

FACULTY OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY (APK)

MODULE: BIC2A01: BIOCHEMICAL TECHNIQUES AND ENZYMOLOGY

JUNE EXAMINATION

DATE: June 2020

TIME: 08:00-11:00

EXAMINER 1 (Section A)
EXAMINER 2 (Section B)

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INTERNAL MODERATORS

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TIME 3 HOURS

MARKS 100

NUMBER OF PAGES: 8 PAGES

INSTRUCTIONS: ANSWER ALL THE QUESTIONS.
DO NOT USE RED INK.
PLEASE HAND IN YOUR QUESTION PAPER WITH YOUR EXAM BOOK.

REQUIREMENTS: ANSWER ALL THE QUESTIONS IN YOUR EXAM BOOKS PROVIDED
ANSWER SECTION A (TECHNIQUES) AND SECTION B
(ENZYMOLOGY) IN TWO SEPARATE EXAM BOOKS

Additional Information:

pKa Values

Carboxyl group : 2.2

Amino group : 9.4

Side Chains : Tyr (10.46); Cys (8.37); Lys (10.54); Arg (12.48); His (6.04); Asp (3.90); Glu (4.07)

SECTION A [50]**Question 1****[20]****Multiple choice questions:**

1. The correct order for the basic features of a mass spectrometer is...
 - a) ionisation, acceleration, deflection, detection
 - b) acceleration, deflection, detection, ionization
 - c) acceleration, ionisation, deflection, detection
 - d) ionisation, deflection, acceleration, detection
2. Which of the following species will be deflected to the greatest extent?
 - a) $^{35}\text{Na}^{2+}$
 - b) $^{37}\text{Na}^{+}$
 - c) $^{35}\text{Na}^{+}$
 - d) ^{37}Na
3. Which of the following is not a use for mass spectrometry?
 - a) investigating the elemental composition of planets
 - b) calculating the molecular mass of organic compounds
 - c) calculating the isotopic abundance in elements
 - d) confirming the presence of O-H and C=O in organic compounds
4. Which one of the following statements about the mass spectrum of CH_3Br is correct?
 - a) there is just one peak for the molecular ion with an m/z value of 44
 - b) the last two peaks have abundances in the ratio 3:1 and occur at m/z values of 94 and 96
 - c) there is just one peak for the molecular ion with an m/z value of 95
 - d) the last two peaks are of equal size and occur at m/z values of 94 and 96
5. Which one of the following pieces of information cannot be obtained from an infra-red spectrum?
 - a) The presence of C=O bonds
 - b) the molecular mass
 - c) the identity of a compound through comparison with other spectra
 - d) the presence of O-H bonds

6. Proton nmr is useful for investigating the structure of organic compounds because...
- a) organic compounds contain carbon atoms
 - b) hydrogen atoms are found in nearly all organic compounds
 - c) organic compounds have low boiling points
 - d) organic compounds are mostly covalent
7. Signals in a proton nmr spectrum do not provide information about...
- a) the environment of different hydrogen atoms in a molecule
 - b) the number of chemically different hydrogen atoms on adjacent atoms
 - c) the molecular mass of an organic molecule
 - d) the relative number of hydrogen atoms in a particular environment
8. The proton nmr of 1-bromopropane will consist of...
- a) two doublets and a sextet
 - b) a singlet, a doublet and a triplet
 - c) a doublet and a septet
 - d) a singlet, two doublet and a triplet
9. The molecule $\text{HOCH}_2\text{CH}_2\text{OH}$ will have an nmr spectrum consisting of...
- a) a triplet and a doublet
 - b) a singlet and a doublet
 - c) two singlets
 - d) two doublets
10. Which compound has a molecular ion at $m/z = 58$, an infra red absorption at 1650cm^{-1} and just one singlet in its nmr spectrum?
- a) 2-methylpropane
 - b) $\text{CH}_3\text{CH}_2\text{CHO}$
 - c) Butane
 - d) CH_3COCH_3
11. The isomer of C_4H_8 which produces an nmr spectrum with four different signals is...
- a) $(\text{CH}_3)_2\text{C}=\text{CH}_2$
 - b) $\text{CH}_2=\text{CHCH}_2\text{CH}_3$
 - c) Cyclobutane
 - d) $\text{CH}_3\text{CH}=\text{CHCH}_3$

12. Which one of the following hydrocarbons produces an nmr spectrum with more than one peak?
- a) Cyclobutane
 - b) Butane
 - c) Ethane
 - d) Methane
13. Indicate which of the following is a true statement about mass analysers used in mass spectrometry.
- a) the energy of ions is directly proportional to their charge.
 - b) low energy ions are substantially deflected.
 - c) a quadrupole filter uses a large magnet to deflect ions.
 - d) ions are deflected in a simple trajectory in a quadrupole filter.
14. HPLC methods include
- a) liquid/liquid (partition) chromatography
 - b) Gas/solid (adsorption) chromatography
 - c) ion exchange and Thin layer chromatography
 - d) all of the above
15. Column efficiency is measured in terms of number of plates which is
- a) inversely related to the square of the peak width
 - b) directly related to the square of the peak width
 - c) inversely related to the cube root of the peak width
 - d) directly related to the square of the peak width
16. In reversed phase HPLC, there is a
- a) non polar solvent/polar column
 - b) non polar solvent/non-polar column
 - c) any of the above
 - d) polar solvent/non-polar column
17. Which of the following statements is true for a refractive index detector in HPLC?
- a) It does not respond to many solutes
 - b) It is more sensitive than a UV detector
 - c) It can only be used for isocratic elutions
 - d) none of above

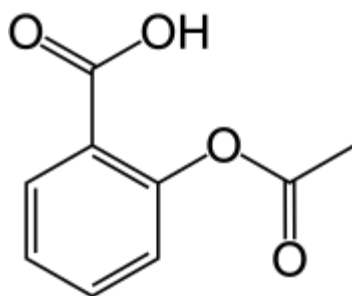
18. Which of the following(s) is/are the advantage of HPLC over traditional LPLC (low-pressure liquid chromatography)?
- a) Greater sensitivity and reusable columns
 - b) Ideal for ionic species and large molecules
 - c) Sample recovery
 - d) All of the above
19. Which of the following wavelength ranges is associated with UV spectroscopy?
- a) 400 - 100nm
 - b) 0.8 - 500 μ m
 - c) 380 - 750nm
 - d) 0.01 - 10nm
20. In a chromatographic separation, which of the following indices is most appropriate for the qualitative identification of a substance?
- a) Retention factor R_f
 - b) Relative retention factor R_{rel}
 - c) Resolution
 - d) Retention time

QUESTION 2**[3]**

1. 20 Carbamazepine tablets were found to weigh 10.000g in total. The tablets were ground to a fine powder using a pestle and mortar. A 0.3000g sample of the powder was boiled with 25ml ethanol for a few minutes. The hot mixture was stirred in a closed flask for 10 minutes and filtered through sintered glass. The flask and filter were washed with ethanol and the washings combined with the filtrate. The cooled mixture was made up to 100ml with ethanol. 5ml of the ethanol extract was diluted to 250ml with ethanol and the absorbance of the resulting solution was found to be 0.588 absorbance units at a wavelength of 285nm. What was the content of a single tablet if the $A_{1\%}^{1cm}$ was 490?

QUESTION 3**[15]**

Given the following structure of Aspirin. Please answer the following question



- a) Draw the H-NMR spectrum of aspirin. [4]
- b) State the set of hydrogen equivalent as per your spectrum. [3]
- c) Draw and label the chemical structure of the most abundant picks that one will observe using Mass Spectrometry. [4]
- d) Explain how the LC-MS directly identify proteins present in a mixture. [4]

QUESTION 4

[12]

- 1. Using the principles of Ion-Exchange, Affinity chromatography and gel filtration chromatography, Explain how one will identify and separate a particular protein in a mixture. [10]
 - 2. Salting in –Salting out is one technique of concentrating protein. Explain how one will concentrate protein using this technique [2]
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SECTION B [50]**Question 1****[13]**

1. Enzymes that have different structures but the same catalytic function. Frequently they are oligomers made from different polypeptide chains and are called
2. The model that explain that the active site is flexible and the catalytic group(s) of the enzyme is (are) brought into proper alignment by the substrate is called
3. Inactive precursors of some enzymes that are activated through hydrolysis reactions are called.....
4. The action of disrupting the three dimensional shape of a protein is termed
5. The protein portion of a conjugated enzyme is called a(n)
6. An uncompetitive inhibitor binds to
7. Enzymes increase the by lowering the, but they do not affect the aspects of reactions.
8. A co-enzyme covalently attached to enzyme is known as a
9. Since proteins are limited in their ability to catalyze oxidation-reduction reactions, enzymes often employ..... to assist with catalysis.
10. Some serine proteases are believed to have developed by convergent evolution, because the..... sequences of some serine proteases show no resemblance to those of others.
11. If an enzyme-catalyzed reaction has a low rate at low pH and high rate at higher pH, this implies that a group on either the enzyme or the substrate must be..... for an efficient reaction.

Question 2**[22]**

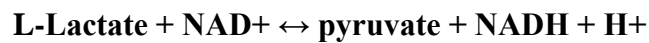
1. Explain the strategies commonly employed by enzymes to accelerate the rates of biochemical reactions. [10]
2. Give a pointwise description of the catalytic mechanism of RNase A. [6]
3. Why would RNase A degrade RNA but not DNA. [2]
4. How is “Transition state theory” used as a tool to understanding how enzymes function? What is the relationship between the kinetics and the thermodynamics of a reaction? Which of these parameters are altered by enzymes? [4]

Question 3**[7]**

1. The lineweaver-Burk plot is a linearization of the Michaelis Menten equation. Derive the Lineweaver-Burk Relationship starting from Michaelis Menten equation. [4]
2. What are the possible errors associated with using the Lineweaver-Burk plot for the analysis of kinetic data? [3]

Question 4**[8]**

1. The reaction indicated below is catalysed by the enzyme lactate dehydrogenase:



In this reaction it is found that NAD^+ binds tightly to the enzyme but there is no detectable binding of lactate. What does this suggest about the reaction mechanism? [3]

2. Explain how the enzyme **carbonic anhydrase** uses a metal ion to facilitate the following reaction: $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HCO}_3^- + \text{H}^+$ [5]