Boron-doped diamond (BDD), a working electrode material developed for use with HPLC-electrochemical detection, promises to open up new areas for analysis with electrochemical detectors.

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Electrochemical detection (ECD) with HPLC has proven to be of significant value in measuring biologically and clinically relevant molecules. ECD detectors work by applying a voltage between a working and a reference electrode in a flow cell. As molecules pass through the flow cell, those that can be easily oxidised or reduced at the applied potential react at the working electrode, producing a flow of electrons (see Figure 1). The detector then measures this flow of electrons (see Figure 2). With the electronics available today, it is a highly sensitive measurement. Only those molecules that will oxidise (or reduce) at the electrode at the applied potential are detected, thus giving a high level of selectivity. Because of the inherent high sensitivity and selectivity, and wide dynamic range of the technique, it is used extensively in important areas such as brain research and the diagnosis of specific cancers.

ELECTRODE MATERIALS

Over the years, a number of materials have been used for the working electrodes. These include noble metals (such as Au, Ag and Pt) and various forms of carbon (carbon paste, graphite, and amorphous or glassy carbon). The carbon-based electrodes are considered general-purpose working electrodes and have found extensive use in a broad array of applications (1). All of these carbon-based electrodes share a common microstructure and demonstrate similar behaviour.

Despite the high utility of such graphitic and glassy carbon electrodes, they are limited in the molecules they can detect because of restrictions in the potential ranges that can be used with such materials. (They may even require high overpotential to produce a response. Some compounds simply do not react well while others actually require the working electrode to take part in the reaction mechanism, rather than just acting as an electron source or sink.) Additionally, some chromatographic conditions and sample matrices cause degradation in the electrode’s performance.
that cannot easily be restored during or following the analysis. This is often the case, resulting from adsorption of contaminants in the sample – or even the analyte – onto the electrode surface. An example of such an application is the analysis of thiols and disulfides in biological samples.

An ideal working electrode that can extend the utility and robustness of ECD would have many of the properties of the carbon electrodes but would be able to operate at more extreme (either higher or lower) potentials than typical carbon-based electrodes, without suffering from the high noise resulting from oxidation of water in the mobile phase.

During the 1980s, various forms of carbon became widely used as electrodes for general electrochemistry. This was due to the simplicity of their fabrication, their relatively low cost, and their ability to produce electrodes of high surface area. Applications included electroanalysis, energy storage devices, and electrocatalysis and reaction, as well as flow injection analysis and HPLC with ECD.

In the mid-1980s, techniques were developed for low-pressure diamond synthesis. Although diamond is mechanically resilient and a strong material, unfortunately, with its $sp^3$ orbital structure, it is notoriously inert and unsuitable for use as a working electrode material. Fortunately, methods became available for the inclusion of metal dopants in these diamond films, rendering the inherently insulating diamond film conductive. One such dopant is boron, forming boron-doped diamond (BDD). Typically, electrodes of boron-doped diamond are constructed on a supporting substrate – often silica, glassy carbon or metals. The polycrystalline, thin film is formed by chemical vapour deposition.

Considerable work has been published, originally on the properties of these materials and later on their use in numerous applications. Pioneering work in the early 1990s was conducted by Swain (2,3) and colleagues in the US, and Fujishima (4,5) and co-workers in Japan. Despite the extensive and impressive work of these groups, as well as others, the use of BDD as a material for analytical electrochemistry has remained primarily the preserve of research laboratories.

**THE BDD ELECTRODE**

Several features of the BDD electrode make it a favourable working electrode material for use in HPLC-ECD. As previously noted, diamond itself is an excellent insulator. When moderately doped with boron, the material behaves as a semiconductor, but at high levels of boron doping, diamond takes on metal-like properties, making it a suitable material for a working electrode. BDD electrodes have low capacitance (resulting in lower inherent noise), a uniform surface, high chemical and structural stability, and resistance to fouling. When used as an electrode, BDD can operate with a wider range of working potentials than glassy carbon.

**Enhanced Surface Stability**

The surface stability of the diamond makes it resistant to surface modification. It is common for thin-film carbon-based electrodes to change their properties over time (for example, oxygen termination versus hydrogen termination and so on), requiring extensive polishing or electrochemical processing to restore the original behaviour. Even at high potentials, the surface of the BDD working electrode remains inert and has a long working life without changing its characteristics. Because of the inherent characteristics of the surface, there is little or no fouling due to adsorption of contaminants from the analyte or sample matrix.

**‘Chip’ Electrode Design**

To take advantage of the properties of the BDD electrode, a thin-film amperometric cell design was chosen. The cell uses a maintenance-free palladium reference electrode (ESA Biosciences). The BDD is deposited on a wafer, which is then cut to the proper size and shape; the wafer is also coated with a conductive backing layer. This electrode chip is then placed into the ESA 5040 Analytical cell. Contact with the electrode and
sealing against a gasket is made with the pin assembly that makes continuous contact with the working electrode (see Figure 3, page 23). The cell is then connected to and controlled by a potentiostat, such as the Coulochem III detector.

APPLICATIONS OF THE TECHNOLOGY

Thiols and disulfides are widely recognised as biologically important molecules. For example, glutathione controls the potential of living cells and is involved in the metabolism of drugs. However, our knowledge of the role of thiols has been hampered by the difficulty in generating reliable analytical data. A number of methods are available, including those found in numerous publications describing the use of HPLC-ECD. Although electrochemical detection is a viable and desirable detection modality, it has not gained widespread use because of the problems when using carbon electrodes. Thiols are reactive and readily adsorbed; disulfides require a high potential and suffer from a poor signal-to-noise ratio. Contaminants in the mobile phase and sample matrix, unless painstakingly removed, cause a rapid degradation of response.

Choosing the optimal potential for oxidation of thiols, disulfides and thioethers requires the generation of a hydrodynamic voltammogram. This is done by injecting a given amount of the compounds onto the HPLC-ECD system at different applied potentials. The signal is then plotted as a function of the applied potential, and the optimal potential (typically where the response plateaus) is chosen. The optimum applied potential for this analysis is found to be +1,400 mV (versus Pd reference) (see Figure 4). This is well within the potential window of the BDD electrode, but too high for conventional glassy carbon working electrodes.

The BDD electrode demonstrates very good stability over time and multiple runs. The response is typically stable for at least 65 hours for standards with 1.5 per cent relative standard deviation (RSD) for reduced glutathione (GSH), and 6.5 per cent RSD for glutathione disulfide (GSSG). Routine analyses of plasma extracts do not adversely affect performance. Typical chromatograms are shown in Figure 5.

BROADENING THE SCOPE OF HPLC-ECD

Despite the high sensitivity and selectivity of HPLC-ECD, it has been limited in the scope of addressable molecules by the working electrode material. The BDD electrode broadens the range of molecules now addressable by this technique. Unlike other working electrode materials, BDD electrodes do not suffer from fouling and do not degrade when subjected to prolonged high-oxidation potentials. BDD is a robust and rugged working electrode material, well suited to the measurement of thiols and disulfides.

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References