Applied Biochemistry and Bioengineering

VOLUME 3

Analytical Applications of Immobilized Enzymes and Cells

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Preface

The inherent advantages of immobilized enzymes as reusable components in biospecific analytical systems have long been recognized. The great advances since the mid-1960s, and particularly over the last decade, in the methodology of covalent fixation of proteins as well as in the theory underlying the behavior of immobilized biocatalysts, described in the earlier volumes of this publication, have been accompanied by parallel developments in chemical and clinical analysis. These efforts, aided by recent progress in microbiology and electronics, have led to the emergence of several novel approaches based on immobilized enzymes (and currently also on whole microbial cells) acting in conjunction with a sensing device; thus various types of enzymic or microbial electrodes and continuous, automated analytical procedures utilizing enzyme tubes or columns are gaining prominence. In biomedical areas solid-phase enzyme-linked immunoassay methods of a high degree of sophistication are increasingly being used; in these assays the molecular recognition properties of antibodies are combined with the high sensitivity associated with enzyme-based analytical methods. The advantages of the newly emerging solid-phase analytical techniques notwithstanding, their acceptance has been relatively limited—for reasons which reflect in no small measure some of the pragmatic tenets of analytical chemistry and chemists. It is the aim of this volume, "Analytical Applications of Immobilized Enzymes and Cells," to present a survey of recent developments as well as trends arising from interdisciplinary interactions and overlap, and thus to help bridge the gap that still exists between research and application.

The first part of this volume is devoted to systems which have been brought to a high degree of development in terms of both methodology and instrumentation, and which are (or in principle can be) utilized in routine analysis, i.e., enzyme tubes, enzyme thermistors, and enzymic or microbial electrodes; a theoretical analysis of electrode design concludes this section. The second part of the book contains chapters on solid-phase enzyme immunoassays and on techniques for *in vivo* monitoring of metabolites. The concluding section contains an economic evaluation of the use of high-purity enzymes in analysis and an assessment of the significance of recent advances in electronics, particularly in microprocessors

and computer science, in relation to future developments in enzyme-based analysis.

The major editorial efforts for this volume were carried out by Leon Goldstein; however, the order of the volume editors was kept the same as for Volumes 1 and 2 to avoid any confusion in library cataloging of Volume 3.

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Open Tubular Heterogeneous Enzyme Reactors in Continuous-Flow Analysis

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I. INTRODUCTION

The use of enzymes in analytical chemistry has made spectacular advances over the last score of years. Nevertheless, the relatively high cost and limited stability of enzymes frequently hampered efforts to exploit fully the high specificity of these powerful biocatalysts. Hence, the development of techniques that allowed for the sequestering of enzymes without loss of catalytic activity gave rise to the expectation that enzyme immobilization would offer many convenient ways to utilize enzymes in a reusable form. It is believed that immobilized enzymes in combination with appropriate sensors will give rise to "reagentless" analytical procedures that are much simpler and more reliable than classical methods, and which lend themselves readily to use in automated analyzers.

The content of the present volume bears witness to the multitudinous approaches that have evolved from the endeavor to explore the advantages of bound enzyme technology in analytical chemistry. In this chapter we attempt to give an account of the development, properties, and applications of narrow bore tubes, with immobilized enzymes at the inner wall, that are used in continuous-flow analyzers of the type manufactured by Technicon Corporation. Such tubular wall reactors are appropriately termed open tubular heterogeneous enzyme reactors and are designated by the acronym OTHER. They are also described among others by names

such as "bound enzyme coils" or "enzyme tubes." Several types of such enzymic appliances are commercially available for continuous-flow biochemical analyzers. Figure 1 depicts a photograph of an immobilized enzyme coil used as a plug-in module for glucose assay on Technicon's SMAC high-speed analyzer. Details of glucose analysis by using this particular enzymic appliance are given later in this chapter.

The first published reports on the use of "tube-supported enzyme derivatives" in continuous-flow analyzers appeared in 1970 (Hornby *et al.*, 1970; Sundaram and Hornby, 1970). The approach taken by Hornby and co-workers was to treat chemically the tube inner wall so that the tube inner surface becomes activated first. The reactive functions at the surface serve as bridging groups for the subsequent immobilization of the enzyme. As a result, the tube inner wall supports a monomolecular enzyme layer. The amount of enzyme immobilized per unit of length may be increased by etching the tube in order to enhance the surface area available. This approach to the preparation of bound enzyme tubes has given rise to some very elegant and elaborate chemical methods for the treatment of a variety of tube surfaces to generate reactive groups.

The enzymic activity that can be "packed" into a given length of enzyme tubes prepared by such a procedure is quite limited, however, in comparison to that obtained with a packed bed in a conduit having comparable dimensions. Owing to the relatively low catalytic activity of the wall, long tubes are needed to obtain appreciable conversion of the substrate and thus sufficient sensitivity of analysis. On the other hand, sample throughput is limited by axial dispersion of the sample zone that can reach considerable magnitude in liquid flow through long open tubes. The range of linearity may also be restricted even at relatively low substrate concentrations when the observed reaction rate is controlled by nonlinear enzyme kinetics.

Another way of preparing open tubular heterogeneous enzyme reactors originated from early work on coating the inner wall of narrow bore tubes with an adsorbent layer for use in gas chromatography (Horváth, 1963). For the preparation of the first OTHERs, the inner wall of nylon tubes was coated with a layer of cellulose-bound trypsin (Cs. Horváth and R. T. Light, unpublished results) in the late 1960s. Subsequently, other methods were developed to prepare tubes with a relatively thick porous annulus (Horváth *et al.*, 1972, 1973a,b; Horváth and Solomon, 1972). Such tubes, which were first developed for medical applications, show exceptionally high stability and catalytic activity so that the observed reaction rate is determined by the radial transport of substrate to the enzymic wall, i.e., the reaction is diffusion controlled with tubes having dimensions of practical interest, even if they are operated with segmented