CHUKA UNIVERSITY

UNIVERSITY EXAMINATIONS

EXAMINATION FOR THE AWARD OF MASTER OF SCIENCE (CHEMISTRY)

CHEM 841: ADVANCED SEPARATION TECHNIQUES

STREAMS: MSC **TIME: 3 HOURS**

DAY/DATE: MONDAY 02/12/2019 **2.30 P.M. – 5.30 P.M.**

INSTRUCTIONS: ANSWER ALL QUESTIONS

QUESTION ONE (20 MARKS)

(a) (i) The zinc from a 2.50g sample of plant tissue is extracted into an aqueous solution and diluted to 50ml in a voltametric flask. The sample is analyzed by voltammetry with a limiting current of 0.583 mA. A 5.00mL a liquot of a solution of zinc is added, resulting in a limiting current of 1.35 mA. Calculate the amount of zinc in the plant tissue, reporting your result as mg zinc per gram of tissue

[1 mark]

(ii) A sample of pottery being considered for import is leached for 24 hours using 50.0ml of 4% acetic acid. A 40.00ml aliquot is transferred to an electrochemical cell and 10.00ml of a 0.200 mM standard solution of is added. A stripping analysis of the solution yields peak currents of 1.81 for lead and 2.18 for cadmium.

Analysis of a standard solution that is 0.0600 mM in and 0.0500mM in gives peak currents of 2.39 and 2.71, respectively. Calculate the concentration of in the original leachate

[½ marks]

(iii) The following data were obtained for the reduction of an analyte using steady-state voltammetry (linear sweep voltammetry while stirring the solution). Show that this data is consisted with an electrochemical reversible reaction.

Applied potential (v)	Current (MA)
- 0.385	0.0
- 0.444	1.0
- 0.465	2.0
- 0.489	4.0
- 0.511	6.0
- 0.535	8.0
- 0.556	9.0
- 0.573	10.0
- 0.596	10.0

[1½ marks]

(b) (i) Explain how you can analyze the monomer/dimer percentage in an aggregation prone sample by SEC, if the protein is not visible under UV [2 marks]

(ii) Explain how you can identify one aggregate from the other contaminants directly using the SEC

[1 mark]

(iii) Comment on the following statement:
"Flow rates have an effect on protein aggregation" [1½ marks]

(iv) Describe how the % of aggregation based on a SEC profile can be calculated [1½ marks]

(v) Suggest guidelines which can be used to analyze protein complexes (for example to analyze the oligomeric of proteins) by SEC using total cell lysates instead of purified proteins

[½ mark]

(vi) Distinguish between aggregate formation and oligomer formation [½ mark]

(vii) Explain why there was a need for multidimensional chromatography [3 marks]

(viii) Compare capillary electrophoresis with high performance liquid chromatography [2 marks]

(c) (i) Discuss with help of a suitable diagram electroosmotic mobility [2½ marks]

(ii) (I) In a hydrodynamic injection, a pressure difference of is applied for 25 to a 75cm long capillary tube with an internal diameter of 50 Assuming that the buffers viscosity is , Calculate the volume and length of sample injected in a nano litres [1 mark]

(II) Suppose that the limit of injection to be less than 0.2% of the capillary's length using information from ii (I) above, calculate the maximum injection time for a hydrodynamic injection.

[1½ marks]

QUESTION TWO (20 MARKS)

(a) (i) Consider A 50cm column with a plate height of 1.5mm that provides a theoretical plate number of 333 at a flow rate of 3ml min^{-1}, V_m=1.0 ml. calculate the solute retention time, retention volume, peak capacity of the column, zone velocity for each solute and retardation factor for each solute when portion ratio is 1, 2, 5 and 10

[1 mark]

(ii) Chromatograms with a standard test mixture were obtained using porous alumina; assume the total porosity is 0.75. the inlet pressure was 22.5 atm for all columns. Operating conditions and retention times are tabulated below. Calculate capacity factor for each solute, the average linear velocity, plate height, reduced plate height, reduced column length for each test column.

[1 mark]

Test substance retention time (tR), sec	Column 1	Column 2	Column 3
Nitrobenzene	538	182	91
Anisole	232	76	36
Biphenyl	168	56	26
Toluene	124	40	19
Dead or voidtime (tm)	104	34	16
Column length (Lcm)	50.0	13.5	9.0
Number of theoretical plates (N)	3200	4450	5000
Diameter of the packing (dp), m	20	10	6.5
Column bore (dc) cm	0.2	0.5	0.5

(iii) Suggest the courses of action which are available and penalties which may accrue for decreasing the plate height and yet increasing the resolution. [1 mark]

(b) (i) State four characteristics which make HPLC exceptional method for the separation and analysis of chemical mixtures compared to the other chromatographic techniques

[1 mark]

(ii) Distinguish the following techniques: Reversed – phase chromatography (RPC), Normal – phase chromatography (NPC), Non-aqueous reversed-phase chromatography (NARP), size – exclusion chromatography (SEC)

[1 mark]

(iii) Discuss the guidelines for selecting column conditions in liquid chromatography

[1 mark]

(iv) Explain how the values of A, B, C terms in Van Deemter equation can be determined experimentally

[1 mark]

(v) Suggest ways which resolution in chromatography can be improved [½ mark]

(vi) Outline three objectives which might be taken into account when developing or improving a chromatographic procedure

[½ mark]

(vii) State eight possible causes of peak distortion or tailing [½ mark]

(viii) Give three reasons why a chemist should understand the effects of the sample size on HPL separation before doing analysis. [½ mark]

(c) Write short notes on the following in relation to liquid chromatography

(i) Mobile – phase filtration [½ mark]

(ii) Mobile – phase degassing [½ mark]

(iii) Vacuum and in-line degassing [1 mark]

(d) (i) Discuss the sample size effects in chromatography [1 mark]

(ii) Briefly explain why it's necessary to carefully control the temperature during analysis with HPLC

[1 mark]

(iii) State nine characteristics of an ideal HPLC detector [1 mark]

(iv) List four general techniques that are used in HPLC detection [1 mark]

(e) (i) Outline the advantages and disadvantages of Buck property detectors [1 mark]

(ii) Give reasons for the use of silica in the form of either particle or monoliths as a support for the production of HPLC packing's

[1 mark]

(iii) Discuss the importance of column selectivity in liquid chromatography [1 mark]

(f) (i) List eight different interactions that can affect column selectivity [1 mark]

(ii) State and explain two possible problems with reverse phase chromatography which require attention during analysis

[1 mark]

QUESTION THREE (20 MARKS)

(a) (i) Why is it that, GC and HPLC are the most frequently used for the detection of pesticide residues and their metabolites in the environmental samples. [2 marks]

(ii) Give some of the drawbacks of these two techniques (GC and HPLC) as techniques for analyzing of pesticides in environmental samples. [2 marks]

(iii) Outline the strength and weakness of capillary electrophoresis for analyzing pesticide's in environmental samples and how these limitations can be overcomed.

[2 marks]

(iv) Describe briefly drawbacks of liquid phase microextraction (LPME) and solid phase microextraction (SPME) as a miniaturized techniques for extraction of synthetic pyrethroid insecticides (SPS) from water. [2 marks]

(v) Describe the principle of dispersive liquid-liquid microextraction (DLLMME) [2 marks]

(vi) Discuss how efficiency of dispersive liquid – liquid microextraction (DLME) of pyrethroids from aqueous media can be optimized. [2 marks]

(vii) Describe how the applicability of dispersive liquid – liquid microextraction (DLLME) for determining pyrethroids in the ground water samples can be tested. [2 marks]

(viii) Explain how effect of ground water sample composition on the formance of the dispersive liquid – liquid microextraction can be checked. [2 marks]

(b) Discuss the pitfalls in the capillary electrophoresis analysis of aggregates of Beta Amyloid Peptides [1 mark]

(c) (i) Compare ultra-high performance liquid chromatography (UHPLC) and High performance liquid chromatography

[1 mark]

(ii) State and explain different types of contaminants in water that affect HPLC results

[1 mark]
