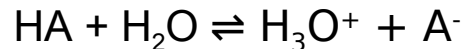


# Biochemical buffers

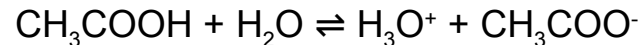
# Acids and bases

In the definition by Brønsted and Lowry (1923), an acid can donate protons whereas bases can accept them.

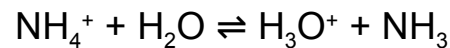


Examples are:

acetic acid and acetate



ammonium ion and ammonia



The strength of an acid is determined by its dissociation constant  $K$  which describes the relative proton affinity of the conjugated acid-base pairs.

$$K = \frac{[\text{H}_3\text{O}^+][\text{A}^-]}{[\text{HA}][\text{H}_2\text{O}]} \quad \text{If the concentration of water is included one gets: } K_a = K[\text{H}_2\text{O}] = \frac{[\text{H}_3\text{O}^+][\text{A}^-]}{[\text{HA}]}$$

Acids with  $K < 1$  are weak acids, acids with  $K > 1$  are strong acids.

# Water and pH

If water was considered as an acid-base pair, it would have the dissociation constant:



$$K = \frac{[\text{H}^+][\text{OH}^-]}{[\text{H}_2\text{O}]} \quad \text{or} \quad K_w = K[\text{H}_2\text{O}] = [\text{H}^+][\text{OH}^-]$$

**Ion product of water**

at 25°C,  $K_w$  is  $10^{-14} \text{ mol}^2\text{L}^{-2}$ ; and because  $[\text{H}^+] = [\text{OH}^-] \rightarrow [\text{H}^+] = [\text{OH}^-] = (K_w)^{1/2} = 10^{-7} \text{ mol/L}$

$$\text{pH} = -\log [\text{H}^+]$$

$$[\text{H}^+] = 10^{-7} \text{ mol/L} \quad \rightarrow \quad \text{neutral solution}$$

$$\rightarrow \text{pH} = 7$$

$$[\text{H}^+] > 10^{-7} \text{ mol/L} \quad \rightarrow \quad \text{acidic solution}$$

$$\rightarrow \text{pH} < 7$$

$$[\text{H}^+] < 10^{-7} \text{ mol/L} \quad \rightarrow \quad \text{basic solution}$$

$$\rightarrow \text{pH} > 7$$

**The concentration of protons (pH) of a solution can be determined using a pH meter.**

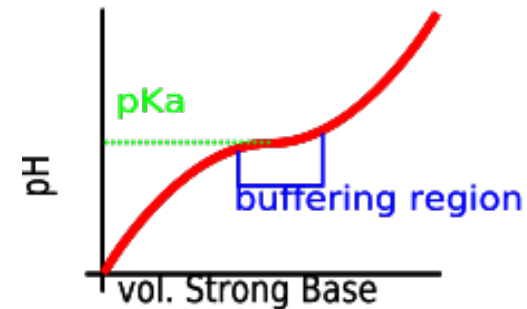
**Using the Henderson-Hasselbalch equation,  
the concentration of an acid and its conjugated  
base can be determined from the pH.**

$$\text{pH} = \text{pK} + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

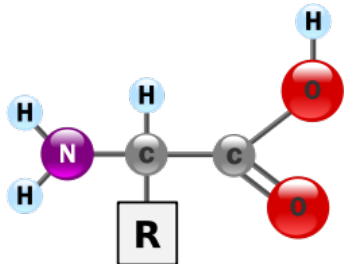
# buffer systems

The titration curve of an acid reveals its buffering range, and the  $pK_a$  of the acidic group can be determined

## Weak Acid

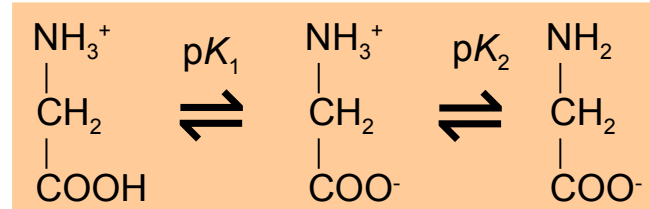


Amino acids have at least two functional groups with acidic and basic properties.

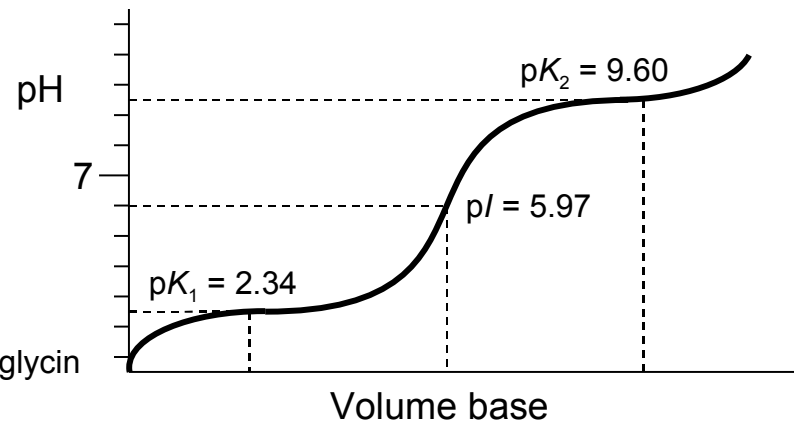


R = H → Glycin

A third reactive group might exist within the side chain R.

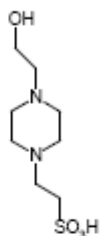


Titration curve of glycin

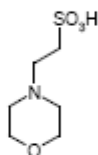


# Buffer systems in the lab

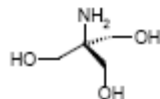
HEPES



MES



TRIS



Buffer system

**Zwitterionic buffers**

Glycylglycine - piperazine -  
2HCl - NaOH

TRIS - maleic acid - NaOH

MES - NaOH

MOPSO - NaOH

MOPS - KOH

TRIS - HCl

DISPO - NaOH

HEPES - NaOH

TAPSO - NaOH

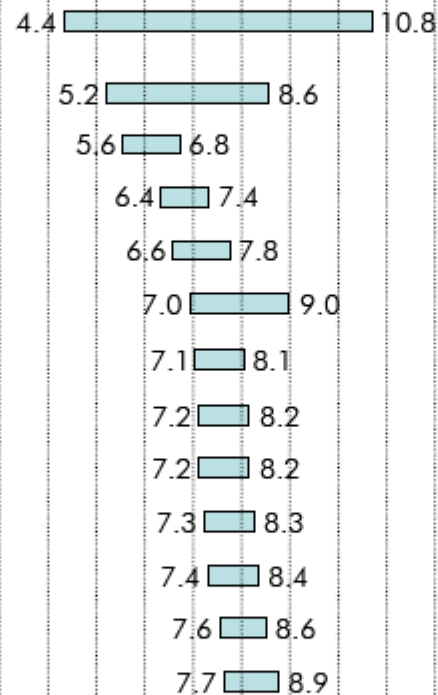
POPSO - NaOH

HEPPSO - NaOH

TRICIN - NaOH

BICIN - NaOH

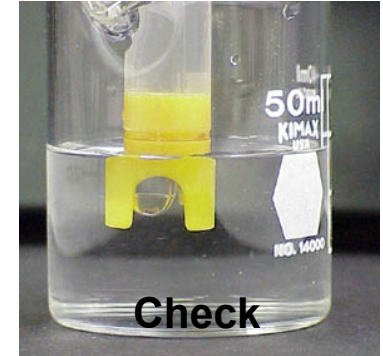
pH 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14



# pH meter



Electrode is stored in storage solution



Handling a pH meter

## Calibrating a pH meter

1. Rinse the electrode with MilliQ water and dry
2. Place electrode in pH 7 standard buffer solution and set pH to 7
3. Rinse the electrode with MilliQ water and dry
4. Place electrode in second standard buffer solution; if you wish to adjust an acidic solution, choose pH 4 standard solution, if you wish to adjust a basic solution, choose pH 10
5. Rinse the electrode with MilliQ water and dry
6. Place electrode in your solution and adjust pH
7. After use, rinse properly with MilliQ water and store electrode in storage solution