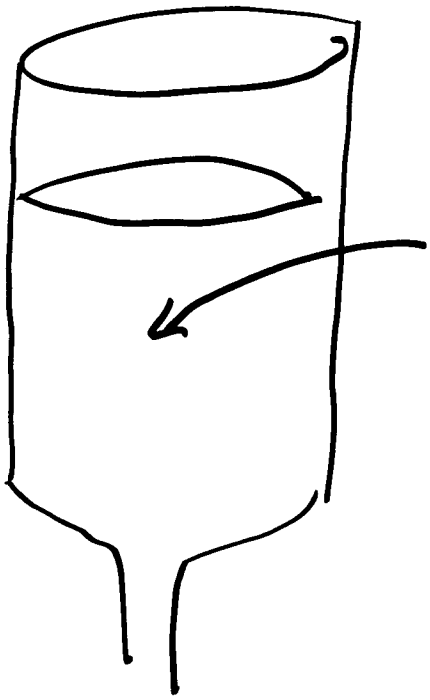
COLUMN CHROMATOGRAPHY

- Differential separation of a mixture on a matrix



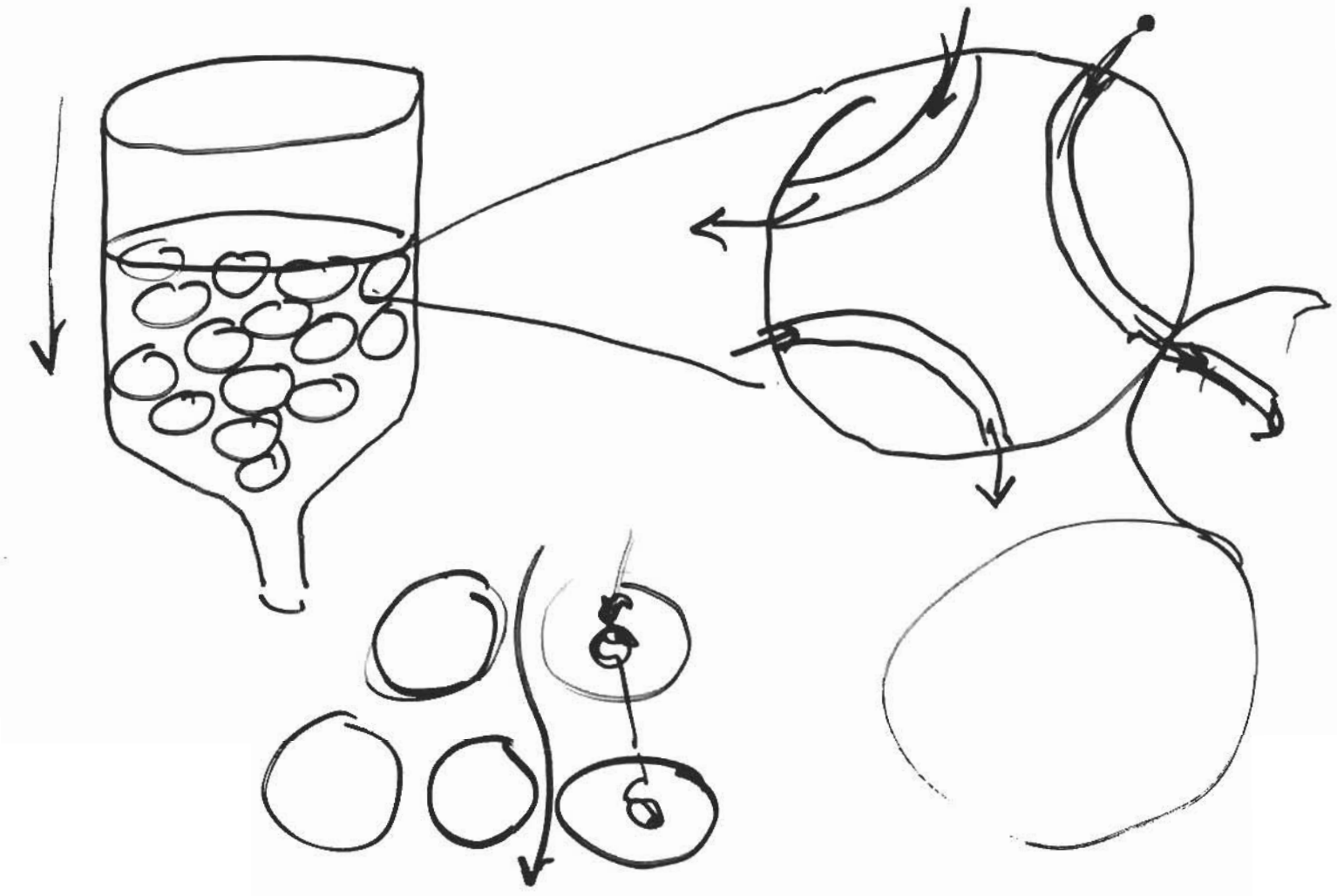
1. EQUILIBRATE
2. ADD SAMPLE
3. (WASH)
4. ELUTE

- SIZE ✓
- CHARGE ✓
- AFFINITY

SIZE-EXCLUSION CHROMATOGRAPHY

- Separates based on size

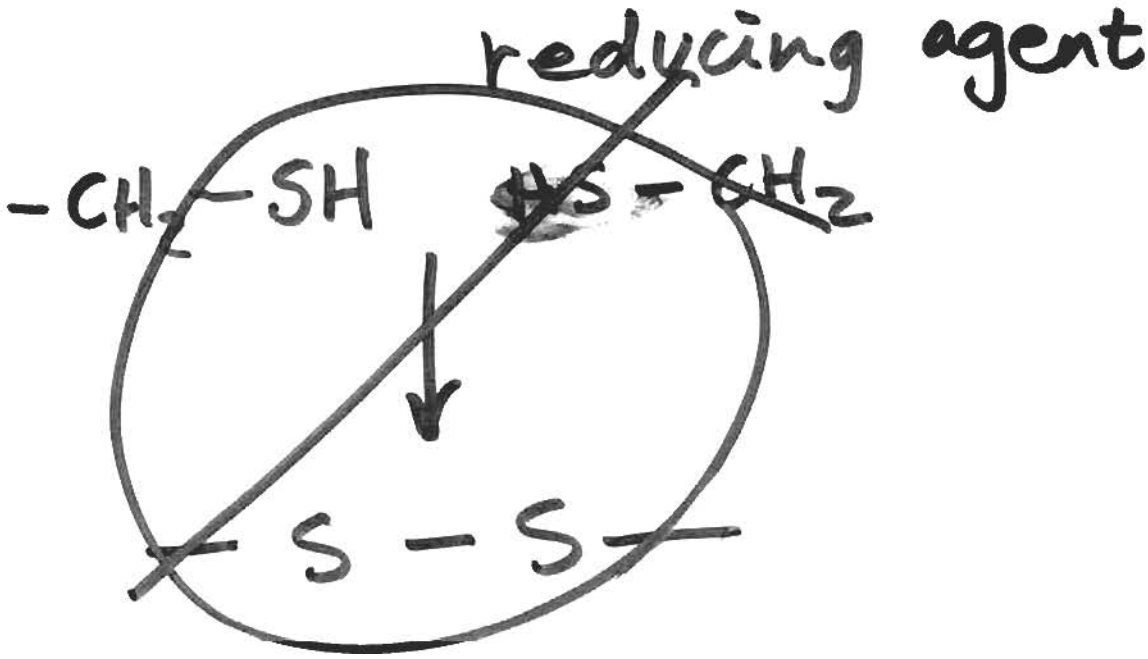
Today: DESALTING COLUMN (PD-10)



DESALTING COLUMN (PD-10)

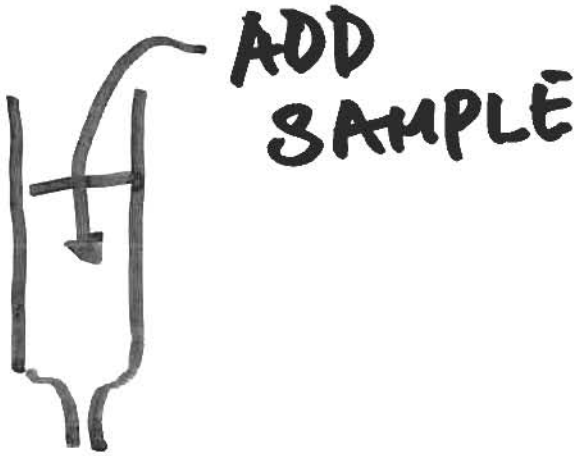
1. EQUILIBRATE with COLUMN BUFFER

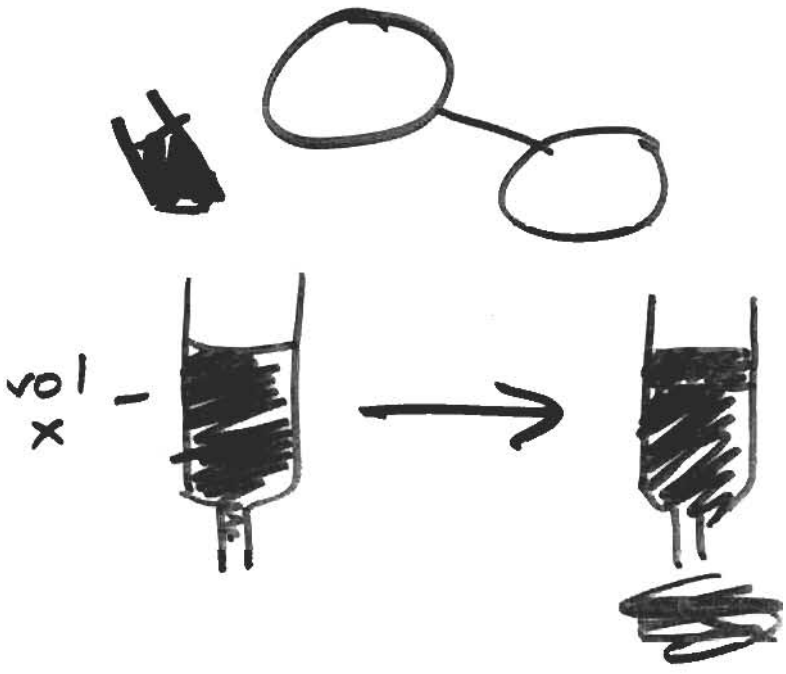
Tris-HCl	pH 7.5
NaCl	100mM
$MgCl_2$	co-factor
DTT	1mM



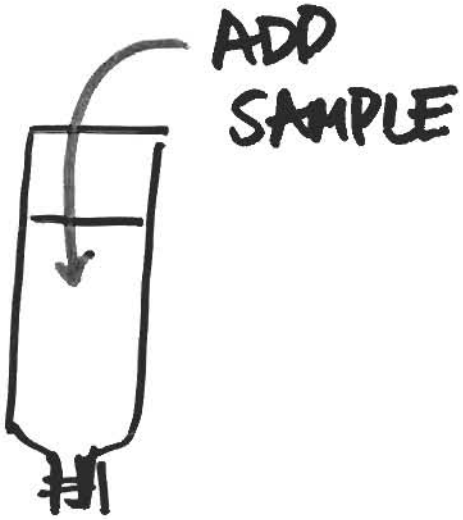
2. ADD SAMPLE

3. ELUTE





3. ELUTE with COLUMN BUFFER.



#1 #2 #3 #4 #5

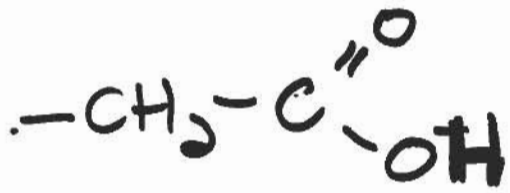
X = =

pK's of amino acids

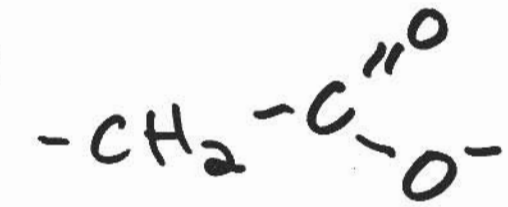
Acidic

ASPARTATE pK 4.5

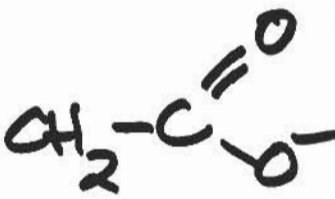
pK



@ pH 4.5



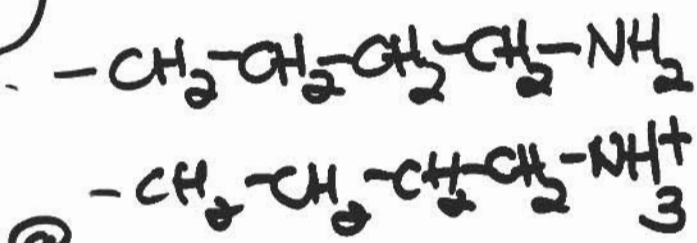
@ 5.5 more



(10X)

Basic

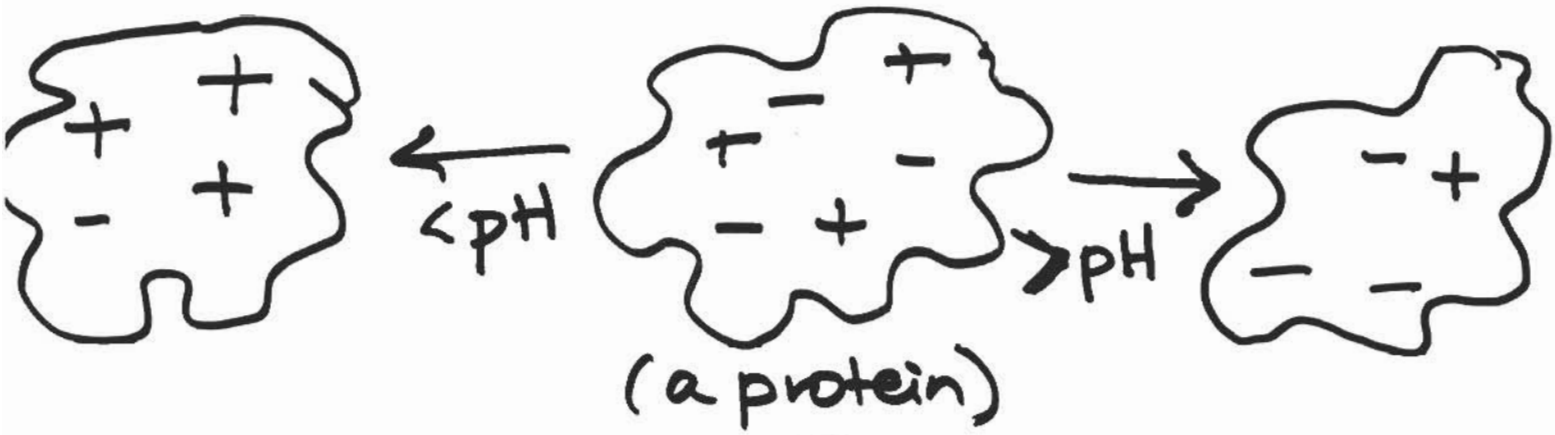
LYSINE pK 11



@ pH 11

@ pH 10 more

(10X)



(a protein)

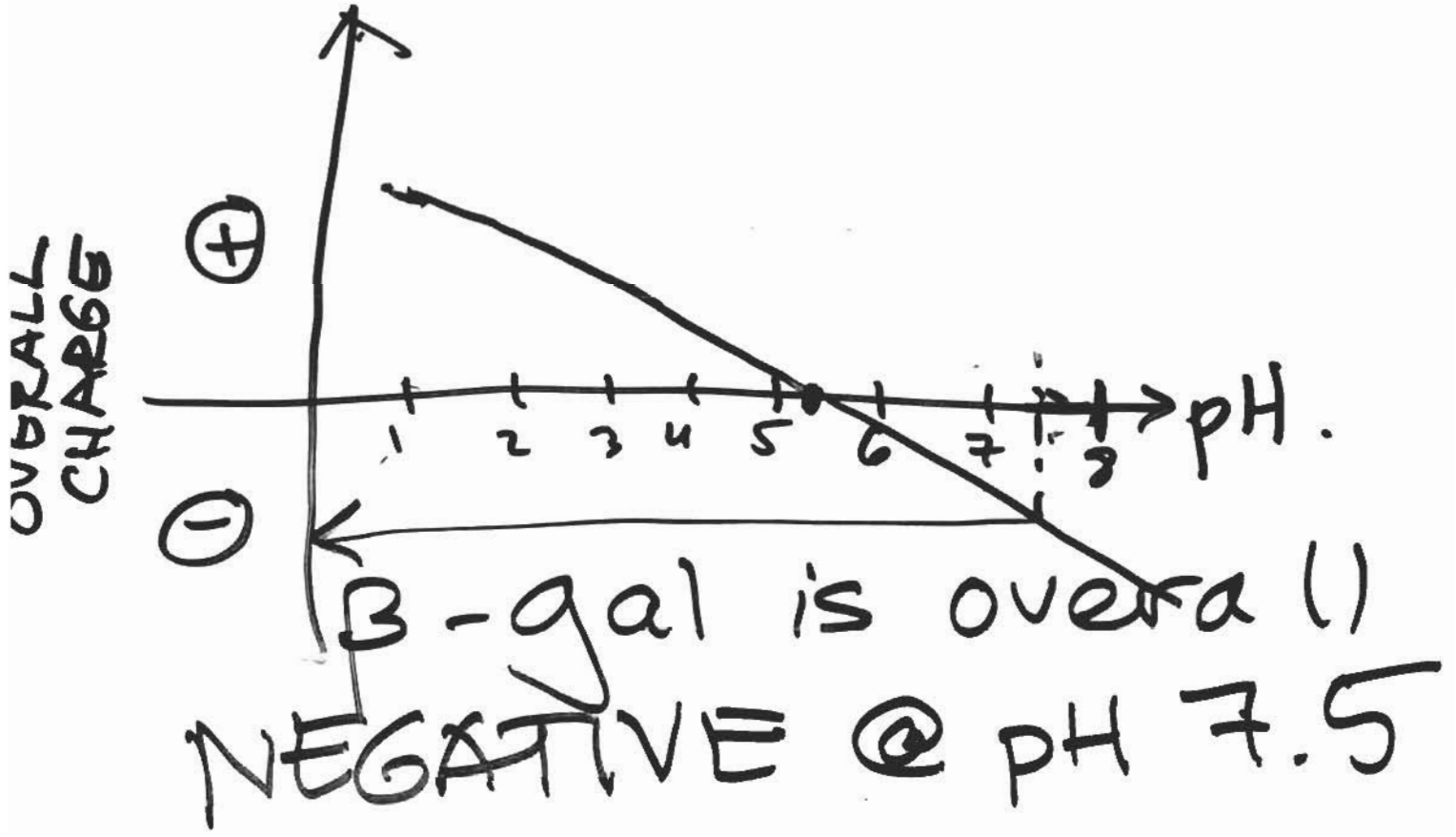
pI

ISOELECTRIC POINT

OVERALL POSITIVE

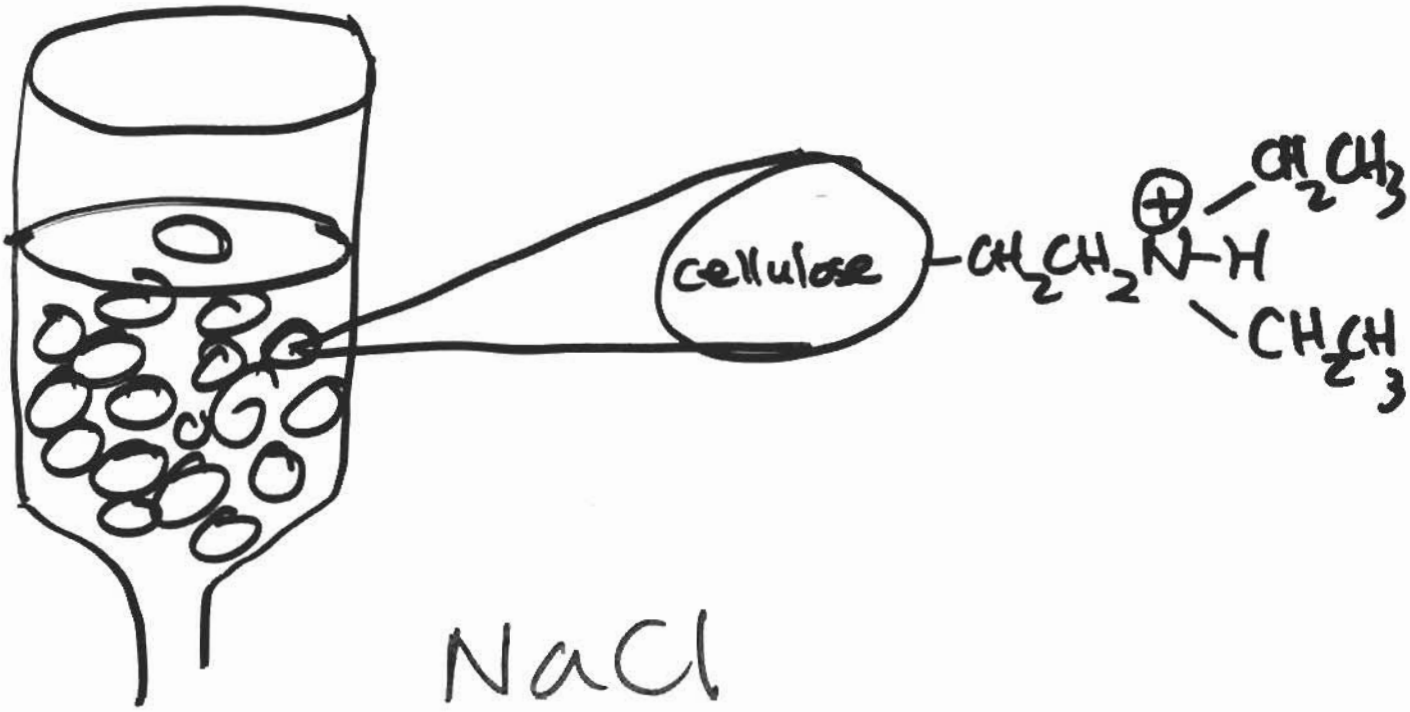
OVERALL NEGATIVE

OVERALL NEUTRAL

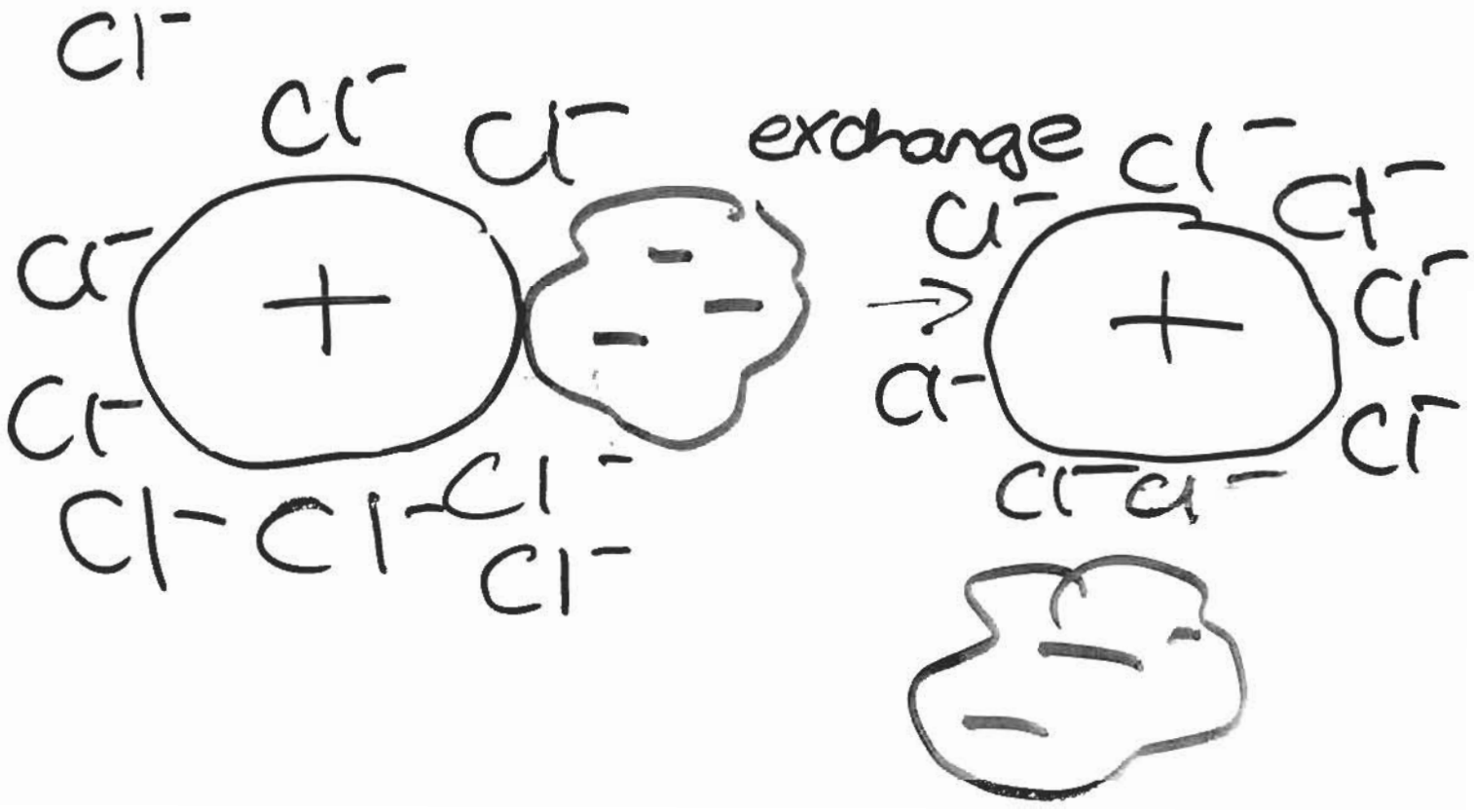


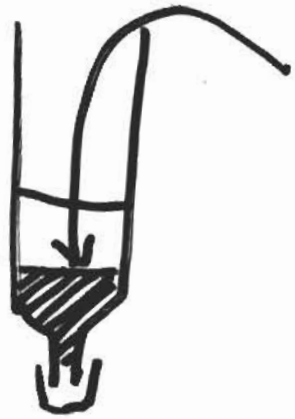
B-gal is overall NEGATIVE @ pH 7.5

ANION EXCHANGE CHROMATOGRAPHY



NaCl



1. 

1mL 50% SLURRY
(½ Buffer ½ beads)
let it PACK (ie do not disturb)

2. EQUILIBRATE (with COLUMN BUFFER) 5x
BE CAREFUL! 500µl.

3. ADD SAMPLE
→ COLLECT FLOWTHROUGH (FT).

4. WASH (0.1M FRACTION)

5. 0.4M SALT BUFFER ELUTION

+ 1.0M SALT