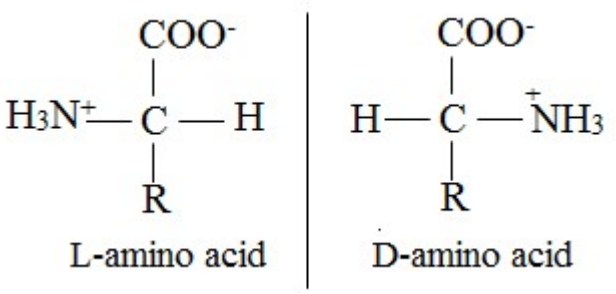
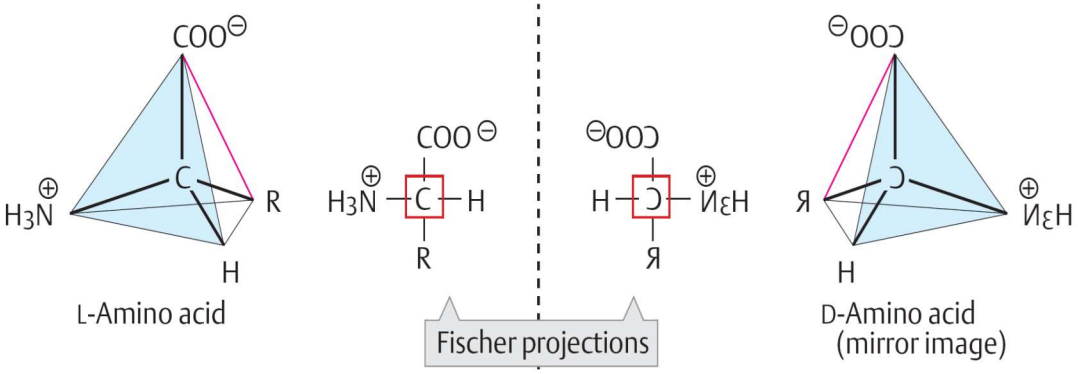
Optical properties of amino acids:

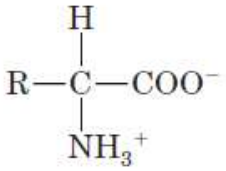
The α-carbon of each amino acid is attached to four different chemical groups and is, therefore, a chiral or optically active carbon atom. Glycine is the exception because its α-carbon has two hydrogen substituents and, therefore, is optically inactive. Amino acids that have an asymmetric center at the α-carbon can exist in two forms, designated D and L that are mirror images of each other. The two forms in each pair are termed stereoisomers, optical isomers, or enantiomers. All amino acids found in proteins are of the L-configuration. However, D-amino acids are found in some antibiotics and in plant and bacterial cell walls.





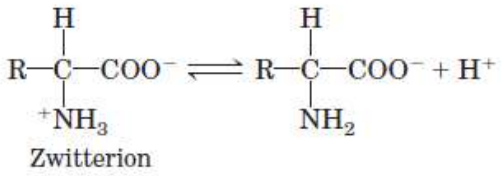
What are zwitterions?

An amino acid has COOH and NH_2 groups in the same molecule. Therefore, in water solution, the COOH donates a proton to the NH_2 so that an amino acid actually has the structure.

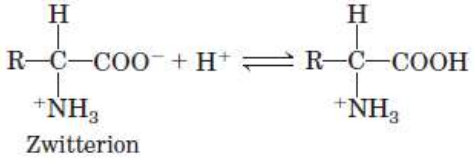


Compounds that have a positive charge on one atom and a negative charge on another are called zwitterions, from the German word zwitter, meaning "hybrid". Amino acids are zwitterions, not only in water solution but also in the solid state. They are therefore ionic compounds that is, internal salts. Un-ionized $RCH(NH_2)COOH$ molecules do not actually exist, in any form.

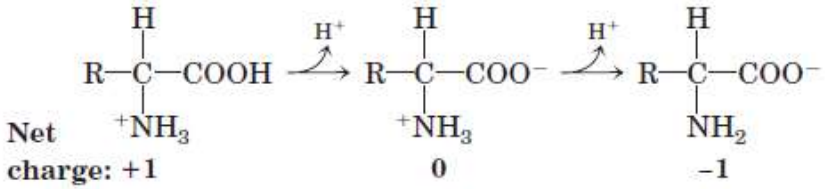
When an amino acid is dissolved in water, it exists in solution as the dipolar ion, or zwitterion. A zwitterion can act as either an acid (proton donor):



Or a base (proton acceptor):



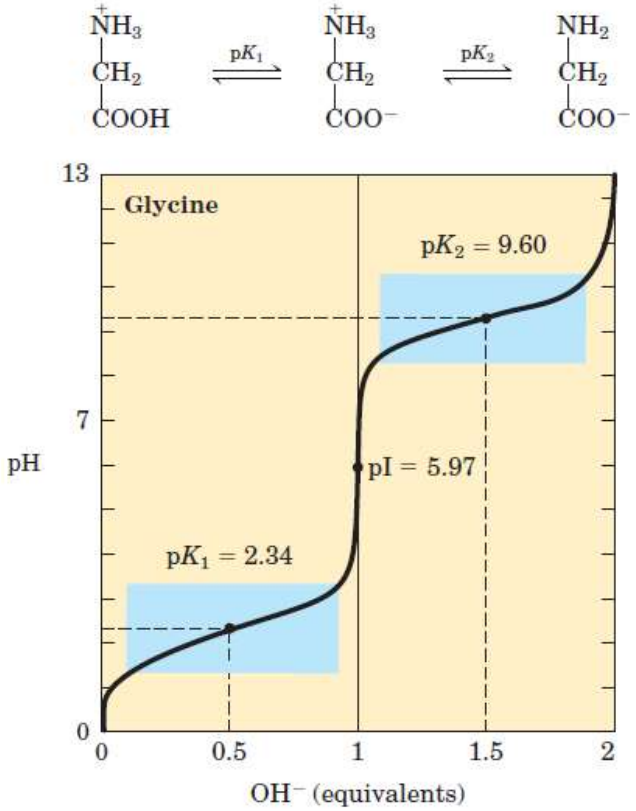
Substances having this dual nature are amphoteric and are often called ampholytes (from amphoteric electrolytes). A simple mono-amino mono-carboxylic amino acid, such as alanine, is a diprotic acid when fully protonated it has two groups, the COOH group and the NH_3 group, that can yield protons:



Acidic and Basic Properties of Amino Acids:

Amino acids and proteins have conspicuous acid–base properties. The amino acids have two or, for those with ionizable side groups, three acid–base groups. The following figure shows

the titration curve of the diprotic form of glycine. The plot has two distinct stages, corresponding to deprotonation of two different groups on glycine.



At very low pH, the predominant ionic species of glycine is the fully protonated form, NH_3^+-CH_2-COOH. At the midpoint in the first stage of the titration, in which the COOH group of glycine loses its proton, equimolar concentrations of the proton-donor (NH_3^+-CH_2-COOH) and proton-acceptor (NH_3^+-CH_2-COO^-) species are present. At the midpoint of any titration, a point of inflection is reached where the pH is equal to the p*Ka* of the protonated group being titrated. For glycine, the pH at the midpoint is 2.34, thus its COOH group has a p*Ka* (labeled pK_1 in the Fig.) of 2.34. As the titration proceeds, another important point is reached at pH 5.97. Here there is another point of inflection, at which removal of the first proton is essentially complete and removal of the second has just begun. At this pH glycine is present largely as the dipolar ion NH_3^+-CH_2-COO^-. We shall return to the significance of this inflection point in the titration curve (labeled pI in the Fig.) shortly. The second stage of the titration corresponds to the removal of a proton from the NH_3 group of glycine. The pH at the midpoint of this stage is 9.60, equal to the

p*Ka* (labeled pK_2 in the Fig.) for the NH_3 group. The titration is essentially complete at a pH of about 12, at which point the predominant form of glycine is $H_2NCH_2COO^-$

From the titration curve of glycine we can derive several important pieces of information. First, it gives a quantitative measure of the p*Ka* of each of the two ionizing groups: 2.34 for the COOH group and 9.60 for the NH_3 group. The second piece of information provided by the titration curve of glycine is that this amino acid has two regions of buffering power. One of these is the relatively flat portion of the curve, extending for approximately 1 pH unit on either side of the first p*Ka* of 2.34, indicating that glycine is a good buffer near this pH. The other buffering zone is centered around pH 9.60. Within the buffering ranges of glycine, the following Henderson-Hasselbalch equation can be used to calculate the proportions of proton-donor and proton-acceptor species of glycine required to make a buffer at a given pH.

$$\mathrm{pH} = \mathrm{p}K + \log\left(\frac{[\mathrm{A}^-]}{[\mathrm{HA}]}\right)$$

Another important piece of information derived from the titration curve of an amino acid is the relationship between its net electric charge and the pH of the solution. At pH 5.97, the point of inflection between the two stages in its titration curve, glycine is present predominantly as its dipolar form, fully ionized but with no net electric charge. The characteristic pH at which the net electric charge is zero is called the isoelectric point or isoelectric pH, designated pI. For glycine, which has no ionizable group in its side chain, the isoelectric point is simply the arithmetic mean of the two p*Ka* values:

$$\mathrm{pI} = \frac{1}{2}(\mathrm{p}K_1 + \mathrm{p}K_2) = \frac{1}{2}(2.34 + 9.60) = 5.97$$

Electrophoresis:

Electrophoresis is a separation technique based on the movement of charged ions under the influence of an electrical field. This technique is primarily used for the separation of amino acids and peptides on the basis of their charge. All amino acids contain ionizable groups that cause the amino acids, in solution, to act as charged polyelectrolytes that can migrate in an

electric field. The amino acids with a net positive charge will migrate toward the negative electrode. Those with a negative net charge will move toward the positive electrode.

As is evident in the Figure above, glycine has a net negative charge at any pH above its pI and will thus move toward the positive electrode (the anode) when placed in an electric field. At any pH below its pI, glycine has a net positive charge and will move toward the negative electrode (the cathode). The farther the pH of a glycine solution is from its isoelectric point, the greater the net electric charge of the population of glycine molecules. At pH 1.0, for example, glycine exists almost entirely as the form H_3NCH_2COOH, with a net positive charge of 1.0. At pH 2.34, where there is an equal mixture of H_3NCH_2COOH and H_3NCH_2COO, the average or net positive charge is 0.5. The sign and the magnitude of the net charge of any amino acid at any pH can be predicted in the same way.

Example: Predict the direction of migration in an electrophoresis for the amino acids (Ser, His, Val and Asp) at pH 2.0, 4.0, 6.0 and 12?