



SOUTH EASTERN KENYA UNIVERSITY

UNIVERSITY EXAMINATIONS 2016/2017

SECOND SEMESTER EXAMINATION FOR THE DEGREE OF BACHELOR OF SCIENCE IN BIOCHEMISTRY AND MOLECULAR BIOLOGY

BCH 201: BIOCHEMICAL TECHNIQUES AND INSTRUMENTATION I

DATE: 20TH APRIL, 2017 TIME: 8.00-10.00 A.M

INSTRUCTIONS TO CANDIDATES

(a) Answer ALL the Questions in Section A

(b) Answer ANY TWO Questions in Section B

(c) Illustrate your answers with well labeled diagrams where appropriate

SECTION A (30 MARKS)

1. The amino acid glycine, which has a pK_a of 9.6, is often used as the main ingredient of a buffer in biochemical experiments.
 - a. Give the reversible equilibrium equation of the glycine buffer. **(0.5 marks)**
 - b. In what pH range can glycine be used as an effective buffer? **(0.5 marks)**
 - c. In a 0.1 M solution of glycine at pH 9.0, what fraction of glycine has its amino group in the $-NH_3^+$ form? **(1 mark)**
 - d. How much 5 M KOH must be added to 1.0 L of 0.1 M glycine at pH 9.0 to bring its pH to exactly 10.0? **(1 mark)**
 - e. When 99% of the glycine is in its $-NH_3^+$ form, calculate the numerical relation between the pH of the solution and the pK_a of the amino group. **(1 mark)**
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2. Outline the various applications of thin layer chromatography. **(3 marks)**
3. Give the advantages and limitations of using cellulose acetate membrane (CAM) in electrophoresis. **(3 marks)**
4. Explain the benefit double beam photometric devices have over single beam ones. **(2 marks)**
5. Outline **three** methods of determining protein concentration. **(3 marks)**
6. The molecular mass of the lactate dehydrogenase (LDH) enzyme is 140 kDa. Its molar extinction coefficient is $\epsilon_{280} = 1.6 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$. The optical absorption of a given LDH solution at 280 nm is $E_{280} = 0.8$. Calculate the following:
 - a. The molar concentration (μM) of the solution. **(1 mark)**
 - b. The percentage concentration (w/v) of (a) above. **(1 mark)**
 - c. The percentage concentration (mg/mL) of (b) above. **(1 mark)**
7. Give **three** examples of enzymatic reactions that can be assayed spectrophotometrically. **(3 marks)**
8. Briefly outline a routine protocol for precipitating DNA. **(3 marks)**
9. Give **two** proteases used in determination of amino acid composition of proteins and state their specificity. **(3 marks)**
10. Briefly explain how 2D electrophoresis employs the physiochemical properties of proteins. **(3 marks)**

SECTION B (40 MARKS)

11. Describe the titration curve of phosphoric acid. **(20 marks)**
12. Discuss the continuous and discontinuous classification of gel electrophoretic methods. **(20 marks)**
13. a. Describe the significant variables for deoxyribonucleic acid precipitation. **(10 marks)**
 b. Describe methanol/chloroform method of protein precipitation. **(10 marks)**
14. Describe the phases of ion exchange chromatography. **(20 marks)**