

FAST, COMPLETE COORDINATION OF NUTRITIONAL-BASED METAL CHELATES THROUGH LIQUID-ASSISTED GRINDING

Brayden N. Thornton*, Jantz Arbon*, Connor W. Pack*, Brandon J. Burnett†

Department of Chemistry and Biochemistry, College of Science, Weber State University, Ogden, UT 84408

Abstract

We present the coordination of nutritional-based metal chelates through a Liquid-Assisted Grinding (LAG) approach as a function of water stoichiometry and grinding time in order to find the most optimized conditions. We focused on nutritional-based samples including chelates made from zinc, magnesium, and calcium coordinated to citric acid and malic acid. We also compared the effect on chelation based on relative solubility of mineral salt using zinc oxide versus zinc carbonate coordinating with citric acid. This is the first investigation into the efficiency of LAG into these industrially important mineral chelates.

*Corresponding author: brandonburnett@weber.edu

*Undergraduate research co-authors

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Introduction

Mineral chelates, or minerals coordinated to polydentate organic acids, signify a substantial market in the nutritional supplement and agriculture industries.¹ Compared to simple inorganic mineral oxides or carbonates, mineral chelates are more readily adsorbed by living systems.^{1,2} The traditional solvothermal synthesis for such industrial mineral chelates involves dissolution of the mineral salt and organic acid in water to react, followed by separation of the product chelate from the reaction mixture.

A liquid-assisted grinding (LAG) approach has been recently explored for creating coordination-based chemicals.³ LAG-based syntheses involve mechanically grinding reactants with a stoichiometric amount of liquid solvent to aid in molecular mobility.^{3,4} The mechanical energy is used to drive the reaction, instead of thermal energy transfer. This strategy has become attractive due to decrease reaction times and increased yields compared to traditional solvothermal reactions.³⁻⁶ For example, Garci et al. developed a grinding method to synthesize platinum squares from K_2PtCl_4 and chelating agents resulting in high yields within hours, compared to the traditional synthetic route which took longer and produced only 35% yield overall.⁵ Furthermore, this technology is completely scalable, from lab scale to production scale.⁷

To date, this approach has not been extended to nutritional or agricultural applications. We hypothesized that LAG could benefit these industries by reducing the reaction time and simplifying the post-reaction workup. We investigated the effect of water stoichiometry and grinding time on the percent chelation of chelates created from zinc oxide, magnesium oxide, and calcium oxide with citric acid and malic acid, as analyzed by Fourier transform infrared/attenuated total reflectance (FTIR/ATR) spectroscopy.

In this communication, we report our efforts in finding the minimum amount of time and water needed to produce fully chelated samples. To our knowledge, this is the first investigation into the efficiency of LAG into industrially important mineral chelates. Additionally, we report the effect of mineral salt solubility on the amount of water needed for complete chelation using LAG synthetic strategy.

Experimental Methods

Materials – Research grade zinc oxide, zinc carbonate, magnesium oxide, calcium oxide, citric acid, malic acid, and sodium dicyanamide were all obtained from commercial sources and used without further purification.

Instrumentation – All liquid-assisted grinding (LAG) syntheses were performed on a Retsch Mixer Mill MM400. Fourier transform infrared/attenuated total reflectance (FTIR/ATR) spectroscopy was performed on a Nicolet iS50. 40 scans, with background correction between each sample was performed on each sample. The ATR function of this instrument was important as we needed FTIR information without dissolving the samples in water first.

LAG Synthesis of Mineral Chelates – Triplicate samples were synthesized and followed the same general synthetic procedure. A stoichiometric amount of mineral salt and organic acid were introduced into a 2-mL polypropylene reaction vial along with a stainless-steel ball (3-mm diameter). Varied amounts of water were added, and the mixture was ground in the ball mill at a frequency of 30 Hz for varied amounts of time. After the designated reaction time, the samples were dried in a desiccating oven at 40°C to dry off any residual water. The analysis of chelation followed the published method.⁶ An equal mass amount of sodium dicyanamide was then added to the dried products as an internal standard. The mixture was then shaken again in the ball mill for 2 minutes at 20 Hz to achieve homogeneity and stored under desiccation until analyzed. The samples were then analyzed by FTIR/ATR.

Synthesis of Zinc Citrate (Zinc Oxide with Citric Acid) – ZnO (24.4 mg, 0.300 mmol), and citric acid (38.4 mg, 0.200 mmol) were added to a 2-mL polypropylene reaction vial along with distilled water ranging from 0 – 25 μ L (0 – 1.39 mmol) and a stainless-steel ball. The resulting mixture was ground for 1-10 min, and then dried overnight. Sodium dicyanamide (62.8 mg, 0.706 mmol) was added to the dried sample and ground again to homogeneously mix and stored under desiccation until analyzed.

Synthesis of Zinc Citrate (Zinc Carbonate with Citric Acid) – $ZnCO_3$ (37.6 mg, 0.300 mmol), and citric acid (38.4 mg, 0.200 mmol) were added to a 2-mL polypropylene reaction vial along

with distilled water ranging from 0 – 25 μL (0 – 1.39 mmol) and a stainless-steel ball. The resulting mixture was ground for 1-10 min, and then dried overnight. Sodium dicyanamide (62.8 mg, 0.706 mmol) was added to the dried sample and ground again to homogeneously mix and stored under desiccation until analyzed. It should be noted that a small internal pressure in the reaction vial was observed post-grinding, created by the release of CO_2 during the reaction.

Synthesis of Zinc Malate (Zinc Oxide with Malic Acid) – ZnO (27.1 mg, 0.333 mmol), and malic acid (44.7 mg, 0.333 mmol) were added to a 2-mL polypropylene reaction vial along with distilled water ranging from 0 – 25 μL (0 – 1.39 mmol) and a stainless-steel ball. The resulting mixture was ground for 1 – 10 min, and then dried overnight. Sodium dicyanamide (71.8 mg, 0.806 mmol) was added to the dried sample and ground again to homogeneously mix and stored under desiccation until analyzed.

Synthesis of Magnesium Citrate (Magnesium Oxide with Citric Acid) – MgO (12.1 mg, 0.300 mmol) and citric acid (38.4 mg, 0.200 mmol) were added to a 2-mL polypropylene reaction vial along with distilled water ranging from 0 – 25 μL (0 – 1.39 mmol) and a stainless-steel ball. The resulting mixture was ground for 1 – 10 min, and then dried overnight. Sodium dicyanamide (50.5 mg, 0.567 mmol) was added to the dried sample and ground again to homogeneously mix and stored under desiccation until analyzed.

Synthesis of Magnesium Malate (Magnesium Oxide with Malic Acid) – MgO (23.5 mg, 0.333 mmol) and malic acid (44.7 mg, 0.333 mmol) were added to a 2-mL polypropylene reaction vial along with distilled water ranging from 0 – 25 μL (0 – 1.39 mmol) and a stainless-steel ball. The resulting mixture was ground for 1 – 10 min, and then dried overnight. Sodium dicyanamide (58.2 mg, 0.284 mmol) was added to the dried sample and ground again to homogeneously mix and stored under desiccation until analyzed.

Synthesis of Calcium Citrate (Calcium Oxide with Citric Acid) – CaO (16.8 mg, 0.300 mmol) and citric acid (38.4 mg, 0.200 mmol) were added to a 2-mL polypropylene reaction vial along with distilled water ranging from 0 – 25 μL (0 – 1.39 mmol) and a stainless-steel ball. The resulting mixture was ground for 1 – 10 min, and then dried overnight. Sodium dicyanamide (55.2 mg, 0.806 mmol) was added to the dried sample and ground again to homogeneously mix and stored under desiccation until analyzed.

Calcium Malate (Calcium Oxide with Malic Acid) – CaO (18.7 mg, 0.333 mmol) and malic acid (44.7 mg, 0.333 mmol) were added to a 2-mL polypropylene reaction vial along with distilled water ranging from 0 – 25 μL (0 – 1.39 mmol) and a stainless-steel ball. The resulting mixture was ground for 1 – 10 min, and then dried overnight. Sodium dicyanamide (63.4 mg, 0.926 mmol) was added to the dried sample and ground again to homogeneously mix and stored under desiccation until analyzed.

Traditional Synthesis of Zinc Citrate (Zinc Oxide with Citric Acid) – ZnO (24.4 mg, 0.300 mmol), and citric acid (38.4 mg, 0.200 mmol) were added to a 5-dram capped glass vial along with 3.00 mL distilled water and a magnetic stir bar. The resulting mixture was stirred on a magnetic stirrer at room temperature for until reaction completion (about 45 minutes, as observed visually by the

disappearance of solid ZnO in the solution). Liquid aliquots of the sample were taken at 35, 40, 45 minutes and analyzed by FTIR/ATR to confirm that over 99% chelation was achieved at 45 minutes of stirring. Complete samples were dried for 48 hours in a 40°C desiccating oven. An identical sample was heated at 80°C in a water bath while stirring via magnetic stirrer until reaction completion (about 25 minutes, as observed visually by the disappearance of solid ZnO in solution). Again, liquid aliquots of the sample were taken at 15, 20, and 25 minutes and analyzed by FTIR/ATR to confirm that over 99% chelation was achieved at 25 minutes of stirring. Complete samples were dried for 48 hours in a 40°C desiccating oven.

Analysis by FTIR/ATR – For each mineral chelate sample studied by LAG synthesis, triplicate analyses were performed and the integrated signal for the carbonyl stretch of free acid (1637 cm^{-1} – 1776 cm^{-1}) was compared to the integrated signal of the mineral chelate (1518 cm^{-1} – 1637 cm^{-1}) according to the published method.⁸ Sodium dicyanamide was used because of its unique absorbance at 2228 cm^{-1} which lies well outside of the region of interest of the mineral chelates, and does not interact with the dried chelate. The integrated signal of the free acid and mineral chelate were divided by the integrated signal of the sodium dicyanamide to normalize any spectral differences and allow independent determination of both free acid and mineral chelate. All of the data for each sample (nine total data points for each sample) were then averaged for further analysis.

Results and Discussion

A representative overlay of spectra showing the results of LAG synthesis at 1 minute of milling with different equivalents of water used in a zinc oxide and citric acid reaction is shown in Figure 1a. As additional amounts of water were added at the same time interval, the signal due to free acid decreased as the signal for the mineral chelate increased until there was essentially no free acid signal left. Figure 1b shows a representative overlay of spectra showing different time intervals of grinding with the same ~1 molar equivalent of water in the same zinc oxide and citric acid reaction. Similar to Figure 1a, as time is increased, as long as there was sufficient water, the signal for the mineral chelate increased and the signal of the free acid decreased until there was essentially no free acid signal left.

Figure 2 shows the % chelation of the six different mineral chelate systems we investigated as a function of reaction time and stoichiometric equivalent of water used. We chose zinc, magnesium, and calcium oxides as our mineral salts, and citric acid and malic acid as our chelating agents, as the resulting chelates are nutritionally and agriculturally important.⁸ It's important to note that some amount of added water was important for the chelation reaction to proceed, as dry samples never produced detectable amounts of chelated products, even at extended grinding times. Upon the addition of around 0.2 molar equivalent of water into the reaction, mineral chelate began to be produced, and extending the amount of grinding time increased the yield. Additionally, as more than 1 molar equivalent of water was added into the reaction, the amount of time needed to produce products decreased.

The optimal reaction setups for LAG synthesis of all six min-

eral chelates are summarized in Table 1. This table shows a relative minimum of grinding time and molar equivalent of water to achieve over 99% chelation in a small, lab scale setup. While the amount of time needed to achieve 99% chelation for all samples were similar, the magnesium and calcium needed less water compared to zinc. This may be due to more favorable Lewis acid-base interactions between the hard-base oxygen and the hard-acid magnesium and calcium over the intermediate-acid zinc.⁹ For zinc citrate synthesized from zinc oxide and citric acid, optimal chelation was achieved with 5 minutes of grind time and 3.5 molar equivalents of water. As a reference, it took about 45 minutes for complete chelation when we synthesized zinc citrate from zinc oxide and citric acid using a more traditional setup at room temperature. Complete chelation using the traditional setup could only be sped up to about 25 minutes by heating the sample in a 70°C water bath while stirring. This is consistent with literature values of traditional synthesis times.¹⁰ It should also be noted that the drying time of the LAG synthesis is significantly faster than that of the traditional synthesis.

We also wanted to investigate how the relative solubility of

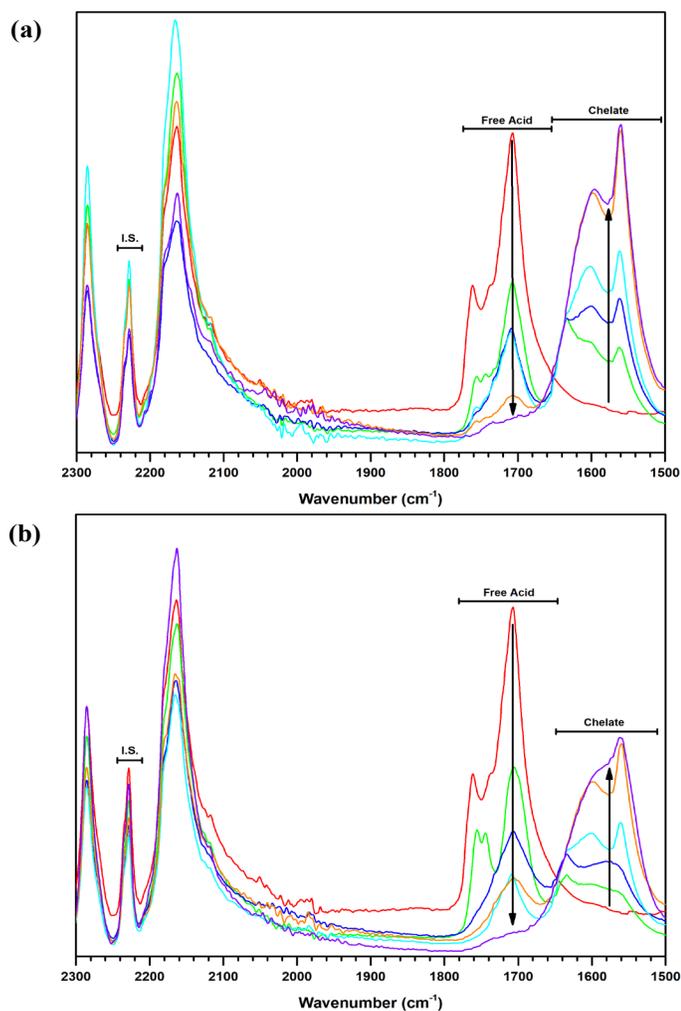


Figure 1 Overlaid FTIR-ATR spectra of zinc citrate chelate LAG reaction. FTIR-ATR spectra of the results of the chelate LAG reaction between zinc oxide and citric acid performed (a) at one minute with different equivalents of water ranging from 0 equivalents to 4.5 equivalents and (b) different reaction times ranging from 0 minutes to 15 minutes with 1 equivalent of water.

the mineral salt would affect the effectiveness of LAG chelation. Zinc oxide has a KSP value of 4×10^{-40} , whereas zinc carbonate has a KSP value of 9.1×10^{-50} .¹¹ Figure 3 shows the % chelation of zinc citrate using zinc carbonate and citric acid. The optimal reaction setup for LAG synthesis of zinc citrate from zinc carbonate was achieved at 10 minutes and 4.5 molar equivalents of water. It consistently took increased amounts of water at each time interval for the zinc carbonate samples to achieve above 99% chelation compared to the zinc oxide samples.

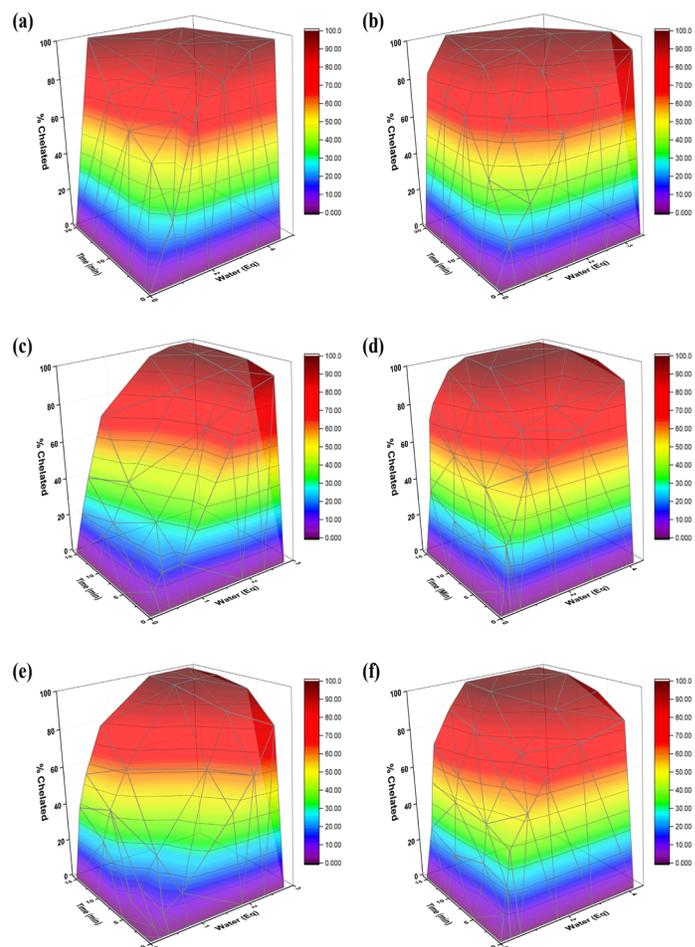


Figure 2 %Chelation Results of Mineral Chelates through LAG Synthetic Strategy.

Results of the % chelation from the LAG synthesis from zinc oxide, magnesium oxide, or calcium oxide with either citric acid or malic acid to create (a) zinc citrate, (b) zinc malate, (c) magnesium citrate, (d) magnesium malate, (e) calcium citrate, and (f) calcium malate.

Table 1. Optimal Reaction Setups for LAG Synthesis of Mineral Chelates from Metal Oxides*

Sample	Grinding Time (min)	Water (molar eq.)
Zinc Citrate	5	3.5
Zinc Malate	10	2.5
Magnesium Citrate	5	2.5
Magnesium Malate	10	1.5
Calcium Citrate	10	2.0
Calcium Malate	10	1.5

* Optimal Reaction Setup is defined as a relative minimum of grinding time and molar equivalent of water to achieve over 99% chelation

Conclusions

In conclusion, we report the use of a LAG strategy to obtain nutritionally and agriculturally important chelates using stoichiometric amounts of water. All reactions were performed at room temperature, and produced products faster than the traditional solution-based reaction. Additionally, the dry time after reaction was significantly reduced by the LAG synthetic strategy over a traditional route. Finally, we found that a mineral salt reactant with higher KSP needs less water to chelate than a corresponding mineral salt reactant with lower KSP, highlighting the importance of reactant interactions with water during the process. While only applied on the lab scale, we believe that these results could be scaled up to production scale and have the potential of saving time and money for nutritional and agricultural industries. These changes address several green chemistry principles outlined by the American Chemical Society, and help move toward a more sustainable future for this type of chemistry.¹²

Acknowledgment

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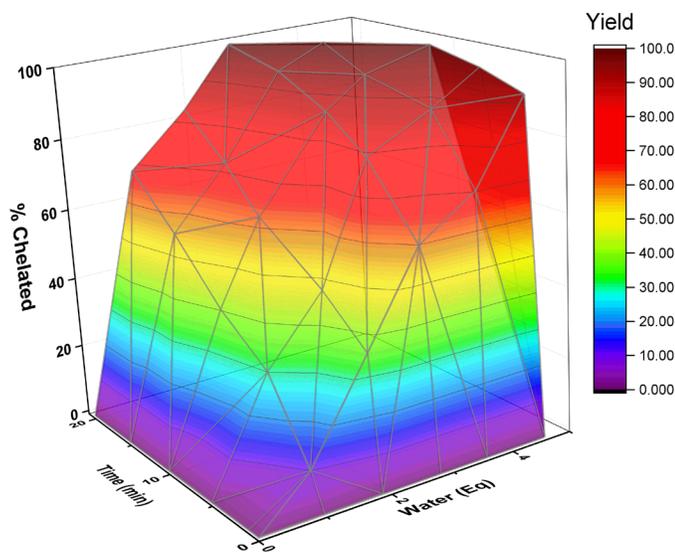


Figure 3 %Chelation Results of Zinc Citrate from Zinc Carbonate through LAG Synthetic Strategy.

Results of the % chelation from the LAG synthesis from zinc carbonate and citric acid to create zinc citrate.

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