eJBio

Electronic Journal of Biology, 2016, S2: 25-30

Zn^{2+} with Clofibric Acid: A Peroxisome Proliferator-Activated Receptors-Alpha (PPARα) Ligand

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Citation: Hamada YZ, Darboe A. Zn^{2+} with Clofibric Acid: A Peroxisome Proliferator-Activated Receptors-Alpha (PPARα) Ligand. Electronic J Biol, S:2

Received: February 07, 2017; **Accepted:** March 09, 2017; **Published:** March 15, 2017

Research Article

Abstract

Peroxisome proliferator-Activated Receptors-Alpha (PPARα) ligands are numerous. One such ligand is Clofibric Acid (CA). CA has a chemical structure that has the 2-phenoxy-2-methylpropanoic acid feature. This feature has the right chelation size to bind many metal ions such as Fe^{3+}, Cr^{3+}, Cu^{2+} and Zn^{2+}. Using potentiometry and spectroscopy, we have found that CA chelates the first member of metal ions of group number 12 in the periodic table (i.e., Zn^{2+}). This chelation occurred in aqueous media in 0.1 M $NaNO_3$ at 25°C. The concentration of Zn^{2+}=2.0 milli molar (2.0 mM). It appeared that, the molar extinction coefficient λmax of free CA is equal to 950 ± 9 $M^{-1}cm^{-1}$ compared to that of 1756 ± 61 $M^{-1}cm^{-1}$ for the Zn/CA complex in 1:1 ratio. Further confirmation using IR spectroscopy for the chelation of Zn^{2+} occurred in the solid phase. In this special issue of Electronic Journal of Biology (eJBio), we have presented two reports for the reaction of the ferric metal ion with CA.

Keywords: Clofibric acid; Zn^{2+}; IR; Potentiometry; UV-Vis-absorption

1. Introduction

A detailed literature survey was conducted on August 29, 2016 of all American Chemical Society (ACS) journals to search for relevant papers that dealt with the first member of group 12 metal ions (i.e., Zn^{2+}) and CA. When the term “Zinc” was used in the title of the paper 6,169 papers appeared. When the term “Zinc” was searched again within the abstract of the paper, about 12,009 papers appeared and when the same term was used anywhere in the papers 95,966 papers appeared. The process was repeated with the term Zinc and aqueous solutions. Finally, the process was repeated with the same term, Zinc and CA. These detailed literature reviews reveal that none of the publications that appeared dealt with the interaction of CA with Zinc metal ion, in particular in aqueous media. Figure 1 of the supplementary material shows these data.

For comparison, on August 29, 2016 also, the Directory of Open Access Journals (DOAJ) was used as a non-ACS search engine for articles. It was found that this index contained more than 2.2 million articles. The DOAJ is one of the citation indexes used to cite the Electronic Journal of Biology (eJBio) among other indexes. Surprisingly, only a single article was found that dealt with the key search term Clofibric acid/aqueous solutions [1]. Although ACS and non-ACS search engines have a plethora of literature on the toxicological, biological, medicinal, and chemical information about CA and PPAR, there are no chemical studies of CA with many metal ions under room temperature and in aqueous media [1-9]. Articles 1-9 are just an example of the massive amount of work that was done on PPAR and/or CA. We have recently published few studies of CA with the chromium (III) metal ion (Cr^{3+}) and the copper metal ion (Cu^{2+}) [10,11]. We have also presented two recent reports for the reaction of $Fe^{3+}$with CA [12,13].

Zinc is an essential component of nearly 300 enzymes [14-16]. It is found only in +2 oxidation state due to the extra stability associated with the filled d-orbital, thus biological redox reactions are not possible. Its biochemistry is different from the rest of the first row transition metal ions such as iron, manganese and copper due to the partially filled d-orbital of these metal ions. It is second to iron as the most abundant trace element in humans [15,16].

The following are some objectives of the current paper: (1) to determine the extent of CA binding to Zn^{2+} (2), to identify the nature of the zinc-complexes, and (3) measure the voltage potential of the reaction mixture. As it has been stated before, one can make potentiometry a most useful tool to study metal ions in aqueous solutions [17-20].

2. Experimental Section

2.1 Materials

Solutions of CA were prepared using 99% purity Sigma reagent grade, $C_{10}H_{11}ClO_3$, formula weight 214.6 g.mol^{-1}. Zinc nitrate hexa-hydrate, $Zn(NO_3)_2 \bullet 6H_2O$, 98% extra pure Acros organics, formula weight 297.46 g.mol^{-1}. Primary standard potassium hydrogen phthalate (KHP, 99.99%) and solid sodium hydroxide pellets (NaOH, 98%) were purchased from Fisher Chemical Co. We are showing the structural formula of CA in Scheme 1. All pH values of all solutions were adjusted using 0.1271 ± 0.0043 mol.L^{-1} sodium hydroxide (NaOH) solution that was standardized to the phenolphthalein indicator endpoint. The pH values were measured using either the Orion Membrane pH meter (model 250A) or (model 720) connected to a combination Orion-glass electrode in 0.1 mole.L^{-1} ionic strength. The ionic strengths were adjusted by the addition of the appropriate 10% v/v of 1.0 M $NaNO_3$ solution.

2.2. Calibration of potentiometry

Each day the pH-electrodes were calibrated using the standard methods shown on the electrode catalogue using two pH-buffer standard solutions; standard buffer solution pH=4.00 and standard buffer solution pH=7.00. However, the pH-meter, the pH-electrode, and the working solutions are further calibrated by titrating standard solution of phosphoric acid (H_3PO_4). The choice of phosphoric acid is due to the fact that it has three titratable protons in the acidic, the neutral, and the basic buffer regions. H_3PO_4 has the following pKa-values: 1.92, 6.71 and 11.52 at 25°C and in 0.1M ionic strength as reported in the stability constants Database by Martell and Smith [21]. Supplementary Figure 2 shows the potentiometric titration of free H_3PO_4. We have overlapped two titrations to show data consistency.

2.3. Solutions used in potentiometry

Sodium Hydroxide (NaOH) solution was always the titrant. We have prepared the NaOH solutions from NaOH laboratory grade pellets in carbonate free water. The detailed methods used to prevent the contamination of the titrant with atmospheric CO_2 and to standardize this NaOH solution had been described elsewhere [17-20]. For any data set generated we have calculated the arithmetic mean, the standard deviation, using Excel software. For example, the concentration of NaOH was found to be 0.1271 ± 0.0043 mol.l^{-1}.

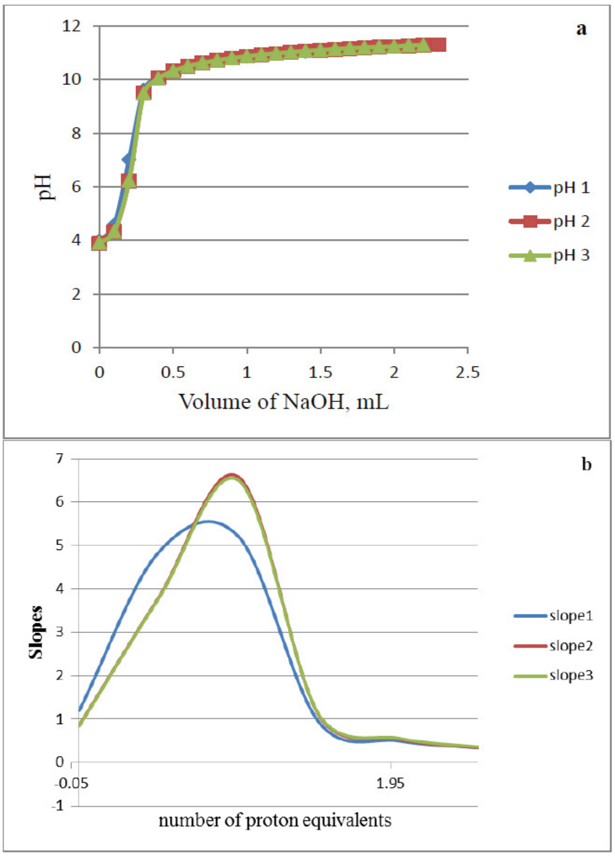

Figure 1. Potentiometric titration graphs of free clofibric acid in aqueous solutions, 25ºC, (I = 0.10 M, $NaNO_3$) $NaNO_3$ was added to adjust the ionic strength of the solutions to 0.10 M). (a) pH vs., Vol. (b) Slopes vs. number of equivalents.

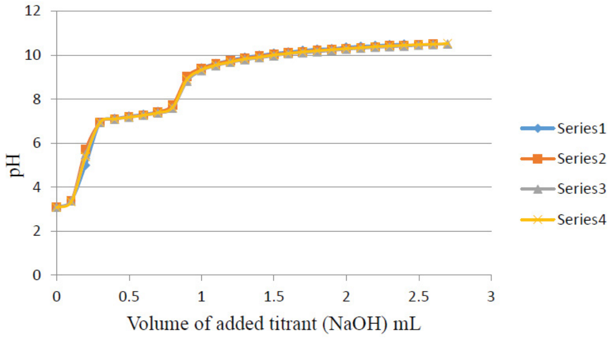

Figure 2. Potentiometric titration graphs of $Zn(NO_3)_2$ with CA in 1:1 ratio in aqueous solutions, 25ºC, (I=0.10 M, $NaNO_3$ was added to adjust the ionic strength of the solutions).

2.4. Potentiometry

The details regarding conduction potentiometry have been reported in previous studies [17-20]. In brief, the CA solution was first added to the titration vessel, followed by the addition of the Zn^{2+} metal ion solution. We adjusted the ionic strength of the solution to 0.1 M by the addition of 10 mL of 1.0 M $NaNO_3$ solution. We adjusted the final volume to 100 µL by adding DI. H_2O. This combination gave [Zn^{2+}] to be in the range of 2.0 to 2.5 mmol.l^{-1}. We have added the NaOH solution in the 100 l aliquots by means of very accurate Eppendorf micro-pipette. It appeared that the initial pH-values of the different titrations in the 1:1, or the 1:2 or the 1:3 ratios Zn^{2+}: CA was in the range of 3-4 and the final pH-values were in the range of 10-11. Each titration took about 5 to 6 hours to complete.

2.5. Ultraviolet and visible spectroscopy

We have gathered the UV-Vis spectroscopy spectra by using the T60 high-performance spectrophotometer that was purchased from Advanced ChemTech (Louisville, KY). Samples were prepared in D.I. water at 25ºC. The UV-Vis spectrum was scanned from 250 to 550 nm using quartz cuvettes with optical path length of 1 cm. A reference cuvette filled with D.I. water as the control. The concentration of Zn^{2+} was 3.03 × 10^{-4} mol.L^{-1}. The UV-Vis spectra were collected at the pH values of 3.15.

2.6 Infrared spectroscopy

The IR spectra were conducted using the Nicolet iS10 spectrophotometer that is connected to OMNIC software version 8.1. The spectrophotometer was purchased from Thermo Fisher Scientific (Madison, WI). We have prepared the samples neat in their solid state at room temperature. We have scanned the entire IR spectrum from 400 cm^{-1} to 4000 cm^{-1} using the provided Attenuated Total Reflectance (ATR) accessory cell compartment. We have set the following data parameters before any data collection: number of sample scans and the number of background scans was set at 32 with resolution of 4.000 cm^{-1} and Laser frequency of 15798.7 cm^{-1}.

In a typical IR spectrum, the X-axis was set as Wavenumbers in units of 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. We are not trying to correlate the solution chemistry with that of the solid state IR measurements. The IR was collected on the neat solid phase due to the solid state requirements of this spectroscopic technique.

3. Results and Discussion

3.1 Potentiometry of free CA and free zinc metal ion Zn^{2+}

Figure 1 shows the potentiometric titration plots of free CA, we have published another free CA titration curve but these plots shown in Figure 1 is what we have gathered when we reacted CA with zinc in this study. As we have shown before, CA releases one proton out of the carboxylic acid group [10-12]. No data of this ligand (CA) has been reported in the NIST standard reference database of critically selected stability constants of metal complexes [21]. We were able to measure and report the pKa-value for CA in aqueous solutions. CA has a pKa value of 4.32 ± 0.06 [10].

Figure 3 of supplementary material shows the potentiometric titration plots of free Zn^{2+}. This figure shows also the mathematical treatments of the raw potentiometric data. These mathematical treatments are calculated by taking the first derivatives or (slopes) versus the number of measured equivalents of added titrant. It is clear that a divalent metal ion such as Zn^{2+} consumes a net of two proton equivalents upon titration. This is because 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 [22,23]. The number of equivalents is defined as the number of milli-moles of added titrant, NaOH in this case, per number of milli-moles of Zn^{2+} ion present in solution.

$[Zn(H_2O)_6]^{2+} \rightarrow [Zn(H_2O)_5(OH)]^{+} + H^{+}$
(First proton equivalent) Eq. 1

$[Zn(H_2O)_5OH]^{+} \rightarrow Zn(OH)_2 \downarrow$Precipitate $+ H^{+}$
(Second proton equivalent) Eq. 2

3.2 Potentiometry of Zn^{2+} with CA

Figure 2 is the potentiometric titration graph of the Zn^{2+}:CA in 1:1 molar ratio. This graph contains a total of four individual plots to show data consistency. This graph shows the exact locations of the minor and the major inflection points. The steepest slope of each plot gives the exact number of protons consumed. For example, the titration plots of the Zn^{2+}:CA in 1:1 molar ratio indicated that the major inflection point has a net of 2.70 ± 0.09 protons consumed. By examining these plots in Figure 3, clearly there has been an interaction between the metal ion Zn^{2+} and CA due to the shift in the location of the inflection points to 2.70 equivalents; compared to 2.0 equivalents in the titration of the free Zn^{2+} ion as shown in supplementary Figure 3.

Since the Zn^{2+}:CA in 1:1 ratio replicas overlapped at 2.70 ± 0.09 equivalents, it is clear that an extra 0.7 equivalents of protons has been released from the reaction of Zn^{2+} with CA. One proton was clearly released from the CA. The source of the other two protons is from the aqua ligand attached to the zinc metal ion (Zn^{2+}). It is established in the literature that such hydroxo-complexes have been seen previously [10-12,21-24]. The most plausible species to be formed in solution will be the ternary Zinc hydroxo-clofibrate complexes $[Zn^{2+}(clofibrate^{-})(OH^{-})_2]^{1-}$. We are proposing the solution structure of this ternary Zinc complex in Scheme 2.

Titrations of the 1:2 and 1:3 Zn^{2+}: CA reaction mixtures are shown in Figures 3 and 4, respectively. It is clear that the presence of an extra mole of CA will increase the net number of protons released into solutions from the Zn^{2+}/CA reaction mixture. An extra 0.92 protons were released from the extra mole of CA present in solution. When the number of moles of CA was increased to 3 (i.e., in the 1:3 titration ratio, Figure 4), it appeared that an extra 0.63 moles of protons was released into solution. A complete summary of the potentiometric data is shown in Table 1.

The potential response of all of these runs (1:1, 1:2 and

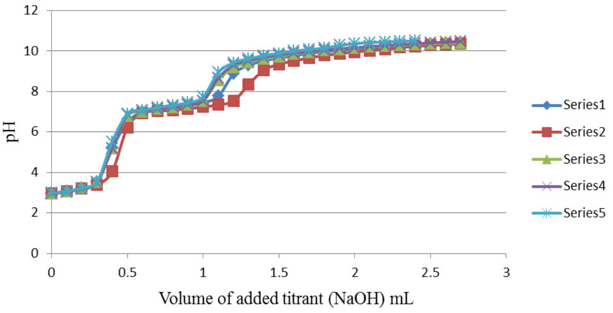

Figure 3. Potentiometric titration graphs of $Zn(NO_3)_2$ with CA in 1:2 ratio in aqueous solutions, 25ºC, (I=0.10 M, $NaNO_3$ was added to adjust the ionic strength of the solutions).

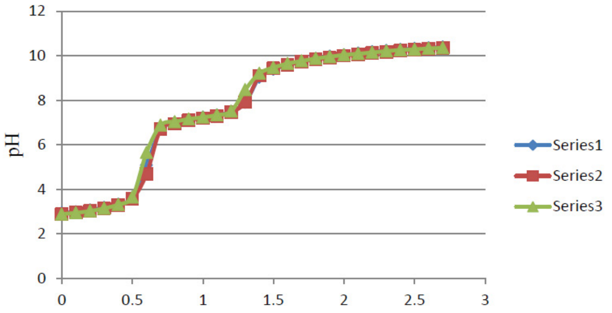

Figure 4: Potentiometric titration graphs of $Zn(NO_3)_2$ with CA in 1:3 ratio in aqueous solutions, 25ºC, (I=0.10 M, $NaNO_3$ was added to adjust the ionic strength of the solutions).

Table1. Potentiometric titration data for (Zn^{2+}, 0.04449 M) with (CA 0.001835M) in different molar ratios, 25°C.

Zn^{2+}/CA mole ratio	Number of titration runs	Equivalents of NaOH titrant	Proposed species	Remarks
1:0[a]	3	2.02 ± 0.07 [a]	$Zn(OH)_2$	Two H^+ were released
0:1[b]	3	1.04 ± 0.04 [b]	Mono anion of clofibric acid $(CA)^-$	Clofibric acid is a mono-protic acid
1:1	4	2.70 ± 0.09	$[ZnCA(OH)_2]^-$	An Oligomer (Zn-CA-OH) complex
1:2	5	3.62 ± 0.31	$[ZnCA(OH)_2]^-$	An Oligomer (Zn-CA-OH) complex
1:3	3	4.25 ± 0.19	$[ZnCA(OH)_2]^-$	An Oligomer (Zn-CA-OH) complex

[a] 0.1335 mmol of Zn^{2+} (3 mL * 0.044485M) were titrated to generate the free Zn^{2+} curve shown in Figure 3 of supplementary material

[b] 0.0367 mmol Clofibric acid (20 mL * 0.001835 M) were titrated to generate the free clofibric acid curve shown in Figure 1

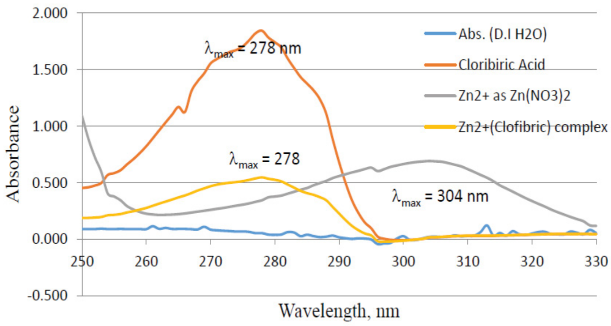

Figure 5: UV-Vis absorption spectra of Zn^{2+} and CA in 1:1.5 ratio. Blue is DI H_2O as control, Red CA only, Grey Zn^{2+} only, and Yellow Clofibric acid and the Zn^{2+}/CA complex in 1:1.5 ratio in aqueous solutions 25ºC. [Zn^{2+}] concentration of the metal ion in the complex was 3.03×10^{-4} M.

1:3 ratios) showed an almost perfect fit to Nernst Equation [21-23]. Figure 4 of supplementary material is only a representative graph showing the correlation of net solution voltage (mV) vs. the volume of added titrant (ml) in the 1:2 titration ratios. All 1:1, 1:2, and 1:3 titrations showed similar behavior. The voltage changes span the range of about -200 mV to +250 mV. Figure 5 of supplementary material is showing the almost perfect linear regression of the observed pH-values and the net potential in units of mV.

3.3 UV-Vis absorption spectra for free CA, free Zn^{2+} and that of Zn^{2+}:CA

The UV-Vis absorption spectra for the free CA, the free Zn^{2+}, and that of the Zn^{2+} to CA in 1 to 1.5 molar ratios are shown in Figure 5. The zinc concentration in the complex was [Zn^{2+}]=3.03×10^{-4} M. This graph is showing the three spectra overlaid to show the sift in UV-Vis absorption due to the reaction of the Zn^{2+} metal ion with CA in aqueous solutions at room temperature. The summary of these spectra is given in Table 2. To the best of our knowledge, this is the first study to show the UV-Vis absorption spectra for Zn^{2+} or CA, or both reacting together under ambient conditions. A most recent accessed of *NIST* chemistry web book data base on 11-2-2016 showed no such data exist in the database [25].

It is somewhat surprising to observe an electronic transition for the Zn^{2+} metal ion because of the fact that it is found only in +2 oxidation state due to the extra stability associated with the filled d-orbital (zinc has a d^{10} electronic configuration), thus no UV-Vis absorption is expected for this colorless metal ion. However, due to the presence of the nitrate as the counter ion associate with the Zn^{2+} metal ion, we have observed this peak for Zn^{2+} with a maximum absorption at 302 nm (λ_{max}=302 nm). As mentioned in the experimental section, we have used zinc ion in the form of (Zinc nitrate hexa-hydrate, $Zn(NO_3)_2 \bullet 6H_2O$). This peak at 302 nm is due to either $n \rightarrow \pi^*$ electronic transition or $\pi \rightarrow \pi^*$ electronic transition for the nitrate counter ion, but definitely not for the d → d transition of Zn^{2+} because this transition is forbidden due to the extra stability of zinc d^{10} configuration.

The peak of the free CA appeared at 279 nm. It is clear that the reason for the appearance of this peak for the colorless CA solution is the $\pi \rightarrow \pi^*$ electronic transition of the aromatic ring of CA. The molar extinction coefficient for free CA is in the range of 950 $M^{-1}cm^{-1}$, $\varepsilon\lambda\mathbf{max}_{=279}$=(950 ± 9). On the other hand, the molar extinction coefficient for Zn^{2+}-CA complex is in the range of $\varepsilon\lambda\mathbf{max}_{=277}$=1756 ± 61 $M^{-1}cm^{-1}$. The dramatic change in the values of the molar extinction coefficient for the free CA compared to that of Zn^{2+}-CA reaction mixture is a clear indication of strong interaction and chelation [22,24,25].

3.4 Infrared of the Zn^{2+}:CA reaction mixture

The IR spectra of CA and that of Zn: CA in 1:1 molar ratio is shown in Figure 6. There are at least ten characteristic peaks that appeared for the free CA. We have given a detailed account of these peaks in our recent paper [11]. The most important peak is that of the carbonyl of the carboxylate group C=O that has the characteristic peak of 1702 cm^{-1}. This peak remained in its location upon binding to zinc but was drastically diminished and broadened due to the metal ion binding and chelation.

4. Conclusion

Since we started to study CA and other fibrates, we have learned that literature evidence indicated few research articles appeared for CA with essential and toxic metal ions [10-12]. NIST standard reference database of critically selected protonation constants and stability constants of ligands and their metal complexes does not contain these data for CA. The shift of the location of the inflection point of free Zn^{2+} titration from 2.0 equivalents compared to that of the zinc/CA complex in all ratios is an evidence of Zn^{2+} binding and chelation. Based on the number of protons

Table 2: Maximum absorption of the UV-Vis-peaks that appeared for free Zn^{2+}, free CA, and Zn-CA reaction in 1:1.5 ratio aqueous solutions at 25°C. [Zn^{2+}] concentration of the metal ion in the complex was 3.03×10^{-4} M.

Species/Property	λ max (nm)	Absorbance	ελ max
CA	279 ± 1	1.83 ± 0.02	950 ± 9
Zn^{2+}	302 ± 3	0.70 ± 0.01	14.3 ± 0.1
Zn^{2+}:CA in 1:1.5 ratio	277 ± 2	0.53 ± 0.02	1756 ± 61

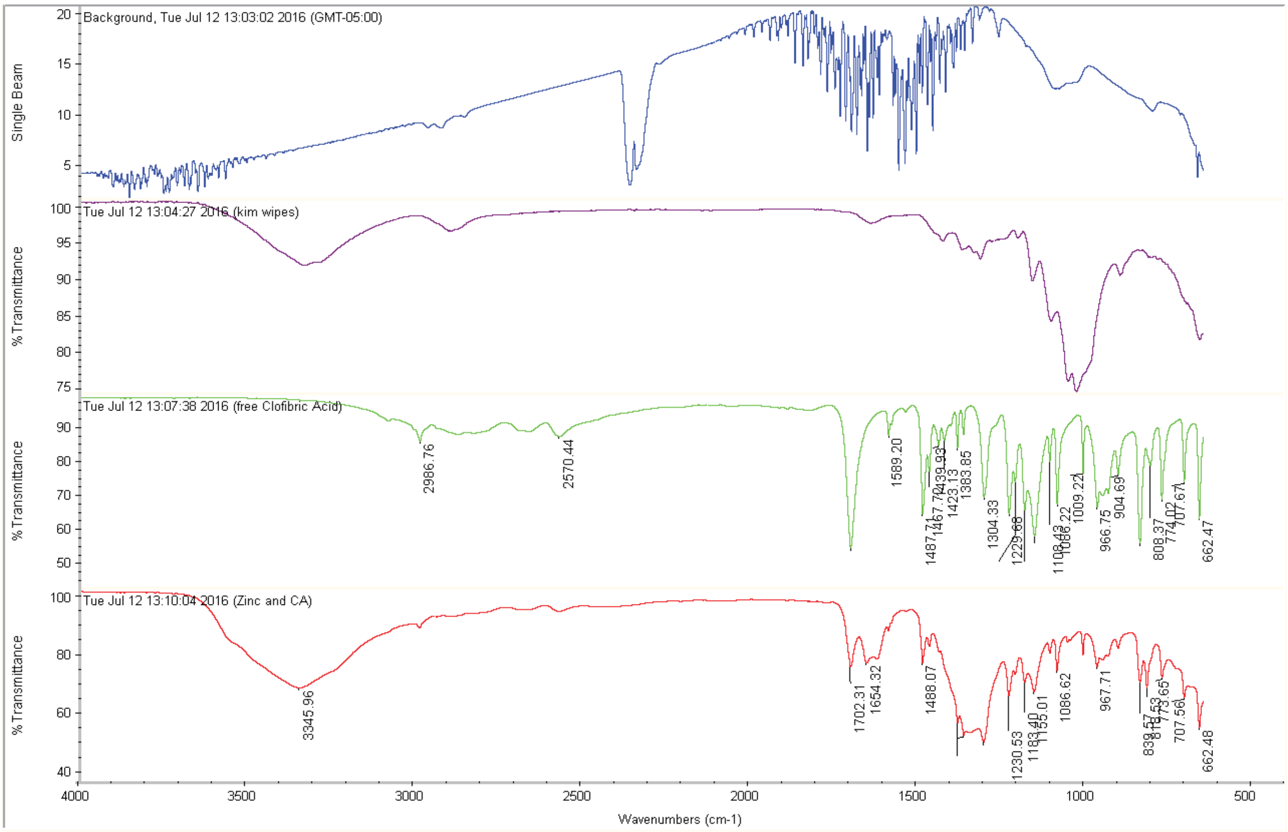

Figure 6: IR spectra of solid $Zn(NO_3)_2$/CA well-mixed in a mortar until complete homogeneity; the y-axis is displayed as %Transmittance. The C=O peak of free CA that appeared at 1702 cm^{-1} was diminished with significant change in peak intensity due to binding to the Zinc-ion.

consumed in aqueous solution, we are proposing the formation of the ternary hydroxo-clofibrate zinc complex with the formula $[Zn^{2+}(clofibrate^{-})\ (OH^{-})_2]^{1-}$ according to the description in schemes 2. Further calculations are underway to identify the other species that might be present.

Based on the shift of the UV-Vis absorption spectra of the free CA and that of the free Zn^{2+} metal ion compared to that of the zinc/CA complex is further indication of a binding and chelation. The exact binding sites cannot be confirmed by using either the potentiometric data or the UV-Vis data changes presented above. However, the change in the intensity and the broadness of the most characterized carbonyl peak in the IR spectra at 1702 cm^{-1} is the indication of the participation of the carboxylate group along with that of the phenoxy oxygen to satisfy the chelate effect usually observed after the first participation of the first binding tooth.

It is worth mentioning here to say that the appearance of the peak at 302 for the free aqueous $Zn(NO_3)_2$ solution is due to either, the n→π* electronic transition, or the π→π* electronic transition for the nitrate counter ion, but definitely, not for the d → d transition of Zn^{2+}. This is due to the fact that Zn^{2+} has an electronic forbidden transition due to the d^{10} configuration.

We believe that the outcome of the data presented in the current report is novel and useful due to the identification of the ternary or mixed Zn^{2+}-Hydroxo-CA complexes in aqueous solutions at room temperature with a unique molar extinction coefficient of 1756 $M^{-1}cm^{-1}$ compared to that for the free Zn^{2+} and free CA. We have chemically identified a novel ternary Zn^{2+}-CA-OH chelate under ambient conditions. We are in the process of measuring the stability constant of CA with these essential meal ions (Fe^{3+}, Cr^{3+}, Cu^{2+} and Zn^{2+}) which will be more appropriate for journal's audience such Inorganica Chimica Acta, or Journal of Coordination chemistry or Journal of solution chemistry.

Acknowledgement

We would like to acknowledge the financial support from NSF under Grant # HRD-1332459. Also we would like to acknowledge the financial support of the ACS-SEED summer program to Aishah Darboe.

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