# TWO-TERMINAL VOLTAMMETRIC MICROSENSORS

## BACKGROUND OF THE INVENTION

The United States government has rights in this invention by virtue of grants from the Office of Naval Research and the Defense Advanced Research Projects Agency.

This invention is in the general area of microelectrochemical devices and is specifically a two-terminal, voltammetric microsensor.

There is an ongoing need to provide stable, microelectrochemical sensor analogous to and integrable with traditional silicon wafer technology. Devices of this sort have been described, for example, in U.S. Pat. No. 4,895,705, U.S. Pat. No. 4,717,673 and U.S. Pat. No. 4,936,956 to Wrighton, et al., the teachings of which are specifically incorporated herein.

These devices still require separate reference and indicator electrodes in order to produce reproducible results.

It is therefore an object of the present invention to provide microelectrochemical sensors having both the indicator(s) and reference functions located on the same microelectrode.

It is a further object of the present invention to provide methods for making and using microelectrochemical sensors having both the indicator(s) and reference functions located on the same microelectrode.

## SUMMARY OF THE INVENTION

A voltammetric sensing system is prepared as a two terminal device with surface-confined, internal, chemically insensitive, redox reagent-based reference and chemically sensitive redox reagent-based indicator, having a voltammetric response as a function of chemical concentration. The preferred embodiment is a two-terminal voltammetric microsensor with an internal reference consisting of two electrodes: a counter electrode which is significantly larger in area than the working electrode, and a working electrode which is derivatized with at least two, surface-confined, reversibly electroactive, redox reagents, one reagent having an electrochemical response that is insensitive to the medium and that serves as a reference, and the other reagent(s) having electrochemical response(s) sensitive to analyte(s), serving as indicators(s) for the analyte(s). The working electrode may consist of more than one microelectrode, if they are shorted together and operated as one terminal of the two terminal device.

Current at the working electrode is recorded as its potential is swept versus that of the counter electrode in a two-terminal linear sweep voltammogram. If the counter electrode is large relative to the microelectrode, its potential does not change. The counter electrode area must be at least $10^2$ to $10^3$ times the working electrode area, depending on the accuracy of voltammetric detection desired. The area must be even larger if the working electrode has greater than monolayer coverage of electroactive material. Sensing is accomplished by measuring the potential difference(s) between the current peak(s) for the indicator reagent(s) and that for the reference reagent.

The key advantages of the device are that the sensor requires no separate reference electrode and that it functions as long as current peaks for the reference and indicator reagents can be detected. The device can be



utilized in a variety of sensing applications, including in vivo biomedical sensing. The small size of the working electrode with internal reference makes it minimally invasive and an external patch electrode can be used as the counter electrode. Other applications include sensing of industrial gases, sensing of anaesthetic gases, sensing in wastewater streams, and air pollution monitoring.

A pH sensor is described in detail, using ferrocenyl and quinone thiols bound to a gold electrode as the reference and sensing reagents, respectively. This device measures acidity at very high acid concentrations. A CO sensor is also described, using ferrocenyl thiol and a ferrocenyl ferraazetine disulfide bound to a gold microelectrode surface as the reference and sensing reagents, respectively. This device measures accumulated exposure to CO.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1A** illustrates cross-sectional views of a two-terminal, voltammemtric microsensor showing idealized response to a species L which binds to the indicator molecule M. FIG. **1B** are linear sweep voltammograms that reveal a difference between the current peak for oxidizing the reference molecule, R, relative to that for M or M-L, depending on the presence of L.

FIG. **2** illustrates a cross-sectional view of a two-terminal, voltammetric microsensor based on the self-assembly of a ferrocenyl thiol and a quinone thiol serving as reference and indicator, respectively.

FIG. **3** is a graph of $E_{\frac{1}{2}}$ versus SCE for the surface—confined ferrocene thiol as a function of pH (FIG. **3A**) and a graph of the difference between cathodic peak potentials for surface-confined acyl ferrocenium and quinone as a function of pH from two-terminal, voltammetric scans (FIG. **3B**). All data are from voltammograms recorded at 500 mV/s in 1.0 M $NaClO_4$ in buffered solution.

FIG. ***4a–4d*** show the cyclic voltammetry (500 mV/s) for a Au macroelectrode derivatized with alkyl ferrocene thiol and quinone thiol in buffers, from top to bottom: 1.0 M $NaClO_4$ buffered to pH 11 with phosphate; 0.1 M $CHClO_4$; 1.0 M $HClO_4$; 10 M $HClO_4$. The reference is the average position of the oxidation and reduction waves for the ferrocene system.

FIGS. **5A** and **5B** are linear sweep voltammograms (500 mV/s) and their first derivatives for oxidation of alkyl ferrocene thiol and hydroquinone thiol confined to an Au macroelectrode, in 0.1 M $HClO_4$ (FIG. **5A**) and in 3 M $HClO_4$ (FIG. **5B**). The potential scale is relative to the Fc oxidation wave.

FIG. **6A** illustrates a cross-sectional view of a two-terminal, voltammetric microsensor for CO formed using as the reference molecule, ferrocenyl dithiol, a redox molecule having a chemically insensitive formal potential, and ferrocenyl ferraazetine disulfide as the indicator molecule, a redox molecule having a CO dependent formal potential. FIG. **6B** is a linear sweep voltammogram where the reference wave (the wave at more negative potential) which is insensitive to CO remains constant in potential, while the indicator wave shifts 120 mV positive.

## DETAILED DESCRIPTION OF THE INVENTION

The two-terminal, voltammetric microsensor is formed of two components: