***Original Research Article***

# Optimization of curcumin method and Usage of natural curcumin in determination of boron in steel

## ABSTRACT

**Boron containing alloy steels are mainly used in constructional, automotive and many other applications. The presence of even trace amounts of boron affects greatly on the properties of steel such as hardness and machinability. Therefore, it is important to determine it accurately and precisely in the manufacture of these alloys. Due to the drawbacks of common advanced techniques such as Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES), the universally accepted method for determination of boron in steel is the spectrophotometric method using curcumin as an auxiliary agent.**

**Although curcumin is the most sensitive spectrophotometric reagent for the above analysis, synthetic curcumin is relatively expensive and it tends to degrade rapidly once it is exposed to air. Furthermore this method is time consuming. Therefore, this study was carried out in order to improve and optimize the available spectroscopic method and to investigate the possibility of using natural curcumin extracted from turmeric in the above analysis.**

**The results of the study implied that natural curcumin extracted and purified from turmeric can be used as accurately and precisely as synthetic curcumin in the analysis of boron in steel. A boron-steel alloy sample found to be containing 0.26±0.01 percent of boron with synthetic curcumin gave a concentration of 0.25±0.00 percent of boron with natural curcumin, indicating no significant difference in the determination. A steel sample found to be containing 0.27±0.01 percent of boron with filter paper ashing step gave a concentration of 0.26±0.01 percent of boron without filter paper ashing step indicating no significant difference. Altering the refluxing step of current procedure significantly changes the concentration of boron indicating that the refluxing step cannot be optimized further.**

*Keywords: [*boron, steel, curcumin, turmeric

## 1. INTRODUCTION

Even the presence of trace amounts of boron affects greatly on the properties of steel. The major function of boron is to increase the hardness of steel. It gives a better machinability compared with boron-free steel with same hardness.[1]. Boron-added steel alloys are less susceptible towards distortion and quench cracking under thermal treatments. They are mainly used in construction, automotive and many other applications. Boron containing steel alloys are cheaper than alloys with any other element. It is also reported that it is easy to work with boron-added steel because the addition of other elements such as Ni, Cr, Mo makes it difficult to work with.

To maintain the desired hardness levels usually boron is added combined with strong nitride forming elements like Al, Zr and Ti. The presence of excess amounts of boron reduces the toughness and hardness. [2].

As the trace amounts of boron in steel affects greatly on the properties of steel, it is essential to determine it very accurately and precisely. Researchers have tried several techniques in order to determine the amounts of boron present in steel. Some of those techniques are Atomic Absorption

Spectrometry (AAS), Inductively-coupled plasma Atomic Emission Spectrometry (ICP-AES), Spectrophotometric techniques etc.

Boron shows very low sensitivity for AAS. Its characteristic concentration is very high even when the effective $N_2O$-$C_2H_2$ flame atomizer is used. This may be due to the low atomization efficiency of boron. It is also very difficult to determine boron in a graphite tube furnace due to its ability of carbide formation. [3].

Many studies have been carried out using ICP-AES for determination of boron in steel. This can be performed for boron either directly in solution which is produced by dissolution of the steel sample or for boron separated from the matrix. Direct analysis involves errors and low accuracy due to the presence of other elements in steel.[4]. Use of ICP could solve the low efficiency of atomization which is associated with AAS. Due to the strong atomic emission compared with the primary source, the function of hollow cathode lamp is deteriorated.[3]. Some ICP-AES studies have been done by volatilization of boron as methyl borate. But the vapor cannot be introduced directly to the plasma because methanol vapor quenches the plasma.

Due to all these drawbacks and failures of the above mentioned techniques as well as the other available techniques, the worldwide accepted most sensitive and most accurate method for determination of boron in steel is the spectrophotometric method using curcumin.
Spectrophotometric analysis of boron is normally carried out using curcumin, carmine, methylene blue, 1-(salicylideneamino)-8-hydroxynaphthalene disulphonic acid etc.[5] Among these spectrophotometric reagents, curcumin is the most sensitive one for determination of boron in steel.

The detailed procedure according to the American Society for Testing and Materials (ASTM)[5] is described under methodology. This procedure mainly contains three steps. They are dissolution of the steel sample, separation of boron to remove interferences, color development process.

Steel samples are dissolved by refluxing in a sulfuric acid solution. It takes about one hour. Then having filtered the solution it includes two filter paper ashing steps at 400 °C and 600 °C. This takes a long time as well as it needs a lot of cost. Due to the interferences of other elements boron is separated before determination. These separation methods include distillation as methyl borate8, isolation of boron as boric acid by pyrohydrolysis, removal of iron by precipitation or extraction. Among the above methods distillation as methyl borate is the accepted and most accurate method. Though the distillation is more time consuming than other techniques it is applicable for all ranges of boron. It is free from interfering components. It takes a minimum of about three hours for complete distillation.

In the color development process, the distilled sample is reacted with curcumin. Curcumin forms a red colored rosocyanine complex with boric acid in the presence of acid (Figure 1). The ratio of curcumin to boric acid is 2:1 in this complex. [6].

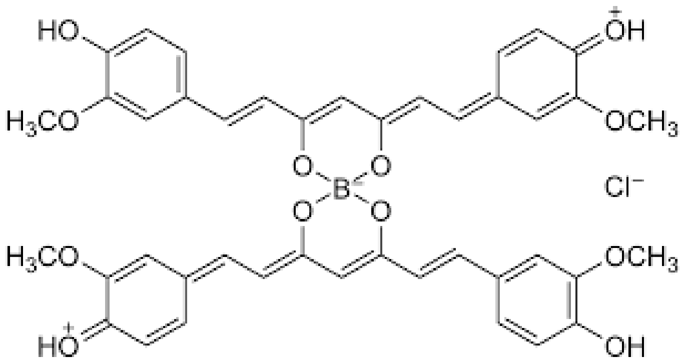

Figure 1: Rosocyanine complex

Though curcumin is the most sensitive spectrophotometric reagent for determination of boron in steel, commercially synthesized curcumin is relatively expensive and once it is exposed to air it oxidizes rapidly. Therefore, as the second part of this study, the possibility of using natural curcumin extracted from turmeric was investigated.

Curcumin is a major phytochemical found in turmeric (*Curcuma longa*).(Figure 2). Curcumin and the related compounds demethoxycurcumin (DMC) and bis-demethoxycurcumin (BDMC) are altogether known as curcuminoids. [7]The most commonly employed method for extraction of curcuminoids is the solvent extraction followed by column chromatography. Several organic solvents are used in the extraction process such as acetone, ethanol, hexane, ethyl acetate. [8]. In this study acetone was used as the solvent for extraction.

Figure 2: Turmeric(*Curcuma longa*)

## 2. MATERIAL AND METHODS

### 2.1Reagents:

Aqueous Sulfuric acid solution (1:4)
Acetone-water solution (1:1)
Sulfuric acid-acetic acid solution (1:1)
Sodium hydroxide solution (20 g/L) in $H_2O$
Hydrogen peroxide solution (1:4)
Curcumin solution (1.25 g/L) in $CH_3COOH$
Boron standard solution (100 ppm) in $H_2O$

### 2.2Current procedure for determination of Boron in steel (ASTM E350-18)

Specimen (2.50 ± 0.01 g of steel) was placed in a round bottom flask. $H_2SO_4$ (50.0 mL) was added and then reflux condenser was inserted. Then it was heated below boiling for 30 minutes. $H_2O_2$ (4.00 mL) was added and heating was continued until dissolution was completed. Solution was cooled. A filter paper was treated with methanol and then with water. Washings were discarded. Then the solution was filtered into a 100-mL volumetric flask. Filter paper was washed with $H_2SO_4$ solution ten times keeping the total volume in the flask approximately 50 mL. Filtrate was reserved.

$Na_2CO_3$ (0.20 ± 0.01 g) was sprinkled over the paper and it was transferred into a crucible. Then the paper was dried and heated up to 400 $^{o}$C until carbon was removed. After it was cooled and $Na_2CO_3$ (1.00 g) was added and fused at 600 $^{o}$C for 25 minutes and then it was cooled.

$H_2SO_4$ solution was added drop wise to the fused mass until dissolution was completed, keeping the crucible covered during additions. Then this solution was transferred to the reserved filtrate rinsing the crucible with $H_2SO_4$ solution into the volumetric flask. It was diluted to volume with $H_2SO_4$ solution and mixed.
Aliquot from the above prepared solution (5.00 mL) was transferred into a round bottom distillation flask. Flask was connected to the condenser and methanol (30.00 mL) was added through the methanol addition tube. Distillation flask was placed in a water bath and an evaporating dish

containing NaOH (5.00 mL) was placed at the end of the condenser so that deliver tip was immersed in NaOH.(Figure 3).

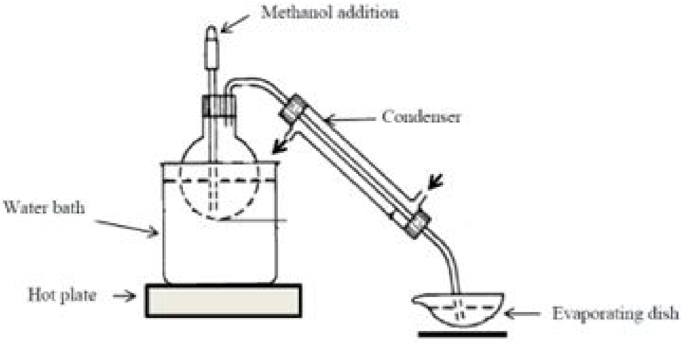

Figure 3: Distillation apparatus for boron in steel

Water bath was heated to 75 ± 2 $^{o}$C and solution was distilled until no more than 5 mL of solution remained in the distillation flask. Then the evaporating dish was placed in a water bath and evaporated just to dryness.

### Color development process

Curcumin solution (3.00 mL) was transferred into the evaporating dish and it was swirled to dissolve the residue. Then sulfuric-acetic acid solution (3.00 mL) was added and mixed. The solution was allowed to stand for 15 minutes. Then acetone-water solution (30.0 mL) was added and then the content was transferred into a 100-mL volumetric flask. Then it was diluted to the volume and mixed.

Absorbance was measured at 555 nm with reference to acetone-water solution.

### Optimization of the method

In the optimization studies several parameters and conditions were changed, and the performance of the method was tested. The altered parameters and steps are shown in the Table 1.

The optimized method was validated using boron standards.

## 2.3 Extraction and separation of curcuminoids from turmeric

### Extraction of curcuminoids from turmeric

Dried powder of turmeric, *Curcuma longa* (25.10±0.01 g) was extracted with acetone (150.0 mL) using Soxhlet apparatus for 8 hours. After completion of extraction, the brown color extract was cooled. Then it was concentrated using rotary evaporator.

### Separation of curcuminoids using column chromatography

The extracted crude (3.20±0.01 g) was dissolved in dichloromethane (10.0 mL) and subjected to column chromatography in silica gel glass column. Then the loaded sample was eluted using a polarity gradient solvent system using hexane and dichloromethane. Fractions (20.0 mL) were collected. Same colored fractions were mixed together and concentrated using rotary evaporator.

Purity of the separated curcumin was determined using FTIR analyses.

Finally, the possibility of using natural curcumin in the analysis of boron in steel was evaluated.

## 2.4 Analysis of Boron in steel using Atomic Absorption Spectroscopy (AAS)

According to the spectrophotometric method, boron concentration of steel samples used in the analysis ranged between 2 ppm to 3 ppm. Therefore a boron solution (2 ppm) was prepared using boron standard (1000 ppm). Then it was measured using AAS under the conditions below,
Lamp current: 20.0 mA
Flame type: Nitrous oxide-acetylene
Wave length: 249.8 nm
Slit width: 0.2 nm
Working range: 340 – 1400 ppm

## 3. RESULTS AND DISCUSSION

### 3.1 Alterations of the ashing temperatures

Percentages of boron in two different steel samples (A & B) under different ashing temperatures were analyzed.

Table 1 (a) Different ashing temperatures used on A & B for Boron analysis.

| Sample | 1st ashing temperature ($^oC$) | 2nd ashing temperature ($^oC$) |
|---|---|---|
| A | 400 | 400 |
| B | 400 | 600 |

(b) Amount of boron according to the above analyses.

| Sample A(% of boron) | Sample B(% of boron) |
|---|---|
| 0.26 ± 0.00 | 0.27 ± 0.01 |

Under the first step of optimization, filter paper ashing temperatures were altered as given in the Table 1: (a). Usually, steel samples are dissolved completely in an acidic solution and then it is filtered using filter papers to remove undissolved matter. In order to obtain a high recovery, the filter paper is ashed and dissolved in the same solution. Initially it is heated up to 400 $^oC$ in order to remove carbon and then it is ashed at 600 $^oC$. In this study the secondashing temperature was reduced to 400 $^oC$ and the rest of the procedure was continued in the same way.

According to the statistical analysis, two means are not significantly different. This indicated that the second filter paper ashing step can be altered without any effect to the recovery of boron. In here, it is assumed that boron is distributed evenly throughout the steel rod. Method blanks were done in order to determine whether there is any addition of boron from glassware and other chemicals used.

### 3.2 Analysis carried out without the filter paper ashing step.

Table 2: Calculated boron percentages with and without filter paper ashing step

| % of boron with filter paper ashing | % of boron without filter paper ashing |
|---|---|
| 0.27 ± 0.01 | 0.26 ± 0.01 |

Final result is includes as mean±standard deviation.

The two filter paper ashing steps of the current procedure are very time consuming and experimental cost is very high. According to the statistical calculations, the two means are not statistically different indicating that curcumin method can be performed without two filter paper ashing steps.
Then the main time-consuming step is the distillation process of boron as methyl borates. It takes about three hours for complete distillation using the apparatus shown in Figure 3. This step is essential in order to remove interferences from other metallic and non-metallic substances present in steel. Therefore, researchers are searching for ways to reduce the distillation time.
In this study as an alteration the distillation process was tried using the rotary evaporator instead of the above-mentioned apparatus. Obtained results are shown below.

### 3.3 Reduction of distillation time

Table 3: Calculated boron percentages under normal distillation time and reduced distillation time (without filter paper ashing)

| % of boron under normal distillation time | % of boron under reduced distillation time |
|---|---|
| 0.27 ± 0.01 | 0.20 ± 0.02 |

After the steel sample is dissolved, Boron is extracted by distillation as methyl borates. Methyl borate is volatile. Therefore, the distillate was collected into a NaOH solution (0.5M, 5.0 mL). Because with NaOH it forms sodium borate and it is non-volatile.

According to the calculations, two data sets are significantly different from each other. Recovery of boron with reduced distillation time is very low when compared with that of normal distillation time.

According to previous studies, the principal factors affecting the reproducibility of method are reaction temperature, reaction time, rate of evaporation, prevailing humidity conditions and loss of boron as volatile methyl borate.[9]. Prolong heating and bath temperatures above 80 $^{o}$C would lead to loss of boron.

According to all the statistical calculations and results obtained, current curcumin method for determination of boron in steel can be performed without two filter paper ashing steps followed by distillation using the apparatus for three hours to obtain a same accuracy and efficiency. Optimized curcumin method is less time consuming and less laborious, and also experimental cost can be reduced as two filter paper ashing steps are no longer needed.

Above optimized method was validated using standard boron solutions.

### 3.4 Extraction and separation of curcumin from turmeric

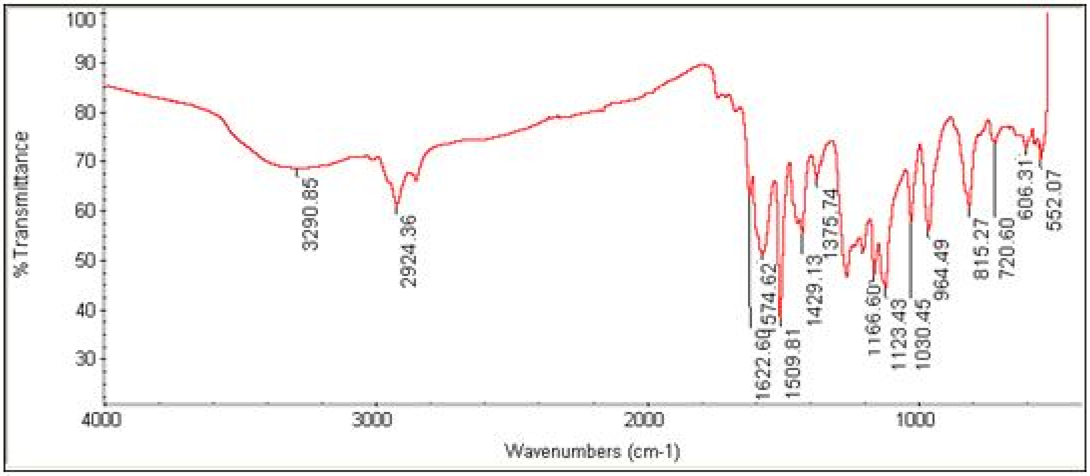

Figure 4: FT-IR spectrum for extracted crude from turmeric.

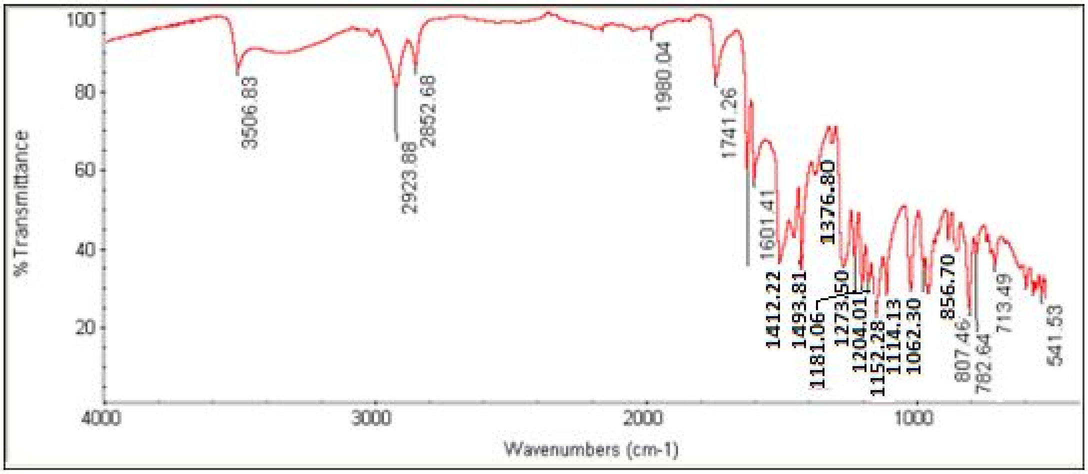

Figure 5: FT-IR spectrum for curcumin separated from turmeric.

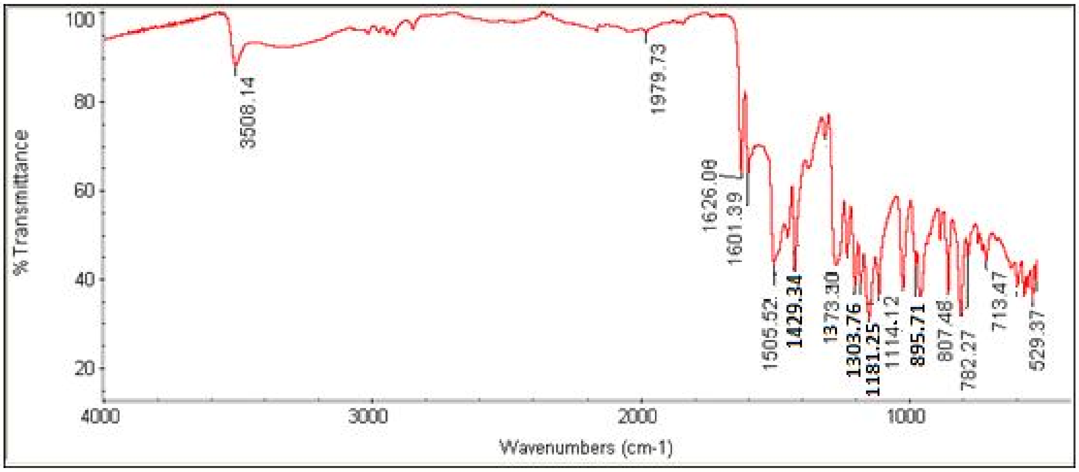

Figure 6.: FT-IR spectrum for commercially synthesized curcumin.

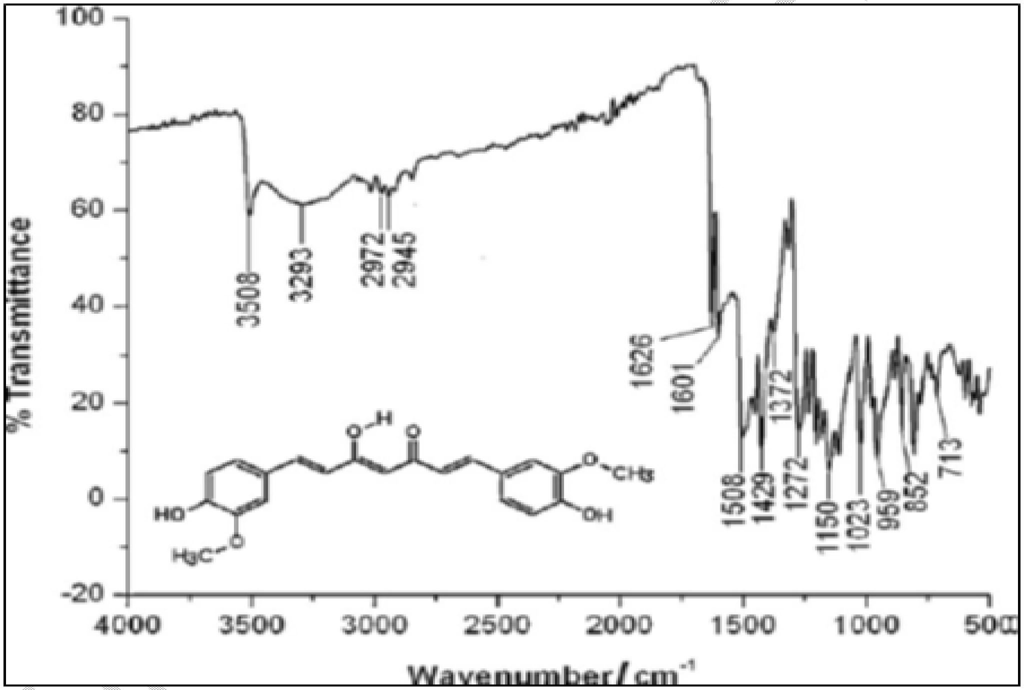

Figure 7: FT-IR spectrum reported for standard curcumin. [10]

According to the FTIR analyses spectra of commercially synthesized curcumin and standard curcumin are identical to each other. And the spectrum of curcumin extracted and separated from turmeric is more or less similar to the spectrum of standard curcumin. But it shows an additional peak at 1741 $cm^{-1}$ and the intensity of the peaks at 2923 $cm^{-1}$ and 2852 $cm^{-1}$ is higher than that of standard curcumin. Peak at 2923 $cm^{-1}$ is due to C-H asymmetric/symmetric stretching [7,13]. and the peak at 1741 $cm^{-1}$ is due to an unknown interference.

Turmeric contains lot of carbohydrates, essential oils, resins, fiber and etc. So when extracted in to acetone other substances may also be extracted together with curcuminoids. And the three curcuminoids (curcumin, demethoxycurcumin and bis-demethoxycurcumin) are almost identical with slight variations of the structure. Curcumin shows a diketone structure and it may undergo keto-enol tautomerization under the conditions given in the laboratory such as temperature and the solvent systems used. Above variations of the FTIR spectrum may be due to these factors.

### 3.5 Determination of the possibility of using extracted curcumin in the analysis

Table 4: Calculated percentages of boron using synthetic curcumin and curcumin extracted from turmeric

| % of boron using synthetic curcumin | % of boron using natural curcumin |
|---|---|
| 0.26 ± 0.01 | 0.25 ± 0.00 |

Usually commercially available curcumin is a mixture of three curcuminoids. In this study the commercial sample used as from Acros-organics TM and it contains ≥ 98% curcumin. Other curcumin sample was extracted and separated from turmeric. According to the statistical calculations, the means of two data sets are not significantly different.

Above study proves that curcumin extracted from turmeric can be used as efficiently and accurately as commercially synthesized curcumin in the analysis of boron in steel.

For large scale industrial determination of boron, extraction and separation of curcumin from turmeric is cost effective than purchasing of synthetic curcumin. The long-term storage of commercially available curcumin is difficult because once the container is opened it oxidizes rapidly. Storage temperature of curcumin is -20 °C and temperature fluctuations cause the degradation of curcumin. Therefore, for routine analyses, it is cost effective to extract curcumin when it is needed rather than purchasing a large amount at once.

### 3.6 Determination of boron in steel using AAS

As the results implied the steel samples used for this study contain boron in the range of 2 to 3 ppm. But the working range of the AAS (GBS Savanta) for boron at 249.8 nm is 340-1400 ppm. The sensitivity of the instrument for boron is 7.5 ppm. Therefore, boron present in the studied steel samples cannot be detected using AAS.

Therefore, it is confirmed that the spectrophotometric method is more sensitive and accurate than the AAS method in determination of boron in steel.

## 4. CONCLUSION

The findings of the study implies that the currently available spectrophotometric method in determination of boron in steel using curcumin can be performed without the two filter paper ashing steps to obtain same results with no significant difference with the results obtained with the two filter paper ashing steps. Therefore, as hypothesized, the current method can be optimized so that it would be less time consuming.

Natural curcumin extracted and separated from turmeric can be used as synthetic curcumin without any significant effect on the accuracy of the analysis.

However, other conditions of the method cannot be improved further and current conditions are found to be optimum.

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