

Kinetics and Thermodynamics of Aspartic Acid Deamination

By
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Personal Section

My introduction to science research started in elementary school with a Saturday program at CW Post College. It was there, in a college laboratory, that I was first introduced to the concepts of energy, genetics and chemistry. In middle school I conducted environmental observation studies for our school science fairs. Following these experiences I then eagerly enrolled in a science research class when it was offered at the high school. However my initial exploration into the area of sustainable energy began with an English paper on genetically modified foods. It was there that I began to understand the extent of world hunger and its relationship to the looming energy crisis. This was followed by further exploration into alternative energy for my science research class. After I wrote a paper about alternative fuels for submission to a science essay competition I came to understand that energy is the root cause of many of the world's problems. Problems of pollution, food shortages, bad or insufficient water, lack of transportation, terrorism, genocide and global warming all have an energy dimension. I have come to believe that these problems could be ameliorated or even eliminated if we had an abundant and inexpensive source of renewable energy. I also realized that I enjoyed helping people and research into this topic was a means to impact the future.

Building foundations in the field of alternative energy requires first acquiring technical tools in the hard sciences and engineering. In high school, I have been fortunate to have had good educational resources to begin this process. I doubled up on science and math honors courses and AP chemistry and physics in order to develop a broader knowledge base. I was able to locate a professor, Dr. Rodney Finzel at Hofstra University, who was conducting research related to this

field. After meeting with me he agreed to become my mentor and sponsor me in the Hofstra University High School Summer Science Research Program. I spent two summers conducting research in which I attempted to address an inexpensive means of generating ammonia through the recycling of protein wastes such as offal and sewage. I picked ammonia because it is one of the key chemicals used in the world today, being essential for production of fertilizer, but rising in price because it is currently made from natural gas. I enjoyed the laboratory experience immensely. What contributed to the fun was spending time with like minded students. We each worked on our projects independently but still enjoyed the camaraderie of the lab. The professors were available to answer questions, as were the college students working in the labs. In the lab I encountered the everyday problems of research. When my samples evaporated after heating I had to redesign a seal for the instruments I used. When I did not get a measurement reading on the instruments I was using I had to modify my sample composition. In order to do a statistical analysis I had to learn new concepts in mathematics. In addition, I had to learn the mathematical part of physical chemistry, kinetics and thermodynamics, in order to understand my data. This entire process led me to learn new skills and knowledge in statistical analysis, experimental design, and data collection. Conducting scientific research is stimulating and energizing. I plan to study environmental science, chemistry, biology and engineering as a foundation for future research. I hope to use my education to improve people's quality of life.

Research Section

Abstract

Recycling must increase as it becomes more costly to obtain raw materials from nature. With ammonia-derived fertilizer more expensive, many portions of the world will be unable to afford food. Low cost ways of generating ammonia are needed. The purpose of this project is to optimize production of ammonia and organic acids from the deamination of aspartic acid waste. Aspartic acid is a common component of protein wastes, such as sewage or offal. Such low cost waste can benefit from breakdown into more useful organic acids and ammonia. In this study, heated aspartic acid deaminated in solution to produce ammonia and malic, maleic and fumaric acids. An ammonium electrode was used to measure the amount of ammonia produced. Measuring ammonia derived from the deamination as a function of time and temperature allowed for the computation of rate constants. The aspartic acid was found to deaminate initially at a single rate constant at each of four temperatures. These rate constants were used to find an activation energy. A high pressure liquid chromatograph (HPLC) was used to determine the amounts of each of the organic acids produced. Although the deamination process approximated a normal reaction initially, there is evidence that a second competing process takes place over longer time periods. Nevertheless, the results show that it should be possible to optimize the production of ammonia while minimizing the degradation of the resulting useful acids. Low cost methods to produce ammonia for fertilizer may someday be important to global agriculture.

Introduction

This work addresses the question of how one can best obtain ammonia through hydrolysis of aspartic acid. More specifically, how can the deamination of aspartic acid be

optimized to produce a favorable mix of both ammonia and the resulting valuable organic compounds?

Our society is currently reliant upon nonrenewable energy sources. As petroleum grows more difficult to find, alternative processes that can produce carbon-based materials are becoming more vital. Recycling must increase as it becomes more costly to obtain raw materials from nature. The deamination of aspartic acid is a process that combines the best aspects of these two concepts. Aspartic acid can be a source of ammonia (for fuel and fertilizer) and several important deamination byproducts - malic, maleic and fumaric acids.

Aspartic acid is present in most protein wastes. This means it has the potential to be an important component in recycling processes. The aspartic acid deamination byproducts have many uses. Malic acid can be used in place of citric acid for sour flavoring in candy. Fumaric acid is used by cells to produce energy from food and its ester is often used to treat the disease psoriasis. In addition, its fruity flavor is commonly used to replace tartaric acid in beverages. Maleic acid, while not as popular as its isomer, fumaric acid, can be used as a reactant in Diels-Alder reactions to produce a cyclohexane ring for very little input energy¹¹.

Ammonia, a pungent colorless gas, is perhaps the most important chemical produced in the world today. The expansion of the world's population from 1.6 billion in 1900 to its current size of over 6 billion would not have been possible without synthetic production of ammonia. Fertilizer produced from ammonia has been necessary for increased food production and thus population growth. The chemist Justus von Liebig demonstrated that plant growth is limited when soil is lacking absorbable nitrogen⁸. Nitrogen in the air is useless to plants and is normally made available to them naturally through nitrogen fixation by soil bacteria and lightning strikes. Natural fixation of nitrogen is inefficient, incapable of supporting today's intensive farming². If

we had to depend entirely on natural nitrogen fertilizers, half the world's population would not survive. Less than 100 years ago the chemists Fritz Haber and Carl Bosch invented the first practical ammonia synthesis process using hydrogen and nitrogen³. Ready availability of synthetic plant nutrients dependent on this process transformed global agriculture over the past century. Only recently have difficulties started to appear in the synthetic production of ammonia. The cost of the input hydrogen has risen dramatically, due to the depletion and increased price of the natural gas (and coal) used to make it. With fertilizer becoming more costly, many portions of the world will be unable to afford food; which is why low cost ways of generating ammonia are needed.

Other amino acids are able to deaminate, but aspartic acid was chosen for this study because existing research papers led to the assumption that useful rate constants could be obtained¹. Qian, advises that in excess of 200C, amino acids decompose through paths other than deamination⁶. Li and Brill found that under conditions of high temperature and pressure glycine, leucine, isoleucine, valine, and alanine favor decarboxylation; while the rest of the amino acids deaminate⁴. Sato suggested that aspartic acid has a fast decomposition rate when compared with other amino acids and that hydrolysis enhanced this rate⁷. This previous research, while helpful, was limited to excessively high temperatures or enzyme-aided reactions. Thus, aspartic acid appeared to be a good material for further study.

A process that could make both commercially viable organic compounds and ammonia from protein waste is highly desirable. By measuring the various quantities of ammonia produced from the deamination of aspartic acid as a function of time and temperature, rate constants can be determined that will allow the derivation of activation parameters. These then allow the prediction of ammonia production for masses, times and temperatures other than those

measured. This information is useful for optimizing the amount of ammonia produced while minimizing the degradation of the resulting compounds.

Materials and Procedures

Typically, three samples of aspartic acid were prepared at a time. A small amount (about .5g) of Sigma 99% Pure Aspartic Acid was measured out and weighed using a Mettler AE100 Massing Device. Each sample was put into an empty stainless steel high-pressure Parr reaction vessel chamber first to avoid losing mass and 12mL of water was then added to each chamber. This number was based upon solubility guidelines to ensure that all of the aspartic acid would react. Each chamber top was reattached and firmly secured. To heat the samples at a constant temperature, a Fisher Scientific Convection Current Oven 825F was first heated to a preset temperature that was five degrees above the desired temperature. The oven was then opened and the vessels were hooked quickly onto the oven rack. The oven was closed and the temperature setting was lowered five degrees as the internal temperature of the oven would normally have dropped to the desired temperature by this time. The initial temperature used for study was 200C. The second, third and fourth experiment sets were conducted at lower temperatures (180°C, 160°C and 140°C) to avoid paths other than deamination⁶. The samples were heated for a predetermined time ranging from a half hour to eighty hours. When the time expired, the vessels were placed in an ice bath for cooling and then opened. These vessels were kept vertical throughout this process to prevent any accidental addition or loss of water. The contents of each vessel were decanted into separate labeled beakers. Each vessel was then flushed with 20mL of nanopure water to capture any remaining ammonia. This water was then added to the respective beakers.

Standard solutions of 1, 10, 100 and 1000 PPM ammonia were prepared from a standard solution to which sodium chloride was added. These solutions were measured with a Mettler Toledo Seven Multi Ammonium electrode to establish a calibration under the same conditions as the aspartic acid samples. These calibrations were remade every week. Three different concentrations (10, 100 and 1000 ppm) were initially considered adequate because all the data points fell within the range of the calibration curve and previous lab experience had shown that three data points produced satisfactory calibration accuracy. However, as the data range shifted lower, a 1 ppm standard was added. The standards were measured before every set of samples. The voltages from the ammonium electrode were recorded for each standard. A calibration chart of voltages vs. ppm ammonia was made and used for sample analysis. Similar calibration methods were used for a Hanna Instruments 8417 pH meter.

Following calibration of the ammonium electrode and pH meter, each sample solution was poured into a 100mL flask and 2mL of 6M sodium chloride were added. This was to ensure equal background ion concentration each time. The samples were then diluted to 100ml using nanopure water. The solutions were stirred and then placed back in their original beakers for testing. First the pH was measured. Because the ammonium electrode does not operate well at a lower pH, sodium hydroxide was added until the pH was above 4. Once the pH had been raised, the ammonium electrode measurement was performed. It had been previously established that the addition of NaOH did not affect the electrode calibration. The samples were stored in a laboratory refrigerator at about 5°C for further analysis.

A Hewlett-Packard 1050 DAD High Pressure Liquid Chromatograph (HPLC) System was used for further analysis on several processed samples to determine the amounts of

compounds other than ammonia. Based on Figure 1 (adapted from Sato⁷), malic, maleic and fumaric acids were used as standards because they were the expected byproducts.

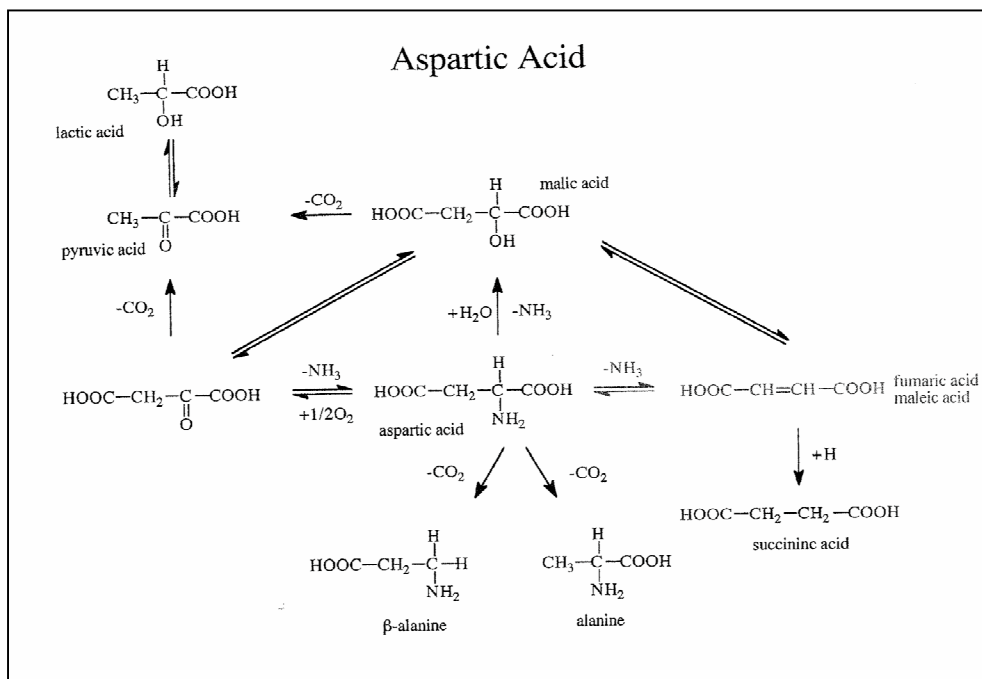


Figure 1:
Aspartic Acid
Reaction
Pathways

A Rezex ROA- Organic Acid column was used with a 0.01% phosphoric acid solution as a solvent. This column was chosen for its ability to separate organic materials. The low concentration of the phosphoric acid solution was used because any higher concentration of organic solvent would have caused the silica beads to swell. The column length necessitated keeping the pressure low. The flow rate was eventually set at 0.25ml/min with a temperature of 30C. Spectrophotometry of each of the standards was used to determine an ideal wavelength (both the UV and the visible spectrum were available) to use. All of the standards had a similar and maximum level of peak absorption near 200 nanometers. The lowest wavelength setting available on the HPLC, 200 nanometers, was the most error prone. Accordingly, a wavelength of 205 nanometers was used to improve the signal to noise ratio. Samples were centrifuged through a 0.22 micrometer nylon filter before they were injected. Aliquots of 10.0 microliters were used

to inject both the standard solutions and the heated samples. The samples were injected using a manual injection port. As a result, the retention times varied slightly. To accommodate this variation, an order of appearance for each of the standards was established using a standard mixture. This order was used to identify the compounds in the sample breakdown pattern. A small number of samples were selected for analysis to verify the reaction pathways.

Results

The data collected from the ammonium electrode were used to calculate the amount of ammonia produced under different experimental conditions (time and temperature). Data obtained from the ammonium electrode are shown in Figures 2 and 3.

Date	Grams Aspartic Acid	Temperature (C)	Time (minutes)	PPM Ammonia
7/6/2006	0.4998	200	120	361.155
7/6/2006	0.5000	200	120	351.577
7/6/2006	0.5089	200	120	350.006
7/6/2006	0.5002	200	60	334.675
7/6/2006	0.5001	200	90	351.819
7/6/2006	0.4997	200	60	315.488
7/6/2006	0.5004	200	90	340.811
7/11/2006	0.4998	200	90	333.801
7/11/2006	0.5001	200	90	341.460
7/11/2006	0.4995	200	90	330.786
7/12/2006	0.5000	200	90	318.870
7/12/2006	0.5001	200	90	344.570
7/12/2006	0.5002	200	90	344.570
7/12/2006	0.4997	200	120	361.245
7/12/2006	0.4998	200	120	351.322
7/12/2006	0.5009	200	120	340.089
7/13/2006	0.5000	200	120	335.180
7/13/2006	0.5002	200	120	317.310
7/13/2006	0.5000	200	60	309.927
7/13/2006	0.5000	200	60	313.126
7/13/2006	0.5000	200	60	297.456
3/16/2006	0.5000	200	60	316.816
3/16/2006	0.5000	200	60	309.885
7/17/2006	0.5000	200	155	376.306
7/17/2006	0.5000	200	155	350.003
7/17/2006	0.5000	200	155	369.551
7/18/2006	0.5000	200	150	374.650
7/18/2006	0.5000	200	150	358.080
7/18/2006	0.5000	200	150	369.600

Figure2: Measured Ammonia Concentration for 200C Preparations

Date	Grams Aspartic Acid	Temperature (C)	Time (minutes)	PPM Ammonia
7/22/2007	0.2998	180	90	110.657
7/22/2007	0.3007	180	90	103.589
7/23/2007	0.3005	180	120	161.489
7/23/2007	0.3003	180	120	152.084
7/23/2007	0.3001	180	120	154.847
8/6/2007	0.2998	180	85	74.101
8/6/2007	0.3002	180	85	84.964
8/6/2007	0.3003	180	85	96.096
8/6/2007	0.3003	180	85	91.603
7/18/2007	0.3005	180	1135	130.474
7/18/2007	0.3001	180	1135	130.474
7/18/2007	0.3001	180	1135	128.613
7/18/2007	0.3000	180	1135	124.972
7/17/2007	0.3005	160	120	23.132
7/17/2007	0.3003	160	120	22.422
7/17/2007	0.3003	160	120	24.619
7/17/2007	0.3000	160	120	25.716
7/25/2007	0.2999	160	180	69.703
7/25/2007	0.3005	160	180	71.330
7/26/2007	0.3002	160	85	12.274
7/26/2007	0.3005	160	85	9.467
7/26/2007	0.3005	160	85	10.146
8/1/2007	0.3001	160	120	21.049
8/1/2007	0.3001	160	120	18.739
8/1/2007	0.3002	160	120	17.262
8/1/2007	0.3004	160	120	15.367
7/26/2007	0.2998	160	4815	162.785
7/26/2007	0.2999	160	4815	151.021
7/26/2007	0.3002	160	4815	156.341
7/26/2007	0.3003	160	4815	175.465
10/27/2006	0.2997	140	120	11.426
10/27/2006	0.2998	140	120	13.067
10/27/2006	0.3001	140	120	11.873
10/27/2006	0.3003	140	120	10.997
10/27/2006	0.2999	140	180	21.406
10/27/2006	0.3002	140	180	18.188
10/27/2006	0.3003	140	180	21.716
10/30/2006	0.3003	140	300	46.279
10/30/2006	0.3003	140	300	43.029
10/30/2006	0.3003	140	300	51.619
10/30/2006	0.3003	140	300	41.491
7/19/2007	0.3006	140	144	2.867
7/19/2007	0.3005	140	144	2.179
7/19/2007	0.3003	140	144	2.299
8/1/2007	0.2999	140	917	32.388
8/1/2007	0.3000	140	917	28.636
8/1/2007	0.3001	140	917	28.636
8/2/2007	0.3002	140	1047	45.284
8/2/2007	0.3003	140	1047	45.908
8/2/2007	0.3004	140	1047	44.975

Figure3: Measured Ammonia Concentration for 180C, 160C, and 140C Preparations

The collection dates, starting masses, temperature, heating time and ppm evolved ammonia are indicated. The samples were initially taken in triplicate and later in quadruplicate to establish sample variation error.

In order to find the rate constant, the following calculation was instituted. The ppm values were first converted into their molarities through stoichiometry. Molarities were then compared with the expected maximums also based on stoichiometry.

The concentration dependence of the reaction rate¹⁰ takes the form

$$R=kA$$

Where R is the reaction rate, k is the rate constant (the units are seconds⁻¹) and A is the concentration of the reactant (aspartic acid). Since $R= dA/ dt$, integration gives $\ln (A) - \ln (A_0) = -kt$. In terms of reaction product, this becomes $\log ((\text{Conc.}-\text{Conc.}_\infty)/ (-\text{Conc.}_\infty))$ which has been plotted vs. heating times in Figure 4 for the four temperatures. The colors used in Figure 4 correspond to the colors in the data tables, Figure 2 and 3. Instead of plotting the individual measured data points, the data were averaged at each time interval. This increased the R² value for the trend line. A weighted trend line was plotted through the averaged data points; the slope (in red type) gives the rate constant. The standard error of the mean for each of the averaged data points was also calculated and indicated in Figure 4. The preferred rate constant for 200°C was $4.51\text{E-}05 \pm 2.24\text{E-}05$ seconds⁻¹, for 180°C was $2.22\text{E-}05 \pm 5.24\text{E-}06$ seconds⁻¹, for 160°C was $6.42\text{E-}06 \pm 1.44\text{E-}08$ seconds⁻¹, and for 140°C was $5.72\text{E-}07 \pm 1.44\text{E-}08$ seconds⁻¹.

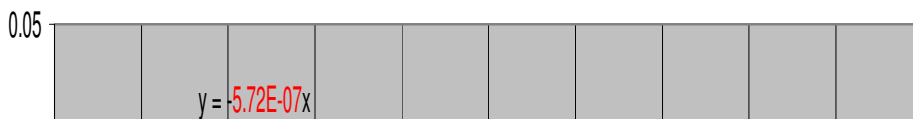


Figure 4: Ammonia Concentration at Four Temperatures

A quantitative expression for relating the rate constant to the activation energy was proposed by Arrhenius. The equation is

$$k = s e^{-\Delta H_a / RT}$$

where k is the rate constant, and both s and ΔH_a are constants. ΔH_a is the heat of activation or activation energy and s is called the frequency factor. The measured rate constants at several temperatures can be used to solve for s and ΔH_a which can then be used to estimate rate constants that would be obtained at other temperatures. If the log of this expression is taken, the resulting expression is in the form of the standard Arrhenius plot of $\log(k)$ vs. $1/T$. On this plot, the slope represents the heat of activation. The slope and the intercept are respectively ΔH_a and s . Figure 5 is the Arrhenius plot using the two sets of four rate constants determined above.

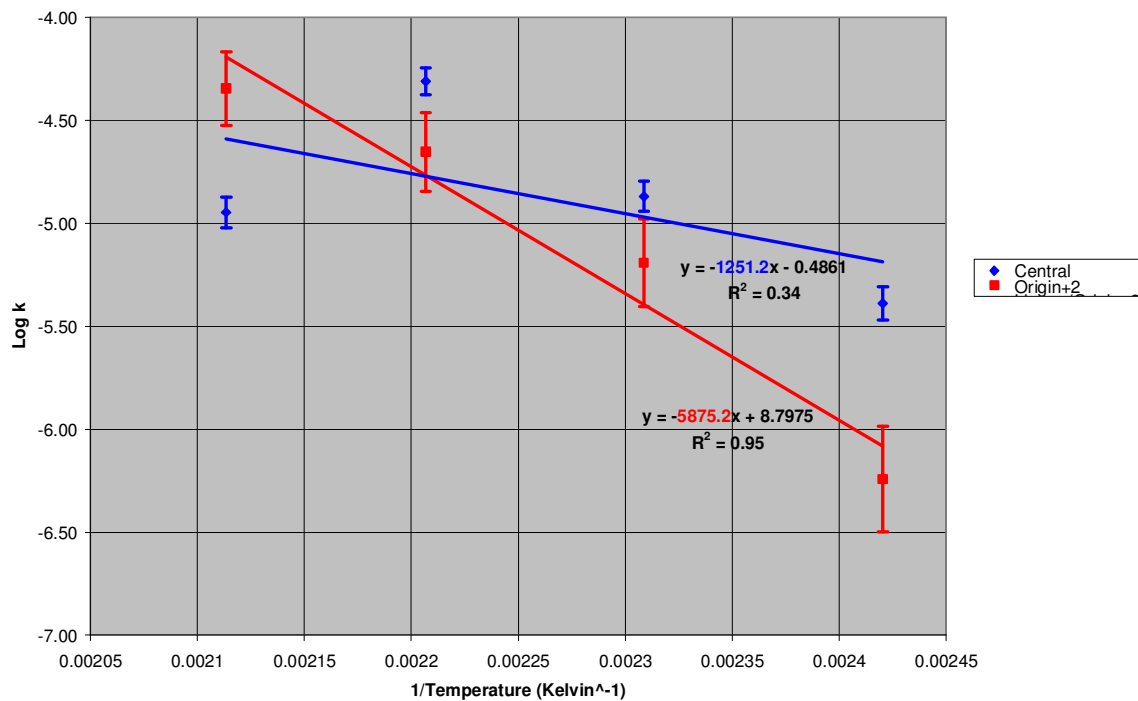


Figure 5: Arrhenius Plot

From the preferred trend line slope (labeled “Origin+2”) an activation energy of 112 kJ/mole is obtained, with an error of 34 kJ/mole. Spartan⁹ was used to estimate a theoretical value for the aspartic acid activation energy of 148 kJ/mole. The Spartan values for the activation energy are compared with those from the two sets of rate constants in Figure 6.

	Spartan	origin+2	center
Plot units	7730	5875	1251
kcal//mole	35.37	26.88	5.72
erg/mole	1.48007E+12	1.12489E+12	2.39531E+11
kJoule/mole	148.0074077	112.4894593	23.95307464

Figure 6: Predicted and Measured Aspartic Acid Activation Energy

The “Origin+2” set is in better agreement with the theoretical estimate than the “Central” set. In both sets, the 200C data shows evidence of an alternative reaction pathway.

From the HPLC it was possible to find the amounts of malic, maleic, aspartic and fumaric acid as well as to derive the amount of ammonia from those amounts. In general, not all of the aspartic acid was converted and the reaction appears to favor the production of compounds in the following order Fumaric>Maleic>Malic. Average results for two cases are shown in Figure 7.

	Mols Malic	Mols Maleic	Mols Aspartic	Mols Fumeric	Mols Ammonia
80 Hr Avg	0.0000146	0.0005805	0.0000114	0.0016490	0.0382176
Std Err	0.0000022	0.0000034	0.0000002	0.0000040	0.0000036
2 Hr Avg	0.0000314	0.0004074	0.0000735	0.0017432	0.0371600
Std Err	0.0000020	0.0000090	0.0000034	0.0000140	0.0000581

Figure 7: Averaged 160C Reaction Results from HPLC

Although standards were run, no standard peak absorbency calibration curve was used on the data because all standards had approximately the same absorbency and such a slight difference would not have significant impact on the results. To get these numbers a series of calculations was used. Each of the acids’ peaks was identified within each readout from each sample injected. The area count for each of these peaks was used to make a new normalized total area. Each individual acid’s area percent was found. These were then multiplied by the initial number

of moles (taken from the starting gram mass). To get the number of moles of aspartic acid that reacted, the aspartic acid moles found from the HPLC runs were subtracted from the starting aspartic acid moles. This number was greater than or equal to the number of moles of ammonia.

Discussion

The lack of agreement between the activation energy calculated from the four data points and the theoretical estimate suggests that more data (at different temperatures) should be collected to establish a larger set of rate constants. If the Arrhenius curve is linear below 140C, then it is likely that 200C represents an upper limit for the process model to be valid. Repeating some of the data points would also confirm the nonlinear behavior suspected here.

For a certain portion of the data, the nanopure water contained an unknown plastic ion. This ion was found towards the beginning of the study, when the nanopure water was tested using an LC//MS. The test showed no major impurities except for a large peak in a location common to plastic ions. Because of the plastic ion peak, the nanopure water was replaced and tested (pure) for the remaining measurements. It is believed that the plastic ion did not react with the samples because several of the samples from before and after were tested in the LC/MS. These samples showed no significant difference in composition.

During the beginning of the investigation occasionally one or two of the vessels would leak. The leakage was obvious because everything evaporated. This was more of an annoyance than an experimental effect since there was nothing left to analyze or record. Accordingly, Figure 4 does not always show triplicate or higher sample numbers at a given heating time. A new gasket approach for the vessels was instituted which stopped any further leaks.

It was hypothesized that a temperature-dependent rate constant can be found for the decomposition of aspartic acid, which would allow control over the production of ammonia.

Two different approaches were used to derive rate constants. In the first approach, the data sets of Figures 2 and 3 were fit with a rate constant function. The rate constants obtained from this did not produce a satisfactory Arrhenius plot (Figure 5, data labeled “central”). This problem may have occurred because a competing reaction was taking place at 200C. This was confirmed with some long heating time measurements (not shown) that demonstrated a decreased ammonia production relative to the central measurements. Accordingly, the second approach was to use only the first two time intervals for each and supplement this with the supposition that the curve had to go through zero at zero time. This data set is labeled “origin + 2”. These are the data points that were ultimately used and they gave a much more consistent result for the activation energy shown in Figure 5. The new data demonstrated that for each temperature ammonia was evolved at a rate that could be fit with a rate constant function. These rate constant functions did produce a good fit to the Arrhenius curve.

It appears that the samples processed at the highest temperature (200C) were not producing ammonia at an expected rate. One possible explanation is that the aspartic acid at 200C was decomposing or decarboxylating as opposed to deaminating. It was suggested that this reaction might occur by Qian⁶. The data from the lower temperatures, which agree with the theoretical model, is accordingly believed to represent more useful information. With the added caution of operating below a temperature limit, the hypothesis was thus proven.

The HPLC measurements are inconclusive and do not agree with the electrode data. The HPLC data indicates more ammonia was produced than indicated by the electrode data. This may be indicative of a competing reaction; the amount of ammonia was calculated under the assumption that none of the aspartic acid was decarboxylating or decomposing. The column used was not selected to measure these other possible products, so it is not surprising that they were

not detected. As another interesting point, it was noted that the HPLC always found leftover aspartic acid. This is consistent with results from the electrode measurements in that theoretic maximums were never reached.

Conclusions

Aspartic acid deamination is a complex process which under some conditions follows linear decomposition rules. The process at higher temperatures and longer reaction times appears to be of a higher order. The linear portion of the deamination curve has been determined, as well as some initial estimates of the nonlinear portions at the higher temperatures and longer times.

Using an HPLC to determine an ion concentration is an experimental method in this application and is completely secondary to any data found from the ammonium electrode. While this method does have the potential to provide more information than an ion specific electrode (because it is also possible to determine the percentage of multiple constituents), it is not supported by reproducible data or any literature. The experience here has been that it can fail if the reaction is too complex. If other molecules are produced in the reaction which can not all be measured by a particular HPLC column then the procedure of subtracting molar amounts has too many unknowns.

These results indicate it should be possible to refine the deamination process to optimize the production of ammonia while minimizing the degradation of amino acids. Careful attention must be paid to temperatures and heating times. The deamination curve is more complex than originally thought and further analysis would better define the nonlinear shape of the curve.

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