UNIVERSITY COLLEGE LONDON

University of London

EXAMINATION FOR INTERNAL STUDENTS

For The Following Qualifications:-

B.Eng.   *M.Eng.*

Biochemical Eng E129: Bioprocess Recovery and Purification

COURSE CODE	:	**BENGE129**
UNIT VALUE	:	**1.25**
DATE	:	**30-APR-04**
TIME	:	**10.00**
TIME ALLOWED	:	**3 Hours**

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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.

Define the following:

Rejection coefficient
Sigma factor
Homogenisation
Cut off point
Breakthrough curve [10]

Homogenisers and disk-stack centrifuges are two examples of equipment originally designed for operation in a different process industry. How do the demands of the biological process industries impact on the design and operation of such items? [15]

2.

If you were asked to assess the suitability of an expanded bed absorption (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 differences 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]

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 and why? Support your answer with appropriate equations. [10]

3.

In what bioprocess applications might you decide to use membrane separation in preference to centrifugal separation? What engineering criteria would be used to determine which of the two methods of separation would most likely be the more appropriate?

[10]

The performance of a membrane is affected by a number of parameters and design variables. Provide a list of the key features that will determine the clarity of filtrate and the throughput realised. [10]

Fouling of membranes is a severe issue and limits the performance of the unit operation. How can fouling affect both the throughput and separation of a membrane? In what ways could you alter the operating conditions of a membrane process in order to minimize the impact of fouling? [5]

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4.

High – performance liquid chromatography (HPLC) has been used to separate two proteins, bovine serum albumin (BSA) and myoglobin (Mb) from solution. Pilot runs in a laboratory type of HPLC indicate that the capacity factors k_1 for BSA and k_2 for Mb are 0.9 and 1.2, respectively. The column length and inside diameter of the HPLC are 0.6 m and 7.5×10^{-3} m, respectively. The relationship between the heights equivalent to a theoretical plate (HETP) and the flow velocity is:

$$HETP = A + B/u + Cu$$

where u is the flow velocity and $A = 9 \times 10^{-5}\ m^2/s$, $B = 2.2 \times 10^{-9}\ m^2/s$ and $C = 1.8$ s.

1) What resolution of the two proteins may be expected when the flow velocity is 3×10^{-4} m/s? [5]

2) To achieve a complete separation of the two proteins, the resolution should be higher than 1.5. What should the flow velocity be to achieve a resolution of 1.5? [5]

3) At scale – up, a large HPLC column with the same characteristics of packing material is to be used. The column length and inside diameter are 1.5 m and 0.108 m, respectively. To achieve the same resolution of 1.5 as the laboratory – scale column, what should the flow velocity be? [5]

4) The minimum flow rate for the large column is 10 L/h. Does this have any impact on the operation and performance of the column? If the performance of a large column is not satisfactory, discuss the reasons why and suggest possible solutions. [10]

5.

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]

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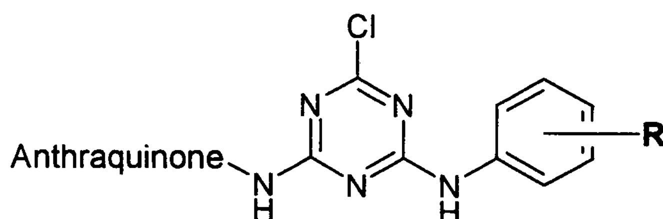
ii) A number of derivatives of Cibachron blue (C) were synthesised, and their affinities for the NADH cofactor utilising enzyme alcohol dehydrogenase (ADH), measured as shown below:



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]

6.

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]

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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