The other 9, called essential amino acids, are biosynthesized only in plants and microorganisms and must be obtained in our diet. The division between essential and nonessential amino acids is not clear-cut, however. Tyrosine, for instance, is sometimes considered nonessential because humans can produce it from phenylalanine, but phenylalanine itself is essential and must be obtained in the diet. Arginine can be synthesized by humans, but much of the arginine we need also comes from our diet.

PROBLEM 26-1 How many of the α-amino acids shown in Table 26.1 contain aromatic rings? How many contain sulfur? How many contain alcohol groups? How many contain hydrocarbon side chains?

PROBLEM 26-2 Of the 19 L amino acids, 18 have the *S* configuration at the α carbon. Cysteine is the only L amino acid that has an *R* configuration. Explain.

PROBLEM 26-3 The amino acid threonine, (2*S*,3*R*)-2-amino-3-hydroxybutanoic acid, has two chirality centers.

(a) Draw threonine, using normal, wedged, and dashed lines to show dimensionality.

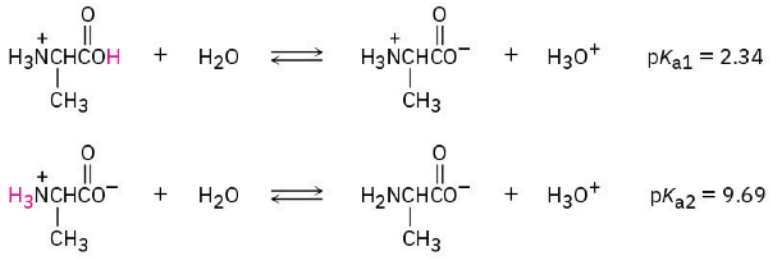
(b) Draw a diastereomer of threonine, and label its chirality centers as *R* or *S*.

26.2 Amino Acids and the Henderson–Hasselbalch Equation: Isoelectric Points

According to the Henderson–Hasselbalch equation (**Section 20.3** and **Section 24.5**), if we know both the pH of a solution and the pK_a of an acid HA, we can calculate the ratio of [A^-] to [HA] in the solution. Furthermore, when pH = pK_a, the two forms A^- and HA are present in equal amounts because log 1 = 0.

$$\text{pH} = \text{p}K_a + \log\frac{[A^-]}{[HA]} \quad \text{or} \quad \log\frac{[A^-]}{[HA]} = \text{pH} - \text{p}K_a$$

To apply the Henderson–Hasselbalch equation to an amino acid, let's find out what species are present in a 1.00 M solution of alanine at pH = 9.00. According to **TABLE 26.1**, protonated alanine [$^+H_3NCH(CH_3)CO_2H$] has pK_{a1} = 2.34 and neutral zwitterionic alanine [$^+H_3NCH(CH_3)CO_2^-$] has pK_{a2} = 9.69:



Because the pH of the solution is much closer to pK_{a2} than to pK_{a1}, we need to use pK_{a2} for the calculation. From the Henderson–Hasselbalch equation, we have:

$$\log\frac{[A^-]}{[HA]} = \text{pH} - \text{p}K_a = 9.00 - 9.69 = -0.69$$

So

$$\frac{[A^-]}{[HA]} = \text{antilog}(-0.69) = 0.20 \quad \text{and} \quad [A^-] = 0.20[HA]$$

In addition, we know that

$$[A^-] + [HA] = 1.00\ \text{M}$$

Solving the two simultaneous equations gives [HA] = 0.83 and [A^-] = 0.17. In other words, at pH = 9.00, 83% of alanine molecules in a 1.00 M solution are neutral (zwitterionic) and 17% are deprotonated. Similar calculations can be done at other pH values and the results plotted to give the titration curve shown in **FIGURE 26.2**.

Each leg of the titration curve is calculated separately. The first leg, from pH 1 to 6, corresponds to the dissociation of protonated alanine, H_2A^+. The second leg, from pH 6 to 11, corresponds to the dissociation of zwitterionic alanine, HA. It's as if we started with H_2A^+ at low pH and then titrated with NaOH. When 0.5

equivalent of NaOH is added, the deprotonation of H_2A^+ is 50% complete; when 1.0 equivalent of NaOH is added, the deprotonation of H_2A^+ is finished and HA predominates; when 1.5 equivalents of NaOH is added, the deprotonation of HA is 50% complete; and when 2.0 equivalents of NaOH is added, the deprotonation of HA is finished.

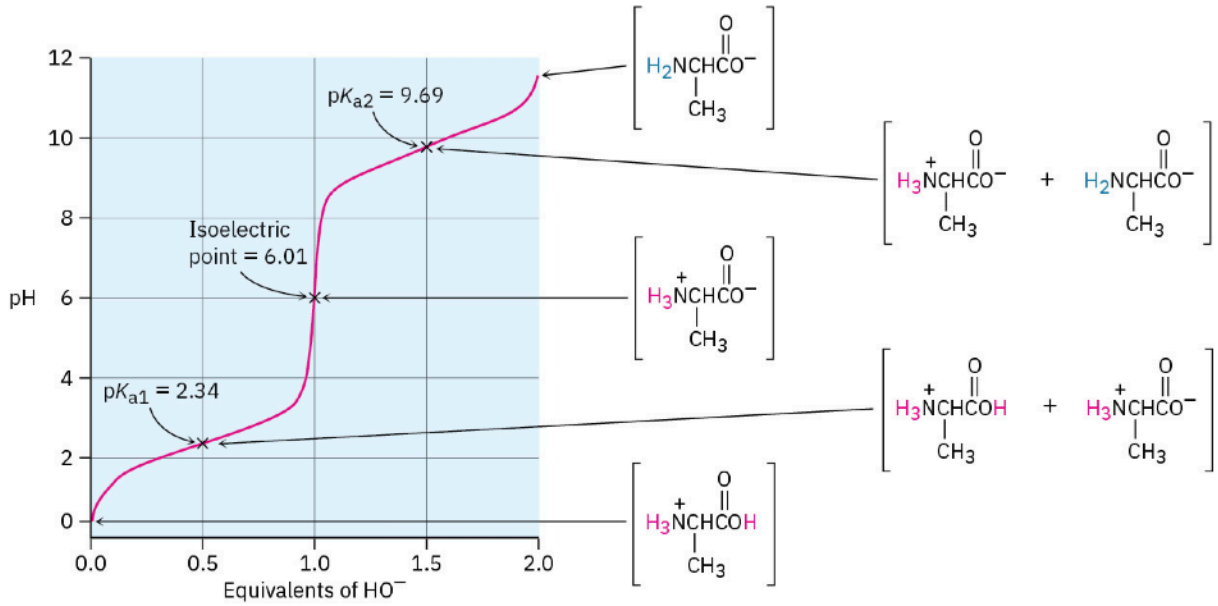
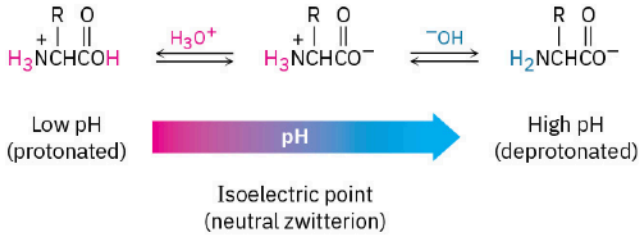


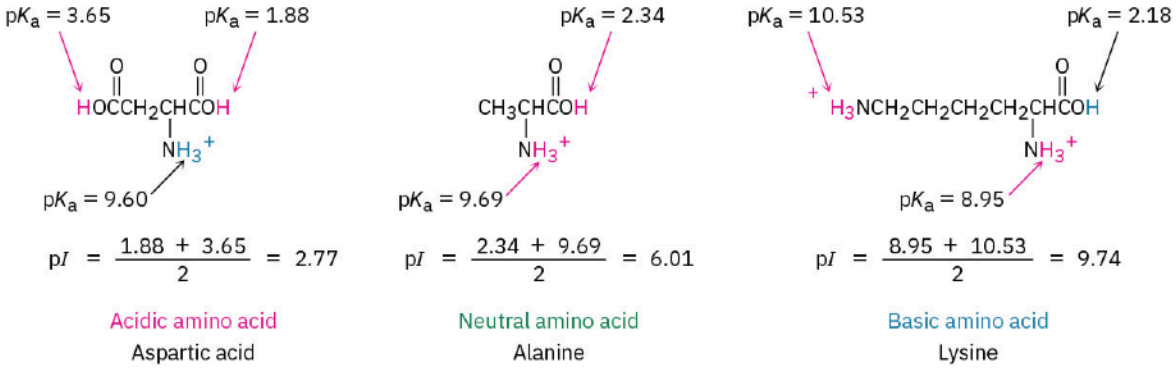
FIGURE 26.2 A titration curve for alanine, plotted using the Henderson–Hasselbalch equation. Each of the two legs is plotted separately. At pH < 1, alanine is entirely protonated; at pH = 2.34, alanine is a 50 : 50 mix of protonated and neutral forms; at pH = 6.01, alanine is entirely neutral; at pH = 9.69, alanine is a 50 : 50 mix of neutral and deprotonated forms; at pH > 11.5, alanine is entirely deprotonated.

Look carefully at the titration curve in **FIGURE 26.2**. In acid solution, the amino acid is protonated and exists primarily as a cation. In basic solution, the amino acid is deprotonated and exists primarily as an anion. In between the two is an intermediate pH at which the amino acid is exactly balanced between anionic and cationic forms, existing primarily as the neutral, dipolar zwitterion. This pH is called the amino acid's **isoelectric point (p*I*)** and has a value of 6.01 for alanine.



The isoelectric point of an amino acid depends on its structure, with values for the 20 common amino acids given previously in **TABLE 26.1**. The 15 neutral amino acids have isoelectric points near neutrality, in the pH range 5.0 to 6.5. The two acidic amino acids have isoelectric points at lower pH so that deprotonation of the side-chain $-CO_2H$ does not occur at their p*I*, and the three basic amino acids have isoelectric points at higher pH so that protonation of the side-chain amino group does not occur at their p*I*.

More specifically, the p*I* of any amino acid is the average of the two acid-dissociation constants that involve the neutral zwitterion. For the 13 amino acids with a neutral side chain, p*I* is the average of pK_{a1} and pK_{a2}. For the four amino acids with either a strongly or weakly acidic side chain, p*I* is the average of the two lowest pK_a values. For the three amino acids with a basic side chain, p*I* is the average of the two highest pK_a values.



Just as individual amino acids have isoelectric points, proteins have an overall pI due to the cumulative effect of all the acidic or basic amino acids they may contain. The enzyme lysozyme, for instance, has a preponderance of basic amino acids and thus has a high isoelectric point (pI = 11.0). Pepsin, however, has a preponderance of acidic amino acids and a low isoelectric point (pI ~ 1.0). Not surprisingly, the solubilities and properties of proteins with different pIs are strongly affected by the pH of the medium. Solubility in water is usually lowest at the isoelectric point, where the protein has no net charge, and is higher both above and below the pI, where the protein is charged.

We can take advantage of the differences in isoelectric points to separate a mixture of proteins into its pure components. Using a technique known as ***electrophoresis***, a mixture of proteins is placed near the center of a strip of paper or gel. The paper or gel is moistened with an aqueous buffer of a given pH, and electrodes are connected to the ends of the strip. When an electric potential is applied, the proteins with negative charges (those that are deprotonated because the pH of the buffer is above their isoelectric point) migrate slowly toward the positive electrode. At the same time, those amino acids with positive charges (those that are protonated because the pH of the buffer is below their isoelectric point) migrate toward the negative electrode.

Different proteins migrate at different rates, depending on their isoelectric points and on the pH of the aqueous buffer, thereby effecting a separation of the mixture into its components. **FIGURE 26.3** illustrates this process for a mixture containing basic, neutral, and acidic components.

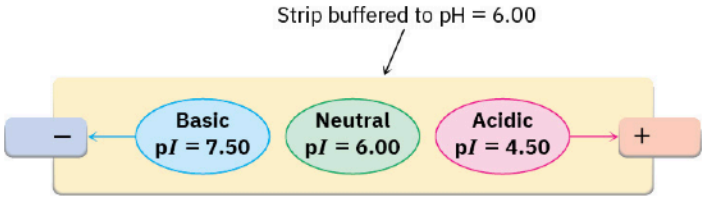


FIGURE 26.3 Separation of a protein mixture by electrophoresis. At pH = 6.00, a neutral protein does not migrate, a basic protein is protonated and migrates toward the negative electrode, and an acidic protein is deprotonated and migrates toward the positive electrode.

PROBLEM 26-4 Hemoglobin has pI = 6.8. Does hemoglobin have a net negative charge or net positive charge at pH = 5.3? At pH = 7.3?

26.3 Synthesis of Amino Acids

α-Amino acids can be synthesized in the laboratory using some of the reactions discussed in previous chapters. One of the oldest methods of α-amino acid synthesis begins with α bromination of a carboxylic acid by treatment with Br_2 and PBr_3 (the Hell–Volhard–Zelinskii reaction; **Section 22.4**). S_N2 substitution of the α-bromo acid with ammonia then yields an α-amino acid.