LABORATORY REPORT

GROUP NUMBER: w3

EXPERIMENT NUMBER: 4

TITLE: Acid-Base Chemistry – Titration of an Amino Acid

DATE: March 3, 2000.

OVERALL REPORT GRADE 4.0

THIS IS A VERY POOR REPORT. READ THE COMMENTS AND COME SEE ME.

ROLE ASSIGNMENTS

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ABSTRACT

A series of three acid-base titrations were conducted: HCl-NaOH**,** Na_2CO_3-HCl, and Alanine-NaOH; two pK_a's were determined for the alanine: pK_{a1}=2.43 with a confidence interval of 0 (this will be explained in the analysis) and pK_{a2} = 8.32, confidence interval of 0.28 as determined from the 95% Confidence test. Titration curves were constructed by titrating the sample with either NaOH or HCl, reading the pH meter at every interval and recording the data points in order to make semi logarithmic WHY? plots of pH versus moles of titrant. NOT NEEDED IN SUMMARY NaOH and HCl were restandardized and their concentrations were 1.00±0.05M. The manufacturer's nominal value for both were 1.000±0.005M. Though the manufacturer's values have a smaller uncertainty, they are essentially the same, our value of 1.00M lies within any possible confidence interval from the manufacturer that is centered around 1.000M. In order to perform a regression analysis, the original graphs of pH versus mol of titrant were modified so that a linear region could be determined. An altered form of the Henderson-Hasselbach equation was used (log([A^-]/[HA]) versus pH in order to obtain a linear relationship for the determination of the pK_a's of the amino acid.

A LOT OF EXTRANEOUS STUFF INDICATED IN YELLOW. YOU DO NOT GIVE THE LITERAURE PKA VALUES FOR THE AA WHICH IS YOU ACCURACY COMPARISON

GRADE FOR SUMMARY PAGE 1.5

RESULTS

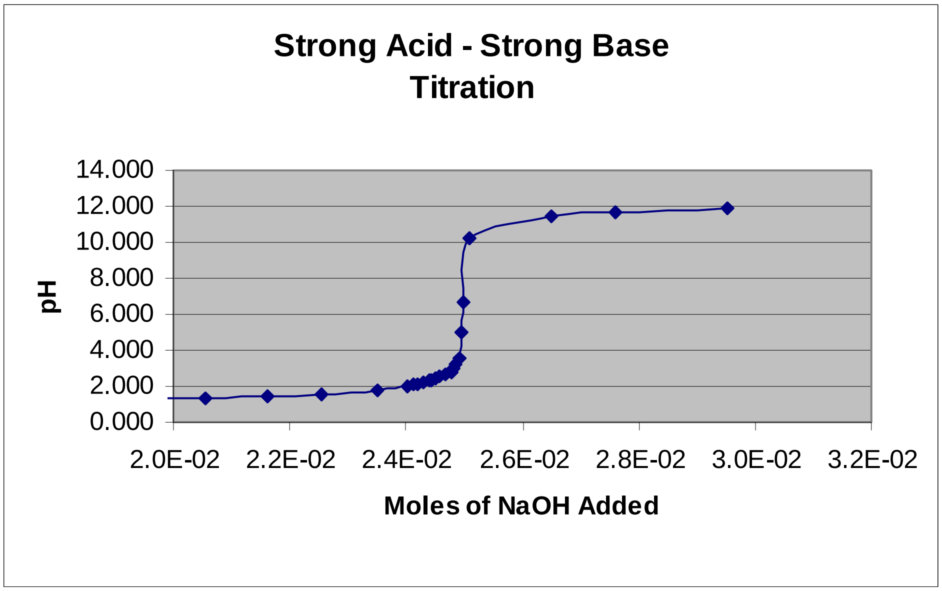
Table 1. Molarities of Secondary Acid and Base Standards

	Experimental Value (M)	Nominal Value (M)
HCl	1.00 +/- 0.05	1.000 +/- 0.005
NaOH	1.00 +/- 0.05	1.000 +/- 0.005

The experimental value obtained for the molarities of the secondary acid and base standards was 1.00 ± 0.05 M for both the HCl and NaOH. This value was obtained in two steps. First, an acid-base titration of HCL with NaOH revealed that 10.0 mL of NaOH was required to titrate 10.0 mL of HCl to the endpoint, which yields a ratio of 1.0. The only I DISAGREE uncertainty in this step came from reading the pipettes (± 0.05 mL).DO YOU MEAN BURETS? After determining this ratio, the Na_2CO_3 primary standard was titrated to find the experimental values for the secondary standards (which are equal because of their 1-to-1 ratio. The calculation is as follows: (1.3252 g Na_2CO_3)*(1 mol Na_2CO_3/105.99 g Na_2CO_3)*(2 mol NaOH/1 mol Na_2CO_3)(1/0.2491 L NaOH) = 1.00 ± 0.05 M. I HOPE YOU MEAN HERE MOL AND L OF HCL, NOT NAOH! SINCE SODIUM CARBONATE IS A BASE I DOUBT IF YOU COULD NEURALIZE IT WITH NAOH SLOPPY! The manufacturer's nominal value was 1.000 ± 0.005 M.

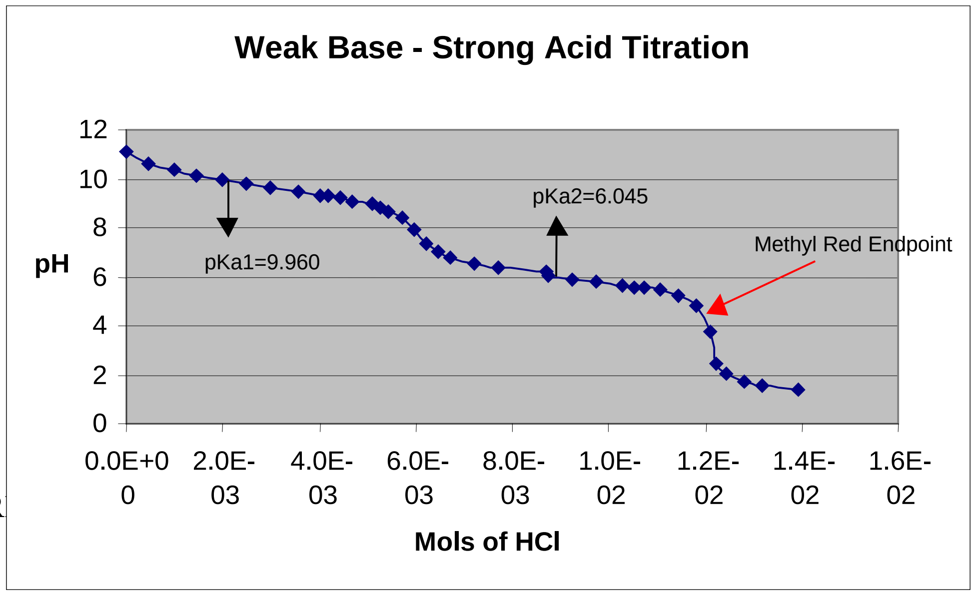
Figure 1. HCl-NaOH Titration Curve

WHERE?



A titration of HCl with NaOH revealed that 2.5E^{-2} moles of HCl was neutralized after 2.5E^{-2} moles of NaOH has been added. This value corresponds exactly to the end point as determined by the phenolphthalein indicator.

Figure 2. Na_2CO_3-HCl Titration Curve



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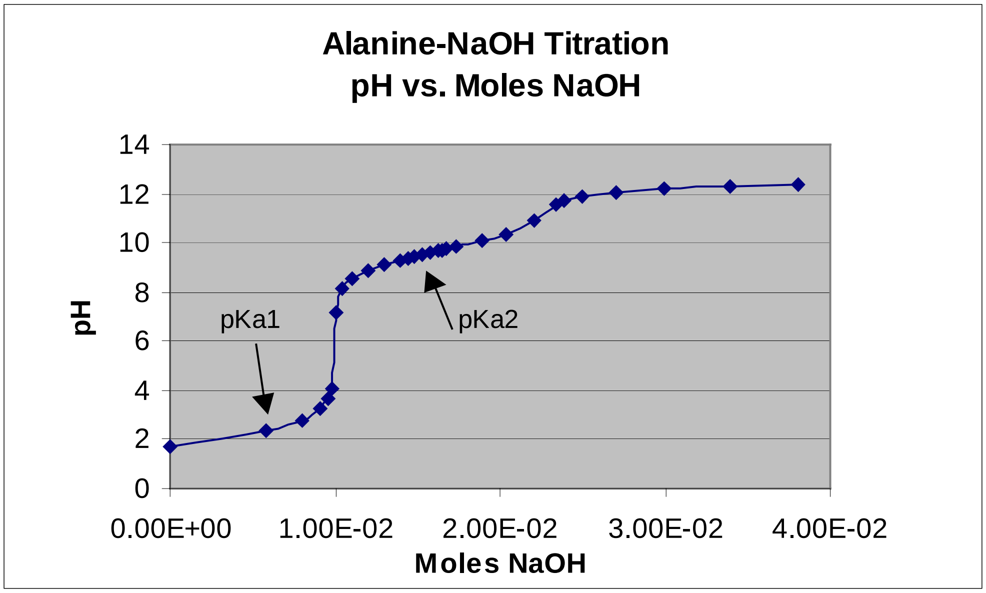
For the titration of Na_2CO_3 with HCl, $pKa_1 = 9.960$ and $pKa_2 = 6.045$. The red arrow indicates the value of the Methyl Red endpoint, which occurs between pH 4 and 5.

Figure 3. Alanine-NaOH Titration Curve

THIS IS UNACCEPTABLE. DID YOU NOT CHECK TO MAKE SURE YOU HAD INCORPORATED THE GRAPHS?



Arrows indicate the pKa values of the alanine amino acid titration with NaOH using a methyl red indicator, WHY DID YOU USE INDICATOR IN THIS TITRATION? IT DOES NOT CORRESPOND TO ANY REGION OF INTEREST pKa_1= 2.43 confidence interval of 0 (explanation in analysis) and pKa_2= 8.32 with confidence interval of 0.28. In order to perform a regression analysis, the original graphs of pH versus mol of titrant were modified so that a linear region could be determined. An altered form of the Henderson-Hasselbach equation was used (log([A^-]/[HA]) versus pH in order to obtain a linear relationship for the determination of the pK_a's of the amino acid. The plot thus constructed (Y vs. pH) follows in Figure 5. Several regression analyses were performed to determine the linear regions in the graph. This is done by applying the 95% confidence interval test for the determination of the best confidence interval.

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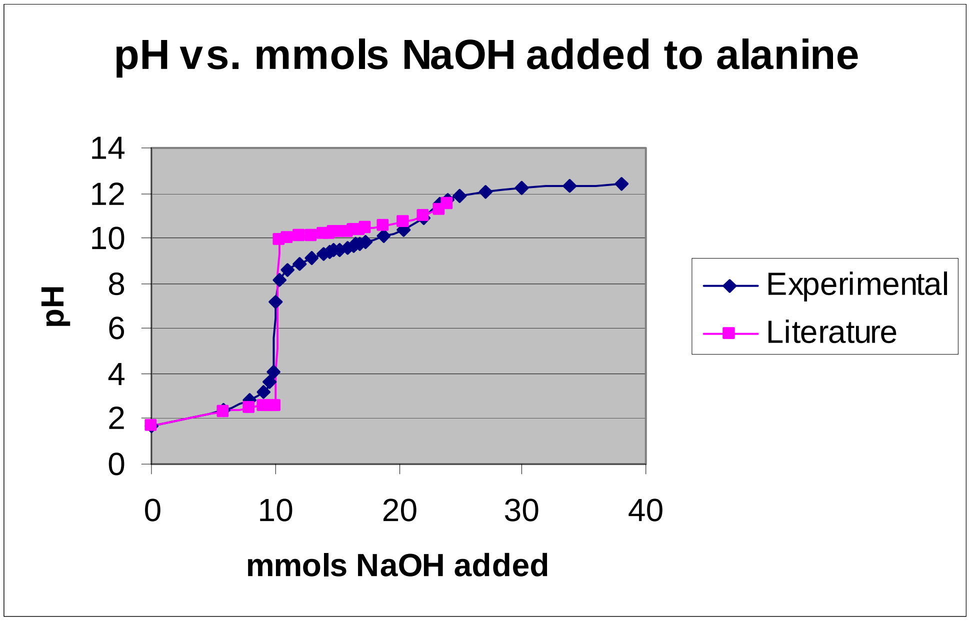
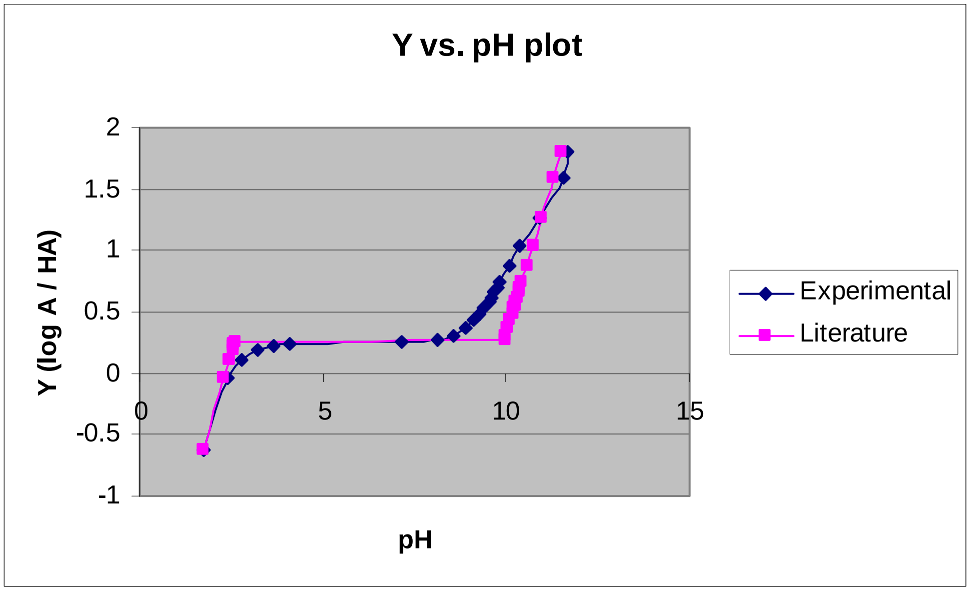
Figure 4: Titration of Alanine with NaOH: pH vs. mmols NaOH added (comparison of experimental and literature values)



Experimental alanine titration curve against literature titration curve is plotted above. The literature titration curve was plotted using the two given pK_a values of 2.35 and 9.69 as a basis. The graph was constructed keeping the x values (mmols of NaOH added) at the same value as the experimental while the y-values (pH) was calculated using the given pK_a values. Points of n=1-6 (where n indicates the nth point) were plotted using pK_a of 2.35, and that of 7 to 20 were plotted using pK_a of 9.69. The literature titration curve has sharp angles because pK_a values can only determine the shape of the graph around where the pK_a would be taken, or the areas roughly in the center between two end points. PK_a values cannot be used to construct a curve that represents the area around the end points. WHY NOT?

It can be seen from this graph, that the titration curve around the first pKa is approximately equal in the two curves, that of the second pKa is very different. The slope around the second pKa is much higher (steeper) in the experimental than in the literature. To quantitatively account for this difference, the Y vs. pH plot for the two values are drawn and presented in Figure 5.

FIGURE 5. Titration of Alanine with NaOH: Y vs. pH (comparison of experimental and literature values)



As stated above, the literature titration curve on this graph has sharp angles because pK_a values can only determine the shape of the graph around where the pK_a would be taken. The two graphs have similar regions around pK_{a1} but very different regions around pK_{a2}. Using regression analysis, the slope of the curve around the pK_{a1}, for the experimental data is calculated to be –0.889±0.00. That of the literature data is –2.35 (exactly). The slope of the curve around pK_{a2} for the experimental data is calculated to be 0.493±0.01. That of the literature data is 1 (exactly). This amounts to errors of 11% and 50% on the slope of the graph, respectively.

HOW CAN I JUDGE WHAT YOU HAVE DONE WITH NO RESULTS? AT THIS POINT IN THE COURSE THERE IS NO EXCUSE FOR SUCH SLOPPY A SUBMISSION

GRADE FOR RESULTS SECTION 1.0

ANALYSIS

The experimental values for the concentrations of the secondary standards, HCl and NaOH, were 1.00 ± 0.05 M for both solutions. The manufacturer's nominal value for both was 1.000 ± 0.005 M. The experimental values are the same as the manufacturer's nominal values and thus, by definition, are not significantly different (i.e., our value of 1.00 M lies within any possible confidence interval from the manufacturer which is centered around 1.000 M). However, the manufacturer was able to obtain smaller uncertainty than was possible in this experiment, due to the use of large volumes of chemicals and more precise instruments than 50 mL pipettes. HOW DO YOU KNOW? DID YOUCALL THEM UP? IF NOT, DO NOT SPECULATE

YOU ARE THE ONLY GROUP TO HAVE DONE SO WELL ON THE STADARDIZATION, NOT ONLY THIS TERM BUT IN ALL PENN BE HISTORY. IT WOULD BE NICE TO KNOW YOUR SECRET SO WE CAN INCLUDE IT IN FUTURE EXPERIMENTS. YOU GIVE NO ANALYSIS. WHERE ARE YOU ORIGINAL DATA? DID YOU DO MORE THAN ONE TRIAL ON THE SODIUM CARBONATE TITRATION? WERE THEY ALL IDENTICAL?

The HCL-NaOH titration curve from the experiment begins at a low pH (1.374pH) that indicates a high concentration of H+ ions. When a volume of 24.51ml of NaOH is added to the HCL, which was originally 25ml of 1M solution, the pH rises from 2.428 to a value of 10.172 at a volume of 25.09 the change in pH with respect to the change in volume is 7.744 : 0.58mL. The strong acid - strong base titration in the appendix also increases approximately 8pH units over a very small volume change. Being that there is only one pKa for both graphs, WHAT DOES THIS MEAN? WHERE IS THERE A PKA?they are both titrations of strong monoprotic acids with one to one molar ratios with their respective base.

The Na2CO3 titration graph in the appendix of the lab manual[1] is a titration of carbonic acid with NaOH. In the case of this lab however, Na2CO3, a weak base, is titrated by HCL. The graphs however both yield the same information, when the carbonic acid is being titrated by a strong base the graph starts at an acidic pH (around 4) and raises to a basic value of about 13. In the lab, the Na2CO3 was titrated by HCl and because of this the graph is reversed form that found in the appendix, meaning that it begins at a basic value (13pH) and falls to an acidic pH of 1.5. The pKa 's for the two graphs are reversed, pKa1 for one graph is pKa2 for the other. NOT TRUE- NAMING THE PKAS HAS NOTHING TO DO WITH HOW YOU DO THE TITRATION The appendix graph has pKa1 and pKa2 values of 6.38 and 10.38 respectively. The lab data graph yields a pKa1 of 9.960 and pKa2 of 6.045. Understanding the revered relationship of the two graphs, the pKa1 value obtained in lab deviated for the book value by 5.25%. Similarly, the pKa2 value obtained in lab differs from the book value by 3.49%. The methyl red endpoint signifies the point at which all of the Na2Co3 has become H2CO3. Every point before with the exception of the initial start of the titration has been an acid base mixture. TO GET THE RESULTS YOU CLAIM YOU HAD TO HIT THE CARBONIC END POINT RIGHT ON. THIS HAS NOTHING TO DO WITH THE PKA WHICH IS NOWHERE NEAR THE ENDPOINT.

The pK_a values of alanine were determined to be pK_{a1}=2.43 with a confidence interval of 0 THERE IS NO WAY YOU CAN GET A CI OF 0. I CAN ONLY CONCLUDE YOU DO NOT UNDERSTAND WHAT YOU ARE REPORTING. and pK_{a2} = 8.32, confidence interval of 0.28 as determined from the 95% Confidence test. Literature values for alanine's $pK_{a1}$and pK_{a2} are 2.35 and 9.69, respectively. Experimental values are 2.43 and 8.32, respectively.[2] The precent error's are 3.29% for pK_{a1} and 14.14% for

[1] Litt, Mitchell. Acid-Base Titrations: Titration of an Amino Acid. *Bioengineering Laboratory Manual*. p. 14, 2000

[2] Litt, Mitchell. Acid-Base Titrations: Titration of an Amino Acid. *Bioengineering Laboratory Manual*. p. 12, 2000

pK_{a2}. NO- YOU HAVE NO ERROR IF YOUR CI IS 0. YOU ARE INFINITELY INACCURATE IF THE STANDARD IS THE LITERATURE VALUE SINCE YOU SAY YOUR VALUE HAS NO UNCERTAINTY. The experiment pK_{a1} in comparison to the literature value is very similar, NOT SO- YOU CANNOT CLAIM INFINITE PRECISION AND THEN SAME VERY SIMILAR. however the second pK_a values doe not show much resemblance to the literature. This will be discussed subsequently.

A regression analysis was conducted on the first three points of the graph WHAT GRAPH? yielding a confidence interval of 8.14. This confidence interval was not suitable because the last point that was included for the test does not even lie in the linear region of the curve, therefore cannot be included. Subsequently, a regression analysis was done using the only two points in the linear region of the curve. This was technically not possible to do since regressions cannot be done on two points. However, there were no other points in the linear region; therefore, a true regression analysis could not have been accomplished, thus a 0 confidence interval was determined. In order to obtain an accurate confidence interval for this first region of the curve, more points should have been recorded along with their pH values. YOU MEAN YOU COULD NOT DETERMINE A CONFIDENCE INTERVAL- THIS IS MUCH DIFFERENT FROM SAYING IT IS ZERO. YOUR PRESENTATION IS INCOHERENT, OBVIOUSLY INCORRECT, AND SHOS LITTLE UNDERSTANDING OR ABILITY TO ANALYZE WHAT IS GOING ON. VERY DISAPPOINTING AT THIS STAGE. IF YOU WERE SO CONFUSED WHY DID YOU NOT REQUEST ASSISTANCE?

In the second linear region of Figure 5, a pK_{a2}=8.32 was yielded. The confidence interval for this pK_{a2} is 0.28. The same method was utilized—testing different points on Figure 5 in order to determine the best confidence interval. In order to obtain this confidence interval points (8.90, 0.31 through 11.51, 1.60) were tested. HOW MANY THIS TIME? Other confidence intervals included: (8.90, 0.37 through 11.60, 1.80 yielded 0.57 confidence; 9.30, 0.52 through 10.37, 1.03 yielded 0.34 confidence); however, as shown one can see that the region between points 8.90, 0.31 through 11.51, 1.60 yield the finest confidence interval.

There are a number of errors that must be accounted. First, the pH meter has difficulty reading pH's in this portion of the pH scale. Basic solutions tend to dissolve the glass, thus the pH meter gives very unstable reading around any pH higher than 9. This was seen when the calibration of the pH meter was done in the first part of the experiment when the pH of the buffer was 10. The meter had a difficult time stabilizing in this area. Such error is only minimal, however. When the pH meter was used to read the pH 10 buffer, a value of 10.03 was read instead of 10.00. This amounts to 0.3% error.

Second, there is an uncertainty on the 50-mL pipette, corresponding to an error of ± 0.02 mL for each reading. AT THIS POINT IN YOUR CAREER, DO YOU NOT KNOW THE DIFFERENCE BETWEEN A BURET AND PIPET? DURING THE EXPERIMENT, DID ANYBODY EVER CALL THAT THING A PIPET?

Neither of these errors, however, can account for the grave experimental percentage error of 14.14% for pK_{a2}. THAT IS CERTAINLY TRUE The most probable explanation for this error is systematic human error, arising from the mistake of not waiting long enough for the pH meter to stabilize. When the pH meter was used to read the pH 10 buffer (giving a value of 10.03 instead of 10.00, as described above), the experimenter waited approximately 30 seconds, during which time the value slowly climbed up to 10. During the course of the actual experiment, however, the experimenter waited an approximately 5 seconds only due to time constraints. As such, it is probable that the pH meter was not able to stabilize and reach its final reading before the reading was taken. This would account for the readings which are consistently below what the literature value would dictate.

In conclusion, three acid-base titrations were conducted and both pK_a values for alanine (pK_{a1}=2.43 with a confidence interval of 0 and pK_{a2} = 8.32, confidence interval of 0.28 as determined from the 95% Confidence test) were determine using a liner regression method and modifications of the Henderson-Hasselbach equation. Restandardazations of both the HCl and the NaOH were done yielding concentrations of 1.00+0.05M for both and were then compared to the nominal manufacturer values of 1.000$\pm$0.005M for both the acid and the base. Our value of 1.00 M lies within any possible confidence interval from the manufacturer which is centered around 1.000 M, thereby definition is not significantly different.

Both educational goals were met. Quantitative measurements of pH using modern instrumentation (pH meter) and classical acid-base indicators (methyl red, phenol red, and phenolphthalein) were used in each titration. Acid-base properties of a biochemical electrolyte, alanine, were studied by use of titration techniques. Additionally, measurements of important equilibrium properties of the reactions involved were studied and analyzed by the techniques developed in the experiment.

I MUST DISAGREE THAT EDUCATIONAL GOALS WERE MET, BASED ON THE ERRORS AND CONFUSION IN YOUR WRITEUP. PLEASE SEE ME ABOUT THIS BEFORE STARTING YOUR PROJECT SO WE CAN TRY TO IMPROVE MATTERS. I CAN ONLY SPECULATE THAT THIS WAS A HURRY-UP JOB WITH NOT MUCH THOUGHT OR EFFORT EXPENDED.
ANALYSIS SECTION GRADE 1.5

REFERENCES

Litt, Mitchell. Acid-Base Titrations: Titration of an Amino Acid. *Bioengineering Laboratory Manual*. p. 12, 2000