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Master I Applied Microbiology

Course: Separation/Purification of Biomacromolecules (SPBMM)

Tutorial sessions

Exercise 1:

An enzyme was purified in three steps, starting from 1000 mg of a crude extract containing 20,000 total units of this enzyme.

Question: Complete the table below.

Step	Protein (mg)	Enzyme activity (EU)	Fold purification	Yield
Crude extract				
Gel filtration	200	14000		
IEC	15	4500		
Affinity C	0.5	3500		

What conclusion can we draw from this study?

Exercise 2:

The partition coefficient of iodine (I_2) between the two immiscible solvents: tetrachloromethane (CCl_4) and water is equal to 100 at 25°C.

To 10 ml of aqueous iodine solution at 10g/L, 10 ml of CCl_4 is added.

Data: I_2 is more soluble in CCl_4 than in water.

Question: Determine the iodine concentration in CCl_4 and in water, after stirring and decantation.

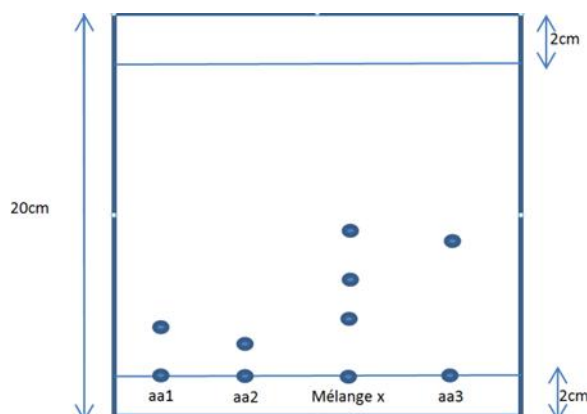
Exercise 3:

Consider the following chromatogram:

1. Calculate the R_f of the different amino acids and the mixture x.

2. After recovering the various amino acids as well as the mixture X, amino acid 1 (aa1) is immersed in Ehrlich's reagent (a solution of 1% para-dimethylaminobenzene in acetone), resulting in an intense violet coloration. Amino acid 3 (aa3) being hydroxyproline, amino acid 2 (aa2) reacts with ninhydrin, yielding a purple coloration.

Deduce the composition of the mixture x and justify your answers.



Sample	Distance
aa1	2.3
aa2	1.2
Mixture x	2.4/3.7/4.8
aa3	4.7

Exercise 4:

We aim to determine the molecular weight of a protein P through size exclusion chromatography. The gel's fractionation limit ranges between 40,000 and 400,000. Calibration of the gel is carried out using various substances, with molecular weights (in daltons) and elution volumes (V_e in ml) indicated in the following table:

MW markers	MW (Da)	V_e (ml)
<i>Dextran</i>	2000000	45
<i>Fibrinogen</i>	340000	60
<i>Catalase</i>	230000	75
<i>Bovine albumin</i>	66000	105
<i>Lactoglobulin</i>	19000	132

1. Recall what the Dalton corresponds to.
2. Protein P exhibits an elution volume of 113 ml. Determine its molecular weight.

Exercise 5:

The elution volume of several proteins with known molecular weights is determined on a gel filtration column.

MW (10^3)	10	20	40	60	80	200	300
V_e (cm^3)	210	188	169	150	140	120	105

The same column is used to study a proteolytic enzyme under different conditions.

Sample	V_e (cm^3)
Enzyme at low concentration	160
Enzyme at high concentration	135
Enzyme at low concentration + soy factor	125
Enzyme in 1% SDS	205

- a) Interpret the results.
- b) The molecular weight of the soy factor is 13,375. Determine the number of units of this factor per enzyme molecule.
- c) Determine the number of soy factor molecules per subunit in the case of the enzyme at low concentration.

Exercise 6:

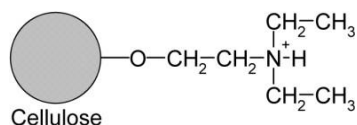
A chromatography gel (Bio-Gel P30) with a volume of 100 cm^3 is poured into a column. The elution volume of hexokinase (96 KDa) is 34 cm^3 . The elution volume of an unknown protein is 50 cm^3 . What is the volume of the column, the volume occupied by the gel, and the relative volume of the unknown protein?

Exercise 7:

A mixture of three amino acids: Asp ($pI=2.87$), Arg ($pI=10.76$), and Leu ($pI=6$) is subjected to cation exchange chromatography. Elution is performed using a buffer at pH 6. In what order can the elution of these amino acids be predicted?

Exercise 8:

Diethylaminoethyl cellulose (DEAE-cellulose) is an anion exchange support obtained by substituting cellulose with diethylaminoethyl groups.

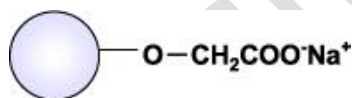


What is the proportion of positively charged DEAE radicals at the following pHs: 2, 7, 9.4, and 12? (pK_a DEAE = 9.4)

Among the following proteins: serum albumin ($pI=4.9$), urease ($pI=5$), chymotrypsinogen ($pI=9.5$), which ones, at pH 7, are retained by DEAE-cellulose? (It is considered that the protein-DEAE-cellulose interactions are solely electrostatic in nature).

Exercise 9:

Carboxymethyl cellulose (CM-cellulose) is a cation exchange resin, obtained by substituting cellulose with carboxymethyl radicals.



What is the proportion of dissociated CM radicals at the following pHs: 1, 4.76, 7, and 9? (pK_a CM = 4.76)

Among the following proteins: ovalbumin ($pI = 4.6$), cytochrome C ($pI = 10.65$), lysozyme ($pI = 11$), which ones are retained by CM-cellulose at pH 7? (It is considered that the protein-CM-cellulose interactions are solely electrostatic in nature).

Exercise 10:

We want to separate glutamic acid, leucine, and lysine by chromatography on a polystyrene resin substituted with sulfonate radicals ($-SO^-$). The isoelectric points of the amino acids are, respectively, 3.22; 5.98; 9.75 at 25°C. The three amino acids are deposited on the column at pH 2, then the pH is gradually raised to 7.

Which amino acids are eluted and in what order? (It is considered that the amino acid-resin interactions are solely electrostatic in nature and that the ionic strength of the eluents is negligible).

Exercise 11:

A peptidase was analyzed by SDS-PAGE electrophoresis in the presence of various markers. The results are as follows:

Protein	MW	Migration (mm)
Bovine albumin	66000	12.9
Carbonic anhydrase	29000	52.5
Beta lactoglobulin	18400	74
Lysozyme	14300	86.4
Myoglobin	17800	75.7
Ovalbumin	45000	31.4
Peptidase	?	49.3
Transferrine	78000	4.9
Trypsinogene	24000	61.6

Determine the MW of the peptidase

Exercise 12:

We have a mixture of three proteins A, B, and C. Protein A has a molecular weight of 100,000 and is composed of 4 identical subunits. Proteins B and C have a molecular weight of 50,000 and only possess a single subunit. If they are subjected to SDS-PAGE electrophoresis, how many bands of different proteins will be revealed? What molecular weights do these bands correspond to when compared to a standard curve obtained with proteins of known molecular weights?

Exercise 13:

Proteins are treated with SDS and then subjected to polyacrylamide gel electrophoresis at pH 7.

1. Towards which pole do the proteins migrate?
2. The migration distance, d , of 4 proteins with known molecular weights is measured. Plot $\log MW$ against d . What do you notice?

Proteins	MW	$d(mm)$
Ovalbumin	43000	37.6
Trypsin	23300	59.5
Myoglobin	17200	70.0
Cytochrom C	13500	79.0

3. For the following 4 proteins, under the same electrophoresis conditions, we found:

Proteins	$d(mm)$
Lysozyme	77.5
Serum albumin	21.5
Carbonic anhydrase	52.0
Pepsin	45.0

Using the previous graph, determine the molecular weight of these proteins.

4. The molecular weight of hemoglobin is 64,000. After electrophoresis, only one band was found with a migration distance of 73 mm. Explain these results.