OLLSCOIL NA HÉIREANN, GAILLIMH

THE NATIONAL UNIVERSITY OF IRELAND, GALWAY

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SUMMER EXAMINATIONS, 2000

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**Higher Diploma in Applied Science, (Analytical Biochemistry/Chemistry)**

**Master of Science, (Analytical Biochemistry/Chemistry)**

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*Fourth Paper*

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Dr. T. Higgins
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Time Allowed: ***Three*** hours

(Answer ***Five*** Questions)

1. Detail the steps involved in the development and validation of a laboratory-based immunoassay for determining the concentration of the steroid hormone progesterone in serum. What assay design and components would you ideally use and why?

2. Answer (a) **and** (b):

   a) Describe some of the more important parameters that must be considered in order to obtain accurate and reproducible concentration measurements using ion selective electrodes.

   b) "The increased sensitivity of the pulsed voltammetric methods over linear sweep methods can be attributed to the minimisation of the contribution of the charging current to the total current". Discuss.

3. Answer (a) **and** (b):

a) Briefly outline the uses of fluorescence in biochemistry.

b) Aminopeptidase B is an enzyme that removes N-terminal basic amino acids, such as arginine and lysine, from di- and tri-peptides. Describe how you would use fluorescence, during purification, to detect the activity of this enzyme (using the substrate arginine-aminomethyl coumarin). How might the activity be quantified

4. Give an account of the applications of HPLC in peptide chemistry

5. Answer (a), (b) **and** (b):

a) What advantages does the 'sodium deoxycholate / trichloroacetate' modification of the method of Lowry, Bensadoun and Weinstein have over the classical approach to this method for measuring protein?

b) How does the sensitivity of the Bensadoun and Weinstein method differ from the Bradford dye-binding assay?

c) A 200μg/ml solution of Bovine serum albumin (BSA) yields an $A_{680nm}$ of 0.312, as determined by the Bensadoun and Weinstein method. Calculate the protein contents (mg/ml) of the following three samples:

i) A: 1/10 dilution $\Rightarrow$ $A_{680nm}$ of 0.178

ii) B: 1/15 dilution $\Rightarrow$ $A_{680nm}$ of 0.231

iii) C: 1/150 dilution $\Rightarrow$ $A_{680nm}$ of 0.153

6. Answer (a), (b) **and** (c):

a) Outline some of the techniques you might use to purify a 60kDa β-glucosidase with an isoelectric point (pI) of 3.5 and a carbohydrate content of 30%.

b) How would you monitor the elution of protein and β-glucosidase during anion-exchange chromatography at ph 6.0?

c) Incubation of β-glucosidase ( a 1/300 diluted sample) with 1.0mM para-nitrophenyl -β-glucopyranosidase at 50°C, pH 5.0 for 15min yields an $A_{405nm}$ of 0.489, using the data in the table below, calculate the enzyme activity in International Units or in μmole/ml/min.

| Para-nitrophenol Standard | $A_{405nm}$ |
|---|---|
| 0.0 | 0.016 |
| 0.25 | 0.131 |
| 0.50 | 0.261 |
| 0.75 | 0.408 |
| 0.1 | 0.509 |
| 0.15 | 0.758 |

7. Answer (a) **and** (b)

a) Distinguish between the following types of atomic spectrometry:
   i) atomic absorption
   ii) atomic emission
   iii) flame photometer
   iv) graphite furnace

b) Calculate the iron content in a diethyldithiocarbamate extract using the following data:

| Absorbance of sample | Iron added, μg/200ml |
|---|---|
| 0.120 | none |
| 0.284 | 2.0 |
| 0.484 | 4.0 |
| 0.677 | 6.0 |

8. The chromatogram of a mixture of industrial solvents, shown below, was obtained in a gas chromatograph

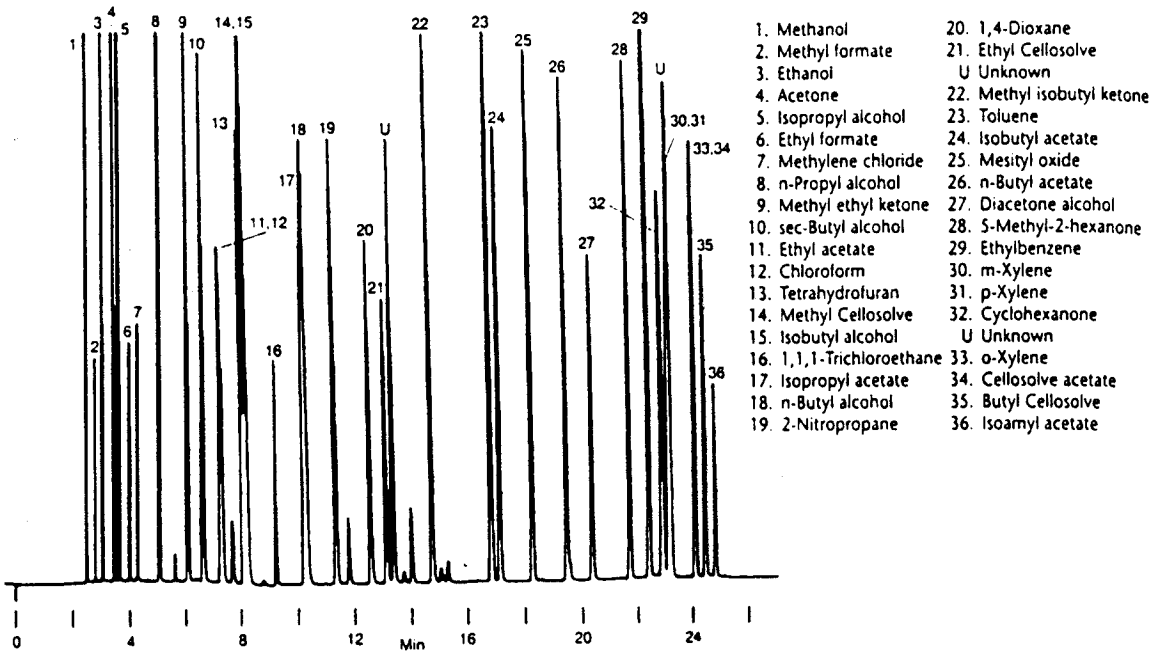
a) What kind of column was used and why?

b) What type of detector was used and why?

c) Why was the analysis not performed at a constant temperature?

d) Why was the sample 'split 200:1'?

e) At least two of the peaks are unknowns ('U'); suggest ways of identifying these unknowns.




| | |
|---|---|
| Column | SPB–1, 30 m × 0.32 mm ID, 1.0 μm film |
| Oven | 30°C (8 minutes) to 125°C at 4°C/minute, hold 5 minutes |
| Carrier | Helium, 25 cm / second |
| Detector | FID, 250°C |
| Injection | 0.2 μl, approximately equal amounts of each component, split 200:1, 250°C |