

Questions – Chapter 03

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

Gravitational force Electrostatic force Friction force Retardation forces

2- For polyacrylamide gel polymerization, what do you need?

Acrylamide Ammonium persulfate and TEMED Bisacrylamide Styrene

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

10% 12.5% 20% 30%

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

5- What is the effect of SDS on proteins?

Protein conformation is affected Protein charge is affected Proteins become positively charged Disulfide bridges are broken

6- What does PAGE stand for?

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

7- In proteomics, how 1D SDS PAGE can be used with mass spectrometry?

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

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

Arginine Glycine Lysine Aspartic acid

9- Glutamic acid has pK_1 (-COOH) = 2.1, pK_2 (-NH₂) = 9.47 and pK_R (-R) = 4.07. What is the net electric charge of Glu at pH = 3?

Positive Negative Zero

10- What is the approximate pI of Glu?

3.1

5.8

6.8

2.1

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

Toward the anode Toward the cathode They do not move

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

Amphoteric Acidic “Carrier” of the current Buffering

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

PAGE IEF Size-based Liquid chromatography

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

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

The slope of the titration curve at p/ 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

16- For what reason(s) can information on the p/ be valuable?

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

17- What is necessary involved in chromatography?

An analyte A mobile phase A stationary phase A liquid

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

The components of serum Some chlorophylls Some carotenoids Caffeine

19- In chromatography, what can explain band broadening?

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

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

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

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

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

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

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

24- What type of chromatography is usually coupled to mass spectrometry?

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

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

- IEF
- SCX
- SEC
- RP