Loughborough University

23CGP062
Downstream Processing

Semester 2 2023/24 In-Person Exam paper

This examination is to take place in-person at a central University venue under exam conditions. The standard length of time for this paper is **3 hours**.

You will not be able to leave the exam hall for the first 30 or final 15 minutes of your exam. Your invigilator will collect your exam paper when you have finished.

Help during the exam

Invigilators are not able to answer queries about the content of your exam paper. Instead, please make a note of your query in your answer script to be considered during the marking process.

If you feel unwell, please raise your hand so that an invigilator can assist you.

You may use a calculator for this exam. It must comply with the University's Calculator Policy for In-Person exams, in particular that it must not be able to transmit or receive information (e.g. mobile devices and smart watches are **not** allowed).

Attempt **THREE** questions in total. Each question carries 25 marks.

Candidates should show full working for all calculations and derivations.

1. (a) Provide three examples of crystals. Provide three common shapes of crystals. State two properties at molecular level which are common to these different crystals.

[3 marks]

(b) Explain the reasons why crystallisation technology is widely used in the downstream pharmaceutical manufacturing from the point of view of both the properties of the crystals and the crystallisation process.

[4 marks]

(c) Provide the definition of a supersaturated solution and supersaturation. Explain the relation between supersaturation and crystallisation processes.

[6 marks]

(d) Celecoxib is a nonsteroidal anti-inflammatory drug (NSAID) used to treat mild to moderate pain and help relieve symptoms of arthritis, such as inflammation, swelling, stiffness, and joint pain. The solubility of Celecoxib in water and propanol at 313 K is shown in Table Q1. Antisolvent crystallisation is used to get Celecoxib crystals. Based on the experimental information, the final solution for crystallisation process should contain at least 20 volume % of water in a mixture of water and propanol to keep the stability of the compound.

(i) Estimate the maximum yield for antisolvent crystallisation at constant temperature of 333 K. Design the crystallisation process with the data in Table Q1 to further increase this yield and explain the advantages and disadvantages of your newly designed crystallisation processes.

[6 marks]

Continued/…

Table Q1 Solubility of Celecoxib in water and propanol mixture at 313 K - 333 K

Volume percentage of water in mixture solvents	Solubility of Celecoxib in mixture solvents (mg / ml)		
	At 273 K	At 303 K	At 333 K
0 %	0.1	0.2	0.3
20 %	0.3	0.6	0.7
40 %	1.0	1.2	1.5
60 %	1.2	1.4	1.7
80 %	1.4	1.6	1.9
100 %	1.6	1.9	2.2

(ii) Provide four techniques to be used to measure the polymorph and purity of these crystal products obtained from your designed crystallisation processes. Explain the importance of the stable polymorph and high purity for pharmaceutical companies.

[6 marks]

2. (a) An important consideration when drawing up a downstream recovery scheme is where the product is, i.e. whether the target species is extracellular, intracellular or the cells themselves. State what 4 other points you would consider when preparing a downstream recovery scheme. Provide a brief explanation why each of these is important. [6 marks]

(b) Name two techniques that can be used in industrial scale operations for cell rupture. Explain briefly the principle of one of these. Compare and contrast these techniques stating an advantage and a disadvantage for each. [5 marks]

(c) Aqueous 2-phase extraction technique is applied to recover an enzyme from a bulk of 200 litres which initially contains 2.8 units of enzyme/ml. A polypropylene- dextrose mixture is added and two phases form. The enzyme partition coefficient is 3.75.

(i) What volume ratio of upper to lower phases should be chosen to achieve 90% recovery of enzyme in a single extraction step? [4 marks]

(ii) What is the concentration factor for 90% recovery? [4 marks]

(d) (i) What is the first step of a downstream recovery scheme? Name 2 unit operations that work on different principles and can be used for this step. [2 marks]

(ii) Consider a case where the product of interest is the cells themselves and the cell containing broth at the end of fermentation is highly viscous. State two challenges relating to the first step of downstream processing. How would you perform this first stage to address these challenges? [4 marks]

3. (a) Explain the reasons for 'salting-in' and 'salting-out' of proteins during the salting-out precipitation of proteins from colloidal solutions. Provide the change in protein solubility with salt concentration profile of a typical salting-out precipitation process and show both phenomena on the diagram.

[5 marks]

(b) A preliminary laboratory experiment on salting-out precipitation of a recombinant monoclonal antibody produced by CHO cells shows that the solubility values of the antibody in 1.5 M (molar) and 2.5 M ammonium sulphate solutions are 18 g L^{-1} and 6 g L^{-1}, respectively. The molecular weight of the antibody is 155,000 kg. $kmol^{-1}$ while that of ammonium sulphate is 132 g mol^{-1}.

(i) Determine the value of the salting-out constant, K_s. [4 marks]

(ii) Predict the solubility of the antibody in water in g L^{-1}. [4 marks]

Relevant equation:

$$\ln S = B - K_S C_S$$

where the symbols have their usual meaning.

(c) A hormone is being recovered from 10 L of a biological fluid by affinity adsorption. The adsorption process follows a linear isotherm and the concentration of hormone in the fluid is 0.01 g L^{-1}. 80% of the hormone is adsorbed in the batch mode if 10 mL of affinity adsorbents are used. If 50 mL of adsorbents are used,

(i) Determine the concentration of bound/ adsorbed and unbound/ unadsorbed hormones. [5 marks]

(ii) Determine the amount of hormone recovered from the biological fluid. [2 marks]

(d) Lysozyme is purified from egg white solution, which also contains ovalbumin and conalbumin proteins. At pH 7, lysozyme is positively charged while other proteins are negatively charged. If the isoelectric pH of lysozyme, ovalbumin, and conalbumin are 11, 4.6, and 6.1, respectively, name a chromatography technique that can be used to separate only lysozyme from the egg white solution. Explain the process with a schematic diagram. [5 marks]

4. (a) (i) Explain why a co-current configuration is better suited to the spray drying of heat sensitive products than a counter-current configuration. [6 marks]

(ii) Also, explain why a counter-current configuration is better suited for drying non-heat sensitive products. [3 marks]

(iii) How and why would recirculation flows of product within a counter-current spray dryer affect product quality? [2 marks]

(b) A food powder is to be manufactured by co-current spray drying. 50 kg h^{-1} (wet weight) of feed liquid will be dried from a wet basis moisture content of 50% to a wet basis moisture content of 5% (equivalent to an ERH/water activity of 10%). Air will be taken from outside the factory (assumed to have a temperature of 25°C and relative humidity of 50%), and electrically heated to 220°C before introducing into the spray dryer. The outlet gas temperature will be 100°C.

Assuming adiabatic operation of the spray dryer, calculate/estimate:

(i) The equivalent dry basis moisture contents of the feed and product. [2 marks]

(ii) The inlet and outlet absolute humidities of the gas. [4 marks]

(iii) The mass flowrate of air required on both a dry and wet basis. [4 marks]

(iv) The temperature of droplets immediately after atomisation. [2 marks]

(v) The temperature of the dried powder at the spray dryer outlet. [2 marks]

A psychrometric chart is attached (Figure Q4).

Q4 Continued/…

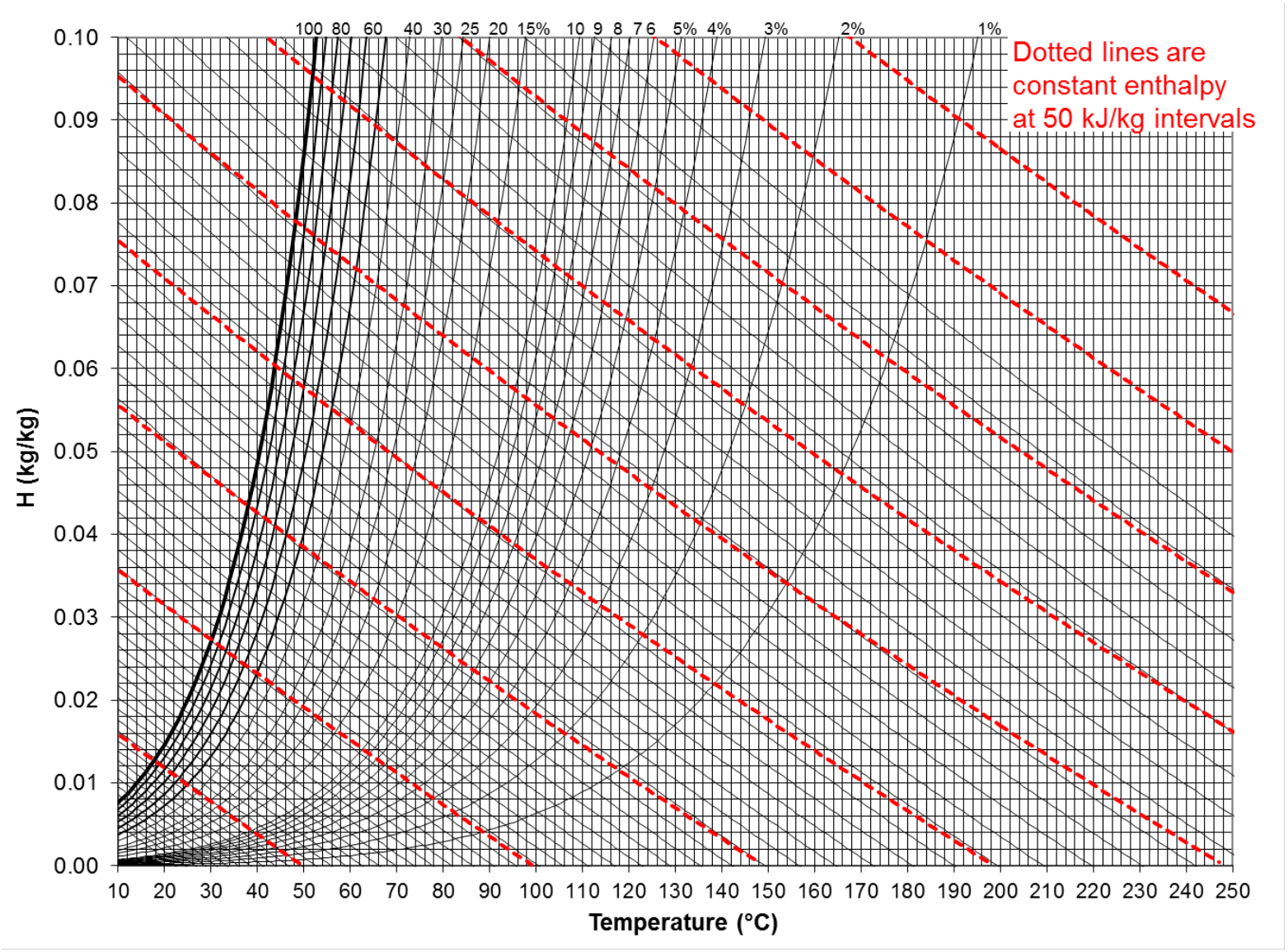

Figure Q4. Psychrometric chart for the air-water vapour system

END OF PAPER

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