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Extract poly phenol oxidase enzyme to use as biological reagent in the determination of some heavy metals

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Abstract: In this paper, we assessed the reaction between poly phenol oxidase enzyme and heavy metals. Poly phenol oxidase solved in acetyl acetone to obtain solid and aqueous complexes via bio method. The Cu (II), Co (II), Ni(II) and Fe(II) complexes are biologically active due to their coordination properties. These complexes act as good chelating agents and have high pharmacological potential. Complex of Co (II) with poly phenol oxidase has red color at λmax = 500nm, where in this case poly phenol oxidase has been extract from eggplant. Cu (II) formed complex, where in this case poly phenol oxidizes extracted from banana. its complex has green color at λmax = 740nm . Fe (II) withpoly phenol oxidase has yellow color at λmax = 480nm, where poly phenol oxidase, has been extract from banana. Ni (II) formed complex with poly phenol oxidase has been extract from banana. It has green color and λmax = 630nm. Beside, we focused on the effect of the metals concentration on optical density. The solid complexes were synthesized and characterized by infrared spectra. All the complexes are colored and thermally stable solids. All samples show the characteristic of absorption band in the 3412 cm^{-1} . PPO show the characteristic of absorption band in 2928 cm^{-1} for O-H stretching vibration and this band disappear completely in Fe- complex and Cu- complex but decrease in case Co-complex. Another characteristic absorption band in 1628 cm^{-1} for O-H stretching vibration and this band disappear completely in Fe- complex and Cu-complex but decrease in case Co-complex. PPO has two characteristic absorption

bands in the 1423 cm^{-1} and in 1159 cm-1 for Scissor-like bending of the CH_2 groups and C-OH stretching frequency respectively and band at 767 cm^{-1} arises from the rocking vibration of CH_2 groups in a straight chain of five carbon atoms or longer. In all complexes appear characteristic absorption at 455-451-464 cm^{-1} that indicate on oxygen -metal complex.

Keywords: Cu (II), (II), Co (II), Ni (II) and Fe (II) complexes, IR, UV -poly phenol oxidase

1. INTRODUCTION

The synthesis of solid complexes from bio method thanks to their applicability in medicine ,pharmacy is in vogue[1].The metal complexes play a pivotal role in the pharmacological properties of some drugs[1]Cu (II) ,Co (II) ,Ni(II) and Fe(II) complexes have stipulated the curiosity of researchers, who have focused on their coordination and biological applications. Complexes of Cu (II), Co (II), Ni (II) and Fe(II) with poly phenol oxidase are used as models to study the pharmacological effects of drugs. We synthesized Cu (II), Co (II), Ni (II) and Fe (II) complexes[2]. Poly phenol oxidase act as bio ligand. This paper focuses on novel method to assure the determination of Cu (II) and Co (II) with natural poly phenol oxidase (PPO) which extract from banana and eggplant. The enzyme[3]PPO it's M.W = 128 two binding site for oxygen[4] and for aromatic compounds have phenolic substrates. PPO is an oxygen transferring enzyme. Oxygen catalyze the dehydrogenation of catechols to the orthohydroxylation of phenols to catechols and orthoquinones [5]. some polyphenols like stilbenoid, flavonoids, copper chelators and free radical scavengers[6]. Tyrosinase is used in research to treat skin disorders[7]. Oxidases (PPO) are enzymes, belonging to a group of copper containing metalloproteinase and are members of oxido reductases that catalyze the oxidation of a wide range of phenolic compounds by utilizing molecular oxygen[8].

2. INSTRUMENTALS

a-2910 HITAC2j-0012 spectrophotometer, the equipment is classified as class A of EN61326. The program has the following measurement modes.

a- Wavelength scan 200-800 nm; b- Time scan; c- Photometry

b- pH - meter 1351

c- Melting equipment 5Mp10

d- Stirring equipment

f- **Infrared spectroscopy:** Ftir Perkinelmer model spectrum 100

3. EXPERIMENTAL

3.1 Materials and Methods: $Cu(NO_3)_2.3H_2O$, $Co(Cl)_2.6H_2O$, Fe SO_4, Ni $(NO_3)_2$. Fresh fruits and vegetables of eggplant, and banana from the local market sijar. Sodium phosphate, acetyl acetone and nitric acid. All chemicals were analytical grade.

3.1.1- Preparation of crude PPOs: The extrication process occur via homogenizing 500 gm of sliced banana and eggplant with 1000ml cold sodium phosphate buffer (0.2 M, pH 7)[9]. The precipitation occur via adding 100 volume of cold acetone (-5C°) with stirring for one hours. This enzyme was dissolved in 50 ml acetyl aceton[10].

3.1.2 Preparation of aqueous complexes: Preparation different concentrations of $Co(Cl)_2.5H_2O$;$Cu(NO_3)_2.3H_2O$, Ni $(NO_3)_2$, $Fe(NO_3)_2$ (0.001- 0.002- 0.003- 0.004) molar. Tack 10 ml of metal solutions and add 5 ml of PPOs(0.02g) which dissolved in 10 ml of conc. 98% acetyl acetone[11] with 1ml of 0.2M sodium phosphate buffer(pH =7) in flask 25 ml and completely with distal water in case Cu- complexes, Ni- complexes, Co- complex and Fe- complexes. Late the complexes for one hours. to determination wave length maximum for each complex.

3.1.3 Synthesis of solid complexes: Therefore, PPOs-metal complexes were prepared 1:2 molar ratio. The [Cu (L) 2] $^{2+}$[Co (L)2]$^{2+}$ and [Fe (L)2] $^{2+}$complexes. PPO n this process 0.04 g PPO was added to 0.02g of metal salt string for 3 hours and add 1 ml of acetyl acetone. A dark blue colored obtained for solid Cu(II)- complex , orange colored solid Fe(II)- complex solid and a dark green solid Ni(II)- complex determination melting point for each complex and characterized by infrared spectra.

4. RESULTS AND DISCUSSION

4.1. Spectrophotometric studies of PPO- complexes: The spectral studies for PPO which dissolved in 10 ml acetyl acetone which give brown color. We measured spectra for this solution from 200-700nm. We obtained band at 403 nm at room temperature in 1cm quartz cell show **Figure (1).**

4.1.1. Validity of Beer's law: The validity of Beer's law for systems was studied by measuring the absorbance values of series of solution having different metals and ligand concentrations in aqueous medium. The obtained results are shown in **Figure (2).**

4.1.2. Effect of concentration of metal ion: Figures (3-6) show a well - defined peaks were observed on the increases with increase concentration for Co- complexes, Cu- complexes, Ni-complexes and Fe- complexes. The concentrations' 0.002- 0.003- 0.004 M for Co -complex, Cu-complex, Ni- complex and Fe- complex are the best[12].

4.1.3. Effect of the contact time on PPOs- complexes: The optical densities of PPOs complexes at 630,740, 480,500 for PPOs- Ni (II), PPOs- Cu (II), PPOs-Fe (II) and PPOs-Co (II) complexes respectively were measured at different time intervals and aging time of prepared metal solutions is 10 minutes .at pH = 7.5 and pH = 3. It can be conclude that:

- At pH <7 the optical density = 0 means that no complexes formed in acidic medium at different contact time for PPOs- Ni(II), PPOs- Cu(II), and PPOs-Co(II) complexes but Fe-complex formed at- pH= 3
- At pH = 7.5 complexes start in formation for PPOs- Ni(II), PPOs- Cu(II), and PPOs-Co(II) complexes but Fe- complex formed at- pH= 3

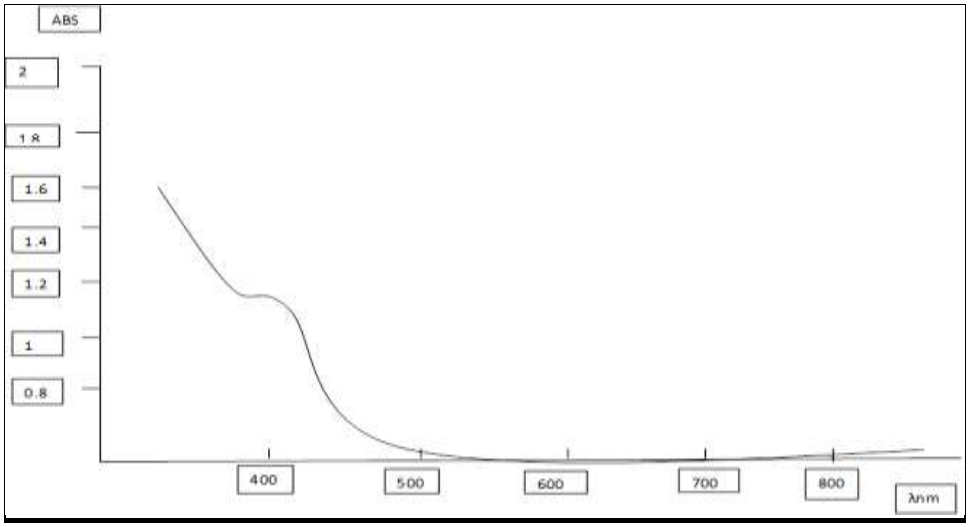

Fig.(1): Optical density of the poly phenol oxidase in acetyl acetone at room temperature

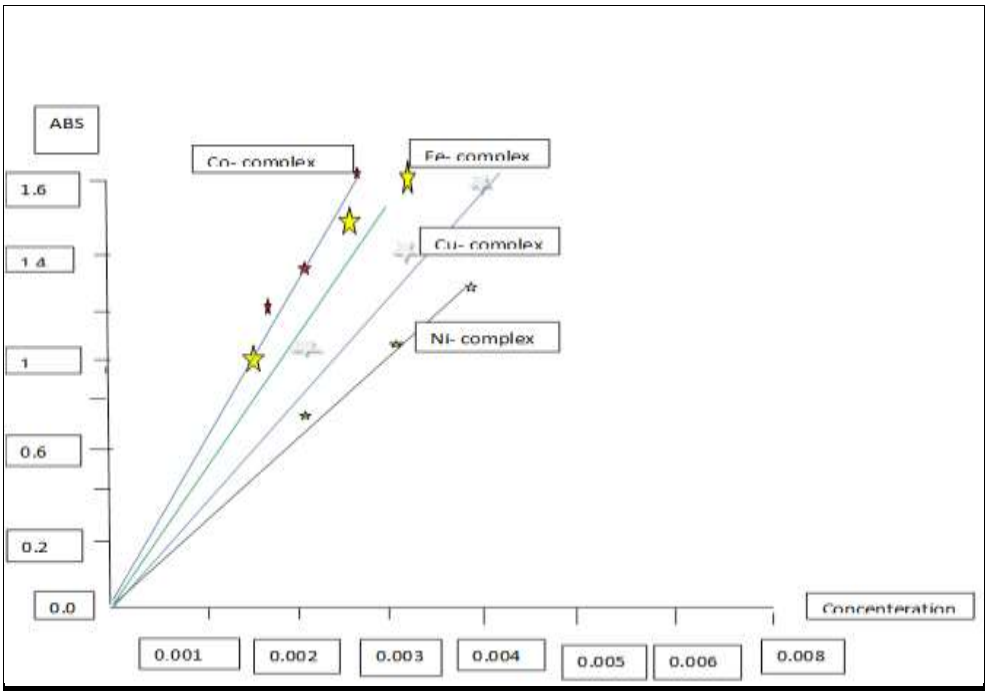

Fig. (2): Confirmation of Beer's law for different complexes**.**

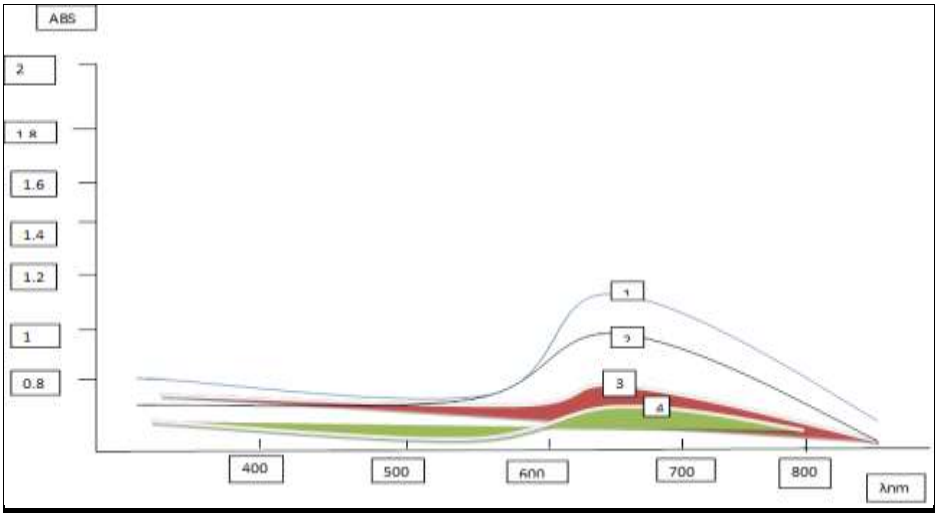

Fig.(3): Effect of different concentrations of Ni(II) ion on absorption spectra of Ni-complexcurve(1)[Ni(II)]=0.004M,curve(2)[Ni(II)]=0.003M,curve(3),[Ni(II)]=0.002M,curve (4) [Ni(II)]=0.001M at pH =7.

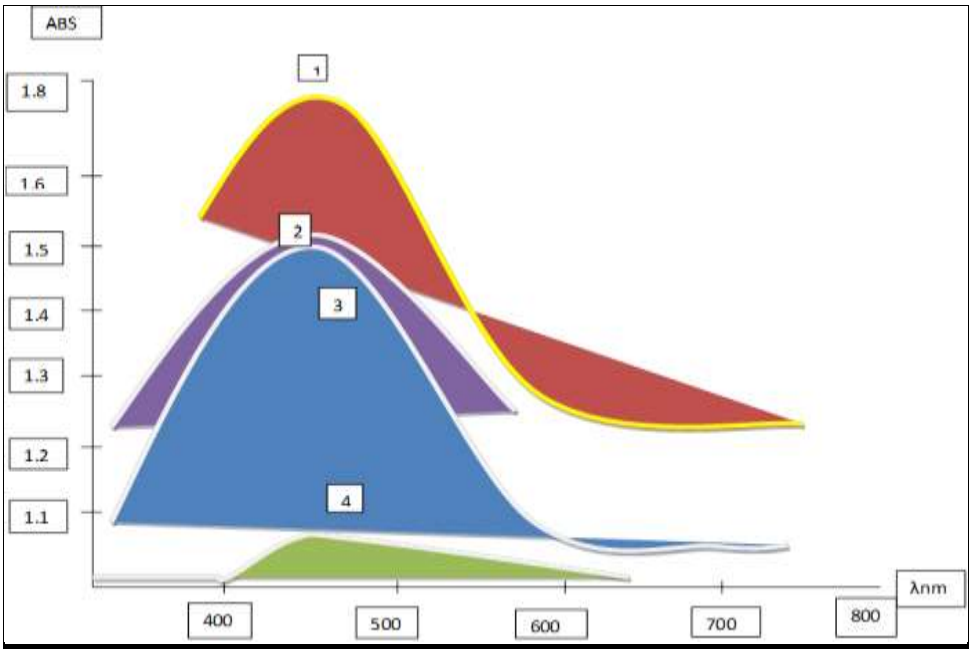

Fig.(4): Effect of different concentrations of Fe(II) ion on absorption spectra of Fe- complex curve(1)[Fe(II)]=0.004M,curve(2)[Fe(II)]=0.003M,curve(3),[Fe(II)]=0.002M,curve(4) [Fe(II)]=0.001M at pH =2.

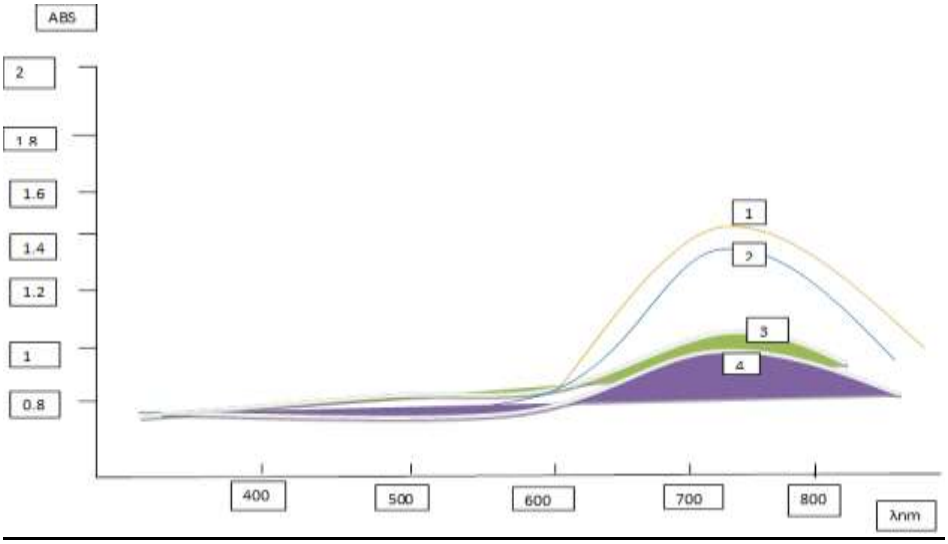

Fig.(5): Effect of different concentrations of Cu(II) ion on absorption spectra of Cu- complex. curve(1)[Cu(II)]=0.004M,curve(2)[Cu(II)]=0.003M,curve(3),[Cu(II)]=0.002M,curve(4) [Cu(II)]=0.001M at pH =7.

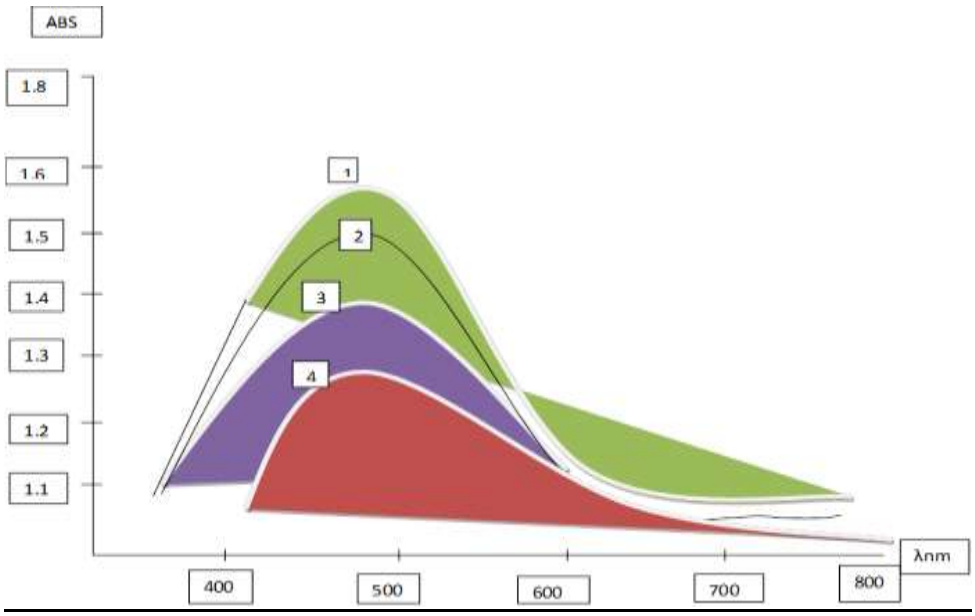

Fig.(6): Effect of different concentrations of Co(II) ion on absorption spectra of Co- complex curve(1)[Co(II)]=0.004M,curve(2)[Co(II)]=0.003M,curve(3),[Co(II)]=0.002M,curve(4) [Co(II)]=0.001M at pH =7.

These ligands donate pairs of electrons to the metal ions act as lewis bases. Since these complexes contains two types of the ligands. They have 4 coordinate bonds arranged square planer about the metal center: Cu $(NO_3)_2.3H_2O$, Co $(Cl)_2.6H_2O$, Fe SO4, Ni $(NO_3)_2$ is a brightly colored substances, whereas the products have a deferent color[13,14]. Electrons in partially filled d-orbital's can absorb light of visible wavelengths. The absorption has got low energy visible light occurs due to splitting of the d-orbital's energy levels of the M^{2+}ion and an interaction between the donated electron pair of the

ligands with the lobes of the 8d-orbitals on the metal center[15]. However, if the electron pairs of the ligands approach a dxy orbital along these same axes, the approach is along a nodal plane and the interaction is less unfavorable, causing the energy of the system to not rise as much. This leads to a splitting in the energy of the d-orbitals[16].

4.2. Solid complexes: All the solid complexes are thermally stable solids and colored. Table (1) show in the physicals properties of the PPO and its complexes. Melting point for these complexes are determination and poly phenol oxidase show **table (1).**

Table (1): Physical properties of enzyme and its complexes

Compounds	***λmax***	***Color solid complexes***	***Color aqueous***	***Melting point***
PPOs	403nm	brown	yellow	280C^0
Cu- complexes	740nm	Dark blue	Dark green	240 C^0
Co- complexes	500nm	violet	red	280 C^0
Fe- complexes	480 nm	orange	yellow	220 C^0

In all samples appear characteristic absorption band in the 3412 cm^{-1} for water[17] show **Fig.(7-10)**. PPOs appear characteristic absorption band in 2928 cm^{-1} for O-H stretching vibration and this band disappear completely in Fe- complex and Cu- complex but decrease in case Co-complex show **Fig. (7-10).**

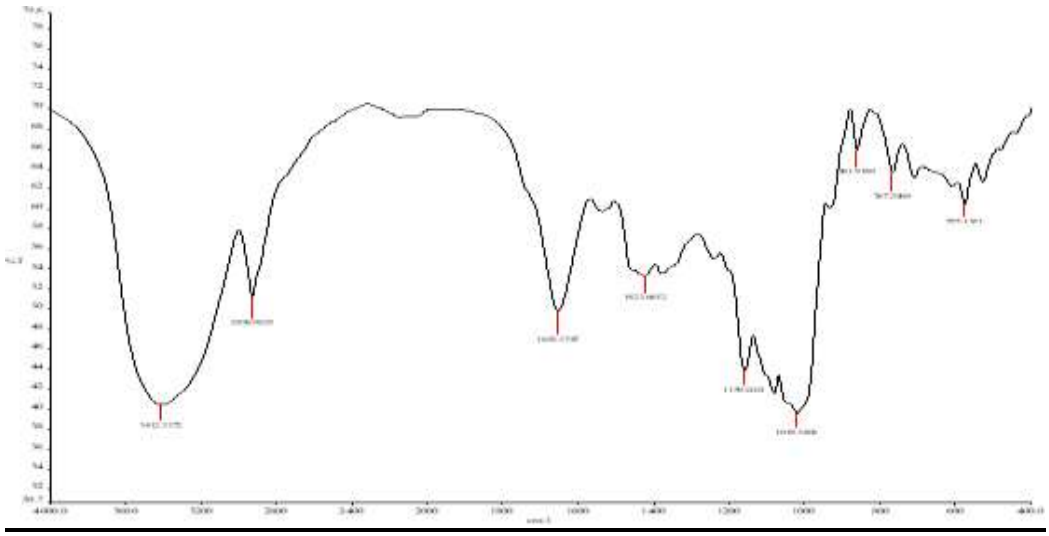

Fig.(7):IR spectra for poly phenol oxidase

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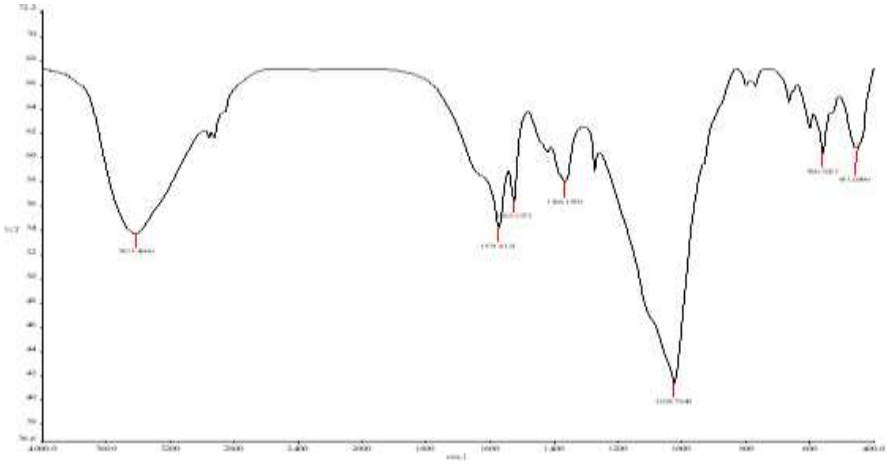

Fig. (8):IR spectra for Fe- PPOs complex

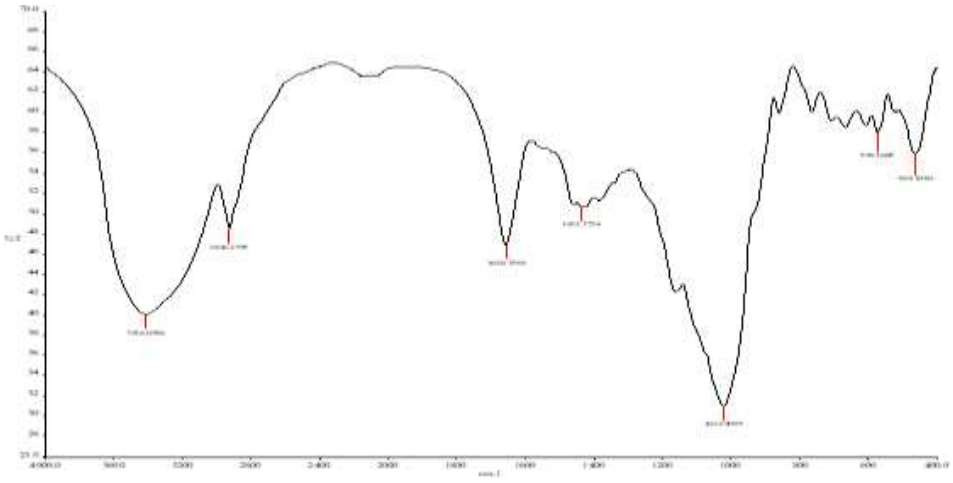

Fig. (9):IR spectra for Co- PPOs complex

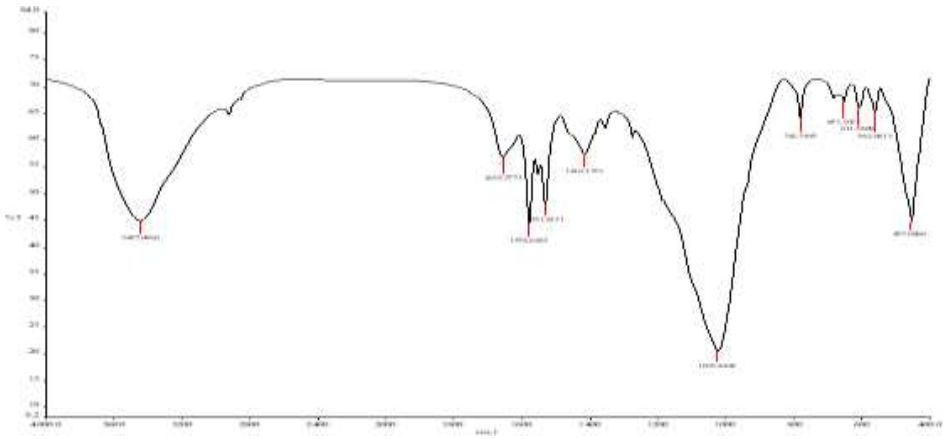

Fig. (10): IR spectra for Cu- PPOs complex

4.3 DISSOCIATION

In all samples appear characteristic absorption band in the 3412 cm^{-1} for water[17] show **Fig.(7-10)**. PPOs appear characteristic absorption band in 2928 cm^{-1} for O-H stretching vibration and this band disappear completely in Fe- complex and Cu- complex but decrease in case Co-complex show **Fig. (7-10).** Another characteristic absorption band in 1628 cm^{-1} for O-H stretching vibration and this band disappear completely in Fe- complex and Cu- complex but decrease in case Co-complex. PPOs has two characteristic absorption bands in the 1423 cm^{-1} and in 1159 cm-1 for Scissor-like bending of the CH_2 groups and C-OH stretching frequency respectively and band at 767 cm^{-1} arises from the rocking vibration of CH_2 groups in a straight chain of five carbon atoms or longer show **Fig(6).** In all complexes appear characteristic absorption at 455-451-464 cm^{-1} show **Fig (8-10).** That indicate on oxygen -metal complex.

5. CONCLUSION

The Cu (II), Co (II), Ni(II) and Fe(II) complexes are biologically active due to their coordination properties. These complexes act as good chelating agents and have high pharmacological potential. Complex of Co (II) with poly phenol oxidase has red color at λmax = 500nm, where in this case poly phenol oxidase has been extract from eggplant. Cu (II) formed complex, where in this case poly phenol oxidizes extracted from banana. its complex has green color at λmax = 740nm . Fe (II) with poly phenol oxidase has yellow color at λmax = 480nm, where poly phenol oxidase, has been extract from banana. Ni (II) formed complex with poly phenol oxidase has been extract from banana. It has green color and λmax = 630nm. Beside, we focused on the effect of the metals concentration on optical density. The solid complexes were synthesized and characterized by infrared spectra. The analytical data of these complexes indicate metal ligand bonding. All samples show the characteristic of absorption band in the 3412 cm^{-1} . PPO show the characteristic of absorption band in 2928 cm^{-1} for O-H stretching vibration and this band disappear completely in Fe-complex and Cu- complex but decrease in case Co-complex. Another characteristic absorption band in 1628 cm^{-1} for O-H stretching vibration and this band disappear completely in Fe- complex and Cu- complex but decrease in case Co-complex. PPO has two characteristic absorption bands in the 1423 cm^{-1} and in 1159 cm-1 for Scissor-like bending of the CH_2 groups and C-OH stretching frequency respectively and band at 767 cm^{-1} arises from the rocking vibration of CH_2 groups in a straight chain of five carbon atoms or longer. In all complexes appear characteristic absorption at 455-451-464 cm^{-1} that indicate on oxygen -metal complex.

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