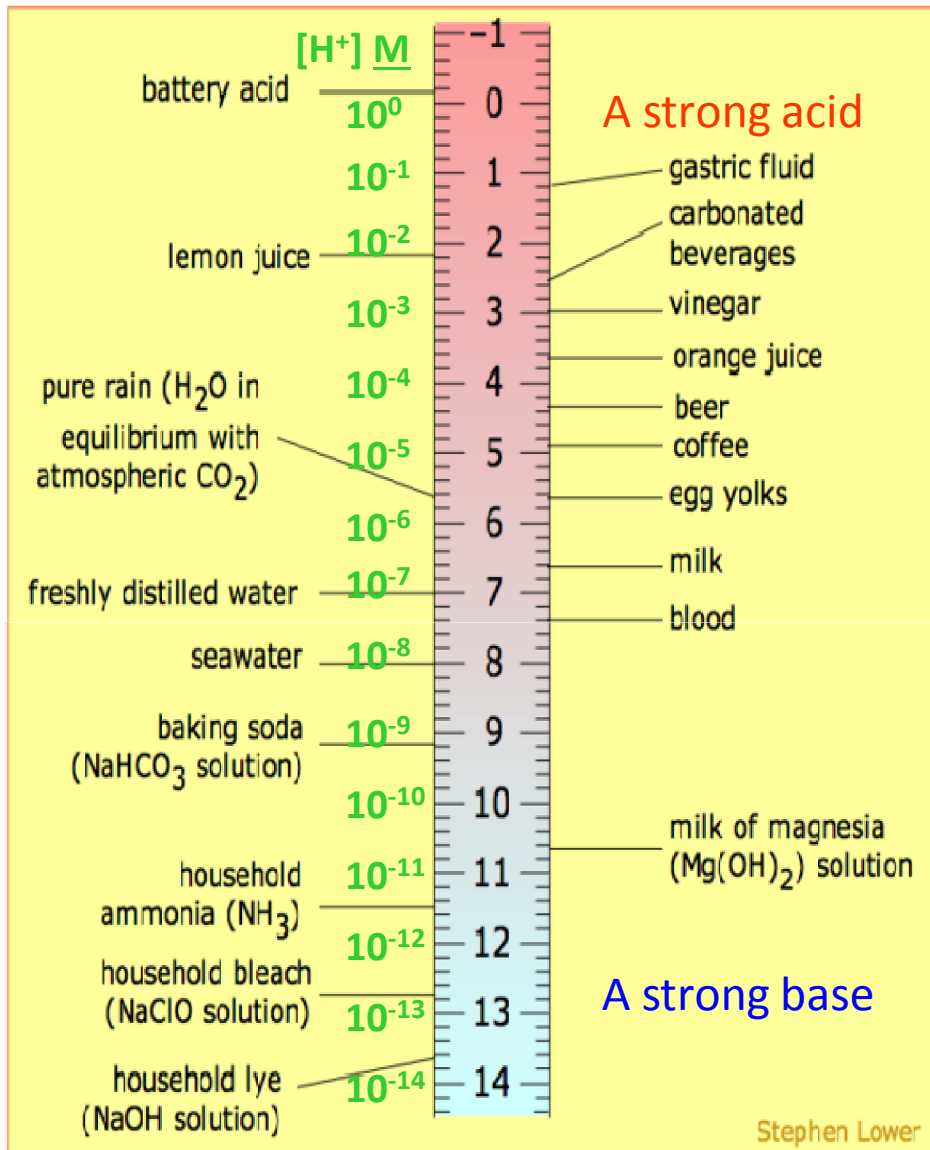


Acids, pH, and Buffers

Some Basic Chemistry for Biological Science

Understanding biological buffers

By: Dr Mehdi Imani ¹



pH definitions and maintenance

- **Most living cells have a very narrow range of tolerance for pH, i.e. $[H^+]$.**
- The $[H^+]$ concentration will be important (either explicitly or implicitly) for many other topics in biology.
- $[H^+]$ is controlled in all biological organisms, and in virtually all biochemical experiments.
- Each pH unit represents a factor of 10 difference in $[H^+]$.

Understanding biological buffers

By: Dr Mehdi Imani ²

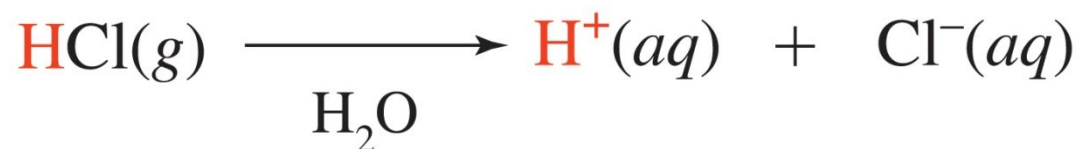
Why is pH important in bioscience?

- pH affects solubility of many substances.
- pH affects structure and function of most proteins - including enzymes.
- Many cells and organisms (esp. plants and aquatic animals) can only survive in a specific pH environment.

Definitions

Acids,

- The Swedish chemist, Svante **Arrhenius**, first described ***acids*** as substances that dissociate, producing hydrogen ions (H^+) when dissolved in water.
- In the early twentieth century, ***Brønsted and Lowry*** redefined ***acids*** as a compound that donates a proton.



Dissociates into ions

Understanding biological buffers
By: Dr Mehdi Imani

Bases

- **Arrhenius** described **bases** as compounds that dissociate to form a metal ion and a hydroxide ion (OH^-) when dissolved in water. Arrhenius bases are formed from Group 1A and 2A metals. Hydroxide bases are characterized by a bitter taste and a slippery feel.
- The **Brønsted–Lowry** definition of a **base** is a compound that accepts a proton.

Understanding biological buffers

By: Dr Mehdi Imani

Buffer definition

Understanding biological buffers

By: Dr Mehdi Imani ⁶

For biological systems:

- Ionization of a strong acid is **TOO BIG!**
- Ionization of water itself is way TOO LITTLE!
- Ionization of a weak acid is **JUST RIGHT!**

Buffer uses

1. Biological systems such as blood and saliva
2. Eye drop
3. Shampoo pH 6 to prevent bacteria growth
4. Baby lotions

Homeostasis

Maintaining Physiological pH with Bicarbonate Buffer

- The bicarbonate system helps our bodies maintain its optimal physiological pH.
- The ability of an organism to maintain its internal environment by adjusting such factors as pH, temperature, and solute concentration is called *homeostasis*.

Understanding biological buffers
By: Dr Mehdi Imani

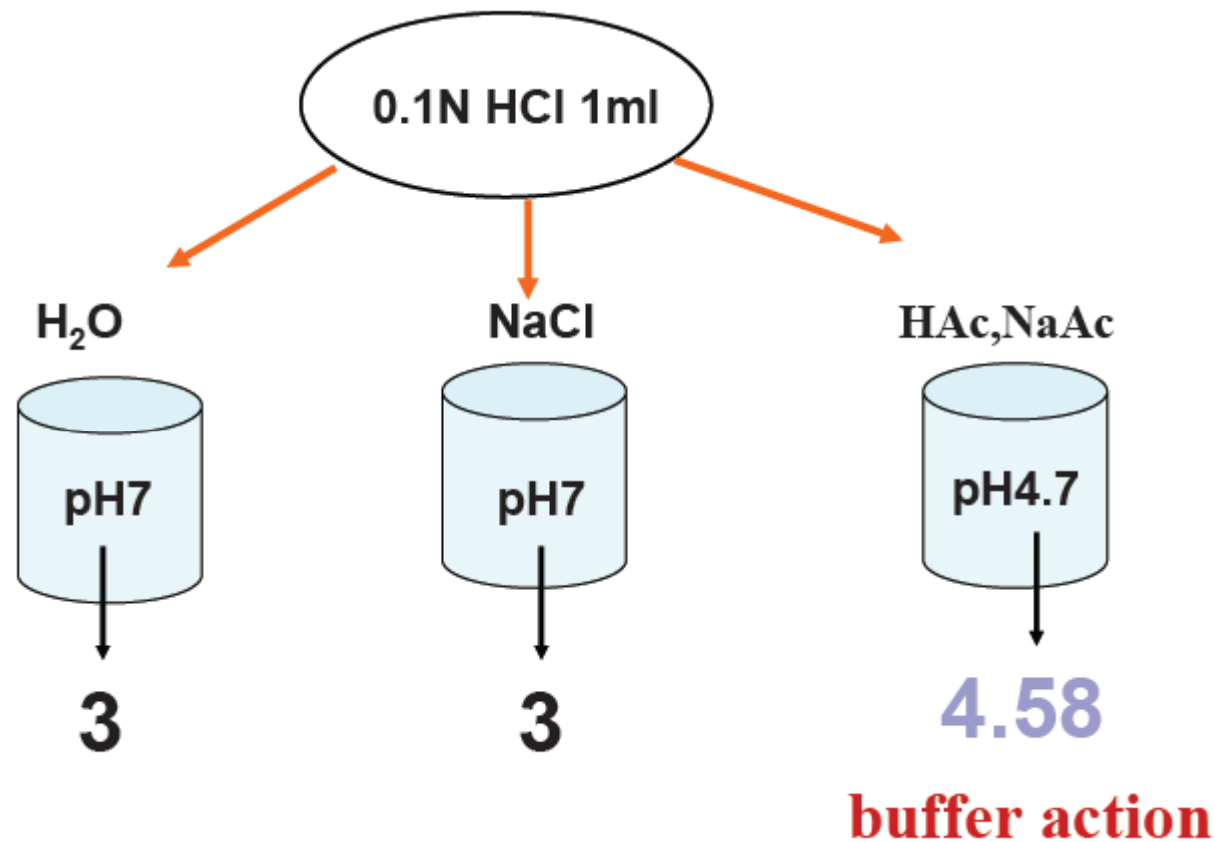
Factors in choosing a buffer

1. Be sure it covers the pH range you need
 - Generally: pK_a of acid ± 1 pH unit
 - Consult tables for ranges or pK_a values
2. Be sure it is not toxic to the cells or organisms you are working with.
3. Be sure it would not confound the experiment (e.g. avoid phosphate buffers in experiments on plant mineral nutrition).
4. Calculation using the Henderson-Hasselbach equation.

Understanding biological buffers

By: Dr Mehdi Imani ¹⁰

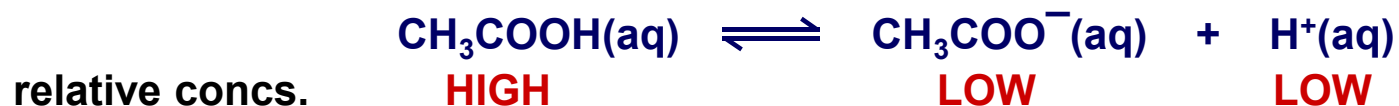
How a Buffer Works



Understanding biological buffers

*By: Dr Mehdi Imani*¹¹

How a Buffer Works (cont.)



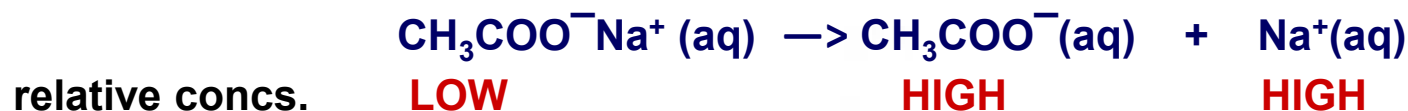
NB A strong acid can't be used as it is fully dissociated and cannot remove $\text{H}^+(\text{aq})$



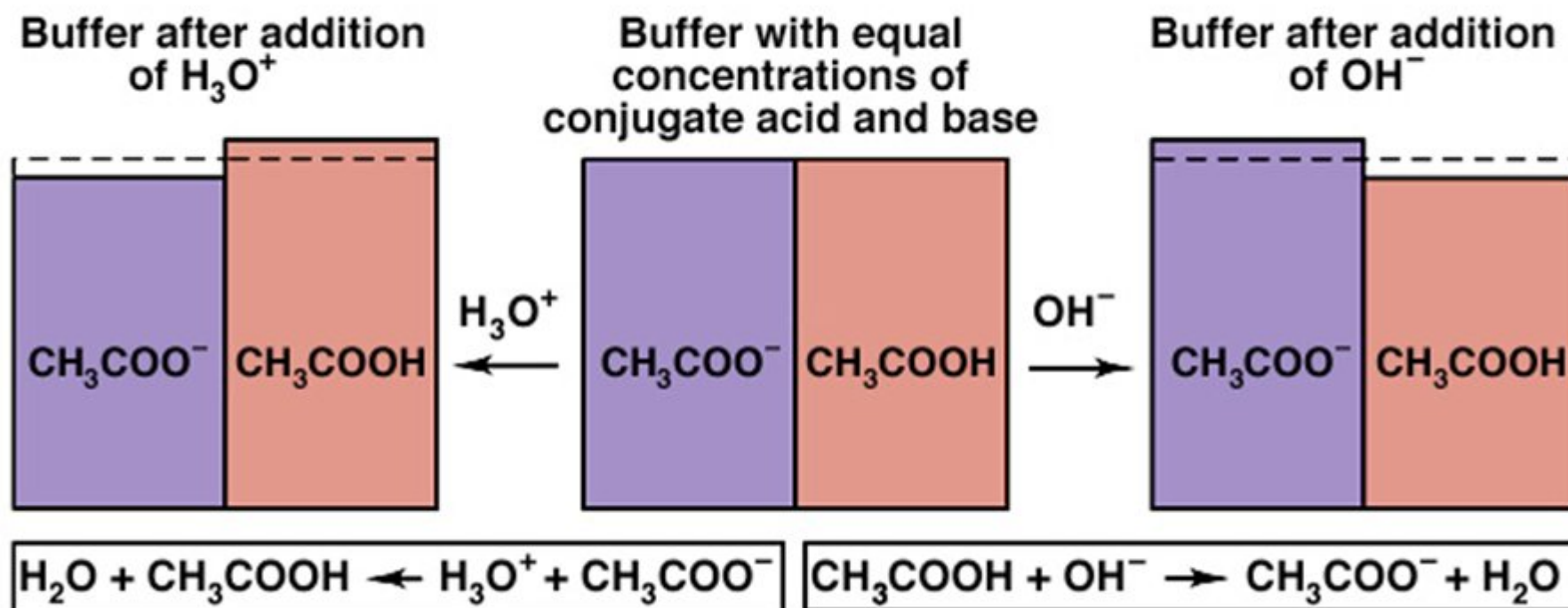
Adding acid

Any H^+ is removed by reacting with CH_3COO^- ions to form CH_3COOH via the equilibrium. Unfortunately, the concentration of CH_3COO^- is small and only a few H^+ can be “mopped up”. A much larger concentration of CH_3COO^- is required.

To build up the concentration of CH_3COO^- ions, sodium ethanoate is added, which dissociates completely.



How a Buffer Works (cont.)



Henderson-Hasselbalch Approximation

$$K_1 = \frac{[A^-][H^+]}{[HA]} \Rightarrow [H^+] = K_1 \frac{[HA]}{[A^-]}$$

$$-\log[H^+] = -\log\left(K_1 \frac{[HA]}{[A^-]}\right) \Rightarrow -\log[H^+] = -\log K - \log \frac{[HA]}{[A^-]}$$

$$-\log \frac{[HA]}{[A^-]} = +\log \frac{[A^-]}{[HA]} = \log \frac{\text{نمک (اسید) باز}}{\text{اسید}}$$

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

Understanding biological buffers

By: Dr Mehdi Imani ¹⁴

- **A Weak Acid and Its Salt**

$$\text{pH} = \text{p}K_a + \log \frac{[\text{salt}]}{[\text{acid}]}$$

**Dissociation
exponent**

Buffer equation or
Henderson-Hasselbalch
equation

Understanding biological buffers

By: Dr Mehdi Imani ¹⁵

In vivo buffer systems

- Blood

- ① Primary buffers : **Plasma** ;

- NaHCO_3 -- H_2CO_3 , NaHPO_4 -- NaH_2PO_4 , protein

- ② Secondary buffers : **Erythrocytes** ;

- hemoglobin-oxyhemoglobin, K_2HPO_4 - KH_2PO_4

- Lacrimal fluid

- pH: 7.4 (range 7 – 8 or slightly higher)

- Urine

- pH: 6.0 (range 4.5 – 7.8)

- below normal...hydrogen ions are excreted by the kidney.

- above pH 7.4...hydrogen ions are retained by action of the kidney.

Understanding biological buffers

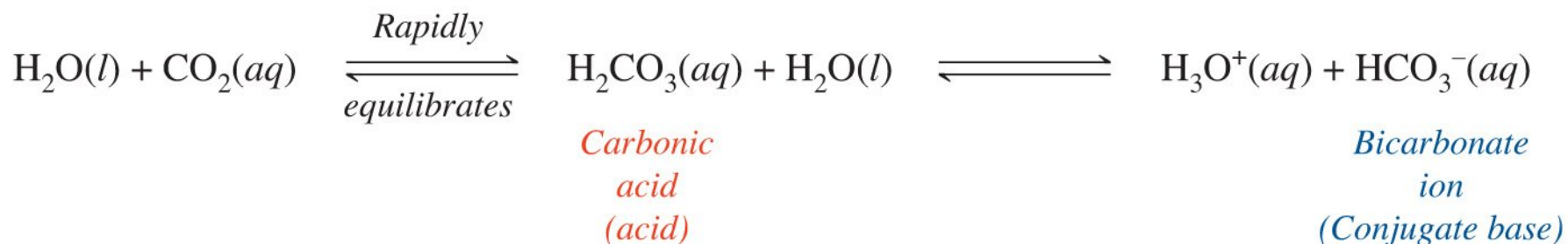
By: Dr Mehdi Imani ¹⁶

Steps for buffer development

- ① Select a weak acid having a pK_a approximately equal to the pH at which the buffer is to be used.
- ② Calculate the ratio of salt & weak acid required to obtain the desired pH.
- ③ Consider the individual conc. Of the buffer salt & acid needed to obtain a suitable buffer capacity
 - * Individual conc. : 0.05 ~ 0.5M
 - * buffer capacity : 0.01 ~ 0.1

The bicarbonate buffer system

Reaction pK is 6.1



© 2011 Pearson Education, Inc.

- The bicarbonate buffer system can be denoted by showing the acid and its conjugate base like this: $\text{H}_2\text{CO}_3/\text{HCO}_3^-$. Sometimes the conjugate base is shown as an ionic compound like this: $\text{H}_2\text{CO}_3/\text{NaHCO}_3$.

Understanding biological buffers
By: Dr Mehdi Imani

ADJUSTING THE pH OF A SOLUTION.

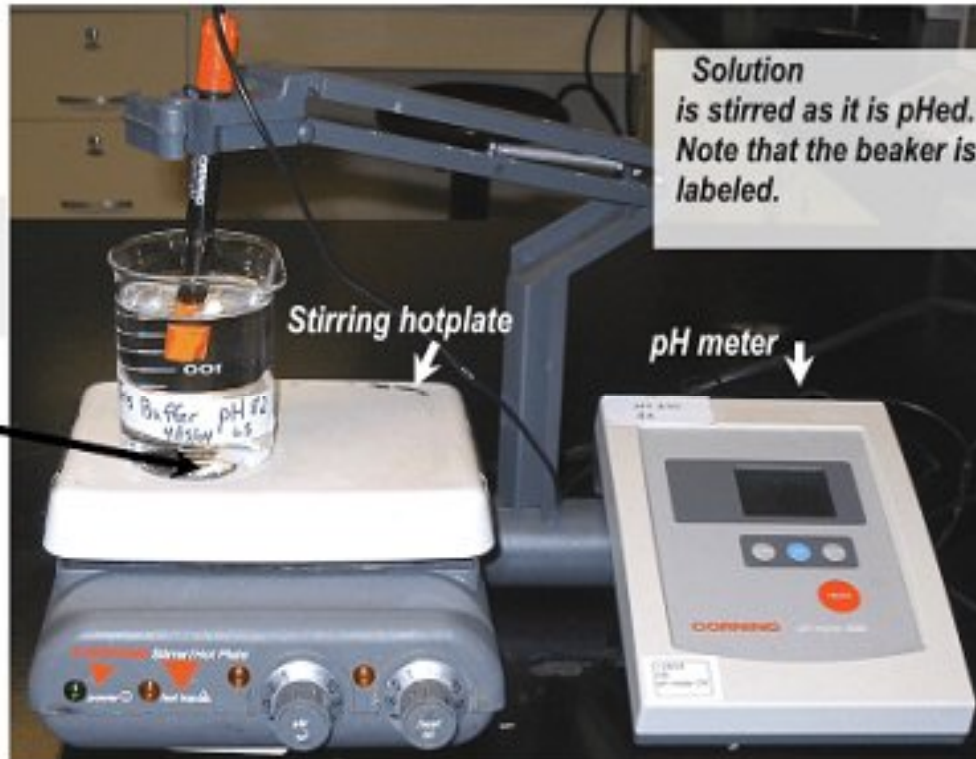
BE CAREFUL NOT TO CRASH
THE ELECTRODE WITH THE
STIR BAR.

Stir bar

Stirring hotplate

Solution
is stirred as it is pHed.
Note that the beaker is
labeled.

pH meter

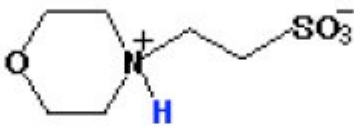
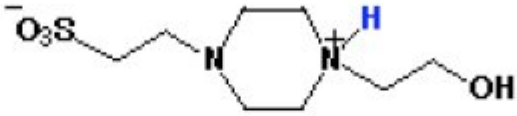
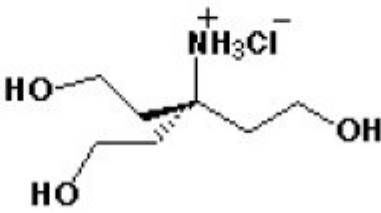


Understanding biological buffers

By: Dr Mehdi Imani 19

pH vs Temperature for Tris Buffer

pH of Tris Buffer (0.05 M)		
5°C	25°C	37°C
7.76	7.20	6.91
7.89	7.30	7.02
7.97	7.40	7.12
8.07	7.50	7.22
8.18	7.60	7.30
8.26	7.70	7.40
8.37	7.80	7.52
8.48	7.90	7.62
8.58	8.00	7.71
8.68	8.10	7.80
8.78	8.20	7.91

NAME	STRUCTURE	pK _a at 20°C	ΔpK _a (°C)
MES [2-(N-morpholino) ethanesulfonic acid]		6.15	-0.011
PIPES [piperazine-N,N'-bis (2-ethanesulfonic acid)]		6.8	-0.0085
Sodium dihydrogen phosphate	NaH ₂ PO ₄	7.21	---
HEPES [N-2-hydroxyethyl piperazine-N'- 2-ethanesulfonic acid]		7.55	-0.014
TRIS [tris(hydroxymethyl) aminoethane] hydrochloride		8.3	-0.031

Understanding biological buffers

By: Dr Mehdi Imani ²¹

Some useful links on buffer calculators!!!!

<http://www.biomol.net/en/tools/buffercalculator.htm>

Buffer Calculator

Buffer:

Volume: mL

pH:

Concentration: mM

☒ Ionic Strength is defined if checked

Ionic Strength: Set the ionic strength to mM

With:

Temperature: Use the buffer at °C

Make the buffer at °C

Programmed by Dr. Rob Beynon, University of Liverpool, UK

...g biological buffers
By: Dr Mehdi Imani ²²

<http://dbr.csoft.net/chem/bufcalc.php>

Buffer Calculator

- Input the pK_a values, the desired pH, and buffer strength
- Get recipes and other important info about your buffer

Enter pK_a values, separated by commas:

8.3
pH 7.1 Buffer Strength 10 mM Calculate

Optional: Free base name Tris Charge on H_NA 1 pK_w 14 Tris HCl

- Shortcuts
- phosphate
- citrate
- phosphonate
- MOPS
- MES
- Tris HCl
- Tris acetate
- carbonate
- borate

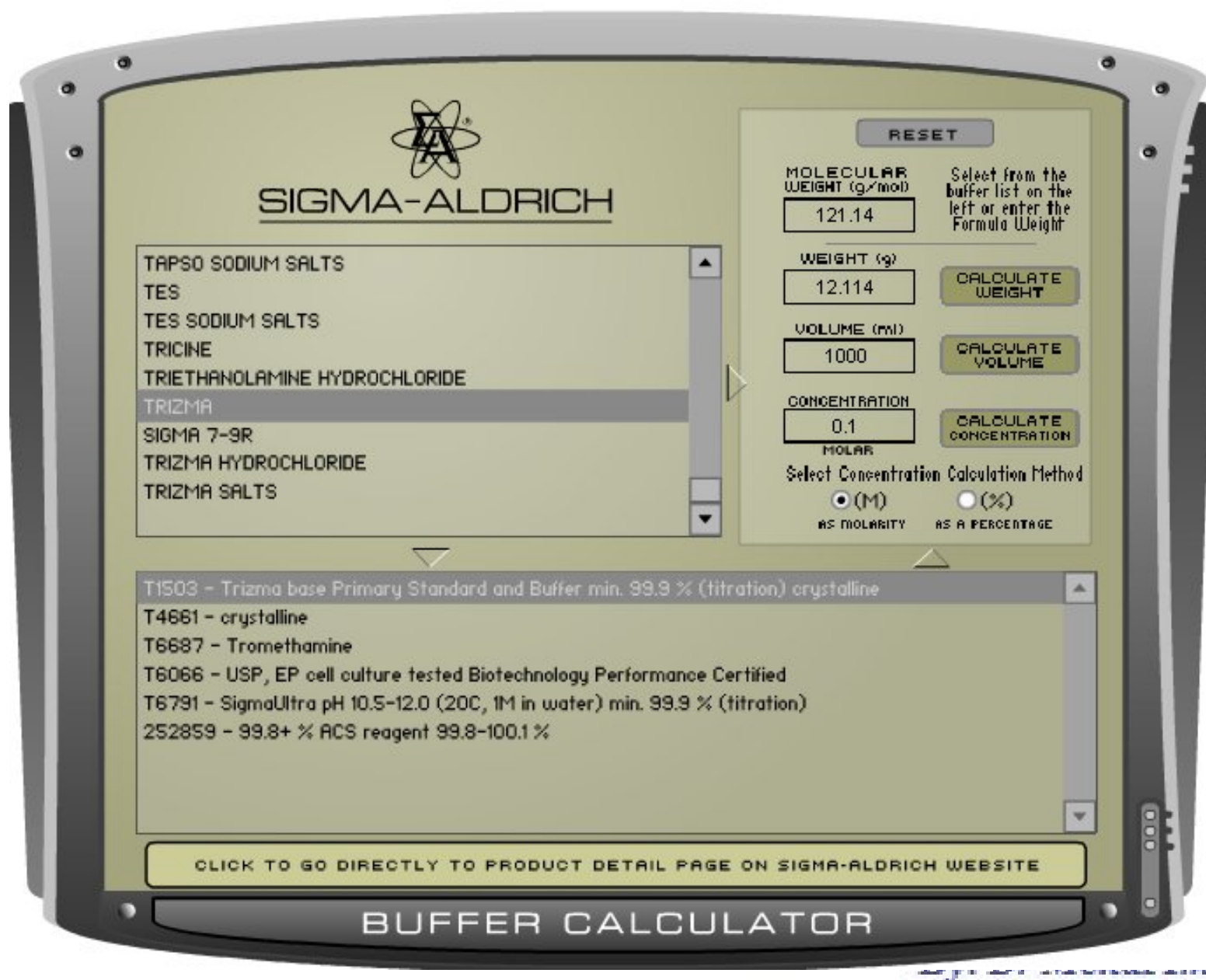
Read [how it works](#)

By [Dave Robinson](#)

Understanding biological buffers

By: Dr Mehdi Imani ²³

<http://www.sigmaaldrich.com/life-science/core-bioreagents/biological-buffers/learning-center/Buffer-Calculator-App.html>



The image shows a digital interface for the Sigma-Aldrich Buffer Calculator. At the top, the Sigma-Aldrich logo and name are displayed. The interface is divided into several sections:

- Buffer Selection:** A list of buffers is shown on the left, including TAPSO SODIUM SALTS, TES, TES SODIUM SALTS, TRICINE, TRIETHANOLAMINE HYDROCHLORIDE, TRIZMA (highlighted), SIGMA 7-9R, TRIZMA HYDROCHLORIDE, and TRIZMA SALTS.
- Calculation Fields:** On the right, there are input fields for:
 - MOLECULAR WEIGHT (g/mol):** 121.14
 - WEIGHT (g):** 12.114
 - VOLUME (mL):** 1000
 - CONCENTRATION (MOLAR):** 0.1
- Buttons:** A "RESET" button is at the top right. Below the input fields are three buttons: "CALCULATE WEIGHT", "CALCULATE VOLUME", and "CALCULATE CONCENTRATION".
- Concentration Method:** A section labeled "Select Concentration Calculation Method" with two radio buttons: "AS MOLARITY" (selected) and "AS A PERCENTAGE".
- Product Details:** A scrollable list at the bottom shows product information for Trizma base, including T1503, T4661, T6687, T6066, T6791, and 252859.
- Footer:** A button at the bottom says "CLICK TO GO DIRECTLY TO PRODUCT DETAIL PAGE ON SIGMA-ALDRICH WEBSITE". The title "BUFFER CALCULATOR" is at the very bottom.