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# Copper-Hydroxo Chelates of Clofibric Acid (CA): Reaction of Cu<sup>2+</sup> with CA

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## Abstract

2-(p-Chlorophenoxy)-2-Methylpropionic acid or (Clofibric acid (CA) chemical formula C<sub>10</sub>H<sub>11</sub>ClO<sub>3</sub>) is a ligand that binds to Peroxisome proliferator-activated receptors-alpha (PPAR $\alpha$ ). This ligand has two centers of attachments that can chelate a variety of metal ions. From our ongoing efforts to study the reaction of CA with many metal ions such as Al<sup>3+</sup>, Zn<sup>2+</sup>, Cr<sup>3+</sup> and Fe<sup>3+</sup>, we are reporting the outcome of the reactions of CA with the hexa-aqua Cu<sup>2+</sup> ion in aqueous solutions under ambient conditions using well established techniques. The main techniques used were potentiometry, UV-Vis, and IR spectroscopies. It appeared that the major species formed from the reaction of CA with Cu<sup>2+</sup> is the ternary [Cu<sup>2+</sup> (CA) (OH)<sub>2</sub>]<sup>-</sup> species. These studies are novel and shed light on this reaction system. UV-Vis spectra of CA, and CA-Cu<sup>2+</sup> complexes are novel. IR spectroscopy proved the participation of the hydroxylate group of CA in the chelation of the Cu<sup>2+</sup> ion.

**Keywords:** Clofibric acid; Copper (II); IR; Potentiometry; UV-Vis

## Introduction

CA is a Peroxisome proliferator-activated receptors-alpha (PPAR $\alpha$ ) ligand. Peroxisome proliferator-activated receptors (PPAR)s constitute a family of receptors members of the steroid receptor superfamily [1-2]. To date, there are three known receptors, namely PPAR ( $\alpha$ ), ( $\beta/\delta$ ), and ( $\gamma$ ). From our ongoing efforts to study the interaction of CA with a variety of metal ions, we have recently published, in this journal, the interaction of CA with the trivalent chromium metal ion Cr<sup>3+</sup> [3]. Moncol et. al. showed the crystal structures and the spectral properties (EPR) for the solid state of the bis Cu(CA)<sub>2</sub>L<sub>2</sub> complex where L is 2-pyridylmethanol. They also showed the dimeric (Cu)<sub>2</sub>(CA)<sub>4</sub>L<sub>2</sub> complexes where L is N,N-

diethylnicotinamide [4]. Most of the works by Moncol and others [5-7] were in the solid state in which they showed the X-ray diffraction patterns of various solid Copper/CA monomer and dimer complexes.

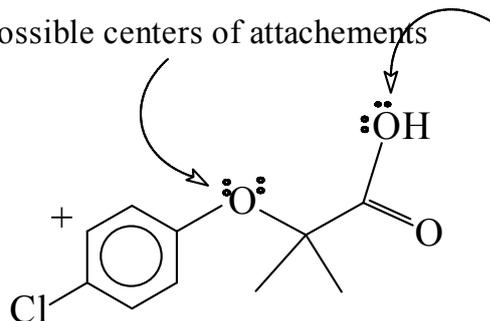
Herein, we are showing aqueous solution studies of CA with hexa-aqua Cu<sup>2+</sup> metal ion under ambient conditions between pH values of 2.5 to 10.5. The main objectives of the current report are (1) To identify the type of Cu<sup>2+</sup>-CA complexes formed, if there is binding, under these ambient conditions in aqueous solutions in the low (1-2) milli molar concentration range and (2) To measure the potential response of the reaction mixture in milli-Volts (mV). Potentiometry is one of the most powerful tools to study metal ions in aqueous solutions under ambient conditions [8-11].

## Experimental Section

### Materials

All solutions were prepared using 99% purity Sigma reagent grade CA, C<sub>10</sub>H<sub>11</sub>ClO<sub>3</sub>, formula weight 214.6 g.mol<sup>-1</sup>, copper sulfate penta-hydrate, Cu(SO<sub>4</sub>) •5H<sub>2</sub>O, formula weight 249.7 g.mol<sup>-1</sup>, and copper nitrate hemipenta-hydrate, Cu(NO<sub>3</sub>)<sub>2</sub> •2.5H<sub>2</sub>O, formula weight 232.6 g.mol<sup>-1</sup> using doubly deionized water to prepare all solutions. **Figure 1** shows the structural formula of CA, or [2-(4-chlorophenoxy)-2-methyl propanoic acid (Chemical formula C<sub>10</sub>H<sub>11</sub>ClO<sub>3</sub>). The pH values of all solutions were adjusted using standardized sodium hydroxide (NaOH) solution. Standardization of this NaOH solution was done to the fourth decimal place (0.1271 ± 0.0043) mol.L<sup>-1</sup>. The pH values were measured using Orion Membrane pH meter (model 720) with a combination Orion-glass electrode. No other ions were added to adjust the ionic strength of the solution; i.e., the potentiometric measurements were done in 0.0 mole.L<sup>-1</sup> ionic strength solutions. The electrode was calibrated using the two points calibrations mentioned in the pH-electrode manual. Using buffer pH 4.00 and buffer pH 7.01 purchased from Fisher scientific.

## Possible centers of attachments



**Figure 1:** Structural formula of 2-(p-Chlorophenoxy)-2-Methylpropionic acid or clofibrac acid (CA). Arrows show the possible centers of metal ion chelation.

## Preparation of the potentiometric titration solutions

In all metal-ligand potentiometric titrations, the NaOH solution was always the titrant. The NaOH solutions were prepared from NaOH laboratory grade pellets in carbonate free water. The methods used to prevent the contamination of the titrant with atmospheric CO<sub>2</sub> had been described elsewhere [8-11]. The NaOH solutions were standardized using primary standard potassium hydrogen phthalate (KHP).

Both NaOH and KHP were purchased from Fisher Chemical Co. Before any KHP titration, the KHP was dried at 110°C for 24 h and stored in a desiccator. A stock indicator solution of about 0.2% phenolphthalein in about 90% ethanol was prepared from reagent grade phenolphthalein. KHP was titrated to the phenolphthalein end point. Twelve runs were carried out to standardize the NaOH solution. Standard statistical treatments of the data such as the arithmetic mean, standard deviation, T-test, and Q-test were conducted using Excel software (**Table 1**).

## Potentiometric titrations

The potentiometric titration solutions were contained in a 250 mL beaker equipped with a magnetic stirring bar. The beaker was covered with a custom made Teflon cover. In brief; the CA solution was added first followed by the addition of the Cu(SO<sub>4</sub>) solution. The total volume of the final titration solution was 100 mL. After standardization, the final concentration of the Cu(SO<sub>4</sub>) solution titrated was  $0.03424 \pm 0.0006$  mmoles.L<sup>-1</sup>. Before each titration, the titration solution mixtures were allowed to stir for 25 minutes to reach equilibrium. The NaOH titrant was added in 100 μL increments using an Eppendorf micropipette with continuous stirring. The time intervals between the additions of the NaOH solution were set to 5 minutes, which was sufficient to get each of the pH values stabilized and reach complete equilibrium. The start pH-value was in the range of 2.5-3.0 and the final pH-value was in the range of 10.3-10.5. Each titration took about 3 to 4

h to complete. All titrations were conducted at room temperature.

**Table 1:** NaOH standardization using phenolphthalein as indicator.

Run Number	Mass of KHP (g)	Mass of KHP (mg)	Number of millimoles	Volume of NaOH (mL)	Molarity of NaOH
1	0.2174	217.4	1.064	8.35	0.1275
2	0.2575	257.5	1.261	9.85	0.128
3	0.1231	123.1	0.6028	4.6	0.131
4	0.2195	219.5	1.075	8.3	0.1295
5	0.24	240	1.175	9.6	0.1224
6	0.2212	221.2	1.083	8.05	0.1345
7	0.2235	223.5	1.094	9.05	0.1209
8	0.2524	252.4	1.236	9.9	0.1248
9	0.2784	278.4	1.363	10.9	0.125
10	0.1991	199.1	0.9749	7.6	0.1283
11	0.1912	191.2	0.9362	7.1	0.1319
12	0.2674	267.4	1.309	10.8	0.1212
				Average (x)	0.1271
				Standard deviation (σ)	±0.0043

## UV-Vis spectroscopy

All UV-Vis spectroscopy measurements were conducted using a T60 high-performance spectrophotometer in connection with UVWIN software version 5.0, both purchased from Advanced ChemTech (Louisville, KY). Samples were prepared in D.I. water at 25°C. The entire UV-Vis spectrum was scanned from 250 to 1100 nm using quartz cuvettes with optical path length of 1 cm. A reference cuvette filled with D.I. water was used with all measurements as the control or as the reference sample. The concentration of the copper solution was  $=1.14 \times 10^{-3}$  mol.L<sup>-1</sup>. In the case of free CA, there was no need to continue the scans beyond the visible region due to the colourless nature of the free CA solutions so that the CA solutions were scanned from 250 nm to 550 nm.

## IR spectroscopy

All IR spectroscopy measurements were conducted using Nicolet iS10 spectrophotometer in connection with OMNIC

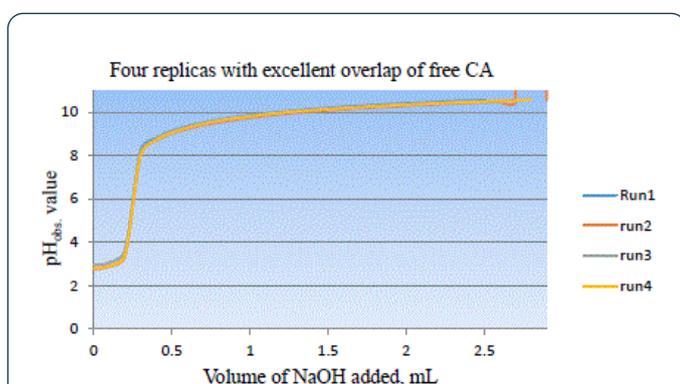
software version 8.1, both purchased from Thermo Fisher Scientific (Madison, WI). Samples were prepared neat in their solid phase at 25°C. The entire IR spectrum was scanned from 400 to 4000  $\text{cm}^{-1}$  using the provided attenuated total reflectance (ATR) accessory cell compartment equipped with a diamond cell that can accommodate various types of samples (solid samples as well as aqueous solution samples). The following data parameters were used in collecting the IR spectra: number of sample scans and the number of background scans was set at 32 with resolution of 4.000  $\text{cm}^{-1}$ , and Laser frequency of 15798.7  $\text{cm}^{-1}$ .

Typical IR spectra were generated in which the X-axis was given as Wavenumbers in  $\text{cm}^{-1}$  and Y-axis was recorded as % Transmittance which can easily be converted and displayed into absorbance by means of the OMNIC software program. Background spectra were collected prior to any sample data collection using polystyrene calibration film at which the fingerprint peaks appeared and were identified. In some cases we have also looked at the IR pattern of the fibers that makes up the lab-cleaning papers (Kimwipes). To ensure data consistency, a duplicate experiment was conducted by mixing  $\text{Cu}(\text{SO}_4)$  with CA in 2:1 ratio to make sure that the observed peaks are not for  $\text{Cu}(\text{SO}_4)$  that is contaminated with CA samples.

## Results and Discussion

### Potentiometric titrations of free CA and free metal ion ( $\text{Cu}(\text{SO}_4)$ )

Our collaborator and research group were the first to report, in 100% aqueous solution at 25°C, the acidity constant of the carboxylic acid functional group of CA to be  $\text{pK}_a = 4.32 \pm 0.06$  [3]. **Figure 2** is the potentiometric titration graph of free CA. This graph contains four plots with excellent overlap.

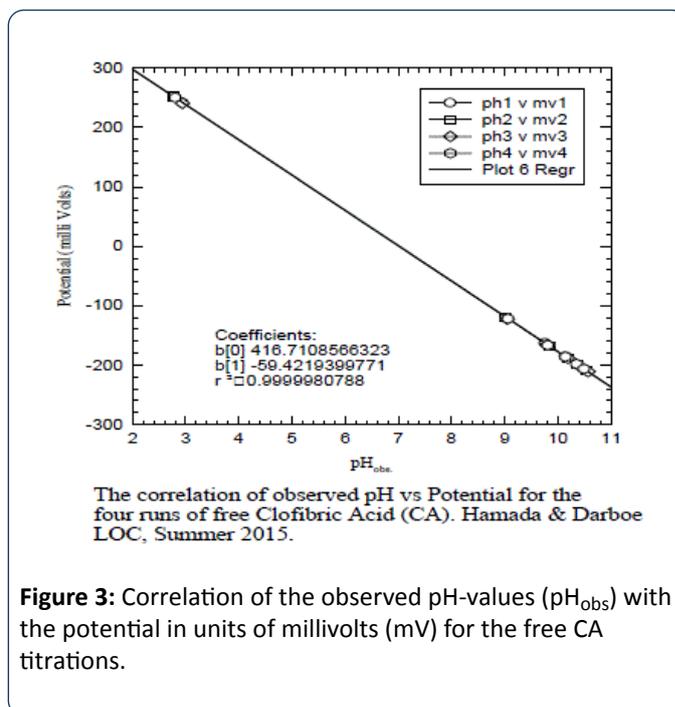


**Figure 2:** Four replicas of the potentiometric titrations of free Clofibric acid (CA) in aqueous solutions, 25°C,  $I=0.0$  M because no other ions were added to adjust the ionic strength of the solutions.

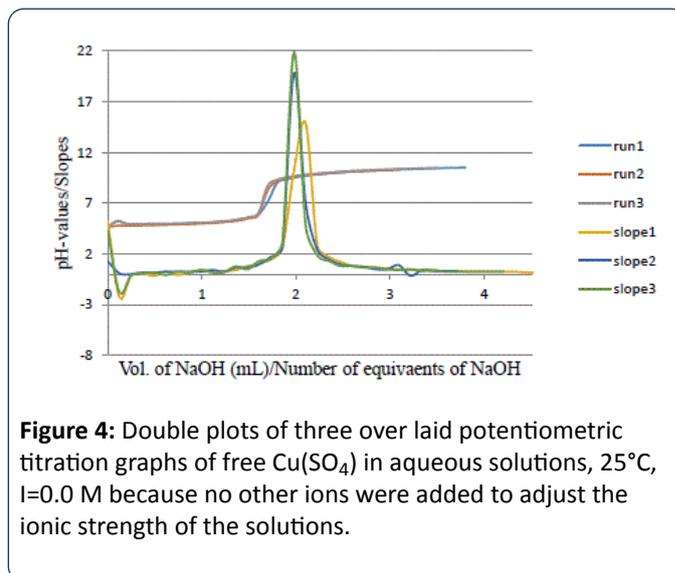
**Figure 3** gives the correlation of the observed pH-values ( $\text{pH}_{\text{obs}}$ ) with the potential in units of mV for the free CA titrations. These figures indicate the agreement with Nernst equation [12, 13]. Careful searching for CA data in National

Institute of Standard and Technology (NIST) databases indicated that CA data have not been report in the NIST standard reference database of critically selected stability constants of metal complexes [12].

**Figure 4** is the potentiometric titration graph of free  $\text{Cu}^{2+}$ . Three titration plots were overlapped to show data consistency.



**Figure 3:** Correlation of the observed pH-values ( $\text{pH}_{\text{obs}}$ ) with the potential in units of millivolts (mV) for the free CA titrations.

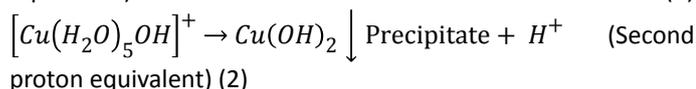
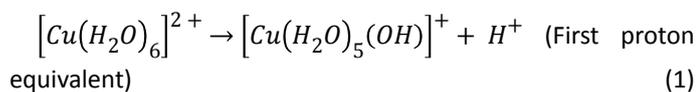


**Figure 4:** Double plots of three overlaid potentiometric titration graphs of free  $\text{Cu}(\text{SO}_4)$  in aqueous solutions, 25°C,  $I=0.0$  M because no other ions were added to adjust the ionic strength of the solutions.

One might explain **Figure 4** as the Double plots of free  $\text{Cu}(\text{SO}_4)$  in aqueous solutions, 25°C, with ionic strength  $I$  of ( $I=0.0$  M) because no other ions were added to adjust the ionic strength of the solutions. The slopes are the mathematical treatment or the first derivatives versus the number of observed equivalents of added titrant (NaOH).

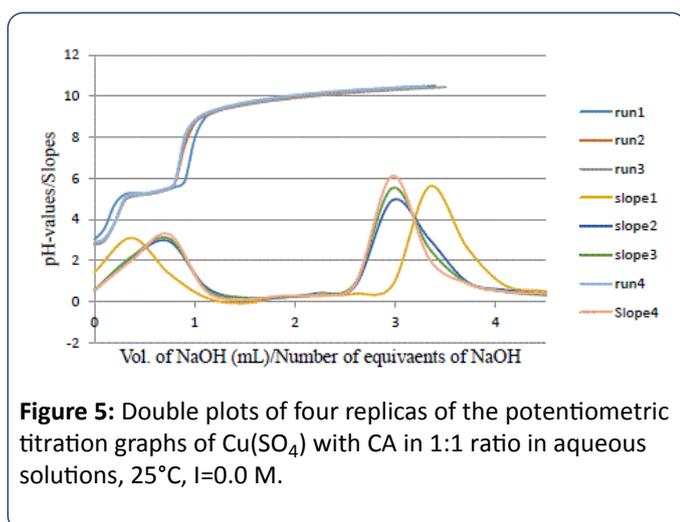
After data treatment and converting the volume of titrant into number of equivalents of titrant, it is clear that a di-valent metal ion such as  $\text{Cu}^{2+}$  releases a net of two proton

equivalents into the aqueous solutions. This is due to the fact that metal ions in aqueous solutions under ambient conditions go through metal ion hydrolysis. This term, metal ion hydrolysis, is defined in equations 1 and 2 [13-16]. The number of equivalents is defined as the number of milli-moles of added titrant (NaOH) per number of milli-moles of  $\text{Cu}^{2+}$  ion present in solution.



### Potentiometric titrations of $\text{Cu}^{2+}$ with CA in various molar ratios

**Figure 5** is the potentiometric titration graph of the  $\text{Cu}^{2+}$ :CA in 1:1 molar ratio. This graph contains a total of four individual plots.



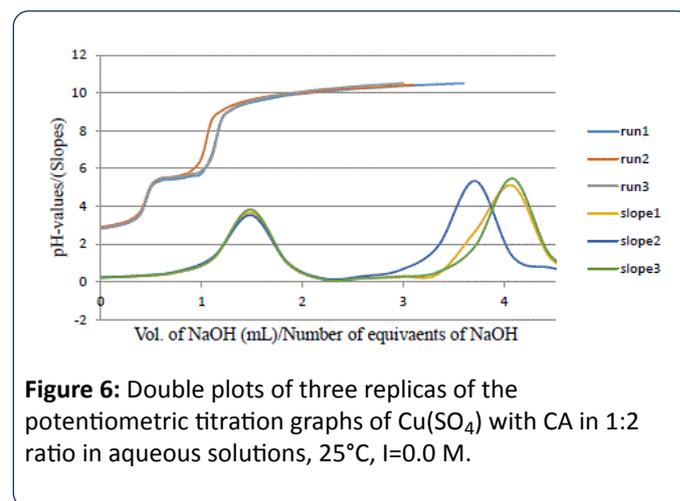
**Figure 5:** Double plots of four replicas of the potentiometric titration graphs of  $\text{Cu}(\text{SO}_4)$  with CA in 1:1 ratio in aqueous solutions, 25°C,  $I=0.0$  M.

This graph shows the exact locations of the inflection points. The location of each inflection point gives the exact number of protons released into the aqueous solution.

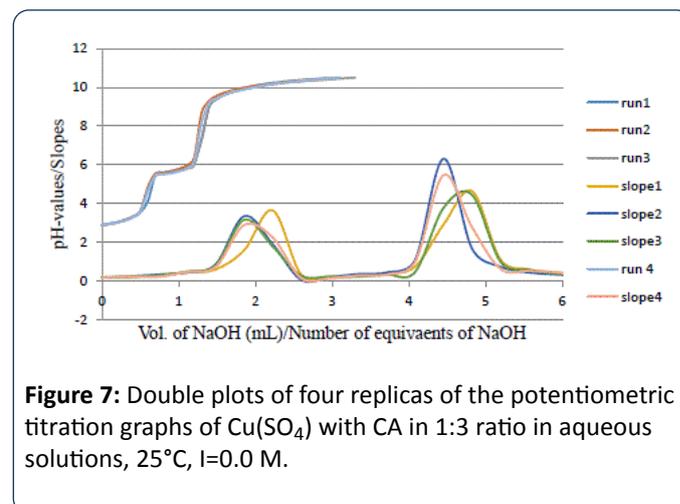
For example, the titration plots of the  $\text{Cu}^{2+}$ :CA in 1:1 molar ratio indicated the release of three protons ( $3.06 \pm 0.19 \text{ H}^+$ ). By examining these plots in this figure, clearly there has been a strong interaction between the  $\text{Cu}^{2+}$  metal ion and CA due to the shift in the location of the inflection points to 3.0 equivalents; compared to  $2.02 \pm 0.07$  equivalents in the titration of the free  $\text{Cu}^{2+}$  ion as shown in **Figure 4**.

For the  $\text{Cu}^{2+}$ :CA in 1:1 ratio, the important point is that three equivalents of protons have been released from the reaction of  $\text{Cu}^{2+}$  with CA into the solutions. One proton was clearly released from the CA. The source of the other two protons must be accounted for. These two protons must come from the aqua ligand attached to the metal ion as it was explained in equations 1 and 2. It is established in the literature that such hydroxo-complexes with  $\text{Cu}^{2+}$  and most metal ions have been seen previously [3-14].

When we conducted the potentiometric titrations between  $\text{Cu}^{2+}$  and CA in 1:2 and 1:3 molar ratios, it appeared that the number of proton equivalents were  $3.97 \pm 0.23$  and  $4.70 \pm 0.22$  respectively. These values indicate that no matter how much CA present in solution with the copper metal ion the same type of Cu-CA species predominates. **Figures 6 and 7** are showing these exact locations of the major inflection points with four and more than four proton equivalents released into solution.



**Figure 6:** Double plots of three replicas of the potentiometric titration graphs of  $\text{Cu}(\text{SO}_4)$  with CA in 1:2 ratio in aqueous solutions, 25°C,  $I=0.0$  M.



**Figure 7:** Double plots of four replicas of the potentiometric titration graphs of  $\text{Cu}(\text{SO}_4)$  with CA in 1:3 ratio in aqueous solutions, 25°C,  $I=0.0$  M.

The proposed and the most plausible species to be formed in solution will be the ternary copper-hydroxo-CA complex  $[\text{Cu}^{2+} (\text{clofibrate}^-)(\text{OH}^-)_2]^{1-}$ . **Table 2** is the summary of all potentiometric experiments conducted in this study. This ternary copper-hydroxo-CA complex accounts for the release of three protons. When the ratio of  $\text{Cu}^{2+}$  to CA is 1:2 there is an extra mole of CA that releases a net of four protons (as seen in the 1:2 titrations). There is another possibility at which the bis-complex or the  $[\text{Cu}^{2+} (\text{clofibrate}^-)_2(\text{OH}^-)_2]^{2-}$  can account for the release of four protons. In the 1:2 potentiometric titrations, the potentiometric techniques cannot differentiate between which complexes are formed just based on the number of protons released.

Further studies are underway to determine which complex is predominant under this experimental 1:2 conditions (**Table 2**).

It is worth mentioning that above pH 6.0 in all titrations, i.e., in the 1:1, 1:2 and 1:3 titration ratios, a light green gelatinous precipitate of  $\text{Cu}(\text{OH})_2$  formed. This indicated that the bidentate binding fashion of CA to  $\text{Cu}^{2+}$  is not strong enough to keep the formed complexes soluble in solutions at the physiological pH value 7.40.

**Table 2:** Potentiometric titration data for ( $\text{Cu}^{2+}$ , 0.03424 millimoles) with (CA 0.001835M) in different molar ratios, 25°C, I=0 M. <sup>a</sup>0.1027 millimoles of  $\text{Cu}^{2+}$  (3 mL \* 0.03424M) were titrated to generate the free  $\text{Cu}^{2+}$  curve shown in **Figure 5**. <sup>b</sup>0.0367 millimoles Clofibrac acid (20 mL \* 0.001835 M) were titrated to generate the Free Clofibrac acid curve shown in **Figure 2**.

$\text{Cu}^{2+}:\text{C}$ A mole ratio	Num ber of runs	Vol. (mL) of NaOH titrant (0.1271 M)	Equival ents of NaOH titrant	Propose d species	Remarks
1:0 <sup>a</sup>	3	1.62 <sup>a</sup>	2.02 0.07 <sup>a</sup> ±	$\text{Cu}(\text{OH})_2$	Two $\text{H}^+$ were released
0:1 <sup>b</sup>	4	0.320 <sup>b</sup>	1.04 0.04 <sup>b</sup> ±	[CA-COO <sup>-</sup> ]	Clofibrac acid is a mono-protic acid
1:1	4	0.83 ± 0.05	3.06 ± 0.19	[CuCA(OH) <sub>2</sub> ]	The ternary (Cu-CA-OH) complexes formed
1:2	3	1.07 ± 0.06	3.97 ± 0.23	[CuCA(OH) <sub>2</sub> ]	The ternary (Cu-CA-OH) complexes formed
1:3	4	1.24 ± 0.03	4.70 ± 0.22	[CuCA(OH) <sub>2</sub> ]	The ternary (Cu-CA-OH) complex is formed

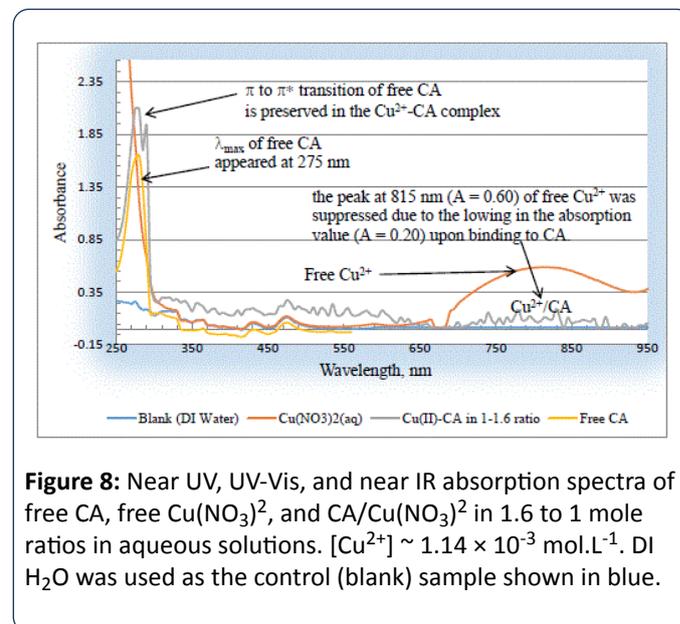
## Electronic spectra of $\text{Cu}^{2+}$ with CA in aqueous solutions

Ultra violet and visible absorption spectroscopy experiments (UV-Vis) were conducted as another tool as further confirmation for the  $\text{Cu}^{2+}/\text{CA}$  binding. **Figure 8** is Near UV, UV-Vis, and near IR absorption spectra of the aqueous solutions of free CA, free  $\text{Cu}(\text{NO}_3)_2$ , and  $\text{CA}/\text{Cu}(\text{NO}_3)_2$  in 1.6 to 1 mole ratios.

The molar concentration of copper solution was  $[\text{Cu}^{2+}] = 1.14 \times 10^{-3} \text{ mol.L}^{-1}$ . DI  $\text{H}_2\text{O}$  was used as the blank (control) sample. From **Figure 8**, one might observe that the  $\lambda_{\text{max}}$  for the free CA peak appeared at 275 nm. This peak is due to the  $\pi \rightarrow \pi^*$  electronic transition of the pi-electrons on the p-Chlorophenoxy ring of free CA. To the best of our knowledge this is the first study to report the absorption pattern of free CA in aqueous solution under ambient conditions.

It is also obvious from **Figure 8** that the electronic transition for the free  $\text{Cu}^{2+}$  ion in aqueous solutions appeared at less energy (or at longer wavelength,  $\lambda_{\text{max}}$  appeared at 815 nm) compared that for the free CA ( $\lambda_{\text{max}}$  for the free CA peak appeared at 275 nm).

The maximum absorption for the free  $\text{Cu}^{2+}$  ion is due to the d-d electronic transition [13]. When the solution of  $\text{Cu}^{2+}$  was mixed with that of CA in 1:1.6 ratio, the peak of  $\text{Cu}^{2+}$  at 815 nm ( $A=0.60$ ) of free  $\text{Cu}^{2+}$  was suppressed due to the lowering in the absorption value to ( $A=0.20$ ) upon binding to CA. The  $\text{Cu}^{2+}$ -CA complex had a bluish-green color.



**Figure 8:** Near UV, UV-Vis, and near IR absorption spectra of free CA, free  $\text{Cu}(\text{NO}_3)_2$ , and  $\text{CA}/\text{Cu}(\text{NO}_3)_2$  in 1.6 to 1 mole ratios in aqueous solutions.  $[\text{Cu}^{2+}] \sim 1.14 \times 10^{-3} \text{ mol.L}^{-1}$ . DI  $\text{H}_2\text{O}$  was used as the control (blank) sample shown in blue.

It is well established that  $\text{Cu}^{2+}$  ion has a  $d^9$  electronic configuration, and the movement of the d-electrons within the visible region is responsible for the characteristic peak at 815 nm. Lowering the absorption value of this characteristic peak for copper is good evidence that CA has bound to the  $\text{Cu}^{2+}$  ion.

Clearly there is ligand to metal charge (or electron) transfer. Further collaborations are needed to measure the EPR pattern for the transformation of  $\text{Cu}^{2+}$  to  $\text{Cu}^+$ .

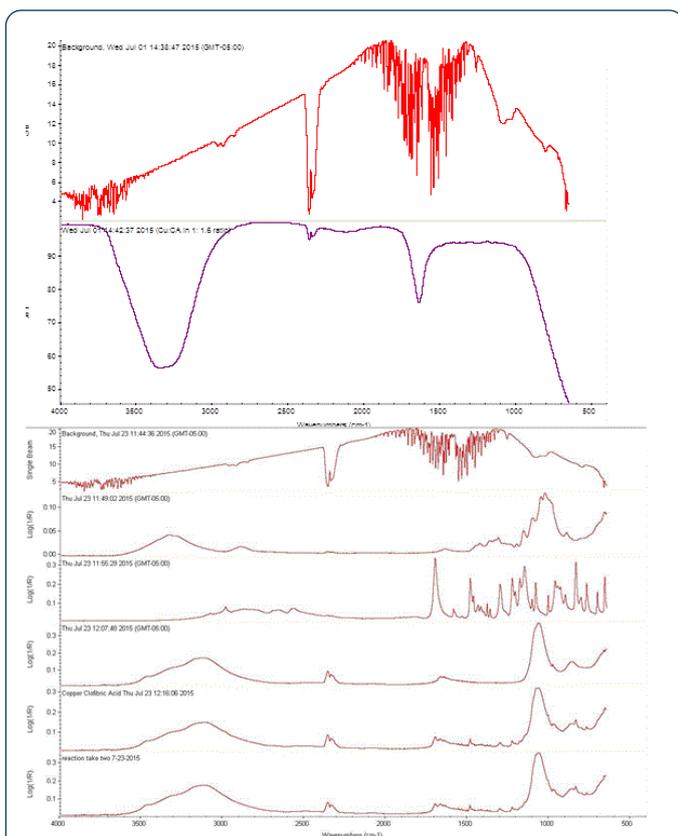
## IR spectra of $\text{Cu}^{2+}$ with CA in the solid state

The characteristic IR peaks for solid phases (neat) of free CA, free  $\text{Cu}^{2+}$  in the form of  $\text{Cu}(\text{SO}_4)$ , and the homogenized mixture of both CA and  $\text{Cu}(\text{SO}_4)$  were collected (**Figure 9**)

**Figures 10-12** are the spectra of these neat samples respectively.

**Table 4** summarizes the main stretching frequency peaks that appeared in the repeated experiment of the reaction of CA and  $\text{Cu}(\text{SO}_4)$ . Interestingly, the very strong carbonyl peak ( $\text{C}=\text{O}$ ) of the carboxylate group of the free CA that appeared at  $1702 \text{ cm}^{-1}$  was lowered and the peak became medium when  $\text{Cu}(\text{SO}_4)$  reacted with CA. This observation is a clear evidence of the participation of the carboxylate group of CA in the chelation of the  $\text{Cu}^{2+}$  ion.

This further confirms our proposed structures depicted in **Figure 13**. The very strong carbonyl peak ( $\text{C}=\text{O}$ ) of the carboxylate group that appeared at  $1702 \text{ cm}^{-1}$  was lowered with no significant change indicating that the de-protonated  $-\text{O}^-$  group of the carboxylic acid of CA is the main center of attachment and not the carbonyl  $\text{C}=\text{O}$  group.



**Figure 9:** IR Raw data of the  $\text{Cu}(\text{SO}_4)/\text{CA}$  solid mixed together. Top in aqueous solution which was the same exact solution used to collect the UV-Vis spectra. Bottom in the solid phase. The bottom set of spectra are arranged bottom to top for the solid reaction mixture of  $\text{Cu}(\text{SO}_4)/\text{CA}$  run# 1 and run #2,  $\text{Cu}(\text{SO}_4)$  only, CA only, Kimwipes only, air only.

**Figures 10-12**

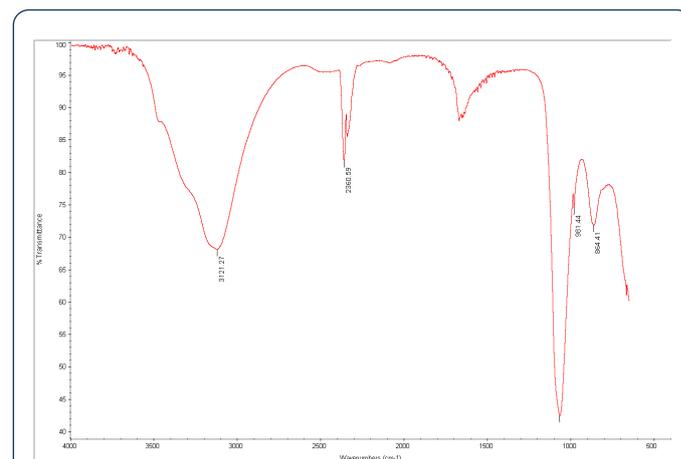
A search of the databases for the IR pattern for CA revealed a simulated or a calculated (theoretical) IR pattern for free CA in the gas phase. To the best of our knowledge, these peaks for free CA and the peaks for  $\text{Cu}(\text{SO}_4)$  with CA are novel and never reported before.

Close examination of the wavenumbers gathered from the IR Peaks of the reaction mixture of  $\text{Cu}(\text{SO}_4):\text{CA}$  in 2:1 ratio shows that most peaks of the CA were lowered in intensity due to copper chelation. We were able to assign each observed peak to its specific functional group. The only peak that was not identified was that at  $1231\text{ cm}^{-1}$ . We do not know to which functional group this stretching frequency is belonging to.

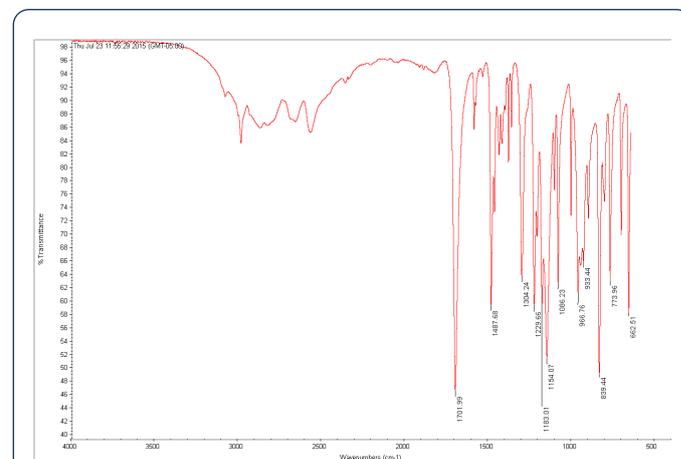
## Conclusion

In a recent paper in this journal, we have reported a detailed literature search which indicated the lack of research articles (experimental and theoretical) for CA with essential metal ions such as copper, iron, zinc and chromium. In another recent paper, we have demonstrated the very large number of research articles and reviews (150,000) that discussed and included copper with various organic ligands but not CA.

Furthermore, NIST standard reference database of critically selected protonation constants and stability constants of ligands and their metal complexes does not contain any data for CA. In the current report, we are showing a careful and detailed experimental potentiometry work that shows that the number of proton equivalents that appeared were three and four. This indicated the formation of the ternary copper-hydroxo-CA chelate complex  $[\text{Cu}^{2+}(\text{clofibrate}^-)(\text{OH}^-)]_2^{1-}$ , and the bis-chelate complex or the  $[\text{Cu}^{2+}(\text{clofibrate}^-)_2(\text{OH}^-)]_2^{2-}$  respectively.



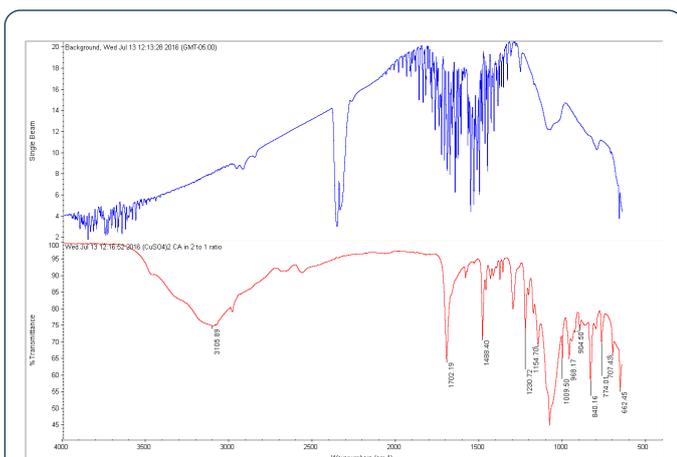
**Figure 10:** IR spectra of solid  $\text{Cu}(\text{SO}_4)$  only; the y-axis is displayed as %Transmittance.



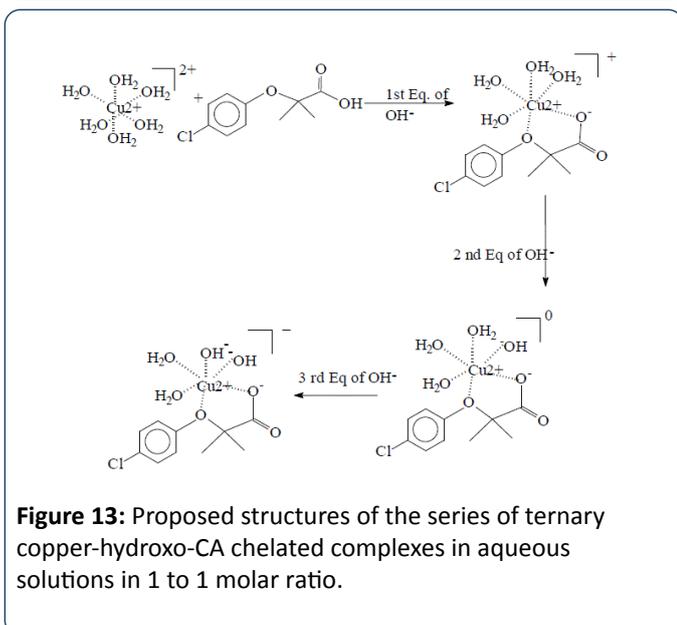
**Figure 11:** IR spectra of solid CA only; the y-axis is displayed as %Transmittance.

**Table 3** shows the selected IR-peaks for  $\text{Cu}(\text{SO}_4)$ , CA, and  $\text{Cu}(\text{SO}_4)/\text{CA}$ .

The IR has shown the participation of the hydroxyl group (O-H) of the carboxylate group of CA in binding the  $\text{Cu}^{2+}$  metal ion. The UV-Vis spectra have showed the lowering of the characteristic peak of free  $\text{Cu}^{2+}$  indicated the reduction of  $\text{Cu}^{2+}$  to  $\text{Cu}^+$ . This observation is in good agreement with the EPR data presented in literature published by Moncol et al. However, the EPR data presented by these authors were done on solid samples.



**Figure 12:** IR spectra of solid  $\text{Cu}(\text{SO}_4)/\text{CA}$  in 2:1 ratio well-mixed using a piston and mortar until complete homogeneity; the y-axis is displayed as %Transmittance. The C=O peak at  $1702\text{ cm}^{-1}$  was lowered with no significant change.



**Figure 13:** Proposed structures of the series of ternary copper-hydroxo-CA chelated complexes in aqueous solutions in 1 to 1 molar ratio.

**Table 3:** Selected IR-peaks for  $\text{Cu}(\text{SO}_4)$ , CA, and  $\text{Cu}(\text{SO}_4)/\text{CA}$ .

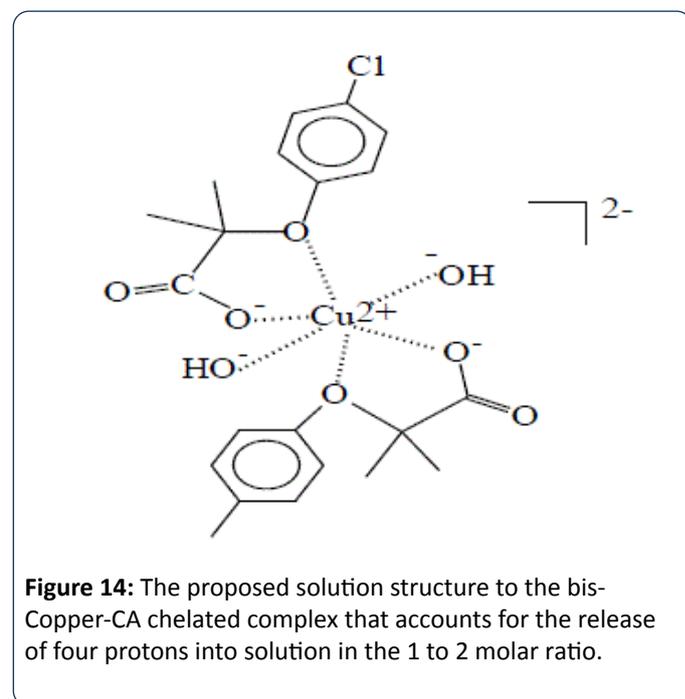
	$\text{Cu}(\text{SO}_4)^2$ only	CA only	$\text{Cu}(\text{SO}_4)^2/\text{CA}$
O-H	$3121\text{ cm}^{-1}$	$2571\text{-}2987\text{ cm}^{-1}$	$3119.4\text{ cm}^{-1}$
C=O	-	$1702\text{ cm}^{-1}$	$1701.4\text{ cm}^{-1}$
O-H ( $\text{Cu}^{2+}$ )	$1066\text{ cm}^{-1}$	-	$1069\text{ cm}^{-1}$
O-H	$864\text{ cm}^{-1}$ ( $\text{H}_2\text{O}$ of crystallization)	$839\text{ cm}^{-1}$	$840\text{ cm}^{-1}$

Due to the fact that CA has two possible centers of attachments when it comes to chelating metal ions, we are proposing the structure of this ternary copper chelate complex in aqueous solution as shown in **Figure 13**. We are also

proposing the aqueous solution structure for the bis- $\text{Cu}^{2+}$ -CA chelate complex as shown in **Figure 14**.

**Table 4:** IR Peaks of the reaction mixture of  $\text{Cu}(\text{SO}_4)/\text{CA}$  in 2:1 ratio. These peaks were repeated twice to ensure data consistency.

$\text{Cu}(\text{SO}_4)/\text{CA}$ in 2:1 ratio/ Peak shape	Wavenumbers Values of all peaks	Assignments
Very broad	$3106\text{ cm}^{-1}$	O-H (water/acid)
Medium	$1702\text{ cm}^{-1}$	C=O
Medium	$1488\text{ cm}^{-1}$	$\text{CH}_3$ Methyl
Medium	$1305\text{ cm}^{-1}$	$\text{CH}_3$ Methyl
Medium	$1231\text{ cm}^{-1}$	Un-identified
Broad	$1155\text{ cm}^{-1}$	C-O-C
Strong	$1086\text{ cm}^{-1}$	C-O-C
Medium/Broad	$968\text{ cm}^{-1}$	O-H (Acid)
Strong	$840\text{ cm}^{-1}$	Aromatic
Broad	$774\text{ cm}^{-1}$	C-Cl
Medium	$662\text{ cm}^{-1}$	Aromatic



**Figure 14:** The proposed solution structure to the bis-Copper-CA chelated complex that accounts for the release of four protons into solution in the 1 to 2 molar ratio.

Further stability constant measurements are needed in this area and they are underway in our laboratory. Furthermore, speciation diagrams are needed to be constructed to sort out which species are the predominant at the physiological pH-value (7.40) and which are less stable compared to other and similar bi-dentate ligands found in NIST standard reference database of critically selected protonation constants and stability constants.

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