BIOINSTRUMENTATION: RESEARCH, DEVELOPMENTS AND APPLICATIONS

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PREFACE

This reference text on bioinstrumentation focuses on the rapid emerging fields of modern biotechnology and advanced electronics. Emphasis is placed on the three major phases of effort from concept to market, namely, research, development and applications. The term *bioinstrumentation* is used increasingly to describe the unique joining of advanced electronics and modern biotechnology. The text consists of contributed chapters prepared by experts directly involved in bioinstrumentation research, development and applications. They describe novel biotechnology-based electronic sensors, such as those used for detection of very low levels of chemical and biological moieties. Rather than present traditional systems in which an electrical instrument is used simply as an observer, the bioinstrumentation described herein provides for direct assay and read-out of bioinstrumentation information. The authors also discuss new biotechnology-based electronic devices used for direct chemical and biological analysis, as in laboratory or process control instruments, and biosensors used to measure chemical and biological moieties in the body and in the environment.

It is hoped this reference text will be of keen interest to a wide audience, including instrument manufacturers, the electronics industry, and many of the increasing number of biotechnology firms, especially those looking for additional applications of their expertise. University professors and government officials, as well as industrial executives, all working in the area of modern biotechnology, advanced electronics, and instrumentation should find this text to be extremely valuable.

Donald L. Wise, Ph.D.

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PART I: BASIC BIOSENSORS

CHAPTER 1

BIOELECTROCHEMICAL SENSORS WITH LIVING CELLS

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1.1 INTRODUCTION

The metabolism of all living cells consists in processing a large variety of molecules, which obviously implies that the cells have developed very effective chemical transduction mechanisms. This capability is particularly evident in the case of unicellulars, but it is also shared by many cells of higher organisms.

The distinctive feature of chemical transduction in living cells is the precise spatial organization of those intracellular components involved in the ordered transformations of molecules. The vectorial character and the high yield of the biochemical reactions in living matter are a consequence of the fact that the catalysts are immobilized in their proper milieu. The same principle was adopted since the early seventies by the chemical analysts, when developing a third generation of chemosensors.

This development was not simply motivated by the desire to attain a better sensitivity, but also by those evolutive constraints like *speed*, *versatility*, and *low cost*, all imposed mainly by economic reasons. These characteristics are best met by electrical measurements and therefore, in order to introduce electroanalytical procedures, new *selective electrodes* have to be conceived. The first generation was that of ion-sensing electrodes, the most familiar being those for the measurement of pH, Na^+ and K^+. The development of synthetic permselective membranes for

dissolved gases led to the second generation, that of gas-sensing electrodes. A third generation that could be distinguished is that for organic compounds. Due to their chemical stability, such compounds are not electroactive. Therefore, prior to their detection, they must be catalytically split into simpler and more reactive molecules. In this way, the ever growing family of chemical *biosensors* have emerged.

When constructing electrodes for organic compounds, the main problem is to immobilize the enzyme in membraneous supports with a minimal loss in activity. However, even if this task is accomplished it entails restrictions in the dynamics of enzyme structures which in turn introduce severe limitations in the functioning of such electrodes. Thus the poisoning effect of various substances is enhanced, so that the cost of such enzyme electrodes will not meet the general economic demands.

A principally simple possibility to circumvent these problems are to use the enzymes in their natural milieu within the cell, that is to immobilize in membraneous supports whole living cells. This is the rationale of focusing the present article to this kind of biosensors, in an effort to reveal the problems related to such systems, as well as their merits and drawbacks. Accordingly, after discussing the basic design of the bioelectrochemical sensors with living cells, we present the sensors with tissue slices. We then tackle the sensing potentialities of the bacterial electrodes. Special attention is paid to the characteristics and technical constraints of these electrodes, aiming to substantiate conclusions concerning their actual and potential utilizations.

1.2 BASIC DESIGN OF BIOELECTROCHEMICAL SENSORS WITH LIVING CELLS

The construction of an electrode with whole living cells is very similar to that of enzyme-membrane probe. No matter if the final result obtained after the chemical transduction is a potential difference or an electrical current, the general requirement is to immobilize in a membraneous support a convenient catalyst, which in this case is represented by living cells, chosen for their metabolic ability to selectively transform an organic substrate into a much simpler and electroactive compound. The metabolical product is electrochemically sensed by the correspondent selective electrode with which the membraneous support containing the cells is attached. Therefore, this support has to be readily permeable for both the organic molecules which are detected, and for the metabolical product (or the oxygen) whose concentration is actually measured. Apart from these, it has to match the geometry of the product-sensing electrode.

The whole living cells, used as catalysts in such applications, are usually bacteria or even animal or plant tissue slices. When compared to enzyme electrodes, both have the great advantage of much less difficult immobilization procedure, and also the common disadvantage of a poorer specificity.

All the works reported until now offer as bacteria immobilization procedures their enclosure either in gels[1-3], or in porous materials,[4,6] or even the use of bacterial paste, i.e. a tiny amount of cells kept at the surface of the main electrode with the aid of a dialysis membrane.[7,8] Naturally, the use of tissue slices simplifies the immobilization requirements, the slices being sandwiched between an inner and an outer support membrane, and the ensemble being mechanically attached to the main electrode.

The immobilized cells metabolically transform the tested substrate into a chemically simpler compound for which the electrical detection has been already performed, so that the whole electro-chemical transduction is complete. Beside this, if aerobic bacteria with specific metabolic affinity towards the organic substance which has to be detected are available, the electro-chemical chemical transduction can then be obtained simply by monitoring the oxygen uptake.

A. Electrochemical Conversion Assembly

Figure 1.1 shows the general description of an experimental set-up involved in the detection of organic substances. This is accomplished with the aid of a specific bioprobe.

The electrodes used in such applications are most frequently the gas-sensing electrodes. Those which are now commercially available are for NH_3, CO_2, SO_2, NO_2 and O_2. Judging from their electrical response, all these can be ranged into potentiometric and amperometric electrodes.

The potentiometric electrodes are based on a pH electrode. The selective sensing capabilities are induced by a synthetic permselective membrane which closes an electrolyte solution to the surface of the electrode. The acid-base equilibrium of the solution is modified by the sensing molecule, so that a pH response appears, which is linearly dependent of the concentration for the given molecule in the tested medium.[9] Therefore, the response of such electrodes corresponds to Nernst equation:

$$\Delta\phi = \frac{RT}{zF}\ell n\left(\frac{a^{out}}{a^{in}}\right) \tag{1.1}$$

which reflect is the functioning of a pH electrode.

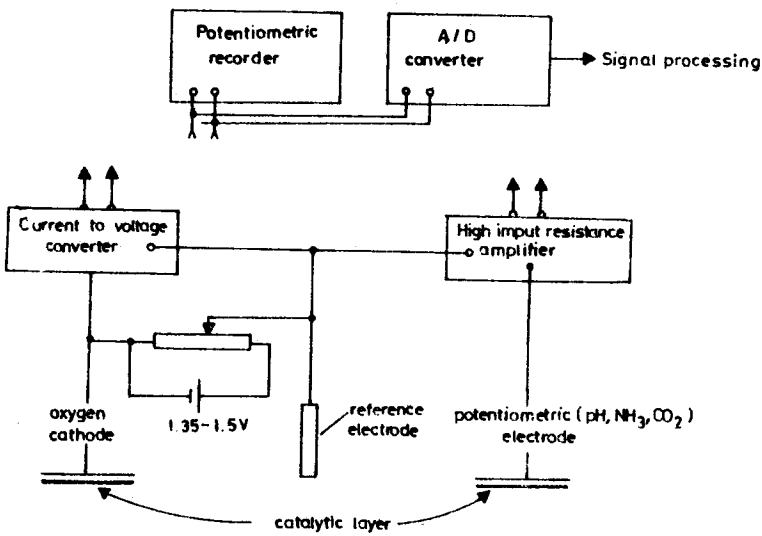

Figure 1.1: The measuring scheme involved in the operation of electrodes with bioprobes, having either current or voltage outputs

The typical amperometric sensor is the Clark oxygen electrode. Its functioning is based on the electrochemical reduction of the oxygen to water, at the surface of a conveniently polarized platinum wire.[10] An oxygen permeable membrane closes an electrolyte solution in which two electrodes are immersed. The potential of the platinum cathode is set versus a reference anode $(Ag/AgCl)$ at a value corresponding to the oxygen reduction to water, for practical reasons chosen in the range $0.6 - 0.8V$.

The reaction scheme is the following:

$$0_2 + 4H^+ + 4e^- \rightarrow 2H_20 \qquad (1.2)$$

(Because a progressive alkalinity of the electrolyte solution thus appears, a buffer is required).

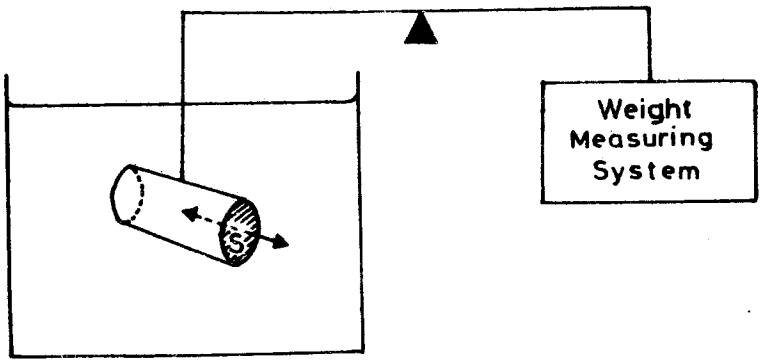

Figure 1.2: The principle of flow measurements by immersion weighing. Inside the immersed vessel, a volume of solution is separated by a membrane (whose permeability has to be measured) from the outer solution having different concentration or composition. The apparent weight of the vessel is measured by attaching it to one arm of a mechanical microbalance.

Due to its consumption, a continuous flux of oxygen occurs, proportional to its content in the tested medium. Thus, the electrical current between the platinum cathode and the reference anode is proportional to the concentration of the 0_2 dissolved in the solution outside the membrane.

B. Permeability Measurements

The permeability characteristics of the membrane supports for the organic compounds to be probed are a limiting factor of the biosensor, so that their measurement is a preliminary condition. There are various physical methods suited for measuring permeability characteristics of artificial membranes: the polarimetric methods, when optically active organic substances, like sugars, are involved, and the powerful tracer techniques, if the chemical compound can be conveniently marked. Apart from these commonly used procedures, we developed in our laboratory a totally different one, namely the immersion weighing method.[11,12] Its principle, presented in Figure 1.2, imposes the measurement of an "apparent" weight, the difference between the real one and the buoyant force, so that the use of the now popular electronic microbalances, irre-

spective of their sensitivity, is excluded.

The apparent mass of the cylinder depicted in Figure 1.2 is given by the equation:

$$M = m_0 + V_i\rho_i - (V_i + v_0)\rho_e \tag{1.3}$$

where m_0 is the real mass of the empty probe and its attachments, V_i is the inner volume of cross-section S, v_0 the volume of the cylinder itself, and ρ stands for densities. The outer solution has a volume $V_e^0 (\geq 100V_i^0)$ and the concentration c_e^0. As for every aqueous salt solution of concentration c : $\rho = \rho_0 + k \cdot c$, where ρ_0 is the density of the pure solvent, and $k = \partial\rho/\partial c$ is obtainable from tables of physical constants, the following equation is obtained:

$$M = m_0 - \rho_0 v_0 + kV_i(c_i - c_e) - kv_0 c_e \tag{1.4}$$

Solvent and solute volume flows J_w and J_s, which can be expressed by means of the correspondent molar permeability coefficients: $J = P(c_i - c_e)$, cause V_i to change:

$$\frac{dV_i}{dt} = S(P_w - P_s)(c_i - c_e) \tag{1.5}$$

The tendency to approach equilibrium causes c_i and c_e to have exponential time variations, so that from the above equations, the time course of M as a double exponential was obtained.[11]

$$M = M_0 + A(1 - \exp(-at)) + B(1 - \exp(-bt)) \tag{1.6}$$

where A, B, a and b are known combinations of the experimental parameters and of $P_w - P_s$. Even if their general expressions are in fact cumbersome to deal with in order to obtain $P_w - P_s$, approximations valid within different accuracy limits can be used. For our purposes, in view of the large V_e^0/V_i^0 ratio, and using as a first approximation $c_i - c_e = \text{const.} = c_i^0$ (the outer solution is pure water), we get from the above equations the practical formula:

$$\frac{dM}{dt} = kS(c_i^0)^2(P_w - P_s) \tag{1.7}$$

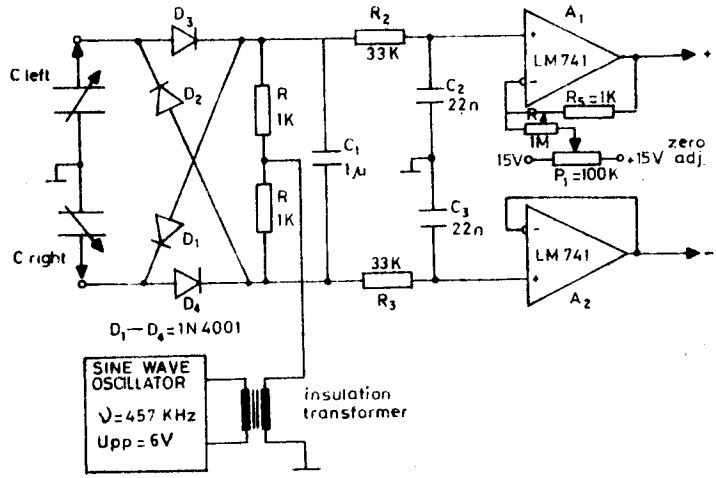

Figure 1.3: Bridge circuit of the differential capacitive transducer. The output signal is approximately proportional with the mechanical displacement δ; $U_0 = 2\sqrt{2}u_i R^2 4\pi^2\nu^2 C_0^2\delta/d$, where: $u_i = 6V$ is the peak-to-peak input signal, with $\nu = 457$ kHz, given by the oscillator; d = 15 mm is the equilibrium distance between the pan and the underneath textolite board; C_0 is the correspondent capacitor value ($\approx 10pF$).[12] (Reproduced with permission)

The diffusional permeability for water alone P'_w can be easily obtained with the same method, if one uses deuterium oxide (heavy water,^2H_2O) for preparing the internal solution, and normal water for preparing the external solution, with the same solute composition. In this case, the time variation of the apparent weight of the cylinder is due to the exchange between external water and internal isotopic water, so that:

$$\frac{dM}{dt} = -P'_w kS\chi(1 + V_i^0/V_e^0) \tag{1.8}$$

Here, χ is the adimensional ratio, expressed in % v/v, of the $^2H_2O/H_2O$ mixtures.

In order to obtain an electrical response, the safest way to convert the mechanical response of the microbalance is by means of the variations in the capacitance of two capacitors formed by placing a copper-plated textolite board under each pan. Obviously, as the capacitance changes are opposite, the capacitive transducer is of a differential type (Figure

1.3).

The above presented transducer allows the measurements of mass flows of $10^{-6} Kg \cdot m^{-2} \cdot s^{-1}$, so that the method is well suited for quantitatively expressing the permeability characteristics of those artificial membranes used as supports in conceiving bioselective electrodes. Its merits are of being applicable for any organic compound, with no pretreatment, and the continuous electrical response which is obtained is well suited for on-line processing.

To sum up the above discussion, it results that the following general scheme is employed for all biosensors with living cells: the probed organic compound enters the compartment, in which the living cells are kept, here it is metabolized and electrochemically active molecules are produced. These are in turn probed by a conventional electrode.

1.3 SENSORS WITH TISSUE SLICES

Starting from the same basic idea, namely to use for the electrochemical transduction the enzymes kept in their natural milieu (the cell), biosensors based on tissue slices showing high catalytic activity have been developed. They are the "youngest" members of the continuously growing family of biosensors, as the first attempt on this line – a hybrid electrode for arginine, based on a thin beef liver tissue slice together with isolated urease, aposed onto the surface of an NH_3- gas electrode – has been presented in 1978.

Since that, several attempts (Figure 1.4) on this line have been reported, the most commonly used tissues being the porcine kidney, like in the biosensor for glutamine reported by Rechnitz et al. [13] or in that one for glucosamine 6-phosphate[14], the rabbit muscle, which presents high catalytic activity for adenosine $5'-$ monophosphate[15], the rabbit liver, which was used in a membrane electrode for guanine[16], or the mouse small intestine used for the electrochemical probing of adenosine.[17]

Not only animal, but also plant tissues were used for constructing membrane biosensors. Thus, a piece of the mesocarp layer from the growing zone of a yellow squash, attached to a carbon dioxide gas electrode, could serve for the electrochemical detection of glutamic acid[8]. Another example is the electrode for pyruvate reported by Kuriyama et al.[19], based on the use of maize-kernel slices in conjunction with a CO_2 - gas electrode.