BIOMOLECULES
SEM-5, CC-12
PART-2, PPT-22

Part-2: Amino Acids-II, Properties

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BIOMOLECULES (PART-2, PPT-22)

Amino Acids-II, Properties

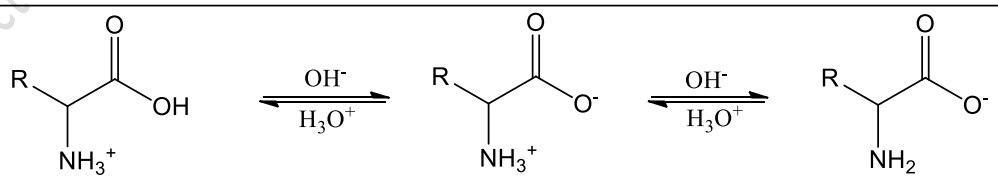
Amino Acids as Dipolar Ions

The physical properties of a typical amino acid such as glycine suggest that it is a very polar substance, much more polar than would be expected on the basis of its formulation as $H_2NCH_2CO_2H$. When measured in aqueous solution, the dipole moment of glycine, μ = 14.0 D, (and other amino acids) is found to have a large value. To account for this large value, it has been suggested that glycine exists, in solution, as an inner salt and therefore, it is to some extent ionic in nature. Such a double charged ion is also known as a zwitterion (from the German, meaning "hybrid ion"), ampholyte or a dipolar ion.

Glycine is a crystalline solid. It does not melt, but on being heated it eventually decomposes at 233 °C. It is very soluble in water (25 g/100 mL, 25 °C) but practically insoluble in nonpolar organic solvents. These properties are attributed to the fact that the stable form of glycine is a zwitterion or inner salt. The dipolar ion structure also accounts for the absence of acidic and basic properties of an amino acid (the carboxyl and amino groups of the same molecule neutralize each other to form a salt). The properties of crystalline glycine, e.g., its melting point and its insolubility in hydrocarbon solvents, also indicate that it exists as the inner salt in the solid state. X-ray analysis has shown that all amino acids exist as dipolar ions.

The dipole moments of the amino acids are comparatively higher than those of amines and carboxylic acids of similar molecular weight (1.7D for propanoic acid and 1.4D for *n*-butylamine). Glycine, as well as other amino acids, is amphoteric, meaning it contains an acidic functional group and a basic functional group. The acidic functional group is the aminium ion $-NH_3^+$ and the basic functional group is the carboxylate ion $-CO_2^-$.

Amino acids contain both a basic group ($-NH_2$) and an acidic group ($-CO_2H$). Consequently, in the dry solid state, amino acids exist as dipolar ions, a form in which the carboxyl group is present as a carboxylate ion, $-CO_2^-$, and the amino group is present as an aminium ion, $-NH_3^+$. Dipolar ions are also called zwitterions. In aqueous solution, an equilibrium exists between the dipolar ion and the anionic and cationic forms of an amino acid (Figure 1).



Cationic form (cA) (predominant in strongly acidic solutions, e.g., at pH 0)

Dipolar ion (DI)

Anionic form (cB) (predominant in strongly basic solutions, e.g., at pH 14)

Figure 1: Different forms of amino acids

Amino Acids as Dipolar Ions and Isoelectric Point

The position of the equilibrium as also the predominant form of the amino acid present in a solution depends on the pH of the solution and on the nature of the amino acid. In strongly acidic solutions all amino acids are present primarily as cations, i.e., the conjugate acid predominating, and in strongly basic solutions they are present as anions, i.e., the conjugate base predominating. For each amino acid there is a particular pH value at which the concentration of the dipolar ion is a maximum.

The isoelectric point (p*I*) is the pH at which the concentration of the dipolar ion is at its maximum and the concentrations of the anions (cB) and cations (cA) are equal, i.e., [cA] = [cB]. Each amino acid has a particular isoelectric point. Proteins have isoelectric points as well. This property of proteins is important for their separation and identification.

Since the net charge is zero, the dipolar ion is electrically neutral and consequently, in this condition, the amino acid does not migrate when placed in an electric field. Since they can behave both as an acid and a base, monoamino-monocarboxylic acids have two pK_a values, one as an acid (when titrated with base) and the other as a base (when titrated with acid). By convention, pK_{a1} is the one corresponding to the group titrated at the most acid region, i.e., the carboxyl group (the change is from carboxylate ion).

The isoelectric point of a monoamino-monocarboxylic acid can easily be calculated. If the isoelectric amino acid is represented as H_3N^+-Z-CO_2^-, then the following equilibria exists.

$$\underset{\text{cA}}{H_3N^+\text{-Z-}CO_2H} \rightleftharpoons \underset{\text{DI}}{H_3N^+\text{-Z-}CO_2^-} + H^+ \text{ ----------Eq. 1}$$

$$\underset{\text{DI}}{H_3N^+\text{-Z-}CO_2^-} \rightleftharpoons \underset{\text{cB}}{H_2N\text{-Z-}CO_2^-} + H^+ \text{ ----------Eq. 2}$$

$$\text{From equation 1: } K_{a_1} = \frac{[DI][H^+]}{[cA]} - - - - Eq.\ 3$$

$$\text{Therefore, [cA]} = \frac{[DI][H^+]}{K_{a_1}} - - - - Eq.\ 4$$

$$\text{From equation 2: } K_{a_2} = \frac{[cB][H^+]}{[DI]} - - - - Eq.\ 5$$

$$\text{Therefore, [cB]} = \frac{K_{a_2}[DI]}{[H^+]} - - - - Eq.\ 6$$

At an isoelectric point (p*I* or pH_i), the [DI] is a maximum and since the net charge is zero,

$$[cA] = [cB]$$

Therefore,

$$\frac{[DI][H_i^+]}{K_{a_1}} = \frac{K_{a_2}[DI]}{[H_i^+]} - - - - Eq.\ 7$$

Simplifying equation 7, one obtains,

$$[H_i]^2 = K_{a_1} x\, K_{a_2} - - - - Eq.\ 8$$

Taking logarithm and applying the definition of pH, one obtains,

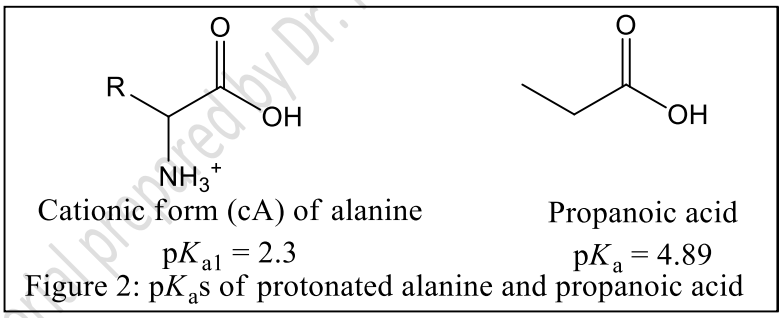
$$2\ pH_i = pK_{a_1} + pK_{a_2} - - - - Eq.\ 9$$

$$pH_i = \frac{pK_{a_1} + pK_{a_2}}{2} - - - - Eq.\ 10$$

Therefore, for mono-acidic and mono-basic amino acids, isoelectric point is the average of the pK_as of the monocation (conjugate acid) and the dipolar ion (zwitterion). The isoelectric point of glycine can easily be calculated from the data pK_{a1} = 2.4 and pK_{a2} = 9.6.

$$pI = \frac{pK_{a_1} + pK_{a_2}}{2} = \frac{2.4 + 9.6}{2} = 6.0\ (isoelectric\ point\ of\ glycine)$$

pK_{a1} corresponds to ionization of the carboxyl group and pK_{a2} corresponds to deprotonation of the aminium ion. Let us consider first an amino acid with a side chain that contains neither acidic nor basic groups - an amino acid, i.e., a neutral amino acid, such as alanine. If alanine is dissolved in a strongly acidic solution (e.g., pH 0), it is present in mainly a net cationic form (conjugate acid). In this state the amino group is protonated (bears a formal +1 charge) and the carboxylic acid group is neutral (has no formal charge). As is typical of α-amino acids, the pK_a for the carboxylic acid hydrogen of alanine is considerably lower (2.3) than the pK_a of an ordinary carboxylic acid (e.g., propanoic acid, pK_a 4.89) (Figure 2).



Cationic form (cA) of alanine — pK_{a1} = 2.3

Propanoic acid — pK_a = 4.89

Figure 2: pK_as of protonated alanine and propanoic acid

The reason for this enhanced acidity of the carboxyl group in an α-amino acid is the inductive effect of the neighbouring aminium ion, which helps to stabilize the conjugate base, a carboxylate anion formed when it loses a proton. Loss of a proton from the carboxyl group in a cationic α-amino acid leaves the molecule electrically neutral (in the form of a dipolar ion). This equilibrium is shown in *left side* of the equation in Figure 3.

The protonated amino group of an α-amino acid is also acidic, but less so than the carboxylic acid group. The pK_a of the aminium group in alanine is 9.7. The equilibrium for loss of an aminium proton is shown in *right side* of the equation in Figure 3. The carboxylic acid proton is always lost before a proton from the aminium group in an α-amino acid.

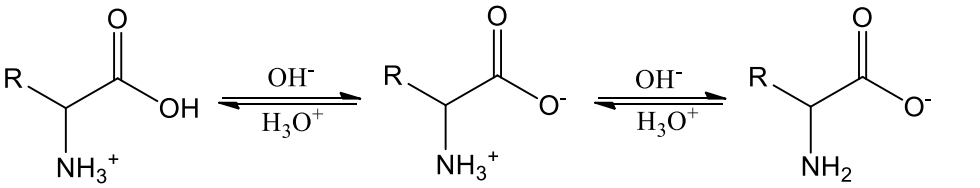


Figure 3: Acid-base equilibrium of a neutral amino acid

The state of an α-amino acid at any given pH is governed by a combination of two equilibria, as shown in the equation in Figure 3 for alanine. The isoelectric point (pI) of an amino acid such as alanine is the average of p$K_{a1}$and pK_{a2}. Therefore,

$$pI = \frac{pK_{a_1} + pK_{a_2}}{2} = \frac{2.3 + 9.7}{2} = 6.0\ (isoelectric\ point\ of\ alanine)$$

When a base is added to a solution of the net cationic form of alanine (initially at pH 0, for example), the first proton removed is the carboxylic acid proton, as is expected. In the case of alanine, when a pH of 2.3 is reached, the carboxylic acid proton will have been removed from half of the molecules. This pH represents the pK_a of the alanine carboxylic acid proton, as can be demonstrated using the Henderson-Hasselbalch equation.

The Henderson-Hasselbalch equation shows that for an acid (HA) and its conjugate base (A^-) when [HA] = [A^-], then pH = pK_a.

$$pH = pK_a - log\frac{[A^-]}{[HA]} \quad \text{[Henderson-Hasselbalch equation]}$$

Therefore, when the acid is half neutralized, [HA] = [A^-],

$log\frac{[A^-]}{[HA]} = 0$, and thus pH = p$K_a$.

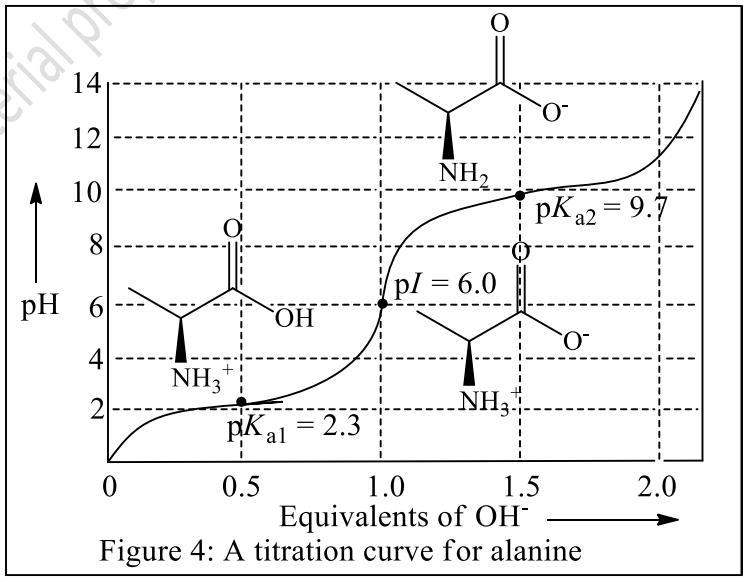


Figure 4: A titration curve for alanine

As more base is added to this solution, alanine reaches its isoelectric point (p*I*), the pH at which all of alanine's carboxylic acid protons have been removed but not its aminium protons. The molecules are, therefore, electrically neutral (in their dipolar ion or zwitterionic form) because the carboxylate group carries a -1 charge and the aminium group a +1 charge. The p*I* for alanine is 6.0.

Now, on continuing addition of base, protons from the aminium ions will begin to be removed, until at pH 9.7 half of the ammonium groups will have lost a proton. This pH represents the pK_a of the aminium group. Finally, as more base is added, the remaining aminium protons will be lost until all of the alanine molecules have lost their aminium protons. At this point (e.g., pH 14) the molecules carry a net anionic charge from their carboxylate group. The amino groups are now electrically neutral.

A titration curve for these equilibria is shown in Figure 4. The graph represents the change in pH as a function of the number of molar equivalents of base. Because alanine has two protons to lose in its net cationic form, when one molar equivalent of base has been added, the molecules will have each lost one proton and they will be electrically neutral (the dipolar ion or zwitterionic form).

The titration curve of glycine: At pH values less than pK_{a1}, $H_3N^+CH_2CO_2H$ is the major species present. At pH values between pK_{a1} and pK_{a2}, the principal species is the zwitterion $H_3N^+CH_2CO_2^-$. The concentration of the zwitterion is a maximum at the isoelectric point p*I*. The pH greater than pK_{a2}, $H_3N^+CH_2CO_2^-$ is the species present in greatest concentration.

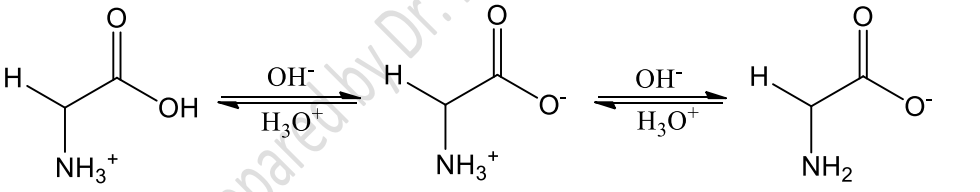


Figure 5: Acid-base equilibrium of glycine

If an amino acid contains a side chain that has an acidic or basic group, the equilibria become different. Let us consider lysine, for example, an amino acid that has an additional -NH_2 group on its ε carbon in addition to the α-NH_2. In strongly acidic solution, lysine is present as a dication because both amino groups are protonated. The first proton to be lost as the pH is raised is a proton of the carboxyl group (pK_{a1} = 2.2), the next is from the α-aminium group (pK_{a2} = 9.0), and the last is from the ε-aminium group (pK_{a3} = 10.5). The equilibria is shown in Figure 6.

Amino Acids as Dipolar Ions

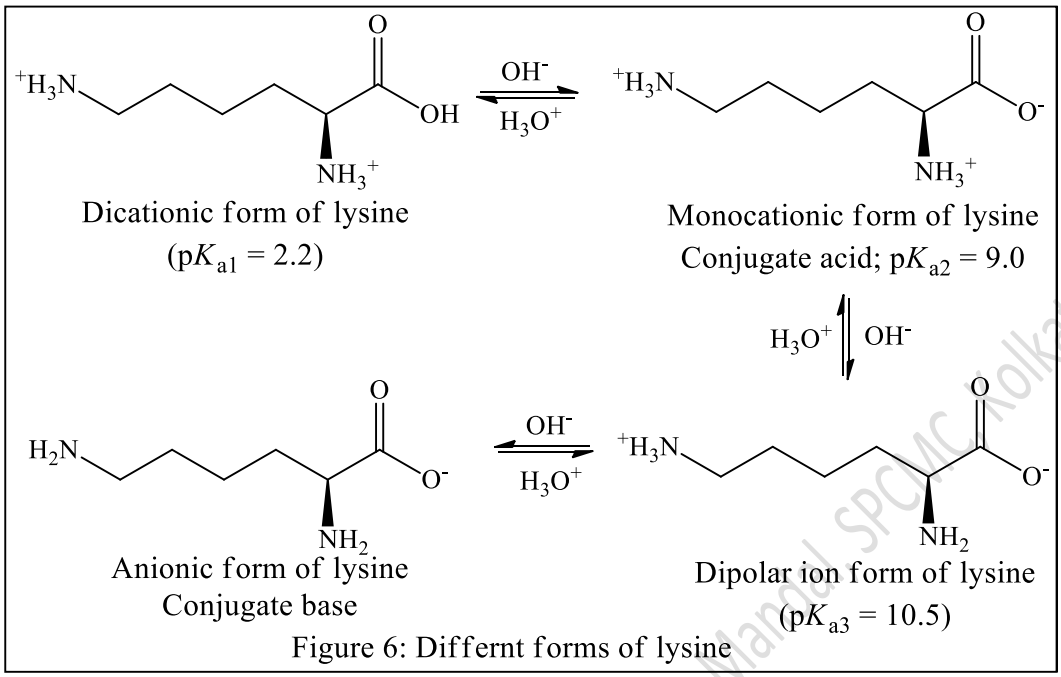


Figure 6: Differnt forms of lysine

The isoelectric point of lysine (as also other basic amino acids) is the average of the pK_{a2} (monocation) and pK_{a3} (the dipolar ion). This, therefore, corresponds to the equilibria among the monocation and monoanion with the dipolar ion. Therefore,

$$pI = \frac{pK_{a_2} + pK_{a_3}}{2} = \frac{9.0 + 10.5}{2} = 9.8\ (isoelectric\ point\ of\ lysine)$$

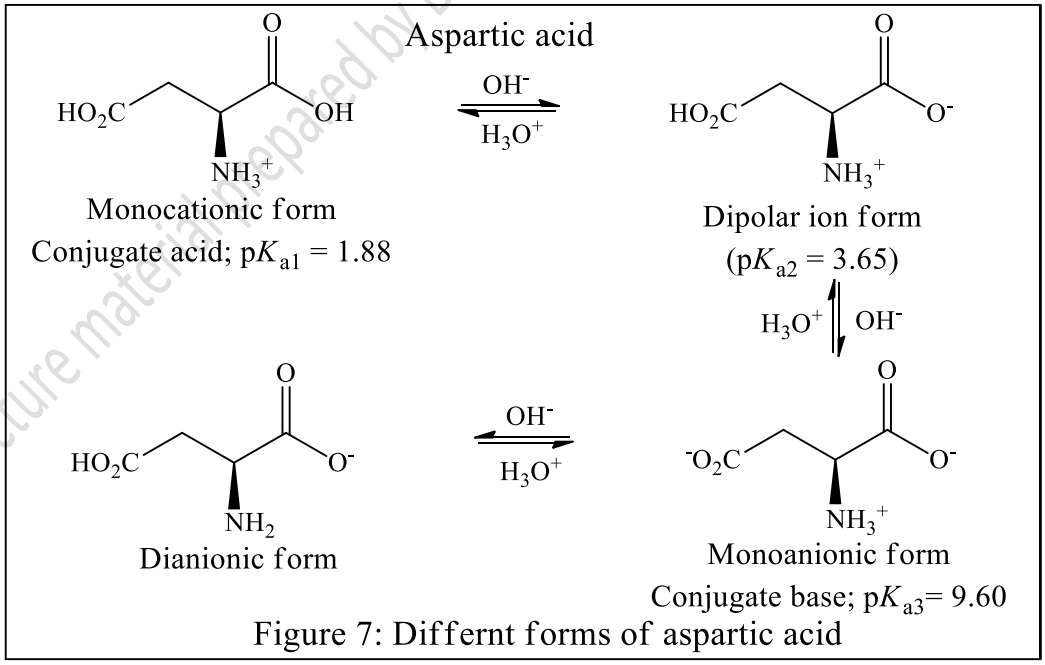


Figure 7: Differnt forms of aspartic acid

The isoelectric point of the amino acids is midway between the pK_a values of the conjugate acid (mono cation) and its dipolar ion (zwitterion). Aspartic acid has an acidic side chain and therefore, a pI of 2.77.

$$pI = \frac{pK_{a_1} + pK_{a_2}}{2} = \frac{1.88 + 3.65}{2} = 2.77\ (isoelectric\ point\ of\ aspartic\ acid)$$

The acidic amino acids (aspartic and glutamic acid) have acidic side chains and basic amino acids (lysine, arginine and histidine) have basic side chains. Individual amino acids differ in their acid-base properties. This is important in peptides and proteins, where the properties of the substance depend on its amino acid constituents, especially on the nature of the side chains.

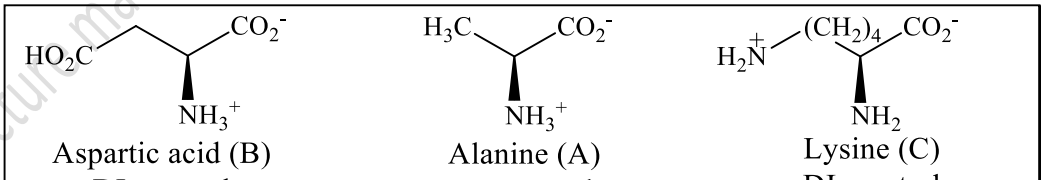
Separation of Amino Acids: Electrophoresis

Electrophoresis is a method of separation and purification that depends on the movement of charged particles in an electric field. Its principles can be introduced by considering the electrophoretic behaviour of some representative amino acids. The medium is a cellulose acetate strip that is moistened with an aqueous solution buffered at a particular pH. The opposite ends of the strip are placed in separate components containing the buffer, and each compartment is connected to a source of direct electric current.

If the buffer solution is more acidic than the isoelectric point (p*I*) of the amino acid, the amino acid has a net positive charge and migrates toward the negatively charged electrode, cathode.

Electrophoresis

Conversely, when the buffer is more basic than the p*I* of the amino acid, the amino acid has a net negative charge and migrates toward the positively charged electrode, anode. When the pH of the buffer corresponds to the p*I*, the amino acid has no net charge and does not migrate from the origin. Thus if a mixture containing alanine, aspartic acid, and lysine is subjected to electrophoresis in a buffer that matches the isoelectric point of alanine (pH 6.0), aspartic acid (p*I* = 2.8) migrates toward the positive electrode, alanine remains at the origin, and lysine (p*I* = 9.7) migrates toward the negative electrode.



Aspartic acid (B) DI, neutral net charge 0 | Alanine (A) DI, neutral net charge 0 | Lysine (C) DI, neutral net charge 0

Figure 8: Structure at the isoelectric point (p*I*)

A mixture of amino acids, e.g., alanine (A), aspartic acid (B), and lysine (C) is placed at the centre of a sheet of cellulose acetate (Figure 9).

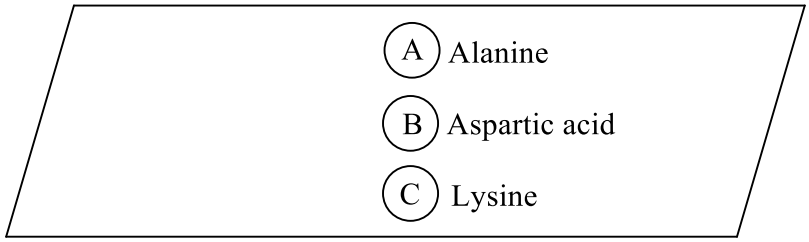


Figure 9: Sheet of cellulose acetate soaked with an aqueous solution buffered at a pH of 6.0.

The sheet is soaked with an aqueous solution buffered at a pH of 6.0. At this pH aspartic acid exists as its -1 ion (as an anion), alanine as its dipolar ion (zwitterion, net charge 0), and lysine as its +1 ion (as a cation) as shown in Figure 10.

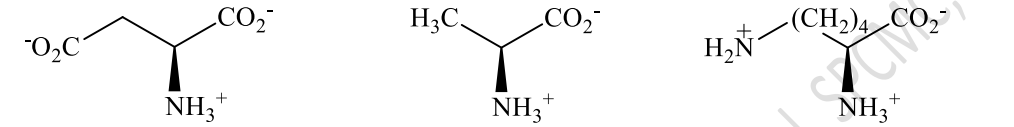
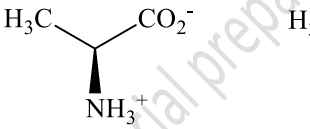
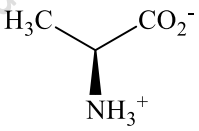
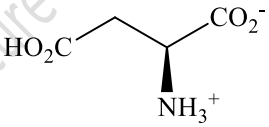
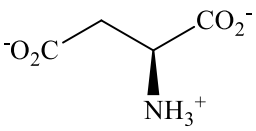
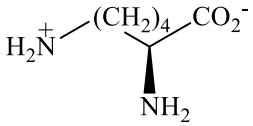
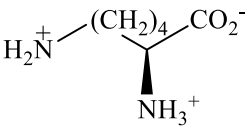

Mono-anion of aspartic acid (net charge -1) | Alanine (neutral; net charge 0) | Mono-cation of lysine (cation; net charge +1)

Figure 10: Structure of amino acids A, B and C at pH = 6

Application of an electric field causes the negatively charged ions to migrate to the positive electrode (anode), and the positively charged ions to migrate to the negative electrode (cathode). The dipolar ion (zwitterion), with a net charge zero, remains at its original position) as shown in Figure 11.

Table 1: Results of electrophoresis of alanine, aspartic acid and lysine at buffer pH = 6.0

Structure at p*I*	Structure at pH = 6	Nature at pH = 6.0	Movement under application of electric field
 Alanine (A) neutral at p*I* = *6.0*		neutral net charge 0	no movement
 Aspartic acid (B) neutral at p*I*		Mono-anion (net charge -1)	movement towards positive electrode (anode)
 Lysine (C) neutral at p*I*		Mono-cation (net charge +1)	movement towards negative electrode (cathode)

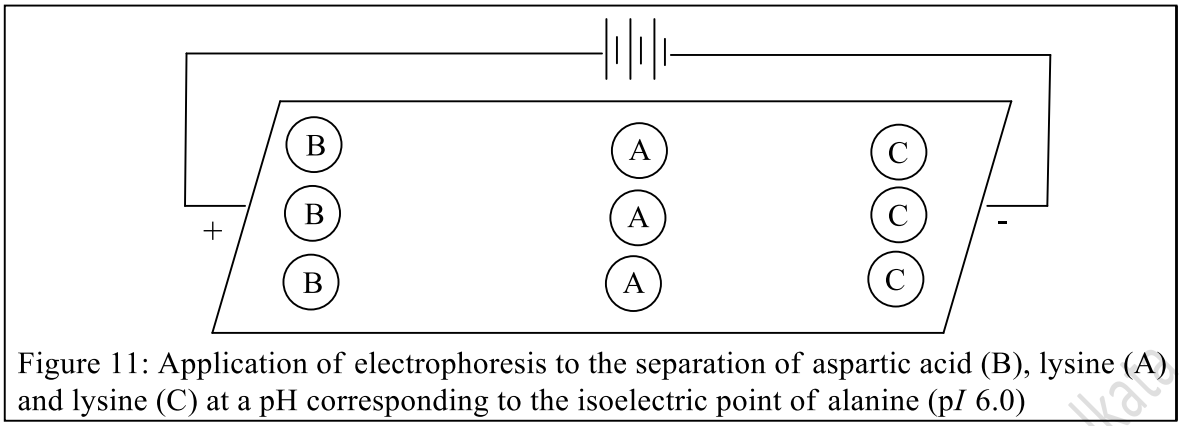


Figure 11: Application of electrophoresis to the separation of aspartic acid (B), lysine (A) and lysine (C) at a pH corresponding to the isoelectric point of alanine (p*I* 6.0)

However, the success of this method in separating mixture of amino acids depends on the nature of amino acids as well as the pH of the buffer medium. Complete Separation of these three amino acids cannot be possible if the sheet is soaked with an aqueous solution buffered at a pH which is equal with the p*I* of either aspartic acid (p*I* 2.8) or lysine (p*I* 9.7).

At pH (= 2.8) which is equal to the p*I* of aspartic acid, it will exist as dipolar ion (zwitterion) and hence, is electrically neutral and do not migrate under the application of electric field. However, alanine (pI 6.0) and lysine (pI 9.7) will exist as +1 ion (monocation) and +2 ion (dication), respectively. Therefore, both of them will migrate towards the positive electrode, cathode. Consequently, alanine and lysine cannot be separated.

On the other hand, at pH (= 9.7) which is equal to the p*I* of lysine, the latter will exist as dipolar ion (zwitterion) and hence, is electrically neutral and do not migrate under the application of electric field. However, aspartic acid (p*I* 2.8) and alanine (p*I* 6.0) will exist as -2 ion (dianion) and -1 ion (monoanion), respectively. Therefore, they will migrate towards the negative electrode, anode. Consequently, aspartic acid and alanine cannot be separated.

Again, if the mixture contains two or more amino acids of the same type, such as neutral or acidic or basic, separation under this method cannot be possible. Electrophoresis is used primarily to analyze mixture of peptides and proteins, rather than individual amino acids, but analogous principles apply. Because they incorporate different numbers of amino acids and because their side chains are different, two peptides will have slightly different net charges at a particular pH.

Thus, their mobilities in an electric field will be different, and electrophoresis can be used to separate them. The medium used to separate peptides and proteins is typically a polyacrylamide gel, leading to the term gel electrophoresis for this technique.