UV-Vis Spectrophotometric Determination of Selected Heavy Metals (Pb, Cr, Cd and As) in Environmental, Water and Biological Samples with Synthesized Glutaraldehyde Phenyl Hydrazone as the Chromogenic Reagent

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ABSTRACT

A simple, sensitive, selective, and non-extractive UV-Vis spectrophotometric method for the determination of cadmium, lead, chromium, and arsenic in biological, soil and water samples using synthesized and characterized phenyl hydrazone; glutaraldehydephenyl hydrazone (GPH) as the chromogenic reagent was developed. GPH was synthesized as new chromogenic analytical reagents for the direct UV-Vis spectrophotometric determination of the selected metals of interest in a slightly acidic pH of 6.5-7.5 and 20 % dimethylformamide (DMF) solution to give stable coloured metal-ligand complexes. The reactions were instantaneous; the wavelengths of maximum absorptions were followed spectrophotometrically and noted. The reagent GPH revealed a wavelength of maximum absorption between 360.0 (Cr) to 395.0 nm for (Pb and As) at a working pH of 6.5 to 7.5 room temperature (37 °C). The reagent GPH had a molar absorptivity (L mol⁻¹ cm⁻¹) ranging from 2.213×10⁴ (Pb) to 2.460×10⁴ (As), a mole ratio of metal to ligand of 2:1, the detection limit (µg/g) ranging from 0.3432 (As) to 0.5250 (Pb) and the metal-ligand complex was stable for 0-48 hours. The reagents had a Beer’s law validity range (mg L⁻¹) of 0.001 to 100. The Sandell’s sensitivities (µg/cm²) ranged from 0000409 (As) to 0.00499 (Pb) for APDH and 0.00452 (Pb) respectively. Large excess of cations and anions as possible interferences up to 15 folds were studied and do not interfere with the determination of the selected metals of interest. The developed method is highly selective for Cd, Pb, and Cr and As and was successfully used for the determination for the said elements in soil, water, and biological samples. The results of the developed methods were comparable with AAS and were found to be in good agreement. The method had very high precision and very good accuracy.

Keywords: Chromogenic reagents, glutaraldehyde phenyl hydrazone, selective, sensitive.

I. INTRODUCTION

The detection and quantification of metals and metal ions, especially transition metals both in solution and solid samples are of increased concern, as a result of increased environmental pollution worldwide. The greatest use of spectrophotometry lies in its application to quantitative measurements. The reason for this stem from the ease with which most spectrophotometric measurements can be made, their sensitivity, precision, the relatively low cost of instrument purchase and operation. This work is aimed at developing a simple, selective, sensitive, and non-extractive UV-Vis spectrophotometric method with synthesized and characterized phenyl-hydrazone (glutaraldehyde phenyl hydrazone) as chromogenic reagents for determining the concentrations of selected heavy metals in soil, water, and biological samples.

II. MATERIALS USED

All the regents and materials used in the synthesis and characterization of the glutaraldehyde phenyl hydrazone
were of analytical grade, sourced from accredited vendors and were used without further purification, unless stated otherwise.

A. Samples Collection and Treatment

The samples (Wastewater, Water and Soil) in triplicates were collected from Challawa Industrial Area of Kamboto Local Government Area of Kano State Nigeria West Africa into polythene bags and transported to the laboratory of Federal College of Agricultural Produce Technology Kano. The wastewater samples collected (500.00 cm$^3$) were filtered using a filter paper (Whatman No. 41) and then each filtered water sample was evaporated nearly to dryness with a mixture of 5.00 cm$^3$ of concentrated H$_2$SO$_4$ and 10.00 cm$^3$ concentrated HNO$_3$ in a fume cupboard and then cooled to room temperature. In order to dissolve the salts, the residue was then heated with 15.00 cm$^3$ of distilled water. After cooling the solution was neutralized with dilute NH$_4$OH solution and the obtained solution was filtered into 50.00 cm$^3$ standard flask and made up to the mark with deionized water [1].

Before digestion of the soil samples, each sample was dried at 65.0 °C for 48.0 hours. All samples were performed in triplicates. Five grams (5.00 g) of the samples in crucibles was placed in a pre-heated muffle furnace at 200-250.0 °C for 30.0 minutes and ashed for eight hours at 500-550.0 °C. Then, the samples were removed from the furnace and cooled. A 2.00 cm$^3$ of 5.00 mol/dm$^3$ of HNO$_3$ was added and evaporated to dryness on a sand bath. The samples were then placed in a furnace and heated to 550.0 °C for 15.0 minutes. The samples were removed from the furnace, cooled and moistened with four drops of distilled water. Then 2.00 cm$^3$ of concentrated HCl was added into the sample was evaporated to dryness, removed, and 5.00 cm$^3$ 2.00 mol/dm$^3$ HCl was again added and the crucible. The solution was filtered through Whatman No. 41 filter paper and the transferred quantitatively to a 50.00 cm$^3$ flask by making it up with deionized water [2].

The biological samples (liver, blood and flesh from animals) and plants samples were collected from Challawa industrial of Kano State Nigeria West Africa. The blood samples of 5.00 cm$^3$, the liver, flesh and plants samples were oven dried and 5.00 g were transferred into crucibles. The samples were then ashed in the muffle furnace at 500-550.0 °C for 8.0 hours in the presence of 5.00 cm$^3$ nitric acid. The contents of each crucible were cooled to room temperature, and 1.50 cm$^3$ of concentrated hydrochloric acid was added and warmed slightly. The solutions were then transferred quantitatively into 50.0 cm$^3$ calibrated flask and made up to the mark with deionized water [3].

B. Samples Collected and their Codes

F = Animal flesh, L = Animal liver, P1 = Spinach, P2 = Cabbage, B = Animal blood, IW = Irrigation water, SW = Wastewater, WS = Farm land Soil, WW = Well water.

C. Effect of Interference or Foreign Ions

The effects of foreign ions (cations; Co, Cu, Al, Fe, Hg, Zn, Ca, and anions like SO$_4^{2-}$, NO$_3^-$) on the determination of the heavy metals were studied using the methods of [4] by measuring the absorbances of the metal-ligand complex containing 1, 5, 10 and 15.0 µg mL$^{-1}$ of the metal of interest.

The criterion for interference is an absorbance value varying by more than ± 5% from the expected value for the metal under observation alone.

III. RESULTS

| TABLE I: Determination of Lead in Soil, Water and Biological Samples with the Developed Method (GPH) with AAS as Reference Method |
| Serial No | Sample Code | Concentration of Pb AAS1 (µg.g$^{-1}$ ±SD GPH) | p-value | Relative Error (%) AAS/GPH |
|-----------|-------------|-----------------------------------------------|---------|---------------------------|
| 1         | F           | 0.007 ± 0.001                                | 0.2297  | 0.00                      |
| 2         | L           | 0.001 ± 0.001                                | 0.0000  | 0.00                      |
| 3         | P1          | 1.018 ± 0.001                                | 0.5878  | +0.00                     |
| 4         | P2          | 1.014 ± 0.001                                | 0.2927  | +0.09                     |
| 5         | B           | 0.001 ± 0.001                                | 0.0000  | 0.00                      |
| 6         | IW          | 0.810 ± 0.001                                | 0.2991  | +0.12                     |
| 7         | SW          | 1.924 ± 0.002                                | 0.2927  | 0.00                      |
| 8         | WS          | 1.173 ± 0.003                                | 0.5242  | +0.08                     |
| 9         | WW          | 0.877 ± 0.002                                | 0.75041 | 0.00                      |

| TABLE II: Determination of Cadmium in Soil, Water and Biological Samples with The Developed Method (GPH) with AAS as The Reference Method |
| Serial No | Sample Code | Concentration of Pb AAS1 (µg.g$^{-1}$ ±SD GPH) | p-value | Relative Error (%) AAS/GPH |
|-----------|-------------|-----------------------------------------------|---------|---------------------------|
| 1         | F           | 0.000 ± 0.000                                | 0.0000  | 0.00                      |
| 2         | L           | 0.020 ± 0.001                                | 0.2297  | 0.00                      |
| 3         | P1          | 0.014 ± 0.003                                | 0.75041 | 0.00                      |
| 4         | P2          | 1.021 ± 0.003                                | 1.0000  | 0.00                      |
| 5         | B           | 0.000 ± 0.000                                | 0.0000  | 0.00                      |
| 6         | IW          | 0.031 ± 0.004                                | 0.59922 | 0.00                      |
| 7         | SW          | 0.055 ± 0.002                                | 0.81481 | 0.00                      |
| 8         | WS          | 0.008 ± 0.004                                | 0.66842 | 0.00                      |
| 9         | WW          | 0.009 ± 0.002                                | 0.79176 | 0.00                      |

| TABLE III: Determination of Chromium in Soil, Water and Biological Samples with The Developed Method (GPH) with AAS as The Reference Method |
| Serial No | Sample Code | Concentration of Pb AAS1 (µg.g$^{-1}$ ±SD GPH) | p-value | Relative Error (%) AAS/GPH |
|-----------|-------------|-----------------------------------------------|---------|---------------------------|
| 1         | F           | 0.033 ± 0.001                                | 0.45826 | 0.00                      |
| 2         | L           | 0.010 ± 0.002                                | 0.43902 | 0.00                      |
| 3         | P1          | 3.474 ± 0.015                                | 0.93992 | +0.02                     |
| 4         | P2          | 2.042 ± 0.005                                | 0.8779  | -0.04                     |
| 5         | B           | 0.000 ± 0.000                                | 0.0000  | 0.00                      |
| 6         | IW          | 1.998 ± 0.001                                | 0.1237  | -0.10                     |
| 7         | SW          | 2.237 ± 0.009                                | 0.2438  | -0.04                     |
| 8         | WS          | 2.615 ± 0.004                                | 0.2710  | -0.03                     |
| 9         | WW          | 1.038 ± 0.001                                | 0.2926  | +0.09                     |

| TABLE IV: Determination of Arsenic in Soil, Water and Biological Samples with the Developed Method (GPH) with AAS as The Reference Method |
| Serial No | Sample Code | Concentration of Pb AAS1 (µg.g$^{-1}$ ±SD GPH) | p-value | Relative Error (%) AAS/GPH |
|-----------|-------------|-----------------------------------------------|---------|---------------------------|
| 1         | F           | 0.000 ± 0.000                                | 0.0000  | 0.00                      |
| 2         | L           | 0.000 ± 0.000                                | 0.0000  | 0.00                      |
| 3         | P1          | 0.012 ± 0.001                                | 0.0000  | 0.00                      |
| 4         | P2          | 0.014 ± 0.002                                | 0.3415  | 0.00                      |
| 5         | B           | 0.000 ± 0.000                                | 0.0000  | 0.00                      |
| 6         | IW          | 0.011 ± 0.001                                | 0.2297  | +0.09                     |
| 7         | SW          | 0.010 ± 0.002                                | 0.5878  | 0.00                      |
| 8         | WS          | 0.009 ± 0.004                                | 0.5878  | +1.11                     |
| 9         | WW          | 0.028 ± 0.002                                | 0.8000  | 0.00                      |
| Sample | Cđ added | Ph added | GPH method | Found (µg/ml) | Recovery (%) ± SD |
|--------|----------|----------|------------|--------------|-------------------|
| F      | -        | 50.0     | ND         | 49.99        | 99.98±0.2         |
|        | 100.0    | 99.99    | ND         | 99.99±0.1    |                   |
|        | 500.0    | 499.99   | ND         | -            |                   |
| L      | -        | 50.0     | ND         | 49.99        | 99.98±0.2         |
|        | 100.0    | 99.98    | ND         | 99.98±0.2    |                   |
|        | 500.0    | 499.99   | ND         | -            |                   |
| P1     | -        | 50.0     | ND         | 49.97        | 99.94±0.3         |
|        | 100.0    | 99.98    | ND         | -            |                   |
|        | 500.0    | 499.96   | ND         | -            |                   |
| P2     | -        | 50.0     | ND         | 49.99        | 99.98±0.3         |
|        | 100.0    | 99.98    | ND         | 99.98±0.2    |                   |
|        | 500.0    | 499.99   | ND         | -            |                   |
| B      | -        | 50.0     | ND         | 49.99        | 99.96±0.3         |
|        | 100.0    | 99.99    | ND         | 99.99±0.1    |                   |
|        | 500.0    | 499.99   | ND         | -            |                   |
| IW     | -        | 50.0     | ND         | 49.98        | 99.96±0.3         |
|        | 100.0    | 99.99    | ND         | 99.99±0.1    |                   |
|        | 500.0    | 499.98   | ND         | -            |                   |
| SW     | -        | 50.0     | ND         | 49.98        | 99.96±0.3         |
|        | 100.0    | 99.99    | ND         | 99.99±0.1    |                   |
|        | 500.0    | 499.98   | ND         | -            |                   |
| WS     | -        | 50.0     | ND         | 49.98        | 99.96±0.3         |
|        | 100.0    | 99.99    | ND         | 99.99±0.1    |                   |
|        | 500.0    | 499.98   | ND         | -            |                   |
| WW     | -        | 50.0     | ND         | 49.98        | 99.96±0.3         |
|        | 100.0    | 99.98    | ND         | 99.98±0.2    |                   |
|        | 500.0    | 499.97   | ND         | -            |                   |

Average of five replicate determinations of each Sample (n = 5). ND = Not Detected. SD = Standard Deviation.

| Sample | As added | Cr added | Method GPH | Found (µg/ml) | Recovery (%) ± SD |
|--------|----------|----------|------------|--------------|-------------------|
| F      | -        | 50.0     | ND         | 49.99        | 99.96±0.3         |
|        | 100.0    | 99.98    | ND         | 99.98±0.2    |                   |
|        | 500.0    | 499.96   | ND         | -            |                   |
| L      | -        | 50.0     | ND         | 49.99        | 99.98±0.2         |
|        | 100.0    | 99.98    | ND         | 99.98±0.2    |                   |
|        | 500.0    | 499.96   | ND         | -            |                   |
| P1     | -        | 50.0     | ND         | 49.97        | 99.94±0.3         |
|        | 100.0    | 99.97    | ND         | 99.97±0.3    |                   |
|        | 500.0    | 499.95   | ND         | -            |                   |
| P2     | -        | 50.0     | ND         | 49.98        | 99.96±0.3         |
|        | 100.0    | 99.98    | ND         | 99.98±0.2    |                   |
|        | 500.0    | 499.96   | ND         | -            |                   |
| B      | -        | 50.0     | ND         | 49.98        | 99.96±0.3         |
|        | 100.0    | 99.97    | ND         | 99.97±0.3    |                   |
|        | 500.0    | 499.96   | ND         | -            |                   |
| IW     | -        | 50.0     | ND         | 49.98        | 99.96±0.3         |
|        | 100.0    | 99.98    | ND         | 99.98±0.2    |                   |
|        | 500.0    | 499.95   | ND         | -            |                   |
| SW     | -        | 50.0     | ND         | 49.98        | 99.96±0.3         |
|        | 100.0    | 99.99    | ND         | 99.99±0.1    |                   |
|        | 500.0    | 499.95   | ND         | -            |                   |
| WS     | -        | 50.0     | ND         | 49.98        | 99.96±0.3         |
|        | 100.0    | 99.98    | ND         | 99.98±0.2    |                   |
|        | 500.0    | 499.96   | ND         | -            |                   |
| WW     | -        | 50.0     | ND         | 49.96        | 99.92±0.3         |
|        | 100.0    | 99.98    | ND         | 99.98±0.3    |                   |
|        | 500.0    | 499.94   | ND         | -            |                   |

Average of five replicate determinations of each Sample (n = 5). ND = Not Detected. SD = Standard Deviation.
IV. DISCUSSIONS

Organic compounds containing different functional groups have been used as analytical chromogenic reagents in the heavy metal analysis in real samples [3] and [5]. The chromogenic reagent used in this study is glutaraldehyde phenyl hydrazone. The ligand generally was soluble and stable in dimethylformamide (DMF), dimethyl sulfoxide (DMSO) and 1,4-dioxane for forty eight hours at room temperature without heating. The colour formation and development was instantly. To avoid precipitation of the DMF, DMSO and 1,4-dioxane content of the final solution, the solvents used must not be below 20.00 % total volume as recommended by [6], [2] and [1].

The ligand: glutaraldehyde phenyl hydrazone tends to reduce the maximum wavelength of the absorptions of the metal complexes at the same working concentrations which tend to give better absorption for spectrophotometric determinations of metals in the UV-Vis region. The colour reactions were instantaneous and the method does not involve heating of the reaction mixture or pre-extraction of the components. The reagents gave colour reactions with metal ions in slightly acidic and slightly neutral regions. For the selection of the working wavelength, a number of solutions were made by mixing equal volume of the reagent concentration (1.00×10^{-3} mol/dm^{3}) and (1.00×10^{-4} mol/dm^{3}), of the salt of metal of interest in a 25.00 cm^{3} volumetric flask and made to the mark with deionized water. The absorbances were scanned between 190-800.00 nm in a 1.00 cm^{3} cuvette to determine the maximum wavelength (\lambda_{max}) of absorption using the UV-Vis spectrophotometer [7].

The quantitative determination of the selected heavy metals (Cd, Pb, Cr and As) in the soil, water and biological samples was based on the measurement of increase and reduction of the absorbance of the [Metal-GPH] [8].

Method validation: under the optimized conditions, the calibration curves were constructed by plotting the absorbance signal against the concentrations of each analyte subjected according to the general procedure [5]. The solutions were transferred into the optical cell of 1.00 cm^{3} for the measurement of each metal ion spectrophotometrically at the respective absorption maximum against a reagent blank prepared under similar conditions. The calibration graphs follow a straight-line equation \text{Y} = ac + b; where c is the concentration of the solution, \text{Y} is the measured absorbance, a and b are the constants. The Beer’s law equation for the reagent GPH is: \lambda_{max} (Cd-GPH 387.0 nm) \text{nm} = 0.2419 X + 0.017 ; r = 0.8109, \lambda_{max} (Pb-GPH 395.0 nm) \text{nm} = 0.2549X + 0.091; r = 0.7581, \lambda_{max} (Cr-GPH 360.0 nm) \text{nm} = 0.2551 X + 0.089; r = 0.8651, \lambda_{max} (As-GPH 395.00 nm) \text{nm} = 0.2654 X + 0.074 ; r = 0.922 which indicated that the methods are moderately sensitive as described by [9], [10], [7], and [4].

The effect of pH on formation the metal-reagent complex was studied based on the method reported by [7] and [11]. In a series of 10.00 cm^{3} volumetric flasks, 2.00 cm^{3} solution (1.00×10^{-3} mol/dm^{3}), of the salt of metal of interest, 3.00 cm^{3} of the hydrazone solution (1.00×10^{-4} mol/dm^{3}) and 4.00 cm^{3} of the buffer of varying pH were added and made up to the mark with deionized water and the absorbances were measured against the ligand blank at the scanned and recorded wavelength. A plot was then made between absorbance and pH in the UV-Vis range. The pH with the highest absorbance was determined as the working pH for each metal of interest. It was noted that the metal-ligand complexes had higher absorbances at pH between 6.5-7.5 which was slightly acidic to almost neutral and was resolved to be the working pH for the study.

The precision of the developed quantitative Uv-Vis spectrophotometric method for determination of selected heavy metals (Cd, Pb, Cr and As) in soil, water and biological samples with the synthesized phenyl hydrazones; glutaraldehyde hydrazone (GPH) as chromogenic reagents is described. The coloured metal-ligand complexes were soluble and stable with higher absorbances in dimethyl formide (DMF) at pH of 6.5-7.5 having maximum absorbances (\lambda_{max}) of; Cd-GPH (387.00 nm), As-GPH (395.00 nm), Pb-GPH (395.00 nm), Cr-GPH (360.00 nm). The complexation of the phenyl hydrazones with the Cd, Pb, Cr and As markedly altered the wavelength of absorption of the complexes and this phenomenon can be utilized in the spectrophotometric determination of these metals [12] and [13]. Five replicate concentrations of the cadmium, lead, chromium and arsenic in the samples under observations were evaluated. The relative standard deviation (n = 5) was between 0.00 - 3.00 % for the proposed method and all the metals analysed which is in agreement with the results of [13]-[17] and [5] indicating that the developed method is highly precise and reproducible as shown in Table V to VIII, respectively.

The effect of possible interring species (cations and anions) which are generally associated with the determination of those metals under study at 1:5, 1:10 and 1:15 folds was evaluated. The interfering species were studied by measuring the absorbance of the reaction mixtures with and without the various species. An error of \pm 5.00 % in the absorbance reading was considered tolerated limit. From all the interfering cations and anions studied at the various folds, their corresponding absorbances were within the tolerable limits of the \pm 5.00 % [17] and [5]. The performance of the developed methods was evaluated by determining the concentrations of cadmium, lead, chromium, and arsenic in biological, environmental, soil, water, and waste samples, the results presented in Table I to IV. The present method was simple, rapid and very sensitive for non-extractive.

Evaluation of the developed method for its accuracy and precisions with Atomic Absorption Spectroscopy (AAS) as reference method was reported. The developed method GPH were applied for the quantitative determination of cadmium, lead, chromium, and arsenic in environmental, biological, soil, water, and waste samples, and were evaluated by adding known amount of cadmium, lead, chromium and arsenic salts at 50, 100 and 500 µg mL^{-1} in five replicates (n = 5). The recoveries were 99.2 to 99.99 % as shown in Table V to VIII which are in good agreement with the works of [1], [3], [18], [4], [13], [6] and [8]. The result indicates that the found values are in a very concordance indicating the good accuracy of the developed method. The samples were digested according to standard methods already reported.
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