Problems in Adapting a Glucose-Oxidase Electrochemical Sensor into an Implantable Glucose-Sensing Device

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Taking into account the analytic patterns of different types of glucose-oxidase electrochemical sensors, the specific problems which have to be solved for such a sensor to be implantable are outlined. Of particular interest is the lifetime of enzymatic membrane and the dependence of the sensor responses to oxygen concentration and hydrodynamics. DIABETES CARE 5: 184–189, MAY–JUNE 1982.

The continuous in vivo monitoring of the glucose concentration in blood should improve greatly treatment of diabetes. Such an implantable glucose sensor, needing neither blood withdrawal nor addition of reagent, should lead to an implantable artificial pancreas, i.e., a glucose-controlled insulin delivery system.[1,2]

The so-called enzyme electrodes, which in a small volume combine the enzyme reaction and electrochemical detection, present the highest selectivity and versatility for such reagentless metabolite determinations.[2–4] Among them, glucose electrodes have been the most extensively studied and have led to, at least, two commercially available devices, i.e., Yellow Springs Instrument Model 23 A[5] and Solea Tacussel model ENGL 1,[6] based on the research work of Clark et al.,[7,8] and Thévenot, Coulet, Gautheron et al.,[9,10] respectively. All of these electrodes use β-D-glucose oxidation by dissolved oxygen in the presence of immobilized glucose oxidase (GOD, E.C. 1.1.3.4): they differ by their GOD immobilization technique and by the type of electrochemical detector pressed against the GOD membrane or film. This article analyzes the contribution of the different parts of the glucose-oxidase electrochemical sensor for the control of its analytical patterns and discusses the specific difficulties which have to be solved for such a sensor to be implantable.

RESULTS

Glucose-oxidase enzymatic membranes. In several commercially available glucose analyzers (Beckman, Technicon, Leeds and Northrup, Owens-Illinois) GOD is either a continuous flowthrough with sample and buffer or immobilized on a reaction loop or cartridge.[11] For such an analyzer to become a reagentless specific electrode, GOD has to be immobilized as a thin layer pressed against the electrochemical detector. Since the pioneer work of Clark et al.[7,8] and Updike et al.[12,13] on glucose electrodes, five different types of immobilization procedures have been used and throughly studied (Table 1). Although similar immobilization procedures yield very different electrode stabilities, a few comments can be made on the published data: (1) the higher the temperature, the lower the stability and no long-term experiments have been performed at 37°C, (2) GOD covalent binding, either by copolymerization or by reaction with activated membranes, yields the most stable glucose electrodes, (3) nonenzymatic proteins, such as albumin, gelatin, or collagen are used by most of the authors; indeed, such a proteinaceous environment seems to enhance GOD stability.[14]

In fact, the stability of such GOD-membranes should be examined from three different view points: mechanical, chemical and enzymatic (Table 2). A poor mechanical stability, encountered, for example, with thin copolymerized membranes, will affect the overall stability of the glucose electrode. Further difficulties appear for an implant: biocompatibility of the membrane and of hydrogen peroxide generation—an inactivator of many enzymes[15], encapsulation by fibroblasts and giant cells,[16] and finally, long-term stability at 37°C in human fluids or tissues.

Oxidation of glucose by dissolved oxygen is an irreversible process (Table 3): thus, on the contrary to sensors working at equilibrium, such as ion-selective electrodes, glucose electrodes reach a steady state in the presence of glucose. This steady state may be controlled either by the oxidation reaction with high temperature dependence (6–10%/°C) or by substrate diffusion with low temperature dependence (2–4%/°C).[34,35,37,39,47,50]

Under such heterogeneous kinetics, the glucose electrode consumes what it is supposed to monitor: this is a characteristic common to Clark's oxygen sensor. Whatever, the electrochemical detector associated to the GOD membrane, factors

TABLE 1
Immobilization procedure of glucose oxidase and stability of glucose electrodes

Immobilization procedure	Membrane material	Storage temp. (°C)	Electrode stability	Authors
Solution entrapment	Cellophane	38	2 mo	Clark et al.[7,8,17]
	Cellophane	25	1–2 wk	Guilbault et al.[18,19]
	Cellophane	Un.	60 h	Mindt et al.[20,21]
	Cellulose triacetate			
	Polyvinyl alcohol			
	Cellophane	Un.	Un.	Mahenc et al.[22]
Polyacrylamide gel	No	Un.	3 wk	Updike et al.[12,13]
	Cellulose acetate	25	8 days	Notin et al.[23]
		4	3 mo	
	Cellophane	25	6 mo	Guilbault et al.[18,24]
	Cellophane	Un.	1 wk	Mosbach et al.[25]
Copolymerization	Polyacrylamide polyacrylic ac.	25	10 mo	Guilbault et al.[18]
	Albumin + glutaraldehyde	25	4 mo	Guilbault et al.[26,27]
	Albumin + glutaraldehyde	Un.	2 mo	Tranh Minh et al.[28]
	Albumin + glutaraldehyde	Un.	3 wk	Scheller et al.[29,30]
	Albumin + glutaraldehyde	37	2 days	Wingard et al.[31]
	Collagen + glutaraldehyde	25	6 mo	Y.S.I.[5,11]
	Gelatin + glutaraldehyde	4	7 mo	Thomas et al.[32]
	Triazenylcellulose	Un.	2 wk	Martiny et al.[33]
Covalently bound activated metal	Carbon paste	5	1–2 mo	Wilson et al.[34,35]
	Platinum			
	Graphite			
	Glassy carbon	4	1–2 wk	Thomas et al.[36,54]
Covalently bound activated membrane	Teflon + albumin + formaldehyde	37	2 wk	Updike et al.[37]
		4	6 mo	
	Collagen (acylazide)	20–30	6 mo	Thévenot et al.[9,10,38]
		4	30 mo	

Un., unknown

TABLE 2
Stability of glucose-oxidase membranes

- Factors affecting GOD membrane stability
  - Mechanical
    - Distance membrane/electrochemical detector
    - Permeability to glucose, oxygen, hydrogen peroxide
    - Rheological parameters
  - Chemical
    - Resistance towards hydrolases and proteases
    - Resistance to microbial degradation
  - Enzymatical
    - GOD thermal denaturation
    - GOD chemical denaturation
    - GOD washing out
    - Effect of microenvironment and hydrogen peroxide generation
- Problems for implants
  - Biocompatibility
    - GOD membrane
    - Hydrogen peroxide generation
  - Implant encapsulation by fibroblasts and giant cells
  - Long-term mechanical, chemical and enzymatic stability of GOD membranes at 37°C, in whole blood, lymph or tissue

TABLE 3
Heterogeneous kinetics with glucose-oxidase membranes

- Equilibrium or steady state?

  $$\text{D glucose} + O_2 \xrightarrow{GOD} \text{gluconate} + H_2O_2 + H^+$$

  at pH 7.0 $K_{app.} = 1.6 \times 10^{24}$
  - Two possibilities:
    - End point titration in flow-through reaction loops of glucose monitors
    - Steady state in glucose electrodes
- Rate limiting step: two possible regimes
  - Mass transport:
    - Either external diffusion (flow rate)
    - Or internal one (permeability to glucose and oxygen)
  - Chemical reaction limited by glucose and oxygen
- Problems for implants
  - Stability of mass transfer reactions
    - Blood or fluid flow rate
    - Possible membrane coating or encapsulation
  - Stability of oxygen level in blood, lymph, or tissue.

affecting external diffusion, i.e., fluid flow rate near the membrane, or internal diffusion, i.e., permeability to substrates, should be maintained constant as well as oxygen concentration level in or near the membrane. In the case of implantable glucose electrodes both conditions are difficult to realize: the latter may be indirectly and partially fulfilled by using a GOD membrane or an external membrane much more permeable to oxygen than to glucose.[40,41] Table 3 outlines these considerations.

Electrochemical detectors in glucose electrodes. Oxidation of glucose in the presence of a GOD membrane may be monitored by the evolution of three reaction constituents, i.e., oxygen depletion, gluconic acid and hydrogen peroxide formation.

As shown in Table 4, detection of gluconic acid via a pH electrode seems to be the worst method resulting in poor sensitivity, selectivity and linearity of calibration curves.

Oxygen and hydrogen peroxide detection have both been used by many different research groups.

A Clark-type oxygen electrode is insensitive to all types of interfering substances, they are obviously very sensitive to variations of partial pressure of oxygen within the fluid in contact with the electrode, unless a differential system is used, i.e., two electrodes differing only by their GOD activity.[12,13,37,44a,44b] One should also remember that the signal related to glucose concentration is derived from the diminution of the initially high current of the oxygen electrode: such systems are less sensitive, unless a well-balanced differential device is used.

Amperometric detection of enzymatically generated hydrogen peroxide is probably the most developed system. It seems to be the only one present in commercially available glucose electrodes.[5,6] Starting from a very low background current in the absence of glucose, this detector is very sensitive. The lowest detection limit reaches 10 nM[9,10] and a higher linear range has been obtained for the calibration curve, i.e., 2.3–4.5 concentration decades.[9,10,18]

Since hydrogen peroxide amperometric detection is very

TABLE 4
Electrochemical detectors used in glucose electrodes

Electroch. detector	Added reactant	Calibration curves: Type	Calibration curves: Detect limit.	Calibration curves: (Nb. of conc. decades)	Precision (%)	Authors
pH (gluconic ac.)	No	pH-pH_0 vs. log C	10^{-3}	Non lin.	Un.	Mosbach et al.[25]
		pH-pH_0 vs. C	2×10^{-3}	0.5	Un.	Enfors et al.[45]
Clark (O_2)	No	$I_0 - I$ vs. C	10^{-4}	2	Un.	Clark et al.[7]
(D)	No	$I_0 - I$ vs. C	10^{4}	2	2–4	Updike et al.[12,13,37]
	No	$I_0 - I$ vs. C	2×10^{-4}	1.7	Un.	Notin et al.[23]
	O_2	$I_0 - I$ vs. C	2×10^{-3}	1	2	Than Minh et al.[28]
	No	$I_0 - I$ vs. C	or 10^{-3}	0.5	Un.	Thomas et al.[42]
	No	$\left[\frac{dI}{dt}\right]_{max}$ vs. C	10^{-4}	Non lin.	1–5	Thomas et al.[32]
	No	$I_0 - I$ vs. C	5×10^{-4} or 5×10^{-3}	0.7	Un.	Gondo et al.[43]
Galv. cell (D)	No	$I_0 - I$ vs. C	3×10^{-3}	0.5	Un.	Bessman et al.[44]
Pt cathode (O_2 and H_2O_2)	No	$I_0 - I$ vs C	5×10^{-5}	1.3	Un.	Guilbault et al.[26]
(D)	No	I vs. C	10^{-4}	2	Un.	Clark et al.[8]
Pt Anode (H_2O_2)	No	$\left[\frac{dI}{dt}\right]_{max}$ vs. C	5×10^{-5}	1.9	2	Y.S.I.[5,46]
	No	I vs. C	5×10^{-4} or 5×10^{-3}	2	5	Scheller et al.[4,29,30]
(D)	No	I vs. C	10^{-5}	1.5	Un.	Martiny et al.[33]
	No	I vs. C	5×10^{-4}	2.3	Un.	Guilbault et al.[18,27]
(D)	No	I vs. C	10^{-8}	4.5	2–4	Thévenot et al.[9,10]
	No	I vs. C	10^{-7}	4	1–3	Solea Tacussel[6]
Pt Cath.	I^-	I vs. C	10^{-4}	1.2	Un.	Mell et al.[47,48]
(I_2 for H_2O_2)	+ Mo(VI)		5×10^{-4}	0.9	2	Wilson et al.[34,35]
I.S.E.	I^-	E vs. log C	3×10^{-4}	0.5	Un.	Guilbault et al.[19]
(I^- for H_2O_2)	+ peroxidase					
Pt anode	quinone	I vs. C	10^{-3}	1.3	6	Williams et al.[49]
(O_2 replacement)	2-6 DPIP	I vs. C	Un.	Un.	Un.	Mindt et al.[20,21]
	$Fe(CN)_6^{3-}$	I vs. C	5×10^{-4}	1.1	5	Mahenc et al.[22]

Un., unknown; (D) differential detector; 2-6 DPIP, 2-6 dichloroindophenol.

sensitive to naturally occurring electron donors, such as ascorbate, urate, tyrosine, etc., two methods have been developed to increase the selectivity of the glucose electrode towards such electrochemically interfering substances. Either the response is compensated by a nonenzymatic detector[8–10,33] or the platinum anode is covered by a cellulose acetate membrane with pores that will exclude ascorbate and most other potential interfering substances in 14 times diluted blood samples.[5]

Table 4 also presents a few other electrochemical detectors either detecting indirectly hydrogen peroxide by its reaction with added iodine,[19,34,35,47,48] or replacing oxygen by other electron acceptors and detecting amperometrically their reaction products.[20–22,49] All these alternatives present no interest for in vivo measurements since they require addition exagenous reagents.

Because of its lower dependence on oxygen concentration in the sample, we favor hydrogen peroxide detection in potentially implantable glucose electrodes. As seen above, either a selection or a compensation of interfering electron donors have to be realized. Furthermore, as glucose levels in blood, i.e., 50–1000 mg/dl or 3–55 mM, are higher than the apparent K_M or $S_{0.5}$ of typical glucose oxidase membranes, i.e., 4–10 mM,[34,47,51] calibration curves may not be linear. If one accepts a decrease of the electrode sensitivity, these calibration curves can become linear in a much higher concentration range, if an external diffusion barrier to glucose is placed in front of the GOD membrane.[4]

DISCUSSION

Taking into account the stability, selectivity, sensitivity, and linearity of glucose-oxidase electrochemical sensors, in vitro determinations of glucose seem relatively easy to perform with a 2–5% precision. If such a sensor is to be implanted into a blood vessel or in tissue, a large number of problems arise and some of them are not yet solved. Indeed, besides the general problem of implanting a physico-chemical sensor[16] that generates hydrogen peroxide,[15] there are many difficulties to take into account: (1) GOD membranes should be resistant to physical, chemical, and enzymatic denaturation for several weeks if not months of implantation at 37°C, (2) hydrodynamic properties of the membrane and of the membrane-solution interface, i.e., the fluid flow rate should not vary in the electrode vicinity, (3) electrode response should be independent of the variations of oxygen level inside the fluid: permeability of oxygen in the membrane should be higher than for glucose, (4) electrode response should vary linearly with glucose level in the hyper- and hypoglycemia range, i.e., from 20 to 2000 mg/dl or 1–100 mM, and have a low temperature dependence (2–4 %/°C), (5) if amperometric detection is used, which can be appreciated for its simplicity, sensitivity and proportionality between signal and glucose concentration in a very large range,[9,10] the electrode response should be made insensitive to endogenous electroactive electron donors by using a differencial device, i.e., a compensating electrode,[8–10] (6) the whole system, membrane plus detector, should require minimal calibration and zero adjustment, (7) finally, the scaling down of the glucose electrode should not modify the geometrical, physical, and enzymatic characteristics which control its analytical properties.

A possible strategy for solving most of these problems consists in the detailed study of the properties of the GOD membrane either freely stirred or mounted on the electrochemical detector. In a previous paper, we have presented a simple device for monitoring the various hydrogen peroxide fluxes generated by the GOD membrane or flowing through it.[51] Following Wilson's[34,35] or Gough's[40,52] approach to such heterogeneous kinetics, the influence of hydrodynamics should be carefully studied.

Last but not least one should pay special attention to the overall stability of the glucose electrode and especially of its GOD membrane, taking into account the relations between GOD stability, nature of the membrane and the way GOD has been coupled. This is obviously an acute problem if the glucose electrode is to be implanted for more than 24–48 h. Much improvement has been made in the last decade,[53] but still several laboratories are active in that field.

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