

Some Remarks on the Meaning of Isoelectric Point of Glycine

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Introduction

One of the most important characteristics of proteins, their components aminoacids, colloids and minerals is the so-called *isoelectric point (IEP)*.

At the IEP, the values of the electric charge of protein and its conductivity are the smallest and the proteins show the smallest mobility; therefore, many of them are precipitated or transitioned from the form of sol into the form of gel. At this point, proteins have smallest viscosity and swelling ability as well as osmotic pressure. The knowledge of these properties is useful for the separation of proteins by electrophoresis or for the successful flotation of minerals.

Usually, the IEP is given as concrete pH value [1, 2], while in reality, it should be a range, sometimes very wide.

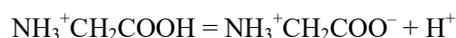
The problem will be discussed below on an example of glycine (*monoaminomonocarboxylic acid*), which is widely used in medicine [3], analytical chemistry [4, 5] and industry in the electrochemical deposition of metals and alloys [6].

Glycine and Ion Equilibria

The IEP value is the pH at which the solution of glycine contains only zwitterion $\text{NH}_3^+\text{CH}_2\text{COO}^-$ and the concentrations of cationic and anionic forms are equal to zero. For glycine, the isoelectric point is reported as equal to 6.05 [7]. However, in reality, the transition of

$\text{NH}_3^+\text{CH}_2\text{COOH}$ (acidic solution) into $\text{NH}_3^+\text{CH}_2\text{COO}^-$ and further into $\text{NH}_2\text{CH}_2\text{COO}^-$ (basic solution) does not happen instantaneously at the strictly determined pH, but in some pH range. Therefore, the calculation of that range is interesting from both theoretical and practical points of view. In our estimations, we assumed that more than 95% of glycine present in the solution occurred in the zwitterion form.

Following equilibria exist in the solution of glycine:



The equilibrium constants are, respectively, equal to [7]:

$$K_1 = \frac{[\text{H}^+][\text{NH}_3^+\text{CH}_2\text{COO}^-]}{[\text{NH}_3^+\text{CH}_2\text{COOH}]} = 4,47 \cdot 10^{-3}$$

$$K_2 = \frac{[\text{H}^+][\text{NH}_2\text{CH}_2\text{COO}^-]}{[\text{NH}_3^+\text{CH}_2\text{COO}^-]} = 2,0 \cdot 10^{-10}$$

The solution of the two equations above together with the mass balance equation:

$$[\text{NH}_3^+\text{CH}_2\text{COO}^-] + [\text{NH}_2\text{CH}_2\text{COO}^-] + [\text{NH}_3^+\text{CH}_2\text{COOH}] = c$$

give the following expression determining the fraction of zwitterion in the solution

$$\left(\alpha = \frac{[NH_3^+CH_2COO^-]}{c} \right) \text{ (where } c \text{ is the total}$$

concentration of glycine), depending on pH

$$\alpha = \frac{[H^+]K_1}{[H^+]^2 + K_1K_2 + K_1[H^+]} \quad (1)$$

or taking into account that $[H^+] = 10^{-pH}$

$$\alpha = \frac{10^{-pH} K_1}{10^{-2pH} + K_1K_2 + K_110^{-pH}} \quad (2)$$

As it follows from the last two equations, the relative fraction of zwitterion concentration $NH_3^+CH_2COO^-$ in the solution as well as the relative fractions of cations $NH_3^+CH_2COOH$ and anions $NH_2CH_2COO^-$ do not depend on the initial concentration of glycine, c , and depend only on the pH of the electrolyte.

If α is equal to unity, from the last equation one obtains the expression for the calculation of the isoelectric point value:

$$pI = -\frac{1}{2}(\lg K_1 + \lg K_2), \quad (3)$$

which coincides with the formula given in literature [7].

From Fig. 1, which shows the relative concentrations of the zwitterion, cations and anions of glycine, depending on pH of the solution, it follows that the relative fraction of glycine zwitterion α is close to unity; not only at the specific pH, but also in the relatively wide pH range. In the considered case, that range spans from 4 to 8, which is in agreement with the titration curve [7, p. 61] (Fig. 2).

Introduction of a small amount of alkali into aminoacetic acid solution after the neutralization of the carboxyl group of glycine dramatically changes the pH value from 4 to 8.

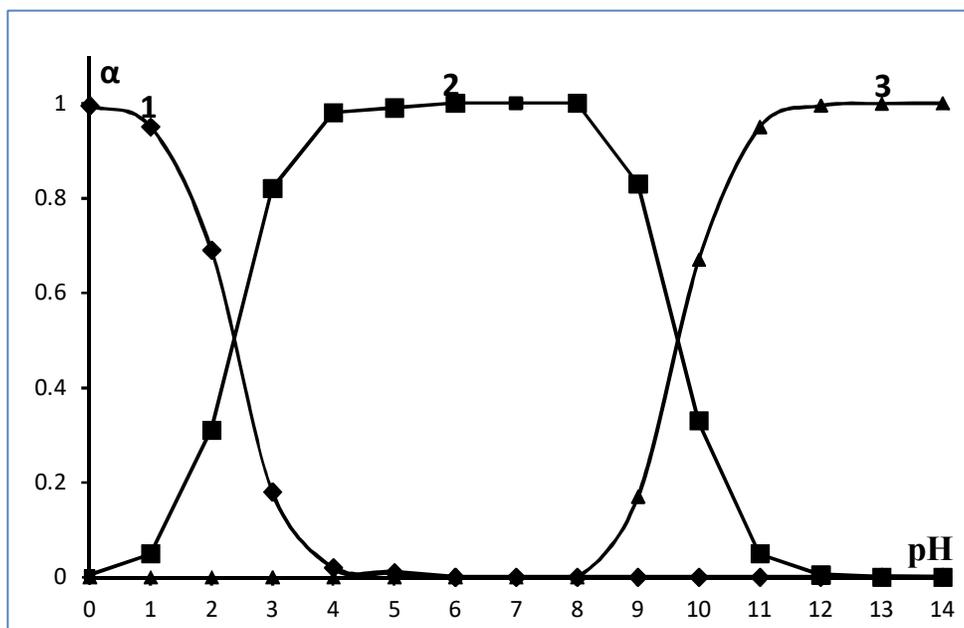


FIG. 1 The relative fractions of concentration of $NH_3^+CH_2COOH$ (1), $NH_3^+CH_2COO^-$ (2) and $NH_2CH_2COO^-$ (3) in the solution of glycine at various pH values.

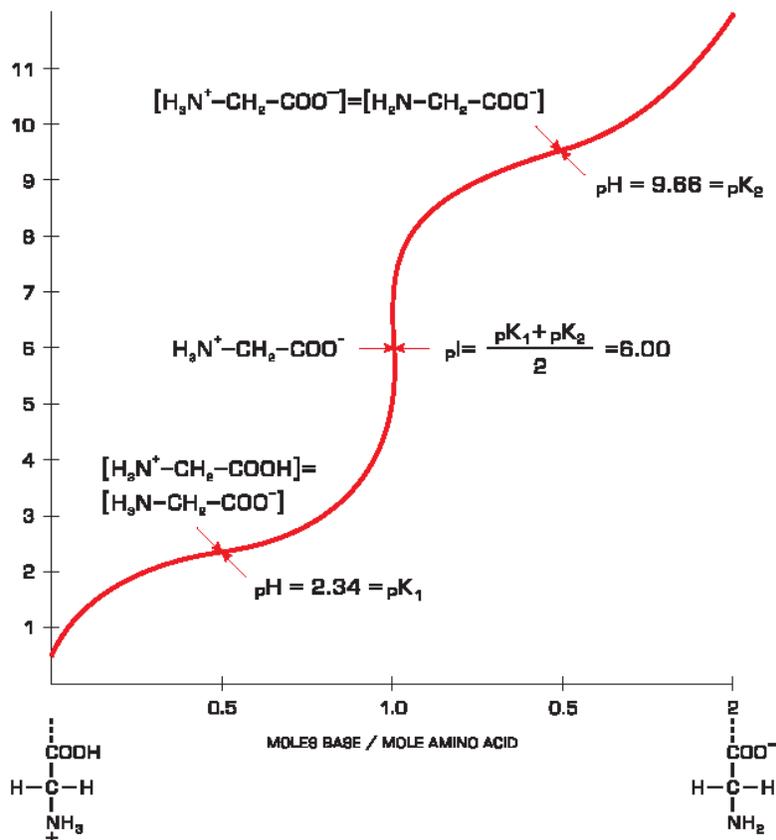


FIG. 2 The titration curve of glycine [7].

Final Remarks

The solution of Eq. (2) for $\alpha \geq 0.95$ results in the pH interval between 3.47 and 8.4, at which the above given relative fraction of zwitterion in solution is really observed.

Apparently, similar reasoning can be applied to other amino-acids. Thus, for the amino-acids, the characteristic value of the isoelectric point should not be given as one point only, but rather as a pH range in which zwitterion with the relative fraction near to unity exists.

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