Chemistry HL Internal Assessment

Research question: How does angle of rotation of an amino acid (L-leucine) change with variation in pH in an aqueous medium?

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RESEARCH QUESTION

How does angle of rotation of an amino acid(L-leucine) change with variation in pH in an aqueous medium?

INTRODUCTION

Proteins are important biomolecules that contribute to structure, communication and transport to perform certain functions[1]. These polymers are made of 2-amino acids which are the monomer units. They consist of an amino group (-NH_2), a carboxylic acid group(-COOH) and an 'R' group(or H atom) bonded to the same Carbon atom. Since the Carbon chain is numbered starting from the C in the carboxylic acid group, the amino group is attached to the 2nd Carbon hence giving the molecule the name 2-amino acid. About 20 different amino acids are found to be present in naturally occurring proteins and are covalent in nature which also contain charged groups which result in the acid-base behaviour; hence termed as a zwitterion.

In aqueous medium, amino acids exist as zwitterions (with positive and negative charges). The pH of this state of amino acid, where it is electrically neutral, is known as the isoelectric point. The amino acid acquires a positive charge when pH is decreased below this point and a negative charge when increased above this point. Also, the 2nd carbon in 2-amino acids is a chiral centre because it is attached to 4 different groups (except in glycine) and will thus show optical activity.

The charge and structure of the molecule will affect the optical activity of the molecule[2]; I wanted to find out the how the angle of rotation of an amino acid would vary with variation in pH, hence charge. I have chosen to experiment on the amino acid: L-leucine, as it is easily available and its isoelectric point is 6.04 which is fairly close to 7.00. It would be easy to comprehend the variation of pH on either side of this isoelectric point.

In my experiment, I prepared a 2% solution of L-leucine in distilled water. I added drops of 0.1M HCl or 1M NaOH to aliquots of the 2-amino acid solution to attain required pH values and measured their angle of rotation with respect to an optically inactive solvent: distilled water.

BACKGROUND KNOWLEDGE

The general structure of an amino acid is as shown in *Figure 1(a)* below. The 2nd Carbon makes 4 covalent bonds out of which 3 are always the same, which are: a hydrogen atom (H), an amino group (NH_2) and a carboxylic acid group (COOH). The 20 common amino acids differ

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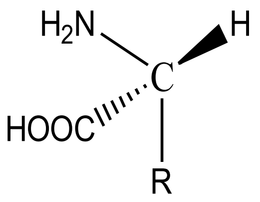
[1] Brown, C., & Ford, M. (2014). *Higher Level Chemistry* (2nd ed.). Edinburgh Gate, Harlow: Pearson Education Limited.

[2] Dill, D. (2008, January 22). Atoms, light, and their interaction. Retrieved from http://quantum.bu.edu/notes/GeneralChemistry/AtomsLightAndTheirInteraction.pdf

from each other because of Carbon's 4th covalent bond- the R side chain which decide the shape of their protein and behaviour of amino acids in different environments.[3]

An amino acid can be predominantly acidic, neutral, or basic as it has an amino group providing basic properties and a carboxylic acid group providing acidic properties. Thus usually, the amino group gains a positive charge while the carboxylic acid group holds a negative charge. In an aqueous solution, an amino acid molecule is neutral although it has different charge, and is known as a zwitterion. The amino acid molecules are also referred to as internal salts due to the interaction between the basic amino and acidic carboxylic acid group. A proton(H^+) is transferred from the acidic carboxylic acid group to the basic amino group. Oxygen attached to the hydrogen in COOH hence gains a negative charge and N in $-NH_2$ group gains a positive charge.



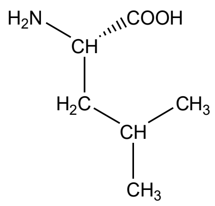


Figure 1(a): General structure of an amino acid.   1(b) Structure of L-leucine.

Amino acids(except glycine are optically active as the 2nd Carbon atom in an amino acid is a chiral centre thus providing for no plane of symmetry in the molecule. This allows for change in orientation of incident light, hence showing optical activity.

I am evaluating the optical activity of the amino acid L-leucine by looking into the angle of rotation of the amino acid for different values of pH. The angle of rotation indicates the shift in plane of plane polarised monochromatic light, for a constant distance and concentration of a product as light passes through a solution of this compound.

When the pH of L-leucine is lowered below its isoelectric point it behaves as an acid and attains a positive charge because the $-COO^-$ group in the zwitterion will gain a proton(H^+) to form a cation and $-NH_3$ will have a positive charge. When the pH is increased above isoelectric point, the zwitterion behaves as an base to have a negative charge(at COO^-) because $-NH_3{}^+$ will lose a H^+ to give a $-NH_2$ group[4]. This amphiprotic nature results in change in orientation and size of electron cloud of the amino acid molecule which will definitely affect the angle of rotation of the molecule. The electron cloud of the molecule plays in important role here because[5] light interacts with the electron cloud upon passing through the

[3] Bylikin, S., Horner, G., Murphy, B., & Tarcy, D. (2014). *Chemistry: course companion*. Oxford: Oxford University Press.
[4] MyTutor. (n.d.). How do amino acids change at different pH? Retrieved from https://www.mytutor.co.uk/answers/6730/A-Level/Chemistry/How-do-amino-acids-change-at-different-p-H/
[5]Dill, D. (2008, January 22). Atoms, light, and their interaction. Retrieved from http://quantum.bu.edu/notes/GeneralChemistry/AtomsLightAndTheirInteraction.pdf

solution. Thus I decided to vary the pH of L-leucine in order to determine its change in angle of rotation.

HYPOTHESIS

Under acidic conditions, the angle of rotation of L-leucine would decrease as the acidity the molecule decreases. This is because the positive charge of L-leucine increases, which causes a reduction in the size of its electron clouds.

Under alkaline conditions, the angle of rotation of L-leucine increases as alkalinity of the molecule increases. This is because the negative charge of L-leucine increases along with the size of the molecule's electron clouds.

METHOD

Equipment:

1. Two 250×10^{-3} dm^3 standard flasks: one with 0.1M HCl solution and another with 1M NaOH solution,
2. Dropper
3. Leucine in powdered form
4. Distilled water
5. Laurent's half shade polarimeter which has a least count of 0.01°
6. Low pressure sodium vapour lamp
7. A pH probe of uncertainty 0.01
8. Vernier lab quest
9. Glass rod for stirring

Experimental setup:

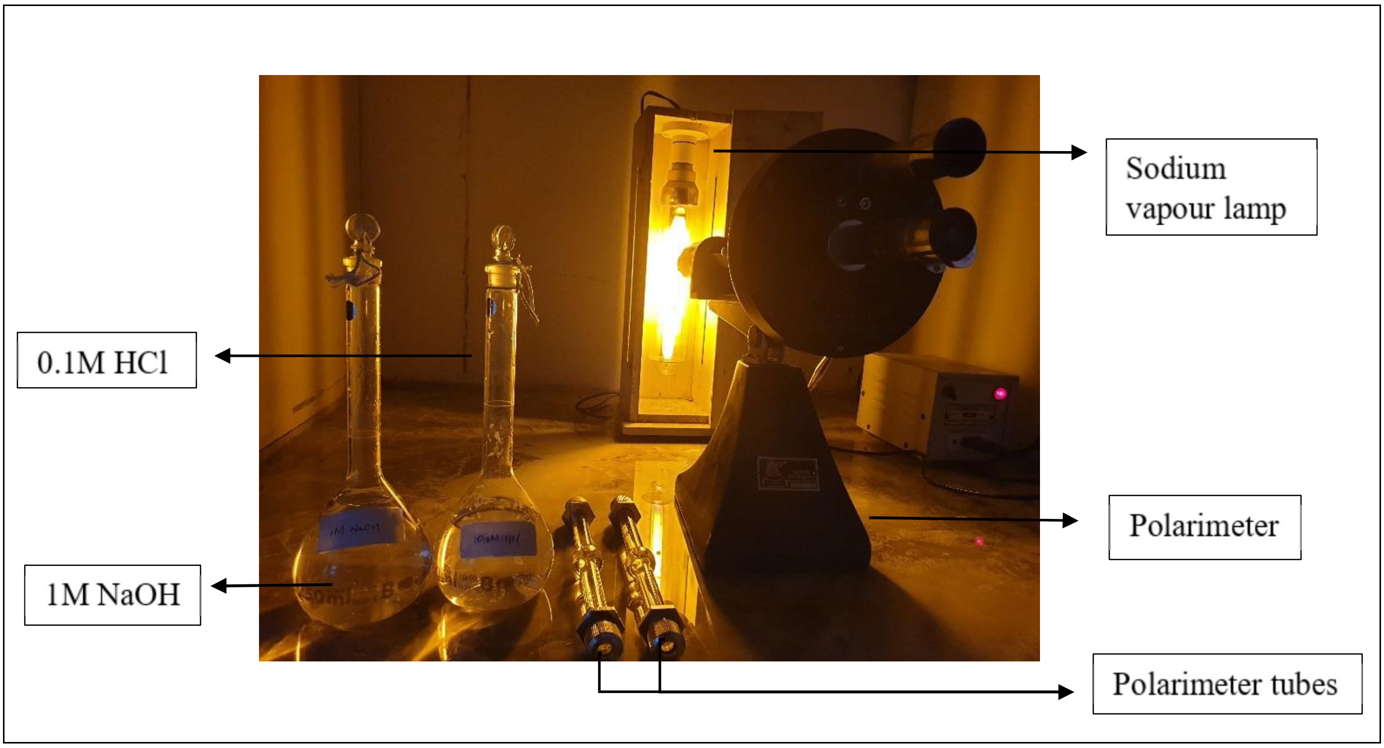


Figure 2:Picture of the actual experimental setup

Variables:

Independent variable: pH of amino acid(leucine) solution.

Since amino acid is an optically active compound, its structure decides its angle of rotation. The carboxylic acid usually gets deprotonated while the amino group is protonated, resulting in a polar amino acid molecule with charge separation. Under acidic conditions, the amino acid gains a positive charge because the amino group is protonated and under basic conditions the carboxylic acid group is deprotonated, causing the amino group to have a negative charge[6]. This charge separation definitely affects the orientation of the molecule due to electron-electron repulsion which will in turn affect the plane by which light is rotated. Hence I am varying the pH to experimentally determine its effect on specific rotation.

[6] MyTutor. (n.d.). How do amino acids change at different pH? Retrieved from https://www.mytutor.co.uk/answers/6730/A-Level/Chemistry/How-do-amino-acids-change-at-different-p-H/

Dependent variable: Angle of rotation of leucine.

It is a property of optically active compounds that depends on the structure of the molecule that incident light passes through. I am measuring this quantity as I vary the pH of leucine which will affect the orientation of groups present in the molecule.

Controlled variables: Length of tube, Distance between source of light and polarimeter, Type of plane polarised light passing through solution, Concentration of solution, Amino acid used.

The above variables need to be kept constant to ensure similar results in future experiments. The type of plane polarised light which is incident on the solution of L-leucine must be kept constant as the plane of different plane polarised light is rotated differently by a solution. The concentration of solution must be constant too to ensure the number of molecules incident light passes through is constant.

The amino acid is kept constant as different amino acids will have different orientations on charge variations as each one's structure and composition is different. Also, I chose to work with leucine as its isoelectric point is close to 7.0, making it easier to collect a set of readings for pH values on either side of 7.0 .

<u>Method I used:</u>

1. I prepared 0.1M solution of HCl and 1M solution of NaOH in a 250×10^{-3} dm^3 standard flask. These solutions are used to vary the pH of the solution of leucine by adding the required quantity.
2. I then took 5g of leucine to dissolve it in distilled water in a standard flask of volume 250×10^{-3} dm^3 .
3. The pH of this solution was measured. This pH is the value at which L-leucine is a zwitterion.
4. Keeping the above solution aside, I filled the polarimeter tube with distilled water carefully to ensure the absence of air bubbles in the tube to increase the accuracy of the readings.
5. I placed the tube in the polarimeter and measured the angle indicated by the scale. This value will behave as a reference value for its respective successive readings. Distilled water does not cause a rotation in the plane of plane polarized light. Hence subtracting the polarimeter reading from the solution's will give the value of by how much the solution in question has rotated plane polarized light.
6. After taking an aliquot of the solution of L-leucine(at isoelectric point) I added drops of HCl to it to test for a pH of 3.80 which I measured using a pH probe.
7. I allowed the probe to measure pH of the solution for at least 1 minute so that the value stabilises.

8. I filled a polarimeter tube(identical to tube filled with distilled water) with this solution of pH 3.80 now and again made sure to allow no air bubbles.
9. I measured the readings shown on the main and vernierss scale(of the polarimeter) for this pH and took a minimum of 3 trials, to minimise error, for pH values(such as: 3.98, 6.80, etc).
10. To collect readings using the same method as above for alkaline conditions, I added drops of NaOH instead of HCl to get the desired pH value.

Calculations for preparing the required solutions:

A. Solution of leucine
   5g of Leucine powder in 250×10^{-3} dm^3 of distilled water.

B. 0.1M HCl[7]
   2.5×10^{-3} dm^3 of stock solution of HCl was added to 50×10^{-3} dm^3 of distilled water and was eventually made up to 250×10^{-3} dm^3 in a standard flask. Adding of the acid to water is to avoid spurting out of the denser acid.

C. 1M NaOH
   Molar mass of NaOH[8]= 40g;

$$c = \frac{n}{v} = \frac{w}{m \times v}$$

$$w = 01 \times 40 \times 250 \times 10^{-3} = 10$$

Hence I weighed 10g of NaOH pellets and dissolved it in distilled water up to the mark in the 250×10^{-3} dm^3 standard flask.

Risk Assessment:

Safety Considerations:

- I added acid to water and not water to acid to prevent spurting of acid.
- Lab coats, gloves and goggles were worn at all times and especially while handling concentrated HCl and the NaOH pellets. GHS symbol of:
  - HCl: Danger[9]
  - NaOH: Danger[10]

[7] Molarity Calculator & Normality Calculator for Acids & Bases. (n.d.). Retrieved from https://www.sigmaaldrich.com/chemistry/stockroom-reagents/learning-center/technical-library/molarity-calculator.html
[8] IB Chemistry Data Booklet
[9] Hydrochloric Acid(HCl) All Grades. (2016, January 21).
[10] Sodium Hydroxide Safety Data Sheet. (2012, March 26).

Ethical Considerations: No ethical considerations as such.

Environmental Considerations:

- HCl was poured down the drain only upon neutralisation with baking soda as it is a very corrosive inorganic acid.
- NaOH was not directly poured into the drains but was neutralised with HCl before disposal.

DATA COLLECTION AND PROCESSING

Example of calculation:

$$\text{Total reading} = \text{Main Scale Reading} + (\text{Least Count } \times \text{Coincidence Vernier scale Division})$$

$$\text{TR} = 164 + (0.01 \times 10) = 164.1^{o}$$

The main scale reading and coincidence Vernier scale division were measured using a polarimeter.

Table 1: showing calculations for average angle of rotation for specific pH values

Trial number	pH of amino acid ±0.01	Total reading ± 0.05		Angle of rotation(d-a) ± 0.1	Average angle of rotation ±0.3
		Distilled water(d)	Amino acid(a)		
1.	3.80	162.09	156.05	6.04	6.00
		162.00	156.07	5.93	
		162.08	156.04	6.04	
2.	3.98	161.09	157.03	4.06	5.04
		161.04	156.04	5.00	
		161.09	155.04	6.05	
3.	6.80	164.04	159.03	5.01	4.65
		164.03	159.05	4.98	
		164.00	160.03	3.97	
4.	9.20	162.00	159.05	2.95	3.99
		164.04	160.05	3.99	
		164.06	159.02	5.04	
5.	9.80	163.02	158.03	4.99	4.37
		163.08	158.03	5.05	
		162.08	159.00	3.08	
6.	10.88	162.00	157.08	4.92	4.98
		163.05	157.00	6.05	
		163.02	159.06	3.96	

ANALYSIS

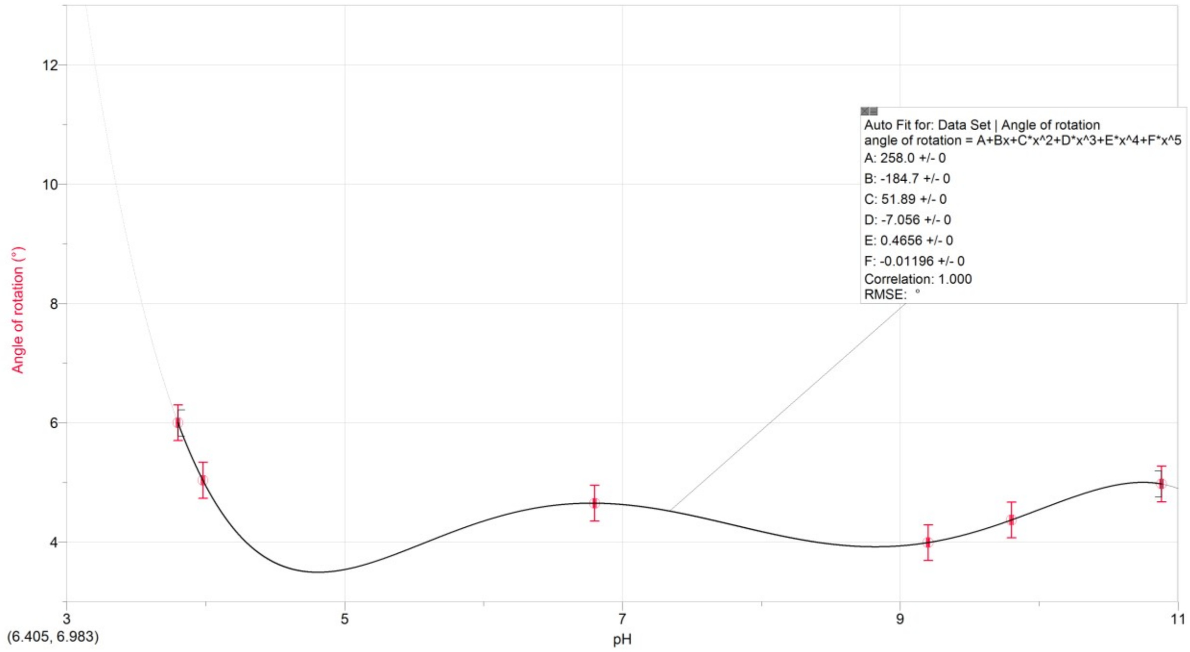


Figure 3: Graph of pH against Angle of rotation

<u>Calculation of random error:</u>

Example of error calculation:

Say true value of angle of rotation= 6° from the formula= $angle\ of\ rotation\ of\ distilled\ water - angle\ of\ rotation\ of\ solution\ of\ L - leucine$

Table 2: showing an example for error calculation

pH	Total reading ± 0.05		Angle of rotation(d-a) ± 0.1	Absolute uncertainty(true-experimental value)
	Distilled water(d)	Amino acid(a)		
3.80	162.09	156.05	6.04	0.04
	162.00	156.07	5.93	0.07
	162.08	156.04	6.04	0.04

$$Mean\ absolute\ uncertainty = \frac{0.04 + 0.07 + 0.04}{3} = 0.05$$

$$Perecentage\ uncertainty = \frac{0.05}{6} \times 100\% = 0.83\%$$

Actual error propagation in experiment:

Table 3: showing error calculation in the experiment performed

pH	True value= average angle of rotation (in ˚)	Total reading ± 0.05 (in ˚)		Angle of rotation(d-a) ± 0.1 (in ˚)	Absolute uncertainty(true-experimental value)	Percentage uncertainty(in %)
		Distilled water(d)	Amino acid(a)			
3.80	6.00	162.09	156.05	6.04	0.05	0.83
		162.00	156.07	5.93		
		162.08	156.04	6.04		
3.98	5.04	161.09	157.03	4.06	0.68	13.43
		161.04	156.04	5.00		
		161.09	155.04	6.05		
6.80	4.65	164.04	159.03	5.01	0.46	9.82
		164.03	159.05	4.98		
		164.00	160.03	3.97		
9.20	3.99	162.00	159.05	2.95	0.70	17.46
		164.04	160.05	3.99		
		164.06	159.02	5.04		
9.80	4.37	163.02	158.03	4.99	0.86	19.76
		163.08	158.03	5.05		
		162.08	159.00	3.08		
10.88	4.98	162.00	157.08	4.92	0.72	14.40
		163.05	157.00	6.05		
		163.02	159.06	3.96		

Hence, average % uncertainty= 12.6%

EVALUATION

Optical activity is shown by molecules with an asymmetric Carbon atom. Light from the sodium vapour lamp passes through the front end of the polarimeter tube which contains a filter that makes the light plane polarised. This plane polarised light passes through the solution of L-leucine in the polarimeter tube and it will undergo a change in the angle of its plane as it hits the optically active molecules. The magnitude of angle of rotation obtained from the polarimeter readings is affected by molecular structure.

The change in angle of rotation of the plane polarised light by L-leucine is observed with respect to the angle of rotation of plane polarised light by distilled water. The plane of plane polarised light is rotated because the light interacts with the electron cloud[11] of the zwitterion of L-leucine which causes a change in orientation of its oscillations and this change depends on the nature of electron cloud. Since the positive and negative charges are over amino and carboxylic acid groups in L-leucine(under acidic or alkaline conditions), the electron cloud is due to more than 1 atom which means the density of the electron cloud is high. This causes it to become mobile and vibrate when plane polarised light interacts with the electric field of the electron clouds. Thus greater the size of the electron cloud, more will it alter the oscillations of the light passing through it; hence a greater shift in the plane of plane polarised light passing through the optically active solution of L-leucine.

The graph above in *Figure 3* shows the relation between pH of the solution of L-leucine and its corresponding angle of rotation. The curve is a quintic equation. As the acidity of the solution increases, the angle of rotation increases[12]. Both amino and carboxylic acid groups are protonated at acidic pH, hence carrying a net positive charge. A similar trend in increase of angle of rotation is seen when pH increases(in alkaline medium) and when the molecules carry a negative charge as a whole.

L-leucine's optical activity is pH dependant because of its amphoteric nature. This in turn favours salt formation which partially contributes to the rotatory power of the amino acid molecule[13]. Additionally, the zwitterion characteristic of L-leucine provides for its isoelectric state because of which the optical activity is affected such that the graph of pH versus angle of rotation plateaus near the isoelectric point and this is as shown by the graph I have obtained in *Figure 3*.

It is also seen that at high pH values of 9.80 and 10.88 there is an accumulation of negative charge which increases the electrostatic attraction at the chiral centre in the molecules of L-leucine. This affects the overall optical activity which results in the increase of angle of rotation of L-leucine as pH increases in the alkaline medium.

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[11] Dill, D. (2008, January 22). Atoms, light, and their interaction. Retrieved from http://quantum.bu.edu/notes/GeneralChemistry/AtomsLightAndTheirInteraction.pdf

[12] Hayashi, K., Fujii, Y., Saito, R., Kanao, H., & Hino, T. (1966). The Influence of Measurement Parameters on the Specific Rotation of Amino Acids. *Agricultural and Biological Chemistry*, *30*(12), 1221–1232. doi: 10.1271/bbb1961.30.1221

[13] Golub, M. A., & Pickett, E. E. (1954). Effect of temperature and pH on the optical rotation of proteins. *Journal of Polymer Science*, *13*(71), 427–440. doi: 10.1002/pol.1954.120137102

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