UNIVERSITY COLLEGE LONDON

University of London

EXAMINATION FOR INTERNAL STUDENTS

For The Following Qualifications:-

Eng.D. *M.Sc.*

Bioprocessing G1: Downstream Processing

COURSE CODE	:	**BENGEG01**
DATE	:	**11-MAY-05**
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.

Define the following:

Trans-membrane pressure drop
Hollow fibre microfiltration device
Homogenisation
Filter aid
Settling velocity

[10]

Tubular bowl and disk stack centrifuges are often used in the recovery of biological materials. Comment on how the design of these items impact on the operation and their respective utility for a range of typical materials including rDNA products. [15]

2.

Your company wishes to use expanded bed adsorption. It is unclear as to the mechanisms of the process and to the rules for scale up. Provide a design brief that explains the basis of this method of protein processing. [15]

Comment on the practical utility of gradient elution at industrial scale. [5]

In most bioprocess a number of individual chromatographic stages are used in order to achieve the final end product specification. Why is this the case? Comment on the likely positioning of gel filtration within a process sequence. [5]

3.

"Membranes are often used in preference to centrifugal separation"

To what extent is this a valid statement?

Defend your answer with examples drawn from across a typical bioprocess sequence. [15]

How would you select the right membrane and correct geometry for a given application? [10]

PLEASE TURN OVER

4.

A successful chromatographic procedure has been developed as seen in Figure A. Subsequently the possibility of speeding the separation up was examined by increasing the flow rate the chromatogram seen in figure B was generated.

i) Calculate the resolution of the two chromatograms, which of the two has the higher number of theoretical plates? [10]

Figure A

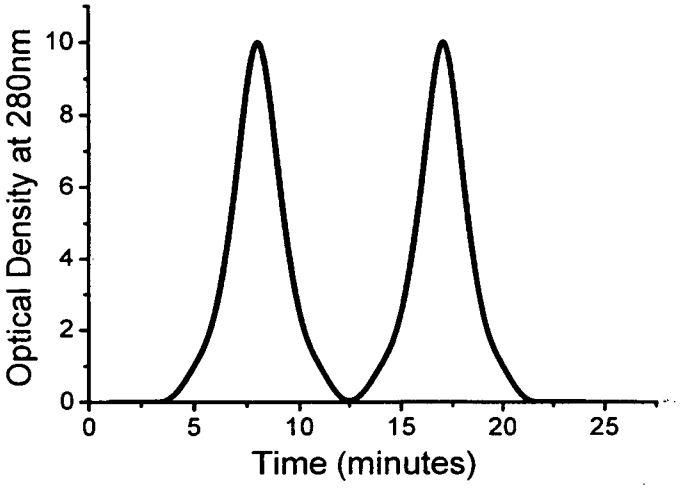
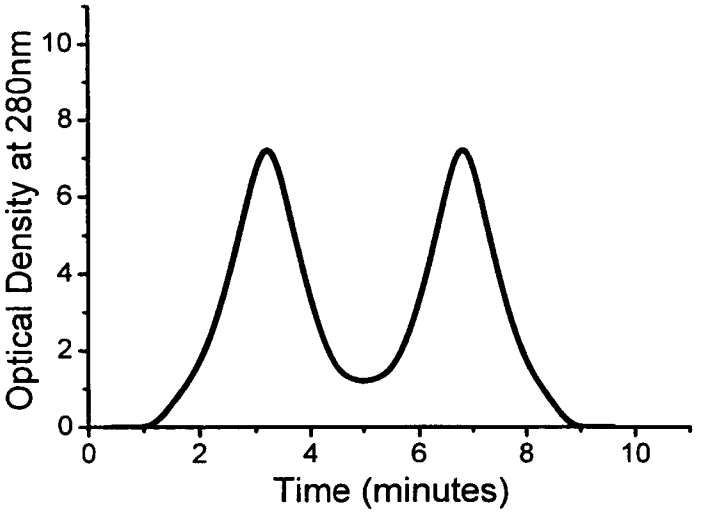


Figure B



ii) Use rate theory with particular reference to the van Deemter equation to explain what is observed? [10]

iii) What factors not covered by the van Deemter equation should be considered if this separation was scaled-up? [5]

PLEASE TURN OVER

5.

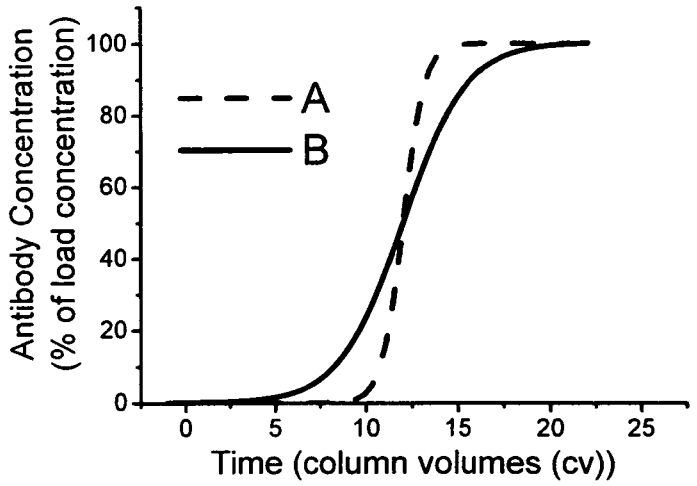
A newly developed synthetic affinity ligand for antibody adsorption is being both ligands are immobilised to the same chromatography beads. The synthetic matrix has shown comparable binding capacity when studied using batch equilibrium experiments in the laboratory. The two matrices were then packed into chromatography columns and the breakthrough curves obtained are shown below.

i) Give binding capacities (g l^{-1}), throughput (g h^{-1}) and productivity (g h^{-1} l^{-1}) assuming loading to 2.5% breakthrough for both columns. (Answers should be given to 2 significant figures). [10]

ii) From the data shown in the breakthrough curve why did the batch equilibrium experiments show the capacity of the two matrices to be equal? [2]

iii) Why are equilibrium experiments unsuitable to accurately determine capacity of chromatography matrices? [3]

iv) An error in the laboratory means it is uncertain which breakthrough curve corresponds to which matrix. Data have also been gathered on the affinity of antibodies for the 2 matrices, the data are shown below. Using this data which breakthrough curve corresponds to the synthetic matrix and which to protein A, giving reasoning. [5]



Operating Conditions: Linear Velocity in the column 150cm h^{-1} (for the whole column cycle), Column Height 15cm, Column diameter 1.6cm, antibody concentration in the load material 2g l^{-1}.
Column cycle: Load (X? column volumes) + wash (3 cv) + elution (2 cv) + regeneration (6 cv) + equilibration (4 cv).
Affinity Data
Synthetic Ligand K_D - $1x10^{-6}M$
Protein A K_D - $1 \times 10^{-9}M$
(K_D – dissociation rate / association rate)

CONTINUED

v) Sketch the effect of reducing the flowrate by 50% on the breakthrough curves, briefly give your reasoning. [5]

6.

A pilot scale counter-current extraction process is to be used to recover a new antibiotic, F911, from a fermentation broth containing 40 g kg^{-1} of the antibiotic. The operation is to be performed in a series of centrifugal extractor units using 100 kg hr^{-1} of feed and an equal flow rate of fresh butan-1-ol as the extracting solvent. At the pH and temperature of operation, the equilibrium distribution coefficient for F911is 2.

(a) Describe the design and operation of centrifugal contactors when used in liquid-liquid extraction processes. List the key advantages of centrifugal contactors over alternative phase contacting devices. [12]

(b) If it is required to reduce the concentration of F911 in the raffinate to less than 1 g kg^{-1} use a graphical method to calculate the number of theoretical stages required. [6]

(c) Further laboratory studies have shown that using an alternative solvent, ethyl acetate, results in a doubling of the F911 equilibrium distribution coefficient. What affect will this have on the number of stages required and the operation of the extraction process? [7]

A sheet of graph paper is supplied.

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7.

You are investigating the precipitation of a highly labile plasmid DNA material in a $10m^3$ vessel (vessel height equals diameter) equipped with a stirrer operating at 100 rpm (stirrer diameter equals one third vessel diameter) and the subsequent precipitate recovery in a disc stack centrifuge. The viscosity of the precipitate suspension is 2 centipoise and its density 1g/mL. A total mixing time of 30 minutes is used before passing the material at a flowrate of $2m^3 h^{-1}$ to a disc stack centrifuge. Overhead air pressure is used to give a low shear feed system. The centrifuge performs well with a partial discharge every 10 minutes of a precipitate sediment equivalent to one tenth of the bowl volume.

A change in fermentation protocol has led to a 100-fold increase in the level of plasmid DNA. As a result the viscosity of the resultant precipitate suspension has increased 20-fold.

You need to address the following questions to deal with the design evaluation:

i) Characterise the operation of the current vessel in terms of mean velocity gradient and Camp number. Comment on suitability of these values. [9]

ii) What will be the new value of mean velocity gradient and Camp number when processing the new material? How might the properties of the precipitate change? [4]

iii) Describe how the operation of the feed system and the centrifuge will have to be changed to recover the precipitate [4]

iv) Recommend a procedure to evaluate the preparation of a precipitate for the new material at bench scale prior to carrying out a full-scale trial (an actual calculated design of a small scale vessel is NOT required). You will need to comment on the interaction between precipitate preparation and its centrifugal recovery as well as the effect of these stages on subsequent plasmid DNA purification by high resolution chromatography. [8]

To note 1 centipoise equals 0.001 Nsm^{-2}

END OF PAPER