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Transformation of Humulone By UV-Visible Spectroscopy with Introduction of Magnesium

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TRANSFORMATION OF HUMULONE BY UV-VISIBLE SPECTROSCOPY WITH INTRODUCTION OF MAGNESIUM

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TRANSFORMATION OF HUMULONE BY UV-VISIBLE SPECTROSCOPY WITH INTRODUCTION OF MAGNESIUM

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Abstract

In the brewing industry, there are four main ingredients that are required to produce beer: water, yeast, barley, and hops. Hops are used in many different stages of brewing. The key reason for their use is the conversion of humulone (α-acids) during the boiling process to cis- and trans-iso-humulone (iso-α-acid). These compounds are the main contributors to the bitterness in beer. Humulone undergoes an acyloin condensation to form a 5-membered ring. Magnesium ion concentration and the pH of the solution are important mediators of this reaction. We envisioned exploration of this mechanism using UV-vis spectroscopy and polarimetry to determine the binding constant of metals to the humulone. The results, coupled with computational analysis, indicated that Mg^{2+} binds to the anion of humulone resulting in structural changes that promote the rearrangement.

Table of Contents

Abstract *3*
Introduction *5*
Review of Related Literature *6*
History of Hops **7**
Hops Chemistry **8**
Isomerization of Humulone **9**
Methods used to analyze Hops **11**
Summary **13**
Project Design *14*
Data Collection Procedures: **15**
Data Analysis Procedures: **17**
Results/Discussion *18*
Conclusion *32*
Future work **34**

Introduction

Beer is probably one of the oldest and most widely used recipes in the world. The brewing process can be dated back to the ancient Egyptians around 5,000 B.C., when they first started brewing with dates, pomegranates and other herbs. Since then, ingredients in the brewing process has evolved immensely, one of them being hop oil. Hops has been around for since ancient times (Verzele, 1986). The hop plant itself is vine like, that has serrated sinuate shape leaves that slightly cover and are attached to these seed cone flowers. The seed cone flowers are the part of the plant that holds the *Humulus lupulus*. *Humulus lupulus* is more commonly known as hop oil and has been used for many different purposes. For example, during the medieval times, the hop plant was used as a decorative plant. It was not until around the 9th century that hops oil was found to have a very important use. The use was found by German monks, when they were experimenting with the plants while they were brewing. They found that the hop oil not only adds a bitter flavoring to the beer, but also helps stabilize the beer which it helps keep beer stored for longer (Verzele & Van Boven, 1971). Additionally, in hops the two main components are the α-and β-acids, which are more commonly referred to as humulone and lupulone. Out of the two components, humulone has a larger presence. The focus of the research is to study the transformation of humulone by UV- visible spectroscopy with the introduction of magnesium cations. The purpose of this research is to confirm the mechanism of the humulone isomerization, as well as to determine the influence of various factors such as metal cations and pH on the rate of this isomerization. For this reason, the background of brewing, hops chemistry, methods that have been used on humulone and the presence of metals is talked about in further detail below.

Review of Related Literature

Beer is a very popular drink that is consumed by millions, however the average person does not fully comprehend the entire brewing process. The brewing process that we know today requires about seven steps, which include malting, milling, lautering, boiling, fermenting, conditioning, and filtering (Goese, 1999). Originally the brewing process dates back to around the 5th millennium B.C. when brewing was mainly used with barley and other herbs (Johnson, 2016). Shortly after its discovery, brewing and consuming beer was found in nearly every culture across the world. Each of those cultures developed unique recipes and methods for brewing beer. For the most part, the brewing process involved different cereals, grains and flavoring herbs like juniper, dandelions, ground ivy and cedar etc. (Verzele, 1986). Abbot Adalhard was one of the first people to record the specific cultivation of the hop plant, most likely for its use in beer production. Adalhard was from the Benedictine monastery in Corbie, France and recorded how to take care and use the hop plant for the next generation, which gave brewers the chance to understand the potential of hops and enjoy the result of hoppy beer (Sharpe, 1981). Hop plants were not particularly popular in Europe, which made it hard to come across by, but due to the high demand of the plant by the 15th century hop plants could be found all over Europe. Besides adding a bitter flavor to beer, it was found that hops can also preserve beer for a lengthy amount of time, which made it easier to store and transport the beer. Lastly, though everyone does not enjoy the bitterness that the hops impart to beer, a small amount of hops is used in every beer because it helps balance out the malty flavors that are present. Without the addition of hops, the beer would be too sweet (Sharpe, 1981).

History of Hops

Humulus lupulus is the scientific name for the plant commonly known as hops. The hop plant produces flowers that have the appearance of a green upside-down pinecone. Before hops were used in the brewing process, they were used for a couple of other things. For example, in medieval times the hop plant was used as a decorative plant and mostly found in gardens (Verzele, 1986). Furthermore, young hop shoots were also a popular ingredient to put into salads by the Greeks; the plant is still commonly used in salads and other dishes in Central Europe today. Fibers of the hop plant (a plant that can grow up to 25 feet in one year) have been used to make linen in Sweden. An extract of the hop plant has been used as a temporary hair dye in Russia for brunettes (Edwardson, 1952). Lastly, physicians would use hops to help cure different forms of maladies and illnesses. The plant was known to "free the blood of all impurities, tumors, and flatulence, to cure the itch and other skin disease, and to relieve the liver and spleen" (Edwardson, 1952, p. 160). The physicians would also use the antibacterial agents of the hop stems to help inhibit the growth of tuberculosis bacterium (Edwardson, 1952). The discovery of hops is probably one of the most significant events to have happened in the brewing history.

For the most part it has been presumed that the use of hops in beer was not used until the 12th century, when monks in Germany were experimenting with their brewing process and decided to add hops as an additive to see what would happen. It was thought that through their experimenting, the monks had determined that hops give off a bitterness flavor to beer. Also, that the monks determined that the hops oil helped stabilize the beer which allowed the beer to be store longer and boost the preservative value of the beer (Verzele, 1986). However, this is not necessarily true and there has been reports that hops were used in beer as far back as the 9th century. The documents mentioned that there was actually a hop garden owned by Pepin

Charlemagne III, who was the King of Franks from 751 through 768 B.C that was mentioned in his son's will. With the use of hops during this time, it threatened the use of gruit that was used for flavoring before hops had been used as an additive. It was not until about the late 1400s that hopped beer was first imported in the United Kingdom (Acitelli, 2017).

Hops Chemistry

As part of his research, Edwardson had determined the botany of hops, which helped him to understand and explain how hops can be broken down. Based on his work, a cone-like inflorescence (the hop flower or cone) and is composed of scales that are covered in hair fibers (Edwardson, 1952). Miniscule sacks line the inner scales of the cone. These so-called lupulin glands produce large amounts of oils. The chemical components of the oils that come from hops have been studied since the 1970s. It has been determined that hops have two main chemical components that make up the oil, humulone and lupulone. These are known as the α-and β-acids. Each of these chemical compounds has at least 4 derivatives denoted by small structural changes. (Goese, 1999). Furthermore, through the studies conducted by Goese, Edwardson, Johnson and Verzele, it has been found that humulone and its derivatives are not only the main component of hops, but also the dominant compound that results in the bitter taste and stability of beer (Goese, 1999). Goese found two pathways to the formation of humulone in the hop plant. The two pathways are the mevalonate pathway and the deoxyxylulose pathway. Each is located in different parts of the plant cells. The deoxyxylulose pathway only works in the plastids, where it supplies the materials to build the organic compounds hemiterpenes, monoterpenes, diterpenes and tetraterpenes. The mevalonate pathway only works in the cytoplasm where it supplies the materials for the compounds phytosterols and sesquiterpenes (Goese, 1999). Goese's experiments on the biosynthesis of humulone determined that humulone is an antibacterial bitter

acid that results from the breakdown of hops. He used several different isotopic enrichments of feed stocks and studied the $C^{13}C^{13}$ coupling patterns to identify several precursors for humulone such as isovaleryl-CoA, malonyl-CoA and dimethylallyl pyrophosphate. Furthermore, his research helped concludee that humulone has a symmetrical biological intermediate.

Before M. Verzele (1986) started his research on humulone, earlier analyses that were performed had concluded that methods like Nuclear Magnetic Resonance (NMR) and Counter Current Distribution (CCD) did not have enough power to expose traces of alternative α-acids in hops. This discovery was part of the reason Verzele (1986) decided to explore the use of High Performance Liquid Chromatography (HPLC). From his research, he determined that HPLC had the power that the other methods lacked to explore hops. Also, HPLC helped Verzele (1986) to analyze hops which aided him to develop the structural chemistry of hops. His work helped to support an earlier discovery by F. Rigby where he determined that the α-and β-acids are a mixture of homologs and analogues. Rigby (1972) had previously named the major three derivates of both humulone (co-humulone, humulone, and ad-humulone) and lupulone (co-lupulone, lupulone and ad-luplone).

In short, though there has been a lot of research done on hops chemistry, there is still so much more to explore like how the isomerization of hops works and what happens when outside sources are introduced.

Isomerization of Humulone

In chemistry, there is a process called isomerization, which occurs when one molecule is transformed into a different molecule using the same atoms. Isomerization is very important because it is the process of "converting straight chain alkanes into branched alkanes" (Taiguchi,

2014). His work was important because formation of branched alkanes was vital for the high-quality production of the sample. (Taiguchi, 2014).

Studying hop oil components is a long process that is very laborious. To start the process, a hop oil extract that is enriched in humulone was weighed out on a balance scale. This is then combined with benzene and o-phenylenediamine. The mixture was swirled to dissolve everything and then left to sit until crystals of the humulone-o-phenylenediamine formed (Johnson, 2016). Once the crystals have formed, the solution was vacuum filtrated to separate the liquid from the solid crystals (Johnson, 2016). The crystals were dissolved again in benzene and the process repeated numerous times until the crystals reached the ideal color (Johnson, 2016). The purified humulone-o-phenylenediamine complex was then treated with hydrochloric acid to separate the complex. The humulone was then extracted with ether and the ether evaporated to give pure humulone.

The isomerization was accomplished by Zhang and coworkers (2018) by treating the humulone oil with hydroxide ion (OH^-) and heat, which moves hydrogens (H) around to form a double oxygen bond on the cyclohexane ring (Zhang, 2018). After nixing this with acid, a ketonized anion intermediate is formed, which basically transforms the cyclohexane ring into a cyclopentane (Zhang, 2018). From this point, two structures are formed, either cis-iso-humulone or trans-iso-humulone (Simpson 1993). Cis-iso-humulone and trans-iso-humulone are the two main complexes that are formed when hops are boiled during the brewing process (Simpson 1993). The two isomers have similar properties, but differ in their ability to rotate polarized light. When humulone is used, especially in small amounts, even though there are two types of isomeric complexes that come from hops being broken down, six iso-humulone complexes are formed (due to the presence of the three major derivatives of humulone). In other words, those

six iso-humulones that are formed include humulone, co-humulone and ad-humulone and their respective iso-humulone compounds (L. O. Spetsig, 1964)

Methods used to analyze Hops

Through all the research that has been collected from the multiple authors and their respective projects like Aberl, Ivanova, Koller, Verzele, Zhang and Johnson, several methods have been used to analyze different areas of hop chemistry. The methods include the European Brewing Convention method (EBC), ultra-violet matrix assisted laser desorption ionization orbitrap mass spectrometry (UV-MALDI), polarimetry, and ultraviolet-visible spectroscopy (UV-vis).

The European Brewing Convention Method is more commonly known as the EBC method. This method is a quantitative headspace method, where the gases above a sample are collected and injected into a gas chromatograph that uses mass spectrometer as the detector. This method helps analyze hops oil, by using four trap cycles to reduce detection limits. Another EBC method can measure a sample of beer that is placed into a cuvette which is then placed in a spectrophotometer at a wavelength of 430 nanometers (nm) (Aberl, 2012).

UV- MALDI Orbitrap Mass Spectroscopy is an analytical technique which ionizes the samples into charged molecules. The ions can then be separated and the ratio of the sample's mass (m/z) to charge can be measured. This method can be used as either a matrix-assisted laser desorption/ionization (MALDI), which is a time-of-flight analyzer (TOF) that measures the mass to charge ratio of the ions it creates (Ivanova, 2014).

Polarimetry is a technique that measures the optical rotation of both inorganic and organic compounds that are present in a sample. This technique works by passing polarized light

through a sample. The angle of the polarized light changes as it passes through compounds that can interact with it. While lupulones and many other molecules do not interact with polarized light, the humulones can interact and be detected (Koller, 1969).

Ultraviolet-visible spectroscopy is more commonly referred as UV-vis. UV-vis is used to analyze the absorption and wavelength of samples (Zhang, 2018). The instrument uses ultraviolet light to determine the amount of energy as light that is absorbed by the compound (Boland, 2019). This method can also be used to note the absorbance of light in the visible region of the spectrum, which gives rise to the observed colors of the chemicals in the sample (Verzele, 1986 & 1971). This technique can be used with humulone because this method is a "conventional analytical technique" that can help determine the bitterness level of beer. By comparing the amount of humulone's absorption, the concentration of humulone and bitterness of the sample can be determined (Zhang, 2018).

So, in short, the EBC method measures only the wavelength on the sample (Aberl, 2012). The UV-MALDI ionizes a sample into charged molecules and is measures the ratio of mass to charge (m/z) (Ivanova, 2014). Polarimetry is the measurement of the polarization of transverse waves that are present in the sample (Koller, 1969). The UV-vis measures absorption by using light in the visible and adjacent range (Verzele, 1986). The reason that the UV-vis was picked for this research is because it is the best technique to measure and study the isomerization and pKa of humulone.

The Introduction of Metals

Through all the research that has been analyzed for this topic, only one research experiment was found that involved the interactions of metals with humulone. During his experiment, Johnson

(2016) introduced five different metals, calcium (Ca), magnesium (Mg), zinc (Zn), sodium (Na) and copper (Cu) to determine if the presence of iso-humulone could be detected. From the five metals, only magnesium had the biggest impact and overall had the highest detected amount in every trial. Through his experiments, Johnson also explored changes in pH to see if they had any effect on the formation of iso-humulone from humulone. It was observed that magnesium was not affected by the pH changes. It was discovered that magnesium had some influence on the rotation, absorption and structure of iso-humulone, which helped to further study the isomerization of humulone (Johnson, 2016).

Summary

Multiple techniques and methods have been used to test hops and iso-humulone. Brewing has been around a long time and has been evolving ever since the process started. Hops chemistry has been around a lot longer than people realize. Furthermore, multiple variables go into the chemistry of how hops oil is broken down. When metals were tested to see what kind of effect they would have on the formation of iso-humulone and if iso-humulone could be made at all. Magnesium cations had the greatest effect indicating that magnesium likely binds to the humulone and assists in the isomerization to iso-humulone. Further research is needed to look more into how magnesium plays a part in the mechanism of humulone isomerization and if it can be used in the brewing industry to improve the production of beer. Also, more research is needed on how other techniques correspond to each other when working with hops or if the techniques above are the only ones that seem to work with the compound. To conclude, since Johnson had studied the isomerization of humulone to iso-humulone, this research was started by wanting to study the difference of the isomerization and pKa of humulone with the introduction of magnesium cations. Due to Johnson's (2016) discovery, magnesium cations were used during

this experiment because it was really the only metal that was found to be able to detect iso-humulone at high amounts every time no matter what the pH was.

Project Design

The purpose of this project is to learn more about the isomerization of humulone. Since the first step in the mechanism of the isomerization of humulone to iso-humulone is the formation of the anion of humulone, studying the formation of that anion is the focus of this project. This can be most easily accomplished by determining the acidity of humulone. The acidity can be quantified by measurement of the pKa of humulone. In addition, if magnesium cations bind to humulone and increase the rate of the first step in the mechanism, the pKa should change. If the pKa drops, this would indicate that magnesium increases the rate of isomerization. If the pKa increases, then the rate of the reaction will decrease.

Materials:

Glassware

- Pipettes
- Beakers
- Erlenmeyer flasks
- Buchner funnel
- Buchner flask
- Glass stirring rod
- Magnetic stir rod
- Spatula
- Cuvette
- Parafilm

Instruments

- pH Meter
- NMR
- UV-visible Spectroscopy
- Balance
- Vacuum pump
- Ethanol

Chemicals

- Sodium Hydroxide (NaOH)
- Hydrochloric acid (HCl)
- Hops oil
- Benzene
- o-phenylenediamine
- D.I. Water
- Magnesium Chloride (MgCl)
- Methanol (MeOH)
- Sodium Chloride (NaCl)

Data Collection Procedures:

Mechanism of Hop Oil Isomerization

The first step in the project is to make an o-phenylenediamine-humulone complex. To make the o-phenylenediamine-humulone complex, approximately 30 grams (g) of hops extract was obtained. Then, the extract was added into a 250 milliliter (mL) Erlenmeyer flask and 60 mL of dry liquid benzene was added. The hops solution was then warmed on a hotplate to completely dissolve the hops. About 5 grams of o-phenylenediamine was added to the solution which turned the liquid to a dark brass color. The hops solution was then cooled to room temperature with a cap on the flask. Once the solution was completely cooled, yellow crystals formed. It was also typical to see a dark brown layer of crystals on the surface especially after the first round of dry benzene. The humulone solution was then mashed up with a glass rod vacuum filtered through a Büchner funnel. The crystals were weighed once they were dry and transferred to a clean and dry 250 mL Erlenmeyer flask. This process was repeated at least three additional times, each time resulting in crystals that were lighter in color. The final color of the crystals was pale-yellow. The purified o-phenylenediamine-humulone complex was stored in a sealed vial in the freezer.

The humulone was isolated from this complex by dissolving it in 50 mL of diethyl ether and placing it in a separatory funnel. Then, 50 mL of 6M hydrochloric acid was added to the funnel. The mixture was shaken gently and allowed to separate. The ether was then removed from the funnel, washed with water once, then dried over magnesium sulfate. After gravity filtering, the ether was removed by rotary evaporation to give pure humulone. This was stored in the freezer in a sealed vial wrapped in aluminum to protect it from sunlight.

pKa of Humulone Procedure One

Following the separation of humulone from hops, the next step was to study it by UV-vis spectroscopy. The humulone was dissolved and then diluted with water since the concentration is too high to see on the UV-vis spectroscopy. To make the humulone solution, approximately 0.1 of the humulone crystals were dissolved in 20 mL of methanol since humulone is not soluble in water. Once the humulone was dissolved in the methanol, it was added to 400 mL of water. This was the stock humulone solution. The concentration, as determined by UV-vis spectroscopy, was too great to be measured accurately, the absorbances for the major signals in the spectrum were too great.

Then, a 100-mL portion of that solution was diluted with another 400 mL of water. This working solution was divided into separate flasks so the pH could be adjusted in each solution. A 0.1 M solution of NaOH and 0.01 M of HCl were used to adjust the pH of the solution. One or two drops of the acid or basee were added at a time and each time the pH was measured using a pH meter. A sample of the solution was then placed into a cuvette and its absorption spectrum measured in the UV-vis spectrometer. This process was repeated until solutions from pH 3 to 6 were obtained. This range was chosen because humulone has a pKa in the 4 to 5 range. This experiment was completed numerous times to confirm the results were consistent.

This process was repeated in the presence of magnesium cations. The procedure was followed as before, but 0.045 grams of magnesium chloride was added to the final 400 mL of water before it was added to the humulone solution.

pKa of Humulone Procedure Two

For this experiment, instead of using NaOH and HCl to adjust the pH of the humulone solution, ten pH buffers were made with specific pH values: 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0. A humulone stock solution was made in methanol, which consisted of 0.07 grams (g) of humulone and 30 milliliters (mL) of methanol. For the first trial, 5 mL of each buffer was added to 17 mL of the humulone / methanol solution. When the absorbance was evaluated for each buffer, it was found that the buffer solution itself absorbed light in the region of interest. Specifically, the buffers absorbed all light from the wavelengths 200 nm to 390 nm. The methanol and buffers were tested themselves to see if the problem resided in one of the samples, but the spectra came back normal. This experiment was tested seven times before it was determined that the humulone being used wasn't one hundred percent humulone. The melting point was taken and came out to be 58 degrees Celsius. The melting point indicated that there was still a little bit of o-phenylenediamine-humulone complex still in the compound which made it impure. This was the reason why a large absorbance existed in the UV-vis spectrum.

Data Analysis Procedures:

UV-visible Spectroscopy

A cuvette, or sample holder, was filled with each solution to be analyzed. That cuvette was then placed in the UV-vis spectrometer and the absorbance spectrum measured from 190nm to 800nm. The spectrum was saved and examined for absorbance maxima. At each maxima, the absorbance was noted.

Spectra were also compared to each other to determine if a change had occurred at a different pH.

Results/Discussion

At the start of this experiment, an initial trial that was conducted twice as shown in Figure 1, 2 and 3. The spectra in Figure 1 is a combination of samples from a humulone/water and an humulone/magnesium solution. The spectrum from Figure 2 and 3 are the separate humulone/water and humulone/magnesium solutions respectively. From this trial, two solutions were prepared with humulone that was separated from hops oil. Each sample was dissolved in methanol and diluted with water before the pH was fluxed with diluted sodium hydroxide and hydrochloric acid. Since this was the first trial in the experiment many measurements at multiple wavelengths were looked at to get an idea of where the humulone was transforming the most. The humulone/water solution showed wavelength maxima at 225, 285, 325, and 365 nm. The humulone/magnesium solution was also evaluated at those wavelengths plus 256 nm.

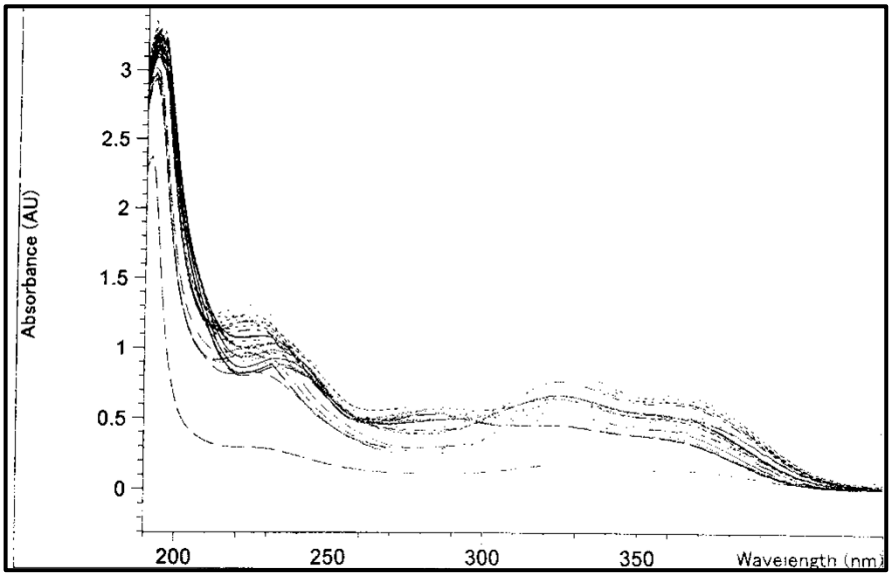

Figure 1: UV-vis spectra of humulone and water solution and humulone with magnesium. pH values range from 3.03 to 10.72. Spectra taken based on Procedure 2.

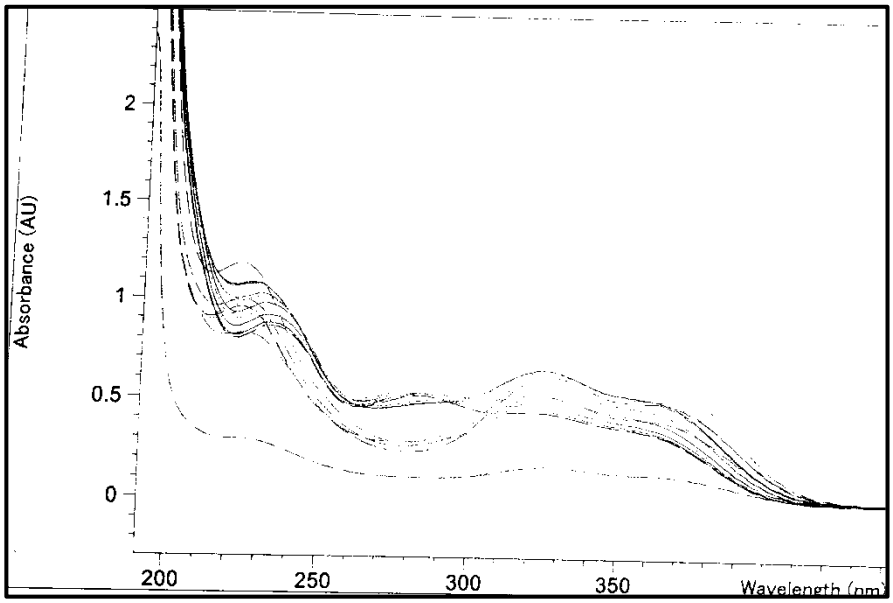

Figure 2: Humulone and water solution separated from the spectra in Figure 1. pH values range from 3.03 to 10.72. Spectra taken using Procedure 2.

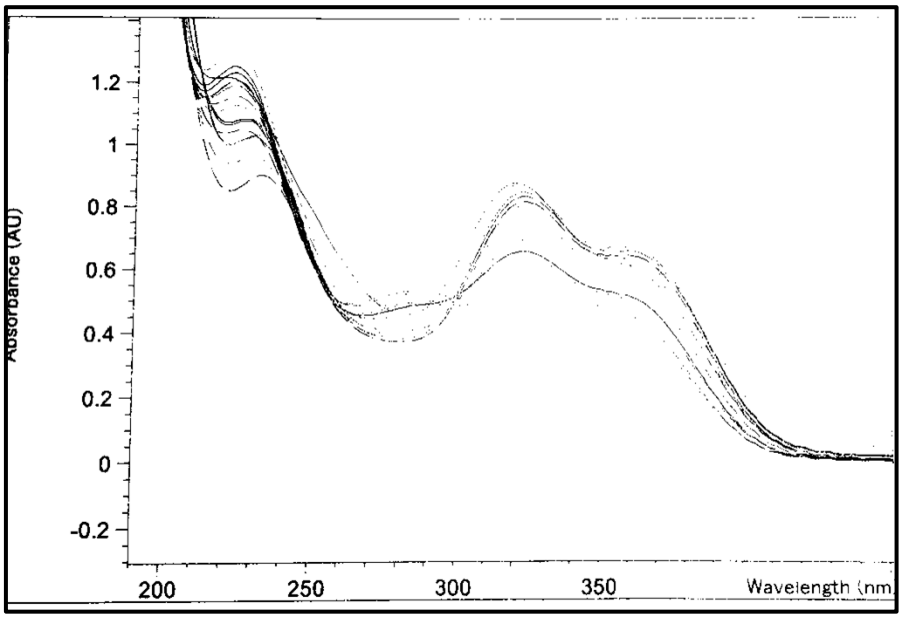

Figure 3: Humulone / magnesium solution separated from the spectra in Figure 1. pH values range from 3.78 to 7.18.

After repeated experimentation, the technique was improved and the spectra shown in Figure 4 and 5 were obtained for the humulone/water solution and the humulone/magnesium solution, respectively. Compared to the first three spectra a difference in the absorbance levels can be seen between the spectra. The main difference between these spectra is the concentration difference between the trials. The first trial started at a higher concentration then as the trials went on the concentration got lower since it became apparent that the measurement of the absorption spectrum of humulone worked better at lower concentrations.

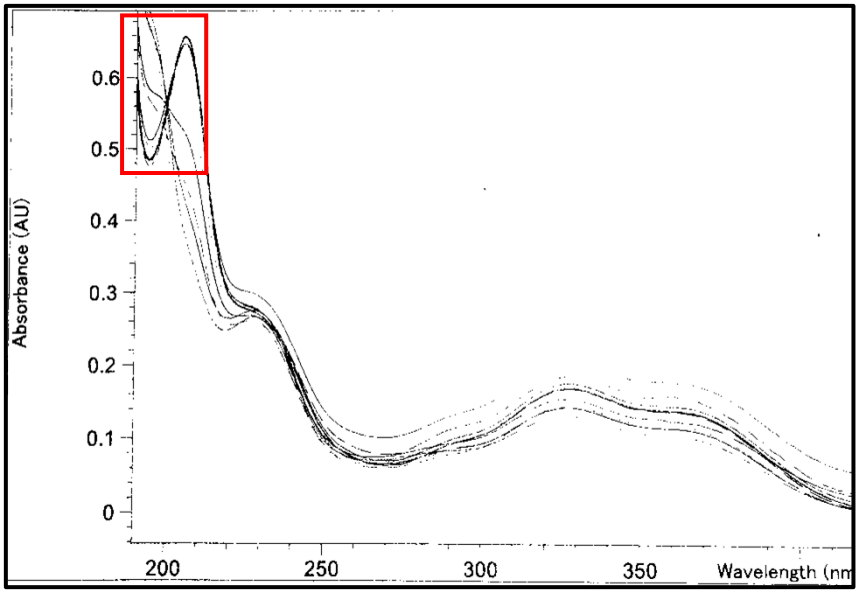

Figure 4: UV-vis spectra of humulone with Magnesium. pH values ranged from pH 3.06 to 6.22.

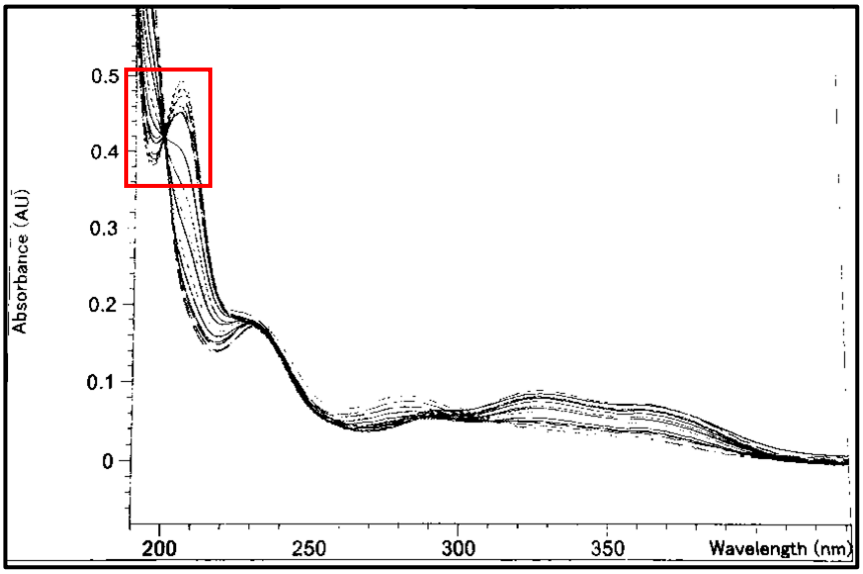

Figure 5: UV-vis spectra of humulone and water solution. pH values ranged from pH 2.99 to 5.59.

Plots of the data from the absorbance spectra were graphed six times. Most of the data was not useful expect for one data set that is shown in Figures 6. This plot was made to determine if one or more of the wavelengths could be used to determine the pKa. Figure 6 shows that the wavelengths at 298, 330 and 372 nm did change dramatically as the pH changed in the humulone solution. The data from the six graphs were not useful for determination of the pKa since there were not enough points to accurately calculate an inflection point in the curve. Also, most of the data did not display the expected sigmoidal curve when plotted. The slope of the sigmoid was also important as the greater the slope would provide a more accurate measurement of the midpoint of the curve. That midpoint on the sigmodal curve is the point where the humulone is 50% protonated and 50% unprotonated. Thus, the midpoint is the measure of the pKa of the humulone.

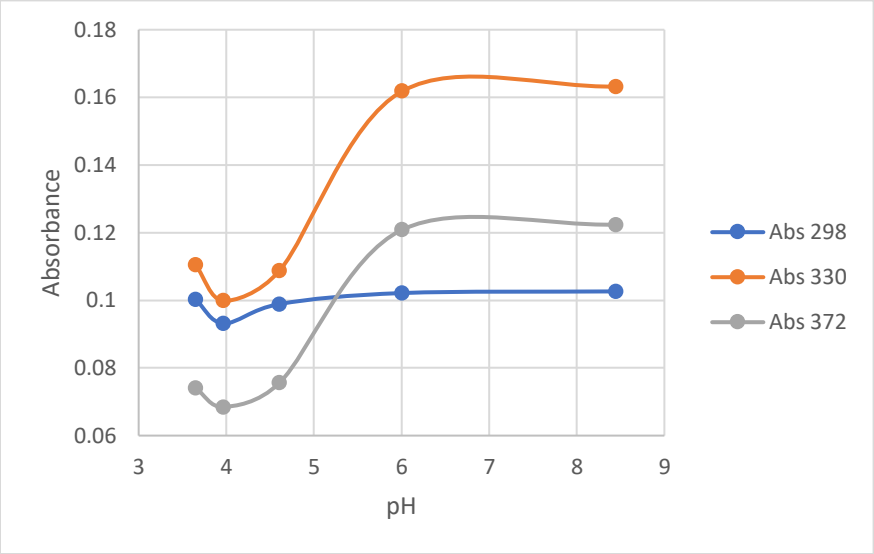

Figure 6: Humulone and magnesium solution from pH 3.65 through 8.45. Absorbance values collected from 298, 330 and 372 wavelengths (nm). (keep)

To determine the experimental pKa, the absorbance values that corresponded with the peaks around 206 nm like the peaks that are enclosed in the red box in Figure 4 and 5. The values were placed in a excel sheet to form a graph like that shown in Figure 7. Then the absorbance values in Figure 7 were taken with the corresponding pH's and graphed with a calculated sigmoidal curve. From the points in the sigmoidal curve, the computer-generated line was "fit" to the data by adjusting each of the five variables in the equation. The curve was fit based on minimizing the error in the calculated line to the data points. When the error was minimized, the midpoint of the sigmoidal curve, represented by variable C was determined. This was equivalent to the pKa of humulone. For the data represented in Figure 7 and 8, the experimental pKa was determined to be 4.26.

$$A + \left(\frac{D}{\left(1 + \left(\frac{x}{C}\right)^{B}\right)^{E}}\right) \quad (1)$$

Where A is the minimum, B is the slope, C is the midpoint, D is the maximum and E is the asymmetry point. (above figure 12)

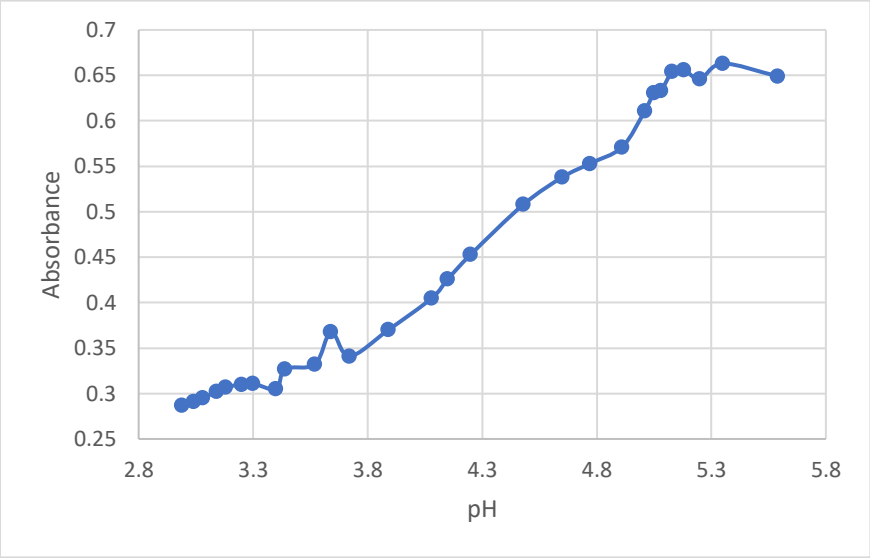

Figure 7: Humulone and water solution from pH 2.99 through 5.59 at 206 nm.

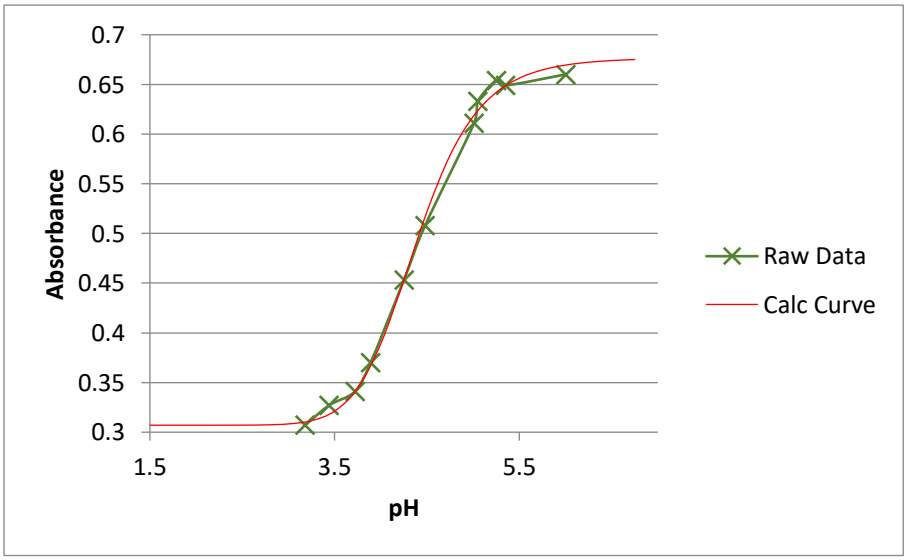

Figure 8: Values taken from Figure 12 to represent the sigmoidal curve. These data are overlaid with a computer-generated sigmoidal curve generated as a logistical equation.

The values with this trial did not line up with the computer-generated sigmoidal curve as nicely as other trials did, but the resulting curve still maintained the sigmoidal shape. By using equation (1), the experimental pKa was determined to be 4.08.

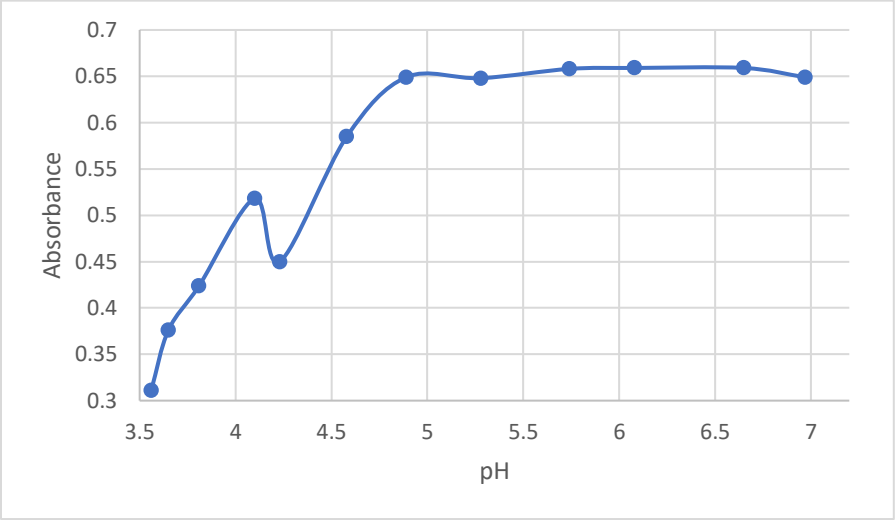

Figure 9: Humulone and water solution from pH 3.56 through 6.97 at 206 nm. Values are from spectra taken using Procedure 1.

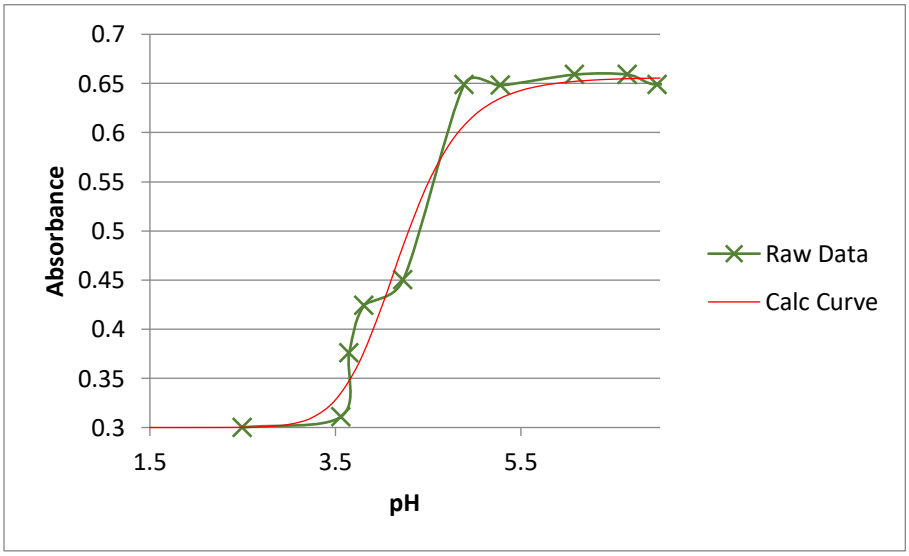

Figure 10: Values taken from Figure 14 to represent the sigmoidal curve that is present. Variability in the data lead to error in the determination of the pKa.

The values with this trial do not line up with the sigmoidal curve as nicely as other trials do and do not really hold a sigmoidal curve either. This trial turned out to be a little flatter than most of the other trials, but by using equation (1), the experimental pKa was determined to be 4.48. This was in close agreement to the pKa found in the literature

Figure 11 exhibit a flattened rise in the absorbance at the pKa that can be seen Figure 12. This is illustrated by the minimized slope on the sigmoidal curve. These trials were performed on humulone that had likely oxidized or isomerized prior to the measurement of the pKa. This further emphasized the need to prepare and analyze the humulone within as short a time as possible. Different from Figure 16, the values from Figure 18 showed an experimental pKa of 3.98.

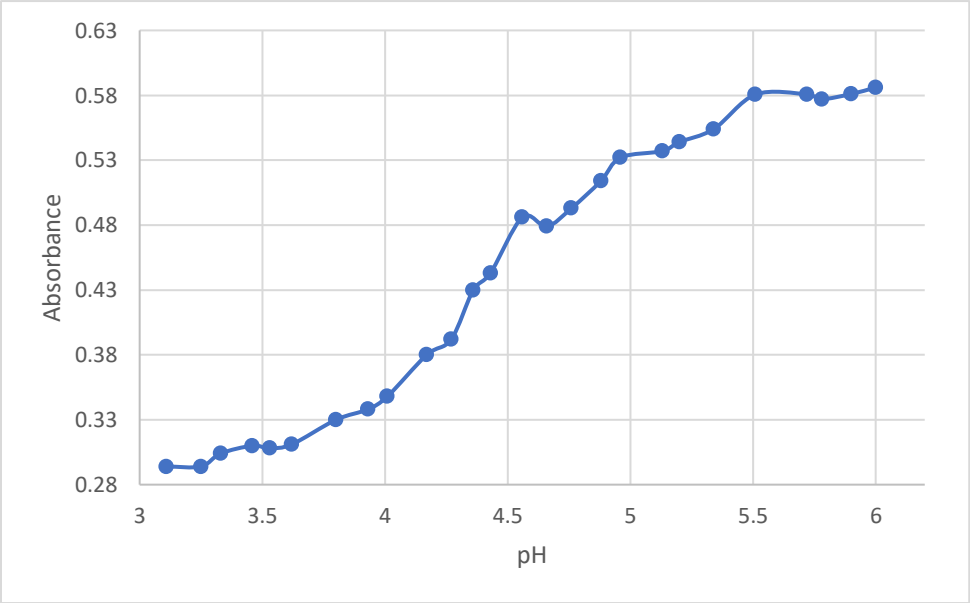

Figure 11: Humulone and water solution from pH 3.11 through 6.00 at 205 nm. Values are from spectra taken using Procedure 1.

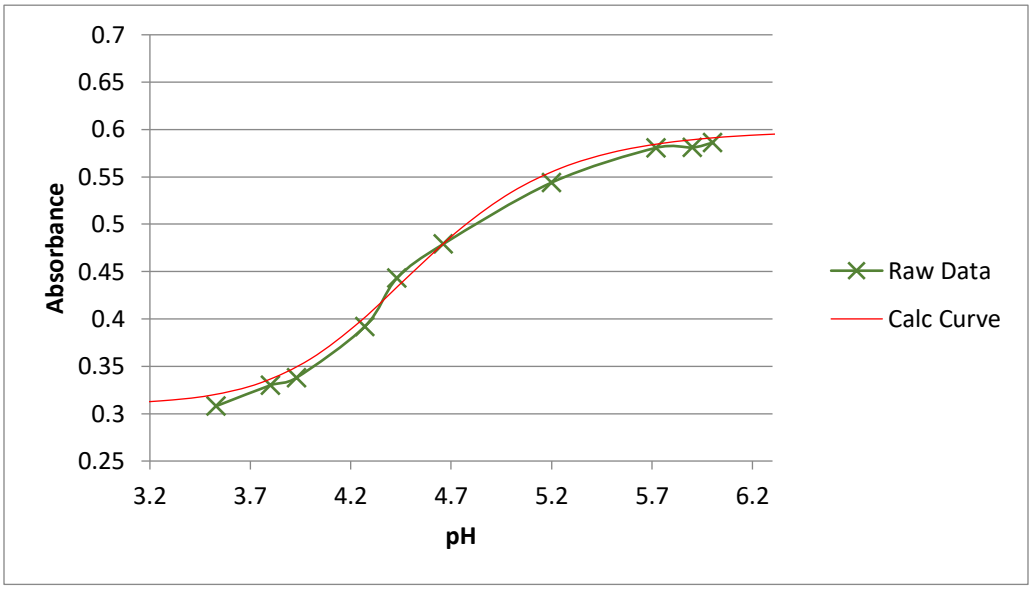

Figure 12 : Values taken from Figure 16 to represent the sigmoidal curve that is present.

A freshly prepared solution of humulone was used for Figure 13 and 14. As was noted above, a diluted humulone solution had a finite lifetime before it was oxidized or decomposed to the point that it could not adequately be measured. Furthermore, this trial was the first run with magnesium in the humulone solution. With this new solution, the absorbance values lined up well on the calculated sigmoidal curve and gave an experimental pKa of 4.27. Compared to the previous trials that are represented by Figure 12, 14, 16, and 18, this trial with the introduction of magnesium had the best fit to the calculated sigmoidal curve.

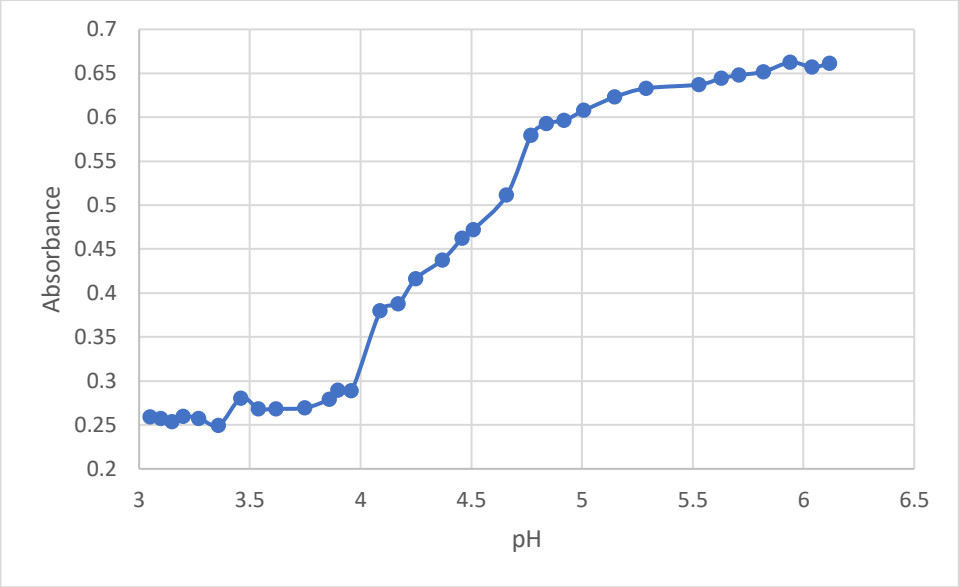

Figure 13: Humulone and magnesium solution from pH 3.05 through 6.12 at 206 nm. Values are from spectra taken using Procedure 1.

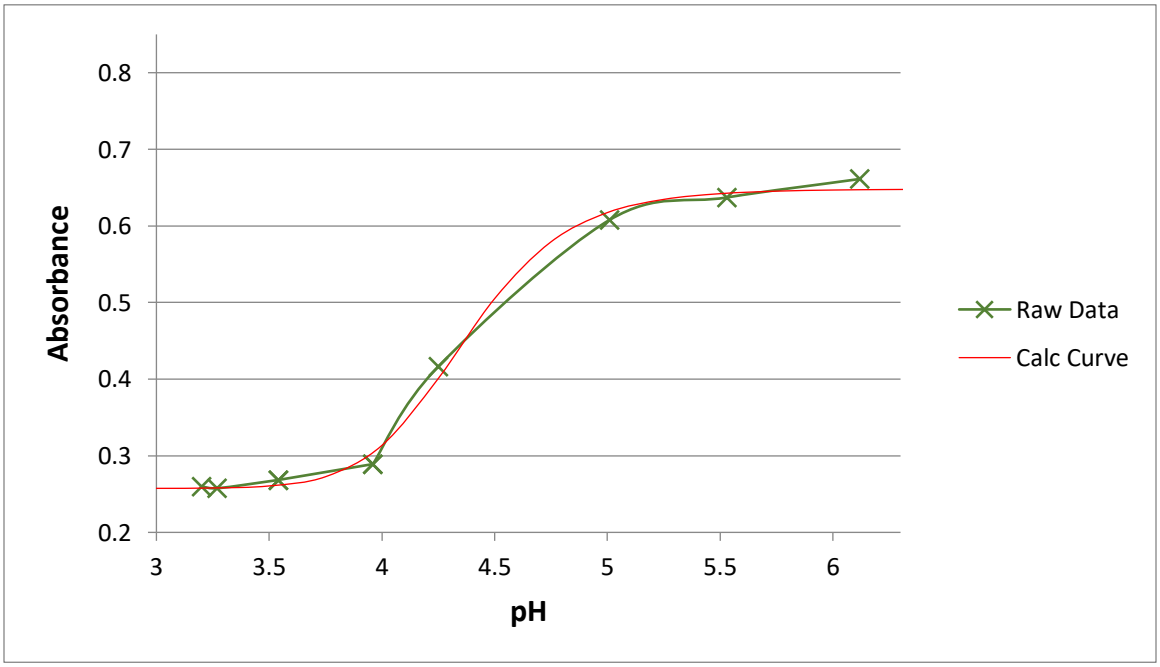

Figure 14: Values taken from Figure 20 to represent the sigmoidal curve that is present.

The trial that is represented in Figure 15 and 16 was generated from a set of data that spanned a wider pH range. This is evident in Figure 16 as a longer maximum absorbance at the top of the curve. Furthermore, since the data did not stretch far along the minimum absorbance, the bottom portion of the sigmoidal curve was not complete. This caused some issues when the computer generated sigmoid was fit to the data. However, the experimental pKa was calculated to be 4.060. The trial that is represented in Figure 15 and 16 is very similar to the data obtained for Figure 17 and 18. The big difference between these two are that this graph above is steeper than the graph in Figure 16. The experimental pKa turned out to be very similar to the trial in Figure 15 and 16 and was low at 3.68.

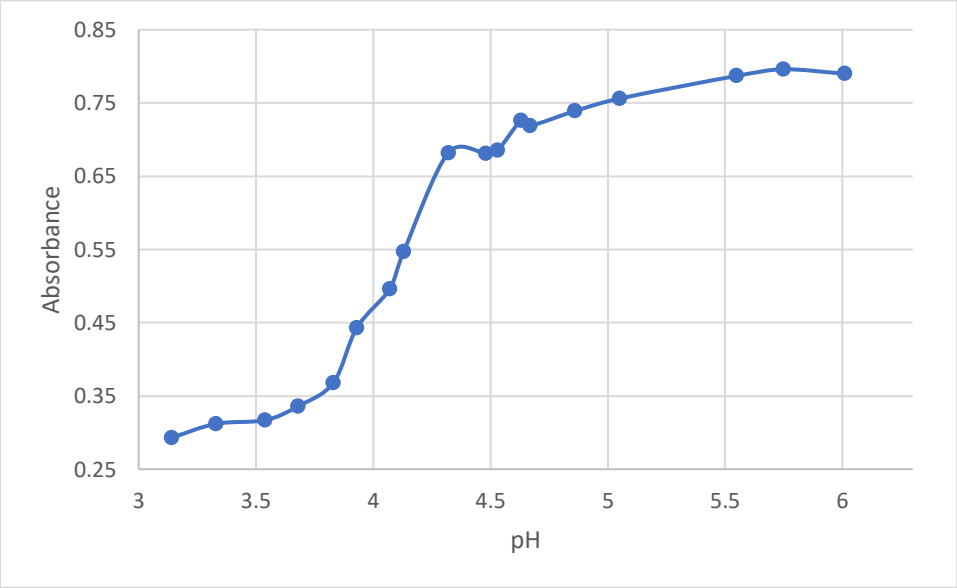

Figure 15: Humulone and magnesium solution from pH 3.14 through 6.01 at 206 nm. Values are from spectra taken using Procedure 1.

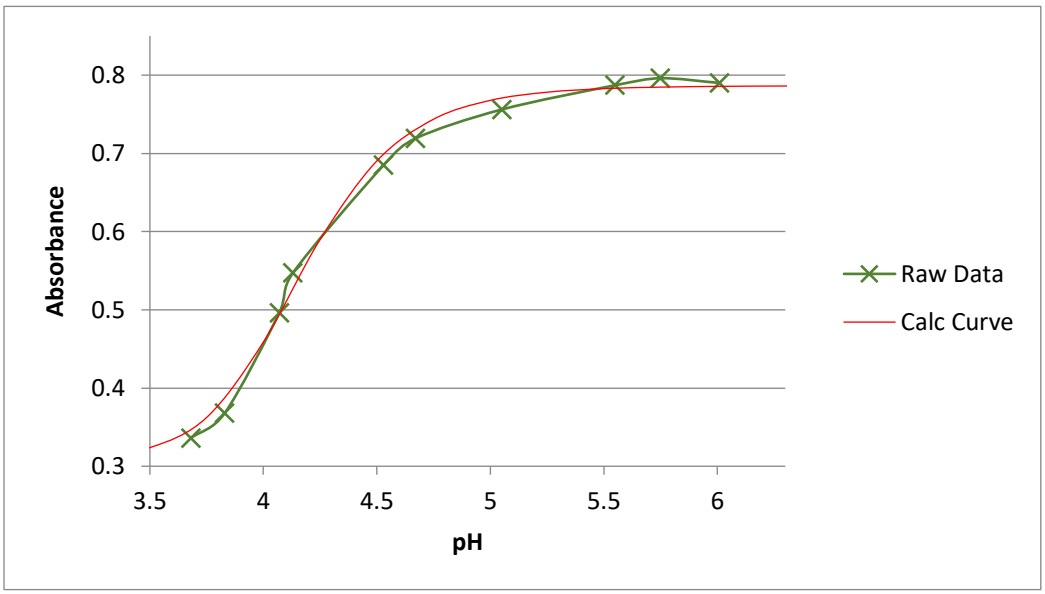

Figure 16: Values taken from Figure 22 weree fit to a computer generated sigmoidal curve.

An adjustment was made as the data in Figure 17 and 18 was obtained. A piece of aluminum foil was wrapped around the Erlenmeyer flask with the humulone/magnesium solution. This was done to see if light was causing a reaction in the solution. The absorbance values obtained followed the calculated curve very well and gave a 4.06 experimental pKa. The second trial with the aluminum foil around the Erlenmeyer flask did not turn out as well as the first run in Figure 18, but the experimental pKa was very similar at around 3.81.

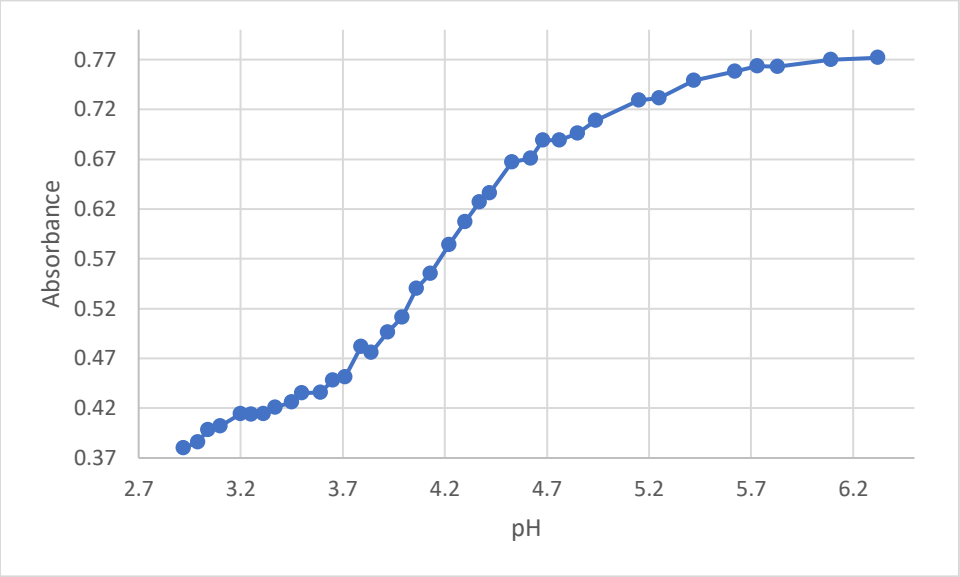

Figure 17 : Humulone and magnesium solution from pH 2.92 through 6.32 at 206 nm. Values are from spectra taken using Procedure 1.

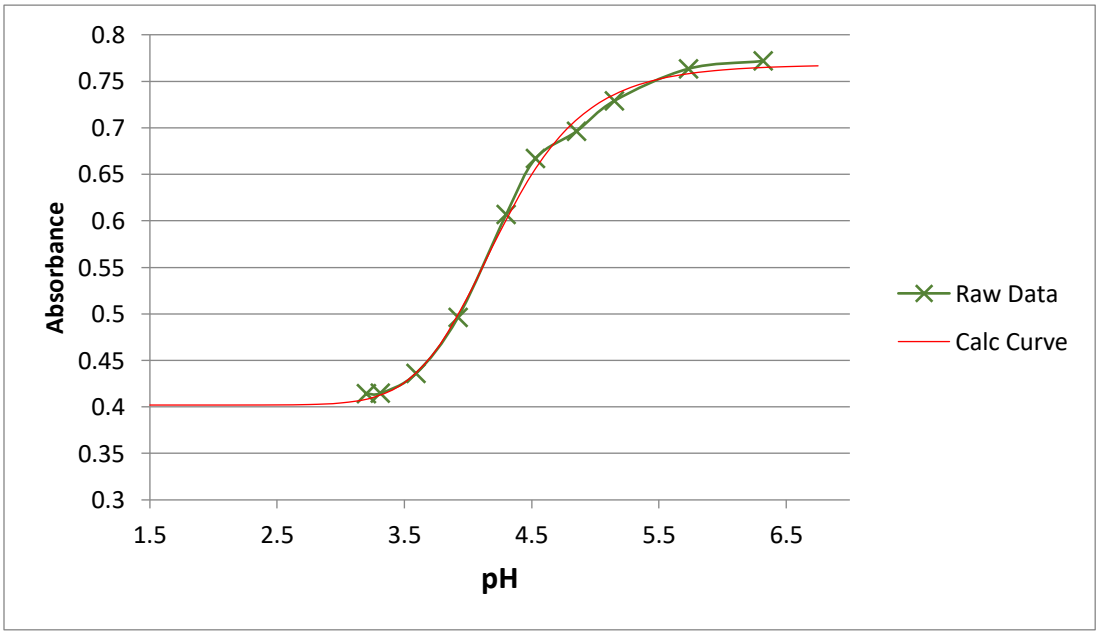

Figure 18: Plot of data from Figure 26 and the best fit of the computer generated sigmoidal curve.

Table 1 lists the pKa values for each trial completed in this study. Thus, for every humulone and humulone/magnesium solution, a calculated pKa was determined. The average pKa for the trials for the humulone solution without magnesium was determined to be 4.214 ± 0.171. The trials for the humulone solution with magnesium can be seen on the right side of the table and the average pKa turned out to be 3.904 ± 0.162. Further examination of the plots and the data obtained using Procedure 1, revealed that there were some data that were not well represented. Specifically, trials 2 and 4 on the humulone side and trials 2 and 4 on the Mg-humulone side were removed from the calculation of the average pKa to determine if the overall data was improved. Examination of the data revealed that the results were not statistically significant due to the overlap of the average and standard deviation.

Table 1: varying pKa values that correspond to the graph above

Humulone		Mg-Humulone		
Trial	pKa	Trial	pKa	Change in pKa
1	4.255	1	4.06	0.195
2	4.083	2	3.686	0.397
3	4.478	3	4.06	0.418
4	3.98	4	3.81	0.17
5	4.273			4.273
Average	4.214		3.904	0.31
Standard Deviation	0.171		0.162	

When the poor data was removed, the average pKa for the trials for humulone without magnesium was determined to be 4.335 ± 0.101. The trials for humulone with magnesium

Table 2: varying pKa values that correspond to the graph above

Humulone		Mg-Humulone		
Trials	**pKa**	**Trials**	**pKa**	**Change in pKa**
1	4.255	1	4.06	0.195
2	4.478	2	4.06	0.418
3	4.273	3	3.81	0.463
Average	4.335		3.977	0.358
Standard Deviation	0.101		0.118	

showed the average pKa turned out to be 3.977 ± 0.118. With the bad curves or outliers removed, neither of the values overlapped, which means that statistically there is significance.

Conclusion

From the multiple trials that were completed for this experiment, it was possible to determine the experimental pKa for humulone without magnesium and humulone with magnesium as seen in Table 1 and 2. With Table 1 and the experimental pKa for humulone without magnesium was 4.214 ± 0.171. For humulone with magnesium the experimental pKa was 3.904 ± 0.162. As seen in Table 1, the values overlap which means that statistically there is no significance. In other words, there is no difference between the two set of data and that magnesium did not have an effect on the isohumulone transformation. By removing the "bad" curves from the calculations the pKa for humulone without magnesium was 4.335 ± 0.101 as seen in Table 2. For humulone with magnesium the experimental pKa was 3.977 ± 0.118. By removing the bad curves from that data, the values did not overlap which means that statistically there is significance and there is a difference between the two sets.

The solvent system used in this study differs from actual work. Thus, the ionic strength and other components found in work are not considered in the model solvent system. Previous results have shown that the isomerization does not occur in the absence of magnesium cations and is slower as the pH decreases. Our computational analysis has shown that the magnesium cation interacts with humulone to form a humulone-Mg complex. It is believed that this complex distorts the humulone and enhances the rate of isomerization. Furthermore, magnesium cations seem to stabilize the formation of the anion in the first step of the mechanism of isomerization. So, the magnesium cations increases the rate to the point that humulone will become deprotonated and will result the pKa being lowered. In our model solvent system, the exact pH is difficult to determine as the solvent system is largely comprised of methanol.

A noticeable increase in the yellow color of the solution occurs as the solution becomes more basic. The UV-vis spectrum of humulone in the model solvent system shows a maximum at 190 nm. As the pH of the model system is adjusted, the absorbance at that maximum decreases while a new maximum appears at 206 nm. The isosbestic point at 200nm between these changes allows us to confirm that the concentration of humulone was not changing during the adjustment of the pH. A plot of the absorbance at 206nm versos the pH produced a sigmoidal curve that was fitted to a logistical equation. The pKa values were then obtained and listed in Table 1. When the UV-vis data was obtained in the presence of 0.5 M magnesium chloride, the pKa values shifted, indicating that the magnesium assisted in the formation of the humulone anion. The difference in pKa values was 0.358 and is related to the binding constant of magnesium to humulone.

Future work

The next step to do in this research project is to convert the difference in the pKa values to a binding constant for magnesium-humulone. Furthermore, the concentration of magnesium may play a role in the binding and will be explored by adjusting magnesium concentrations and remeasuring the pKa value.

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