

THE RELEVANCE OF PARTICLE SIZE IN PROTEIN PROCESSING

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Part 1. Introduction

Useful biological materials, such as proteins, often occur in a complex mixture of other materials. This is true both for materials for which a production route has to be devised, such as fermentation, and of materials which are to be extracted from natural resources, such as blood plasma.

Hence, the preparation of such materials in a useful form normally requires a sequence of separation and purification operations. The choice of the most appropriate operations and their linkage into a viable and economic processing chain is described by the term "process synthesis".

A schematic of the separation processes typically involved in an overall separation and purification sequence for a product produced by fermentation is shown in Figure 1.

It is important that each step in such a multi-step scheme is operated with the highest possible efficiency, for the value of the product is often very high. It may be seen that many of the separation process options are controlled by the size and surface electrical properties of the product to be recovered: centrifugation, filtration, membrane microfiltration, ultrafiltration, precipitation, adsorption, extraction, gel filtration and crystallisation.

Due to the complexity of mixtures of biological materials, process synthesis has until recently relied heavily on qualitative judgements based on experience and on extensive laboratory and pilot plant testing. However, recent developments in methods for the characterisation of biological materials, coupled with mathematical descriptions of the individual processes, are leading to quantitative predictive approaches to the design of such bioseparation schemes. Such approaches can lead to better separation schemes and a reduction in time from initial process concept to full-scale product production.

This note gives examples of some of these developments. First, it is shown how photon correlation spectroscopy (PCS) can be used to choose the optimum conditions for the microfiltration of protein solutions. Secondly, it is shown in part 2 (MRK383a), how measurement of electrophoretic mobility and hence determination of zeta potential can be used to test descriptions of the surface properties of proteins and hence validate predictions of their properties over a wide range of solution conditions. Thirdly, it is shown in part 3 (MRK384a), how a knowledge of protein size and protein zeta potential can result in the successful prediction of process operation, with examples from the ultrafiltration and ion exchange of proteins.

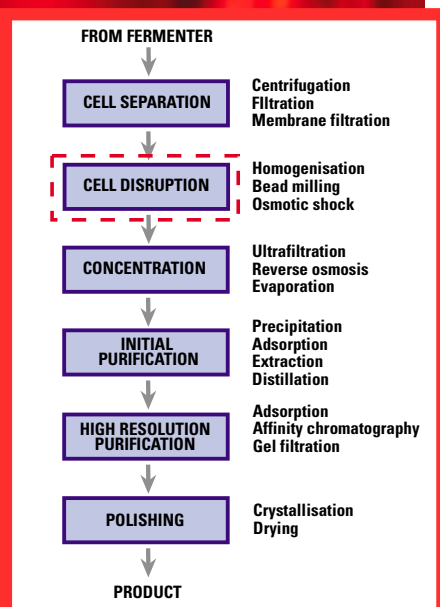


Fig1. Schematic of the options for recovery and purification of biological products from fermentation broths.

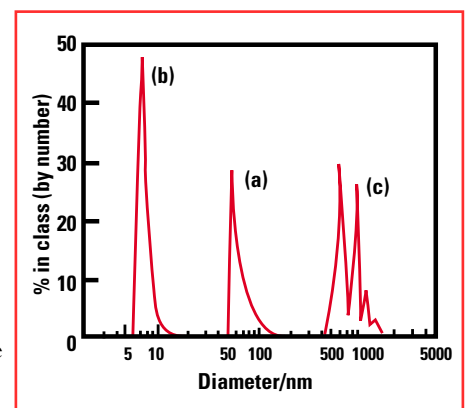


Fig2. Size distribution of YADH as a function of ionic strength in phosphate buffer at pH 7.5:(a) 0.001M, (b) 0.01 M, (c) 0.1M. YADH concentration = 0.25 g/l

Sizing of proteins in solution and successful microfiltration

Membrane microfiltration is now an established unit operation in the biotechnological industries. Microfiltration membranes are well suited to the separation of particles in the size range 0.1 to 10.0 μ m. Thus, an important application of such a process is in the removal of microbial cells from fermentation broths. Another significant biotechnological application of membrane microfiltration is in polishing and sterilization of product solutions. In both of these examples it is important that the product in solution, for example, a protein, passes freely through the membrane so that product losses are small. Thus, the size of the protein in solution is of critical importance. For efficient product recovery it is essential that the processing conditions are such that protein aggregation is minimum, for aggregates may be removed by the microfilter.

Photon correlation spectroscopy is a good method of determining the size and degree of aggregation of proteins in solution. It has the advantage of allowing measurements to be made directly on the solutions used for filtration. A suitable instrument is the Malvern Zetasizer 1000 HS or 3000 HS system equipped with a 50mW Uniphase 532nm laser. Such a system allows sizing to 1nm under favourable conditions. This instrument can be used to find the optimum solution conditions for

the microfiltration of solutions of the enzyme yeast alcohol dehydrogenase (YADH) (2), which has a molecular weight of 150,000, an isoelectric point in the range 5.4–5.8 and a pH range of stability from 6.0 to 9.0. It had initially proved very difficult to microfilter solutions of this enzyme with a high yield. PCS measurements were made to determine the degree of aggregation of the protein as a function of ionic strength and pH. Figure 2 shows that the enzyme exists in solution as essentially discrete molecules at a buffer concentration of 0.01M, but is extensively aggregated at either higher or lower buffer concentration. Figure 3 shows that the enzyme exists in solution as essentially discrete molecules at pH 7.5, but is aggregated at either higher or lower pH.

Hence, PCS identifies that the best solution conditions for transmission of the enzyme through a microfiltration membrane are a buffer concentration of 0.01M at pH 7.5. Use of these conditions did indeed result in successful process operation with a high recovery of the enzyme.

Determination of the size of proteins in solution may also aid the optimisation of other widely used separation processes, in particular precipitation followed by filtration or centrifugation. In these cases it is important to ensure that sufficient aggregation has taken place to allow successful separation.

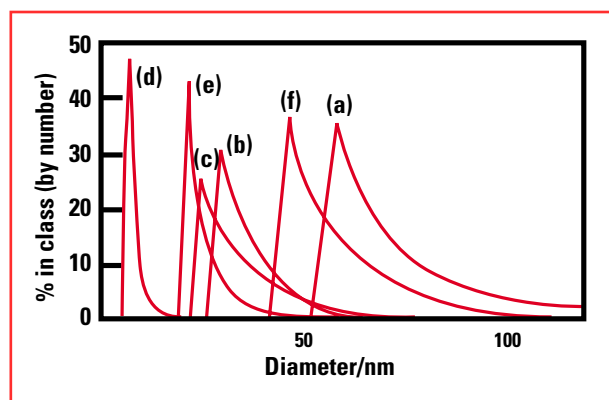


Fig3. Size distribution of YADH as a function of pH in phosphate buffer of ionic strength 0.01M:(a) pH 6.0, (b) pH 6.5, (c) pH 7.0, (d) pH 7.5, (e) pH 8.0 and (f) pH 9.0
YADH concentration = 0.25g/l

References

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2. W.R. Bowen and N.J. Hall, Properties of microfiltration membranes: mechanisms of flux loss in the recovery of an enzyme, Biotechnology and Bioengineering 46 (1995) 28-35.

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