Real Time Kinetic Reaction Rate of Zinc Bisglycinate Determined by Use of FT-Raman Spectroscopy

Clayton Ericson, R. Charles Thompson
Albion Advanced Nutrition, Clearfield, Utah USA

Abstract

Determining reaction kinetics for any chemical reaction can be challenging. For zinc bisglycinate, reaction kinetics is especially challenging, because it has no known chromophore. We have created a simple method to determine kinetic reaction rates by utilizing FT-Raman technology. We used a Nicolet FT-Raman module with a high energy Nd: YVO_4 laser giving us a range of power settings from 0.5 to 2.0 watts to generate Raman scatter. In an aqueous environment the laser's energy accelerates the reaction of zinc oxide and glycine in their proper molar ratios. Using the FT-Raman spectrophotometer to determine reaction bisglycinate reaction kinetics, we collected data at 15 second time intervals after initiating the reaction. Changes in several frequencies is associated with structure are monitored over the set time interval, which allows us to model the reaction kinetics. Intensity or peak height of a selected frequency monitored over time can be graphed, and the rate order of the reaction then may be determined. This method could provide a useful technique to model reaction kinetics for other compounds.
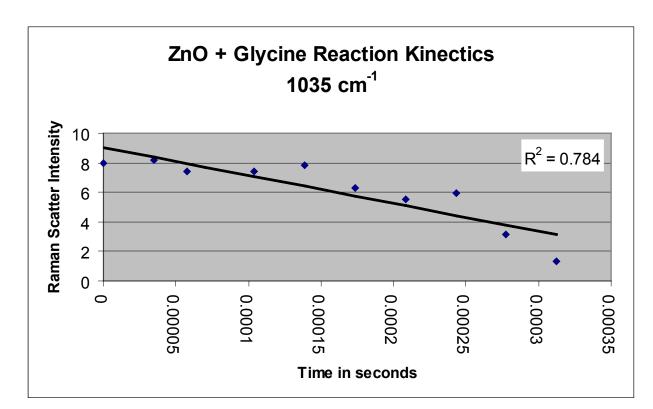
Introduction

With the advancement of new health technologies and the desire for healthier life styles, a much larger selection of nutritional supplements are being sold to benefit health conscious consumers. Proposed regulatory guidelines for validating nutritional supplements have many nutrition supplement manufacturers rushing to get processes and methods in place.
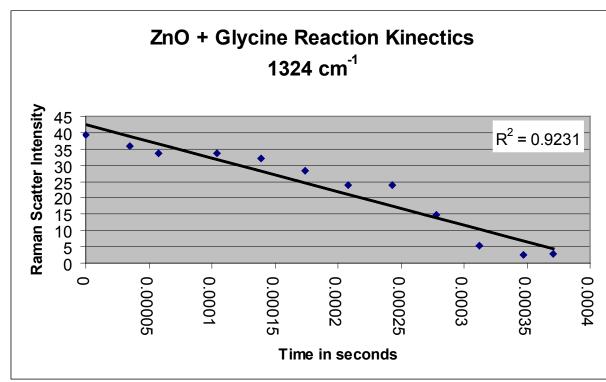
Mineral chelation is one area of on-going research where work is being done to authenticate the proof of chelation and to validate methods for quantification[1,2]. One supplement, zinc bisglycinate chelate, manufactured by Albion Advanced Nutrition has benefited consumers with a better zinc supplement by providing a valid proof of the chelate's structure[1,2]. Many years of clinical trials, research, and product improvements have resulted in the submission of articles for peer reviewed journal publications that demonstrate an increased bioavailability with respect to regular inorganic zinc sources[3].
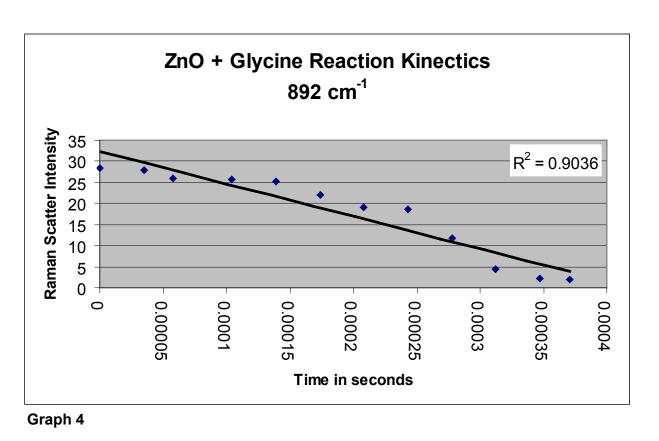
The manufacturing of mineral chelates like zinc bisglycinate has always been difficult. One reason for this is the difficulty in quantifying the chelated product during the synthesis process while in a liquid state. One current proof of chelation for zinc bis-glycinate uses FT-IR spectroscopy, where the sample must be in a powder or a crystalline state. Other methods such as UV/Visible spectrophotometry are difficult because zinc bisglycinate does not have a suitable chromophore for UV/Visible detection. For this reason we are developing a new method using FT-Raman technology to measure the chelation process. Raman spectroscopy has certain advantages over infrared. One is the simpler spectra usually observed because of reduced overtone or combination bands, which are an order of magnitude weaker in the Raman than in the infrared. These overtones and combinations are usually too weak to be observed. A second advantage is the ability to use a wider choice of solvents for solution studies. In particular, water can be used, as well as other solvents that have more clear regions in the Raman than in the infrared.
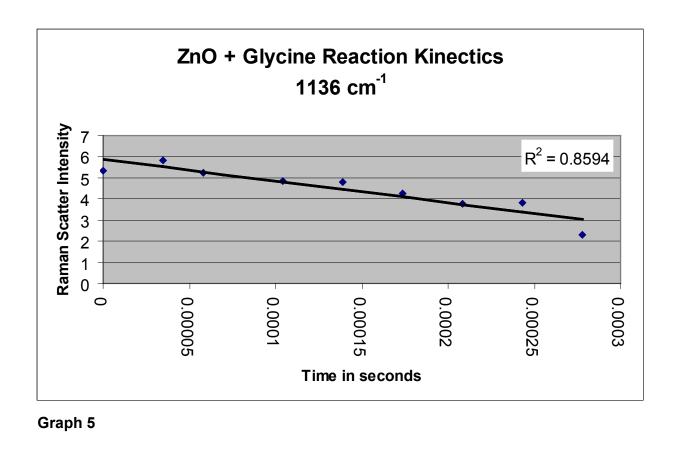
The FT-Raman can measure the intensity of bonds formed during real time reactions, with the corresponding disappearance of the original band assignments of the reactants. FT-Raman makes it possible to monitor reaction kinetics by selecting important structural frequencies and measuring intensity and change of each frequency throughout a controlled reaction.

Our kinetic reaction model was designed to be carried out in a small glass tube that can be placed into the FT-Raman sample holder. The contents in the glass tube can therefore be monitored by gathering a Raman spectrum every 4 seconds during the reaction procedure. The intensities or peak heights of each significant frequency are extrapolated from the FT-Raman scans, and the changes in these intensities are graphically plotted versus time. Mathematical models such as linear regression can then be used to fit the curve and determine the overall rate order for the reaction kinetics.
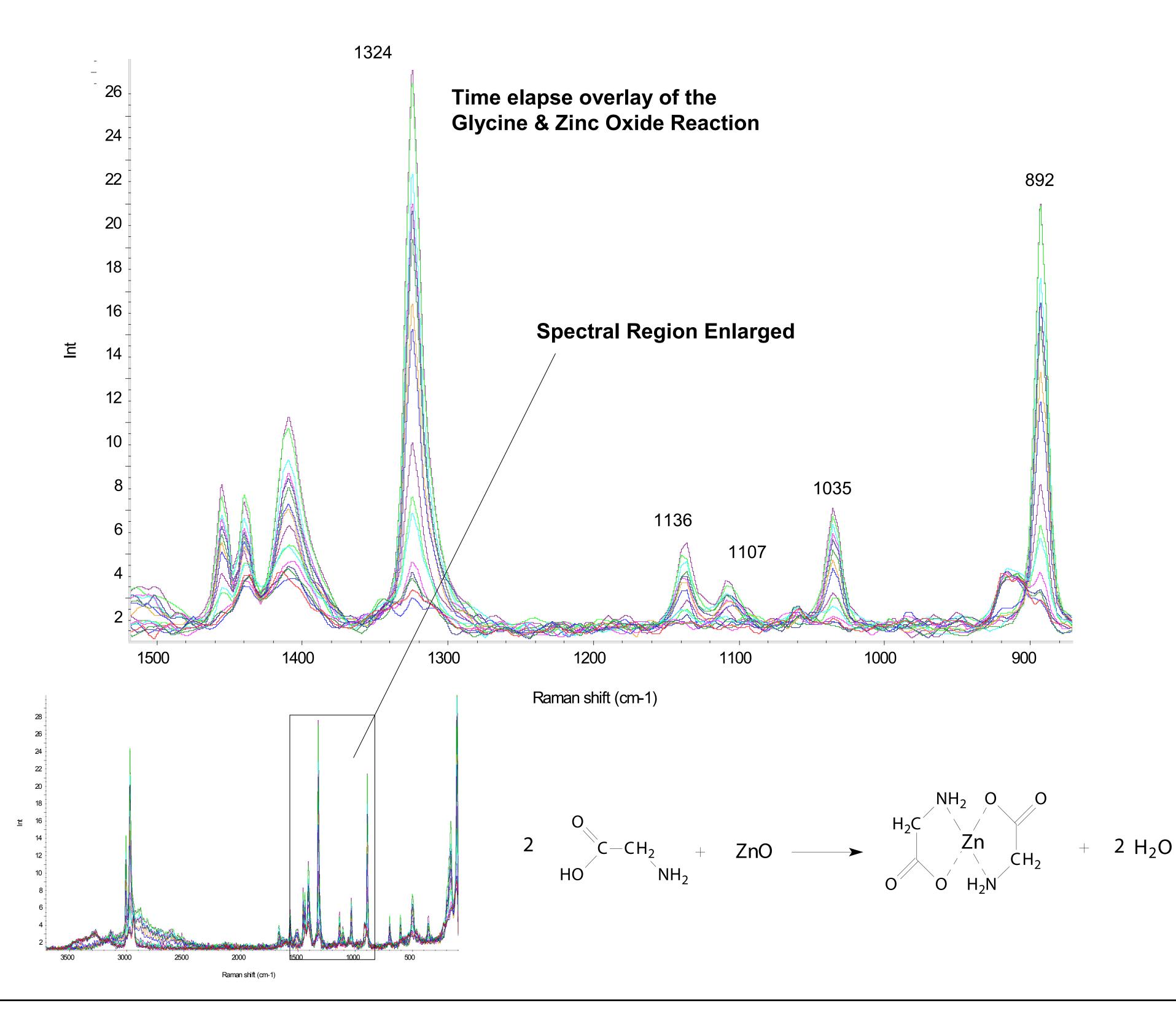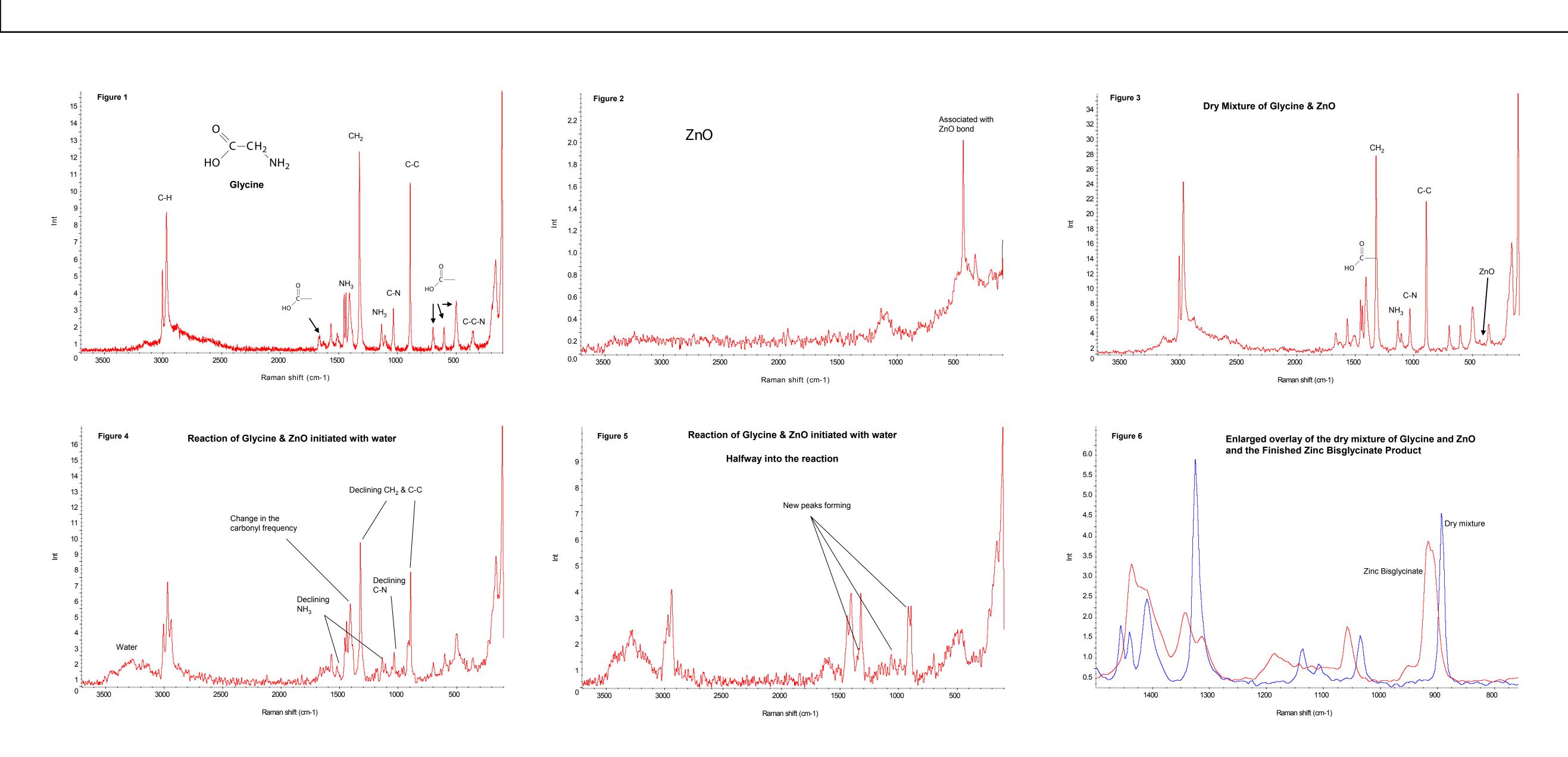
Procedure

The reaction kinetics of zinc oxide, glycine, and water were monitored with a Raman module as part of a Thermo Nicolet FT/IR 6100 optics bench. The reaction mixture was stoichiometricaly balanced to model the above reaction.

One mole of zinc oxide, dry mixed with two moles of glycine (Gly) was thoroughly ground and mixed using a mortar and pestle. We then took 60mg of the ZnO/Gly dry-mix and placed it into a 5mm x 25mm glass tube. 35mg of anhydrous sodium sulfite was placed on top of the ZnO/Gly mixture as a wick to provide a delay in the initial reaction slowing the flow of water into the reaction mixture. The Raman spectrometer was then started and 35 μl of water added to the ZnO/Gly mixture. A Raman spectrum was then gathered every 4 seconds for 60 seconds from 4000cm^{-1} to 100cm^{-1}. A total of fifteen Raman spectra were obtained over the specified spectral range in a one minute time frame.

We then plotted the peak height with respect to time for five peaks, which were associated with previously identified functional groups in the Raman spectrum of glycine (Figure 1). We also determined that there were no substantial peaks associated with the zinc oxide or with sodium sulfite in this spectral range (Figure 3). With the spectral changes of the chosen peaks plotted with respect to time, we then attempted to determine rate constant and reaction order of the reaction with respect to the chosen peaks and functional groups associated with them.

Results

Glycine constitutes 70% of zinc bisglycinate. FT-Raman scans of glycine, zinc oxide, and dry mixtures containing both were gathered to determine and identify which FT Raman peaks make up the backbone of glycine, as well as, to correlate the transition of frequencies during a reaction of zinc oxide with glycine[4,5,6].

The scan of glycine in figure 1 has several areas showing backbone structure across the scan. The fingerprint region contained the peaks of most interest particularly the area from 1500 cm^{-1} to 800 cm^{-1}. The peak 1409 cm^{-1} has been identified as being a carbonyl (O-C-O) symmetric stretch[4]. 1324 cm^{-1} is associated with a carbon-methylene (C-CH$_2$) wag[4]. A band assignment at 1137 cm^{-1} has been identified as an amine (NH$_3$) deformation[4]. The peak at 1035 cm^{-1} is believed to be the carbon-nitrogen (C-N) stretch[4,5]. And lastly, peak 892 cm^{-1} is a carbon-carbon (C-C) stretch[4].

Figure 2 shows FT-Raman scan of ZnO. It is believed that the peak at 438 cm^{-1} is the bond stretch between zinc and oxygen. It is also believed that most metal to oxygen bonds are located in that same region from 500 cm^{-1} to 300 cm^{-1}(1). Figure 3 shows the dry mix of glycine and zinc oxide. The small peak at 438 cm^{-1} is zinc oxide. Since zinc only makes up 30 % of the mixture, it is difficult to monitor this peak during the reaction step.

When water is introduced to the sample, the energy from the laser causes the two powders to react with each other. One problem we encountered was a weak frequency signal caused when the dry powder mixed with water. We had to be careful to limit the amount of water going into the system by using a micropipette which controlled the amount of water introduced into the reaction. Using small amounts of water in our reactions keeps the concentration ratio of reactants to water high, but even at higher concentration the signal is still weakened when the water passed through the laser's energy beam.

In figure 4, water passing through the area where the laser's beam is projected initiates a full reaction. The carbonyl frequency begins to shift as would be expected when zinc binds to the carbonyl oxygen. Further indication of chelation is the disappearance of the amine deformation peaks showing that the nitrogen is binding to zinc by a coordinate covalent bond. And last, the carbon-carbon, carbon-nitrogen frequencies of the glycine backbone disappear.

In figure 5, the reaction nears the half way point where clearly new peaks begin to appear. One peak at 1354 cm^{-1} is believed to be a carbon-methylene frequency. The peak at 1063 cm^{-1} is believed to be the carbon-nitrogen bond. And the peak at 915 cm^{-1} is believed to be the carbon-carbon bond.

Figure 6 is a spectral overlay of the dry mixture of glycine and zinc oxide, and a dry zinc bisglycinate standard. Figure 6 dramatically shows the spectral differences as reactants appeared before the reaction, and what changes occurred in the finished product after the reaction.

In an attempt to mathematically model a kinetic reaction rate, we used the FT-Raman software to setup a program that would take one scan every four seconds. An overlay of these scans from start to finish resulted in a dramatic presentation as shown in center of this poster. Peaks at 1324 cm^{-1}, 1136 cm^{-1}, 1107 cm^{-1}, 1035 cm^{-1}, and 892 cm^{-1} begin gradually diminishing until they disappear. New frequencies are shown to appear at 1063 cm^{-1}, 915 cm^{-1}, and other locations in the spectrum that are not in the enlarged view.

Computing kinetic reaction rates requires all peak height intensities from 1324 cm^{-1}, 1136 cm^{-1}, 1107 cm^{-1}, 1035 cm^{-1}, and 892 cm^{-1}, shown in the enlarged region of the middle graph, to be graphed with respect to time with a linear regression analysis.

Graphs 1 through 5 are linear plots of time versus peak height intensity of the identified peaks obtained from each scan taken over the course of the entire modeled reaction. R-squared values were calculated and reported accordingly; 0.9231 at 1324 cm^{-1}, 0.8594 at 1136 cm^{-1}, 0.6533 at 1107 cm^{-1}, 0.7840 at 1035 cm^{-1}, and 0.9036 at 892 cm^{-1}.

The outcomes of the R-squared results do not appear to fit a first order kinetic rate because of the linear fit of the curves. When the data was calculated with a model second order kinetic rate, R-squared values did not improve. Close examination of the curves exhibit different results for each frequency or different part of the amino acid. However, there are striking resemblances when comparing the curves among themselves. For example, peak 1324 cm^{-1} which is the carbon-methylene wag has nearly the same slope as the 892 cm^{-1} carbon-carbon stretch. Within the molecule, the carbon-carbon bonds are not exchanging electrons and forming new bonds as the nitrogen or the oxygen ends of the molecule. A real change or difference comes about when the plotted curves of the carbonyl (O-C-O), amine (NH$_3$), and the carbon-nitrogen (C-N) peaks are compared to each other. The peaks disappear in what appears to be a stepwise trend. These peaks do not follow second order kinetic rates, but it has been suggested they may follow zero order.

We have some difficulty with getting good reproducibility of the curves from experiment to experiment, however, peaks at 1324 cm^{-1} and 892 cm^{-1} seem to be most reproducible. We think that the difficulty in reproducibility is caused by the water addition in the procedures. We also had difficulties in fitting a mathematical equation to the kinetic reaction curves; however, our team is currently trying to correct these problems.

Conclusions

In conclusion we feel that our FT-Raman kinetics experiments went very well. Our original intentions of producing a mathematical model for the overall reaction kinetics proved to more difficult than originally anticipated. We do, however, feel we have gained much insight and knowledge into the reaction of zinc oxide with glycine. Our studies with respect to the changes in the functional groups of glycine when reacted with zinc oxide support the bonding model for zinc bis-glycinate, which has been confirmed by x-ray crystallography.

We are currently trying to refine our physical procedure for the addition of water to our reaction, in an attempt to obtain more reproducible results, which we hope will lead to a mathematical model for the overall reaction kinetics of the reaction of zinc oxide and glycine.

References

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Acknowledgements

Time elapse overlay of the Glycine & Zinc Oxide Reaction

Graph 1 — ZnO + Glycine Reaction Kinetics 1035 cm^{-1}, $R^2 = 0.784$

Graph 2 — ZnO + Glycine Reaction Kinetics 1107 cm^{-1}, $R^2 = 0.6533$

Graph 3 — ZnO + Glycine Reaction Kinetics 1324 cm^{-1}, $R^2 = 0.9231$

Graph 4 — ZnO + Glycine Reaction Kinetics 892 cm^{-1}, $R^2 = 0.9036$

Graph 5 — ZnO + Glycine Reaction Kinetics 1136 cm^{-1}, $R^2 = 0.8594$

Figure 1 — Glycine; Figure 2 — ZnO; Figure 3 — Dry Mixture of Glycine & ZnO; Figure 4 — Reaction of Glycine & ZnO initiated with water; Figure 5 — Halfway into the reaction; Figure 6 — Enlarged overlay of the dry mixture of Glycine and ZnO and the Finished Zinc Bisglycinate Product