

# The Synthesis, Characterization and Spectroscopic Study of Glutaraldehydiphenyl Hydrazone as Chromogenic Reagent for Spectrophotometric Determination of Selected Toxic Heavy Metals (Pb, Cr, Cd and As) in Water and Biological Samples.

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## Abstract

In this work, synthesis, characterization and spectrophotometric study of glutaraldehydiphenyl hydrazone (GDP), a chromogenic reagent for determination of selected heavy metals in water and soil samples has been carried out. The results obtained showed product yield of 79.0%, nitrogen content of 18.91% and melting points of 129-131°C. Infrared spectra data obtained showed absorptions bands at regions around 3492.5, 3451 for -NHR groups and stretches at 3085 and 1604 for -CH and C=N respectively. The nuclear magnetic resonance (<sup>13</sup>C-NMR) spectrum showed peaks around 111.0-141.0 for aromatic carbons, 129.3ppm for cyano carbon atom and 40.0, 39.2 and 31.7ppm for three methylene carbons. The <sup>1</sup>HNMR spectrum revealed peaks at 1.64-1.69 ppm for multiplet -CH<sub>2</sub>- protons, 2.214-2.386 ppm for quartet -CH<sub>2</sub>- protons, 7.331-7.345 ppm for -CH protons, 7.015 ppm for -NH signal and 6.59-7.34 ppm for aromatic protons. The spectral analysis of the glutaraldehydiphenyl hydrazone complexes revealed wavelength ranging between 360.0 (Cr) to 395.0 nm for (Pb and As) as maximum absorption at a working pH of 6.5 to 7.5 at 37°C. The reagent showed a molar absorptivities (L mol<sup>-1</sup> cm<sup>-1</sup>) ranging from 2.213 x 10<sup>4</sup> (Pb) to 2.460 x 10<sup>4</sup> (As), a mole ratio of metal to ligand of 2:1, detection limits (µg/g) ranging from 0.3432 (As) to 0.5250 (Pb) and the metal-ligand complex was stable for 0-48 hours. Additionally, the compounds Beer's law validity ranges from 0.001 to 100 (mgL<sup>-1</sup>). The spectrophotometric results were compared with atomic absorption spectroscopy results and the results were satisfactory with good precision for the GDH method.

**Keywords:** Absorption, Complex, Protons, Carbon, Spectra, Ligand, Analysis.

## 1. Introduction

The detection of metal ions especially transition metals both in solution and in solid materials is of increased concern as a result of increased environmental pollution worldwide. Metals have continued to play important role in biological activities of all living organisms for nutrients' balance especially where they are present in substances in tolerable and permissible limits [1]. In particular, transition metals deficiency causes many clinical disorders and physiological abnormalities in the human body and some metal ions may cause serious health problems and toxicity to humans, animals as well as to plants [2]. Researchers were able to

resolve many industrial and biological problems with the aid of complexation of metal ions [3]. 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. A molecule or an ion exhibits absorption in the visible or ultra-violet region when the radiation causes an electronic transition in molecules containing one or more chromophoric groups. The colour of a molecule may be identified by substituents called auxochromic groups, which displace the absorption

maxima towards longer wavelength (bathochromic shift). The colour determining factors in many molecules is the introduction of conjugated double bonds by means of electrons donor and electron acceptor groups [4]. Hydrazones have interesting analytical properties due to the presence of several potential coordination sites. Also, hydrazones have attracted much attention as analytical reagents, their applications as photometric and fluorimetric analytical reagents for the determination of metal ions, have been reviewed. The formation of a highly fluorescent chelate through the combination of a metal ion and an organic ligand has often proved to be sensitive and specific method for the determination of metal ions particularly those which are difficult to measure by atomic absorption spectroscopy.

Organic reagents containing the atomic arrangement like hydrazones have been widely used for the spectrophotometric determination of metal ions because of their great complexing capability to form coloured complexes with transition metal ions [5]. Notably, such compounds are capable of behaving as tridentate chelating agents, considering the donor atoms involved and the steric restrictions imposed which can make them to be selective in their reactions with metals ions, and such ligands have shown significant possibilities as analytical reagents. The availability of heavy metal ions in biological materials is usually influenced by a number of factors including physical factors such as temperature, adsorption and phase association, biological factors like biochemical adaptation, physiological adaptation and species characteristics and chemical factors [6]. However, it is expedient to note that, it is the chemical factor affects mostly the formation of more ions in the course of their reactions biological systems [7]. Therefore, this work is aimed specifically on synthesis, characterization and evaluation of Glutaraldehydephenyl hydrazone as a chromogenic reagent for spectrophotometric determination of selected toxic heavy metals (Pb, Cr, Cd and as) in water, environmental and biological samples.

## 2. Materials and Methods

### **Instruments for Spectral study and heavy metal analysis:**

All absorbance measurements were made on a U/V-Vis spectrophotometer matched with a 1.0cm quartz cell, (Model-1800) double beam Jenway (England, UK). An air-acetylene Atomic Absorption spectrophotometer (AAS) for comparing results. pH meter (Hanna, U.S.A) with combination of electrodes, A Shimadzu (Model-prestige 21) FTIR spectrophotometer, range 7500-350  $\text{cm}^{-1}$  to record FTIR spectrum, An Agilent-NMR-vnmrs 400.

**Reagents/Chemicals for synthesis and analysis:** The following reagents were used; N, N-dimethylformamide, 1,4-Dioxane, Dimethyl sulfoxide, Potassium dichromate, Universal Buffer, Cadmium chloride, glutaraldehydephenyl hydrazine, Lead nitrate, Arsenic trioxide. All the chemicals used were of analytical reagent grade of (>85%) and distilled and deionized water were used in the preparation of all reagents and solubility tests.

**Preparation of buffer solutions:** The required pH buffer solutions were prepared by dissolving 1.0 M sodium acetate

and 0.1M hydrochloric acid (pH 0.5 - 3.0), 0.2 M sodium acetate and 0.2 M acetic acid (pH 3.5 - 6.0), 1.0 M sodium acetate and 0.2 M acetic acid (pH 6.5 - 7.5), 2.0 M ammonia and 2.0 M ammonium chloride (pH 8.0 - 12.0) in distilled water. Equal volumes of these solutions were mixed to get the desired and adjusted pH [8]. All metals show maximum and constant colour development in basic phosphate buffer medium pH range 6.5 - 7.5.

**Determination of working pH range:** In a series of 10.0  $\text{cm}^3$  volumetric flasks, 2.0  $\text{cm}^3$  solution ( $1.0 \times 10^{-4}$  M), of the salt of each heavy metal, 2.0  $\text{cm}^3$  of the hydrazone solution ( $1.0 \times 10^{-4}$  M) and 4.0  $\text{cm}^3$  of buffer solution of varying pH were added and made up to the mark with deionised water and the absorbance were measured against the ligand blank at a determined 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 [9].

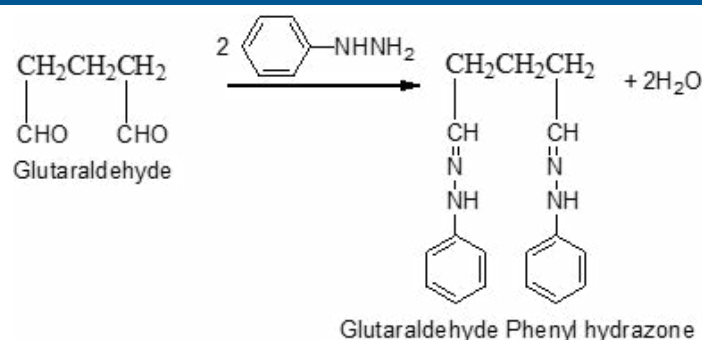
**Determination of solubility of the ligands:** The solubility of the glutaraldehydephenyl hydrazone was tested in different organic and aqueous solvents. The common organic solvents tested include N, N-dimethylformamide (DMF), demethyl sulfoxide (DMSO) and 1, 4-dioxane.

**Solutions for spectrophotometric analysis:** Standard stock solutions of the metals of interest (1000 ppm), of Pb, Cd, As, and Cr were prepared by dissolving requisite quantities of the salt of Lead nitrate, cadmium, and Arsenic oxide. The solutions were serially diluted to the required working standards of interest. The various chromogenic reagents in  $1.0 \times 10^{-4}$  M were prepared in each solvent (N, N-dimethylformamide, demethyl Sulphur oxide and 1, 4-dioxane) and diluting it to 100  $\text{cm}^3$  in volumetric flask [10].

**Working concentrations of the reagent solution:** Known aliquot of metal ion solution was taken into a set of 25.0  $\text{cm}^3$  volumetric flask containing 5.0  $\text{cm}^3$  of buffer solution of different pH. Different known aliquots of the reagent solutions were added to each of the flasks and the contents were made up to the mark with distilled water. The absorbances of each solution were measured against the corresponding reagent blank solution. Based on the results, the required concentrations of the reagent for complete colour development were determined and extrapolated [10].

### **Synthesis and characterization of the glutaraldehydephenyl hydrazone:**

The reaction mixture containing glutaraldehyde (0.050mol, 4.7ml in 20ml ethanol) and phenyl hydrazine (0.1mol, 9.85g in methanol) dissolved in hot condition was taken into a round bottom flask and the mixture was refluxed for 8hours. On cooling, the reaction mixture gave a dark brown coloured product which was collected by filtration. The compound was recrystallized twice with ethanol and the melting point was determined ( $^{\circ}\text{C}$ ). The compound was characterized by FTIR and NMR spectral studies. The preliminary purity of the products obtained was confirmed by elemental analysis [11].



**Scheme 1: Synthesis of glutaraldehydephenyl hydrazone.**

**Determination of nitrogen content of the synthesized glutaraldehydephenyl hydrazone:** Preliminary investigation of the chemical and elemental constituents present in the product was carried out. The nitrogen content of the compound was carried out by Khjedal methods as reported in Jamaluddin (2012). About 0.2g of the sample was placed in Khjedal flask and digested with 4.0ml concentrated sulphuric acid. A tablet of khjedal was added to the mixture and the mixture was then heated in a fume cupboard until it became clear. The content was cooled and transferred quantitatively into a distillation apparatus; few anti-bombing granules were added. Calculated quantity of sodium hydroxide solution (40%) was added and the mixture was heated to boiling, and the end of the condenser was dipped into a collection flask containing 100ml 0.1M hydrochloric acid. The distillation was continued until all the ammonia gas evolution ceased and the excess acid was titrated against a standard 0.1M sodium hydroxide using phenolphthalein indicator [12].

**Evaluation of the analytical properties of the synthesized glutaraldehydephenyl hydrazone:** The following analytical parameters were analysed on the synthesized hydrazones to ascertain its analytical properties.

**Determination of the working wavelength:** For the selection of the working wavelength, a number of solutions were made by mixing equal volume of the reagent concentration ( $1.0 \times 10^{-4}$  M) and ( $1.0 \times 10^{-4}$  M), of the salt of each metal in a 25.0 cm<sup>3</sup> volumetric flask and made to the mark with deionized water. The absorbance was scanned between 190-800 nm in a 1.0 cm<sup>3</sup> cuvette to determine the maximum wavelength of absorption ( $\lambda_{\text{max}}$ ) using the UV-Visible spectrophotometer. The wavelength of maximum absorbance for each complex was noted [13].

**Stability of the chromogenic reagent:** To establish the maximum storage time of the chromogenic reagent solution without loss of reactivity, analysis was performed to establish the stability of the organic ligand over time. About 1.0 cm<sup>3</sup> of  $5 \times 10^{-4}$  M of each of the metals was taken into 10.0 cm<sup>3</sup> volumetric flask and then 5.0 cm<sup>3</sup> of  $5.0 \times 10^{-4}$  M of the hydrazones complex was added. The pH of these solutions was adjusted to the range of 6.5 - 7.5 using acetate buffers. Then, each solution was made to the mark with deionized water and the flasks were stored at room temperature. The stability of these reagents was thereafter followed

spectrophotometrically after 0, 3, 6, 9, 12, 24, 48, 72, 96 and 120 hours [13].

**Determination of working wavelength of the metal-ligand complexes:** For each metal, 1.0 cm<sup>3</sup> of  $5.0 \times 10^{-4}$  M of salt solution was taken into 25 cm<sup>3</sup> volumetric flask and then 5.0 cm<sup>3</sup> of  $5.0 \times 10^{-4}$  of the hydrazones complex was added and mixture was thoroughly stirred. The pH of the solutions was then adjusted to range of 6.5 - 7.5 using acetate buffers and each solution was made to the mark with deionized water. The absorbance was then determined in the entire UV-visible region against reagent blank. The spectra of the reagent were also measured in the same wavelength region against the organic solvent of used. The plot of absorbance against wavelength was also extrapolated on the same graph and the working wavelength was chosen where there was maximum difference between the absorbance due to complex and reagent [13].

**Applicability of Beer's law:** The known aliquots of 10.0 cm<sup>3</sup> solutions, each containing constant volume of 4.0 cm<sup>3</sup> of buffer (desired pH), 3.0 cm<sup>3</sup> of  $1.0 \times 10^{-4}$  M of hydrazone and 2.0 cm<sup>3</sup> of corresponding salt of each metal of interest ranging from  $0.1 \times 10^{-4}$  to  $1.0 \times 10^{-4}$  M. The absorbances were measured at optimal wavelength for each metal and a graph was plotted between the concentration of the metals and their absorbances. A straight-line graph passing through the origin obeys Beer's and the correlation coefficient which indicates the linearity between the two variables, the molar absorptivity coefficient and Sandell's sensitivity of the ligand-metal complexes was obtained from the plot [13].

**Composition and stability constants of the hydrazone-metal complexes:** The molar ratio methods were used to determine the composition of the complexes as reported in Humaira *et al* [14]. To 25.0 cm<sup>3</sup> volumetric flask, 5.0 cm<sup>3</sup> of buffer solution was transferred into 10.0 cm<sup>3</sup> of metal ion solution and varying volumes of the solutions were added and the contents of each flask were made up to the mark with double distilled water. The absorbance of each solution was measured at a chosen wavelength against the solution blank containing 5.0 cm<sup>3</sup> buffer solution and the required volume of each reagent. From the plot between the absorbance and the volume of the reagent, the composition of the complex was computed.

Sample collection and treatment: The sample (waste water and soil) in triplicates were collected into sterilized polythene bags from mechanic workshop village in Otukpo Metropolis in Benue State, Nigeria and were transported to the laboratory of Federal University of Health Sciences Otukpo, where they were stored in refrigerator at regulated temperature for analysis.

### 2.1. Preparation of sample

• **Water sample:** Each of the collected waste water collected (500.0 cm<sup>3</sup>) was filtered using a filter paper (Whatman No.41) and then each filtered water sample was evaporated nearly to dryness with a mixture of 5.0 cm<sup>3</sup> of concentrated H<sub>2</sub>SO<sub>4</sub> and 10.0 cm<sup>3</sup> concentrated HNO<sub>3</sub> in a fume cupboard and then cooled to room temperature. In order to dissolve the salts, the residue was then heated with 15.0 cm<sup>3</sup> of distilled

water and after cooling, the solution was neutralized with dilute NH<sub>4</sub>OH solution and the obtained solution was filtered into 25.0 cm<sup>3</sup> standard flask and made up to the mark with distilled water according to the procedure reported in Adi *et al* [15].

• **Soil sample:** The soil samples were placed in a furnace and heated to 400°C for 15 minutes and thereafter were removed from the furnace, cooled and moistened with drops of distilled water. Then, 2.0 cm<sup>3</sup> of concentrated HCl was added and the sample was evaporated to dryness, removed, and 5.0 cm<sup>3</sup> 2.0 M HCl was again added and the mixture was swirled. The solution was filtered using Whatman filter paper (#42) and then it was transferred quantitatively into a 50 cm<sup>3</sup> flask and was made to the 50 cm<sup>3</sup> with distilled water [16].

### 3. Results and Discussion

**Table 1: Physicochemical properties of the synthesized glutaraldehydiphenyl hydrazone**

FI-IR Analysis		<sup>13</sup> C-NMR		<sup>1</sup> H-NMR	
Absorption (cm <sup>-1</sup> )	Inference	δ (ppm)	Inference	δ (ppm)	Inference
3492.5, 3451	N-H stretch (due to the two -NHR groups)	111.0 to 141.0	Due to aromatic carbon atom	1.64–1.69(m)	Due to multiplet CH <sub>2</sub> - protons
3085	=CH stretch	129.3	Due to Cyano carbon atom.	2.214-2.386(q)	Due to quartet -CH <sub>2</sub> protons=C-H protons
3034	Phenyl N-H group	40.0, 39.2 and 31.7	Due to the three-methylene carbon.	7.3317.345, 7.015	Due to -NH signal
1604	C=N stretch (Cyano group)			6.59-7.34	Due to aromatic protons

**Table 2: FT-IR and NMR properties of the synthesizes glutaraldehydephenyl hydrazone.**

Properties	Result
Yield (%)	84
Melting point (°C)	129-131
Colour	Dark brown
Nitrogen content (%)	19.01

The elemental analysis of the nitrogen content of the synthesized compound yielded 19.01 % (calculated value 19.15%). The data shows good agreement between experimental and calculated values as reported by Jamaluddin and Zanat [12]. The Infrared spectra (IR) of glutaraldehydiphenyl hydrazone (FT-IR) shown in (Figure 1) showed the presence of string bands and shoulders assigned to C=N, NH<sub>2</sub> and phenyl C=C. Additionally, the NMR spectra showed peaks associated to cyano carbon atoms, aromatic carbon atoms and NH<sub>2</sub>. From the results of the IR and NMR analysis, the structure shown for hydrazones can be suggested to be their composition and the IR spectrum of the compound (in Figure 1) showed peaks at 3492.5 cm<sup>-1</sup> and 3451 which are attributed to the amino (-NHR) groups in the compound. The spectrum also revealed a peak at 3085 cm<sup>-1</sup> assignable to the =CH groups and 3034 cm<sup>-1</sup> phenyl N-H groups and a peak at 1604.0 cm<sup>-1</sup> assignable to the cyano

group respectively as shown in figure 2 and 3, and in table 2.

The NMR spectra of Glutaraldehydephenyl hydrazone (in DMSO) showed signals between 111-140 ppm associated to aromatic carbon atoms, while signal at 129.3 ppm is assignable to cyano carbon atom. The three methylene carbon atoms signals appeared at 40, 39 and 31 ppm. The protons signal between 6.59-7.34 ppm are assignable aromatic protons, 1.64-2.38 ppm are for aliphatic protons, while N-H signal and olefinic proton were observed at 2.38 and 6.83 ppm respectively. The <sup>13</sup>C-NMR and <sup>1</sup>H-NMR spectra of glutaraldehydephenyl hydrazone (in DMSO) are shown in figure 3 and 4 respectively. The <sup>13</sup>C-NMR spectrum showed signals between 111.0-141.0 ppm associated to aromatic carbon atoms, while signal at 129.3 ppm is assignable to cyano carbon atom. The three methylene carbon atoms signals appeared at 40.0, 39.0 and 31.7 ppm. The <sup>1</sup>H-NMR

spectrum revealed signals between 1.64-1.69(m) ppm due to multiplet  $-CH_2-$  protons, 2.214-2.386(q) ppm are associated to quartet  $-CH_2-$  protons atoms while 7.331-7.345

ppm are due to  $-C-H$  protons. In addition, the peak at 7.015 ppm was due to  $-NH$  atoms and the 6.59-7.34 ppm is for the assignable aromatic protons as shown in figure 2 and 3.

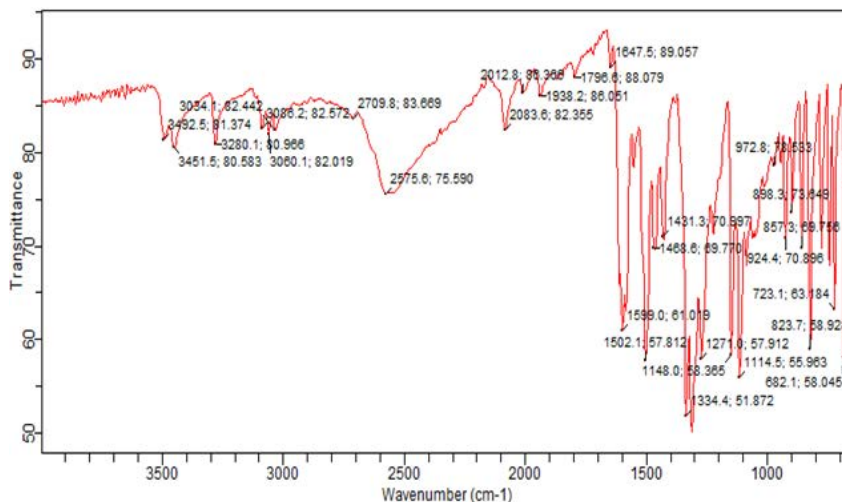


Figure 1: IR spectrum of glutaraldehydiphenyl hydrazone

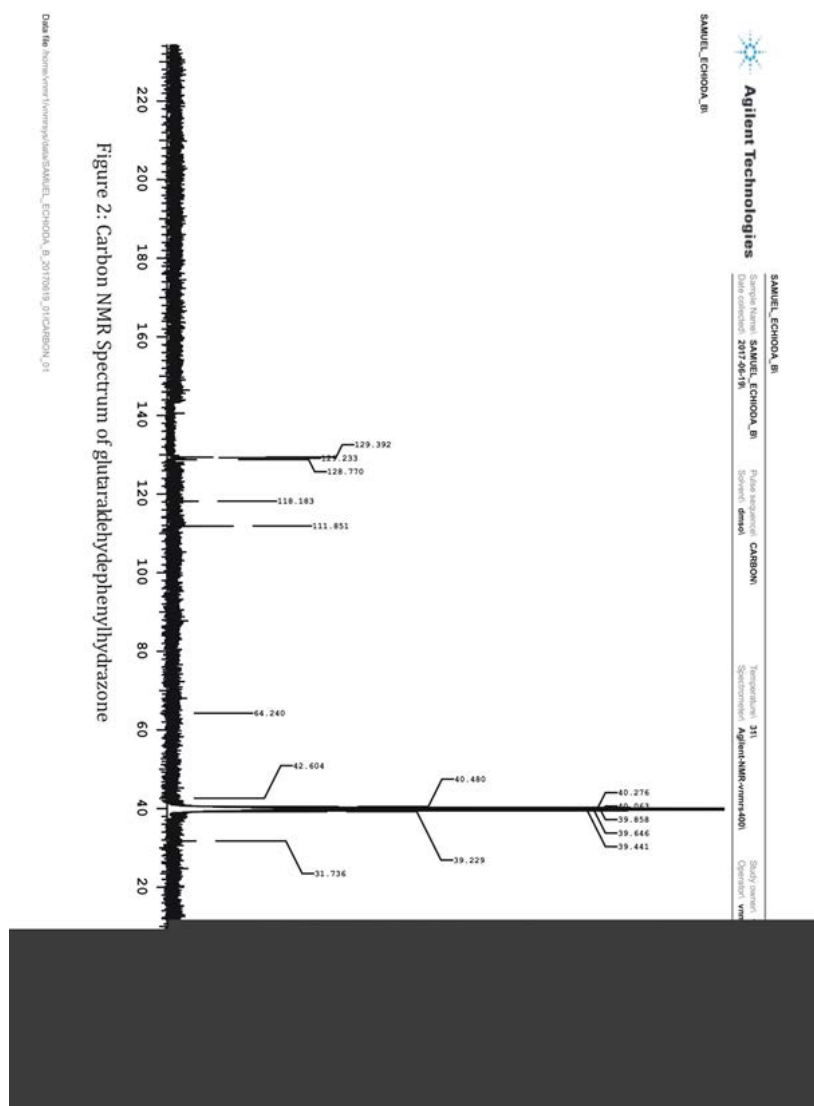


Figure 2: Carbon NMR spectrum glutaraldehydiphenyl hydrazone

Table 3: Solubility of the synthesized hydrazone in various solvents.

Solvent used	Solubility of (GDPH)	Absorbance of GDPH
Water	Less soluble	----
Ethanol	Soluble	----
Dimethylformide	Soluble	0.511
Dimethylsulfoxide	Soluble	0.487
1,4-dioxane	Soluble	0.352
Methanol	----	0.319
Chloroform	-----	0.327

Table 4: Analytical characteristic of metal complexes of glutaraldehydephenyl hydrazone (metal-GPH)

characteristics	Cd-GPH	As-GPH	Pb-GPH	Cr-GPH
Selected wavelength ( $\lambda_{max}$ ) nm	387.0	395.0	395.0	360.0
Selected (pH)	6.5-7.5	6.5-7.5	6.5-7.5	6.5-7.5
Working temperature (°C)	37±2	37±2	37±2	37±2
Molar absorptivity ( $\epsilon$ ) ( $Lmol^{-1}cm^{-1}$ )	2.261x10 <sup>4</sup>	2.460 x10 <sup>4</sup>	2.213 x10 <sup>4</sup>	2.274x10 <sup>4</sup>
Sandell's sensitivity ( $\mu g/cm^2$ )	0.00442	0.00406	0.00452	0.00440
Mole ratio (M: L)	2:1	2:1	2:1	2:1
Detection Limit ( $\mu g/g$ )	0.4933	0.3432	0.5250	0.3825
Quantification limit ( $\mu g/g$ )	1.4948	1.040	1.5909	1.1590
Complex Stability (h)	0-48	0-48	0-48	0-48
Beer's Law validity range/ $mgL^{-1}$	0.001-100	0.001-100	0.001-100	0.001-100

Table 5: Result of concentrations of heavy metals.

Name of Sample	AAS method (ppm) ( $\bar{x} \pm SD$ )	GPH method (ppm) ( $\bar{x} \pm SD$ )
Waste water (ww)	0.878±0.0003	0.877±0.060
Waste water sand (wws)	1.173±0.0002	1.173±0.030

Note:  $\bar{x}$  is mean from the set of results; SD is the standard deviation for the set of data with n = 3.

Organic compounds containing different functional groups have been used as chromogenic reagents in the trace metal analysis. The chromogenic reagent used in this study is glutaraldehydiphenyl hydrazone. The ligand generally is soluble and stable in dimethylformamide (DMF), dimethylsulfoxide (DMSO) and 1, 4-dioxane for between thirty-six to forty-eight hours at room temperature without heating. The colour formation and development were instantly as shown in table 2. To avoid precipitation of the DMF, DMSO and 1, 4-dioxane content of the final solution, the solvents used was not below 20% of the total volume as recommended by Cristofol *et al* [11]. Glutaraldehydiphenyl hydrazone tend 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 which agrees with the report of Okoye *et al* [10].

The working pH for the metal-reagent complex was selected

based on the method reported by Nityananda *et al*, it could be seen 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 [9]. The sensitivity of the spectrophotometric method is often described in terms of the molar absorptivity ( $\epsilon$ ,  $L mol^{-1}cm^{-1}$ ) of the metal-ligand complex. The awareness of the sensitivity is very important in spectrophotometric determination of trace metals. Sanagi *et al* have suggested a relation between sensitivity and molar absorptivity [17]. They stated the following criteria for describing the sensitivity. Low sensitivity  $\epsilon < 2 \times 10^4 L mol^{-1} cm^{-1}$ , Moderate sensitivity  $\epsilon = 2 - 6 \times 10^4, L mol^{-1} cm^{-1}$  and High sensitivity  $\epsilon > 6 \times 10^4, L mol^{-1}cm^{-1}$ . From the data generated the molar absorptivity for GPH ranged 2.213 x 10<sup>4</sup> for Pb-GPH to 2.460 x 10<sup>4</sup> L mol<sup>-1</sup> cm<sup>-1</sup>. Importantly, the values obtained have indicated that they could be classified as moderately sensitive and the reactions and measurements could be made highly selective by using appropriate masking agents

[12, 14]. The values of the molar absorptivity were higher than the values reported in Fahrudin et al, Jamaluddin and Tasnima and Srikanth and Chandra and Saritha *et al* and were improved by using appropriate masking agents [12]. The mole ratio of metal to ligand in the complexes was 2:1 and the detection limit was the smallest concentration that can be detected with a high degree of certainty [18, 19]. Based on the standard deviations of the reagent blank and the slope of the calibration curve of the analyte, the detection limit for glutaraldehydiphenyl hydrazone (GPH) ranged from 0.3432 µg/g (As) - 0.5250 µg/g (Pb). The stability of the colour complex is influenced by experimental conditions like temperature and pH when the various concentrations have been maintained. The solutions formed were clear and free from precipitate as precipitates tend to scatter as well as absorb light. The reagent complex for metal-GPH for the metals studied (Cd, As, Pb, and Cr) were stable for 0-48 hours spectrophotometrically as tabulated in table 4. The preliminary investigations on GPH as possible chelating reagents for the spectrophotometric determination of heavy metals in waste water and soil samples was carried out and the results were compared with the results from atomic absorption spectroscopy (AAS). The results obtained were satisfactory with good precision and accuracy as shown in table 5 [20-56].

#### 4. Conclusion

It is a new and adopted approach that serves as an alternative for standard method in determining the concentrations of heavy metals. The present method was simple, inexpensive, sensitive, rapid and reasonably selective without the need for heating or extraction. The hydrazone was easy to synthesize and purify with available chemicals. The most favorable characteristic of these reagents is that it forms water soluble complexes which facilitate the determination of metals in biological, soil, water and waste water samples. The method offers a good procedure for speciation analysis of heavy metals, although, many sophisticated techniques, such as pulse polarography, High performance chromatography, ICP-MS, AAS are available for the determination of heavy metals at trace levels in numerous complex materials.

#### Conflict of Interest

The authors have declared that, there is no conflicts of interest for this article

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