USE OF IMMobilIZED ENZYMES IN CHEMICAL ANALYSIS

GEORGE G. GUILBAULT

Department of Chemistry
University of New Orleans
New Orleans, Louisiana 70122

Accepted May 1, 1978

Immobilized enzymes are becoming increasingly popular as analytical reagents because of their reusability, stability, and sensitivity to many inhibitors that would seriously interfere in assays using soluble enzymes. In this article, some of the kinetic and catalytic effects of immobilized enzymes in analysis will be discussed. The shift of the activity-pH profile curves on immobilization, the changes in temperature dependence, the inhibitor constants ($K_i$), Michaelis constants ($K_m$), and the maximum velocity ($V_{max}$), plus others, will be discussed. Finally, the use of these immobilized enzymes in fluorometric and electrochemical monitoring systems will be shown, and the future of these reagents in various areas will be discussed.

A survey of enzyme electrodes will be presented as an example of the use of immobilized enzymes. Application of immobilized enzyme technology to the assay of BUN, glucose, uric acid, amino acids, ethanol, and other metabolites will be discussed.

INTRODUCTION

Excellent chemical analysis can be performed with enzymes, which are biological catalysts; the real advantages of immobilized enzymes are many in analyses using electrochemical probes or other methods of analysis. One advantage of the immobilized enzyme is a pH shift; i.e., the pH optimum can be shifted to that region at which one wants to make a measurement, by choosing the right support for immobilization. Take an enzyme with a narrow pH range of, say, 6–8; this can be shifted on immobilization down to the acidic side or, conversely, up to the basic side. The enzymes are furthermore much more stable. In some work at Edgewood Arsenal, Maryland, we actually heated our enzymes to 150°F and brought them back down to room temperature, with very little loss of activity. No soluble enzyme could be treated in this fashion.

One advantage often overlooked is that better selectivity can be realized with the enzyme when immobilized; this insolubilized reagent

becomes much more selective for an inhibitor, and only the most powerful inhibitor can actually attack the enzyme. We demonstrated this several years ago in an immobilized cholinesterase alarm for the assay of organophosphorus compounds in air and water. No other common interferants disturbed the alarm—it responded only to organophosphorus compounds.

In 1961 at Edgewood Arsenal, I first experimented with some soluble enzymes, such as glucose oxidase, and developed an electrochemical assay for glucose. This led to the use of immobilized enzymes with a commercially available ion-selective electrode sensor to form one self-contained sensor that could be used to measure either organic or inorganic compounds which are primary or secondary substrates for the immobilized enzyme. The base sensor can be glass; i.e., the cation response can be measured (the ammonium ion, for example), or the pH change in a penicillin electrode can be measured, as done by Mosbach and Papariello and others. Or a gas membrane can be used as a base sensor, such as the ammonia or the CO₂ membrane. Next are the polarographic sensors which measure peroxide or oxygen, or any of the solid membrane electrodes, i.e., the cyanide electrode. For example, the enzyme can be placed on top of a flat glass electrode sensor; a membrane is then put over the outside of this sensor to hold the enzyme in and keep things like catalase and bacteria out. This protects the enzyme from bacterial spoilage, which is one of the primary reasons for loss of enzyme activity.

With potentiometric devices, we can measure the response either by a steady-state (i.e., equilibrium) method measuring millivolts or microamperes, or by a rate method which senses the change in millivolts or microamperes per minute. Measurements of substrate can be performed by either a steady-state or a rate method. But measurements of enzyme activity must be done by a rate method. This is a point often hazy in the literature—one can find many claiming that they are measuring enzymes by steady-state methods. This is impossible by basic definition of enzyme activity. Enzymes are catalysts and have to be measured by a rate method, but this may be either an interrupted or a continuous measurement of rate. In Table 1 is presented a compilation of enzyme electrodes—this list is not by any means complete. It is the first in a series of tables that were published in a recent book of mine, *Handbook of Enzymatic Analysis* (Dekker, New York, 1977). In this table are listed the enzymes that act on these various materials, and some of the base sensors that might be useful. Take as a typical example glucose, which can be assayed with glucose oxidase:

\[
O_2 + \text{glucose} \xrightarrow{\text{oxidase}} H_2O_2 + \text{gluconic acid} \tag{1}
\]

One can measure the uptake of oxygen with a gas membrane electrode, a technique pioneered by Clark and perfected by Hicks and Updike, or