Chapter 3 Exercises

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CH-419 – Protein mass spectrometry and proteomics

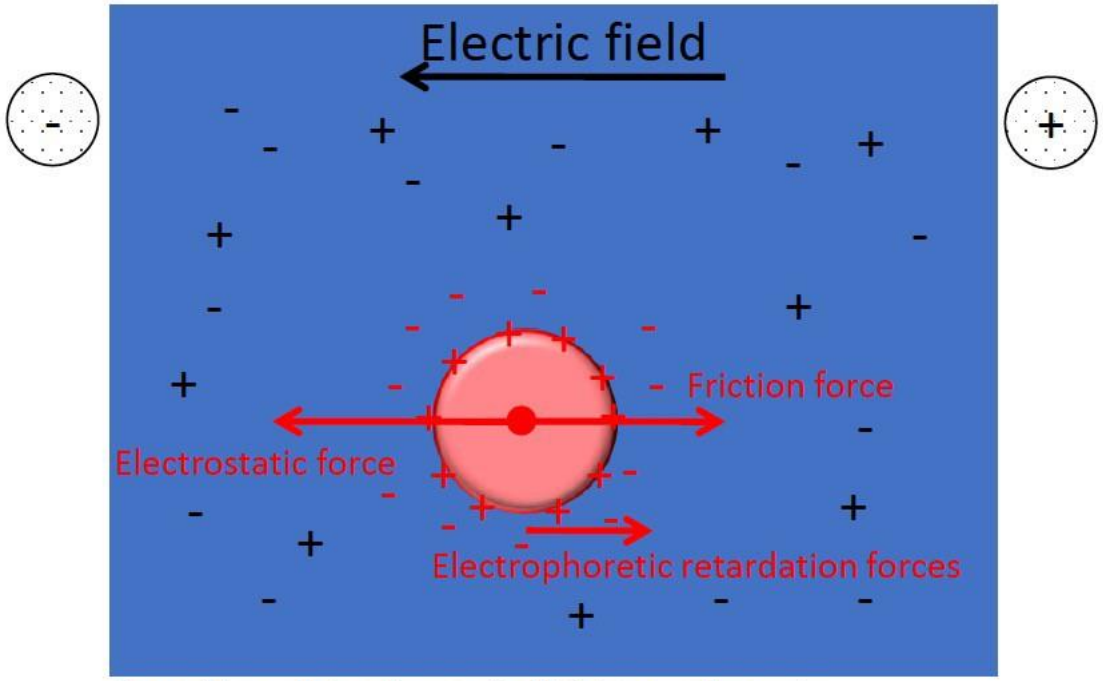
April 27, 2022

Question 1

What force(s) do(es) apply in electrophoresis?

- □ Gravitational force
- □ Electrostatic force
- □ Friction force
- □ Retardation forces

Forces in electrophoresis



http://en.wikipedia.org/wiki/Electrophoresis

Answers:

1) Electric field exerts **electrostatic force** on the particle through the carried charge
2) Hydrodynamic **friction force** affects all bodies moving in viscous fluids
3) Electrophoretic **retardation forces** are applied to the ions in the diffuse layer

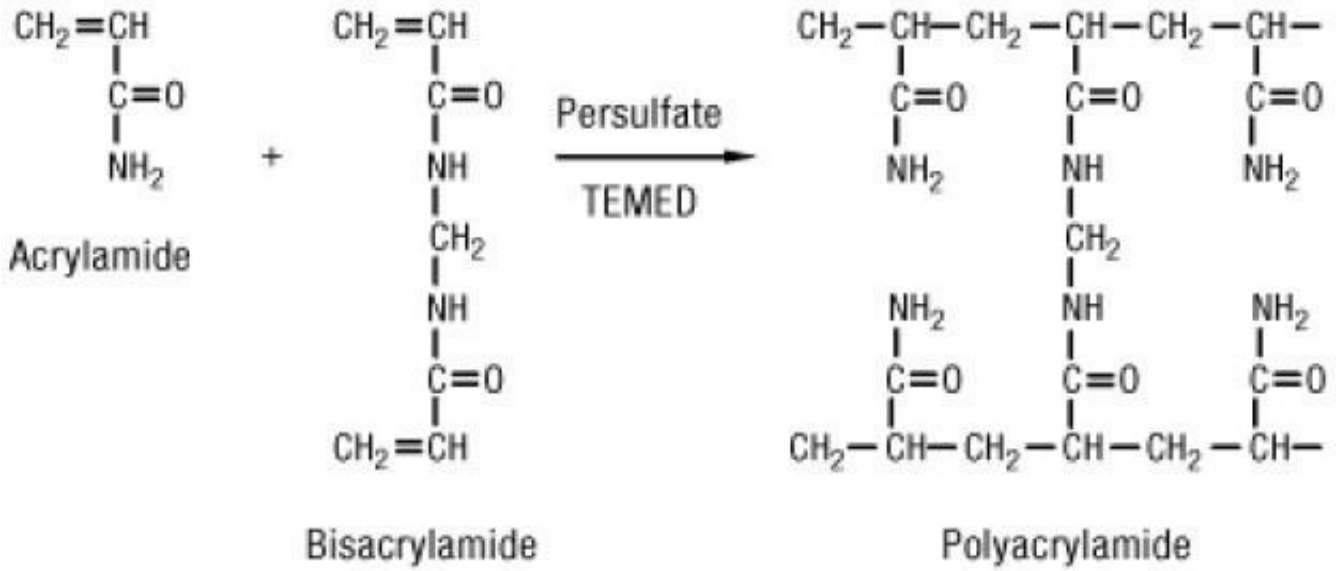
Question 2

For polyacrylamide gel polymerization, what do you need?

- □ Acrylamide
- □ Ammonium persulfate and TEMED
- □ Bisacrylamide
- □ Styrene

Polyacrylamide gel polymerization

Lecture slide(s): 11



Answer:
Acrylamide, bisacrylamide, ammonium persulfate and TEMED are needed

Question 3

What percentage of acrylamide would you recommend to separate proteins of 4-40 kDa?

□ 10% □ 12.5% □ 20% □ 30%

Polyacrylamide gel choice

Lecture slide(s): 12

Protein size	Gel acrylamide percentage
4–40 kDa	20%
12–45 kDa	15%
10–70 kDa	12.5%
15–100 kDa	10%
25–200 kDa	8%

Answer: 20% acrylamide

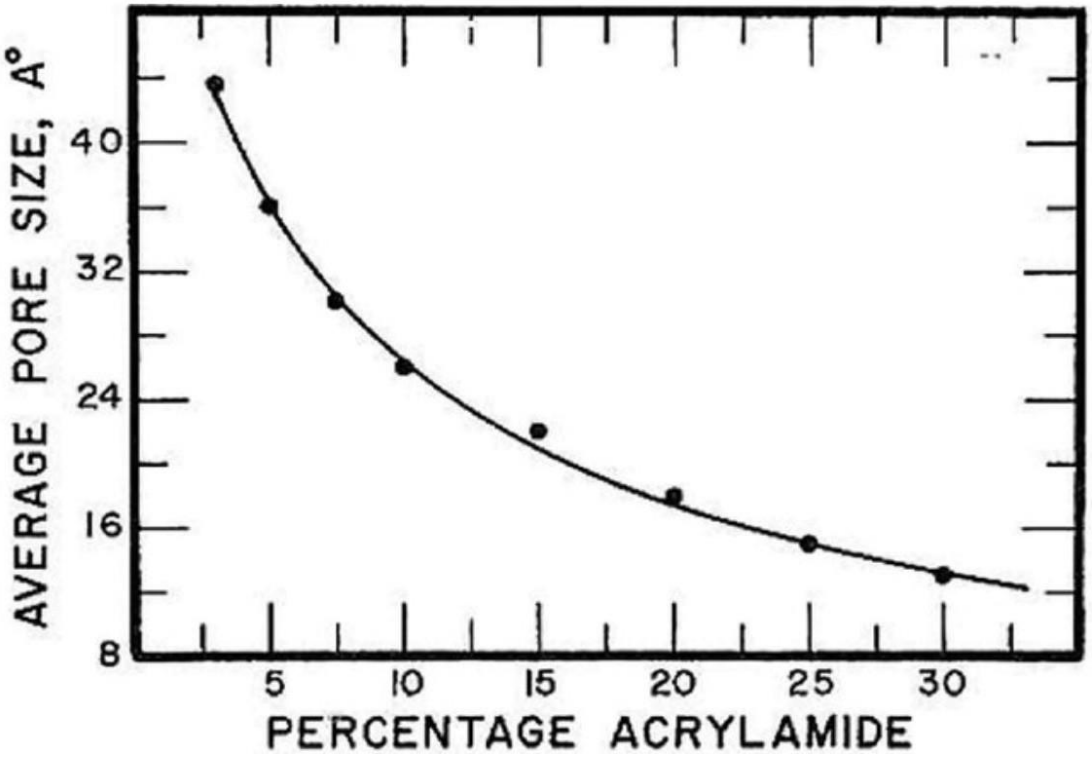
Question 4

In PAGE, what is the effect on the pore size when the percentage of acrylamide increases?

- □ The pore size increases
- □ The pore size decreases
- □ The pore size is not affected
- □ None of the those

Acrylamide % and pore size

Lecture slide(s): 12



Answer:
As acrylamide concentration **increases**, pore size **decreases**

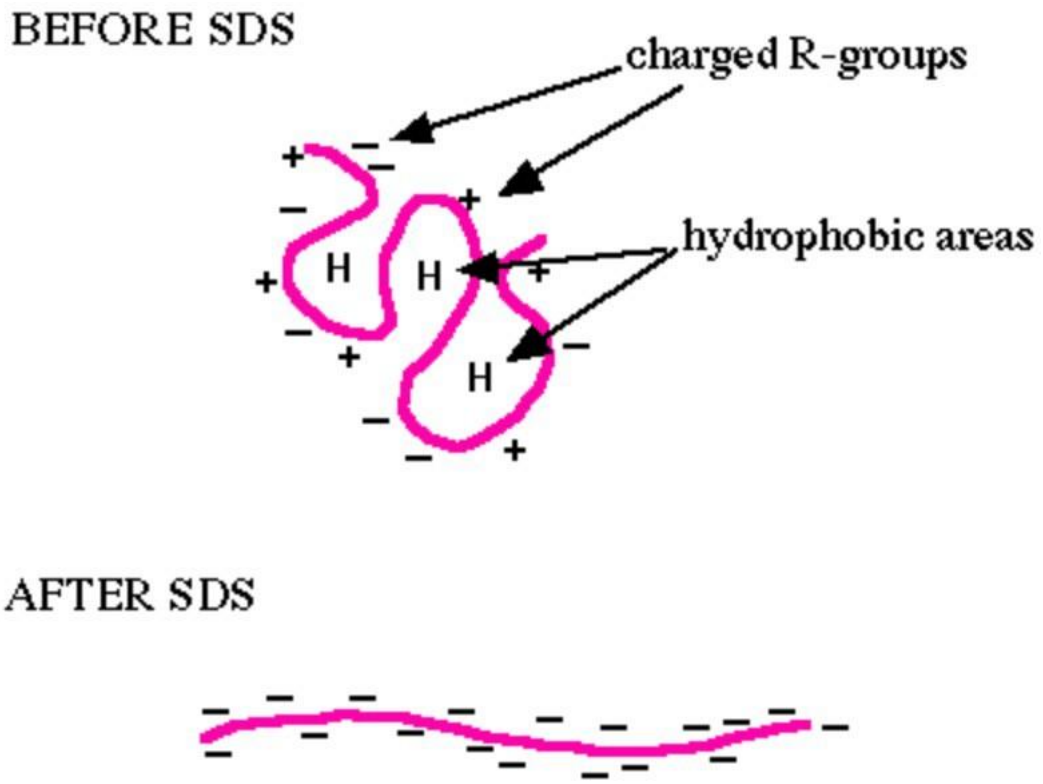
Question 5

What is the effect of SDS on proteins?

- □ Protein conformation is affected
- □ Protein charge is affected
- □ Proteins become positively charges
- □ Disulfide bridges are broken

What SDS does to protein

Lecture slide(s): 13



- Introduction of negative charges onto protein
- Linearization of protein. Removal of secondary, tertiary structures. NOT disulfide bonds!

Question 6

Lecture slide(s): 14-17

What does PAGE stand for?

- □ Polymer aggregated gel electrophoresis
- □ Polyacrylamide gel electrophoresis
- □ Polyamine gel electrophoresis
- □ None of those

Answer: **P**oly**A**crylamide **G**el **E**lectrophoresis

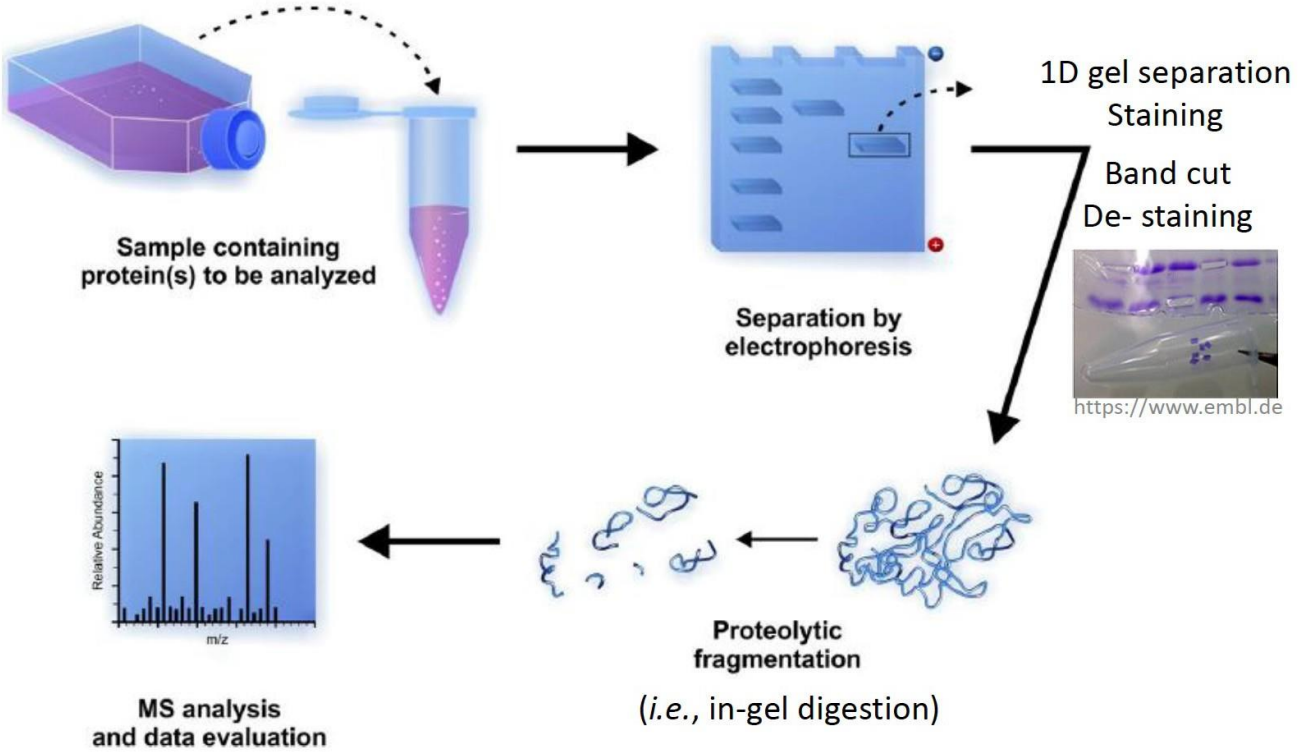
Question 7

In proteomics, how 1D SDS PAGE can be use with mass spectrometry?

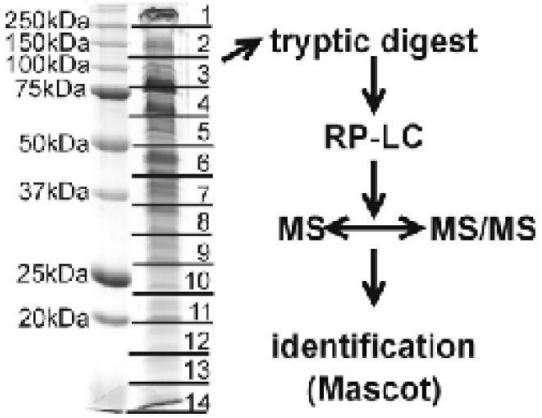
- □ After in-solution digestion
- □ With in-gel digestion
- □ Both are not compatible
- □ In the procedure, bands are cut after staining

1D SDS PAGE and MS

Lecture slide(s): 14



doi: 10.3390/biom3040923



https://www.biochem.mpg.de

Answers:

1) With in-gel digestion
2) In the procedure, bands are cut after staining

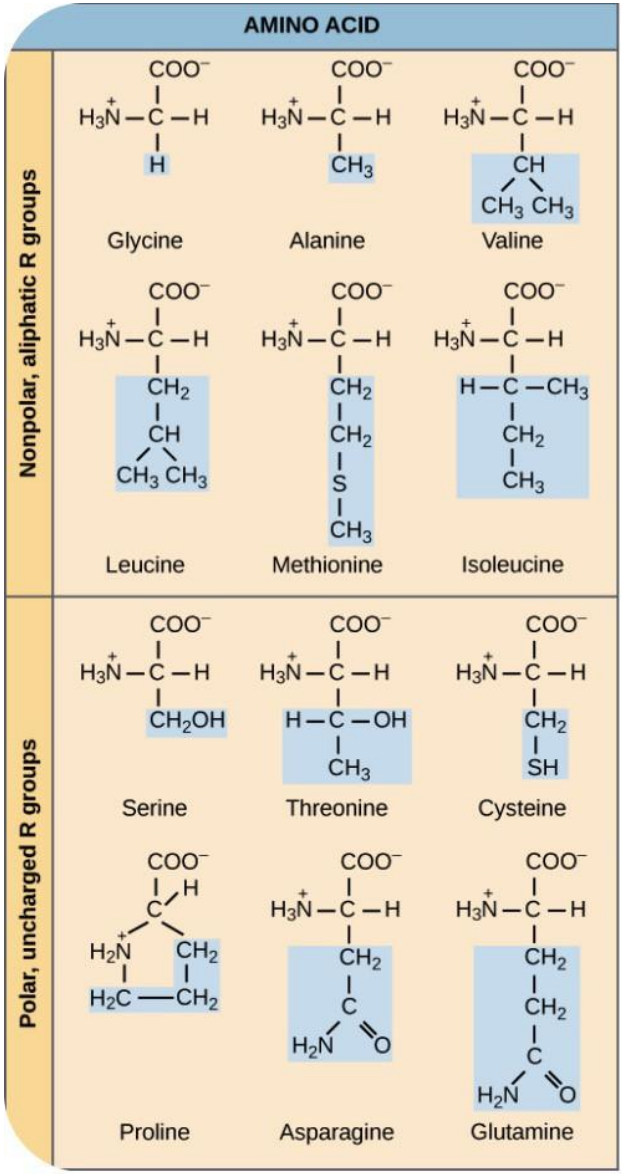
Question 8

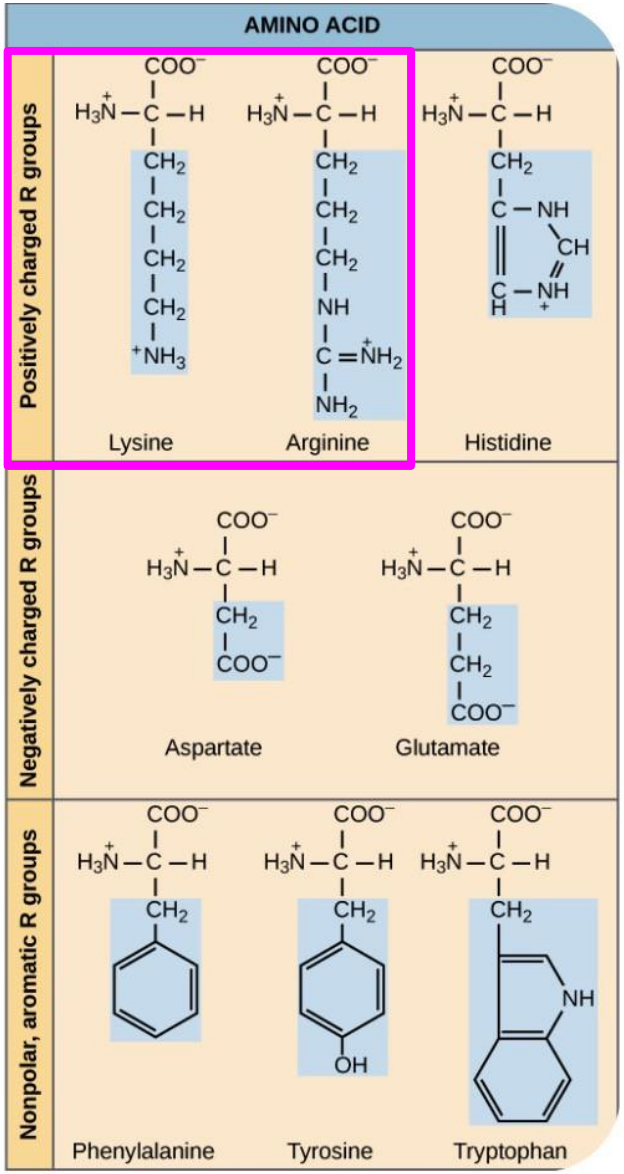
What amino-acid(s) do(es) present positively charged lateral chains?

□ Arginine □ Glycine □ Lysine □ Aspartic acid

Amino acids chart

Refer to chapter 1





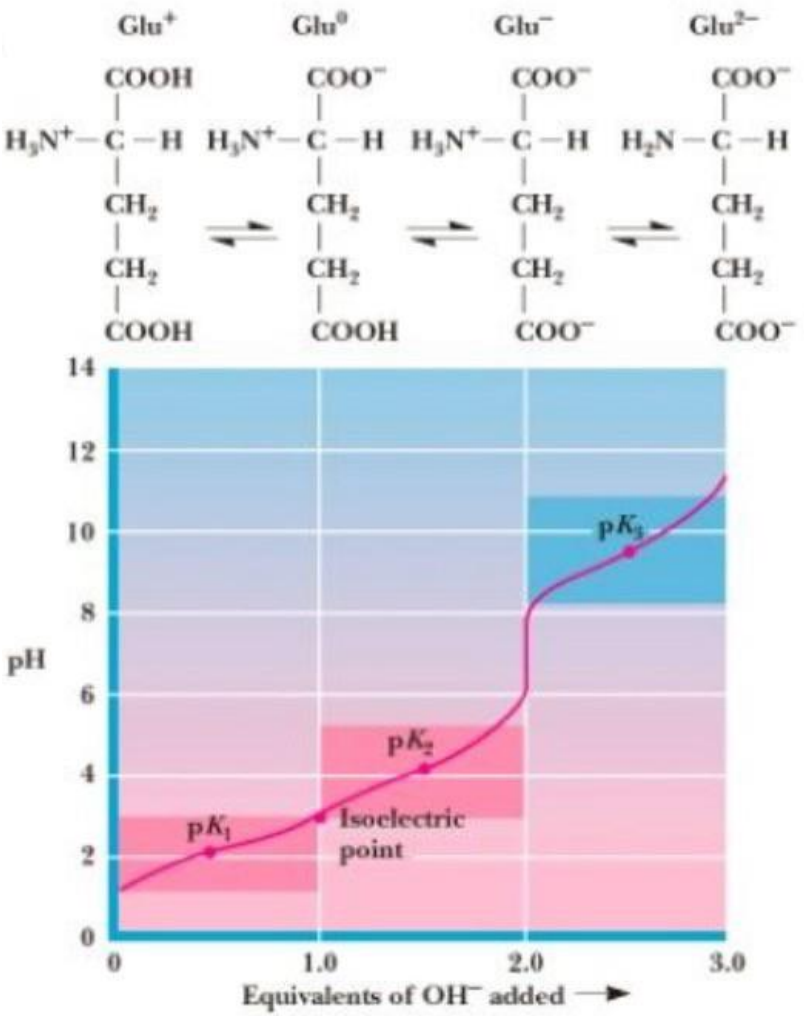
Answer:
1) Arginine
2) Lysine

Question 9

Glutamic acid has pK1 (-COOH) = 2.1, pK2 (-NH2) = 9.47 and pKR (-R) = 4.07. What is the net electric charge of Glu at pH = 3?

□ Positive □ Negative □ Zero

Charge of Glu at pH 3



- We are given the values of 3 pKs: pK_1 (-COOH) = 2.1, pK_2 (-NH_2) = 9.47 and pK_R (-R) = 4.07
- At pH 3, we are below the pK of the R group, the NH_2 group and the above the C terminus COOH group
- If we are below the pK, the molecule is in its protonated form, but if above, the deprotonated form
- Therefore, we have NH3 (1+ positive charge), COOH (uncharged) and COO- (-1 charge)
- Answer: Charge is 0 at pH 3

Question 10

What is the approximate pI of Glu?

□ 3.1 □ 5.8 □ 6.8 □ 2.1

Approximate pI of Glu

- The isoelectric point (pI) is defined as the pH at which a molecule carries no net charge
- We see that for glutamic acid, this happens between the pH of 2.1 and 4.07, which are the pK_1 and the pK_R respectively
- So to calculate the pI, we find the average between the pK_1 and the pK_R
- Answer: $pI = (pK_1 + pK_R)/2 = 6.17/2 = \sim 3.1$

pH	NH_2	COOH (C term)	COOH (R group)	Net charge
< 2.1	+	0	0	+
2.1< pH <4.07	+	-	0	0
4.07< pH <9.47	+	-	-	-
> 9.47	0	-	-	2-

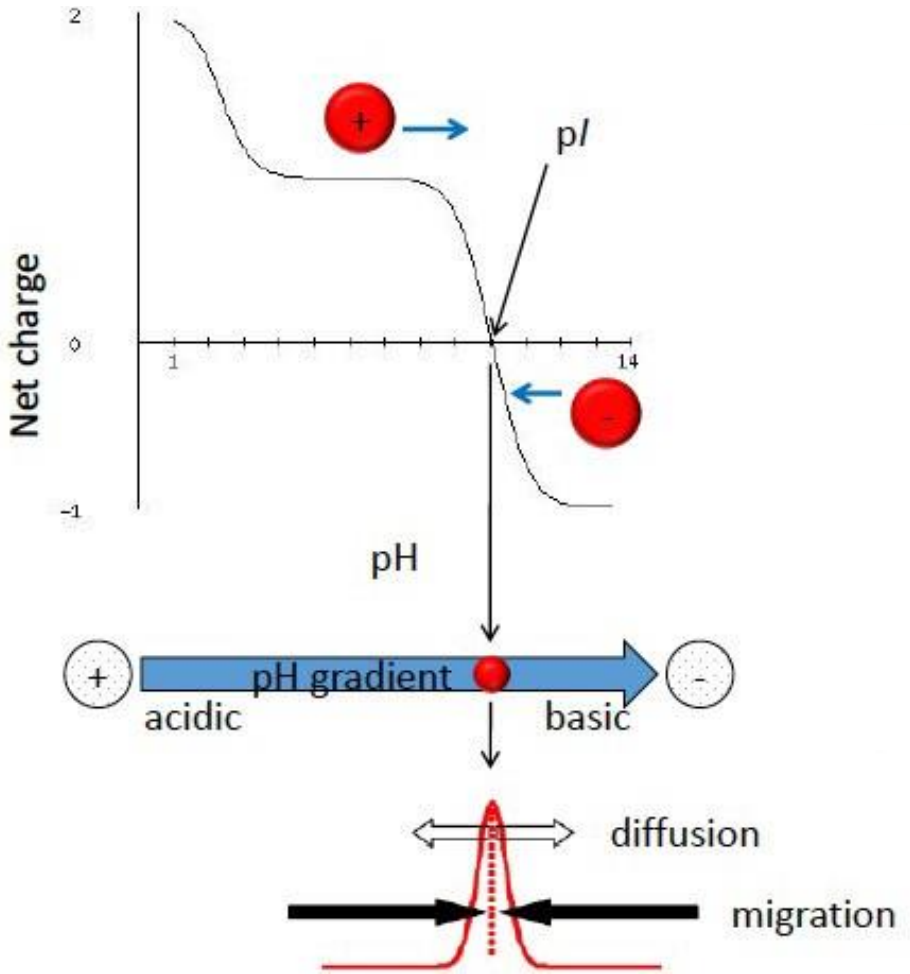
Question 11

During isoelectric focusing, in which direction do positively charged ions move?

- □ Toward the anode
- □ Toward the cathode
- □ They do not move

Positively charged ions move towards the negative terminal

Lecture slide(s): 25



- pH is low at the anode. At low pH, we always have more positive charges, so the anode is positively charged
- At the cathode, the pH is high, we will always have more negative charges
- Therefore, since the positively charged ions would move towards the negative terminal, they will move towards the cathode

Question 12

What property(ies) do(es) present carrier ampholytes?

- □ Amphoteric
- □ Acidic
- □ “Carrier” of the current
- □ Buffering

Carrier ampholytes and amphoteric compounds

- Amphoteric: a compound that can react as both a base and an acid
- Ampholytes: amphoteric molecules that contain both acidic and basic groups and will exist mostly as zwitterions in a certain range of pH
- The 3 main requirements for a good carrier ampholyte:

1. Compound must be amphoteric
2. To be “carrier” of the current (a good conducting species)
3. Good buffering species

Lecture slide(s): 26

Question 13

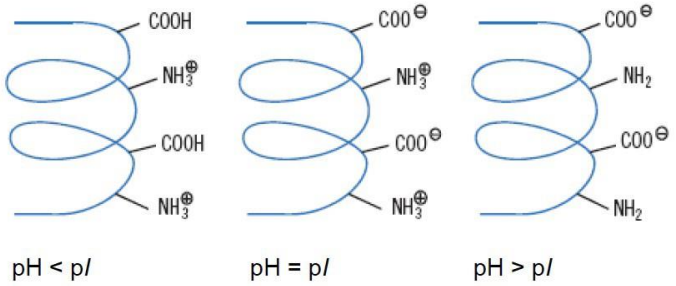
In 2D gel electrophoresis, what is the principle of the first dimension of the separation?

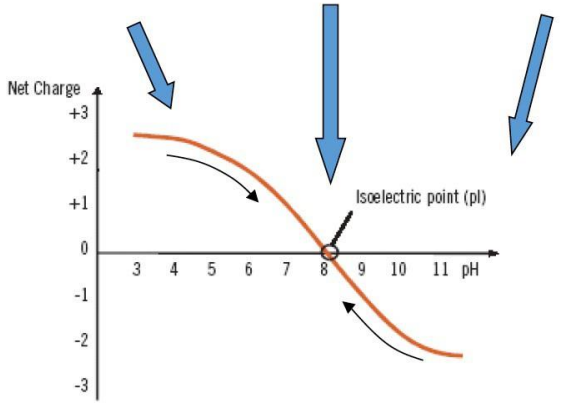
- □ PAGE
- □ IEF
- □ Size-based
- □ Liquid chromatography

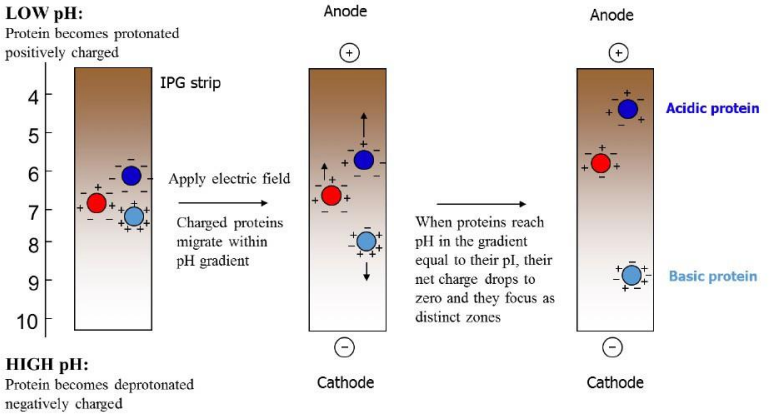
2D gel electrophoresis 1st dimension: IEF

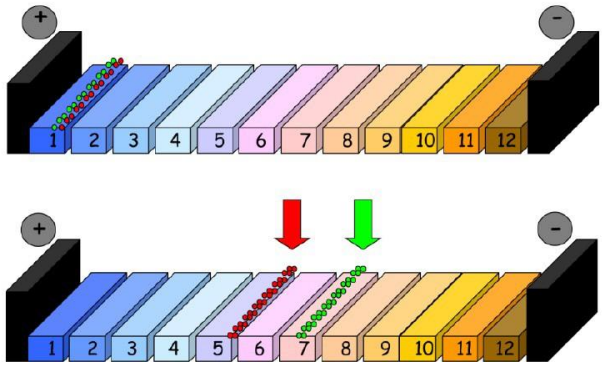
Lecture slide(s): 34

IEF peptide separation for proteomics: IPG IEF, first dimension of protein 2D-PAGE









Question 14

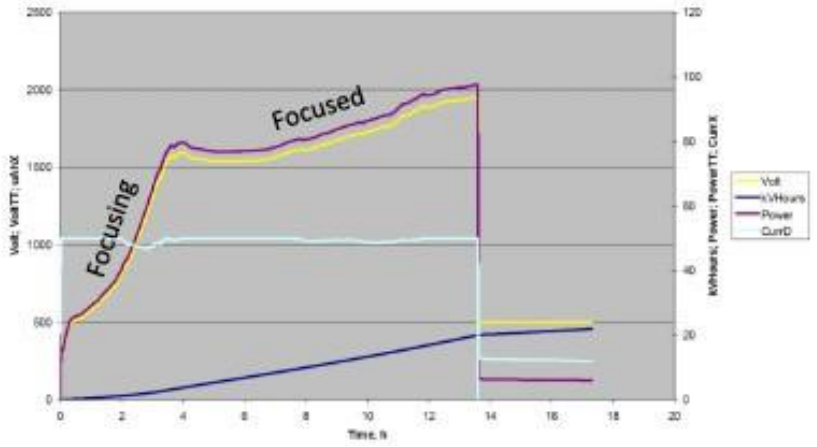
What advantage(s) do(es) off-gel electrophoresis present with respect to classical IEF?

- □ Diffusion is absent
- □ Focusing is much faster
- □ Separated analytes are recovered in solution
- □ Both proteins and peptides can be separated

Diffusion is not absent… also not necessarily faster!

Numerical studies to characterize off-gel IEF in a 3-compartement device:

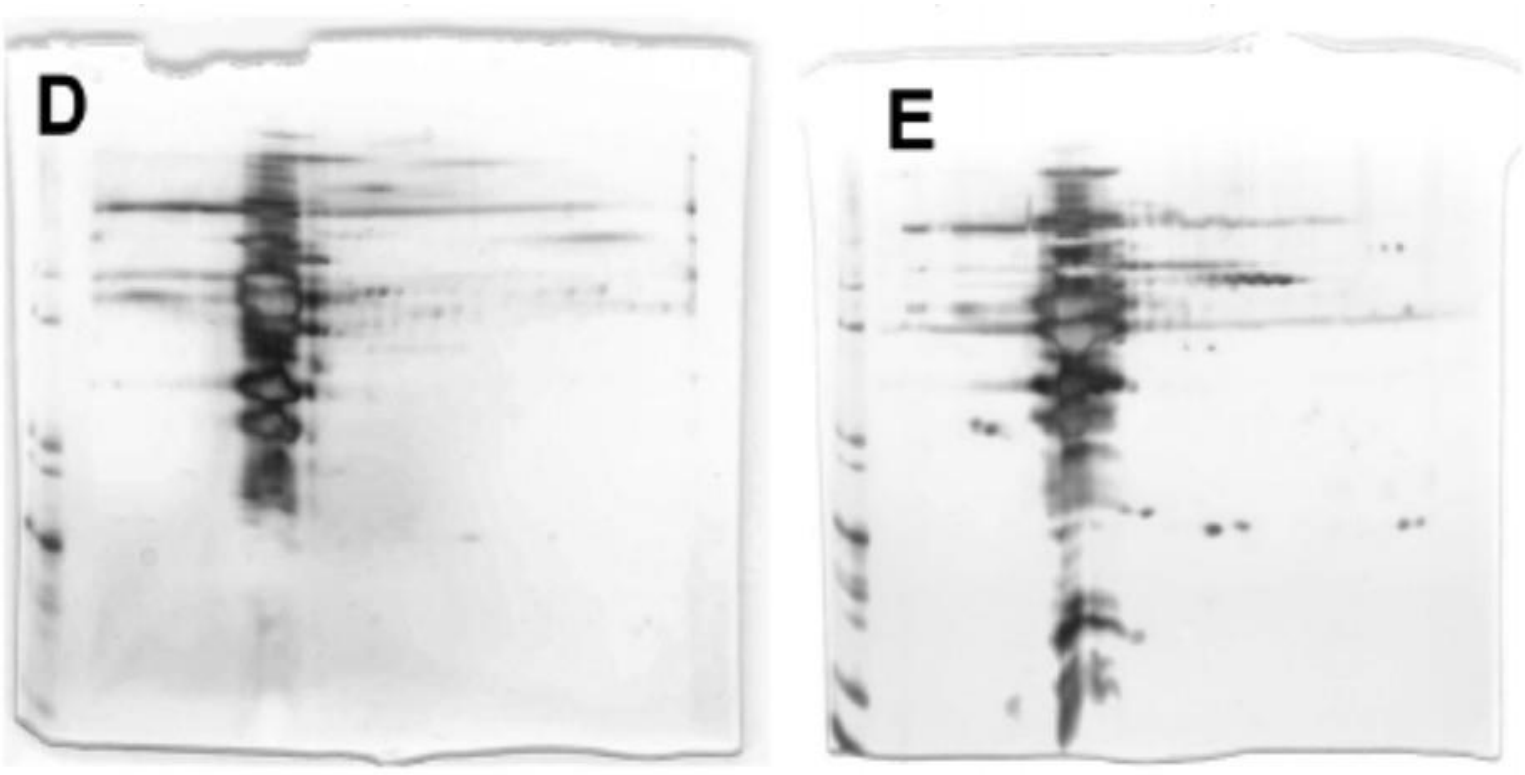
- Electric field vectors: the electric field arriving from the gel enters the well on the left end side, and occupies the full volume of the well
- Concentration isovalues of angiotensin II: focusing time depends on slope at p*I*; however diffusion ("vertical focusing") reduce the difference



Lecture slide(s): 45

OGE can be used to separate proteins, but the separations are often not great

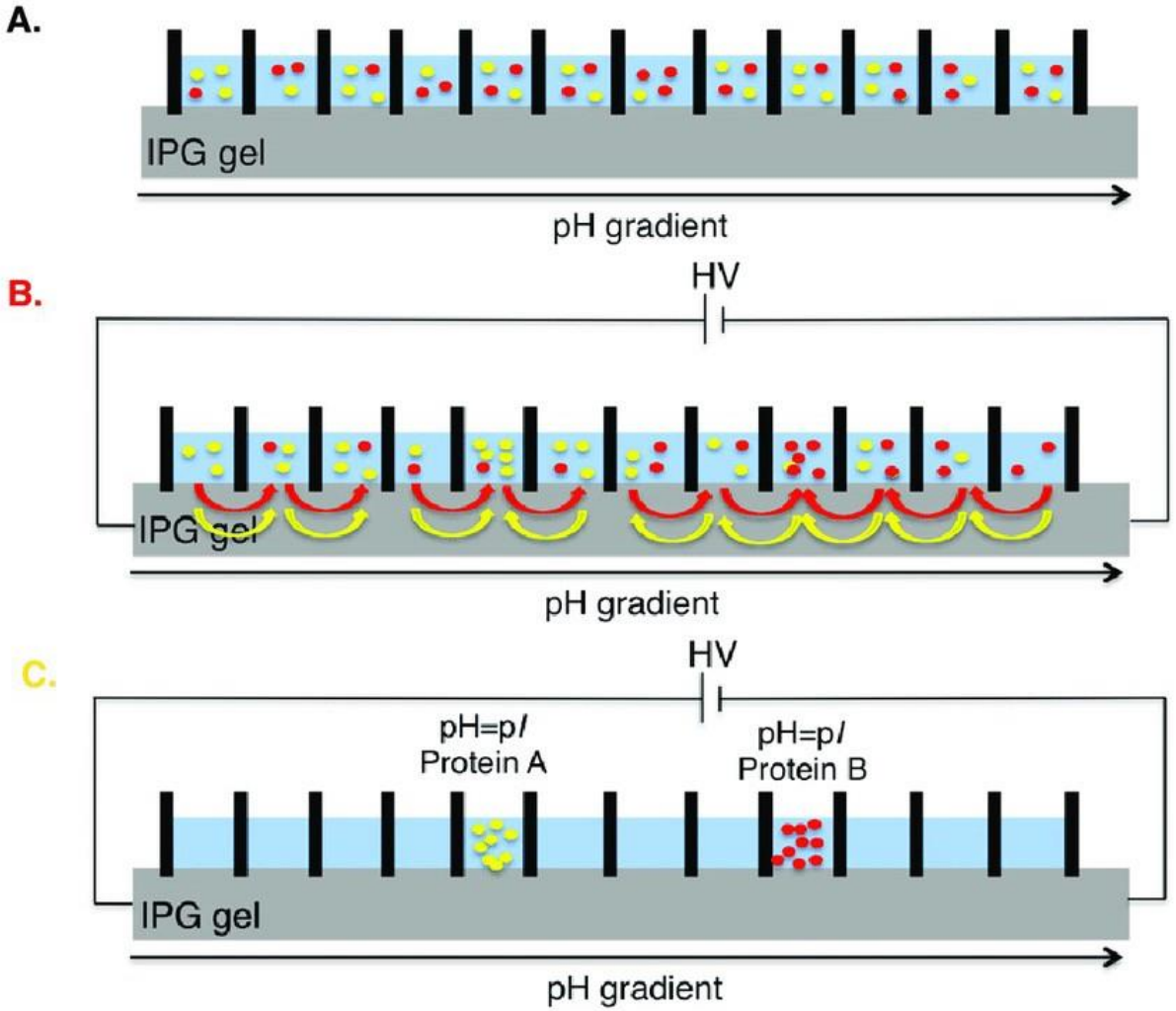
- Collection of fractions from plasma protein separated by OGE, followed by 2D gel electrophoresis to analyze fractions



Heller, M. *et al. Electrophoresis* (2005)

OGE recovers analytes in solution

- This is one big advantage compared to classical IEF



Question 15

What can explain an analyte did not efficiently focus during IEF?

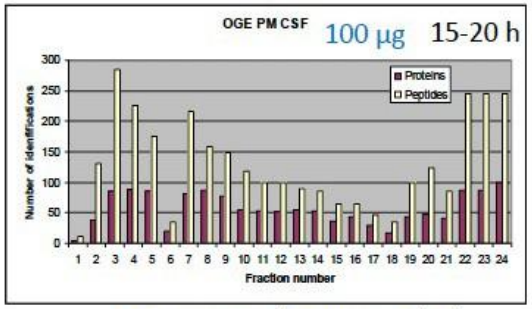
- □ The slope of the titration curve at pI for this analyte is steep
- □ Too many salts were present in the sample
- □ The analyte is a protein
- □ Voltage was stopped for 30 minutes before sample recovery

Influence of salt and voltage in IEF

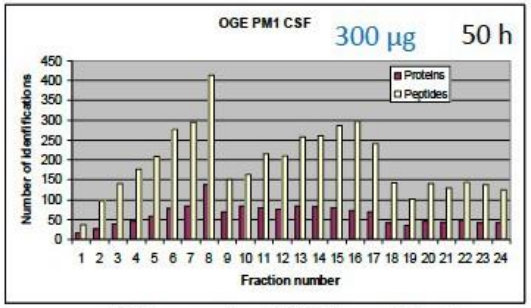
- Impairs separation efficiency
- Samples must be desalted prior to IEF

Influence of salts

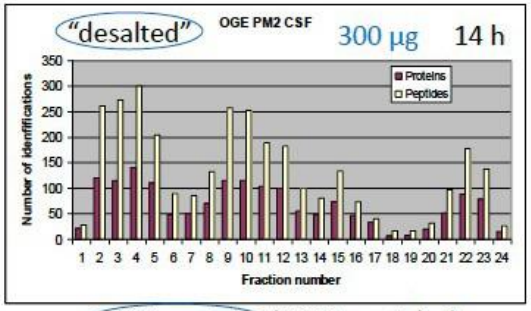
Digests of *post-mortem* cerebrospinal fluid separated by OGE (TOF/TOF MS)



364 proteins (3590 peptides)



273 proteins (5186 peptides)



582 proteins (4019 peptides)

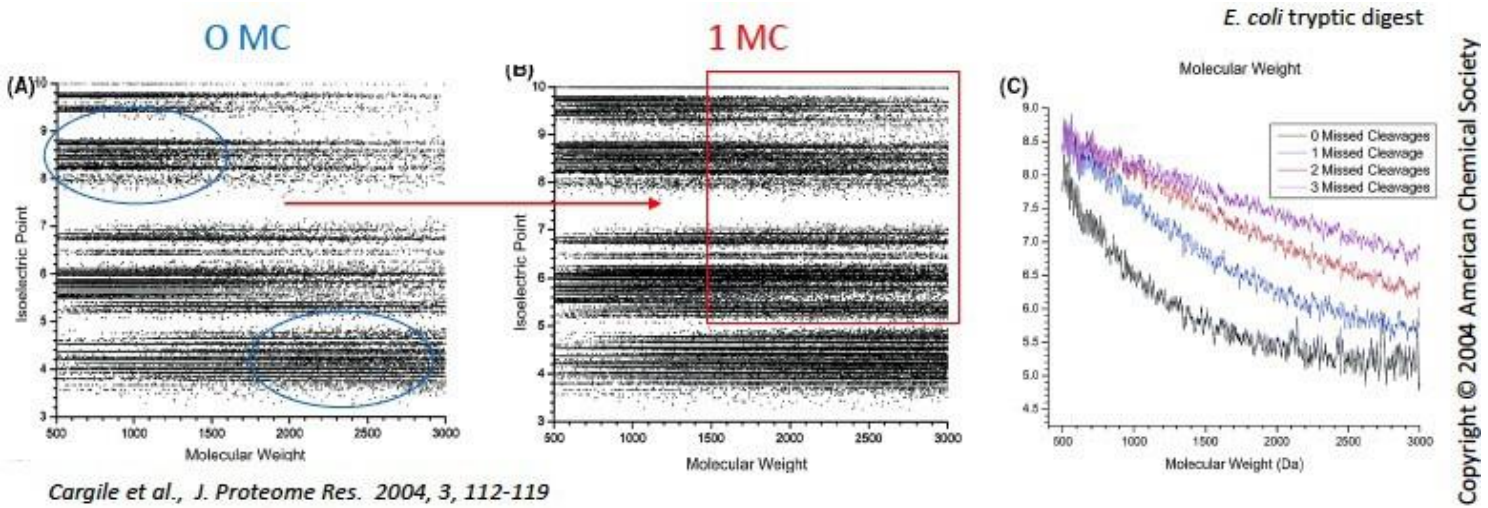
- If the voltage is stopped 30 minutes prior to recovery, then diffusion dominates, and the focused samples can diffuse and become out of focus

Question 16

For what reason(s) can information on the pI be valuable?

□ MS data validating/filtering
□ Optimizing protein digestion conditions
□ Phosphopeptide selection
□Mass of peptide/protein is not anymore needed for their identification

MS data validating and filtering



Cargile et al., J. Proteome Res. 2004, 3, 112-119

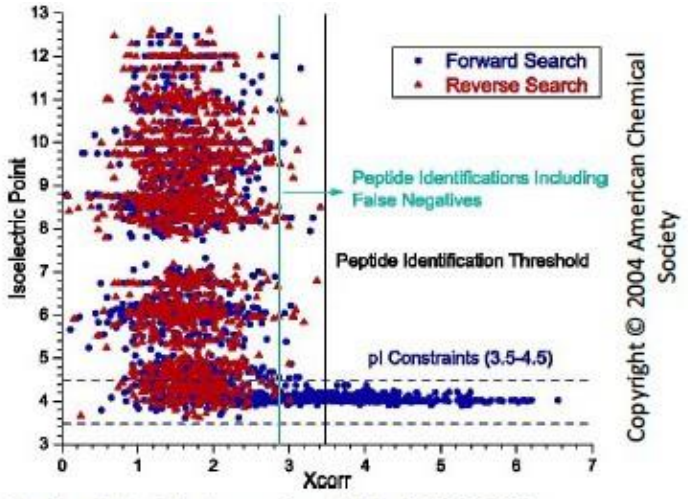
⇒ data is spread out over the entire mass/p*I* plot

⇒ MC lead to an increase in the absolute number of peptides, but also an increase in the medium to high molecular weight range of the middle to high p*I* (6.0-9.5)

⇒ as the peptide increase in mass, the average p*I* decreases (fig. c)
⇒ as the number of MC increases, the average p*I* increases (fig. c)

Lecture slide(s): 50

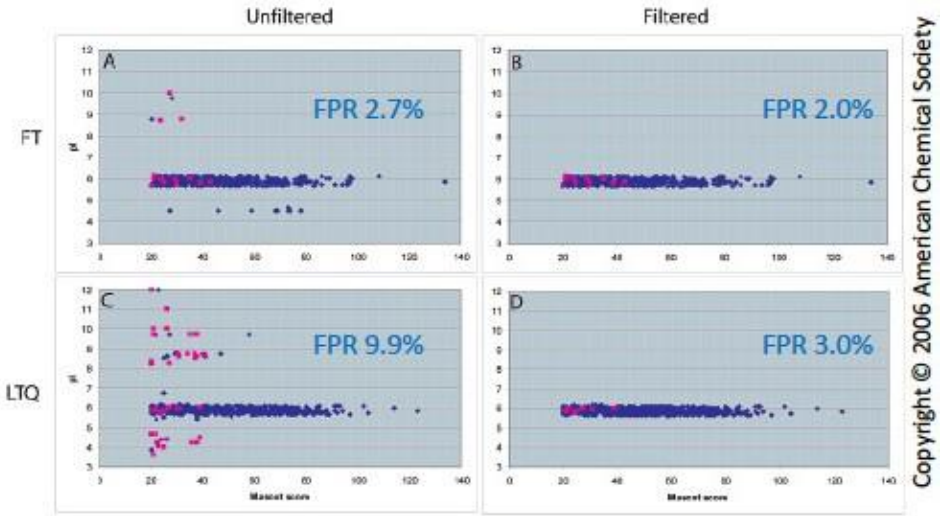
MS data validating and filtering



Cargile et al., J. Proteome Res. 2004, 3, 1082-1085

Reducing of false positive rate by filtering of peptides based on p*I*

=> Importance of p*I* calculation algorithms



Krijgsveld et al., J. Proteome Res. 2006, 5, 1721-1730

Lecture slide(s): 51

Enrichment of phosphopeptides for phosphoproteomics

Lecture slide(s): 53

Anal. Chem. **2007,** *79,* 2007–2014

Selective Enrichment and Fractionation of Phosphopeptides from Peptide Mixtures by Isoelectric Focusing after Methyl Esterification

Chong-Feng Xu,[†] Huaibin Wang,[‡] Daming Li,[§] Xiang-Peng Kong,[‡] and Thomas A. Neubert*[†]

Electrophoresis 2007, *28*, 2044–2052

Chien-Wen Hung[1]
Dieter Kübler[2]
Wolf D. Lehmann[1]

[1]Central Spectroscopy, German Cancer Research Center, Heidelberg, Germany
[2]Biomolecular Interactions Group, German Cancer Research Center, Heidelberg, Germany

Research Article

p*I*-based phosphopeptide enrichment combined with nanoESI-MS

Journal of Proteome Research 2007, 6, 1153-1157

research articles Journal of proteome research

Isolation of Phosphopeptides by p*I*-Difference-Based Electrophoresis

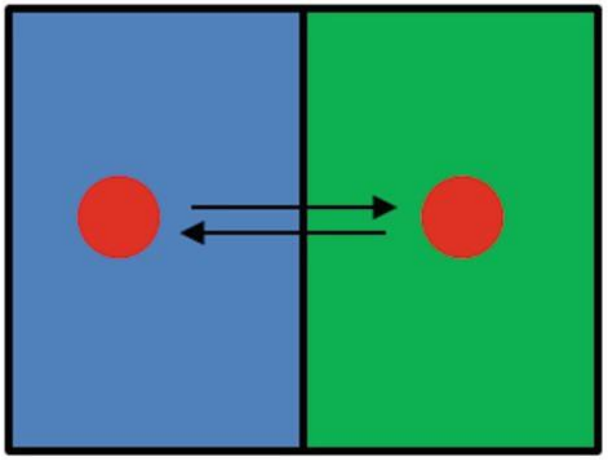
Yingda Xu, Robert Sprung, Sung Won Kwon, Sung Chan Kim, and Yingming Zhao*

Question 17

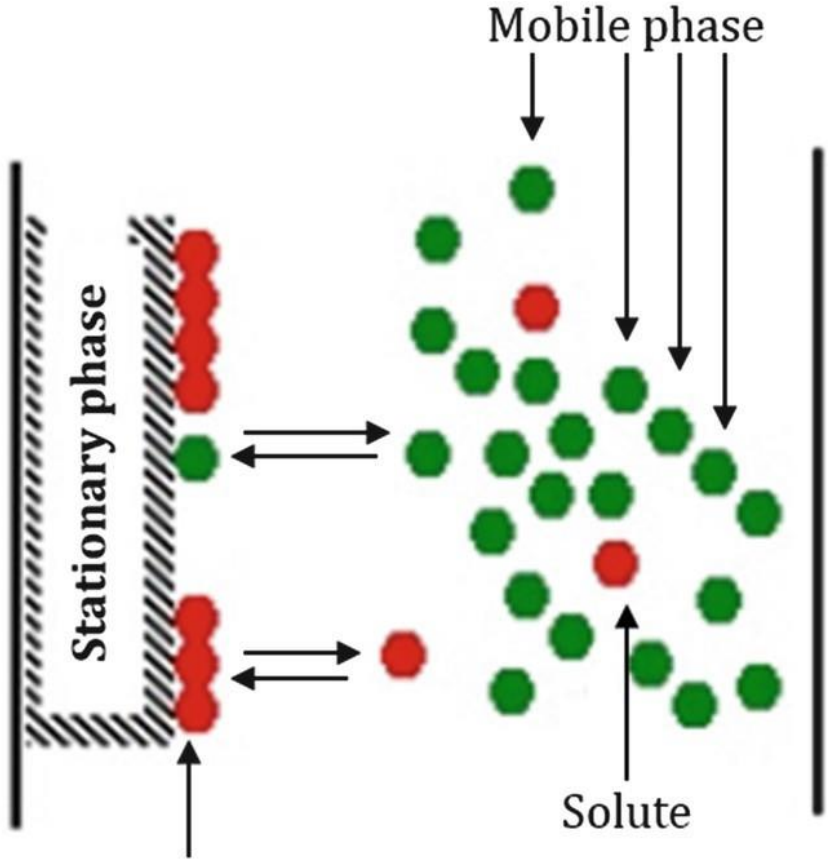
What is necessary involved in chromatography?

- □ An analyte
- □ A mobile phase
- □ A stationary phase
- □ A liquid

The staples of chromatography: a mobile phase, a stationary phase and an analyte



Partition chromatography is based on the solute partitioning between the two liquid phases



Solute dissolved in liquid phase is coated on the surface of stationary phase

Lecture slide(s): 57

Question 18

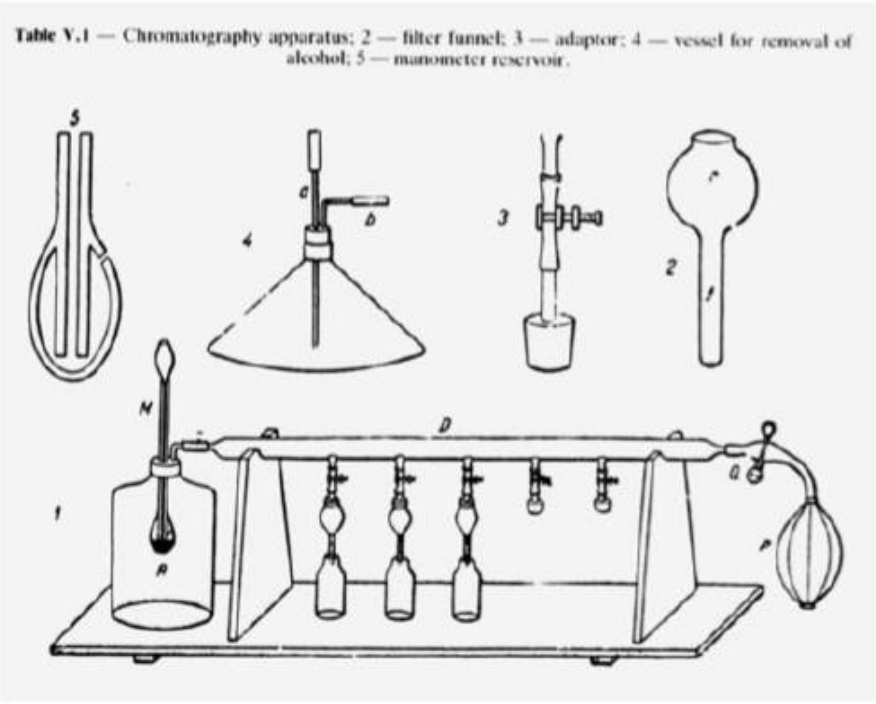
What was M.S. Tswett able to separate using chromatography?

- □ The components of serum
- □ Some chlorophylls
- □ Some carotenoids
- □ Caffeine

He used chromatography to separate chlorophylls and carotenoids

Lecture slide(s): 58

History of chromatography



Tswett-Apparatus (1905)

Michail Semjonowitsch Tswett

He used liquid-adsorption column chromatography with calcium carbonate as adsorbent and petrol ether / ethanol mixtures as eluent to separate chlorophylls and carotenoids

Question 19

In chromatography, what can explain band broadening?

- □ Multiple path of the analyte
- □ Perpendicular diffusion
- □ Mass transfer between phases
- □ High plate number

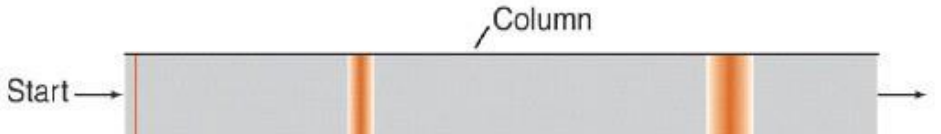
Multiple paths of analyte, longitudinal diffusion and mass transfer between phases lead to band broadening

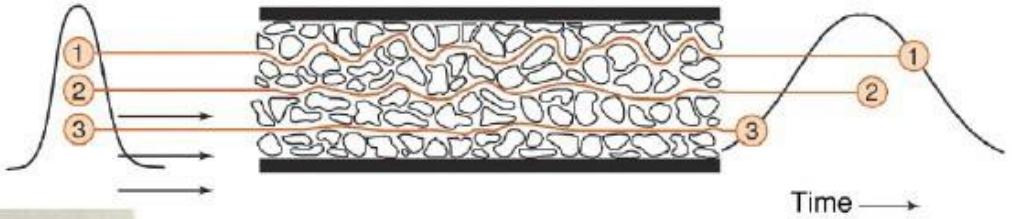
After injection, a narrow band is broadened during its movement through the column by:

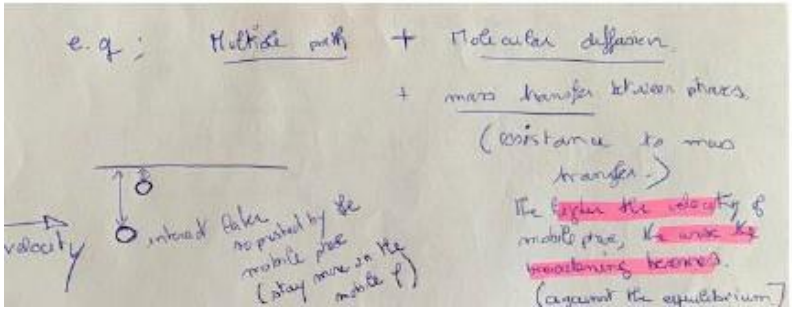
-multiple path of analyte

-Longitudinal diffusion

-mass transfer between phases







The higher the column band broadening, the smaller the number of components that can be separated.

The sharpness of the peak is an indication of how good, or efficient a column is.

Lecture slide(s): 67

Question 20

What factor(s) can affect the chromatographic resolution?

- □ The column length
- □ The flow rate
- □ The pore size of the packing material
- □ None of those

Question 20

Lecture slide(s): 74

Factors affecting resolution

1. Column length
2. Column diameter
3. Flow-rate
4. Uniformity of column packing
5. Uniformity of stationary phase (packing material)
6. Sample size
7. Suitability of stationary phase
8. Suitability of mobile phase
9. Pressure
10. Gradient elution

Question 21

What type of chromatography(ies) would you recommend to separate proteins?

- □ Size exclusion
- □ Reversed-phase
- □ Affinity
- □ Strong-anion exchange

Size- Exclusion Chromatography

- Also known as Gel-Filtration Chromatography
- Separation of protein by size/ hydrodynamic radius (proteins are often of different sizes)
- Polymerized agarose beads containing pores

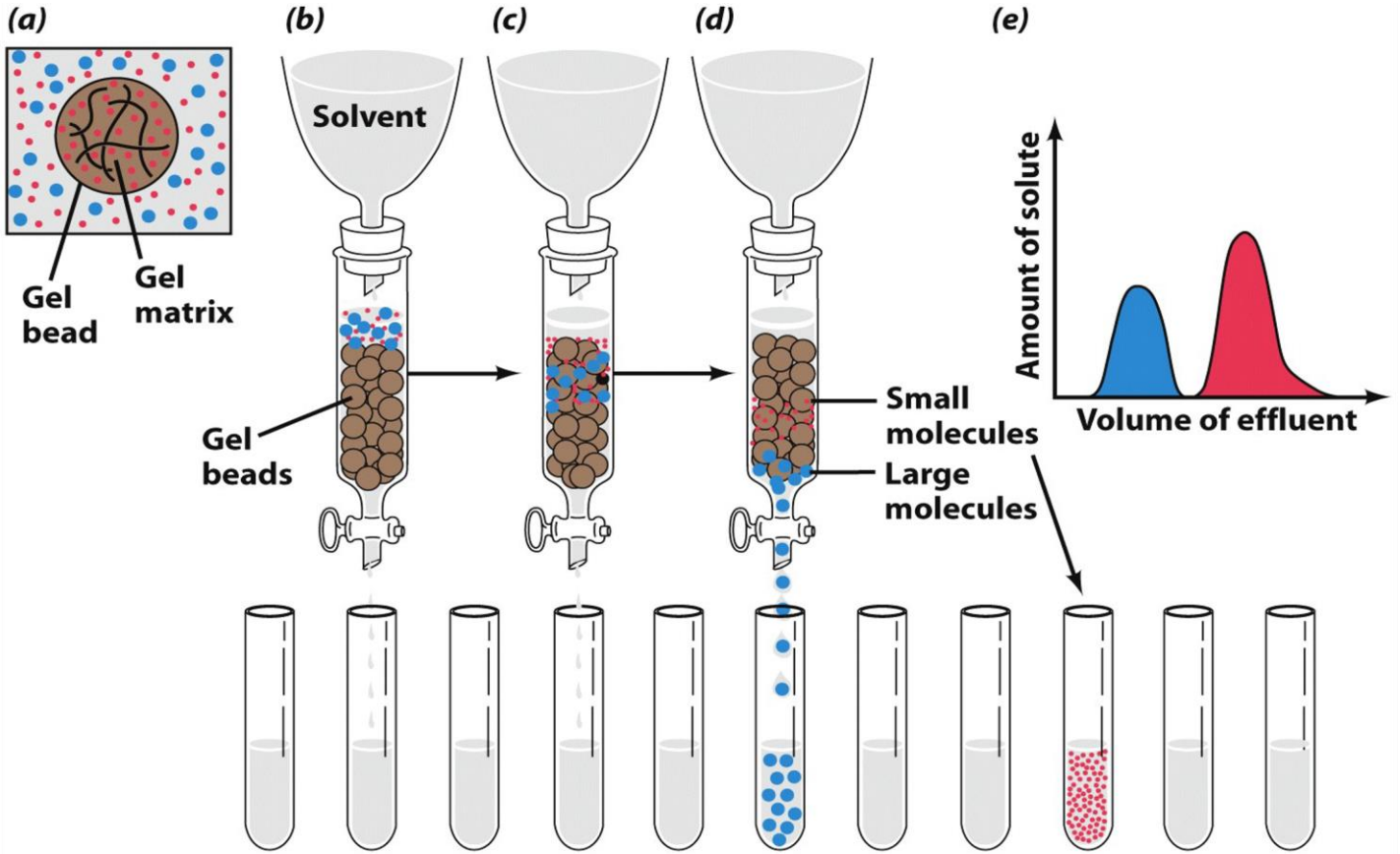


Figure 6-9

Separation by molecular mass by size exclusion chromatography

- V_o = Volume of Column
- V_e = Elution Volume
- (V_e / V_o) = Relative Elution Volume

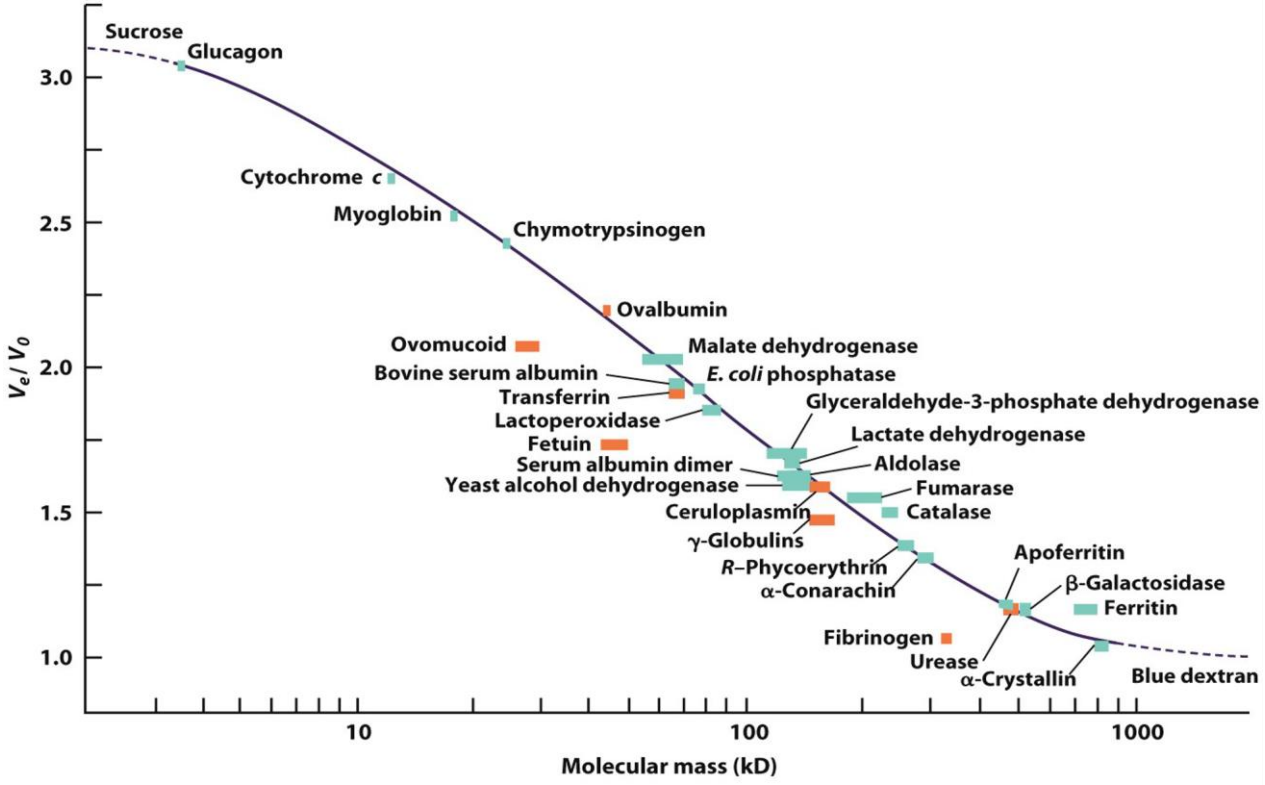


Figure 6-10

Affinity Chromatography

- Ligand chemically attached to polymer beads
- Protein binds ligand, other components washed out
- Competitor added to elute protein from ligand

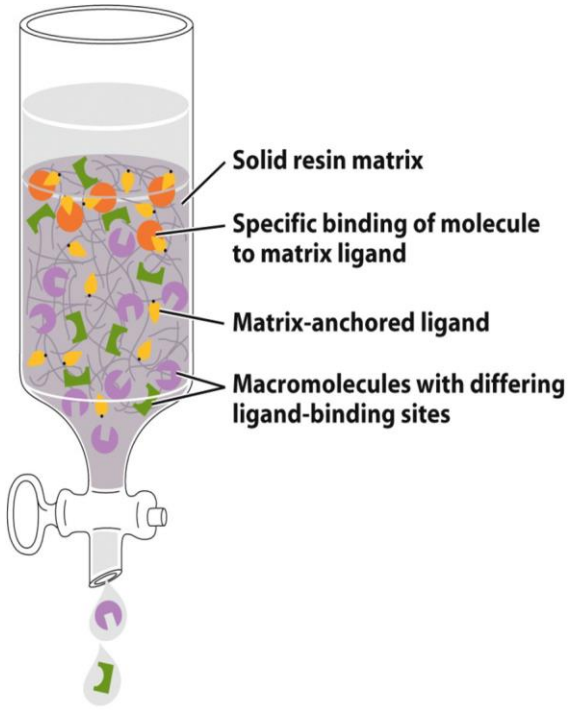
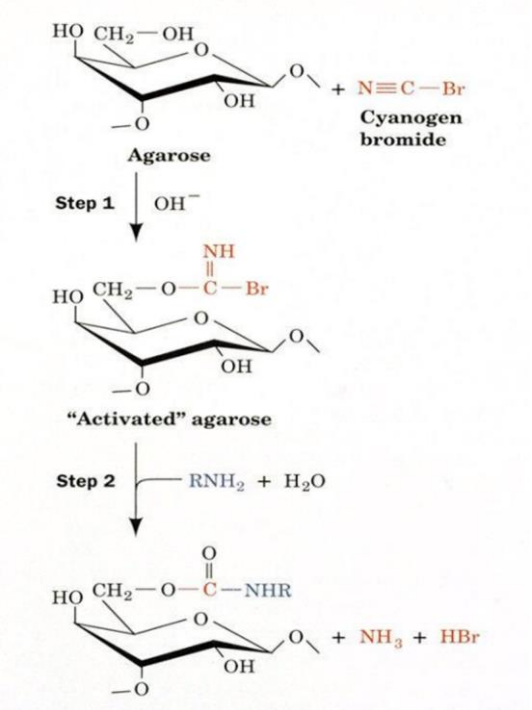
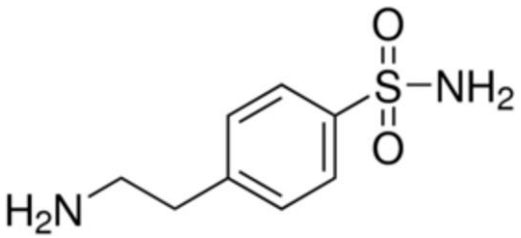


Figure 6-12

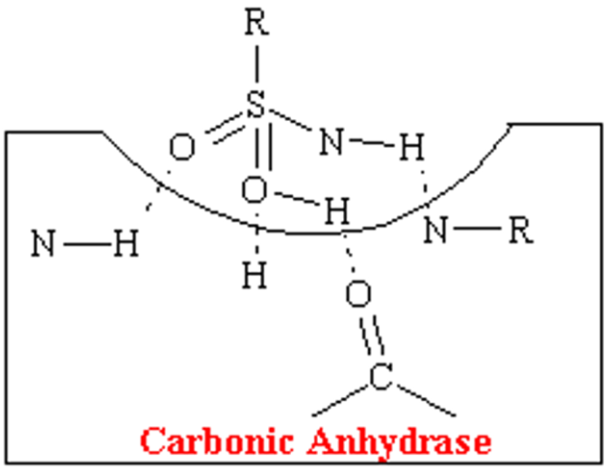


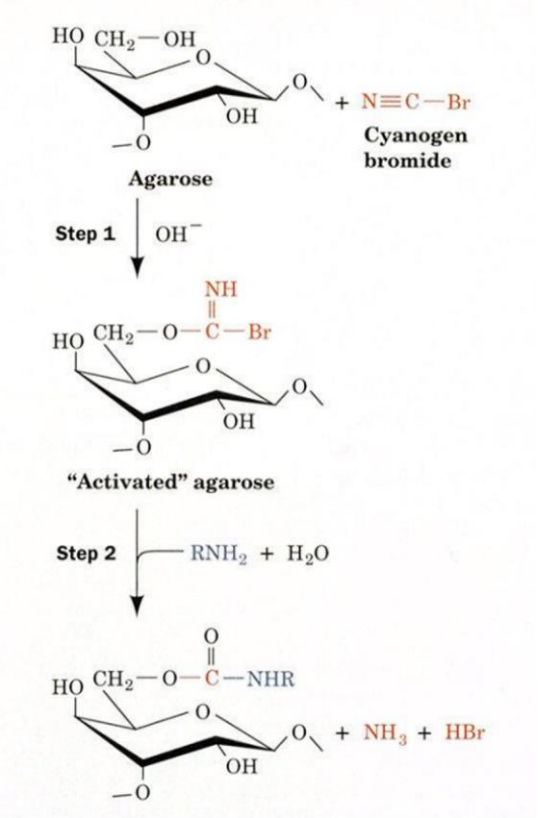
Example: Affinity Purification of hCAII

- R= p- (aminomethyl)benzenesulfonamide
- hCAII binds tightly to specific sulfonamide through H-bonding mechanism
- Carbonic anhydrase inhibitors which can be found in diuretic medication work through the same mechanism.



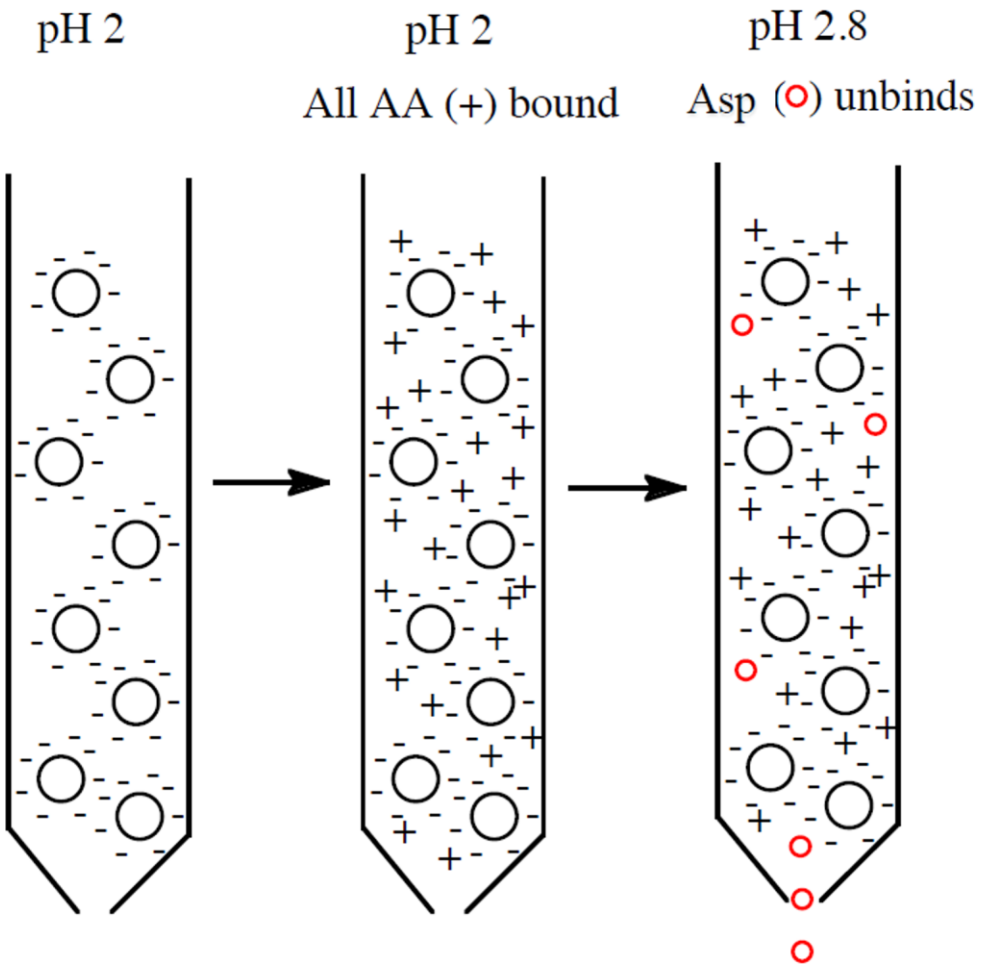
p- (aminomethyl)benzenesulfonamide





Ion-Exchange Chromatography is also used for purifying proteins

- Differences in pI (isoelectric point) of different peptides/ proteins leads to dissociation from beads at different pH/ salt concentration
- We can push the pH slightly below or above the pI of the protein to introduce a negative or positive charge
- Works well for simple protein mixtures, but not well suited for complex mixtures

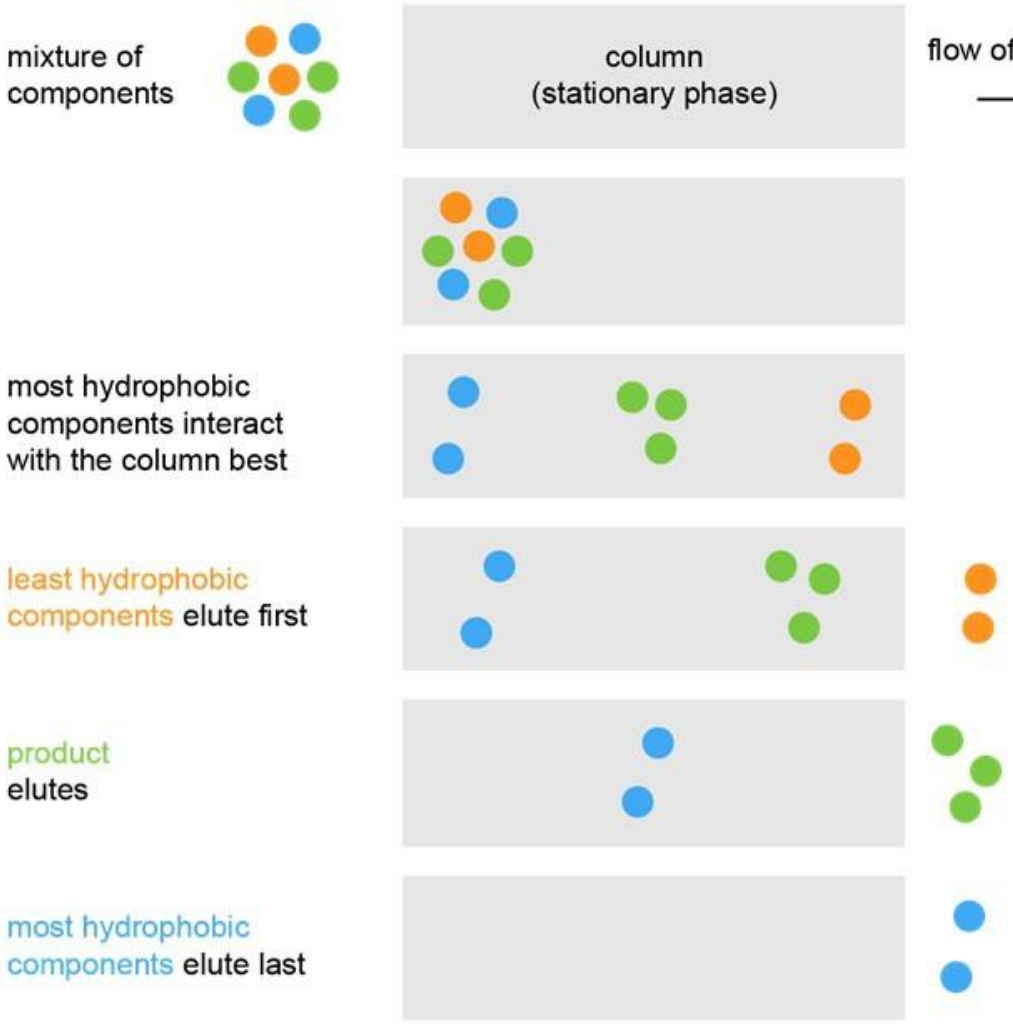


Question 22

For what type of chromatography is hydrophobicity of the analytes relevant?

- □ Size exclusion
- □ Strong-cation exchange
- □ Partition
- □ Reversed-phase

Hydrophobicity is important in methods which separates by polarity



- In Reversed Phase chromatography, we use a non-polar stationary phase and a polar mobile phase (can be gradient from polar to non-polar)
- We separate by polarity
- Reversed Phase, HILIC and Normal Phase are all examples of Partitioning chromatography
- A: Reversed Phase and Partitioning

Question 23

Why HPLC was developed?

- □ To speed up the separation process
- □ To cope with smaller particle sizes
- □ To separate more sample
- □ To accommodate nano-flow rate

Answer:

- □ To speed up the separation process (high pressure)
- □ To separate more sample (automated fractionation)

Question 24

What type of chromatography is usually coupled to mass spectrometry?

□ Size exclusion
□ Strong-cation exchange
□ Affinity
□ Reversed-phase

Answer:
□Reversed-phase (can retain most organic analytes, works for broad range of compounds, lots of freedom to stationary phase and mobile phase)

Question 25

What separation techniques may be complementary to RP-LC to separate peptide mixtures?

- □ IEF
- □ SCX
- □ SEC
- □ RP

Answer:

All the above except for size exclusion chromatography. This is because we cannot resolve analytes of very similar masses well with SEC and therefore SEC is not suitable for the separation of a peptide mixture