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A Highly Sensitive Enzymatic Catalysis System for Trace Detection of Arsenic in Water

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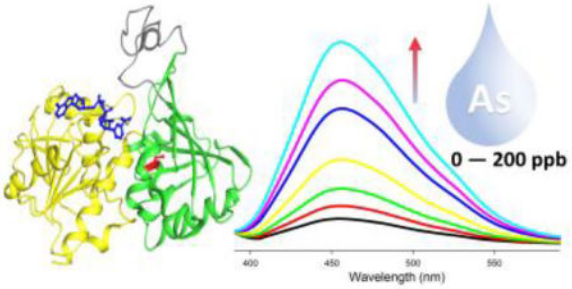
Abstract

Arsenic (As) is an extremely toxic element that exists in the environment in different chemical forms. The detection of arsenic in potable water remains a challenging task. This study presents a highly sensitive enzymatic catalysis system for trace sensing of inorganic arsenic in water. This is the first enzyme-catalyzed fluorescence assay capable of detecting arsenic at concentrations below the allowable level adopted by the World Health Organization (10 ppb in drinking water). The enzyme catalytically produces fluorescent NADH in the presence of arsenate, which enables facile detection of arsenate at concentrations in the 0 – 200 ppb range. Calibration curves made at a set time interval allow accurate determination of unknown arsenic samples. This method holds potential for interfacing with automated analytical sampling systems to allow arsenic determinations in environmental health applications.

Graphical abstract



Detect Arsenic (As): The enzyme catalytically produces fluorescent NADH in the presence of arsenate and enables facile detection of arsenic with concentrations in the 0 – 200 ppb range. This method holds potential for a convenient benchtop arsenic assay with concentrations near and below the allowable level adopted by the World Health Organization (10 ppb in potable water).

Keywords

Arsenic; Sensing; Enzyme; Catalysis; Fluorescence

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Arsenic is one of the World Health Organization's (WHO's) 10 chemicals of major public health concern.[1] The chief soluble arsenic species present in the environment include arsenite (As^{III}) and arsenate (As^{V}) salts. Only smaller amounts of organic forms, for example, dimethylarsinic acid (DMA), dithioarsenate (DTA) and monomethylarsinic acid (MMA) are found in natural waters.[2] Exposure to arsenic may cause reduced IQ in children, as well as cause bladder and lung cancer, and skin and circulatory system damage.[3] As many as 200 million people are exposed to drinking water with arsenic concentrations that exceed the WHO guideline of 10 ppb.[4] In some countries, measured arsenic concentrations in contaminated ground water have been observed at levels up to 1000 ppb.[5] Public health concerns have prompted intensive efforts to develop sensitive detection methods. Current technology for trace arsenic analysis relies on expensive instrumental analyses, such as ICP-MS. Qualitative colorimetric methods are also used.[6] These methods either require highly trained technicians to operate expensive instruments or produce highly toxic chemicals, such as arsine gas. Harrop et al. developed fluorescent molecular sensors for arsenic; however, these sensors can only function in organic solvents.[7] Other methods have also been proposed, such as the use of nanomaterials,[8] a surface-enhanced Raman scattering (SERS) based assay,[9] bacterial biosensors,[10] and genetically modified *E. coli*.[11] Although significant progress has been made, these techniques have not yet met the demands of throughput, sensitivity or ease of operation. Consequently, the development of inexpensive quantitative assays for trace arsenic detection has been widely sought.

Goode and Matthews reported an enzyme assay nearly 40 years ago, which uses GAPDH and a kinetic measurement assay for arsenate.[12] The necessary kinetic analysis of the fast reaction at high enzyme concentrations does not lend the method to routine benchtop analyses. Herein, we report an inexpensive GAPDH (from rabbit muscle) sensing system based on a low concentration enzyme-catalyzed fluorescence assay for arsenic in aqueous solution. The detection range is lower than the WHO guideline of 10 ppb. Figure 1 shows the enzyme, coenzyme and substrate used in the enzyme catalysis system. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has long been known as a ubiquitous glycolytic enzyme. Figure 2 (top) outlines the catalysis mechanism for conversion of glyceraldehyde-3-phosphate (G3P) to the corresponding phosphoglycerate.[13] The thiolate cysteine of the holoenzyme initially attacks the carbonyl group of substrate (G3P), creating a hemithioacetal intermediate. This results in the formation of the acyl-enzyme intermediate after hydride transfer from the hemithioacetal to the coenzyme (NAD^+). NADH is released and rapidly substituted by another NAD^+ to yield an NAD^+-acyl-enzyme adduct. In the presence of an acyl acceptor (phosphate or arsenate), the NAD^+-acyl-enzyme reacts to release stable 1,3-diphosphoglycerate or hydrolytically labile 1-phospho-3-arsenoglycerate. Since arsenate is a much better nucleophile than phosphate, and the arsenoglycerate hydrolyzes very rapidly at slightly basic pH, the resulting holoenzyme will re-enter the catalytic cycle and amplify production of fluorescent NADH in the presence of arsenate. This provides a convenient assay for detection of arsenate by absorbance or fluorescence based measurements.

The GAPDH catalysis system could therefore potentially afford an efficacious benchtop assay for arsenic with a low detection limit, since the immediate product of the "arsenolysis"

reaction, 1-arseno-3-phosphoglycerate (APG), undergoes rapid hydrolysis (Figure 2) in aqueous solution to regenerate arsenate.[14] This will drive the catalytic cycle until the NAD^+ is consumed or enzyme deactivation occurs. In other words, even though only trace amounts of arsenic are present, the catalytic cycle will continuously generate NADH in the presence of excess NAD^+.

In a standard analysis, an aqueous solution of $HAsO_4^{2-}$ was added into 2 mL of HEPES (50 mM) buffered solution containing GAPDH (14 nM), G3P (75 μM), NAD^+(30 μM), EDTA (0.5 mM) and DTT (30 μM) at pH = 8.5. The reaction was then allowed to proceed at room temperature (~23 °C) and the production of NADH monitored by its fluorescence emission (Ex = 340 nm) as a function of time (Figure 3). The addition of 5 ppb of arsenic leads to a substantial increase in fluorescence intensity of the enzymatic catalysis system. Thus, the system possesses the capacity for monitoring arsenic levels well below the WHO allowable concentration of 10 ppb.

The arsenic concentration was observed to alter the enzymatic catalysis efficiency as expected. Reactions were carried out with different amounts of arsenic in the system. Figure 4 shows how the variation in the arsenate concentration influences fluorescence at 460 nm (supporting information, full spectra in Figure S1-S8). The calibration curve (Figure 5) shows that the enzymatic catalysis efficiency at 20 minutes incubation depends directly on the concentration of arsenic. The more arsenic present in the system, the more NADH produced, as indicated by the fluorescence intensity. Importantly, the system demonstrates a dynamic range for arsenic (5–200 ppb) covering that of most interest in environmental water analyses. The reaction without adding arsenic (blank) also exhibits a non-negligible background fluorescence, which indicates a low level of NADH generated in the system. This is not unreasonable because the catalytic reaction will produce at least one equivalent of NADH even without any acyl acceptor present. Note that the complete conversion of NAD^+ to NADH is not observed at low arsenic concentrations, since the reaction plateaus after 90 minutes. This is likely due to enzyme deactivation at long reaction times, and attributed to the ~90 minute lifetime of DTT preservative at pH 8.5.[15] Indeed, we found similar results over the course of a year with the use of three different samples of GAPDH enzyme. In light of the direct arsenic concentration dependency and the useful dynamic range of the enzymatic catalysis system, a calibration curve was created at a convenient 20 min incubation period to quantify the arsenic in unknown water samples (supporting information, Figure S10). As shown in Figure 5, the predicted concentrations of unknown blind samples (8.1 ± 1.9 ppb and 126 ± 4 ppb) are quite close to the known concentrations (7.5 ppb and 125 ppb). San Diego tap water, which is routinely analyzed by the city for arsenic contamination, was sampled at two locations and the values were estimated as significantly less than 5 ppb.

While the enzyme kinetics of GAPDH is complex, the calibration curve constructed for the dilute enzyme assay at short incubation times is approximately linear. The enzymatic catalysis reactions were also carried out in the presence of different concentrations of arsenic at higher enzyme concentrations. Then complete conversion of NAD^+ to NADH was observed relatively rapidly. The high background and rapid reaction found at the higher concentrations of enzyme reduces the useful As sensing dynamic range (supporting

information, Figure S9). Tolerance of the enzymatic catalysis system was tested in the presence of K_2HPO_4 as a potential interference due to the ubiquitous presence of phosphate in water. Addition of K_2HPO_4 (0.1 mM) doesn't result in any significant change in fluorescence of the enzymatic catalysis system (supporting information, Figure S11) compared with the blank during the time scale of this experiment (supporting information, Figure S1). The concentration of phosphate tested was 33 times the EPA allowed concentration for fresh water rivers and streams. Other potential interferents (Fe^{3+}, $Cr_2O_7^{2-}$, nitrate) were not problematic at levels likely to be encountered in fresh water (see supporting information for details).

To demonstrate the potential applicability of the described enzymatic catalysis system for natural waters that may contain both arsenate (As^V) and arsenite (As^{III}), a proof-of-concept experiment was performed. Arsenite $NaAsO_2$ (As^{III}) was initially oxidized to arsenate by a brief 1 minute exposure of the $NaAsO_2$ solution to 2% ozone in air; the solution was then added to the GAPDH catalysis system after removing the residual ozone by purging the solution with argon for 5 minutes. As expected, the emission profile observed by the addition of oxidized $NaAsO_2$ solution is nearly identical to that produced by direct addition of an equivalent amount of Na_2HAsO_4 (supporting information, Figure S14).

In summary, we have reported a highly sensitive enzymatic catalysis system for trace sensing of inorganic arsenic in potable water. This provides for a simple benchtop enzyme-catalyzed fluorescence assay capable of detecting inorganic arsenic at levels below the allowable level adopted by the WHO for safe drinking water (10 ppb). We showed that concentrations in the 0 – 200 ppb range are easily detected by the assay. The enzyme and substrates employed are relatively inexpensive and the assay itself requires only a simple fluorimeter. We believe that this method will lead to its application in automated analytical sampling systems for determination of arsenic in environmental and health and safety applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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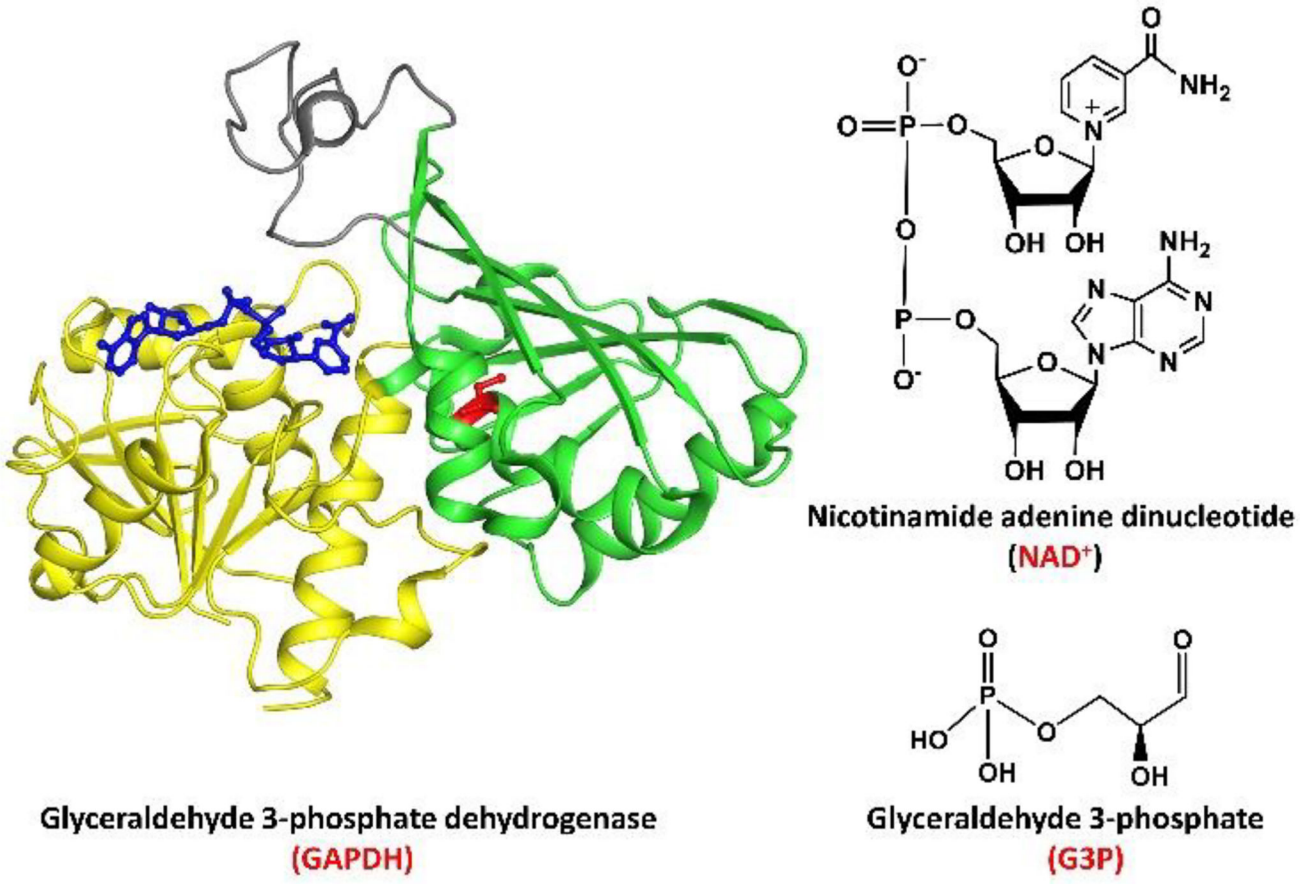

Figure 1.
Molecular structures of enzyme (GAPDH), coenzyme (NAD^+), and substrate (G3P) used in the enzyme catalysis system for arsenic detection.

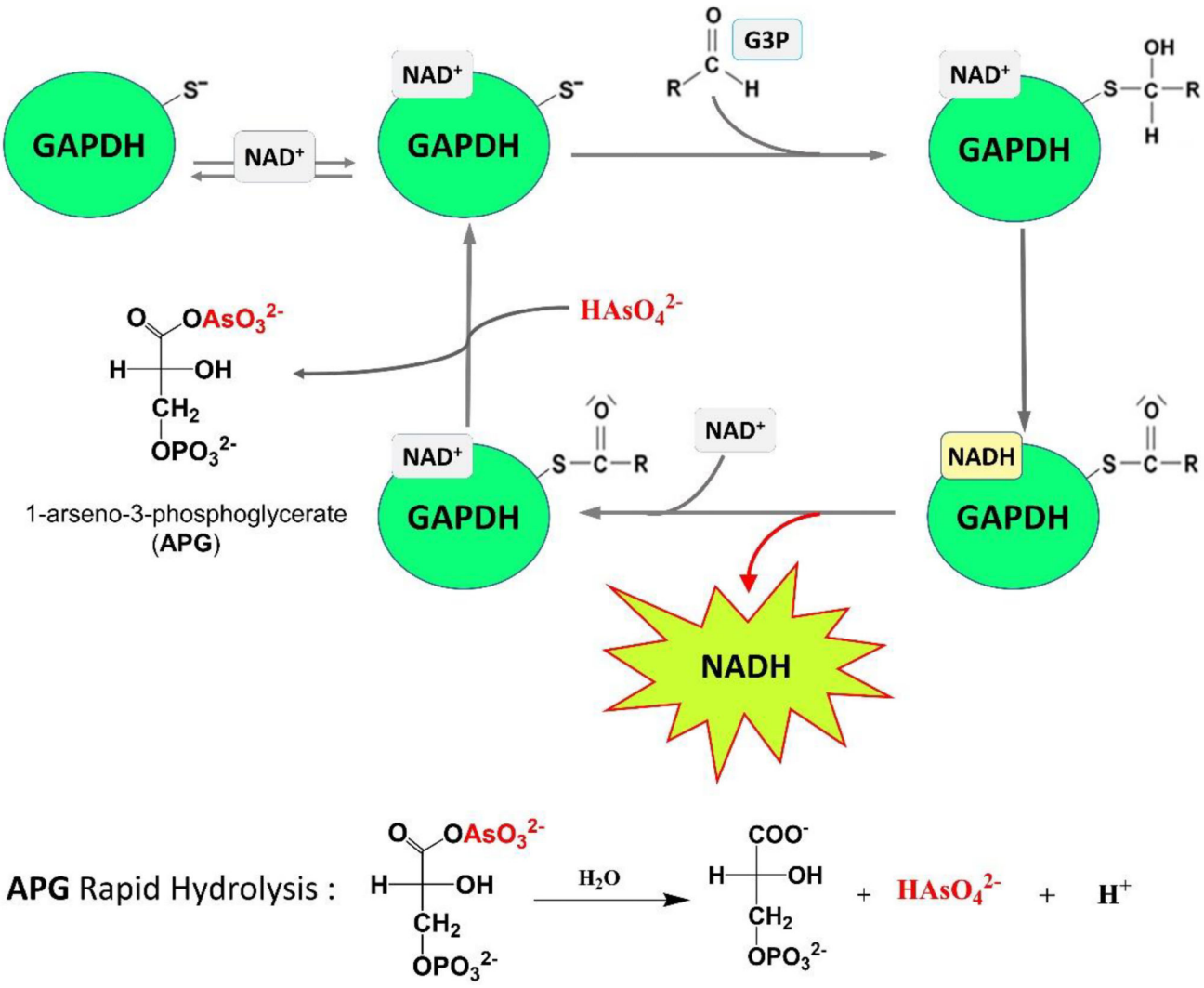

Figure 2.

Scheme which illustrates the enzyme catalytic mechanism for arsenic sensing (top); hydrolysis reaction of 1-arseno-3-phosphoglycerate (bottom).

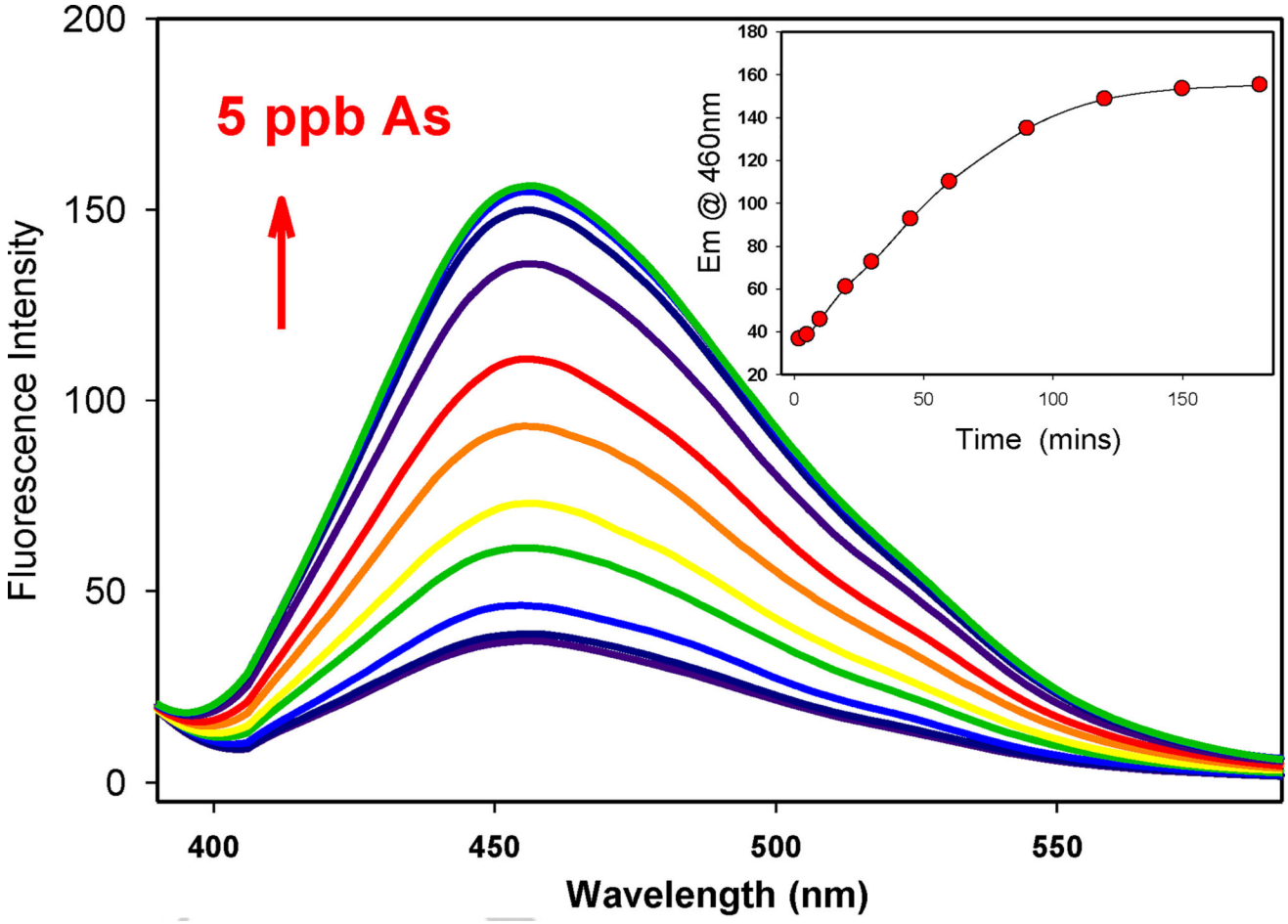

Figure 3.

Fluorescence spectra of the enzyme catalysis system (NAD^+: G3P: GAPDH: DTT) in the presence of 5 ppb of As (as Na_2HAsO_4) in buffered (50 mM HEPES) solution.

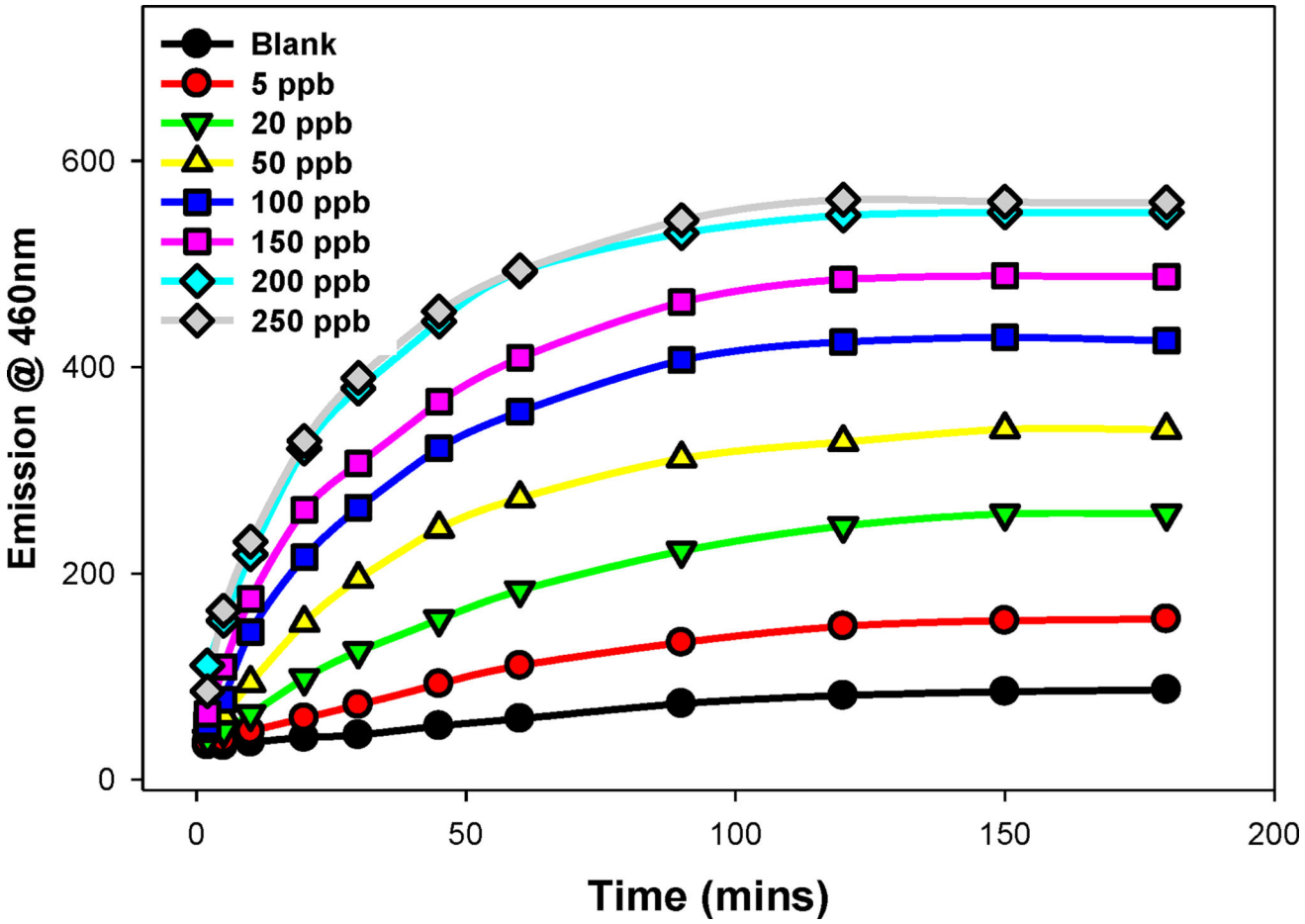

Figure 4.

Emission intensity (λ_{ex} = 340 nm, λ_{em} = 460 nm) of GAPDH enzyme catalysis system in the presence of As (0–250 ppb, as Na_2HAsO_4) as a function of time (0–180 minutes).

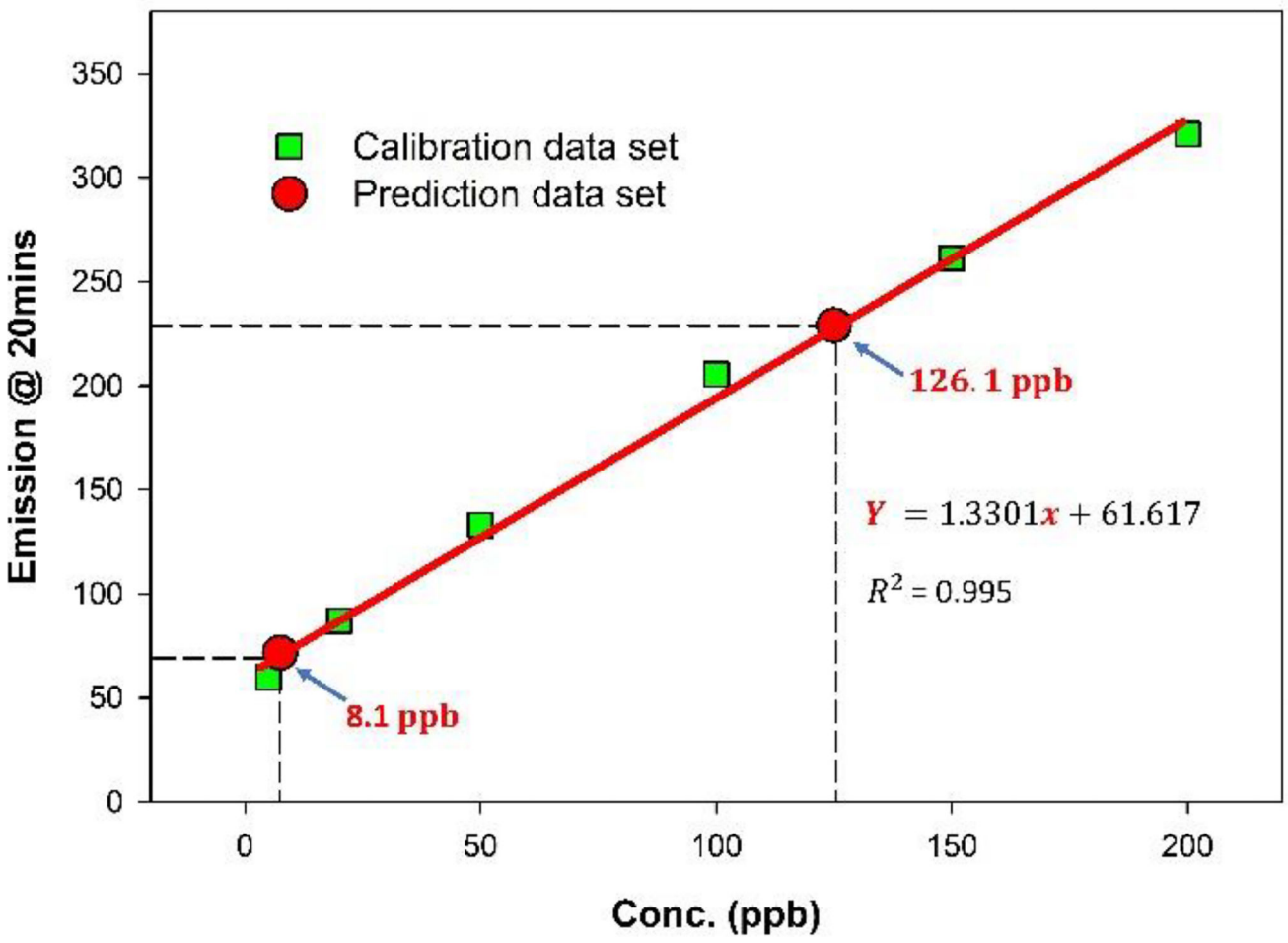

Figure 5.

Prediction of unknown arsenic samples by a calibration curve created at a convenient 20 min incubation period. The analyzed values of 8.1 ± 1.9 ppb and 126 ± 4 ppb agree well with the known concentrations of 7.5 ppb and 125 ppb, respectively.