 1 Voltage, V	-3.2
Lens 2 Voltage, V	-63.5
Focus Lens Voltage, V	17.1
1. Diffraction Aperture Voltage, V	-54.9
2. Diffraction Aperture Voltage, V	-207
Quadrupole Voltage, V	-2.5
Hexapole Voltage , V	1.9
Argon Flow Rate in Nebulizer, L/min	0.76
Lens 3 Voltage, V	-188.2
3. Diffraction Aperture Voltage, V	-74.5
Argon Flow Rate to Cool Torch, L/min	18.0

Table 2.3 (continued)

Argon Flow Rate to Produce Plasma, L/min	0.86
Sampling Depth	13
Forward Power, Watt	1400

2.3.4. Ultrasonic Water Bath

Ultrasonic pre-treatment before microwave digestion of NIST 1573a Tomato Leaves SRM was carried out with Elma S40 H ultrasonic water bath. The weighed sample was transferred into PTFE digestion bomb. 5.0 mL HNO_3 was added and sonicated for 20 minutes at room temperature. Then 2.0 mL of H_2O_2 was added and the mixture was sonicated for another 20 minutes at room temperature.

2.3.5. Microwave Oven

Milestone Ethos PLUS microwave dissolution system with a 10 digestion bombs set was used for digestion of NIST 1573a Tomato Leaves SRM. The temperature program given in Table 2. 4 was followed.

Table 2. 4 Temperature program for microwave digestion

Step	Temp (°C)	Ramp Time (min)	Hold Time (min)	Power, W
1	100	10	5	700
2	150	5	10	700

2.4. Procedures

2.4.1. Analytical Procedure

Sample transportation was carried out by Varian PSD 120 programmable sample dispenser. Ta treated graphite tubes were used as atomizer. 15.0 µL sample solution and 5.0 µL of each chemical modifier, 0.01 mol/L $Ca(NO_3)_2$ and 0.05 mol/L citric acid were co-injected into the graphite tube which make a total volume of 25.0 µL. Temperature program recommended in instrument cookbook (Table 2.5) was optimized as in Table 2.6 and a transient signal was obtained. For Ta coated tubes, ashing and atomization temperatures were found optimum as 1100 and 2700 °C, respectively. Peak height values were collected for all measurements and peak area values were calculated by Excel (Microsoft 2010).

Table 2. 5 The recommended temperature program in the instrument cookbook (Varian SpectrAA software, 1997-2006)

Step	Temp (°C)	Ramp Time (s)	Hold Time (s)	Gas Flow (L/min)
Drying	85	5	-	0.3
Drying	95	15	-	0.3
Drying	120	10	-	0.3
Ashing	1000	5	1	0.3
Ashing	1000	-	2	0.0
Atomization	3000	1	5	0.0
Cleaning	3000	-	2	0.3

Table 2. 6 Optimized temperature program for the method

Step	Temp (°C)	Ramp Time (s)	Hold Time (s)	Gas Flow (L/min)
Drying	90	5	5	0.3
Drying	150	15	-	0.3
Ashing	Varied	0.4	5	0.3
Atomization	Varied	0.8	5	-
Cleaning	2800	2	-	0.3

2.4.2. Coating Procedure

The coating of pyrolytic graphite tubes with refractory carbide forming elements (Ta, W, Zr, Pd, Ru, Os) was treated in the following manner with reference to a method by *Lima et al.* **(Lima, Krug, Jackson, 1998).** 50.0 µL of 1000 mg/L solution was introduced into the graphite tube each time and two different temperature programs were applied. Five injections were followed with the temperature program given in Table 2.7. Two more injections were followed with the temperature program given in Table 2.8. After deposition stage two different conditioning steps, one is at mild temperature (Table 2.9) and the other is at high temperature (Table 2.10) were applied consecutively. These conditioning steps were aimed at transforming oxides and oxycarbides to refractory carbides.

During coating procedure, since relatively large volumes of solution in high concentration were used, windows of the graphite furnace assembly were removed to prevent damaging of the lenses.

Table 2. 7 Temperature program for coating procedure at mild temperature range

Step	Temp (°C)	Ramp Time (s)	Hold Time (s)	Gas Flow (L/min)
1	90	5	10	0.3
2	150	15	15	0.3
3	600	10	5	0.3
4	1000	5	5	0.3

Table 2. 8 Temperature program for coating procedure at high temperature range

Step	Temp (°C)	Ramp Time (s)	Hold Time (s)	Gas Flow (L/min)
1	90	5	10	0.3
2	150	15	15	0.3
3	600	10	5	0.3
4	1000	5	5	0.3
5	1400	5	5	0.3
6	2000	2	3	0.3

Table 2. 9 Temperature program for tube conditioning at mild temperature range

Step	Temp (°C)	Ramp Time (s)	Hold Time (s)	Gas Flow (L/min)
1	150	1	5	0.3
2	600	5	5	0.3
3	1100	5	5	0.3
4	1400	5	5	0.3

Table 2. 10 Temperature program for tube conditioning at high temperature range

Step	Temp (°C)	Ramp Time (s)	Hold Time (s)	Gas Flow (L/min)
1	150	1	5	0.3
2	600	5	5	0.3
3	1100	5	5	0.3
4	1400	5	5	0.3
5	1500	1	2	0.3
6	1600	1	2	0.3
7	1700	1	2	0.3
8	1800	1	2	0.3
9	1900	1	2	0.3
10	2000	1	2	0.3

2.4.3. Cleaning Cycle

To minimize problems arising from memory effect, such as peak broadening and tail increase, a separate cleaning cycle was applied after every second boron determination. In this cycle, 30.0 µL of 10.0 g/L NaF solution was injected onto tube and the temperature program given in Table 2. 11 was applied. This temperature programme was optimized as in Table 2.12 in the course of the work.

Table 2. 11 Temperature program for cleaning cycle at the beginning of the work

Step	Temp (°C)	Ramp Time (s)	Hold Time (s)	Gas Flow (L/min)
1	100	5	20	0.3
2	150	5	10	0.3
3	1000	0.5	5	0.3
4	2700	0.9	5	0.3
5	2800	2	-	0.3

Table 2. 12 Optimized temperature program for cleaning cycle

Step	Temp (°C)	Ramp Time (s)	Hold Time (s)	Gas Flow (L/min)
1	100	10	10	0.3
2	120	10	-	0.3
3	150	5	-	0.3
4	1500	5	2	0.3
5	2700	0.9	2	0.3

2.5. Accuracy Check

The accuracy of the method was confirmed by performing EnviroMAT-Waste Water EU-L-1 CRM and NIST 1573a Tomato Leaves analysis. The analysis results are given in section 3.2.

CHAPTER 3

RESULTS AND DISCUSSION

This chapter consists of three parts. First part includes the method development studies. The role of matrix modification for boron determination was presented and modification techniques were optimized. At the end, calibration plots and analytical figure of merits for Ta and W coated tubes are provided.

Second part includes the studies for accuracy check of the method. For this purpose, Certified Reference Material (CRM) and Standard Reference Material (SRM) analyses were performed.

Third part includes real sample analysis. Water samples collected from Bigadiç and Emet were analyzed by the developed method and the results are provided.

3.1. Method Development

At first, boron solutions prepared in 1.0% HNO_3 were introduced into the graphite furnace with no modification and the recommended temperature programme given in Table 2.5 was applied. No analytical signal could be produced by this way as it is seen in Figure 3.1. These data showed that B was lost from the tube prior to atomization cycle.

According to many authors and manufacturers, use of chemical modification has been indispensable for boron determination by ETAAS. It does not only prevent loss of boron but increase sensitivity as well (**Burguera, Burguera, Rondon, Carrero, 2001; Ferraresi, Fornasari, 1997).**

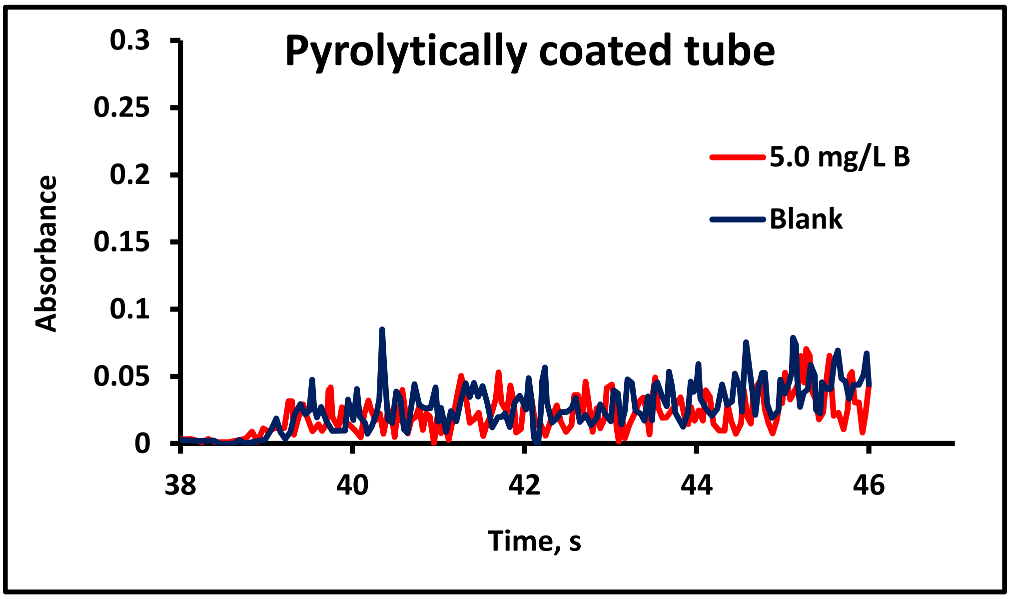


Figure 3. 1 Signal for 15.0 µL 5.0 mg/L B solution on pyrolytically coated tube with ashing and atomization temperatures of 1000 °C and 3000 °C, respectively, using conditions in Table 2.5

3.1.1. Chemical Modification

Both permanent and temporary modifiers were used in this study. Permanent modifiers contained refractory carbide forming elements (W, Zr, Ta, Rh, Pd & Os) for coating the graphite surface. Temporary modifiers were co-injected into the atomizer with analyte solution. Metal nitrate salts and organic acids were examined as temporary modifiers. $Mg(NO_3)_2$ was another temporary modifier as the medium of all standard and sample solutions.

3.1.2. Use of Permanent Modifiers

3.1.2.1. Refractory Carbide Forming Elements

Jiang and Yao experienced poor sensitivity of boron determination in their study that even 10 mg/L B solution could not produce a signal on an uncoated tube **(Jiang, Yao, Yankuang, 1988, cited in Wiltshire, Bolland, Littlejohn, 1994).** The reason was attributed to refractory boron carbide formation. Refractory carbide formation does not only cause analyte loss but also it is the reason of serious memory effects. Once it is formed on the tube surface, it is almost impossible to dissociate and atomize boron completely.

In this study, graphite tubes were treated with 1000 mg/L solutions of Os, Pt, Rh, Ta, W and Zr individually. The signal for 5.0 mg/L B solution on W coated tube is shown in Figure 3.2. It was realized that refractory carbide formation is not the only major cause for analyte loss but in addition boron cannot be stabilized up to high temperatures. A study by *Byrne et al.* **(Byrne et al., 1994)** using electrothermal vaporization inductively coupled plasma mass spectrometer (ETV-ICP-MS) proposes that molecular boron compounds, principally boron oxides begin to form at around 800 ^{0}C and they are lost from the tube up to atomization temperature which makes up the major loss. Therefore, another chemical modification was required to retain boron to high temperatures.

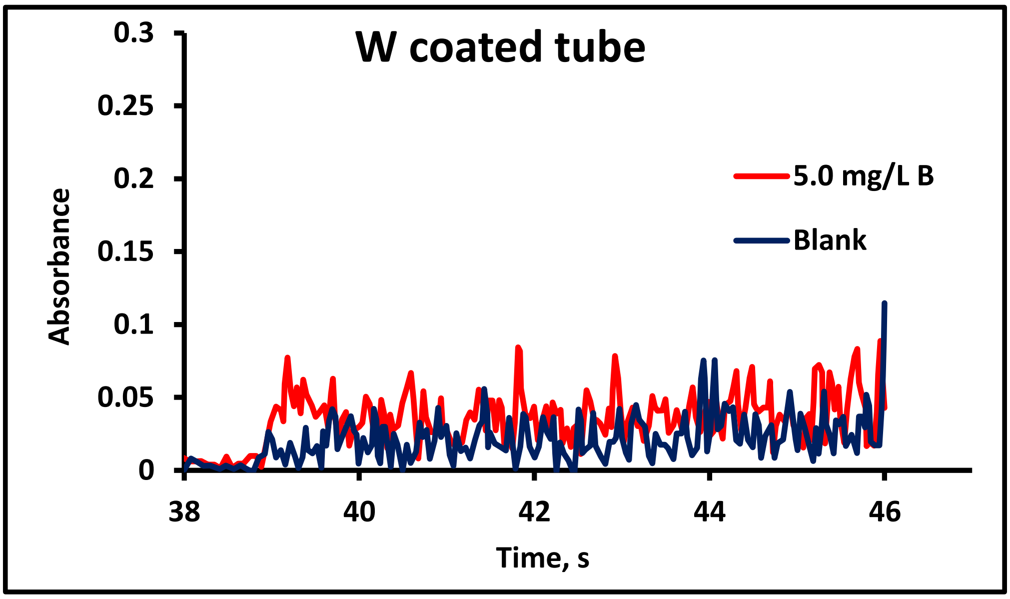


Figure 3. 2 Signal for 15.0 µl 5.0 mg/L B solution on tungsten coated tube with ashing and atomization temperatures of 1000 °C and 3000 °C, respectively, using conditions in Table 2.5

3.1.2.2. Metal Nitrate Salts

Slavin et al. (Slavin, Carnrick, Manning, 1982) reported in their study that use of metal nitrates as modifiers, specifically $Mg(NO_3)_2$, stabilizes analyte elements. The possible mechanism is formation of magnesium oxide at first hand. Then MgO can block the active carbon sites on graphite layer at which low temperature vaporization of B species occurs. This process is continued until vaporization or dissociative desorption of MgO occur at around 1000 – 1200 °C. Then vaporization of boron oxides is possible.

To investigate the proposed effect, 5.0 mg/L B solution was prepared in 1000 mg/L $Mg(NO_3)_2$. 15.0 µL of this solution was injected on pyrolytically coated tube and the resulting signal is given in Figure 3.3. It was realized that boron could be stabilized in $Mg(NO_3)_2$ medium. Therefore, in all following studies B solutions were prepared in 1000 mg/L $Mg(NO_3)_2$.

Additionally, 15.0 µL of 5.0 mg/L B solution was injected on W treated tube. The absorbance signal, which is given in Figure 3.4, was at least two times higher; in addition better peak shape was obtained compared to the one on the pyrolytically coated tube.

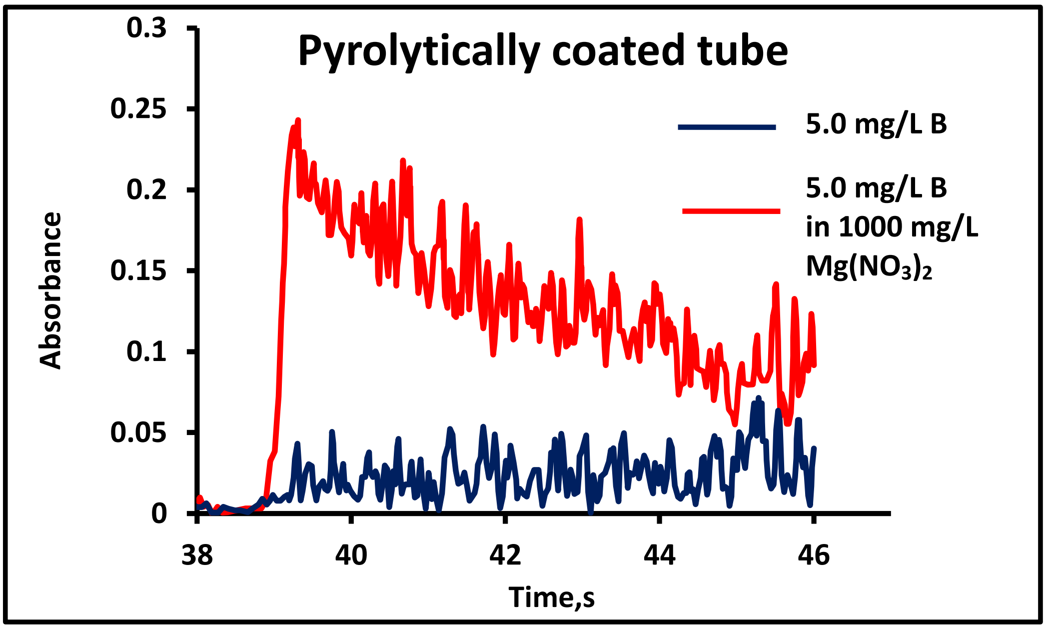


Figure 3. 3 Signals for 15.0 µL 5.0 mg/L B solutions on pyrolytically coated tube with ashing and atomization temperatures of 1000 °C and 3000 °C, respectively, using conditions in Table 2.5

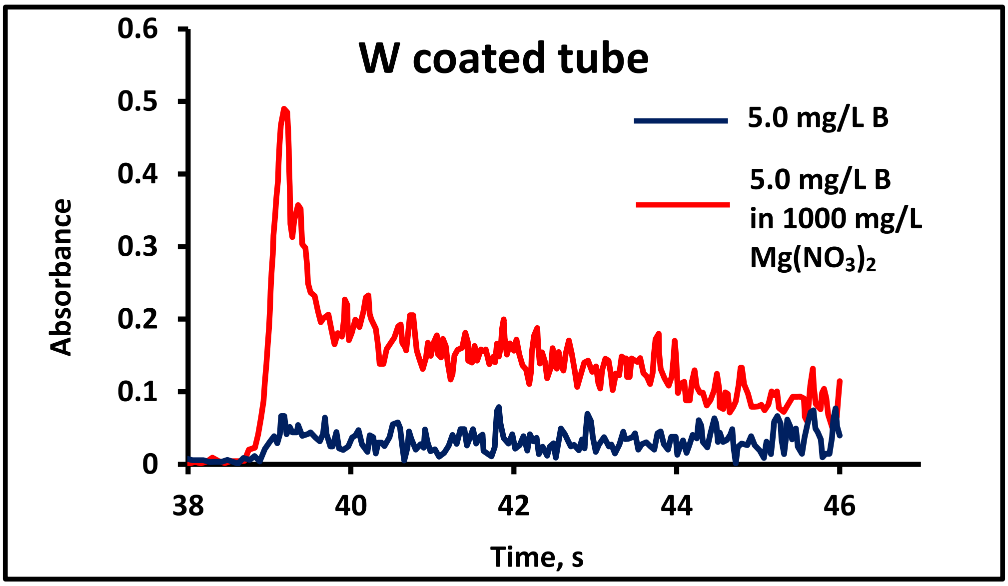


Figure 3. 4 Signals for 15.0 µL 5.0 mg/L B solutions on tungsten coated tube with ashing and atomization temperatures of 1000 °C and 3000 °C, respectively, using conditions in Table 2.5

3.1.3. Optimization of Temperature Program

3.1.3.1. Optimization of Ashing Temperature

Optimum ashing temperatures for each coated tube were determined by plotting ashing curves. To do this, ashing temperature was varied while atomization temperature was set at 2700 °C.

Choosing an ashing temperature as high as possible is desirable to get rid of matrix constituents. This is especially important for complex matrices such as blood, urine and food samples. The ashing curves for several pyrolytically coated tube surfaces additionally treated and coated with different modifiers are given in Figure 3.5.

Peak height values were used for the construction of graphs. At least two replicate measurements were taken for each data point. Optimum ashing temperatures for each coated surface are illustrated in Table 3.1. Relatively high signals were obtained for Ta and W coated tubes at 1100 and 1000 °C, respectively. Besides, it is clearly seen that suitable ashing temperature range is more extended to high values for W coated surface; this will alow a more efficient ashing procedure. An ashing temperature as high as 1800 °C may be employed for W coated surface while an ashing temperature of 1400 °C causes loss of almost all analyte species for other coated surfaces. This indicates W modified graphite tubes may be used for difficult matrices where higher ashing temperatures are needed.

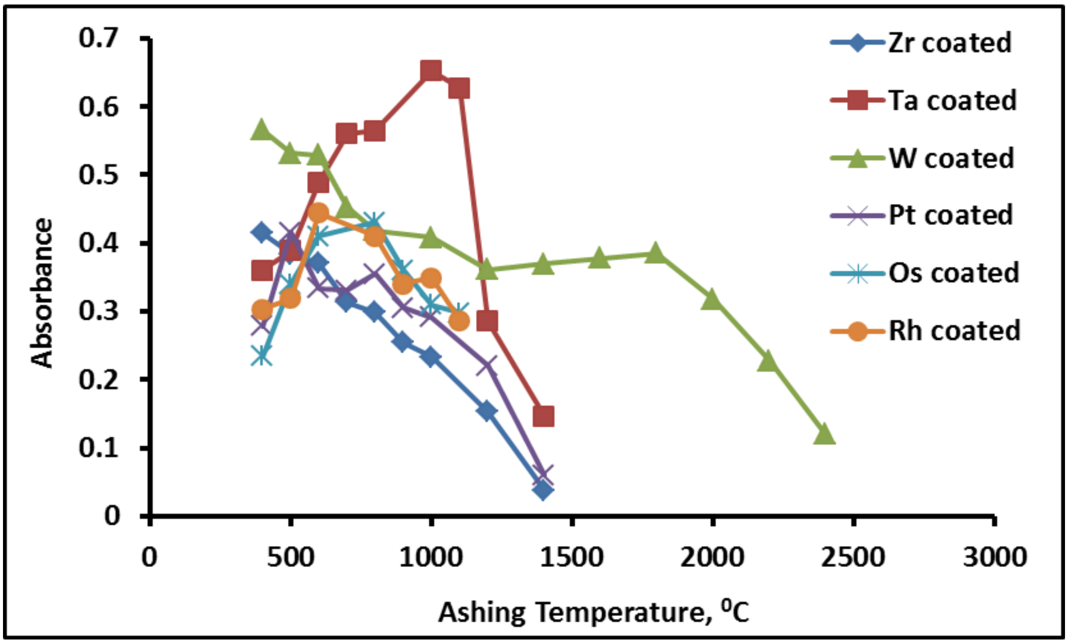


Figure 3. 5 Ashing curves for 15.0 µL 5.0 mg/L B solution on modified tubes using conditions in Table 2.6 and atomization temperature as 2700 °C

3.1.3.2. Optimization of Atomization Temperature

Optimum atomization temperature for each coated tube was determined by plotting atomization curves, which are given in Figure 3.6. Previously determined optimum ashing temperatures were set for each coated surface while atomization temperature was varied. As boron has an involatile nature and atomization of boron is mostly through molecular boron compounds (oxides and carbides), higher atomization temperatures are required. For almost all modified surfaces (except Rh), an atomization temperature of 2700 °C was chosen as optimum. Optimum atomization temperatures for all coated surfaces are given in Table 3.1. Despite temperatures above 2700 °C gives higher signals for some coated surfaces (Zr, Pt), higher temperatures were not preferred due to decrease in signal to noise ratio, elevation of blank signal, as well as disruption of analytical signal shape and decrease in tube life time.

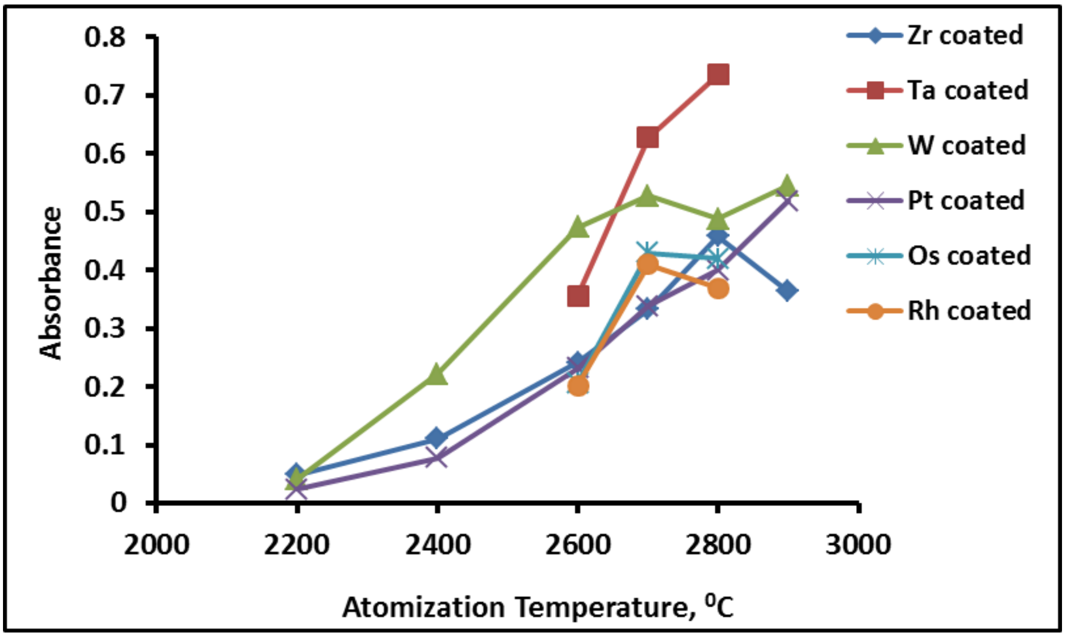


Figure 3. 6 Atomization curves for 15.0 µL 5.0 mg/L B solution on modified tubes using conditions in Table 2.6 and optimum ashing temperatures for each modified tube as given in Table 3.1

Then optimum ashing and atomization temperatures were set for each coated surface and 15.0 µL of 5.0 mg/L B solution prepared in 1000 mg/L $Mg(NO_3)_2$ was injected. At least two replicate measurements and a blank were run. Peak height values, which are given in Figure 3.7, were determined.

According to Figure 3.7, tantalum coated tube is superior to the others as it produces a higher absorbance signal as well as a better peak shape at a higher ashing temperature. For all following studies, Ta coated tubes were preferred.

Table 3. 1 Optimum ashing and atomization temperatures for each coated tube

Atomizer	Ashing Temp (°C)	Atomization Temp (°C)
Zr coated	800	2800
Pt coated	800	2800
Os coated	800	2700
Rh treated	1000	2700
W treated	1000	2700
Ta treated	1100	2700

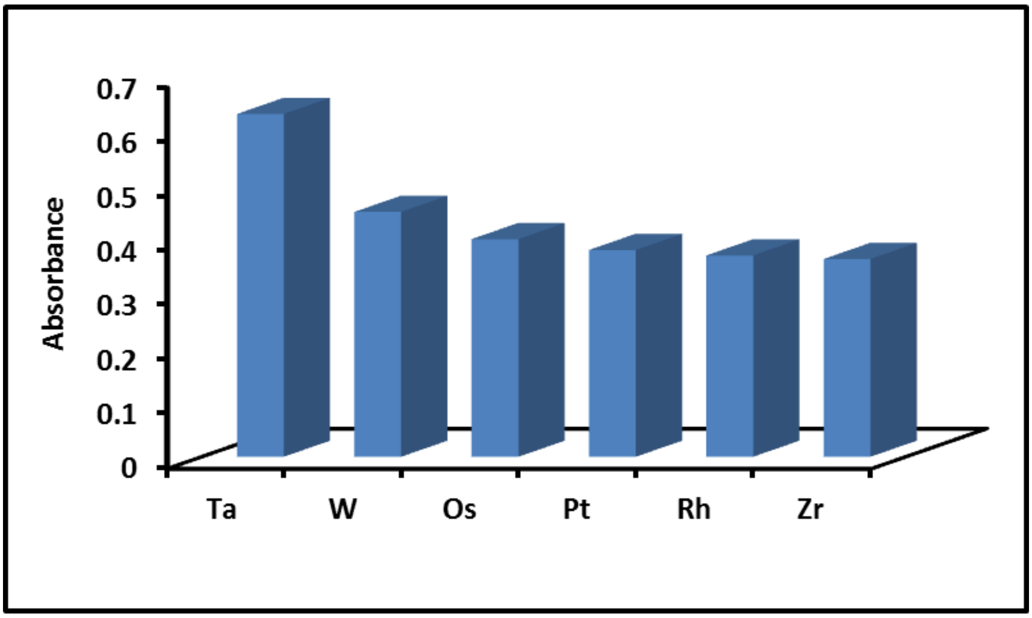
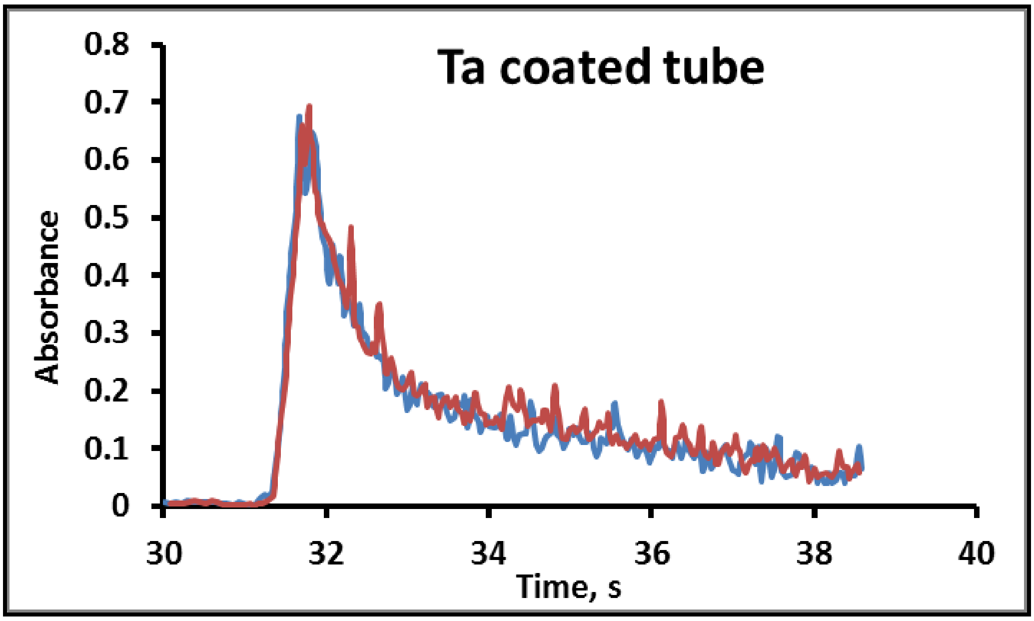


Figure 3. 7 Peak heights for 15.0 µL 5.0 mg/L B solution at optimum ashing and atomization temperatures listed in Table 3.1 and using conditions given in Table 2.6

Analytical signals for two replicate measurements of 5.0 mg/L B solutions on Ta and W treated surfaces are given in Figure 3.8



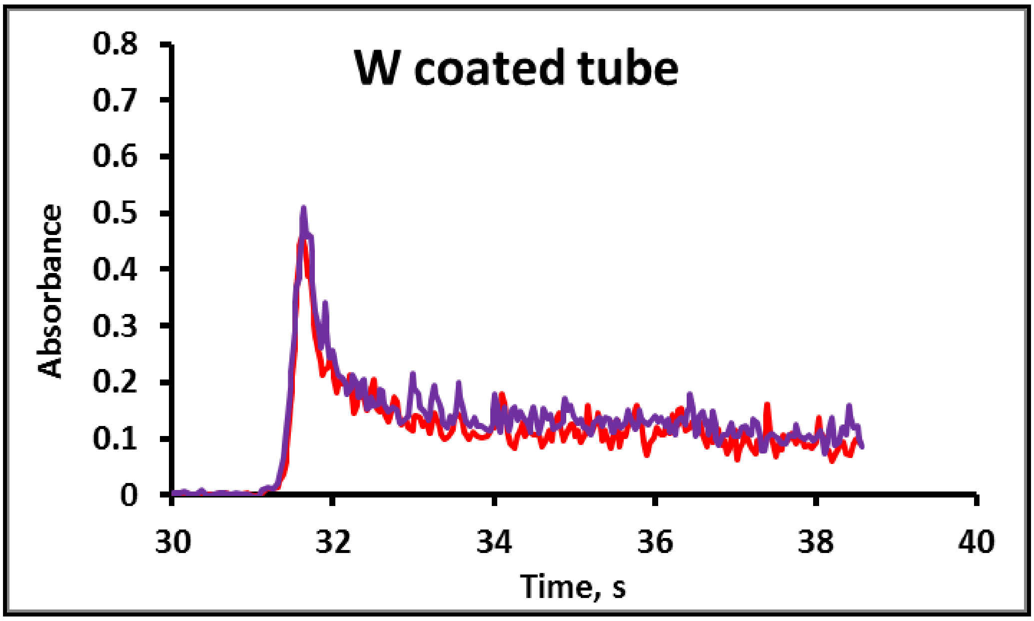


Figure 3. 8 Analytical signals in duplicate for 15.0 µL 5.0 mg/L B solution on Ta and W treated surfaces using conditions given in Table 2.6 and Table 3.1

3.1.4. Use of Temporary Modifiers

For elements such as boron which are susceptible to losses at pyrolysis stage, combination of modifiers has been utilized to increase sensitivity further. *Wiltshire et al.* **(Wiltshire, Bolland, Littlejohn, 1994)** used a mixture of calcium and magnesium nitrate for boron determination. By this way, ashing temperature was increased to 1200 °C and a characteristic mass of 0.8 ng was obtained. In another study, nitrates of strontium and nickel besides citric acid were used as modifiers; a detection limit of 0.048 mg/L was obtained and the method was used for determining trace amounts of boron in metallic samples **(Matsusaki, Yamaguchi, Nomi, Sata, 1996).**

In this study, metal nitrate salts and organic acids were examined as temporary modifiers. They were added simultaneously into the atomizer together with analyte solution. The purpose was to have an enhancement on analytical sensitivity.

3.1.4.1. Metal Nitrate Salts

5.0 µL of 0.01 mol/L nitrate solutions of Mg, Ca, Ni and Pd were co-injected with 15.0 µL of 5.0 mg/L B solution individually. The enhancement in peak heights is compared in Figure 3.9.

$Ca(NO_3)_2$ use provided a 1.5 fold increase in absorbance signal compared to no temporary modifier use. The signal is given in Figure 3.10.

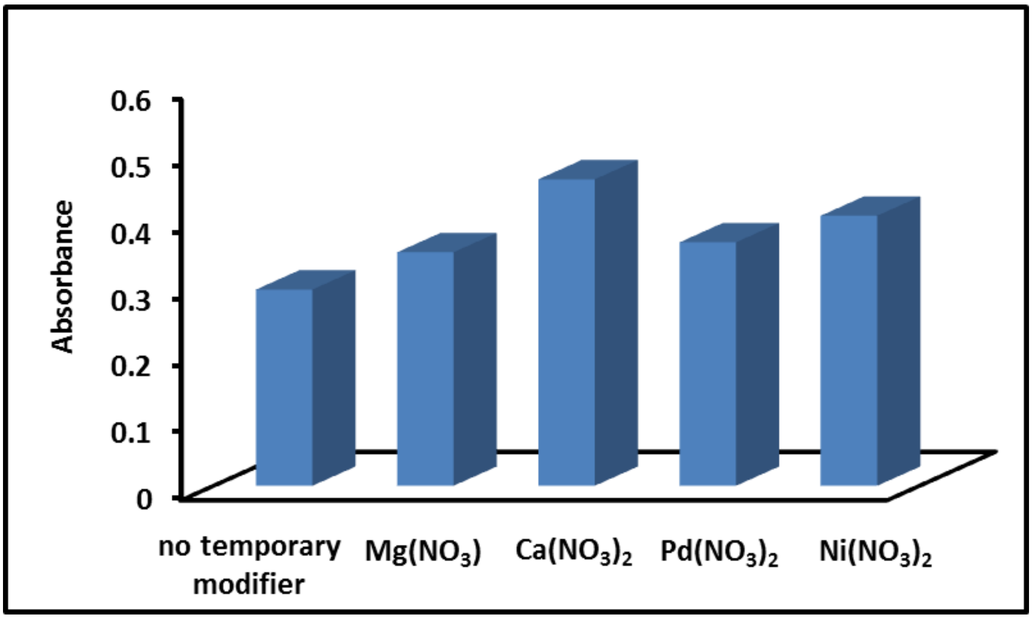


Figure 3. 9 Effect of 5.0 µL 0.01 mol/L of each metal nitrate solution as temporary modifier on peak height using Ta coated tube and conditions given in Table 2.6

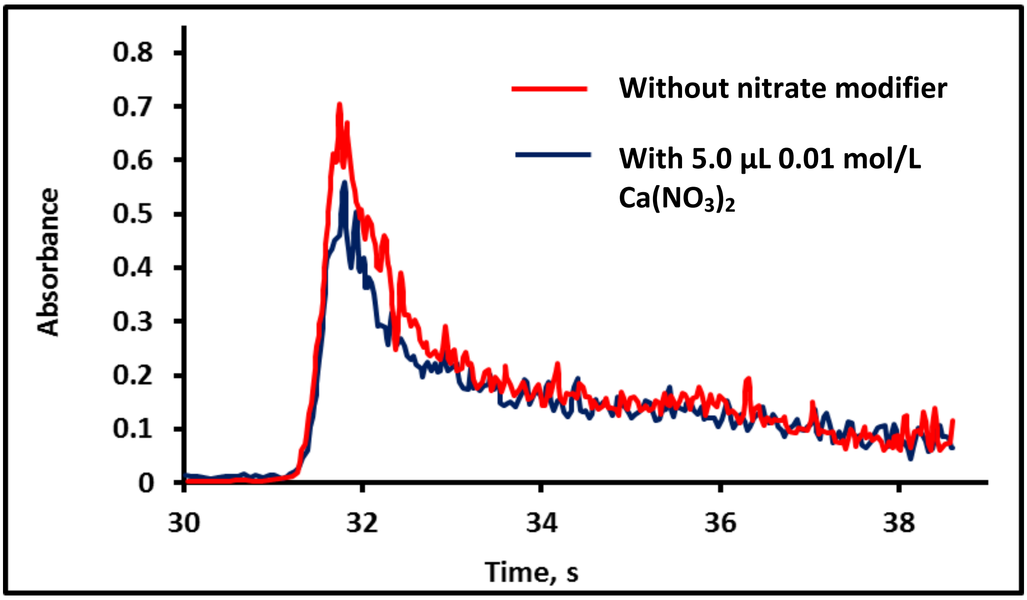


Figure 3. 10 Signal for 15.0 µL 5.0 mg/L B solution with and without temporary modifier $Ca(NO_3)_2$ using Ta coated tube and conditions given in Table 2.6

3.1.4.1.1. Optimization of $Ca(NO_3)_2$ Concentration

Effect of varying $Ca(NO_3)_2$ concentration on absorbance signal of 15 µL 5 mg/L B solution was studied. 0.01, 0.02, 0.03, 0.04 and 0.05 mol/L $Ca(NO_3)_2$ solutions were prepared and each was co-injected with analyte solution individually. $Ca(NO_3)_2$ volume was kept constant at 5.0 µL for all injections. Peak height values for each concentration are compared in Figure 3.11. Two replicate measurements and a blank were run for each data point; 0.01 mol/L was chosen as the optimum concentration.

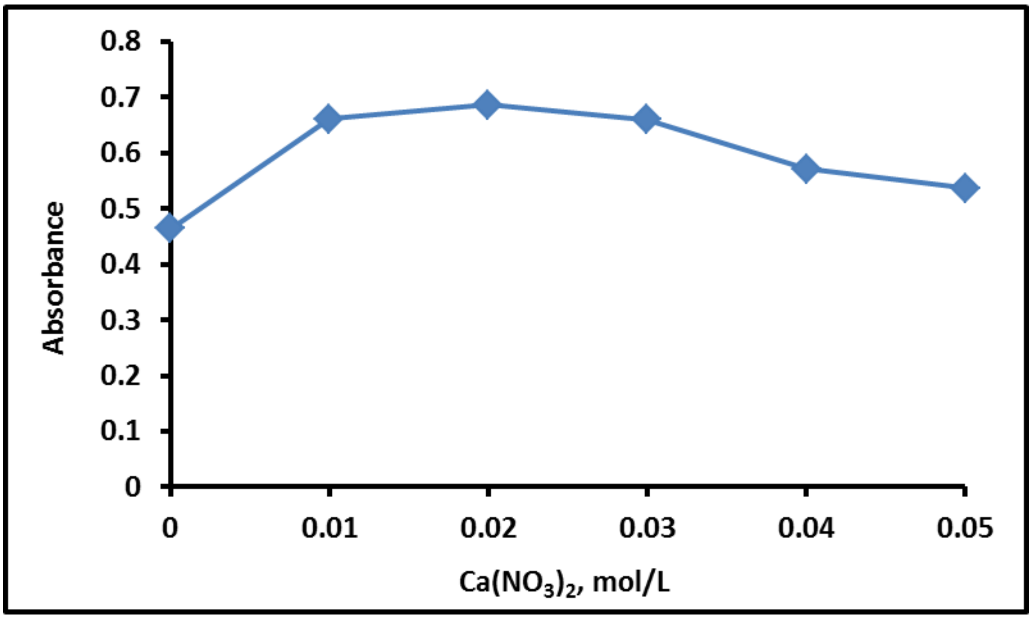


Figure 3. 11 Effect of $Ca(NO_3)_2$ concentration on absorbance signal of 15 µL 5 mg/L B solution using Ta coated tube and conditions given in Table 2.6

3.1.4.2. Organic Acids

Atomization of B in electrothermal atomic absorption spectrometry (ETAAS) is mostly through molecular dissociation rather than by sublimation of B **(Wiltshire, Bolland, Littlejohn, 1994).** As already mentioned, these molecular compounds are mainly boron oxides, which have high dissociation energies.

Addition of a reducing agent for breaking these high energy boron – oxygen bonds was already studied and provided an enhancement in sensitivity for boron determination **(Matsusaki, Yamaguchi, Nomi, Sata, 1996).**

5.0 µL of 0.05 mol/L solutions of ascorbic, citric, oxalic or tartaric acids were individually co-injected with 15.0 µL of 5.0 mg/L B solution and 5.0 µL of 0.01 mol/L $Ca(NO_3)_2$ as temporary modifier. Variation of absorbance signal after each organic acid use is compared in Figure 3.12.

Using citric acid as a temporary modifier provided about 1.5 fold increase in absorbance signal. The absorbance signal is given in Figure 3.13.

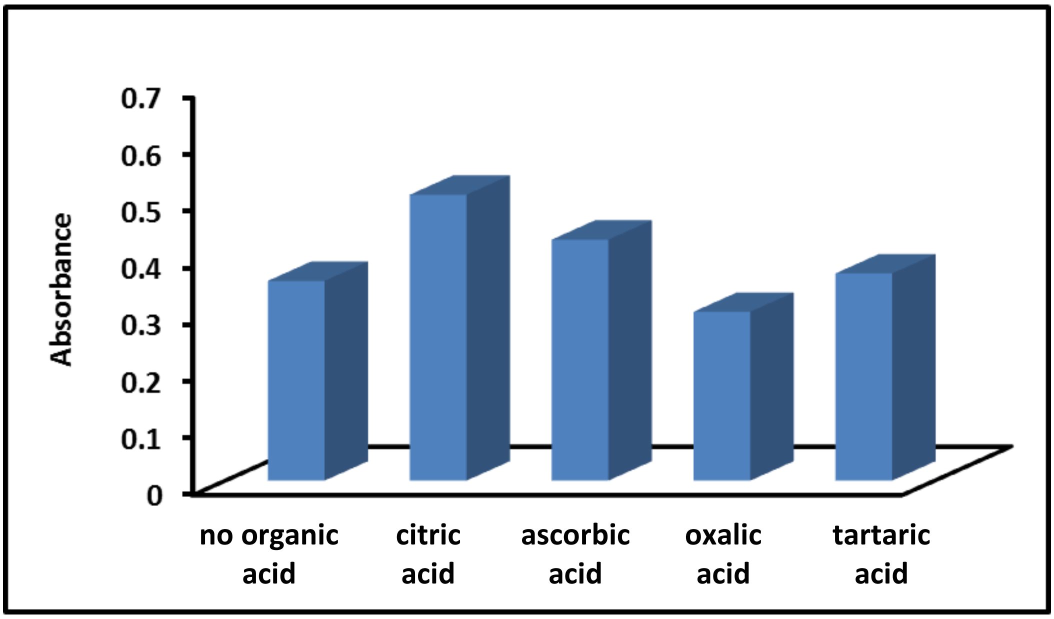


Figure 3. 12 Effect of 5.0 µL 0.05 mol/L each organic acid as temporary modifier on peak height using Ta coated tube and conditions given in Table 2.6

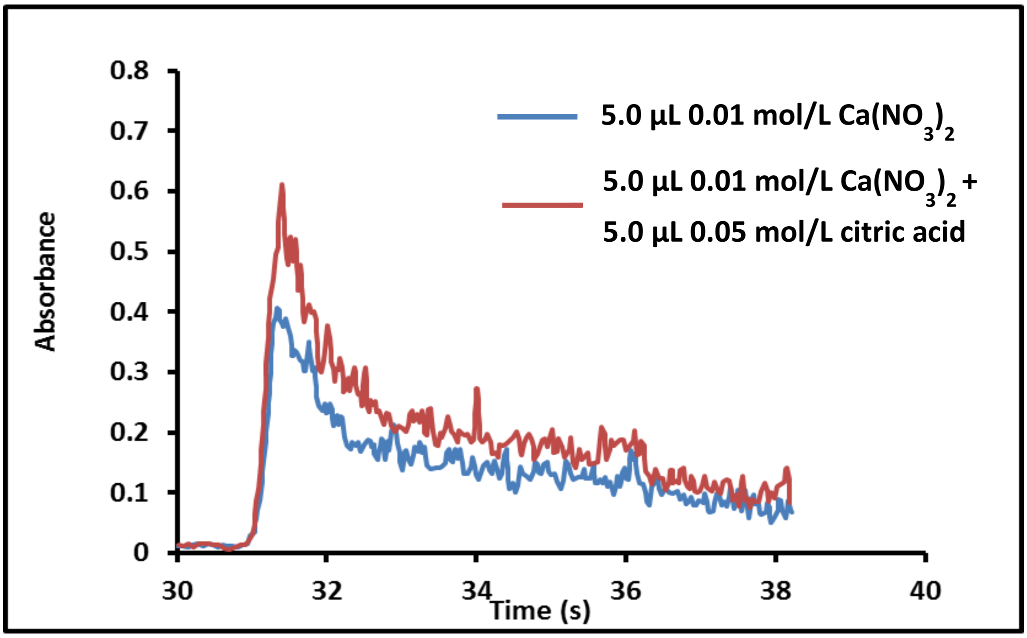


Figure 3. 13 Signal for 15.0 µL 5.0 mg/L B solution with respective temporary modifiers using Ta coated tube and conditions given in Table 2.6

3.1.4.2.1. Optimization of Citric Acid Concentration

Effect of varying citric acid concentration on absorbance signal of 15 µL 5 mg/L B solution was studied. 0.05, 0.10, 0.15 and 0.20 mol/L citric acid solutions were prepared and each was co-injected individually in addition to 5.0 µL 0.01 mol/L $Ca(NO_3)_2$. The volume of citric acid solution was also kept constant at 5.0 µL. Peak height values for each concentration is illustrated in Figure 3.14. Two replicate measurements and a blank were run for each data point. 0.05 mol/L was chosen as the optimum citric acid concentration.

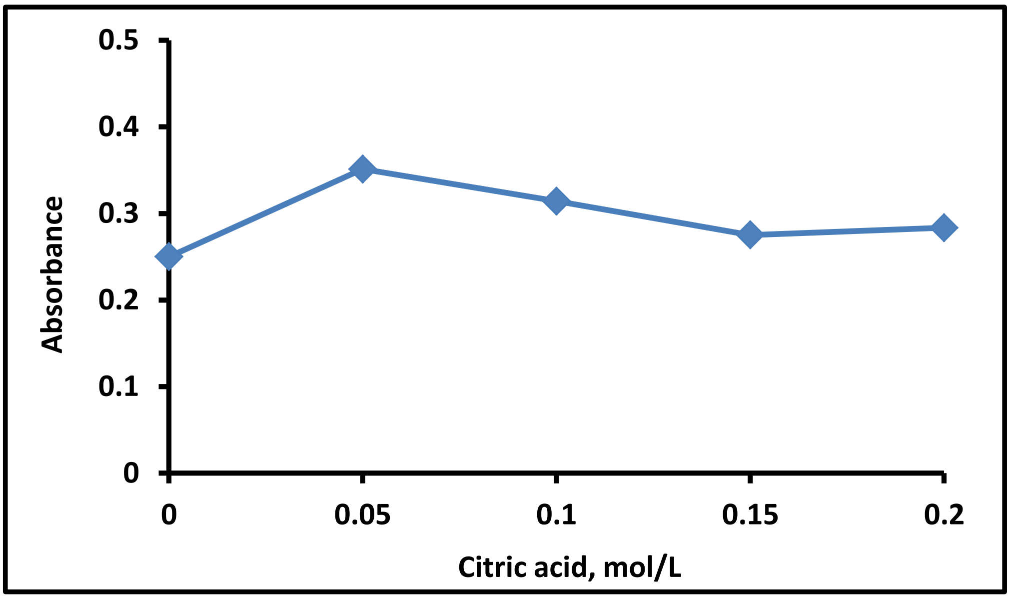


Figure 3. 14 Effect of citric acid concentration on peak height of 15 µL 5 mg/L B solution using Ta coated tube and conditions given in Table 2.6

3.1.5. Metal Nitrate Salt as Medium of Solution

Up to this point, all boron solutions were prepared in 1000 mg/L $Mg(NO_3)_2$. After determining $Ca(NO_3)_2$ as a more effective temporary modifier than $Mg(NO_3)_2$, the idea of replacing the medium of solutions with $Ca(NO_3)_2$ was apparent. 5.0 mg/L boron solutions were prepared in either 1000 mg/L $Mg(NO_3)_2$ or 1000 mg/L $Ca(NO_3)_2$ separately. For both cases 5.0 µL of 0.01 mol/L $Ca(NO_3)_2$ were used as the temporary modifier. The absorbance signals for each case are illustrated in Figure 3.15. Use of 1000 mg/L $Mg(NO_3)_2$ as solution medium was decided owing to higher peak height obtained.

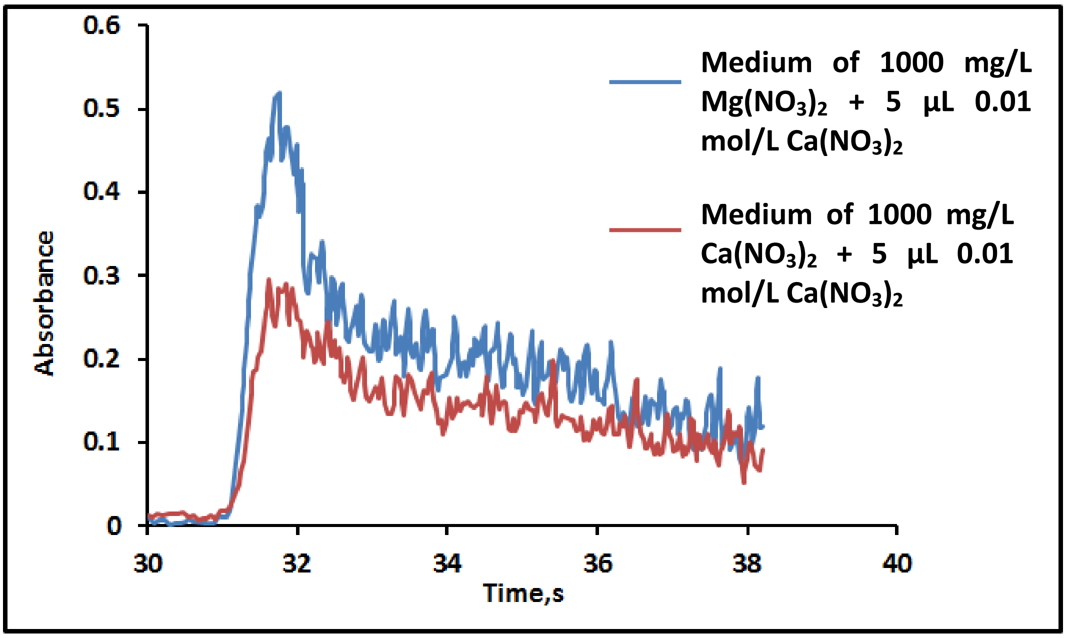


Figure 3. 15 Signals for 15.0 µL 5.0 mg/L B solution in either 1000 mg/L $Mg(NO_3)_2$ or 1000 mg/L $Ca(NO_3)_2$ medium using 5.0 µL 0.01 mol/L $Ca(NO_3)_2$ as temporary modifier, Ta coated tube and conditions given in Table 2.6

3.1.6. Cleaning Cycle

Memory effect is a serious problem for boron determination by ETAAS. Absorbance profiles after several injections of boron solution revealed that the signal tail tended to increase and flatten. Blank analysis was always performed between sample injections to minimize this effect. However, that was not alone enough to get rid of the problem. Blank signal was even getting higher in the course of analysis. It was almost a half of analyte signal as shown in Figure 3.16.

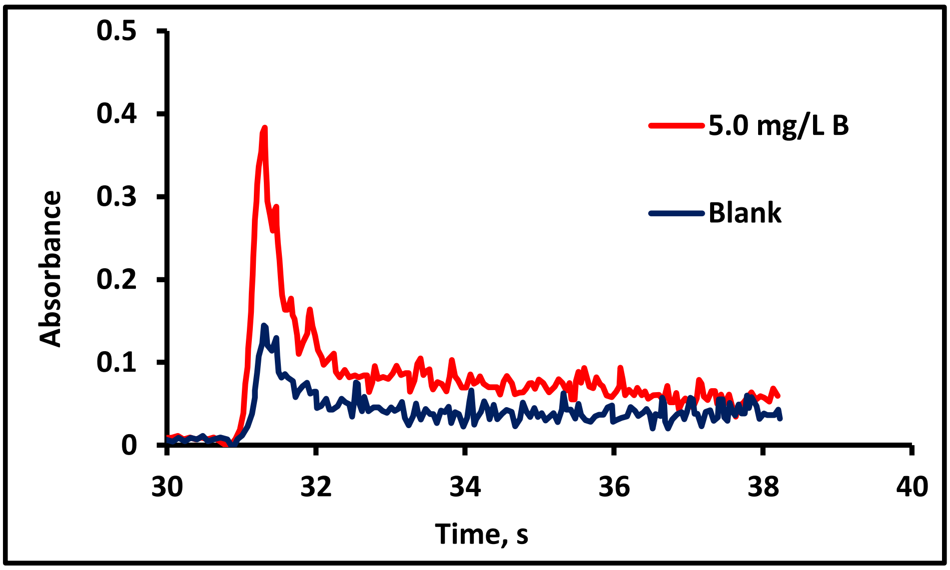


Figure 3. 16 Blank and 15.0 µL 5.0 mg/L B solution signals on W coated tube with $Ca(NO_3)_2$ as temporary modifier using conditions given in Table 2.6 and 3.1

In order to minimize this problem, the effects of some solutions were investigated which were injected during the cleaning cycles. They were expected to form volatile boron compound, boron trifluoride (BF_3). 2.0 g/L of each NH_4F HF and NaF solutions were examined.

Firstly, 30.0 µL of 2g/L NH_4F HF solution were injected in duplicate on a tube with a boron history of 20 firings. The absorbance signal at 2700 °C is shown in Figure 3.17. These signals would indicate that almost all the boron was removed from the tube. However, thereafter 30.0 µL of 2 g/L NaF solution were injected onto same tube in duplicate. The absorbance signal at 2700 °C is shown in Figure 3.18. This proves that NaF solution is effective in liberating boron from the tube surface.

When the graphite tube was replaced and coated with Ta, 2 g/L NaF solution was injected before any boron solution injections. The signal was as shown in Figure 3.19. This signal assures that a new tube brings no boron signal. When Figure 3.19 is compared with Figure 3.18, the severity of memory effect from the tube can be understood more clearly.

To have an atomizer, which is free of memory effects as much as possible, cleaning cycle was applied since the beginning of the method development studies. A cleaning cycle was performed after every second boron injection. A concentration of 10.0 g/L NaF was preferred in 30.0 µL volume for each cycle and the temperature program in Table 2.12 was applied. This procedure assured a longer lifetime for tubes and more reliable data.

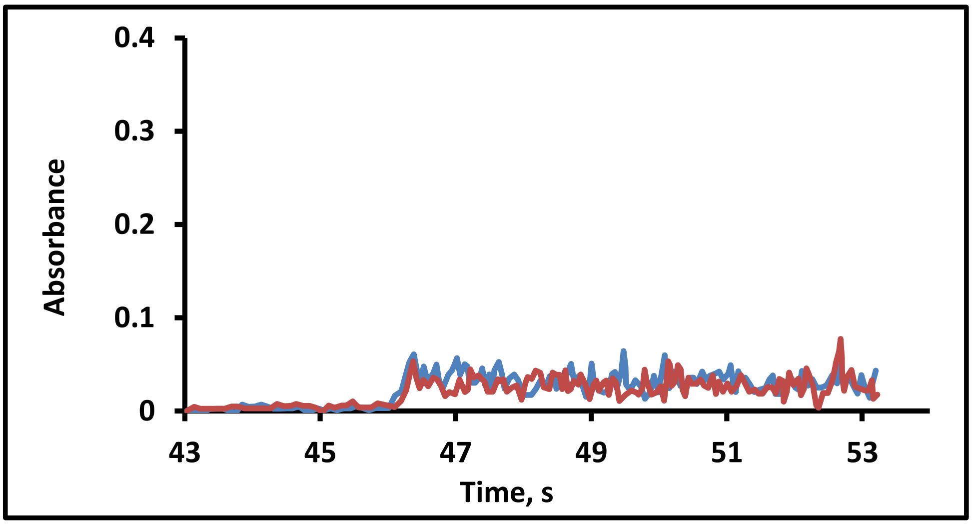


Figure 3. 17 The absorbance signals for duplicate sampling at 2700 °C when 30.0 µL 2.0 g/L NH_4F HF were applied on Ta coated tube with a boron history of 20 firings using conditions given in Table 2.11

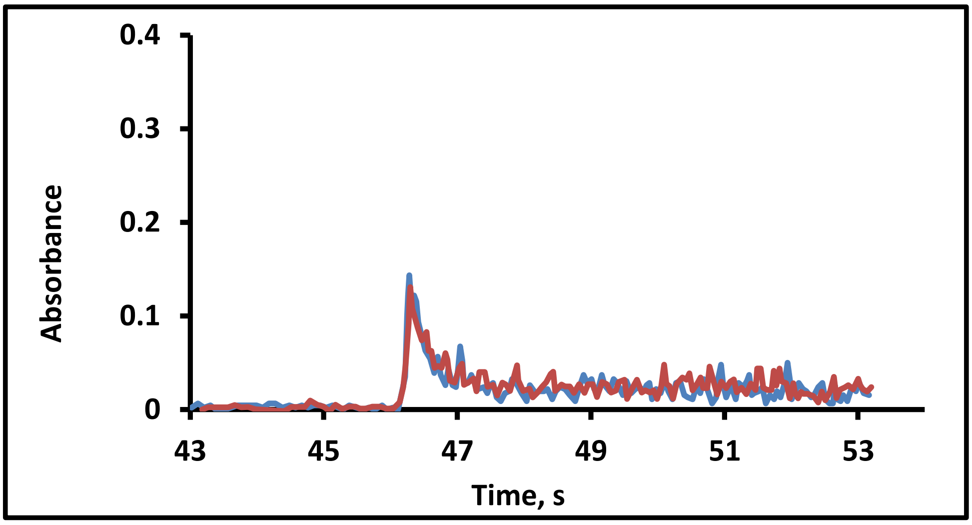


Figure 3. 18 The absorbance signals for duplicate sampling at 2700 °C when 30.0 µL 2.0 g/L NaF were applied on Ta coated tube with a boron history of 20 firings using conditions given in Table 2.11

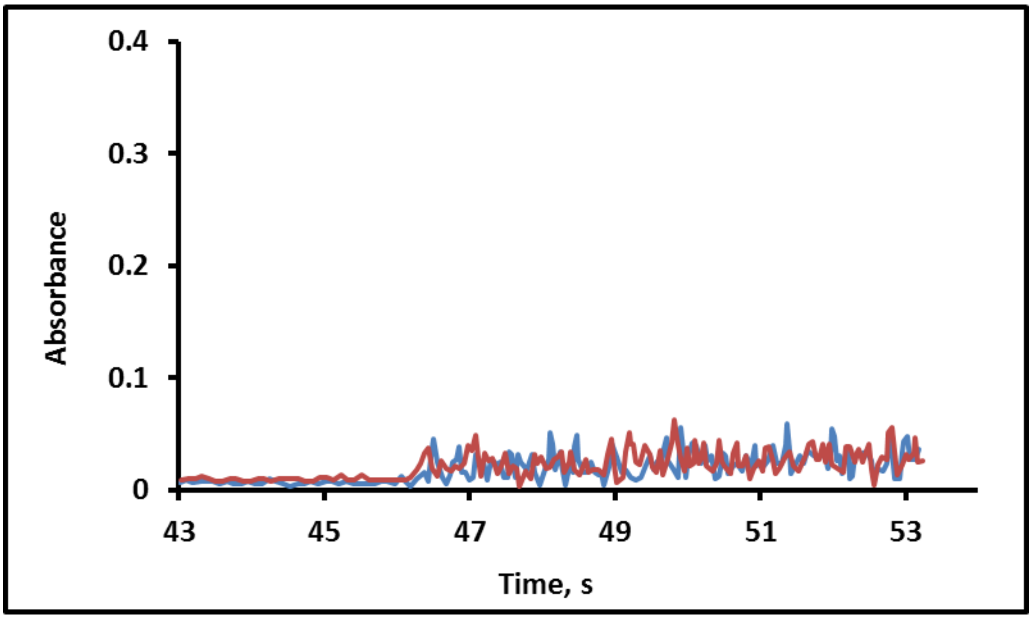


Figure 3. 19 The absorbance signals for duplicate sampling at 2700 °C when 30.0 µL 2.0 g/L NaF were applied on an unused Ta coated tube using conditions given in Table 2.11

3.1.7. The Developed Method

As optimization studies were completed, the method has been shaped. Chemical modification reagents and optimized amount or concentration for each are shown in Table 3.2.

Table 3. 2 Chemical modification for proposed method

Parameter	**Concentration & Amount**
Modifier (as medium of solution)	1000 mg/L $Mg(NO_3)_2$
Permanent modifier (as tube coating reagent)	1000 mg/L Ta solution – 350 µg deposit
Temporary modifier	5.0 µL 0.01 mol/L $Ca(NO_3)_2$
Temporary modifier	5.0 µL 0.05 mol/L Citric acid
Cleaning reagent	30.0 µL 10.0 g/L NaF solution

3.1.8. Calibration Graphs and Analytical Signals

After the optimization studies were completed, analytical signals of sample solutions ranging from 0.5 mg/L to 10.0 mg/L were obtained to determine the linear range and calibration plot for both Ta coated and W coated surfaces.

Despite peak height values were used for simplicity in optimization studies, calibration plot and calculation of analytical figures of merit studies were conducted based on peak area values as they are more reliable. The area under the curve during the atomization stage was calculated by Microsoft Excel 2010. The concerned time interval is between 31.0 to 36.2 seconds of the temperature program as it is shown in Figure 3.20. Data points are the average of two replicate measurements, which are blank corrected. Regarding the linear range both Ta coated and W coated tubes gave a linear range of 0.5 to 5.0 mg/L.

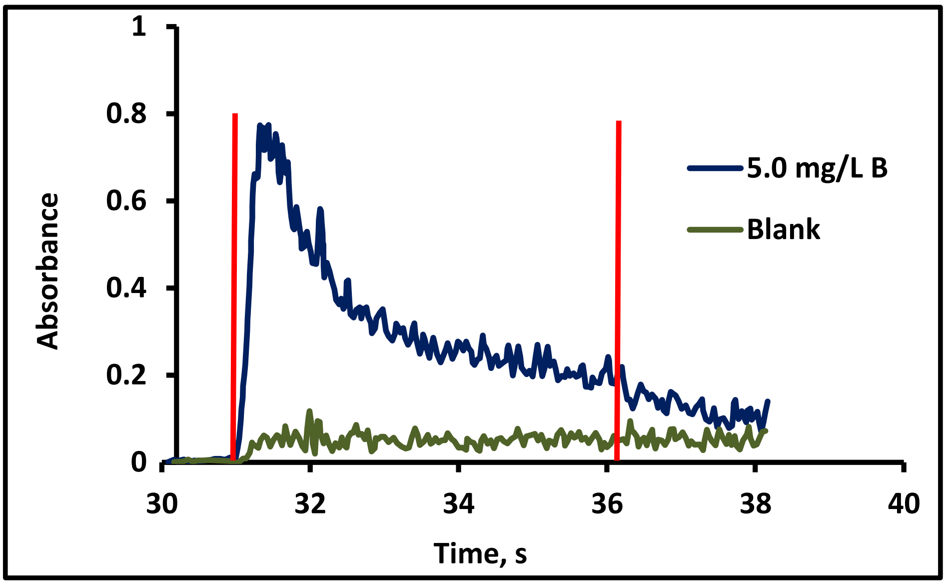


Figure 3. 20 Peak area of concern for the time interval between 31.0 and 36.2 seconds

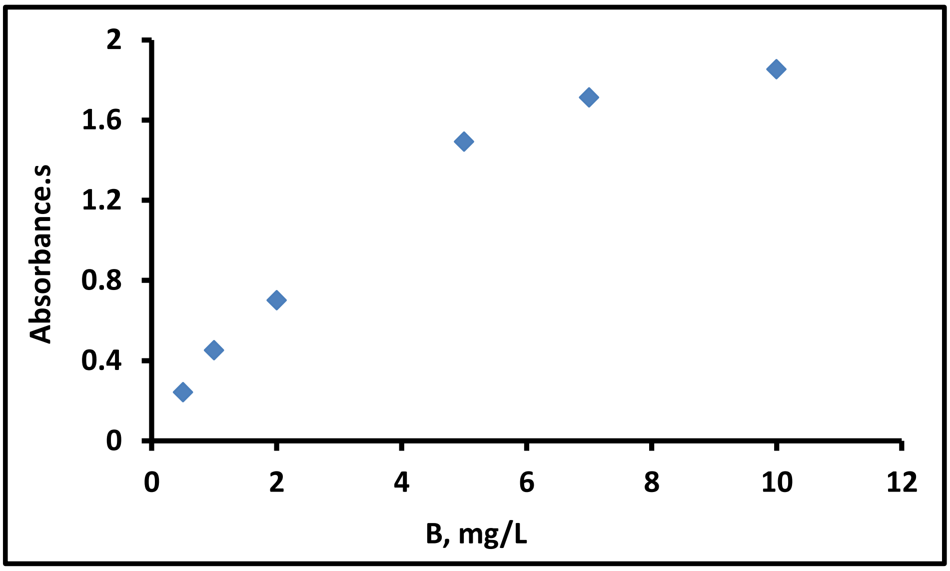


Figure 3. 21 Calibration plot for Ta coated graphite tube using conditions given in Table 2.6. Ashing and atomization temperatures were used 1100 °C and 2700 °C, respectively

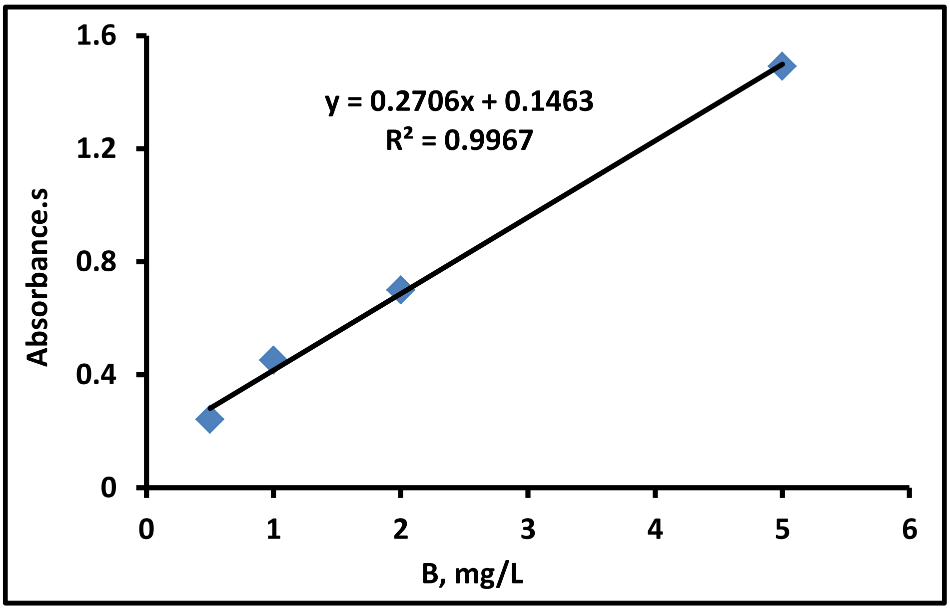


Figure 3. 22 Linear portion of the calibration plot and best line equation for Ta coated graphite tube using conditions given in Table 2.6. Ashing and atomization temperatures were used 1100 °C and 2700 °C, respectively

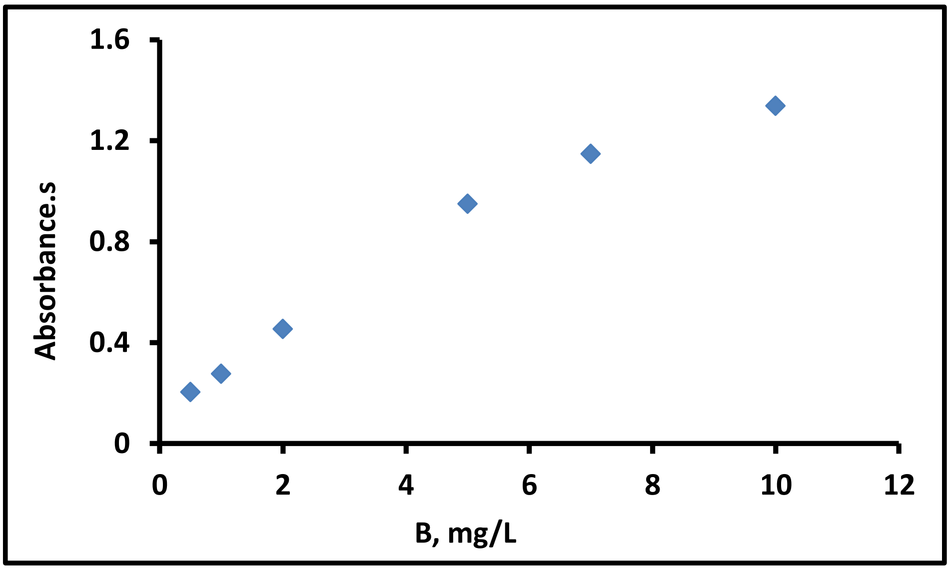


Figure 3. 23 Calibration plot for W coated graphite tube using conditions given in Table 2.6. Ashing and atomization temperatures were used 1000 °C and 2700 °C, respectively

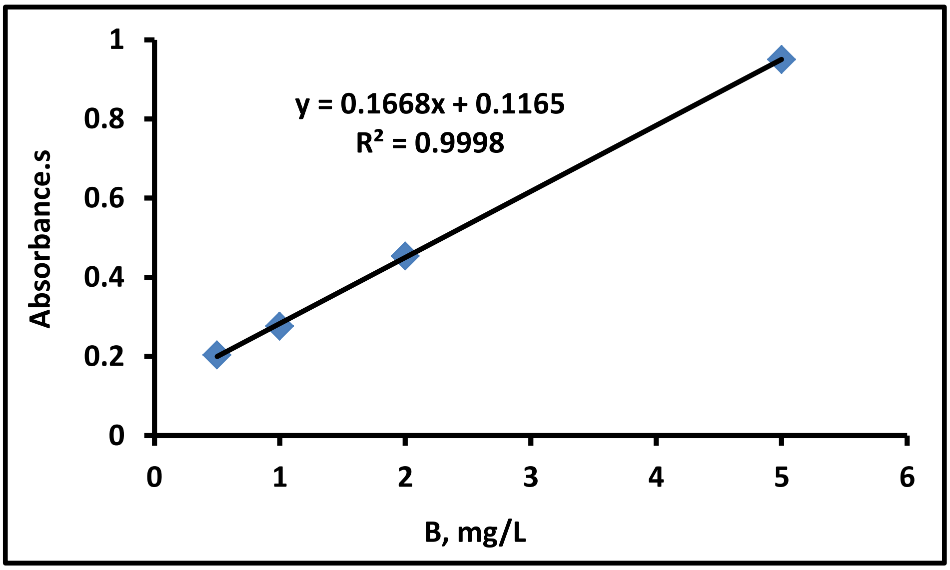


Figure 3. 24 Linear portion of the calibration plot and best line equation for W coated graphite tube using conditions given in Table 2.6. Ashing and atomization temperatures were used 1000 °C and 2700 °C, respectively

3.1.9. Analytical Figures of Merit

Limit of detection (LOD), Limit of quantification (LOQ), characteristic concentration (C_0) and characteristic mass (m_0) for both Ta and W coated tubes were calculated and given in Table 3.3.

LOD and LOQ values were calculated from 3s/m and 10s/m formulas, respectively, where s was the standard deviation of 7 replicate measurements of 0.5 mg/L B solution. Characteristic concentration, (C_0) is the concentration of analyte which produces a peak absorbance of 0.00436 (or 1% absorbance). Characteristic mass, (m_0) is the mass of analyte which produces a peak absorbance of 0.00436 (or 1% absorbance). m_0 is calculated by multiplying C_0 with sample volume.

Table 3. 3 Analytical figures of merit for Ta and W coated graphite tubes using conditions given in Table 2.6 and Table 3.1

	Ta coated graphite tube	**W coated graphite tube**
Limit of Detection, (LOD), mg/L	**0.088**	**0.13**
Limit of Quantification, (LOQ), mg/L	**0.29**	**0.43**
Characteristic Concentration, (C_0), mg/L	**0.012**	**0.019**
Characteristic Mass, (m_0), pg	**186**	**288**
Linear Range, mg/L	**0.5 – 5.0**	**0.5 – 5.0**

3.2. Accuracy Check for the Method

Accuracy of the method was tested by two reference materials, which are EnviroMAT-Waste Water Low EU-L-1 CRM and NIST 1573a Tomato Leaves SRM.

Since waste water sample requires no preparation steps, 1.0 mL was taken and diluted to 10.0 mL. To provide 1000 mg/L $Mg(NO_3)_2$ medium, 10.55 mg of $Mg(NO_3)_2.6H_2O$ were dissolved with addition 0.10 mL of concentrated HNO_3. Direct calibration method was used with three replicates and one blank measurement. Analysis result given in Table 3.4 is very close to the certified value. % RSD value of 0.83 was determined for the analysis. Student t-test was applied at both 95% and 99% confidence levels. It is concluded that there is a significant difference between the values at 95% confidence level. However, null hypothesis at 99% confidence level can be accepted owing to no significant difference between the experimental and certified values.

Table 3. 4 Certified values and analysis result for EU-L-1 Waste Water CRM

Standard Reference Material	Certified Value, mg/L	Analysis Result, mg/L	Confidence Interval, mg/L	Tolerance Interval, mg/L
EU-L-1 Waste Water	25.0 ± 1.0	24.1 ± 0.2	24.0 – 26.0	18.0 – 32.0

0.20 g of NIST 1573a Tomato Leaves SRM was weighed and digested in microwave oven after addition of 5.0 mL concentrated HNO_3 and 2.0 mL H_2O_2. For microwave digestion, program given in Table 2.4 was used. After digestion, some precipitate was observed and therefore the solution was filtered. The analysis result, which is given in Table 3.5, was significantly lower than the certified value. Therefore, standard addition technique was applied in order to control any suppression effect. The result of standard addition technique was almost the same with direct calibration technique. This means that no suppression effect was present but some of the B remained undissolved in the precipitate. Therefore, another digestion method was examined.

Bock **(Bock, 1979)** states that when organic substances are to be dissolved with HNO_3 + H_2O_2, they are usually treated with nitric acid alone to start with, being warmed or evaporated to dryness, before the peroxide is added. This suggestion was coupled with ultrasound pre-treatment step in this study. Firstly, onto 0.20 g of NIST 1573a Tomato Leaves SRM, 5.0 mL of concentrated HNO_3 was added and the mixture was kept in ultrasound water bath for 20 min at room temperature. Then, 2.0 mL of H_2O_2 was added into the mixture and the contents were kept in ultrasound water bath for another 20 min at room temperature. Despite no heating was applied, a temperature increase around 20 °C was observed at the end of the process. After these steps, the mixture was digested in microwave oven using the program given in Table 2.4. Then, it was observed that the entire sample was dissolved with this second digestion method. The digest was kept on hot plate to volatilize acid until the volume was halved. Later, the digest was diluted to 5.0 mL using deionized water. Direct calibration technique was applied for determination of boron concentration. Three replicates and one blank were run. The analysis result, which is shown in Table 3.6, is close to the certified value. % RSD was calculated as 2.50. Student t-test at 95% confidence level shows no significant difference between the values.

Table 3. 5 Analysis results for NIST 1573a Tomato Leaves for direct calibration and standard addition techniques after first digestion method

SRM	Certified Value, mg/kg	Direct Calibration Technique, mg/kg	Standard Addition Technique, mg/kg
NIST 1573a Tomato Leaves	33.3 ± 0.7	20.6 ± 1.0	21.1 ± 2.1

Table 3. 6 Analysis result for NIST 1573a Tomato Leaves after second digestion method

	Certified Value	Analysis Result
B, mg/kg	33.3 ± 0.7	32.0 ± 0.8

3.3. Analysis of Water Samples

3.3.1. Natural Water Samples from Balıkesir, Bigadiç

Bigadiç, which is a district of Balıkesir, has rich boron reserves. The natural water and soil in the region have high levels of boron. Water samples from five different local fountains were collected. Firstly, they were analyzed by ICP-OES for the purpose of comparison of results. Then, the analysis was performed by the developed method for ETAAS. All the results were calculated based on direct calibration method. Two replicates and one blank were run for each sample. The analysis results for the developed method which are given in Table 3.7 are compatible with ICP-OES results. Student t-test at 95% confidence level proves that there is no significant difference between the results of two techniques.

Table 3. 7 Analysis results for drinking water samples from Bigadiç by ICP-OES and ETAAS

Water Samples	ICP-OES, mg/L	ETAAS, mg/L
Olukpınar	4.31 ± 0.21	4.27 ± 0.25
Belediye Önü	4.15 ± 0.20	4.48 ± 0.02
Küçükpınar	9.46 ± 1.43	8.87 ± 0.09
Cumalık	16.91 ± 0.86	16.69 ± 0.47
İnce Memed	5.05 ± 0.37	4.63 ± 0.23

3.3.2. Tap Water Sample from Kütahya, Emet

Kütahya, Emet is another important site of Turkey with rich boron reserves. The local water is rich in boron. A tap water sample running in a petrol station in Emet was analyzed. The analysis result, which is given in Table 3.8 confirms predictions about high content of boron in water. The water sample was also analyzed by ICP-OES and ICP-MS for a comparison study. The results from three different techniques are in good agreement. Student t test was applied at 95% confidence level between the developed method and ICP-OES and ICP-MS methods, separately. The tests assure that there is no significant difference between the results of ETAAS and ICP-OES as well as ETAAS and ICP-MS.

Table 3. 8 Analysis result for tap water sample from Emet

	ETAAS	**ICP-OES**	**ICP-MS**
B, mg/L	19.93 ± 0.21	19.35 ± 0.02	18.38 ± 0.75

CHAPTER 4

CONCLUSION

In this study, the main purpose was the development of a sensitive analytical method for boron determination in water samples by electrothermal atomic absorption spectrometry.

First part mainly deals with chemical modification studies to stabilize boron in the atomizer. Refractory carbide forming elements (Ta, W, Zr, Pd, Ru, Os) were used to prevent boron carbide formation. However, by this way boron determination was not possible. The main problem was loss of analyte at low temperatures. To retain the analyte to higher temperatures, metal nitrate salts were used. Preparing analyte solutions in 1000 mg/L $Mg(NO_3)_2$ could produce analytical signals for the first time. Then use of modified surfaces was studied again and Ta and W coated surfaces were chosen superior to the others. Use of metal nitrate salt helped to increase ashing temperature. Maximum ashing temperatures were determined as 1100 °C and 1000 °C for Ta and W coated tubes, respectively. Optimum atomization temperature was 2700 °C for each surface. Other than permanent modifiers, temporary modifiers were also used in this study. Co-injection of 5.0 µL 0.01 mol/L $Ca(NO_3)_2$ with sample solution provided 1.5 fold increase in absorbance signal. With Mg-Ca as modifier, it is possible that magnesium and calcium oxides were produced by decomposition of nitrate modifiers or alternatively magnesium and/or calcium borate was formed. These oxides and borates could have blocked the active carbon sites until vaporization or dissociative desorption of MgO and CaO occurred at around 1200 °C. Vaporization of boron oxide would then be possible.

Another temporary modifier was organic acids. As atomization of boron is mostly through vaporization of boron oxide species, organic acids were utilized as reducing agents to break high energy boron-oxygen bonds. Addition of 5.0 µL 0.05 mol/L citric acid as temporary modifier enhanced the absorbance signal another 1.5 fold.

Despite coating the tube with carbide forming elements and using other modifiers, memory effect was an obvious problem. One formed, B atoms have high affinity for graphite surface and they recondense, which cause a long tail in the signal. Blank signals were high and as many blanks were run successively to eliminate memory effect, the tube life time was short. To remove boron on the tube as volatile boron trifluoride, 10.0 g/L NaF was used as cleaning agent. After every two solution injections, 30.0 µL of NaF solution were applied following a specific temperature programme. This practice not only suppressed blank signals but also extended the tube life time. Number of tube firing was increased almost three times from forty to around one hundred-twenty firings.

Then the method was released and the analytical figures of merit were evaluated. LOD values of 0.088 mg/L and 0.13 mg/L and characteristic masses of 186 pg and 288 pg were calculated for Ta and W coated surfaces, respectively.

In the second part, accuracy of the method was confirmed by EnviroMAT-Waste Water EU-L-1 CRM and NIST 1573a Tomato Leaves SRM analyses. Results for B were in good agreement with certified values. Moreover, student t-test showed no significant difference at 99% CL for EnviroMAT-Waste Water CRM and 95% CL for NIST 1573a Tomato Leaves SRM analyses.

Lastly, developed method was applied for determination of boron in water samples from Balıkesir, Bigadiç and Kütahya, Emet. Natural water samples from Bigadiç were analyzed by both the developed method and ICP-OES technique for a comparison study. The compatibility of experimental means were assured by performing student t-test at 95% CL. Tap water sample from Emet was analyzed by three of the techniques, ETAAS, ICP-OES and ICP-MS. The t-test at 95% CL proves that there is no significant difference between the results from ETAAS and ICP-OES as well as ETAAS and ICP-MS.

After the analysis, it was confirmed that boron in water samples from the regions with boron reserves are significantly high. As the developed method by ETAAS is in good agreement in results with more sensitive techniques ICP-OES and ICP-MS, it is concluded that determination of boron in water samples by ETAAS can be performed successfully. The method is advantageous not only because its simplicity but also economical meaning.

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