

## COPPER DETERMINATION USING ABTS AND GUAIACOL

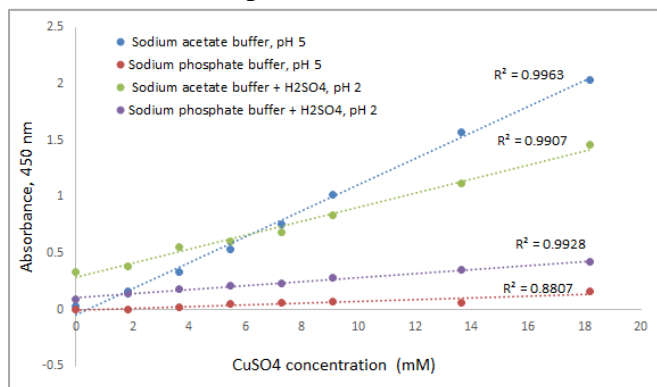
In order to remove metals and treat the waste mining waters, different detection methods of metals are developed. Uv-vis spectrophotometry is still an important instrumental method for the determination of heavy metal ions in their inorganic or organic complexes. Various spectrophotometric methods have been proposed for the determination of copper contents of the various samples including natural waters, and mining waters.

In this report, results obtained from two spectrophotometric methods for copper detection, in synthetic water, using ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and guaiacol reactive solutions are described.

### DETECTION OF COOPER USING ABTS AND GUAIACOL REAGENTS

Experiments regarding the detection of copper ions, from copper sulfate solution, using two different reagents as ABTS and guaiacol, were performed. These methods are developed in order to determine the metal concentration from natural mining waters and different other samples, as well as recovery of copper and its further applications. The spectrophotometric methods create a way to determine metal concentration even in living cells or organisms and offer the possibility to find the easiest procedures of recovery and remediation of waste mining waters.

We performed experiments of copper detection using ABTS and guaiacol reactive solutions, in two types of buffers and two different pHs.

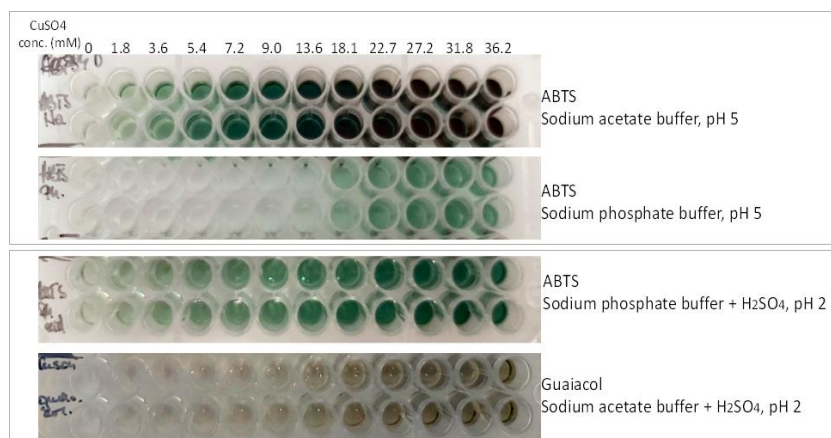


**Figure 1.** Log-linear plots of absorbance (450 nm) versus CuSO<sub>4</sub> solution concentration (mM) on ABTS and hydrogen peroxide reagents in presence of sodium acetate and sodium phosphate buffers (pH 2.0 and 5.0). The experiment was performed in duplicate and repeated two times.

Calibration curves with known concentrations of copper were realized. It was started from a copper sulfate solution prepared in distilled water. The reaction was performed in microtiter plates at two different pH (2.0 and 5.0), with ABTS (Figure 1, Figure 2). In Figure 1 it can be observed

that the lowest detected concentration of copper by ABTS in sodium acetate buffer pH 5.0 is lower than 1.8 mM, with the linear response with log (concentration) between 0-18 mM.

The reaction occurred is faster where the concentration of copper is higher, therefore the absorbance of ABTS reduced is higher. The linear response with log (concentration) between 0-18 Mm is available for all experiments performed for copper detection, using ABTS.



**Figure 2.** Colorimetric detection overview of copper, in sodium acetate and sodium phosphate buffer (pH 2.0 and 5.0) using ABTS and guaiacol reagents

In sodium acetate buffer pH 2.0, the reaction works in negative control as well (data not shown). Thus, at pH 2.0 the results even though show an increase of absorbance with increasing copper concentration, are not valid, and the method cannot be considered for copper detection. When the sodium phosphate buffer was used, at pH 5.0 and 2.0, the values of the slopes are smaller than the slopes obtained for sodium acetate buffer. These results signify that the reactions are slow and the method is less sensitive. The ABTS radicals may be neutralized either by direct reduction via electron transfers or by radical quenching via hydrogen atom transfer (an acid medium facilitates the mechanism of electron transfer) [1]. Thermodynamically, a compound can reduce ABTS if it has a redox potential lower than ABTS (+0.68V). The redox potential of  $\text{Cu}^{2+}$  is +0.34 V [2]. In some studies, was shown that an increase in the amount of copper sulfate in the reacting solution, increases the rate of decomposition of hydrogen peroxide solution using phosphate buffer.

The detection of copper was realized using guaiacol solution as well. Guaiacol is a phenolic compound with a methoxy group. Starting from the fact that guaiacol is oxidized by peroxidase (an enzyme that is dependent on iron or copper) in presence of hydrogen peroxide and this reaction is the basis of a widely colorimetric assay, we introduced this compound for the determination of copper [3]. The detection limit of  $\text{Cu}^{2+}$  was around 7 mM when guaiacol was used. A brown compound was formed, the intensity of color increasing with the increase of copper concentration. A summarized view regarding detection limits of copper for the methods used is shown in Table 1.

**Table 1.** Detection limits of copper ions ( $\text{Cu}^{2+}$ ) starting from ( $\text{CuSO}_4$ ) in sodium acetate and sodium phosphate (pH 2.0 and 5.0) using ABTS and guaiacol reagents.

Ions	Added as	Reactive solution	Buffer solution	pH	Detection limit (mM)
$\text{Cu}^{2+}$	$\text{CuSO}_4$	ABTS	Sodium acetate	5.00	<1
$\text{Cu}^{2+}$	$\text{CuSO}_4$	ABTS	Sodium phosphate	5.00	18
$\text{Cu}^{2+}$	$\text{CuSO}_4$	ABTS	Sodium phosphate + $\text{H}_2\text{SO}_4$	2.00	2
$\text{Cu}^{2+}$	$\text{CuSO}_4$	guaiacol	Sodium acetate	5.00	7

These experiments will be improved, in order to be accurate in the discrimination between copper and other metal ions from mining waters.

## CONCLUSIONS

The preliminary results show that ABTS and guaiacol can be successfully used for copper detection at different pHs, in synthetic water, with sensitivity lower than 1 mM for ABTS and 7 mM for guaiacol. The combining of different pH, buffers and reactive solutions is a mean for discrimination between copper and other metals found in mining water samples. The method developed could be used for copper detection in living cells and organisms, this fact conducting to copper recovery methods.

## REFERENCES

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