

Aqueous Solutions NMR, UV-Vis and Potentiometric Titrations of Cd²⁺ and Hg²⁺ with Clofibrac Acid

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Research Article

Abstract

In this study, we are presenting the reactions of the two metal ions Cd²⁺ and Hg²⁺ which are in the same group-family as zinc metal ion, i.e., group number 12, with the organic ligand Clofibrac acid (CA). It appeared that the reaction of Cd²⁺ with CA in aqueous solutions at 25°C in 0.1 M ionic strength (NaNO₃) formed a series of dimer complexes while that of the Hg²⁺ formed a series of monomer complexes. The difference in behavior of the two metal ions, although in the same family, is attributed to the difference in their ionic size. The detailed potentiometric, NMR and UV-Vis spectroscopic data for these reaction systems are discussed in this report. This paper is the first in a series that we are launching to celebrate the 80th birthday of the Chemistry Nobel Laureate Roald Hoffmann of the chemistry department at Cornell University.

Keywords: Aqueous clofibrac acid; Cd²⁺; Hg²⁺; Potentiometry; ¹H-NMR; UV-Vis-absorption spectroscopy.

1. Introduction

It appeared from our previous studies on CA that not much research has been done on the reaction of CA with essential or toxic metal ions [1-4]. Clofibrac acid (CA) is a Peroxisome proliferator-activated receptors- α (PPAR α) ligand. It has a chemical structure that is characterized by the presence of the 2-phenoxy-2-methylpropanoic acid moiety which has the right ring chelating size to chelate large metal ions such as Cd²⁺ and Hg²⁺. We have published recently some articles that dealt with the reactions of CA with a variety of essential metal ions. The metals were the zinc metal ion (Zn²⁺) and the ferric ion (Fe³⁺) in aqueous media [1,2]. In addition, we have published studies on the reactions of CA with the chromium (III) metal ion (Cr³⁺) [3] and the copper metal ion (Cu²⁺) [4].

Cadmium (Cd²⁺) has been considered a toxic metal ion for over 70 years. It has no known essential role in biology except in the replacement of the zinc metal ion at the catalytic site of a particular carbonic anhydrase enzyme in certain marine diatoms [5]. It

is considered to be a human carcinogen [5]. Mine workers are the one class of population with the largest risk of exposure along with cigarette smokers [5,6]. One cigarette contains approximately 2.0 μ g of cadmium, 2-10% of which is transferred to primary cigarette smoke. Cd²⁺ finds its way into humans via water, food and inhalation [6]. People of the highest risk for cadmium exposure in addition to mine workers are: paint makers, paint sprayers, pesticide makers, pharmaceutical workers, photoelectric cell makers, pigment makers, plastic products makers, smelters and textile printers [6]. Cadmium is mainly bound to metallothionein and excreted very slowly from the body.

Cadmium is not a pollution concern within the North American population. However, the east region of the Nile Delta in Egypt has the most severe pollution concern [7]. A detailed study that was conducted by very large research collaborative groups in both the USA and Egypt connected the relation of pancreatic cancer and serum cadmium levels [7]. They concluded that the occurrence of pancreatic cancer in people living in the east of the Nile Delta region is significantly associated with high levels of serum cadmium and farming. This study was published in *Environmental Health Prospective*, a prestigious health related journal in 2006 [7]. An earlier work by Cherian in the same journal, showed ten different thiol-containing chelators such as Propanethiol 1,2-dimercaptopropane and seven other compounds along with EDTA [8]. In this chelation study, the author showed effective chelation of cadmium without increased renal cadmium deposition [8]. Other researchers from Switzerland, Ireland and Spain in one concerted effort published a detailed paper measuring the stability constants of Cd²⁺ with the di-carboxylic acids Malate and Succinate [9].

One does not need to be an expert in metal toxicology to recognize the adverse effect of mercury (Hg²⁺). Mercury is toxic in any form (metallic, inorganic, organic, or organometallic); even inhaling the vapor of metallic mercury is toxic. Exposure to mercury or its compounds have adverse health effects such as: neurological, renal, respiratory, immune, dermatological, reproductive and developmental [10].

We have conducted a detailed library search of the many papers that dealt with the reaction of Mercury. When the term Mercury was searched within the title of the paper in all of the American Chemical Society Journals about 4 K papers appeared. When the term was searched again within the abstract about 6 K papers appeared and ~101 K papers appeared if the term mercury was anywhere in the paper. We repeated the same search using the term Mercury coupled with aqueous solutions and again the term Mercury coupled with CA. As the detailed library search showed, there are many papers that dealt with the very toxic metal Hg^{2+} . It will suffice herein to cite one paper as an example for the many papers that dealt with Hg^{2+} . The Lippard group at the chemistry department of MIT published an article that selectively detected Hg^{2+} in aqueous solutions [11]. To the best of our knowledge, there are no studies that discussed the reaction of CA with the carcinogenic Cd^{2+} metal ion and the very toxic Hg^{2+} . The main objectives of this study are 1-assess if CA binds to Cd^{2+} and Hg^{2+} metal ions under ambient conditions and 2-identify the identity of the formed complexes if they are of a monomeric or dimeric or oligomeric or polymeric nature.

2. Experimental Section

2.1 Materials and method

Solutions of CA were prepared using 99% purity (Sigma reagent grade), $\text{C}_{10}\text{H}_{11}\text{ClO}_3$, formula weight 214.6 $\text{g}\cdot\text{mol}^{-1}$. This sample of CA was a generous donation from Professor Badr of the University of Missouri-Kansas City School of Pharmacy. Cadmium metal ion (Cd^{2+}) was prepared from cadmium nitrate tetra hydrate, $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 98% extra pure Fisher brand, formula weight 308.47 $\text{g}\cdot\text{mol}^{-1}$. The Mercury metal ion (Hg^{2+}) was prepared from mercury nitrate mono hydrate, $\text{Hg}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$, ACROS Organics. Primary standard potassium hydrogen phthalate (KHP, 99.99%) and solid sodium hydroxide pellets (NaOH, 98%) were purchased from Fisher Chemical Co. We have shown the structural formula of CA in our previous work [1-4]. Sodium hydroxide (NaOH) solution was standardized to four digits to the right of the decimal point. pH values were measured using the Orion Membrane pH meter (model 250A) connected to a combination Orion-glass electrode in 0.1 $\text{mol}\cdot\text{L}^{-1}$ ionic strength. We have adjusted the ionic strengths by the addition of 10% v/v of 1.0 M NaNO_3 solution.

2.2. Potentiometry

The detailed methods used to carry out potentiometry in atmospheric CO_2 -free solutions and to standardize all solutions had been described in many of our previous publications [12-18]. For all data sets generated, the arithmetic mean and the standard deviation were calculated using Excel software.

For each individual experiment, CA solutions were first added to the titration vessel, followed by the

addition of the specified metal ion solution. The ionic strength of the solution was adjusted to 0.1 M by the addition of 10 ml of 1.0 M NaNO_3 solution. The final total volume was 100 ml. NaOH solution was added in 100 μl aliquots by means of a calibrated and accurate Eppendorf micro-pipette. Each titration took about 2.5 to 3.5 h to complete.

2.3. Ultraviolet and visible spectroscopy

The UV-Vis spectroscopy spectra were collected using the T60 high-performance spectrophotometer that was purchased from Advanced ChemTech (Louisville, KY). Samples were prepared in D.I. water at 25°C. 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 was set as the control. The concentration of metal ion was $\sim 2 \times 10^{-4}$ $\text{mol}\cdot\text{L}^{-1}$. All UV-Vis spectra were collected at the pH values of 3.5.

2.4 NMR spectroscopy

The NMR spectra were conducted using the JEOL 400 MHz unit in the Chemistry Department at the University of Memphis, Memphis, Tennessee. The aqueous solution samples of free CA and the Cd^{2+} :CA complexes in 1:1 ratio and that of Hg^{2+} :CA in 1:1 ratio complexes were prepared in the high quality Norell-NMR tubes using D_2O to lock and shim the signal. These NMR tubes were a generous gift from Prof. Ted Burkey of the Chemistry Department at the University of Memphis. Professor Burkey also assisted in the experimental set up and data acquisition/collection.

In a typical NMR spectrum, the X-axis was set as ppm units and Y-axis was recorded as % abundance in which we have used D_2O as internal reference that showed its characteristic peak at 4.80 ppm. Other organic peaks (aliphatic and aromatic protons) were identified as shown in Supplementary Material.

3. Results and Discussion

3.1 Potentiometry of free Cd^{2+} and Cd^{2+} with CA in 1:1 and 1:2 ratios

Figure 1 shows the potentiometric titration plots of free Cd^{2+} . The free CA titration curve has been published in previous studies [1,3,4]. It is established that CA releases one proton out of the carboxylic acid group. No data of this ligand (CA) has been reported in the NIST standard reference database of critically selected stability constants of metal complexes [19].

Figure 2 shows the potentiometric titration plots of Cd^{2+} and Clofibrac Acid (CA) in 1:1 molar ratio. Cd^{2+} concentration is 2.0×10^{-3} M in aqueous solutions at $25^\circ\text{C} \pm 1.0^\circ\text{C}$ in 0.1 M ionic strength (NaNO_3). Figure 3 shows the first derivatives (Slopes) of the potentiometric titrations of Cd^{2+} and CA in 1:1 molar ratio shown in Figure 2. The Cd^{2+} concentration is 2.0×10^{-3} M, CA concentration is 2.0×10^{-3} M in aqueous solutions. These slopes indicated the presence of an inflection point at 2.5 H^+ equivalents as indicated by

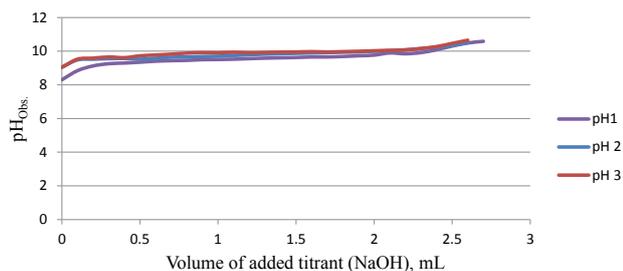


Figure 1. Potentiometric titration of 2 mM (2.0×10^{-3} M) free Cd^{2+} in the form of $\text{Cd}(\text{NO}_3)_2$ in aqueous solutions at $25^\circ\text{C} \pm 1.0^\circ\text{C}$ in 0.1 M ionic strength (NaNO_3).

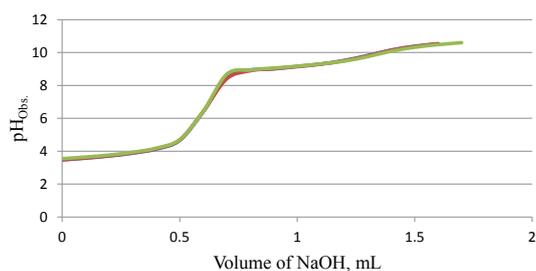


Figure 2. Potentiometric titration of Cd^{2+} and Clofibric Acid (CA) in 1:1 molar ratio. Cd^{2+} Concentration is 2.0×10^{-3} M in aqueous solutions at $25^\circ\text{C} \pm 1.0^\circ\text{C}$ in 0.1 M ionic strength (NaNO_3).

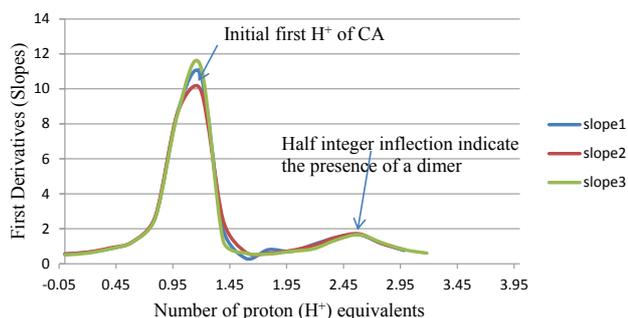


Figure 3. First derivatives (SLOPES) of the potentiometric titrations of Cd^{2+} and CA in 1:1 molar ratio shown in Figure 2. The Cd^{2+} Concentration is 2.0×10^{-3} M, CA Concentration is 2.0×10^{-3} M in aqueous solutions. These slopes indicated the presence of a minor inflection point at 2.5 H^+ equivalents. Arrow shows the location of the inflection point.

the arrow in the figure. This location at 2.5 equivalents is unequivocal evidence of binding of Cd^{2+} to CA. It is also strongly suggesting the dimerization of the Cd^{2+} :CA complex(es) in solution.

It is established in the literature that the movement of the location of the inflection point of the titrated free ligand from its original location (CA had a peak at 1.0 equivalent) to a new location after mixed with a metal ion is an indication of metal binding (the 2.5 H^+ equivalents is the new location upon Cd^{2+} binding to CA) [12-19]. The value of the inflection point (2.5 in this case) indicates the dominant complexes present. Clearly, in this case, a dimer species is formed. A dimer complex will release a half integer number of protons (H^+) into solutions [12-19].

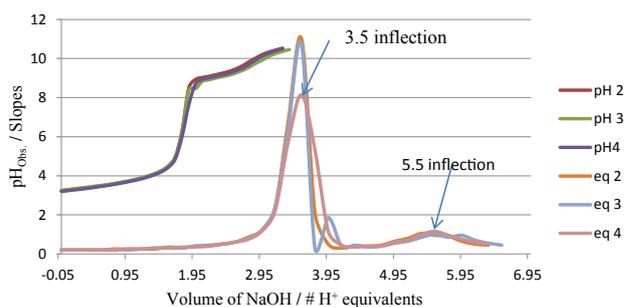


Figure 4. Double plots of both potentiometric titrations and the first derivatives (Slopes) of the potentiometric titrations of Cd^{2+} and CA in 1:2 molar ratio. Cd^{2+} Concentration is 2.0×10^{-3} M, CA Concentration is 4.0×10^{-3} M in aqueous solutions. These slopes indicated the presence of a major inflection at 3.5 H^+ equivalents and a minor inflection at 5.5 H^+ equivalents confirming the presence of a dimer complex. Arrows indicate the major and the minor inflection points.

To further confirm whether or not the dimer species is the dominant form, the amount of CA was doubled to see if the dimer would persist. Figure 4 shows the double plots of both potentiometric titrations and the first derivatives (Slopes) of the potentiometric titrations of Cd^{2+} and CA in 1:2 molar ratios. Cd^{2+} concentration is 2.0×10^{-3} M, CA concentration is 4.0×10^{-3} M in aqueous solutions. These slopes indicated the presence of a major inflection at 3.5 H^+ equivalents and a minor inflection at 5.5 H^+ equivalents further confirming the presence of dimer complex or complexes. Arrows indicate the major and the minor inflection points.

3.2 Potentiometry of free Hg^{2+} and Hg^{2+} with CA in 1:1 and 1:2 ratios

Figure 5a shows the potentiometric titration of 2 mM (2.0×10^{-3} M) free Hg^{2+} in the form of $\text{Hg}(\text{NO}_3)_2$ in aqueous solutions at $25^\circ\text{C} \pm 1.0^\circ\text{C}$ in 0.1 M ionic strength (NaNO_3). Figure 5b shows the slopes of the potentiometric titrations of free Hg^{2+} . This graph contains a total of four individual plots to show data consistency. This graph shows the exact locations of the major inflection points at 2 H^+ equivalents which one might expect for the di-valent Hg^{2+} ion.

Figure 6a is the potentiometric titration of 2 mM (2.0×10^{-3} M) Hg^{2+} :CA in 1:1 molar ratio in aqueous solutions at $25^\circ\text{C} \pm 1.0^\circ\text{C}$ in 0.1 M ionic strength. Figure 6b shows the slopes of the potentiometric titrations of the plots in the top graph Figure 6a. It appeared that a net of four proton equivalents were released into the solutions. Since the free Hg^{2+} released a net of 2 H^+ equivalents while CA releases one H^+ equivalent (see above), it is clear that an extra 1.0 equivalent of proton has been released from the reaction of Hg^{2+} with CA. One proton was clearly released from the CA and three protons came from the aqua ligands attached to the mercury metal ion Hg^{2+} . The main observation from this set of experiments is that in contrast to the Cd^{2+} data presented above, the Hg^{2+} did not form dimeric complex (es). Instead the large diameter of Hg^{2+} formed a series of monomeric

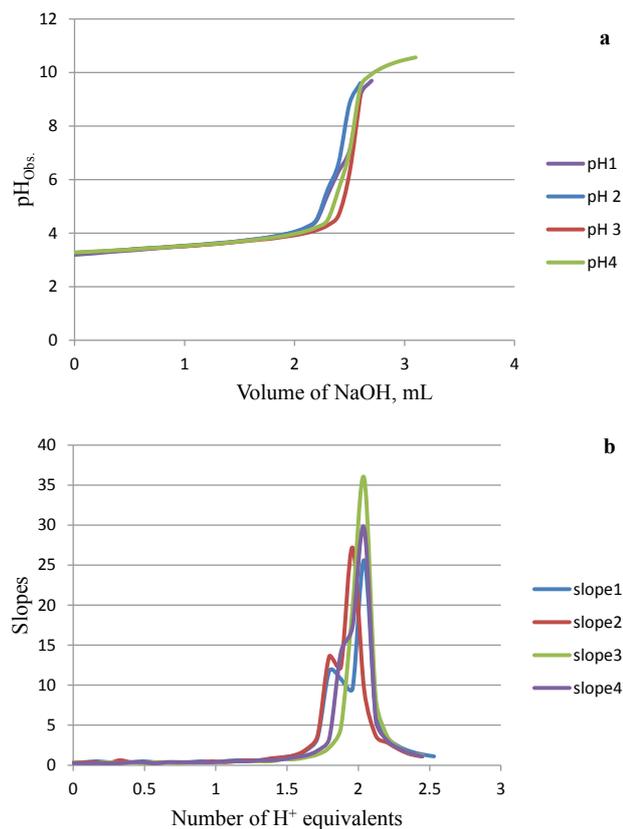


Figure 5. (a) Potentiometric titration of 2 mM (2.0×10^{-3} M) free Hg²⁺ in the form of Hg(NO₃)₂ in aqueous solutions at 25°C ± 1.0°C in 0.1 M ionic strength (NaNO₃). (b) First derivatives (Slopes) of the potentiometric titrations of free Hg²⁺.

complex(es). Figure 7a shows the potentiometric titration of 2 mM Hg²⁺:CA in 1:2 molar ratio and Figure 7b shows the slopes of the top graphs of the potentiometric titrations which further confirmed the presence of monomeric complexes.

3.3 UV-Vis absorption spectra for free CA, free Cd²⁺ and that of Cd²⁺:CA

The UV-Vis absorption spectra for the free CA, the free Cd²⁺, and that of the Cd²⁺ to CA in 1:1 molar ratios are shown in Supplementary Figures. In this figure, the UV-Vis absorption spectrum of DI H₂O as a control is shown in which no significant absorption was observed. The absorption spectra of free Cd²⁺ in the form of Cd(NO₃)₂, that of the free CA, and that of Cd²⁺:CA in 1:1 molar ratio in aqueous solutions are also shown on the same graph of this Supplementary Figures 0 and 1.

Clearly, the UV-Vis absorption spectra of the free Cd²⁺ observed is due to the presence of the nitrate anion, [NO₃]⁻, as the counter ion and not for the d → d electronic transition because it is well established that a d¹⁰ metal ion such as Zn, Cd and Hg do not possess a d → d electronic transition [20]. To the best of our knowledge, this is the first study to show the UV-Vis absorption spectra for free Cd²⁺ and/or Cd²⁺ with CA reacting together in aqueous solution under ambient conditions. A most recent accessed of NIST

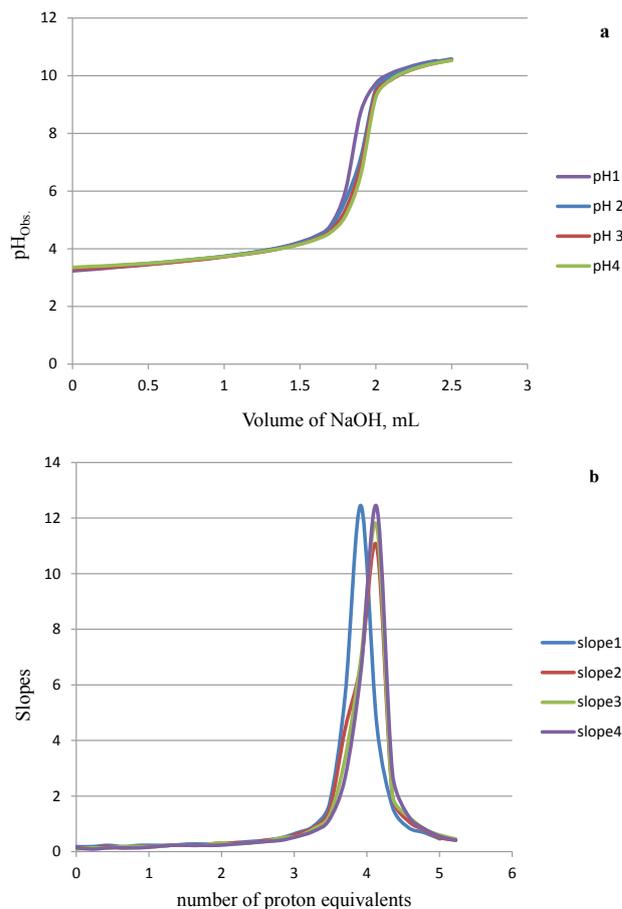


Figure 6. (a) Potentiometric titration of 2 mM (2.0×10^{-3} M) Hg²⁺:CA in 1:1 molar ratio in aqueous solutions at 25°C ± 1.0°C in 0.1 M ionic strength (NaNO₃). (b) First derivatives (slopes) of the potentiometric titrations of the plots in graph (a).

chemistry web book data base on 9-18-2017 showed no such data exist in the database [21].

The peak for Cd²⁺ had a maximum absorption at 305 nm ($\lambda_{max}=305$ nm). As stated in the experimental section, Cd²⁺ was in the form of (cadmium nitrate tetra-hydrate, Cd(NO₃)₂•4H₂O). This peak at 305 nm is due to either n → π* electronic transition or π → π* electronic transition for the [NO₃]⁻ anion. The peak of the free CA appeared with a maximum absorption at 275 nm. It is clear that the reason for the appearance of this peak for the colorless CA solution is the π → π* electronic transition of the chlorinated aromatic ring of CA. Because CA had a chloride (Cl) atom at the para-position, it is possible that there is n → π* electronic transition.

The fact that the electronic absorption spectra for the Cd²⁺:CA in 1:1 molar ratio overlapped with that of the free Cd²⁺ ion indicated that the peak at 305 nm of the Cd²⁺:CA complex is due to the nitrate anion present in solution that overshadowed that of the free CA that appeared at 275 nm. The overlap of the peaks of free Cd²⁺ ion at 305 nm with that of the Cd²⁺:CA complex does not indicate a binding or no binding. The potentiometric profile previously showed that there is strong binding between Cd²⁺ and CA as explained in

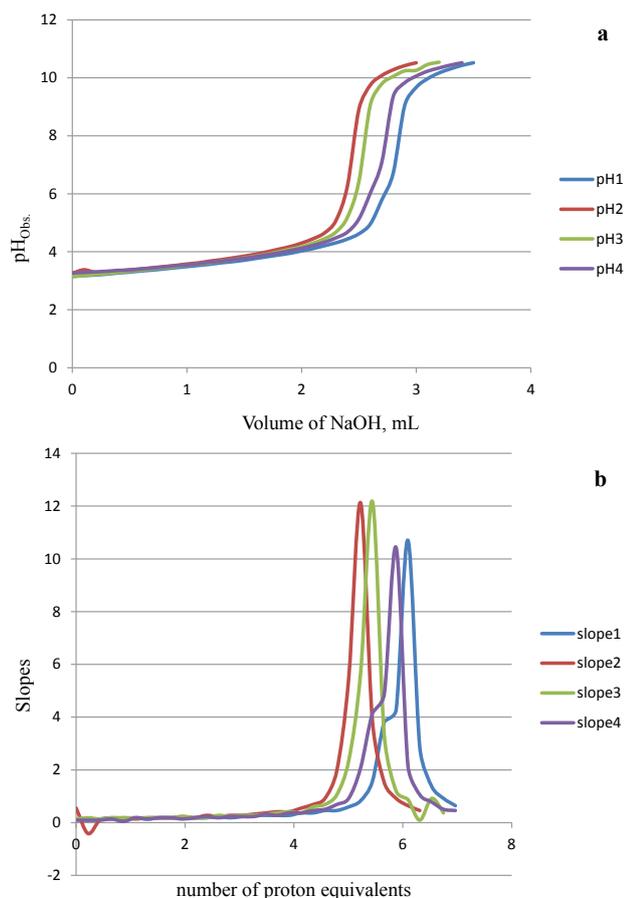


Figure 7. (a) Potentiometric titration of 2 mM (2.0×10^{-3} M) Hg^{2+} :CA in 1:2 molar ratio in aqueous solutions at $25^\circ\text{C} \pm 1.0^\circ\text{C}$ in 0.1 M ionic strength (NaNO_3). (b) First derivatives (slopes) of the potentiometric titrations of the plots in graph (a).

section 3.1 and figure 3 discussed above.

3.4 NMR of the free CA, the Cd^{2+} :CA and the Hg^{2+} :CA reaction mixtures in D_2O

The NMR spectrum of free CA, that of Cd^{2+} :CA in 1:1 molar ratio and that of Hg^{2+} :CA in 1:1 ratios are shown in Supplementary Figures 2-4, respectively. There are at two sets of ^1H -NMR peaks that appeared for the free CA, the aliphatic protons and the aromatic protons. The two methyl proton peaks were averaged at $(1.41 \pm 0.044 \text{ ppm})$ of the three spectra. There were two different types of aromatic protons the ortho-protons (H_o) and the meta-protons (H_m). The two methyl peaks of CA had a chemical shift at 1.39 ppm. That of Cd^{2+} CA complexes appeared at 1.38 (very broad), and methyl peaks appeared at 1.45 ppm (sharp) for the Hg^{2+} -CA complex. We do not know why there are differences in bandwidths of the two peaks. Perhaps this will open up a point of research for the NMR-specialized scientists.

4. Conclusion

From the potentiometric profiles of the free CA, that of the Cd^{2+} :CA in 1:1 and 1:2 ratios, it appeared that a dominant dimer complex was formed. From the potentiometric profile of the free CA, that of the

Hg^{2+} :CA in 1:1 and 1:2 ratios, it appeared that a dominant monomer complex was formed. The shift of the location of the inflection point of free Hg^{2+} titration from 2.0 equivalents compared to that of the Hg^{2+} :CA complex in all ratios is an evidence of Hg^{2+} binding and chelation. The 1:1 titrations shifted the inflection to 4.0 equivalents of protons. Based on the number of protons released into the solution, we are proposing the formation of the ternary hydroxo-CA mercury complex with the formula $[\text{Hg}^{2+}(\text{clofibrate})_2(\text{OH}^-)_2]^{2-}$ according to the description in Scheme 1. This complex is the most plausible to account for the release of 4.0 H^+ -equivalents. This structure is symmetric which agrees with the sharp methyl peak observed in the ^1H -NMR experiment of the Hg^{2+} -CA complex. We will not attempt to draw the structure of the dimer Cd^{2+} -CA complex, but it is expected that it is not as symmetrical as that of the Hg^{2+} -complex due to the broad line with of the methyl groups from the ^1H -NMR experiment of the Cd^{2+} -CA complex (Scheme 2).

Although, the ^1H -NMR experiments showed minor differences in the values of the chemical shifts upon binding to CA, there is a clear indication in differences between the binding of Cd^{2+} to CA compared to that of Hg^{2+} to CA from the shapes of the two methyl peaks. These observations further confirm the potentiometric work for the differences in the formation of a dimer versus monomer respectively. The NMR-experiment showed a de-shielding effect of the para-chloro atom on the adjacent protons Hm. The ortho-protons were more shielded as indicated from the values of the chemical shifts. The chemical shifts of the ortho-protons (H_o) were averaged at $6.78 \pm 0.026 \text{ ppm}$, while that of the meta-protons (H_m) were averaged at $7.19 \pm 0.015 \text{ ppm}$. All differences in behavior between the cadmium and mercury metal ions are attributed to the differences in their ionic radii. We hope that we have shown that the carcinogenic Cd^{2+} and the very toxic Hg^{2+} are binding to the important PPAR- α ligand CA. The toxicologists and pharmacologists have the ball in their filed to assess the physiological effects of these aqueous solutions metal-CA complexes. Further, the NMR specialized scientists have the opportunity to study these Cd^{2+} -CA and Hg^{2+} -CA systems that were not studied before.

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