CHUKA  **UNIVERSITY**

UNIVERSITY EXAMINATIONS

EXAMINATION FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE IN

BIOC 241: ADVANCED LABORATORY TECHNIQUES I

STREAMS: **TIME: 2 HOURS**

DAY/DATE: TUESDAY 10/04/2018 **2.30 P.M – 4.30 P.M**

INSTRUCTION:

- **Answer all questions in section A and any two in section two in section B**

SECTION A: Answer all questions (5marks each)

1. How would you prepare 10ml of a 0.01 M phosphate buffer, pH 7.40 =, from stock solutions of 0.10M KH2PO4 and 0.25M K2HPO4? pKa of KH2PO4 = 7.20.[5marks]

2. For each of the following methods of separation proteins, describe the principle of the method, and tell what property of proteins allows their separation by this technique.

   (a) ion-exchange chromatography. [5marks]

   (b) Size-exclusion (gel filtration) chromatography.

   (c) Affinity chromatography

3. State the principle and application of spectrophotometry. [5marks]

4. Briefly explain how Geiger-Mueller counter measures the β-radiation. [5marks]

5. Suppose during your practical lesson, you conduct thin layer chromatography (TLC) and find that compound A travels 3.0cm while compound B travels 7.0 from the bottom of the plate. If the solvent travels 10.0cm from the bottom and the sample was spotted 1.0cm above the bottom of the plate, find the retention/retardation factor of both compounds and explain their implications. [5marks]

6. How does the shape of a titration curve confirm the fact that the pH region of greatest buffering power for an amino acid solution is around its pka? [5marks]

SECTION B: ANSWER ANY OTHER TWO QUESTIONS (20MARKS EACH)

1. (a) A biochemist is attempting to separate a DNA –binding protein (protein X) from other proteins in a solution. Only three other proteins (A,B and C) are present. The proteins have the following properties.

	Pl(isoelectric point)	Size M_r	Bind to DNA
Protein A	7.4	82,000	Yes
Protein B	3.8	21,500	Yes
Protein C	7.9	23,000	No
Protein X	7.8	22,000	Yes

What type of protein separation techniques might she use to separate

(i) Protein X from protein A? [2marks]
(ii) Protein X from protein B? [2marks]
(iii) Protein X from protein C? [2marks]

(b) Explain the principle and procedure of gel filtration chromatography. [14marks]

2. (a) Explain the following terms used to describe radioactive compounds. [14marks]

(i) Specific activity

(ii) Radiochemical purity [5marks]

(b) Discuss the principle and instrumentation of Scintillation spectrophotometry. [15marks]

3. (a) Outline the principle and factors that effect application of thin layer chromatography (TLC) analytical technique. [10marks]

(b) Discuss the preparatory and operational steps required to perform a thin layer chromatography to analyze a complex samples. [10marks]
