

With Solvent Extraction Method, and via new Organic Reagent 2-(Benzo thiazolyl azo)-4,5- Diphenyl Imidazole for Spectrophotometric Determination of Copper (II) in different Samples

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Abstract:

The new organic reagent 2-[Benzo thiazolyl azo]-4,5-diphenyl imidazole was prepared and used as complexing agent for separation and spectrophotometric determination of Cu^{2+} ion in some samples include plants, soil, water and human blood serum. Initially determined all factors effect on extraction method and the results show optimum pH was ($\text{pH}_{\text{ex}}=9$), optimum concentration was $40\mu\text{g}/5\text{mL}\text{Cu}^{2+}$ and optimum shaking time was (15min.), as well stoichiometry study appears the complex structure was 1:1 Cu^{2+} : BTADPI. Interferences effect of cations were studied. Synergism effect shows MIBK gave increasing in distribution ratio (D). Organic solvent effect appears there is no any linear relation between dielectric constant for organic solvent used and distribution ration (D). Thermodynamically found the reaction was Endothermic reaction, with $\Delta H_{\text{ex}}= 0.0131 \text{ KJ.mole}^{-1}$, $\Delta G_{\text{ex}}=-54.20 \text{ KJ.mole}^{-1}$, $\Delta S_{\text{ex}}= 167.84 \text{ J.mole}^{-1}$. Beer's law was obeyed over the concentration 1-30 $\mu\text{g}/5\text{mL}$, and $\epsilon=922.90 \text{ Lmol}^{-1}.\text{cm}^{-1}$, with detection limit 1.7×10^{-5} and Sandell's sensitivity $6.8\times 10^{-7} \text{ gcm}^{-2}$.

Key words: Solvent extraction, Copper, Spectrophotometric determination.

Introduction:

Separation and determination of metal ions have been one of the most important topics of analytical Chemistry. Developing highly functional Chelating agents has been a great concern of many analytical Chemists. Investigations into developing new type of Chelating agents have produced many functional results having high sensitivity. New chelating resin was prepared by coupling Amberlite XAD-4 with 1-amino-2-naphthole through an azo spacer. The resulting sorbent has been characterized by FT-IR, element analysis and thermo gravimetric analysis and studied for preconcentrating of Cu (II) using FAAS for metal monitoring, and the

optimum pH value for sorption of the Cu^{2+} was 6.5[1]. An extraction and sensitive differential pulse anodic stripping voltammetric method at hanging mercury drop electrode is described for the determination of Zn, Cu, Pb ($\mu\text{g g}^{-1}$) and Cd (ng g^{-1}) metal ions in water and 0.1M HCl extracts for Kakade, anise, Cumin, Caraway and black pepper [2]. The significant spectral overlap $(\text{Di})_{0.5} = 0.5667$ which is about 75.3% overlapping of the UV/Vis, absorption spectra of Fe (II) and Cu(II) complexes necessitates chemometric assisted methods for simultaneous analysis of these ions in the pharmaceutical mixture. These metal ions have been analyzed simultaneously by UV/Vis.

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spectrophotometric method where 8-hydroxyquinoline was used as a chromogenic reagent. The methods were successfully applied for analyzing synthetic mixtures and commercial pharmaceutical preparation [3]. The synergistic extraction of Cu (II) with N-phenyl benzo hydroxamic acid (PBHA) and tri-n-octyl phosphinoxide or tributyl phosphate was investigated in CHCl_3 . The effect found in presence of natural ligand is due to formation of the adduct $\text{Cu}(\text{PBHA})_2\text{S}$ in CHCl_3 . The synergic coefficients were determined which have higher value in presence of TOPO than TBP, since TOPO has the higher basicity than TBP [4]. A solid phase extraction method was developed for pre-concentration and spectrophotometric determination of copper in water samples by using lead 4-benzyl piperidine dithiocarbamate complex ($\text{Pb}(4\text{-BPDC})_2$), Cu in large volume liquid phase quantitatively replaces lead on colorless ($\text{Pb}(4\text{-BPDC})_2$) complex and naphthalene solid phase mixture forming $\text{Cu}(4\text{-BPDC})_2$, $\lambda_{\text{max}}=437\text{nm}$, linearity is $0.4\text{--}10\text{mgL}^{-1}$ of Cu, $\epsilon=8.197\times 10^3\text{L.mol}^{-1}\text{.cm}^{-1}$ [5]. Spectrophotometric determination of Cu (II) based on the reaction at pH4-9 between the synthesized acetophenone-p-chloro phenyl thiosemicarbazone (A-p-CIPI) and Cu(II) forming a green complex $\text{Cu}^{+2}:\text{A-p-CIPI}$ (1:2) that floats quantitatively with oleic acid surfactant, $\lambda_{\text{max}}=600\text{nm}$ in both aqueous and surfactant layers, $\epsilon=5.5\times 10^3$ and $1.3\times 10^4\text{Lmol}^{-1}\text{.cm}^{-1}$ in aqueous and surfactant layers, Beer's law is obeyed over the range $0.25\text{--}6.35\text{mgL}^{-1}$ with $\text{DL}=0.021\text{mgL}^{-1}$ for a standard aqueous solution of Cu (II) with a concentration of 3.82mgL^{-1} , Sandell's sensitivity was $0.244\mu\text{g cm}^{-2}$ [6]. Spectrophotometric method has been developed for the determination of Cu(II) by using 2,3,4-trihydroxyacetophenonephenylhydrazo

ne (THAPPH) as an analytical reagent. The metal ion has formed 1:2 (M:L) bluish green coloured complex with THAPPH in HCl-KCl buffer of pH 2.5. Beer's law was obeyed in the range $0.04\text{--}0.64\mu\text{g/ml}$ of Cu(II) at $\lambda_{\text{max}}=385\text{nm}$. $\epsilon=1.0053\times 10^5\text{L.mol}^{-1}\text{.cm}^{-1}$ and Sandell's sensitivity ($0.0006265\mu\text{gcm}^{-2}$), SD (0.0024), RSD (0.47%), confidence limit (± 0.0017), and standard error (0.0008). The interfering effect of various cations and anions has been also studied. The reliability of the method was assured by analyzing the standard alloys, Brass, Bronze, and Phosphor-Bronze. This method was successfully employed for the determination of Cu(II) in food and medicinal leafy samples and inter compared the experimental values using AAS and statistically evaluated using F-test.[7].

Materials and Methods:

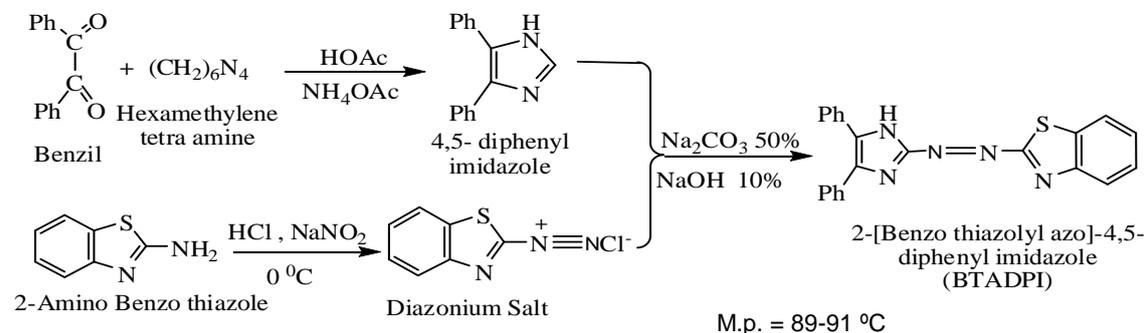
Apparatus:

All apparatus used was Double beam (UV-Vis) Spectrophotometer Shimadzu (UV-1700) (Japan), Signal beam (UV-Vis) Spectrophotometer Shimadzu (UV.100-02) (Japan). PH-meter, WTW (Germany), Stuart Melting point Apparatus (Japan), As well FT-IR8400S (CE), Shimadzu Corporation. Element analysis Carried out by Micro analytical unit, 1108 C.H.N elemental analysis Atomic Absorption Spectroscopy AA-6300 used also to comparable the results.

Chemicals and reagents:

All Chemicals used in this study were analytical reagents grade (Fluka, Merck & BDH). The stock standard Copper(II) solution 1mg/mL was prepared by dissolving 0.3928gm of $\text{CuSO}_4.5\text{H}_2\text{O}$ in distilled water contain 1mL of concentration H_2SO_4 and diluting to 100mL . $1\times 10^{-2}\text{M}$ Stock dithiazone solution prepared by dissolving (0.0256gm) of dithiazone in

10mL of CCl_4 . The stock organic reagent Solution $1 \times 10^{-2}\text{M}$ was prepared by dissolving 0.0381gm of BTADPI in 10mL Chloroform other working solutions prepared by appropriate dilution with suitable solvent.



General procedure:

Extraction experiment was carried out for Cu^{+2} ions in aqueous solutions by 5mL of $1 \times 10^{-4}\text{M}$ organic reagent BTADPI dissolved chloroform shaking the two layers for fixed time separate organic layer from aqueous layer and determine remainder quantity of Cu^{+2} in aqueous phase spectrophotometrically by dithiazone method [8]. Also determine Cu^{+2} ions transferred to the organic phase to share in complex formation, at later calculate distribution ratio (D). On the other hand, determined λ_{max} for complex in organic solvent for spectrophotometric determination of Cu^{+2} ions in different samples, environmental and vital samples digested by dry digestion method [9], 5mL digested solution shaken with 5mL of $1 \times 10^{-4}\text{M}$ organic reagent dissolved in chloroform for (15min) at $\text{pH}_{\text{ex}}=9$, and add 2mL of

Preparation of organic reagent:

Preparation of 2-(Benzo thiazolyl azo)-4,5-diphenyl imidazole (BTADPI) consisted of processing in three steps from commercially available material. As below:

$1 \times 10^{-2}\text{M}$ sodium fluoride, after separated the two layers taken the absorbance of organic phase $\lambda_{\text{max}}=494\text{nm}$ against organic reagent as blank.

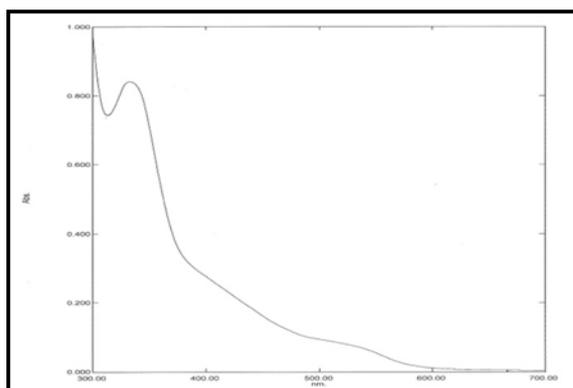
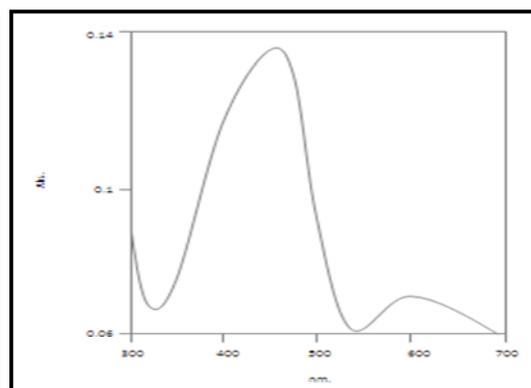
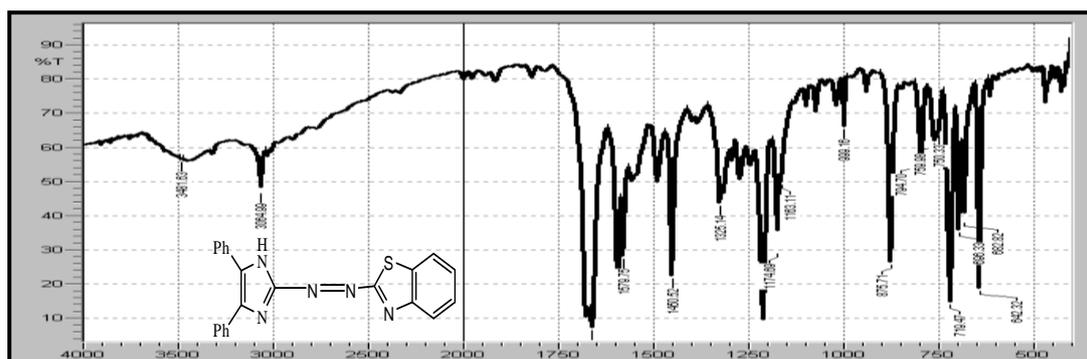
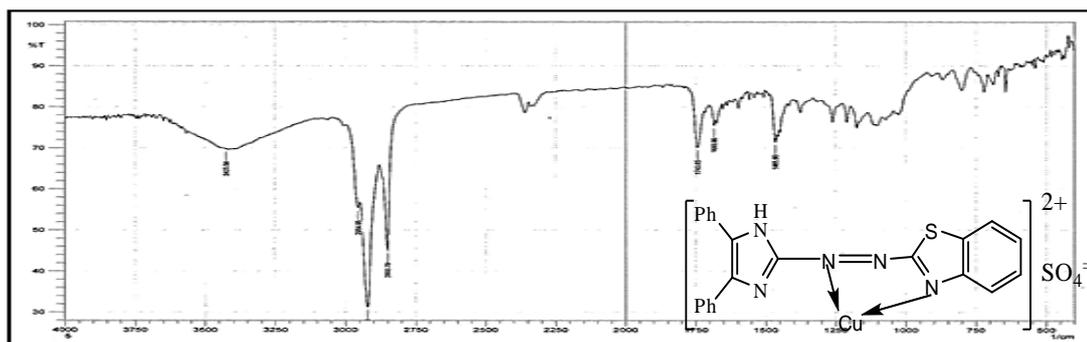
Results and Discussion:

Spectrophotometric Studies:

UV.-Vis spectrum as in Figures (1,2) and Table(1) shows $\lambda_{\text{max}}=332\text{nm}$ for organic reagent and $\lambda_{\text{max}}=494\text{nm}$ for its complex with Cu^{2+} these results show complex formation between organic reagent and Cu^{2+} ions [10,11]. While IR-spectrum as in Figures (3,4) and Table(1) the spectrum of Cu^{2+} complex with organic reagent show shift for absorbance peaks (-N-H, C-H, C=N, -N=N-, C-S) in the spectrum of organic reagent and appear new peak by effect the coordination with Cu^{2+} and SO_4^{2-} , H_2O [12,13].

Table(1): Spectrophotometric studies & analysis of element for organic reagent & its complex with Cu^{2+}

Compound	Analysis of elements %				UV-Vis λ_{nm}	Infrared cm^{-1}							
	C	H	N	Cu		-N-H	C-H	C=N	N=N-	C-S	Cu-	H ₂ O	SO ₄ ²⁻
$\text{C}_{22}\text{H}_{15}\text{N}_5\text{S}$	70.05	4.01	19.11	-	332	3550-3200 (s) 3300 (w)	3065 (s) 3000 (w)	1600 1580 (imidazole) 1685 (thiazole)	1450, 1500	1230 (s)	-	-	-
$[\text{Cu}^{2+}(\text{C}_{22}\text{H}_{15}\text{N}_5\text{S})]\text{SO}_4$	44.54	2.43	16.23	29.46	494	-	2954, 2850	1465 (imidazole) 1683 (thiazole)	1743	1250	700-800	3425	1100

**Fig. (1): UV.-Vis. Spectrum of organic reagent BTADPI****Fig. (2): UV.-Vis. Spectrum of Cu^{2+} complex with BTADPI****Fig.(3): Infrared Spectrum of organic reagent BTADPI****Fig. (4): Infrared Spectrum of Cu^{2+} complex with organic reagent BTADPI**

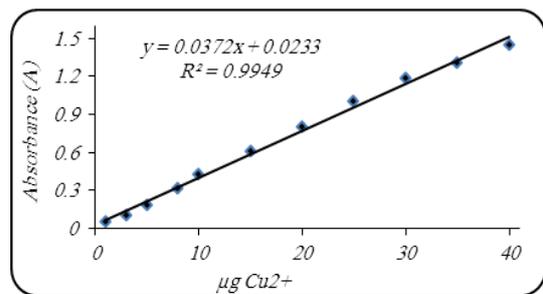


Fig. (5): Calibration curve of Cu²⁺ with dithiazone

Effect of pH

pH of aqueous solution plays major role for complex formation and extraction methods, extracted Cu²⁺ from aqueous phase at pH range 5→10, the results show maximum (D) at pH=9 as in Table (2) and Figure (6). The results appear enhancement in (D) with pH increasing to pH=9 after that the decline in (D) value with increasing pH more than (9).

pH	D	%E
5	3.44	77.47
6	5.66	84.98
7	7.88	88.73
8	10.47	91.28
9	12.33	92.49
10	6.31	86.32

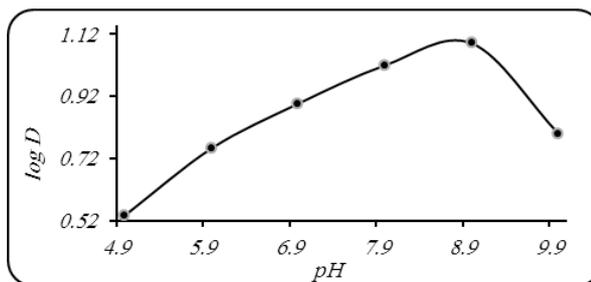


Fig. (6): Effect of pH

[Cu²⁺]=50µg/5mL, [BTADPI]=1×10⁻⁴M in CHCl₃, Shaking time=10min., Temp.=25°C

Effect of metal ion concentration

Extraction experiments of different amount of Cu²⁺ in aqueous solutions, the results show (D) increase with Cu²⁺ concentration increase and

maximum (D) appear at Cu²⁺ concentration (40µg/5mL) because reached to more stable ion pair complex as in equilibrium:



µg Cu ²⁺	D	%E
20	1.32	56.89
30	3.28	76.63
40	12.33	92.49
50	5.37	84.30
60	3.28	76.63

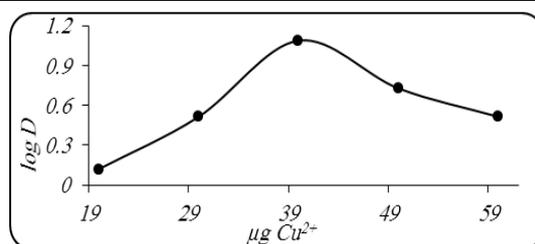


Fig. (7): Effect of Cu²⁺ concentration

pH=9, [BTADPI]=1×10⁻⁴M in CHCl₃, Shaking time=10min., Temp.=25°C

Effect of shaking time:

The results of kinetic side study show that (D) change as a function for

shaking time, and the optimum shaking time was 15 min. This is giving higher (D) as in Table (4) and Figure (8).

Table (4): Effect of shaking time

Time	D	%E
5	4.07	80.27
10	12.33	92.49
15	14.38	93.33
20	7.10	87.66
25	3.22	76.30

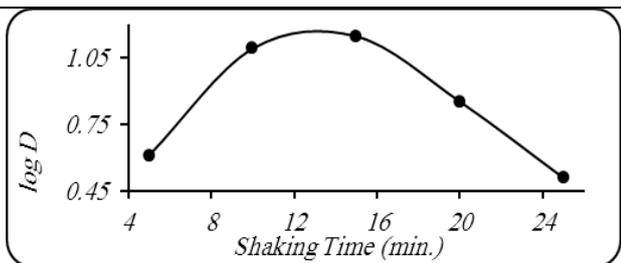


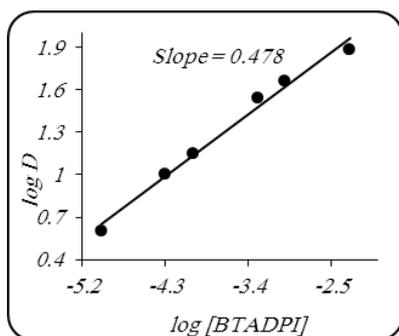
Fig. (8): Effect of shaking time

[Cu²⁺]=40µg/5mL, pH=9, [BTADPI]=1×10⁻⁴ M in CHCl₃, temp=25°C

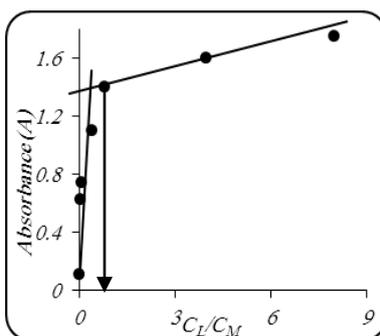
Stoichiometry of the complex

Three methods(Slope analysis method, Mole ratio method, Continuous Variation Method)have been applied to ascertain the

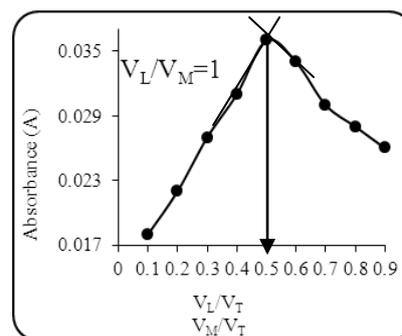
stoichiometric composition of complex and the 1:1 (Cu²⁺:BTADPI) complex was indicated by these methods as in Figure (9, 10, 11).



Fig(9):Slope analysis method



Fig(10):Mole ratio method



Fig(11):Continuous Variation Method

[Cu²⁺]= 40µg/5mL , Shaking time =15 min. , Temp.=25°C , pH=9

Effect of foreign ions:

The effect of some foreign ions, which often accompany copper (II), was examined by carry out the determination of 40µg/5mL of Cu²⁺ in presence of a number of other ions. The results in Table (5), indicate the

foreign ions giving high interfere with the determination of copper (II).Then must be used masking agents when determination copper ion in foundation of these foreign ions.

Table (5): Effect of foreign ions

M ⁿ⁺	D	%E
Cr ³⁺	3.83	79.32
Ni ²⁺	8.56	89.55
Pb ²⁺	7.46	88.18

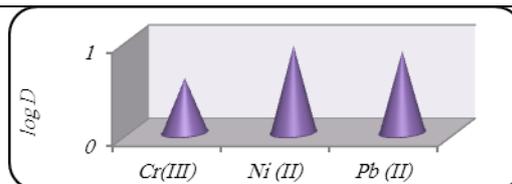


Fig. (12): Effect of foreign ions

[Cu²⁺]=40µg/5mL,[BTADPI]=1×10⁻⁴ M in CHCl₃,[Mⁿ⁺]=40µg/5mL,pH=9,Shaking time=15min., Temp.=25°C

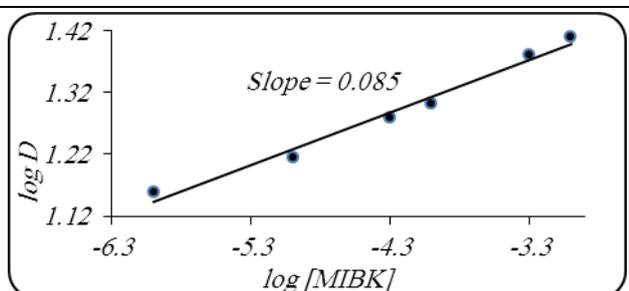
Synergism effect

The extraction of Cu(II) with 1×10^{-4} M BTADPI and $1 \times 10^{-3} \rightarrow 1 \times 10^{-6}$ M MIBK in chloroform was carried out. The logarithm of (D) was plotted against the logarithm of MIBK concentration the plot gave a straight line with slope

0.085 as in Table (6) and Figure (13) show (D) showing that the extracted species was $[\text{Cu}(\text{BTADPI})(\text{MIBK})]^{2+} \text{SO}_4^-$, higher D (synergistic distribution ratio) value were observed in presence of MIBK as compared with D in the absence of MIBK.

Table (6): Synergism effect

[MIBK]	D	%E
1×10^{-3}	25.66	96.25
5×10^{-4}	24.00	96.00
1×10^{-4}	20.05	95.25
5×10^{-5}	19.00	95.00
1×10^{-5}	16.39	94.25
1×10^{-6}	14.38	93.50

**Fig. (13): Synergism effect**

$[\text{Cu}^{2+}] = 40 \mu\text{g}/5\text{mL}$, $[\text{BTADPI}] = 1 \times 10^{-4} \text{M}$ in CHCl_3 , $\text{pH} = 9$, Shaking time = 15 min., Temp. = 25°C

Organic Solvent effect:

Results in Table (7) indicate there is no any linear relation between dielectric constant (ϵ) for organic

solvents used and distribution ratio (D), these show the effect of organic solvents structure on extraction method.

Table (7): Organic Solvent Effect

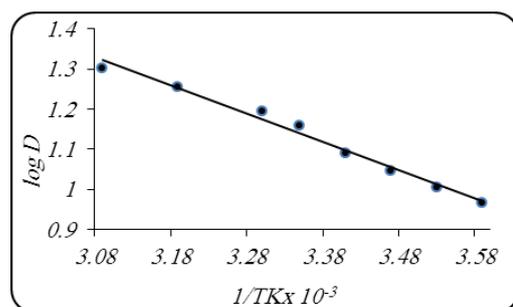
Organic Solvents	ϵ	D	%E	ΔG_t	$K_A \times 10^4$ liter/mo l	$K_{ex} \times 10^4$	ΔG_{ex} KJ/mol
Nitro benzene	35.74	8.32	89.27	-0.04	7.40	57.74	-29.98
Dichloro metha e	9.0	4.64	82.30	-0.27	4.48	18.01	-27.35
Chloroform	4.806	14.38	93.50	-0.54	12.21	172.43	-32.45
Carbone tetrachloride	2.38	3.38	77.20	-1.13	3.48	9.55	-25.91

$[\text{Cu}^{2+}] = 40 \mu\text{g}/5\text{mL}$, $[\text{BTADPI}] = 1 \times 10^{-4} \text{M}$ in different organic solvent, $\text{pH} = 9$, Shaking time = 15 min., Temp. = 25°C

Temperature effect:

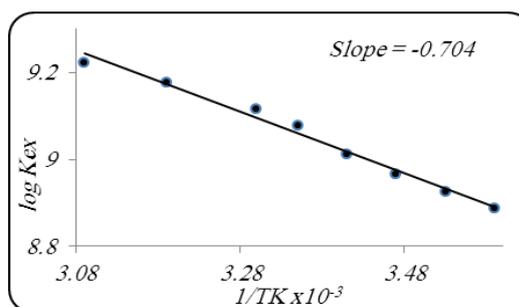
Thermodynamically the reaction between Cu^{2+} ions and organic reagent

BTADPI was endothermic; the results presented in Figure (14, 15).

**Fig. (14): Effect of Temperature**

$[\text{Cu}^{2+}] = 40 \mu\text{g}/5\text{mL}$, $[\text{BTADPI}] = 1 \times 10^{-4} \text{M}$ in CHCl_3 , $\text{pH}_{ex} = 9$, Shaking time = 15 min.

$\Delta H_{ex} = 0.0131 \text{ KJ.mole}^{-1}$, $\Delta G_{ex} = -54.20 \text{ KJ.mole}^{-1}$, $\Delta S_{ex} = 167.84 \text{ J.mole}^{-1}$

**Fig. (15): Effect of Temperature**

Spectrophotometric determination of Copper (II) by organic reagent BTADPI:

Calibration Curve and Sensitivity:

UV.-Vis. Spectra of Cu²⁺ ions and the organic reagent BTADPI in chloroform was presented in Figure (2) show λ_{max}=494nm.Under the optimum conditions described in the recommended procedure, the calibration curve Figure (16) was constructed with ten standard solutions containing 1-30μg/5mL of copper (II), the formula for the best line of calibration $y = 0.0031x + 0.00019$, and the correlation coefficient was obtained with the method of least squares $R^2 = 0.999$, the molar absorptivity of the complex determined from the linear portion of Beer's law plot $\epsilon=922.90L.mol^{-1}.cm^{-1}$, and Sandell's sensitivity $6.8 \times 10^{-7} gcm^{-2}$.

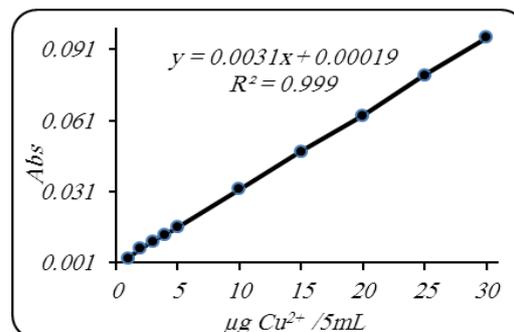


Fig. (16): Calibration curve of Cu²⁺ with BTADPI

Application:

Soil samples

The soil contains copper at the range 10-200ppm and this different return to nature of soil according to WHO measurements[14,15]Spectrophotometric determination of Cu²⁺ ions in different soil samples by BTADPI as the results in Table (8) and Figure(17).

Table (8):The quantity of Copper(II) in soil

No. of sample	Sample name	Copper quantity		
		ppm*	%RSD	AAs**
1	Al-Najaf	34.8	4.60	35.1
2	Al-Abasia	86.0	0.38	87.2
3	Al-Mishkhab	78.0	1.29	79.5
4	Al-Manadira	94.0	0.35	95.0

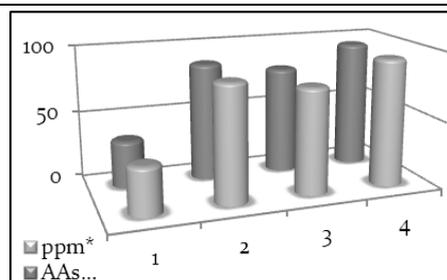


Fig.(17): The quantity of Copper(II) in soil

*ppm: copper quantity determined by this Spectrophotometric determination method

**AAs: copper quantity determined by Atomic absorption method

Plant samples

Spectrophotometric determination of Cu²⁺ ions in different plant samples by BTADPI as the results in Table (9)

and Figure(18),The range of copper in plants according to WHO measurements is 5-30ppm[14,15].

Table (9):The quantity of Copper(II) in plant

No. of sample	Sample name	Copper quantity		
		ppm*	%RSD	AAs**
1	Visit	9.0	8.0	9.2
2	MyriusCommunis	15.0	2.27	15.2
3	L.Japonica	28.5	1.18	29.0
4	C.Sativus	27.0	1.25	27.6
5	Aplumgravealens	15.0	2.27	15.3
6	Ledpidiumsativum	23.4	1.15	23.6
7	Lactuce sativa	25.4	1.06	26.0
8	ViciaFaba	14.6	0.45	14.6
9	Phaseolus Sp.	13.0	2.68	13.2
10	Juglans Sp.	25.3	0.79	25.6
11	Pistacia Sp.	28.0	1.20	28.3
12	Prunusamygdalus	28.5	4.90	28.8

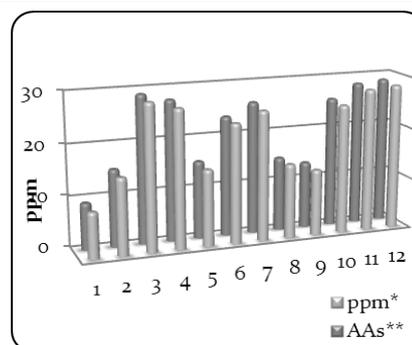


Fig.(18): The quantity of Copper(II) in plant

Water samples

Spectrophotometric determination of Cu^{2+} ions in different water samples by BTADPI as the results in Table (10)

and Figure(19). The range of copper in water according to WHO measurements is 2ppm[14,15].

Table (10):The quantity of Copper(II) in water

No. of sample	Sample name	Copper quantity		
		ppm*	%RSD	AAs*
1	Al-Najaf (drink water)	0.42	3.27	0.43
2	Al-Mishkhab (river)	0.7	10.52	0.76
3	Al-Kufa (river)	0.16	2.12	0.18

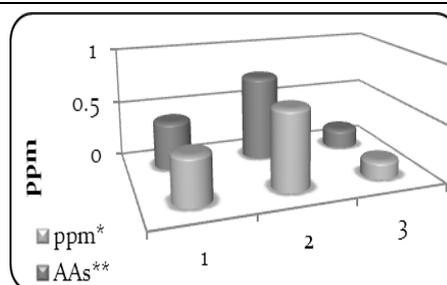


Fig.(19): The quantity of Copper(II) in water

Human blood serum samples

Spectrophotometric determination of Cu^{2+} ions in different human blood serum samples by BTADPI as the

results in Table (11) and Figure(20).The range 1.1-1.5mg/L according to WHO measurements [14,15].

Table (11):The quantity of Copper(II) in human blood serum

No. of sample	Sex	Age	Copper quantity		
			ppm*	%RSD	AAs**
1	Male	4	2.7	3.84	2.7
2	Male	14	2.3	6.15	2.5
3	Male	34	2	2.04	2.1
4	Female	28	1.6	1.12	1.8
5	Female	49	1.2	9.09	1.4

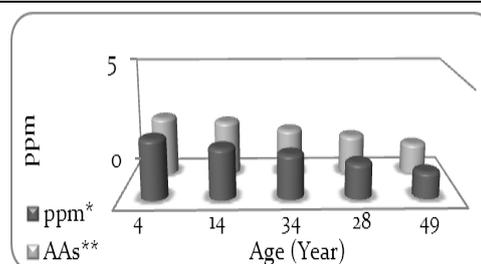


Fig.(20): The quantity of Copper(II) in human blood serum

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بواسطة طريقة الاستخلاص بالمذيب وباستخدام الكاشف العضوي الجديد 2-
[بنزوثيرازوليل أزو]-4,5-ثنائي فنيل اميدازول للتقدير الطيفي للنحاس (II) في
نماذج مختلفة

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الخلاصة:

الكاشف العضوي الجديد 2-[بنزوثيرازوليل أزو]-4,5-ثنائي فنيل اميدازول تم تحضيره واستخدامه ككاشف عضوي للفصل والتقدير الطيفي لأيونات النحاس Cu^{2+} في نماذج مختلفة تضمنت عينات نباتية وتربة ومياه ومصل دم الإنسان. وفي البداية تم تحديد كافة العوامل المؤثرة على عملية الفصل وإشارات النتائج إلى أن الدالة الحامضية المثلى لعملية الاستخلاص كانت ($\text{pH}_{\text{ex}}=9$) والتركيز الأمثل لأيون Cu^{2+} في المحلول المائي $40\mu\text{g}/5\text{mL}$ وزمن الرج الأمثل (15 دقيقة). أما دراسة التركيب الأكثر احتمالاً أظهرت أن تركيب المعقد كان $\text{Cu}^{2+}:\text{BTADPI} = 1:1$. كما تم دراسة تأثير التداخل للأيونات الموجبة ودراسة تأثير توليف المذيب Synergism بينت أن MIBK أعطى ارتفاعاً في نسب التوزيع (D) أما دراسة تأثير المذيب العضوي أظهرت عدم وجود أي علاقة خطية بين ثابت العزل الكهربائي (ϵ) للمذيبات العضوية المستخدمة وقيم نسب التوزيع (D) وفي الجانب الثرموديناميكي للدراسة فقد أظهر أن التفاعل كان ماص للحرارة Endothermic reaction وقيم الدوال الثرموديناميكية كانت $\Delta S_{\text{ex}} = 167.84 \text{ J.mole}^{-1}$ وكانت المطاوعة لقانون بير في مدى التركيز $1-30\mu\text{g}/5\text{mL}$ وقيمة الامتصاصية المولية كانت $922.90 \text{ Lmol}^{-1} \text{ cm}^{-1}$ وإن حد الكشف هو $1.7 \times 10^{-5} \text{ mgL}^{-1}$. أما حساسية ساندل فكانت $6.8 \times 10^2 \text{ gcm}^{-7}$.