

Non-faradaic Electrochemical Sensors: Principles and Practice

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In this paper the principles of non-faradaic electrochemistry are outlined, and non-faradaic electrochemical methods are described which provide: (i) a solution to the problem of devising a real-time biomass probe; and (ii) in certain instances a means of measuring enzymic behaviour *in situ*.

The linear, non-faradaic or 'passive' electrical properties of a biological or other system are completely characterized by its conductance (siemens) and capacitance (farads), reflecting, respectively, the in- and out-of-phase portions of the alternating potential difference caused in response to the application of an alternating current. These macroscopic properties depend in part on the size and geometry of the electrodes, and reflect the intrinsic properties of the system, permittivity and conductivity (see, for example, references 1–3). For plane-parallel electrodes of area A separated by a distance d , the relationship between the conductivity σ' and conductance G is $\sigma' = G(d/A)$, where (d/A) is known as the cell constant and has units of length^{-1} . The capacitance C is related to the permittivity ϵ' by $\epsilon' = C(d/A\epsilon_0)$, where ϵ_0 is an experimental constant equal to $8.854 \times 10^{-12} \text{ F m}^{-1}$, such that a cubic electrochemical cell of unit dimensions containing water (which has a permittivity of 78.4 at 298 K) has a capacitance of some 6.94 pF. By 'linear', we mean that the measured conductance and capacitance are independent of the magnitude of the exciting field, and that excitation by a current at a frequency f Hz leads only to an alternating potential difference at the same frequency.

Use of the Linear Dielectric Properties for Sensing Microbial Biomass

The dielectric properties of cellular suspensions themselves (as opposed to those of the suspending medium or the electrodes) are generally characterized by three major areas of frequency-dependence, known (in order of increasing frequency) as the α -, β - and γ -dispersions.² The β -dispersion, centred in the radiofrequency region of the electromagnetic spectrum, is caused predominantly⁴ by the charging of the large membrane capacitance C_m displayed by all intact cells. This is typically of the order of $1 \mu\text{F cm}^{-2}$, and is due to the possession by cells of non-micellar phospholipid membranes of molecular thickness. For spherical cells of radius r , present at a volume fraction P , the permittivity at low radiofrequencies exceeds that of the background by a value given by $9PrC_m/4\epsilon_0$. For non-spherical cells the factor $9/4$ is different. Hence, by measuring the dielectric permittivity of cell suspensions at low radiofrequencies, it is possible to design a biomass probe that is specific for viable cells (as necromass, particles, emulsions and gas bubbles do not have intact bilayer-type cell membranes; references 5–8). We have therefore developed a biomass probe (the bugmeter) suitable for the real-time estimation of biomass in fermentors *in situ*. The (steam-sterilizable) probe consists of four gold electrodes in an insulating matrix suitable for insertion in a standard 25 mm port. The outer two electrodes apply alternating current of a suitable frequency in the range 0.1–10 MHz while the inner two pick up the alternating potential difference. Biofouling can be obviated by the manual or automatic application of electrolytic cleaning pulses, while a similar system without cleaning pulses can be used to assess its extent.⁹ The bugmeter is suitable for use in all types of fermentations, and has been applied to various prokaryotic and eukaryotic microbes,^{4,5} pitching control in breweries,¹⁰ plant cells,¹¹ animal cells,¹² immobilized cells,¹³ the solid-substrate

tempe fermentation,^{14,15} and (as it measures biomass possessed of an intact cell membrane, and not necromass lacking one) in assessing cytotoxicity.¹⁶ The approach also represents a convenient means to control the biomass content of continuous microbial cultures.¹⁷

Non-linear Dielectric Properties of Cell Suspensions: Applications in Sensing

The above is based on 'classical' dielectric theory, which assumes that the system of interest contains structures, which, when excited by the field, relax only by frictional interactions with the (solvent) heat bath in which they are embedded, which obey the fluctuation-dissipation theorem, and which therefore give a linear response. None of these assumptions is likely to be true of enzymes *in vivo*.^{18,19}

We have constructed a dual-cell, non-linear dielectric spectrometer, and have applied it to the study of resting cell suspensions of *S. cerevisiae*.²⁰ Substantial, odd harmonics were generated by these cells when stimulated by modest sinusoidal electrical fields (about 2 V cm^{-1} , 20 Hz). The generation of these harmonics occurred only in living cells, and in a cell concentration-dependent manner. The ability to generate a third harmonic was observable only within narrow voltage and frequency windows, and was strongly inhibited by low concentrations of sodium metavanadate, suggesting that it might be ascribed largely to the H^+ -ATPase present in the plasma membranes of these cells, the k_{cat} of which is close to that of the optimum exciting frequency. In glycolysing cells, the third harmonic disappeared and was replaced by strong second and fourth harmonics. The ability to distinguish populations of enzymes which are turning over from those which are not (by whether they generate even- or odd-numbered harmonics) opens up many possibilities for the study of metabolism *in vivo*.²¹ These non-linear dielectric properties can also be observed as the generation of 'beat' frequencies when cells are excited by more than one sinusoid at a time.²² Non-linear dielectric spectroscopy might provide a convincing mechanism by which to account for the many reports of the ability of very weak electromagnetic fields to affect biological activity,²³ and should lead to a variety of novel biosensors.

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Recent Advances in Applied Electrochemical Gas Sensors

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Electrochemical gas sensors have been in commercial and research use since the work of Clarke.¹ Various groups have developed this technology for specific applications and some of the major advances have been seen in the work of Bergman,² Niedrach and Alford;³ latterly this technology has diversified, producing a body of literature and patents which now represents a significant academic as well as theoretical body of knowledge.

Applications of the technology are widespread, a fact to which this symposium itself is a tribute. In being selective and presenting some of the work in which Neotronics has been involved one must not overlook the wide contributions which have been made by others, but the commercial position of much of the work renders it unavailable to the wider academic sphere.

When dealing with the commercial exploitation of this technology strictly in the field of environmental gas detection (as opposed to medical or process control), one must acknowledge the contributions to the field from the following: development of permeable membranes for dissolved oxygen measurement; use of gas permeable membranes with sputtered electrode for atmospheric monitoring; and the development of porous PTFE electrodes using platinum black for fuel cells. These advances have enabled development of the electrochemical gas sensor into a sophisticated, accurate and reliable device. Typically, these are three electrode devices with an internal reference or pseudoreference operated by an external potentiostatic circuit of the general form shown in Fig. 1. Here

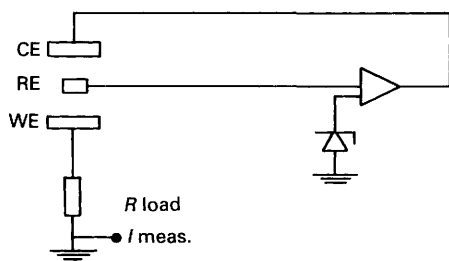


Fig. 1 Potentiostatic circuit. CE = counter electrode; RE = reference electrode; WE = working electrode

the secondary (counter) electrode potential is varied with respect to the working electrode such that the reference potential is at a constant potential to the working electrode.

From this technological base, many companies are now developing these sensors into devices which have greater

applicability. The commercial pressures forcing this development are providing many research groups with the opportunity to widen knowledge in other areas. The primary objectives which commercial companies are addressing at present are as follows: extending the range of gases to which this type of sensor is sensitive; reducing the size of this type of sensor to enable its use in smaller instrumentation without loss of performance; developing the electronic control and monitoring circuitry for this type of sensor to allow better performance and 'fail-safe' operation; improving the performance of existing sensors by the development of new catalyst formulations with particular properties; improving the understanding of the basic electrochemistry of these systems in order to deal with application-related problems.

Recent Advances in Sensor Technology at Neotronics and Co-operating Institutions

Whilst some of the work being undertaken at Neotronics is of a commercially sensitive nature, much can be described here in at least general terms.

Extension of the Range of Gases Measured

As a result of a collaboration with the group led by Pletcher⁴ at the University of Southampton, it has been possible to extend the range of this type of sensor to cover carbon dioxide. This has only been possible thus far in non-aqueous solvents, for example in the work of Alberty and Barron.⁵ In the work at Southampton an aqueous solution containing $[\text{Cu}(\text{DAP})_2]^{2+}$ [bis(propylene-1,3-diamine copper(II))] was used as a 'moderator'-like compound enabling the $\text{Cu}^{\text{II}}/\text{Cu}^{\text{I}}$ couple to be accessed as an electrochemically active system. Upon dissolution in the electrolyte, the CO_2 causes the $[\text{Cu}(\text{DAP})_2]^{2+}$ to decompose, liberating Cu^{II} ions; these are then reduced to Cu^{I} which are themselves readily re-oxidised by atmospheric oxygen to Cu^{II} . The removal of the CO_2 atmosphere results in the reformation of the complex. This work is described in a patent.^{6,7}

As an adjunct to the transfer of technology from the laboratory to the commercial sphere, much materials science and catalyst development work was undertaken to enable a reliable and reproducible product to be marketed.

Collaboration with the University of Strathclyde has also resulted in interesting developments. Professor Hitchman's group has not only provided the chemistry of an NO sensor, but has also, in the course of a general study of the chemistry and electrochemistry of NO and NO_2 on precious metal surfaces,

established an understanding of this system which will lay the theoretical basis for much further work.⁸

Miniaturization of Sensors

The reduction in size of these devices is not so attractive technologically as some of the other recent developments in this field, but has lead, nevertheless, to a much greater understanding of the processes and chemistry of these systems.

Several companies have now produced smaller versions of their sensors. As a result of several years' endeavour Neotronics have made certain advances in this area with a sensor that is approximately 10% of the volume of the standard sensor of this type. This has been achieved by the following: reduction in physical size of the electrodes; corresponding reduction in the output levels of the signal; better stability of the electronics; and better understanding of the linearity limitations of the sensors. This last point is the stumbling block which has hindered much development in this field. The basic problem is that in reducing the physical size of the sensors, the linearity and low temperature performance falls off. This is illustrated in Figs. 2 and 3.

Fig. 2 shows the linearity of a standard sensor (CO is chosen as a typical example) compared with a miniature sensor made from the same materials. Fig. 3 is the same comparison for the low temperature performance. It can be seen that in both instances the miniature sensor is inferior. It is not clear at first sight why this should be so, as the catalyst surface area at the working electrode and output currents are in proportion and thus one might expect identical performance. This can easily be established by examination of the coefficient of variation curves for these sensors which show the working electrode to have the same surface characteristics in both instances.

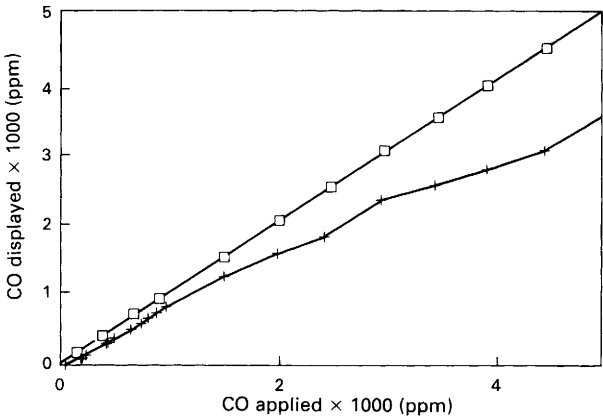


Fig. 2 Linearity of carbon monoxide sensor: □, standard; +, mini

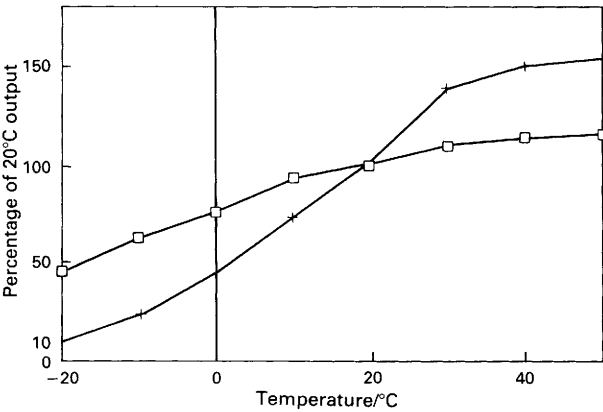
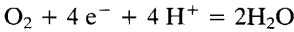


Fig. 3 Temperature behaviour of carbon monoxide sensors: □, standard; +, mini

An explanation of this effect was eventually discovered in the secondary electrode. The reaction occurring at the secondary is oxygen reduction:



In the case of the standard sensor, activity of the catalyst on the secondary electrode is sufficient to balance the working electrode reaction over the temperature range. However, with the miniature sensor, the activity of the secondary is insufficient, because of the fact that a wick carrying electrolyte into the electrode region reduces the available area disproportionately. A more active reduction catalyst at the secondary proved to be the required solution in this instance. This example serves to illustrate the difficulties encountered when simple size and geometry changes are made in this type of system.

Improvement in Electronics

The sensor will only be as good as the electronics used to drive it. Normally these sensors are supplied with a potentiostatic circuit of the type shown in Fig. 1. One variant of many systems can be seen, viz., the reference; in most instances pseudoreference potential is maintained against a reference diode potential by variation of the secondary potential. The signal, corresponding to the current due to oxidation of the gaseous species on the electrode surface of the working electrode, is measured by recording the potential developed across a sensing resistor. This particular arrangement suffers a drawback when large currents pass through the cell, owing to change in the potential at the reference electrode caused by the sensing resistor. This method has the advantage of not being subject to existing patents. One problem with this arrangement is that it is capable of giving the appearance of functioning when there is no sensor fitted, or worse, when the sensor has malfunctioned. Some work at Neotronics has resulted in an electronic circuit to check the status of the sensor by monitoring its response to a potential pulse. With an on-board microprocessor it is possible to monitor the performance of the sensor at frequent intervals and give an alarm when the performance of the sensor is no longer acceptable.

New Catalyst Formulations

Although some manufacturers produce their own catalysts from readily available starting materials, others rely on the major catalyst producing companies such as JMC, Degussa, Platina, etc. These companies have experience in the production of catalysts for the automotive and chemical industries, many of which are applicable to gas sensors. One promising area of research currently being investigated by Neotronics and the University of Salford is that of ion implantation techniques to produce true alloys and mixed catalysts. This work is in its early stages, but it is hoped that following an academic study by Colligon and co-workers¹⁰ there will be a development of useful technology in this field.

One example of the effect that the catalyst can have is in the use of ruthenium catalysts for chlorine reduction. Chlorine can be reduced on platinum on carbon electrodes, but this results in a massive cross-sensitivity of these sensors to hydrogen sulphide and sulphur dioxide, both of which are found in the same applications as chlorine (see Table 1). By changing the

Table 1 Percentage cross sensitivity

Gas	Sensor type				
	Cl ₂	H ₂ S	SO ₂	RuO ₂ ⁻	Ru
Cl ₂	100	-10	0	9	100
H ₂ S	-720	100	98	-22	-36
SO ₂	-170	13	100	0	-10
H ₂	-10	5	0	0	0
CO	-20	20	0	0	0
NO ₂	180	-16	-280	100	94
NO	5	0	0	-1	1

catalyst to ruthenium dioxide, the improvements in these figures are impressive. However, pure ruthenium has a very different set of cross-sensitivities. This is curious, as those involved in the automotive catalyst industry experience little difference in the reactivity of ruthenium dioxide and ruthenium.

Improvements in Basic Electrochemistry

As can be seen from the above discussion, the basic understanding of these systems is patchy. Some electrochemical sensors are well understood: others, however, are in need of much basic research. As noted above, Hitchman's work at Strathclyde¹¹ has shown that a detailed understanding of these systems is possible. For example, it has been assumed that sensors of this type³ are limited in their output current by the rate of diffusion of the measurand species through the entrance hole or pore and over the working electrode. As can be seen in Section 2, this is not the case. Here the limiting factor was found, empirically, to be the activity of the secondary electrode.

Conclusion

In this brief review of the work currently being undertaken at Neotronics and its co-operating bodies, one can see that it is a field which poses more questions than answers. It is a fertile

mix of both the academic quest for basic understanding and the commercial need for devices which will protect life and the environment. This field will need the attention of both industry and academia in order to understand the function of these sensors and design the next generation of instrumentation for safety and environmental protection.

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