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GRAPHICAL DETERMINATION OF DISSOCIATION CONSTANT, pKa OF NON – POLAR AMINO ACIDS

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ABSTRACT
The dissociation constant (pKa) of non – polar amino acids including (alanine, glycine, valine phenylalanine and tryptophan) were determined by potentiometric titration technique. The pKa values obtained by extrapolation for alanine, glycine, and valine were 10.29, 9.87 and 9.91 respectively. The implications of the results are discussed and recommendations were made.

Keywords: Dissociation constant, amino acids, non-polar, potentiometry.

INTRODUCTION

Aliphatic amino acids are the most common α – amino carboxylic acid with general formula $RCH(NH_2)COOH$). Except glycine, the aliphatic amino acids contain a chiral centre (Sovago *et al.*, 1993) while two chiral centres are present in molecule of isoleucine. It is the characteristic of these aliphatic amino acids that they do not contain any donor group in the side chain and thus their protonation and complexation equilibrium are very similar to those of glycine.

Aliphatic amino acids generally act as bidentate ligands yielding mono, bis and tris complexes with most metal ions. Such ligands contain two donor groups (the amino and carboxylate groups), and therefore two hydrogen ions can dissociates from the fully protonated cations of the amino acids. Dissociation of these protons occurs stepwise, but in well separated processes and the aliphatic amino acids can appear in three different forms in different pH ranges: the cationic, the neutral or Zwitterions and the anionic (Sovago *et al.*, 1993).

The graphical approach, in this paper could serve as a reference for the determination or ascertaining the dissociation constants of aliphatic amino acids and other weak organic acids. This approach is the most recent from the available literature and is much simpler and mere scientific compared to the conventional calculation method being used (Angelici, 1997, Csaba *et al.*, 1999 and Lehninger, 1975).

MATERIALS AND METHODS

The determination of the pKa was carried our by first measuring the pH of the reaction mixture prepared by adding into 400 cm^3 beaker containing magnetic stirring bar, 90 cm^3 distilled water, 100 cm^3 of 0.04 mol dm^{-3} potassium trioxonitrate(V) and 10cm^3 of 0.08 mol dm^{-3} of glycine respectively (Angelici, 1977). An aliquot of standardized 0.1mol dm^{-3} sodium hydroxide from a burette was added into the reaction mixture and after each addition of the aliquot, the corresponding stable reading of pH was recorded using Jenway pH meter model 3320. The same procedure was repeated for alanine and valine respectively

RESULTS AND DISCUSSION

The equation Ka = [H+] [A]/[HA] used by Csaba *et al* (1999) for the calculation of pKa was modified to pH = - log [HA]/[A^-] + pKa where, [A^-] deprotonated amino acid and [A] protonated amino acids to conform to the general equation of straight – line graph; y = mx + c, where y = pH, m = slope, x = log [HA]/[A] and C = intercept = pKa. Thus the pKa is obtained as the intercept of the graph of pH versus – log [HA]/[A] as shown in Figures 1,2 and 3 for glycine, alanine and valine respectively.

The values obtained were 9.87 (glycine), 10.29 (alanine), and 9.90 (valine). The pKa value of 9.87 for glycine (Figure 1) obtained in this study was similar to the value of 9.80 reported by Stryer (1988). However, the value (9.87) was comparatively higher than the reported value of 9.6 (Sovago *et al.,* 1993). The value of 10.29 obtained for alanine (Figure 2) was higher than the recommended literature value reported by Sovago *et al* (1995) but closer to 9.87 and 9.90 reported by Robert and Melvin (1982 , 1983) and Stryer (1988) respectively.

Figure 3 shows the pKa value of 9.99 for valine and it is higher than the recommended value of 9.54 reported by Sovago and Gergely (1993) and 9.60 also reported by Stryer (1988) but closer to 9.72 reported by David and Macheal (2000).

The results obtained in this study were similar to those obtained by using Henderson-Hasselbalch equation. The accuracy and simplicity of this method will to some extent make determination of pKa easier and less cumbersome. The accurate determination of pKa is of paramount importance as it determines the buffering activity of the physiological buffers in the body. In addition the results could serve as qualitative test for the amino acids.

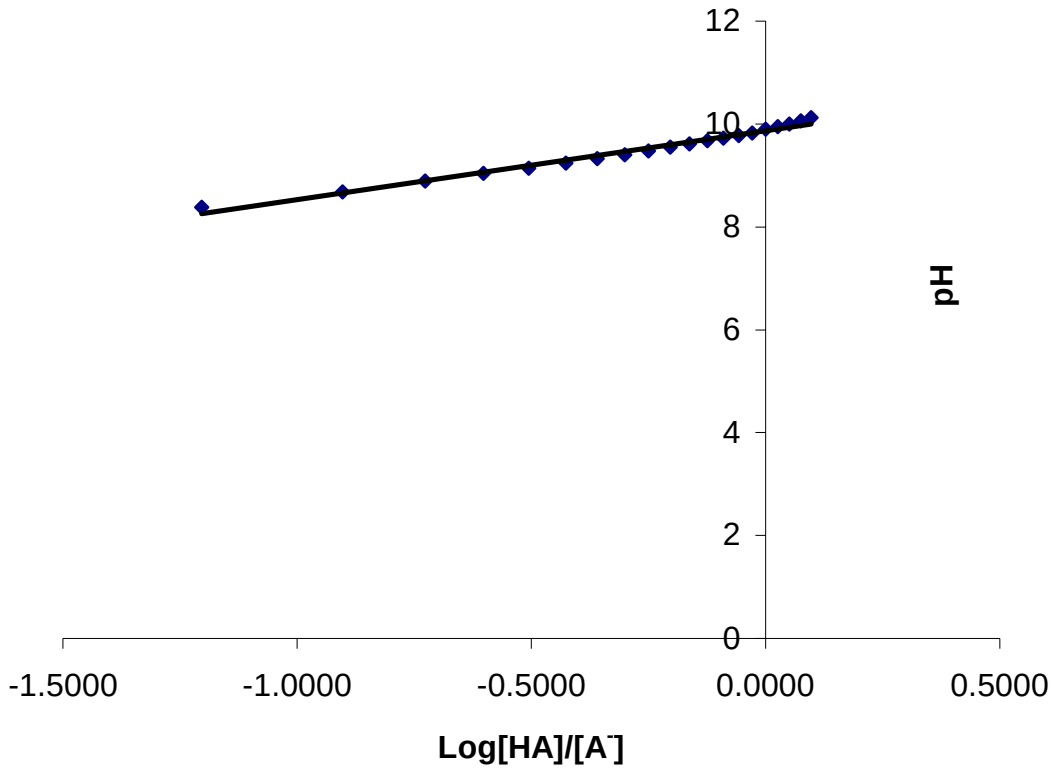

Fig. 1: Plot of pH versus Log [HA] / [A$^-$] for pKa of Glycine

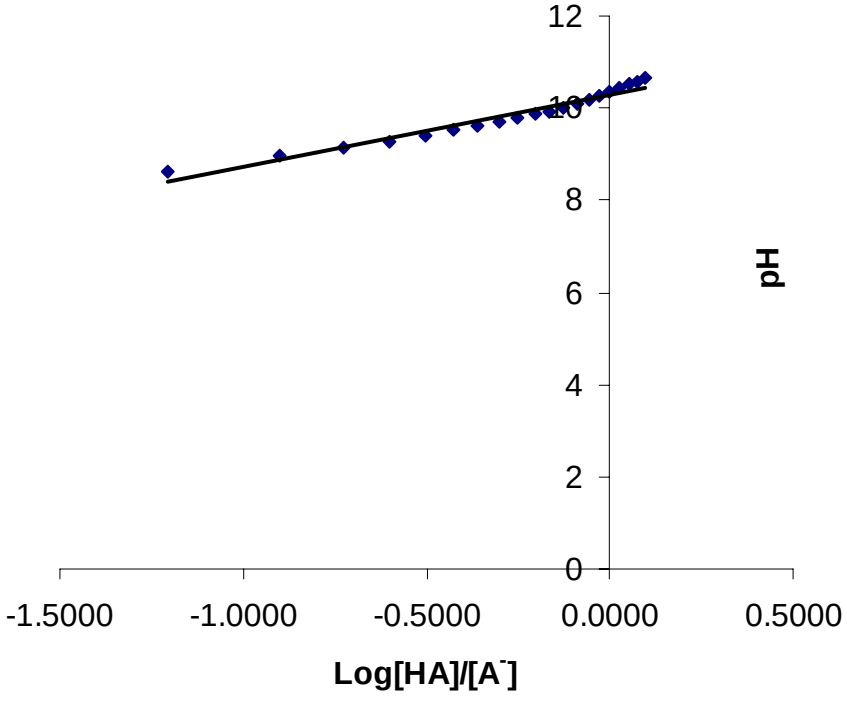

Fig. 2: Plot of pH versus Log [HA] / [A$^-$] for pKa of Alanine

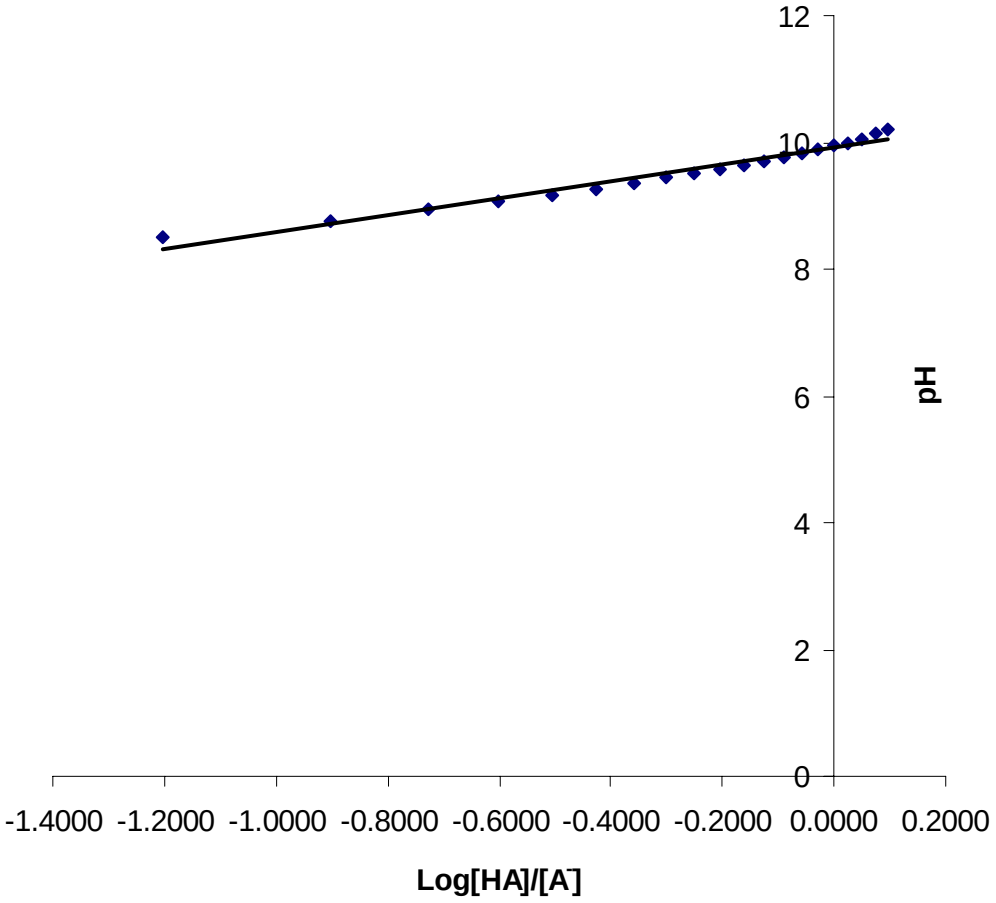

Fig. 3: Plot of pH versus Log [HA] / [A$^-$] for pKa of Valine

CONCLUSION

The acid dissociation constant of non polar amino acids determined graphically for the first time from the available literature were found to be similar with corresponding calculated values reported in the literature.

Therefore the graphical approach is recommended for easier determination of the pKa because of its simplicity, accuracy and being more scientific.

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