

UNIVERSITY OF FORT HARE

BCH 314

EXAMINATIONS QUESTION PAPER

June 2025

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Time : 3 HOURS

Subject: Theory of Biochemical Techniques

Marks: 100

This paper consists of 3 pages including cover page

Internal Examiner

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Instructions

Answer all Questions.

QUESTION ONE [20]

- 1.1. What factors should you consider when selecting a buffer for a biochemical experiment? [2]
- 1.2. How are accuracy and precision evaluated in biochemical experiment analysis, and what role does a calibration curve play in evaluating accuracy? [3]
- 1.3. Discuss ten critical factors that must be evaluated when selecting an appropriate analytical method for biochemical analysis and explain the significance of each. [10]
- 1.4. How would you prepare 500 mL of a 2M KOH solution, given that the Molecular Mass of KOH is 56.1056 g/mol [5]

QUESTION TWO [12]

- 2.1. Describe the principle behind differential centrifugation and how it separates macromolecules in biochemistry. Give examples of how it's applied in biochemistry research. [5]
- 2.2. Compare density-gradient centrifugation versus differential centrifugation and provide examples of where they would be used. [3]
- 2.3. What factors should be considered when selecting a centrifuge for isolating mitochondria from liver cells? [4]

QUESTION THREE [27]

- 3.1. What are the key factors to consider when planning a protein purification strategy, and why? [6]
- 3.2. What are the differences between the purification strategy for affinity-tagged recombinant proteins as compared to untagged proteins? [2]
- 3.3. State the principles of and evaluate the advantage and disadvantages of ion-exchange chromatography (IEX) and hydrophobic interaction chromatography (HIC). [6]
- 3.4. A protein elutes at 15 ml in a gel filtration column with a void volume (V_0) of 10 ml and a total column volume (V_c) of 30 ml. Calculate its K_{av} . [4]
- 3.5. A protein binds to a DEAE column at pH 8.0 but not at pH 6.0. What can you deduce about the protein's pI, from these results? [3]

3.6. Compare the advantages and disadvantages of gas chromatography versus liquid chromatography? [6]

QUESTION FOUR [4]

4.1. Sephadex G-75 has an exclusion limit of 80 000 Daltons for globular proteins. If you used a Sephadex G-75 column to try and separate alcohol dehydrogenase (MW 150 000) from β -amylase (MW 200 000) what result would you obtain and why?

[2]

4.2. Would you be able to separate β -amylase from bovine serum albumin (MW 66 000) using a Sephadex G-75 column? Justify your answer.

[2]

QUESTION FIVE [10]

5.1. Describe the principle of Salting-in and Salting-out and give an example of how this can be used in protein purification? [10]

QUESTION SIX [10]

6.1. You are characterizing a partially purified protein suspected to exist in two forms:

- A monomer (50 kDa)
- A dimer (100 kDa) held together by non-covalent interactions.

You subject the protein to SDS-PAGE and Sephadex Exclusion Chromatography (SEC) using a 10mM Tris/HCl pH 8 running buffer, separately. The SDS-PAGE gel shows a single band at 50 kDa, while the SEC elution profile shows two peaks corresponding to ~50 kDa and ~100 kDa.

Explain the seemingly contradictory results from these two techniques. Which method more accurately reflects the protein's native state, and why? How would you design an experiment to confirm the dimer's existence? [10]

QUESTION SEVEN [17]

7.1. Compare Hydrophobic Interaction Chromatography and Reverse Phase Chromatography, making specific reference to the principle of binding, the solid matrices used, the mobile phases used to bind and elute molecules to and from the column and what each of the two chromatography systems can be used for. [10]

7.2. What are the differences between HPLC and GC? In your answer, make reference to the principle of separation, the columns, the solid matrix used, the mobile phases used and the detectors. [7]

End of Examination

