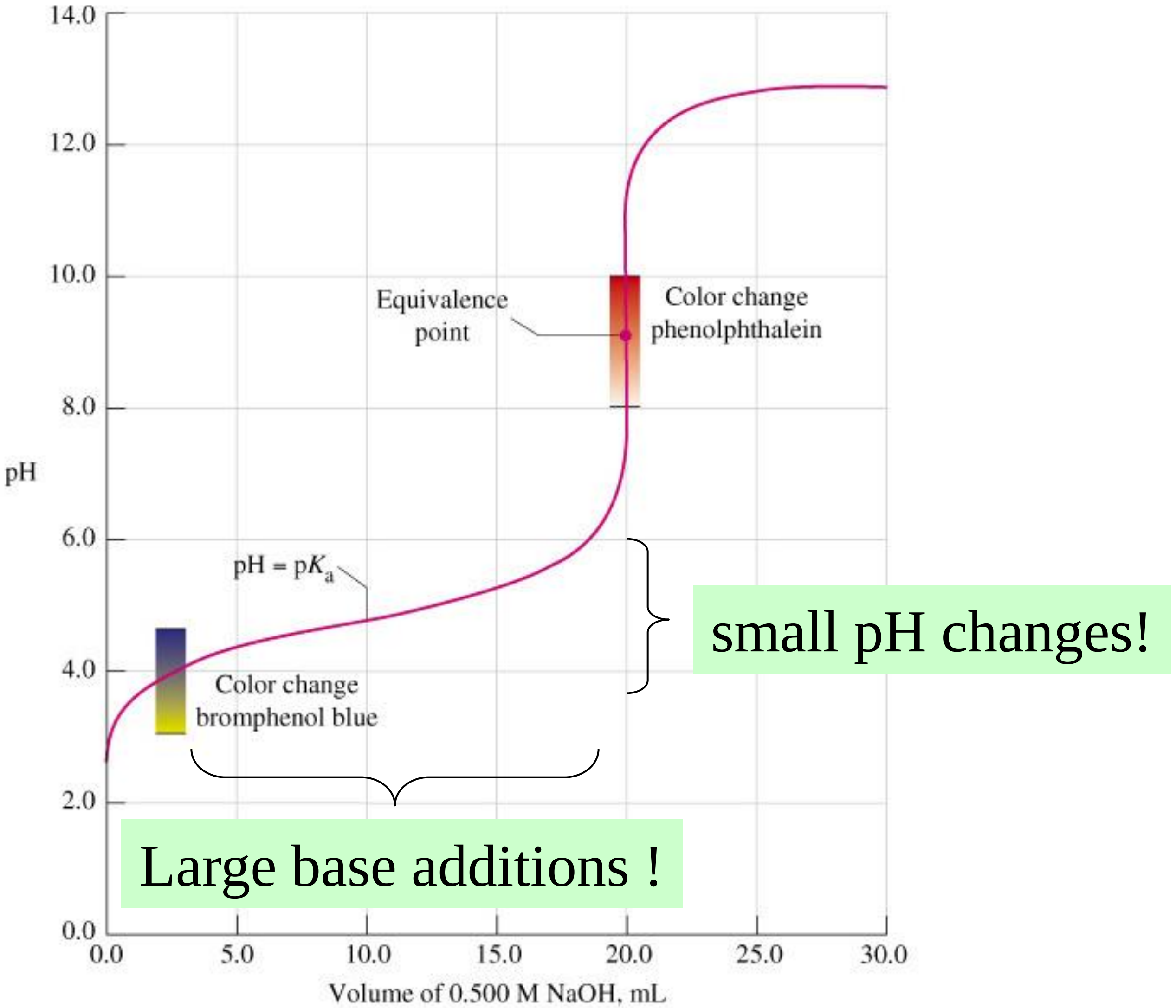
Let's consider again the 50% neutralization condition



Buffer… !!

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***definition*:**

Weak acids or bases

in the presence of

Their salt with strong acids/bases

Buffers minimize pH variations in the pH region close to *pKa o pKb* (of the weak species)

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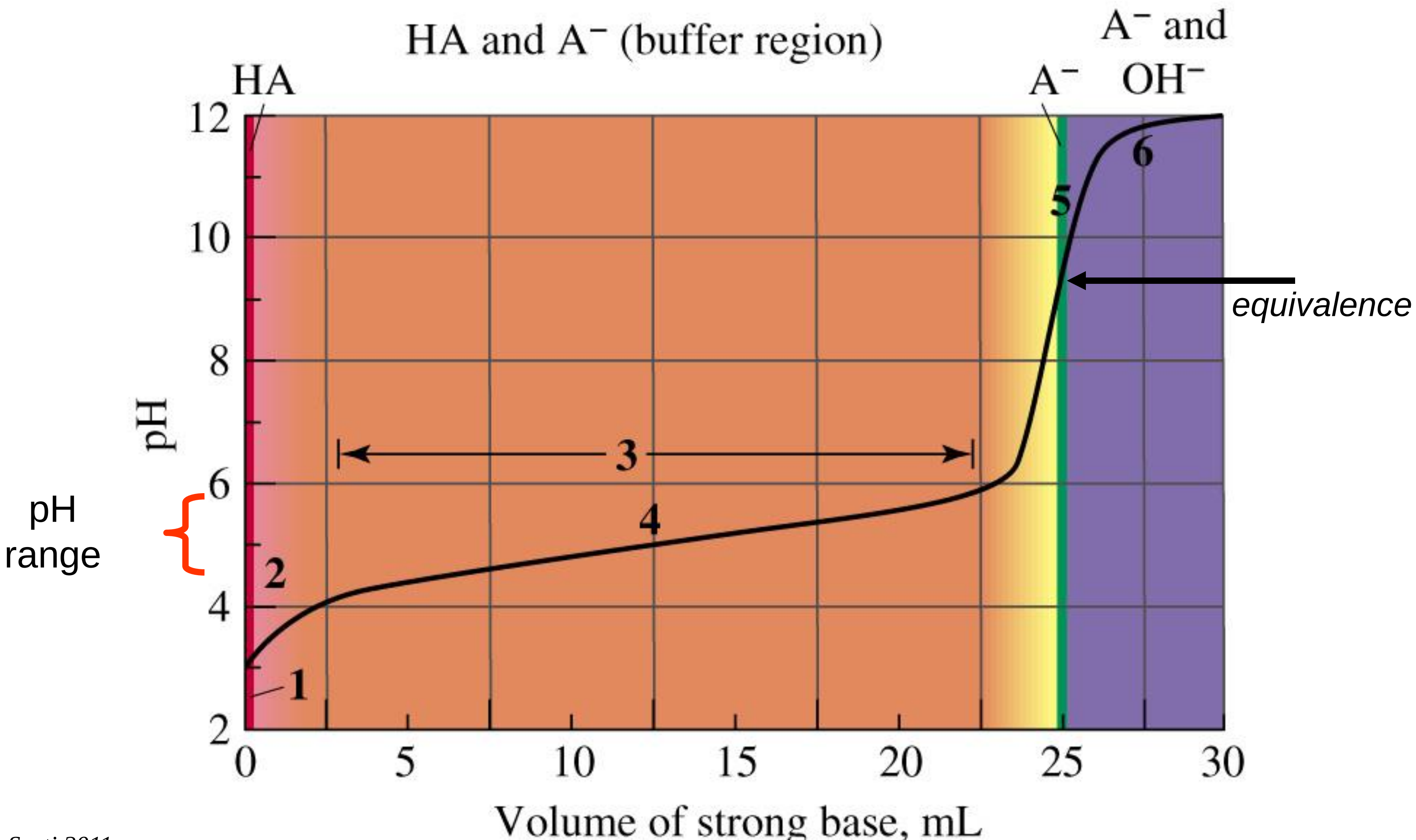

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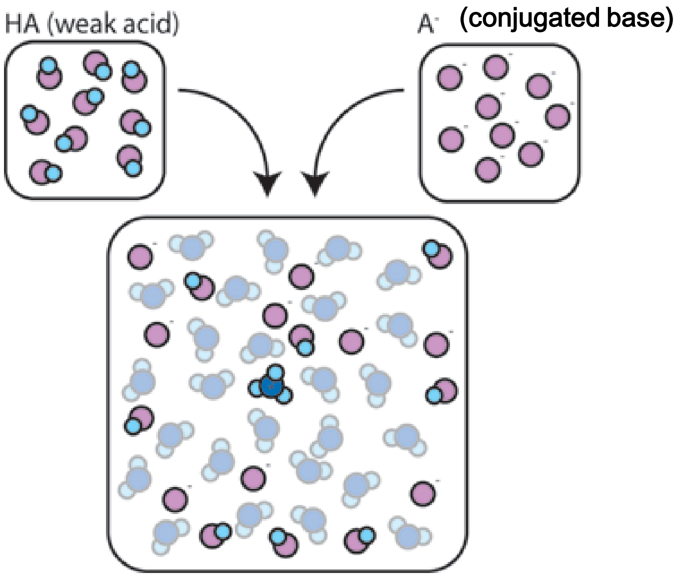
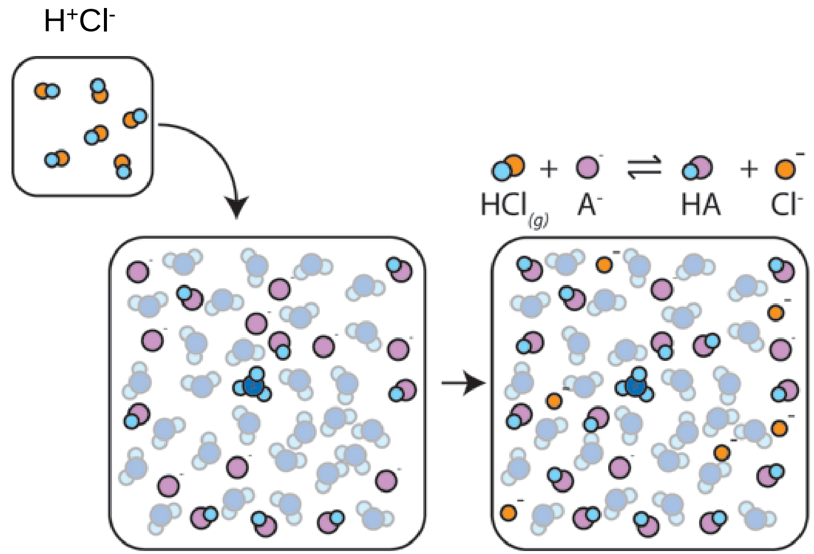
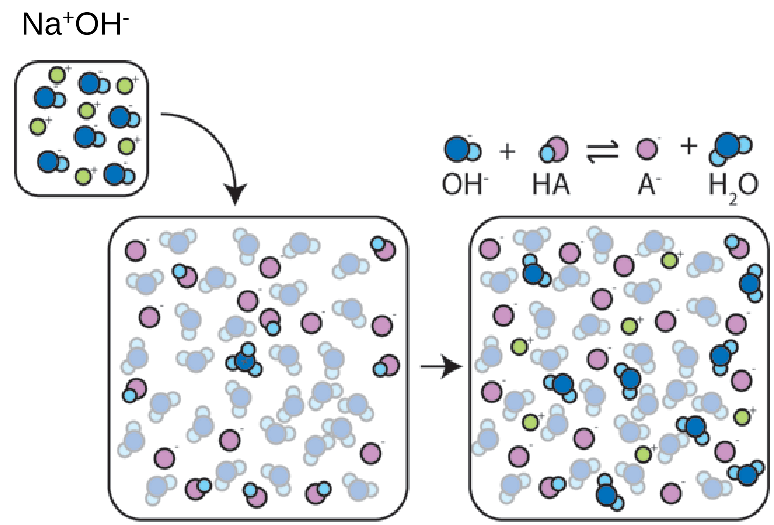
Schematic representation







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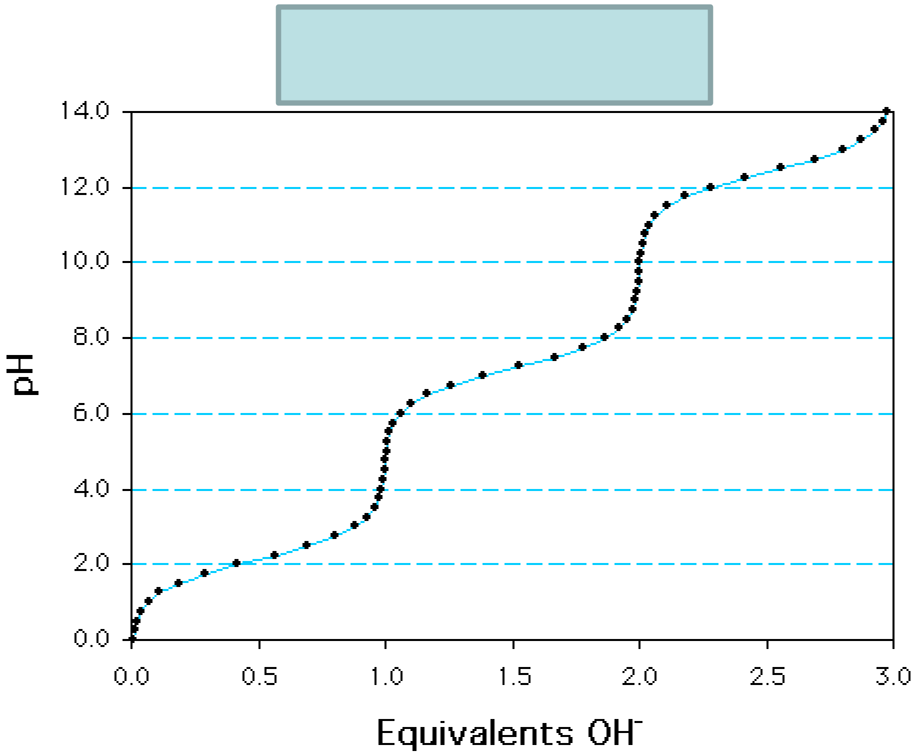
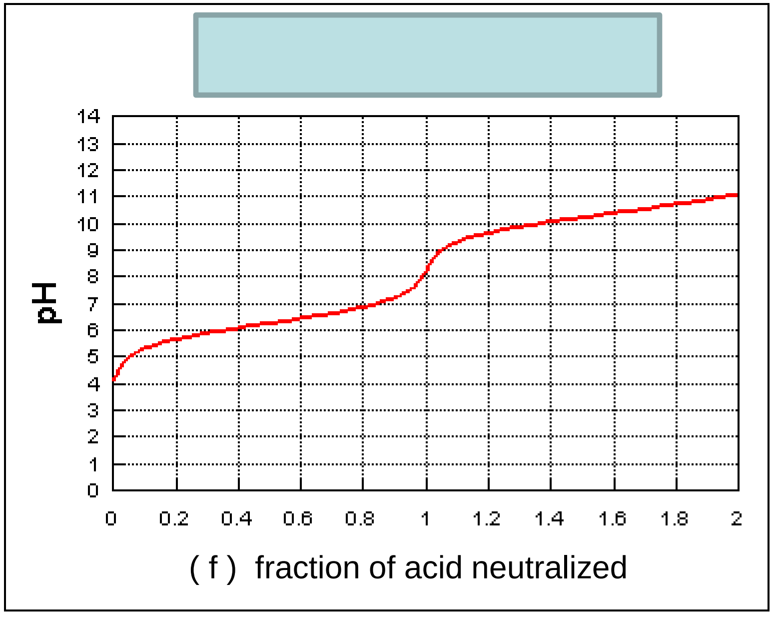


14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
pH
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Equivalents OH⁻


pH
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
(f) fraction of acid neutralized

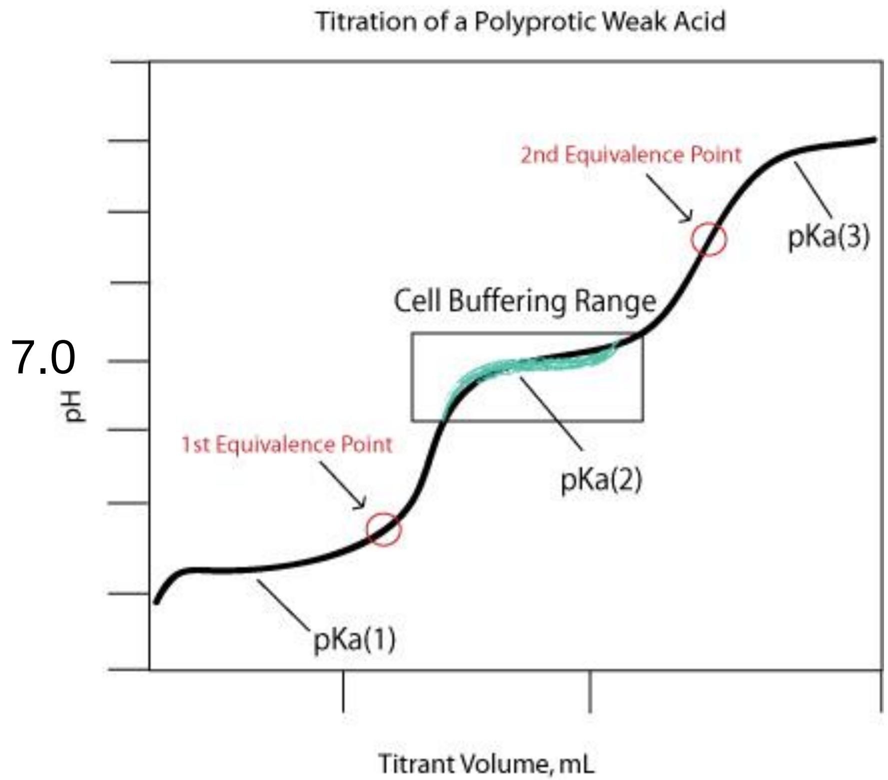
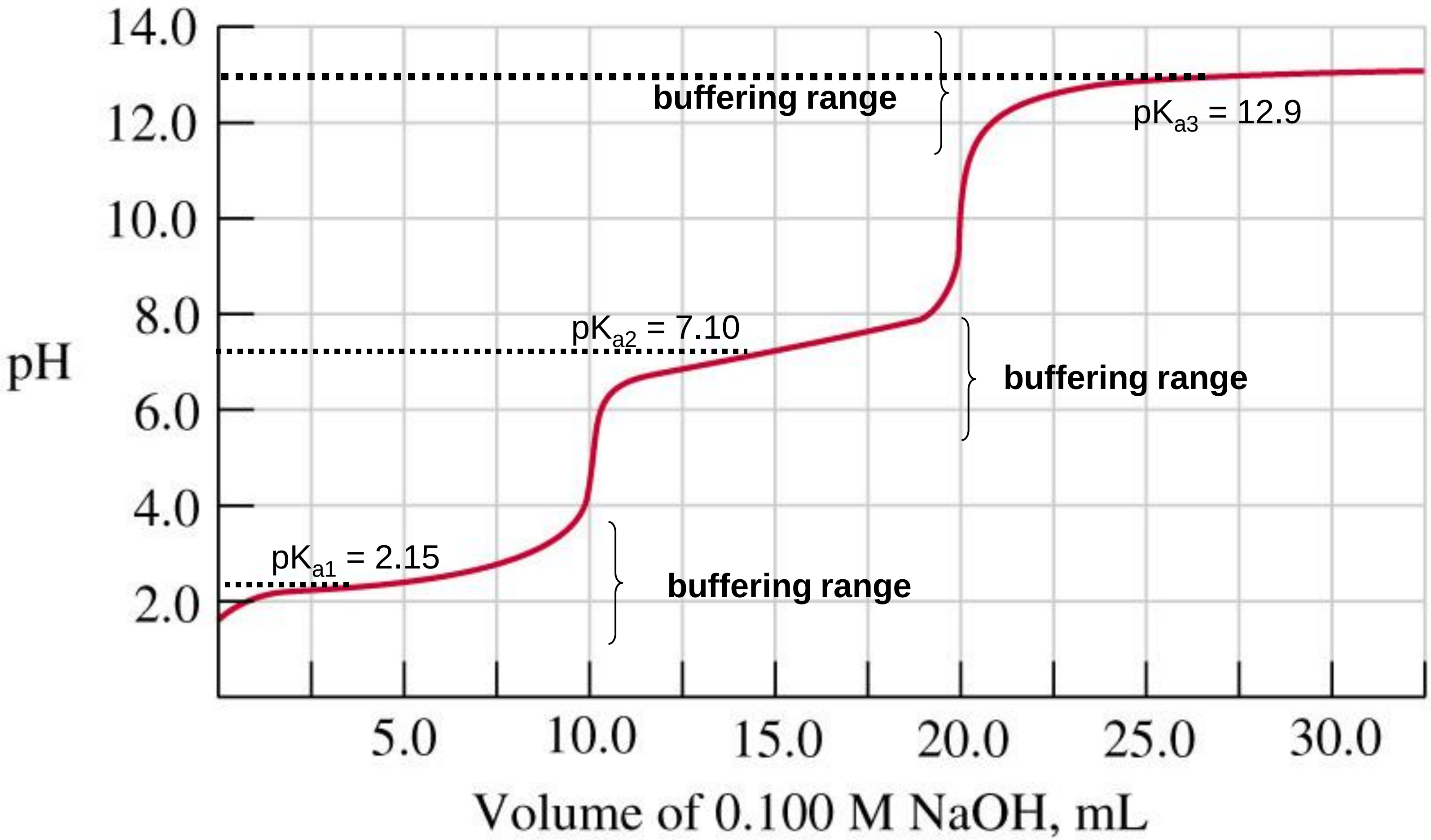


Fig. 2 Polyprotic Acid Titration

H_3PO_4 buffers in the 3 pH regions of pH = pK_{a1}, pK_{a2}, pK_{a3}



Piccole variazioni di pH a fronte di grosse variazioni di base aggiunta!

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Henderson Hasselbalch equation

$$HA \rightleftarrows A^- + H^+$$

$$K_a = \frac{[H^+][A^{-1}]}{[HA]}$$

si fa il log ad entrambi i lati dell'equazione

$$\log K_a = \log[H^+] + \log\frac{[A^-]}{[HA]}$$

riarrangiandola

$$-\log[H^+] = -\log K_a + \log\frac{[A^-]}{[HA]}$$

$$-\log[H^+] = pH; -\log K_a = pK_a$$

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

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Henderson Hasselbalch

$$pH = pKa + \log \frac{Cs}{Ca}$$

Buffering range = pKa ± 1

Buffer capacity up to 1/50 ÷ 1/25 [species] *(residual)*

Ex. 0.1 M HCO_3^-/H_2CO_3 *(down to 2 – 4 mM)*

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$[H^+]$ HOMEOSTASIS in our body

buffer (Cs/Ca)	[Cs]/[Ca] mM	pKa	Control	velocity
HCO_3^-/H_2CO_3	27/1.3	6.1	Chem. eq.	instantaneous
$HPO_4^{2-}/ H_2PO_4^-$	2/0.5	~ 7.1	Chem. eq.	instantaneous
$Prot.^{(H+)}/prot.^{(-)}$	variable	6.0÷9.0	Chem. eq.	instantaneous
Body (organs) control				
lungs	Levels of $CO_2\uparrow + H_2O \rightleftarrows H_2CO_3$			Fast (min)
kidney	Levels of H^+ / OH^-			slow (h)
Intestin	Levels of HCO_3^-			slow (h)
skin	Levels of acidic equivalents			slow (h)

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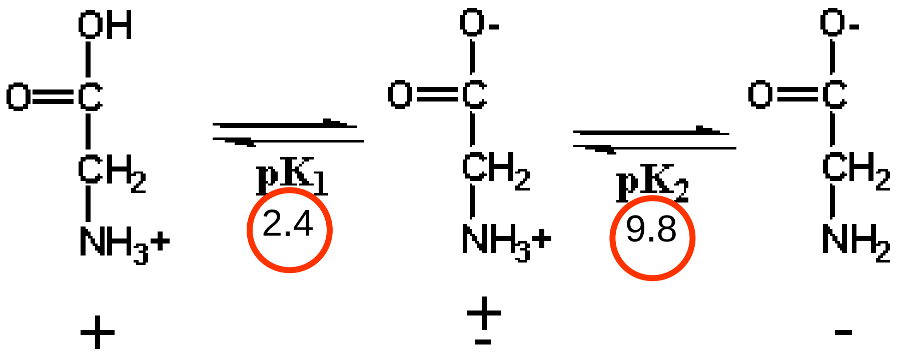
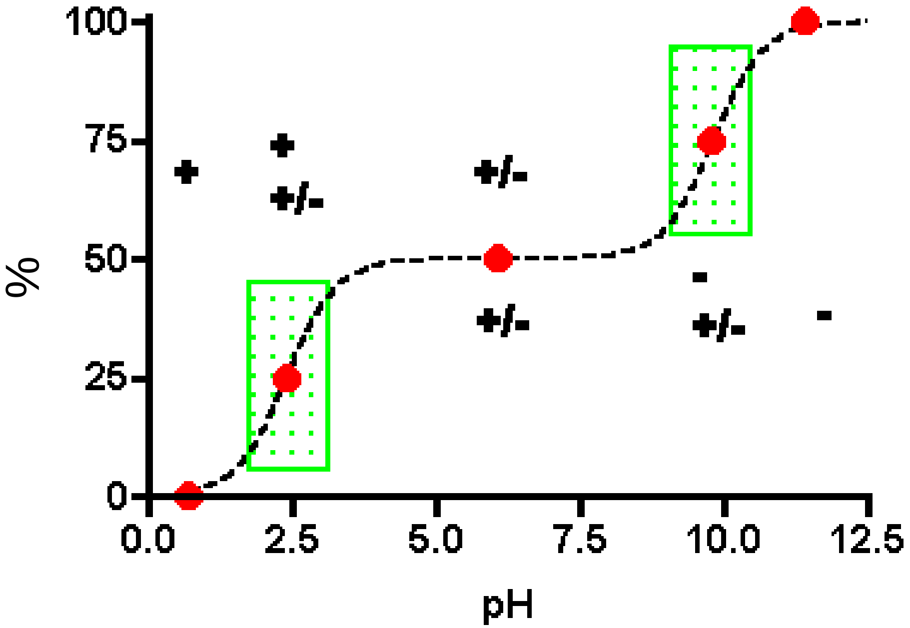
Glicine: an aminoacid, is a buffer ! …



Titration curve



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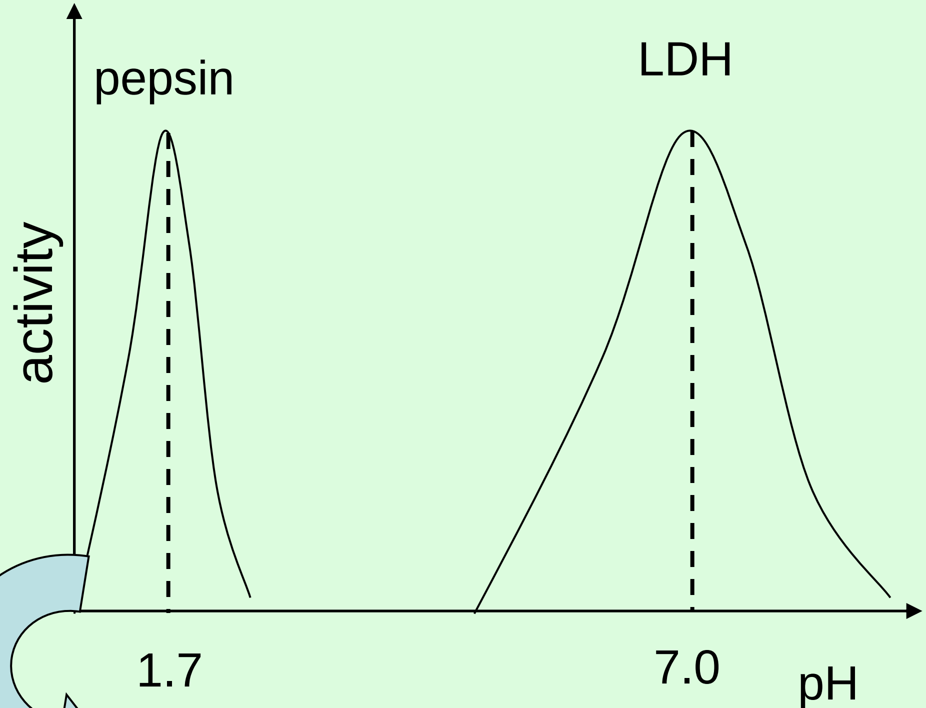
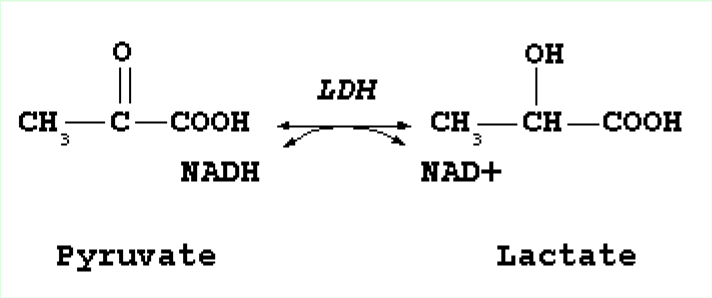
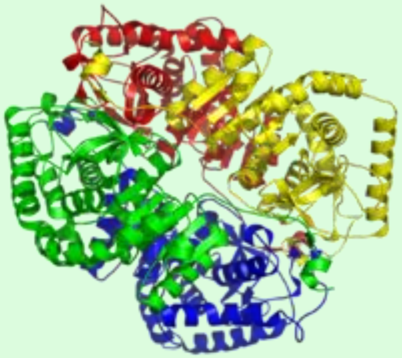
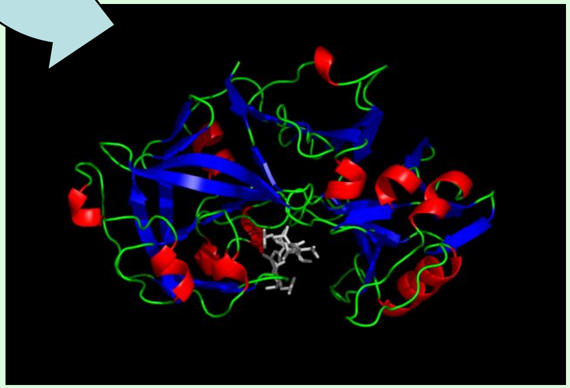
pH & enzymatic activity









M.W. 34500 Da

Hydrophobic—aromatic

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1 10 20

H-Ile-Gly-Asp-Glu-Pro-Leu-Glu-Asn-Tyr-Leu-Asp-Thr-Glu-Tyr-Phe-Gly-Thr-Ile-Gly-Ile-

21 30 40

Gly-Thr-Pro-Ala-Gln-Asp-Phe-Thr-Val-Ile-Phe-Asp-Thr-Gly-Ser-Ser-Asn-Leu-Trp-Val-

41 50 60

Pro-Ser-Val-Tyr-Cys-Ser-Ser-Leu-Ala-Cys-Ser-Asp-His-Asn-Gln-Phe-Asn-Pro-Asp-Ser-

61 P 70 80

Asp-Ser-Thr-Phe-Glu-Ala-Thr-Ser-Gln-Glu-Leu-Ser-Ile-Thr-Tyr-Gly-Thr-Gly-Ser-Met-

81 90 100

Thr-Gly-Ile-Leu-Gly-Tyr-Asp-Thr-Val-Gln-Val-Gly-Gly-Ile-Ser-Asp-Thr-Asn-Gln-Ile-

101 110 120

Phe-Gly-Leu-Ser-Glu-Thr-Glu-Pro-Gly-Ser-Phe-Leu-Tyr-Tyr-Ala-Pro-Phe-Asp-Gly-Ile-

121 130 140

Leu-Gly-Leu-Ala-Tyr-Pro-Ser-Ile-Ser-Ala-Ser-Gly-Ala-Thr-Pro-Val-Phe-Asp-Asn-Leu-

141 150 160

Trp-Asp-Gln-Gly-Leu-Val-Ser-Gln-Asp-Leu-Phe-Ser-Val-Tyr-Leu-Ser-Ser-Asn-Asp-Asp-

161 170 180

Ser-Gly-Ser-Val-Val-Leu-Leu-Gly-Gly-Ile-Asp-Ser-Ser-Tyr-Tyr-Thr-Gly-Ser-Leu-Asn-

181 190 200

Trp-Val-Pro-Val-Ser-Val-Glu-Gly-Tyr-Trp-Gln-Ile-Thr-Leu-Asp-Ser-Ile-Thr-Met-Asp-

201 210 220

Gly-Glu-Thr-Ile-Ala-Cys-Ser-Gly-Gly-Cys-Gln-Ala-Ile-Val-Asp-Thr-Gly-Thr-Ser-Leu-

221 230 240

Leu-Thr-Gly-Pro-Thr-Ser-Ala-Ile-Ala-Ile-Asn-Ile-Gln-Ser-Asp-Ile-Gly-Ala-Ser-Glu-

241 250 260

Asn-Ser-Asp-Gly-Glu-Met-Val-Ile-Ser-Cys-Ser-Ser-Ile-Asp-Ser-Leu-Pro-Asp-Ile-Val-

261 270 280

Phe-Thr-Ile-Asp-Gly-Val-Gln-Tyr-Pro-Leu-Ser-Pro-Ser-Ala-Tyr-Ile-Leu-Gln-Asp-Asp-

281 290 300

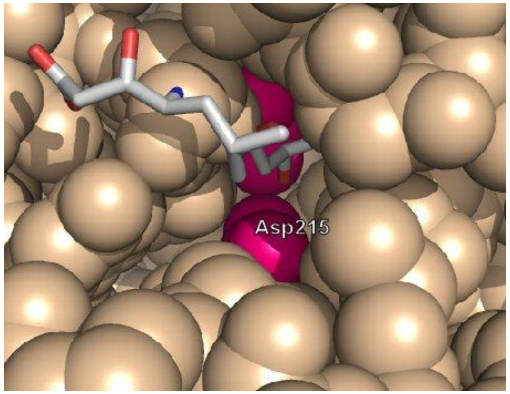
Asp-Ser-Cys-Thr-Ser-Gly-Phe-Glu-Gly-Met-Asp-Val-Pro-Thr-Ser-Ser-Gly-Glu-Leu-Trp-

301 310 320

Ile-Leu-Gly-Asp-Val-Phe-Ile-Arg-Gln-Tyr-Tyr-Thr-Val-Phe-Asp-Arg-Ala-Asn-Asn-Lys-

321 327

Val-Gly-Leu-Ala-Pro-Val-Ala-OH



Active site

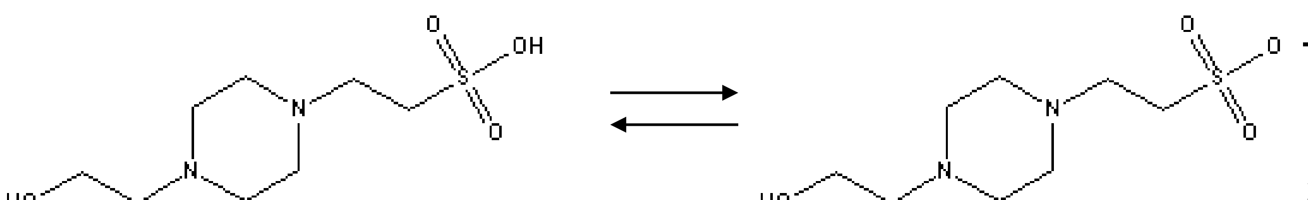
Pepsin A – primary structure – *active site asp 32, 215*

Common buffers (lab/clinical)

substances	ΔpH (range)
citrate	3.0 ÷ 6.2
acetate	3.6 ÷ 5.6
succinate	3.8 ÷ 6.0
cacodilate	5.0 ÷ 7.4
phosphate mono/bibasic	5.0 ÷ 8.0
barbiturate	6.8 ÷ 9.2
borate	8.0 ÷ 10.0
glycine	8.6 ÷ 10.6
bicarbonate	9.2 ÷ 10.6

Common buffers (biological-cellular)

substances	ΔpH (range)
MES	5.6 ÷ 6.8
PIPES	6.1 ÷ 7.3
MOPS	6.6 ÷ 7.8
HEPES	7.2 ÷ 8.2
TRIS	7.0 ÷ 9.0
TRICINA	7.4 ÷ 8.8
TRIZMA	7.0 ÷ 9.2



$+ \ H^+$

HEPES

pKa ≈ 7.7

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