

(1) a microelectrode (referred to herein as the working electrode) having at least one chemically sensitive redox material and a chemically insensitive redox material located thereon; and

(2) a larger counterelectrode.

The redox materials are characterized as "surface confined", i.e., in electrical contact with the microelectrode, whether in a liquid electrolyte or in a solid state electrolyte.

As shown schematically in FIG. 1, the sensor 10 consists of a working microelectrode 12 and a counterelectrode 14, with two redox active materials 16 and 18 for detecting a chemical species L, where the indicator material 16 is sensitive to the presence of L and the reference material 18 is not. As shown in FIGS. 1B, 15 interaction of L with the indicator material 16 alters the difference in redox potential measured between the indicator 16 and the reference 18.

Detection is accomplished by measuring the potential difference, ΔE , associated with current peaks for oxidation (or reduction) of microelectrode-confined redox reagents, where the magnitude of ΔE can be related to the concentration of analyte. One of the electrode-bound reagents has an electrochemical response that is insensitive to variations in the medium and serves as the reference. At least one of the electrode-bound reagents is chemically sensitive and serves as the indicator. Current peaks for oxidation or reduction of the reference and indicator are determined from two-terminal, linear sweep voltammograms using a counterelectrode having an area much larger than the sensor electrode.

The key advantage of the device is that it does not require a reference electrode, since the indicator and reference molecules are both on the sensor electrode. A second advantage is that the sensor function is based on peak-to-peak separations, ΔE , rather than on peak amplitudes. Accordingly, the sensor can be useful as long as peaks can be located, even when slow decomposition of the electrode-bound reagents occurs.

The Counterelectrode

A relatively large surface area counterelectrode is needed so that the linear sweep of applied voltage yields only a change in potential of the sensor electrode. For example, for a working microelectrode with about one monolayer of redox active material, the counterelectrode is 10^3 times larger than the sensor electrode the counterelectrode will move only approximately 1 mV upon application of a one volt potential difference.

The counter electrode area should be at least 10^2 to 10^3 times the working electrode area for a working electrode with about one monolayer electroactive material, and for coverages of redox reagents above monolayer levels the counter electrode size should scale approximately linearly with the number of monolayers of electroactive material on the working electrode:

$$\text{counter electrode area} = [(>10^2 - 10^3) (\text{working electrode area})] \times [\text{number of monolayers of redox material}]$$

For biomedical applications the counter electrode can be a transdermal patch electrode. All that is required in any application is that it be sufficiently large, and that it be made of a material which is chemically inert to the medium so that its potential does not change significantly during the course of measurement. Suitable materials in many applications include platinum

and gold, and, slightly less versatile, stainless steel and carbon.

The Working Microelectrode

The selection criteria for use as reference and indicator reagents on the working electrode are:

1. Reversible redox activity with well-defined cyclic voltammetry oxidation and/or reduction peaks. Examples include:

a) redox molecules and material with molecule-based redox processes: e.g., ferrocenes, polyvinyl ferrocene, quinone, viologen, polyviologen, polyviologen-quinone, polycation charge compensated with electroactive metal complex (polyvinylpyridine H^+M^-).

b) inorganic materials and metal oxides: e.g. Prussian Blue, $Ni(OH)_2$, RuO_x .

Type a) redox reagents having molecular redox properties are generally preferable because they tend to exhibit the highest degree of Nernstian electrochemical reversibility but other types of materials, such as those discussed above, can be used.

2. One redox reagent, the reference, must have a redox potential which is insensitive to the chemical medium. Examples include ferrocenyl thiol, polyvinylferrocene, viologen, polyviologen, and polythiophene.

What qualifies as a suitable reference redox reagent can vary from application to application or medium to medium.

3. The other one or more redox reagent(s) must have redox potential(s) sensitive to the analyte. Examples include:

pH sensitive: quinone thiol (as shown in FIGS. 3 and 4); monoquaternized N-alkyl-4,4'-bipyridinium, RuO_x , and $Ni(OH)_2$.

CO sensitive: ferrocenyl ferrocene disulfide (as shown in FIG. 3);

alkaline metal cation sensitive: 1,1'-(1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diyl dimethyl), ferrocenyl thiol, and other ferrocene derivatives containing covalently attached cryptands. These materials are described, for example, Hammond, et al., *J. Chem. Soc. Perkin. Trans. I* 707 (1983); Medina, et al., *J. Chem. Soc. Chem. Commun.* 290 (1991); Shu and Wrighton, *J. Phys. Chem.* 92, 5221 (1988). Included are examples such as the above ferrocenyl ferrocene and ferrocenyl cryptand, in which an ordinarily chemically insensitive redox center (ferrocene) is covalently linked to a chemical recognition site in such a way as to make its redox potential chemically sensitive. Also suitable are molecules or polymers in which the sensor and reference functionalities are covalently linked such as 1-hydro-1'-(6-(pyrrol-1-yl)hexyl)-4,4'-bipyridinium bis(hexafluorophosphate), as described by Shu and Wrighton, *J. Phys. Chem.* 92, 5221 (1988).

4. The redox reagents must be durably surface confusable. Examples include:

a) spontaneously self-assembling (adsorbing to the working electrode) molecules: ferrocenyl thiols, quinone thiol, ferrocenyl isonitriles, porphyrinyl isonitrile, ferrocenyl ferrocene disulfide, ferrocenyl dithiocarbamates, on gold or platinum; ferrocenyl carboxylates on indium tin oxide.

Self-assembling reagents are preferred because they automatically yield single monolayers and it is easy to mix sensor and reference reagents on single microelectrodes.