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Spectrofluorimetric Determination of Arsenic(III) Using Dansylated Peptide

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The potential of dansylated peptide in determination of arsenic(III) was investigated. Fluorescence intensity of dansyl-*D*-Ala-Gly-OH (DAG) was quenched during addition of arsenic(III) in aqueous solution. The fluorescence spectrum of DAG was measured at pH 12 with excitation and emission wavelengths of 331 nm and 524 nm. Limit of detection of arsenic(III) by DAG is 0.15 µM. The presence of foreign ions at 1:1 molar ratio of arsenic(III)-interfering ions did not have significant effect on determination of arsenic(III). The developed detection method was also applied on analysis of electroplating waste samples.

Key Words: Fluorescent chemosensor, Dipeptide, As(III), Quenching.

INTRODUCTION

Arsenic was found in various levels in environment such as soils[1], plants[2], animals[3], foods[4] and water[5,6]. Heavy metal ions can be found in assorted oxidation state in solution. Arsenic have two soluble inorganic forms which are arsenite, As(III) and arsenate, As(V). Arsenic(III) is more toxic than As(V)[7]. Arsenic has been classified as carcinogenic (class A) and the latest maximum contaminant level in drinking water is 10 µg/L[8]. Indeed arsenic is dominants to bad health impact such as hyperkeratosis or even arsenic poisoning[9]. Diverse ways have been carried out to detect/remove this toxic metal. A lot of development of analysis or reagents which has field deployable, low cost, high sensitivity and low limit of detection for determination of arsenic has been developed. Recently, a review paper reported three different types of measurement techniques for arsenic determination. The techniques are spectroscopy, electrochemistry and chromatography[10]. Other methods such as precipitation, adsorption or cementation also reported can be used for removal of arsenic[11]. However it is difficult to choose which method is the best for analysis measurement. Recently amino acid and peptide had gain interest for researchers to explore the capabilities for metal ion determination. Previously our research group had discussed the development of amino acid and peptide for metal ion detection[12].

Peptidyl fluorescent promises a good chemosensor for metal ion detection. Previous researchers have designed peptide as a host molecule which have properties such as phytochelatin[13], ratiometric fluorescent peptide[14], biological function[15] or Ni(II)-Binding (ATCUN) Motif[16] for metal ion. The length of the host can be short as dipeptide, tripeptide or oligopeptide. Meanwhile fluorophore will be attached into the host molecule as a label for fluorescent measurement. Among the peptidyl fluorescent example are; Napth-Cys-Lys-Gly[13] and Dansyl-Gly-Gly-His-Gly[16]. The aim of this study is to explore the usage of small peptide as a host and couple with dansyl as ligand for As(III) detection in solution. Fluorescence measurement was used to characterize analytical performance *i.e.*, amount of reagent, pH study, dynamic range, reproducibility, interference and validation. The validation of developed method was established with inductively coupled plasma optical emission spectrometry (ICP-OES).

EXPERIMENTAL

Double deionized water (distilled) was used throughout the experiment as solvent. DAG *ca.* 95 % (Sigma Aldrich), 1000 ppm of As(III) solution in 1 M hydrochloric acid (Fisher Scientific), 1000 ppm of As(III), Fe(II), Fe(III), Co(II), V(IV), Ni(II), Zn(II), Cr(III), Cu(II), Sb(IV) and Se(IV) are prepared from AAS standard solution. 0.001 M of $Na_2(SO_4)$ (14.2 mg, 142.04 g/mol) and NaCl (5.84 mg, 58 g/mol) were dissolved in a 100 mL of diionized water in volumetric flask. 2-[4-(2-hydroxyethyl)piperazin-1-yl] ethane sulfonic acid 99.5 % (HEPES) (Sigma Aldrich). Different concentration of As(III) solutions were prepared from stock solution of 1000 ppm. pH adjustments were carried out using 10 % acetic acid and 0.01 M NaOH solution.

Absorption spectra were determined uisng UV-VIS Shimadzu Spectrophotometer UV-1DAG50PC. Fluorescence spectra measurements were performed on a Shimadzu Spectrofluorophotometer SRF-5301 PC. Perkin Elmer Optima 2000DV model of inductively coupled plasma-optical emission spectrometry (ICP-OES) was used for metal ion analysis. All pH measurements were made with a Mettler Toledo S20 SevenEasy™ pH Meter. All of the measurements were operated at room temperature at about 25 ºC. All glassware were soaked for overnight in hundred percent Decon solution, rinse with hot and diionized water and dried in the oven at 80 ºC. Optically four sides clear cuvette was cleaned following the same procedure. pH meter was routinely calibrated prior to use. Working solutions were prepared by appropriate dilution.

Absorption and fluorescence measurements: DAG solution was prepared by dissolving solid DAG in double deionized water. UV absorption spectrum of DAG in aqueous solution was measured over the range of 310-350 nm and fluorescence of the DAG were measured over the range of 350 to 800 nm with excitation at 331 nm in the presence and absence of metal ions. All reactions were performed at 25 ºC. Wavelength of 524 nm was used for fluorescent measurement.

RESULTS AND DISCUSSION

Molecular weight of Dansyl-D-Ala-Gly-OH (DAG) is *m/z* 379.1 and the molecular structure is shown in Fig. 1. DAG consists of fluorophore (dansyl group) and peptide. The C-terminal of peptide is attached to hydroxy group.

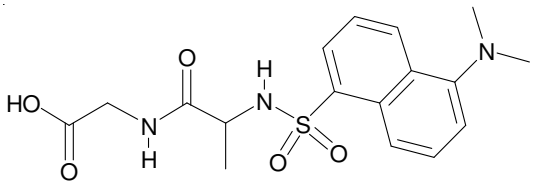


Fig. 1. Molecular structure of DAG

UV absorption spectrum: UV absorption of DAG in double deionized water is shown in Fig. 2. Solution of DAG in double deionized water is in a form of colourless solution. UV absorption spectrum was measured over the range 310 to 350 nm. Wavelength of 331 nm is used as excitation wavelength for fluorescent study.

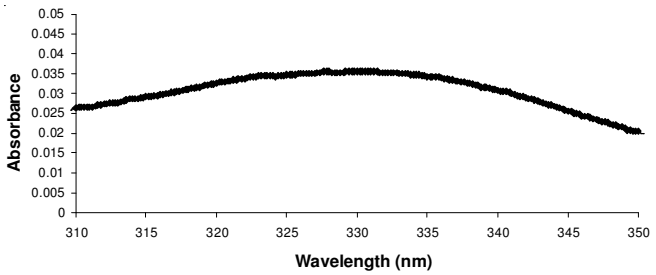


Fig. 2. UV absorption spectrum of DAG (10 microM, 10 mM HEPES, pH 5) in deionized water.

Emission spectra: Fluorescence spectra of solvent and DAG in double deionized water based on excitation at 331 nm is shown in Fig. 3 and maximum emission was observed at 524 nm. The signal was strong in the visible region and relatively has long emission wavelength. The interfering peak observed is due to light scattering phenomena[17].

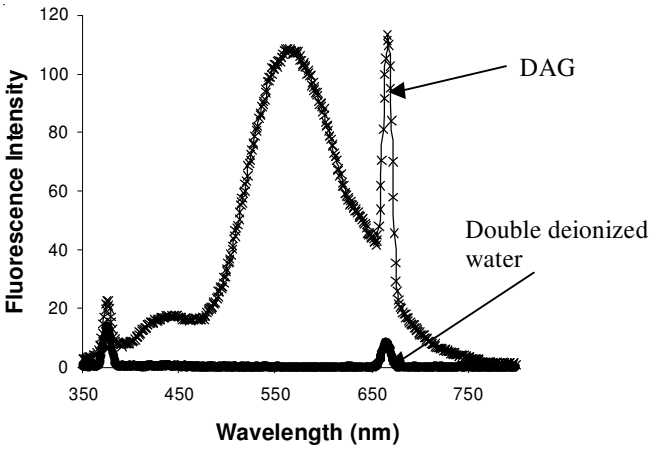
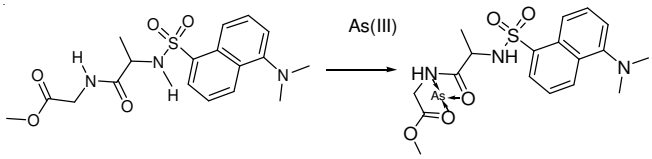


Fig. 3. Fluorescence emission spectra of double deionized water and DAG (10 microM, 10 mM HEPES, pH 5) in double deionized water. Excitation at 331 nm

Proposed binding mode between As(III) and DAG: The complexation between DAG and As(III) is proposed in **Scheme-I**. It is proposed that an electrostatic interaction occur between negative charge of N and O in DAG with positive charge of As(III). The same peptide-metal interaction was also reported before[18].



Scheme-I: Proposed binding mode of DAG with As(III)

Fluorescence study on interaction of DAG and metals: The selectivity of DAG towards metal ion at 1:1 (DAG: metal ion) concentration ratio was studied and significant change in fluorescence intensity was observed upon addition of As(III) (Fig. 4). Upon addition of metal ions [Fe(II), Co(II), V(V), Ni(II), Zn(II), Cr(III)] into DAG, it resulted in a quenching of fluorescence intensity. Among them, As(III) was able to decrease the fluorescence intensity, 50 % from its original intensity. This phenomena was known as Turn-off or fluorescence quenching. This result provided a proof for the formation of a complex of DAG with As(III). Similar result has been reported during addition of As(III) even at different molar ratio[13]. The quenching may be attributed to interaction of cation with the chromophore in dansyl group.

pH study: The effect of pH on fluorescence intensity at constant concentration of DAG has been carried out by adjusting the pH of the solution with 10 % acetic acid and 0.01 M sodium hydroxide. Fluorescence of DAG is very sensitive towards pH. Intensity of maximum emission changed significantly when the pH value is altered. As can be observed in Fig. 5 the emission is very low at pH 2. As the pH increased, the fluorescence emission gradually started to increase. The fluorescence showed highest fluorescence intensity in basic

solution. In acidic condition, protonation of dimethyl amino group of dansyl could cause the fluorescence intensity to decrease[19]. This could be explained based on the charge transfer between amino and naphthyl group which could not occur. As a result, the fluorescence was quenched[20].

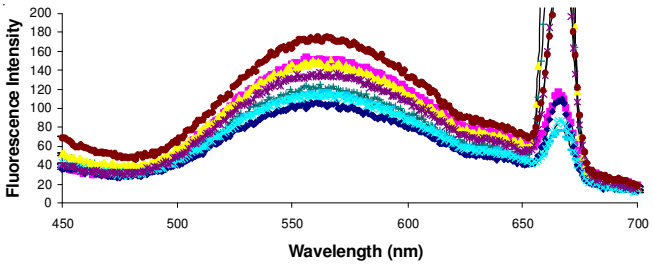


Fig. 4. Fluorescence emission spectra of DAG (10 microM, 10 mM HEPES, pH 5) with addition of 10 microM of (Fe(II), Co(II), V(V), Ni(II), Zn(II), Cr(III), As(III))

Upon increasing pH values, the presence of base was able to deprotonate the sulphonamide group of dansyl which increased the electron density on the napthyl. As a result, the fluorescence intensity increased. Maximum intensity shift to the lower wavelength after pH 8 and this is known as hypsochromic shift. At pH 12, 524 nm was observed to be the wavelength for maximum fluorescence intensity and this pH is used for further experiment.

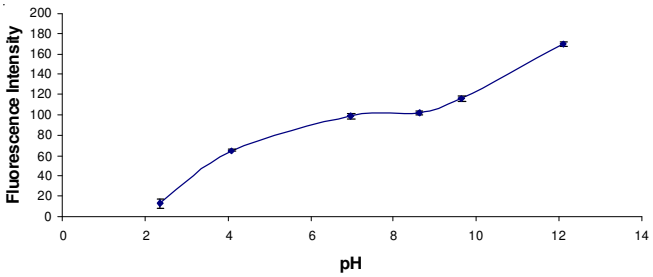


Fig. 5. Maximum fluorescence intensity of DAG (5 µM, 5 mM HEPES) at different pH values

Reagent concentration: Effect of different concentration of DAG at constant concentration of As(III) was studied and 1.5 µM of DAG was observed to give a maximum fluorescent different as shown in Fig. 6.

Dynamic range: According to the above results, the optimized condition for As(III) are; 1.5 M DAG, 1.5 mM HEPES and pH 12 in aqueous solution. Meanwhile wavelength of 524 nm was used for fluorescence different calculation.

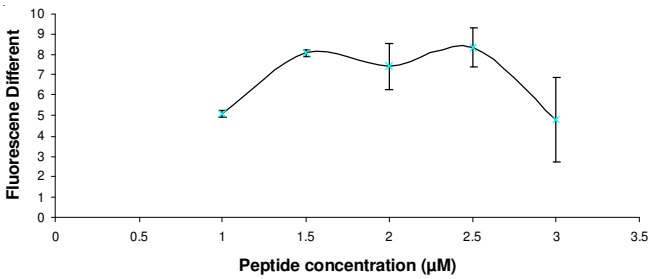


Fig. 6. Fluorescence different between DAG and complex DAG-As(III). Concentrations of DAG are (1.0, 1.5, 2.0, 2.5 and 3.0 µM) in (1.0, 1.5, 2.0, 2.5 and 3.0 mM HEPES). As(III) concentration is 5 µM at pH 12

As shown in Fig. 7 the fluorescence different increased with increasing As(III) concentrations. The calibration plot of fluorescent different against As(III) concentration shows a good linear relationship ($R^2 = 0.9674$) for As(III) concentration in the range of 0.19 to 9.8 µM. A typical calibration line with the analytical regression features of $Y = 0.7876X + 1.3984$.

For the purpose of limit of detection determination, it was calculated using $\alpha + 3\beta$ and the value is 0.15 µM, where a is the fluorescence measurement of blank sample and β is the standard deviation of α. The dynamic range for As(III) detection obtained is between 0.19 to 9.8 µM As(III).

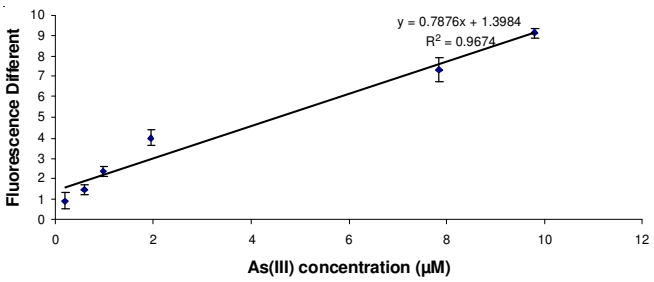


Fig. 7. Fluorescence different between DAG and complex DAG-As(III) at 524 nm. Concentration of DAG is 1.5 µM, 1.5 mM HEPES at pH 12. As(III) concentration is between 0.19 to 9.8 µM

Reproducibility: In order to evaluate reproducibility of DAG, 10 replicate of measurements were carried out using 7.84 µM of As(III). The percentage of relative standard deviation is 9.66 % and this RSD value indicate DAG has acceptable reproducibility for As(III) determination.

Interference: A few transition and non metal ions have been tested for interference study for detection of As(III) as shown in Fig. 8. It was reported that As(III) analysis suffers interfere by Cu(II)[10]. Arsenic and copper have been reported in the form of intermetallic form as this could be the reason for decreasing of fluorescence intensity[21]. However, the presence of all tested foreign ions did not interfere the fluorescence intensity of complex DAG-As(III) based on the insignificant value of percentage interference.

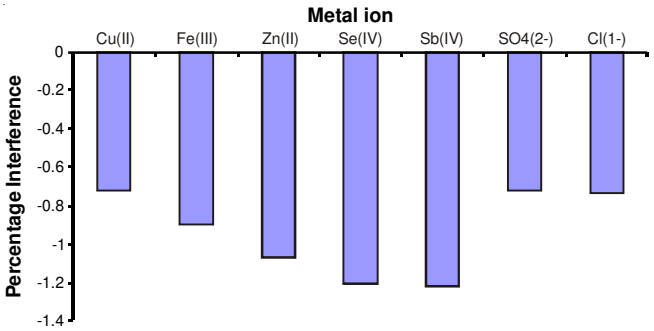


Fig. 8. Percentage Interference for 1:1 molar ratio of complex DAG-As(III) :Interfering Ions.

Validation: The validity of the constructed method was evaluated using electroplating waste. The waste solution was spiked with 5 and 10 µM As(III) followed by addition of DAG. Then, the same spiked solution was analyzed using ICP-OES without the addition of DAG. Significant difference was observed between the developed method and ICP-OES and

TABLE-1
DETERMINATION OF ARSENIC(III) IN ELECTROPLATING WASTE USING DAG

Sample	Fluorescent (μM)	ICP-OES (μM)	Recovery (%) fluorescent	Recovery (%) ICP-OES
DAG + Waste + 5 microM As(III)	12.12	4.82	242.4 ± 4.66	96.4 ± 1.47
DAG + Waste + 10 microM As(III)	19.41	10.29	194.1 ± 0.71	102.9 ± 0.54

this may be due to interference effect from the unknown foreign ions in the tested waste.

Conclusion

In this paper, dipeptide with fluorescence properties was applied to detect As(III) in basic aqueous media. Under optimized condition, the quantification of As(III) by DAG using spectrofluorometric method was satisfactory in a linear range of 0.19 to 9.8 μM As(III) with a detection limit of 0.15 μM As(III). Acceptable reproducibility was obtained for detection of As(III) based on the current developed method. No significant interference was observed from the foreign ions tested. The proposed method has a potential to be developed for the analysis of the inorganic arsenic species in waste water.

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