Synthesis and Characterization of the new 2- [2-(5-Nitro Thiazolyl) Azo]-4,6-Dibromo phenol (NTADBrP) as an Analytical Reagent for Determination of Copper (II) with Anticancer Study

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Abstract

The new azo reagent 2- [2⁻ -(5-nitro thiazolyl) azo] -4,6-Di bromo phenol (NTADBrP) was prepared and examined by using, UV-Vis, infrared spectra, H-NMR and mass spectrum. A sensitive and selective spectrophotometric method is proposed for the rapid determination of copper (II) using (NTADBrP) as anew spectrophotometric reagent. The reaction between this reagent and copper (II) is instantaneous at 589 *nm* (λ_{max}) and pH=9 to form red complex having a mole ratio 1 : 2 (metal : Reagent) and the absorbance remains stable for over 24 hr. Beer's law is obeyed in the range of (1-40) $\mu g.ml^{-1}$ with a detection limit of 0.3419$\mu g.ml^{-1}$ The precision and accuracy were obtained to be R.S.D%=0.57%,Re%=97.02% and %E_{rel}=2.98 %. By utilizing appropriate masking agents, the effect of ions (Co^{+2}, Ni^{+2}, Cr^{+3}, Pb^{+2}, Hg^{+2}, Cd^{+2}, Ag^{+1}) that interfere with the reagent (NTADBrP) was evaluated, as well as its impact on the proliferation of laryngeal cancer cell lines and normal cells.

1. Introduction

Organic reagents, which are compounds with high molecular weights that are poorly soluble in water, due to the nature of their covalent bonds, and they are characterized by having effective groups to give colored macro compounds in most cases that qualifies them to interact with many elements in the periodic table. It gave very important results [1] . A large number of polymeric dyes were prepared from the condensation of azo dyes with formalin. In the field of industry, heterocyclic azo compounds have played an important role. Thiazolyl azo reagents have been widely used as dye-generating reagent. In the field of analytical chemistry, it has exploited the dominant color characteristic of this type of compounds and their complexes formed with metal ions in their aqueous and organic solutions in spectroscopic analyzes called spectral reagents [2] . Copper is one of the important and essential nutrients for human health as well as the growth of animals and plants.1-7 Copper is required for normal metabolic processes. Copper combines with certain proteins to produce enzymes that act as catalysts to help a number of body functions. Copper helps provide energy required by biochemical reactions. Although copper is an essential micronutrient and is required by the body in very small amounts, excess copper in the human body can cause stomach and intestinal distress such as nausea, vomiting, diarrhea, and stomach cramps. The lowest level at which these adverse effects occur has not been well defined. People with Wilson's disease, a rare genetic disorder, are more sensitive to the effects of copper. Copper is also a commonly occurring element in natural waters. Most copper contamination in drinking water happens in the water delivery system, as a result of corrosion of the copper pipes or fittings.1 [3]. It was crucial to establish copper's function in the body because it is a necessary component of enzymes that transport oxygen and electrons and plays a role in redox processes [4] , which are involved in the mobilization of hemoglobin formation [5]. The presence of copper in the serum is a common illness sign [6]. Ion selective electrodes[7] flame atomic absorption spectrometry, electromagnetic atomic absorption spectroscopy [8], differential pulse adsorption stripping voltammetry, and others are used to determine the presence of copper [9] and the HPLC reverse phase [10] .There are many methods used for the determination of copper (II), including : Using the reagent 3-{2-[2-(2-hydroxyimino-1-methylpropylideneamino)-(ethylamino]-ethylimino}-butan-2-oneoxime),which gave a stable complex with a ratio of (1:1) at room temperature and at a wavelength 570 nm, and the value of the molar absorption coefficient was 0.16 $\times 10^{-4}$ liters.mol^{-1}. cm^{-1}, and the estimation range ranged from (0.2-225) ppm. Cancer is classified as a non-communicable and non-hereditary disease characterized by the uncontrolled growth of its cells. There are several types of cancer that are classified according to the type of cell that was affected first [11]. In this study, a 2-[2⁻ -(5-nitro thiazolyl) azo] -4,6-Di bromo phenol (NTADBrP) was used as an

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analytical reagent for the micro determination of Cu the mothed and it was discovered to be simple, rapid, and sensitive for the determination of these metal ions.

2. Materials and Methods

Instruments

(FTIR)Spectra (4000-400cm^{-1}) in KBr disks were recorded on a SHIMADZU FTIR-8400S fourier transform infrared spectrophotometer (Japan), absorption spectra were measured on aT80 UV-Vis spectrophotometer and absorbance were measured on Apel PD-303UVvisible spectrophotometer using 1 cm quartz cells, pH –meter was used to adjusted and measure the pH of the solution, melting point were measured using SMP30 Stuart, UK.

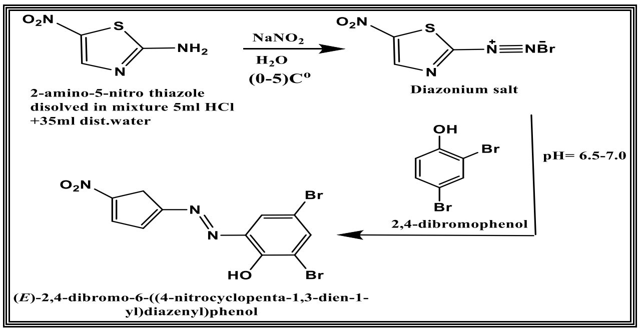
Materials

Ethanol Absolute(99.9% , Germeny) , Methanol Absolute(99.8% ,Germeny), Sodium hydroxide (97% , Alpha Chemika) , Hydrochloric acid(37% , J.T.Baker) , Sodium nitrite (96%, Alpha Chemika) , Dimethyl sulphoxide (98% , J.T.Baker) , Acetone (99.9% , J.T.Baker) , Sodium chloride(98% , CARLO ERBA) , Cupric chloride dehydrate(99% , Alpha Chemika) ,Glacial acetic acid(Analar, Germeny) , 2,4-dibromophenol (97% , Alpha Chemika) , 2-amino 5-nitro thiazole (99% , Glanthum) .

Methodology

Synthesis of 2- [2-(5-nitro thiazolyl) azo] -4,6-Di bromo phenol (NTADBrP)

The above reagent was prepared based on the method proposed by the researcher with some modifications to the method of work where (0.01) mole of the thiazole derivative was dissolved in a mixture consisting of 9 ml of concentrated hydrochloric acid and the mixture was cooled to 3 ° C. Then a solution of sodium nitrite prepared by dissolving (0.01mol) in 30 ml of distilled water was added drop by drop to the mixture with constant stirring, noting that the temperature did not rise above 3℃, then the solution was left for 20 minutes with constant stirring to complete the process. A brown orange dysonium salt solution was added dropwise with constant stirring to the solution consisting of 0.01 mol of 4,2-dibromophenol and dissolved in a mixture of 25 ml of ethanol and 15 ml of a 10% solution of sodium hydroxide. Then, the coloration of the mixture was observed in a dark red color, and after completing the addition, two hours later, a quantity of cooled water was added, and the medium was made at PH = 6.5-7, then the solution was left for 24 hours. As the reagent precipitates. It is filtered, washed with distilled water, recrystallized with absolute ethanol, then kept in an opaque bottle, and then its melting point was measured between (199-201)℃ .



Scheme 1: Describes the equations for the synthesis of the Organic Reagent (NTADBrP)

Standerd solution

Copper solution

Stock Cu (II) solution: A solution of Cu (II) (1000 μg/ml^{-1}) was prepared by dissolving (0.2680 g) of ($CuCl_2$.$2H_2O$) in 100 ml deionized water. Other standard Cu (II) solutions were prepared by diluting the stock solution with distilled water.

(NTADBrP) Solution

A standard solution of 3 * 10^{-4} M (NTADBrP) was prepared by dissolving (0.0306 g) in 250 ml of absolute ethanol.

Gerneral Procedure

The pH of the medium was adjusted using glacial acetic acid buffer solution, ammonium acetate 0.01 M, and ammonia (2-12). Transfer 2 ml of the sample solution, which has fewer than 100 Cu (II) in it, into a 10 ml calibrated flask, and use ammonium acetate buffer to bring the pH to 9. Deionized water was used to dilute the 3 M ethanolic (NTADBrP) solution to the proper concentration. After 5 minutes, the solution's absorbance at 589 nm and 25 C was measured in comparison to the comparable reagent blank made under the same circumstances but without copper.

3. Results and Discussion

FT-IR Spectra of the Reagent (NTADBrP) and its complex

Researchers have examined the NTADBrP-Cu(II) combination and reagent NTADBrP fig(1) infrared spectroscopy data. Certain distinguishing characteristics between the spectra of the reagent and the coordination Cu(II) - complex have been found. The following list of key developments and conclusions includes some of these:-

1- The spectra of the free reagent exhibits a broad and weak (O-H) absorption band at 3332.76 cm-1. This shows strong hydrogen bonds between molecules [12,13]. The meta hydroxyl group is responsible for the Cu(II)-Complex's extremely broad and feeble absorption band at 3317.34 cm-1 (O-H)..

2- Two faint bands were found in the spectrum of the free reagent at 2839.18 cm-1 and 2815.88 cm-1, which are attributed to (C-H) aromatic and aliphatic compounds, respectively. Both for the reagent and the Cu(II)-Complex, these bands are stable in both their position and their intensity.

3- The thiazole ring's (C=N) appearance appears at 1679.24 cm-1 in the reagent's spectrum[14,15]. This band changes somewhat in form and switches to a lower frequency at 1596.95.4 cm-1. These variations point to a link between the copper ion and the nitrogen of the heterocyclic thiazole ring[16].

4- The azo group (N=N) in the reagent's spectrum causes two absorption bands at 1504.37 cm-1 and 1458.08 cm-1. These bands' locations in the Cu(II)-complex spectrum are moved to lower frequencies at 1542.95 cm-1 and 1411.80 cm-1 with a reduction in strength. This may be a sign that the coordination with the copper ion has been affected. [17-19]

5- In the spectra of the reagent, there are two bands at 1265.22 cm-1 and 1211.21 cm-1 that are caused by the (C-S) of the thiazole ring [20]. The fact that these bands in the copper complex are unaffected indicates that the heterocyclic ring's sulfur atom is not involved in coordination.

6- The reagent's spectrum's modest absorption band at 840.91 cm-1 is caused by (C-Br).

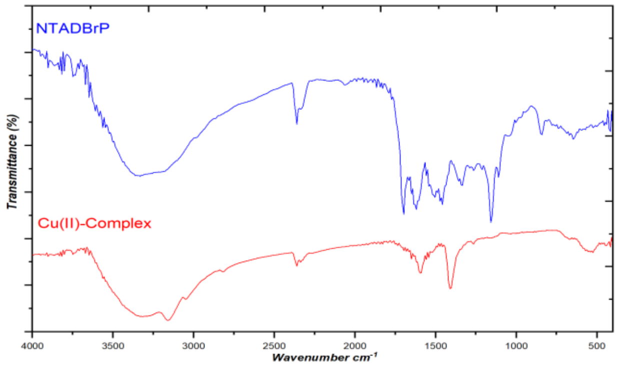


Fig 1: FT:IR spectrum of Reagent(NTADBrP) and Cu(II) complex

Physical of Properties (NTADBrP)

NTADBrP is a dark-red powder that is soluble in organic solvents such as ethanol, methanol, DMSO, acetone, and fig. 2 shows the UV-Vis spectra of the reagent in 100% ethanol solution. It is only marginally soluble in water.

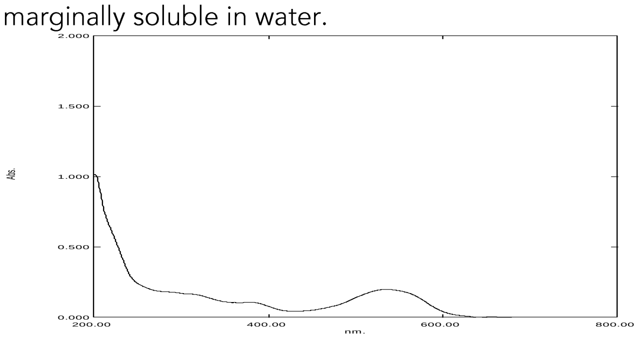


Fig 2: absorption spectra UV-Vis of the reagent (NTADBrP) =3 $\times 10^{-4} M$

Best Solvent of the Reagent (NTADBrP)

A study was conducted to find out the solubility of the reagent in some solvents, namely ethanol, acetone, dimethyl sulfoxide, methanol. By dissolving (0.00306) g of the reagent in one of the mentioned solvents in a volumetric bottle of 25 ml to prepare it at a concentration of (3 x 10^{-4}) M, then a spectral survey of the reagent solution versus the solvent as a reference. The purpose of this study is to know the best solvent for the reagent under study and to use it To do all the experiments, Where it was observed that the solvents of acetone and dimethyl sulfoxide are affected with the reagent due to a change in the spectrum of the reagent and the emergence of new peaks and the absence of other peaks. The toxicity of methyl alcohol is not preferred, as the fig 3.

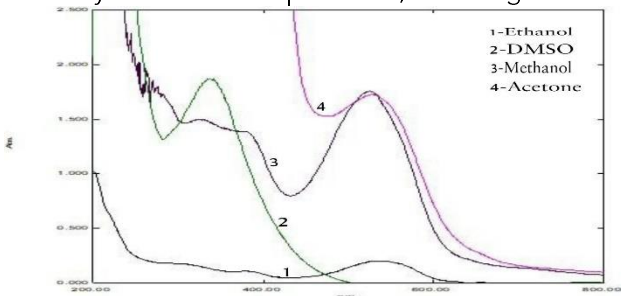


Fig3: absorption spectra UV-Vis of the reagent (NTADBrP) in different solvents

Optimum concentration of the reagent (NTADBrP)

Cu (II) solution of constant concentration (20 μg.ml[1]) is placed in a volumetric flask with a capacity of 10 ml. The effect of reagent concentration (3 x 10^{-4} M) on absorbance of the complex was studied by varying the volume of reagent (0,5-4.0) ml. It was discovered that the formation was complete and gave maximum absorbance with 2 ml of the reagent.

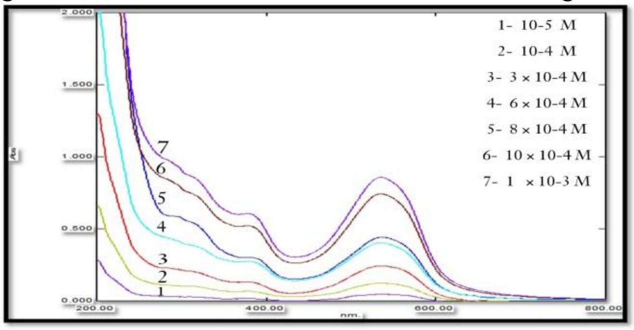


Fig 4 : absorption spectra UV-Vis of the reagent(NTADBrP) in different concentration

Absorption spectra and characteristics of the reagent and complex

NTADBrP is a dark-red powder that is soluble in organic solvents such ethanol, methanol, DMS, and acetone. Fig 4 shows the UV-Vis spectra of the reagent in a 100% ethanol solution. displays the UV-Vis absorption spectra of the compound (NTADBrP) and (NTADBrP)-Cu (II) . The reagent's maximum absorbance (λ_{max}) is at 535 nm, while the copper complex(λ_{max}) is at 589 nm. The reagent forms a red complex with copper (II) right away in an aqueous solution with a pH of less than 9. The absorbance reached its peak in 5 minutes and was stable for at least 24 hours at room temperature. As in the Fig(5).

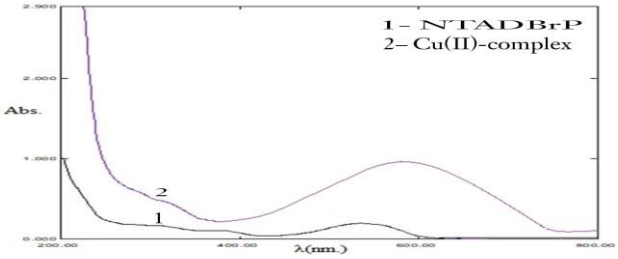


Fig 5: Absorption spectra of copper ion 20 ppm with the reagent at pH=9

H1-NMR spectrum for organic reagent

The nuclear magnetic resonance spectrum of the thiazolyl azo reagent (NTADBrP) was studied using dimethyl sulfur dioxide as a solvent DMSO_d6 and TMS as a standard reference. (δ =7.32 - 7.01) belongs to the aromatic ring protons. Also, a single sign appeared in the site (δ =10.4) belonging to the hydroxyl group. The spectrum of the detector also showed a band at the chemical displacement (δ =2.32_2.22) which returns the solvent [21]. The fig 6 shows the nuclear magnetic resonance spectrum of the organic reagent.

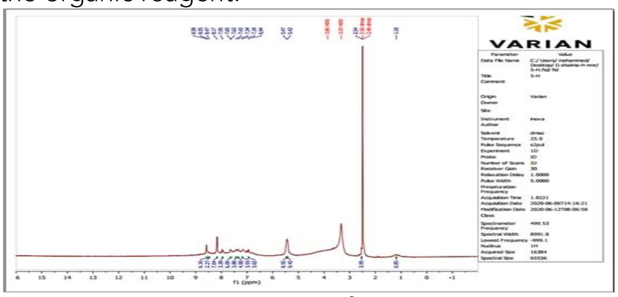


Fig6: H1- NMR spectrum for organic reagent (NTADBrP)

Mass spectrometry of the organic reagent

The mass spectrum of the new organic reagent NTADBrP was recorded, giving a group of peaks shown in the fig 7 , where a main peak appeared at m/z = 409 due to the molecular weight of the ligand with formula($C_9H_8N_4O_3Br_2$) . While other peaks appeared at (323, 304, 257 , 157 $^m/_{Z+}$) the ions return to [$C_9H_4N_4O_3SBr_2$] , [$C_9H_6N_4O_3SBr_1$] , [$C_9H_8N_2O_3SBr$] , [$C_9H_7NOSBr_1$] , [C_6H_5Br].

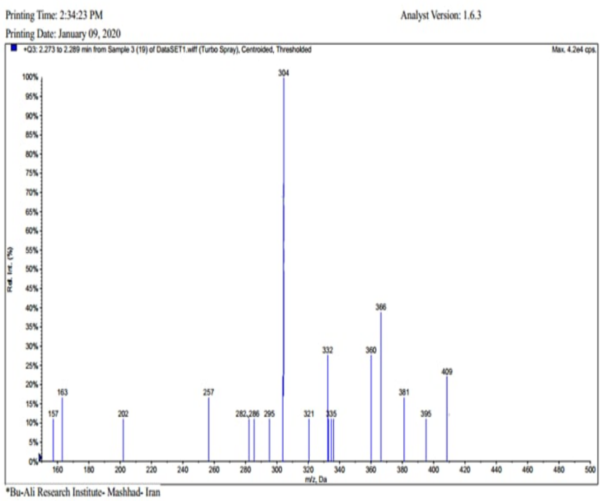
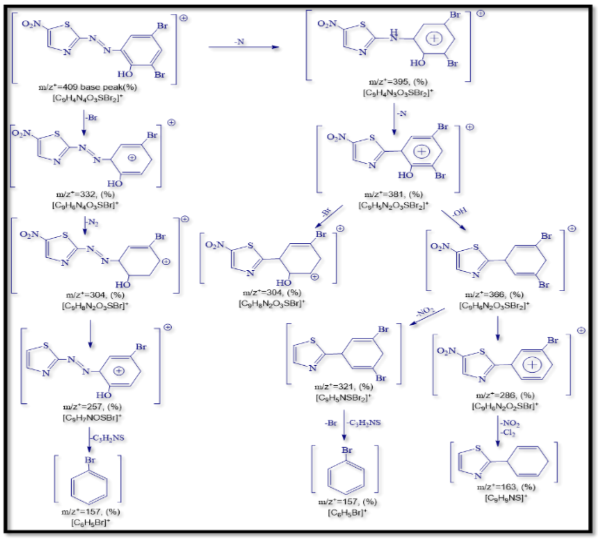


Fig 7: Mass spectrum of the Organic reagent NTADBrP



Scheme 2: Suggested mass fractionation pathways for organic reagent

Optimum Conditions

Effect of pH on Complex Formed with Cu (II)

The pH of the solution affects the Cu-ability complexes to absorb light. After studying this relationship over the pH range of (2–12) and adjusting it with dilutions of NH_3 and HCl, the ideal pH range for the complex was discovered to be between (7–10). The correlation between complex absorbance and pH is depicted in fig 8. At pH< 5, a drop in absorbance may be caused by the synthesis of copper hydroxide as a result of the reaction between the hydrogen ion and the ion pair of electrons on the nitrogen atom in the thiazole ring. This reaction also results in the formation of the azolium cation at pH >8.5.

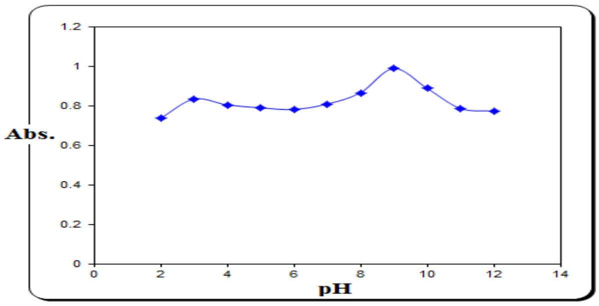


Fig 8: Effect of pH on the Cu complex (Cu=20ppm), (2ml, $3 \times 10^{-4}M$) of Reagent

As seen in Fig 8, the absorbance increased with increasing pH and peaked at pH 9 for the Cu (II) complex, due to partial dissociation of the complexes at higher pH, the absorbance steadily declined. As a result, pH 9 was chosen as the optimal pH for full Cu(II) complex production.

The Temperature and Time Effect

The complex's absorbance reaches a maximum value in 5 minutes, which is sufficient for the maximum absorbance of Cu (II), and it stays stable for 24 hours. The influence of temperature on the complex was investigated in the range of 10 to 40℃. Temperatures above 50℃ were avoided because the complex would be disspciated or evaporated.

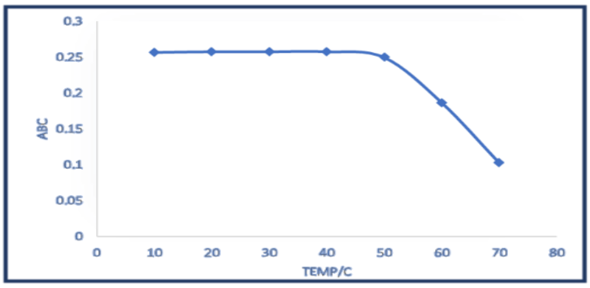


Fig 9: Effect of temperature on the absorbance of Cu (II) complex (Cu=20ppm), (2ml , $3 \times 10^{-4} M$) of Reagent

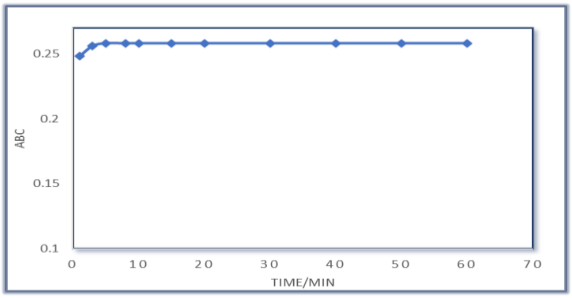


Fig10: Effect of time on the absorbance of Cu(II) complex (Cu=20ppm), (2ml , $3 \times 10^{-4} M$) of Reagent

Complex Composition and Stability Constant

Both the continuous variation and the mole ratio approaches were used to determine the composition and stability constant. At pH 9, both procedures revealed that the molar ratio of Cu(II) ions to the organic reagent is 1:2 (metal:Reagent). Using the following equations, the stability constant is determined to be (K_{st} = 33 × 10^7) and the complex α is found to be 0.315 .

$$K_{st} = \frac{1}{K_{ins}} \text{———————————} (1)$$

$$K_{ins} = \frac{(\alpha C)(n\alpha C)^2}{C(1-\alpha)} \;.\; \alpha = \frac{A_m - A_s}{A_m} \text{———————}(2)$$

Where: α=degree of dissociation.
c =total concentration of the complex,
n =mole ratio = (2 to Cu (II)) .
Am= absorbance of a solution containing reagent two times excess than the amount of copper.
As= absorbance of a solution containing a stoichiometric amounts [reagent][Copper].

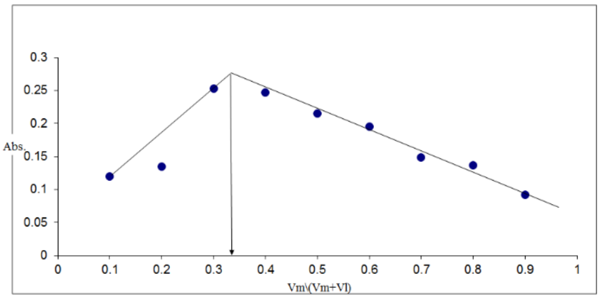


Fig11: continuous variation method for Cu complex with (NTADBrP) at PH= 9

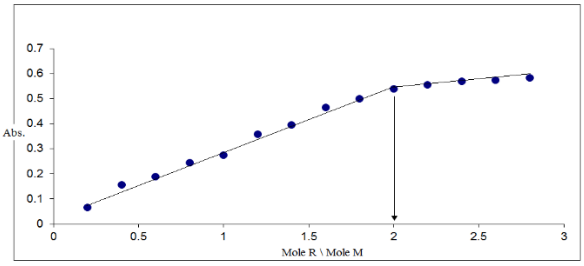


Fig12: mole ratio method for Cu complex with (NTADBrP) at PH=9

Characteristics of Analysis

According to the experimental protocol, the calibration curve was made, and a good correlation coefficient was found. The analytical parameters for this Spectrophotometric analysis of Cu (II) with this reagent are summarized in Table 1.

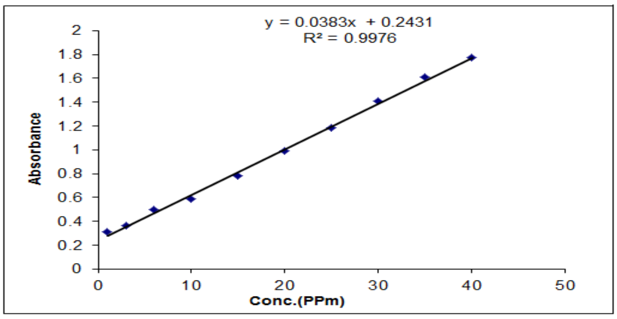


Fig 13: calibration curve of Cu complex

Table 1: Analytical data and some analytical parameters for the proposed Cu(II) determination technique .

Analytical Parameter	Cu(II) /ppm
λmax	589nm
Regression equation	Y=0.0383x+0.2431
Molar absorptivity ($L.mol^{-}cm^{-}$)	2433.8
Sandell Sensitivity	0.0261 L^{-1}.gm.cm
Corroation coefficient (r)	0.9976
Detection limit(ppm)	0.3419
Percent Relative error %	2.98%
Percent Recovery %	97.02
Composition of complex (M: L)	1:2
Linear dynamic range(ppm)	(1-40)
Standerd deviation	0.001
Relative. Standard. Deviation %	0.57%

According to these results, this technique is highly accurate and suitable for spectrophotometrically detecting Cu (II).

Copper(II) Complex was Investigated by Interference

The ions (Co^{+2}, Ni^{+2}, Cr^{+3}, Pb^{+2}, Hg^{+2}, Cd^{+2} , Ag^{+1}) formed complexes with the reagent NTADBrP during the reaction with copper, and the effects of these ions were examined as indicated in Table 2 .

Table 2: Effect of foreign ions on the determination of Cu(II) in concentration (20ppm) and suitable masking agents			
Foreign ion 40 ppm	Error %	Masking agent () ml, [] M	Error % after adding masking agent
Ag^{+}	6.2	Thiourea (2), [0.5]	- 0.3
Co^{2+}	-17.5	$Na_2S_2O_3$ (0.7), [0.01]	-0.4
Ni^{2+}	2.2	KSCN (0.6), [0.5]	- 0.4
Cr^{3+}	-19.4	$NaNO_2$ (0.4), [5× 10^{-4}]	0.0
Pb^{2+}	5.2	KI (0.3), [7.5 × 10^{-4}]	0.7
Hg^{2+}	3.2	Thiourea (0. 5)[0.01]	0.2
Cd^{2+}	-12.2	Thiourea (1) [0.01]	0.0

Investigate cancer cell biological activities

There are several types of cancer that are classified according to the type of cell that was affected first. In this study, MCF-7 laryngeal cancer cells and healthy cell line WRL.68 were used for comparison and for the purpose of demonstrating their effectiveness on human body cells and the possibility of using them as cancer drugs. We will review in detail the effect of the prepared reagent under study on cancer cells and on healthy cells. The test (MTT) was used for the biological examination of all cells, and the results showed that the type and concentration of the prepared compound are of great importance in determining the percentage of cytostatics. In this study, cancer cells and toxic throat cancer line cells were used to compare the degree of its impact on human body cells and the potential for using medicines. All cells are biologically screened using the test (MTT). The results showed that the proportion of cell inhibition is mostly dependent on the composite record type and concentration. According to the table below, the study reagent's effect on the growth of laryngeal cancer cell lines and healthy cells at 400 μg/ml^{-1} is the best inhibitory concentration compared to the concentration used where other effective good amount inhibitory migraine I_{50} concentration is equal to 67.84 μg/ml^{-1} and less effect on healthy cells where needed to kill cells sound at 273.1 μg/ml^{-1} .

Table 3: Effect of reagent on the cells of the laryngeal cancer cell line MCF-7 and its comparison with the normal cell line WRL-68 for the same concentration using MTT test				
Con.(m/ml)	Cancer Line Cell MCF-7		Normal Line Cell WRL-68	
	Mean	SD	Mean	SD
400.00	28.94	5.67	65.39	6.90
200.00	37.85	1.74	73.23	1.20
100.00	61.50	2.49	93.60	2.10
50.00	90.05	2.42	95.33	1.18
25.00	95.45	0.68	95.22	0.82
12.5	96.64	1.36	95.95	1.03

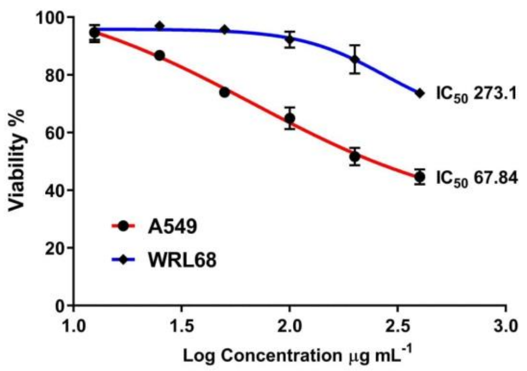


Fig 14: Half -inhibitory concentration of Throat cancer cell line cells and normal line cells WRL68

4. Conclusion

In this work 2- [2⁻-(5-nitro thiazolyl) azo] -4,6-Di bromo phenol (NTADBrP) was used for determination of microamounts of Cu(II). The40) ar absorptivity of this complex are 2433.8 L.mol^{-1}.cm^{-1}. Most foreign ions do not interfere with ion study when masked using different masking agents. Beca13: the (NTADBrP) can rapidly react with Cu(II) at room temperature, this method can determine this ion spectrophotometrically without heating or extraction. It is sensitive, selective, rapid, easy and convenient method for determining the ion Cu(II). MCF-7 laryngeal cancer cells and healthy cell line WRL-68 were used to show their effectiveness on human body cells and the extent to which they can be used as cancer drugs. The MTT test was used for biological examination of all cells. Cell inhib] [ion rate.

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