# UNIVERSITY COLLEGE LONDON

*University of London*

## EXAMINATION FOR INTERNAL STUDENTS

*For The Following Qualification:-*

*Eng.D.*

**Bioprocessing G1: Downstream Processing**

| | | |
|---|---|---|
| COURSE CODE | : | **BENGEG01** |
| DATE | : | **20-MAY-03** |
| TIME | : | **10.00** |
| TIME ALLOWED | : | **3 Hours** |




**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.** The following specification for a microfiltration membrane has been provided to you by a new supplier. Provide an engineering critique of this supporting your analysis by theory wherever possible showing how you would assess each element of the specification, i.e. membrane, module and performance data.
*"The membrane material we recommend for your protein concentration step is our latest cellulose acetate material in the form of a hollow-fibre geometry. Typical fluxes will be in the range of 100–200 L/m$^2$/h. The membrane has a MW cut-off at 25,000 making it ideal for the total rejection of your product at 30,000 whilst ensuring high transmission of all species below this site"* [15]

Having selected your membrane unit it is now necessary to decide upon whether to operate batch or fed-batch. Summarise the features of both ready for a process decision to be made. [10]

**2.** The table below shows an experiment in which a 15kDa antibody fragment (Fab) and a 60kDa monoclonal antibody (MCA) were compared as immunoaffinity chromatography ligands for antigen X.

| | Fab column | MCA column |
|---|---|---|
| Silica used (g) | 1.0 | 1.0 |
| Ligand used (mg) | 4.0 | 4.0 |
| Ligand coupled (mg) | 3.4 | 2.0 |
| Capacity for antigen X (mg) | 0.80 | 0.15 |
| Antigen X recovered (mg) | 0.62 | 0.12 |
| % of maximum recovery | | |

i) Comment on and compare the quantity of ligand coupled in each column. [5]

ii) Comment on and compare the capacity for antigen X in each column. [5]

iii) Calculate the % maximum recovery in each case. [3]

iv) Comment on and compare the % recovery and total yield of antigen X in each column in terms of accessibility of the antigen-binding surface of each ligand. [6]

v) What characteristics constitute an ideal support for affinity chromatography? [6]

**3.** i) Centrifuges are widely used in the recovery of proteins and in the separation of solids during manufacture. For each of the following duties determine which design of machine would be most appropriate and justify your answers.

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a) Removal of a small concentration of fine particulates prior to chromatographic separation. [5]

b) Recovery of the product phase from a fermentation of E.coli expressing an excreted product which is highly labile. [5]

c) Recovery of a voluminous precipitate phase generated by ethanol precipitation. [5]

i) Explain the origins and use of Sigma ($\Sigma$) theory and comment on the necessity for the inclusion of correction factors in such analysis. [10]

**4.** Expanded bed chromatography (EBA) seeks to replace conventional process flowsheets. Develop an engineering critique of the method to address the perceived advantages and disadvantages of the technology. [15]

Using a mass balance derive an expression that relates the height of expansion in EBA system with the average bed voidage. Comment also on how the voidage will change with liquid velocity and explain why this occurs [10]

5. A pharmaceutical intermediate, AX-415, is to be recovered from a bioconversion medium by liquid-liquid extraction. Laboratory scale tests have previously shown that the molecule has a single pK value of 5.0, is rather prone to acid hydrolysis and has an equilibrium distribution coefficient of 12 when extracted with hexane.

a) Outline a complete process flow sheet for the isolation of AX-415 justifying your choice of operations and commenting on the likely pH and composition of each stream. [11]

b) Explain why any solvent extraction step is likely to be situated early in the downstream process sequence. [6]

c) Following removal of the biocatalyst, the aqueous medium contains 85 g $kg^{-1}$ of AX-415 and can be provided at a flow rate up to 400 kg $hr^{-1}$. Calculate the number of theoretical stages required to achieve recovery greater than 95% w/w of AX-415 if the flow rate of the extraction solvent, hexane, to be used is 300 kg $hr^{-1}$. Clearly state any assumptions made. [8]

A sheet of graph paper is supplied.

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6. The broth from a 10000 L (total volume) fermenter is to be clarified by continuous centrifugation. The resulting clarified broth will be treated by precipitation and centrifugation to concentrate up an extracellular product. You are required to put in place a laboratory-based strategy to evaluate the proposed design and specification (see below) of the industrial scale operation. This strategy is to include:

i) a flowsheet to define the full large scale process sequence. [3]

i) the design of a 100 mL batch scale precipitation reactor with specifications for its operation. [12]

ii) an evaluation of the potential interactive effects across the whole process which would need to be examined at the laboratory scale. Credit will be given for prioritisation of these interactive effects to be studied. [10]

*Process specification:*

*Clarified fermentation broth (density 1000 kg/m$^3$, viscosity 0.003 Nsm$^{-2}$) is fed to a 10000 L stirred tank vessel (vessel height = diameter; impeller diameter is one third vessel diameter, impeller speed = 50 rpm) Precipitant (2000 L of ethanol) is added to the broth to effect precipitation (final total volume 9000 L). After 1 h the precipitate is to be recovered in a continuous disc stack Centrifuge operating at 1500 L/h. Precipitate is to be stored and subsequently redissolved and filtered prior to chromatographic separation. Final product is to be used for therapeutic purposes (repeat injectables).*

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7 A small laboratory column of inner diameter 0.02 m contained a packed bed of 0.5 m high Sephadex will be used to separate a mixture of bovine serum albumin (BSA) and myoglobin (Mb). The studied results will be used to scale up the process using a large column of inner diameter of 0.06 m and height of 0.8 m.

a) What experiments do you need to run and what data do you need to collect to generate the constants in the Van Deemter equation? [6]

b) If the Van Deemter equation for the small column has achieved:

$$H = A + \frac{B}{u} + Cu$$

c) where A = $6.2\times10^{-5}$ m, B = $2\times10^{-9}$ $m^2$ $s^{-1}$, and C = 1.13 s. At what flow velocity is the column most efficient use of the column? [5]

d) To achieve the same resolution for the large column, what flow velocity should you choose? [10]

e) If running at your chosen flow velocity, the resultant resolution is not as good as expected, give suggestions (no more than three) to improve your resolution as well as explaining why such changes would help. [4]

**END OF PAPER**