TECHNOLOGICAL UNIVERSITY DUBLIN
TALLAGHT CAMPUS

**Higher Certificate in Science**
**Bachelor of Science**

**Applied Biology**
**Bioanalysis**

**Full Time**

**Semester** Three : January  2024

Chromatography Techniques & Measurement Systems

*Internal Examiners*
Dr Sinead Currivan-Macdonald
Dr Eugene Hickey

*External Examiners*
Dr Annamarie Rogers

**Day**
**Date**
**Time**

**Instructions to Candidates**

**Answer question one (compulsory) in Section A and two questions in Section B.**

**Total questions to be answered is three.**
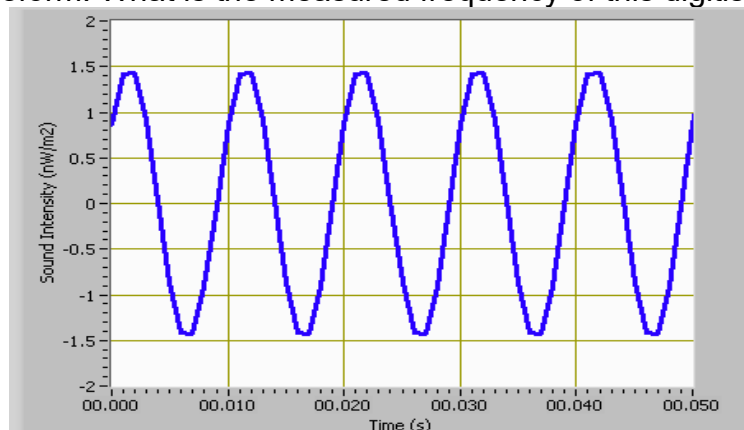
**All questions are worth equal marks (100).**

**Total marks = 300**

## Question 1                                        (compulsory)

Answer any eight from twelve parts. Each part is worth 12.5 marks.

a) Distinguish between **first order** and **second order** sensors and give an illustrative example of each.

b) What process turns a **information** into **knowledge**?

c) The detector in the Scanning Electron Microscope operates at a temperature of 295K. It has an electrical resistance of 200kΩ and a bandwidth of $\Delta f$ = 50kHz. Calculate the Johnson Noise from the detector using $V_n = (4k_BTR\Delta f)^{\frac{1}{2}}$. Note that Boltzmann's constant is $1.38\times10^{-23}$J/K.

d) Write a paragraph discussing how filters can be a useful tool in reducing noise within a signal

e) Give four advantages for converting a signal into digital format as opposed to keeping it in analogue format.

f) Calculate the frequency in hertz of the signal shown below. This signal was digitised with a sample taken every 0.01s. Do a rough sketch in your answer copy (no graph paper needed) of the input signal, the samples taken, and the digitised waveform. What is the measured frequency of this digitised waveform?



g) A signal contains frequencies up to 20khz. What is the minimum sampling rate needed to capture this signal correctly?

h) Why is essential to filter a signal before it is converted to digital format?

i) An ADC has a range of 0V to 5V and digitises to 10 bits (1024 levels). What is the digital resolution of this ADC?

j) A signal is digitised capturing 100s seconds of data with a sampling rate of 2.5khz and a sampling depth of 10 bits. What is the size in Bytes of the raw data file of this signal?

k) Write a paragraph discussing the USB connection protocol.

l) Distinguish between **raster graphics** and **vector graphics**. For the following types of image format, state whether they are raster or vector:
- PNG
- Bitmap
- GIF

**(8x12.5 = 100 marks)**

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# Section B - S. Currivan-Macdonald
## Answer 2 questions from section B.

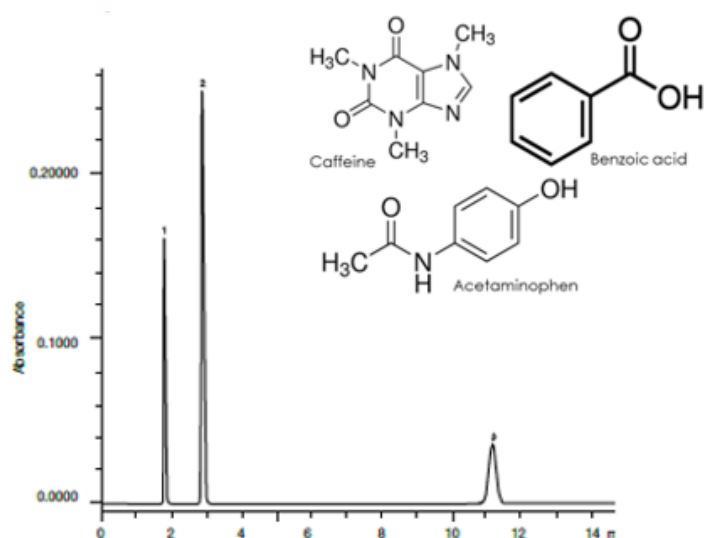Question 2: (100 marks) **Answer all parts** (each worth 10 marks).

a) What is chromatography defined as?

b) What is the correlation between particle size and efficiency?

c) What is the difference between isocratic and gradient elution in HPLC?

d) Define the following terms:
   *a.* Resolution
   b. Efficiency
   c. End-capping

e) Why is controlling pH important in reversed-phase HPLC?

f) If I increase the efficiency of my column, what affect should be observed in the resulting chromatogram?

g) In gas separations with a liquid coated open tubular column, what type of separation mechanism would you expect?

h) What factor is used to control retention in GC?

i) A GC column gave a plate height of 0.015 mm (15 μm), and an efficiency of 750, 000. What is the length of the column?

j) Name 2 detectors commonly found on a GC and note if they are universal or selective detectors.

Question 3. (100 marks) **Answer all parts**

a) What are the main causes of band broadening within liquid chromatography using particle packed columns? Show how each is related to observed peak efficiency as a function of mobile phase flow rate. Within your answer sketch a typical van Deemter plot, showing the combined response and individual A, B and C terms. (30 marks)

b) Compare and contrast **two** chromatographic modes from the list below. Within your answer briefly describe the stationary phase, mobile phase, and the typical analyte chemistry.

(40 marks)

| a. | Normal phase | d. | Affinity |
|----|--------------|----|----------|
| b. | Ion exchange | e. | Reversed phase |
| c. | Size exclusion | | |

c) In RPLC, three analytes are separated on a C18 column, using a mobile phase of 70 % ACN. Based on the following data, which compound will elute first? Within your answer justify your decision in terms of structure, chemistry, and polarity. (30 marks)



| Analyte | Log P | Molecular weight (g/mol) |
|---------|-------|--------------------------|
| Caffeine | -0.1 | 194.19 |
| Benzoic acid | 1.9 | 122.12 |
| Acetaminophen | 0.5 | 151.16 |

Question 4: (100 marks) **Answer all parts**.

a) Draw a schematic of a GC system and identify the role of each component. Note two detector types commonly found and describe the operation principles of one of those detectors.

(40 marks)

b) Compare and contrast the use of a packed column versus a capillary column in GC.

(20 marks)

c) A temperature-programmed analytical method uses a 30 m column with $d_c$ = 0.25 mm and $\beta$ = 250 for analysis of an 18-component mixture. A list of retention times and peak widths obtained by analysing the mixture using this column is provided in the Table below.

(40 marks)

| Peak Number | 1 | ... | 10 | 11 | 12 $C_{14}H_{30}$ | 13 | 14 | 15 $C_{15}H_{32}$ | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | |
| $t_R$ (min) | 6.233 | ... | 11.811 | 12.039 | 17.350 | 18.000 | 18.311 | 20.195 | 25.389 | 26.389 | 27.233 |
| $w_h$ (min) | 0.118 | ... | 0.118 | 0.118 | 0.118 | 0.118 | 0.118 | 0.118 | 0.118 | 0.118 | 0.118 |

i. Calculate *Efficiency* using a suitable metric and using the information provided in the Table.

ii. Calculate *Resolution* of peaks 13 and 14 using the information provided in the Table.

iii. Discuss any potential advantages and disadvantages of replacing the column described above with a 60 m column with $d_c$ = 0.25 mm and $\beta$ = 250.