Introduction to Amperometric Biosensor Configurations

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A brief perspective is given on the way electrochemical biosensors are used in commercial practice to help the novice classify the vast literature in this field. Single use, intermittent use, and continuous use applications are defined and compared.

Coupling oxidoreductase and dehydrogenase enzyme reactions to electrodes (amperometric and potentiometric) has been an attractive approach to developing sensors for thirty years. There has recently been considerable commercial success for glucose sensors (especially for medical purposes, but also in the food industry), and that market is well past $\$10^8$/year for electrochemical sensors and $\$10^9$/year for all sensors. The second most common analyte is lactate and it remains a distant second, probably a market at least 100-fold smaller than for glucose. In spite of thousands of publications on numerous other analytes (glutamate, amino acids, choline, etc.) and a continuing stream of highly repetitive papers on glucose and lactate, the technology remains in its infancy in many respects and the commercial challenges are very severe.

There are three principal means by which amperometric biosensors are employed in analytical systems. For simplicity, let's assume the common redox cross reaction catalyzed by an enzyme,

$$R_A + O_B \xrightarrow{enz} O_A + R_B$$

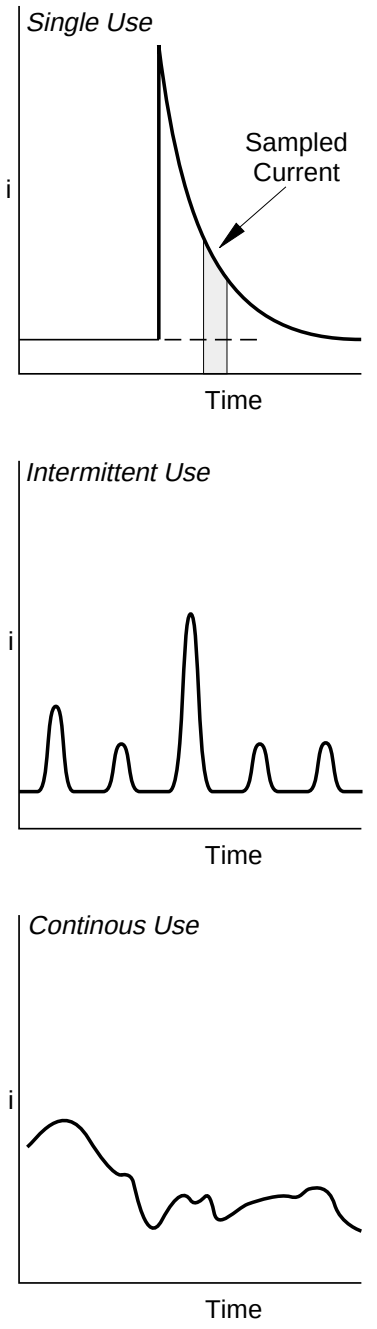
If R_A is the analyte, it is oxidized by O_B (present in large excess) to form O_A and R_B. Either the consumption of O_B or the production of R_B is determined as an indication of the analyte R_A (concentration or amount) originally present. As simple as this appears, there can be numerous problems associated with an inadequate supply of O_B, enzyme inhibitors in the sample, instability of the enzyme over time, irreproducibility of the electrode kinetics for reoxidizing R_B or reducing O_B, redox active interferences which either react at the electrode and/or couple with the reagent couple O_B/R_B, and inadequate temperature control. Several of these problems are mitigated when the analyte (substrate) concentration is high, the enzyme is rugged, and the enzyme kinetics are fast. These criteria are pretty well met by glucose oxidase and (in view of the clear commercial need) it is not surprising to find that everything else suffers by comparison. Glucose dehydrogenase is also a viable choice, but its use is less widespread.

There are three distinct configurations for using such amperometric sensors. These are depicted in ***F1*** based on the assumption that the reduced reagent, R_B, is reoxidized as the means to generate current related to the analyte O_A turned over to R_A.

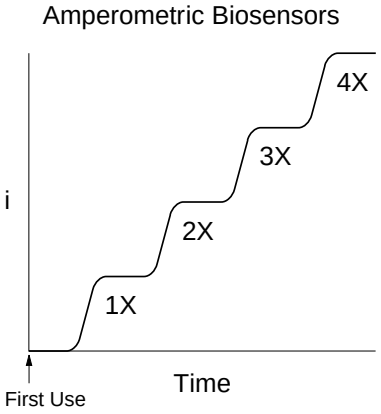
Single-Use Sensors

The single use approach represents 99% of the commercial value and probably less than 1% of the published papers (there are, however, a substantial number of patents). These are the glucose electrode strips available (in the USA) in virtually every pharmacy, supermarket, and discount store. The electronics is virtually free (<$50) by laboratory standards and actually free in many cases due to the fierce competition between the three major suppliers. The electrochemical cell and all the components are typically a $0.50 investment (it is amazing what volume can do for price). A free instrument with digital readout, loaded with software, and using a throw-away cell is not a common notion to chemists. As one who regularly spends $20,000 or more for an analytical instrument, such a possibility is mind-boggling.

The strips are typically two or three electrodes, some are screen printed and some are metal films. The sample application triggers a clock. At a fixed time after the reaction has been initiated (some 10s of seconds), a potential is applied and current is measured as RB is oxidized back to OA. The current is



F1 *The three configurations for using amperometric biosensors.*



F2 *The most "popular" figure in continuous-use biosensor papers.*

sampled and the data converted into concentration units for display.

Intermittent-Use Sensors

The second approach is to use a sensor intermittently. In this case, there is often a carrier stream and the samples are processed sequentially. In continuous flow systems, the current is measured. In batch systems (stopped flow), the rate of increase in current is sometimes processed. Instruments based on intermittent use are typically benchtop units which can be automated and cost a minimum of several thousand U.S. dollars ($\$10^3$-$10^4$). Unlike for the single use devices, pumps, autosamplers, and computers drive the cost and relatively few instruments are sold.

Continuous-Use Sensors

In the first two configurations we bring the sample to the electrode. It is also possible to bring the electrode to the sample in the form of a sensor which is dipped into or implanted into the sample. The sensor puts out a continuous signal reflecting (hopefully) the analyte concentration as a function of time.

T1 summarizes several of the features of these three schemes. The single-use units provide modest performance with very low up front cost, but rather high cost per data point. Intermittent units give excellent performance, but have a high up front cost. Intermittent units can provide detection limits even three orders of magnitude better than for single- or continuous-use sensors. This is because the background current can be precisely measured and because hydrodynamic electrochemistry is far superior to electrochemistry in static solutions. Calibration is easy for intermittent use instruments and not at all favorable for single- or continuous-use units.

Continuous-use biosensors are very low in cost both for the apparatus and for the data rate achieved. They give poor performance in detection limit and calibration in use is typically not favorable (or even possible). They are potentially very attractive, but in reality have been very unattractive.

F2 shows what I have called the most popular figure in continuous use biosensor papers. This is an idealized version, but the concept is universal. This figure is extremely misleading because frequently "first use" is very close to "first made." A more appropriate view is shown in ***F3***, which considers the practical issues in the manufacture of sensors which real customers might want to buy. There are three critical time periods (T1, T2, and T3) which very rarely are given any consideration in academic papers. The temperature and relative humidity during these time periods is frequently very critical. To develop a commercially practical device, T1 must be at the very least 60 days, T2 between 2 and 10 days, and T3 again at least 60 days (some would want 120).

Overall, if the sensor cannot be manufactured at least four months before it is put into use, it will have virtually no commercial value. We find that shipping a highly temperature sensitive sensor with a "cold pack" often causes the shipping expense to exceed the cost of the sensor and presents a very unfavorable situation both for ourselves and our customers. I believe that ignoring these issues is a primary reason that continuous use biosensors remain an academic curiosity where the primary product is publishable work and not useable sensors.

Conclusion

My academic colleagues always give me a very curious look when I point out these facts. In truth, we want nothing more than reliable biosensors which our company can build, sell, and ship. Having tested numerous schemes from the published literature, we find that

T1
A summary of the features of single use, intermittent use, and continuous use amperometric sensors.

	Complexity of Apparatus	QA in Use	Calibration	Precision	Accuracy	Cost vs. Data Rate
Single Use	Low	Poor	No	Poor (Adequate)	Poor (Adequate)	Very High
Intermittent Use	Moderate	Excellent	Easy	Good	Good	Moderate
Continuous Use	Very Low	Poor	No (External)	Good	Poor	Low

most fall very short of what is needed.

My purpose in writing this short article is first to give a perspective on the three common modes of use for amperometric biosensors and second to ask more of my academic colleagues to consider T1, T2, T3, and F3 when describing a new sensor approach.

For those unfamiliar with the current state of biosensor design, I recommend the following journals as a starting point: Biosensors & Bioelectronics; Analytical Chemistry Electroanalysis; Anal. Chim. Acta; and the Journal of Electroanalytical Chemistry.

Examining issues of these journals over the last year or two will reveal a number of interesting approaches from laboratories around the world. I remain quite skeptical about continuous use sensors. It is time to make them work or direct funding to more productive areas. Meanwhile, single and intermittent use sensors are proving to be very practical.

F3
Practical issues in the manufacture of sensors inevitably involve stability, both in storage and use.