UNIVERSITY of LIFE SCIENCES in Lublin

FACULTY OF VETERINARY MEDICINE

Buffer, solutions

The aim:

- to familiarize with the properties of solutions

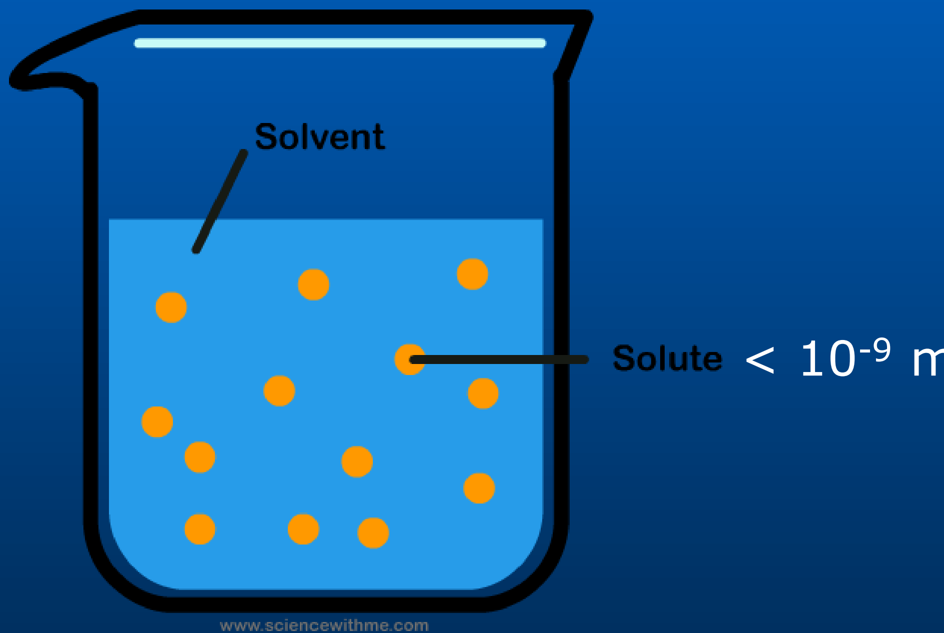
- to familiarize with the mechanism of buffers action and the properties of buffer solutions

Solutions

1. True solution
2. Colloidal solution
3. Suspension

I. True solution

It is a homogeneous mixture of two or more substances in which substance dissolved (solute) in solvent has the particle size of less than 10^{-9} m.



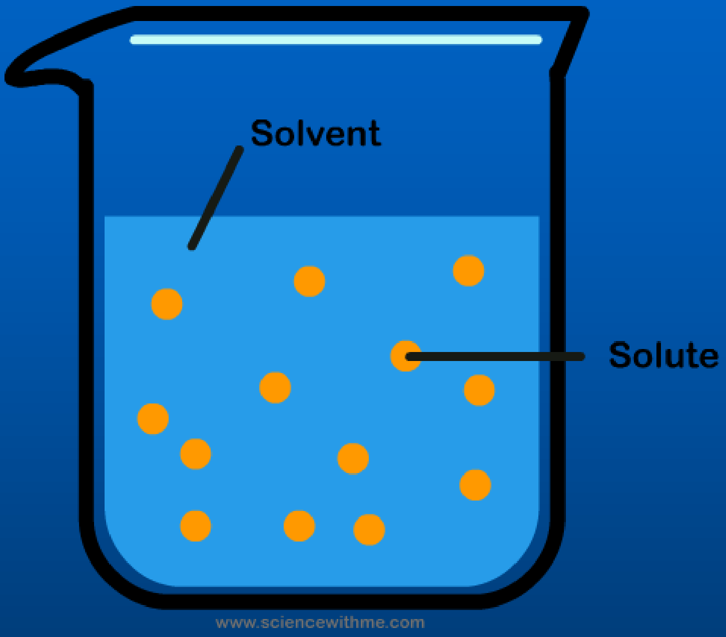
Particles of true solution cannot react with each other.

I. True solution

Conventionally, it is assumed that:

the ingredient which is in greater amount – is a solvent (dispersion phase),

compound in smaller amount – is a dissolved substance (dispersed phase).



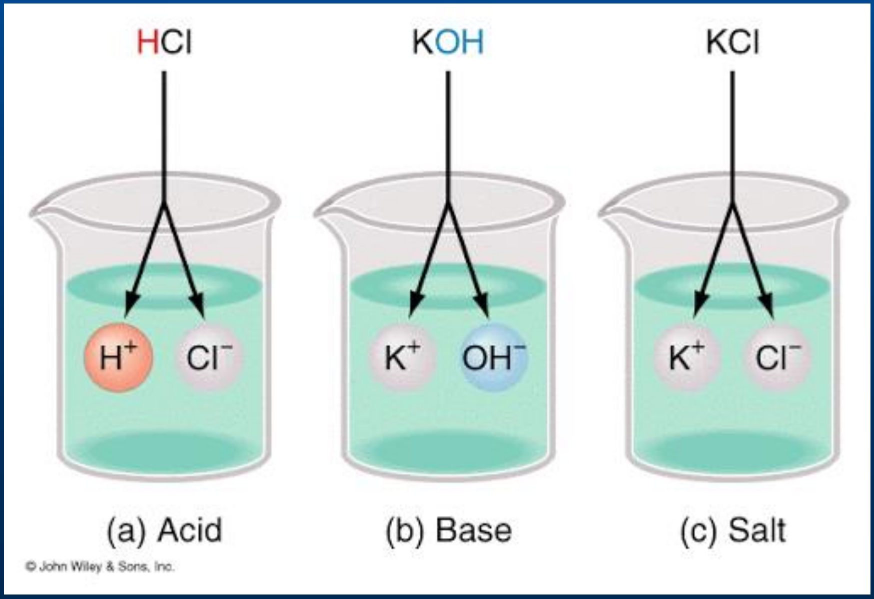
Molecules of the dissolved substance are surrounded by solvent molecules – solvation (if the solvent is water - hydration).

I. True solution

Water solutions are a fundamental component of the living matter and the environment in which there is life.

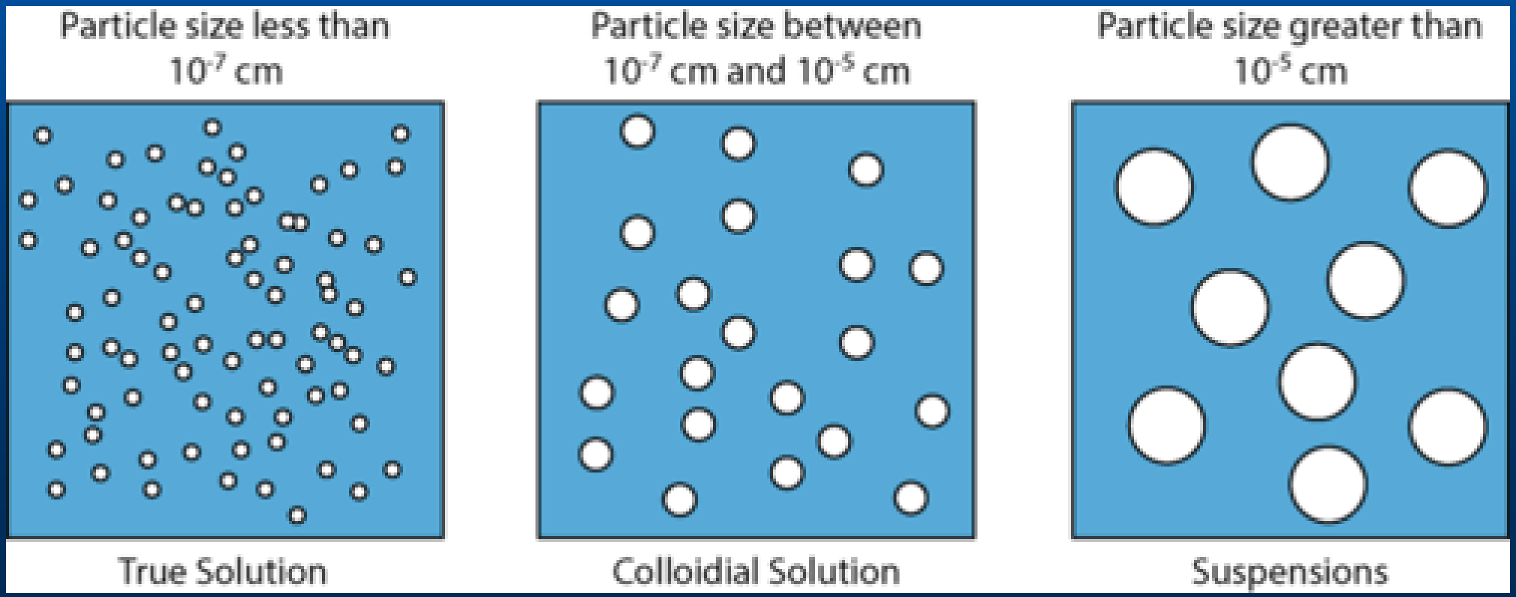
Examples:

- Salt water (sodium chloride)
- Aqueous ammonia
- Acids
- Bases



II. Colloidal system

Colloidal solution has the molecules so small that it does not form a suspension, but also too large to form a true solution. Colloids: 10^{-9} – 10^{-7} m



II. Colloidal system

Properties:

- Colloidal particles are visible under the electron microscope,
- We can separate them in ultracentrifuges.

Example: blood plasma of animals

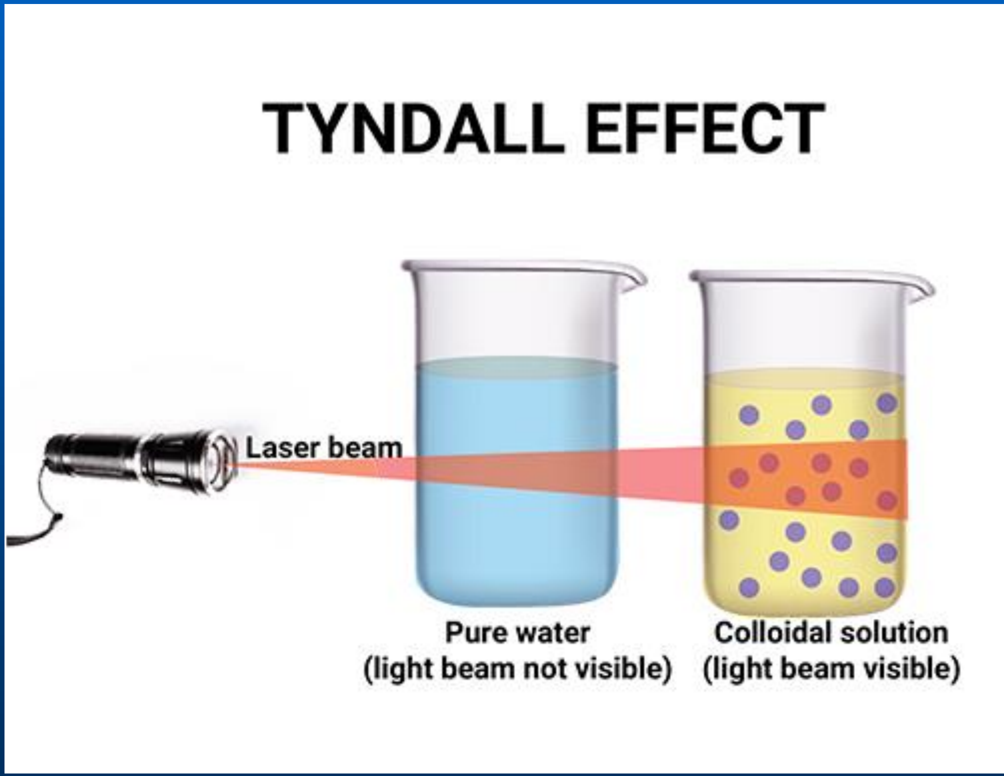
Colloids show some unique properties:

- Brownian motion - is the random motion of particles suspended in a medium (a liquid or a gas).
- Tyndall effect

II. Colloidal system

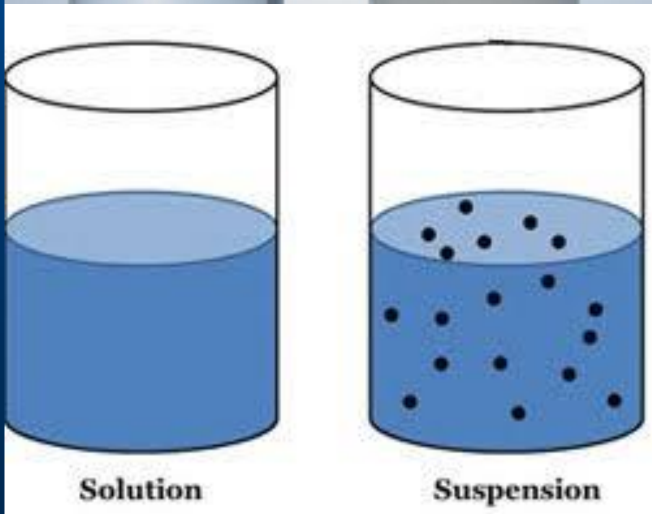
Tyndall effect:

light scattering by particles in a colloid or in a very fine suspension.



III. Suspensions

These are the heterogenous and biphasic systems, in which the diameter of particles of the substance dispersed in the solvent exceeds 100 nm ($>10^{-7}$ m).



Examples:

soup, mud, water in the lake, drugs, etc.

Solubility

Solubility - is the max number of grams of a substance which can be dissolved in 100 g of solvent (Temp., Pres. = Const).

Solubility of the substance is equal to the concentration of the saturated solution.

The opposite is a dilute solution - this solution can accept more solute.

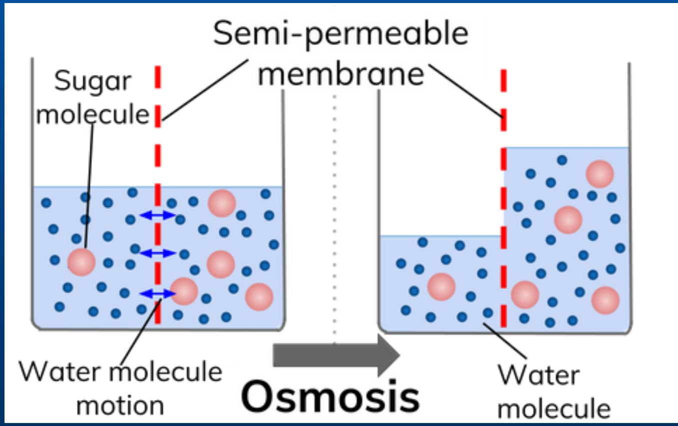
Solubility

Substance solubility depends on:

1. Type of the dissolved substance (solute)
2. Type of the solvent
3. Temp. (solubilty of solids in liquids increases with temperature; gases - decreases)
4. Pres. (solubility of gases in liquids increases with pressure)

Important processes for the living organism

Osmosis – is the spontaneous movement of solvent molecules through a semi-permeable membrane into a region of higher solute concentration.



Dialysis – is the movement of solute particles through a semi-permeable membrane.

Semi-permeable membranes – permeate the molecules of water and other solvents, while they do not permeate many molecules of dissolved substances, especially macromolecules.

It does not mean that naturally occuring membranes are only the mechanical sieves that permeable small molecules and retain larger particles.

They selectively permeate one group of molecules, do not allowing other molecules (even of the same size) to cross the membrane, at the same time.

Buffers

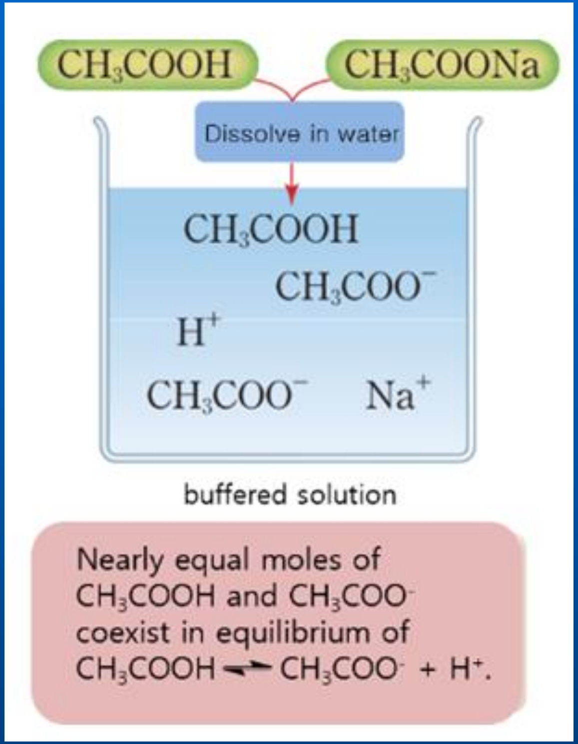
A buffer solution (pH buffer / hydrogen ion buffer) is an aqueous solution consisting of:

- a mixture of a weak acid and its conjugate base
- a mixture of a weak base and its conjugate acid

The mechanism of action of acetate buffer

Acetate buffer:

- CH_3COOH (weak acid)
- CH_3COONa (salt, conjugated base)



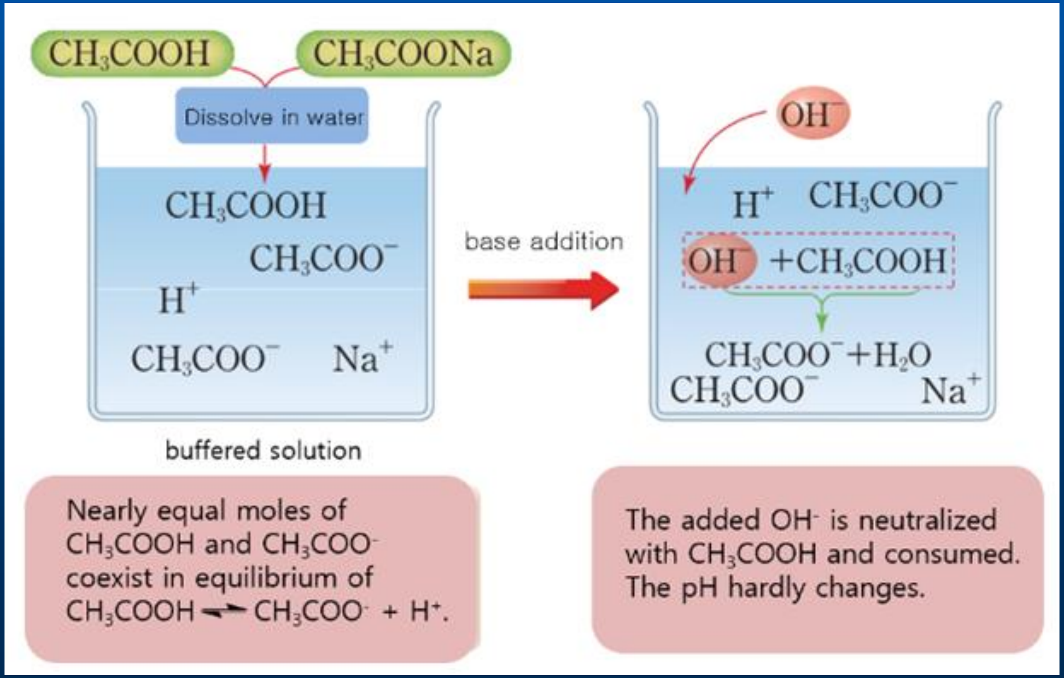
Acetic acid present in the buffer solution is practically in undissociated form and acts as proton donor. While, sodium acetate has the ion form: Na^+ and CH_3COO^-, wherein CH_3COO^- ions (as a strong base) are proton acceptors.

The mechanism of action of acetate buffer

The proton donor CH_3COOH (the acid according to the Brønsted–Lowry theory) protects the solution from pH changes during adding the base to the system.

Adding excess of the OH^- ions turns to the undissociated water:

$$CH_3COOH + \mathbf{OH^-} \Leftrightarrow CH_3COO^- + H_2O$$

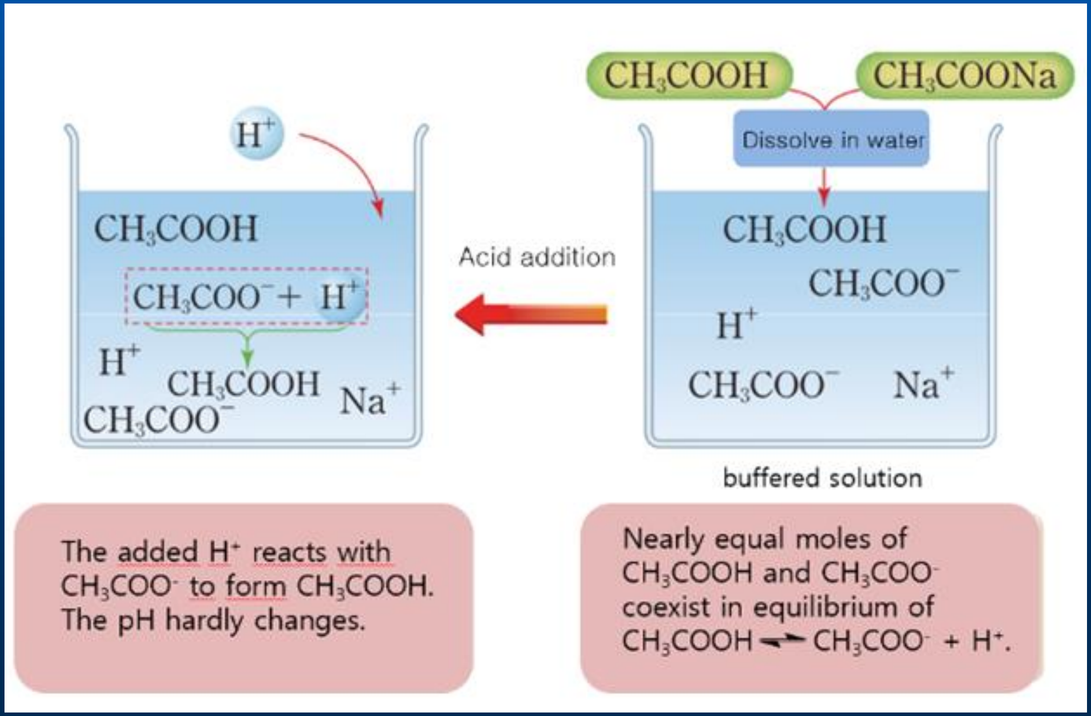


The mechanism of action of acetate buffer

The proton acceptor CH_3COO^- ions (the base according to the Brønsted–Lowry theory) protects the solution from pH changes during adding small amount of acid to the system (hydrogen ions, oxonium ions - H_3O^+):

$$CH_3COO^- + \mathbf{H_3O^+} \Leftrightarrow CH_3COOH + H_2O$$

During the reaction, poorly dissociated acetic acid is created and pH of the solution practically does not change.

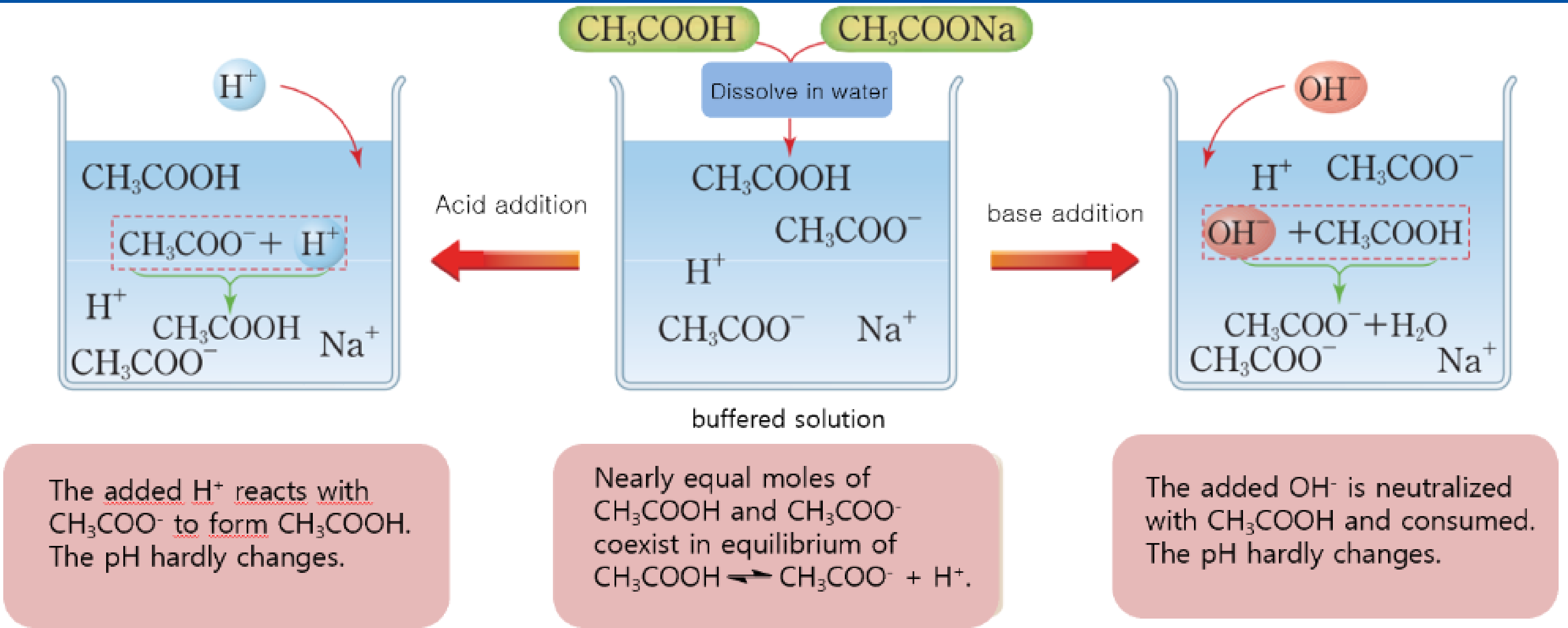


The mechanism of action of acetate buffer

In this way, buffer system works on both sides - counteracts the increasing and decreasing pH value.

$$CH_3COOH + \mathbf{OH^-} \Leftrightarrow CH_3COO^- + H_2O$$

$$CH_3COO^- + \mathbf{H_3O^+} \Leftrightarrow CH_3COOH + H_2O$$



The mechanism of action of phosphate buffer

Phosphate buffer:

- NaH_2PO_4
- Na_2HPO_4

Sodium dihydrogen phosphate (V) present in the buffer solution has an ion form – sodium ions (Na^+) and dihydrogen phosphate ions ($H_2PO_4^-$) and acts as proton donor.

While, sodium hydrogen phosphate (V) consists of: sodium ions (Na^+) and hydrogen phosphate ions (HPO_4^{2-}). HPO_4^{2-} ions as a base are proton acceptors.

The mechanism of action of phosphate buffer

The proton donor: $H_2PO_4^-$ (the acid according to the Brønsted–Lowry theory) protects the solution from pH changes during adding the base to the system.

Adding excess of the OH^- ions turns to the undissociated water:

$$H_2PO_4^- + \mathbf{OH^-} \Leftrightarrow HPO_4^{2-} + H_2O$$

The proton acceptor HPO_4^{2-} ions (the base according to the Brønsted–Lowry theory) protects the solution from pH changes during adding small amount of acid to the system (hydrogen ions, oxonium ions - H_3O^+):

$$HPO_4^{2-} + \mathbf{H_3O^+} \Leftrightarrow H_2PO_4^- + H_2O$$

During the reaction dihydrogen phosphate ion is created and pH of the solution practically does not change.

What are the buffers of living organisms?

Biological buffer

It is an organic substance that has a neutralizing effect on hydrogen ions. In this way, a biological buffer helps maintain the body at the correct pH so that biochemical processes continue to run optimally.

- Blood: carbonic acid / bicarbonate buffer (H_2CO_3 / HCO_3^-) and hemoglobin buffer
- Blood plasma: protein buffer (NH_3^+ / COO^-)
- Cells, tissues: phosphate buffer

How to measure the pH ?

The mechanism of action of an indicator

To precisely conduct the neutralization reaction, it is necessary to use the indicators. Because the indicator changes its color depending on the pH of the solution.

Even a very small amount of acid or base causes an immediate color change of the indicator.

Change the color of an indicator is caused by its dissociation or withdrawal of the dissociation rate.

The mechanism of action of an indicator

The conclusion is that the:

color of the solution depends on the color of ions or the color of the undissociated indicator molecules.

Therefore, the indicators that we generally use are weak acids or weak organic bases.

The mechanism of action of an indicator

Conventional indicators can be divided into:

1) monochromatic
2) dichromatic

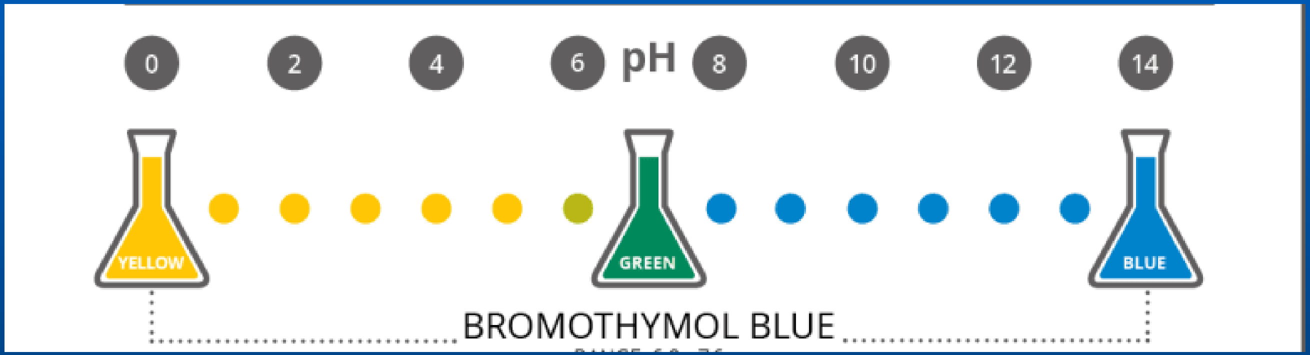
Each indicator used in neutralization reaction is characterised by so-called the range of color change depending on pH.

The mechanism of action of an indicator

Example of dichromatic indicator:

- **Bromothymol blue**

It changes the color between pH 6.2 - 7.6.



It means that this indicator in the solutions of pH = 6.2 or less is yellow.

The solutions of pH within the range 6.2 – 7.6 are green.

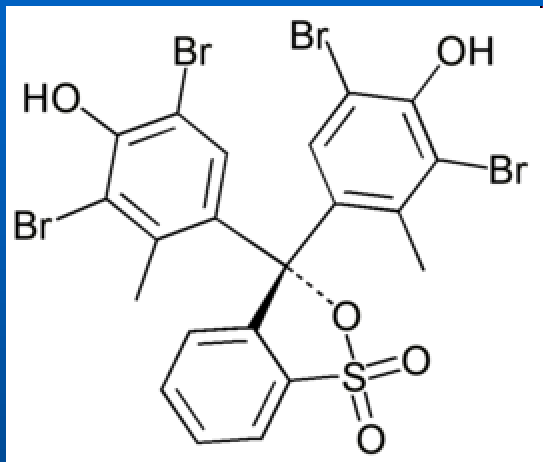
Green is the range of color change of bromothymol blue.

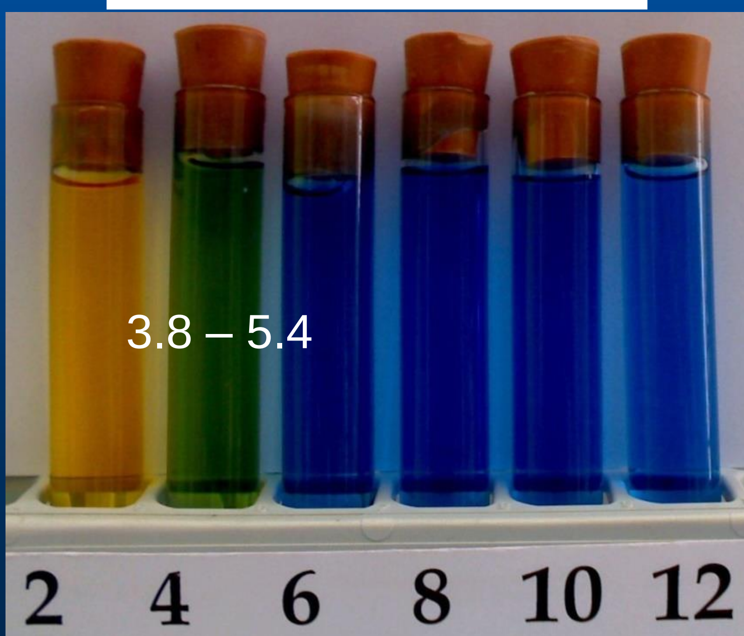
If the solution has pH 7.6 or higher, after adding bromothymol blue it becomes blue.

Indicator	pH range for color change
malachite green	0.0 – 2.0
brilliant green	0.0 – 2.6
methyl green	0.1 – 2.3
methyl violet	0.1 – 2.7
cresol red	0.2 – 1.8
thymol blue	1.2 – 2.8
dinitrophenol	2.4 – 4.0
methyl yellow	2.9 – 4.0
methyl orange	3.1 – 4.4
bromocresol green	**3.8 – 5.4**
methyl red	4.2 – 6.3
litmus	4.5 – 8.3
bromocresol red	5.2 – 6.8
bromothymol blue	6.2 – 7.6
phenol red	6.4 – 8.0
cresol red	7.2 – 8.8
phenolphthalein	8.3 – 10.0
thymolphthalein	9.3 – 10.5
tropeolin O	11.0 - 13.0

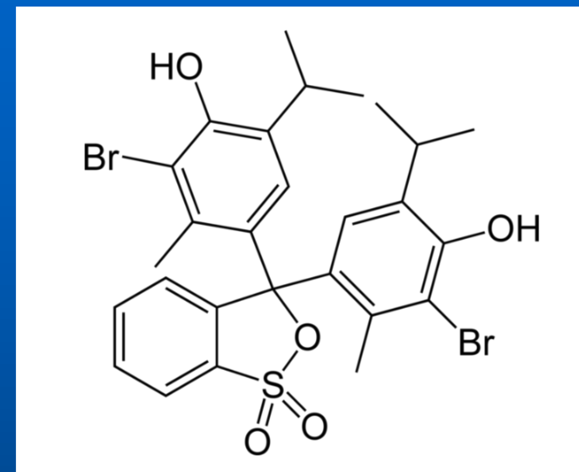
Selected indicators

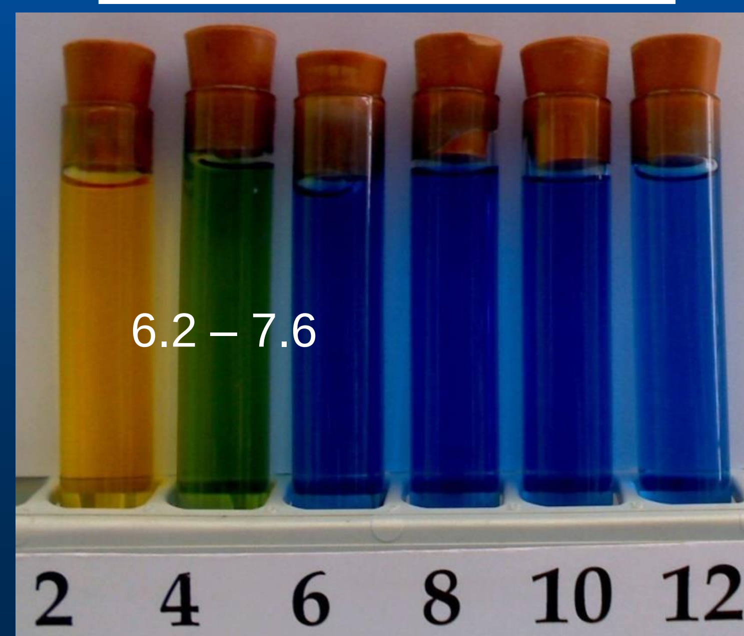
Bromocresol green





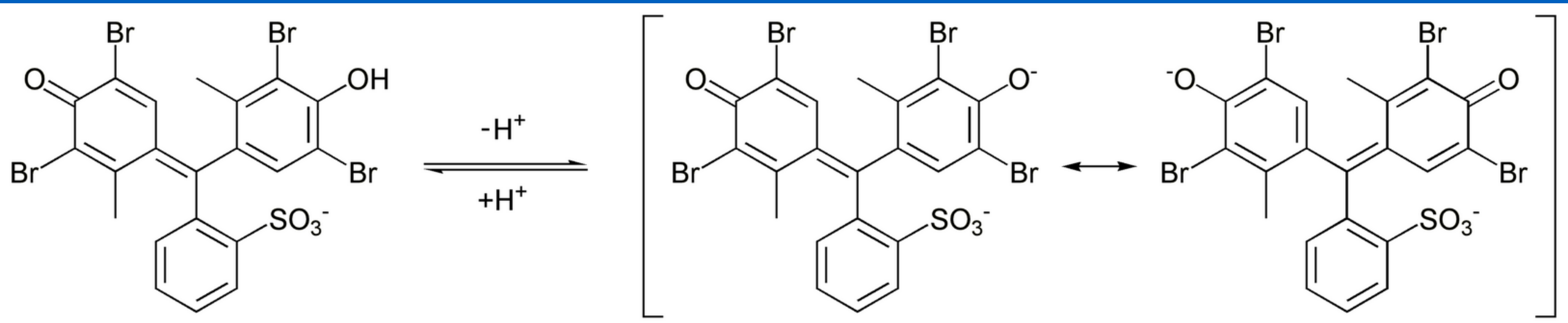
Bromothymol blue



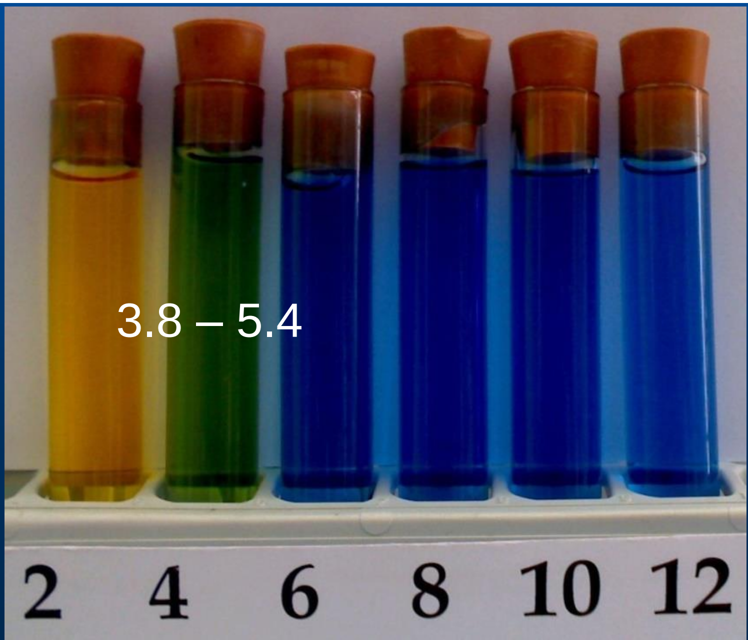


Bromocresol green

Yellow at pH <3.8 **Blue** at pH >5.4

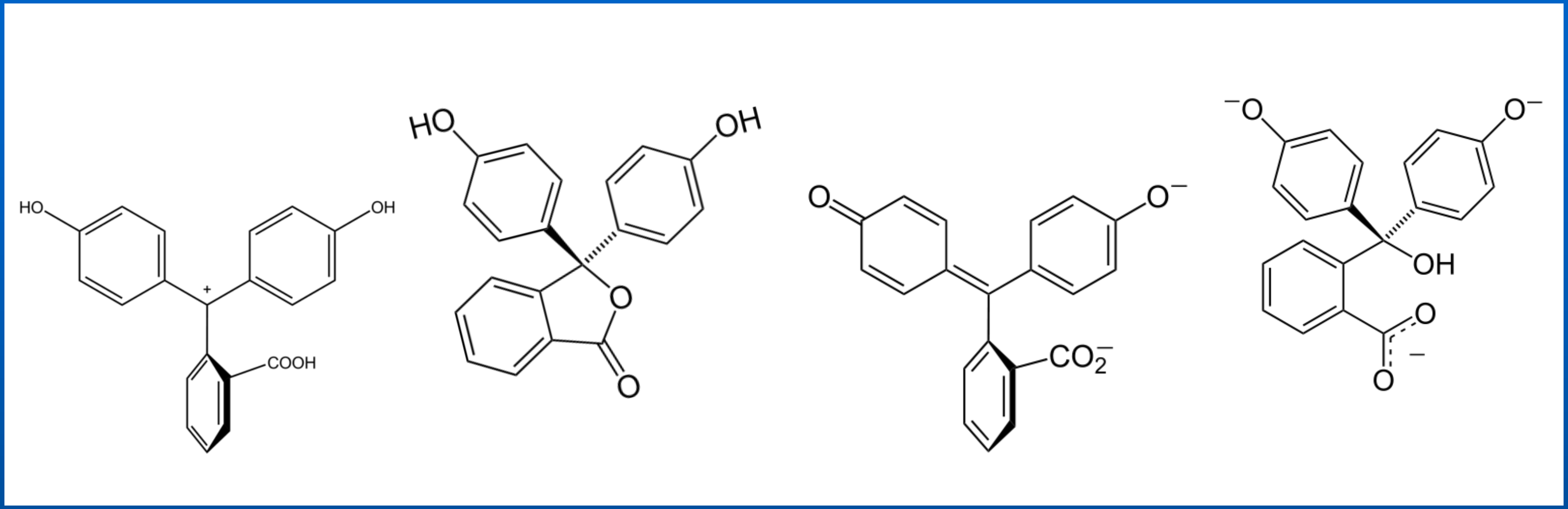


In aqueous solution, bromocresol green will ionize to give the monoanionic form (yellow), that further deprotonates at higher pH to give the dianionic form (blue), which is stabilized by resonance.



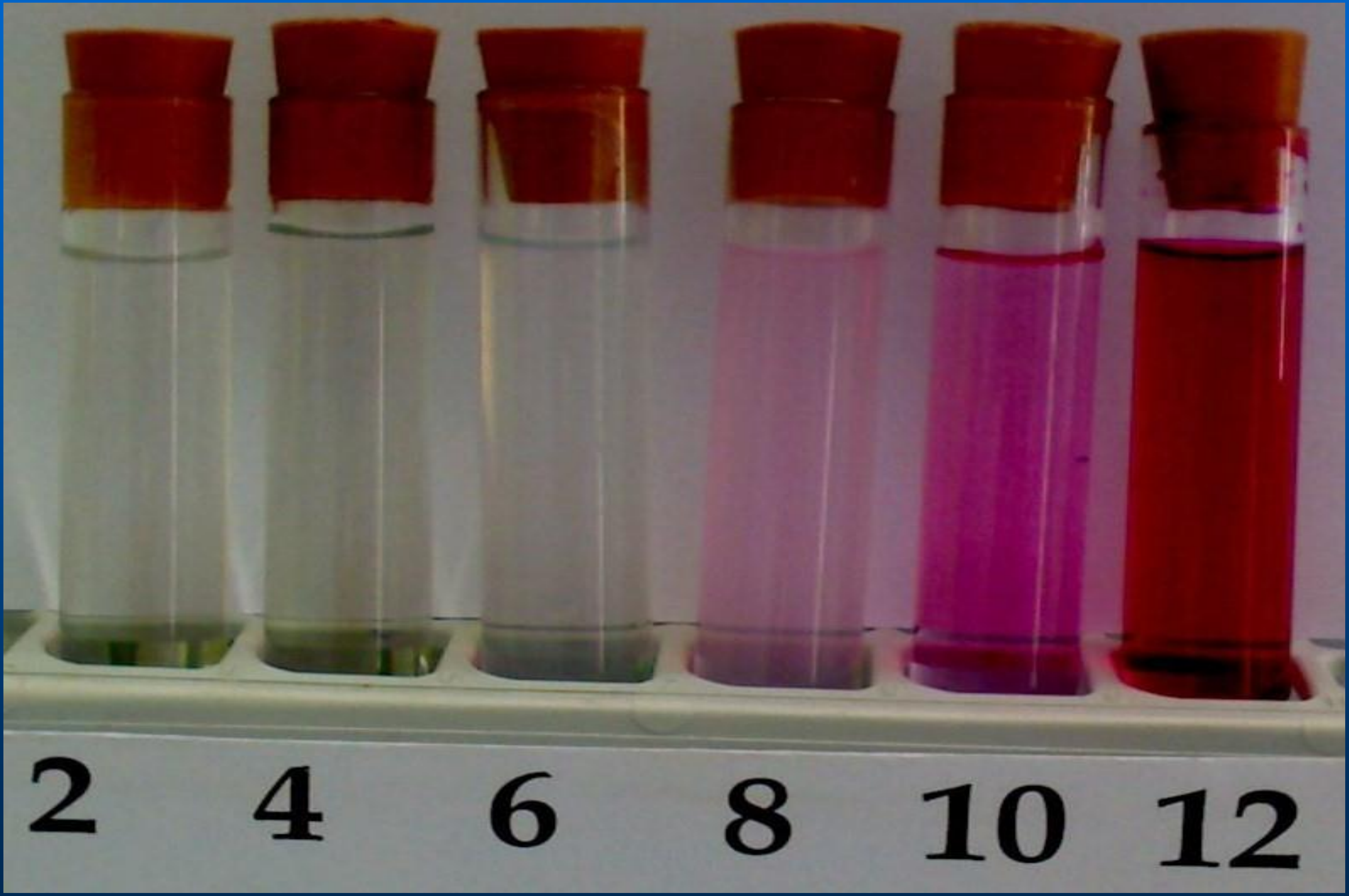
PHENOLPHTALEIN

structure



pH	< 0	0 − 8.2	8.2 − 12.0	>12.0
color	orange	colorless	from pink to fuchsia	colorless

PHENOLPHTALEIN



Task 1

The purpose: to prepare and to calculate the percentage and molar concentration of the copper sulfate solutions.

Procedure:

1. Add the following volume of the $CuSO_4$ solution and distilled water to 5 tubes (I, II, III, IV, V) according to the table below:

Tube No.	$CuSO_4$ cm^3	H_2O cm^3	Concentration	
			%	mol/dm^3
I	2	1		
II	1	1		
III	1	2		
IV	0.5	4.5		
V	0.1	0.9		

2. Calculate the final concentration (percentage and molar) of the copper sulfate solutions.

Concentration of stock solution was 1%

Molar mass of $CuSO_4$ = 159.61 g/mol

Task 1

Calculate the final concentration (percentage and molar) of the copper sulfate solutions.
Concentration of stock solution was 1%
Molar mass of $CuSO_4$ = 159.61 g/mol

Tube No.	$CuSO_4$ cm^3	H_2O cm^3	Concentration	
			%	mol/dm^3
I	2	1		

I step - C_p calculation:

We add 2 cm^3 $CuSO_4$ to 1 cm^3 H_2O and obtain 3 cm^3

The concentration is then:

$$C_p = 2/3 \times 1\% = 0.67\%$$

Task 1

Calculate the final concentration (percentage and molar) of the copper sulfate solutions.
Concentration of stock solution was 1%
Molar mass of $CuSO_4$ = 159.61 g/mol

Tube No.	$CuSO_4$ cm^3	H_2O cm^3	Concentration	
			%	mol/dm^3
I	2	1	0.67	

II step - C_M calculation:

1) C_M = ?

$$C_M = \frac{C_p \times d}{100\,\% \times M}$$

$d = 1\ g/cm^3 = 1000\ g/dm^3$

Task 1

Calculate the final concentration (percentage and molar) of the copper sulfate solutions.
Concentration of stock solution was 1%
Molar mass of $CuSO_4$ = 159.61 g/mol

Tube No.	$CuSO_4$ cm^3	H_2O cm^3	Concentration	
			%	mol/dm^3
I	2	1	**0.67**	**0.04**

II step - C_M calculation:

1) C_M = ?

$$C_M = \frac{C_p \times d}{100\,\% \times M}$$

$d = 1\ g/cm^3 = 1000\ g/dm^3$

Task 2

The purpose: to prepare the buffer solutions of a selected pH.

Procedure:

1. Add 0.1 mol/dm^3 acetic acid and 0.1 mol/dm^3 sodium acetate to 5 tubes (as described in the table below):

Tube No.	CH_3COOH 0.1 mol/dm^3 cm^3	CH_3COONa 0.1 mol/dm^3 cm^3	Calculated pH value (for 18°C)	pH value measured potentiometrically
1	9	1		
2	7	3		
3	5	5		
4	3	7		
5	1	9		

2. Mix the tubes precisely.

3. Calculate the pH of the buffer solutions from each tube using the Henderson-Hasselbalch equation.

4. Measure the pH by pH-meter to confirm the accuracy of the calculations.

Task 2

Calculate the pH* of the buffer solutions from each tube using the **Henderson-Hasselbalch equation:**

$$pH = pK_a - \log \frac{[CA]}{[CS]}$$

pK_a - is a negative logarithm of the acid dissociation constant

C_A – acid concentration

C_S – salt concentration

*****pH is the negative of the logarithm to base 10 of the activity of the hydrogen ion.*

Task 2

Calculate the pH of the buffer solutions from each tube using the **Henderson-Hasselbalch equation:**

$$pH = pK_a - \log \frac{[CA]}{[CS]}$$

Tube No.	CH_3COOH 0.1 mol/dm^3 cm^3	CH_3COONa 0.1 mol/dm^3 cm^3	Calculated pH value (for 18°C)	pH value measured potentiometrically
1	9	1		

We know that pK_a for CH_3COOH is 4.76

But C_A and C_S in the buffer should be calculated:

$C_A = n_A / V_{buffer}$
n_A – moles of acid
V_{buffer} – volume of buffer

$C_S = n_S / V_{buffer}$
n_S – moles of salt
V_{buffer} – volume of buffer

Task 2

for Tube No 1:

1) We calculate C of acid in the buffer:

V_A = 9 cm^3 = 0.009 dm^3
$C_{A(initial)}$ = 0.1 mol/dm^3

$n_A = V \times C$ = 0.0009 moles

$C_A = n_A / V_{buffer}$
V_{buffer} = 1 + 9 = 10 cm^3 = 0.01 dm^3

$C_A = n_A / V_{buffer}$
C_A = 0.0009 / 0.01 = 0.09 M

2) We calculate C of salt in the buffer:

V_S = 1 cm^3 = 0.001 dm^3
$C_{S(initial)}$ = 0.1 mol/dm^3

$n_S = V \times C$ = 0.0001 moles

$C_S = n_S / V_{buffer}$
V_{buffer} = 1 + 9 = 10 cm^3 = 0.01 dm^3

$C_S = n_S / V_{buffer}$
C_S = 0.0001 / 0.01 = 0.01 M

$$pH = pK_a - \log \frac{[CA]}{[CS]} = 4.76 - \log \frac{[0.09]}{[0.01]} = 3.81$$

Task 2 - results

CH_3COOH 0.1 mol/dm^3 (cm^3)	CH_3COONa 0.1 mol/dm^3 (cm^3)	CH_3COOH (mol)	CH_3COONa (mol)	CH_3COOH (mol/dm^3)	CH_3COONa (mol/dm^3)	**pH**
9	1	0.0009	0.0001	0.09	0.01	**3.81**
7	3					
5	5					
3	7					
1	9					

Task 3

The purpose: to observe the effect of the dilution of buffer solution on its pH.

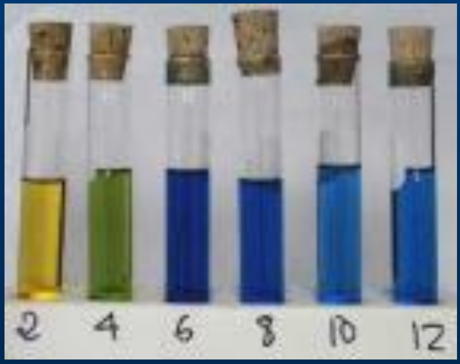
Procedure:

1. Add 0.1 mol/dm^3 acetate buffer (pH=4.7) and distilled water to 5 tubes

Tube No.	Buffer cm^3	H_2O cm^3	Dilution	Molarity	Color of the indicator
1	2	0			
2	0.5	1.5			
3	0.2	1.8			
4	0.02	1.98			
5	0	2			

2. Calculate the dilution and molarity of the buffer solutions deriving from each tube.
3. Add 5 drops of bromocresol green to each tube.

Question: Does the dilution has an influence on the buffer pH?



Task 4

The purpose: to evaluate the capacity of the acetate buffer.

Procedure:

1. Add 0.1 mol/dm³ sodium acetate, 0.1 mol/dm³ acetic acid and distilled water in appropriate volume to 4 tubes:

Tube No.	CH_3COOH 0.1 mol/dm³ cm³	CH_3COONa 0.1 mol/dm³ cm³	H_2O cm³	Dilution	The volume of consumed NaOH (drops)
1	2	2	0		
2	1	1	2		
3	0.5	0.5	3		
4	0	0	4		

2. Calculate the dilution of the buffer solution.

3. Add 5 drops of bromocresol green to each tube.

4. Add 0.1 cm³ of 0.1 mol/dm³ NaOH to tube No. 4.

5. Add by drops 0.1 mol/dm³ NaOH to other tubes, until the color has become the same as in the tube No. 4. Note the volume of the consumed NaOH in each tube!

Question: Does the dilution has an influence on the buffer capacity?