The Effect of Amino Acids on the Solubility of Copper (II) Citraconate

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Abstract

Zinc and copper salts are known to be important in general human nutrition and surface wound healing. The success formulations that provide zinc and copper(II) ions locally at the wound site may depend on the bioavailability of zinc and copper(II) ions. A study of the influence that amino acids have on the solubility of zinc citraconate may be relevant to such therapy. In this research, copper(II) citraconate was combined with varying molar amounts of amino acids (glycine, alanine and serine) and each solution was titrated with Na_2EDTA to determine the total copper(II) ion content in the solutions, and therefore the percent of zinc citraconate that dissolved and the percent copper(II) titrated. It is hoped that this research may help aid future development of medical copper (II) salt treatments.

Introduction

The purpose of this research is to determine the interaction of strong and weak complexing agent and it effect on free copper (II) ion content, which can lead to useful insight for development of applications.

It has been shown that zinc enhances the treatment of the common cold. Initial research with zinc gluconate and glycine showed a modest increase in free zinc ion content (93% vs.91%). (Novick, et. al. 1996) Will copper (II) complexes act the same? In an experiment by Haertl, 1963, the control of metal nutrition in plants with synthetic chelating agents was studied. Metal chelates are utilized in plant metabolism and have

been proven to be beneficial in the treatment of some metal deficiencies and for the stimulation of growth. Zinc provides an essential nutrient in plant nutrition.

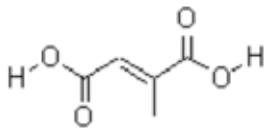
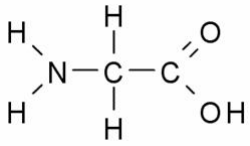
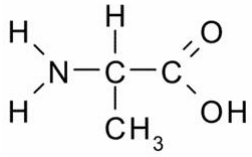
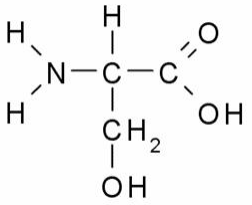
Zinc complexes are important in many biological and industrial contexts. A study by Keefer, et. al., 1998, showed that a topical preparation containing a relatively high zinc concentration could significantly encourage autolytic burn wound debridement (burn wound healing). Zinc ion containing compounds maintain bacteriostatic activity and have an advantage of improving the autolytic digestion of necrotic tissue. This advantage can enhance healing of these wounds in burn patients.

For every situation, a different form of zinc is needed. In some cases, free zinc ions (weakly complexed) or strongly complexed zinc ions are more beneficial, and in other cases, strong complexes are needed for the removal of zinc while weak complexes are needed for the delivery of zinc. Strong complexing agents (e.g. citraconate) and weak complexing agents (e.g. amino acids) are well known. The interaction of the strong and weak complexing agents and effect on free zinc ion content is not known. Previous research in this group has shown an increase of zinc citrate solubility with the addition of amino acids with glycine, 0 - 50 molar excess: range of 10% - 70% soluble.

Experimental

Methods and Materials

Copper (II) carbonate, citraconic acid and all amino acids were used as received. Disodium ethylenediaminetetraacetate (Na_2EDTA) was dried for 1 hour at 100°C before use. Table 1 shows the chemical structure and composition of citraconic acid, and the amino acids used. The amino acids used in this study were glycine, alanine and serine.

			
Citraconic acid	Glycine	Alanine	Serine
Table 1 shows the chemical structure and composition of citraconic acid, glycine, alanine and serine.			

Preparation of Zinc Citraconate

Copper (II) carbonate (3.0000g) and citraconic acid (3.4256g) were weighed out in separate beakers to yield 5 g copper (II) citraconate. Citraconic acid was stirred in 20 mL Nanopure H_2O until completely dissolved. Copper (II) carbonate was stirred in 20 mL Nanopure H_2O on medium heat. Citraconic acid solution was added to stirred copper (II) carbonate solution by pipette. Mixture was left to stir overnight and filtered. Filtrate was placed in rotary evaporator and the solids were dried in a desiccator to yield potentially copper (II) citraconate.

Preparation of Solutions

Approximately one gram copper (II) citraconate was combined with varying molar amounts of amino acid and weighed out into beakers. Table 2 gives the theoretical grams used to make each solution of copper (II) citraconate and each amino acid used in this study glycine, alanine and serine. Approximately 20 mL Nanopure water was added and the mixture stirred to overnight. It was then transferred into a 25 mL volumetric flask and diluted to 25 mL. Mixtures were set aside for 48 hours and filtered. The residue was weighed and the remaining solution was titrated.

Glycine	75.07 g/mol		Alanine	89.1 g/mol		Serine	105.09 g/mol	
Ratio	Cu(Citr) (g)	Glycine (g)	Ratio	Cu(Citr) (g)	Alanine (g)	Ratio	Cu(Citr) (g)	Serine (g)
0	1.0000	0.0000	0	1.0000	0.0000	0	1.0000	0.0000
1	1.0000	0.0751	1	1.0000	0.0891	1	1.0000	0.1051
2	1.0000	0.1502	2	1.0000	0.1782	2	1.0000	0.2102
3	1.0000	0.2252	3	1.0000	0.2673	3	1.0000	0.3153
4	1.0000	0.3003	4	1.0000	0.3564	4	1.0000	0.4204
5	1.0000	0.3754	5	1.0000	0.4455	5	1.0000	0.5255
10	1.0000	0.7507	10	1.0000	0.8909	10	1.0000	1.0509

Table 2 Molar ratios and masses used of copper (II) citraconate and each amino acid. (Glycine, alanine and serine)

Analysis of Total Zinc Ion Content (Titrations)

Preparation of 0.0100 M Na_2EDTA

Na_2EDTA was dried at 100^{o}C for one hour. Approximately 3.7251 g was weighed out and stirred to dissolve with 700 mL – 800 mL nanopure water. Mild heat was used when needed. It was then transferred into a 1000 mL volumetric flask and diluted to 1000 mL. This was then transferred into a 4 L bottle. This was repeated for a total of 4 times. Na_2EDTA was standardized with 0.01706 M Zn (Acetate).

Preparation of 0.10% Xylenol Orange Indicator

Approximately 0.0100 g of Xylenol orange was weighed out and 10 mL Nanopure water was added and was shaken until dissolved. Solution was made every 24-48 hours.

Preparation of pH 5.5 Buffer

Approximately 4.1 g sodium acetate was weighed out into a 1.00 L polypropylene bottle. It was filled to the max with Nanopure water and using a pH meter and glacial acetic acid, the pH was adjusted to pH 5.50.

Titration

Small aliquots of filtered solution (~2 mL) were measured into a flask. Next, 25.0 mL of pH 5.5 acetate buffer and 3-4 drops of Xylenol orange indicator was added. Xylenol orange indicator changed the color of solution to blue when free Cu^{2+} is present. Using an automatic titrator, the solution was titrated with 0.0100 M Na_2EDTA. At endpoint, the color of the solution changed to green. The milliliters delivered were recorded. This procedure was continued for each zinc citraconate and amino acid (glycine, alanine and serine) molar ratio in triplicate.

Calculation of Percent Dissolved Zinc Citraconate

All titration results were entered into an Excel spreadsheet. Based on the stoichiometry of 1:1 Cu^{2+}: $EDTA^{2-}$, moles copper, grams copper (II) citraconate, and percent dissolved copper (II) citraconate was calculated.

Results

Synthesis of Copper (II) Citraconate

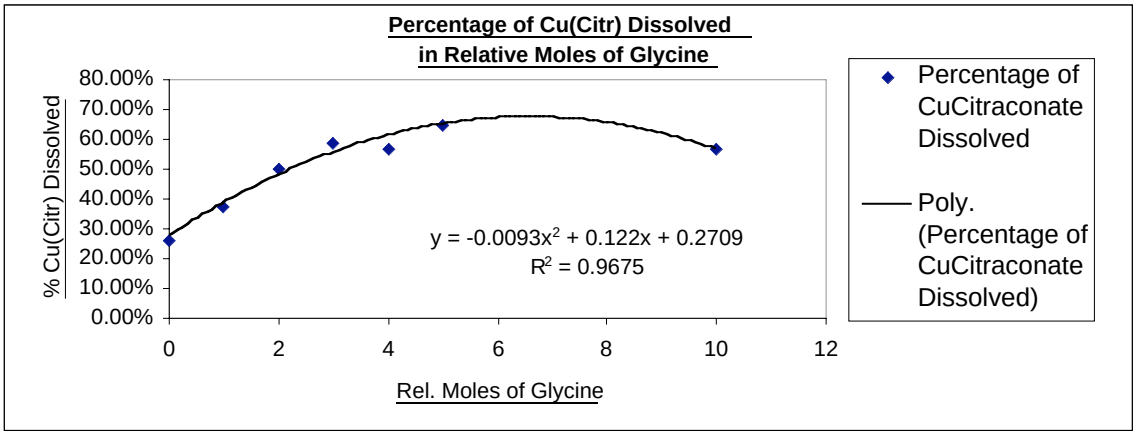
The copper (II) citraconate synthesized was found to not be 100% pure. Elemental analysis for pure copper content, by ashing to CuO, resulted in an experimental percent

copper of 48.29%. Theoretical percent zinc for copper (II) citraconate is 33.16%. Therefore, it was concluded that a mixed zinc salt was prepared and Infrared Spectrum (IR) analysis revealed no presence of carbonate anions.

Copper (II) Citraconate and Glycine

While titrating copper (II) citraconate at a 1:0 ratio, the percent copper dissolved starts at ~26% in glycine. Adding glycine gradually increases the solubility of copper (II) citraconate. The maximum solubility of 65% was seen at a glycine to total copper molar ratio of 5:1. (Graph 1)

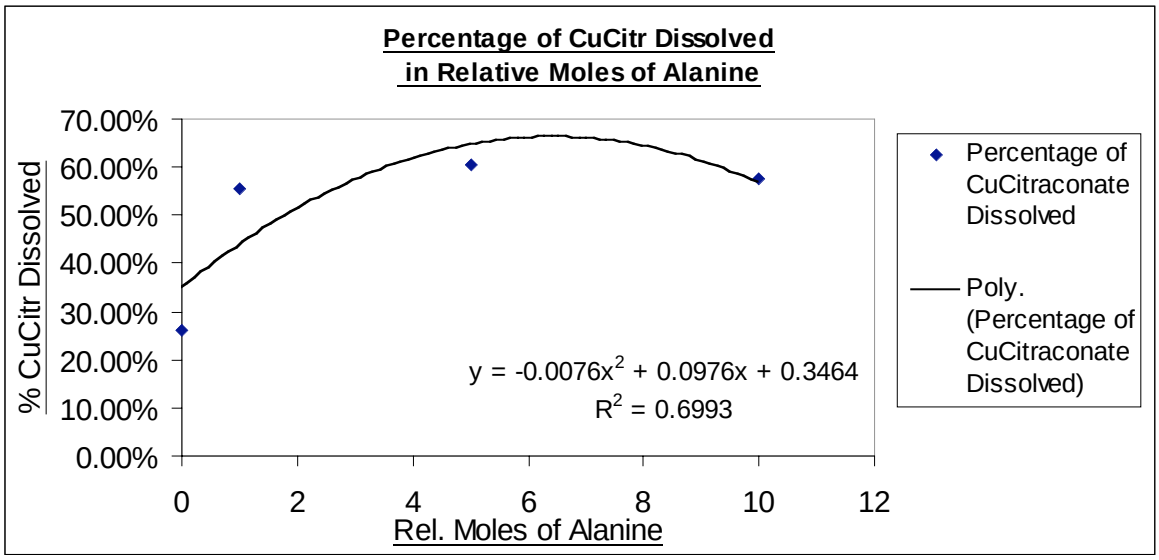
Graph 1 Percentage of Zinc titrated in relative moles of glycine.



Zinc Citraconate and Alanine

Adding alanine gradually increases the solubility of copper (II) citraconate. The maximum solubility of 60% was seen at alanine to mixed zinc salt molar ratio of 5:1. (Graph 2) Composite K_{sp} of mixed salt is 1.47×10^{-4}. Alanine is less polar than glycine, and serine is more polar that glycine. The order in solubilites is not clear, which is

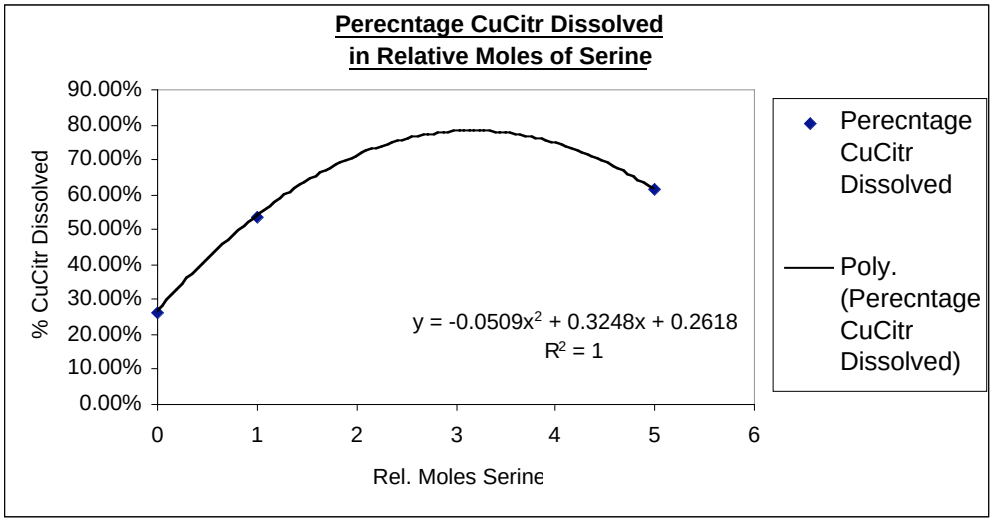
possibly due to impure starting material, using the mixed copper salt instead of pure copper (II) citraconate.



Graph 2 Percentage of zinc titrated in relative moles of alanine.

Zinc Citraconate and Serine

Adding serine increases the solubility of copper (II) citraconate. The maximum solubility of 61% was seen at a serine to total zinc molar ratio of 5:1. (Graph 3)



Graph 3 Percentage of zinc titrated in relative moles of serine.

Theoretical Information About the System Studied

Equilibrium	pK	Symbol
$H_2citr \Leftrightarrow H^+ + Hcitr^-$ (HL)	2.23	K_{citr1}
$Hcitr^- \Leftrightarrow H^+ + citr^{-2}$ (L)	5.46	K_{citr2}
$H_2gly^+ \Leftrightarrow H^+ +$ Hgly (zwitterion) (HL')	2.34	K_{gly2}
Hgly $\Leftrightarrow H^+ + gly^-$ (L')	9.60	K_{gly3}
	logK	
$Zn^{+2} + HL \Leftrightarrow ZnHL$	*To be determined*	K_{HL}
$Zn^{+2} + L \Leftrightarrow ZnL$	1.8	K_{L1}
$Zn^{+2} + HL' \Leftrightarrow ZnHL'$	0.12	$K_{HL'}$
$Zn^{+2} + L' \Leftrightarrow ZnL'$	4.90†	$K_{L'1}$
$ZnL' + L' \Leftrightarrow ZnL'_2$	9.01†	$K_{L'2}$
Other Possible Equilibria		
(Citr → gly) Ligand Exchange		
$ZnL + L' \Leftrightarrow ZnL' + L$	3.10†*	
$ZnHL + HL' \Leftrightarrow ZnHL' + HL$	*To be determined*	
$ZnL + HL' \Leftrightarrow ZnHL' + L$	*To be determined*	
Glycine Addition to Zinc Citraconate Complexes		
$ZnHL + HL' \Leftrightarrow Zn(HL)HL'$	*To be determined*	
$ZnL + HL' \Leftrightarrow Zn(L)HL'$	*To be determined*	
$ZnL + L' \Leftrightarrow ZnLL'$	*To be determined*	

Table 3 List of equilibria with unknown and known constants of the major species in solution at pH 5.5.
† May not be important at pH 5.5, [L'] = 0.0132% of total [glycine]
Not important: K < 1
L= Citraconate
HL' = Glycine (zwitterion)

Table 3 lists all known equilibrium constants that are important for this system obtained from the NIST database. First of all, the acid ionization constants for the ligands are important because of the form of the ligand (neutral, monoanion, dianion, zwetterion, etc.) present at pH 5.5 indicates which zinc ion complexes will dominate in solution at

that pH. As can be seen form Table 3, the first ionizations of citraconic acid and protonated glycine are similar, pK = 2.23 and 2.34, respectively. The second ionization of citraconic acid is about the same as the pH used for titration. As can be seen in Graph 4, the dominant species of citraconic acid at pH 5.5 is expected to be Hcitr-. Similarly, a speciation diagram of glycine vs. pH indicates that the dominant form at pH 5.5 is the zwitterion: $^{+}H_3NCH_2COO^{-}$. Therefore, these are considered to be that most important ligands, Hcitr- and $^{+}H_3NCH_2COO^{-}$, for zinc ions at pH 5.5. Interestingly, the equilibrium constant for formation of $Zn(Hcitr)^{+}$ is not known, and remains a future goal of this research.

<u>Modeling the Zinc Citraconate : Glycine System</u>

Total Metal Ion Concentration (ignoring mixed ligand complexes)

$$T_M = [Zn] + [ZnH_2L] + [ZnHL] + [ZnL] + [ZnHL']$$

$$T_M = [Zn]\left(1 + \frac{K_{H_2L}}{K_{Suc_1}K_{Suc_2}}[H]^2[L] + \frac{K_{HL}}{K_{Suc_2}}[H][L] + K_L[L] + K_{HL'}[HL']\right)$$

Figure 1 All important possible metal ion forms found in solution.

Total Ligand Concentration (ignoring mixed ligand complexes)

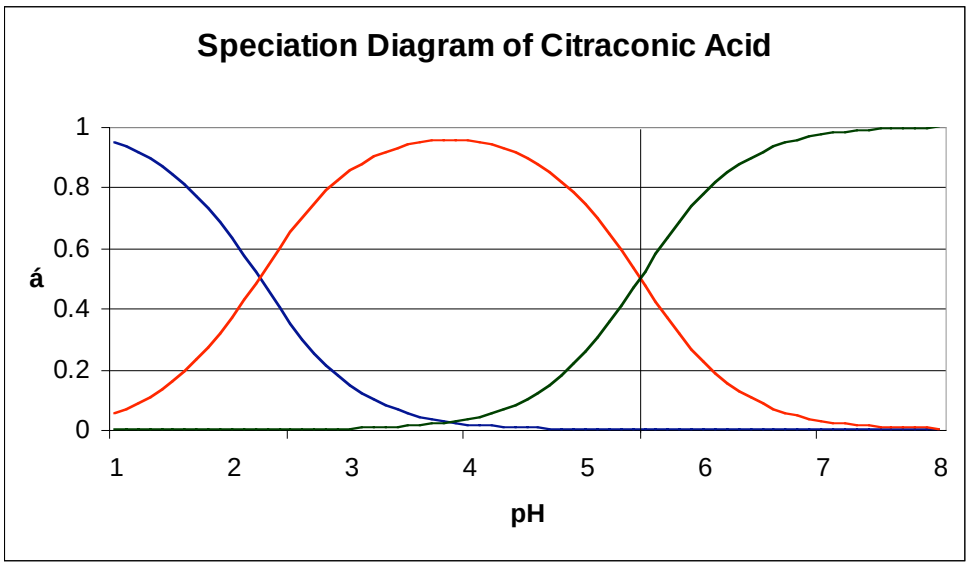
$$T_L = [H_2L] + [HL] + [L] + [ZnHL] + [ZnL]$$

$$T_L = [L]\left(1 + \frac{1}{K_{Suc_1}K_{Suc_2}}[H]^2 + \frac{1}{K_{Suc_2}}[H] + \frac{K_{HL}}{K_{Suc_2}}[H][Zn] + K_L[Zn]\right)$$

Figure 2 All important possible ligand forms in solution.

Figures 1 and 2 show the equations solved for total metal ion content (Figure 1) and total ligand content (Figure 2) based on consideration of all possible simultaneously occurring equilibria. Both equations are expressed in terms of the minimum variables

necessary. The variables are T_M (total Zn^{2+} calculated from titrations) and T_L (deduced from T_M stoichiometrically), [H] (calculated from pH 5.5), [Zn] (concentration of free Zn^{2-}) and [L] (concentration of $citr^{2-}$, deduced from speciation diagram). For complete solution of these equations, determination of free [Zn^{2+}] via ion specific electrode titration is necessary. This is another future goal of this research. If the equations exactly match the experimental data, then we will know that mixed ligand complexes are not important in solution. If there is a discrepancy between theory and experiment, then we can suspect that mixed ligand complexes are important.



Graph 4 The percent distribution of citraconic acid at different pH levels. Legend: blue = H_2citr, red = $Hcitr^-$, green = $citr^{-2}$

Conclusion

Calculations were done to find the percent copper (II) citraconate dissolved in each amino acid (glycine, alanine and serine) molar ratio (Graphs 1-3). Speciation diagrams of citraconic acid and glycine were calculated using known equilibrium

constants (Table 3). The enhancement of solubility cannot solely be due to the protonation of salt anion by free amino acid; pK's of anions and amino acids are comparable.

Since the percent yield was low during synthesis of copper (II) citraconate, several different samples were made over the time period of this research. The attempted synthesis of copper (II) citraconate was partially successful. Several IR's were taken at different times when copper (II) citraconate was synthesized, which showed the presence of citraconic acid. Samples of copper (II) citraconate were ashed and the results were used to calculate the percent copper found in the copper (II) citraconate sample. The calculation showed that 48.29% copper was present. This was compared to the theoretical calculations that showed 33.16% of copper should be present.

Copper dissolved did show general trends seen in-group previously. The percent of copper dissolved increased as amino acid ratio increased. The addition of amino acid dramatically enhances solubility of copper (II) citraconate prepared. Titration results indicate some promising trends. Solutions prepared with glycine, serine and alanine show a steady increase in salt solubility as amino acid is increased.

Future Work

In the near future, a new method will be created to synthesis pure zinc citraconate and copper (II) citraconate. A complete analysis of the pure zinc citraconate and copper (II) citraconate salt will be done before completing all titrations with each amino acid, glycine, alanine and serine. It is expected that the percent zinc and percent copper dissolved will increase as amino acid ratio increases. Using an ion specific electrode, free

[Zn^{2+}] will be measured in all solutions. It is expected that we will see an increase in free [Zn^{2+}] as the amino acid and zinc salt ratio increases.

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