So \([H^+]\) is maintained at

\[
[H^+] = \frac{k_{HA}[HA]}{[A^-]} = \frac{k_{HA}[HA]}{k_{HA}[NaA][Na^+]}
\]

even if some HCl or KOH is added.

The new protons from the HCl combine with \(A^-\) ions from the \(NaA\), forming \(HA\). The new \(OH^-\) from the KOH absorb protons from \(HA\) which the \(OH^-\) once formed.

This works as long as the amounts of \(HA\) and \(NaA\) are similar and sufficient.

In blood, a buffer of carbonic acid \(H_2CO_3\) and bicarbonate \(HCO_3^-\) (with counterions \(Na^+, K^+, \) etc.) keeps \(7.35 < pH < 7.45\).
Incidentally, since

\[ K_{HA} = \frac{[H^+][A^-]}{[HA]} \]

it follows that the pH (8.26) is

\[ pH = -\log_{10} [H^+] = -\log_{10} \frac{K_{HA}[HA]}{[A^-]} \]

\[ = pK_{HA} + \log_{10} \frac{[A^-]}{[HA]} \]  \hspace{1cm} (HM) \]

since \( pK_{HA} = -\log_{10} K_{HA} \) (8.12), chemists call (HM) the **Henderson-Hasselbalch equation**.

It is proteins are chains of amino acids.

There are 20 different amino acids in local living organisms — although a few of them can be modified.
The side chains of glutamic acid and of aspartic acid contain carboxylic groups that remain ionized at all physiological pH's.

Aspartic acid (asp)  
\[
\text{H}_3\text{N} - \text{CH} - \text{COO}^-  \\
|   \text{CH}_2  \\
|   \text{COO}^-  \\
\text{D}
\]

Glutamic acid (gln)  
\[
\text{H}_3\text{N} - \text{CH} - \text{COO}^-  \\
|   \text{CH}_2  \\
|   \text{COO}^-  \\
\text{E}
\]

Acidic side chain  
\[
\text{COOM} \rightleftharpoons \text{COO}^- + \text{H}^+
\]

The side chains of lysine and arginine are protonated at physiological pH's. They are basic.
Lysine (Lys)  
\[ \text{basic side chain} \]

\[ -\text{NH}_3^+ \rightleftharpoons -\text{NH}_2 + \text{H}^+ \]

Histidine is protonated below about pH 6.

Histidine (His)  

Arginine (Arg)  

Histidine is protonated below about pH 6.
For each of these five amino acids

\[ -12.5 \leq K_{eq} = \frac{[H^+] [R]}{[H^+R]} \leq -3.7 \]

arginine aspartic acid

\[ P_\alpha = \frac{[H^+R]}{[H^+R] + [R]} \]

\( P_\alpha \) is the probability that the side chain is protonated. So

\[ P_\alpha = \frac{1}{1 + \frac{[R]}{[H^+R]}} = \frac{1}{1 + \frac{k_{eq}}{[H^+]}} \]

Since \( pH = -\log [H^+] \), it follows that

\[ 10^{pH} = \frac{1}{[H^+]} \]

so in an environment of acidity \( pH \),

\[ P_\alpha = \frac{1}{1 + 10^{pH} K_{eq}} \]

is the probability the side chain will be protonated.
Now by (8.12)

\[ pK = -\log_{10} K_{eq} \]

\[ 10^{-pK} = K_{eq} \]

so

\[ p\alpha = \frac{1}{1 + 10^{\frac{pH-pK}{pK}}} \]

\[ = \frac{1}{1 + 10^{x}} \]

(\text{where } x = pH - pK) \text{ is the probability that a side chain of } pK \text{ will be protonated at } pH. \text{ Now the average change } \langle q \rangle \text{ on an acidic residue is }

\[ \langle q \rangle = (-e) (1 - p\alpha) \]

\[ (-\text{COO}^-) \]

and on a basic residue is

\[ \langle q \rangle = -e \ p\alpha \]

\[ (-\text{NH}_3^+) \text{ where } e > 0. \]
Note that if $pK = pH$, then $x = 0$ and

$$P = \frac{1}{1+1} = \frac{1}{2}$$

so the probability of protonation is 50%.

The local pH near each amino acid (aa) determines $P_{aa}$ that $a_{aa}$.

One may **titrate** a solution of a given protein as in Fig. 8.1 on page 313 for the number of protons dissociated from each ribonuclease molecule as the pH rises:

![Graph showing dissociated protons vs pH with an isolectric point and zero net charge at pH 7.4]
Put an electric field \( E \) across a solution of a given protein solution at a given pH. There will be a force \( qE \) on the protein. By Stokes's law (4.14) the viscous friction coefficient is

\[
\gamma = 6\pi \eta R
\]

where \( \eta \) is the viscosity of the fluid and \( R \) the radius of the protein. The migration of the protein is electrophoresis. Its speed is more complex than \( v = qE/\eta \).

The probability \( P_\alpha \) of the protonation of residue \( \alpha \)

\[
P_\alpha = \frac{1}{1 + 10^{x_\alpha - \phi}}
\]

changes rapidly from 1 to 0 as \( x_\alpha = \phi \text{H} - \phi \text{K}_\alpha \) passes 0.

\[ x_\alpha = \phi \text{H} - \phi \text{K}_\alpha \]
So as the pH rises, the charge on each protein will jump down in steps of e from some positive value $\text{ne}$ to zero and then to negative values. At low pH, the protein will move with $\mathcal{E}$, then will stop when the pH is at the protein's isoelectric point, and then will move against $\mathcal{E}$ as the pH increases further.

Linus Pauling (et al.) used this technique in 1949 to separate the $\beta$-globin chains of normal (wild-type) hemoglobin from those of sickle-cell hemoglobin. These 146 aa proteins
differ only by the mutation of glutamic acid to valine at position 6. G10 is negatively charged for pH > $pK_{G10} = 4.25$, so it carries a negative charge at all physiological pH’s. But valine is neutral and hydrophobic. The mutant proteins clump in fibers of 14 interwound helical strands that give the red blood cell a sickle shape. These deformed, stiff red blood cells get stuck in capillaries and are destroyed, causing anemia. At pH = 6.9, the wild-type and sickle-cell hemoglobins have opposite charges.

A different mutation, also at
position 6 of the \( \beta \) chain, causes hemoglobin-C disease. Here glutamic acid, which has charge \(-e\), is replaced by lysine, with charge \(+e\).

There also are three kinds of hemoglobin-\( M \) disease caused by histidine \(58 \rightarrow\) tyrosine or hist 63 \( \rightarrow \) tyrosine or valine, 67 \( \rightarrow \) glutamic acid. These mutations are on residues near a heme where \( O_2 \) binds; they cause cyanosis — incomplete oxygenation of hemoglobin.

Amphiphiles are molecules that have a hydrophobic part and a hydrophilic part. The detergent
sodium dodecyl sulfate (SDS)

\[ \text{charged} \quad N_\text{a}^+ \quad \text{non-polar} \quad \text{hydrocarbon} \]

\[ \text{pol} \quad \text{ar} \quad \text{charged} \quad \text{hydrocarbon} \]

\[ \text{Na}_2 \text{SO}_4 \quad (\text{CH}_2)_11 \text{CH}_3 \]

(aka sodium lauryl sulfate) is an ionic surfactant used in toothpaste, shampoo, shaving cream, and in SDS-PAGE (SDS polyacrylamide gel electrophoresis).

Surfactants reduce surface tension by forming a monomolecular layer on the surface of the water, with the hydrocarbon tails in the air, the negatively charged sulfate groups in the water, and the \( \text{Na}^+ \) in a layer of counterions.

\[ \text{water} \quad \text{Na}_2^+ \quad \text{Na}_3^+ \quad \text{Na}_4^+ \]
Phosphatidylcholine, a phospholipid,

\[
\begin{align*}
\text{monopolar} & \quad \text{electric dipole} \\
R \quad \overset{\text{O}}{\underset{\text{CH}_2}{\text{H}}} \quad \overset{\text{O}}{\underset{\text{R'}}{\text{C}}} \\
\end{align*}
\]

is another example of an amphiphile. It occurs in the phospholipid bilayer of a cell's membrane. Lecithin is a phospholipid found in yolks of eggs, which with olive oil makes an emulsion, known as mayonnaise.

\[
\begin{align*}
\text{oil} & \quad \text{water} \\
\text{water, acetic acid} & \quad \text{water, vinegar} \\
\text{mustard, KCl} & \quad \text{oil}
\end{align*}
\]