EXPERIMENTAL METHODS IN MODERN BIOTECHNOLOGY

Editors

Ibrahim Ali Noorbatcha

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Preface

The rapid growth of biotechnology as the interdisciplinary field involving scientists and engineers has given rise to new challenges in carrying out experiments in diverse areas ranging from examining the biological systems to the purification and production of products of commercial importance. The idea of writing a book on Modern Experimental Methods in Biotechnology in such a diverse area is a very daunting task. However, there is a need for such a reference book for senior undergraduate students or beginning researchers in biotechnology, providing an integrated view of the experimental techniques along with the underlying principles, exemplified with case studies or sample results, and pointing out the advantages and limitations of the methods. A modest beginning towards this direction has been attempted in this book by combining the expertise and the experience of the researchers at the Department of Biotechnology Engineering, International Islamic University Malaysia and their collaborators who have been doing or teaching these experiments for the past one decade. For example, a selected set of experiments ranging from straight forward liquid-liquid separation process to the more latest direct nucleation control approach for the control of crystal size distribution in crystallization processes have been described in detail. In addition to extraction and purification of some selected compounds, a set of experiments to screen for metabolic disorders and anti-cancer activities are described with suitable examples. Any biotechnology experimental methods books will be incomplete without the inclusion enzyme assay methods, which has been described in this book as a set of three simple experiments. A flavor for the use of computers in biotechnology is provided in a chapter on homology modeling. Even though the list of experiments included in this book is not exhaustive, the reader will find that the experiments covered are fully self contained with good explanation of the theory behind the experiments and details about how to carry out these experiments. A more exhaustive coverage of a wide variety of other experiments in biotechnology will be included in the later editions.

I. A. Noorbatcha
M. I. A. Karim
H. M. Salleh

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CHAPTER 15

Direct Nucleation Control: A Novel Approach for the Control of Crystal Size Distribution in Crystallization Processes

Mohd Rushdi Abu Bakar, Zoltan Karman Nagy, Ali Nauman Saleemi and Christopher David Rielly

1. Introduction

Crystallisation from solution is an important unit operation used in various industries as a technique for separating solid materials in purified forms. It is a technique of choice for solid-liquid separation due to its capability of producing high purity materials. In biopharmaceutical industry, the crystallisation operation is often critical because it determines the product properties, such as the crystal size distribution (CSD), morphology and polymorphic form. The CSD is of primary importance since it influences subsequent downstream operations such as filtration and drying, and the product therapeutic performance such as dissolution rate, bioavailability and stability (Abu Bakar, 2010). Driven by the United States Food and Drug Administration's (FDA) Process Analytical Technology (PAT) initiative and the Quality-by-Design (QbD) concept, the development of control approaches, which can improve the manufacturing of products with desired properties, has become of significant interest.

PAT-based control approaches involve the use of *in situ* analysers that are able to measure and monitor product quality and critical process properties in real-time. This real-time process information is integrated into a control framework to provide an optimal control strategy with the aim of producing products with consistent desired quality. In addition to the provision of kinetic data that are required in the development of a robust process, real-time monitoring also allows appropriate remedial actions to be taken during the process if undesirable product quality or process property are identified (Yu *et al.*, 2003).

Lasentec focused beam reflectance measurement (FBRM) is one of the *in situ* analysers that has been used extensively in the crystallization processes to provide qualitative as well as quantitative information about crystallisation process, which comprises of nucleation and crystal growth (Abu Bakar *et al.*, 2009a; Abu Bakar *et al.*, 2009b; Barrett & Glennon, 2002; Chew *et al.*, 2007a; Doki *et al.*, 2004; Howard *et al.*, 2009; Nagy *et al.*, 2008; Simon *et al.*, 2009). FBRM uses a laser beam sent through fibre optics to an immersion probe tip where it is finely focused by a rotating lens, which causes the beam to scan in a circular path through a sapphire window at a fixed high speed. The beam then passes into the solution under study and when it hits a solid particle suspended in the solution, light is scattered in many directions, but only light scattered back towards the probe is collected. The solid particle continues to back-scatter the light until the beam reaches the opposite edge of the crystal. The time period of the backscattering (Δt) is recorded and multiplied by the scan speed of the beam (v_b) to give the distance between one edge of the solid particle to the other (s). This distance measured by FBRM is called a chord length. Fig. 1 shows a schematic diagram of backscattered light pulses detection and chord length measurement.

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