Due to the pivotal role of glucose in physiological processes, much effort has been devoted to the development of methods for detecting glucose in food and biological matrices. However, the development of direct methods has been hindered by the lack of a suitable chromophore or electrophore, and hence more complicated methods have been adopted for glucose determination. Two of these methods, pulsed amperometric determination and enzyme-based biosensors, are discussed in this article.

**Pulsed Amperometric Detection**

Electrochemical detection using a fixed potential works best with molecules for which the products of the electron transfer reaction are stabilized by delocalization through a conjugated π system. Such stabilization is not possible for glucose, and hence the free radicals formed by oxidation or reduction are high energy; that is, these processes have high activation energies. Therefore, a large overpotential is required, which is a disadvantage in electrochemical detection. Catalytic oxidation at moderate potentials by platinum and gold electrodes is possible through a mechanism that involves adsorption of glucose to a clean electrode surface \( F_1 \). Unfortunately, the products of this catalytic oxidation can remain adsorbed to the electrode surface, which blocks the catalytic sites and prevents further oxidation. Consequently, the behavior of glucose at platinum and gold electrodes at fixed potential is characterized by a decrease in the activity of the electrodes \( F_2 \).

The deleterious effects of product adsorption can be reversed and the catalytic activity of the electrode surface can be restored by a two-step cleaning process following a detection step \( F_3 \) \( (1) \). The first step is at a potential more positive than the potential used for detection, and the second step lies at a more negative potential. The effect of these two potential steps can be understood by examining the rotating disk voltammogram of a gold electrode in 0.1 M sodium hydroxide in the absence (a) and in the presence of glucose (b) \( F_4 \). When no glucose is present, there are two major features in the rotating disk voltammogram. The anodic current on the forward scan at A is due to the formation of a surface oxide layer, and the cathodic current on the reverse scan at C is due to dissolution of this layer. The addition of glucose leads to a considerable increase in the anodic current at G. The dissolution of the oxide layer proceeds more readily because the aldehyde and alcohol groups at the bare electrode surface have higher activation energies than the aldehyde groups in the oxide layer. The current at G is still larger in the presence of glucose due to oxidative desorption of the adsorbed oxidation products. The dissolution of the oxide layer...

Some of the more commonly used methods for the electrochemical determination of glucose are discussed, with particular emphasis on pulsed amperometric detection and enzyme-based sensors.
on the reverse scan allows the catalytic oxidation to recommence, and this is shown by the oxidative current at H.

The optimum detection potential for glucose is in the potential region in which there is no oxide formation and no oxygen reduction (oxygen reduction occurs at potentials more negative than -0.1 V). The adsorbed oxidation products that result from this catalytic oxidation can be oxidatively desorbed by applying a more positive potential. However, the application of this potential also leads to the formation of an oxide layer, which leads to passivation of the surface. The catalytic activity of the electrode surface is restored by the application of a more negative potential, causing the dissolution of the oxide layer. Detection of glucose (and other sugars) using this triple-pulse potential wave form is referred to as Pulse Amperometric Detection (PAD) or Pulsed Electrochemical Detection (PED) (1) (the latter term is also used for other pulsed detection techniques). The effectiveness of PAD is illustrated in F2, which compares the reproducibility of the chromatograms using PAD (F2A) and constant potential detection (F2B). It can be readily seen that the peak currents for the constant potential detection decrease with time, whereas those for PAD remain constant.

Catalytic oxidation of glucose has also been reported using electrodes modified with copper salts or fabricated from copper metal (2). The optimum potential for the detection of glucose is independent of the method used to make the copper electrode, which suggests a common mechanism (3) (it has been proposed that this mechanism involves the copper(III) oxidation state (4)). One major advantage of the copper electrodes is that oxidation products are not adsorbed. Therefore, there is no need for pulsed potential wave forms, and a constant potential can be used for detection (F5).

The catalytic oxidations discussed above are specific only for the appropriate functional group—that is, the alcohol or aldehyde groups. Therefore, there is no discrimination between glucose and other sugars or alcohols inherent in PAD, and chromatographic separation is required. The other commonly used method for the determination of glucose is based on the reaction of glucose with various enzymes. This approach has the advantage of the specificity inherent in enzymes (5).

**Enzyme-Based Sensors**

The enzyme most widely used for glucose detection is glucose oxidase. The active site of this enzyme is a flavin adenine nucleotide (FAD), which exists in one of two forms—oxidized (FAD) or reduced (FADH$_2$). FAD oxidizes glucose to gluconic acid, and the FADH$_2$ generated by this reaction can be oxidized to FAD by oxygen (hydrogen...
peroxide is a by-product of this reaction). This mechanism is shown in F6.

A schematic diagram for an enzyme-based sensor is shown in F7. The enzyme is immobilized on the surface of an electrode, and this immobilized layer is covered by a membrane. The function of the membrane is to provide stability, and it can also be used to prevent potential interferants from reacting with the enzyme. The electrode assembly is placed in the solution containing the analyte, which can readily diffuse through the membrane, and into the immobilized enzyme layer.

In order to be used as a glucose sensor, glucose oxidase activity must be converted into an analytical signal. The earliest glucose sensors were based on measurements of either the decrease in the oxygen (reactant) concentration or the detection of hydrogen peroxide (product) (the concentrations of both oxygen and hydrogen peroxide can be determined electrochemically). However, the accuracy of oxygen concentration measurements is limited by the natural variations in oxygen concentration. The detection of hydrogen peroxide by its oxidation at a platinum electrode requires a potential of +0.5 - +0.6 V (vs. silver/silver chloride), and hence is subject to interference by ascorbic and uric acids.

An alternative approach is to use oxidants other than oxygen to regenerate the oxidized form of FAD. The ideal method would be direct electron transfer between the active site and the electrode surface, but this is not possible for glucose oxidase since the active site is embedded within the protein. Hence, electron transfer must be achieved through the use of mediators. Commonly used mediators include ferrocene, potassium ferricyanide, and ruthenium and osmium complexes. The cyclic voltammograms of a sensor based on ferrocene is shown in F8 (6). In the absence of glucose (A), the reversible cyclic voltammogram of ferrocene is seen. The addition of glucose is accompanied by an increase in oxidation current (B), which is characteristic of catalytic oxidation of glucose oxidase by ferrocene. However, there are
some problems associated with this method. Since mediators are small molecules, they can diffuse out of film immobilized on the electrode surface, resulting in loss of catalytic activity. In addition, the oxidized mediator and oxygen can compete for oxidation of the active site.

One approach that has been well studied at BAS is that of “wired” enzyme electrodes (7-12). The mediators for these systems are osmium bipyridine complexes, which are cationic and bind electrostatically to the anionic glucose oxidase. This allows facile exchange of electrons between the osmium centers of the complexes and the active site of the enzyme. One of the coordination sites of the osmium complex is occupied by the N-atom of an imidazole or pyridine moiety of a polyvinylimidazole or polyvinylpyridine polymeric unit (F9). These polymeric units react with a diepoxide to form a cross-linked, three-dimensional redox polymer which remains immobilized on the surface of the electrode (F10). Electron transfer between the active site and the electrode surface is achieved by “electron-hopping” between the osmium centers attached to polyvinyl units; hence, the active sites are considered to be “wired” to the electrode surface.

Some typical results for the characterization of “wired” glucose oxidase are shown in F11-13 (9). The redox polymer for these studies was based on a polyvinylimidazole polymer bound to an osmium center coordinated to methyl-substituted bipyridine ligands. The hydrodynamic voltammograms of this electrode in the absence and in the presence of glucose are shown in F11. When there is no glucose present, the hydrodynamic voltammogram is characteristic of a surface-bound redox species (the osmium complex); however, the addition of glucose causes an increase in the oxidation current that is characteristic of a catalytic process. The effect of oxygen on the variation of the limiting current with glucose concentration is shown in F12. At low glucose concentration, the competition with oxygen causes a significant decrease in the catalytic current, but there is essentially no effect at higher glucose concentrations. The effects of interferents (50 µM ascorbate, 0.4 mM urate and 1 mM acetaminophen) is shown in F13. Lack of interference from these molecules can be attributed to the low potential required for oxidizing the osmium mediators (i.e., it is not positive enough to cause oxidation of any interferants).

Detection of Hydrogen Peroxide

As mentioned above, one method for the determination of glucose concentration is the mea-
measurement of the concentration of hydrogen peroxide generated by the reoxidation of the active site of the enzyme. Oxidation of hydrogen peroxide at a platinum electrode is prone to interference from ascorbic acid and uric acid, and hence other methods for hydrogen peroxide determination have been investigated (10,11,13-18). One approach has been the use of an enzyme electrode based on the reduction of hydrogen peroxide by horseradish peroxidase. This enzyme is different from glucose oxidase in that direct electron transfer between the active site and the electrode surface is possible (“mediatorless” electron transfer) (13-15). However, there are advantages to using mediator-based enzyme electrodes, some of which have been illustrated by studying a system based on a “wired” peroxidase electrode consisting of cross-linked polyvinylpyridine (10).

The hydrodynamic voltammograms for the “wired” peroxidase electrode with no coordinated osmium complexes in the absence (a) and in the presence (b) of hydrogen peroxide are shown in F14. The addition of hydrogen peroxide causes a much larger increase in the oxidation current (F15) (about two orders of magnitude larger). In the first case, electron transfer can only occur between the electrode surface and the active sites of enzymes that are in direct contact with the electrode surface, whereas in the second case, active sites throughout the whole immobilized layer are “wired” to the electrode surface. Many more active sites are therefore available for the catalytic process, and the current is correspondingly greater.

The “wired” peroxidase electrode has been used for detection of glucose following separation by liquid chromatography, and its performance has been compared with
that of a platinum electrode (11). The experimental setup is shown schematically in \textbf{F16}. Note that the eluant from the column first passes through an IMmobilized Enzyme Reactor (IMER) containing glucose oxidase, which generates the hydrogen peroxide to be detected at the electrode. A solution containing ascorbic acid and uric acid in addition to glucose was passed through the chromatography column. The chromatograms recorded using the platinum electrode and the peroxidase electrode are shown in \textbf{F17A} and \textbf{F17B}, respectively. The sensitivity and detection limit for glucose is better for the peroxidase electrode. This electrode is also more stable and less susceptible to interferants due to the lower potential required for its operation. In addition, the equilibration time required for the peroxidase electrode is less than that required for the platinum electrode.

\textbf{Conclusion}

Although the glucose sensors discussed above give good results in the research laboratory, there is no guarantee that the technology involved can be adapted for commercial use. The factors that must be taken into consideration when developing a commerically viable sensor have been previously discussed in this journal (19).

\textbf{References}

Experimental setup for the detection of glucose using an IMER followed by detection of hydrogen peroxide after chromatographic separation.

Chromatograms of 1 nmol glucose (G), 0.23 nmol ascorbic acid (AA), and 0.24 nmol of uric acid (UA) at A) a platinum electrode, and B) a “wired” peroxidase electrode.