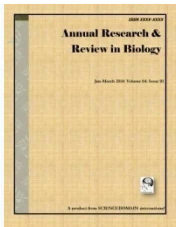

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An Enzymatic Method for Zinc Determination in Wastewater

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Authors' contributions

This work was carried out in collaboration between all authors. Author ND designed the study. Authors AAG, HN and YD managed the analyses of the study and performed the statistical analysis, wrote the protocol and the first draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: In this study, an enzymatic zinc determination method was based on the regaining of the activity of apo carbonic anhydrase by the zinc is presented in the sample.
Study Design: This method determines the amount of zinc in the waste water of the environment can be used in order to find out how much contaminated. For this purpose, wastewater from seven different areas and three drinking waters in Erzurum were taken and zinc concentrations were determined.
Methodology: Carbonic anhydrase was purified by Sepharose-4B-L tyrosine sulphanilamid affinity chromatography from bovine erythrocytes. The zinc in this structure was removed by dialysis against dipicolinic acid resulting in apo carbonic anhydrase obtained at a ratio of 100%. The activity of the enzyme was determined by the esterase activity on p-nitrophenyl acetate. For comparison purposes, the same samples were analyzed in an atomic absorption (AAS).
Results: When we compared the results of the AA and the enzymatic method, we

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observed there is a good correlation between the two methods.
Conclusion: The enzymatic method can be used for determination of zinc in wastewater.

Keywords: Wastewater; zinc determination; apoenzymes; carbonic anhydrase.

1. INTRODUCTION

Carbonic anhydrase (E.C. 4.2.1.1) (CA) is a zinc, containing metallo-enzyme, which catalyzes the hydration of CO_2 and the dehydration of H_2CO_3. When the zinc, covalently bound to active site, is removed, the apocarbonic anhydrase (apoCA) is obtained, resulting in no enzymatic activity [1]. The apoCA can show activity when the Zn^{2+} is added to the reaction medium (this is the basic principle of the method). This method was first tried by Kobayashi et al. [2]. They used the Zn^{2+} in fruit juices and water for the reactivation of apoCA and determined the activity by means of esterase action [1].

Zinc is an essential and beneficial element in human growth. Concentrations above 5 ppm can cause a bitter astringent taste and opalescence in alkaline waters. Zinc most commonly enters into the domestic water supply from the deterioration of galvanized iron and dezincification of brass. In such cases, lead and cadmium may also exist because there are impurities of the zinc used in galvanizing. Zinc in water also may result from industrial waste pollution [3].

Wastewater refers to the liquid waste discharged from homes, commercial premises and industrial plants to individual disposal systems or to municipal sewer pipes. The steady increase in the amount of water usage and wastewater produced by urban communities and industries throughout the world pose potential health and environmental problems. Countries are seeking safe, environmentally sound and cost-efficient ways to treat and dispose of wastewaters. Wastewater has more hazardous metal ions than drinking water [4].

Recently, Demir et al. obtained the apoenzyme at a high purity (100%) [5]. After then, this re-activation method was used to determine the zinc level in the Pleural fluid, fruit juice and vegetable [6-9]. This work demonstrates the utility of the enzymatic method of zinc determination in the analysis of wastewater. The present study was conducted with a correlation for wastewater material.

2. MATERIALS AND METHODS

2.1 Preparation of ApoCa from Bovine Erythrocytes

Carbonic anhydrase was purified using Sepharose-4B-L tyrosine sulphanilamid affinity chromatography from bovine erythrocytes [10]. For obtaining apoenzymes, carbonic anhydrase was dialyzed against 0,075M dipicolinic acid. By using this method, almost 100% pure apoenzyme was obtained. 5mL of the apoenzyme reagent can be prepared by using 100mg of CA. There salting reagent may be sufficient for about 100 determinations. In addition, CA is a rather stable enzyme and has a longer constitution life [11]. For instance, only 5% of activity loss is detected after one year standing.

CA activity in eluates obtained during the purification was determined by the method of Wilbur and Anderson as modified by Rickly et al. [12-13]. For the preparation of a standard curve in serum, Zn^{2+} determination, the esterase action of CA was used. In this method, 4-nitrophenyl acetate is hydrolyzed to 4-nitrophenyl by CA and the absorbance of the product is measured at 348 nm. Reaction mixtures in a 3mL cuvette contained 0.1mL of apoenzyme solution, 1.0mL of Tris-SO_4 (0.05 mol/L, pH: 7.4), 0.4mL Zn^{2+} contained standard and 1.5mL of substrate. Three minutes later, the absorbance of the sample and blank cuvette (it contains distilled water instead of standard) were measured at 348 nm at 25ºC [14].

2.2 Preparation of Standard Curves

A standard stock solution of Zn^{2+} was prepared by dissolving the zinc metal in sulfuric acid. This solution was diluted and adjusted to a pH at 7.4 by adding solid Tris for the preparation of a standard curve. The relationship between apoCA and Zn^{2+}concentrations in reactivations analysis was determined by measuring the activities at different Zn^{2+} concentrations with a fixed amount of apoCA (3.6 x 10^{-5} M). A graphical representation of absorbance versus Zn^{2+}concentration was given in Figure 1.

2.3 Zinc Determination in Wastewater

In order to show the applicability of this method to Zn^{2+} determination of wastewater and drinking water, measurements were made on 10 samples taken from 10 areas in Erzurum city. The water was heated in a boiling water bath for 1h in tubes and then it was centrifuged. The samples (1mL) were diluted with deionized water (3mL) and were deproteinized with trichloroacetic acid (sample/TCA: 1/1). The resulting contents were centrifuged (for 15 min. 1500xg). The same samples were used for zinc determination both with AAS and enzymatic method [15,4].

3. RESULTS AND DISCUSSION

The contaminated water with low concentrations of heavy metals could be caused very serious problems. They may accumulate in the food chain. For this reason, the development of new methods for quantifying trace metals is required and challenged. Most of the sensitive and selective methods recently available such as ICP-MS, ICP-AES and GF-AAS are too expensive and are not practically applied in developing countries [2].

It is critical for an analytical method to exhibit high sensitivity, selectivity, and ease of using in order to measure the concentration of trace metal ions in environmental science, life sciences, energy science, and materials science. Preparative procedures for the separation and concentration of the trace elements are usually required, because only trace amounts are present, and other species in the same sample solution may interfere with analysis of the element's composition. In this study, we tried an alternative non-interfere method based on enzymatic principal for Zn^{2+} in wastewater. In another study, zinc have also determined spectrophotometrically by connecting Zinc. Determination of the actual amount of zinc content with a low concentration of 50% was barely able to determine. Compared to our results we perform the enzymatic determination of zinc sensitivity of the method is obvious. Thus, an alternative to the work done and the high sensitivity of a new enzymatic method of determination of zinc believe that we have developed [16].

Standard curve obtained with constant apoCA (20ppm) and changing Zn^{2+} concentrations are presented in Fig. 1.

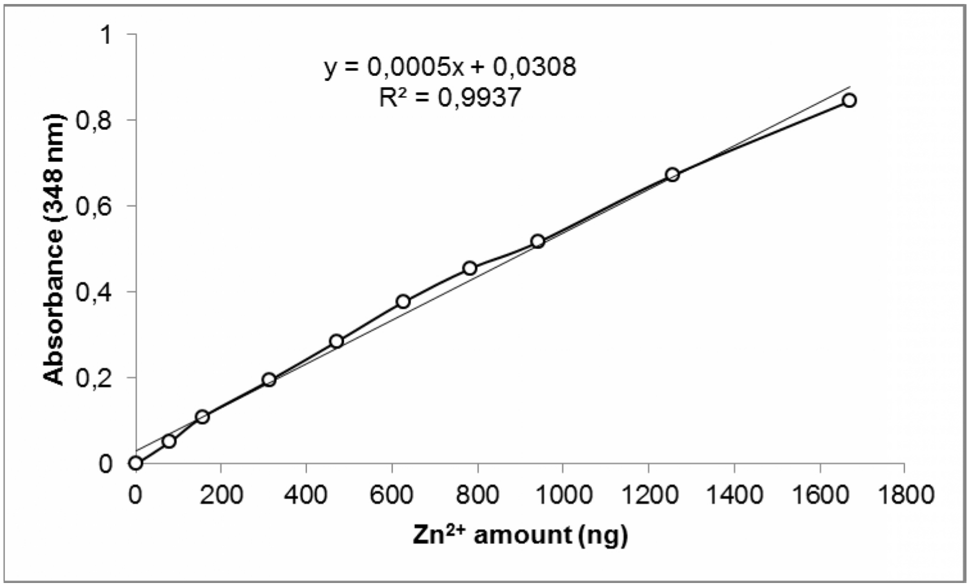

Fig. 1. Standard curve obtained with constant apoCA ($3.6x10^{-5}$mol/L) and changing Zn^{2+} concentrations

The Table 1 shows the statistical analysis of the results of seven wastewater and three drinking water samples.

Table 1. Statistical evaluation of the results

Water samples	Zn^{2+} concentration (mg/L) with enzymatic method X ± (n=10)	Zn^{2+} concentration (mg/L) with atomic adsorption method X ± (n=10)	Student's t-test t	p	Correlation r	*p*
1	0.511±0.085	0.507±0.0025	0.713	>0.05	0.995	>0.5
2	0.374±0.005	0.370±0.014	0.543	>0.05	0.993	> 0.5
3	0.435±0.13	0.432±0.007	0.270	>0.05	0.969	> 0.5
4	0.316±0.011	0.310±0.02	0.480	>0.05	0.999	> 0.5
5	0.471±0.019	0.470±0.015	0.071	>0.05	0.984	> 0.5
6	0.996±0.021	0.998±0.009	0.197	>0.05	0.984	> 0.5
7	0.738±0.007	0.735±0.004	0.191	>0.05	0.967	> 0.5
8	0.977±0.009	0.970±0.016	0.627	>0.05	0.987	> 0.5
9	0.662±0.007	0.588±0.032	0.476	>0.05	0.999	> 0.5
10	0.450±0.008	0.455±0.015	0.360	>0.05	0.998	> 0.5

When compares the results of the AA and enzymatic method, it can be easily seen from the *r* (>0.9) and *p* value that there is a good correlation between the two methods. In addition, the t-test result shows the correlation. In student t test, p values are bigger than 0.05, so this method can be safely used the determination of zinc amount in waste water.

Furthermore, the stability of apoCA as a protein and the high activity of bovine CA are important advantages of this method.

4. CONCLUSION

The techniques suitable for the quantification of zinc ions in wastewater, ICP-MS, ICP-AES and GF-AAS are likely to be the most widely used. However, these techniques are reliable and sensitive, they suffer from the limitation of being rather costly (seeing instrument purchase and care), time-consuming, and not always readily available. Thus, simple spectrophotometric techniques, which tend to be less expensive is feasible alternatives to those methods requiring more sophisticated instrumentation [16]. In conclusion, the enzymatic method can easily be used in wastewater zinc determination. This method is cheap and can be used all laboratory On the other hand, studies on its application to auto analytic equipment and routine use are continuing.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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