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RESEARCH ARTICLE

Potentiometric and Spectrophotometric Studies of (E)-2-(mercapto-phenylamino-methylene)-3-oxo-N-p-tolyl-butanamide (MPMPB) with Some Metal Ions.

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Abstract

Complexes of V(V), Co(II), Ni(II), Cu(II), Pd(II) and Cd(II) with (MPMPB) have been investigated in solution using Potentiometric and spectrophotometric methods. The composition of the complexes was determined to be 1:1 and/or 1:2 (M: L), the optimum conditions favouring the formation of the coloured complexes were studied extensively; protonation constants and their stability constants of the complexes were calculated. Adherence to Beer's law was observed for the $\mu g m L^{-1}$ concentration range. Moreover, the solid complexes have been characterized by elemental analyses, electronic spectra, thermal method (TGA and DTA), IR and X-ray powder diffraction. The antimicrobial activities of the ligands and their complexes have been studied by screening the compounds against the *bacteria S. marcescens (-ve), P. eruginosa (-ve), E. coli (-ve), S. aurous (+ve), B. cereus (+ve) and M. luteus* and also the fungi *C. albicans (-ve), G. Candidum (-ve), A. flavus (+ve), F.oxysporium (-ve), S. brevicaulis (+ve) and T.rubrum* and results have been compared with the standard drug,

INTRODUCTION

2-(Mercapto-phenylamino-methylene)-3-oxo-N-p-tolyl-butanamide (MPMPB) as derivative of acetoacetanilide [1]. It is noticed that the preparation of metal complexes of various transition metals with different derivatives of β - ketoanilides compounds and their structural investigations have not been carried out much. It was therefore considered worthwhile to undertake this study systematically.

It is of growing interest that the coordination compounds and metal chelates possessing nitrogen and oxygen donor atoms, show strong biological properties [2, 3].

Metals play a vital role in all living systems. Any malfunctioning of these metals can initiate a number of physiological abnormalities and symptoms of clinical disorders. Transition metal ions are responsible for proper functioning of different enzymes. Copper is one of the trace elements essential to the healthy life of humans and animals [4, 5]. Human diet usually includes 2 to 5 mg of copper per day. The adult body contains 100–150 mg, the muscle mass having 64 mg and the liver 18 mg of copper, [6]. Copper(II) usually forms stable complexes with N, O, and S donor ligands. For example, Cu(II) makes square planar complex with 4-methyl-imidazole [7].

Metal coordination complexes have been extensively used in clinical applications as enzyme inhibitors [8], anti-bacterial [9, 10], antiviral [11–13] and as anti-cancerous [14–16]. Different kinds of metals have been employed in these complexes including platinum, gold, vanadium, iron, molybdenum, cobalt, tin, gallium, copper and many others [15]. Metal complexes of adenine (A) have been shown to possess anticancer activity [16–20].

The present study investigates the physico- chemical properties of the complexes of chelating agents with transition and inner transition metals on both solution and solid states and will be characterized from elemental analysis, magnetic, UV- visible Diffusive reflectance, IR, NMR, etc.

The potentiometric method has been used extensively in many branches of solution chemistry. It is by for the most accurate and widely applicable technique currently available for the study of ionic equilibria [21]. The stability of (MPMPB) in aqueous buffer solutions has been studied as a function of pH. The rate of (MPMPB); loss and the mode of degradation are dependent upon the pH of solution.

This work aims to study the complex formation of MPMPB with V(V), Co(II), Ni(II), Cu(II), Pd(II) and Cd(II) in mixed ethanol—water solutions using Potentiometrie and spectroscopic methods to throw some light on their compositions, structures, and analytical applications.

2. Experimental

2.1. Apparatus

All The pH measurements were carried out on VWR scientific model 2000 digital analyzer accurate total 0.01 pH unit. The absorption spectra of solutions were measured at room temperature with a Perkin-Elmer Lambda 3B recording spectrophotometer, using 1-cm matched quartz cells in the wavelength range 200-800 nm. The C, H, N elemental analyses were done at the microchemical analysis Unit, Assiut University and. FT- IR spectra (400- 4000 cm^{-1}) were recorded as KBr disces using a Shmiadzu IR 479 spectrophotometer. Thermogravimetric analysis of TGA was recorded on Shimadzu analyser 50 in a dynamic nitrogen atmosphere (100 ml/min) at a heating rate 10°C/min. X-ray diffraction data were collected at room temperature (~25°) on a Philips 1710 diffractometer at Assiut University. The patterns were run with cutarget and Ni filter (λ= 1.5418 N) at 40 KV and 30 mA in the range of 2θ= 40-60, 4-80 with scanning speed of 3.6 deg min^{-1}.

2.2. Reagents and Materials

The solutions of V(V), Co(II), Ni(II), Cu(II), Pd(II) and Cd(II) ions (Merck and BDH) as nitrates were prepared and titrated complexmetrically by EDTA [22]. Were obtained by dissolving the accurate weight of metal nitrate in the appropriate volume of bi-distilled water**.** More dilute solutions used for spectral measurements were obtained by accurate dilution**.**

Stock standard solution of ($1x10^{-3}$ M) of (MPMPB) ligand was prepared by dissolving 0.0085 gm of pure Ligand in ethanol and diluted with the same solvent to 10 mL volumetric flask (at 25 °C). (MPMPB) working solutions were prepared daily by sequential dilution of the stock standard solution using bidistilled water.

Sodium hydroxide (Merck) was prepared in bidstilled water as fresh solution.The universal buffer solution were prepared as recommended [23], and used to control the hydrogen ion concentration and ionic strength.

2.3 General Experimental procedure

Calvin-Bjerrum, technique as adopted by Irving and Rossoti [24] or Kather and Munshi [25], were used to determine the dissociation constants of the ligand (MPMPB) and the formation constants of their metal complexes with at 25 ± 0.1^0C in aqueous solutions. The solutions were titrated potentiometrically with 0.2 M standard free sodium hydroxide solution standardized against standard potassium hydrogen phthalate a = 0.001 M.HNO_3 , b = a + 0.001 M (MPMPB) and c = b + 0.001 M metal nitrate solution. The total volume was adjusted to 50 cm^3 by adding doubly-distilled water in each case. The titration's were performed at 25 ± 0.1 ^oC and Ionic strength of I = 0.1M $NaNO_3$.

The solid complexes were prepared by mixing equimolecular amounts of the lignad and metal salt in alcohol. The mixture was then stirred for about two hours. The solid complexes were isolated by addition of ether and then filtered immediately. Recrystallization of the solid complexes from ethanol/ether, to ensure the isolation of pure compounds was carried out. The solid complexes were then filtered off, washed and dried over P_2O_5. Their structures were confirmed by elemental microanalyses IR, electronic absorption spectra, and thermogravimetric analysis and as well as x-ray powder diffraction analysis.

Results and Discussion

The titration curves are shown in Fig. 1, and the average number of proton attached per ligand, $\bar{n}H$ was calculated [24].

$$\bar{n}H = Y + \frac{(V_1 - V_2)(N^0 + E^0)}{(V_0 - V_1)(Tcl^0)} \quad \text{---}\rightarrow 1)$$

Where Y= 3 (number of dissociable protons in the ligand), V◦ is the initial volume, V_1 and V_2 are the volume of alkali required to reach the same pH in mineral acid (HNO_3) and (HNO_3 + MPMPB), respectively. T_CL° is the total concentration of ligand, N° is the normality of the alkali and E° is the initial concentration of free acid.

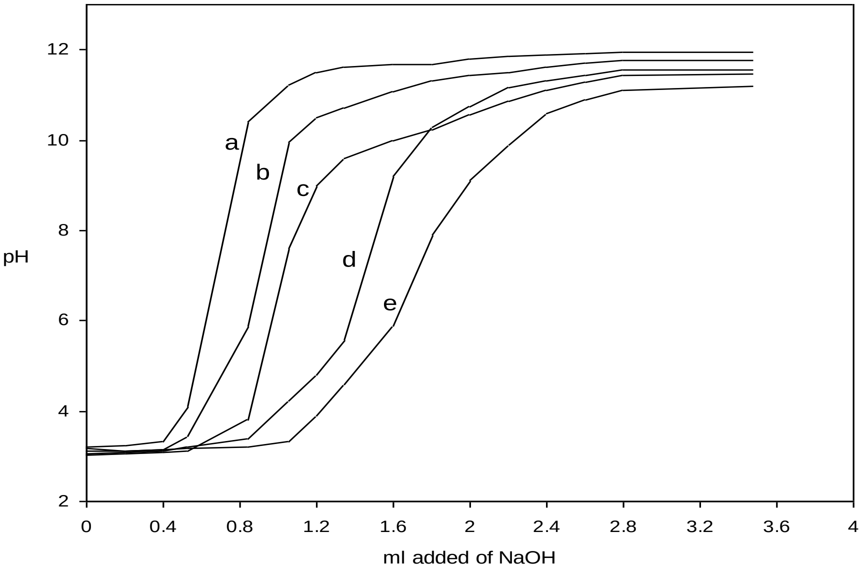


Fig.1.Titration curve of (MPMPB) with different metal ions at I = 0.1 $MNaNO_3$ (a): acid solution, (b): solution (a) + ligand, (c): solution (b) + Ni (II) ion, (d): b+ Cu (II) ion, (e): b+ V (III) ion.

The proton - ligand formation curve obtained by plotting $\bar{n}H$ against pH at the ionic strength I = 0.1 M $NaNO_3$ as shown in Fig. 2.The values of $\log K_1^H$, $\log K_2^H$ and $\log K_3^H$ (the first, second and third proton dissociation constants of (MPMPB) ligand are the pH values corresponding to half $\bar{n}H$ values such as $\bar{n}H$ = 0.5, 1.5 and 2.5, respectively.

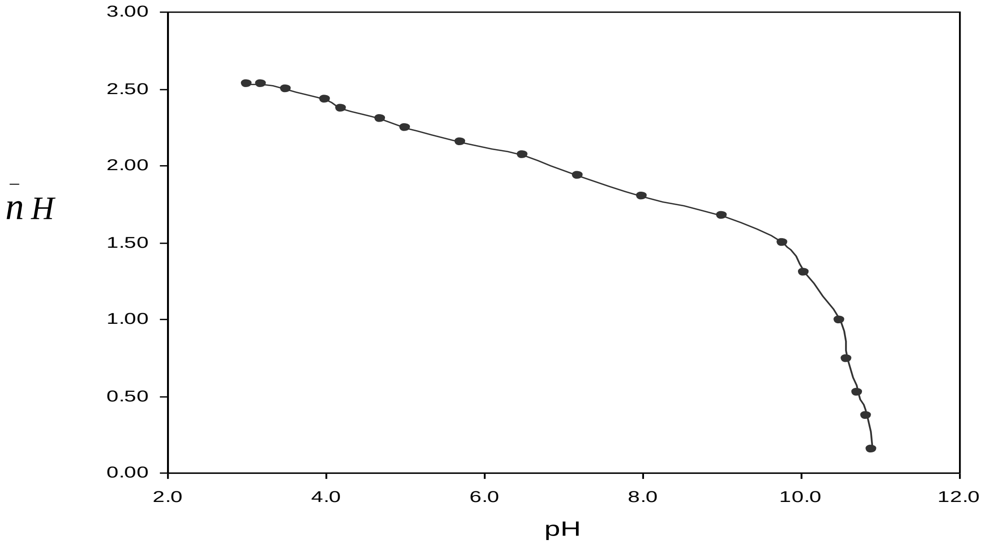


Fig.2.Protonation constants curve of (MPMPB) at 0.1 M (aqueous - ethanolic) solution at 25 ºC.

It is worth mentioning that the ligand do not hydrolysis under the experimental conditions .This is indicated by the rapid attainment of equilibrium during the titration time. The titration curves of the metal - ligand solutions (C) are well separated from the ligand solution (b) in Fig. 1. Thus replacement of H^+ ion is due to complexation. From these titration curves, $\bar{n}$ (average number of ligand molecules attached per metal ion) and pL (free ligand exponent) values were calculated using Irving and Rossoti equations [24].

$$\bar{n} = \frac{(V_3 - V_2)(N^0 + E^0)}{(V_0 + V_2)\,\bar{n}HTcM^o} \text{ -------- --} \rightarrow (2)$$

$$pL = Log\left[\frac{(1 + K_1{}^H[H^+] + K_2{}^H[H^+]^2 + K_3{}^H[H^+]^3 + ----)}{(Tcl^0 - \bar{n}TcM^0)} \times \frac{V_0 + V_3}{V_0}\right] \text{ ------} \rightarrow (3)$$

Where V_1, V_2, V3 are the amounts of alkali reach the same pH in the free acid, free acid + ligand and free acid + ligand + metal, respectively. T_cM° denotes the total concentration of metal present in the solution. The $\bar{n}$ values were plotted against the corresponding pL values to get the formation curves of the metal complexation equilibria. The formation curves are shown in Fig.3.

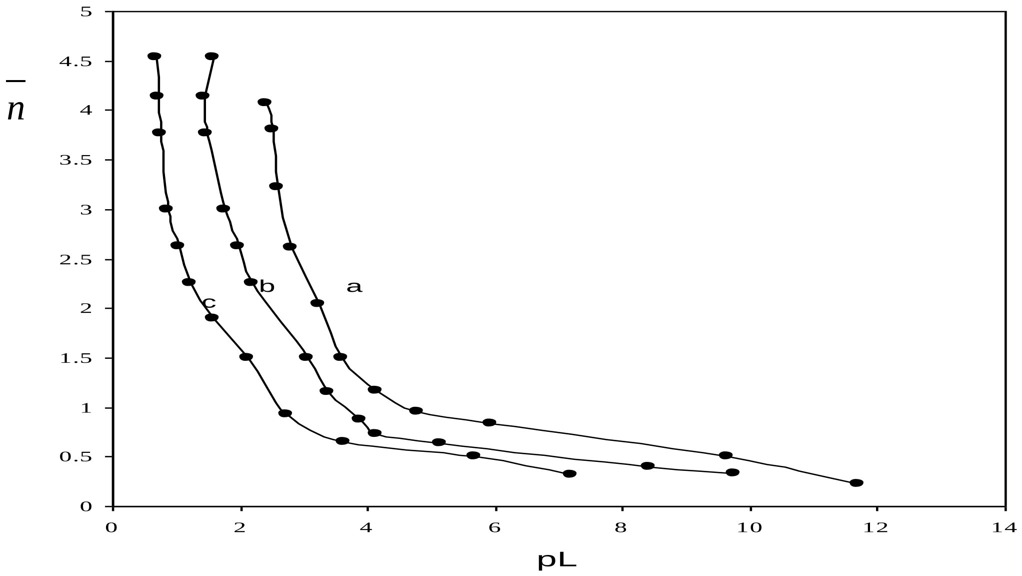


Fig.3. Formation curves of binary metal ion complexes with (MPMPB) ligand at I = 0.1 M $NaNO_3$ (a): Pd(II) ion, (b): Cu(II) ion and (C): Co(II) ion.

From these formation curves, the values of stability constants at the ionic strength I = 0.1M listed in Table 1, were determined using the half - integral method [24].

Table(1): Protonation constants and stability constants of metal ions with (MPMPB) ligand at ionic strength = 0.1M $NaNO_3$ at 25°C.

No	Metal ion	$LogK_{1\,(1:1)\,M:L}$	$LogK_{2\,(1:2)\,M:L}$	$LogK_{3\,(1:3)\,M:L}$
1	H^+ (ion)	10.70	9.8	3.5
2	Pd(II)	9.67	4.34	----------
3	Cu(II)	8.42	3.05	-------

4	Co(II)	5.67	2.11	-------
5	Ni(II)	5.42	2.05	-------
6	Cd(II)	4.36	1.58	-------
7	V(V)	7.37	2.78	-------

The three proton- ligand dissociation constants for (MPMPB) are 3.5, 9.8 and 10.7, listed in Table 1. These were assigned as follows: 3.5 belong to the protonation in imines(NH) group of 2-mercapto-phenylamino, 9.8 due to the deprotonation of thiol or mercapto (SH) group and 10.7 attributed to deprotonation (NH) group of N-P-tolybutamide. However Ionization (MPMPB) takes place in three steps that can be represented by the following equilibria: The sites are shown as follow:

$$H_3L \overset{K_1}{======} H_2L^- + H^+$$

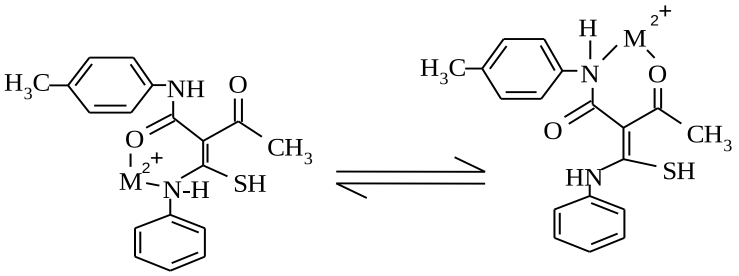
$$K_{BH^+}\ (protonation\ of\ (NH)\ group of\ 2 mercapto - phenyl\ a\min o\ pK_{a1}$$

$$H_2L \overset{K_2}{======} H_2L^- + H^+$$

$$(deprotonation\ of\ (SH)\ thiol\ or\ mercapto\ group\ \ pK_{a2}$$

$$HL \overset{K_3}{======} L^- + H^+$$

$$(deprotonation\ of\ (NH)\ group of\ N - p - tolybu\tan amide\quad pK_{a3}$$



(MPMPB) - Metal ion complex

Where M = V(V), Co(II), Ni(II), Cu(II), Pd(II) and Cd(II)

The order of stability constants of the different binary complexes formed between (MPMPB) ligand and transition metal ions investigated in this study is in the expected Irving - Williams order [26] for (1:1) metal to ligand at I = 0.1 M $NaNO_3$ + 50 % alcoholic solution:

Pd (II) > Cu (II) > V (V) > Co (II) > Ni (II)) > Cd (II).

3.2- Spectrophotometric studies:

3.2.1- Determination of the Metal Complexes.

The stoichiometry of the Cu (II) and Pd (II) complexes was studied by applying the molar ratio [27], continuous variation [28] methods. Figures, (4, 5) shown the absorption spectra of molar ratio and continuous variation methods. The results proved the 1:1 or/and 1:2 (M: L) complexes were formed are shown in figs.(6,7). The formation and stability constants of the complexes of these metal ions are listed in table (3).The results concise to in potentiometeric method.

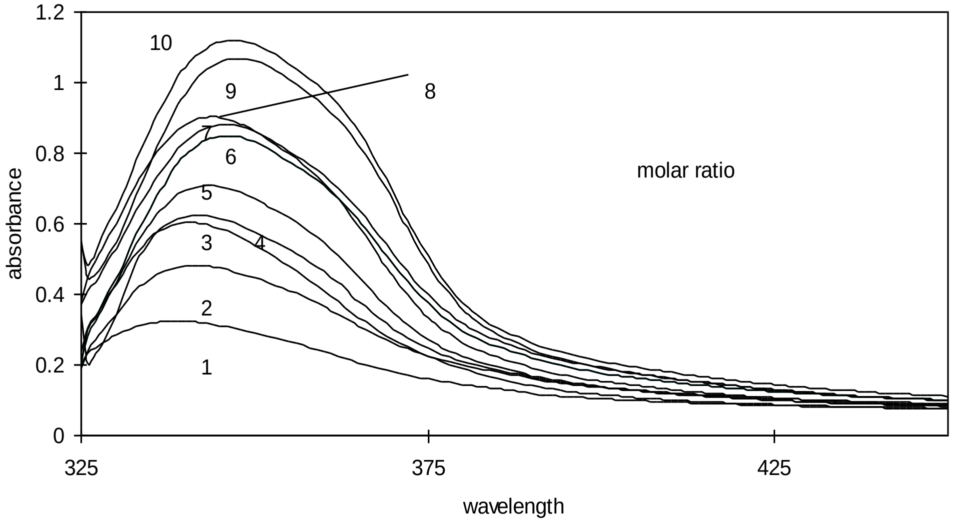


Fig.4. Absorption spectra of molar ratio for Cu (II)-MPMPB) complexes at 25ºC.and λ_{max} =345 nm.

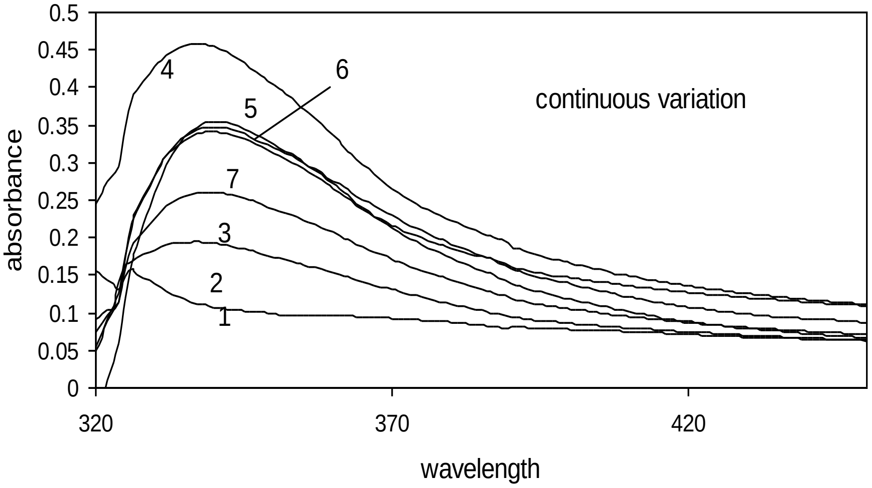


Fig.5. Absorption spectra of continuous variation for Cu (II) - (MPMPB) complexes λ =340 nm

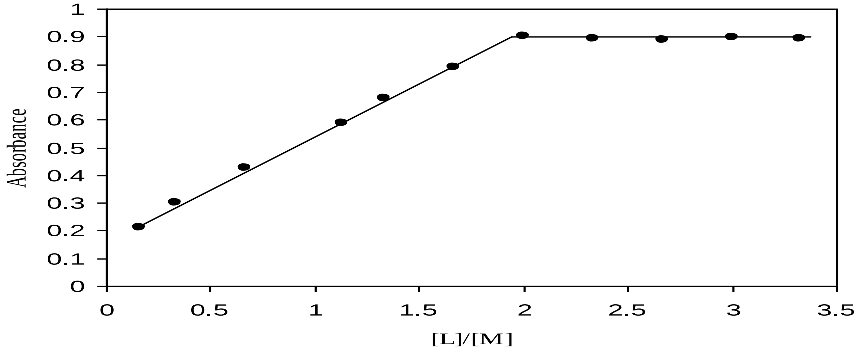


Fig.6.Absorbance molar ratio relationship for Cu (II)- (MPMPB) complex at 25ºC and 25ºC.and λ_{max} =345 nm.

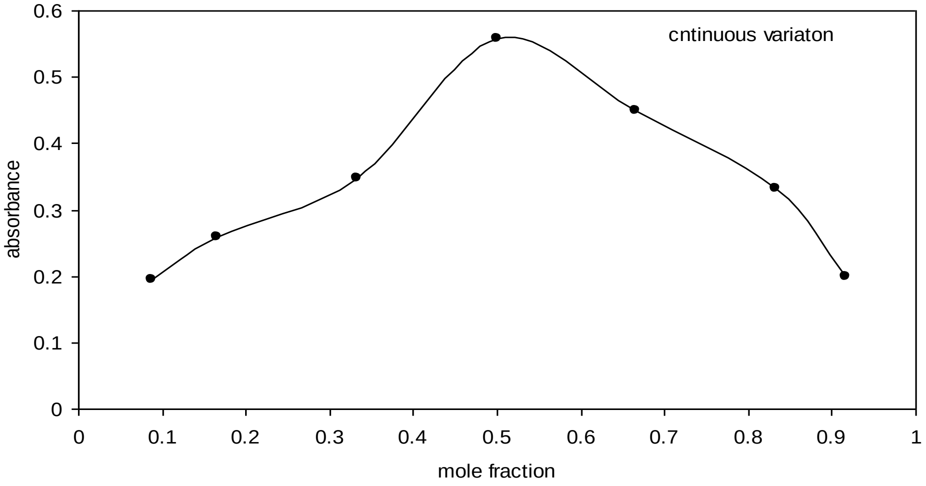


Fig.7. Absorbance mole fraction relationship for Cu (II) - (MPMPB))

3.2.2- Calibration Curve:

In order to use the complexes of the metal ions Cu(II), Pd(II), with (MPMPB) for microdetermination of metal ions. Solutions were prepared in which the concentration of the ligand was kept constant at 1x10^{-3} M and that of the metal ion was regularly varied. Absorbance of the obtained mixture is measured at the corresponding λ_{max} of the formed chelate. A graph of absorbance versus the concentration (μg/ml) of the tested metal ion is plotted in Fig.8.The curves indicate that, the absorbance is a linear function of the concentration of the metal ions studied. The linearity range obeyed beer's low form 0.31-3.5 and 0.31-9.5(μgml^{-1}) for Cu(II) ion and Pd(II) ion, respectively. Molar absorptive was 15,000 and 25,000 L mol^{-1} cm^{-1} for Cu(II) and Pd(II), respectively. The curves pass through the origin, denoting a perfect validity of beer's law. Deviation are observed at high concentrations of metal ions. This behaviour clearly indicates that at such metal ion concentrations, the amount of lignad is not sufficient to transform the metal ions to complexes of the adequate stoichiometry.

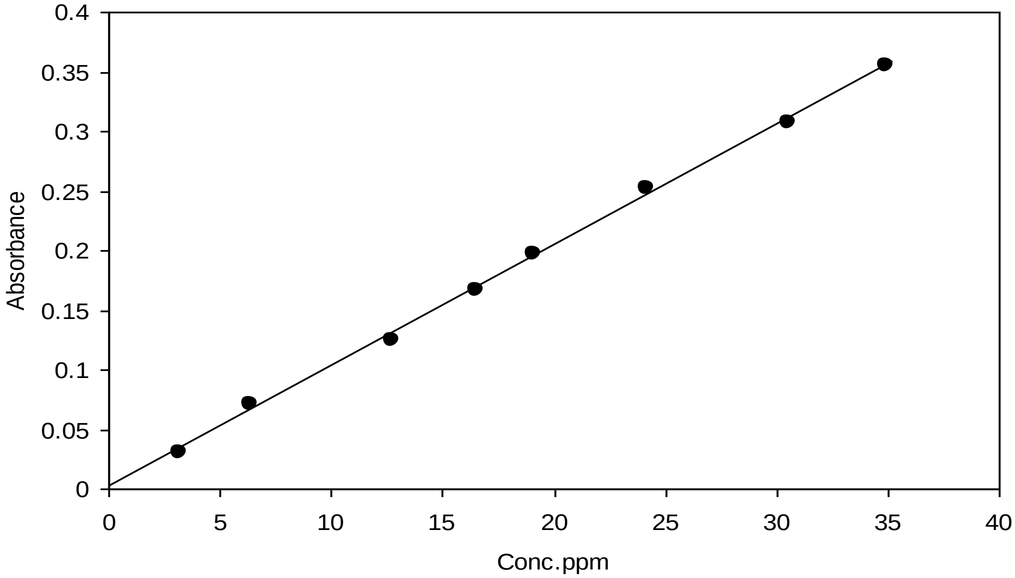


Fig.8. *Absorption spectra of 1x10^{-5} M of (MPMPB) with different Concentrations of Cu (II) ion.*

4- Microchemical analysis of L (MPMPB) and their metal complexes

The results of analysis of the solid metal complexes of L with of V(V), Co(II), Ni(II), Cu(II), Pd(II) and Cd(II) are illustrated in Table (3).

Table (3): Microchemical analysis data of L (MPMPB) and their metal complexes**.**

Compound	Colour	M.wt	C% Calc. (found)	H% Calc. (found)	N% Calc. (found)	S% Calc. (found)
L (MPMPB) $C_{18}H_{18}N_2O_2S$	Cotton yellowish	326.42	66.23 (66.21)	5.56 (5.54)	8.58 (8.56)	9.82 (9.80)
$NH_4[V L (H_2O)_4] 2H_2O$	Black	503.40	42.95 (42.51)	6.81 (6.64)	8.35 (8.10)	6.37 (6.10)
$[CoL H_2O)_2]Cl_2.4H_2O$	greenish	564.34	38.30 (38.04)	5.35 (5.30)	4.96 (4.92)	5.68 (5.64)
$[Ni L (H_2O)_2] Cl_2$	Yellow	455.99	47.41 (47.08)	3.97 (3.95)	6.14 (6.10)	7.03 (6.98)
$[Cu L (H_2O)_2 Cl_2] 4H_2O$	Brown	568.71	38.01 (37.75)	5.31 (5.28)	4.92 (4.89)	5.63 (5.59)
$[Pd L (H_2O)_4] Cl_2$	Black	574.84	37.57 (37.52)	3.13 (3.11)	4.87 (4.90)	5.56 (5.59)
$[Cd L (H_2O)_4] Cl_2$	White Yellowish	563.73	38.35 (38.00)	4.29 (4.10)	4.96 (5.00)	6.00 (5.17)

5-Infrared Spectra of Solid Complexes *of* (MPMPB).

The infrared spectra of investigated solid complexes, it's observed the influenced bands are shifts to lower and higher frequencies by complexation The IR spectra in fig. 9 shows, the band at 3246 cm^{-1} in the free ligand which corresponding to υ(NH) group is shifts to higher frequencies in the spectra of the complexes due to coordination with Pd(II), Cu(II), Co(II), Cd(II), V(V), Ni(II) metal ions at 3435cm^{-1}, 3442 cm^{-1}, 3446 cm^{-1}, 3275 cm^{-1}, 3436 cm^{-1} and 3434 cm^{-1}, respectively. The two bands at 1677, 1600 cm^{-1} in the IR spectra of (MPMPB) were attributed to stretching C=O are shifts to lower frequencies and only one band exchanges appears in spectra of the complexes at 1676cm^{-1}, 1660 cm^{-1}, 1652 cm^{-1}, (1644,1606) cm^{-1}, 1600 cm^{-1} and 1630, 1600 cm^{-1} for Pd(II), Cu(II), Co(II), Cd(II), V(III) and Ni(II) metal ions, respectively.

On the other hand the carbonyl group (C=O) stretching vibration appeared of lower frequencies in spectra of the complexes relative to its position in free ligand in all cases depending upon the nature of the metal ion.

Two new bands appears for the all chelates at 560-509 cm^{-1} region corresponding to stretching [M-O] metal-oxygen bond and 456-439 cm^{-1} due to stretching [M-N] metal-nitrogen bond. The important IR bands are listed in table 4.

Table4. Relative IR bands of ligand (MPMPB) and their metal chalets.

Compound	ν NH		ν C=O		ν M←O	ν M←N
L (MPMPB) $C_{18}H_{18}N_2O_2S$	3246	3181	1677	1617	-	-
NH_4 [V L $(H_2O)_4$]. $2H_2O$	3436	-	1646	1626	560	439
[Co L $(H_2O)_2$]Cl_2.$4H_2O$	3446	-	1652	1600	520	456
[Ni L $(H_2O)_2$] Cl_2	3434	3318	1635	1600	511	442
[CuL $(H_2O)_2$ Cl_2] $4H_2O$	3442	-	1660	1601	509	440
[Pd L $(H_2O)_4$] Cl_2	3435	3247	1676	1602	519	456
[Cd L $(H_2O)_4$] Cl_2	3275	3151	1644	1606	510	442

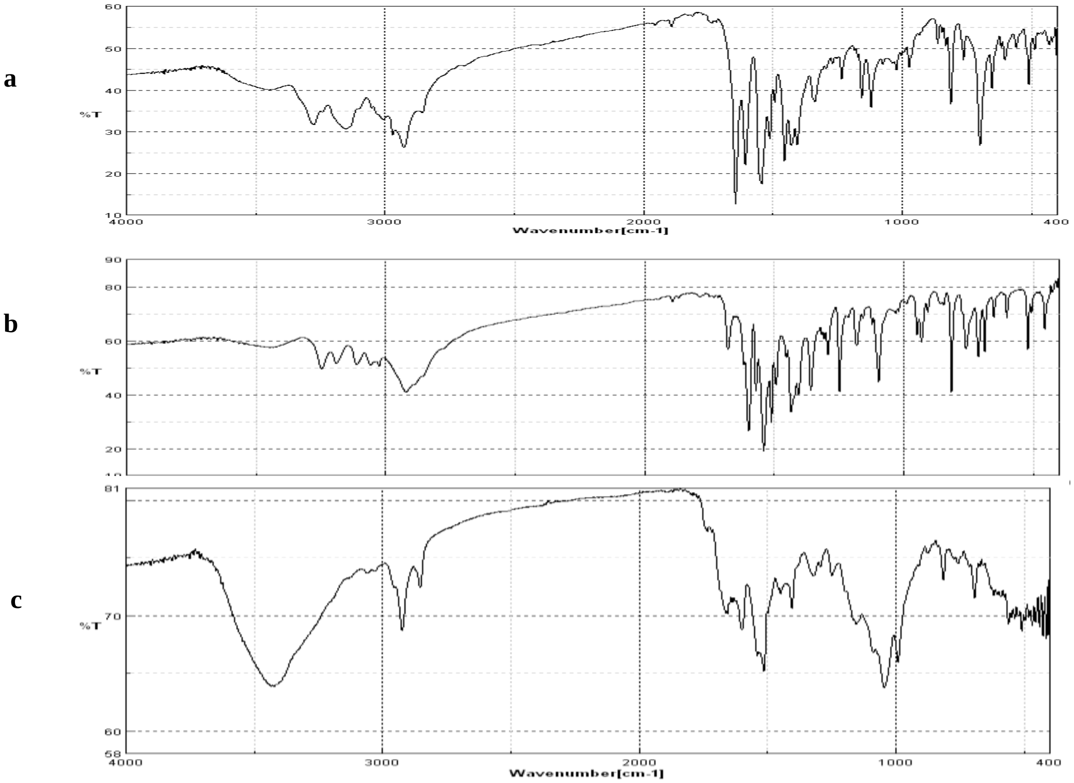


Fig.9. Infrared spectra of the free ligand L and its metal complexes (a) Free ligand (L), (b) Ni(II)-LComplex and (c) Cu(II)-LComplex.

7- Thermogravimetric Analysis of (MPMPB) -Metal ion Complexes:

Thermogravimetric analysis curves (TGA) of two representatives compounds such as [Cu L $(H_2O)_4$]Cl_2.2H_2O and [Pd L $(H_2O)_4$] Cl_2 in the temperature range from ambient to 800 at heating rate 15°C/min.

The [Cu L $(H_2O)_4$]$Cl_{2.}$ 2H_2O complex thermogram (Fig.10) shows that the complex decomposes completely up to 800°C, with mass loss on four steps and the final residue is CuO, which is a stable product. The first step amounted to 18.80% in the temperature range between 70-160°C, this corresponds to the removal of the four coordinated water molecules and the two crystalline water molecules. The mass loss in the second step amounted to 11.90% in the temperature range 200-250°C, which accordance with the elimination of two HCl molecules. The third step from 260-420°C, with a mass loss of 17.4% corresponds to the decomposition of the non-coordinated part of ligand p-toluidine. The four step amounted to 35.60% occurs in the temperature range 430-720°C, corresponds to the decomposition of the coordinated part of the ligand 2-(Mercapto-phenylamino) methylene)-3-oxobutanal. The structure of the metal complex can be represented as follows:

For [Pd L $(H_2O)_4$] Cl_2 complex thermogram, shows that the complex decompose completely up to 800°C, with weight losses on two mainly steps. And finally give PdO oxide as a stable product.

The first step amounted to (~ 10.52%) equal three water molecules at temperature range (60-210°C) this can be attributed to removal of four coordinated water molecules. The second step amounted to (25.65%) temperature range between 220-330°C which can be corresponding to the removal 3-oxo-*N*-*p*-tolyl butyramide block. The third amounted to (18.56%) can be attributed to the elimination of 2-(Mercapto-phenylamino) at temperature range between (332-545%). Remain PdO amounted to (21.1%) up to 800°C.

Structure Cu(II)- L (MPMPB) binary complex.

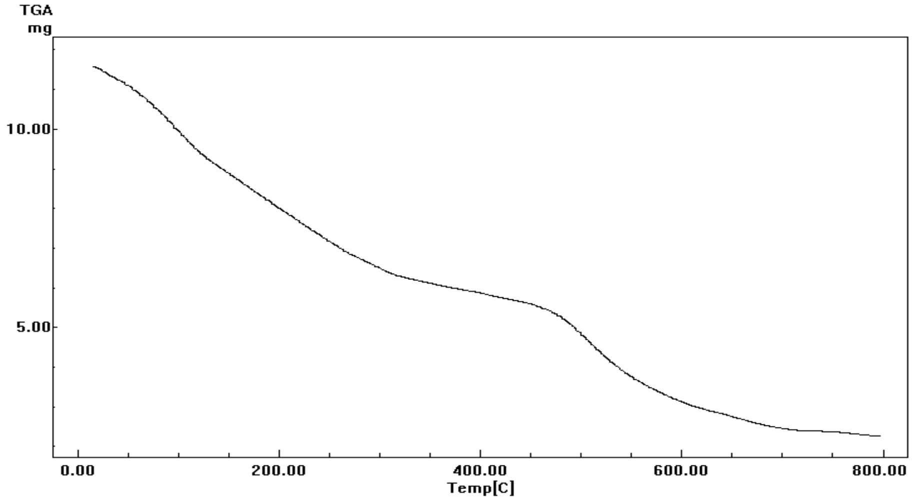


Fig. 10**.** Thermogravimetric analysis curve of Cu (II) - (MPMPB) complex.

4- X-Ray Powder Diffraction:

The X-ray diffraction pattern for Cu(II)- (MPMPB). Complex is shown in Fig.10 indicates it’s crystalline. But for Co(II) and Pd(II) with (MPMPB) complexes X-ray diffraction pattern reveals it's crystalline - amorphous.

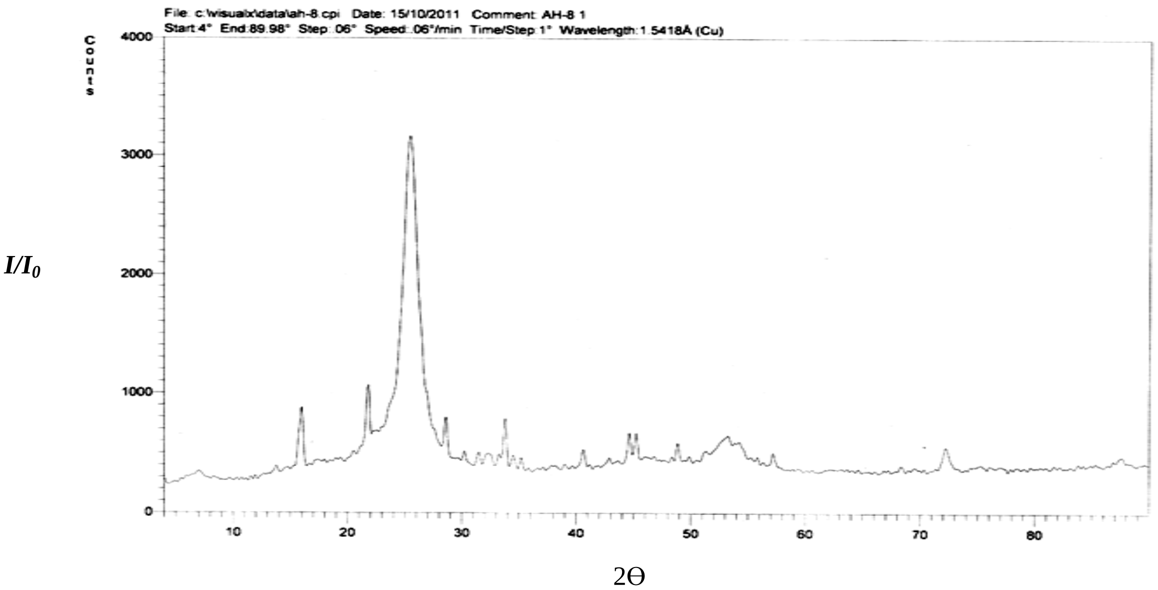


Fig.11. X-ray powder diffraction for Cu (II) – (MPMPB) complex.

5- Antimicrobial Activity studies

(I)Antibacterial activity

The results of antibacterial activity of (MPMPB) ligand and their Cu(II) and Pd(II) solid binary complexes.The organisms selected for the studies were *Serratia marcescens (-ve), Pseudomonas eruginosa (-ve), Escherichia coli (-ve), Staphylococcus aurous (+ve), Bacillus cereus (+ve) and Micrococcus luteus.* The antibacterial activity was evaluated by the inhibition zone technique. The results of antibacterial screening of L and their Cu (II) and Pd (II) binary complexes at concentration of all samples are 2%. Cu(II) binary complexe was more antibacterial activity than the parent ligand, but they show less activity than chloroamphenicol as antibacterial standard.

Pd(II) with (MPMPB) ligand L complex do not show any significant effect.

(II)Antifungal activity

The antifungal activity of ligand (MPMPB) and their Cu(II) and Pd(II) solid binary complexes were carried out against pathogenic fungi , namely *Candida albicans (-ve), Geotrichum Candidum (-ve), Aspergillus flavus (+ve), Fusarium oxysporium (-ve), Scopulariopsis brevicaulis (+ve) and Trichophytin rubrum* by radial growth methods. The solution of the test compounds was prepared as 2% for all samples. The linear growth of the fungus was recorded by measuring the diameter of the fungus colony after 24 h and the percentage inhibition was calculated as 100(C- T)/ C, where T are the diameter of the fungus colony in the control and text plates, respectively. The results of antifungal activity of the ligand and Cu(II), Pd(II) binary complexes are compared with the conventional fungicide clotriamazole as antifungal standards. Pd(II) showed no significant effects except the highest antifungal activity to *Candida albicans (-ve).*The antifungal screening data, reveal that the Cu(II)binary complexes are more fungi toxic than the parent ligand. The toxicity increased with increasing concentrations.

6- Conclusion.

A simple, rapid and sensitive Potentiometric and Spectrophotometric methods have been developed for determination of investigated metal ions based on chelating reaction with (MPMPB) reagent in aqueous solution. New binary complexes with (MPMPB) in aqueous solution and solid state were studied in this work.

According to the results presented in the article, one can be noticed that potentiometric investigation of complexation in water solvent, more information about pH dependency of complexation could be obtained. Using the obtained data, selection of proper choice of parameter to predict selective and sensitive method.

Potentiometric method is excellent method for calculation of stability constants of metal ligand complexes. (MPMPB) forms 1:1 and/or 1:2 metals to ligand complexes.

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