UNIVERSITY OF FORT HARE

BCH 314

EXAMINATIONS

June 2023

.....

Time: 3 HOURS

Subject: Theory of Laboratory Techniques

Marks: 100

This paper consists of 3 pages including cover page

Internal Examiner

Dr. N. Nqumla

Mr N Mazomba

External Examiner

Dr. NN Phasha

Instructions

Answer all questions.

Subminimum of 40%

QUESTION 1 (7)

1.1. List criteria used for select a buffer for biochemical reaction (4)
1.2. Calculate the pH of the buffer solution that contains 0.2 M acetic Acid (pKa = 3.76) and 0.25 M Sodium acetate (3)

QUESTION 2 (13)

2.1. The fasting blood glucose levels were measured from 10 different patients before they had breakfast. The values obtained were 3.5; 4.0; 5.0; 5.5; 4.8; 3.8; 2.9; 4.6; 3.5 and 2.5 mM.
Calculate the precision of the data set expressed as:
i) one standard deviation of the mean (4)
ii) the coefficient of variation of the mean. (3)

2.2. The acceptable fasting blood glucose concentration for a non-diabetic person is 3.5 mM. Does the experimental data set for the fasting blood glucose concentration in Question 2.1, agree with the known value within experimental error at a 95% or 99 % confidence level? Assume $t_{95\%} = 2.776$ & $t_{99\%} = 4.604$. (6)

QUESTION 3 (10)

a) For the pelleting of the Nuclei fraction from a muscle homogenate, an ultracentrifuge is operated at a speed of 3 000 r.p.m. If the maximal radius of the rotor is 75 mm, what is the relative centrifugal field at the bottom of the centrifuge tube? (4)

b) You need to separate the following two proteins, Protein A (MW 45 600 Da; pI 3.5) and Protein B (MW 48 000; pI 8.0).
(i) Describe what principle you would use to separate the two proteins and provide the pH of the buffer/s you would use. Motivate your choice of method and pH. (4)
(ii) How would you monitor the purification of the two proteins? (2)

QUESTION 4 (8)

a) What is the principle of SDS-PAGE and how can it be used to determine the relative molecular mass of a protein. (5)
b) A relative molecular mass for a protein was obtained using SDS-PAGE and gel exclusion chromatography. The value obtained from SDS-PAGE was 32 000 and from gel exclusion, 96 000. Why are these values different and what do they tell us about the structure of the protein? (3)

QUESTION 5 (14)

a) Compare Ultraviolet Spectroscopy versus Fluorescence Spectroscopy. (10)
b) Describe the process one would follow in order to identify a protein spot obtained in 2D gels. (4)

QUESTION 6 (6)

a) Give the definition of isoelectric point of a protein? (2)

b) What is the difference between SDS-PAGE and Native PAGE? How can one calculate the MW of an unknown protein using SDS-PAGE? (4)

QUESTION 7 (16)

a) 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)

b) 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. (6)

QUESTION 8 (16)

a) a) Describe how the Triple Antibody Sandwich and Double Antibody Sandwich ELISA methods are used to determine the presence of a diseased state. In your answer explain how these methods are used to detect the presence of Hepatitis B virus and thc Potato Leaf Roll virus. (8)

b) Describe the principle of competitive ELISA and give an example of where it is used. (5)

c) What is a Western Blotting assay principle (3)

QUESTION 9 (10)

A sample of unknown peptide was divided into two aliquots. One aliquot was treated with trypsin and the other with cyanogen bromide. Given the following sequences (N-terminal to C-terminal) of each of the fragments obtained from these digestions, deduce the amino acid sequence of the original peptide on the basis of this information. Clearly indicate all steps and the reasons for your conclusions at each step. (10)

Trypsin Digest:

a) Asn-Thr-Trp-Met-Ile-Lys
b) Gly-Tyr-Met-Gln-Phe
c) Val-Leu-Gly-Met-Ser-Arg

Cyanogen Bromide treatment:

a) Gln-Phe
b) Val-Leu-Gly- Met
c) Ile-Lys-Gly-Tyr-Met
d) Ser-Arg-Asn-Thr-Trp-Met

TOTAL [100]

END OF EXAMINATION

APPENDIX

Equations

$$s = \sqrt{\frac{\sum(x - \bar{x})^2}{n-1}}$$

$$\bar{x} = \frac{\sum x_i}{n}$$

$$CV = \frac{s}{\bar{x}} \times 100\%$$

$$\mu = \bar{x} \pm \frac{ts}{\sqrt{n}}$$

$$G = \omega^2 r$$

$$\omega = \frac{2\pi s}{60}$$

$$RCF = \frac{G}{g} = \frac{\omega^2 . r}{981}$$

$$t_{calc} = \frac{(known\ value - \bar{x})}{s}\sqrt{n}$$

$$K_{av} = \frac{V_e - V_0}{V_c - V_0}$$

Values of Student's t

Degrees of freedom	Confidence level (%)		
	50	95	99
2	0.816	4.303	9.925
3	0.765	3.182	5.841
4	0.741	2.776	4.604
5	0.727	2.571	4.032
6	0.718	2.447	3.707

	Amino Acid	pKa Value		
	Name	Alpha Carboxy	+Alpha Amino	Side Chain
Non-Polar Amino Acids	Glycine	2.34	9.60	
	Alanine	2.34	9.69	
	Valine	2.32	9.62	
	Leucine	2.36	9.60	
	Isoleucine	2.36	9.68	
	Methionine	2.28	9.21	
	Phenylalanine	1.83	9.13	
	Tryptophan	2.38	9.39	
	Proline	1.99	10.60	
Polar Amino Acids	Serine	2.21	9.15	
	Threonine	2.63	9.10	
	Cysteine	1.71	10.78	8.33
	Tyrosine	2.2	9.11	10.07
	Asparagine	2.02	8.84	
	Glutamine	2.17	9.13	
Acidic Amino Acids	Aspartic Acid	2.09	9.82	3.86
	Glutamic Acid	2.19	9.67	4.25
Basic Amino acids	Lysine	2.18	8.95	10.79
	Arginine	2.17	9.04	12.48
	Histidine	1.82	9.17	6.04