# UNIVERSITY COLLEGE LONDON

University of London

## EXAMINATION FOR INTERNAL STUDENTS

For The Following Qualification:-

*M.Sc.*

**Biochem Eng G23: Integrated Downstream Processing**

| | | |
|---|---|---|
| COURSE CODE | : | **BENGEG23** |
| DATE | : | **30-APR-04** |
| TIME | : | **10.00** |
| TIME ALLOWED | : | **3 Hours** |

04-C0120-3-50



**TURN OVER**

*Answer **FOUR QUESTIONS.*** **Only the first four answers will be marked.**
*ALL questions carry a total of **25 MARKS** each, distributed as shown* [ ]

**1.**

Centrifuge designs each attempt to increase the clarity of product achieved by either reducing the distance required for a particle to sediment and/or maximizing the forces available to achieve a high velocity of sedimentation. Provide a brief on how the following designs of machine each achieve these goals and then comment on the relative extent to which these machines realise the targets of high clarification and efficient dewatering: tubular bowl, multi – chamber and disk stack. [20]

Given the requirement to recover a delicate protein precipitate formed by ammonium sulphate precipitation which of the above machines do you think would be the most appropriate? [5]

**2.**

If you were asked to assess the suitability of an expanded bed adsorption (EBA) process how would you set about comparing it with a conventional process? Your answer should include sketch diagrams of potential flowsheets that demonstrate the difference that the adoption of EBA might make. An engineering analysis of the differences in performance and the process characteristics that might result upon adoption of the alternative processing technology is also needed. [15]

Using appropriate expressions demonstrate how the diameter of the matrix particle used in EBA affects the maximum velocity of operation that can be achieved. Given that the particles used in such columns are not mono-sized what characteristic of the particle size distribution would you use in order to determine the maximum throughput that was possible in the case of expanded bed operation. [10]

**3.**

Conventional filtration using pre – coats and body feed is still widely used in elements of the biological process industries. How does the addition of particles, where one is expecting to achieve the removal of solids, actually enhance the separation? [5]

With the use of suitable figures illustrate how the choice of pre – coat will affect the ultimate output of such a filtration process. [5]

Given the need to operate a rotary vacuum filtration system discuss whether constant pressure of constant flux operation would be the most appropriate to adopt. Provide a brief analysis of the suitability or otherwise of rotary vacuum filtration using both pre – coat and body feed to enhance the separation performance when handling a high value excreted biological product. [15]

**PLEASE TURN OVER**

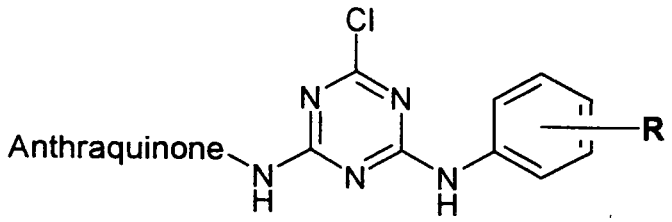
**4.**

i) Immobilised metal affinity chromatography with $Ni^{2+}$ - NTA as the affinity ligand, was used to purify two enzymes (**A** and **B**) of similar size and shape, both containing the His-Gly-Ala-Ser-His amino-acid sequence with the following results:

| | Enzyme loaded (mg) | Enzyme eluted (mg) |
|---|---|---|
| **A** | 120.0 | 99.0 |
| **B** | 120.0 | 5.0 |

Briefly give two reasons to explain why enzymes **A** and **B** give the results shown above. [8]

ii) A number of derivatives of Cibachron blue (C) were synthesised, and their affinities for the NADH cofactor utilising enzyme alcohol dehydrogenase (ADH), measured as shown below:




| **R** | **Apparent $K_d$ (μM)** |
|---|---|
| *m*-$COO^-$ | 0.06 |
| H | 0.2 |
| *o*-$COO^-$ | 0.2 |
| *p*-$COO^-$ | 5.9 |

a) Which of these R groups gives the stronger affinity between ADH and the Cibachron blue derivative? [3]

b) Briefly describe why the Cibachron dye derivatives have an affinity for ADH. [4]

c) Briefly explain why ADH has a stronger affinity for the dye given in your answer to question (a) above, than for the other derivatives. [5]

**PLEASE TURN OVER**

5.

a) By reference to the purification of plasmid DNA (pDNA) for use as a human gene therapy vector, describe the limitations of particulate-based high performance liquid chromatography (HPLC) when operated at large scale. [12]

b) Outline the principles and operating features of membrane chromatography systems and the advantages they offer for the industrial purification of pDNA gene therapy vectors. [13]

6.

a) Describe the design features and respective advantages of mixer-settler units and centrifugal phase contactors for use in industrial liquid-liquid extraction processes. [10]

b) By reference to Part (a) explain why centrifugal phase contactors are most commonly used in industrial bioprocesses. [6]

c) Your company wishes to evaluate a continuous, counter-current liquid-liquid extraction process for the recovery of a novel antibiotic compound. The antibiotic is present in the clarified broth at a level of 85 g $kg^{-1}$ and the broth can be supplied at a mass flow rate up to 400 kg $hr^{-1}$. The broth is to be extracted with butyl acetate. Based on laboratory tests the equilibrium distribution coefficient of the antibiotic in a broth–butyl acetate two-phase system is 5. Calculate the number of theoretical stages required to recover greater than 95% w/w of the antibiotic if the mass flow rate of butyl acetate to be used is 300 kg $hr^{-1}$. Clearly state any assumptions made. [9]

A sheet of graph paper is supplied.

**PLEASE TURN OVER**

7.

a) Describe all the stages in going from a protein molecule to a fully aged precipitate about to enter the settling region of a continuous flow centrifuge. In particular you should show how the process environment at each stage is likely to affect protein structure and the precipitate particle structure. [10]

b) It is proposed to respecify the agitator speed in a precipitation reactor in the hope that this leads to significantly larger particles and hence easier centrifugal recovery.

Prepare a detailed report, giving all calculations, showing how you would test out, at laboratory scale, the likely effectiveness of this design change. [15]

Design details of stirred precipitation reactor:

reactor height = diameter = 3 m
current agitator speed = 200 rpm
impeller diameter = 1 m
suspension density = 1100 kg $m^{-3}$
suspension viscosity = 0.010 Ns $m^{-2}$
Proposed new agitator speed for large scale reactor = 100 rpm

Test reactors are of 100 ml volume and of the same geometry as the full scale reactors The test reactor have variable speed drives.

**END OF PAPER**