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Detailed Potentiometric Study of Al^{3+} and Cr^{3+} with Malic Acid in Aqueous Solutions

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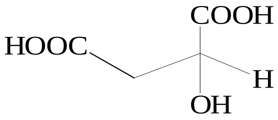
Abstract: It appeared that malic acid solubilized both Al^{3+} and Cr^{3+} in aqueous solutions at all pH-values in 0.1 M $NaNO_3$ at 25 °C. The detailed potentiometric measurements indicated that these free tri-valent metal ions released a net of three protons ($3H^+$'s) into the solution. Free malic acid released a net of ($2H^+$'s) into the solution from the two carboxylates. However, in the presence of metal ions malic acid effectively releases a net of three protons ($3H^+$'s) into the solution; two from the two carboxylates and the third from the alcoholic group. The reaction mixture of Al^{3+}:malic acid indicated the formation of a dimeric species. The proposed structure of this dimeric species is in good agreement with what has been shown in the literature. We are presenting a dimeric species that may play an important role in malate transportation across cell membrane. Formation of the Al^{3+}-malic acid complexes cover the span of a total of 400 mV; from +250 mV to -150 mV. The Cr^{3+}-malic acid reaction mixture indicated the formation of a dimeric species as well.

Keywords: Aqueous solutions, Dimeric species, Malic acid, Al^{3+}, Cr^{3+}, and Speciation diagrams.

1. INTRODUCTION

Aluminum is the most abundant metal in the earth's crust; it constitutes about 8.1% of the earth's crust weight [1,2]. The mean occurrence of aluminum is about 81 kg per ton of earth's crust, while the mean occurrence of chromium is about 100 grams per ton of earth's crust [1]. Although, aluminum is more abundant than most essential and non-essential trivalent metal ions [1-3], nature did not utilize the Al^{3+} ion. One of the factors that may explain this phenomenon is the fact that the aluminum ion possesses single oxidation state of 3+ while most of the other trivalent metals possess Redox chemistry. It has been established that aluminum became accessible to living organisms upon the release of Al^{3+} ion from the soil by the effect of acid rains [4]. Research of aqueous solution chemistry of aluminum (or any other metal ion in that regards such as Cr^{3+}) can be categorized into two main broad categories (1) Speciation of metal ion among biologically active ligands [5-9], and (2) Synthesis of new chelating agents to treat the metal ion over-loads and/or toxicity if it has toxicity effects [10-13].

Fairly recently, Matsumoto *et al.* have shown evidence for the plasma membrane localization of aluminum-activated malate transporter protein (ALMT1) [14]. These researchers discovered that ALMT1 is a strong candidate for the gene that is responsible for the malate efflux from the cytoplasm to the apoplasm after high levels of aluminum exposure [14]. Until now, citrate or malate has been identified as the organic acid anions secreted by roots in plants to detoxify aluminum in high aluminum-containing soils [15]. Some reports have investigated reactions of malic acid with metal ions [16-19]. The main objectives of the current report are 1- To collect the potentiometric titration plots for malic acid with Al^{3+} and Cr^{3+}, 2- To show the type of metal complexes formed under the ambient conditions of the study, and 3- To measure the potential response of the reaction mixture in milli-volts and relate this to Nernst Equation. These objectives will confirm if malic acid is a candidate to un-leach these metal ions from the soil and if one of the formed metal complexes plays a role in the transport of these metal ions across cell membrane [14-19]. Potentiometry is one of the most powerful tools to study metal ions in aqueous solutions at ambient conditions [16-21].



Scheme 1: Structural formula of the hydroxycarboxylic acid (D, L-Malic acid) used in this study. The racemic mixture D, L-Malic acid was used for ease of data treatment.

2. EXPERIMENTAL SECTION

2.1. Materials

All solutions were prepared using Fisher reagent grade malic acid, $C_4H_5O_5$, formula weight 192.12 g.mol^{-1}, chromium nitrate nona-hydrate, $Cr(NO_3)_3 \bullet 9H_2O$, formula weight 400.15 g.mol^{-1}, and aluminum nitrate nona-hydrate, $Al(NO_3)_3 \bullet 9H_2O$, formula weight 375.13 g.mol^{-1}, using doubly deionized water to prepare all

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solutions. 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 $NaNO_3$ solution.

2.2. 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_2 had been described elsewhere [9,13,16]. 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 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.

2.3. 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 malic acid solution was added first followed by the addition of the metal ion solution (either Al^{3+} or Cr^{3+}). To adjust the ionic strength of the solution to 0.1 M; we have added the appropriate amount of 1.0 M $NaNO_3$. 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 20-25 minutes for equilibrium. The NaOH titrant was added in the 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 2-3 and the final pH-value was in the range of 10-11. Each titration took about 5.5 to 6.5 hours to complete. All titrations were conducted at room temperature.

3. RESULTS AND DISCUSSION

3.1. Free Metal Ions and Free Malic Acid

Potentiometric titration experiments of free malic acid had proven the literature values for the acidity constants of the functional groups involved. Kiss, *et al.* reported pKa1 = 3.16 and pKa2 = 4.57 for free malic acid in aqueous solutions at 25 °C, 0.2 M NaCl. Kramer, *et al.* reported the respected values of 3.46 and 5.10 at 25 °C, 0.1 M ionic strength. We have chosen to used the more reliable numbers published and complied by Martel, *et al.* in the *National Institute of Standard and Technology* (NIST) [12]; the two pKa-values of the two carboxylates are 3.24 and 4.68. Figure **1** is the speciation diagram of free malic acid generated in aqueous solutions using Hyperquad simulation and speciation (Hyss) software program [20]. pKw value of 13.78 was taken from the literature [21]. Clearly Martell's pKa-values lie between the previously published values [12,17,18]. This gave more confidence in the data presented in Figure **1**. It is worth mentioning here that although malic acid has two titratable carboxylate groups, the alcoholic group does not release its proton unless malic acid is reacting with a strong Lewis acid (the metal ion) [7,12,16-19, 22-26]. The next section gives more details on this point at which multiple examples prove the chelation of the alcoholic group participation to a variety of metal ions.

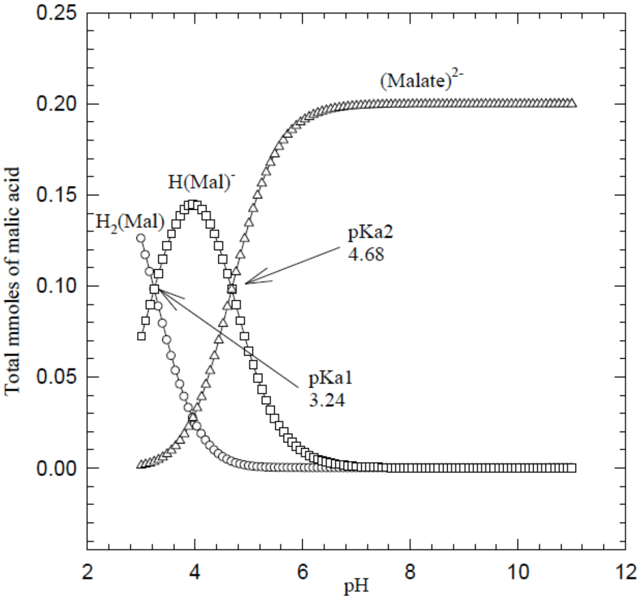


Figure 1: Speciation diagram of free malic showing the pKa values of the two carboxylates. Total mmoles of acid is = 0.2 mmoles at 25°C, pKw = 13.78 from Ref. no. [21].

3.2. Titrations of Cr^{3+} with Malic Acid

Figure **2** is the potentiometric titration graph of the free malic acid, free Cr^{3+} solution, and Cr^{3+}:malic acid in 1:1 molar ratio. This graph contains a total of seven individual plots. This graph shows the exact locations of the inflection points for each plot presented. The location of each inflection point of every plot gives the exact number of protons released into the aqueous solution. For example, the titration plot of free malic acid has an up-hill buffer region, which is common for many hydroxy carboxylates due to the closeness of the two pKa-values of the two carboxylates 3.24 and 4.68. This buffer region is terminated with a very steep inflection point at 2.0 equivalents. The number of equivalents is defined as the number of milli-moles of added titrant per number of milli-moles of malic acid. This confirms the already known fact that malic acid is a di-protic acid.

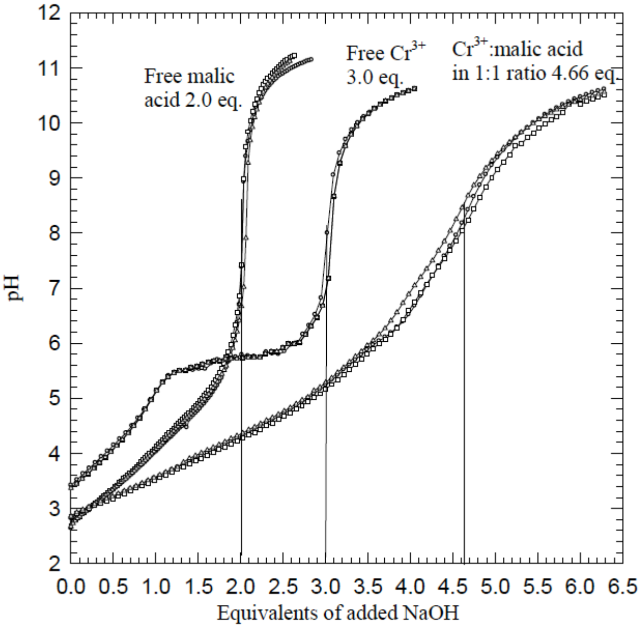


Figure 2: Titration curves of free malic acid, free Cr^{3+} solution, and that Cr^{3+}: malic in 1:1 ratio.

When free samples of Cr^{3+} solutions are titrated the second set of plots in the graph shown in Figure **2** are generated. The major inflection point appeared at 3.0 equivalents. The number of equivalents here is defined as the number of milli-moles of added titrant per number of milli-moles of Cr^{3+}. By examining the last bundle of plots in this figure, clearly there has been a strong interaction between the metal ion Cr^{3+} and malic acid due to the shift in the location of the inflection points to 4.5 equivalents (with a hard to define inflection point). Three plots were overlapped to show data consistency.

Further mathematical treatments of the plots shown in Figure **2** were needed to confirm the exact locations of the inflection points, particularly with the un-easily defined inflection for the Cr^{3+}:malic acid in 1:1 ratio. Figure **3** is the mathematical treatments graphs for Figure **2**. This mathematical treatment is the first derivatives (slopes) versus the number of observed equivalents.

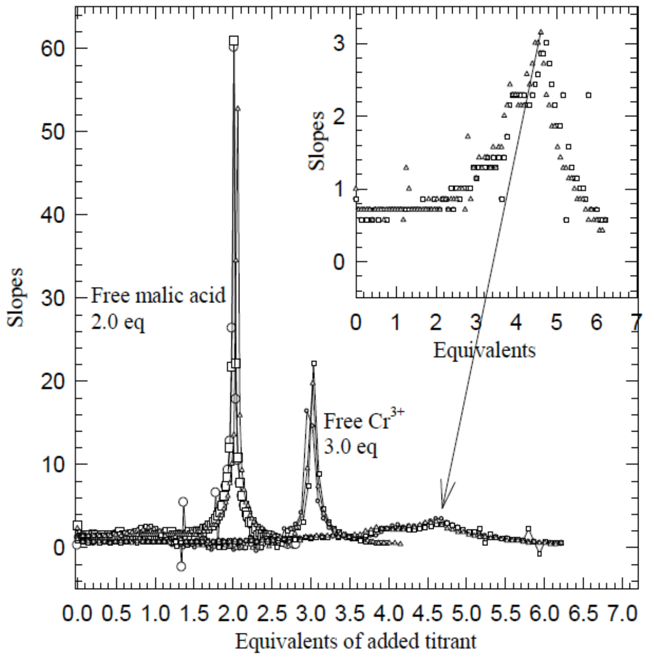


Figure 3: Triplicate titrations of free malic acid, Free Cr^{3+}, and Cr^{3+}: malic acid in 1:1 ratio. Equivaelnt (eq.) is number of mmoles of titrant per mmoles of Cr^{3+}. The inset shows the number of eq. of the 1:1 titration.

For the Cr^{3+}:malic acid in 1:1 ratio, the three replicas overlapped on 4.56 to 4.66 equivalents. The important point here is that more than four equivalents of protons have been released from the reaction of Cr^{3+} with malic acid into the solutions. Two protons were clearly released from the malic acid. The source of the other two protons must be accounted for. It has been established in the literature that the alcoholic group of the hydroxy carboxylates such as lactate, malate, and citrate participate in the chelation of the metal ions [22-27]. Hence, malic acid effectively can release a net of three protons the two carboxylate protons plus the alcoholic proton. The fourth proton has 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 [28,29]. The proposed and the most plausible species to be formed in solution will be the mixed chromium hydroxo-malate complex $[Cr^{3+}(malate^{3-})(OH^{-})]^{1-}$.

Table 1: The Exact Number of Protons Released into the Aqueous Solutions from the Reactions of Al^{3+}: Malic Acid in Different Molar Ratios. Also Shown are the Number of Protons Titrated from the Free Al^{3+} and Free Malic Acid as Standards

	Free Al^{3+}	Free Malic	1:1.3 ratio	1:2.3	1:3.1
# of runs (replicas)	11 replicas	4 replicas	3	4	3
# of H^+ Equivalents	3.02±0.08	2.05±0.12	3.90±0.09	6.59±0.19	8.59±0.14
Remarks	Al^{3+} releases three H^+'s	Malic acid is a di-protic acid	Four H^+'s released	Six and a half H^+'s released	Eight and a half H^+'s released
Proposed complexes	–	–	At least a one-to-one hydroxo-complex formed	A dimeric metal complex	A dimeric metal complex

3.3. Titrations of Al^{3+} with Malic Acid and Nernst Equation

Table **1** is the summary of all the potentiometric titrations of the free malic acid, free Al^{3+} solution, and Al^{3+}:malic acid in 1:1, 1:2, and 1:3 molar ratios. There are a total of 25 titrations in this table. Similar to the Cr^{3+}:malic acid system, and from the number of proton equivalents released one can predict the type of species complex formed in solution. For example when four protons are released from the Al^{3+}:malic acid in 1:1 ratio we expect that the $[Al^{3+}(malate^{3-})(OH^{-})]^{1-}$ complex has been formed. Because the number of proton equivalents is based on the number of milli-moles of Al^{3+} present in solution, a one-half (1/2) proton released could be accounted for if a dimeric species have been formed. From the 1:2 and 1:3 titration systems; 6.5 and 8.5 proton equivalents have been released into the solution. Such one-half integer equivalent has been observed previously [9,24,25,30].

Figure **4** shows the linear regression of the relation between the observed pH-values and the potential in milli-volts (mV) for the Al^{3+}:malic acid in 1:3 ratio. Three runs were overlapped to show consistency of the work presented. Similar graphs could be plotted for the 1:1 and the 1:2 reaction ratios. This graph shows that the formation of the dimer Al^{3+}/malic acid complex and the $[Al^{3+}(malate^{3-})(OH^{-})]^{1-}$ complex covers the span of 400 mV from +250 mV to -150 mV. The slope of this linear regression = -59.69, which is in a good agreement with Nernst equation [31].

4. CONCLUSION

The literature evidence overwhelmingly favors the formation of dimeric species when it comes to the interaction of malate/hydroxy carboxylates with variety of metal ions [7-9,12,13,26,32-36]. Table **2** shows the selected references from the literature (not the full list) of various metal ions in different oxidation states with malate/other hydroxy carboxylates that formed the dimer complexes. Based on the number of protons released into the solution, we are proposing the formation of the mixed hydroxo-malate metal complex namely $[M^{3+}(malate^{3-})(OH^{-})]^{1-}$ and the dimeric metal-malate complex namely $[[M_2^{3+}(malate^{3-})_2](OH^{-})_3]^{3-}$ according to the presentations in Schemes **2** and **3**.

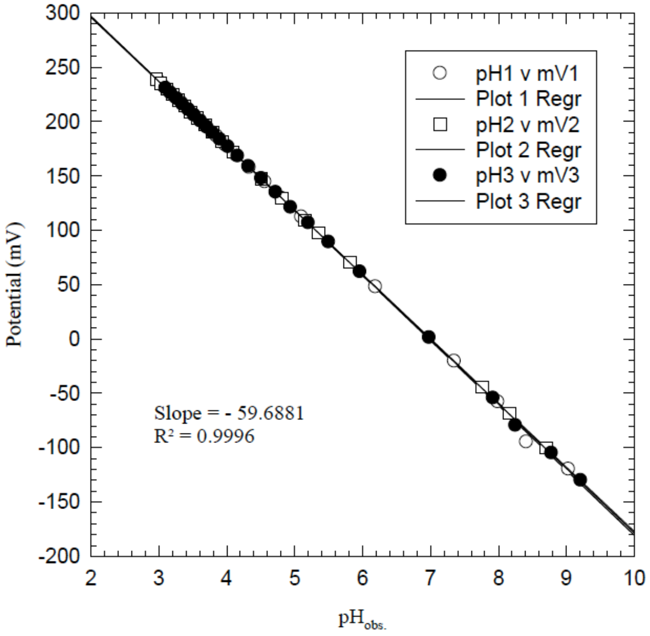


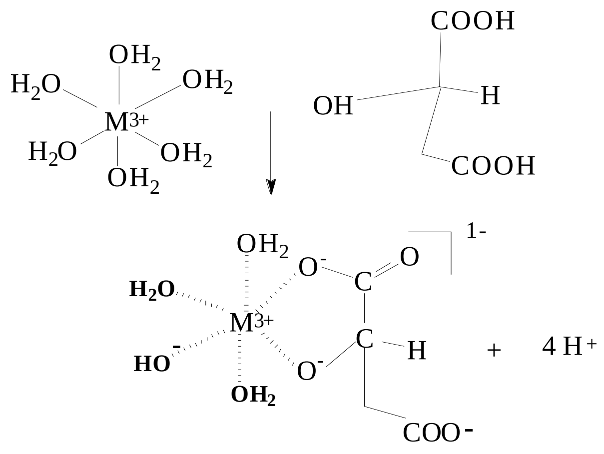
Figure 4: Linear regression of the observed pH-values with total solution potential in millivolts of the Al^{3+}: malic acid in 1:3 ratio in triplicate.

As published by Yamaguch *et al.* [14], aluminum-activated malate transporter protein plays a major role in the transport of malate. We are presenting a dimeric species that may play an important role in malate transportation across cell membrane. The anionic nature of this dimeric species is obvious, which may be a reasonable model for the actual species anchored into the cleft of the cell receptor. We are sure that some researchers will try to crystallize this dimeric species.

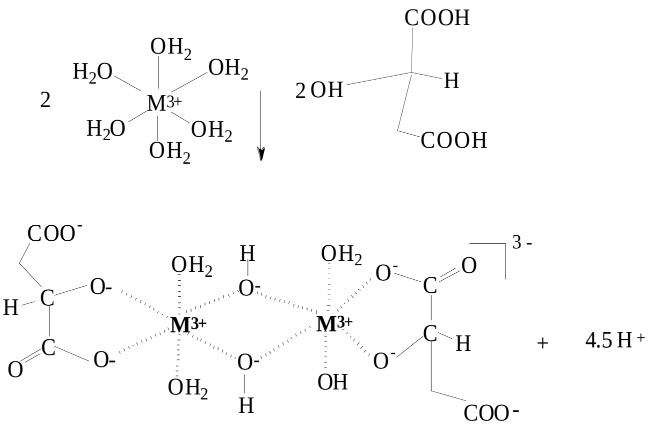
Table 2: Selected References from the Literature (Not the Full List) for Various Metal Ions in Different Oxidation States with Malate/Other Hydroxy Carboxylates that Formed the Dimer Complexes

Metal ion	Nature of the study	Year of Publication	Ref. #
Al^{3+}	Aqueous solutions equilibrium/mass spectra	2003	[7]
Al^{3+}	Aqueous solutions equilibria	1994	[8]
Al^{3+}	Aqueous solutions equilibria	2006	[9]
All metal ions	Aqueous solutions equilibria	2001	[12]
Al^{3+}	Aqueous solutions equilibria	2010	[13]
Fe^{3+}	Crystal structure	1994	[26]
V^{5+}	Crystal structure and solution studies	2001	[32]
UO_2^{3+}	Aqueous solutions/ Crystal structure	2013	[33]
Co^{2+}	Crystal structure, EPR, and IR, UV/Vis study	2003	[34]
V^{5+}	Crystal structure	2004	[35]
In^{3+}/U^{6+}	Aqueous solutions equilibrium constant study	1972	[36]

We hope that we have shed light on this important type of research that will help the science community.



Scheme 2: Proposed structure for the metal-malate one-to-one complex.



Scheme 3: Proposed structure for the metal-malate dimer complex.

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SUPPLEMENTARY DATA

The supplementary Figures can be downloaded from the journal website with the article.

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