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Ternary Metal-Hydroxo Chelate of Cr^{3+} with Clofibric Acid (CA): A Peroxisome Proliferator-Activated Receptors-Alpha (PPARα) Ligand

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Abstract

Fibrates are the only marketed PPARα ligands that are effective in lowering triglycerides. Their chemical structures are characterized by the presence of the 2-phenoxy-2-methylpropanoic acid moiety. Clofibric acid (CA) is a known ligand for PPARα. Using potentiometric titrations, UV-Vis, IR and speciation diagrams it appeared that CA chelates Cr^{3+} in aqueous solutions in 0.1 M $NaNO_3$ at 25°C in the 2.0 milli molar concentration range. The proposed solution structure of the (Cr^{3+}-CA) species is in a good agreement with what has been shown in the literature. Formation of the Cr^{3+}-CA complexes cover the span of a total of 400 mV; from +150 mV to -250 mV. Cr^{3+}-CA reaction mixtures indicated the formation of the ternary Cr^{3+}-CA-OH chelate. The pKa value for CA is 4.32 ± 0.06.

Keywords: Clofibric acid; Chromium (III); IR; Potentiometry; UV-Vis; Speciation diagrams

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Introduction

Peroxisome proliferator-activated receptors (PPAR)s constitute a family of receptors; members of the steriod receptor superfamily [1-4]. To date, there are three known receptors, namely PPAR (α), (β/δ), and (γ). Since their discovery, PPARs have been shown to be expressed in monocytes/macrophages, the heart, vascular smooth muscle cells, endothelial cells, and in atherosclerotic lesions. Since PPARs are nuclear transcription factors; they regulate multiple genes involved in energy production, glucose and lipid metabolism [4-8]. Polymorphisms in these receptors influence the pathology of numerous diseases including obesity, diabetes, atherosclerosis, inflammation and cancer [1-8]. Furthermore, PPARs can be activated by a vast number of compounds including synthetic drugs such as the clofibrate and anti-diabetic thiazoldinedione classes, polyunsaturated fatty acids, and a number of eicosanoids, including prostaglandins, lipoxygenase products, and oxidized low density lipoprotein [6, 9].

Yokoyama et al. [7] have investigated the inhibitory effect of clofibric acid (CA), a ligand for PPARα, on growth of ovarian malignancy *in vivo* and *in vitro* experiments using OVCAR-3 and DISS cells derived from human ovarian cancer and aimed to elucidate the molecular mechanism of its antitumor effect. CA treatment significantly suppressed the growth of OVCAR-3 tumors xenotransplanted s.c. and significantly prolonged the survival of mice with malignant ascites derived from DISS cells as compared with control. The results in this study indicated that CA produced potent antitumor effects against ovarian cancer in conjunction with a reduction of angiogenesis and induction of apoptosis [7].

The detailed literature review conducted in the current study revealed that a very limited number of publications have appeared that dealt with the interaction of CA with metal ions, especially in aqueous solutions [10]. Moncol et al showed the crystal structures and the spectral properties (UV-Vis, EPR) for both the bis $Cu(CA)_2$ complex and the dimeric $(Cu)_2$:CA complexes [10]. Ghauch et al. studied the effect of CA on metallic Iron (Fe^0) and other iron plated surfaces such as plated Fe^0 (mFe^0: Fe^0/Pd^0, Fe^0/Ni^0) [11]. This paper by Ghauch et al. was not biologically relevant because

iron was in its metallic form and not in aqueous environment. Although there is a wealth of toxicological, biological, medicinal, and chemical information about CA and PPAR [10-66] yet, there is lack of chemical studies of CA with essential and toxic metal ions under ambient conditions.

We have initiated a series of studies of CA with a series of metal ions such as Fe^{3+}, Cu^{2+}, Zn^{2+}, and Cr^{3+}. Clearly, these metals are essential metal ions that are found in the biological sphere with various degrees of abundance [67]. The choice of Cr^{3+} to be presented in the current report was due to the fact that Cr^{3+} is considered to be an essential metal that forms what is known as "the low-molecular-mass chromium-binding complex" (LMMCr) [68], which plays a role glucose metabolism [69, 70].

Fibrates are the only marketed PPARα agonists that are effective in lowering elevated serum triglycerides. Their chemical structures are characterized by the presence of the 2-phenoxy-2-methylpropanoic acid moiety. The main objectives of the current report are 1) To show whether CA binds with Cr^{3+} or not?; 2) To identify the type of metal-complexes formed, if there is binding, under the ambient conditions, and 3) To measure the potential response of the reaction mixture in milli-Volts. Potentiometry is one of the most powerful tools to study metal ions in aqueous solutions at ambient conditions [71-74].

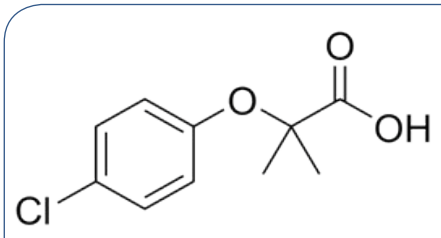
Experimental Section

Materials

All solutions were prepared using 99% purity Sigma reagent grade CA, $C_{10}H_{11}ClO_3$, formula weight 214.6 g.mol^{-1} and chromium nitrate nona-hydrate, $Cr(NO_3)_3 \bullet 9H_2O$, formula weight 400.15 g.mol^{-1}, using doubly deionized water to prepare all solutions. **Scheme 1** shows the structural formula of CA, or [2-(4-chlorophenoxy)-2-methyl propanoic acid (Chemical formula $C_{10}H_{11}ClO_3$). The pH values of all solutions were adjusted using ~ 0.1 mol.L^{-1} sodium hydroxide (NaOH), solution that was standardized to the fourth decimal place. The pH values were measured using Orion Membrane pH meter (model 720) with a combination Orion-glass electrode in 0.1 mole.L^{-1} ionic strength using the appropriate amounts of 1.0 M $NaNO_3$ solution.

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.



Scheme 1 Structural formula of Clofibric acid (CA), or [2-(4-chlorophenoxy)-2-methylpropanoic acid] (Chemical formula $C_{10}H_{11}ClO_3$).

The methods used to prevent the contamination of the titrant with atmospheric CO_2 had been described elsewhere [70-74]. 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 110ºC for 24 hours 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. Typically, thirteen-fifteen 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.

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 a typical titration; the CA solution was added first followed by the addition of the Cr^{3+} metal ion solution. To adjust the ionic strength of the solution to 0.1 M the appropriate amount of 1.0 M $NaNO_3$ was added. The total volume of the final titration solution was 100 mL. The final concentration of the metal ion titrated was in the range of 2.0 to 2.5 mmoles.L^{-1}. Before each titration, the titration solution mixtures were allowed to stir for 25 minutes to reach equilibrium. The NaOH titrant was added in the 100 L 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 3-4 and the final pH-value was in the range of 10-11. Each titration took about 5 to 6 hours to complete. All titrations were conducted at room temperature.

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 25ºC. The entire UV-vis spectrum was scanned from 200 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. The concentration of the metal was = 4.3×10^{-2} mol.L^{-1}. The UV-Vis spectra were collected at the pH values of 3.00.

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 in D.I. water at 25ºC. The entire IR spectrum was scanned from 400 to 4000 cm^{-1} using the provided attenuated total reflectance (ATR) accessory cell compartment equipped with a diamond cell that can accommodate all kind of samples (solid samples or 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, and Laser frequency of 15798.7 cm^{-1}. Typical IR spectra

were generated in which the X-axis was given as Wavenumbers in cm^{-1} and Y- axis was recorded as % Transmittance.

Results and Discussion

Potentiometric titrations of free CA and free metal ions

Potentiometric titration experiments of free CA showed that the acidity constants of the carboxylic acid functional groups present to be pKa = 4.32 ± 0.06 at 25°C, 0.1 M $NaNO_3$. To the best of the researcher's knowledge, this is the first time to report the acidity constant of CA in aqueous solutions at room temperature in 0.1 M ionic strength. **(Figure 1)** is the potentiometric titration graph of free CA. This graph contains three overlapped plots to show data consistency. **(Figure 2)** is the speciation diagram of free CA generated in aqueous solutions using Hyperquad simulation and speciation (Hyss) software program [75]. pKw value of 13.78 was taken from the literature [76]. CA releases one proton due to the fact that CA has a sole titratable functional group; the carboxylic acid group. This confirms the fact that clofibric acid is a mono-protic acid. These data of this ligand has not been reported in the NIST standard reference database of critically selected stability constants of metal complexes [77].

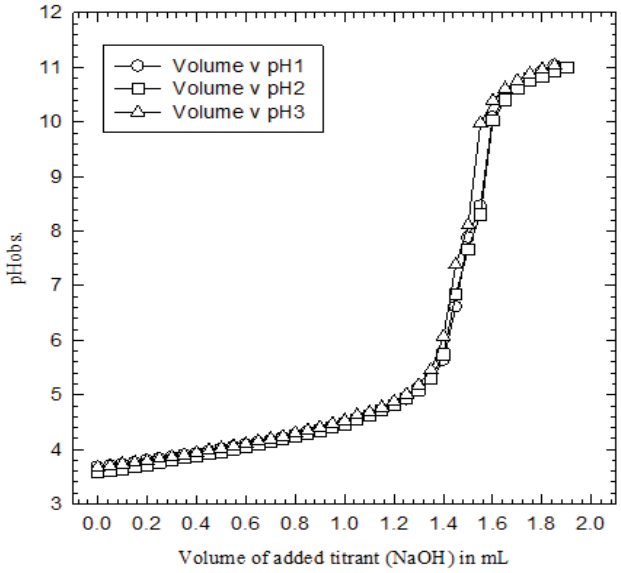


Figure 1 Potentiometric titration graphs of free Clofibric acid (CA) in 0.1 M NaNO3, 25°C. Three plots were overlapped to show data consistency.

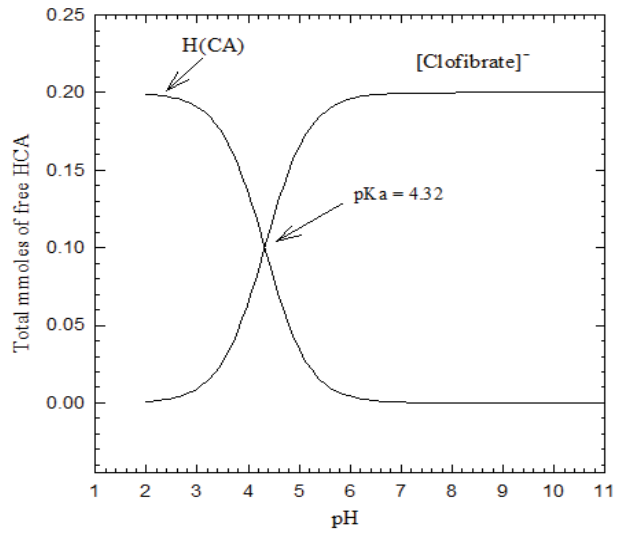


Figure 2 Speciation diagram of free CA. The cross point is the protonation constant (pKa) value of 4.32 ± 0.06.

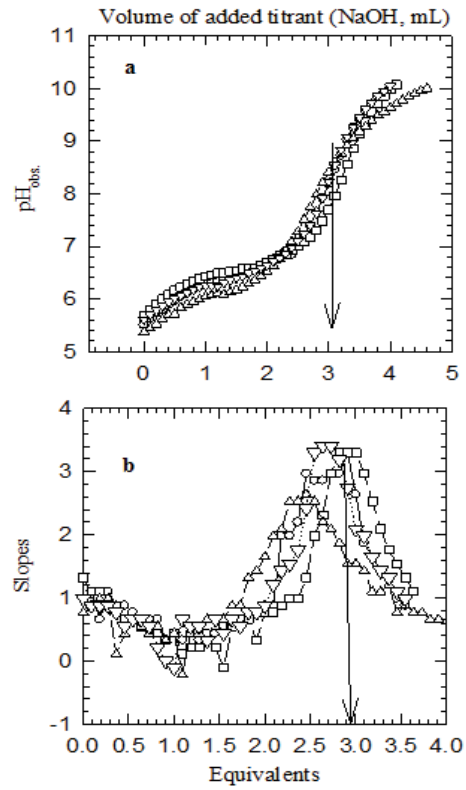
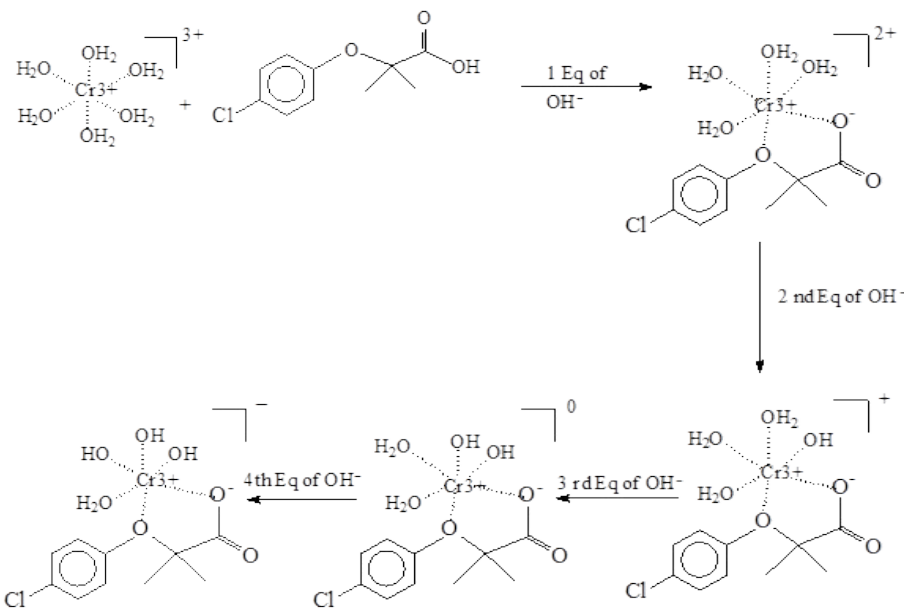


Figure 3 a) Potentiometric titration graphs of free Cr^{3+}, 0.1 M $NaNO_3$, 25°C. Four titration plots were overlapped to show data consistency. b) Slopes or the first derivatives versus the number of observed equivalents.

(Figure 3a) is the potentiometric titration graph of free Cr^{3+}. Four titrations plots were overlapped to show data consistency. **(Figure 3b)** is the mathematical treatments graphs for **(Figure 3a).** This mathematical treatment is the first derivatives (slopes) versus the number of observed equivalents. After data treatment and converting the volume of titrant into number of equivalents of titrant, it is clear that a tri-valnet metal ion such as Cr^{3+} releases a net of three 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 1-3 [78-80] and it is valid for any metal ion such as the chromium in its trivalent oxidation state. The number of equivalents is defined as the



Scheme 2 The proposed aqueous solution structures for the proposed Cr^{3+}-Clofibrate ternary chelates.

number of milli-moles of added titrant per number of milli-moles of Cr^{3+} ion present in solution.

$Cr(H_2O)_6^{3+} \rightarrow Cr(H_2O)_5(OH)_2^{+} + H^{+}$ Eq. 1

$Cr(H_2O)_5OH^{2+} \rightarrow Cr(H_2O)_4(OH)_2^{+} + H^{+}$ Eq. 2

$Cr(H_2O)_4(OH)_2^{+} \rightarrow Cr(H_2O)_3(OH)_3 + H^{+}$ Eq. 3

Titrations of Cr^{3+} with CA

(**Figure 4a)** is the potentiometric titration graph of the Cr^{3+}:CA in 1:1 molar ratio. This graph contains a total of three individual plots. 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 Cr^{3+}:CA in 1:1 molar ratio indicated the release of four protons. By examining these plots in this figure, clearly there has been a strong interaction between the metal ion Cr^{3+} and CA due to the shift in the location of the inflection points to 4.0 equivalents; compared to 3.0 equivalents in the titration of the free Cr^{3+} ion as shown in **(Figure 3)**. **(Figure 4b)** is the mathematical treatments graphs for **(Figure 4a)**. This mathematical treatment is the first derivatives (slopes) versus the number of observed equivalents.

For the Cr^{3+}:CA in 1:1 ratio, the three replicas overlapped at 4.00 equivalents. The important point here is that four equivalents of protons have been released from the reaction of Cr^{3+} with CA into the solutions. One proton was clearly released from the CA. The source of the other three protons must be accounted for. These three protons have to come for the aqua ligand attached to the metal ion. It is established in the literature that such hydroxo-complexes with Cr^{3+} have been seen previously [72, 79, 80]. The proposed and the most plausible species to

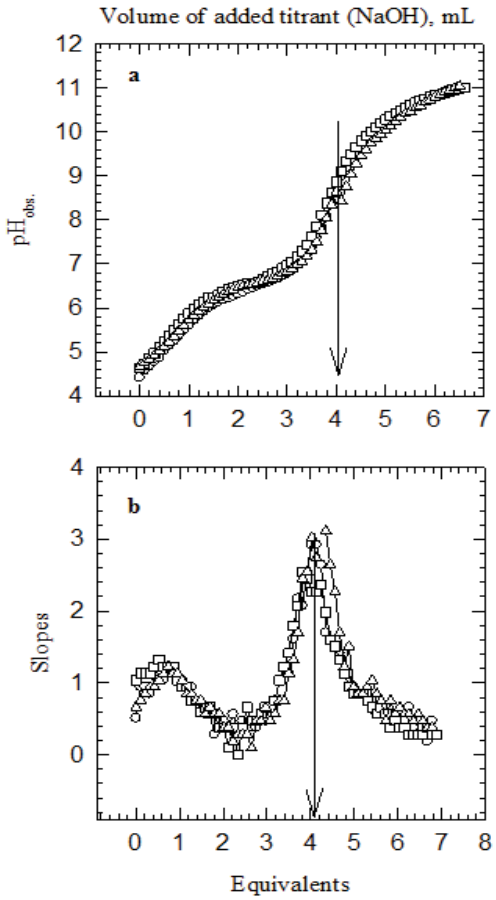


Figure 4 a) Potentiometric titration graphs of Cr^{3+}:CA in 1:1 molar ratio, 0.1 M $NaNO_3$, 25°C. Three titration plots were overlapped to show data consistency. b) Slopes or the first derivatives versus the number of observed equivalents.

be formed in solution will be the ternary chromium hydroxo-clofibrate complex $[Cr^{3+}(clofibrate^{-})(OH^{-})_3]^{1-}$. We are proposing the structure of this ternary chromium complex in aqueous solution in **Scheme 2**.

Conclusion

The literature evidence overwhelmingly indicated the lack of research articles for CA with essential and toxic metal ions. 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 [77]. Based on the number of protons released into the solution, we are proposing the formation of the ternary hydroxo-clofibrate metal complex with the formula $[Cr^{3+}(clofibrate^{-})(OH^{-})_3]^{1-}$ according to the description in **Schemes 2**. 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 Cr^{3+}-Hydroxo-CA complexes in aqueous solutions at room temperature. Also, the reported pKa value in the literature for CA was done in a 50/50 (v/v) acetonitrile/water solvent mixture [81]. The pKa value measured in the current study is in close proximity to the literature value 4.622, reference 81, versus 4.32 of the current study. We have chemically identified a novel ternary Cr^{3+}-CA-OH chelate under ambient conditions. More toxicity and biological studies are needed in these areas to test the effect of these Cr^{3+} chelate compared to the effect of the free CA alone on PPARα.

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