SPECTROPHOTOMETRIC DETERMINATION OF THE IONIZATION CONSTANTS OF METHIONINE, CYSTINE AND CYSTEINE

Hamad M. Adress Hasan[a]*, Ibrahim H. Habib[b] and Ahlam M. Ali[a]

Keywords: amino acids, ionization constant, spectrophotometry, cystine, cysteine, methionine.

A simple, rapid and sensitive method has been applied on three amino acids viz., methionine, cystine and cysteine, to estimate the ionization constant. Spectrophotometric measurements were carried out on the absorbance of each amino acid at different pH values. The results showed that the cystine and cysteine have one pK_a, while the methionine gave two different pK_a values by using this method.

* Corresponding Authors
E-Mail: Drhamadmhasan85@yahoo.com
[a] Chemistry Department, Faculty of Science, Omar El – Mukhtar University, Libya.
[b] Al-Dogy Research Center, Cairo, Egypt.

INTRODUCTION

Amino acids may have positive, negative, or zero net charge. Charged and uncharged forms of the ionisable COOH and NH_3^+ weak acid groups exist in solution in the following protonic equilibria.

$$\underset{(A)}{RCOOH} \leftrightarrows \underset{(B)}{RCOO^- + H^+} \quad (1)$$

$$RNH_3^+ \leftrightarrows RNH_2 + H^+ \quad (2)$$

While both RCOOH and RNH_3^+ are weak acids, RCOOH is a far stronger acid than RNH_3^+, at physiologic pH 7, carboxyl groups exist almost entirely as $RCOO^-$ and amino groups predominantly as RNH_3^+ form.

Molecules that contain an equal number of ionizable groups of opposite charge and that therefore bear no net charge are termed zwitter ions. Amino acids in blood and most tissues thus should be represented as (B). Structure A cannot exist in aqueous solution because at any pH low enough to protonate the carboxyl group the amino group would also be protonated.[1] Similarly, at any pH sufficiently high for an uncharged amino group to predominate, a carboxyl group will be present as $RCOO^-$. The uncharged representation (A) is, however, often used for reactions that do not involve protonic equilibrium.

The acid strengths of weak acids are expressed as their pK_a. The net charge on an amino acid-the algebraic sum of all the positively and negatively charged groups present-depend upon the pK_a values of its functional groups and on the pH of the surrounding medium. Altering the charge on amino acids and their derivatives by varying the pH facilitate the physical separation of amino acids, peptides and proteins.[2] The isoelectric species is the form of a molecule that has an equal number of positive and negative charges and thus is electrically neutral. The isoelectric pH, also called the pI is the pH midway between pK_a values on either side of the isoelectric species. For amino acids such as alanine that has only two dissociating groups, there is no ambiguity, the first pK_a (COOH) is 2.35, and the second pK_a (NH_3^+) is 9.69, the isoelectric pH (pI) of alanine is thus 6.02.

The environment of a dissociable group affects its pK_a, the pK_a values of the ionisable groups of free amino acids in aqueous solutions provide only an approximate guide to the pK_a values of the same amino acids when present in proteins. A polar environment favors the charged form, $RCOO^-$ or RNH_3^+, and nonpolar environment favors the uncharged form, RCOOH or RNH_2.[3]

A nonpolar environment thus raises the pK_a of the carboxyl group (making it a weaker acid) but lowers that of a protonated amino group (making it a stronger acid). The presence of adjacent charged groups can reinforce or counteract solvent effects. The pK_a of a functional group thus will depend upon its location within a given protein. Variations in pK_a can in compass whole pH units, pK_a values that diverge from those listed by as much as three pH units are common at the active sites of enzymes. An extreme example, a buried aspartic acid of thioredoxin has a pK_a, above 9 a shift over six pH units.[2]

Experimental

A stock solution of cysteine (10^{-4} mol) was prepared by dissolving 0.00302 g of cysteine in distilled water by gentle heating till complete dissolution. The solution was cooled and diluted to 250 mL. A stock solution of cystine (10^{-4} mol) was prepared by dissolving 0.0060 g of cystine in distilled water by gentle heating till complete dissolution. The solution was cooled and diluted to 250 mL. A stock solution of methionine (10^{-3} mol) was prepared by dissolving 0.0373 gm of methionine in 250 mL distilled water. More dilute solutions were prepared by diluting the stock solutions.

Buffer solutions

The buffer used was of the universal type (99) by taking 100 mL of an acid mixture containing 0.04 mol of H_3BO_4, H_3PO_4 and CH_3COOH acids and adding the required volume of 0.2 N NaOH to give the desired pH (Table 1).

Table 1. Constituting Universal buffer.

No.	pH	No.	pH
1	2.374	7	8.0
2	3.100	8	9.299
3	4.69	9	10.487
4	5.107	10	11.099
5	6.385	11	11.990
6	7.482		

Determination of pKa metal complexes by half height method

This method [3] depends on the fact that the limiting absorbance (A_1) represents the complete conversion of the compound from one form to other. Since pK_a is equal to the pH value at which the two forms exist in equivalent amounts, the pH corresponding to half the height of the absorbance-pH curve, $A_{½}$ is equal to pK_a. The $A_{½}$ value is given by the relation

$$A_{½} = (A_1 - A_{min}/2) + A_{min} \quad (1)$$

where

A_1 = maximum absorbance,

A_{min} = minimum absorption.

The absorbance of the amino acid was measured by a spectrophotometer type 800 DU.

Results and Discussion

The absorption spectra were recorded to investigate the spectral properties of the species likely to exist in such media and to determine the ionization constant (pKa) values of the acidic groups present.

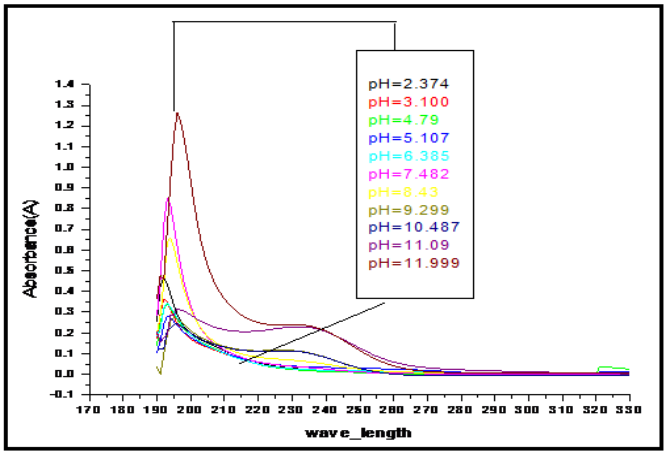

Figure 1. Electronic spectra of $1.3x10^{-4}$ mol MET at different pH.

Britton – Robinson universal buffers, were used to control the pH over the range (2.5- 12.0). From the Figures (1, 2 and 3), it is apparent that the maximum absorption of the ligand increases as pH of the buffer increases. The values of the maximum absorptions of the investigated ligands at different pH values are listed in table 2. These bands are due to absorption of the non- ionized form liable to exist in such solutions and may be assigned to $\pi - \pi^*$ electronic transition within the ligand molecule influenced by intermolecular charge transfer.[4] The

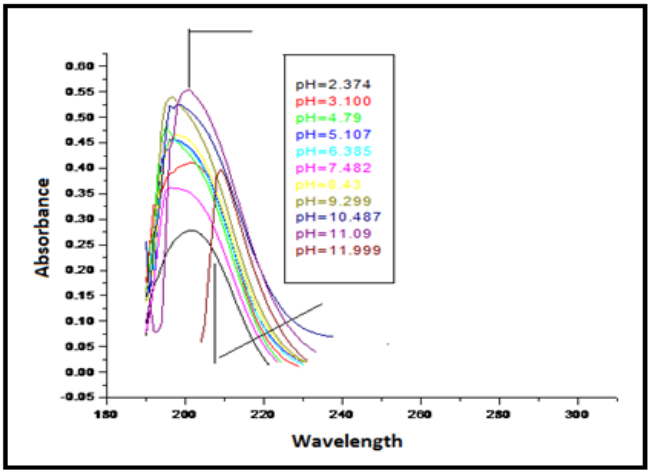

Figure 2. electronic spectra of $3x10^{-4}$ mol cystine at different pH.

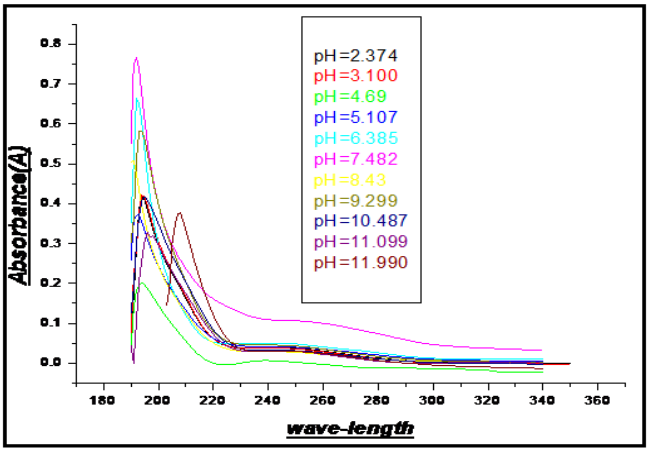

Figure 3. electronic spectra of $3x10^{-4}$ mol cysteine at different pH.

Table 2. Absorptions of the amino acids at different pH values.

pH	Absorbance		
	Cysteine	Cystine	Methionine
2.374	0.4654	0.4185	0.2782
3.100	0.3641	0.4218	0.4103
4.69	0.3427	0.2016	0.4802
5.107	0.2796	0.3719	0.4568
6.385	0.3463	0.6651	0.4549
7.482	0.8528	0.767	0.3609
8.43	0.6582	0.5088	0.4651
9.299	0.2759	0.5818	0.5388
10.487	0.2443	0.4183	0.5261
11.099	0.3182	0.3281	0.5541
11.990	1.2665	0.3777	0.3992

spectra in alkaline solutions are characterized by the presence of a strong band absorbing maximally at the same range, which may be assigned to the absorption of the ionized form liable to exist at high pH values as a result of acid base equilibrium. It has been noticed in this investigation that the absorption bands assigned to the ionized form increase gradually by increasing of pH, attaining the maximum value at pH nearly 12.0.

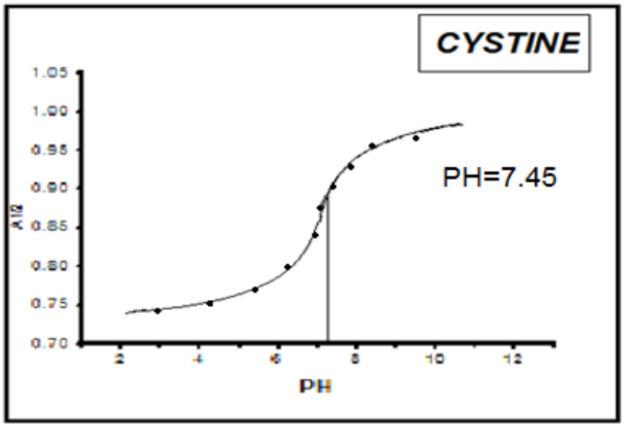

Figure 4. Absorbance and pH relationship for cystine.

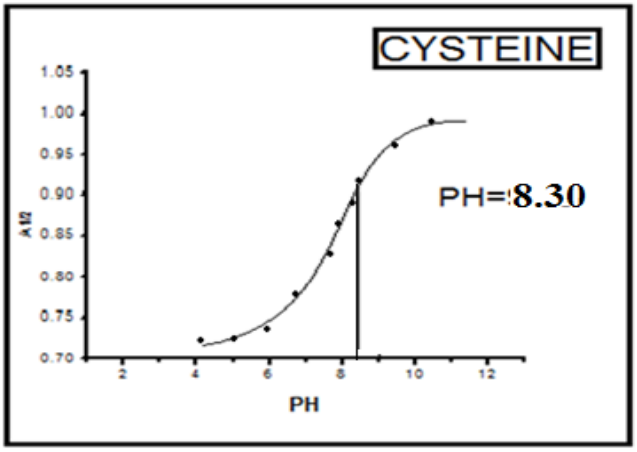

Figure 5. Absorbance and pH relationship for cysteine.

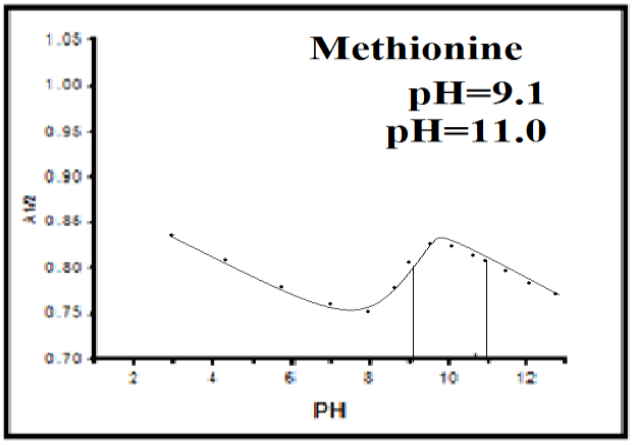

Figure 6. Absorbance and pH relationship for methionine.

The absorbance-pH curves show that the absorbance attains a limiting value at the extreme pH values in highly acidic or alkaline solutions indicating the existence of only one ionization step which is the ionization of NH_2 or COOH groups.

The variation of absorbance with pH is used for the calculation of ionization constants (pKa values) of the investigated ligands using the half height method.[4] The ionization of strong acidic carboxylic group is apparent at lower pH value, due to the high stability of the corresponding anion by resonance. They do not impart any spectral changes as the ionizable proton is not conjugated with the π–system of the molecule. In case methionine two maximal bands at 200 and 235 nm appeared. The two maxima increase with an increase in pH.

In the case of cystine one maximal band is apparent at 200 nm. This band becomes more intense with the increase of pH. On the other hand, in solution with pH > 9, the position of the maximum absorption band is red shifted with the increase of pH. This is an indication of the formation of an anionic species in alkaline solutions. The observations in case of cysteine are similar to those in cystine with two maxima at 200 and 235 nm and an increase in absorbance with an increase in pH.

The absorbance values of the studied amino acid are given in table (2) and shown in figures 4, 5, and 6.

Conclusions

1. Only one pK value was observed spectrophotometrically for cystine and cysteine.

2. In the case of cystine, the pK value obtained spectrophotometrically is 7.45 units. The sample must be dissolved in an acid for spectral measurements. The variation in the media from water to acid for solubility and the insolubility of the cystine zwitterion in aqueous solution leads to differences in the pK values.[5,6] The pKa of cysteine was 8.30.

3. In the case of methionine, two pK values are obtained spectrophotometrically, 8.96 and 11.030. The first pK is attributed to the deprotonation of the carboxylic group.[7] The second pK is smaller by 0.3-0.6 units than those obtained by pH-measurements and this may be due to protonation of zwitterions.[7]

References

[1]Kreil, G., *Ann. Rev. Biochem*,.**1997**, *66*, 337. https://doi.org/10.1146/annurev.biochem.66.1.337

[2]Zhangand, F. Y., Corey, E. J., *Org . Lett*., **2000**, *2*, 1097-1100. https://doi.org/10.1021/ol0056527

[3]Issa, M., Zewail, A. H., *J. Chem. U.A.R*., **1971**, *14*, 461.

[4]H.T.S.Britton, H. T. S., *Hydrogen Ions*,4th Ed., Chapman and Hall, London, **1972**.

[5]Hawkins, J., Perrin, D. D., *Inorg. Chem.*, **1993**, *2*, 843. https://doi.org/10.1021/ic50008a043

[6]Mizuochi, R., Uehara, A., Kyuno, E., *Bull. Chem. Soc. Jpn.*, 1971, *44*, 1565. https://doi.org/10.1246/bcsj.44.1555

[7]Hallman, P. S., Perrin, D. D., Watt, A. E., *Biochem. J.*, 1971, *121*, 549. https://doi.org/10.1042/bj1210549

Received: 19.06.2017.

Accepted: 17.08.2017.