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Determination of Dissociation Constants of Amino Acids Using 'ORIGIN 50' Program

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ABSTRACT: Potentiometric Studies on the dissociation constants of amino acids were carried out. The dissociation constants of amino acids were determined using 'ORIGIN 50' Program, as an attempt to find an alternative way for determination of the dissociation constants of amino acids, pKa, instead of the conventional calculation methods. The dissociation constants of amino acids determined are; alanine (10.29), arginine (12.02), asparagine (9.39), glycine (9.87), histidine (7.01), lysine (9.28), methionine (9.68), phenyalanine (9.61), proline (10.53), threonine (10.31), tryptophan (9.77), and valine (10.28)

Key Words: Amino acids, Dissociation constant, Potentiometry,

Introduction

Amino acids are organic molecules containing amino group, - NH_2 and carboxylic acid group, - COOH both attached to the same carbon atom called α – carbon. Such amino acids are also known as α – amino acids. Thus an α – amino acid consists of an amino group (- NH_2), a carboxylic group (- COOH), a hydrogen atom (H) and a distinctive R – group bonded to the α – amino carbon atom. The carbon atom to which these groups are attached is called α – amino because it is adjacent to the carbonyl acidic group. Amino acids were earlier discovered as constituents of natural products even before they were recognized as components of proteins; asparagine was discovered in 1806 in juice of asparagus plant and cystine in 1810 in urinary stones. In deed their names are based on the sources from which they were isolated (Akpurieme, 2001).

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The first amino acid isolated from hydrolysis of protein was glycine, obtained in 1820 from gelatin by Braconnot as reported by Lehninger (2000). He also reported threonine as the most recently discovered amino acid isolated from hydrolyzates of fibrin by Rose in 1935 (Lehninger, 2000). Even though a large number of amino acids had already been discovered by the end of 19th Centaury it was not until 1902, with the publication of the works of Hofmeister and of the Fischer (1902) that an explanation was offered for the mode of combination of the amino acids in proteins (Akpurieme, 2001). Their development of the peptide hypothesis of protein structure is regarded as one of the most important events in the history of protein chemistry.

Amino acids are the essential constituents of plants and animal tissues and also occur in plants cells both as free acid or amides. Over 200 different amino acids have been found in higher plants and twenty (20) are known to be the building blocks of protein found in cytoplasm. Some of the isolated amino acids, which are not compounds of proteins, are found in plants as earlier mentioned and others are found in bacteria and animal tissues (Akpurieme, 2001). All proteins are constructed from the same set of 20 amino acids covalently linked in characteristics of linear sequences. Amino acids are therefore the basic structural units of proteins. Among the twenty standard amino acids that are obtained from the hydrolysis of proteins, eight amino acids are said to be essential and cannot be manufactured by the body, therefore must be supplemented with proper nutrition. The essential amino acids include tryptophan, lysine, methionine, phenylalanine, threonine, valine, leucine and isoleucine. The non – essential amino acids include; serine glycine, glutamine, asparagines, cystein, proline, alanine, aspartate and glutamate. These can be manufactures by the body while the other three amino acids namely, arginine, histidine, and tyrosine are called semi essential amino acids especially in infants.

This paper reports the use of an alternative way for determination of dissociation constants of amino acids, pKa, instead of the conventional calculation methods.

Material and Method

The chemicals and solvents used in this work were of Analar grade. All the glass wares used were washed thoroughly with distilled water and dried in an oven. Weighing was carried out on electric metler balance, model AB 54. The pH measurements were carried out using Jenway pH meter model 3320.

Determination of pKa of the Amino Acids

The determination was carried by first measuring the pH of the reaction mixture prepared by adding into 400cm^3 beaker containing magnetic stirring bar 90cm^3 of distilled water, 100cm^3 of 0.04moldm^{-3} potassium trioxonitrate (V) and 10cm^3 of 0.08 mol/dm^3 of glycine respectively. The electrodes of the standardized pH meter were then introduced into the reaction mixture and with stirring aliquots (0.5cm^3) of standardized 0.1 moldm^{-3} sodium hydroxide were added from a burette into the reaction mixture. After each addition of the aliquot the corresponding stable pH reading was recorded. The same procedure was repeated for each of the remaining amino acids (Angelici, 1977).

Results and Discussion

In aqueous solution the aliphatic amino acids exist as zwitterions $H_3\overset{+}{N}CRCOO^-$ the amino group being protonated $(\overset{+}{N}H_3)$ while the carboxyl group is deprotonated $\left(COO^-\right)$. In the case of $a-$amino acids, deprotonation of the ammonium group results in a slightly basic solution pH 9 to 10 to give the specie, $H_2NCHRCOO^-$. The carboxylate group undergoes protonation in acidic media pH 2 to 3 and therefore the two dissociation of the fully anionic, $H_2NCHRCOO^-$ and protonated cationic form, $H_3\overset{+}{N}CHRCOOH$ are completely separated. The values of dissociation constants Ka by convention

are reported as pKa (Sovago et al., 1993; Yamuchi and Odani 1996; Berthon, 1995). The dissociation constants of the amino acids were determined as intercept on Y-axis from the figures 1 – 12. The values of the dissociation constants of the amino acids studied are 9.87 (glycine), 12.02 (arginine), 9.39 (asparagines), 7.09 (Histidine), 10.28 (lysine), 9.68 (methionine), 9.61 (Phenylalanine), 10.31 (Threonine), 9.77 (Tryptophan) and 9.90 (Valine), respectively as shown in Fig. 1 to 12.

The pKa value determined for alanine, 10.29 in Fig. 1, is higher than the recommended literature value reported by Sovago et al (1993) but closer to values of 9.87 and 9.90 reported by Robert and Melvin (1982 – 1983) and Stryer, (1988) respectively, Sovago et al (1993) also reported the tentative value of 9.78. The pKa value obtained for arginine, 12.02 in Fig. 2 is similar to 12.09 recommended by Yamuchi and Odani (1996) but closer to the value of 12.48 reported by Robert and Melvin (1982 – 1983). The pKa value of 9.39 for asparagine, from Fig. 3 is similar to the tentative value of 9.30 reported by Berthon, (1995). Literature values of pKa reported for glycine include 9.80 by Stryer (1988), 9.6 recommended by Sovago *et al* (1993). However in Fig. 1 the pKa value of 9.87 for glycine is similar to the value reported by Stryer (1988) and 0.27 higher than the recommended value by Sovago *et al* (1993). The pKa value of 7.09 for histidine from Fig. 5 is closer to the mean pKa of 7.59 reported Lehninger (2000). The pKa value of 10.28 for lysine from Fig. 6 is lower than the reported value of 10.51 reported by Robert and Melvin (1982 – 1983). The dissociation constant of methionine from Fig. 7 is 9.68 is slightly higher than 9.21 reported by Lehninger (2000). However, the pKa value is similar to value of 9.69 reported by Berthon (1995) in $NaClO_4$ medium but higher than the value of 9.05 recommended Berthon (1995). The pKa value of phenylalanine in Fig. 8 is 9.61, which is closer to the 9.24 reported by Lehninger (2000) and 9.11 by Gergely *et al* (1972). The value of 10.54 obtained for proline dissociation constant from Fig. 9 is similar to 10.6 reported by Lehninger (2000) and also by Robert and Melvin, (1982 – 1983). Fig. 10 gives the pKa value of 10.31 for threonine. This is similar to the 10.43 value of 10.43 reported by Lehninger (2000) and alsoby Robert and Melvin, (1982 – 1983) but higher than the tentative value of 8.97 ± 0.06 reported by Berthon (1995) and 8.98 reported by Gergely et al (1972). Berthon (1995) also reported values of 10.08, 10.73 in organic medium. The value of 9.77 obtained for tryptophan in Fig. 11 is comparable to 9.40 by Lehninger (2000). Finally the pKa of 9.99 for valine in Figure 12 is higher than the recommended value of 9.54 reported by Sovago et al. (1993) and 9.60 also by Stryer (1988) but the value is closer to 9.72 by Lehninger (2000).

Conclusion

The acid dissociation constant of amino acids determined graphically for the first time from the available literature were found to be similar with the corresponding values reported in the literature. Therefore the graphical approach from the Henderson-Hasselbalch equation is recommended for easier determination of the pKa because of its simplicity, accuracy and being more scientific. The accurate determination of pKa is of paramount importance because; the pKa determines the buffering activity of the physiological buffer in the body.

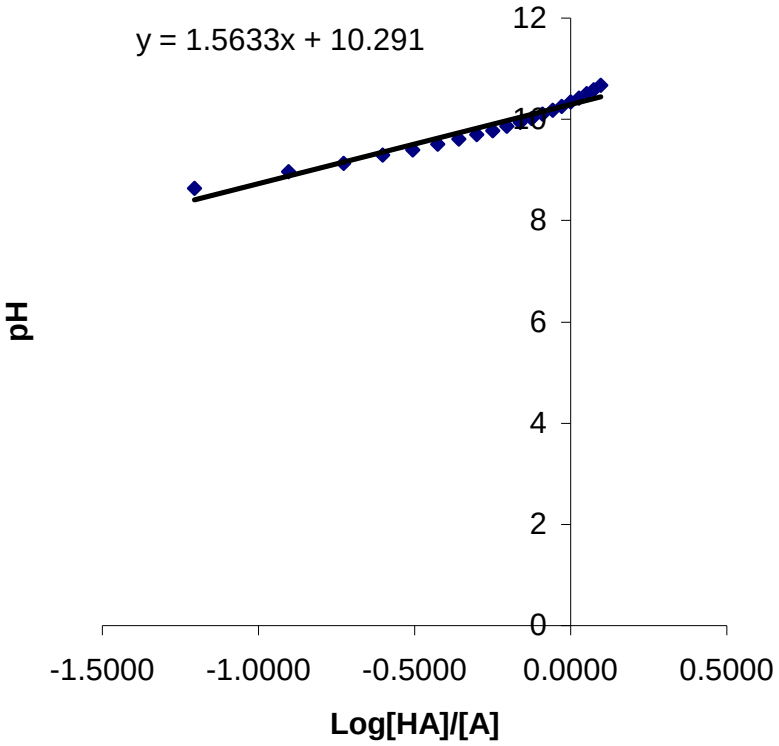

Fig. 1: Plot of pH Versus Log [HA]/[A] for pKa of Alanine

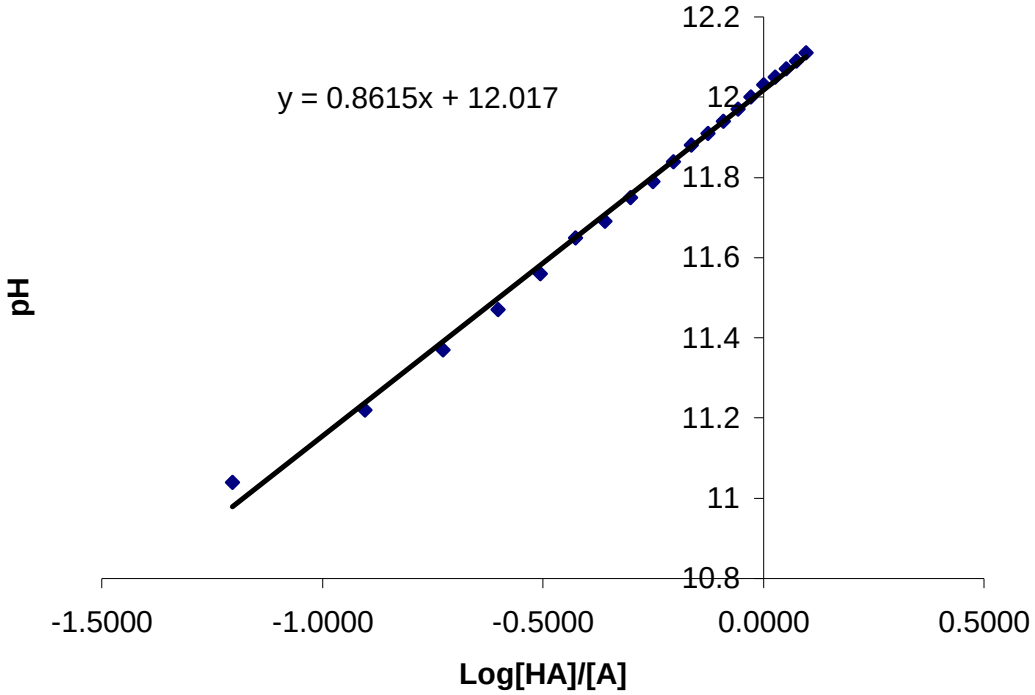

Fig. 2: Plot of pH Versus Log [HA]/[A] for pKa of Arginine

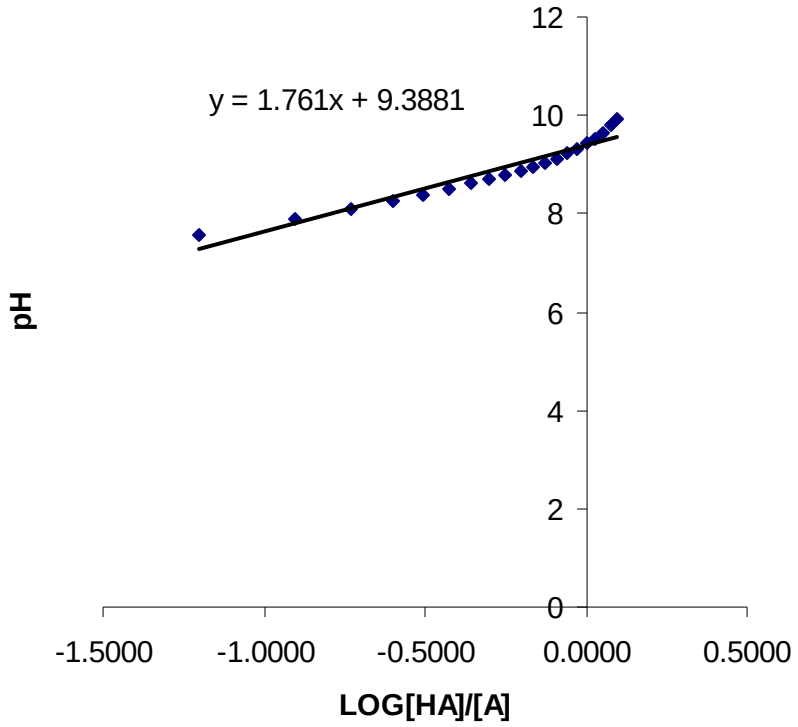

Fig. 3: Plot of pH Versus Log [HA]/[A] for pKa of Asparagine

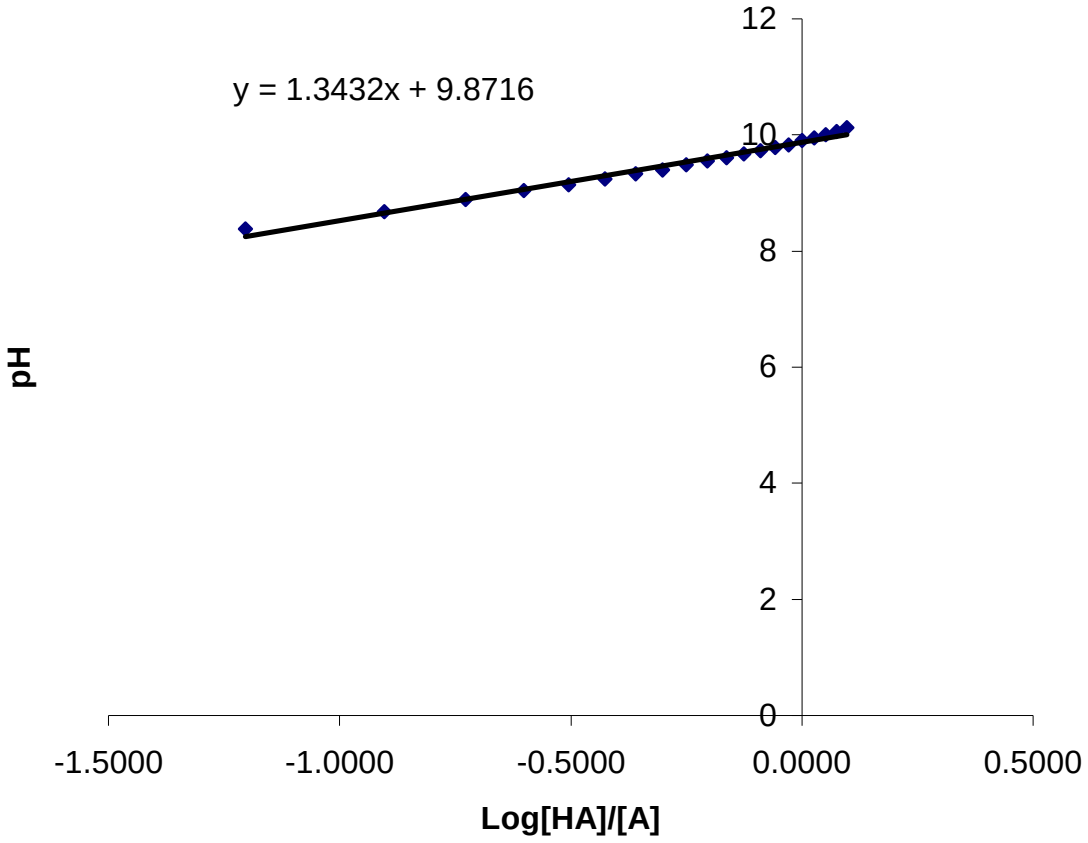

Fig. 4: Plot of pH Versus Log [HA]/[A] for pKa of Glycine

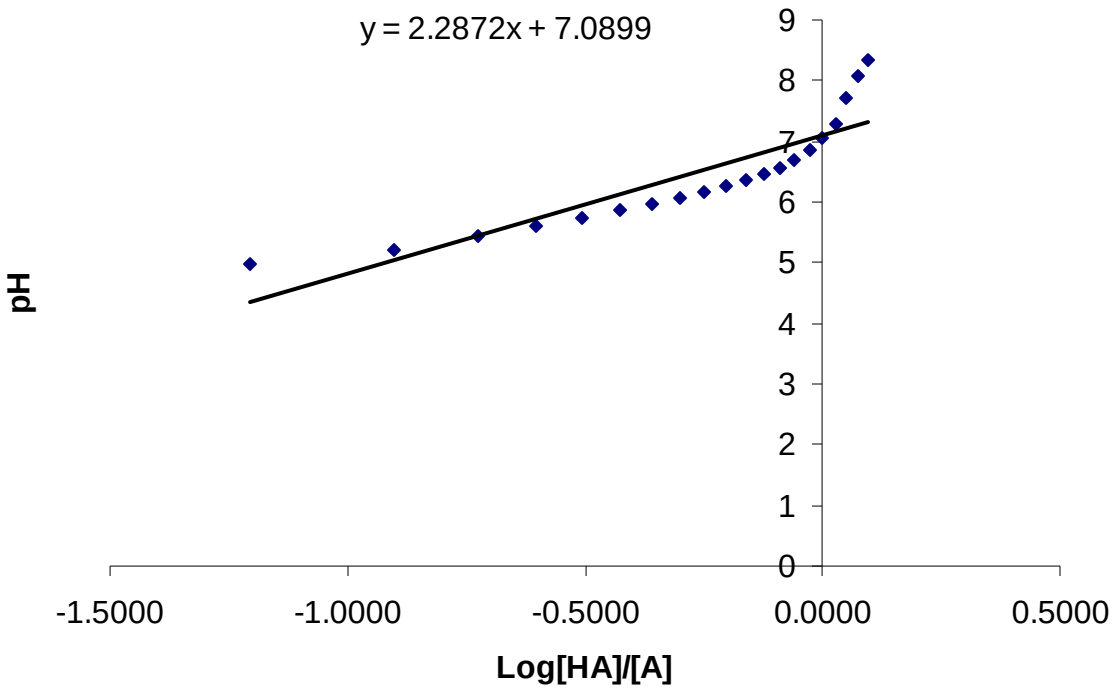

Fig. 5: Plot of pH Versus Log [HA]/[A] for pKa of Histidine

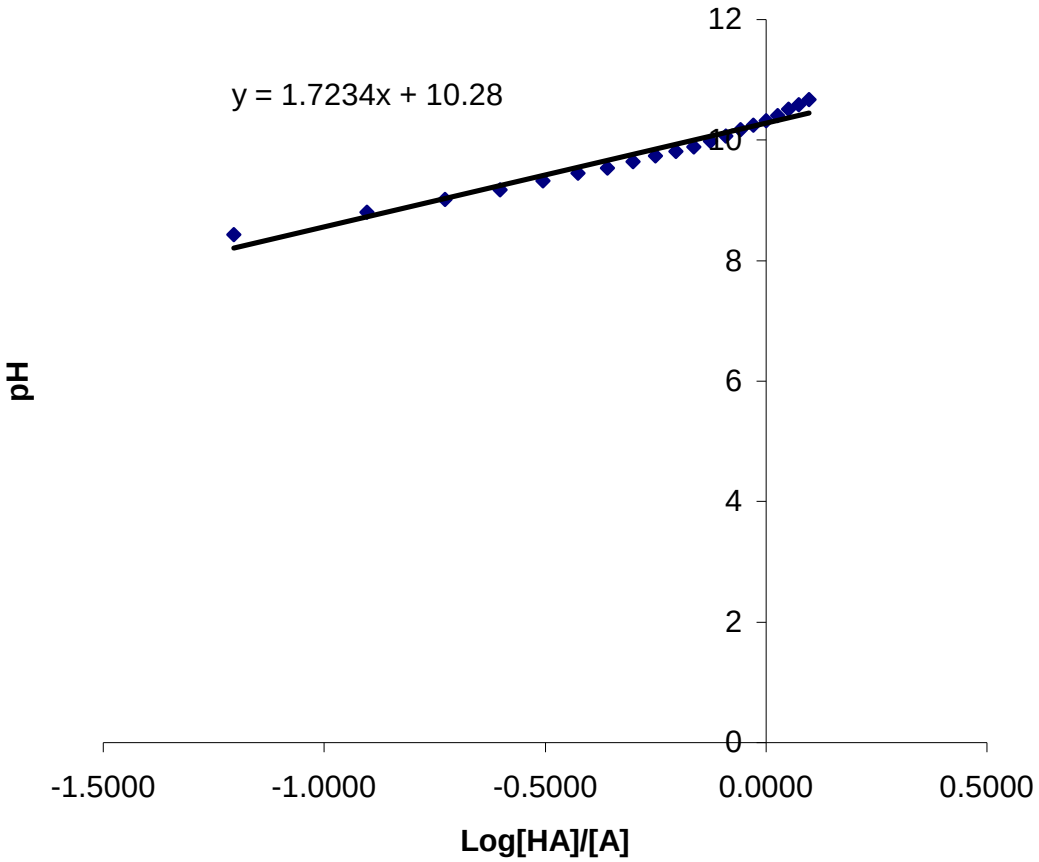

Fig. 6: Plot of pH Versus Log [HA]/[A] for pKa of Lysine

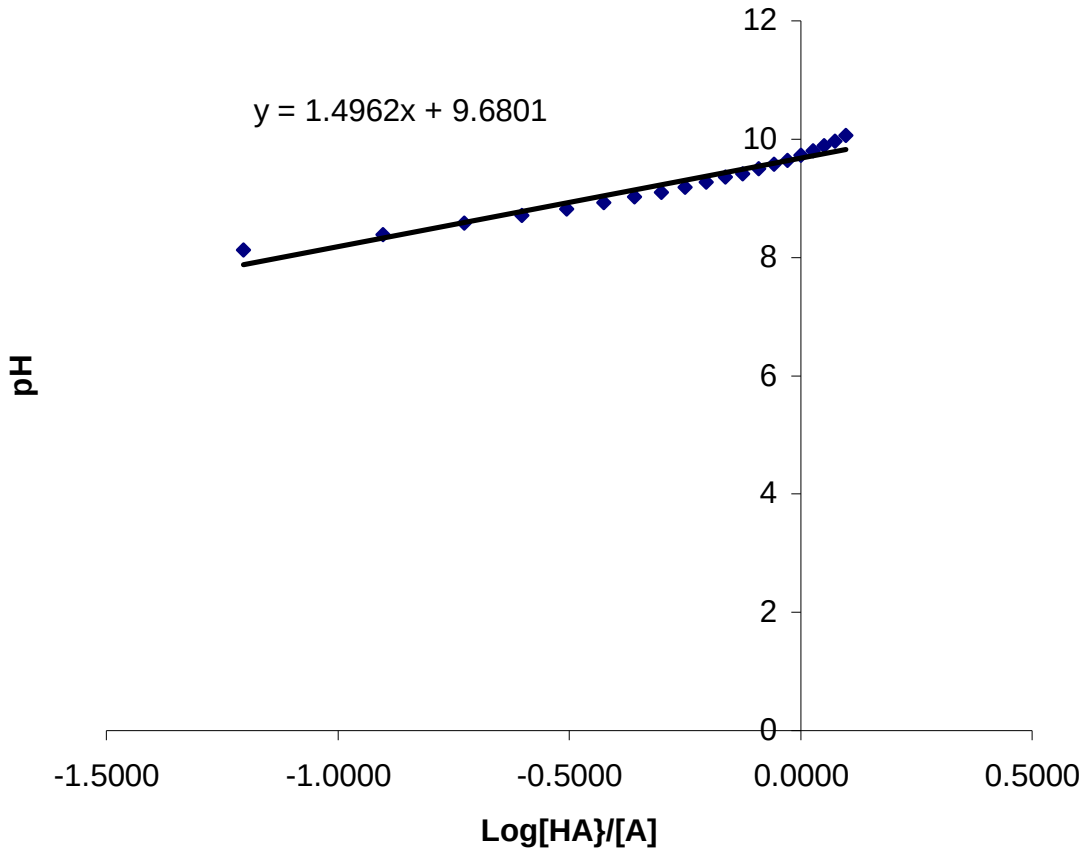

Fig. 7: Plot of pH Versus Log [HA]/[A] for pKa of Methionine

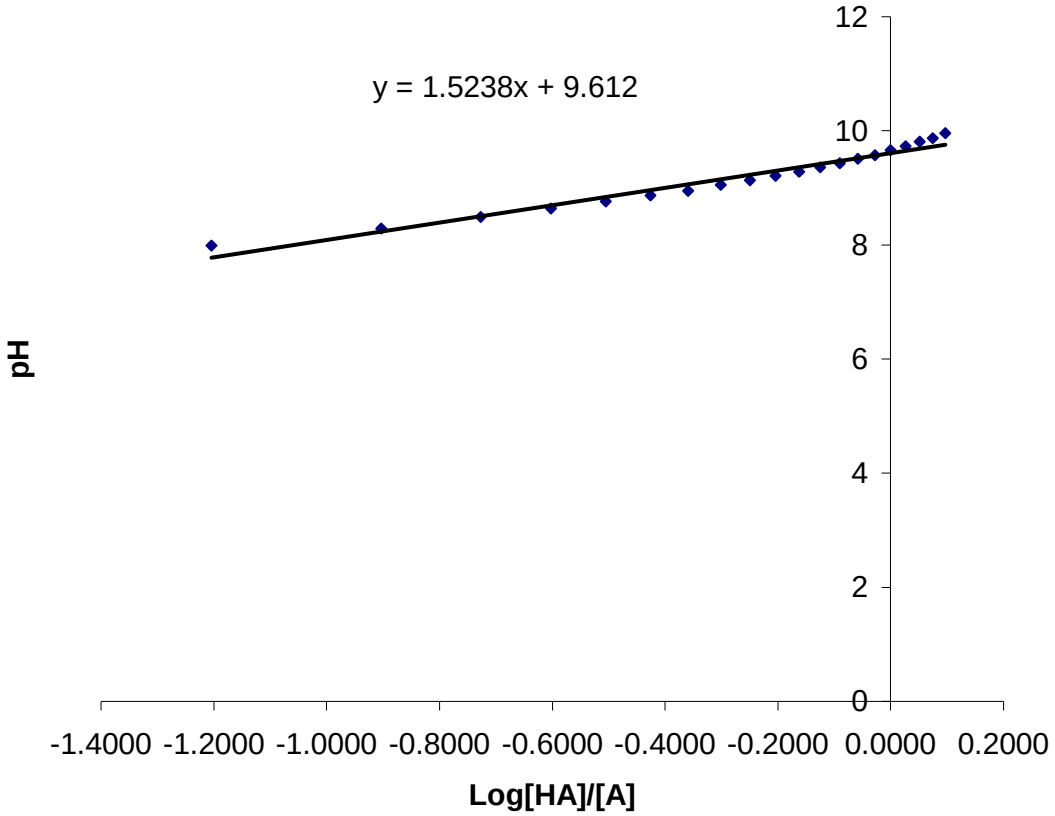

Fig. 8: Plot of pH Versus Log [HA]/[A] for pKa of Phenylalanine

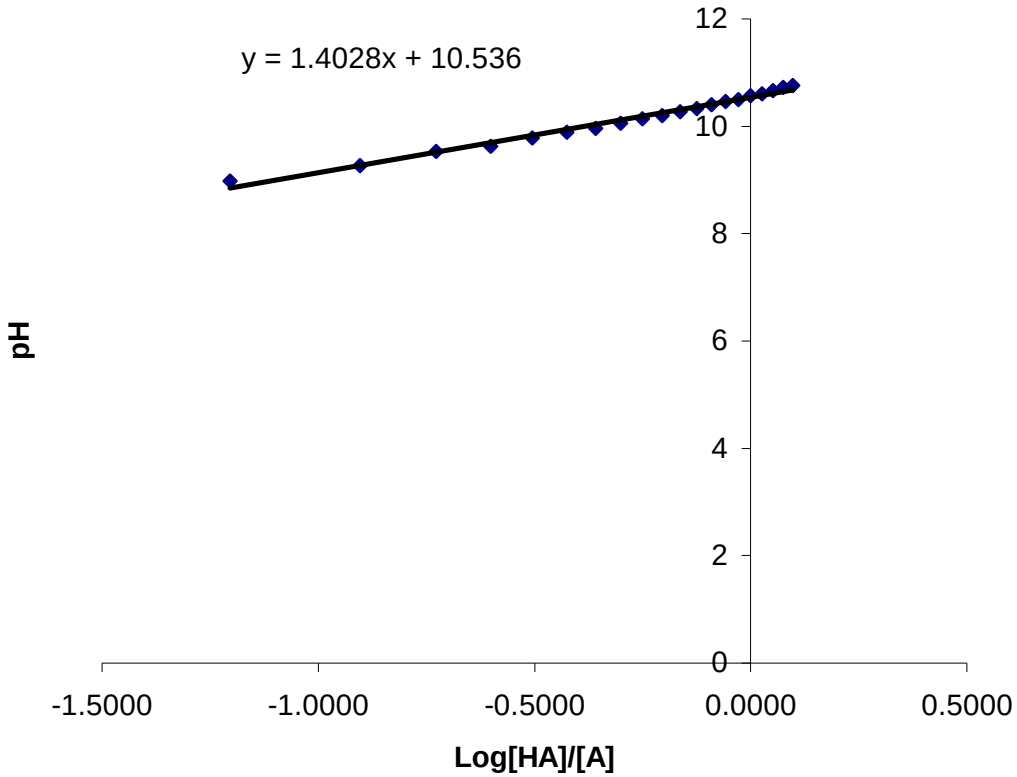

Fig. 9: Plot of pH Versus Log [HA]/[A] for pKa of Proline

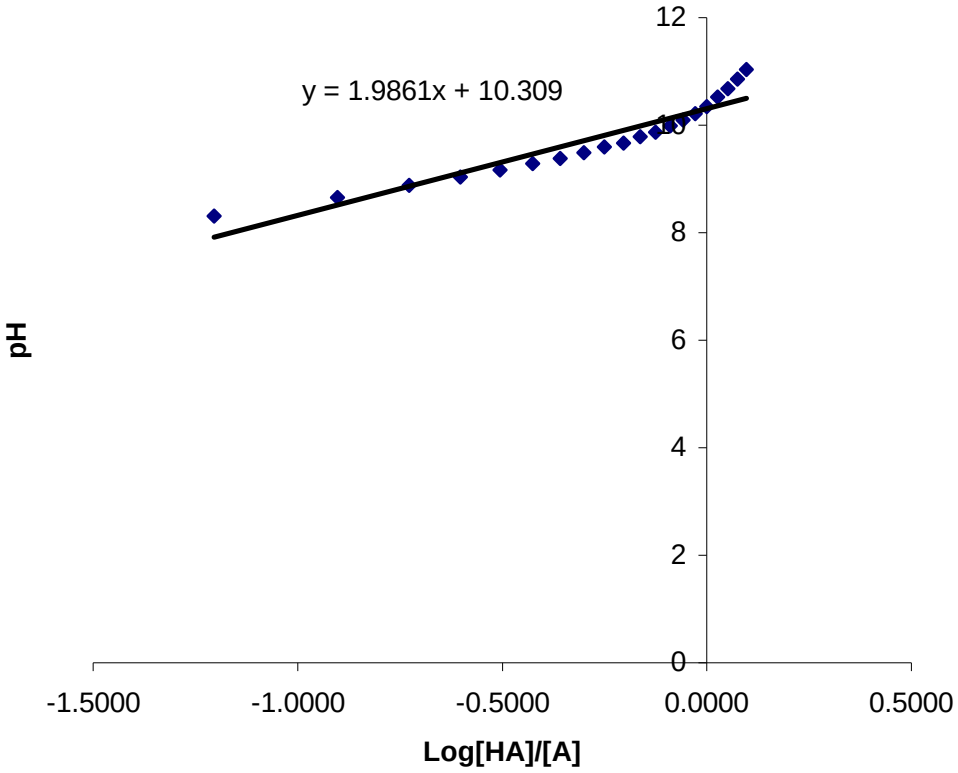

Fig. 10: Plot of pH Versus Log [HA]/[A] for pKa of Threonine

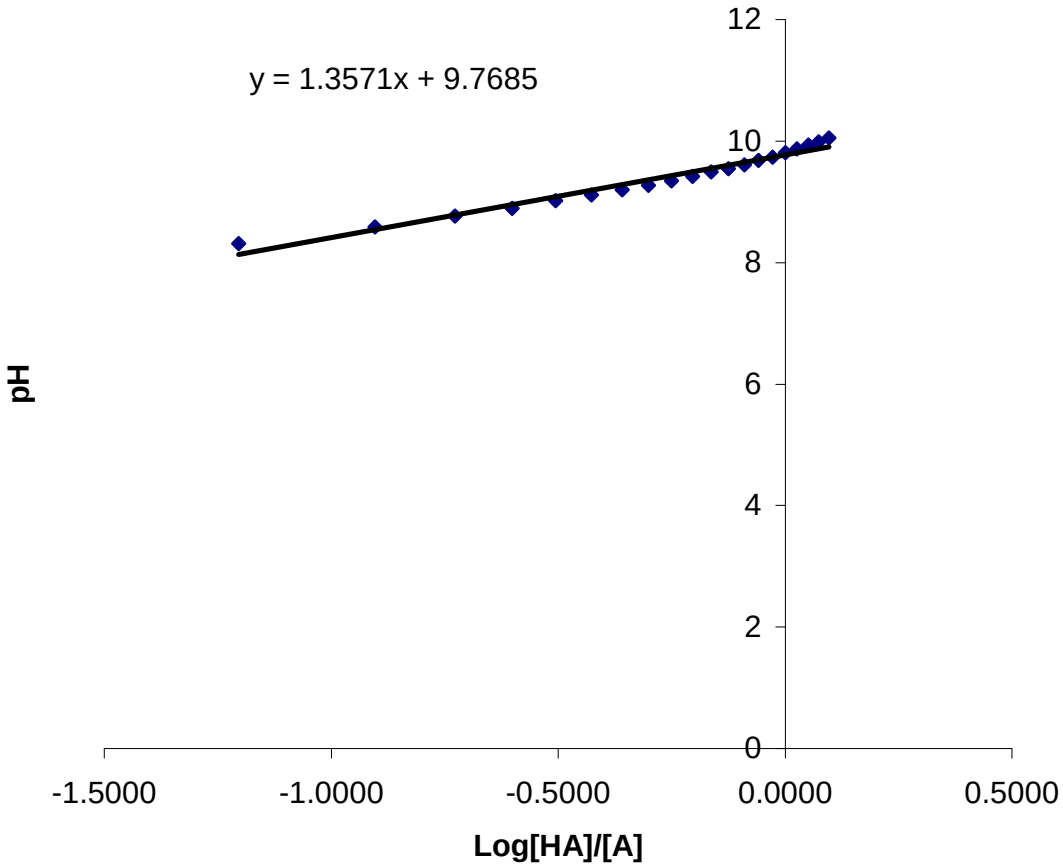

Fig. 11: Plot of pH Versus Log [HA]/[A] for pKa of Tryptophan

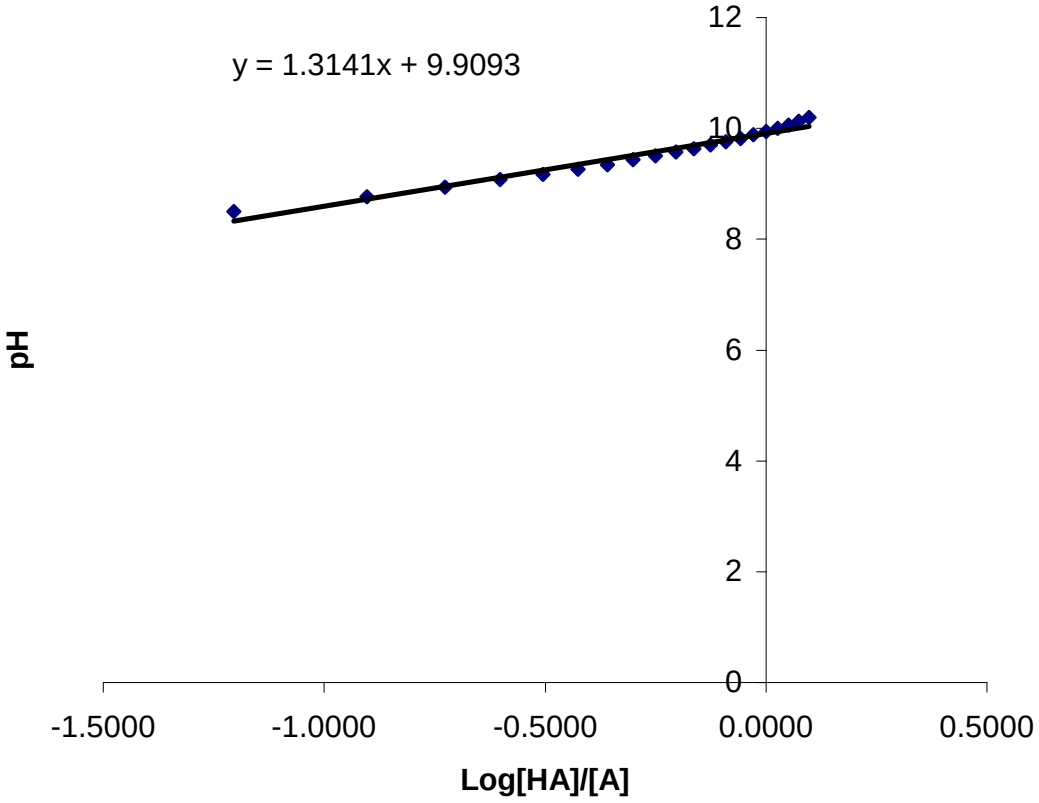

Fig. 12: Plot of pH Versus Log [HA]/[A] for pKa of Valine

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